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Synthesis and Evaluation of PPARδ Agonists That Promote Osteogenesis in a Human Mesenchymal Stem Cell Culture and in a Mouse Model of Human Osteoporosis

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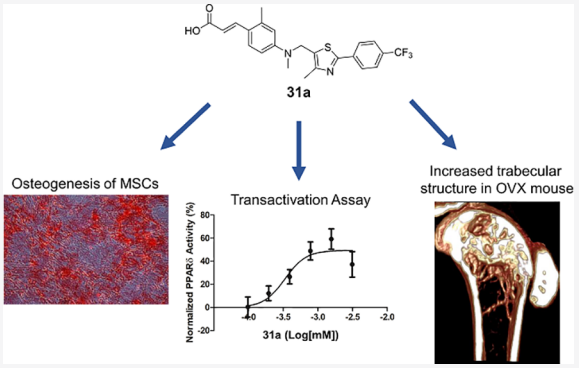
ABSTRACT: We synthesized a directed library of compounds to explore the structure−activity relationships of peroxisome proliferator-activated receptor δ (PPARδ) activation relative to mesenchymal stem cell (MSC) osteogenesis. Our scaffold used para-substituted cinnamic acids as a polar headgroup, a heteroatom and heterocycle core connecting units, and substituted phenyl groups for the lipophilic tail. Compounds were screened for their ability to increase osteogenesis in MSCs, and the most promising were examined for subunit specificity using a quantitative PPAR transactivation assay. Six compounds were selected for in vivo studies in an ovariectomized mouse model of human postmenopausal osteoporosis. Four compounds improved bone density in vivo, with two (**12d** and **31a**) having activity comparable to that of GW0742, a well-studied PPARδ-selective agonist. **31a** (2-methyl-4-[*N*-methyl-*N*-[5-methylene-4-methyl-2-[4-(trifluoromethyl)phenyl]thiazole]]aminocinnamic acid) had the highest selectivity for PPARδ compared to other subtypes, its selectivity far exceeding that of GW0742. Our results confirm that PPARδ is a new drug target for possible treatment of osteoporosis via in situ manipulation of MSCs.



INTRODUCTION

The human skeleton continually renews itself in response to the physical demands of everyday life.[1] This renewal process involves the resorption of old bone by osteoclasts followed by the formation of new bone by osteoblast proliferation. The replacement of old bone with new bone is important for maintaining structural integrity and reducing the risk of fracture.[2] In healthy adults, osteoclast function is balanced by osteoblast activity, resulting in bone homeostasis indicated by unchanging bone mineral density (BMD).[3] The bone remodeling process is controlled by several signaling pathways and hormones including sex hormones. A reduction in sex hormones, such as estrogen in postmenopausal women, results in overactivation of the osteoclasts and the reduction of BMD, which can lead to the development of osteoporosis.[4,5]

Treatment options for osteoporosis are categorized pharmacologically as either antiresorptive or anabolic. This classification depends on whether the pharmaceutical acts through inhibition of the osteoclast or stimulation of osteoblast proliferation, respectively. Clinically, the most prominent front-line therapy involves the inhibition of bone resorption using the bisphosphonate class of drugs. These drugs act through encouraging apoptosis of osteoclasts, therefore decreasing bone turnover and resorption.[6] Although bisphosphonates have been shown to be effective at preventing fracture,[7] they carry possible long-term side effects including an increased risk of atypical femoral fracture and osteonecrosis of the jaw.[8,9] While disputed in the literature, these links to bisphosphonates are thought to be the result of an accumulation of old bone due to the decreased rate of bone turnover.[8]

Other antiresorptive therapies include hormone replacement therapy (HRT), which functions in a way that mimics the effects of estrogen. HRTs include estrogen-only formulations or estrogen−progesterone formulations and are effective at preventing bone loss by increasing osteoprotegerin, a glycoprotein involved in reducing osteoclast activation.[9,10] However, there is evidence that these therapies increase the risk of blood clots, stroke, and heart attack. The estrogen−progesterone combination may also increase the risk of developing breast cancer.[10,11]

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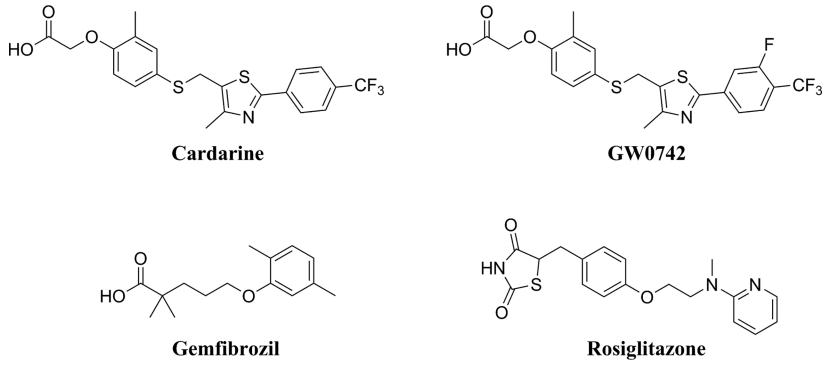

Figure 1. Chemical structures of classical PPAR agonists having selectivity for δ (cardarine and GW0742), α (gemfibrozil), and γ (rosiglitazone) subtypes. Compounds are depicted with their similar acidic "head groups" located on their western edges. In addition to having an acidic head, the identical connecting portion "core" centrally located in cardarine and GW0742, along with the similar lipophilic "tail" groups located on their eastern edges, influenced the design of our initial PPARδ probes.

Osteoclasts play an important role in bone homeostasis by removing older necrotic bone tissue. Decreasing osteoclast function with antiresorptive agents can therefore be problematic over time due to an accumulation of old bone.[12−14] Alternatively, anabolic agents avoid this risk by encouraging bone formation from an increase in osteoblast proliferation and function.[15] Current anabolic therapies include teriparatide, abaloparatide, and romosozumab. Romosozumab, the most recently approved anabolic agent, has a mechanism of action involving inhibition of sclerostin, a Wnt-β-catenin signaling inhibitor.[16,17] This suggests that increasing Wnt signaling can have positive effects toward increasing bone density and treating osteoporosis. This recent finding provides clinical relevance for our preliminary research studying peroxisome proliferator-activated receptor δ (PPARδ) in stem-cell cultures because others have shown that the activation of PPARδ plays a central role in bone remodeling though directing an increase in Wnt signaling.[18] Deploying small-molecule agents to potentially manipulate stem cells *in situ* for therapeutic applications was highlighted within the field of medicinal chemistry about 10 years ago.[19] Progress in devising these types of medicinal chemistry strategies has continued,[20] and recently it was noted that for stem-cell technologies in general, "the only constant is change", while this promising field rapidly evolves in many directions.[21]

PPARs are a family of ligand-activated nuclear receptors that play a role in regulating cell differentiation and development in addition to lipid and glucose metabolism.[22] This family includes three subtypes, PPARα, PPARγ and PPARδ. In regard to mesenchymal stem cell (MSC) differentiation, the activation of PPARγ has been shown to increase adipogenesis at the expense of osteogenesis,[23] whereas the activation of PPARδ increases osteogenesis and bone formation.[18,24] The negative aspects of PPARγ activation can be seen in the clinic when considering the long-term side effects of the thiazolidinedione (TZD) class of drugs. The TZDs are PPARγ agonists and have been associated with an increased risk of fracture and an increased risk of developing osteoporosis.[25]

Currently, there are no selective PPARδ agonists approved for use in the marketplace. This is due in part to lingering concerns previously raised by cardarine (Figure 1), a selective and potent PPARδ agonist[26] that was entered into clinical development to treat metabolic diseases. Cardarine's Phase II trials were halted in 2007 when long-term toxicity studies in rodents demonstrated carcinogenicity at the 2 year juncture.[27,28] Despite warnings and its prohibition by the World Anti-Doping Agency, cardarine continues to be abused by athletes.[29] Notoriously known as "endurabol," it is thought to enhance physical performance.[30] There has been considerable research directed toward understanding the mechanisms for carcinogenicity associated with certain PPARα and γ agents, but "PPARδ agonists have not been extensively investigated and have produced varying and conflicting results".[31] Thus the "biological function of the PPARδ"[32] pathway when activated with agents like cardarine that could be directly or indirectly initiating and/or promoting[31] rodent "tumorigenesis remains complex, conflicting"[32] and, as of today, is still undefined. Furthermore, the connection of PPARδ-related *in vitro* and *in vivo* models to human cancer has been questioned[31−33] and, instead, it has been noted that these particular "adverse effects may be linked to significant off-target" interactions of the ligands when present at "very high doses".[33] Specific studies into the role of PPAR ligands as potential anticancer agents have likewise produced contradictory results, and there is no scientific consensus on whether this pathway's activation or inhibition can be used to effectively promote or prevent human disease.[32,33] For the medicinal chemist, this situation is accompanied by a paucity of systematically delineated toxicity-associated structure−activity relationship (SAR) data. At best, it can be noted "that synthetic ligands that target the same PPAR subtype may not always possess comparable efficacy, safety profiles, and clinical outcomes".[32] From this backdrop, the goals of our early-stage drug discovery program became four-fold: (i) Assess if small-molecule drug-induced activation of the PPARδ receptor in stem-cell cultures is a viable mechanistic target to increase bone density; (ii) Establish preliminary efficacy-related SAR associated with this novel indication wherein certain molecular features in our probe compounds have been systematically altered from those present in cardarine; (iii) Determine if efficacy can be maintained *in situ* by using an animal model of human osteoporosis to study a series of representative compounds; and, (iv) Identify a promising group of candidate compounds that can also serve as probes for long-term toxicity SAR. In line with the guidelines initially stipulated by the U.S. FDA,[34] it is our view that long-term toxicity studies will need to be initiated in the future as an early step toward the preclinical development of any PPARδ-derived small-molecule drug for the potential prolonged treatment of any chronic disease.

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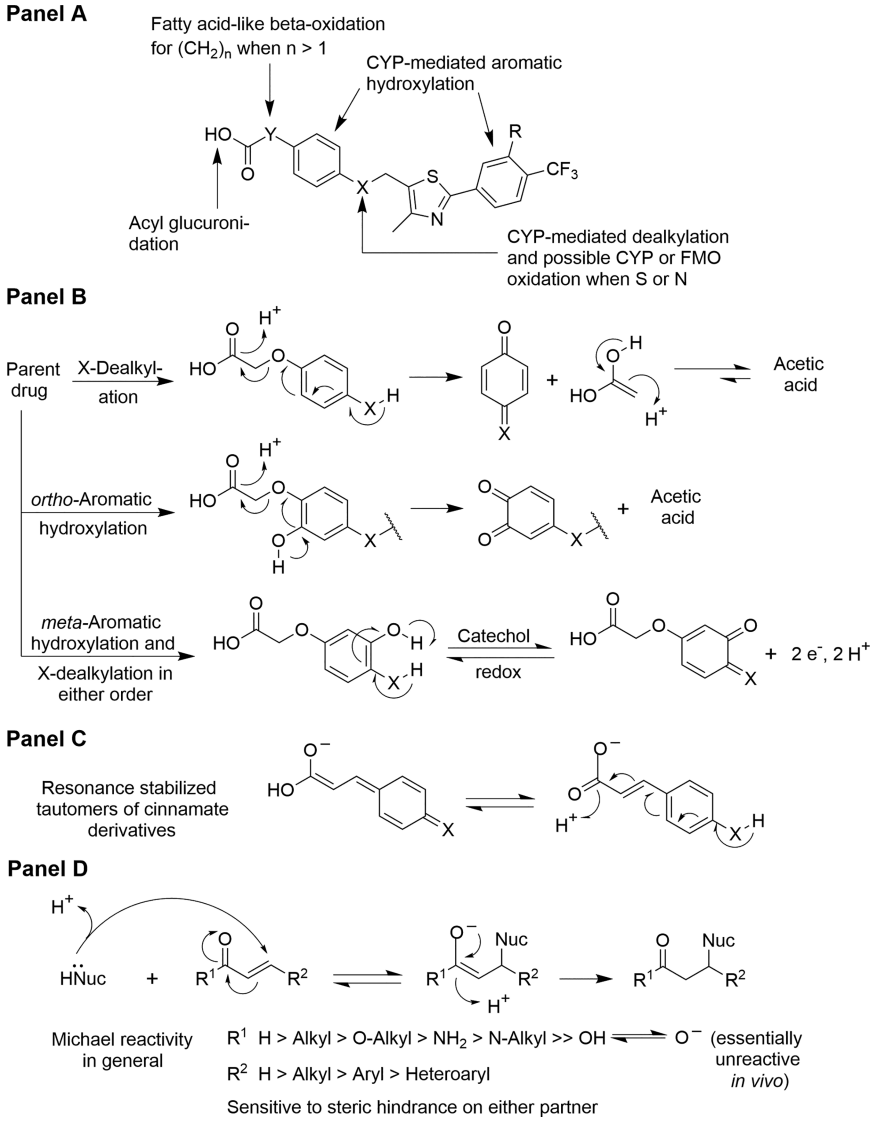

Figure 2. Metabolism considerations used during the design of PPARδ probes. (A) Generalized composite of metabolic possibilities for the typical three-ring scaffold. When β-oxidation is prevented, metabolic switching to aromatic hydroxylation followed by glucuronidation and acyl glucuronidation becomes the most probable biotransformation pathway. (B) Less probable and thus less predominant pathways should they happen to occur. As shown, however, these pathways could potentially generate reactive metabolites that might be responsible for off-target toxicity upon long-term dosing. (C) Stability present in cinnamic acid that circumvents the reactivity of its potential metabolites analogous to those in panel B. (D) Typical Michael reaction that can sometimes cause off-target toxicity by this alternate pathway. Note that cinnamic acid resides at the lower end of reactivity in terms of both R^1 and R^2 adducts. See the additional discussion in text for all panels.

During consideration of our manuscript's submission, two extensive review articles were published. The first by Takada and Makishima surveyed PPAR agonist and antagonist ligand patents (2014−2020).[35] In addition to the long-standing possibility of treating metabolic syndromes like dyslipidemia and type 2 diabetes, many synthetic ligands are being developed to potentially treat new indications like mitochondrial disease, inflammatory/autoimmune disease, cancer and neurological disorders. These authors also offer a brief discussion about "bone disease" while referring to the earliest[36] of our four issued patents in this arena.[36−39] The second paper by Kadayat et al. provides an excellent overview and perspective for the medicinal chemistry associated with PPARδ agonists all the way from drug design and fundamental SAR, to several drug candidates now undergoing clinical study for a variety of diseases.[40] They likewise mention our patent for bone disease relative to new indications.[36] These reviews will be discussed further in the section entitled Translational Consideration of Toxicity Relative to Use in Humans.. At this point, however, it is interesting to note that both reports clearly demonstrate that the PPARδ field has finally begun to again push forward, this time with many new compounds for several new indications. Furthermore, the summary offered by Kadayat et al. refers to the ambiguous nature of the historical toxicity, exactly as we do in terms of the PPARδ pathway's questionable involvement and the lack of chemical structural dependency when relying upon only a single compound's anecdotal data,[41] namely, what was observed for cardarine several years ago.

In addition to the previously noted increase in Wnt signaling and bone formation reported by others while using cardarine,[18] members of our group simultaneously reported that GW0742 can attenuate the negative effect that nicotine has on MSC osteogensis.[24] GW0742 (Figure 1) is another potent and selective PPARδ agonist[26,42] that has nearly the same structure

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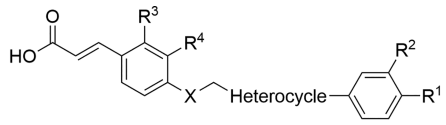
as cardarine. The three-ring scaffold displayed by both of these structures is significantly different from that present in either a classical PPARα or PPARγ agonist (respectively represented in Figure 1 by gemfibrozil[43] and rosiglitazone[44]). This distinct scaffold has been referred to as having an acidic "head", a connecting "core", and a lipophilic "tail".[40,45] We have used it as the starting point for the design of our PPARδ-agonist-directed library. To distinguish the library from cardarine, we immediately considered replacing the sulfur-connecting atom with an oxygen or nitrogen atom, both of which have precedent for retaining efficacy.[26,46−49]

Part of our directed library compound design was also based on metabolic and pharmacokinetic (PK) considerations. Cardarine is reported to have a half life of ca. 10 h in rats and nearly double that in humans,[50] but its detailed disposition has not been fully reported, in particular, with regard to the possibility that it could produce low levels of localized toxic metabolites that become insidious upon long-term dosing. A composite of metabolic possibilities for PPARδ ligands is summarized in Figure 2A.[26,45] By analogy to other agents,[45] cardarine's aryl-thioether, head-core-linkage region resides in a metabolic soft spot[51,52] such that the aryl moiety may be susceptible to CYP-450-mediated aromatic hydroxylation, whereas the sulfur may be subject to CYP-450-mediated S-dealkylation and to CYP-450 or flavin-containing monooxygenase (FMO)-mediated S-oxidation.[53] The latter can complicate PK profiling because the S atom becomes asymmetric, whereas the dealkylated metabolite could contribute to toxicity because of the resulting aryl-sulfhydryl group's inherent reactivity.[54] The dealkylated metabolite also sets up the possibility for it to form a sulfur version of the even more reactive *para*-quinone-type chemical species[55] upon the loss of acetic acid (Figure 2B, upper arrows) via a pathway that could initially involve the transient formation of the acid's tautomer.[56] Initial rescue from such reactive intermediates in mammals by glutathione can be compromised by acute overdose or by continual exposure in sensitive tissues, the latter potentially making a relevant association with cardarine's carcinogenicity being observed only after long-term dosing.[57,58]

Although the steps shown subsequent to metabolic dealkylation might be considered to occur spontaneously,[59−61] we have not found any evidence to support this toxic series of conversions in the case of PPAR ligands like cardarine or even within the chemical literature associated with any of the simpler 2-(4-X-phenoxy)acetic acid compounds. Thus if quinone-like materials are formed at some low level, then this is likely prompted by some additional biochemical interaction if not by another complete biotransformation step such as the previously mentioned possibility for aromatic hydroxylation leading to catechol-like arrangements wherein redox chemistry becomes applicable[59,62,63] (Figure 2B, lower arrow). One can imagine that the metabolite resulting from a common CYP-450-mediated X-dealkylation for any of the three heteroatom substitutions could also follow the proposed toxic pathways shown in Figure 2B. In this regard, whereas less data is available for S-dealkylations, this initial step for the constructs having X = O and N is known to be highly sensitive to steric inhibition such that this type of metabolic-driven toxicity compared with X = S is likely not highly probable when simultaneously considered against competing metabolic possibilities.[64,65] Nevertheless, because the proposed culprit quinone-related pathways remain as at least a possibility across all three types of X, we next considered replacing the entire phenoxyacetic acid with a suitable mimic that can further help to avoid such pathways. A cinnamic acid moiety has precedent for affording PPARδ agonist efficacy when used as a headgroup.[26,45,66,67] Of note to us was the display of a carboxylic acid similarly located two atoms away from a phenyl ring, wherein its double bond is rich in electron density, somewhat like the lone electron pairs' density present on the O atom involved in the analogous two-atom link within the phenoxyacetic acid group. Previous modeling studies suggest that either the E or Z isomers can be deployed because of their ability to isomerize to the more active form at the PPARδ binding site, presently thought to be the Z or cis isomer.[26] In terms of metabolism, cinnamic acid is similar to phenoxyacetic acid in that it is not as prone to β-oxidation degradation when compared with simple alkanoic acids. Opposite to phenoxyacetic acid, cinnamic acid withdraws electrons from its aryl ring, making it less sensitive to aromatic hydroxylation. Finally, the hypothetical series of conversions that might lead to the collapse of the phenoxyacetic acid headgroup produces a less fragile species in the case of cinnamic acid because the latter is stabilized by direct conjugation with the carboxylic acid moiety (Figure 2C).

Among these potential benefits, however, we also noted that as an α,β-unsaturated carbonyl system, our switch to a cinnamic acid headgroup could itself lead to Michael addition reaction[68] off-target toxicity[69] (Figure 2D). Fortunately, when such carbonyls are part of a carboxylic acid group, Michael reactivity is significantly diminished compared with aldehydes and ketones, especially so when deployed within biological systems[69−73] where strong bases are not present to activate the initial step for a donor partner.[68,69] Indeed, cinnamic acid and its derivatives are used extensively in the food and cosmetic industry without issues stemming from Michael addition reactions.[74−76] Nevertheless, to ensure that we were not swapping out the hypothesized toxic head region in cardarine for yet another possibly toxic and potentially problematic pathway, we performed experiments to confirm the safety of deploying cinnamic acid in our specific molecular context. These studies are described in the Preliminary Toxicity Assessments section. In the end, our final library design used *trans*-cinnamic acid as a constant headgroup while several other structural features of the overall scaffold were systematically examined. Figure 3 summarizes the compounds that were synthesized to explore the PPARδ agonist efficacy SAR as it relates to osteogenesis. This series additionally represents a



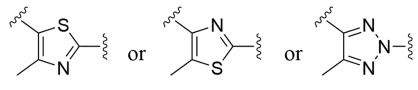
X = O, S, N-H, N-Me, N-Et, N-iPr, N-Pr
R^1 = H, or CF_3
R^2 = H, F, or CF_3
R^3 = H, Me, Et, or iPr
R^4 = H or Me
Heterocycle =  or or

Figure 3. General scaffold design outlining the structural modifications explored herein.

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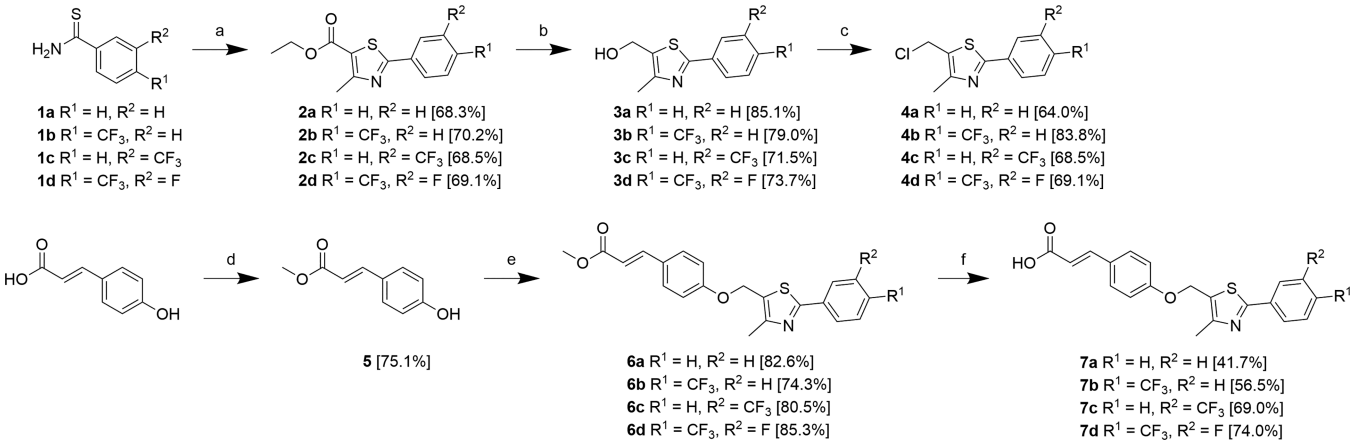
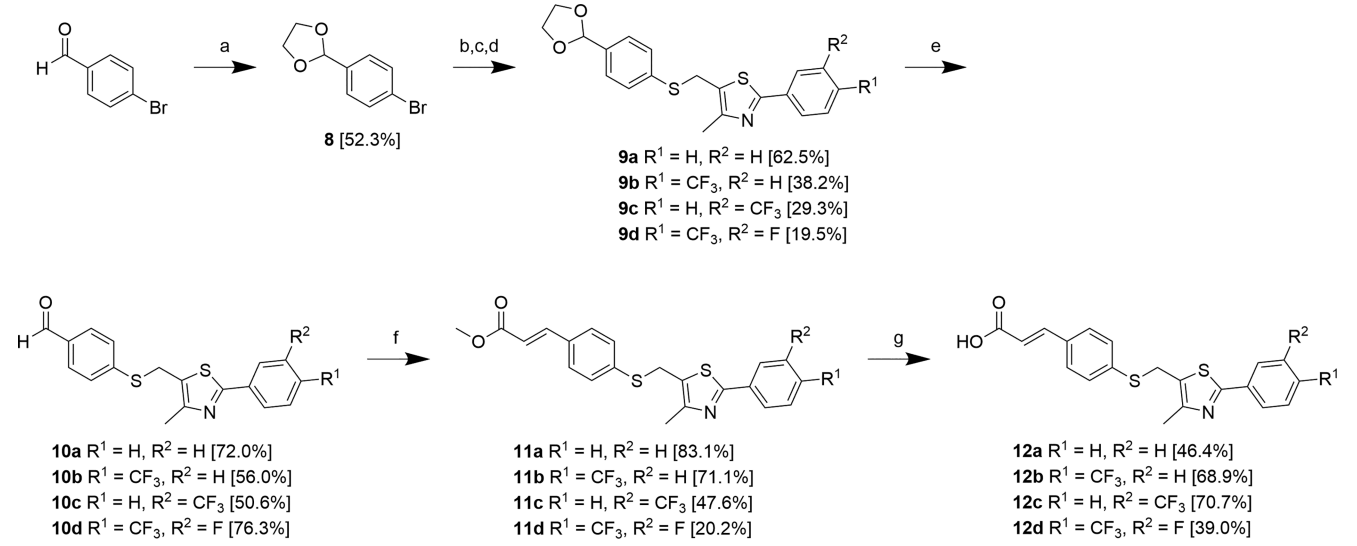
Scheme 1. Synthesis of Oxygen Linker Series[a]



[a]Reagents and conditions: (a) ethyl 2-chloroacetoacetate, 95% EtOH, reflux; (b) LAH, THF, 0 °C; (c) $MeSO_2Cl$, TEA, DCM, 0 °C; (d) H_2SO_4, MeOH, reflux; (e) **4**, Cs_2CO_3, MeCN, rt; (f) NaOH, 95% EtOH, rt.

Scheme 2. Synthesis of Sulfur Linker Series[a]



[a]Reagents and conditions: (a) ethylene glycol, pTSA, toluene, Dean−Stark reflux; (b) *t*-BuLi, THF, −78 °C; (c) sulfur, THF, −78 °C to rt; (d) **4**, THF, 0 °C; (e) HCl, THF, rt; (f) methyl (triphenylphosporanylidene)acetate, THF, 40 °C; (g) NaOH, 95% EtOH, rt.

family of compounds from which to select probes that can contribute toward delineating long-term toxicity SAR as may be useful to us or others in the future.

Our synthesized library reevaluated some key literature SAR conclusions, such as the role of sulfur as a linker heteroatom compared with oxygen and nitrogen, along with an assessment of more novel structural changes such as trying two different heterocycles in the core region (Figure 3). The first series of synthesized compounds included combinations of different linker heteroatoms with various trifluoromethyl and fluorine substitutions on the terminal phenyl-ring tail. The SAR conclusions from this series (Table 1) influenced the structure design for a second series of compounds while holding the initially optimized features constant (Table 2). An MSC differentiation assay giving comparative values of osteogenesis or adipogenesis was used as an initial screen to determine which compounds were the strongest inducers of osteogenesis. Adipogenesis was prompted for gross comparisons of PPAR selectivity by administering rosiglitazone, a standard PPARγ agonist (Figure 1). These results (Tables 1 and 2) led to the selection of seven hit compounds (**7b,d**, **12b,d**, **16b**, **23b**, and **31a**—chemical structures shown on Table 3) that exhibited varying osteogenic profiles while allowing for representative structural comparisons in the core and lipophilic tail components. They were tested in a luciferase reporter assay for each of the three PPAR subunits. This assay generated dose−response curves and statistically derived EC_{50} values for quantitative assessments of PPAR selectivity as well as potency (Table 3). Six of these hit compounds (**7d**, **12b,d**, **16b**, **23b**, and **31a**) were additionally tested in ovariectomized (OVX) mice for which the *in vivo* results (Figures 5 and 6) suggest that four compounds (**7d**, **12d**, **23b**, and **31a**) have potential for treating osteoporosis in mammals. Taken together, the composite of testing results indicates that two compounds (**12d** and **31a**) can be considered as the preferred lead agents. Of these, **31a** (2-methyl-4-[*N*-methyl-*N*-[5-methylene-4-methyl-2-[4-(trifluoromethyl)phenyl]thiazole]]aminocinnamic

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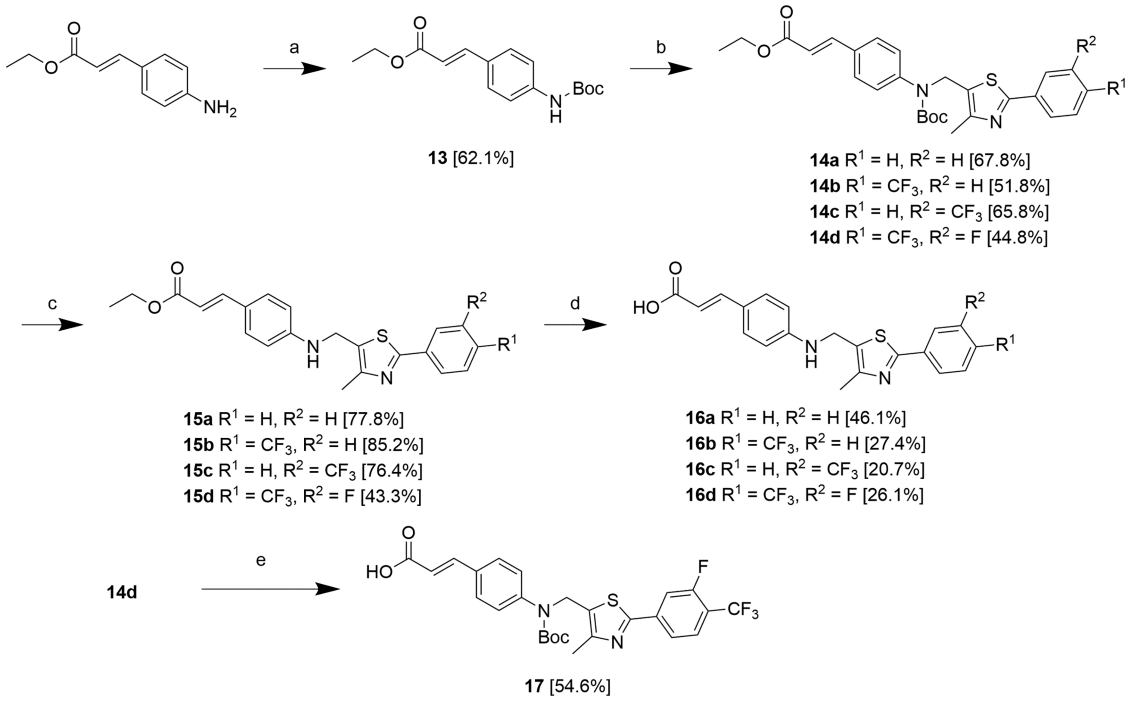
Scheme 3. Synthesis of N−H Linker Series and Compound 17[a]



[a]Reagents and conditions: (a) BOC_2O, THF, 35 °C; (b) **4**, NaH, DMF, 0 °C to rt; (c) TFA, DCM, rt; (d) NaOH, 95% EtOH, rt; (e) NaOH, 95% EtOH, rt.

acid) exhibits extremely high selectivity for PPARδ, much greater than that of the prototypical standard GW0742.

RESULTS AND DISCUSSION

Chemistry. The first series of analogs investigated a SAR for the linker heteroatom located para- to the *E*-olefin (X-position, Figure 3) along with trifluoromethyl and fluorine substitutions on the terminal phenyl ring (R^1 and R^2, Figure 3). The X-position heteroatom had previously been explored in the literature as either an oxygen or sulfur for a similar scaffold in the development of GW0742.[26] We decided to repeat this comparison for our scaffold in addition to a comparison with a nitrogen linker in the form of either N−H or *N*-methyl. Our intended use of PPARδ agonists as agents for controlling MSC differentiation was also unexplored in the literature, and we elected to repeat certain SAR conclusions from other studies to determine whether they remained similar for our new indication.

The synthesis of the alkyl chloride intermediates **4a−d** (Scheme 1) was completed as previously described[26] and involved the formation of ester **2** using a Hantzsch thiazole synthesis from commercially available thiobenzamides and ethyl 2-chloroacetoacetate. The resulting ester was reduced using lithium aluminum hydride (LAH) to give alcohol **3**. The alcohol was chlorinated using methanesulfonyl chloride to give the desired "eastern-half" intermediates **4a−d** as alkyl chlorides. These key alkyl chloride intermediates were used in various coupling reactions with the appropriate linker heteroatom. Target compounds **7a−d** containing the X-position oxygen linker heteroatom were synthesized from commercially available *p*-coumaric acid by Fischer esterification to give methyl 4-hydroxycinnamate **5**[77] followed by coupling to intermediates **4** using cesium carbonate to give potential ester prodrug compounds **6** and base hydrolysis to give the carboxylic acid target compounds **7a−d**.

The series of target compounds with an X = sulfur were synthesized from the commercially available 4-bromobenzaldehyde by conversion of the aldehyde using ethylene glycol to give acetal **8** (Scheme 2). The next step involved a one-pot reaction[78] using *t*-BuLi for lithium−halogen exchange of the bromine followed by the formation of the thiolate using sulfur powder and lastly, coupling to the alkyl chloride intermediate **4** to give intermediate **9**. Despite the multiple reactions occurring in this one-pot conversion, the overall yields remained favorable, and no attempts at isolated stepwise improvements were deemed necessary. The acetal intermediates **9** were deprotected using HCl to give aldehydes **10** followed by a Wittig reaction using methyl (triphenylphosphoranylidene)-acetate to give *E*-olefins **11**. The subsequent base hydrolysis of esters **11** gave the carboxylic acid targets **12a−d**.

The synthesis of target compounds containing an X-position nitrogen linker began with *tert*-butyloxycarbonyl (BOC) protection of the commercially available ethyl 4-amino-cinnamate to give **13** (Scheme 3). BOC-protected amine **13** was then coupled to alkyl chlorides **4** to give intermediates **14** followed by the removal of the BOC group using trifluoro-acetic acid (TFA) to give the secondary amine prodrugs **15** and base hydrolysis to give target compounds **16a−d** containing the NH linker atom. In one instance, base hydrolysis was completed without the removal of the BOC group to give an unanticipated test compound **17** that then contained an *N*-BOC linker moiety from the **14d** intermediate.

The synthesis of **16a−d** was originally attempted without BOC protection of 4-aminocinnamate by coupling directly to alkyl chloride **4**. This was found to be problematic due to the formation of a disubstituted side product **18** despite using only 1 equiv of **4b** and the weak base $CsCO_3$. The intermediate **18**

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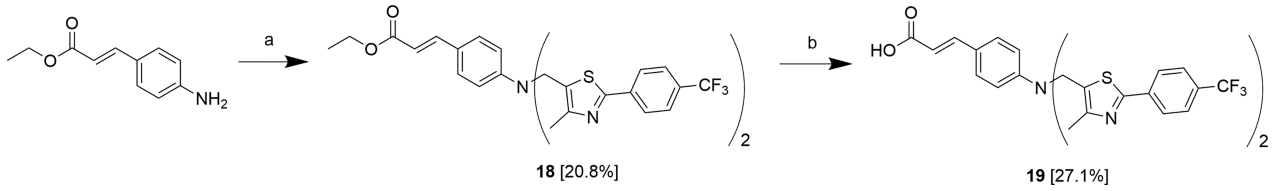
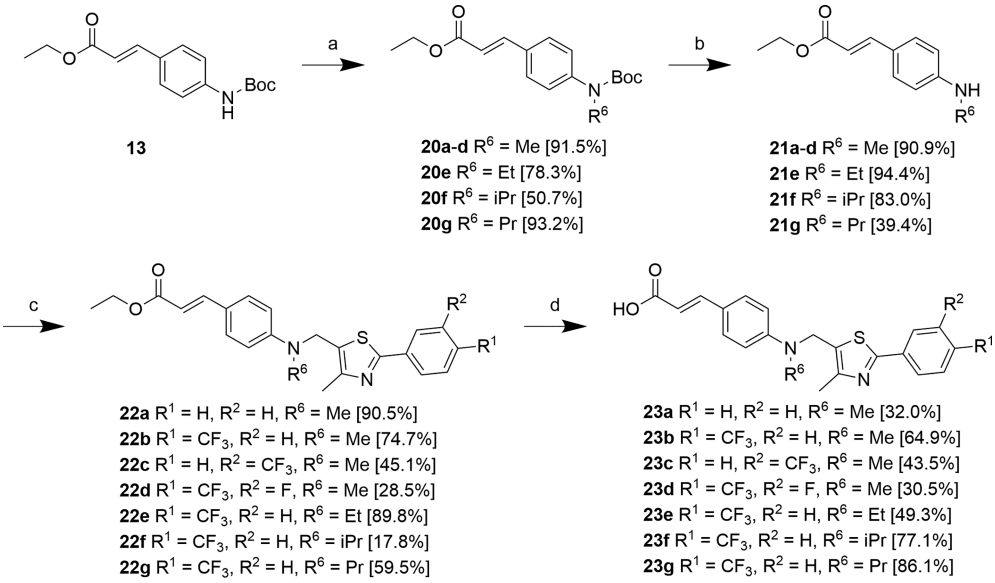
Scheme 4. Synthesis of Disubstituted Test Compound 19[a]



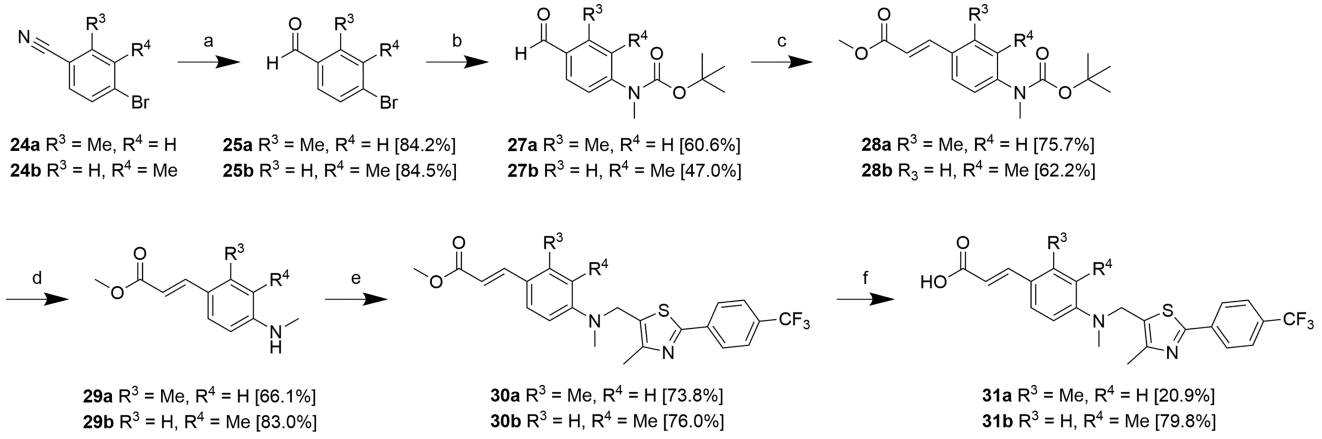
[a]Reagents and conditions: (a) **4b**, NaH, NaI, MeCN, rt; (b) NaOH, 95% EtOH, rt.

Scheme 5. Synthesis of *N*-Me Linker Series and other *N*-Alkyl Targets[a]



[a]Reagents and conditions: (a) R_6-I, NaH, DMF, 0 °C to rt; (b) TFA, 0 °C to rt; (c) **4** (**a**, **b**, **c**, or **d**), NaI, NaH, DMF, 0 °C to rt; (d) NaOH, 95% EtOH, rt.

Scheme 6. Synthesis of Targets with R^3 or R^4 Methyl Substitution[a]



[a]Reagents and conditions: (a) DIBAL-H, toluene, −78 °C; (b) MeNBOC **26**, $Pd_2(dba)_3$, Cs_2CO_3, 1,4-dioxane; (c) methyl-(triphenylphosphoranylidene)acetate, THF, 55 °C; (d) TFA, DCM, 0 °C to rt; (e) **4b**, NaH or DIPEA, DMF, 0 °C to rt; (f) 3 N NaOH, 95% EtOH, THF, rt.

was later intentionally synthesized using 2 equiv of **4b** and NaH as the base (Scheme 4). This led to the synthesis of another initially unanticipated test compound **19** after base hydrolysis.

A similar synthetic strategy involving BOC protection was utilized in the synthesis of the *N*-Me linker series, as well as the later *N*-alkyl series, to avoid any possible dialkylated side-product formation. BOC-protected cinnamate **13** was alkylated using the appropriate alkyl iodide to give series **20** (Scheme 5). The BOC group was removed using TFA to give intermediates **21** followed by a nucleophilic substitution involving alkyl chlorides **4** to give ethyl ester **22**. Lastly, base hydrolysis of the esters provided targets **23a−g**.

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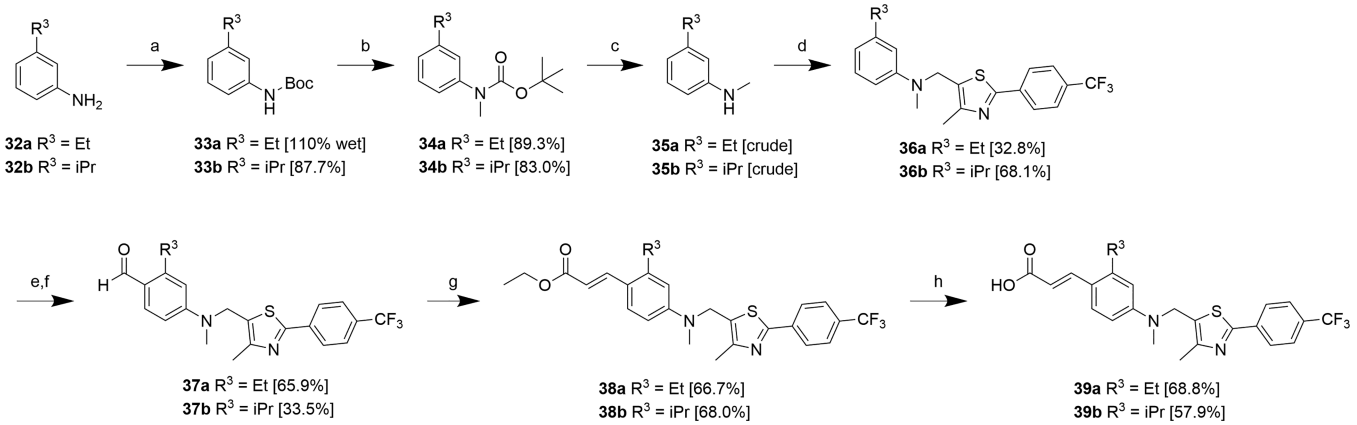
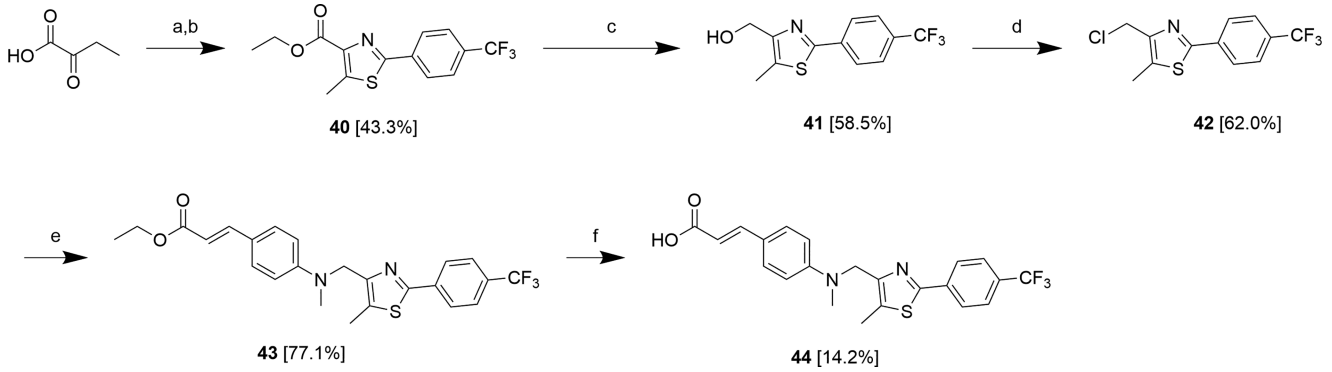
Scheme 7. Synthesis of R^3-Substituted Ethyl and Isopropyl Targets[a]



[a]Reagents and conditions: (a) di-*tert*-butyl dicarbonate, THF, reflux; (b) NaH, MeI, DMF, reflux; (c) TFA, DCM, 0 °C to rt; (d) **4b**, NaH, NaI, DCM, 0 °C to rt; (e) $POCl_3$, DMF, 0 °C to rt; (f) H_2O (ice); (g) triethyl phosphonoacetate, NaH, THF, 0 °C to rt; (h) 3 N NaOH, 95% EtOH, THF, rt.

Scheme 8. Synthesis of the Thiazole Regioisomer Target[a]



[a]Reagents and conditions: (a) Br_2, DCM, rt; (b) **1b**, 95% EtOH, reflux; (c) LAH, THF, 0 °C to rt; (d) $MeSO_2Cl$, TEA, DCM, 4 °C; (e) **21a**, NaI, NaH, DMF, 0 °C to rt; (f) NaOH, 95% EtOH, rt.

The second major series of target compounds had structural features shaped by the SAR results from studying the aforementioned probes in the MSC differentiation assay and selected compounds in the PPAR transactivation assay. Prominent structural features of interest included the retention of the terminal phenyl *para*-trifluoromethyl and *N*-methyl linker atom (R^1 = CF_3, X = *N*-Me). The length of the alkyl chain located at the nitrogen X-position was also explored and was synthesized as previously described (Scheme 5). The other targets synthesized as part of the second series of targets contained various alkylations at the western phenyl headgroup (R^4 and R^5 positions) as well as other targets containing a thiazole regioisomer or triazole heterocycle in the central core region.

Target compounds containing methyl substitutions at either the R^3 or R^4 position were synthesized from the appropriate commercially available 4-bromobenzonitrile **24a** or **24b** (Scheme 6). Stephen aldehyde synthesis of bromides **24** using diisobutylaluminum hydride afforded aldehydes **25**.[79] The BOC-protected methylamine **26** was used in a Buchwald–Hartwig amination of aldehydes **25** to give carbamates **27**.[79] A Wittig olefination of aldehydes **27** was used to exclusively form the *E*-olefins **28**. The BOC group was then removed using TFA to give secondary amines **29** followed by a nucleophilic substitution of **4b** using either NaH or *N*,*N*-diisopropylethylamine (DIPEA) as base to give **30a**,**b**. Lastly, base hydrolysis of the esters gave the target compounds **31a**,**b**.

The probes containing either an ethyl or isopropyl substitution at the R^3 position were synthesized using a different scheme due to the unavailability of the analogous commercially available starting materials. These targets were synthesized from 3-ethylaniline **32a** and 3-isopropylaniline **32b** (Scheme 7). The aniline nitrogen was protected using di-*tert*-butyl dicarbonate to give the BOC-protected amine intermediates **33** followed by methylation using methyl iodide to give carbamates **34** and deprotection of the BOC group using TFA to give the secondary amines **35**. We were unable to obtain pure secondary amines **35**; however, we were able to effectively use these crude intermediates directly in a coupling reaction with alkyl chloride **4b** to give compounds **36** as pure materials. An aryl aldehyde was formed para to the tertiary amine using a Vilsmeier–Haack reaction to give aldehydes **37**. This aldehyde was initially subjected to a Wittig olefination similar to that in Scheme 6. These attempts resulted in low yields, presumably due to the steric hindrance from the larger R^3 alkyl groups. Alternatively, a Horner–Wadsworth–Emmons olefination using triethyl phosphonoacetate and NaH was able to give the desired *E*-olefins **38** in acceptable yields. Lastly, base hydrolysis of the esters afforded the target compounds **39a**,**b**.

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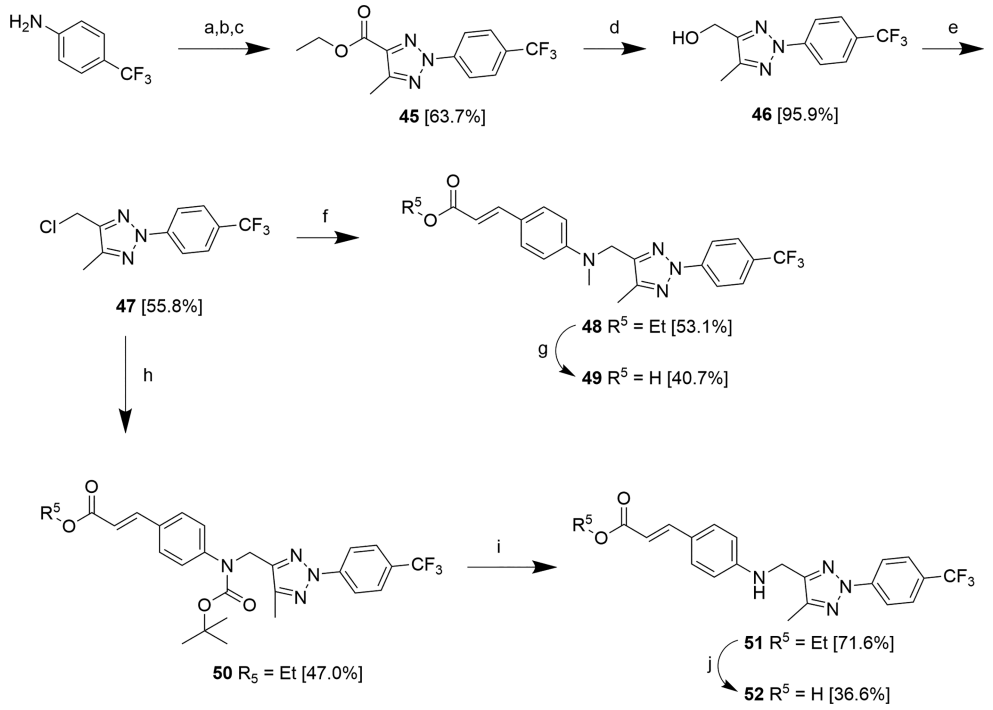
Scheme 9. Synthesis of Triazole Targets[a]



[a]Reagents and conditions: (a) AcOH, H_2O, HCl, $NaNO_2$, 0 °C; (b) ethyl acetoacetate, 95% EtOH, NaOAc, Na_2CO_3, 0 °C; (c) $CuCl_2$, NH_4OAc, 95% EtOH, reflux; (d) 2 M $LiAlH_4$, THF, 0 °C to rt; (e) $MeSO_2Cl$, Et_3N, DCM; (f) **21a**, NaI, NaH, DMF, 0 °C to rt; (g) 3 N NaOH, 95% EtOH, THF, rt; (h) **13**, NaI, NaH, DMF, 0 °C to rt; (i) TFA, DCM, 0 °C to rt; (j) NaOH, 95% EtOH, THF, rt

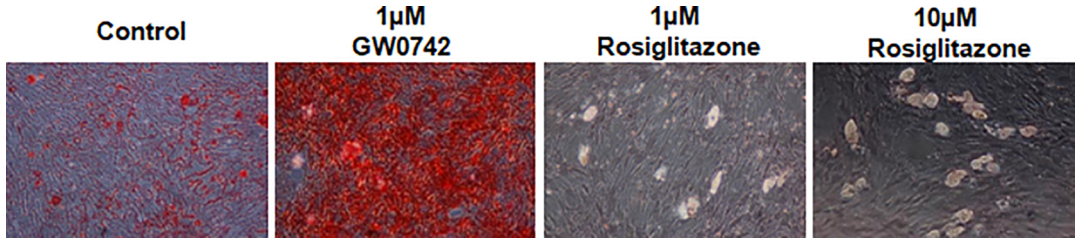


Figure 4. Treatment of MSCs with the PPARδ agonist, GW0742, and the PPARγ agonist, rosiglitazone, results in osteogenesis and adipogenesis, respectively. Typical results showing a robust osteogenic response (+++) to 1 μM GW0742 when visualized using Alizarin Red stain. Alternatively, the moderate (++) to robust (+++) adipogenic responses to 1 and 10 μM rosiglitazone, respectively, are shown by the formation of white lipid droplets.

A regioisomer of the thiazole was also synthesized based on a literature example that suggested that this change within a different overall scaffold can result in an increase in the PPARδ potency and selectivity.[46] Synthesis of the thiazole regioisomer was completed, as described in the literature,[46] using bromine and 4-(trifluoromethyl)thiobenzamide **1b** to give intermediate **40** (Scheme 8). The subsequent steps were completed analogously to those used in the synthesis of the *N*-methyl series. The ester **40** was reduced to the primary alcohol **41** using LAH. The alcohol **41** was chlorinated using methanesulfonyl chloride to give the alkyl chloride **42** followed by a coupling reaction using NaH with **21a** to give **43**. Lastly, ester **43** was hydrolyzed to give the carboxylic acid target **44**.

Two targets having a 1,2,3-triazole heterocycle as part of the core were synthesized by the formation of the triazole ring in the first step using a one-pot reaction (Scheme 9).[80] This reaction utilized *in situ* nitrous acid to form a diazonium salt from the 4-(trifluoromethyl)aniline starting material, the addition of ethyl acetoacetate, and triazole formation using $CuCl_2$ and excess ammonium acetate to give **45**. The targets, containing either N−H or *N*-methyl at the X-position (**49** and **52**), were synthesized using methods analogous to the previously shown schemes for **16** and **23**, respectively.

***In Vitro* Testing.** All target compounds were screened for their ability to promote the osteogenic differentiation of human-bone-marrow-derived MSCs by using Alizarin Red, which stains osteocytes red followed by microscopic examination. This action is indicative of PPARδ activation, whereas observation of adipogenesis is a sign of PPARγ activation. The latter was ascertained before and after staining by microscopic examination wherein the formation of white droplets is readily discernible without the need for staining methods. The divergent roles of PPARγ and PPARδ in MSC differentiation are illustrated in Figure 4, showing the results from treating MSCs with either the PPARγ agonist, rosiglitazone, or the PPARδ agonist, GW0742. This comparative differentiation assay was utilized as an initial screen for our library of compounds, and the degree of osteogenesis or

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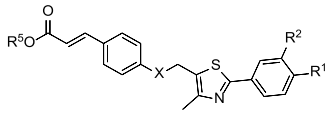
Table 1. Results of the First Series of Target Compounds Using a Comparative MSC Differentiation Assay[a]



compd	X	R^1	R^2	R^5	AD(1 μM)	AD (10 μM)	AD (20 μM)	OS (1 μM)	OS (10 μM)	OS (20 μM)
GW0742					0	0		+++	++	
6b	O	CF_3	H	Me	+++	toxic		0	toxic	
6c	O	H	CF_3	Me	+++	toxic		0	toxic	
7a	O	H	H	H	+++	+++		0	0	
7b	O	CF_3	H	H	++	toxic		++	toxic	
7c	O	H	CF_3	H	+	toxic		+++	toxic	
7d	O	CF_3	F	H	0	+	0	+++	+++	+++
12a	S	H	H	H	+	++		+	0	
12b	S	CF_3	H	H	0	0		+	++++	
12c	S	H	CF_3	H	0	+	++	++	0	0
12d	S	CF_3	F	H	0	0	0	+	++	+++
16a	N−H	H	H	H	++	+++		+	0	
16b	N−H	CF_3	H	H	0	++	+++	+	+++	++++
16c	N−H	H	CF_3	H	+	+++	++++	0	0	0
16d	N−H	CF_3	F	H	0	+	+++	++	0	0
17	*N*-BOC	CF_3	F	H	0	+	+++	++	0	0
19	*N*-ThPh	CF_3	H	H	0	0	toxic	+	0	toxic
23a	*N*-Me	H	H	H	0	++	+++	++	0	0
23b	*N*-Me	CF_3	H	H	0	+	++	+	+++	++++
23c	*N*-Me	H	CF_3	H	0	+	++	++	0	0
23d	*N*-Me	CF_3	F	H	0	0	+	++++	0	0

[a]AD, adipogenesis; OS, osteogenesis. Number of plus signs ("+") indicates an increasing OS response relative to GW0742 or vehicle control or an increasing AD response relative to rosiglitazone or vehicle control. "Toxic" response indicates a significant loss of overall cell numbers; >20% compared with controls upon microscopic examination.

adipogenesis was scored in gradients relative to the osteogenic response from treatment with GW0742 or the adipogenic response elicited by rosiglitazone. For example, the dark-red robust response of GW0742 was assigned "+++", whereas if microscopic examination appeared to be approximately half as red or only very weakly red, scores of "++" or "+" were assigned. Compounds unable to generate any red beyond the nontreated control were assigned a zero value. The objective of this preliminary assay was to identify compounds with promising osteogenic properties and minimal adipogenesis activity. Compounds displaying this profile were further tested in a quantitative manner by using a panel of PPAR-transfected cells that can also assess the subtype specificity. The combined results from these assays allows for SAR correlations between MSC osteogenesis and PPARδ activation.

MSC Differentiation Assay. As previously noted, the results from the MSC differentiation assay were based on microscopic visualization of Alizarin Red staining or lipid droplet formation for osteogenesis (OS) or adipogenesis (AD), respectively. The degree of osteogenesis or adipogenesis is indicated by an increased number of "plus" (+) symbols or by a "0", indicating no response. Figure 4 outlines the qualitative scoring relative to the robust response from standards GW0742 and rosiglitazone. All of the first series of compounds are listed in Table 1 along with their MSC differentiation data, including those for GW0742 as a standard.

To evaluate the importance of the carboxylic acid functional group, intermediates **6b** and **6c** were tested for their ability to increase osteogenesis. Treatment of the MSCs with the acid-masked methyl esters **6b** or **6c** strictly resulted in adipogenesis. Treatment with the analogous unmasked carboxylic acid targets **7b** and **7c** resulted in osteogenesis at 1 μM. This result highlighted the need for the incorporation of the carboxylic acid functional group in the target compound design, and additional ester compounds were not tested in the *in vitro* assays, even though they can still be considered as potential ester prodrugs if later administered *in vivo*.

The *para*-trifluoromethyl ($R^1 = CF_3$) seems to be desirable for osteogenesis, as shown by the response from **12b**, **16b**, and **23b**. A meta-substituted fluorine ($R^2 = F$) in combination with the *para*-trifluoromethyl also seems to increase osteogenesis at lower doses, as seen in **7d** and **23d**. These two phenyl ring arrangements were later further explored by the selection of compounds **6b**, **6c**, **12b**, and **12d** for use in the quantitative PPAR transactivation assay. Because of the promising results from compounds containing the R^1-CF_3, we incorporated this structural feature into the design of further targets.

The specific heteroatom used for the X-position seems to have the least pronounced effect. Each heteroatom (O, S, N−H, or *N*-Me) had at least one example of a target compound with a score of "+++" or better at one or more concentrations. The inclusion of *N*-Me at the X-position seemed to have the greatest effect of increasing osteogenesis with compounds **23b** and **23d**. The success of a methylated nitrogen led to our exploration of other alkylations at this position as part of the second series of target compounds (**23e−g**). However, results from compounds **17** and **19** showed little osteogenesis, indicating that additionally larger groups at this position were unfavorable. The impact of the X-position was further explored in the quantitative PPAR transactivation assay by the selection of **7b**, **12b**, **16b**, and **23b**.

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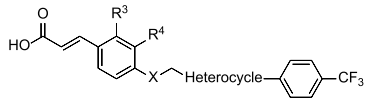
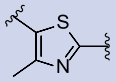
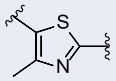
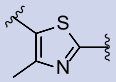
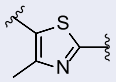
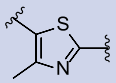
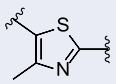
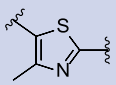
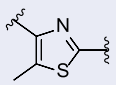
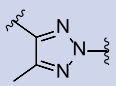
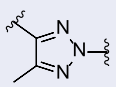
Table 2. Results of the Second Series of Target Compounds Using a Comparative MSC Differentiation Assay[a]



Compd	X	R^3	R^4	Heterocycle	AD (1μM)	AD (10μM)	AD (20μM)	OS (1μM)	OS (10μM)	OS (20μM)
GW0742					0	0		+++	++	
23e	N-Et	H	H		+	+	+	++	++	+
23f	N-iPr	H	H		0	0	0	+	++	+
23g	N-Pr	H	H		0	+	+	++	++	0
31a	N-Me	Me	H		0	0	0	+++	+++	+
31b	N-Me	H	Me		0	0	0	+	++	++
39a	N-Me	Et	H		+	+	++	+++	++	+
39b	N-Me	iPr	H		0	0	0	++	+++	+
44	N-Me	H	H		0	0	0	++	++++	0
49	N-Me	H	H		0	0	0	++	++	+
52	N-H	H	H		+	+	+	++++	+++	+++

[a]AD, adipogenesis; OS, osteogenesis. Number of plus signs ("+") indicates an increasing response relative to GW0742 or vehicle control or an increasing AD response relative to rosiglitazone or vehicle control.

The second series of compounds explored the effect of incorporating alkyl groups at the R^3 position or lengthening them at the *N*-alkyl X-position. This series also examined the effect that R^4 methylation has compared with R^3 methylation along with the impact of two different heterocycles. All of the second series of compounds are listed in Table 2 along with their MSC differentiation data, including those for GW0742 as a standard.

The target compounds **23e**,**f**, containing bulkier N-alkylations than those of the previous **23b**, showed an overall decrease in osteogenesis, indicating that a simple methyl group at this position is preferred. Similar alkylations were also explored at the R^3 position. Methylation at this location (**31a**) resulted in a slight decrease in osteogenesis when compared with **23b** of the previous series. However, it showed a very significant increase in the selectivity by completely avoiding adipogenesis when assessed by microscopic visualization. This observation was further supported later by the PPAR transactivation assay, where no PPARα or PPARγ activity was shown for compound **31a**. Increasing the size of the alkyl substitution at R^3 to ethyl (**39a**) or isopropyl (**39b**) resulted in similar osteogenesis and therefore was not considered to be more favorable than the methyl substitution. The substitution of a methyl group at the R^4 position, compared with R^3, resulted in a decrease in osteogenesis and can be seen when comparing **31b** and **31a**.

Thiazole regioisomer **44** showed an increase in osteogenesis at 1 and 10 μM but a drop-off in activity at 20 μM when

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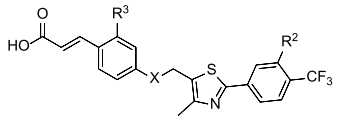
Table 3. Transactivation Activity of Selected Compounds



	X	R^2	R^3	PPARα EC_{50} (μM)[a] (% activation)[b]	PPARδ EC_{50} (μM)[a] (% activation)[b]	PPARγ EC_{50} (μM)[a] (% activation)[b]
Gemfibrozil				11.8 [8.93−15.6] (110%)[c]	inactive[d]	68.4[e] (>52%)
GW0742[f]				2.50[e] (>108%)	0.0269 [0.00940−0.0767] (135%)[c]	9.53 [2.37−38.3] (179%)
Rosiglitazone				3.96[e] (>120%)	8.89 [4.17−18.9] (159%)	0.144 [0.0551−0.377] (100%)[c]
7b[f]	O	H	H	2.23 [0.138−36.1] (135%)	0.274 [0.116−0.652] (171%)	inactive[d]
7d	O	F	H	51.3 [16.5−159] (346%)	0.0959 [0.0533−0.173] (151%)	13.2[e] (>74%)
12b	S	H	H	17.7 [10.0−31.4] (338%)	0.535 [0.0472−6.07] (114%)	17.0 [6.24−46.1] (83%)
12d	S	F	H	0.915 [0.609−1.37] (163%)	0.134 [0.0730−0.246] (160%)	1.30 [0.497−3.40] (114%)
16b	N−H	H	H	6.01 [2.89−12.5] (377%)	1.22 [0.190−7.85] (172%)	5.28 [3.34−8.30] (76%)
23b[f]	*N*-Me	H	H	0.947 [0.650−1.38] (44%)	0.199 [0.114−0.347] (107%)	24.7 [2.78−220] (80%)
31a[f]	*N*-Me	H	Me	inactive[d]	0.335 [0.197−0.568] (59%)	inactive[d]

[a]Activity of reference and test compounds in the cell-based transient transfection assay with the receptor response quantified using a luciferase reporter. Data are representative of three or more independent experiments performed in triplicate ($N = 9$ or more). EC_{50} values were derived from a four-parameter variable slope model best fit, with EC_{50} 95% confidence interval shown in brackets. [b]% activation: the highest activation achieved when compared with the reference compound normalized to 100%. [c]Reference compound response normalized to 100% at the concentration used in the test compound assays. [d]Inactive: compound is considered inactive if EC_{50} is >100 μM or if the % activation is <40%. [e]Maximum value of normalized response could not be determined, preventing a reliable estimation of the EC_{50} 95% confidence interval. [f]Potential toxicity observed at doses significantly higher than efficacious range. See the discussion in Preliminary Toxicity Assessments section.

compared with **23b**. Compound **44** also showed no adipogenesis, indicating a likely increase in selectivity versus PPARγ. The other heterocycle explored was a 1,2,3-triazole that showed a decrease in osteogenesis when comparing compound **49** with the thiazole of **23b**. Interestingly, this same substitution showed an increase in osteogenesis at lower concentrations when the X-position was N−H, as seen when comparing compounds **52** and **16b**.

PPAR Transactivation Assay. We selected seven compounds for the quantitative measurement of PPAR potency and subunit selectivity using a luciferase reporter assay utilizing COS-7 cells transfected with human PPARα, PPARγ, or PPARδ.[81,82] Our selection was based on results from the MSC differentiation assay (Tables 1 and 2) and included compounds **7b,d**, **12b,d**, **16b**, **23b**, and **31a** (chemical structures are shown in Table 3). This series exhibited significant osteogenic activities while allowing for representative structural comparisons for substitutions on the head, varying cores, and substitutions on the lipophilic tail components. Specifically, we selected structurally diverse compounds for maximizing possible SAR comparisons at the R^1, R^2, R^3, and X positions (Figure 3). Effective concentration at 50% response (EC_{50}) values were obtained for each of the three PPAR subunits for each selected target compound. The percent maximum activation (E_{max}) was measured relative to the positive control gemfibrozil for PPARα, rosiglitazone for PPARγ, and GW0742 for PPARδ (structures are shown in Figure 1).

The results generated from this assay (Table 3) showed that substitution of the R^2 position with fluorine results in roughly a 3- to 4-fold increase in PPARδ potency when comparing **7b** with **7d** or **12b** with **12d**. This substitution also shows an increase in selectivity toward PPARα but a 13-fold or greater decrease in selectivity toward PPARγ. We considered this decrease in selectivity important to consider for the design of an osteogenic compound due to the contrasting adipogenic function of PPARγ activation. When considering the optimal X-position heteroatom, a comparison between **7b**, **12b**, **16b**, and **23b** showed that the *N*-Me-containing compound **23b** had the greatest PPARδ potency. Compound **23b** also showed 124-fold selectivity toward PPARδ versus PPARγ compared with 31-fold selectivity for **12b**, 4-fold selectivity for **16b**, and undefined selectivity for **7b**. This result in combination with an excellent osteogenic profile for **23b** in the MSC differentiation assay led us to favor the *N*-Me X-position when designing the structure of later compounds. Lastly, methyl substitution at the R^3 position (**31a**) resulted in a 1.7-fold decrease in PPARδ activity when compared with **23b**. However, this substitution resulted in a further increase in the selectivity, and **31a** had essentially no activity on PPARα or PPARγ. Despite the decrease in the PPARδ activity, the strong increase in the overall selectivity was considered beneficial, and additional compounds were synthesized to explore larger alkyl substitutions at the R^3 position.

***In vivo* Testing.** Six compounds, as described later, were selected for the *in vivo* study involving OVX mice, a mouse

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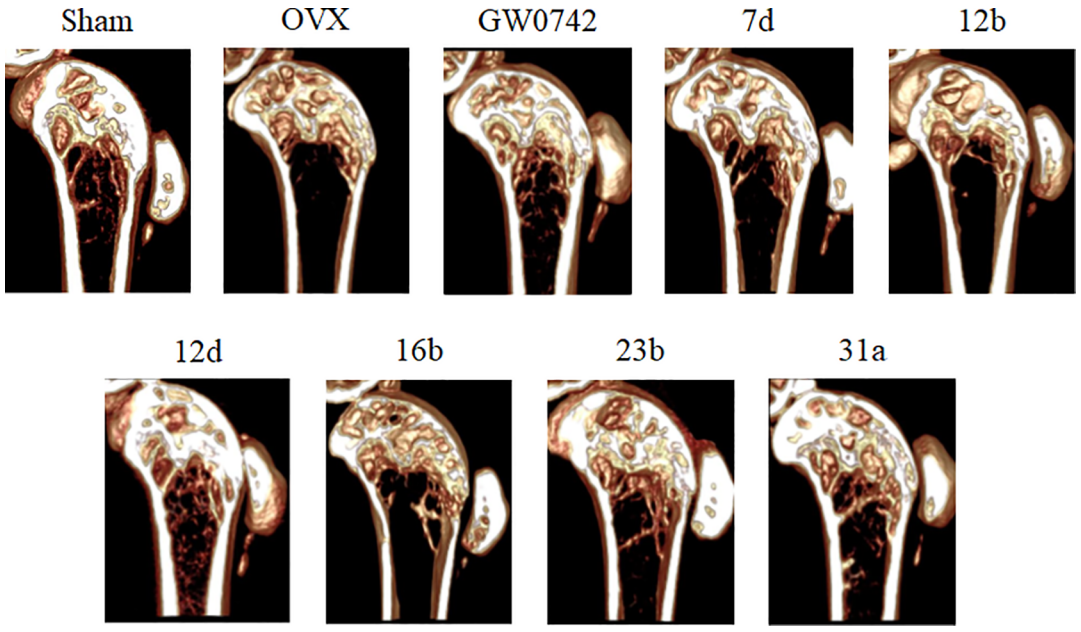

Figure 5. 3-D μCT images of the distal femur.

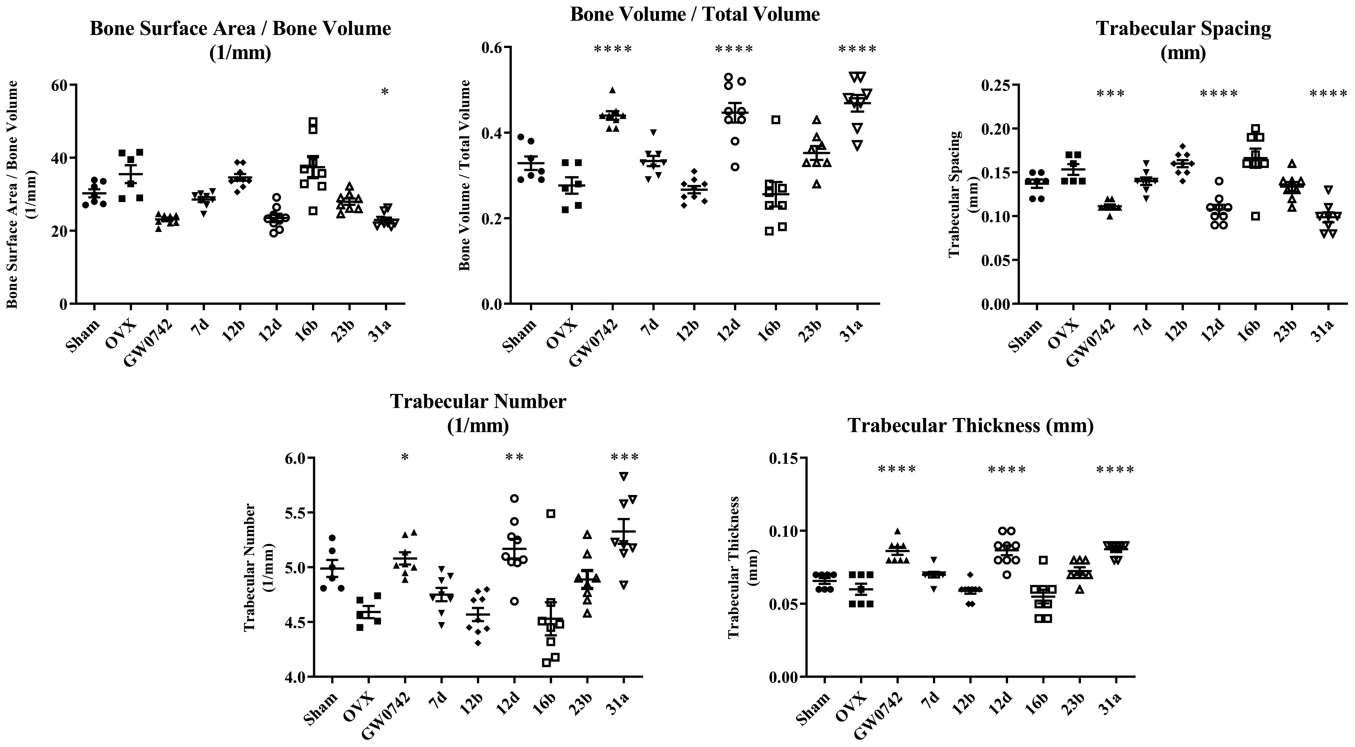

Figure 6. Analysis of the femur microarchitecture of sham, OVX, or test-compound-treated mice after 6 weeks of treatment. *P < 0.0332, **P < 0.0021, ***P < 0.0002, ****P < 0.0001 versus OVX group (ordinary one-way analysis of variance (ANOVA) followed by Tukey multiple comparisons test; Brown−Forsythe and Welch ANOVA were used for the Bone surface area (BSA)/Bone volume (BV)).

model for human postmenopausal osteoporosis.[83] The study used female C57BL/6 mice that were ovariectomized at 12 weeks of age. A sham surgery was also performed on a control group of mice, leaving the ovaries intact. After a recovery period, each group of mice was dosed with an assigned compound or vehicle by oral gavage each day for 6 weeks. This was followed by sacrifice at 22 weeks of age and micro-computer tomography analysis of the femur trabecular structure. The test compounds used in this study were the same as those used in the PPAR transactivation assay, apart from **7b**. Therefore, compounds **7d**, **12b**, **12d**, **16b**, **23b**, and **31a** were all selected based on the same SAR rationale previously explained for the transactivation assay. Compound **7b** was excluded due to the potential for toxicity (Tables 1 and 3; see the later discussion) and for its lower PPARδ potency in the transactivation assay (Table 3). Importantly, these six compounds still allowed for a systematic SAR comparison with all four linker heteroatoms included in the study. These

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compounds were tested for their ability to increase the bone density and trabecular structure in OVX mice compared with the sham group of mice. Comparisons were also made toward the same positive control used in the *in vitro* experiments, GW0742.

A visual inspection of the microcomputer tomography (μCT) images (Figure 5) of the distal femur showed an expected decrease in the trabecular structure of untreated OVX mice, indicating the development of osteoporosis and decreased bone density. OVX mice treated with test compounds showed varying degrees of improvement in the trabecular structure. Compounds **7d**, **12d**, **23b**, and **31a** improved the bone density at least back to normal (sham control) levels. Those treated with **12d** or **31a** showed an even greater improvement in the trabecular structure, comparable to or slightly better than GW0742.

An assessment of bone microarchitecture also highlighted promising improvements in bone density and structure. The ratio of bone volume to total volume (Figure 6) was increased the most for mice treated with compounds **12d** and **31a**, reaching a ratio even higher than those in the sham group. As previously indicated, this indicates that the bone density of the OVX mouse was corrected and increased beyond that of the sham mice. Mice treated with **7d** and **23b** also showed bone volume ratios that returned to sham levels but were not as high as those displayed by **12d** and **31a**. Other assessments of bone density and microarchitecture were also favorable, as shown in Figure 6. Reductions in both the bone surface area/volume ratio and trabecular spacing further reflect enhanced bone density and integrity. Likewise, significant increases in the trabecular number accompanied by moderate increases in the trabecular thickness are indicative of healthy bone remodeling. Finally, the microarchitectures for **12d**, **23b**, and **31a** are distinctive in the orientations of the newly forming trabeculae along the vectors needed to best accommodate stress in this bone's particular gravitational orientation and physical use. This can be discerned by comparing the test agents with the sham control in Figure 5, where it can also be noted that the positive-control standard GW0742 does not appear to be as effective in this regard. The composite of these assessments indicates that treatment with compounds **12d** and **31a**, in particular, results in significant increases in bone density and is able to return measured values to sham levels and beyond, with the results being either comparable to or better than GW0742 in terms of both bone density and healthy microarchitecture.

Preliminary Toxicity Assessments. A specific study was performed at the biochemical level, and general observations were made during the efficacy-related cell culture and *in vivo* studies. In addition to the possibility of toxicity after long-term dosing, of potential immediate concern was the presence of an α,β-unsaturated carbonyl system. The latter may be subject to spurious Michael addition reactions that could lead to off-target toxicity.[68,69] However, when the carbonyl is part of a carboxylic acid or ester functional group, Michael acceptor reactivity is significantly reduced[68−73] (Figure 2D). For example, cinnamic acid and its simple esters have been extensively deployed within the food and perfume industry for many years without incident.[74−76] We saw no evidence of Michael addition side products during our synthetic reactions when basic amines capable of serving as donor partners were also present. Nevertheless, to confirm that this type of reactivity is not insidiously present within the specific constructs of our molecular scaffolds, we conducted biochemical studies under more physiologic conditions.

Biochemical Studies. Compounds were incubated in triplicate with a 10-fold excess of glutathione for up to 24 h at 25 °C in pH 7.4 phosphate buffer with 50% methanol present to ensure uniform solubility across all substrates. Analyses were performed at several time points using LC-MS/MS to monitor the disappearance of substrate and to confirm the structure of any glutathione-conjugated products. Target compounds **23b**, **31a**, **7d**, and **12d** were studied to examine the possibility that different para-substituents on the cinnamic acid portion of our scaffold (X units) may have a differing influence on the reactivity. All four of the test agents remained stable for 24 h compared with a positive-control compound, benzylideneacetone, which underwent 60−70% of the reaction within 4 h. Cinnamic acid was also tested and, not unexpectedly, shown to be nonreactive. Further details of these experiments will be published as part of a larger study that intends to convey a useful SAR across a broad range of scaffolds and appendages associated with α,β-unsaturated carbonyl systems in general.

Cell-based Observations. From the MSC differentiation assay previously described, observations regarding cell toxicity were also made. Cell toxicity was generally not observed for the test compounds within relevant dose ranges of 1 to 20 μM. However, higher levels in the 0.1 to 1 mM range did cause cellular death (>25% overall loss of cells compared with controls) that we defined as toxicity. Interestingly, there was one family of compounds wherein X = O did appear to contribute to cell loss and death in a consistent manner at lower doses, namely, beginning at 10 μM. Thus, these agents have been noted in Table 1 as "toxic" and are likewise defined by a footnote. Whereas this may or may not translate to actual toxicity in humans (see the later discussion), this level of cell-based toxicity in our primary efficacy model interferes with effectively assessing osteogenesis or adipogenesis. Hence we noted these observations as "toxic" and consider them as potential flags for such compounds should they need to be dosed at high levels that could lead to systemic and thus cellular concentrations in the so-noted range.

Similarly, the previously described PPAR transfection assay allows for some speculation regarding the potential toxicity of the seven test compounds. Efficacy results are shown in Table 3 with the possible toxicity noted at higher doses for a few of the compounds by footnote *f*. Cells exhibiting a significant reduction in constitutive *Renilla* luciferase activity may be considered to be experiencing toxicity. Using a light microscope to monitor cell health revealed changes in the cell morphology for PPARδ-transfected cells when treated with 50 μM of GW0742, **7b**, **23b**, and **31a**. However, lower treatment concentrations in the range relevant for efficacious activity did not show changes in cell morphology. This is in general agreement with the toxicity pattern previously noted.

In Vivo Studies. During our 6 week mouse efficacy studies, none of the compounds demonstrated signs of gross toxicity, for example, alteration of weight, behavior, eating or other habits, compared with controls.

Selection of Potential Lead Compounds and Specific Assessment of their Cytotoxicity. From the several compounds showing significant osteogenesis activity in the preliminary human MSC assay, **12d** and **31a** exhibited promising *in vivo* activity in the mouse model that was comparable to or better than that of GW0742, a prototypical PPARδ selective agonist. Compound **31a** proved to be the

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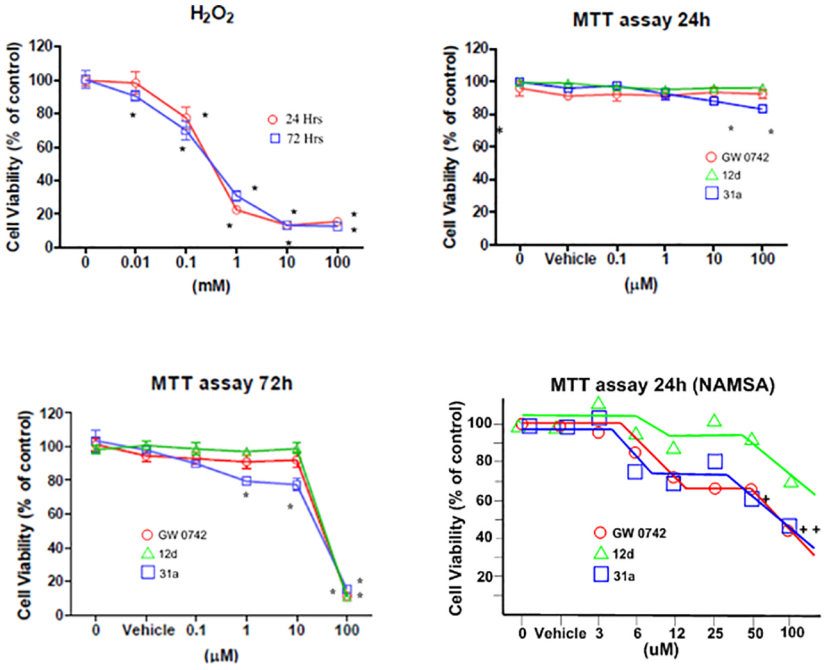

Figure 7. Cytotoxicity studies using MTT analyses of potential lead compounds **12d** and **31a** and of a standard PPARδ agonist GW0742. The top two graphs and the lower left graph are summaries of our studies using HEK 293T/17 cells. The lower right graph is a summary of the results from independent studies conducted by NAMSA (see the accompanying text) using L-929 mouse fibroblast cells. Our experiments were conducted in quadruplicate (N = 4), and graphs of the mean with standard deviations were produced using Prism software. Points at 0 μM have only cell medium. Points at "Vehicle" have no drug while containing the drug vehicle at its highest DMSO concentration (0.1%), equivalent to what would be present after a first dilution of drug stock solution with the cell medium (i.e., a 100 μM drug dose). Points thereafter are increasing drug doses in the vehicle obtained after serial dilutions with additional cell medium. Asterisks (*) denote statistically significant different data points compared with their 0 data points using Graphpad to calculate a two-tailed P value. The upper left plot is for our toxic positive control (H_2O_2), which provides a classic dose-toxicity−response relationship across the concentration range of primary interest for our compounds' efficacies and for what we considered as acceptable toxicity levels (namely, ca. 100 times or more higher than the efficacious dose). Translation to potential toxicity in humans is generally considered to be relevant when the cell viability becomes <70% after 24 h of exposure of the cell culture to a given drug concentration (upper right graph). To further prompt toxicity and assess possible SAR aspects, we also ran our studies for exposure times of 72 h (lower left graph). The NAMSA experiments were conducted in triplicate (N = 3), and their data are routinely reported as the tabulated averages for each concentration tested. These tabulated data are provided in the Supporting Information. For a ready comparison to our summary graphs, we also manually plotted the NAMSA averages using ChemDraw. In this case, the plus symbols (+) denote that the falloff in cell viability at that concentration of drug may be relevant for predicting potential toxicity in humans.

most selective for PPARδ among all of the compounds studied in our transfection assay. Alternatively, **12d**'s selectivity profile was nearly evenly balanced as an agonist across all three PPAR subtypes. Because they represented the most promising candidates from our *in vivo* studies, we decided to pursue **12d** and **31a** as potential lead compounds while also assessing the potential impact of their differences in PPAR selectivity with regard to a toxicity-related SAR. Our first step was to initiate validated Good Laboratory Practice (GLP)-compliant bioanalytical assays for each lead compound using LC-MS/MS instrumentation. These methods are provided in the Experimental Section, and the results are added to each compound's chemical synthesis details. Our next step was to evaluate their cytotoxicity in a more specific manner to determine if their significant difference in PPAR selectivity would have an impact on this critical aspect of their ADMET (absorption, distribution, metabolism, excretion, and toxicity) profiles. Toward this end, we performed cell viability tests using human embryonic kidney cell (HEK 293T/17) cultures assessed by standard MTT analyses.[84,85] These studies were initially conducted for the typical 24 h exposure of the test agent to the HEK 293T/17 cultures. Because our compounds demonstrated only minimal losses in cell viability, these studies were also repeated for 72 h exposure times to further prompt more pronounced toxicity end points. As in all of our prior assays, GW0742 was included as a prototypical PPARδ agonist. Hydrogen peroxide was used as a toxic positive control,[86] non-drug-containing medium was used as a negative control, and our drug-vehicle solution without drug was studied at its highest concentration of dimethyl sulfoxide (DMSO) (0.1% in cell culture medium prior to the addition to the cell culture milieu) that would be given to cells when used to deliver the highest concentration of drug. The results for all tests are summarized in Figure 7 as graphical representations. Tables of the raw data and individual plots for all tests are provided in the Supporting Information.

As expected, cell media and vehicle controls[87] had essentially no effect on the cell viability at 24 or 72 h of exposure. Also, as expected, hydrogen peroxide exhibited a typical dose−response curve, making a transition from >70% cell viability to ca. 60% viability at ca. 100 μM after either 24 or 72 h of exposure.[86] This transition percent range is thought to be relevant when assessing the appropriateness of translating the cell-based toxicity to potential toxicity within humans during clinical studies.[88] After the 24 h of exposure, GW0742 and **12d** had little effect on the cell viability, whereas **31a** began to demonstrate modest but statistically significant toxicity at 10 and 100 μM, despite it having the highest PPAR selectivity

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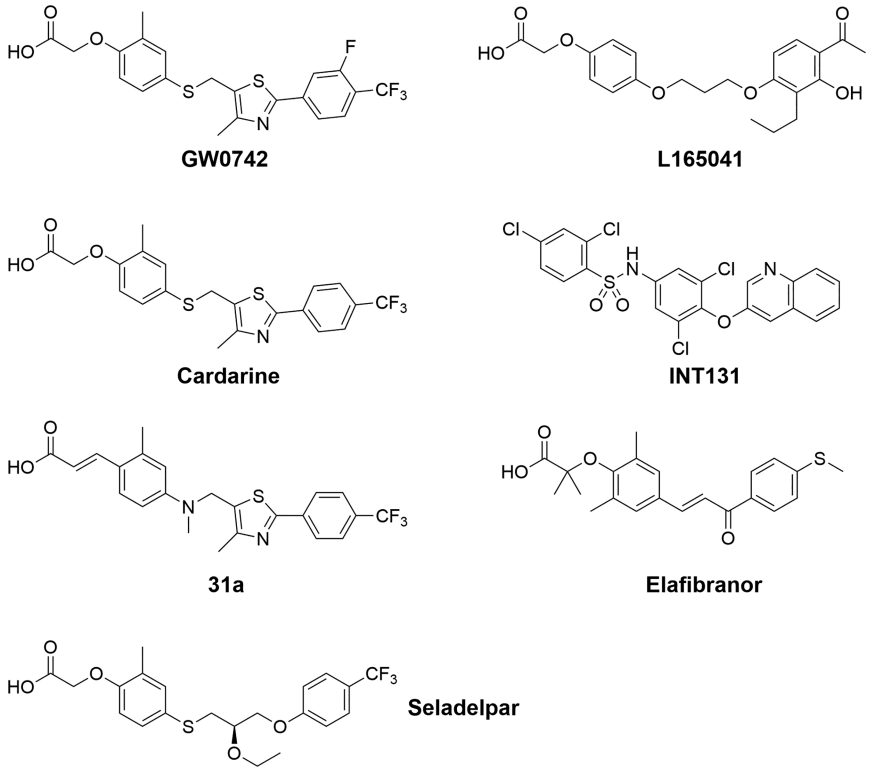

Figure 8. Chemical structures for comparison. GW0742, L165041, cardarine, and **31a** are PPARδ agonists. INT131 is a PPARγ agonist. The latter's acidic sulfonamide group has been situated toward the "west" to be at least somewhat similar to the other structures. All of these compounds have been reported to be osteogenic *in vitro*. Whereas cardarine decreased bone density in rat studies, **31a** maintained its osteogenic properties in mouse studies as reported herein. Elafibranor and seladelpar are PPARδ agonists that have undergone advanced clinical testing. Although cardarine proved to be carcinogenic during 2 year toxicity studies in rodents, both elafibranor and seladelpar have more recently been found to not be problematic.

profile. Importantly, none of the test agents dipped into the relevant toxicity range for potentially flagging the possibility of displaying human toxicity. We then pushed the assay's typical time frame further by extending the test agent exposure to 72 h. All three agents proved to be toxic to cells in the key loss of viability range at doses somewhere higher than 10 μM and lower than 100 μM. In addition to our own cytotoxicity study, we also contracted to have these three compounds independently examined in a similar assay conducted by NAMSA (Northwood, OH). NAMSA is an FDA-certified contract research organization that specializes in GLP toxicity assessments of devices and bioimplantable materials.[89] One of their cytotoxicity assays also lends itself to testing solid and liquid samples/formulations of potential pharmaceutical agents. For comparison, the NAMSA experimental procedure is recorded herein within the Experimental Section immediately after that of our own study's method. A hand-plot summarized from their data is also provided in Figure 7 for ready comparison to our summarized data. Raw data are tabulated in the Supporting Information. The NAMSA results are in close agreement with our findings. Taken together, the data support the overall conclusion that GW0742 and **31a** demonstrate "cytotoxic potential" at 100 μM, with **31a** possibly doing so at a somewhat lower dose as well. Alternatively, **12d** remains nontoxic unless exposure times are elongated past the typical assessment time frame generally utilized for these types of assays. Importantly, even for **31a**, the lower threshold for potential toxicity is ~50 times higher than its efficacious dose range in the MSC assay. Interestingly, PPAR selectivity does not appear to be correlated with less cytotoxicity. If the PPARδ potency taken from the transactivation assay is also taken into consideration analogous to a therapeutic index (TI) by calculating an approximated "cell toxicity index" (CTI = CT_{50}/EC_{50}, wherein the denominator can be taken directly from the transactivation assay and the numerator can be represented by the average from extrapolated estimates derived from the plots of both of the cytotoxicity assays), then all three compounds demonstrate at least 100-fold margins of "cell safety". The calculated CTI values are approximately 1700 for GW0742, 1350 for **12d**, and 250 for **31a**. Although these numbers reflect approximations and their individual values should not be misconstrued with actual TI calculations, together their relative rankings can be informative from a toxicity SAR perspective. For example, it appears that their sequence of relative cell-based "safeties" is directly proportional to the PPARδ potency (and toxicity is indirectly proportional to efficacy). When this observation is then taken together with the noted PPAR selectivity assessment, it seems reasonable to speculate that net cytotoxicity does not derive from a simple overstimulation of the PPARδ pathway and likely does not derive from the overstimulation of either of the other two PPAR pathways either. Similarly, our results begin to hint that toxicity may occur in a stepwise manner relative to increasing dose. This is suggested most clearly for **31a** in our 72 h toxicity–SAR experiment. It then becomes quite apparent in the NAMSA study for all three compounds because their experiments involved an additional three dose levels in the key range between 1 (or 3) μM and 10 (or 12) to 100 μM, namely, additional NAMSA doses at 6, 25, and 50 μM. Taken with the

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previously noted suggestions, these stepped curves imply that there may be multiple pathways leading to net toxicity that are somewhat similar among all three compounds and for which none of the mechanisms are likely linked in a proportional manner to any of the PPAR pathways.

Translational Consideration of Toxicity Relative to Use in Humans. Our preliminary toxicity assessments and specific cytotoxicity experiments have not raised any red flags with regard to moving **12d** or **31a** into preclinical development; however, because of the unfavorable history still looming from cardarine, long-term toxicity studies in rodents (up to at least 2 years) will need to be initiated as an early step toward the preclinical development of any PPARδ drug intended as a prolonged treatment for a chronic illness. Such studies are beyond the scope of early drug discovery efforts, which in our case focused first on simply assessing the possibility to impact favorably upon bone disease and, if so, then secondarily toward identifying potential candidate compounds for future testing. Alternatively, it is noteworthy that commentary applicable to addressing short- and long-term toxicity versus efficacy is afforded in the two recent reviews previously mentioned in the Introduction. In their patent review, Takada and Makishima[35] first refer to favorable effects on bone resulting from treatments with two different PPARδ agonists, namely, GW0742 (our prior work)[24] and L165041.[33,90,91] Likewise, we referred to mechanistic studies by others that also showed cardarine to have favorable effects on bone.[18] On the contrary, Takeda and Makishima next cite studies in rats using cardarine that caused a decrease in bone density.[92] Our studies reported herein used mice wherein we identified several cardarine analogs to have very positive effects on bone. Thus we fully agree with Takeda and Makishima's concluding remarks that the "effects of novel PPARδ agonists in the treatment of bone disorders require careful evaluation", and we further add that our work clearly demonstrates that certain analogs can favorably diverge from cardarine in terms of osteogenic activity.[35] This, in turn, raises the distinct possibility that analogs may also be able to diverge from cardarine in terms of the latter's long-term toxicity. Finally, these authors also discuss the PPARγ agonist, INT131,[93] which can increase bone density[94] by a "mechanism" that "remains unclear".[35] A side-by-side comparison of all of the aforementioned compounds is provided in Figure 8 along with one of our more preferred agents, **31a**. The different structure of the PPARγ compound is immediately evident even after similarly aligning the orientation of its weakly acidic sulfonamide.

In their medicinal chemistry review, Kadayat et al. cite nine PPARδ agonists that have recently "reached early stage clinical trials, with some of them under active development."[40] Several of these are intended for shorter term treatments. This range of compounds moving into the clinic suggests that, in general, the PPARδ agonists as a family are typically nonproblematic in terms of acute or short-term toxicity. As previously discussed, our preliminary results are in accord with this overall assessment. Similarly, for PPAR agonists, in general, "it is well established that drug candidates are nongenotoxic in vitro and in vivo".[31] Alternatively, as we have previously emphasized, it is their potential for long-term toxicity, likely resulting from off-target actions, that remains to be clarified. Importantly in this regard, Kadayat et al. also note that from their listing, two of the more advanced compounds "have cleared the 2 year carcinogenicity studies in rodents",[40] namely, elafibranor[95] and seladelpar[96] (structures also shown in Figure 8). Thus these authors finally conclude "that the tumorigenicity of GW501516 (also known as cardarine) may be a compound-specific not PPARδ-specific phenomenon".[40] This further underscores what we first raised as a general question in the Introduction and then were able to discern above as an even more distinct possibility from the Takeda and Makishima patent report when their general assessments were coupled to our specific results, as reported herein.

CONCLUSIONS

We synthesized a directed library of about 30 small-molecule probes designed to explore the selective activation of PPARδ and the possibility of promoting osteogenesis and bone formation. SARs were examined in the middle and at each end of a classical PPAR scaffold consisting of three independent rings. Probe features were designed by analogy to the structural requirements of known PPARδ agonists as well as with the intention of exploring certain novel modifications. Several compounds showed promising osteogenic activity in human MSC cultures. Six of these compounds were then tested in a PPAR transactivation assay and in an *in vivo* mouse model of human osteoporosis that required the *in situ* activation of inherent stem cells. Results from the transactivation assay provided quantitative assessments of potency and PPAR selectivity, with compound **7d** being the most potent and compound **31a** standing out as the most selective toward PPARδ. Compounds **7d**, **12d**, **23b**, and **31a** promoted improvements in bone density and microarchitecture *in vivo*, with compounds **12d** and **31a**, in particular, showing activity comparable to or better than GW0742, a classic PPARδ agonist. Because of their significant *in vivo* activity, **12d** and **31a** are regarded as our more preferred lead compounds. The extremely high selectivity for PPARδ demonstrated by **31a** is also noteworthy. Although an α,β-unsaturated carbonyl is present, the compounds do not undergo Michael addition reactions under simulated physiologic conditions. Most of the compounds do not display any significant cell-based toxicity within their efficacious dose ranges. No gross toxicity was observed during the *in vivo* studies, which involved drug treatments for up to 6 weeks. Specific cytotoxicity assessments of **12d** and **31a** did not reveal any flags for further consideration as preclinical development compounds. Interestingly, **12d** proved to be less toxic than **31a** despite the latter's high degree of selectivity. Cytotoxicity does not appear to be associated with a simple overstimulation of any of the PPAR pathways and, instead, likely derives from a composite of more than one other type of mechanism. Importantly, because of the clinical history for this class of compound, long-term (2-year) toxicity studies in rodents will need to be initiated early as a key step during preclinical development. Because most osteoporosis treatments currently rely upon the inhibition of osteoclast function, our results support PPARδ as a possible novel mechanistic target for treating osteoporosis or fracture healing through the *in situ* promotion of bone cell proliferation. Thus, continued success of prompting this mechanism through drug development and clinical studies without encountering significant unwanted side effects or toxicity could eventually result in a major paradigm shift for the treatment of osteoporosis, fractures, and other metabolic bone diseases.

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EXPERIMENTAL SECTION

Chemistry. *General Methods.* Chemical reactions were conducted using reagents purchased commercially from Sigma-Aldrich or Fisher Scientific International with the exception of the starting materials 4-(trifluoromethyl)thiobenzamide **1b** and 3-fluoro-4-(trifluoromethyl)thiobenzamide **1d**, which were purchased from Matrix Scientific. TLC was completed using Baker-flex precoated flexible TLC sheets purchased from VWR International (cat. no. JT4463-2) and visualized using shortwave ultraviolet light or by iodine chamber. Flash chromatography was completed using technical-grade silica gel (230−400 mesh particle size, 40−63 μm particle size, 60 Å pore size) purchased from Sigma-Aldrich. Melting points (Mp) were recorded using a Mel-Temp II melting point apparatus equipped with a 250 °C thermometer. Nuclear magnetic resonance (NMR) spectra were recorded using a Bruker Avance III 600 MHz spectrometer with a cryoprobe. Peak locations were referenced using a residual nondeuterated solvent and recorded as the chemical shift (δ) measured in parts per million (ppm). All compounds had >95% purity, as assessed by NMR and C,H,N elemental analysis. Elemental analysis was performed by Atlantic Microlab, and experimental values within ±0.4% of the calculated values were considered acceptable. A few compounds contain traces of solvent that were likewise well-defined by elemental analyses and accounted for as formula weights when conducting biological studies.

*4-Methyl-2-phenylthiazole-5-carboxylic Acid Ethyl Ester (**2a**).* To a suspension of thiobenzamide **1a** (6.05 g, 0.044 mol) in 95% ethanol was added ethyl 2-chloroacetoacetate (6.10 mL, 0.044 mol), and the mixture was stirred at reflux temperature for 26 h. The reaction mixture was concentrated under reduced pressure, and the resulting residue was suspended in ice-cold hexane and stirred for 20 min. The suspension was filtered, and **2a** was collected as a cream-colored solid (7.434 g, 0.030 mol, 68.3%). TLC R_f (25% EtOAc/hexane) = 0.63. Mp 84−87 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.19 (2H, d, J = 7.32 Hz), 7.56 (1H, t, J = 7.32 Hz), 7.53 (2H, t, J = 7.08 Hz), 4.41 (2H, q, J = 7.14 Hz), 2.94 (3H, s), 1.41 (3H, t, J = 7.14 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 171.25, 161.07, 157.83, 133.21, 129.82, 128.10, 122.55, 62.37, 16.29, 14.52.

*4-Methyl-2-phenyl-5-thiazolemethanol (**3a**).* To a stirred solution of ethyl ester **2a** (0.304 g, 1.237 mmol) in anhydrous THF (1 mL) at 0 °C was added 2 M lithium aluminum hydride solution in THF (1.24 mL, 2.48 mmol). The resulting mixture was stirred under inert gas at 0 °C for 1.5 h. The reaction mixture was quenched by the careful addition of water (0.5 mL) followed by dilution with ethyl acetate (2.5 mL), and drying with anhydrous sodium sulfate (0.92 g). The mixture was stirred for 15 min and was filtered and concentrated under reduced pressure to give **3a** as a light-yellow solid (0.215 g, 1.053 mmol, 85.1%). TLC R_f (25% EtOAc/hexane) = 0.11. Mp 101−102 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.88 (2H, d, J = 7.92 Hz), 7.41 (3H, m), 4.79 (2H, s), 2.94 (1H, s), 2.41 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 166.39, 150.22, 133.59, 131.49, 130.07, 129.03, 126.46, 56.79, 15.13.

*5-Chloromethyl-4-methyl-2-phenyl-thiazole (**4a**).* To a stirred solution of alcohol **3a** (4.095 g, 0.019 mol) in anhydrous dichloromethane (100 mL) was added triethylamine (5.50 mL, 0.039 mol). The resulting mixture was cooled to 4 °C, and methanesulfonyl chloride (2.30 mL, 0.029 mol) was slowly added. The mixture was stirred at 4 °C for 24 h and then diluted with dichloromethane (100 mL), washed with saturated $NaHCO_3$ solution, water, and brine, dried with Na_2SO_4, and concentrated. The residue was purified by flash chromatography using 10% ethyl acetate/hexane to give **4a** as a light-yellow solid (2.850 g, 0.013 mol, 64.0%). TLC R_f (25% EtOAc/hexane) = 0.57. Mp 89−90 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.90 (2H, m), 7.43 (3H, m), 4.80 (2H, s), 2.50 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 166.99, 152.68, 133.34, 130.27, 128.97, 127.55, 126.46, 37.53, 15.10. Anal. Calcd for $C_{11}H_{10}NSCl$: C, 59.06; H, 4.51; N, 6.26. Found: C, 59.32; H, 4.58; N, 6.33.

*Methyl 4-Hydroxycinnamate (**5**).* To a stirred solution of *p*-coumaric acid (0.704 g, 4.288 mmol) in anhydrous methanol (10 mL) was added concentrated H_2SO_4 (1 mL), and the solution was heated to reflux temperature for 20 h. The mixture was cooled to room temperature and concentrated under reduced pressure. The residue was dissolved in ethyl acetate, washed with water, dried with Na_2SO_4, and concentrated. The residue was purified by flash chromatography to give **5** as a white solid (0.574 g, 3.221 mmol, 75.1%). TLC R_f (25% EtOAc/hexane) = 0.18. Mp 137−138 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.65 (1H, d, J = 16.02 Hz), 7.44 (2H, d, J = 8.58), 6.86 (2H, d, J = 8.58), 6.31 (1H, d, J = 15.98), 5.57 (1H, s), 3.81 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 168.22, 157.87, 144.85, 130.14, 127.29, 116.01, 115.26, 51.87.

*Methyl 4-[[4-Methyl-2-phenylthiazol-5-yl]methyl]-methoxycinnamate (**6a**).* To a stirred solution of methyl 4-hydroxycinnamate **5** (0.142 g, 0.797 mmol) and chloromethyl **4a** (0.150 g, 0.670 mmol) in anhydrous acetonitrile (5 mL) was added cesium carbonate with partial solubility. The resulting mixture was stirred for 24 h at room temperature at which TLC showed that the chloromethyl **4a** had been consumed. The reaction mixture was concentrated, and the residue was dissolved in ethyl acetate and washed with water and brine, dried with Na_2SO_4, and concentrated. Flash chromatography failed to give a pure product and the crude white solid **6a** (0.202 g, 0.553 mmol, 82.6%) collected was moved to the next step without further purification.

*4-[[4-Methyl-2-phenylthiazol-5-yl]methyl]methoxycinnamic Acid (**7a**).* To a stirred solution of methyl ester **6a** was added dropwise 3 N NaOH. After 20 h, the mixture was acidified with 1 N HCl to pH 1 to 2 and concentrated. The residue was suspended in ethyl acetate and washed with water and brine. The aqueous phase was extracted with a separate portion of ethyl acetate, and the organic phases were combined, dried with Na_2SO_4, and concentrated. The residue was purified by flash chromatography using a 25% EtOAc/hexane to 100% EtOAc mobile-phase gradient to give **7a** as a white solid (0.061 g, 0.173 mmol, 41.7%). TLC R_f (50% EtOAc/hexane) = 0.17. Mp 209−211 °C. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.91 (2H, m), 7.67 (2H, d, J = 8.76 Hz), 7.55 (1H, d, J = 15.96 Hz), 7.49 (3H, m), 7.09 (2H, d, J = 8.82 Hz), 6.41 (1H, d, J = 15.96 Hz), 5.38 (2H, s), 2.46 (4H, s). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 159.85, 130.43, 129.72, 126.40, 115.83, 79.21, 15.43. Anal. Calcd for $C_{20}H_{17}NO_3S$ (with 0.3 EtOH mol per target): C, 67.74; H, 5.54; N, 3.83. Found: C, 67.97; H, 5.54; N, 3.76.

*Ethyl 4-Methyl-2-[4-(trifluoromethyl)phenyl]-thiazole-5-carboxylate (**2b**).* By analogy to the procedure described in example **2a**, 4-(trifluoromethyl)thiobenzamide **1a** (1.065 g, 5.190 mmol) was treated with ethyl-2-chloroacetoacetate (0.75 mL, 5.42 mmol) in 95% ethanol (30 mL) to give **2b** as a cream-colored solid (1.148 g, 3.644 mmol, 70.2%). TLC R_f (25% EtOAc/hexane) = 0.69. Mp 89−89.5 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.10 (2H, d, J = 8.04 Hz), 7.73 (2H, d, J = 8.16 Hz), 4.39 (2H, q, J = 7.14 Hz), 2.81 (3H, s), 1.42 (3H, t, J = 7.08 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.87, 162.17, 161.38, 136.16, 132.59 (q, $^2J_{FC}$ = 33 Hz), 127.18, 126.22 (m), 123.90 (q, $^1J_{FC}$ = 270 Hz), 123.13, 61.61, 17.67, 14.47. Anal. Calcd for $C_{14}H_{12}F_3NO_2S$ (with 0.2 H_2O mol per target): C, 52.73; H, 3.92; N, 4.39. Found: C, 52.59; H, 3.85; N, 4.71.

*4-Methyl-2-[4-(trifluoromethyl)phenyl]-thiazole-5-methanol (**3b**).* By analogy to the procedure described in example **3a**, ethyl ester **2b** (1.320 g, 4.190 mmol) in solution with anhydrous THF (15 mL) was treated with 2 M $LiAlH_4$ solution in THF (2.0 mL, 4.0 mmol) to give **3b** as a yellow solid (0.904 g, 3.308 mmol, 79.0%). TLC R_f (25% EtOAc/hexane) = 0.16. Mp 121.5−122 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.02 (2H, d, J = 8.04 Hz), 7.69 (2H, d, J = 8.16 Hz), 4.86 (2H, s), 2.48 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 164.33, 150.91, 136.74, 132.57, 131.63 (q, $^2J_{FC}$ = 33 Hz), 126.66, 126.08 (m), 124.03 (q, $^1J_{FC}$ = 270 Hz), 57.04, 15.27.

*5-Chloromethyl-4-methyl-2-[4-(trifluoromethyl)phenyl]-thiazole (**4b**).* By analogy to the procedure described in example **4a**, alcohol **3b** (0.883 g, 3.231 mmol) was treated with methanesulfonyl chloride (0.40 mL, 5.168 mmol) and triethylamine (0.90 mL, 6.462 mmol) in anhydrous DCM (25 mL) to give **4b** as a light-yellow solid (0.790 g, 2.708 mmol, 83.8%). TLC R_f (25% EtOAc/hexane) = 0.53. Mp 68.5−69 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.03 (2H, d, J = 8.10 Hz),

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7.70 (2H, d, J = 8.16 Hz), 4.81 (2H, s), 2.52 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 165.02, 153.25, 136.51, 131.92 (q, $^2J_{FC}$ = 33 Hz), 129.06, 126.77, 126.12 (m), 123.99 (q, $^1J_{FC}$ = 270 Hz), 37.32, 15.20. Anal. Calcd for $C_{12}H_9ClF_3NS$: C, 49.41; H, 3.11; N, 4.80. Found: C, 49.43; H, 3.22; N, 4.75.

*Methyl 4-[[4-Methyl-2-[4-(trifluoromethyl)phenyl]-thiazol-5-yl]-methyl]methoxy]cinnamate (**6b**).* By analogy to the procedure described in example **6a**, chloromethyl **4b** (0.331 g, 1.135 mmol) and methyl 4-hydroxycinnamate **5** (0.212 g, 1.133 mmol) were treated with cesium carbonate (0.570 g, 1.749 mmol) in anhydrous acetonitrile (6 mL) to give **6b** as a light-yellow solid (0.365 g, 0.842 mmol, 74.3%). TLC R_f (25% EtOAc/hexane) = 0.32. Mp 153−155 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.04 (2H, d, J = 8.1 Hz), 7.70 (2H, d, J = 8.2 Hz), 7.68 (1H, d, J = 16.0 Hz), 7.52 (2H, d, J = 8.6 Hz), 7.00 (2H, d, J = 8.7 Hz), 6.36 (1H, d, J = 16.0 Hz), 5.24 (2H, s), 3.8 (3H, s), 2.54 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.79, 165.13, 159.81, 152.49, 144.33, 136.67, 131.80 (q, $^2J_{FC}$ = 33 Hz), 129.96, 128.20, 127.61, 126.75, 126.10 (m), 124.02 (q, $^1J_{FC}$ = 270 Hz), 116.08, 115.38, 62.29, 51.82, 15.57. Anal. Calcd for $C_{22}H_{18}F_3NO_3S$: C, 60.96; H, 4.19; N, 3.23. Found: C, 60.79; H, 4.36; N, 3.15.

*4-[[4-Methyl-2-[4-(trifluoromethyl)phenyl]-thiazol-5-yl]methyl]-methoxycinnamic Acid (**7b**).* Methyl ester **6b** (0.117 g, 0.270 mmol) was dissolved in 95% ethanol (5 mL) and THF (5 mL). 3 N NaOH (1.0 mL) was slowly added, and the reaction mixture was stirred overnight followed by neutralization with 1 N HCl to pH 2. The solvent was evaporated under reduced pressure, and the residue was taken up into EtOAc, washed with saturated $NaHCO_3$ and brine, dried with Na_2SO_4, filtered, and concentrated. The crude material was purified with flash chromatography using a 25−50% EtOAc/hexane mobile-phase gradient supplemented with dropwise acetic acid. The recovered product was recrystallized using 95% ethanol to give **7b** as a white solid (0.064 g, 0.152 mmol, 56.5%). TLC R_f (50% EtOAc/hexane) = 0.15. Mp 227−228 °C. ^{1}H NMR (acetone-d_6, 600 MHz): δ 8.18 (2H, d, J = 8.1 Hz), 7.85 (2H, d, J = 8.2 Hz), 7.67 (2H, d, J = 8.8 Hz), 7.63 (1H, d, J = 16.0 Hz), 7.13 (2H, d, J = 8.8 Hz), 6.41 (1H, d, J = 16.0 Hz), 5.44 (2H, s), 2.53 (3H, s). ^{13}C NMR (acetone-d_6, 150 MHz): δ 167.94, 164.82, 160.83, 153.28, 144.93, 137.90, 131.71 (q, $^2J_{FC}$ = 33 Hz), 130.75, 129.46, 128.87, 127.50, 126.93 (m), 125.11 (q, $^1J_{FC}$ = 270 Hz), 117.15, 116.24, 62.85, 15.42. ^{19}F NMR (acetone-d_6, 376 MHz): δ −63.67 (3F, s). Anal. Calcd for $C_{21}H_{16}F_3NO_3S$: C, 60.14; H, 3.85; N, 3.34. Found: C, 60.12; H, 3.85; N, 3.31.

*Ethyl 4-Methyl-2-[3-(trifluoromethyl)phenyl]-thiazole-5-carboxylate (**2c**).* By analogy to the procedure described in example **2a**, 3-(trifluoromethyl)thiobenzamide **1c** (2.00 g, 9.75 mmol) was treated with ethyl-2-chloroacetoacetate (1.35 mL) in 95% ethanol (55 mL) to give **2c** as a cream-colored solid (2.103 g, 6.677 mmol, 68.5%). TLC R_f (25% EtOAc/hexane) = 0.63. Mp 90−91 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.26 (1H, s), 8.14 (1H, d, J = 7.86 Hz), 7.73 (1H, d, J = 7.80 Hz), 7.60 (1H, dd, J_1 = 7.86 Hz, J_2 = 7.80 Hz), 4.38 (2H, q, J = 7.14 Hz), 2.81 (3H, s), 1.41 (3H, t, J = 7.14 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.94, 162.18, 161.34, 133.83, 131.80 (q, $^2J_{FC}$ = 32.7 Hz), 130.03, 129.78, 127.48 (q, $^3J_{FC}$ = 3.5 Hz), 123.81 (q, $^3J_{FC}$ = 271 Hz), 123.69 (q, $^3J_{FC}$ = 3.5 Hz), 122.84, 61.59, 17.65, 14.46. Anal. Calcd for $C_{14}H_{12}F_3NO_2S$: C, 53.33; H, 3.84; N, 4.44. Found: C, 53.05; H, 3.93; N, 4.54.

*4-Methyl-2-[3-(trifluoromethyl)phenyl]-thiazole-5-methanol (**3c**).* By analogy to the procedure described in example **3a**, ethyl ester **2c** (2.053 g, 6.511 mmol) in solution with anhydrous THF (20 mL) was treated with 2 M $LiAlH_4$ solution in THF (3.3 mL, 6.60 mmol) to give **3c** as a yellow solid (1.273 g, 4.658 mmol, 71.5%). TLC R_f (25% EtOAc/hexane) = 0.16. Mp 58−60 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.19 (1H, s), 8.07 (1H, d, J = 7.8 Hz), 7.66 (1H, d, J = 7.8 Hz), 7.56 (1H, t, J = 7.8 Hz), 4.87 (2H, s), 2.49 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 164.43, 150.67, 134.31, 132.36, 131.61 (q, $^2J_{FC}$ = 32.5 Hz), 129.63, 126.52 (q, $^3J_{FC}$ = 3.5 Hz), 123.92 (q, $^1J_{FC}$ = 271 Hz), 123.19 (q, $^3J_{FC}$ = 3.5 Hz), 56.95, 15.20. Anal. Calcd for $C_{12}H_{10}F_3NOS$: C, 52.74; H, 3.69; N, 5.13. Found: C, 52.55; H, 3.64; N, 5.21.

*5-Chloromethyl-4-methyl-2-[3-(trifluoromethyl)phenyl]thiazole (**4c**).* By analogy to the procedure described in example **4a**, alcohol **3c** (1.295 g, 4.739 mmol) was treated with methanesulfonyl chloride (0.55 mL, 7.11 mmol) and triethylamine (1.3 mL, 9.33 mmol) in anhydrous DCM (25 mL) to give **4c** as a light-yellow solid (0.830 g, 2.846 mmol, 60.1%). TLC R_f (25% EtOAc/hexane) = 0.61. Mp 43−44 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.19 (1H, s), 8.07 (1H, d, J = 7.8 Hz), 7.68 (1H, d, J = 7.8 Hz), 7.57 (1H, t, J = 7.8 Hz), 4.81 (2H, s), 2.51 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 165.09, 153.16, 134.21, 131.68 (q, $^2J_{FC}$ = 33 Hz), 129.70, 129.67 128.75, 126.78 (q, $^3J_{FC}$ = 3.6 Hz), 123.90 (q, $^1J_{FC}$ = 271 Hz), 123.30 (q, $^3J_{FC}$ = 3.6 Hz), 37.37, 15.21. Anal. Calcd for $C_{12}H_9ClF_3NS$: C, 49.41; H, 3.11; N, 4.80. Found: C, 49.46; H, 3.16; N, 4.91.

*Ester 4-[[4-Methyl-2-[3-(trifluoromethyl)phenyl]-thiazol-5-yl]-methyl]methoxycinnamate (**6c**).* By analogy to the procedure described in example **6a**, chloromethyl **4c** (0.472 g, 1.618 mmol) and methyl 4-hydroxycinnamate **5** (0.281 g, 1.577 mmol) were treated with cesium carbonate (0.773 g, 2.372 mmol) in anhydrous acetonitrile (10 mL) to give **6c** as a white solid (0.551 g, 1.270 mmol, 80.5%). TLC R_f (25% EtOAc/hexane) = 0.30. Mp 125−127 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.19 (1H, s), 8.09 (1H, d, J = 7.7 Hz), 7.68 (1H, d, J = 7.8 Hz), 7.67 (1H, d, J = 16.0 Hz), 7.56 (1H, t, J = 7.8 Hz), 7.51 (2H, d, J = 8.6 Hz), 6.99 (2H, d, J = 8.7 Hz), 5.24 (2H, s), 3.81 (3H, s), 2.54 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.81, 165.26, 159.79, 152.23, 144.34, 134.20, 131.66 (q, $^2J_{FC}$ = 32 Hz), 129.96, 129.72, 129.67, 128.19, 127.36, 126.72 (q, $^3J_{FC}$ = 3.9 Hz), 125.71 (q, $^1J_{FC}$ = 270 Hz), 123.31 (q, $^3J_{FC}$ = 3.5 Hz), 116.06, 115.39, 62.28, 51.81, 15.51. Anal. Calcd for $C_{22}H_{18}F_3NO_3S$ (with 0.4 H_2O mol per target): C, 59.97; H, 4.30; N, 3.18. Found: C, 59.66; H, 4.29; N, 3.07.

*4-[[4-Methyl-2-[3-(trifluoromethyl)phenyl]-thiazol-5-yl]methyl]-methoxycinnamic Acid (**7c**).* By analogy to the procedure described in example **7a**, methyl ester **6c** (0.207 g, 0.477 mmol) was treated with 3 N NaOH (1 mL) in 95% ethanol (16 mL) to give **7c** as a white solid (0.138 g, 0.329 mmol, 69.0%). TLC R_f (50% EtOAc/hexane) = 0.40. Mp 179.5−181 °C. ^{1}H NMR (acetone-d_6, 600 MHz): δ 8.29 (1H, s), 8.23 (1H, d, J = 7.8 Hz), 7.83 (1H, d, J = 7.8 Hz), 7.76 (1H, t, J = 7.8 Hz), 7.69 (2H, d, J = 8.7 Hz), 7.65 (1H, d, J = 16.0 Hz), 7.14 (2H, d, J = 8.7 Hz), 6.43 (1H, d, J = 16.0 Hz), 5.46 (2H, s), 2.54 (3H, s). ^{13}C NMR (acetone-d_6, 150 MHz): δ 167.93, 164.81, 160.85, 153.12, 145.02, 135.37, 131.73 (q, $^2J_{FC}$ = 32 Hz), 131.14, 130.77, 130.70, 129.09, 128.85, 127.27 (q, $^3J_{FC}$ = 3.3 Hz), 125.01 (q, $^1J_{FC}$ = 270 Hz), 123.11 (q, $^3J_{FC}$ = 3.5 Hz), 117.05, 116.24, 62.86, 15.42. ^{19}F NMR (acetone-d_6, 376 MHz): δ −63.76 (s, 3F). Anal. Calcd for $C_{21}H_{16}F_3NO_3S$: C, 60.14; H, 3.85; N, 3.34. Found: C, 59.96; H, 3.89; N, 3.30.

*Ethyl 4-Methyl-2-[3-fluoro-4-(trifluoromethyl)phenyl]thiazole-5-carboxylate (**2d**).* By analogy to the procedure described in example **2a**, 3-fluoro-4-(trifluoromethyl)thiobenzamide **1d** (1.606 g, 7.195 mmol) was treated with ethyl-2-chloroacetoacetate (1.00 mL, 7.23 mmol) in 95% ethanol (40 mL) to give **2d** as a cream-colored solid (1.657 g, 4.971 mmol, 69.1%). TLC R_f (25% EtOAc/hexane) = 0.72. Mp 101−102 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.86 (1H, d, J = 10.9 Hz), 7.82 (1H, d, J = 7.7 Hz), 7.70 (1H, t, J = 7.7 Hz), 4.40 (2H, q, J = 7.1 Hz), 2.80 (3H, s), 1.41 (3H, t, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 166.23, 161.97, 161.43, 160.16 (d, $^1J_{FC}$ = 254 Hz), 138.56 (d, $^4J_{FC}$ = 8.2 Hz), 128.16 (q, $^3J_{FC}$ = 3.9 Hz), 123.75, 122.39 (q, $^1J_{FC}$ = 271 Hz), 122.34 (d, $^3J_{FC}$ = 3.9 Hz), 120.20 (dq, $^2J_{FC}$ = 33 Hz, $^2J_{FC}$ = 12 Hz), 115.07 (d, $^2J_{FC}$ = 23 Hz), 67.74, 17.62, 14.45. ^{19}F NMR ($CDCl_3$, 376 MHz): δ −61.74 (3F, s), −113.07 (1F, s). Anal. Calcd for $C_{14}H_{11}F_4NO_2S$: C, 50.45; H, 3.33; N, 4.20. Found: C, 50.47; H, 3.32; N, 4.24.

*5-Hydroxymethyl-4-methyl-2-[3-fluoro-4-(trifluoromethyl)-phenyl]thiazole (**3d**).* By analogy to the procedure described in example **3a**, ethyl ester **2d** (4.640 g, 13.92 mmol) was dissolved in THF (50 mL) and treated with a 2 M $LiAlH_4$ solution in THF (7.2 mL, 14.4 mmol) to give **3d** as a yellow solid (2.988 g, 10.26 mmol, 73.7%). TLC R_f (25% EtOAc/hexane) = 0.21. Mp 158−159 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.79 (1H, d, J = 11.2 Hz), 7.75 (1H, d, J = 8.3 Hz), 7.65 (1H, t, J_1 = 7.6 Hz, J_2 = 7.7 Hz), 4.87 (2H, d, J = 5.2 Hz), 2.48 (3H, s), 1.93 (1H, t, J = 5.5 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 162.73, 160.16 (d, $^1J_{FC}$ = 255 Hz), 151.11, 139.20 (d, $^4J_{FC}$ =

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8.2 Hz), 133.37, 127.98 (m), 122.53 (q, $^{1}J_{FC}$ = 271 Hz), 121.83 (d, $^{3}J_{FC}$ = 3.3 Hz), 119.17 (dq, $^{2}J_{FC}$ = 33 Hz, $^{2}J_{FC}$ = 12 Hz), 114.48 (d, $^{2}J_{FC}$ = 23 Hz), 57.05, 15.27. ^{19}F NMR ($CDCl_3$, 376 MHz): δ −61.81 (3F, s), −113.84 (1F, s). Anal. Calcd for $C_{12}H_9F_4NOS$: C, 49.49; H, 3.11; N, 4.81. Found: C, 49.52; H, 3.09; N, 4.79.

*5-Chloromethyl-4-methyl-2-[3-fluoro-4-(trifluoromethyl)-phenyl]thiazole (**4d**).* By analogy to the procedure described in example **4a**, alcohol **3d** (1.502 g, 5.157 mmol) was treated with methanesulfonyl chloride (0.60 mL, 7.74 mmol) and triethylamine (1.40 mL, 10.314 mmol) in anhydrous DCM (50 mL) to give **4d** as a yellow oil (1.357 g, 4.382 mmol, 85.0%). TLC R_f (25% EtOAc/hexane) = 0.68. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.75 (2H, m), 7.65 (1H, m), 4.79 (2H, s), 2.50 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 163.38, 161.02 (d, $^{1}J_{FC}$ = 255 Hz), 159.32, 153.52, 139.00 (d, $^{4}J_{FC}$ = 8.2 Hz), 129.83, 128.02 (q, $^{3}J_{FC}$ = 4 Hz), 123.39 (q, $^{1}J_{FC}$ = 270 Hz), 121.91 (m), 119.47 (dq, $^{2}J_{FC}$ = 33 Hz, $^{2}J_{FC}$ = 12 Hz), 114.60 (d, $^{2}J_{FC}$ = 22 Hz), 37.15, 15.21. ^{19}F NMR ($CDCl_3$, 376 MHz): δ −61.82 (3F, s), −113.62 (1F, s). Anal. Calcd for $C_{12}H_8ClF_4NS$: C, 46.54; H, 2.60; N, 4.52. Found: C, 46.61; H, 2.37; N, 4.44.

*Methyl 4-[Oxo-[5-methylene-4-methyl-2-[3-fluoro-4-(trifluoromethyl)phenyl]-thiazole]]cinnamate (**6d**).* By analogy to the procedure described in example **6a**, chloromethyl **4d** (0.494 g, 1.595 mmol) and methyl 4-hydroxycinnamate **5** (0.287 g, 1.611 mmol) were treated with cesium carbonate (1.037 g, 3.183 mmol) in anhydrous acetonitrile (10 mL) to give **6d** as a white solid (0.614 g, 1.360 mmol, 85.3%). TLC R_f (25% EtOAc/hexane) = 0.46. Mp 180 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.78 (1H, d, J = 11.6 Hz), 7.75 (1H, d, J = 8.3 Hz), 7.66 (2H, m,), 7.51 (2H, d, J = 8.7 Hz), 6.98 (2H, d, J = 8.8 Hz), 6.34 (1H, d, J = 16.0 Hz), 5.23 (2H, s), 3.80 (3H, s), 2.52 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.78, 163.52, 160.17 (d, $^{1}J_{FC}$ = 255 Hz), 159.73, 152.66, 144.29, 139.12 (d, $^{4}J_{FC}$ = 7.5 Hz), 129.97, 128.43, 128.27, 128.00 (m), 122.51 (q, $^{1}J_{FC}$ = 270 Hz), 121.89 (d, $^{3}J_{FC}$ = 3 Hz), 119.48 (dq, $^{2}J_{FC}$ = 33 Hz, $^{2}J_{FC}$ = 12 Hz), 116.14, 115.36, 114.58 (d, $^{2}J_{FC}$ = 22 Hz), 62.24, 51.82, 15.56. Anal. Calcd for $C_{22}H_{17}F_4NO_3S$: C, 58.53; H, 3.80; N, 3.10. Found: C, 58.56; H, 3.83; N, 3.15.

*4-[Oxo-[5-methylene-4-methyl-2-[3-fluoro-4-(trifluoromethyl)-phenyl]thiazole]]cinnamic Acid (**7d**).* By analogy to the procedure described in example **7a**, methyl ester **6d** (0.562 g, 1.245 mmol) was treated with 3 N NaOH (2 mL) in 95% EtOH (20 mL) and THF (10 mL) to give **7d** as a white solid (0.403 g, 0.921 mmol, 74.0%). TLC R_f (50% EtOAc/hexane) = 0.29. Mp 192−194 °C. ^{1}H NMR (acetone-d_6, 600 MHz): δ 7.97 (2H, t, J = 11 Hz), 7.87 (1H, t, J = 7.9 Hz), 7.68 (2H, d, J = 8.7 Hz), 7.65 (1H, d, J = 16.0 Hz), 7.14 (2H, d, J = 8.7 Hz), 6.43 (1H, d, J = 16.0 Hz), 5.46 (2H, s), 2.53 (3H, s). ^{13}C NMR (acetone-d_6, 150 MHz): δ 167.89, 163.30, 161.58 (d, $^{1}J_{FC}$ = 254 Hz), 160.79, 153.41, 144.98, 140.57 (d, $^{4}J_{FC}$ = 8.4 Hz), 130.77, 130.46, 129.14 (q, $^{3}J_{FC}$ = 3.9 Hz), 128.91, 124.51 (q, $^{1}J_{FC}$ = 270 Hz), 123.06 (d, $^{3}J_{FC}$ = 4 Hz), 119.11 (dq, $^{2}J_{FC}$ = 33 Hz, $^{2}J_{FC}$ = 13 Hz), 117.10, 116.24, 114.89 (d, $^{2}J_{FC}$ = 23 Hz), 62.86, 15.43. Anal. Calcd for $C_{21}H_{15}F_4NO_3S$: C, 57.66; H, 3.46; N, 3.20. Found: C, 57.95; H, 3.48; N, 3.20.

*2-(4-Bromophenyl)-1,3-dioxolane (**8**).* 4-Bromobenzaldehyde (4.303 g, 23.26 mmol) was dissolved in anhydrous toluene (50 mL), and ethylene glycol (3.80 mL, 69.18 mmol) was added followed by *p*-toluenesulfonic acid monohydrate (0.303 g, 1.59 mmol). The mixture was heated to a vigorous reflux in a Dean−Stark apparatus and stirred at that temperature for 2 days. The mixture was allowed to cool to rt and was poured in a 75 mL saturated $NaHCO_3$ solution and extracted with 40 mL of toluene. The organic phase was collected and washed with water twice, dried with Na_2SO_4, and concentrated under reduced pressure. The resulting crude mixture was purified using flash chromatography to give **8** as a white solid (2.787 g, 12.17 mmol, 52.3%). TLC R_f (25% EtOAc/hexane) = 0.55. Mp 34−35 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.52 (2H, d, J = 8.4 Hz), 7.36 (2H, d, J = 8.4 Hz), 5.78 (1H, s), 4.10 (2H, m), 4.02 (2H, m). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 137.29, 131.63, 128.30, 123.33, 103.19, 65.43. Anal. Calcd for $C_9H_9O_2Br$: C, 47.19; H, 3.96; N, 0.00. Found: C, 47.00; H, 3.97; N, 0.00.

*2-[4-Thio-[5-methylene-4-methyl-2-phenylthiazole]phenyl]-1,3-dioxolane (**9a**).* Acetal **8** (0.934 g, 4.077 mmol) was dissolved in anhydrous THF (15 mL) under inert gas and cooled to −78 °C. A *t*-BuLi solution in THF (1.7 M, 4.8 mL, 8.55 mmol) was slowly added, and the reaction was stirred for 2 h. Sulfur (0.131 g, 4.078 mmol) was suspended in anhydrous THF (9 mL) and added to the reaction at −78 °C. The mixture was stirred at rt for 1.5 h and then cooled to 0 °C. Thiazole **4a** (0.918 g, 4.103 mmol) was dissolved in THF (15 mL) and added to the reaction mixture. The reaction mixture was allowed to warm to rt and stirred for an additional 2 h. The mixture was quenched with aqueous NH_4Cl, and the organic phase was separated. The aqueous phase was extracted using two portions of EtOAc, and the organic phases were combined, washed with water and brine, and dried over Na_2SO_4. After evaporation of the solvent, a crude product **9a** was isolated as a sticky yellow solid. Attempts to purify the product using flash chromatography were unsuccessful, and the material was moved to the next step (0.941 g, 2.546 mmol, 62.5%). TLC R_f (25% EtOAc/hexane) = 0.23.

*4-Thio-[5-methylene-4-methyl-2-phenylthiazole]benzaldehyde (**10a**).* Crude acetal **9a** (0.476 g, 1.288 mmol) was dissolved in THF (15 mL), and 3 N HCl (5 mL) was added. The reaction mixture was stirred at rt for 2 h and was concentrated under reduced pressure. The residue was neutralized with 1 N NaOH and extracted with EtOAc. The organic extract was washed with water and brine and dried with Na_2SO_4. After evaporation of the solvent, the product was purified with flash chromatography using a gradient of 10−20% EtOAc/hexane. The purified product **10a** was collected as a yellow solid (0.302 g, 0.928 mmol, 72.0%). TLC R_f (25% EtOAc/hexane) = 0.35. Mp 116−117 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 9.95 (1H, s), 7.86 (2H, m), 7.80 (2H, d, J = 8.3 Hz), 7.43 (2H, d, J = 8.3 Hz), 7.41 (3H, m), 4.37 (2H, s), 2.43 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 191.34, 165.94, 151.23, 144.55, 134.30, 133.51, 130.28, 130.17, 129.04, 128.34, 127.31, 126.42, 29.28, 15.34. Anal. Calcd for $C_{18}H_{15}NOS_2$: C, 66.43; H, 4.65; N, 4.30. Found: C, 66.29; H, 4.53; N, 4.19.

*Methyl 4-[Thio-[5-methylene-4-methyl-2-phenylthiazole]]-cinnamate (**11a**).* Aldehyde **10a** (0.251 g, 0.771 mmol) and methyl (triphenylphosphoranylidene)acetate (0.291 g, 0.870 mmol) were dissolved in anhydrous THF (6 mL) under inert gas and stirred at 60 °C for 2 days. The reaction mixture was concentrated, and the resulting residue was taken up into EtOAc, washed with water and brine, and dried with Na_2SO_4. After evaporation of the solvent, the product was purified with flash chromatography using a gradient of 5−15% EtOAc/hexane. The product collected from the column was recrystallized using 95% EtOH to give **11a** as a light-yellow solid (0.244 g, 0.640 mmol, 83.1%). TLC R_f (25% EtOAc/hexane) = 0.36. Mp 102−103 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.87 (2H, m), 7.63 (1H, d, J = 16.0 Hz), 7.44 (2H, d, J = 8.2 Hz), 7.41 (3H, m), 7.34 (2H, d, J = 8.3 Hz), 6.41 (1H, d, J = 16.0 Hz), 4.27 (2H, s), 3.80 (3H, s), 2.35 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.47, 143.99, 137.99, 133.19, 130.51, 130.32, 129.10, 128.86, 128.74, 128.48, 127.35, 126.62, 126.53, 118.09, 51.92, 30.43, 15.07. Anal. Calcd for $C_{18}H_{15}NOS_2$: C, 66.11; H, 5.02; N, 3.67. Found: C, 66.05; H, 4.87; N, 3.56.

*4-[Thio-[5-methylene-4-methyl-2-phenylthiazole]]cinnamic Acid (**12a**).* Methyl ester **11a** (0.200 g, 0.524 mmol) was dissolved in 95% EtOH (15 mL) using heat. The mixture was cooled to rt, and 3 N NaOH was added. The reaction mixture was stirred for 24 h and was concentrated under reduced pressure. The resulting residue was taken up into EtOAc and washed with acidified water (HCl, pH 3) and brine and dried with Na_2SO_4. After evaporation of the solvent, the product was purified by flash chromatography using a gradient of 25−100% EtOAc/hexane. The product collected from the column was recrystallized using 95% EtOH to give **12a** as an off-white solid (0.089 g, 0.243 mmol, 46.4%). TLC R_f (50% EtOAc/hexane) = 0.33. Mp 192.5−193.5 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.90 (2H, m), 7.65−7.61 (3H, m), 7.45 (5H, m), 6.51 (1H, d, J = 16.0 Hz), 4.52 (2H, s), 2.35 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.71, 165.36, 151.94, 144.51, 139.16, 134.56, 133.79, 130.76, 130.55, 129.86, 129.65, 129.56, 126.76, 119.24, 15.18. Anal. Calcd for

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$C_{18}H_{15}NOS_2$ (0.2 H_2O mol per target): C, 65.37; H, 4.66; N, 3.81. Found: C, 64.64; H, 4.57; N, 3.72.

*2-[4-Thio-[5-methylene-4-methyl-2-[4-(trifluoromethyl)phenyl]-thiazole]phenyl]-1,3-dioxolane (**9b**).* By analogy to the procedure described in example **9a**, acetal **8** (0.909 g, 3.968 mmol) was reacted in a one-pot reaction involving a *t*-BuLi solution in THF (1.7 M, 4.7 mL, 7.99 mmol), sulfur (0.130 g, 4.053 mmol), and thiazole **4b** (1.150 g, 3.942 mmol). The crude product was isolated as a sticky yellow solid and was purified by flash chromatography using a 5−20% EtOAc/hexane gradient to give **9b** as a yellow solid (0.658g, 1.504 mmol, 38.2%). TLC R_f (25% EtOAc/hexane) = 0.33. Mp 57−61 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.98 (2H, d, J = 8.1 Hz), 7.66 (2H, d, J = 8.1 Hz), 7.42 (2H, d, J = 8.2 Hz), 7.38 (2H, d, J = 8.4 Hz), 5.78 (1H, s), 4.24 (2H, s), 4.12 (2H, m), 4.04 (2H, m), 2.31 (3H, m). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 163.50, 151.43, 137.36, 136.75, 135.85, 131.48 (q, $^2J_{FC}$ = 32 Hz), 131.10, 130.36, 127.40, 126.57, 126.01 (m), 124.05 (q, $^1J_{FC}$ = 270 Hz), 103.34, 65.46, 30.98, 15.12. Anal. Calcd for $C_{21}H_{18}NO_2S_2$ (with 0.4 H_2O mol per target): C, 56.72; H, 4.26; N, 3.15. Found: C, 56.50; H, 4.00; N, 3.32.

*4-Thio-[5-methylene-4-methyl-2-[4-(trifluoromethyl)phenyl]-thiazole]benzaldehyde (**10b**).* By analogy to the procedure described in example **10a**, acetal **9b** (0.589 g, 1.346 mmol) was treated with 3 N HCl (5 mL), and the crude product was purified with flash chromatography using a gradient of 5−20% EtOAc/hexane to give **10b** as a yellow solid (0.297 g, 0.754 mmol, 56.0%). TLC R_f (25% EtOAc/hexane) = 0.36. Mp 77−79 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 9.94 (1H, s), 7.96 (2H, d, J = 8.1 Hz), 7.79 (2H, d, J = 8.5 Hz), 7.65 (2H, d, J = 8.2 Hz), 7.43 (2H, d, J = 8.3 Hz), 4.36 (2H, s), 2.43 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 191.28, 163.85, 151.76, 144.21, 136.58, 134.39, 131.65 (q, $^2J_{FC}$ = 32 Hz), 130.30, 128.94, 128.37, 126.57, 126.04 (m), 123.99 (q, $^1J_{FC}$ = 271 Hz), 29.19, 15.32. Anal. Calcd for $C_{18}H_{15}NOS_2$: C, 58.00; H, 3.59; N, 3.56. Found: C, 57.83; H, 3.67; N, 3.63.

*Methyl 4-[Thio-[5-methylene-4-methyl-2-[4-(trifluoromethyl)-phenyl]-thiazole]]cinnamate (**11b**).* By analogy to the procedure described in example **11a**, aldehyde **10b** (0.248 g, 0.629 mmol) was treated with methyl (triphenylphosphoranylidene)acetate (0.238 g, 0.712 mmol), and the crude product was purified with flash chromatography using a gradient of 5−15% EtOAc/hexane to give **11b** as a light-yellow solid (0.201 g, 0.447 mmol, 71.1%). TLC R_f (25% EtOAc/hexane) = 0.44. Mp 121−122 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.98 (2H, d, J = 8.1 Hz), 7.65 (3H, m), 7.45 (2H, d, J = 8.2 Hz), 7.35 (2H, d, J = 8.3 Hz), 6.42 (1H, d, J = 16.0 Hz), 4.28 (2H, s), 3.81 (3H, s), 2.36 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.43, 163.68, 151.50, 143.91, 137.80, 136.62, 133.27, 131.61 (q, $^2J_{FC}$ = 32.5 Hz), 130.53, 129.90, 128.74, 126.58, 126.03 (m), 124.01 (q, $^1J_{FC}$ = 270 Hz), 118.16, 51.93, 30.39, 15.19. Anal. Calcd for $C_{18}H_{15}NOS_2$: C, 58.78; H, 4.04; N, 3.12. Found: C, 58.98; H, 4.05; N, 3.13.

*4-Thio-[5-methylene-4-methyl-2-[4-(trifluoromethyl)phenyl]-thiazole]cinnamic Acid (**12b**).* By analogy to the procedure described in example **12a**, methyl ester **11b** (0.178 g, 0.396 mmol) was treated with 3 N NaOH (1 mL), and the crude product was purified by flash chromatography and recrystallized with 95% EtOH to give the product **12b** as a light-yellow solid (0.119 g, 0.273 mmol, 68.9%). TLC R_f (50% EtOAc/hexane) = 0.54. Mp 217 °C. ^{1}H NMR (acetone-d_6, 600 MHz): δ 10.79 (1H, bs), 8.11 (2H, d. J = 8.1 Hz), 7.81 (2H, d, J = 8.1 Hz), 7.64 (3H, m), 7.47 (2H, d, J = 8.5 Hz), 6.52 (1H, d, J = 16.1 Hz), 4.55 (2H, s), 2.39 (3H, s). ^{13}C NMR (acetone-d_6, 150 MHz): δ 167.62, 163.35, 152.54, 144.54, 138.94, 137.98, 133.89, 131.60, 131.51 (q, $^2J_{FC}$ = 32 Hz), 130.64, 129.60, 127.31, 126.88 (q, $^3J_{FC}$ = 4.5 Hz), 125.13 (q, $^1J_{FC}$ = 270 Hz), 119.25, 15.20. ^{19}F NMR (acetone-d_6, 376 MHz): δ −63.67 (s, 3F). Anal. Calcd for $C_{21}H_{16}F_3NO_2S_2$: C, 57.92; H, 3.70; N, 3.22. Found: C, 57.80; H, 3.66; N, 3.29.

*2-[4-Thio-[5-methylene-4-methyl-2-[3-(trifluoromethyl)phenyl]-thiazole]phenyl]-1,3-dioxolane (**9c**).* By analogy to the procedure described in example **9a**, acetal **8** (1.079 g, 4.710 mmol) was reacted in a one-pot reaction involving a *t*-BuLi solution in THF (1.7 M, 5.6 mL, 9.52 mmol), sulfur (0.176 g, 5.488 mmol), and thiazole **4c** (1.390 g, 4.765 mmol). The crude product **9c** was isolated as an orange oil, and attempts to purify the product using flash chromatography were unsuccessful. The material was moved to the next step (0.604 g, 1.381 mmol, 29.3%). TLC R_f (25% EtOAc/hexane) = 0.31.

*4-Thio-[5-methylene-4-methyl-2-[3-(trifluoromethyl)phenyl]-thiazole]benzaldehyde (**10c**).* By analogy to the procedure described in example **10a**, crude acetal **9c** (0.604 g, 1.381 mmol) was treated with 3 N HCl (5 mL), and the crude product was purified with column chromatography using a gradient of 10−20% EtOAc/hexane to give **10c** as a yellow solid (0.275 g, 0.698 mmol, 50.6%). Elemental analysis was completed after NMR experiments, leading to $CDCl_3$ contaminant. TLC R_f (25% EtOAc/hexane) = 0.40. Mp 89−91 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 9.95 (1H, s), 8.15 (1H, s), 8.02 (1H, d, J = 7.8 Hz), 7.81 (2H, d, J = 8.5 Hz), 7.65 (1H, d, J = 7.8 Hz), 7.54 (1H, d, J = 7.8 Hz), 7.44 (2H, d, J = 8.3 Hz), 4.37 (2H, s), 2.44 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 191.32, 163.96, 151.64, 144.24, 134.40, 134.27, 131.60 (q, $^2J_{FC}$ = 33 Hz), 130.33, 129.60, 129.54, 128.65, 128.39, 126.54 (m), 123.90 (q, $^1J_{FC}$ = 271 Hz), 123.13, 29.22, 15.34. Anal. Calcd for $C_{19}H_{14}F_3NOS_2$ (with 1.0 H_2O and 0.1 $CDCl_3$ mol per target): C, 54.17; H, 3.81; N, 3.31. Found: C, 54.11; H, 3.57; N, 3.41.

*Methyl 4-[Thio-[5-methylene-4-methyl-2-[3-(trifluoromethyl)-phenyl]-thiazole]]cinnamate (**11c**).* By analogy to the procedure described in example **11a**, aldehyde **10c** (0.239 g, 0.607 mmol) was treated with methyl (triphenylphosphoranylidene)acetate (0.228 g, 0.682 mmol), and the crude product was purified with flash chromatography using 10% EtOAc/hexane to give **11c** as a light-yellow solid (0.130 g, 0.289 mmol, 47.6%). TLC R_f (25% EtOAc/hexane) = 0.53. Mp 142−143 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.14 (1H, s), 8.01 (1H, d, J = 7.9 Hz), 7.65 (2H, m), 7.53 (1H, t, J = 7.9 Hz), 7.45 (2H, d, J = 8.2 Hz), 7.34 (2H, d, J = 8.3 Hz), 6.41 (1H, d, J = 16.0 Hz), 4.27 (2H, s), 3.80 (3H, s), 2.34 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.45, 163.73, 151.48, 143.95, 137.88, 134.41, 133.25, 131.59 (q, $^2J_{FC}$ = 33 Hz), 130.52, 129.57, 129.55, 129.53, 128.75, 126.44 (m), 123.94 (q, $^1J_{FC}$ = 271 Hz), 123.11 (m), 118.14, 51.92, 30.41, 15.23. Anal. Calcd for $C_{18}H_{15}NOS_2$: C, 58.78; H, 4.04; N, 3.12. Found: C, 58.49; H, 3.90; N, 3.18.

*4-Thio-[5-methylene-4-methyl-2-[3-(trifluoromethyl)phenyl]-thiazole]cinnamic Acid (**12c**).* By analogy to the procedure described in example **12a**, methyl ester **11c** (0.120 g, 0.267 mmol) was treated with 3 N NaOH (1 mL), and the crude product was purified with flash chromatography using 25% EtOAc/hexane supplemented with dropwise acetic acid. The product was recrystallized using 95% EtOH to give **12c** as a light-yellow solid (0.082 g, 0.188 mmol, 70.7%). TLC R_f (50% EtOAc/hexane) = 0.51. Mp 150−151 °C. ^{1}H NMR (acetone-d_6, 600 MHz): δ 8.21 (1H, s), 8.13 (1H, d. J = 7.8 Hz), 7.78 (2H, d, J = 7.8 Hz), 7.71 (1H, t, J = 7.8 Hz), 7.64 (3H, m), 7.46 (2H, d, J = 8.3 Hz), 6.52 (1H, d, J = 16.0 Hz), 4.54 (2H, s), 2.38 (3H, s). ^{13}C NMR (acetone-d_6, 150 MHz): δ 167.64, 163.37, 152.37, 144.54, 138.95, 135.43, 133.89, 131.69 (q, $^2J_{FC}$ = 33 Hz), 131.22, 131.08, 130.65, 130.54, 129.60, 127.05 (m), 125.00 (q, $^1J_{FC}$ = 270 Hz), 122.92 (m), 119.25, 15.18. ^{19}F NMR (acetone-d_6, 376 MHz): δ −63.79 (3F, s). Anal. Calcd for $C_{21}H_{16}F_3NO_2S_2$: C, 57.92; H, 3.70; N, 3.22. Found: C, 57.81; H, 3.72; N, 3.23.

*2-[4-Thio-[5-methylene-4-methyl-2-[3-fluoro-4-(trifluoromethyl)-phenyl]thiazole]phenyl-1,3-dioxolane (**9d**).* By analogy to the procedure described in example **9a**, acetal **8** (0.915g, 3.99 mmol) was reacted in a one-pot reaction involving a *t*-BuLi solution in THF (1.7 M, 4.7 mL), sulfur (0.129g, 4.022 mmol), and thiazole **4d** (0.959 g, 3.29 mmol). The crude product was purified with column chromatography using a 0−20% EtOAc/hexane gradient to give the product **9d** as an off-white solid (0.354 g, 0.777 mmol, 19.5%). TLC R_f (25% EtOAc/hexane) = 0.40. Mp 105−107 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.72 (1H, m), 7.69 (1H, m), 7.62 (1H, t, J = 7.5 Hz), 7.40 (2H, m), 7.37 (2H, m), 5.78 (1H, s), 4.23 (2H, s), 4.11 (2H, m), 4.03 (2H, m), 2.30 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 161.91, 160.14 (d, $^1J_{FC}$ = 255 Hz), 151.67, 139.21 (d, $^4J_{FC}$ = 7.5 Hz), 137.47, 135.66, 131.31, 131.19, 127.90 (q, $^3J_{FC}$ = 4.5 Hz), 127.45, 122.55 (q, $^1J_{FC}$ = 270 Hz), 121.75 (d, $^3J_{FC}$ = 4.5 Hz), 119.04 (dq, $^2J_{FC}$ = 33 Hz, $^2J_{FC}$ = 13 Hz), 114.47, 114.32, 103.33, 65.49, 30.96, 15.11. Anal.

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Calcd for $C_{21}H_{17}F_4NO_2S_2$: C, 55.38; H, 3.76; N, 3.08. Found: C, 55.41; H, 3.65; N, 3.20.

*4-Thio-[5-methylene-4-methyl-2-[3-fluoro-4-(trifluoromethyl)-phenyl]thiazole]benzaldehyde (**10d**).* By analogy to the procedure described in example **10a**, acetal **9d** (0.354g, 0.7770 mmol) was treated with 3 N HCl (3 mL), and the crude product was purified with flash chromatography using a 5−20% EtOAc/hexane gradient to give the desired product **10d** as an off-white solid (0.244 g, 0.593 mmol, 76.3%). TLC R_f (25% EtOAc/hexane) = 0.33. Mp 122−123 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 9.91 (1H, s), 7.76 (2H, d, J = 8.4 Hz), 7.69 (1H, d, J = 11.2 Hz), 7.65 (1H, d, J = 8.3 Hz), 7.59 (1H, t, J = 7.6 Hz), 7.39 (2H, d, J = 8.4 Hz), 4.32 (2H, s), 2.39 (3H, s), 1.50 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 191.28, 162.30, 160.15 (d, $^1J_{FC}$ = 255 Hz), 152.00, 143.99, 139.02 (d, $^4J_{FC}$ = 7.5 Hz), 134.50, 130.36, 129.92, 128.48, 127.97 (dq, $^3J_{FC}$ = 4.5 Hz, $^3J_{FC}$ = 3.0 Hz), 122.50 (q, $^1J_{FC}$ = 270 Hz), 121.77, 119.30 (dq, $^2J_{FC}$ = 33 Hz, $^2J_{FC}$ = 13 Hz), 114.52, 114.36, 29.19, 15.33. Anal. Calcd for $C_{19}H_{13}F_4NOS_2$: C, 55.47; H, 3.18; N, 3.40. Found: C, 55.64; H, 3.35; N, 3.42.

*Methyl 4-[Thio-[5-methylene-4-methyl-2-[3-fluoro-4-(trifluoromethyl)phenyl]-thiazole]]cinnamate (**11d**).* By analogy to the procedure described in example **11a**, aldehyde **10d** (0.244 g, 0.594 mmol) was treated with methyl (triphenylphosphoranylidene)-acetate (0.216 g, 0.646 mmol), and the crude product was purified with flash chromatography using a 5−20% gradient to give the desired product **11d** as a white solid (0.056 g, 0.12 mmol, 20.2%). TLC R_f (25% EtOAc/hexane) = 0.40. Mp 121−123 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.72 (1H, d, J = 11.2 Hz), 7.69 (1H, d, J = 8.3 Hz), 7.63 (2H, m), 7.44 (2H, d, J = 8.4 Hz), 7.33 (2H, d, J = 8.3 Hz), 6.40 (1H, d, J = 16.0 Hz), 4.27 (2H, s), 3.80 (3H, s), 2.34 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.41, 162.08, 160.14 (d, $^1J_{FC}$ = 255 Hz), 151.77, 143.87, 139.08 (d, $^3J_{FC}$ = 9.0 Hz), 137.61, 133.37, 130.84, 130.59, 128.77, 127.94 (d, $^3J_{FC}$ = 4.0 Hz), 122.52 (q, $^1J_{FC}$ = 271 Hz), 121.75 (d, $^3J_{FC}$ = 4.5 Hz), 119.15 (dq, $^2J_{FC}$ = 33 Hz, $^2J_{FC}$ = 13 Hz), 118.24, 114.48, 114.33, 51.94, 30.35, 15.18. Anal. Calcd for $C_{22}H_{17}F_4NO_2S_2$: C, 56.62; H, 3.67; N, 3.00. Found: C, 56.54; H, 3.73; N, 3.02.

*4-Thio-[5-methylene-4-methyl-2-[3-fluoro-4-(trifluoromethyl)-phenyl]thiazole]cinnamic Acid (**12d**).* Methyl ester **11d** (0.053 g, 0.113 mmol) was treated with 3 N NaOH (1 mL), and the crude product was purified with flash chromatography using a 25% EtOAc/hexane mobile phase followed by recrystallization of the column product with 95% ethanol to give **12d** as a white solid (0.020 g, 0.044 mmol, 39.0%). TLC R_f (25% EtOAc/hexane) = 0.20. Mp 180−182 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.88 (2H, m), 7.83 (1H, m), 7.64 (3H, m), 7.47 (2H, m), 6.52 (1H, d, J = 15.9 Hz), 4.56 (2H, s), 2.39 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.58, 161.85, 160.73 (d, $^1J_{FC}$ = 254 Hz), 152.76, 144.53, 140.61 (d, $^4J_{FC}$ = 8.8 Hz), 138.78, 133.95, 132.75, 130.67, 129.63, 129.08 (q, $^3J_{FC}$ = 4.5 Hz), 123.63 (q, $^1J_{FC}$ = 269 Hz), 122.87 (d, $^3J_{FC}$ = 3 Hz), 119.26, 118.84 (dq, $^2J_{FC}$ = 33 Hz, $^2J_{FC}$ = 12 Hz), 114.66, 114.51, 30.34, 15.19. Anal. Calcd for $C_{21}H_{15}F_4NO_2S_2$: C, 55.62; H, 3.33; N, 3.09. Found: C, 55.35; H, 3.44; N, 3.04. LC-MS/MS analysis shows >98% chromatographic purity (total ion current) with retention time = 6.13 min and with m/z = 454 (for M + 1).

*Ethyl 4-(N-tert-Butoxycarbonyl)aminocinnamate (**13**).* Ethyl 4-aminocinnamate (1.270 g, 6.641 mmol) and di-*tert*-butyl dicarbonate (1.480 g, 6.781 mmol) were dissolved in THF (20 mL) under inert gas and heated to reflux at 65 °C overnight. The mixture was cooled to room temperature and concentrated under reduced pressure. The resulting residue was taken up into EtOAc and washed with sat. $NaHCO_3$, water, and brine, dried with Na_2SO_4, and concentrated. The crude product was purified with flash chromatography using a 10−20% EtOAc/hexane mobile-phase gradient to give the desired product **13** a light-orange solid (1.200 g, 4.122 mmol, 62.1%). TLC R_f (25% EtOAc/hexane) = 0.46. Mp 93−96 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.63 (1H, d, J = 16.0 Hz), 7.46 (2H, d, J = 8.6 Hz), 7.39 (2H, d, J = 8.5 Hz), 6.58 (1H, bs), 6.34 (1H, d, J = 16.0 Hz), 4.25 (2H, q, J = 7.1 Hz), 1.52 (9H, s), 1.33 (3H, t, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.39, 152.46, 144.20, 140.40, 129.27, 129.22, 118.42, 116.65, 81.19, 60.54, 28.43, 14.49. Anal. Calcd for $C_{16}H_{21}NO_4$: C, 65.96; H, 7.27; N, 4.81. Found: C, 65.81; H, 7.42; N, 4.93.

*Ethyl 4-[N-(tert-Butoxycarbonyl)-N-[(5-methylene-4-methyl-2-phenyl)thiazole]]aminocinnamate (**14a**).* Ethyl 4-(*N-tert*-butoxycarbonyl)aminocinnamate **13** (1.336 g, 4.586 mmol), thiazole **4a** (1.027 g, 4.590 mmol), and NaI (0.690 g, 4.603 mmol) were dissolved in anhydrous DMF (30 mL) under inert gas and cooled in an ice bath. NaH (60% dispersion in mineral oil, 0.281 g, 7.025 mmol) was carefully added to the reaction mixture by briefly exposing the system to air. The reaction mixture was stirred for 3 h at room temperature and quenched with a 50% dilution of sat. $NaHCO_3$. The mixture was extracted with three portions of ether that were combined, washed with brine, dried with Na_2SO_4, and concentrated. The crude material was purified by column chromatography using a gradient of 100% hexane to 25% EtOAc/hexane to give the desired product **14a** as a yellow oil (1.488 g, 3.109 mmol, 67.8%). TLC R_f (25% EtOAc/hexane) = 0.39. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.87 (2H, m), 7.64 (1H, d, J = 16.0 Hz), 7.46 (2H, d, J = 8.5 Hz), 7.39 (3H, m), 7.15 (2H, bd, J = 8.0 Hz), 6.39 (1H, d, J = 16.0 Hz), 4.94 (2H, s), 4.25 (2H, q, J = 7.1 Hz), 2.19 (3H, s), 1.47 (9H, s), 1.32 (3H, t, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.00, 166.24, 154.12, 150.93, 143.69, 143.49, 133.67, 132.70, 130.02, 128.99, 128.71, 128.16, 127.49, 126.41, 118.57, 81.61, 60.65, 45.87, 28.42, 15.13, 14.43. Anal. Calcd for $C_{27}H_{30}N_2O_4S$ (with 0.2 H_2O mol per target): C, 67.25; H, 6.35; N, 5.81. Found: C, 67.02; H, 6.55; N, 5.58.

*Ethyl 4-[N-[5-Methylene-4-methyl-2-phenylthiazole]]-aminocinnamate (**15a**).* BOC-protected amine **14a** (1.354 g, 2.749 mmol) was dissolved in anhydrous DCM (15 mL) and cooled in an ice bath. Trifluoroacetic acid (3 mL) was slowly added, and the mixture was stirred at room temperature for 3 h. The reaction mixture was then washed with a chilled saturated $NaHCO_3$ solution, water, and brine and dried with Na_2SO_4. After filtration and evaporation of the solvent, the crude product was purified with flash chromatography using a gradient of 10−25% EtOAc/hexane. The purified product **15a** was collected as a yellow solid (0.810 g, 2.140 mmol, 77.8%). TLC R_f (25% EtOAc/hexane) = 0.23. Mp 112−113 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.86 (2H, m), 7.60 (1H, d, J = 15.9 Hz), 7.39 (5H, m), 6.63 (2H, d, J = 8.6 Hz), 6.23 (1H, d, J = 15.8 Hz), 4.47 (2H, s), 4.37 (1H, bs), 4.23 (2H, q, J = 7.1 Hz), 2.48 (3H, s), 1.32 (3H, t, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.83, 165.86, 150.16, 149.10, 144.89, 133.63, 130.02, 129.49, 129.01, 126.38, 124.65, 113.75, 113.00, 60.30, 40.19, 15.44, 14.50. Anal. Calcd for $C_{22}H_{22}N_2O_2S$: C, 69.81; H, 5.86; N, 7.40. Found: C, 69.57; H, 5.88; N, 7.53.

*4-[N-[5-Methylene-4-methyl-2-phenylthiazole]]aminocinnamic Acid (**16a**).* Methyl ester **15a** (0.721 g, 1.905 mmol) was dissolved in a mixture of THF (20 mL) and 95% EtOH (10 mL). A 3 N NaOH solution (3 mL) was added, and the reaction mixture was stirred for 24 h. The reaction was found to be incomplete, as determined by TLC, and an additional 3 N NaOH (3 mL) was added. The mixture was stirred for an additional 24 h (48 h total) and was neutralized and then acidified with 1 N HCl (to pH 3). Organic solvents were removed under reduced pressure, and the residue was extracted with EtOAc. The organic extract was washed with brine, dried with Na_2SO_4, and concentrated. The crude material was purified by flash chromatography using a 25−30% EtOAc/hexane gradient supplemented with dropwise amounts of acetic acid to give the desired product **16a** as a yellow solid (0.308 g, 0.879 mmol, 46.1%). TLC R_f (50% EtOAc/hexane) = 0.15. Mp 205−207 °C. ^{1}H NMR (acetone-d_6, 600 MHz): δ 10.41 (1H, bs), 7.90 (2H, m), 7.56 (2H, d, J = 15.9 Hz), 7.45 (5H, m), 6.76 (2H, d, J = 8.6 Hz), 6.24 (1H, d, J = 15.9 Hz), 6.16 (1H, bs), 4.61 (2H, m), 2.47 (3H, s). ^{13}C NMR (acetone-d_6, 150 MHz): δ 168.36, 165.08, 151.07, 150.59, 146.12, 134.77, 132.01, 130.75, 130.58, 129.83, 126.71, 124.33, 113.57, 113.46, 40.12, 15.40. Anal. Calcd for $C_{20}H_{18}N_2O_2S$: C, 68.55; H, 5.18; N, 7.99. Found: C, 68.46; H, 5.20; N, 8.06.

*Ethyl 4-[N-(tert-Butoxycarbonyl)-N-[(5-methylene-4-methyl-2-[4-(trifluoromethyl)phenyl]thiazole]]aminocinnamate (**14b**).* By analogy to the procedure described in example **14a**, ethyl ester **13** (1.342 g, 4.606 mmol) and thiazole **4b** (1.345 g, 4.611 mmol) were treated with NaI (0.693 g, 4.623 mmol) and NaH (60% dispersion in

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mineral oil, 0.288 g, 7.20 mmol). The crude product was purified by flash chromatography using a gradient of 100% hexane to 25% EtOAc/hexane to give the desired product **14b** as a yellow solid (1.302 g, 2.384 mmol, 51.8%). TLC R_f (25% EtOAc/hexane) = 0.45. Mp 51−54 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.99 (2H, d, J = 8.2 Hz), 7.65 (3H, m), 7.48 (2H, d, J = 8.5 Hz), 7.16 (2H, bd, J = 8.1 Hz), 6.40 (1H, d, J = 16.0 Hz), 4.95 (2H, s), 4.26 (2H, q, J = 7.1 Hz), 2.21 (3H, s), 1.47 (9H, s), 1.33 (3H, t, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.00, 164.25, 154.13, 151.53, 143.62, 143.41, 136.80, 132.81, 131.53 (q, $^2J_{FC}$ = 33 Hz), 129.60, 128.76, 127.43, 126.60, 126.01 (m), 124.03 (q, $^1J_{FC}$ = 270 Hz), 118.69, 81.75, 60.70, 45.87, 28.41, 15.15, 14.44. Anal. Calcd for $C_{28}H_{29}F_3N_2O_4S$: C, 61.53; H, 5.35; N, 5.13. Found: C, 61.63; H, 5.53; N, 5.27.

*Ethyl 4-[N-[5-Methylene-4-methyl-2-[4-(trifluoromethyl)phenyl]-thiazole]]aminocinnamate (**15b**).* By analogy to the procedure described in example **15a**, BOC-protected amine **14b** (1.140 g, 2.085 mmol) was treated with trifluoroacetic acid (3 mL), and the crude product was purified with flash chromatography using a gradient of 10−25% EtOAc/hexane. The purified product **15b** was collected as an ivory solid (0.793 g, 1.776 mmol, 85.2%). TLC R_f (25% EtOAc/hexane) = 0.20. Mp 120−122 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.98 (2H, d, J = 8.1 Hz), 7.65 (2H, d, J = 8.2 Hz), 7.60 (1H, d, J = 15.9 Hz), 7.39 (2H, d, J = 8.6 Hz), 6.65 (2H, d, J = 8.6 Hz), 6.24 (1H, d, J = 15.9 Hz), 4.52 (2H, s), 4.24 (2H, q, J = 7.1 Hz), 2.51 (3H, s), 1.32 (3H, t, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.79, 163.90, 150.59, 148.79, 144.77, 136.67, 131.61 (q, $^2J_{FC}$ = 32.5 Hz), 131.22, 130.05, 126.60, 126.05 (m), 125.05, 124.02 (q, $^1J_{FC}$ = 270 Hz), 114.08, 113.18, 60.36, 40.37, 15.47, 14.53. Anal. Calcd for $C_{23}H_{21}F_3N_2O_2S$: C, 61.87; H, 4.74; N, 6.27. Found: C, 61.73; H, 4.77; N, 6.37.

*4-[N-[5-Methylene-4-methyl-2-[4-(trifluoromethyl)phenyl]-thiazole]]aminocinnamic Acid (**16b**).* By analogy to the procedure described in example **16a**, methyl ester **15b** (0.595 g, 1.333 mmol) was treated with 3 N NaOH (2 mL), and the crude material was purified by flash chromatography using a 25−30% EtOAc/hexane gradient supplemented with dropwise amounts of acetic acid to give the desired product **16b** as a yellow solid (0.153 g, 0.365 mmol, 27.4%). TLC R_f (50% EtOAc/hexane) = 0.35. Mp 224−226 °C. ^{1}H NMR (acetone-d_6, 600 MHz): δ 10.42 (1H, bs), 8.12 (2H, d, J = 8.2 Hz), 7.79 (2H, d, J = 8.3 Hz), 7.56 (1H, d, J = 15.8), 7.48 (2H, d, J = 8.6 Hz), 6.77 (2H, d, J = 8.7 Hz), 6.24 (2H, m), 4.65 (2H, s), 2.50 (3H, s). ^{13}C NMR (acetone-d_6, 150 MHz): δ 168.35, 163.07, 151.11, 150.95, 146.07, 138.17, 134.09, 131.35 (q, $^2J_{FC}$ = 31.8 Hz), 130.76, 127.22, 126.85 (m), 125.15 (q, $^1J_{FC}$ = 270 Hz), 124.46, 113.61, 113.58, 40.17, 15.40. ^{19}F NMR (acetone-d_6, 376 MHz): δ −63.64 (s, 3F). Anal. Calcd for $C_{21}H_{17}F_3N_2O_2S$ (with 0.4 mol H_2O per target): C, 59.26; H, 4.22; N, 6.58. Found: C, 59.41; H, 4.58; N, 6.36.

*Ethyl 4-(N-tert-Butoxycarbonyl)-[N-[5-methylene-4-methyl-2-[3-(trifluoromethyl)phenyl]thiazole]]aminocinnamate (**14c**).* By analogy to the procedure described in example **14a**, ethyl ester **14** (1.313 g, 4.507 mmol) and thiazole **4c** (1.315 g, 4.509 mmol) were treated with NaI (0.680 g, 4.538 mmol) and NaH (60% dispersion in mineral oil, 0.277 g, 6.925 mmol). The crude product was purified with flash chromatography using a 100% hexane to 15% EtOAc/hexane gradient to give **14c** as a viscous yellow oil (1.621 g, 2.966 mmol, 65.8%). TLC R_f (25% EtOAc/hexane) = 0.35. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.18 (1H, s), 8.05 (1H, d, J = 7.8 Hz), 7.65 (2H, m), 7.55 (1H, t, J = 7.8 Hz), 7.49 (2H, d, J = 8.5 Hz), 7.17 (2H, d, J = 8.1 Hz), 6.41 (1H, d, J = 16.0 Hz), 4.98 (2H, s), 4.28 (2H, q, J = 7.1 Hz), 2.23 (3H, s), 1.49 (9H, s), 1.35 (3H, t, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.00, 164.36, 154.15, 151.38, 143.63, 143.41, 134.40, 132.82, 131.56 (q, $^2J_{FC}$ = 32 Hz), 129.62, 129.56, 128.77, 126.44 (m), 123.93 (q, $^1J_{FC}$ = 271 Hz), 123.15 (m), 118.70, 81.75, 60.70, 45.85, 28.43, 15.13, 14.45. Anal. Calcd for $C_{28}H_{29}F_3N_2O_4S$: C, 61.53; H, 5.35; N, 5.13. Found: C, 61.39; H, 5.48; N, 5.01.

*Ethyl 4-[N-[5-Methylene-4-methyl-2-[3-(trifluoromethyl)phenyl]-thiazole]]aminocinnamate (**15c**).* By analogy to the procedure described in example **15a**, BOC-protected amine **14c** (1.30 g, 2.378 mmol) was treated with trifluoroacetic acid (3 mL), and the crude material was purified using flash chromatography and a 10% to 25% EtOAc/hexane gradient to give **15c** as a light-yellow solid (0.811 g, 1.817 mmol, 76.4%). TLC R_f (25% EtOAc/hexane) = 0.23. Mp 133−135 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.14 (1H, s), 8.02 (1H, d, J = 7.9 Hz), 7.63 (1H, d, J = 7.8 Hz), 7.60 (1H, d, J = 15.9 Hz), 7.52 (1H, m), 7.39 (2H, d, J = 8.6 Hz), 6.64 (2H, d, J = 8.6 Hz), 6.24 (2H, d, J = 15.9 Hz), 4.51 (2H, s), 4.23 (2H, q, J = 7.1 Hz), 2.51 (3H, s), 1.32 (3H, t, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.80, 163.89, 150.44, 148.92, 134.40, 131.57 (q, $^2J_{FC}$ = 32 Hz), 130.94, 130.05, 129.57, 129.52, 126.42 (m), 124.90, 123.92 (q, $^1J_{FC}$ = 271 Hz), 123.10 (m), 113.97, 113.08, 60.33, 40.29, 15.46, 14.51. Anal. Calcd for $C_{23}H_{21}F_3N_2O_2S$: C, 61.87; H, 4.74; N, 6.27. Found: C, 61.87; H, 4.79; N, 6.26.

*4-[N-[5-Methylene-4-methyl-2-[3-fluoro-4-(trifluoromethyl)-phenyl]thiazole]]aminocinnamic Acid (**16c**).* By analogy to the procedure described in example **16a**, ethyl ester **15c** (0.629 g, 1.409 mmol) was treated with 3 N NaOH (2 mL), and the crude product was purified using flash chromatography with a 25% EtOAc/hexane mobile phase that was supplemented with dropwise amounts of glacial acetic acid. A yellow−white solid was collected (0.474 g) and recrystallized using 95% ethanol to give the desired product **16c** as a light-yellow solid (0.122 g, 0.292 mmol, 20.7%). TLC R_f (50% EtOAc/hexane) = 0.33. Mp 171−173 °C. ^{1}H NMR (acetone-d_6, 600 MHz): δ 8.22 (1H, s), 8.13 (1H, d, J = 7.9 Hz), 7.77 (1H, d, J = 7.8 Hz), 7.70 (1H, t, J = 7.8 Hz), 7.56 (1H, d, J = 15.8 Hz), 7.47 (2H, d, J = 8.6 Hz), 6.76 (2H, d, J = 8.7 Hz), 6.24 (2H, d, J = 15.8 Hz), 4.64 (2H, s), 2.50 (3H, s). ^{13}C NMR (acetone-d_6, 150 MHz): δ 168.41, 163.08, 150.95, 150.93, 146.09, 135.61, 133.69, 131.67 (q, $^2J_{FC}$ = 32 Hz), 131.05, 130.76, 130.47, 126.88 (m), 125.02 (q, $^1J_{FC}$ = 270 Hz), 124.45, 113.61, 113.58, 40.17, 15.38. ^{19}F NMR (acetone-d_6, 150 MHz): δ −63.76 (3F, s). Anal. Calcd for $C_{21}H_{17}F_3N_2O_2S$: C, 60.28; H, 4.10; N, 6.69. Found: C, 60.16; H, 4.15; N, 6.65.

*Ethyl 4-(N-tert-Butoxycarbonyl)-[N-[5-methylene-4-methyl-2-[3-fluoro-4-(trifluoromethyl)phenyl]thiazole]]cinnamate (**14d**).* By analogy to the procedure described in example **14a**, ethyl ester **13** (1.274 g, 4.373 mmol) and thiazole **4d** (1.314 g, 4.250 mmol) were treated with NaI (0.677 g, 4.520 mmol) and NaH (60% dispersion in oil, 0.222 g, 5.55 mmol) to give a crude yellow−orange oil that was purified using flash chromatography with a 100% hexane to 15% EtOAc/hexane gradient, yielding the desired product **14d** as a yellow solid (1.075 g, 1.90 mmol, 44.8%). TLC R_f (25% EtOAc/hexane) = 0.43. Mp 64−66 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.73 (2H, m), 7.64 (2H, m), 7.49 (2H, d, J = 8.4 Hz), 7.16 (2H, d, J = 8.2 Hz), 6.41 (1H, d, J = 15.9 Hz), 4.97 (2H, s), 4.27 (2H, q, J = 7.14 Hz), 2.22 (3H, s), 1.47 (9H, s), 1.34 (3H, t, J = 7.14 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 166.85, 162.60, 159.52 (d, $^1J_{FC}$ = 254 Hz), 153.99, 151.66, 143.45, 143.22, 139.10 (d, $^1J_{FC}$ = 8.4 Hz), 132.75, 130.29, 128.67, 127.79, 127.78 (d, $^1J_{FC}$ = 3.3 Hz), 127.28, 122.42 (q, $^1J_{FC}$ = 269 Hz) 121.67, 119.00 (dq, $^2J_{FC}$ = 33 Hz,, $^2J_{FC}$ = 13 Hz) 118.65, 114.32 (d, $^1J_{FC}$ = 22 Hz), 81.72, 60.60, 28.30, 15.02, 14.33. Anal. Calcd for $C_{28}H_{28}F_4N_2O_4S$: C, 59.57; H, 5.00; N, 4.96. Found: C, 59.30; H, 5.13; N, 4.95.

*Ethyl 4-[N-[5-Methylene-4-methyl-2-[3-fluoro-4-(trifluoromethyl)phenyl]thiazole]] Aminocinnamate (**15d**).* By analogy to the procedure described in example **15a**, BOC-protected amine **14d** (0.684 g, 1.473 mmol) was treated with trifluoroacetic acid (5 mL), and the solid yellow crude product was purified using flash chromatography with a 100% hexane to 35% EtOAc/hexane gradient, yielding the desired product **15d** as a yellow solid (0.296 g, 0.637 mmol, 43.3%). TLC R_f (25% EtOAc/hexane) = 0.20. Mp 138−140 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.77 (1H, d, J = 11.2 Hz), 7.72 (1H, d, J = 8.3 Hz), 7.63 (2H, m), 7.42 (2H, d, J = 8.6 Hz), 6.68 (2H, d, J = 8.6 Hz), 6.28 (1H, d, J = 15.9 Hz), 4.51 (2H, s), 4.26 (2H, m), 2.52 (3H, s), 1.34 (3H, t, J = 7.14 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 169.86, 162.15, 160.01 (d, $^1J_{FC}$ = 255 Hz), 150.74, 148.53, 144.60, 139.8 (d, $^1J_{FC}$ = 8.3 Hz), 132.02, 129.93, 129.21, 127.80 (d, $^1J_{FC}$ = 4.2 Hz), 125.02, 122.39 (q, $^1J_{FC}$ = 270 Hz), 121.62, 118.97 (dq, $^2J_{FC}$ = 33 Hz, $^2J_{FC}$ = 13 Hz), 117.96, 114.25 (d, $^1J_{FC}$ = 22 Hz), 60.25, 40.28, 15.36, 14.39. Anal. Calcd for $C_{23}H_{20}F_4N_2O_2S$: C, 59.48; H, 4.34; N, 6.03. Found: C, 59.48; H, 4.24; N, 6.00.

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*4-[N-[5-Methylene-4-methyl-2-[3-fluoro-4-(trifluoromethyl)-phenyl]thiazole]]aminocinnamic Acid (**16d**).* By analogy to the procedure described in example **16a**, ethyl ester **15d** (0.098 g, 0.211 mmol) was treated with 3 N NaOH (1.0 mL), and the crude material was purified by flash chromatography using a 25−40% EtOAc/hexane gradient supplemented with dropwise amounts of acetic acid to give the desired product as a yellow solid (90.6 mg). The product was recrystallized using 95% ethanol to give **16d** as a cream-colored crystalline solid (0.024 g, 0.055 mmol, 26.1%). TLC R_f (50% EtOAc/hexane) = 0.30. Mp 213−214 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.89 (2H, d, J = 9.8 Hz), 7.82 (1H, t, J = 7.71 Hz), 7.57 (1H, d, J = 15.9 Hz), 7.48 (2H, d, J = 8.6 Hz), 6.76 (2H, d, J = 8.6 Hz), 6.24 (1H, d, J = 15.9 Hz), 4.66 (2H, s), 2.51 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 168.34, 161.56, 159.88, 151.28, 150.83, 146.08, 140.81 (d, $^4J_{FC}$ = 8.5 Hz), 135.33, 134.01, 130.77, 129.03 (d, $^3J_{FC}$ = 4.1 Hz), 124.52, 123.64 (q, $^1J_{FC}$ = 270 Hz), 122.81, 122.79 118.66 (dq, $^2J_{FC}$ = 33 Hz, $^2J_{FC}$ = 13 Hz), 114.56, 114.41, 113.61, 40.13, 15.39. Anal. Calcd for $C_{21}H_{16}F_4N_2O_2S$: C, 57.79; H, 3.70; N, 6.42. Found: C, 57.74; H, 3.72; N, 6.28.

*4-[N-(tert-Butoxycarbonyl)-N-[5-methylene-4-methyl-2-[3-fluoro-4-(trifluoromethyl)phenyl]thiazole]]aminocinnamic Acid. (**17**).* By analogy to the procedure described in example **16a**, ethyl ester **14d** (0.206 g, 0.365 mmol) was treated with 3 N NaOH (1.0 mL), and the crude product was purified by flash chromatography using a 20−35% EtOAc/hexane gradient supplemented with dropwise amounts of acetic acid to give the desired product as a white solid. The solid was recrystallized with 95% EtOH giving **17** as a crystalline white solid product (0.107 g, 0.199 mmol, 54.6%). TLC R_f (50% EtOAc/hexane) = 0.46. Mp 212−214 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.92 (2H, m), 7.84 (1H, t, J = 7.8 Hz), 7.68 (2H, d, J = 8.4 Hz), 7.66 (1H, d, J = 15.9 Hz), 7.34 (2H, d, J = 8.4 Hz), 6.51 (1H, d, J = 15.9 Hz), 5.12 (2H, s), 2.23 (3H, s), 1.47 (9H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.62, 162.52, 160.73 (d, $^1J_{FC}$ = 253 Hz), 154.52, 152.70, 144.50, 144.42, 140.69 (d, $^3J_{FC}$ = 8.6 Hz), 133.43, 132.15, 129.53, 129.06 (d, $^3J_{FC}$ = 4.1 Hz), 128.29, 123.64 (q, $^1J_{FC}$ = 269 Hz), 122.96, 119.38, 118.91 (dq, $^2J_{FC}$ = 33 Hz, $^2J_{FC}$ = 13 Hz), 114.74, 114.58, 81.67, 46.12, 28.39, 15.15. Anal. Calcd for $C_{26}H_{24}F_4N_2O_4S$: C, 58.20; H, 4.51; N, 5.22. Found: C, 57.93; H, 4.63; N, 5.08.

*Ethyl (E)-3-[4-[Bis[(4-methyl-2-(4-(trifluoromethyl)phenyl)-thiazol-5-yl)methyl]amino]phenyl]acrylate (**18**).* Ethyl 4-aminocinnamate (0.315 g, 1.647 mmol), thiazole **4b** (0.969 g, 3.321 mmol), and NaI (0.556 g, 3.709 mmol) were dissolved in anhydrous DMF (20 mL) under inert gas and cooled in an ice bath. NaH (60% in mineral oil, 0.130 g, 3.250 mmol) was added by briefly exposing the reaction to air. The reaction mixture was stirred at rt for 24 h and subsequently quenched with water and diluted with ether and saturated $NaHCO_3$ solution. Three ether extracts were obtained, combined, dried with Na_2SO_4, filtered, and concentrated. Purification of the material failed using flash chromatography, and the crude material **18** (0.240 g, 0.342 mmol, 20.8%) was moved to the next step.

*(E)-3-[4-[Bis[(4-methyl-2-(4-(trifluoromethyl)phenyl)thiazol-5-yl)-methyl]amino]phenyl]acrylic Acid (**19**).* Ethyl ester **18** (0.238 g, 0.339 mmol) was dissolved in 95% EtOH (5 mL) and THF (5 mL). The mixture was cooled in an ice bath, and 3 N NaOH (1 mL) was added. The reaction mixture was stirred at rt overnight and was concentrated under reduced pressure. The resulting residue was taken up into EtOAc and washed with acidic water (HCl, pH 3) and brine, dried with Na_2SO_4, filtered, and concentrated. The subsequent crude material was purified using flash chromatography with a 25% EtOAc/hexane mobile phase supplemented with dropwise amounts of acetic acid. A yellow solid was collected and recrystallized using a mixture of EtOAc and EtOH to give the product **19** as a light-yellow solid (0.062 g, 0.092 mmol, 27.1%). TLC R_f (50% EtOAc/hexane) = 0.50. Mp 199−200 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.97 (4H, d, J = 8.2 Hz), 7.72 (1H, d, J = 15.8 Hz), 7.65 (4H, d, J = 8.3 Hz), 7.51 (2H, d, J = 8.9 Hz), 6.90 (2H, d, J = 8.9 Hz), 6.30 (1H, d, J = 15.8 Hz), 4.73 (4H, s), 2.47 (6H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 171.32, 164.07, 150.93, 149.21, 146.67, 136.64, 131.71 (q, $^2J_{FC}$ = 32 Hz), 130.50, 130.18, 126.61, 126.07 (q, $^3J_{FC}$ = 3 Hz), 125.11, 123.10, 114.22, 113.51, 46.95, 15.65. Anal. Calcd for $C_{33}H_{25}F_6N_3O_2S_2$: C, 58.83; H, 3.74; N, 6.24. Found: C, 58.57; H, 3.88; N, 5.96.

*Ethyl 4-[N-(tert-Butoxycarbonyl)methyl]aminocinnamate (**20**).* Ethyl 4-(*N*-*tert*-butoxycarbonyl)aminocinnamate **13** (0.935 g, 3.211 mmol) was dissolved in anhydrous DMF (17 mL) under inert gas and cooled in an ice bath. NaH (60% dispersion in oil, 0.198 g, 4.95 mmol) was added, and the mixture was stirred for 60 min followed by the addition of iodomethane (0.60 mL, 9.63 mmol). The reaction mixture was stirred overnight at room temperature and quenched with a 50% aqueous solution of $NaHCO_3$ and extracted twice with ether. The ether extracts were combined and washed with brine, dried with Na_2SO_4, and concentrated. The crude material was purified by flash chromatography using a 10% EtOAc/hexane mobile phase, giving the product **20** as an off-white solid (0.897 g, 2.937 mmol, 91.5%). TLC R_f (25% EtOAc/hexane) = 0.53. Mp 44−45 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.65 (1H, d, J = 16.0 Hz), 7.47 (2H, d, J = 8.5 Hz), 7.27 (2H, d, J = 8.5 Hz), 6.38 (1H, d, J = 16.0 Hz), 4.25 (2H, q, J = 7.1 Hz), 3.27 (3H, s), 1.46 (9H, s), 1.33 (3H, t, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.19, 154.48, 145.60, 143.97, 131.21, 128.40, 125.34, 117.84, 80.97, 60.62, 37.10, 28.43, 14.46. Anal. Calcd for $C_{16}H_{21}NO_4$: C, 66.86; H, 7.59; N, 4.59. Found: C, 66.60; H, 7.52; N, 4.45.

*Ethyl 4-(N-Methyl)aminocinnamate (**21**).* Ethyl 4-[*N*-(*tert*-butoxycarbonyl)methyl]aminocinnamate **20** (0.775 g, 2.538 mmol) was dissolved in anhydrous DCM (20 mL) and cooled in an ice bath. Trifluoroacetic acid (5 mL) was slowly added, and the mixture was allowed to warm to room temperature. The mixture was stirred for 90 min, and the solvent was removed under reduced pressure. The residue was taken up into EtOAc and washed with chilled saturated $NaHCO_3$ and brine, dried with Na_2SO_4, filtered, and concentrated. The crude material was purified by flash chromatography using 10% EtOAc/hexane to give the product **21** as a yellow solid (0.474 g, 2.307 mmol, 90.9%). TLC R_f (25% EtOAc/hexane) = 0.37. Mp 49−50 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.61 (1H, d, J = 15.8 Hz), 7.38 (2H, d, J = 8.5 Hz), 6.56 (2H, d, J = 8.6 Hz), 6.22 (1H, d, J = 15.8 Hz), 4.24 (2H, q, J = 7.1 Hz), 4.08 (1H, bs), 2.87 (3H, s), 1.32 (3H, t, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 168.01, 151.18, 145.26, 130.01, 123.47, 112.86, 112.20, 60.21, 30.41, 14.53. Anal. Calcd for $C_{12}H_{15}NO_2$: C, 70.22; H, 7.37; N, 6.82. Found: C, 70.03; H, 7.25; N, 6.71.

*Ethyl 4-[N-Methyl-N-[5-methylene-4-methyl-2-phenylthiazole]]-aminocinnamate (**22a**).* Ethyl 4-(*N*-methyl)aminocinnamate **21** (0.200 g, 0.974 mmol), thiazole **4a** (0.238 g, 1.063 mmol), and NaI (0.180 g, 1.200 mmol) were dissolved in anhydrous DMF (15 mL) and cooled in an ice bath. NaH (60% dispersion in oil, 0.080 g, 2.000 mmol) was added, and the reaction mixture was stirred at room temperature for 3.5 h. The reaction mixture was diluted with ether and washed with a 10% aqueous $NaHCO_3$ solution. The organic phase was collected, and the aqueous phase was extracted with ether three times. The combined organic extracts were washed with brine, dried with Na_2SO_4, filtered, and concentrated. Purification by silica gel plug was unsuccessful, and the yellow oil crude product **22a** (0.346 g, 0.881 mmol, 90.5%) was moved to the next step. TLC R_f (25% EtOAc/hexane) = 0.35.

*4-[N-Methyl-N-[5-methylene-4-methyl-2-phenylthiazole]]-aminocinnamic Acid (**23a**).* Ethyl ester **17a** (0.205 g, 0.522 mmol) was dissolved in 95% ethanol (5 mL) and THF (5 mL). The solution was cooled in an ice bath, and 3 N NaOH (1 mL) was added and the mixture was stirred at room temperature overnight. The reaction mixture was concentrated, and the residue was taken up into EtOAc and acidified water (HCl, pH 3). The organic phase was collected and washed with brine, dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography using a 25% EtOAc/hexane mobile phase containing dropwise amounts of acetic acid. The collected product was recrystallized using a mixture of EtOH and EtOAc to give the product **23a** as a light-yellow solid (0.061 g, 0.167 mmol, 32.0%). TLC R_f (50% EtOAc/hexane) = 0.36. Mp 196−197 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.88 (2H, m), 7.58 (1H, d, J = 15.8 Hz), 7.54 (2H, d, J = 8.8 Hz), 7.42 (3H, m), 6.90 (2H, d, J = 8.9 Hz), 6.27 (1H, d, J = 15.9 Hz), 4.85 (2H, s), 3.10 (3H,

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s), 2.48 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 168.38, 165.15, 151.46, 150.95, 145.89, 134.74, 130.75, 130.71, 129.91, 126.82, 124.24, 114.07, 113.71, 49.09, 38.46, 15.57. Anal. Calcd for $C_{21}H_{20}N_2O_2S$: C, 69.12; H, 5.53; N, 7.69. Found: C, 69.03; H, 5.69; N, 7.76.

*Ethyl 4-[N-Methyl-N-[5-methylene-4-methyl-2-[4-(trifluoromethyl)phenyl]thiazole]]aminocinnamate (**22b**).* By analogy to the procedure described in example **22a**, ethyl 4-(*N*-methyl)aminocinnamate **21** (0.430 g, 2.094 mmol) and thiazole **4b** (0.661 g, 2.265 mmol) were treated with NaI (0.336 g, 2.241 mmol) and NaH (60% dispersion in oil, 0.132 g, 3.300 mmol). Separation of the starting material and the desired product by flash chromatography was unsuccessful, and the yellow−orange oil **22b** (0.721 g, 1.565 mmol, 74.7%) was moved to the next step. TLC R_f (25% EtOAc/hexane) = 0.36.

*4-[N-Methyl-N-[5-methylene-4-methyl-2-[4-(trifluoromethyl)-phenyl]thiazole]]aminocinnamic Acid (**23b**).* By analogy to the procedure described in example **23a**, ethyl ester **22b** (0.555 g, 1.205 mmol) was treated with 3 N NaOH (1 mL), and the crude product was purified by flash chromatography using a 25% EtOAc/hexane mobile phase containing dropwise amounts of acetic acid. The collected product was recrystallized using EtOAc to give the product **23b** as a yellow solid (0.338 g, 0.782 mmol, 64.9%). TLC R_f (50% EtOAc/hexane) = 0.40. Mp 210−212 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 10.46 (1H, bs), 8.10 (2H, d, J = 8.1 Hz), 7.79 (2H, d, J = 8.3 Hz), 7.59 (1H, d, J = 15.9 Hz), 7.55 (2H, d, J = 8.9 Hz), 6.90 (2H, d, 8.9 Hz), 6.29 (1H, d, J = 15.9 Hz), 4.88 (2H, s), 3.11 (3H, s), 2.51 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 186.32, 163.07, 151.42, 151.28, 145.80, 138.05, 132.54, 131.38 (q, $^2J_{FC}$ = 33 Hz), 130.69, 127.27, 126.84 (m), 125.14 (q, $^1J_{FC}$ = 270 Hz), 124.27, 114.06, 113.64, 49.09, 38.48, 15.49. Anal. Calcd for $C_{22}H_{19}F_3N_2O_2S$: C, 61.10; H, 4.43; N, 6.48. Found: C, 61.11; H, 4.41; N, 6.49.

*Ethyl 4-[N-Methyl-N-[5-methylene-4-methyl-2-[3-(trifluoromethyl)phenyl]thiazole]]aminocinnamate (**22c**).* By analogy to the procedure described in example **22a**, ethyl 4-(*N*-methyl)aminocinnamate **21** (0.247 g, 1.203 mmol) and thiazole **4c** (0.407 g, 1.395 mmol) were treated with NaI (0.213 g, 1.421 mmol) and NaH (60% dispersion in oil, 0.085 g, 2.125 mmol). Separation of the starting material and the desired product by flash column chromatography was unsuccessful, and the yellow solid **22c** (0.250 g, 0.543 mmol, 45.1%) was moved to the next step. TLC R_f (25% EtOAc/hexane) = 0.45.

*4-[N-Methyl-N-[5-methylene-4-methyl-2-[3-(trifluoromethyl)-phenyl]thiazole]]aminocinnamic Acid (**23c**).* By analogy to the procedure described in example **23a**, ethyl ester **22c** (0.240 g, 0.521 mmol) was treated with 3 N NaOH (1 mL), and the crude product was purified by flash chromatography using a 25% EtOAc/hexane mobile phase containing dropwise amounts of acetic acid. The collected product was recrystallized using EtOH to give the product **23c** as a yellow solid (0.098 g, 0.226 mmol, 43.5%). TLC R_f (50% EtOAc/hexane) = 0.35. Mp 217 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.21 (1H, s), 8.12 (1H, d, J = 7.8 Hz), 7.76 (1H, d, J = 7.8 Hz), 7.69 (1H, t, J = 7.8 Hz), 7.59 (1H, d, J = 15.9 Hz), 7.55 (2H, d, J = 8.6 Hz), 6.91 (2H, d, J = 8.8 Hz), 6.28 (1H, d, J = 15.8 Hz), 4.88 (2H, s), 3.12 (3H, s), 2.51 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 168.28, 163.09, 151.29, 151.24, 145.79, 135.51, 132.20, 131.66 (q, $^2J_{FC}$ = 33 Hz), 131.05, 130.69, 130.52, 126.94 (q, $^3J_{FC}$ = 4 Hz), 125.01 (q, $^1J_{FC}$ = 270 Hz), 124.26, 122.89 (q, $^3J_{FC}$ = 4 Hz), 114.05, 113.62, 49.08, 38.47, 15.47. Anal. Calcd for $C_{22}H_{19}F_3N_2O_2S$: C, 61.10; H, 4.43; N, 6.48. Found: C, 60.92; H, 4.45; N, 6.45.

*Ethyl 4-[N-Methyl-N-[5-methylene-4-methyl-2-[3-fluoro-4-(trifluoromethyl)phenyl] thiazole]]aminocinnamate (**22d**).* By analogy to the procedure described in example **22a**, ethyl ester **21** (0.2885 g, 1.405 mmol) and thiazole **4d** (0.4754 g, 1.535 mmol) were treated with NaI (0.2384 g, 1.590 mmol) and NaH (0.0697, 2.904 mmol). The crude product was purified by flash chromatography with a 5−20% EtOAc/hexane mobile-phase gradient, giving the desired product **22d** as an orange solid (0.192 g, 0.401 mmol, 28.5%). TLC R_f (25% EtOAc/hexane) = 0.43. Mp 142 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.73 (1H, m), 7.67 (1H, m), 7.63 (2H, m), 7.45 (2H, d, J = 8.8 Hz), 6.76 (2H, d, J = 8.8 Hz), 6.26 (1H, d, J = 16 Hz), 4.68 (2H, s), 4.26 (2H, q, J = 7.14 Hz), 3.05 (3H, s), 2.50 (3H, s), 1.33 (3H, t, J = 7.14 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.81, 161.97, 160.97 (d, $^1J_{FC}$ = 254 Hz), 150.57, 150.09, 144.69, 139.29 (d, $^4J_{FC}$ = 8.6 Hz), 131.88, 129.99, 127.85, 124.07, 123.42 (q, $^1J_{FC}$ = 270 Hz), 121.70 (d, $^3J_{FC}$ = 3.5), 119.16 (dq, $^2J_{FC}$ = 14 Hz, $^2J_{FC}$ = 33 Hz), 114.40 (d, $^2J_{FC}$ = 23 Hz), 113.97, 112.85, 60.33, 49.15, 38.42, 15.57, 14.53. Anal. Calcd for $C_{24}H_{22}F_4N_2O_2S$ (with 0.3 water mol per target): C, 59.57; H, 4.71; N, 5.79. Found: C, 59.19; H, 4.55; N, 5.74.

*4-[N-Methyl-N-[5-methylene-4-methyl-2-[3-fluoro-4-(trifluormethyl)phenyl]thiazole]]aminocinnamic Acid (**23d**).* By analogy to the procedure described in example **23a**, ethyl ester **22d** (0.157 g, 0.328 mmol) was treated with 3 N NaOH (1 mL), and the crude product was purified by flash chromatography with a gradient of 25−50% EtOAc/hexane containing dropwise amounts of acetic acid to give the product **23d** as an orange solid (0.045 g, 0.100 mmol, 30.5%). TLC R_f (50% EtOAc/hexane) = 0.36. Mp 214 °C. ^{1}H NMR (acetone-d_6, 600 MHz): δ 7.86 (2H, m), 7.81 (1H, m), 7.60 (1H, d, J = 16 Hz), 7.56 (2H, d, J = 8.9 Hz), 6.91 (2H, d, J = 8.9 Hz), 6.29 (1H, d, J = 16 Hz), 4.89 (2H, s), 3.12 (3H, s), 2.52 (3H, s). ^{13}C NMR (acetone-d_6, 150 MHz): δ 168.30, 161.56, 159.86 (d, $^1J_{FC}$ = 255 Hz), 151.61, 151.21, 145.74, 140.72 (d, $^4J_{FC}$ = 9 Hz), 133.73, 130.69, 129.04 (m), 124.52 (q, $^1J_{FC}$ = 270 Hz), 124.33, 122.87 (d, $^3J_{FC}$ = 3.3 Hz), 118.85 (dq, $^2J_{FC}$ = 33 Hz, $^2J_{FC}$ = 12 Hz), 114.60 (d, $^2J_{FC}$ = 23 Hz), 114.14, 113.63, 49.16, 38.54, 15.47. Anal. Calcd for $C_{22}H_{18}F_4N_2O_2S$: C, 58.66; H, 4.03; N, 6.22. Found: C, 58.43; H, 4.09; N, 6.21.

*Ethyl 4-[N-Ethyl-N-(tert-butoxycarbonyl)]aminocinnamate (**20e**).* To a stirred solution of ethyl 4-(*N-tert*-butoxycarbonyl)-aminocinnamate **13** (1.459 g, 5.01 mmol) in anhydrous DMF (20 mL) under inert gas at 0 °C was added NaH (60% dispersion in mineral oil, 0.317 g, 13.21 mmol). The mixture was stirred for 1 h, iodoethane (1.2 mL, 14.9 mmol) was then added, and the mixture continued to stir at room temperature overnight. The mixture was then diluted with 50% sodium bicarbonate solution and extracted with ether three times. The ether extracts were combined and washed with brine, dried with Na_2SO_4, filtered, and concentrated. The residue was concentrated then purified by flash chromatography using a gradient of 10−15% EtOAc/hexane to give the desired product **20e** as a yellow solid (1.253 g, 3.923 mmol, 78.3%). TLC R_f (25% EtOAc/hexane) = 0.56. Mp 58−60 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.67 (1H, d, J = 16.0 Hz), 7.49 (2H, d, J = 8.4 Hz), 7.23 (2H, d, J = 8.4 Hz), 6.40 (1H, d, J = 16.0 Hz), 4.28 (2H, q, J = 7.1 Hz), 3.71 (2H, q, J = 7.0 Hz), 1.44 (9H, s), 1.35 (3H, t, J = 7.1 Hz), 1.17 (3H, t, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.19, 154.30, 144.44, 143.99, 131.82, 128.54, 126.93, 118.04, 80.62, 60.65, 44.93, 28.46, 14.47, 14.10. Anal. Calcd for $C_{18}H_{25}NO_4$: C, 67.69; H, 7.89; N, 4.39. Found: C, 67.62; H, 7.90; N, 4.42.

*Ethyl 4-(N-Ethyl)-aminocinnamate (**21e**).* By analogy to the procedure described in example **21**, ethyl ester **20e** (1.143 g, 3.579 mmol) was treated with trifluoroacetic acid (6 mL), and the crude product was purified by flash chromatography using a 5−10% EtOAc/hexane mobile phase to give the desired product **21e** as a light-yellow solid (0.741 g, 3.380 mmol, 94.4%). TLC R_f (25% EtOAc/hexane) = 0.43. Mp 65−67 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.61 (1H, d, J = 16.0 Hz), 7.39 (2H, d, J = 8.5 Hz), 6.61 (2H, d, J = 8.5), 6.22 (1H, d, J = 16.0 Hz), 4.25 (2H, q, J = 7.1 Hz), 3.22 (2H, q, J = 7.2 Hz), 1.33 (3H, t, J = 7.1 Hz), 1.28 (3H, t, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.89, 145.00, 143.07, 132.70, 130.03, 129.23, 113.58, 60.29, 39.11, 14.54, 14.49. Anal. Calcd for $C_{13}H_{17}NO_2$: C, 71.21; H, 7.81; N, 6.39. Found: C, 71.01; H, 7.75; N, 6.33.

*Ethyl 4-[N-Ethyl-N-[5-methylene-4-methyl-2-[4-(trifluormethyl)-phenyl]thiazole]]amino Cinnamate (**22e**).* Ethyl ester **21e** (0.362 g, 1.651 mmol), thiazole (0.593 g, 2.032 mmol), and NaI (0.392 g, 2.615 mmol) were combined in a two-necked flask fitted with a reflux condenser. The system was flushed with nitrogen, and anhydrous acetonitrile (30 mL) was added. The reaction mixture was cooled in an ice bath, and *N*,*N*-diisopropylethylamine (0.43 mL, 2.469 mmol) was added. The reaction mixture was heated to reflux temperature overnight. The mixture was allowed to cool, and the solvent was

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removed under reduced pressure. The residue was taken up into DCM, washed with water and brine, and dried with Na_2SO_4. After filtration, the filtrate was concentrated, and the resulting crude product was purified by column chromatography with a 5−25% EtOAc/hexane mobile-phase gradient to give the product **22e** as a yellow solid (0.704 g, 1.483 mmol, 89.8%). TLC R_f (25%EtOAc/hexane) = 0.40. Mp 108−109 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.92 (2H, d, J = 8.2 Hz), 7.59 (3H, m), 7.40 (2H, d, J = 8.9 Hz), 6.69 (2H, d, J = 8.9 Hz), 6.22 (1H, d, J = 16 Hz), 4.58 (2H, s), 4.22 (2H, q, J = 7.1 Hz), 3.48 (2H, q, J = 7.1), 2.49 (3H, s), 1.30 (3H, t, J = 7.1 Hz), 1.21 (3H, t, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.90, 163.49, 149.74, 149.02, 144.78, 136.84, 132.18, 131.42 ($^2J_{FC}$ = 32 Hz), 130.09, 126.49, 125.99 ($^3J_{FC}$ = 3.7 Hz), 124.05 ($^1J_{FC}$ = 270 Hz), 123.54, 113.55, 112.55, 60.31, 46.95, 45.39, 15.62, 14.54, 12.38. Anal. Calcd for $C_{25}H_{25}F_3N_2O_2S$: C, 63.28; H, 5.31; N, 5.90. Found: C, 63.02; H, 5.35; N, 5.90.

*4-[N-Ethyl-N-[5-methylene-4-methyl-2-[4-(trifluormethyl)-phenyl]thiazole]]aminocinnamic Acid (**23e**).* By analogy to the procedure described in example **23a**, ethyl ester **22e** (0.132 g, 0.278 mmol) was treated with 3 N NaOH (1 mL). The crude product was purified by flash chromatography with a gradient of 15−45% EtOAc/hexane with dropwise amounts of acetic acid to give the desired product **23e** as an orange solid (0.061 g, 0.137 mmol, 49.3%). TLC R_f (50% EtOAc/hexane) = 0.43. Mp 198 °C. ^{1}H NMR (acetone-d_6, 600 MHz): δ 8.09 (2H, d, J = 8.9 Hz), 7.78 (2H, d, J = 8.2 Hz), 7.58 (1H, d, J = 16 Hz), 7.52 (2H, d, J = 8.9 Hz), 6.86 (2H, d, J = 8.9 Hz), 6.27 (1H, d, J = 16 Hz), 4.82 (2H, s), 3.62 (2H, q, J = 7 Hz), 2.51 (3H, s), 1.24 (3H, t, J = 7 Hz). ^{13}C NMR (acetone-d_6, 150 MHz): δ 168.45, 162.97, 150.80, 150.05, 145.83, 138.08, 133.86, 131.43 (q, $^2J_{FC}$ = 32 Hz), 130.80, 127.21, 126.82 (q, $^3J_{FC}$ = 4 Hz), 126.03 (q, $^1J_{FC}$ = 270 Hz), 123.88, 113.76, 113.36, 47.22, 45.92, 15.49, 12.52. Anal. Calcd for $C_{23}H_{21}F_3N_2O_2S$ with 0.17 mol per target $CDCl_3$: C, 59.60; H, 4.53; N, 6.00. Found: C, 59.56; H, 4.75; N, 5.96.

*Ethyl 4-[N-Isopropyl-N-(tert-butoxycarbonyl)]aminocinnamate (**20f**).* By analogy to the procedure described in example **20e**, ethyl 4-aminocinnamate-BOC **13** (0.840 g, 2.885 mmol) was treated with NaH (60% dispersion in mineral oil, 0.262 g, 6.550 mmol) and 2-iodopropane (1 mL, 10.1 mmol). The crude material was purified by flash chromatography with a gradient of 5−10% EtOAc/hexane to give the desired product **20f** as a yellow oil (0.488 g, 1.464 mmol, 50.7%). TLC R_f (25% EtOAc/hexane) = 0.58. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.68 (1H, d, J = 15.9 Hz), 7.50 (2H, d, J = 8.3 Hz), 7.10 (2H, d, J = 8.3 Hz), 6.42 (1H, d, J = 16.0 Hz), 4.50 (1H, sept, J = 6.8 Hz), 4.27 (2H, q, J = 7.14 Hz), 1.38 (9H, s), 1.34 (3H, t, J = 7.14 Hz), 1.13 (6H, d, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.11, 154.70, 144.00, 141.47, 133.11, 130.37, 128.35, 118.54, 80.16, 60.69, 48.93, 28.49, 21.69, 14.48.

*Ethyl 4-(N-Isopropyl)aminocinnamate (**21f**).* By analogy to the procedure described in example **21**, ethyl ester **20f** (1.05 g, 3.149 mmol) was treated with TFA (8 mL). The crude product was purified by flash chromatography to give the desired product **21f** as a white solid (0.609 g, 2.613 mmol, 83.0%). TLC R_f = 0.11 (25% EtOAc/hexane). Mp 112−115 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.59 (1H, d, J = 15.8 Hz), 7.35 (2H, d, J = 8.5 Hz), 6.54 (2H, d, J = 8.6 Hz), 6.20 (1H, d, J = 15.8 Hz), 4.23 (2H, q, J = 7.1 Hz), 3.95 (1H, bs), 3.67 (1H, sept., J = 6.3 Hz), 1.32 (3H, t, J = 7.1 Hz), 1.22 (6H, d, J = 6.3 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 168.03, 149.32, 145.23, 130.12, 123.27, 112.94, 112.73, 60.21, 44.24, 22.96, 14.55. Anal. Calcd for $C_{14}H_{19}NO_2$: C, 72.07; H, 8.21; N, 6.00. Found: C, 72.03; H, 8.30; N, 5.98.

*Ethyl 4-[N-Isopropyl-N-[5-methylene-4-methyl-2-(4-trifluoromethylphenyl)thiazole]]aminocinnamate (**22f**).* By analogy to the procedure described in example **22a**, *N*-isopropyl ester **21f** (0.490 g, 2.100 mmol) and thiazole **4b** (0.745 g, 2.554 mmol) were treated with NaI (0.41g, 2.735 mmol) and NaH (0.107 g, 2.675 mmol). Attempts to purify the crude sample by flash chromatography failed, and the crude product **22f** was moved to the next step without further purification (0.181 g, 0.370 mmol, 17.8%). TLC R_f (25% EtOAc/hexane) = 0.35. Mp 80−82 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.94 (2H, d, J = 7.9 Hz), 7.60 (3H, m), 7.40 (H, d, J = 8.9 Hz), 6.76 (2H, d, J = 8.9 Hz), 6.22 (1H, d, J = 15.8 Hz), 4.49 (2H, s), 4.28 (1H, sept., J = 6.3 Hz), 4.23 (2H, q, J = 8.14 Hz), 1.32−1.29 (9H, m). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.86, 163.27, 149.91, 148.34, 144.69, 136.93, 134.83, 131.27 (q, $^2J_{FC}$ = 32 Hz), 129.91, 127.57, 126.38, 125.95 (q, $^3J_{FC}$ = 3.5 Hz), 124.06 (q, $^1J_{FC}$ = 270 Hz), 113.81, 113.61, 60.31, 49.14, 41.65, 20.12, 15.71, 14.51.

*4-[N-Isopropyl-N-[5-methylene-4-methyl-2-(4-trifluoromethylphenyl)thiazole]]aminocinnamic Acid (**23f**).* By analogy to the procedure described in example **23a**, *N*-isopropyl ester **22f** (0.168 g, 0.344 mmol) was treated with 3 N NaOH (1 mL), and the crude product was purified by flash chromatography with a gradient of 5−30% EtOAc/hexane to give the desired product **23f** as a white solid (0.122 g, 0.266 mmol, 77.1%). TLC R_f (50% EtOAc/hexane) = 0.52. Mp 208−210 °C. ^{1}H NMR (acetone-d_6, 600 MHz): δ 10.28 (1H, bs), 8.09 (2H, d, J = 8.1 Hz), 7.77 (2H, d, J = 8.3 Hz), 7.56 (1H, d, J = 15.9 Hz), 7.51 (2H, d, J = 8.9 Hz), 6.89 (2H, d, J = 9.0 Hz), 6.26 (1H, d, J = 15.8 Hz), 4.68 (2H, s), 4.41 (1H, sept., J = 6.6 Hz), 2.52 (3H, s), 1.33 (6H, d, J = 6.6 Hz). ^{13}C NMR (acetone-d_6, 150 MHz): δ 168.24, 162.68, 151.03, 149.56, 145.67, 138.24, 136.40, 131.20 (q, $^2J_{FC}$ = 32 Hz), 130.61, 127.10, 126.79 (q, $^3J_{FC}$ = 3.5 Hz), 125.17 (q, $^1J_{FC}$ = 270 Hz), 124.48, 114.57, 114.15, 49.93, 41.98, 20.04, 15.60. Anal. Calcd for $C_{24}H_{23}F_3N_2O_2S$: C, 62.60; H, 5.03; N, 6.08. Found: C, 62.30; H, 5.09; N, 6.03.

*Ethyl 4-[N-Propyl-N-(tert-butoxycarbonyl)]aminocinnamate (**20g**).* By analogy to the procedure described in example **20e**, ethyl 4-aminocinnamate-BOC **13** (2.147 g, 7.374 mmol) was treated with NaH (60% dispersion in mineral oil, 0.314 g, 7.850 mmol) and 1-iodopropane (2.2 mL, 22.558 mmol). The crude material was purified by flash chromatography with a gradient of 100% hexane to 10% EtOAc/hexane to give the desired product **20g** as a yellow solid (2.290 g, 6.868 mmol, 93.2%). TLC R_f (25% EtOAc/hexane) = 0.59. Mp 56 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.66 (1H, d, J = 16 Hz), 7.48 (2H, d, J = 8.4 Hz), 7.22 (2H, d, J = 8.4 Hz), 6.39 (1H, d, J = 16 Hz), 4.26 (2H, q, J = 7.14 Hz), 3.60 (2H, t, J = 7.4 Hz), 1.56 (2H, sex., J = 7.4 Hz), 1.43 (9H, s), 1.34 (3H, t, J = 7.14 Hz), 0.87 (3H, t, J = 7.4 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.13, 154.51, 144.53, 143.95, 131.81, 128.51, 127.03, 118.02, 80.54, 60.60, 51.53, 28.41, 21.92, 14.44, 11.26. Anal. Calcd for $C_{19}H_{27}NO_4$: C, 68.44; H, 8.16; N, 4.20. Found: C, 68.46; H, 8.22; N, 4.22.

*Ethyl 4-(N-Propyl)aminocinnamate (**21g**).* By analogy to the procedure described in example **21**, ethyl ester **20g** (2.080 g, 6.238 mmol) was treated with trifluoroacetic acid (8 mL), and the crude material was purified by flash chromatography with a gradient of 10−20% EtOAc/hexane to give an orange solid **21g** (0.573 g, 2.455 mmol, 39.4%). TLC R_f (25% EtOAc/hexane) = 0.43. Mp 78 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.61 (1H, d, J = 16 Hz), 7.38 (2H, d, J = 8 Hz), 6.61 (2H, d, J = 8 Hz), 6.23 (1H, d, J = 16 Hz), 4.25 (2H, q, J = 7.14 Hz), 3.13 (2H, t, J = 7.3 Hz), 1.68 (2H, sex., J = 7.3 Hz), 1.33 (3H, t, J = 7.14 Hz), 1.00 (3H, t, J = 7.3 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.97, 149.60, 145.11, 130.06, 129.20, 124.13, 133.22, 60.28, 46.01, 22.50, 14.55, 11.67. Anal. Calcd for $C_{13}H_{17}F_4NO_2$(with 0.13 mol $CDCl_3$ per target): C, 68.17; H, 7.69; N, 5.63. Found: C, 68.09; H, 7.50; N, 5.53.

*Ethyl 4-[N-Propyl-N-[5-methylene-4-methyl-2-[4-(trifluormethyl)phenyl]aminocinnamate (**22g**).* By analogy to the procedure described in example **22a**, ethyl ester **21g** (0.237 g, 1.014 mmol) and thiazole **4b** (0.325 g, 1.115 mmol) were treated with NaI (0.231 g, 1.542 mmol) and NaH (0.045, 1.896 mmol). The crude material was purified by flash chromatography with a gradient of 10−30% EtOAc/hexane to give **22g** as an orange solid (0.294 g, 0.603 mmol, 59.5%). TLC R_f (25% EtOAc/hexane) = 0.35. Mp 128−129 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.97 (2H, d, J = 8.2 Hz), 7.64 (2H, d, J = 8.2 Hz), 7.60 (1H, d, J = 16 Hz), 7.43 (2H, d, J = 8.9 Hz), 6.75 (2H, m), 6.24 (1H, d, J = 16 Hz), 4.66 (2H, s), 4.23 (2H, q, J = 7.14 Hz), 3.39 (2H, t, J = 7.4 Hz), 2.51 (3H, s), 1.71 (2H, sex., J = 7.6 Hz), 1.32 (3H, t, J = 7.14 Hz), 0.98 (3H, t, J = 7.4 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.89, 163.61, 149.79, 148.99, 144.73, 136.68, 131.92, 131.60 ($^2J_{FC}$ = 32 Hz), 130.05, 126.58, 126.01 ($^3J_{FC}$ = 3.9 Hz), 124.92 ($^1J_{FC}$ = 270 Hz), 123.67, 113.64, 112.69, 60.32, 53.18,

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47.66, 20.62, 15.57, 14.53, 11.56. Anal. Calcd for $C_{26}H_{27}F_3N_2O_2S$: C, 63.92; H, 5.57; N, 5.73. Found: C, 63.71; H, 5.55; N, 5.65.

*4-[N-Propyl-N-[5-methylene-4-methyl-2-[4-(trifluormethyl)-phenyl]]aminocinnamic Acid (**23g**).* By analogy to the procedure described in example **23a**, ethyl ester **20c** (0.165 g, 0.337 mmol) was treated with 3 N NaOH (1 mL). The crude material was purified by flash chromatography with a gradient of 15−50% EtOAc/hexane supplemented with dropwise amounts of acetic acid to give the desired compound **23g** as an orange solid (0.134 g, 0.290 mmol, 86.1%). TLC R_f (50% EtOAc/hexane) = 0.36. Mp 205 °C. ^{1}H NMR (acetone-d_6, 600 MHz): δ 10.41 (1H, bs), 8.09 (2H, d, J = 8.2 Hz), 7.78 (2H, d, J = 8.2 Hz), 7.57 (1H, d, J = 16 Hz), 7.52 (2H, d, J = 8.9 Hz), 6.85 (2H, d, J = 8.9 Hz), 6.25 (1H, d, J = 16 Hz), 4.85 (2H, s), 3.50 (2H, t, J = 7.5 Hz), 2.51 (3H, s), 1.73 (2H, sex., J = 7.5 Hz), 0.98 (3H, J = 7.5 Hz). ^{13}C NMR (acetone-d_6, 150 MHz): δ 168.36, 162.95, 150.85, 150.25, 145.83, 138.09, 133.72, 131.43 ($^2J_{FC}$ = 32 Hz), 130.76, 127.21, 126.83 ($^3J_{FC}$ = 4 Hz), 126.04 ($^1J_{FC}$ = 270 Hz), 123.82, 113.71, 113.37, 53.49, 47.80, 21.17, 15.51, 11.49. Anal. Calcd for $C_{24}H_{23}F_3N_2O_2S$: C, 62.60; H, 5.03; N, 6.08. Found: C, 62.20; H, 5.04; N, 6.03.

*4-Bromo-2-methylbenzaldehyde (**25a**).* 4-Bromo-2-methylbenzonitrile **24a** (1.859 g, 9.483 mmol) was dissolved in anhydrous toluene (40 mL) under nitrogen and was cooled to −78 °C. Reagent grade DIBAL-H (1.70 mL, 9.538 mmol) was slowly added, and mixture was stirred for 30 min. Anhydrous methanol (3 mL) was carefully added followed by 2 M H_2SO_4 (9 mL) dropwise. The mixture was stirred overnight at rt. The mixture was diluted with ethyl acetate (50 mL), and the organic phase was collected, dried with Na_2SO_4, and concentrated. The crude material was purified with flash chromatography using 5% EtOAc/hexane to give **25a** as an off-white solid (1.590 g, 7.988 mmol, 84.2%). TLC R_f (25% EtOAc/hexane) = 0.80. Mp 29−30 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 10.21 (1H, s), 7.66 (1H, d, J = 8.2), 7.51 (1H, dd, J_1 = 8.2 Hz, J_2 = 1.9 Hz), 7.44 (1H, d, J = 1.1 Hz), 2.65 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 191.79, 142.53, 134.88, 133.37, 133.04, 129.85, 129.03, 19.42.

*tert-Butyl-N-methylcarbamate (**26**).* Di-*tert*-butyl dicarbonate (10.91 g, 49.98 mmol) was dissolved in anhydrous THF (30 mL) under nitrogen and was cooled in an ice bath. Methylamine (2 M) in THF (50.0 mL, 0.1 mol) was slowly added, and mixture was allowed to warm to rt and was stirred overnight. The solvent was removed using rotary evaporation, and the resulting residue was taken up into 1 M HCl and DCM. The organic phase was collected, and the aqueous phase was extracted with a separate portion of DCM. The organic phases were combined and washed with water, dried with sodium sulfate, and concentrated. Residual solvents were removed using Kugelrohr distillation to give the title compound **26** as a light-yellow oil (6.476 g, 49.37 mmol, 98.7%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 4.43 (1H, bs), 2.73 (3H, d, J = 4.92 Hz), 1.44 (9H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 156.76, 79.27, 28.59, 27.28. Anal. Calcd for $C_6H_{13}NO_2$ (with 0.1 mol H_2O and 0.1 mol DCM per target): C, 51.79; H, 9.55; N, 9.90. Found: C, 51.70; H, 9.34; N, 9.73.

*2-Methyl-4-[N-(tert-butoxycarbonyl)methyl]benzaldehyde (**27a**).* Aldehyde **25a** (1.286 g, 6.461 mmol), *tert*-butyl-*N*-methylcarbamate **26** (1.015 g, 7.737 mmol), and Cs_2CO_3 (2.958 g, 9.078 mmol) were dissolved in anhydrous dioxane (40 mL) under nitrogen. Xantphos (0.175 g, 0.302 mmol) and $Pd_2(dba)_3$ (0.136 g, 0.148 mmol) were measured out in a nitrogen bag and added to the reaction flask. The reaction mixture was refluxed with stirring overnight and was then cooled to room temperature before removal of the solvent under reduced pressure. The resulting residue was taken up into EtOAc and water followed by filtration through Celite. The EtOAc phase was collected, washed with water and brine, and dried with Na_2SO_4. After filtration, the filtrate was concentrated, and the residue was purified using flash chromatography and a 100% hexane to 5% EtOAc/hexane mobile-phase gradient to give the desired product **27a** as a yellow oil (0.976 g, 3.914 mmol, 60.6%). TLC R_f (25% EtOAc/hexane) = 0.53. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 10.20 (1H, s), 7.75 (1H, d, J = 8.4 Hz), 7.28 (1H, d, J = 2.2 Hz), 7.19 (1H, d, J = 1.7 Hz), 3.30 (3H, s), 2.66 (3H, s), 1.50 (9H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 191.66, 154.22, 148.55, 141.43, 132.73, 130.96, 127.39, 122.29, 81.41, 36.92, 28.47, 19.76. Anal. Calcd for $C_{14}H_{19}NO_3$ (with 0.1 H_2O and 0.1 mol DCM per target): C, 65.24; H, 7.53; N, 5.40. Found: C, 65.27; H, 7.18; N, 5.06.

*Methyl 2-Methyl-4-[N-(tert-butoxycarbonyl)methyl]-aminocinnamate (**28a**).* Aldehyde **27a** (0.591 g, 2.371 mmol) and methyl(triphenylphosphoranylidene)acetate (0.876 g, 2.620 mmol) were dissolved in anhydrous THF (10 mL) under nitrogen gas and stirred at 55 °C for 2 days. The reaction mixture was concentrated, and the resulting crude solid was taken up into EtOAc, washed with water and brine, and dried with Na_2SO_4. After filtration, the filtrate was concentrated and purified using flash chromatography with a 5−15% EtOAc/hexane gradient to give the desired product **28a** as a yellow oil (0.548 g, 1.794 mmol, 75.7%). TLC R_f (25%EtOAc/hexane) = 0.53. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.94 (1H, d, J = 15.9 Hz), 7.51 (1H, d, J = 9.1 Hz), 7.11 (2H, m), 6.33 (1H, d, J = 15.9 Hz), 3.81 (3H, s), 3.26 (3H, s), 2.42 (3H, s), 1.47 (9H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.70, 154.56, 145.36, 141.95, 138.32, 130.16, 127.01, 126.78, 123.11, 118.34, 80.87, 51.84, 37.14, 28.46, 20.06. Anal. Calcd for $C_{17}H_{23}NO_4$ (with 0.1 mol H_2O per target): C, 66.47; H, 7.61; N, 4.56. Found: C, 66.26; H, 7.44; N, 4.56.

*Methyl 2-Methyl-4-(N-methyl)aminocinnamate (**29a**).* Methyl 2-Methyl-4-[*N*-(*tert*-butoxycarbonyl)methyl]aminocinnamate **28a** (0.516 g, 1.689 mmol) was taken up into anhydrous DCM (10 mL) under nitrogen gas and cooled in an ice bath. Trifluoroacetic acid (4 mL) was slowly added, and the mixture was stirred at room temperature for 1.5 h. The reaction mixture was concentrated, and the residue was taken up into EtOAc, washed with chilled sat. $NaHCO_3$ and brine, and dried with Na_2SO_4. After filtration, the filtrate was concentrated, and the filtrate was purified using flash chromatography and a 10−20% EtOAc/hexane mobile-phase gradient to give the product **29a** as a yellow solid (0.229 g, 1.116 mmol, 66.1%). TLC (25% EtOAc/hexane) = 0.31. Mp 79−83 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.92 (1H, d, J = 15.7 Hz), 7.47 (1H, d, J = 8.5 Hz), 6.44 (1H, dd, J_1 = 8.5 Hz, J_2 = 2.5 Hz), 6.19 (1H, d, J = 15.8 Hz), 4.11 (1H, bs), 3.78 (3H, s), 2.86 (3H, s), 2.39 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 168.51, 150.94, 142.66, 139.97, 128.13, 122.18, 113.62, 113.10, 110.61, 51.52, 30.41, 20.26. Anal. Calcd for $C_{12}H_{15}NO_2$: C, 70.22; H, 7.37; N, 6.82. Found: C, 70.00; H, 7.26; N, 6.68.

*Methyl 2-Methyl-4-[N-methyl-N-[5-methylene-4-methyl-2-[4-(trifluoromethyl)phenyl]-thiazole]]aminocinnamate (**30a**).* Methyl ester **29a** (0.316 g, 1.540 mmol), thiazole **4b** (0.520 g, 1.782 mmol), and NaI (0.328 g, 2.188 mmol) were dried in vacuum desiccator overnight to ensure dry starting reagents. These starting reagents were combined in a two-necked flask under nitrogen, and anhydrous DMF (15 mL) was added. The reaction mixture was cooled in an ice bath, and NaH (60% dispersion in mineral oil, 0.101 g, 2.525 mmol) was added by briefly exposing the system to air. The mixture was warmed to room temperature and stirred for 2.5 h. The reaction mixture was carefully quenched with water, diluted with ether, and neutralized with $NaHCO_3$. Additional water was added to obtain a clear two-phase solution. The organic phase was collected, and the aqueous phase was extracted with two portions of ether. The organic phases were combined, washed with water and brine, dried with Na_2SO_4. After filtration, the filtrate was concentrated, and the residue was purified by flash chromatography with a 5−20% EtOAc/hexane mobile-phase gradient to give the product **30a** as a yellow−orange solid (0.523 g, 1.136 mmol, 73.8%). TLC (25% EtOAc/hexane) = 0.33. Mp 151−152 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.96 (2H, d, J = 8.2 Hz), 7.93 (1H, d, J = 15.8 Hz), 7.64 (2H, d, J = 8.2 Hz), 7.53 (1H, d, J = 8.8 Hz), 6.66 (1H, dd, J_1 = 8.8 Hz, J_2 = 2.7 Hz), 6.58 (1H, d, J = 2.5 Hz), 6.24 (1H, d, 8.8 Hz), 4.67 (2H, s), 3.79 (3H, s), 3.04 (3H, s), 2.51 (3H, s), 2.43 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 168.35, 163.65, 150.31, 149.90, 142.29, 139.88, 136.73, 131.51 (q, $^2J_{FC}$ = 32.4 Hz), 131.02, 128.18, 126.57, 126.02 (q, $^3J_{FC}$ = 3.5 Hz), 124.03 (q, $^1J_{FC}$ = 271 Hz), 122.88, 114.52, 114.21, 111.08, 51.64, 49.06, 38.33, 20.65, 15.56. Anal. Calcd for $C_{24}H_{23}N_2F_3O_2S$: C, 62.60; H, 5.03; N, 6.08. Found: C, 62.36; H, 5.06; N, 6.05.

*2-Methyl-4-[N-methyl-N-[5-methylene-4-methyl-2-[4-(trifluoromethyl)phenyl]thiazole]]aminocinnamic Acid (**31a**).* Methyl ester **30a** (0.400 g, 0.868 mmol) was dissolved in anhydrous

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THF (10 mL) and 95% ethanol (5 mL) and was cooled in an ice bath. Upon cooling, a precipitate formed but then returned to solution after the addition of 3 N NaOH (1 mL). The reaction mixture was stirred overnight at room temperature and was then concentrated under reduced pressure. The resulting residue was taken up into EtOAc and washed with acidic water (pH 3, HCl) and brine and dried with Na_2SO_4. After filtration, the filtrate was concentrated, and the residue was purified by flash chromatography using a 25% EtOAc/hexane mobile phase that was supplemented with dropwise amounts of acetic acid. The column-purified product was collected and recrystallized using 95% ethanol to give **31a** as a yellow solid (0.081 g, 0.181 mmol, 20.9%). TLC (50% EtOAc/hexane) = 0.46. Mp 228−230 °C. ^{1}H NMR (acetone-d_6, 600 MHz): δ 10.36 (1H, bs), 8.10 (2H, d, J = 8.1 Hz), 7.90 (1H, d, J = 15.8 Hz), 7.79 (2H, d, J = 8.3 Hz), 7.64 (1H, d, J = 8.7 Hz), 6.78 (1H, dd, J_1 = 9.0 Hz, J_2 = 2.8 Hz), 6.75 (1H, d, J = 2.6 Hz), 6.25 (1H, d, J = 15.8 Hz), 4.86 (2H, s), 3.10 (3H, s), 2.52 (3H, s), 2.41 (3H, s). ^{13}C NMR (acetone-d_6, 150 MHz): δ 168.33, 163.07, 151.40, 151.08, 142.79, 140.12, 138.07, 132.66, 131.38 (q, $^2J_{FC}$ = 31.8 Hz), 128.82, 127.27, 126.84 (q, $^3J_{FC}$ = 3.9 Hz), 125.14 (q, $^1J_{FC}$ = 271 Hz), 122.88, 115.10, 114.68, 111.88, 48.97, 38.38, 20.31, 15.49. Anal. Calcd for $C_{23}H_{21}F_3N_2O_2S$ (with 0.2 H_2O mol per target): C, 61.38; H, 4.79; N, 6.22. Found: C, 61.15; H, 4.86; N, 6.18. LC-MS/MS analysis shows >98% chromatographic purity (total ion current) with retention time = 5.21 min and m/z = 447 (for M + 1).

*4-Bromo-3-methylbenzaldehyde (**25b**).* By analogy to the procedure described in example **25a**, 4-Bromo-3-methylbenzonitrile **24b** (2.100 g, 10.71 mmol) was treated with DIBAL-H, and the crude material was purified by column chromatography using a 100% hexane to 5% EtOAc/hexane mobile-phase gradient. The desired product **25b** was collected as a white solid (1.801 g, 9.051 mmol, 84.5%). TLC (25% EtOAc/hexane) = 0.70. Mp 120−122 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 9.95 (1H, s), 7.72 (1H, s), 7.70 (1H, d, J = 8.1 Hz), 7.55 (1H, d, J = 8.0 Hz), 2.48 (3H, d). ^{13}C NMR ($CDCl_3$, 600 MHz): δ 191.56, 139.29, 135.54, 133.38, 132.37, 131.59, 128.41, 23.05. Anal. Calcd for C_8H_7OBr: C, 48.27; H, 3.54; N, 0.00. Found: C, 48.01; H, 3.56; N, 0.13.

*3-Methyl-4-[N-(tert-butoxycarbonyl)methyl]benzaldehyde (**27b**).* By analogy to the procedure described in example **27a**, the reaction with aldehyde **25b** (1.423 g, 7.149 mmol) and *tert*-butyl-*N*-methylcarbamate **26** (1.123 g, 8.561 mmol) using $Pd_2(dba)_3$ (0.149 g, 0.163 mmol) gave a crude product that was purified with flash chromatography using a 100% hexane to 5% EtOAc/hexane mobile-phase gradient to give the desired product **27b** as a light-yellow, almost colorless oil (0.837 g, 3.357 mmol, 47.0%). TLC (25% EtOAc/hexane) = 0.41. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 9.98 (1H, s), 7.75 (1H, s), 7.71 (1H, d, J = 7.7 Hz), 3.17 (3H, s), 2.30 (3H, s), 1.53 (3H, s), 1.34 (7H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 191.85, 154.47, 148.25, 136.77, 135.08, 132.16, 128.46, 128.18, 80.54, 36.69, 28.36, 17.78. Anal. Calcd for $C_{14}H_{19}O_3N$: C, 67.45; H, 7.68; N, 5.62. Found: C, 67.26; H, 7.73; N, 5.67.

*Methyl 3-Methyl-4-[N-(tert-butoxycarbonyl)methyl]-aminocinnamate (**28b**).* By analogy to the procedure described in example **28a**, reaction with aldehyde **27b** (0.736 g, 2.953 mmol) and methyl(triphenylphosphoranylidene)acetate (1.087 g, 3.251 mmol) gave a crude material that was purified using flash chromatography and a 100% hexane to 7% EtOAc/hexane mobile-phase gradient to give the desired product **28b** as a light-yellow oil (0.561 g, 1.837 mmol, 62.2%). TLC (25% EtOAc/hexane) = 0.43. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.66 (1H, d, J = 16 Hz), 7.37 (1H, s), 7.35 (1H, d, J = 7.9 Hz), 7.09 (1H, d, J = 7.9 Hz), 6.42 (1H, d, J = 15.8 Hz), 3.80 (3H, s), 3.14 (3H, s), 2.23 (3H, s), 1.51 (3H, s), 1.33 (6H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.57, 154.86, 144.47, 144.38, 136.17, 133.16, 130.57, 127.92, 126.50, 117.91, 80.17, 51.87, 36.79, 28.39, 17.75. Anal. Calcd for $C_{17}H_{23}O_4N$: C, 66.86; H, 7.59; N, 4.59. Found: C, 66.57; H, 7.57; N, 4.55.

*Methyl 3-Methyl-4-(N-methyl)aminocinnamate (**29b**).* By analogy to the procedure described in example **29a**, methyl ester **28b** (1.359 g, 4.450 mmol) was treated with trifluoroacetic acid (7 mL, 91.48 mmol). Flash chromatography was attempted to purify the crude material along with recrystallization. Purification methods were unsuccessful, and the crude yellow solid **29b** (0.758 g, 3.693 mmol, 83.0%) was moved to the next step. TLC (25% EtOAc/hexane) = 0.36. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.63 (1H, d, J = 16.0 Hz), 7.35 (1H, dd, J_1 = 8.4 Hz, J_2 = 2.0 Hz), 6.57 (1H, d, J = 8.4 Hz), 6.24 (1H, d, J = 15.6 Hz), 3.77 (3H, s), 2.93 (3H, s), 2.13 (3H, s).

*Methyl 2-Methyl-4-[N-methyl-N-[5-methylene-4-methyl-2-[4-(trifluoromethyl)phenyl]-thiazole]]aminocinnamate (**30b**).* By analogy to the procedure described in example **30a**, methyl ester **29b** (0.200 g, 0.974 mmol) and thiazole **4b** (0.340 g, 1.166 mmol) were treated with NaI (0.215 g, 1.434 mmol) and DIPEA (0.255 g, 1.464 mmol). The crude product was purified by flash chromatography with a 5−13% EtOAc/hexane mobile-phase gradient to give the product **30b** as a yellow solid (0.341 g, 0.740 mmol, 76.0%). TLC (25% EtOAc/hexane) = 0.50. Mp 92−94 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.00 (2H, d, J = 8.1 Hz), 7.65 (3H, m), 7.40 (1H, d, J = 1.6 Hz), 7.35 (1H, dd, J_1 = 8.2 Hz, J_2 = 2.0 Hz), 7.04 (1H, d, J = 8.2 Hz), 6.36 (1H, d, J = 16.0 Hz), 4.23 (2H, s), 3.80 (3H, s), 2.72 (3H, s), 2.43 (6H, m). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.85, 164.05, 153.14, 151.00, 144.71, 136.93, 133.26, 131.45 (q, $^2J_{FC}$ = 33 Hz), 131.31, 129.73, 126.91, 126.57, 126.02 (q, $^3J_{FC}$ = 3.5 Hz), 124.07 (q, $^2J_{FC}$ = 270 Hz), 120.39, 116.30, 51.97, 51.79, 41.05, 18.78, 15.50. Anal. Calcd for $C_{24}H_{23}F_3N_2O_2S$ (with 0.2 H_2O mol per target): C, 62.11; H, 5.08; N, 6.04. Found: C, 62.08; H, 5.06; N, 5.93.

*2-Methyl-4-[N-methyl-N-[5-methylene-4-methyl-2-[4-(trifluoromethyl)phenyl]thiazole]]aminocinnamic Acid (**31b**).* By analogy to the procedure described in example **31a**, methyl ester **30b** (0.220 g, 0.478 mmol) was treated with 3 N NaOH (2 mL), and the crude product was purified using flash chromatography and a 25% EtOAc/hexane mobile phase that was supplemented with dropwise amounts of acetic acid. The column product was recrystallized using 95% ethanol to give **31b** as a pale-yellow solid (0.170 g, 0.382 mmol, 79.8%). ^{1}H NMR (acetone-d_6, 600 MHz): δ 10.66 (1H, bs), 8.14 (2H, d, J = 8.1 Hz), 7.81 (2H, d, J = 8.2 Hz), 7.61 (1H, d, J = 16.0 Hz), 7.56 (1H, s), 7.48 (1H, dd, J_1 = 8.2 Hz, J_2 = 1.7 Hz), 7.18 (1H, d, J = 8.3 Hz), 6.43 (1H, d, J = 16.0 Hz), 4.37 (2H, s), 2.75 (3H, s), 2.45 (3H, s), 2.42 (3H, s). ^{13}C NMR (acetone-d_6, 150 MHz): δ 167.93, 163.64, 154.06, 151.91, 145.33, 138.21, 134.06, 132.68, 131.85, 131.37 (q, $^2J_{FC}$ = 32 Hz), 130.50, 127.73, 127.27, 126.85 (q, $^3J_{FC}$ = 3.8 Hz), 125.16 (q, $^1J_{FC}$ = 270 Hz), 121.40, 117.38, 52.07, 41.45, 18.66, 15.45. ^{19}F NMR (acetone-d_6, 376 MHz): δ −63.62 (3F, s). Anal. Calcd for $C_{23}H_{21}F_3N_2O_2S$: C, 61.87; H, 4.74; N, 6.27. Found: C, 61.69; H, 4.58; N, 6.14.

*3-Ethyl-N-(tert-butoxycarbonyl)aniline (**33a**).* 3-Ethylaniline **32a** (3.0 mL, 0.024 mol) and di-*tert*-butyl dicarbonate (5.840 g, 0.026 mol) were dissolved in anhydrous THF (40 mL) under inert gas. The mixture was refluxed at 65 °C overnight. The reaction mixture was then concentrated under reduced pressure, and the resulting residue was taken up into EtOAc, washed with saturated $NaHCO_3$ solution, water, and brine, and dried with Na_2SO_4. After filtration and evaporation of the solvent, the crude product was purified with flash chromatography using 10% EtOAc/hexane. The purified product **33a** was collected as an orange oil containing EtOAc (5.837 g, 0.026 mol, 110%). TLC R_f (25% EtOAc/hexane) = 0.64. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.20 (1H, m), 7.12 (1H, d, J = 7.7 Hz), 6.88 (1H, d, J = 7.5 Hz), 6.43 (1H, bs), 2.62 (2H, q, J = 7.6 Hz), 1.52 (9H, s), 1.22 (3H, t, J = 7.6 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 152.91, 145.47, 138.42, 129.00, 122.77, 118.15, 115.97, 80.53, 29.06, 28.50, 15.70. Anal. Calcd for $C_{13}H_{19}NO_2$: C, 70.56; H, 8.65; N, 6.33. Found: C, 70.27; H, 8.52; N, 6.10.

*3-Ethyl-[N-(tert-butoxycarbonyl)methyl]aniline (**34a**).* 3-Ethyl-*N*-*tert*-butoxycarbonyl)aniline **33a** (4.547 g, 20.55 mmol) was dissolved in anhydrous DMF (50 mL) and cooled in an ice bath. NaH (60% dispersion in mineral oil, 1.234 g, 30.85 mmol) was added, and the mixture was stirred for 45 min followed by the addition of iodomethane (3.8 mL, 61.04 mmol). The reaction mixture was stirred overnight at room temperature and quenched with 50% saturated $NaHCO_3$ and extracted with two portions of ether. The ether extracts were combined and washed with brine, dried with Na_2SO_4, and concentrated. The crude material was purified by flash chromatography using 5% EtOAc/hexane to give the desired product

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34a as a yellow−orange oil (4.318 g, 18.35 mmol, 89.3%). TLC R_f (25% EtOAc/hexane) = 0.62. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.23 (1H, m), 7.07 (1H, s), 7.04 (1H, d, J = 7.9 Hz), 7.01 (1H, d, J = 7.6 Hz), 3.25 (3H, s), 2.66 (2H, q, J = 7.6 Hz), 1.45 (9H, s), 1.23 (3H, t, J = 7.6 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 155.00, 144.85, 143.94, 128.53, 125.26, 125.07, 122.80, 80.26, 37.53, 28.90, 28.50, 15.65. Anal. Calcd for $C_{14}H_{21}NO_2$: C, 71.46; H, 9.00; N, 5.95. Found: C, 71.17; H, 8.89; N, 5.85.

*3-Ethyl-(N-methyl)aniline (**35a**).* 3-Ethyl-[*N*-(*tert*-butoxycarbonyl)methyl]aniline **34a** (3.582 g, 15.22 mmol) was dissolved in anhydrous DCM (40 mL) under inert gas and cooled in an ice bath. Trifluoroacetic acid (10 mL, 0.13 mol) was slowly added, and the mixture was stirred at rt for 1.5 h. The reaction mixture was diluted with DCM and neutralized with chilled saturated $NaHCO_3$. The organic phase was collected and washed with water and brine, dried with Na_2SO_4, and concentrated. The crude material was purified by column chromatography using a gradient of 5−50% EtOAc/hexane and appeared to oxidize upon collection to give the desired product **35a** as a crude black oil with trace EtOAc (2.845 g). This material was used in the next step without further purification. TLC R_f (25% EtOAc/hexane) = 0.61. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 9.66 (1H, bs), 7.35 (1H, m), 7.28 (2H, m), 7.24 (1H, d, J = 7.6 Hz), 2.99 (3H, s), 2.67 (2H, q, J = 7.6 Hz), 1.22 (3H, t, J = 7.6 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 147.25, 137.64, 130.31, 129.07, 121.51, 119.34, 38.08, 28.73, 15.25.

*3-Ethyl-[N-methyl-N-[(5-methylene-4-methyl-2-(4-(trifluoromethyl)phenyl)]thiazole]aniline (**36a**).* 3-Ethyl-(*N*-methyl)aniline **35a** (0.693 g, 5.125 mmol), thiazole **4b** (1.478 g, 5.069 mmol), and NaI (0.790 g, 5.271 mmol) were dissolved in anhydrous DMF (20 mL) under inert gas and cooled in an ice bath. NaH (60% dispersion in mineral oil, 0.314 g, 7.85 mmol) was carefully added to the reaction by briefly exposing the system to air. The reaction mixture was stirred for 3 h at room temperature and quenched with a 50% dilution of sat. $NaHCO_3$. The mixture was extracted with three portions of ether that were combined, washed with brine, dried with Na_2SO_4, and concentrated. The crude material was purified by flash chromatography using a gradient of 5−30% EtOAc/hexane to give the desired product **36a** as an orange oil (0.650 g, 1.66 mmol, 32.8%). TLC R_f (25% EtOAc/hexane) = 0.56. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.96 (2H, d, J = 8.1 Hz), 7.63 (2H, d, J = 8.3 Hz), 7.19 (1H, m), 6.68 (3H, m), 4.61 (2H, s), 2.97 (3H, s), 2.63 (2H, q, J = 7.6 Hz), 2.50 (3H, s), 1.24 (3H, t, J = 7.6 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 163.46, 150.19, 149.15, 145.66, 136.99, 131.30 (q, $^2J_{FC}$ = 33 Hz), 129.40, 126.49, 125.96 (m), 124.08 (q, $^1J_{FC}$ = 270 Hz), 118.11, 113.57, 111.36, 50.01, 38.53, 29.44, 15.81, 15.56. Anal. Calcd for $C_{21}H_{21}F_3N_2S$: C, 64.60; H, 5.42; N, 7.17. Found: C, 64.31; H, 5.48; N, 7.01.

*2-Ethyl-4-[N-methyl-N-[5-methylene-4-methyl-2-[4-(trifluoromethyl)phenyl]thiazole]] Aminobenzaldehyde (**37a**).* Anhydrous DMF (1.0 mL, 12.92 mmol) was added to reaction flask and cooled in an ice bath. $POCl_3$ (0.5 mL, 5.364 mmol) was slowly added, and the mixture was stirred for 15 min. Aniline product **36a** (0.612 g, 1.567 mmol) from the previous reaction was dissolved with anhydrous DMF (1 mL) in a separate flask. The aniline product solution was carefully transferred to the reaction flask containing the Vilsmeier reagent. The reaction mixture was stirred for 2.5 h at 70 °C and then allowed to cool before being poured onto crushed ice. This mixture was neutralized with 3 N NaOH until a pH of 10 was reached. This mixture produced no precipitate and was extracted with two portions of ether. The ether extracts were combined, washed with brine, dried with Na_2SO_4, and concentrated. The crude material was purified by flash chromatography using a gradient of 5−20% EtOAc/hexane to give the desired product **37a** as a yellow−orange solid (0.432 g, 1.032 mmol, 65.9%). TLC R_f (25% EtOAc/hexane) = 0.28. Mp 97−98 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 10.02 (1H, s), 7.96 (2H, d, J = 8.2 Hz), 7.74 (1H, d, J = 8.7 Hz), 7.64 (2H, d, J = 8.3 Hz), 6.70 (1H, dd, J_1 = 8.7 Hz J_2 = 2.6 Hz), 6.58 (1H, d, J = 2.5 Hz), 4.74 (2H, s), 3.12 (3H, s), 3.03 (2H, q, J = 7.5 Hz), 2.53 (3H, s), 1.27 (3H, t, J = 7.5 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 190.26, 163.76, 152.40, 150.51, 149.64, 136.59, 134.89, 131.60 (q, $^2J_{FC}$ = 33 Hz), 130.33, 126.58, 126.02 (m), 123.99 (q, $^1J_{FC}$ = 271 Hz), 123.96, 112.81, 109.78, 48.65, 38.30, 26.68, 16.42, 15.56. Anal. Calcd for $C_{22}H_{21}F_3N_2OS$: C, 63.14; H, 5.06; N, 6.69. Found: C, 62.85; H, 5.19; N, 6.58.

*Ethyl 2-Ethyl-4-[N-methyl-N-[5-methylene-4-methyl-2-[4-(trifluoromethyl)phenyl] thiazole]]aminocinnamate (**38a**).* Triethylphosphonoacetate (0.24 mL, 1.21 mmol) was taken up into anhydrous THF (5 mL) in an ice bath and under inert gas. NaH (60% dispersion in mineral oil, 0.055 g, 1.38 mmol) was added by briefly exposing the system to air. In a separate flask, aldehyde **37a** (0.385 g, 0.920 mmol) was dissolved in anhydrous THF (5 mL) under inert gas. After the triethylphosphonoacetate mixture had stirred for 30 min, the aldehyde solution was slowly added. The reaction mixture was stirred for 3 h at room temperature and upon completion was quenched with sat. NH_4Cl and was extracted with three portions of EtOAc. The combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated. The crude material was purified by column chromatography using a gradient of 10−20% EtOAc/hexane to give the desired product **38a** as a light-yellow solid (0.300 g, 0.614 mmol, 66.7%). TLC R_f (25% EtOAc/hexane) = 0.38. Mp 127−128 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.95 (3H, m), 7.64 (2H, d, J = 8.3 Hz), 7.56 (1H, d, J = 8.8 Hz), 6.65 (1H, dd, J_1 = 8.8 Hz J_2 = 2.7 Hz), 6.60 (1H, d, J = 2.7 Hz), 6.24 (1H, d, J = 15.7 Hz), 4.67 (2H, s), 4.25 (2H, q, J = 7.1 Hz), 3.04 (3H, s), 2.77 (2H, q, J = 7.6 Hz), 2.52 (3H, s), 1.33 (3H, t, J = 7.1 Hz), 1.23 (3H, t, J = 7.6 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.96, 163.57, 150.34, 150.20, 146.00, 141.78, 136.84, 131.45 (q, $^2J_{FC}$ = 32.6 Hz), 131.15, 128.23, 126.53, 125.98 (m), 124.05 (q, $^1J_{FC}$ = 270 Hz), 122.02, 114.81, 112.97, 111.11, 60.31, 49.03, 38.26, 27.10, 16.28, 15.59, 14.55. Anal. Calcd for $C_{26}H_{27}F_3N_2O_2S$: C, 63.92; H, 5.57; N, 5.73. Found: C, 63.66; H, 5.61; N, 5.58.

*2-Ethyl-4-[N-methyl-N-[5-methylene-4-methyl-2-[4-(trifluoromethyl)phenyl]thiazole]]aminocinnamic Acid (**39a**).* Ethyl ester **38a** (0.279 g, 0.571 mmol) was dissolved in 95% EtOH (8 mL) and THF (8 mL). 3 N NaOH (2 mL) was added, and the mixture was stirred overnight at room temperature. The reaction was determined to be incomplete, and an additional 3 N NaOH (2 mL) was added. The mixture was stirred for an additional 24 h and was neutralized with 1 N HCl to pH 3. The solvent was removed under reduced pressure, and the residue was taken up into EtOAc, washed with sat. $NaHCO_3$ and brine, dried with Na_2SO_4, and concentrated. The crude material was purified by flash chromatography using a 25−50% EtOAc/hexane gradient supplemented with dropwise amounts of acetic acid to give the desired product **39a** as a bright-yellow solid (0.181 g, 0.393 mmol, 68.8%). TLC R_f (50% EtOAc/hexane) = 0.45. Mp 216−218 °C. ^{1}H NMR (acetone-d_6, 600 MHz): δ 10.47 (1H, bs), 8.10 (2H, d, J = 8.1 Hz), 7.94 (1H, d, J = 15.7 Hz), 7.78 (2H, d, J = 8.3 Hz), 7.66 (1H, d, J = 8.8 Hz), 6.78 (2H, m), 6.26 (1H, d, J = 15.7 Hz), 4.87 (2H, s), 3.10 (3H, s), 2.77 (2H, q, J = 7.6 Hz), 2.52 (3H, s), 1.20 (3H, t, J = 7.6 Hz). ^{13}C NMR (acetone-d_6, 150 MHz): δ 168.37, 163.09, 151.43, 151.30, 146.39, 142.50, 138.07, 132.64, 131.38 (q, $^2J_{FC}$ = 32 Hz), 128.90, 127.27, 126.85 (m), 125.14 (q, $^1J_{FC}$ = 271 Hz), 121.95, 114.81, 113.80, 112.04, 48.99, 38.39, 27.43, 16.63, 15.51. ^{19}F NMR (acetone-d_6, 376 MHz): δ −63.66. Anal. Calcd for $C_{24}H_{23}F_3N_2O_2S$: C, 62.60; H, 5.03; N, 6.08. Found: C, 62.45; H, 5.07; N, 6.24.

*3-Isopropyl-N-(tert-butoxycarbonyl)aniline (**33b**).* By analogy to the procedure described in example **33a**, 3-isopropylaniline **32b** (1.087 g, 8.039 mmol) was treated with di-*tert*-butyl dicarbonate (1.988 g, 9.109 mmol), and the crude product was purified with flash chromatography using a 5% EtOAc/hexane mobile phase to give the product **33b** as a light-orange solid (1.660 g, 7.054 mmol, 87.7%). TLC R_f (25% EtOAc/hexane) = 0.70. Mp 44−45 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.25 (1H, bs), 7.21 (1H, m), 7.15 (1H, d, J = 7.4 Hz), 6.91 (1H, d, J = 7.5 Hz), 6.47 (1H, br-s), 2.88 (1H, sept., J = 7.0 Hz), 1.52 (9H, s), 1.24 (6H, d, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 152.92, 150.08, 138.40, 128.99, 121.28, 116.85, 116.17, 80.48, 34.31, 28.49, 24.07. Anal. Calcd for $C_{14}H_{21}NO_2$: C, 71.46; H, 9.00; N, 5.95. Found: C, 71.72; H, 9.03; N, 5.86.

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*3-Isopropyl-[N-methyl-N-(tert-butoxycarbonyl)]aniline (**34b**).* By analogy to the procedure described in example **34a**, 3-isopropyl-*N*-(*tert*-butoxycarbonyl)aniline **33b** (3.148 g, 13.38 mmol) was treated with NaH (60% dispersion in oil, 0.805 g, 20.13 mmol) and iodomethane (2.5 mL, 40.16 mmol). The crude material was purified by flash chromatography using a 100% hexane to 5% EtOAc/hexane gradient giving the desired compound **34b** as an orange oil (2.769 g, 11.10 mmol, 83.0%). TLC R_f (25% EtOAc/hexane) = 0.73. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.24 (1H, t, J = 7.8 Hz), 7.09 (1H, bs), 7.03 (2H, dd, J_1 = 7.7 Hz, J_2 = 1.6 Hz), 3.26 (3H, s), 2.89 (1H, sept., J = 6.9 Hz), 1.45 (9H, s), 1.25 (6H, d, J = 6.9 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 155.00, 149.45, 143.91, 128.50, 124.03, 123.61, 122.77, 80.23, 37.54, 34.18, 28.51, 24.09. Anal. Calcd for $C_{15}H_{23}NO_2$: C, 72.25; H, 9.30; N, 5.62. Found: C, 72.53; H, 9.27; N, 5.71.

*3-Isopropyl-(N-methyl)aniline (**35b**).* By analogy to the procedure described in example **35a**, 3-isopropyl-[*N*-methyl-*N*-(*tert*-butoxycarbonyl)]aniline **34b** (2.409 g, 9.661 mmol) was treated with trifluoroacetic acid (7.4 mL, 96.70 mmol). The crude material was purified by flash chromatography using a gradient of 5−50% EtOAc/hexane and appeared to oxidize upon collection to give the desired product **35b** as a crude black oil with trace EtOAc (1.733 g). This material was used in the next step without further purification. TLC R_f (25% EtOAc/hexane) = 0.51. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.36 (1H, m), 7.31 (1H, s), 7.27 (1H, m), 2.99 (3H, s), 2.92 (1H, sept., J = 6.9 Hz), 1.24 (6H, t, J = 6.9 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 151.94, 137.93, 130.31, 127.44, 120.05, 119.33, 37.97, 34.15, 23.78.

*3-Isopropyl-[N-methyl-N-[(5-methylene-4-methyl-2-(4-(trifluoromethyl)phenyl)]thiazole]aniline (**36b**).* By analogy to the procedure described in example **36a**, 3-isopropyl-(*N*-methyl)aniline **35b** (1.352 g, 9.059 mmol) and thiazole **4b** (1.755 g, 6.016 mmol) were treated with NaI (0.990 g, 6.604 mmol) and NaH (60% dispersion in mineral oil, 0.363 g, 9.075 mmol). The crude material was purified by flash chromatography using a gradient of 100% hexane to 5% EtOAc/hexane to give the desired product **36b** as an orange oil (1.657 g, 4.096 mmol, 68.1%). TLC R_f (25% EtOAc/hexane) = 0.58. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.97 (2H, d, J = 8.2 Hz), 7.64 (2H, d, J = 8.3 Hz), 7.21 (1H, t, J = 8.1 Hz), 6.72 (2H, m), 6.68 (1H, m), 4.61 (2H, s), 2.98 (3H, s), 2.87 (1H, sept., J = 6.9 Hz), 2.49 (3H, s), 1.27 (6H, m). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 163.55, 162.66, 150.33, 136.97, 133.67, 131.31 (q, $^2J_{FC}$ = 32.4 Hz), 129.40, 127.58, 126.51, 125.97(m), 124.08 (q, $^1J_{FC}$ = 271 Hz), 116.74, 112.41, 111.69, 50.17, 38.62, 34.65, 24.18, 15.57. Anal. Calcd for $C_{22}H_{23}F_3N_2S$: C, 65.33; H, 5.73; N, 6.93. Found: C, 65.06; H, 5.67; N, 6.83.

*2-Isopropyl-4-[N-methyl-N-[5-methylene-4-methyl-2-[4-(trifluoromethyl)phenyl]thiazole]] Aminobenzaldehyde (**37b**).* By analogy to the procedure described in example **37a**, aniline product **36b** (1.156 g, 2.858 mmol) was treated with Vilsmeier reagent. Purification of the crude material was attempted using flash chromatography with a gradient of 5−10% EtOAc/hexane. The yellow oil **37b** (0.414 g, 0.957 mmol, 33.5%) was moved to the next step without further purification. TLC R_f (25% EtOAc/hexane) = 0.28. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 10.08 (1H, s), 7.96 (2H, d, J = 8.2 Hz), 7.74 (1H, d, J = 8.8 Hz), 7.64 (2H, d, J = 8.3 Hz), 6.72 (1H, d, J = 2.5 Hz), 6.68 (1H, dd, J_1 = 8.7 Hz J_2 = 2.6 Hz), 4.74 (2H, s), 4.01 (1H, sept. J = 6.8 Hz), 3.13 (3H, s), 2.54 (3H, s), 1.28 (6H, d, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 190.30, 163.81, 154.01, 152.53, 150.52, 136.59, 135.09, 131.62 (q, $^2J_{FC}$ = 32.5 Hz), 130.39, 126.60, 126.04 (m), 124.00 (q, $^1J_{FC}$ = 270 Hz), 123.49, 109.64, 108.85, 48.74, 38.37, 28.07, 23.91, 15.58.

*Ethyl 2-Isopropyl-4-[N-methyl-N-[5-methylene-4-methyl-2-[4-(trifluoromethyl)phenyl] thiazole]]aminocinnamate (**38b**).* By analogy to the procedure described in example **38a**, aldehyde **37b** was treated with triethylphosphonoacetate (0.28 mL, 1.41 mmol) and NaH (60% dispersion in mineral oil, 0.065 g, 1.63 mmol). The crude material was purified by flash chromatography using a gradient of 10−15% EtOAc/hexane to give the desired product **38b** as a yellow solid (0.371 g, 0.739 mmol, 68.0%). TLC R_f (25% EtOAc/hexane) = 0.35. Mp 99−100 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.05 (1H, d, J = 15.7 Hz), 7.95 (2H, d, J = 8.2 Hz), 7.63 (2H, d, J = 8.3 Hz), 7.53 (1H, d, J = 8.8 Hz), 6.69 (1H, d, J = 2.6 Hz), 6.65 (1H, dd, J_1 = 8.7 Hz J_2 = 2.6 Hz), 6.23 (1H, d, J = 15.6 Hz), 4.67 (2H, s), 4.25 (2H, q, J = 7.1 Hz), 3.38 (1H, sept., J = 6.8 Hz), 3.05 (3H, s), 2.52 (3H, s), 1.33 (3H, t, J = 7.1 Hz), 1.25 (6H, d, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.93, 163.62, 150.36, 150.22, 149.93, 141.78, 136.80, 131.45 (q, $^2J_{FC}$ = 32.7 Hz), 128.31, 126.54, 126.00 (m), 124.04 (q, $^1J_{FC}$ = 271 Hz), 121.88, 115.40, 111.04, 109.44, 60.33, 49.18, 38.37, 29.22, 23.87, 15.60, 14.55. Anal. Calcd for $C_{27}H_{29}F_3N_2O_2S$: C, 64.52; H, 5.82; N, 5.57. Found: C, 64.45; H, 5.83; N, 5.63.

*2-Isopropyl-4-[N-methyl-N-[5-methylene-4-methyl-2-[4-(trifluoromethyl)phenyl] thiazole]]aminocinnamic Acid (**39b**).* By analogy to the procedure described in example **39a**, ethyl ester **38b** (0.327 g, 0.651 mmol) was treated with 3 N NaOH. The crude material was purified by column chromatography using a 15−50% EtOAc/hexane gradient supplemented with dropwise amounts of acetic acid to give the desired product **39b** as a yellow solid (0.179 g, 0.377 mmol, 57.9%). TLC R_f (50% EtOAc/hexane) = 0.46. Mp 218−220 °C. ^{1}H NMR (acetone-d_6, 600 MHz): δ 10.50 (1H, bs), 8.10 (2H, d, J = 8.2 Hz), 8.04 (1H, d, J = 15.8 Hz), 7.78 (2H, d, J = 8.3 Hz), 7.64 (1H, d, J = 8.8 Hz), 6.81 (1H, d, J = 2.6 Hz), 6.77 (1H, dd, J_1 = 8.8 Hz, J_2 = 2.6 Hz), 6.25 (1H, d, J = 15.6 Hz), 4.88 (2H, s), 3.37 (1H, sept., J = 6.8 Hz), 3.12 (3H, s), 2.53 (3H, s), 1.26 (3H, s) 1.25 (3H, s). ^{13}C NMR (acetone-d_6, 150 MHz): δ 168.36, 163.12, 151.38, 150.21, 142.45, 138.07, 132.73, 131.39 (q, $^2J_{FC}$ = 32.3 Hz), 128.97, 127.27, 126.84 (m), 125.14 (q, $^1J_{FC}$ = 270 Hz), 121.73, 115.37, 111.89, 110.09, 49.13, 38.51, 23.94, 15.54. ^{19}F NMR (acetone-d_6, 376 MHz): δ −63.66. Anal. Calcd for $C_{25}H_{25}F_3N_2O_2S$: C, 63.28; H, 5.31; N, 5.90. Found: C, 63.18; H, 5.33; N, 5.98.

*Ethyl 5-Methyl-2-[4-(trifluoromethyl)phenyl]-1,3-thiazole-4-carboxylate (**40**).* 2-Ketobutyric acid (3.245 g, 0.032 mol) was dissolved in anhydrous DCM (30 mL) under nitrogen gas. Bromine (1.7 mL, 0.033 mol) was slowly added, and the reaction mixture was stirred at room temperature for 15 min followed by evaporation of the solvent under reduced pressure. Toluene was added and immediately removed under reduced pressure. 4-(Trifluoromethyl)thiobenzamide (6.224 g, 0.303 mol) was added to the residue, dissolved in 95% ethanol, and refluxed overnight. The solvent was then removed, and the residue was purified by flash chromatography using a DCM mobile phase to give **40** as a white solid (4.140 g, 13.13 mmol, 43.3%). TLC (25% EtOAc/hexane) = 0.52. Mp 91 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.03 (2H, d, J = 8.1 Hz), 7.67 (2H, d, J = 8.16 Hz), 4.44 (2H, q, J = 7.14 Hz), 2.80 (3H, s), 1.43 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 162.51, 161.90, 145.57, 142.91, 136.08, 131.93 (q, $^2J_{FC}$ = 33 Hz), 126.98, 126.00 (q, $^3J_{FC}$ = 4.1 Hz), 123.93 (q, $^1J_{FC}$ = 270 Hz), 61.44, 14.47, 13.54. Anal. Calcd for $C_{14}H_{12}F_3NO_2S$: C, 53.33; H, 3.84; N, 4.44. Found: C, 53.19; H, 3.93; N, 4.35.

*4-Hydroxymethyl-5-methyl-2-[4-(trifluoromethyl)phenyl]-thiazole (**41**).* Ethyl ester **40** (3.284 g, 10.42 mmol) was dissolved in anhydrous THF (30 mL) under nitrogen gas. The mixture was cooled in an ice bath, and a chilled 2.0 M solution of $LiAlH_4$ in THF (5.2 mL, 10.4 mmol) was slowly added. The reaction mixture was stirred in an ice bath for 15 min before removal from the ice bath to allow warming to room temperature. The reaction mixture stirred for 90 min and was quenched with water and dried with Na_2SO_4. After filtration, the filtrate was concentrated, and the residue was purified by flash chromatography using a 10−50% EtOAc/hexane mobile-phase gradient to give **41** as a yellow solid (1.663 g, 6.086 mmol, 58.5%). TLC (25% EtOAc/hexane) = 0.16. Mp 112 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.99 (2H, d, J = 8.2 Hz), 7.68 (2H, d, J = 8.3 Hz), 4.74 (2H, s), 2.50 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 162.99, 152.58, 136.67, 131.50 (q, $^2J_{FC}$ = 32.5 Hz), 130.70, 126.52, 126.07 (q, $^3J_{FC}$ = 3.5 Hz), 124.05 (q, $^1J_{FC}$ = 271 Hz), 58.64, 11.32. Anal. Calcd for $C_{12}H_{10}F_3NOS$: C, 52.74; H, 3.69; N, 5.13. Found: C, 52.67; H, 3.77; N, 5.11.

*4-Chloromethyl-5-methyl-2-[4-(trifluoromethyl)phenyl]thiazole (**42**).* Alcohol **41** (1.606 g, 5.877 mmol) was dissolved in anhydrous DCM (40 mL) under nitrogen gas, and triethylamine (1.65 mL, 11.838 mmol) was added at room temperature. The mixture was cooled in an ice bath, methanesulfonyl chloride (0.69 mL, 8.915

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mmol) was added, and the reaction mixture was stirred at 4 °C overnight. The reaction mixture was diluted with DCM, washed with sat. $NaHCO_3$ solution, water, and brine, and dried with Na_2SO_4. After filtration, the filtrate was concentrated, and the residue was purified by flash chromatography using a 5−10% EtOAc/hexane gradient to give **42** as a white solid (1.063 g, 3.643 mmol, 62.0%). TLC (25% EtOAc/hexane) = 0.69. Mp 128−129 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.01 (2H, d, J = 8.1 Hz), 7.68 (2H, d, J = 8.2 Hz), 4.73 (2H, s), 2.55 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 162.90, 149.33, 136.56, 133.99, 131.63 (q, $^2J_{FC}$ = 32.6), 126.64, 126.06 (q, $^3J_{FC}$ = 4 Hz), 124.03 (q, $^1J_{FC}$ = 270 Hz), 38.82, 11.65. Anal. Calcd for $C_{12}H_9F_3NSCl$: C, 49.41; H, 3.11; N, 4.80. Found: C, 49.40; H, 3.06; N, 4.80.

*Ethyl 4-[N-Methyl-N-[4-methylene-5-methyl-2-[4-(trifluoromethyl)phenyl]thiazole]]aminocinnamate (**43**).* Ethyl ester **21** (0.419 g, 2.041 mmol), thiazole **42** (0.614 g, 2.105 mmol), and NaI (0.463 g, 3.085 mmol) were all dissolved in DMF (20 mL) and cooled in an ice bath. NaH (60% dispersion in oil, 0.112 g, 4.654 mmol) was added, and the reaction mixture was stirred at room temperature for 2.5 h. The reaction mixture was quenched with 50% $NaHCO_3$ and extracted with ether three times. The ether extracts were combined, washed with brine, and dried with Na_2SO_4. After filtration, the filtrate was concentrated, and the residue was purified by flash chromatography using a 10−25% EtOAc/hexane to give **43** as a yellow solid (0.725 g, 1.574 mmol, 77.1%). TLC (25% EtOAc/hexane) = 0.41. Mp 157−158 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.95 (2H, d, J = 8.2 Hz), 7.65 (2H, d, J = 8.2 Hz), 7.62 (1H, d, J = 15.8 Hz), 7.43 (2H, d, J = 8.7 Hz), 6.84 (2H, d, J = 8.5 Hz), 6.23 (1H, d, J = 15.8 Hz), 4.63 (2H, s), 4.24 (2H, q, J = 7.1 Hz), 3.13 (3H, s), 2.46 (3H, s), 1.32 (3H, t, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.97, 162.37, 151.03, 150.01, 145.02, 136.86, 131.36 (q, $^2J_{FC}$ = 32 Hz), 131.03, 129.84, 126.46, 126.01 (q, $^3J_{FC}$ = 3.8 Hz), 124.07 (q, $^1J_{FC}$ = 270 Hz), 113.23, 112.79, 60.26, 50.91, 38.87, 14.56, 11.62. Anal. Calcd for $C_{24}H_{23}F_3N_2O_2S$: C, 62.60; H, 5.03; N, 6.08. Found: C, 62.32; H, 5.16; N, 6.05.

*4-[N-Methyl-N-[4-methylene-5-methyl-2-[4-(trifluoromethyl)-phenyl]thiazole]]aminocinnamic Acid (**44**).* Ethyl ester **43** (0.666 g, 1.446 mmol) was dissolved in 95% ethanol (10 mL) and THF (10 mL) and cooled in an ice bath. 3 N NaOH (3 mL) was slowly added, and the reaction mixture was stirred for 2 days at room temperature. The reaction mixture was neutralized with acidic water (HCl, pH 3) and diluted with EtOAc. The organic phase was collected, washed with water and brine, and dried with Na_2SO_4. After filtration, the filtrate was concentrated, and the residue was purified by flash chromatography using a 15% EtOAc/hexane to 50% EtOAc gradient supplemented with dropwise amounts of acetic acid to give the desired product **44** as an orange solid (0.089 g, 0.205 mmol, 14.2%). TLC (50% EtOAc/hexane) = 0.47. Mp 232−234 °C. ^{1}H NMR (acetone-d_6, 600 MHz): δ 8.09 (2H, d, J = 8.1 Hz), 7.79 (2H, d, J = 8.1 Hz), 7.57 (1H, d, J = 15.8 Hz), 7.50 (2H, d, J = 8.9 Hz), 6.93 (2H, d, J = 8.9 Hz), 6.24 (1H, d, J = 15.8 Hz), 4.74 (2H, s), 3.20 (3H, s), 2.58 (3H, s). ^{13}C NMR (acetone-d_6, 150 MHz): δ 168.37, 162.34, 152.11, 151.78, 146.03, 138.02, 132.17, 131.36 (q, $^2J_{FC}$ = 33 Hz), 130.51, 127.22, 126.88 (q, $^3J_{FC}$ = 3.9 Hz), 125.14 (q, $^1J_{FC}$ = 270 Hz), 123.38, 113.31, 50.68, 39.20, 11.37. Anal. Calcd for $C_{22}H_{19}F_3N_2O_2S$ (with 0.1 mol H_2O per target): C, 60.60; H, 4.48; N, 6.42. Found: C, 60.56; H, 4.57; N, 6.27.

*Ethyl 5-Methyl-2-[4-(trifluoromethyl)phenyl]-1,2,3-triazole-4-carboxylate (**45**).* 4-Trifluoromethylaniline (2.298 g, 14.26 mmol) was dissolved in acetic acid (5 mL) followed by the addition of water (10 mL) and concentrated HCl (2.5 mL). This mixture was cooled in an ice bath, and a solution of $NaNO_2$ (1.19 g, 17.25 mmol) in water (2 mL) was added. This collective mixture was added to a separate mixture of ethyl acetoacetate (1.80 mL, 14.23 mmol) in 95% ethanol (20 mL) and NaOAc (1.203 g, 14.67 mmol) in 1 N Na_2CO_3 (5 mL) at 0 °C. The resulting mixture was stirred for 2 h at room temperature followed by dilution with water and extraction with two portions of ethyl acetate. The organic extracts were collected and combined, washed with water and brine, dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The concentrated material was dissolved in 95% ethanol (30 mL),and $CuCl_2{\cdot}2H_2O$ (5.214 g, 30.58 mmol) was added followed by NH_4OAc (10.69 g, 0.138 mol). The dark-green mixture was refluxed for 20 h. The reaction mixture was removed from heat and allowed to cool to room temperature. Concentrated HCl was added to form a precipitate that was collected by filtration and washed with 1 N HCl to give an orange solid. This material was recrystallized using 95% ethanol, giving light-orange crystalline needles. The crystallization filtrate was collected and purified using flash chromatography with a mobile-phase gradient of 100% hexane to 20% EtOAc/hexane, giving a light-orange powder. This powder was combined with the crystallization product to give the total desired compound **45** (2.711 g, 9.059 mmol, 63.7%). TLC R_f (25% EtOAc/hexane) = 0.64. Mp 88 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.25 (2H, d, J = 8.5 Hz), 7.75 (2H, d, J = 8.5 Hz), 4.48 (2H, q, J = 7.1 Hz), 2.63 (3H, s), 1.45 (3H, t, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 161.37, 149.58, 141.57, 139.11, 130.21 (q, $^2J_{FC}$ = 33 Hz), 126.78 (q, $^3J_{FC}$ = 3.3 Hz), 123.88 (q, $^1J_{FC}$ = 270 Hz), 119.51, 61.72, 14.47, 11.82. Anal. Calcd for $C_{13}H_{12}F_3N_3O_2$: C, 52.18; H, 4.04; N, 14.04. Found: C, 52.43; H, 4.02; N, 14.12.

*[5-Methyl-2-[4-(trifluoromethyl)phenyl]-2H-1,2,3-triazole-4-yl]-methanol (**46**).* To a stirred solution of ethyl ester **45** (1.818 g, 6.075 mmol) in anhydrous THF (20 mL) at 0 °C was added 2 M $LiAlH_4$ solution in THF (3.10 mL, 6.20 mmol). The resulting neon-green mixture was stirred under argon at rt for 2 h. The reaction mixture was quenched by the careful addition of water (3 mL) followed by dilution with ethyl acetate and the addition of anhydrous sodium sulfate (18 g). The light-yellow mixture was stirred for 20 min, filtered, and concentrated under reduced pressure to give **46** as a white solid (1.498 g, 5.824 mmol, 95.9%). TLC R_f (25% EtOAc/hexane) = 0.20. Mp 105 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.12 (2H, d, J = 8.5 Hz), 7.71 (2H, d, J = 8.6 Hz), 4.82 (2H, d, J = 5.8 Hz), 2.43 (3H, s), 1.94 (1H, t, J = 5.9 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 147.47, 144.98, 141.97, 128.92 (q, $^2J_{FC}$ = 33 Hz), 126.72 (q, $^3J_{FC}$ = 3.4 Hz), 124.06 (q, $^1J_{FC}$ = 270 Hz), 118.38, 56.18, 10.24. Anal. Calcd for $C_{11}H_{10}F_3N_3O$: C, 51.37; H, 3.92; N, 16.34. Found: C, 51.36; H, 3.95; N, 16.24.

*4-(Chloromethyl)-5-methyl-2-[4-(trifluoromethyl)phenyl]-2H-1,2,3-triazole (**47**).* To a stirred solution of alcohol **46** (3.232 g, 12.57 mmol) in anhydrous dichloromethane (50 mL) was added triethylamine (3.50 mL, 25.11 mmol). The resulting mixture was cooled to 4 °C, and methanesulfonyl chloride (1.50 mL, 19.38 mmol) was slowly added. The mixture was stirred at 4 °C for 24 h and then diluted with dichloromethane (50 mL), washed with saturated $NaHCO_3$ solution, water, and brine, dried with Na_2SO_4, and concentrated. The residue was purified by flash chromatography using 5% EtOAc/hexane to give **47** as a white solid (1.928 g, 6.994 mmol, 55.8%). TLC R_f (25% EtOAc/hexane) = 0.71. Mp 71−73 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.13 (2H, d, J = 8.4 Hz), 7.73 (2H, d, J = 8.5 Hz), 4.72 (2H, s), 2.46 (3H, s). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 145.71, 144.65, 141.84, 129.22 (q, $^2J_{FC}$ = 33 Hz), 126.74 (q, $^3J_{FC}$ = 3.8 Hz), 124.01 (q, $^1J_{FC}$ = 270 Hz), 118.59, 35.35, 10.25. Anal. Calcd for $C_{11}H_9F_3N_3Cl$: C, 47.93; H, 3.29; N, 15.24. Found: C, 48.08; H, 3.36; N, 15.32.

*Ethyl 4-[N-Methyl-N-[4-methylene-5-methyl-2-[4-(trifluoromethyl)phenyl]-2H-1,2,3-triazol-4-yl]]aminocinnamate (**48**).* Ethyl 4-(*N*-methyl)aminocinnamate **21** (0.486 g, 2.368 mmol), triazole **47** (0.763 g, 2.768 mmol), and NaI (0.492 g, 3.282 mmol) were dissolved in anhydrous DMF (20 mL) and cooled in an ice bath. NaH (60% dispersion in oil, 0.147 g, 3.675 mmol) was added, and the reaction mixture was stirred at room temperature for 2.5 h. The reaction mixture was carefully quenched with water, diluted with ether, and washed with a 10% aqueous $NaHCO_3$ solution. The organic phase was collected, and the aqueous phase was extracted with ether/sat. $NaHCO_3$ three times. The combined organic extracts were washed with brine, dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography using an isocratic 5% EtOAc/hexane mobile phase to give the desired product as a white solid. This material was recrystallized using a mixture of EtOAc and hexane to give **48** as an ivory white solid (0.559 g, 1.258 mmol, 53.1%). TLC R_f (25% EtOAc/hexane) = 0.40. Mp 92−93 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.10 (2H, d, J = 8.5

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Hz), 7.71 (2H, d, J = 8.6 Hz), 7.62 (1H, d, J = 15.9 Hz), 7.44 (2H, d, J = 8.9 Hz), 6.83 (2H, d, J = 8.9 Hz), 6.24 (1H, d, J = 15.8 Hz), 4.66 (2H, s), 4.24 (2H, q, J = 7.1 Hz), 3.09 (3H, s), 2.32 (3H, s), 1.32 (3H, t, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.89, 150.80, 145.39, 144.86, 141.96, 129.87, 128.84 (q, $^2J_{FC}$ = 33 Hz), 126.69 (q, $^3J_{FC}$ = 3.5 Hz), 124.06 (q, $^1J_{FC}$ = 270 Hz), 123.55, 118.34, 113.57, 112.76, 60.29, 47.55, 38.71, 14.54, 10.71. Anal. Calcd for $C_{23}H_{23}F_3N_4O_2$: C, 62.15; H, 5.22; N, 12.61. Found: C, 62.42; H, 5.15; N, 12.48.

*4-[N-Methyl-N-[4-methylene-5-methyl-2-[4-(trifluoromethyl)-phenyl]-2H-1,2,3-triazol-4-yl]]aminocinnamic Acid (**49**).* Methyl ester **48** (0.543 g, 1.222 mmol) was dissolved in anhydrous THF (10 mL) and 95% ethanol (10 mL) and was cooled in an ice bath. Upon cooling, a precipitate formed but then returned to solution after the addition of 3 N NaOH (3 mL). The reaction mixture was stirred overnight at room temperature. TLC analysis of the reaction progress showed that the reaction was incomplete. An additional 3 N NaOH (1 mL) was added, and the reaction mixture was stirred at room temperature for an additional 24 h. The completed reaction was neutralized with 1 N HCl until pH 4 was reached and was then concentrated under reduced pressure. The resulting residue was taken up into EtOAc and washed with saturated $NaHCO_3$ and brine and dried with Na_2SO_4 and concentrated. The yellow crude product was purified by flash chromatography using a 10−75% EtOAc/hexane mobile-phase gradient that was supplemented with dropwise amounts of acetic acid. The column-purified product was collected and recrystallized using 95% ethanol and EtOAc to give **49** as a crystalline yellow solid (0.207 g, 0.498 mmol, 40.7%). TLC R_f (50% EtOAc/hexane) = 0.48. Mp 236−240 °C. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 11.99 (1H, bs), 8.09 (2H, d, J = 8.6 Hz), 7.89 (2H, d, J = 8.6 Hz), 7.50 (1H, d, J = 15.8 Hz), 7.46 (2H, d, J = 8.8 Hz), 6.85 (2H, d, J = 8.8 Hz), 6.22 (1H, d, J = 15.9 Hz), 4.77 (2H, s), 3.08 (3H, s), 2.32 (3H, s). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 168.18, 150.47, 146.20, 145.18, 144.44, 141.48, 129.76, 127.12 (m), 126.98 (m), 124.04 (q, $^1J_{FC}$ = 270 Hz), 122.31, 118.07, 113.51, 112.34, 46.29, 38.49, 10.17. ^{19}F NMR (acetone-d_6, 376 MHz): δ −63.20 (3F, s). Anal. Calcd for $C_{21}H_{19}F_3N_4O_2$: C, 60.57; H, 4.60; N, 13.46. Found: C, 60.55; H, 4.53; N, 13.40.

*Ethyl 4-[(N-tert-Butoxycarbonyl)-N-[4-methylene-5-methyl-2-[4-(trifluoromethyl)phenyl]-2H-1,2,3-triazol-4-yl]]aminocinnamate (**50**).* Ethyl 4-(*N*-*tert*-butoxycarbonyl)aminocinnamate **13** (0.813 g, 2.790 mmol), triazole **47** (0.846 g, 3.069 mmol), and NaI (0.476 g, 3.176 mmol) were dissolved in anhydrous DMF (25 mL) and cooled in an ice bath. NaH (60% dispersion in oil, 0.215 g, 5.375 mmol) was added, and the reaction mixture was stirred at room temperature for 2.5 h. The reaction mixture was carefully quenched with water, diluted with ether, and washed with a 10% aqueous $NaHCO_3$ solution. The organic phase was collected, and the aqueous phase was extracted with ether/sat. $NaHCO_3$ three times. The combined organic extracts were washed with brine, dried with Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography using an isocratic 5% EtOAc/hexane mobile phase to give the desired product **50** as a white solid (0.696 g, 1.312 mmol, 47.0%). TLC R_f (25% EtOAc/hexane) = 0.48. Mp 99−112 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.06 (2H, d, J = 8.6 Hz), 7.69 (2H, d, J = 8.7 Hz), 7.63 (1H, d, J = 16.0 Hz), 7.47 (2H, d, J = 8.5 Hz), 7.28 (2H, d, J = 8.2 Hz), 6.38 (1H, d, J = 16.0 Hz), 4.97 (2H, s), 4.25 (2H, q, J = 7.1 Hz), 2.35 (3H, s), 1.44 (9H, s), 1.32 (3H, t, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.08, 154.23, 145.26, 144.81, 143.85, 143.79, 141.97, 132.29, 128.76 (q, $^2J_{FC}$ = 33 Hz), 128.58, 126.97, 126.66 (q, $^3J_{FC}$ = 3.5 Hz), 124.07 (q, $^1J_{FC}$ = 270 Hz), 118.39, 118.26, 81.52, 60.68, 44.57, 28.37, 14.46, 10.32. Anal. Calcd for $C_{27}H_{29}F_3N_4O_4$: C, 61.12; H, 5.51; N, 10.56. Found: C, 61.38; H, 5.47; N, 10.42.

*Ethyl 4-[N-[4-Methylene-5-methyl-2-[4-(trifluoromethyl)phenyl]-2H-1,2,3-triazol-4-yl]]aminocinnamate (**51**).* BOC-protected amine **50** (0.482 g, 0.908 mmol) was dissolved in anhydrous DCM (16 mL) and cooled in an ice bath. Trifluoroacetic acid (3 mL) was slowly added, and the mixture was stirred at room temperature for 2 h. The reaction mixture was carefully neutralized and washed with cold saturated $NaHCO_3$ solution. The organic phase was collected, washed with water and brine, and dried with Na_2SO_4. After filtration and evaporation of the solvent, the crude product was purified with flash chromatography using a gradient of 10−25% EtOAc/hexane to give **51** as a white solid (0.280 g, 0.651 mmol, 71.6%). TLC R_f (25% EtOAc/hexane) = 0.31. Mp 126−127 °C. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 8.13 (2H, d, J = 8.5 Hz), 7.72 (2H, d, J = 8.6 Hz), 7.61 (1H, d, J = 15.9 Hz), 7.41 (2H, d, J = 8.6 Hz), 6.71 (2H, d, J = 8.5 Hz), 6.24 (1H, d, J = 15.8 Hz), 4.51 (1H, m), 4.46 (2H, d, J = 5.0 Hz), 4.24 (2H, q, J = 7.1 Hz), 2.41 (3H, s), 1.32 (3H, t, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 167.85, 149.40, 145.40, 144.92, 144.58, 141.94, 130.03, 128.94 (q, $^2J_{FC}$ = 32.5 Hz), 126.73 (q, $^3J_{FC}$ = 3.5 Hz), 124.61, 124.05 (q, $^1J_{FC}$ = 270 Hz), 118.37, 113.77, 113.03, 60.32, 39.00, 14.54, 10.37. Anal. Calcd for $C_{22}H_{21}F_3N_4O_2$: C, 61.39; H, 4.92; N, 13.02. Found: C, 61.30; H, 4.88; N, 13.05.

*4-[N-[4-Methylene-5-methyl-2-[4-(trifluoromethyl)phenyl]-2H-1,2,3-triazol-4-yl]]aminocinnamic Acid (**52**).* Methyl ester **51** (0.190 g, 0.441 mmol) was dissolved in anhydrous THF (8 mL) and 95% ethanol (7 mL) and was cooled in an ice bath. Upon cooling, a precipitate formed but then returned to solution after the addition of 3 N NaOH (2 mL). The reaction mixture was stirred overnight at room temperature. TLC analysis of the reaction progress showed that the reaction was incomplete. An additional 3 N NaOH (1 mL) was added, and the reaction mixture was stirred at room temperature for an additional 24 h. The completed reaction was neutralized with 1 N HCl until pH 4 was reached and was then concentrated under reduced pressure. The resulting residue was taken up into EtOAc, washed with saturated $NaHCO_3$ and brine, dried with Na_2SO_4, and concentrated. The yellow crude product was purified by flash chromatography using a 10−35% EtOAc/hexane mobile-phase gradient that was supplemented with dropwise amounts of acetic acid. The column-purified product was collected and recrystallized using 95% ethanol and EtOAc to give **52** as a crystalline off-white solid (0.065 g, 0.162 mmol, 36.6%). TLC R_f (50% EtOAc/hexane) = 0.25. Mp 216−219 °C. ^{1}H NMR (acetone-d_6, 600 MHz): δ 10.39 (1H, bs), 8.22 (2H, d, J = 8.4 Hz), 7.89 (2H, d, J = 8.6 Hz), 7.56 (1H, d, J = 15.9 Hz), 7.47 (2H, d, J = 8.6 Hz), 6.82 (2H, d, J = 8.7 Hz), 6.23 (1H, d, J = 15.8 Hz), 6.10 (1H, m), 4.59 (2H, d, J = 5.1 Hz), 2.42 (3H, s). ^{13}C NMR (acetone-d_6, 150 MHz): δ 168.39, 151.33, 147.44, 146.24, 146.16, 143.04, 130.73, 128.75 (q, $^2J_{FC}$ = 32.5 Hz), 127.69 (q, $^3J_{FC}$ = 3.4 Hz), 125.16 (q, $^1J_{FC}$ = 270 Hz), 124.14, 118.99, 113.42, 113.36, 38.94, 10.34. ^{19}F NMR (acetone-d_6, 376 MHz): δ −63.29. Anal. Calcd for $C_{20}H_{17}F_3N_4O_2$: C, 59.70; H, 4.26; N, 13.92. Found: C, 59.91; H, 4.39; N, 13.86.

LC-MS/MS Analysis. As a first step toward future *in vitro* and *in vivo* PK studies followed by toxicity studies, the two lead compounds, **12d** and **31a**, were analyzed by LC-MS/MS using an Alliance HT liquid chromatograph (model 2795) equipped with a quaternary pump, a degasser, an autosampler/injector (250 μL syringe volume), and a column oven from Waters connected to a Quattro Micro (triple-quadrupole) MS/MS instrument equipped with an ESCi multimode ionization source from Micromass. MassLynx (version 4.01) software from Micromass was used for data acquisition and handling. A C18 column (Gemini, 5 μm C18 110 Å LC; 50 × 2 mm 5 μm) was eluted with a mobile-phase gradient (0.1% formic acid in water/acetonitrile: [50/50] to [25/75] at 6 min and held for 1.5 min before returning to [50/50] at 7.6 min and re-equilibrating for 10 min between injections). A standard guard column was also in place (Waters, 2.1 × 10 mm 5 μm). The cone voltage (CV) and collision energy (CE) were optimized for both compounds at CV = 35 V and CE = 30. Samples were dissolved in 50/50 aqueous methanol form, and 10 μL injections were conducted across serial dilutions. The latter showed excellent linearity from 100 μM to 100 nM, as may be needed for future bioanalytical assays of both compounds. Representative chromatograms for injections of **12d** and for **31a** at 50 μM are provided in the Supporting Information and contribute further toward confirming both compounds' chemical integrity.

MSC Differentiation Assay. Human-bone-marrow-derived MSCs (purchased from AllCells; passages 2 and 3) were replated in a 24-well plate at a density of 1 × 10^5 cells/cm^2 and cultured in α-

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minimal essential medium (α-MEM) with 20% fetal bovine serum (FBS) for 1 day at 37 °C. On the next day, the medium was switched to an osteogenic medium (Stem X-Vivo, R&D System, Minneapolis, MN) containing 10% FBS, 50 μg/mL ascorbic acid, and 3 mM β-glycerophosphate. Test samples and positive controls (GW0742 for bone; rosiglitazone for fat) were prepared from stock solutions having a 100 mM compound in DMSO by serial dilutions with the cell medium. MSCs were cultured in the osteogenic medium and treated with test samples, positive controls, or vehicle in quadruplicate every other day for 21 days. The resulting cells were stained with Alizarin Red S and visualized by phase-contrast microscopy to determine the cell morphology and to verify the presence of mineralized nodules in red (bone) and white droplets (fat). Images were analyzed by Image-Pro analyzer (Media Cybernetics, Bethesda, MD). The degree of osteogenesis or adipogenesis was scored based on a comparison with the GW0742 or rosiglitazone control and assigned "+" signs based on the intensity of the response: +++, similar to the robust response observed for the positive control; ++, moderate; +, minimal. No plus sign indicates essentially no additional response when compared with nontreated controls.

PPAR Transactivation Assay. *Cell Culture.* The African green monkey fibroblast-like cell line, COS-7 (obtained from American Type Culture Collection CRL-1651), was stored in a liquid-nitrogen container in separate cryogenic vials. Cells were thawed and maintained in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Thermo Fisher Scientific) containing L-glutamine and sodium pyruvate and supplemented with 10% FBS (Gibco, Thermo Fisher Scientific), penicillin (Alkali Scientific), and streptomycin (Alkali Scientific). The cells were incubated at 37 °C with 5% CO_2 and constant humidity. The medium was changed three times a week until the cells were 80% confluent, in which case, cells were split (1:10) into a subculture using 0.25% trypsin (Corning, Fisher Scientific)/0.02% ethylenediaminetetraacetic acid (EDTA) (Sigma-Aldrich) solution. COS-7 cells were maintained in this manner for use in experiments until a passage number of 30 was reached.

Reporter Assay. The reporter assay was a 4 day procedure involving plating of cells, transient transfection, treatment with the test agent, and detection of the luciferase reporter response. The first day involved plating COS-7 cells in a 48-well plate (40 000 cells/well). Cells were obtained from a trypsinized cell culture dish at 80–90% confluence and were counted using trypan blue (Gibco, Thermo Fisher Scientific) and a hemocytometer. Plated cells were then incubated for 24 h at 37 °C with 5% CO_2 and constant humidity.

Cells were then transiently transfected using GeneFect transfection reagent (0.75 μL/well) (Alkali Scientific) with the appropriate plasmid DNA (previously prepared by minipreparation) in a reduced serum medium, Opti-MEM (Gibco, Thermo Fisher Scientific). Plasmid DNA included PPARα, PPARγ, PPARδ, RXRα, pRL-CMV, and pcDNA3.1 (all gifts from Terry Hinds, Jr., University of Toledo) and PPRE (peroxisome proliferator response element) X3-TK-luc (a gift from Bruce Spiegelman, Addgene plasmid no. 1015).[97] Wells that would be treated with the test agent or DMSO vehicle were transfected with PPAR (PPARα at 0.2 μg/well, PPARγ at 0.3 μg/well, or PPARδ at 0.2 μg/well), RXRα (0.1 μg/well), PPRE X3-TK-luc (0.2 μg/well), and pRL-CMV (0.002 μg/well). Six control wells were transfected with empty pcDNA3.1 (0.4 μg/well), PPRE X3-TK-luc (0.2 μg/well), and pRL-CMV (0.002 μg/well). Transfection mixtures were incubated with GeneFect at rt for 30 min before addition to the wells. Cells were incubated for 24 h at 37 °C with 5% CO_2 and constant humidity.

Test Agent Treatments. Test samples and positive controls (gemfibrozil for PPARα, rosiglitazone for PPARγ, and GW0742 for PPARδ) were prepared from stock solutions having 10 mM compound in DMSO by serial dilutions with the cell medium. The vehicle control (no drug) had 1% DMSO to account for the highest drug level tested (100 μM), and it had no observable effects in the assay compared with negative-control treatments using just the cell medium. DMEM was changed in the wells, and cells were treated with test agent at various dilutions in triplicate. Six PPAR-transfected wells along with the six pcDNA3.1-transfected wells were treated with the DMSO vehicle control. Another six PPAR-transfected wells were treated with an appropriate PPAR standard (gemfibrozil, rosiglitazone, or GW0742). Cells were then incubated for 24 h at 37 °C with 5% CO_2 and constant humidity. The treated cells were lysed using passive lysis buffer, and the luciferase activity (Promega, Dual-Luciferase Reporter Assay System) was recorded by a BioTeck Synergy 2 luminometer. The firefly luciferase response was normalized by the constitutive expression of *Renilla* luciferase. The raw data were further normalized by vehicle response (0% response) and PPAR standard (100% response) followed by processing with Prism software (GraphPad, version 5.04). The results for the PPAR standards when used on their respective receptor (i.e., PPARδ-transfected cells treated with GW0742) were processed using a variable slope model where the dose–response curve was forced to run from 0 to 100% response. All other results were derived from a four-parameter variable slope model. EC_{50} values were obtained from Prism based on these models and were based on the line of best fit. The bottom of the dose–response curve was occasionally constrained to a value of zero in scenarios where data points were negative values. For data sets where this constraint was unnecessary, the bottom plateau was determined by Prism.

OVX Mouse Study. The following *in vivo* study was completed in accordance with the IACUC (Institutional Animal Care and Use Committee, approval no. 20170915001). C57BL/6 female mice acquired from Japan SLC (Shizuoka, Japan) were ovariectomized at 12 weeks of age. Sham surgery was performed on a control group of mice at the same age, leaving the ovaries intact. The mice were given a 4 week recovery period that also served as a period to allow for the development of osteoporosis in the OVX mice. Mice were divided into eight groups having eight subjects. The six test agent "stock solutions" and GW0742 as a positive control were freshly prepared each week under aseptic conditions in water at 10 mM concentrations and stored at 2 °C as fine suspensions that were shaken at room temperature prior to daily dilutions in 5% aqueous carboxymethylcellulose (CMC). Each group of mice was administered 150 μL doses of the assigned compound (10 mg per kg of body weight per day) or vehicle by oral gavage for 6 weeks. The sham group, vehicle, and GW0742 control groups and all six of the test agent groups were sacrificed at 22 weeks of age, and the femurs from each mouse were analyzed using microcomputer tomography (μCT).

Cytotoxicity Studies Conducted In-House. Human embryonic kidney cells (HEK 293T/17) were obtained from ATCC (Manassas, VA). Cells were cultured at 37 °C in a 5% CO_2 atmosphere in a cell medium consisting of α-MEM also containing 10% heat-inactivated FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin. Test samples and GW0742 used as a prototypical standard throughout all of our studies were prepared from stock solutions having 100 mM compound in DMSO by serial dilutions with the cell medium. Hydrogen peroxide was similarly diluted and used as a positive control spanning a range from mid-mM to low-μM. Cells were plated at 1×10^4 per well, and the plates were treated with controls and test agents in a first study for the typical 24 h duration and in a second study for a prolonged period of 72 h. Surviving cells were counted using the MTT method, whereby 20 μL of 5 mg/mL MTT in phosphate-buffered saline (PBS) solution, pH 7.4, was added to each well, and the plates were incubated for 2 h. After the removal of this solution, DMSO was added to dissolve formazan products, and the plates were shaken for 5 min. The absorbance of each well was recorded on a microplate spectrophotometer at 570 nm and compared with non-drug-treated wells to derive the percent cell viability. Each study was conducted in quadruplicate, and Prism was used to derive plots of the mean values with error bars reflecting one standard deviation. The latter were also manually calculated for selected data points suspected of being statistically different when compared with their 0 drug data points. The statistical significance between these pairs was assessed using the hand-calculated data as entries for Graphpad to calculate a two-tailed *P* value. Asterisks were added to the plots to denote statistically significant differences between such points and their 0 dose levels. Translation to potential toxicity in humans is generally considered to be relevant when the cell

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viability becomes <70% after 24 h of exposure of the cell culture to a given drug concentration.

Cytotoxicity Studies Conducted by NAMSA, an Extramural Clinical Research Organization (CRO). The test articles (four liquid samples) were evaluated for potential cytotoxicity effects by following the guidelines of ISO 1099-5, "Biological Evaluation of Medical Devices − Part 5: Tests for in Vitro Cytotoxicity". NAMSA remained blinded from the composition and concentration of all test articles throughout the study. Rather than performing an extraction, as is done for devices, each test article was mixed and diluted (1:19 v/v per the client's specification) with single-strength minimum essential medium (1× MEM used for cell culture). This was considered the 100% or "full strength" test article solution/dilution. Three test article solutions were tested at 100% (full strength) and at the following standard dilutions of 50, 25, and 12.5% plus the client's request of additional 6.25 and 3.13% dilutions. The fourth test agent "(4) UT Sol" was tested only at the standard 100, 50, 25, and 12.5% dilutions. A negative control, a reagent control, and two positive controls were similarly diluted for each test agent study. Studies were undertaken independently in triplicate. Each study used monolayers of L-929 mouse fibroblast cells on 96-well plates initially seeded with 1×10^4 cells. Cells were dosed with full-strength and diluted solutions and incubated at 37 °C (humidified) in the presence of 5% CO_2 for 24−26 h. Cell culture and dosing steps used aseptic procedures throughout. Following incubation, the cultures were examined under a phase-contrast microscope to identify any systemic cell-seeding errors, abnormal growth, or significant changes in morphology, any of which could negate the study. The culture medium was then replaced with a 1 mg/mL MTT solution and incubated for an additional 2 h, after which the MTT solution was replaced with isopropanol. The percent viability for the test article was determined from the reagent control. A decrease in the number of living cells results in a decrease in the metabolic activity in the sample. This decrease directly correlates to the amount of blue-violet formazan formed, as monitored by the optical density at 570 nm. The percent viability is compared with the reagent control by the following formula: percent viability = 100× OD_{570c}/OD_{570rc}, wherein OD_{570c} is the blank corrected mean value of the measured optical density of the test or control article dilution and OD_{570rc} is the blank corrected mean value of the measured optical density of the reagent control. For the test to be valid: (i) the average reagent control OD must be ≥0.2; (ii) the average of the reagent control plate columns immediately to the left and right of the test agent columns must not differ by >15% from the overall mean of the two reagent control columns; (iii) the percent viability of the negative control extract must be ≥70% of the reagent control; (iv) the percent viability of a minimum of one dilution from each of the positive controls must be <70% of the reagent control, and for test articles with one or more percent viability readings of <70% of the reagent control, the 50% test article dilution should have at least a similar or higher percent viability as the 100% test article full-strength solution. The lower the percent viability value, the higher the cytotoxic potential. Historical data have established that when the viability is reduced to <70% of the reagent control extract, there is a distinct possibility that the cytotoxic potential may translate to exposure of the agent in humans. Post-testing Notes: Data for the four test agents are tabulated in the Supporting Information accompanied by NAMSA's summary comment pertaining to the overall assessment of potential toxicity in each case based on the historical performance of this model as it translates to prediction in humans. Test article solutions were provided to NAMSA by the University of Toledo (UT) in a blinded manner in terms of the composition and concentration. All of the UT solutions were 2 mM in PBS/methanol (50:50). Labeling followed the compound designations used in this Article and are thus self-explanatory to a reader. The sample labeled "(4) UT Sol" contains no drug and represents our control for the methanol. Its effect at full strength (2.5% methanol) had essentially no effect on the percent viability, noting the variance typically seen during this assay as used across all of our agents. Our two potential lead compounds **12d** and **31a** were tested along with the prototype PPARδ agonist GW0742 (labeled as "(1) UT GW") because we have deployed it as a standard in a common fashion across all of our own assays.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.1c00560.

Molecular formula strings (CSV)

NMR spectral data for all synthesized compounds, LC-MS/MS chromatograms for the two lead compounds **12d** and **31a**, and all cytotoxicity raw data for these same two compounds from both our studies and the NAMSA studies (PDF)

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Notes

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■ ABBREVIATIONS USED

ADMET, absorption, distribution, metabolism, excretion, and toxicity; αMEM, alpha modification of minimum essential medium eagle; μCT, microcomputed tomography; AcOH, acetic acid; AD, adipogenesis; BMD, bone mineral density; BOC, *tert*-butyloxycarbonyl; DCM, dichloromethane; DIBAL-H, diisobutylaluminum hydride; DIPEA, *N*,*N*-diisopropylethylamine; DMEM, Dulbecco's modified Eagle's medium; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; EC_{50}, half-maximal effective concentration; EDTA, ethylenediaminetetraacetic acid; Et, ethyl; Et_3N, triethylamine; EtOAc, ethyl acetate; EtOH, ethanol; FBS, fetal bovine serum; HRT, hormone replacement therapy; Hz, Hertz (unit); iPr, isopropyl; *J*, coupling constant; $LiAlH_4$, lithium aluminum hydride; MeCN, acetonitrile; MeOH, methanol; $MeSO_2Cl$, methanesulfonyl chloride; Mp, melting point; MSC, mesenchymal stem cell(s); NMR, nuclear magnetic resonance; OS, osteogenesis; OVX, ovariectomized; PPAR, peroxisome proliferator-activated receptor; ppm, parts per million; PPRE, peroxisome proliferator response element; Pr, propyl; pTSA, *para*-toluene sulfonic acid; R_f, retention factor; rt, room temperature; SAR, structure−activity relationship(s); *t*-BuLi, *tert*-butyl lithium; THF, tetrahydrofuran; TFA, trifluoroacetic acid; TLC, thin-layer chromatography; TZD, thiazolidinedione

■ REFERENCES

(1) Maïmoun, L.; Sultan, C. Effects of physical activity on bone remodeling. *Metab., Clin. Exp.* **2011**, *60*, 373−388.
(2) Maggioli, C.; Stagi, S. Bone modeling, remodeling, and skeletal health in children and adolescents: mineral accrual, assessment and treatment. *Ann. Pediatr. Endocrinol. Metab.* **2017**, *22* (1), 1−5.
(3) Kanis, J. A.; Johansson, H.; Oden, A.; McCloskey, E. V. Assessment of fracture risk. *Eur. J. Radiol.* **2009**, *71*, 392−397.
(4) Eriksen, E. F. Cellular mechanisms of bone remodeling. *Rev. Endocr. Metab. Disord.* **2010**, *11*, 219−227.
(5) Manolagas, S. C.; O'Brien, C. A.; Almeida, M. The role of estrogen and androgen receptors in bone health and disease. *Nat. Rev. Endocrinol.* **2013**, *9* (12), 699−712.
(6) Russell, R. G. G.; Watts, N. B.; Ebetino, R. H.; Rogers, M. J. Mechanisms of action of bisphosphonates: similarities and differences and their potential influence on clinical efficacy. *Osteoporosis Int.* **2008**, *19*, 733−759.
(7) MacLean, C.; Newberry, S.; Maglione, M.; McMahon, M.; Ranganath, V.; Suttorp, M.; Mojica, W.; Timmer, M.; Alexander, A.; McNamara, M.; Desai, S. B.; Zhou, A.; Chen, S.; Carter, J.; Tringale, C.; Valentine, D.; Johnsen, B.; Grossman, J. Systematic review: comparative effectiveness of treatments to prevent fractures in men and women with low bone density or osteoporosis. *Ann. Intern. Med.* **2008**, *148*, 197−213.
(8) Watts, N. B.; Diab, D. L. Long-term use of bisphosphonates in osteoporosis. *J. Clin. Endocrinol. Metab.* **2010**, *95* (4), 1555−1565.
(9) Papaioannou, A.; Morin, S.; Cheung, A. M.; Atkinson, S.; Brown, J. P.; Feldman, S.; Hanley, D. A.; Hodsman, A.; Jamal, S. A.; Kaiser, S. M.; Kvern, B.; Siminoski, K.; Leslie, W. D. 2010 clinical practice guidelines for the diagnosis and management of osteoporosis in Canada: summary. *CMAJ.* **2010**, *182* (17), 1864−1873.
(10) Delmas, P. D. Treatment of postmenopausal osteoporosis. *Lancet* **2002**, *359*, 2018−2026.
(11) Bolland, M. J.; Grey, A. B.; Gamble, G. D.; Reid, I. R. Effect of osteoporosis treatment on mortality: a meta-analysis. *J. Clin. Endocrinol. Metab.* **2010**, *95*, 1174−1181.
(12) Giusti, A.; Hamdy, N. A. T.; Papapoulos, S. E. Atypical fractures of the femur and bisphosphonate therapy a systematic review of case/case series studies. *Bone.* **2010**, *47*, 169−180.
(13) Russow, G.; Jahn, D.; Appelt, J.; Märdian, S.; Tsitsilonis, S.; Keller, J. Anabolic therapies in osteoporosis and bone regeneration. *Int. J. Mol. Sci.* **2019**, *20*, 83.
(14) Sims, N. A.; Ng, K. W. Implications of osteoblast-osteoclast interactions in the management of osteoporosis by antiresorptive agents denosumab and odanacatib. *Curr. Osteoporos. Rep.* **2014**, *12* (1), 98−106.
(15) Rosen, C. J.; Bilezikian, J. P. Anabolic therapy for osteoporosis. *J. Clin. Endocrinol. Metab.* **2001**, *86* (3), 957−964.
(16) Lim, S. Y.; Bolster, M. B. Profile of romosozumab and its potential in the management of osteoporosis. *Drug Des., Dev. Ther.* **2017**, *11*, 1221−1231.
(17) Poole, K. E.; van Bezooijen, R. L.; Loveridge, N.; Hamersma, H.; Papapoulos, S. E.; Löwik, C. W.; Reeve, J. Sclerostin is a delayed secreted product of osteocytes that inhibits bone formation. *FASEB J.* **2005**, *19* (13), 1842−1844.
(18) Scholtysek, C.; Katzenbeisser, J.; Fu, H.; Uderhardt, S.; Ipseiz, N.; Stoll, C.; Zaiss, M. M.; Stock, M.; Donhauser, L.; Böhm, C.; Kleyer, A.; Hess, A.; Engelke, K.; David, J.-P.; Djouad, F.; Tuckermann, J. P.; Desvergne, B.; Schett, G.; Krönke, G. PPARβ/δ governs Wnt signaling and bone turnover. *Nat. Med.* **2013**, *19* (5), 608−613.
(19) Russell, A. J. Regenerative medicinal chemistry: The in situ control of stem cells. *ACS Med. Chem. Lett.* **2013**, *4*, 365−368.
(20) Davies, S. G.; Kennewell, P. D.; Russell, A. J.; Seden, P. T.; Westwood, R.; Wynne, G. M. Stemistry: The control of stem cells in situ using chemistry. *J. Med. Chem.* **2015**, *58*, 2863−2894.
(21) LeMieux, J. *Gen. Eng. Biotechnol. News* **2019**, *39* (12). https://www.genengnews.com/insights/stem-cell-technology-the-only-constant-is-change/ (accessed June 15, 2020).
(22) Yu, S.; Reddy, J. K. Transcription coactivators for peroxisome proliferator-activated receptors. *Biochim. Biophys. Acta, Mol. Cell Biol. Lipids* **2007**, *1771*, 936−951.
(23) Sun, H.; Kim, J. K.; Mortensen, R.; Mutyaba, L. P.; Hankenson, K. D.; Krebsbach, P. H. Osteoblast-targeted suppression of PPARγ increases osteogenesis through activation of mTOR signaling. *Stem Cells* **2013**, *31* (10), 2183−2192.
(24) Kim, D. H.; Liu, J.; Bhat, S.; Benedict, G.; Lecka-Czernik, B.; Peterson, S. J.; Ebraheim, N. A.; Heck, B. E. Peroxisome proliferator-activated receptor delta agonist attenuates nicotine suppression effect on human mesenchymal stem cell-derived osteogenesis and involves increased expression of heme oxygenase-1. *J. Bone Miner. Metab.* **2013**, *31*, 44−52.
(25) Kahn, S. E.; Zinman, B.; Lachin, J. M.; Haffner, S. M.; Herman, W. H.; Holman, R. R.; Kravitz, B. G.; Yu, D.; Heise, M. A.; Aftring, R. P.; Viberti, G. Rosiglitazone-associated fractures in type 2 diabetes: an analysis from a diabetes outcome program trial (ADOPT). *Diabetes Care* **2008**, *31* (5), 845−851.
(26) Sznaidman, M. L.; Haffner, C. D.; Maloney, P. R.; Fivush, A.; Chao, E.; Goreham, D.; Sierra, M. L.; LeGrumelec, C.; Xu, H. E.; Montana, V. G.; Lambert, M. H.; Willson, T. M.; Oliver, W. R., Jr.; Sternbach, D. D. Novel selective small molecule agonists for peroxisome proliferator-activated receptor δ (PPARδ)-synthesis and biological activity. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1517−1521.

(27) Geiger, L.; Dunsford, W.; Lewis, D.; Brennen, C.; Liu, K.; Newsholme, S. Rat Carcinogenicity Study with GW501516, a PPAR Delta Agonist. *48th Annual Meeting of the Society of Toxicology* **2009**, *108* (1), PS-895. https://web.archive.org/web/20150504013406/http:/www.toxicology.org/AI/PUB/Tox/2009Tox.pdf (accessed November 15, 2019).
(28) Newsholme, S.; Dunsford, W.; Brodie, T.; Brennan, C.; Brown, M.; Geiger, L. Mouse Carcinogenicity Study with GW501516, a PPAR Delta Agonist. *48th Ann. Meet. Society of Toxicology* **2009**, *108* (1), PS-896. https://web.archive.org/web/20150504013406/http:/www.toxicology.org/AI/PUB/Tox/2009Tox.pdf (accessed November 15, 2019).
(29) Thevis, M.; Geyer, H.; Thomas, A.; Schanzer, W. Trafficking of drug candidates relevant for sports drug testing: detection of non-approved therapeutics categorized as anabolic and gene doping agents in products distributed via the internet. *Drug Test. Anal.* **2011**, *3*, 331−336.
(30) Narkar, V.; Downes, M.; Yu, R.; Embler, E.; Wang, Y.-X.; Banayo, E.; Mihaylova, M.; Nelson, M.; Zou, Y.; Juguilon, H.; Kang, H.; Shaw, R.; Evans, R. AMPK and PPARδ agonists are exercise mimetics. *Cell* **2008**, *134*, 405−415.
(31) Pruimboom-Brees, I. M.; Francone, O.; Pettersen, J. C.; Kerlin, R. L.; Will, Y.; Amacher, D. E.; Boucher, G. G.; Morton, D. The development of subcutaneous sarcomas in rodents exposed to peroxisome proliferators agonists: hypothetical mechanisms of action and de-risking attitude. *Toxicol. Pathol.* **2012**, *40*, 810−818.
(32) Cheng, H.; Tan, W.; Low, Z.; Marvalim, C.; Lee, J.; Tan, N. Exploration and development of PPAR modulators in health and disease: an update of clinical evidence. *Int. J. Mol. Sci.* **2019**, *20*, 5055.
(33) Mackenzie, L.; Lione, L. Harnessing the benefits of PPARβ/δ agonists. *Life Sci.* **2013**, *93*, 963−967.
(34) Aoki, T. Current status of carcinogenicity assessment of peroxisome proliferator-activated receptor agonists by the US FDA and a mode-of-action approach to carcinogenic potential. *J. Toxicol. Pathol.* **2007**, *20*, 197−202.
(35) Takada, I.; Makishima, M. Peroxisome proliferator-activated receptor agonists and antagonists: a patent review (2014-present). *Expert Opin. Ther. Pat.* **2020**, *30*, 1−13.
(36) Heck, B.; Kim, D.; Erhardt, P.; Kress, B. Analogs of Peroxisome Proliferator-Activated Receptor (PPAR) Agonists, and Methods of Using the Same. U.S. Patent 9695137 B2, July 4, 2017.
(37) Heck, B.; Kim, D.; Erhardt, P.; Kress, B. Analogs of Peroxisome Proliferator-Activated Receptor (PPAR) Agonists, and Methods of Using the Same. U.S. Patent 10181018 B2, January 15, 2019.
(38) Heck, B.; Kim, D.; Erhardt, P.; Kress, B. Analogs of Peroxisome Proliferator-Activated Receptor (PPAR) Agonists, and Methods of Using the Same. U.S. Patent 10351541 B2, July 16, 2019.
(39) Heck, B.; Kim, D.; Erhardt, P.; Kress, B. Analogs of Peroxisome Proliferator-Activated Receptor (PPAR) Agonists, and Methods of Using the Same. U.S. Patent 10776461 B2, September 15, 2020.
(40) Kadayat, T.; Shrestha, A.; Jeon, Y.; An, H.; Kim, J.; Cho, S.; Chin, J. Targeting peroxisome proliferator-activated receptor delta (PPARδ): a medicinal chemistry perspective. *J. Med. Chem.* **2020**, *63*, 10109−10134.
(41) Erhardt, P. Drug metabolism data: past and present status. *Med. Chem. Res.* **1998**, *8*, 400−421.
(42) Niu, H.-S.; Ku, P.-M.; Niu, C.-S.; Cheng, J.-T.; Lee, K.-S. Development of PPAR-agonist GW0742 as antidiabetic drug: study in animals. *Drug Des., Dev. Ther.* **2015**, *9*, 5625−5632.
(43) Rodney, G.; Uhlendorf, P.; Maxwell, R. The hypolipidaemic effect of gemfibrozil (CI-719) in laboratory animals. *Proc. R. Soc. Med.* **1976**, *69* (Suppl. 2), 6−10.
(44) Cantello, B.; Cawthorne, M.; Haigh, D.; Hindley, R.; Smith, S.; Thurlby, P. The synthesis of BRL 49653 - a novel and potent antihyperglycaemic agent. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1181−1184.
(45) Lagu, B.; Kluge, A.; Fredenburg, R.; Tozzo, E.; Senaiar, R.; Jaleel, M.; Panigrahi, S.; Tiwari, N.; Krishnamurthy, N.; Takahashi, T.; Patane, M. Novel highly selective peroxisome proliferator-activated receptor δ (PPARδ) modulators with pharmacokinetic properties suitable for once-daily dosing. *Bioorg. Med. Chem. Lett.* **2017**, *27*, 5230−5234.
(46) Li, Z.; Chen, Y.; Zhou, Z.; Deng, L.; Xu, Y.; Hu, L.; Liu, B.; Zhang, L. Zhang, L. Discovery of first-in-class thiazole-based dual FFA1/PPARδ agonists as potential anti-diabetic agents. *Eur. J. Med. Chem.* **2019**, *164*, 352−365.
(47) Sierra, M. L.; Beneton, V.; Boullay, A.-B.; Boyer, T.; Brewster, A. G.; Donche, F.; Forest, M.-C.; Fouchet, M.-H.; Gellibert, F. J.; Grillot, D. A.; Lambert, M. H.; Laroze, A.; Le Grumelec, C.; Linget, J. M.; Montana, V. G.; Nguyen, V.-L.; Nicodeme, E.; Patel, V.; Penfornis, A.; Pineau, O.; Pohin, D.; Potvain, F.; Poulain, G.; Ruault, C. B.; Saunders, M.; Toum, J.; Xu, H. E.; Xu, R. X.; Pianetti, P. M. Substituted 2-[(4-aminomethyl)phenoxy]-2-methylpropionic acid PPARα agonists. 1. Discovery of a novel series of potent HDLc raising agents. *J. Med. Chem.* **2007**, *50*, 685−695.
(48) Luckhurst, C.; Stein, A.; Furber, M.; Webb, N.; Ratcliffe, M.; Allenby, G.; Botterell, S.; Tomlinson, W.; Martin, B.; Walding, A. Discovery of isoindoline and tetrahydro-isoquinoline derivatives as potent, selective PPARδ agonists. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 492−496.
(49) Luckhurst, C.; Ratcliffe, M.; Stein, L.; Furber, M.; Botterell, S.; Laughton, D.; Tomlinson, W.; Weaver, R.; Chohan, K.; Walding, A. Synthesis and biological evaluation of N-alkylated 8-oxybenz[c]-azepine derivatives as selective PPARδ agonists. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 531−536.
(50) Sauerberg, P.; Olsen, G.; Jeppesen, L.; Mogensen, J.; Pettersson, I.; Jeppesen, C.; Daugaard, J.; Galsgaard, E.; Ynddal, L.; Fleckner, J.; Panajotova, V.; Polivka, Z.; Pihera, P.; Havranek, M.; Wulff, E. Identification and synthesis of a novel selective partial PPARδ agonist with full efficacy on lipid metabolism in vitro and in vivo. *J. Med. Chem.* **2007**, *50*, 1495−1503.
(51) Bodor, N.; Buchwald, P. Retrometabolism-Based Drug Design and Targeting. In *Burger's Medicinal Chemistry, Drug Discovery and Development*, 6th ed.; Abraham, D., Ed.; John Wiley & Sons, Inc.: New York, 2003; pp 533−608.
(52) Erhardt, P. Esmolol. In *Chronicles of Drug Discovery*; Lednicer, D., Ed.; ACS Books: Washington, DC, 1993; Vol. 3, pp191−206.
(53) Parkinson, A. Biotransformation of Xenobiotics. In *Casarett and Doull's Toxicology - The Basic Science of Poisons*, 6th ed.; Klaassen, C., Ed.; McGraw-Hill, Inc.: New York, 2001; pp 133−224.
(54) Munday, R. Toxicity of thiols and disulphides: involvement of free-radical species. *Free Radical Biol. Med.* **1989**, *7*, 659−673.
(55) El-Dakdouki, M.; Adamski, N.; Foster, L.; Hacker, M.; Erhardt, P. Hypoxia activated prodrugs of a 9-aza-anthrapyrazole derivative that has promising anticancer activity. *J. Med. Chem.* **2011**, *54*, 8224−8227.
(56) Mardyukov, A.; Eckhardt, A.; Schreiner, P. 1,1-Ethenediol: the long elusive enol of acetic acid. *Angew. Chem., Int. Ed.* **2020**, *59*, 5577−5580.
(57) Mitchell, J.; Jollow, D.; Potter, W.; Gillette, J.; Brodie, B. Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. *J. Pharmacol. Exp. Therap.* **1973**, *187*, 211−217.
(58) Armstrong, J.; Steinauer, K.; Hornung, B.; Irish, J.; Lecane, P.; Birrell, G.; Peehl, D.; Knox, S. Role of glutathione depletion and reactive oxygen species generation in apoptotic signaling in a human B lymphoma cell line. *Cell Death Differ.* **2002**, *9*, 252−263.
(59) Erhardt, P.; Smith, R.; Sayther, T.; Keiser, J. Thin-layer chromatography of apomorphine and its analogs. *J. Chromatogr.* **1976**, *116*, 218−224.
(60) Khupse, R.; Erhardt, P. Total synthesis of racemic, natural (−) and unnatural (+) glyceollin I. *Org. Lett.* **2008**, *10*, 5007−5010.
(61) Luniwal, A.; Khupse, R.; Reese, M.; Liu, J.; El-Dakdouki, M.; Malik, N.; Fang, L.; Erhardt, P. Multigram synthesis of glyceollin I. *Org. Process Res. Dev.* **2011**, *15*, 1149−1162.
(62) Griffith, W. Recent advances in dioxolene chemistry. *Transition Met. Chem.* **1993**, *18*, 250−256.

https://doi.org/10.1021/acs.jmedchem.1c00560
J. Med. Chem. 2021, 64, 6996−7032

(63) Nematollahi, D.; Rafiee, M. Electrochemical oxidation of catechols in the presence acetylacetone. *J. Electroanal. Chem.* **2004**, *566*, 31−37.
(64) Erhardt, P. Statistics-Based Probabilities of Metabolic Possibilities. In *Drug Metabolism - Databases and High-Throughput Testing During Drug Design and Development*; Erhardt, P., Ed.; IUPAC Books via Blackwell Science, Ltd.: Oxford, U.K., 1999; pp 185−191.
(65) Wilbury, T. Comparison of Commercially Available Metabolism Databases during the Design of Prodrugs and Codrugs. In *Drug Metabolism - Databases and High-Throughput Testing During Drug Design and Development*; Erhardt, P., Ed.; IUPAC Books via Blackwell Science, Ltd.: Oxford, U.K., 1999; pp 208−222.
(66) Adams, A.; Yuen, W.; Hu, Z.; Santini, C.; Jones, A.; MacNaul, K.; Berger, J.; Doebber, T.; M?ller, D. Amphipathic 3-phenyl-7-propylbenzisoxazoles; Human PPaRγ, δ and α agonists. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 931−935.
(67) Genovese, S.; Foreman, J.; Borland, M.; Epifano, F.; Gonzalez, F.; Curini, M.; Peters, J. A natural propenoic acid derivative activates peroxisome proliferator-activated receptor-β/δ (PPARβ/δ). *Life Sci.* **2010**, *86*, 493−498.
(68) Kürti, L.; Czakó, B. Michael Addition/Reaction. In *Strategic Applications of Named Reactions in Organic Synthesis*; Kurti, L., Czako, B., Eds.; Elsevier Academic Press, Inc.: Burlington, MA, 2005; pp286−287.
(69) Offenbacher, J. Michael Addition. In *Importance of the α,β-Unsaturated Ketone in Methuosis-Inducing Compounds*. MS Thesis, University of Toledo, Toledo, OH, 2015; pp 15−20.
(70) Mather, B.; Viswanathan, K.; Miller, K.; Long, T. Michael addition reactions in macromolecular design for emerging technologies. *Prog. Polym. Sci.* **2006**, *31*, 487−531.
(71) Zhang, J.; Yang, P.; Gray, N. Targeting cancer with small molecule kinase inhibitors. *Nat. Rev. Cancer* **2009**, *9*, 28−39.
(72) Johansson, M. H. Reversible Michael additions: Covalent inhibitor and prodrugs. *Mini-Rev. Med. Chem.* **2012**, *12*, 1330−1344.
(73) Jackson, P.; Widen, J.; Harki, D.; Brummond, K. Covalent modifiers: a chemical perspective on the reactivity of α,β-unsaturated carbonyls with thiols via hetero-Michael addition reactions. *J. Med. Chem.* **2017**, *60*, 839−885.
(74) Heleno, S.; Martins, A.; Queiroz, M.; Ferreira, I. Bioactivity of phenolic acids: metabolites versus parent compounds: a review. *Food Chem.* **2015**, *173*, 501−513.
(75) Pei, K.; Ou, J.; Huang, J.; Ou, S. p-Coumaric acid and its conjugates: dietary sources, pharmacokinetic properties and biological activities. *J. Sci. Food Agric.* **2016**, *96*, 2952−2962.
(76) Taofiq, O.; Gonzalez-Paramas, A.; Barreiro, M.; Ferreira, I. Hydroxycinnamic acids and their derivatives: cosmeceutical significance, challenges and future perspectives, a review. *Molecules* **2017**, *22*, 281.
(77) Ueda, R.; Suzuki, T.; Mino, K.; Tsumoto, H.; Nakagawa, H.; Hasegawa, M.; Sasaki, R.; Mizukami, T.; Miyata, N. Identification of cell-active lysine specific demethylase 1-selective inhibitors. *J. Am. Chem. Soc.* **2009**, *131* (48), 17536−17537.
(78) Ham, J.; Kang, H. A highly efficient synthesis of antiobestic ligand GW501516 for the peroxisome proliferator-activated receptor δ through in situ protection of the phenol group by reaction with a Grignard reagent. *Tetrahedron Lett.* **2005**, *46*, 6683−6686.
(79) Mitchell, D. J.; Seal, J. T.; Thompson, M.; Westaway, S. M.; Brown, S. L. Piperazinyl Derivatives Useful in the Treatment of GPR38 Receptor Mediated Diseases and Their Preparation. PCT Int. Appl. WO 2008000729, 2008.
(80) Zhu, Y.; Ma, J.; Cheng, P.; Zhao, Z.; Gregoire, F. M.; Rakhmanova, V. A. Bicyclic, Substituted Triazoles as Modulators of PPAR and Methods of Their Preparation. U.S. Patent Appl. 2006014785, 2006.
(81) Edwards, L.; Watt, J.; Webster, T. F.; Schlezinger, J. J. Assessment of total, ligand-induced peroxisome proliferator activated receptor γ ligand acitivity in serum. *Environ. Health* **2019**, *18* (45), 1−15.
(82) Costa, M. J.; So, A. Y.-L.; Kaasik, K.; Krueger, K. C.; Pillsbury, M. L.; Fu, Y.-H.; Ptacek, L. J.; Yamamoto, K. R.; Feldman, B. J. Circadian rhythm gene period 3 is an inhibitor of the adipocyte cell fate. *J. Biol. Chem.* **2011**, *286*, 9063−9070.
(83) Jee, W. S. S.; Yao, W. Overview: animal models of osteopenia and osteoporosis. *J. Musculoskel. Neuron Interact.* **2001**, *1* (3), 193−207.
(84) Zheng, Y.; Chen, Y.; Jin, L.-W.; Ye, H.-Y.; Liu, G. Cytotoxicity and genotoxicity in human embryonic kidney cells exposed to surface modify chitosan nanoparticles loaded with curcumin. *AAPS PharmSciTech* **2016**, *17*, 1347−1352.
(85) Mellati, A.; Valizadeh Kiamahalleh, M.; Dai, S.; Bi, J.; Jin, B.; Zhang, H. Influence of polymer molecular weight on the in vitro cytotoxicity of poly(N-isopropylacrylamide). *Mater. Sci. Eng., C* **2016**, *59*, 509−513.
(86) Ohguro, N.; Fukuda, M.; Sasabe, T.; Tano, Y. Concentration dependent effects of hydrogen peroxide on lens epithelial cells. *Br. J. Ophthalmol.* **1999**, *83*, 1064−1068.
(87) Nguyen, S.; Nguyen, H.; Truong, K. Comparative cytotoxic effects of methanol, ethanol and DMSO on human cancer cell lines. *Biomed. Res. & Therapy.* **2020**, *7*, 3855−3859.
(88) Vinken, M.; Blaauboer, B. In vitro testing of basal cytotoxicity: establishment of an adverse outcome pathway from chemical insult to cell death. *Toxicol. In Vitro* **2017**, *39*, 104−110.
(89) See: NAMSA Home Page. https://namsa.com (accessed November 15, 2019).
(90) Berger, J.; Leibowitz, M.; Doebber, T.; Elbrecht, A.; Zhang, B.; Zhou, G.; Biswas, C.; Cullinan, C.; Hayes, N.; Li, Y.; Tanen, M.; Ventre, J.; Wu, M.; Berger, G.; Mosley, R.; Marquis, R.; Santini, C.; Sahoo, S.; Tolman, R.; Smith, R.; M?ller, D. Novel peroxisome proliferator-activated receptor (PPAR)γ and PPARδ ligands produce distinct biological effects. *J. Biol. Chem.* **1999**, *274*, 6718−6725.
(91) Chan, B.; Gartland, A.; Wilson, P.; Buckley, K.; Dillon, J.; Fraser, W.; Gallagher, J. PPAR agonists modulate human osteoclast formation and activity in vitro. *Bone* **2007**, *40*, 149−159.
(92) Mosti, M. P.; Stunes, A. K.; Ericsson, M.; Pullisaar, H.; Reseland, J. E.; Shabestari, M.; Eriksen, E. F.; Syversen, U. Effects of the peroxisome proliferator-activated receptor (PPAR)-δagonist GW501516 on bone and muscle in ovariectomized rats. *Endocrinology* **2014**, *155*, 2178−2189.
(93) Motani, A.; Wang, Z.; Weiszmann, J.; McGee, L. R.; Lee, G.; Liu, Q.; Staunton, J.; Fang, Z.; Fuentes, H.; Lindstrom, M.; Liu, J.; Biermann, D. H.T.; Jaen, J.; Walker, N. P.C.; Learned, R. M.; Chen, J.-L.; Li, Y. INT131: A selective modulator of PPARγ. *J. Mol. Biol.* **2009**, *386*, 1301−1311.
(94) Lee, D.; Huang, H.; Choi, K.; Mantzoros, C.; Kim, Y.-B. Selective PPARγ modulator INT131 normalizes insulin signaling defects and improves bone mass in diet-induced obese mice. *Am. J. Physiol. Endocrin. Metab.* **2012**, *302*, E552−E560.
(95) Cariou, B.; Zair, Y.; Staels, B.; Bruckert, E. Effects of the new dual PPARα/δ agonist GFT505 on lipid and glucose homeostasis in abdominally obese patients with combined dyslipidemia or impaired glucose metabolism. *Diabetes Care* **2011**, *34*, 2008−2014.
(96) Zhang, R.; Wang, A.; DeAngelis, A.; Pelton, P.; Xu, J.; Zhu, P.; Zhou, L.; Demarest, K.; Murray, W.; Kuo, G.-H. Discovery of para-alkylthiophenoxyacetic acids as a novel series of potent and selective PPARδ agonists. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3855−3859.
(97) Kim, J. B.; Wright, H. M.; Wright, M.; Spiegelman, B. M. ADD1/SREBP1 activates PPARgamma through the production of endogenous ligand. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95* (8), 4333−4337.

https://doi.org/10.1021/acs.jmedchem.1c00560
J. Med. Chem. 2021, 64, 6996−7032