Journal of Medicinal Chemistry

pubs.acs.org/jmc

Article

N-Trifluoromethyl Amines and Azoles: An Underexplored Functional Group in the Medicinal Chemist's Toolbox

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Cite This: https://dx.doi.org/10.1021/acs.jmedchem.0c01457

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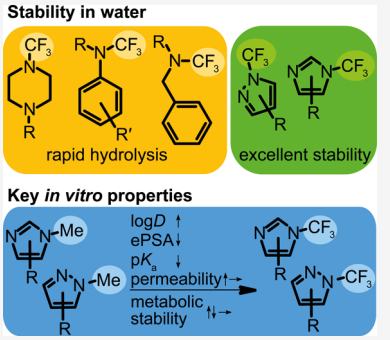
ABSTRACT: Introducing trifluoromethyl groups is a common strategy to improve the properties of biologically active compounds. However, *N*-trifluoromethyl moieties on amines and azoles are very rarely used. To evaluate their suitability in drug design, we synthesized a series of *N*-trifluoromethyl amines and azoles, determined their stability in aqueous media, and investigated their properties. We show that *N*-trifluoromethyl amines are prone to hydrolysis, whereas *N*-trifluoromethyl azoles have excellent aqueous stability. Compared to their *N*-methyl analogues, *N*-trifluoromethyl azoles have a higher lipophilicity and can show increased metabolic stability and Caco-2 permeability. Furthermore, *N*-trifluoromethyl azoles can serve as bioisosteres of *N*-*iso*-propyl and *N*-*tert*-butyl azoles. Consequently, we suggest that *N*-trifluoromethyl azoles are valuable substructures to be considered in medicinal chemistry.

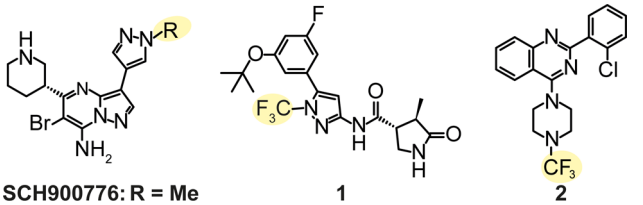


INTRODUCTION

The introduction of a trifluoromethyl group is a popular strategy to modulate the properties of biologically active molecules. For example, exchanging a methyl for a trifluoromethyl can increase metabolic stability and permeability, decrease the basicity of proximal amines, or change the conformation of the molecule.[1] Introducing a trifluoromethyl substituent can also lead to increased potency via the formation of multipolar interactions with carbonyl groups in a protein.[2,3] Moreover, a trifluoromethyl can interact with the hydroxyl group of tyrosine or with carboxylic acid residues.[4,5] The importance of trifluoromethyl substituents in medicinal chemistry is further illustrated by the seventy-two launched drugs that contain at least one trifluoromethyl group.[6] Sixty-nine of these drugs contain the trifluoromethyl moiety attached to a carbon, and three contain an *O*-trifluoromethyl. In contrast, no drug has been launched so far with the trifluoromethyl group attached to nitrogen.[6] This is particularly surprising given, for example, the importance of *N*-methyl azoles and amines in medicinal chemistry. Furthermore, in the AstraZeneca internal compound collection, more than 22 000 compounds contain an *O*-trifluoromethyl moiety, whereas only one *N*-trifluoromethyl azole and nine *N*-trifluoromethyl amines were listed prior to this study.[7] However, a very limited number of reports suggest that *N*-trifluoromethyl azoles, at least, deserve a spot in the medicinal chemist's toolbox. For example, replacing the methyl substituent of the checkpoint kinase 1 (CHK1) inhibitor **SCH900776** (Chart 1) with trifluoromethyl to obtain **MU380** resulted in reduced *N*-dealkylation while keeping a comparable CHK1 potency.[8] The notion that *N*-trifluoromethyl azoles can be important in medicinal chemistry is further supported by the single compound patent protecting sodium/glucose cotransporter 1 (SGLT1) inhibitor **1**, suggesting that this compound has been of significant interest.[9] Despite these reports, *N*-trifluoromethyl azoles are still considered an unusual pharmacophore as noted by Samadder et al.[8] Furthermore, compared to *N*-trifluoromethyl azoles, *N*-trifluoromethyl amines have been even less frequently used in medicinal chemistry[1] with the cannabinoid receptor modulator **2** being a rare example.[10]

The reluctance of medicinal chemists to use *N*-trifluoromethyl amines or azoles could be due to their historically challenging synthesis. Until very recently, synthetic methods for *N*-trifluoromethylation of amines generally required the use of

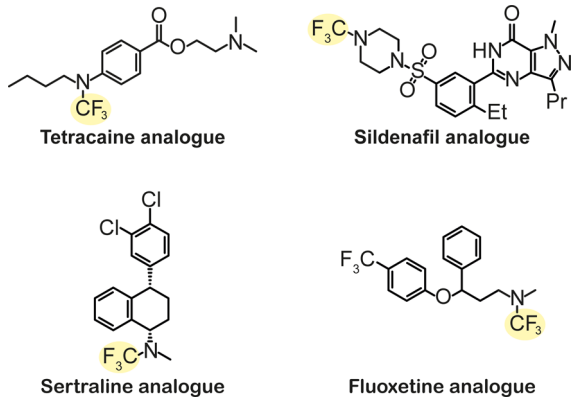
Chart 1. Examples of Reported Druglike Molecules Containing an *N*-Trifluoromethyl Azole or Amine



Received: August 21, 2020

highly toxic HF-based reagents[11] or thermally unstable compounds like *O*-trifluoromethyl benzofuranium reagents[12] (for an overview on methods to synthesize *N*-trifluoromethyl azoles, see the end of the Results and Discussion section). Whereas no significant improvement in the synthesis of *N*-trifluoromethyl azoles has been reported until a very recently described synthesis specific to *N*-trifluoromethyl indoles[13] (*vide infra*), four operationally simple methods for the synthesis of tertiary *N*-trifluoromethyl amines have been published in the last four years (additionally, a synthesis of *N*-trifluoromethyl azepines was recently described[14]). All four methods generate a thiocarbamoyl fluoride intermediate, which is then transformed into the desired *N*-trifluoromethyl amine using silver(I) fluoride. The Schoenebeck group reported the formation of the thiocarbamoyl fluoride intermediate using $(Me_4N)SCF_3$,[15] whereas Yu et al. generated this intermediate from difluorocarbene.[16] Liang et al.[17] reported a procedure using Langlois' reagent (CF_3SO_2Na), and Onida et al.[18] used carbon disulfide and (diethylamino)sulfur trifluoride (DAST). These strategies have been used to synthesize interesting analogues of biologically active compounds (examples shown in Chart 2).[15,17]

Chart 2. Exemplary Structures Synthesized by the Schoenebeck Group[15] and Yi et al.[17]



However, while these new procedures improve the synthetic access to at least tertiary *N*-trifluoromethyl amines, the suitability of these groups in drug design is still in doubt due to their questionable stability in aqueous media. For example *N*,*N*-bisalkyl-*N*-trifluoromethylamines are described to be hydrolytically unstable,[19−21] and there is a single report mentioning the hydrolysis of *N*-ethyl-*N*-trifluoromethylaniline.[19] Pan suggested that reducing the electron density of the nitrogen could result in more stable trifluoromethyl groups.[22] However, there is no systematic study investigating the effect of the electron density of the nitrogen on the stability of the trifluoromethyl group. Moreover, whether the potentially increased stability of electron-deficient *N*-trifluoromethyl groups renders the compounds sufficiently stable for applications in medicinal chemistry is still in question.[22,23] Furthermore, it is unknown whether piperazines, benzylamines, and azoles, which are highly important substructures in medicinal chemistry,[24] are sufficiently stable in aqueous media[25] when bearing an *N*-trifluoromethyl group.

Additional to concerns over aqueous stability, the highly electron-withdrawing trifluoromethyl group could potentially result in other liabilities, such as inducing reactivity toward physiological nucleophiles such as glutathione, resulting in putative toxic conjugates.[26] Moreover, key *in vitro* properties of *N*-trifluoromethyl amines and azoles have not been investigated. Consequently, a systematic investigation of their stability and properties is highly desirable to assess their suitability for medicinal chemistry, as already noted by Meanwell.[1]

We therefore synthesized an array of diverse compounds containing a trifluoromethyl amine or azole and systematically investigated their stability in aqueous media. Since very few examples of secondary *N*-trifluoromethyl amines have been published, with no general synthetic method available,[12,27] we excluded secondary *N*-trifluoromethyl amines from this study. For stable compounds, we explored their *in vitro* properties, with a view to making sound recommendations for using *N*-trifluoromethyl amines and azoles in drug design.

RESULTS AND DISCUSSION

Solution Stability of Tertiary *N*-Trifluoromethyl Amines and Azoles. To evaluate whether *N*-trifluoromethyl groups on amines and azoles are sufficiently stable to be useful for medicinal chemistry, we investigated the stability of piperazine **3**, anilines **4**−**13**, benzylamine **14**, pyrazole **15**, and benzimidazole **16** (Figure 1). This set was selected to include substructures that are often used in drug design.[24] Anilines **4**−**13** contain electron-donating and electron-withdrawing substituents to elucidate the influence of the electron density of the nitrogen on the stability of the *N*-trifluoromethyl moiety.

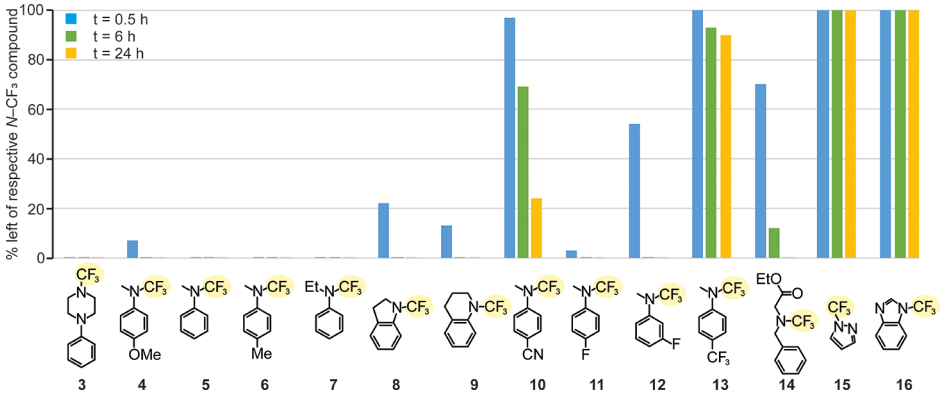


Figure 1. Percentage of *N*-trifluoromethyl amine and azole left (relative to 0 h) after standing in a 2 mM water/dimethyl sulfoxide (DMSO) 4:1 solution at room temperature under air for 0.5, 6, and 24 h (based on their respective integrated absorption between 220 and 350 nm determined by liquid chromatography).

https://dx.doi.org/10.1021/acs.jmedchem.0c01457
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In many company compound collections, samples are stored as 10 mM solutions in DMSO. Consequently, we started by studying the stability of the compounds in DMSO. All compounds investigated are stable as 10 mM solutions in neat DMSO at room temperature under air without protection from sunlight with no trace of decomposition observed by liquid chromatography−mass spectrometry (LCMS) after 1 month. In contrast, in a 2 mM DMSO/H_2O 1:4 mixture,[28] piperazine **3** and benzylamine **14** as well as electron-rich and electron-neutral anilines **4**−**9** hydrolyze to a substantial extent within 0.5 h (Figure 1, for an exemplary liquid chromatogram of aniline **4**, see Figure 2). Anilines **10** and **13**, which bear strong electron-withdrawing substituents, are more stable toward hydrolysis with 24 and 90% of parent detected after 24 h, respectively. Gas chromatography-mass spectrometry (GCMS) analysis of all hydrolysis reactions shows the formation of the corresponding carbamoyl fluoride, which was further supported by fully characterizing the hydrolysis product **17** of aniline **4** by NMR.[19,20,29] For *N*-trifluoromethyl azoles **15** and **16**, no trace of the hydrolyzed compound was detected by LCMS or by NMR even after 1 month.

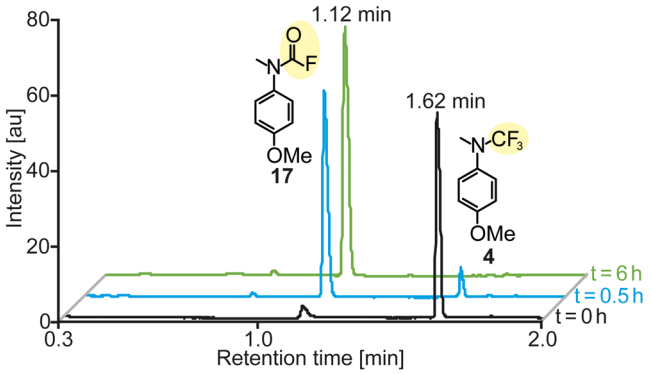

Figure 2. Exemplary liquid chromatogram of 4-methoxy-*N*-methyl-*N*-trifluoromethyl aniline after standing in a 2 mM water/DMSO 4:1 solution at room temperature under air for 0, 0.5, and 6 h.

We next investigated whether the above-observed stability trends can be used to predict the stability of *N*-trifluoromethyl analogues of known bioactive compounds. To do so, we determined the half-lives of compounds containing an *N*-trifluoromethyl piperazine (sildenafil analogue **18a**) or an electron-deficient aniline (sulfamethoxazole derivative **19a** and tetracaine derivative **20a**) at pH 1.0, 7.4, and 10.0. We also investigated two *N*-trifluoromethyl imidazoles (**21a** and **22a**) and three *N*-trifluoromethyl pyrazoles (**23a**, **24a**, and **25a**) with the corresponding *N*-methyl analogues being inhibitors of the hedgehog pathway,[30,31] methionine aminopeptidase,[32] interleukin-1 receptor associated kinase 4 (IRAK4),[33] b-rapidly accelerated fibrosarcoma $(BRAF)^{V600E}$,[34] and pyridoxal-5′-phosphate-dependent transaminase,[35] respectively (Chart 3a).

Piperazine **18a** and the two anilines **19a** and **20a** showed fast hydrolysis at all three pH values investigated, with half-lives of less than 1.5 days at 25 °C (Table 1). For **18a** and **19a**, the corresponding secondary amine was detected as the major product at all three pH values. For **20a**, the corresponding carbamoyl fluoride **26** was the main product at pH 1.0 with a small amount of product where both the carbamoyl fluoride had been further hydrolyzed to the secondary amine and the ester bond cleaved. The latter compound was also the main product at pH 7.4 and pH 10.0.

Chart 3. (a) Structures of *N*-Trifluoromethyl Compounds Synthesized to Determine Their Aqueous Stability and Additional Key *In Vitro* Properties. (b) Hydrolysis of Aniline 20a Results in Formation of Carbamoyl Fluoride 26, Which Can React with Glutathione to Yield Adduct 27

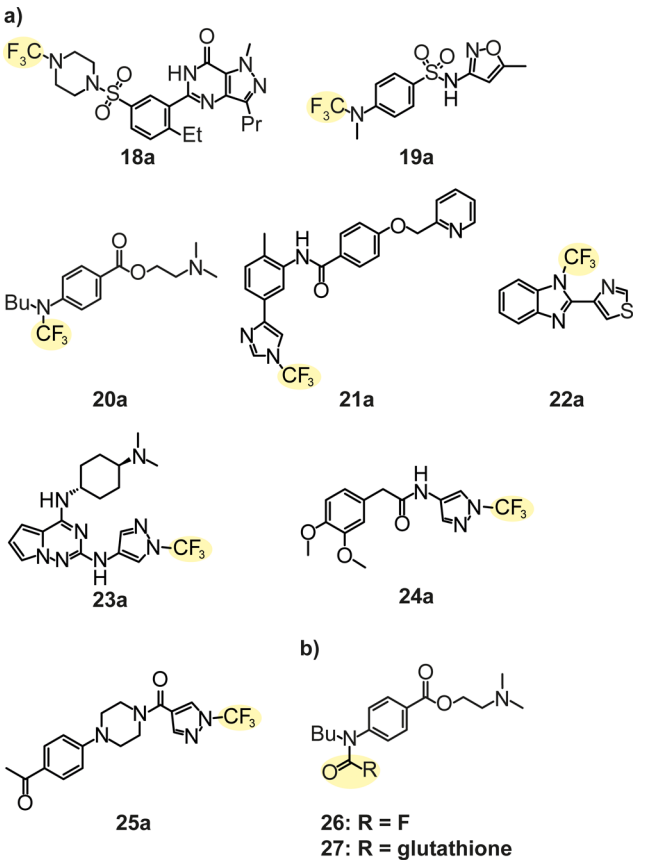

Table 1. Half-Life of *N*-Trifluoromethyl Compounds 18a−25a at 25 °C in 0.1 M HCl Solution (pH 1.0), 20 mM Sodium Phosphate Buffer (pH 7.4), and 20 mM Sodium Carbonate Buffer (pH 10.0)

	half-life at 25 °C		
#	pH 1.0 [d]	pH 7.4 [d]	pH 10.0 [d]
18a	<1.3	<0.8	<0.5
19a	0.2	0.4	0.3
20a	<0.6	<0.6	<0.6
21a	>72	>72	>72
22a	>72	>72	>72
23a	>72	>72	71
24a	12	>72	>72
25a	41	>72	17

Formation of an electrophilic carbamoyl fluoride[36,37] as a potentially reactive hydrolysis product raises concerns in terms of drug safety. To see if a carbamoyl fluoride is liable to react with physiological nucleophiles we studied the reactivity of the carbamoyl fluoride product **26** with glutathione. Carbamoyl fluoride **26** disappeared in the buffer in the presence of glutathione with a half-life of 66 min (compared to a half-life of 237 min in buffer without glutathione), and the corresponding glutathione adduct **27** (Chart 3b) was clearly detected by LCMS. The half-life of 66 min is in the range of half-lives

https://dx.doi.org/10.1021/acs.jmedchem.0c01457
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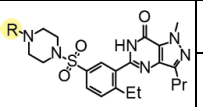
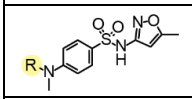
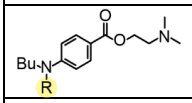
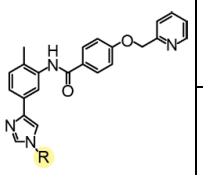
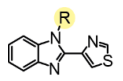
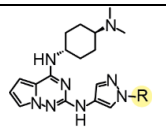
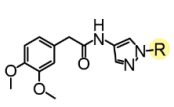
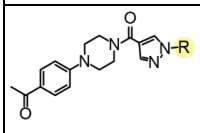
Table 2. Overview of the Change in Key *In Vitro* Properties When Exchanging *N*-Methyl, *N-iso*-Propyl, *N-tert*-Butyl, and *N*-Trifluoromethyl Groups[f]

Structure	R	#	log $D_{7.4}$ [a]	chromlog $D_{7.4}$	ePSA[b] [Å²]	Caco-2 P_{app} [c] [10^{-6}cm/s]	HLM[d] [μL/min/mg]
	CF_3	**18a**	>4.0	>5.3	67 (1)	nd[e]	nd[e]
	CH_3	**18b**	2.7 (0.1)	3.3 (0.1)	73 (0)	nd[e]	nd[e]
	CF_3	**19a**	1.6 (0.1)	1.5 (0.1)	82 (1)	nd[e]	nd[e]
	CH_3	**19b**	0.6 (0.1)	1.3 (0.1)	81 (1)	nd[e]	nd[e]
	CF_3	**20a**	>4.0	4.9 (0.2)	38 (2)	nd[e]	nd[e]
	CH_3	**20b**	2.8 (0.1)	3.3 (0.1)	50 (1)	nd[e]	nd[e]
	CF_3	**21a**	3.7 (0.1)	3.3 (0.0)	73 (1)	61 (27)	47 (11)
	CH_3	**21b**	2.7 (0.1)	2.2 (0.1)	92 (1)	72 (1)	4.8 (1.9)
	CF_3	**22a**	3.0 (0.1)	3.3 (0.0)	39 (2)	58 (18)	39 (18)
	CH_3	**22b**	2.3 (0.0)	1.5 (0.1)	56 (1)	72 (20)	172 (41)
	CF_3	**23a**	2.2 (0.1)	2.2 (0.0)	92 (1)	12 (8)	<3.0
	CH_3	**23b**	0.6 (0.1)	<0.0	98 (0)	2.4 (0.9)	<3.0
	^{i}Pr	**23c**	1.4 (0.1)	1.1 (0.1)	98 (3)	8.4 (1.4)	<3.0
	^{t}Bu	**23d**	1.7 (0.1)	1.5 (0.1)	97 (5)	11 (2)	<3.0
	CF_3	**24a**	2.4 (0.0)	2.2 (0.1)	65 (1)	56 (17)	53 (15)
	CH_3	**24b**	0.9 (0.1)	0.1 (0.0)	71 (0)	42 (5)	<3.0
	^{i}Pr	**24c**	1.6 (0.1)	0.9 (0.1)	67 (1)	44 (5)	<3.3
	^{t}Bu	**24d**	2.0 (0.1)	1.6 (0.1)	64 (1)	45 (11)	<4.1
	CF_3	**25a**	1.9 (0.1)	2.3 (0.1)	61 (0)	71 (9)	<3.0
	CH_3	**25b**	1.1 (0.0)	0.6 (0.1)	72 (1)	42 (5)	<3.0
	^{i}Pr	**25c**	1.8 (0.0)	1.5 (0.0)	66 (0)	40 (12)	<4.2
	^{t}Bu	**25d**	2.2 (0.1)	2.2 (0.0)	65 (2)	66 (12)	<4.5

[a]Due to the limitations in the determination of log $D_{7.4}$ using the shake-flask method, exact values for measured log $D_{7.4}$ > 4 are given as >4.0. [b]Experimentally determined polar surface area. [c]Apical to basolateral passive permeability across the Caco-2 cell monolayer in the presence of inhibitors against the three major efflux transporters: P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and multidrug-associated protein 2 (MRP2). [d]Metabolic stability of the compound measured as the disappearance of the parent compound over time when incubated with human liver microsomes. [e]Due to the low stability of compounds **18a**, **19a**, and **20a** in an aqueous environment, which would interfere with a proper determination of their metabolic stability or Caco-2 permeability, no metabolic stability or Caco-2 permeability was determined for these compounds or for their *N*-methyl analogues. [f]Each experimental value is the mean of at least three independent replicates. The standard deviation is given in brackets.

observed for typical covalent warheads like *N*-acrylamides, vinylsulfonamides, and reactive electrophilic heterocycles.[38−40]

In contrast to the *N*-trifluoromethyl anilines and piperazine, for all *N*-trifluoromethyl azoles investigated, no corresponding carbamoyl fluoride or free azole was detected in aqueous media at all three pH values studied. Furthermore, the determined half-lives are in a range suitable for chemical development (Table 1).[41] We investigated the reactivity of azoles **21a−25a** toward glutathione to exclude the possibility that these compounds have intrinsic electrophilicity. For all *N*-trifluoromethyl imidazoles and pyrazoles investigated, no trace of glutathione adduct was detectable after incubation for 20 h at 37 °C in the presence of glutathione.

Consequently, our data suggests that tertiary *N*-trifluoromethyl amines are likely to be of limited use in medicinal chemistry due to their rapid hydrolysis and the formation of electrophilic carbamoyl fluorides. In contrast, the investigated *N*-trifluoromethyl azoles have excellent stability in aqueous media and show no reactivity toward glutathione.

Comparison of Properties of *N*-Trifluoromethyl and *N*-Methyl Amines and Azoles. We next compared key *in vitro* properties in medicinal chemistry (log *D*, experimentally determined polar surface area (ePSA),[42,43] permeability in human epithelial colorectal adenocarcinoma cells (Caco-2), and metabolic stability) for the *N*-trifluoromethyl compounds **18a−25a** and their *N*-methyl counterparts **18b−25b** (Table 2). For comparing the log *D*, we used the shake-flask method (termed log $D_{7.4}$) and chromatographically determined log $D_{7.4}$[44] (chromlog $D_{7.4}$), since both methods are often used in medicinal chemistry. In the compounds investigated, the exchange of a

https://dx.doi.org/10.1021/acs.jmedchem.0c01457
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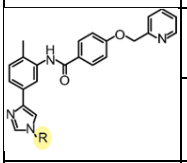
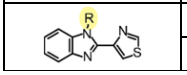
methyl for trifluoromethyl leads to the expected higher lipophilicity as proven by an increased log $D_{7.4}$ and chromlog $D_{7.4}$ and a decreased ePSA. Log $D_{7.4}$ increases by on average 1.1 log units and chromlog $D_{7.4}$ by 1.6 log units. However, the extent of this change can vary significantly and is dependent on both the individual compound and type of log $D_{7.4}$ analysis used.

Changes in permeability and metabolic stability are less consistent. The Caco-2 permeability, for example, can be significantly increased as for **25a** ($p = 0.007$). Stability to human liver microsomes (HLMs) can be significantly increased for the trifluoromethyl analogue as seen for **22a** ($p = 0.004$) or decreased as for **24a** and **21a**. The decreased metabolic stability of the latter two compounds could be due to an increased lipophilicity, rendering the potential metabolic soft spots (benzylic methyl group in **21a** and two methoxy ethers in **24a**) more susceptible toward metabolism.

Additionally, we investigated the change in pK_a when replacing an *N*-methyl with an *N*-trifluoromethyl group on imidazoles and showed that having an *N*-trifluoromethyl group decreases the basicity by at least 3 orders of magnitude (Table 3).

Table 3. Measured pK_a Values of the Corresponding Acids for Compounds 21a/21b and 22a/22b[a]

Structure	R	#	p$K_{a,1}$	p$K_{a,2}$
	CF_3	**21a**	2.5	4.0
	CH_3	**21b**	5.8	3.9
	CF_3	**22a**	<2.0	---
	CH_3	**22b**	4.9	---

[a] p$K_{a,2}$ is likely to correspond to the pyridine motif of compounds **21a** and **21b**.

Comparison of Properties of *N*-Trifluoromethyl, *N*-*iso*-Propyl, and *N*-*tert*-Butyl Azoles. The replacement of *N*-methyl with *N*-*iso*-propyl or *N*-*tert*-butyl is reported to on average lead to similar increases in log $D_{7.4}$ to those we see when replacing an *N*-methyl with an *N*-trifluoromethyl moiety.[45] Consequently, we investigated whether a trifluoromethyl group on an azole could be a suitable bioisostere for an *iso*-propyl or a *tert*-butyl group (Table 2). As expected from the large spread of log $D_{7.4}$ values when exchanging a methyl for a trifluoromethyl group, trifluoromethyl-containing compounds can have lipophilicities similar to those of *iso*-propyl analogues (**25a**) or even significantly higher than that of a *tert*-butyl moiety (**23a** and **24a**). In our set of investigated compounds, the metabolic stability of the trifluoromethyl compounds is either similar to (**23a** and **25a**) or lower (**24a**) than that of the *iso*-propyl and *tert*-butyl compounds. Interestingly, the Caco-2 permeability is significantly higher for the trifluoromethyl derivative **25a** compared to its *iso*-propyl analogue **25c** ($p = 0.01$) despite very similar log $D_{7.4}$ and resembles more the Caco-2 permeability of the more lipophilic *tert*-butyl variant.

Synthesis. The synthesis of *N*-trifluoromethyl amines **5**, **8**, **14**, **18a**, and **20a** is described by Scattolin et al. (Scheme 1),[15] and their procedure was used with slight modifications[46] to access amines **3**, **4**, **6**, **7**, and **9**–**13**. Compound **19a** was synthesized following the procedure of Liang et al.[17] *N*-Methyl analogue **18b** is commercially available, and compounds **19b** and **20b** were synthesized by reductive amination of the corresponding secondary amines.

Whereas *N*-trifluoromethyl pyrazole **15** is commercially available, the *N*-trifluoromethyl benzimidazoles **16** and **22a** were synthesized in a two-step procedure. First, the benzimidazoles **28** and **29** were transformed into their *N*-bromodifluoromethyl analogues, which were then converted into the desired benzimidazoles **16** and **22a**. Imidazole **21a** and pyrazoles **23a**, **24a**, and **25a** were synthesized using commercially available azole building blocks bearing the *N*-trifluoromethyl group via either Buchwald–Hartwig amination (**23a**) or an amide bond formation (**24a** and **25a**) following the procedures described for the synthesis of the *N*-methyl analogues **21b**, **23b**, **24b**, and **25b**.[30−35] The *iso*-propyl and *tert*-butyl analogues of compounds **23a**, **24a**, and **25a** were synthesized following a similar procedure using the respective azole building blocks. *N*-Methyl benzimidazole **20b** was synthesized following the procedure described by Siméon et al.[47]

CURRENT CHALLENGES AND NEED FOR IMPROVEMENTS IN THE SYNTHESIS OF *N*-TRIFLUOROMETHYL AZOLES

Whereas mild conditions using nontoxic reagents have recently been reported for the synthesis of tertiary *N*-trifluoromethyl amines (*vide supra*),[15−18] the synthesis of *N*-trifluoromethyl azoles still mainly requires the use of highly toxic and nongreen reagents.[48] For example, the two-step procedure to synthesize benzimidazole derivatives **22a** and **16** relies on the use of CF_2Br_2,[49−53] a known ozone-depleting reagent.[54] An alternative approach is the alkylation of azoles using trifluoromethyl iodide.[55−58] However, for both approaches, strong bases like NaH or KO^{t}Bu are generally required.

This could explain why these methodologies have so far only been applied to azoles bearing a very limited number of functional groups and not to more druglike structures. An alternative approach was described by Yagupolskii et al. where they transformed 2-methyl-benzimidazole and benzotriazole first into the corresponding dithiocarbamates using carbon disulfide, followed by their conversion into the corresponding *N*-trichloromethyl azoles using chlorine gas and carbon tetrachloride, and finally into the desired *N*-trifluoromethyl azoles using anhydrous HF.[53,59] Kanie et al. showed that the dithiocarbamate of indole could be directly converted into the *N*-trifluoromethyl indole via the use of HF·TEA. However, this strategy was not applied to azoles beyond indole. It still needs the use of special equipment on larger scales and poses a safety risk due to the need for a HF-based reagent.[11] The Toste and Togni groups reported on the synthesis of *N*-trifluoromethyl (benz)triazoles, (benz)imidazoles, (benz)pyrazoles, and tetrazoles,[60−62] but the required hypervalent iodine species are reported to exhibit violent thermal decomposition[60,63,64] severely limiting their use. Another approach was described by the Beier group who reacted a trifluoromethyl azide either with an alkyne or an enamine to the corresponding 1,2,3-triazole,[65,66] which could then be further transformed into various *N*-trifluoromethyl pyrroles and imidazoles.[25] However, the need to use trifluoromethylazide could limit the use of this strategy at least on scale.

Very recently, the Schoenebeck group reported on the synthesis of *N*-trifluoromethyl hydrazine derivatives. Even though these might be promising intermediates to access

https://dx.doi.org/10.1021/acs.jmedchem.0c01457
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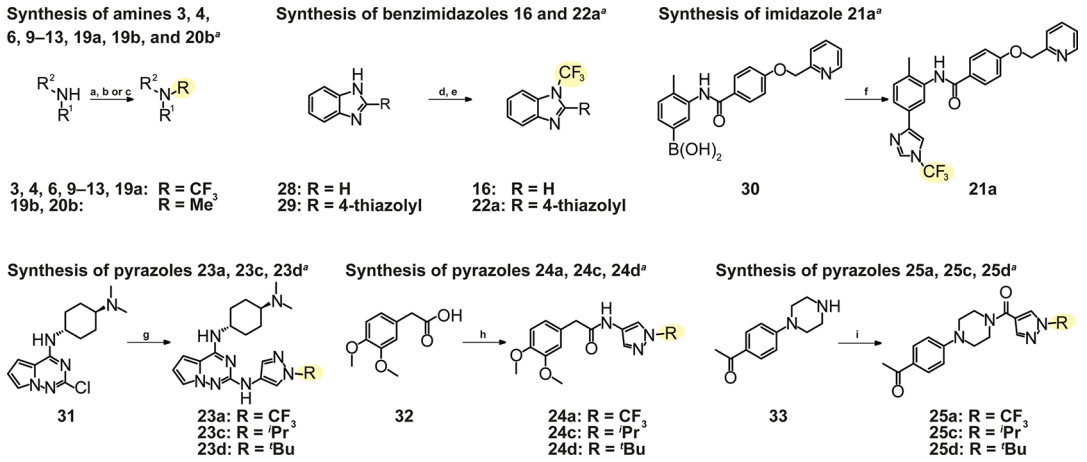
Scheme 1. Synthesis of *N*-Trifluoromethyl Compounds and *N*-Methyl, *N*-*iso*-Propyl, and *N*-*tert*-Butyl Analogues[a]



[a]Reagents and conditions: (a) **3**, **4**, **6**, **9**−**13**, **19a**: corresponding amine, $(NMe_4)(SCF_3)$, dichloromethane (DCM), rt, 15 min *then* AgF, rt, 4−9 h, yields: **3**: 37%, **4**: 24%, **6**: 76%, **7**: 6%, **9**: 58%, **10**: 67%, **11**: 42%, **12**: 55%, **13**: 37%. (b) **19a**: $NaCF_3SO_2$, PPh_3, MeCN, rt, 3 h, *then*, AgF, rt, 3.5 h, 8%. (c) **19b**: $HO(CH_2O)_nH$, $NaBH_3CN$, MeOH, rt to 40 °C, 23 h, 6%. **20b**: $HO(CH_2O)_nH$, $NaBH_3CN$, MeOH, rt to 40 °C, 23 h, 4%. (d) **16**: CF_2Br_2, NaH, NBu_4Br, *N*,*N*-dimethylformamide (DMF), 0 °C to rt, 2 h. **22a**: CF_2Br_2, NaH, NBu_4Br, DMF, 0 °C to rt, overnight. (e) **16**: NMe_4F, sulfolane, 60 °C, 12 h, 2% (over two steps). **22a**: NMe_4F, sulfolane, 60 °C, 12 h, 9% (over two steps). (f) **21a**: 4-bromo-1-(trifluoromethyl)-1*H*-imidazole, $Pd(PPh_3)_4$, Cs_2CO_3, 1,4-dioxane/H_2O 3:1, 150 °C, 5 h, 40%. (g) **23a**: 1-(trifluoromethyl)-1*H*-pyrazol-4-amine, XPhos Pd G3, K_3PO_4, ^{t}BuOH, 80 °C, 15 h, 29%. **23c**: 1-(*iso*-propyl)-1*H*-pyrazol-4-amine, XPhos Pd G3, K_3PO_4, ^{t}BuOH, 80 °C, 6 h, 10%. **23d**: 1-(*tert*-butyl)-1*H*-pyrazol-4-amine, XPhos Pd G3, K_3PO_4, ^{t}BuOH, 80 °C, 6 h, 12%. (h) **24a**: 1-(trifluoromethyl)-1*H*-pyrazol-4-amine, EDC·HCl, HOBt·xH_2O, DCM, rt, 14 h, 80%. **24c**: 1-(*iso*-propyl)-1*H*-pyrazol-4-amine, EDC·HCl, HOBt·xH_2O, DCM, rt, 5 h, 26%. **24d**: 1-(*tert*-butyl)-1*H*-pyrazol-4-amine, EDC·HCl, HOBt·xH_2O, DCM, rt, 23 h, 13%. (i) **25a**: 1-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid, EDC·HCl, 4-dimethylaminopyridine (DMAP), DMF, rt, overnight, 61%. **25c**: 1-(*iso*-propyl)-1*H*-pyrazole-4-carboxylic acid, EDC·HCl, DMAP, DMF, rt, 6 h, 37%. **25d**: 1-(*tert*-butyl)-1*H*-pyrazole-4-carboxylic acid, EDC·HCl, DMAP, DMF, rt, 6 h, 29%.

various *N*-trifluoromethyl azoles, this strategy has so far only been applied to the synthesis of *N*-trifluoromethyl indoles.[13]

Consequently, to encourage the use of *N*-trifluoromethyl azoles in medicinal chemistry, novel synthetic procedures are needed, which avoid the use of explosive, highly toxic, or nongreen reagents. Furthermore, the introduction of the trifluoromethyl moiety on azoles in a late-stage fashion using mild conditions with high functional group tolerance is highly desirable.

CONCLUSIONS

N-Trifluoromethyl amines and azoles are not frequently used in medicinal chemistry,[1,8] which could be due to their challenging syntheses and unknown aqueous stability. With the recently developed mild conditions for the synthesis of *N*-trifluoromethyl amines using nontoxic reagents,[15−18] we set out to investigate the stability of *N*-trifluoromethyl amines in aqueous media. We found that the stability of *N*-trifluoromethyl amines correlates with the electron density of the nitrogen, with electron-deficient anilines being the most stable amines investigated. However, even the electron-deficient anilines investigated underwent significant hydrolysis within hours. Furthermore, the formed hydrolysis product, the corresponding *N*-carbamoyl fluoride, can react with physiological nucleophiles. Consequently, *N*-trifluoromethyl amines might only be suitable for very specific drug discovery endeavors. We therefore strongly recommend stability studies to be conducted on *N*-trifluoromethyl amines made to ensure that any observed pharmacological effect is due to the *N*-trifluoromethyl amine and not due to hydrolysis products like the corresponding carbamoyl fluoride or secondary amine. Furthermore, our study showcases the need to rigorously investigate the aqueous stability and *in vitro* properties of newly accessible compounds to judge their suitability for biological applications.

In contrast, the *N*-trifluoromethyl moiety attached to azoles is significantly more stable with no signs of hydrolysis for any investigated azole. Additionally, none of the five investigated *N*-trifluoromethyl azoles showed any reactivity with glutathione, despite the strongly electron-withdrawing trifluoromethyl group. Exchanging an *N*-methyl for an *N*-trifluoromethyl moiety can be a valuable strategy to increase the lipophilicity and Caco-2 permeability and to modulate the metabolic stability of a compound, depending on the desired profile and on the starting point. Furthermore, we showed that an *N*-trifluoromethyl group could serve as a bioisostere to *N*-*iso*-propyl or *N*-*tert*-butyl azoles with potentially improved Caco-2 permeability.

In recent years, underused moieties like sulfoximines,[67] sulfonimidamides,[68] and phosphine oxides[69] have been suggested as interesting substructures in medicinal chemistry. As an addition to these moieties, we believe that *N*-trifluoromethyl azoles can be a highly valuable substructure and deserve a spot in the toolbox of medicinal chemists. We hope that our study provides inspiration as to when an *N*-trifluoromethyl moiety on an azole can be considered and will facilitate the use of this underused functional group.

EXPERIMENTAL SECTION

General Procedures. All reactions were performed in dried reaction vessels under a N_2 atmosphere. Reactions were monitored by either LCMS (ESI+ and ESI−), GCMS (EI+) or analytical thin-layer chromatography (TLC). TLC was performed on silica-plated glass plates, using Merck silica gel grade 60 F_{254}. Spots on TLC plates were visualized by UV (λ = 254 nm) or by cerium ammonium molybdate staining solution (2.5 g of ammonium molybdate tetrahydrate, 1 g of cerium ammonium sulfate dihydrate in 10 mL of sulfuric acid and 90 mL of water) followed by heating at 640 °C for 10 s. For LCMS analysis,

https://dx.doi.org/10.1021/acs.jmedchem.0c01457
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a GenTech Scientific Waters ACQ equipped with an Acquity ultra performance liquid chromatography (UPLC) system, an HSS C18 column (1.8 μm, 2.1 mm × 50 mm), and an SQ2 detector was used. Acetonitrile and water (modified either with 47 mM ammonia and 6.5 mM ammonium carbonate, pH 10, or with 1 mM ammonium formate and 10 mM formic acid, pH 3) were used as mobile phases. For GCMS analysis, an Agilent 7890A GCMS system equipped with an Agilent HP-5MS column (0.25 μm, 30 mm × 0.25 mm), a 7683B Series injector, a 7683 autosampler, and a 5975C Insert MSD detector was used. For automated flash column chromatography, a Biotage SP-4 system with Biotage prepacked KP-SIL SNAP cartridges was used. For preparative high-performance liquid chromatography (HPLC), a Waters Fraction Lynx system with a Waters Acquity SQD and a Waters binary gradient module 2525, with a flow of 60 mL/min at ambient temperature, was used. The HPLC was equipped with either a Kromasil C8 column (10 μm, 250 mm × 20 mm, HPLC-system A), a Waters Sunfire C18 column (5 μm, 19 mm × 150 mm, HPLC-system B), a Waters Xbridge C18 column (5 μm, 30 mm × 150 mm, HPLC-system C), a Waters Sunfire C18 column (5 μm, 10 mm × 100 mm, HPLC-system D), a Waters Sunfire C18 column (5 μm, 30 mm × 150 mm, HPLC-system E), or a Waters BEH C18 column (1.7 μm, 2.1 mm × 50 mm, HPLC-system F). For preparative SFC, a Waters Prep 100 SFC MS with a Waters mass detector 3100, a TharSFC high-pressure pump, and a Waters quaternary gradient pump 2545, with a flow of 100 g/min at 40 °C and 120 bar, was used. The SFC system was equipped with either a Waters BEH column (5 μm, 30 mm × 250 mm, SFC-system A), a Phenomenex Luna Hilic column (3.5 μm, 3 mm × 100 mm, SFC-system B), or a Waters Sunfire C18 column (5 μm, 19 mm × 150 mm, SFC-system C). NMR spectra were recorded at an uncalibrated temperature of 25 °C on a Bruker Avance III 500 spectrometer with a 5 mm QNP cryoprobe at a frequency of 500 MHz (^{1}H), 125 MHz (^{13}C), or 471 MHz (^{19}F), a Bruker Avance III with a 5 mm QNP cryoprobe at a frequency of 600 MHz (^{1}H) or 151 MHz (^{13}C), or a Bruker Neo with a 5 mm TCI probe with a cryofit at a frequency of 600 MHz (^{1}H) or 151 MHz (^{13}C). ^{13}C NMRs and ^{19}F NMRs were run in the proton decoupled mode. Chemical shifts are reported in parts per million (δ) and referenced from the residual protonium for ^{1}H NMR [($CDCl_3$): δ 7.26 ($CHCl_3$); DMSO-d_6: δ 2.50 (DMSO-d_5); CD_2Cl_2: δ 5.43 ($CHDCl_2$)]. ^{13}C NMR spectra are referenced from the carbon reference of the solvent [($CDCl_3$: δ 77.2; DMSO-d_6: δ 39.5; CD_2Cl_2: δ 54.0)]. COSY, HSQC, and HMBC spectra were recorded to support the structural assignments of the NMR signals. A Waters LCT Premiere mass spectrometer coupled to a Waters Acquity UPLC was used to record high-resolution mass spectra (HRMS). The Waters Acquity UPLC was equipped with either a BEH C18 column (1.7 μm, 2.1 mm × 50 mm, at 45 °C using a gradient from 5 to 90% acetonitrile in water modified with 40 mM ammonia and 5 mM H_2CO_3, pH 10 within 2.5 min or 3 min) or a CSH C18 column (1.7 μm, 2.1 mm × 50 mm at 45 °C using a gradient from 5 to 90% acetonitrile in water modified with 10 mM formic acid and 1 mM ammonium formate, pH 3, within 2.5 min or 3 min). For purity analysis, a GenTech Scientific Waters ACQ equipped with an Acquity UPLC system, an HSS C18 column (1.8 μm, 2.1 mm × 50 mm), and an SQ2 detector with a wavelength range of 220−350 nm was used. Acetonitrile and water (modified either with 47 mM ammonia and 6.5 mM ammonium carbonate, pH 10, or with 1 mM ammonium formate and 10 mM formic acid, pH 3) were used as mobile phases. The purity of all final compounds is 95% or higher unless otherwise stated.

Materials. All solvents and chemicals were used as purchased without further purification. Solvents for reactions were anhydrous (≤50 ppm H_2O) unless otherwise stated. Solvents for extraction and chromatographic purification were of HPLC grade. Where necessary (so noted), solvents where deoxygenated using three cycles of freeze, pump for 1 min, and thaw. The synthesis of *N*-trifluoromethyl amines **5**, **8**, **14**, **18a**, and **20a** is described by Scattolin et al.[15] Compounds **21b**,[30,31] **22b**,[47] **23b**,[33] **24b**,[34] **25b**,[35] **30**,[30,31] and **31**[33] were synthesized according to literature procedures. Compound **18b** is commercially available.

General Procedure for the Synthesis of *N*-Trifluoromethyl Amines. The respective secondary amine (360 μmol, 1.0 equiv) was dissolved in DCM (HPLC grade, 2.6 mL). The resulting solution was added dropwise to tetramethylammonium trifluoromethanethiolate (126 mg, 719 μmol, 2.0 equiv), and the resulting mixture was stirred at room temperature. After 15 min, silver(I) fluoride (228 mg, 1.80 mmol, 5.0 equiv.) was added and stirring at room temperature was continued until LCMS analysis showed complete conversion into the desired *N*-trifluoromethyl amine (typically after 4−6 h). The reaction mixture was purified using automated silica column chromatography with isocratic 100% pentane as the mobile phase.

*1-Phenyl-4-(trifluoromethyl)piperazine (**3**).* It was synthesized according to the general procedure. Colorless solid (31 mg, 37%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.33−7.26 (m, 2H), 6.97−6.88 (m, 3H), 3.27−3.20 (m, 4H), 3.12−3.06 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 151.0 (s), 129.4 (s), 124.6 (q, $^{1}J_{C-F}$ = 256.2 Hz), 120.7 (s), 116.7 (s), 48.6 (s), 44.5 (q, $^{3}J_{C-F}$ = 2.8 Hz). ^{19}F NMR (470 MHz, $CDCl_3$): δ = −68.3 (s). MS (EI): *m*/*z* calcd for $C_{11}H_{13}F_3N_2$ $[M]^+$: 230.1. Found: 230.2. ^{1}H, ^{13}C, and ^{19}F NMR are consistent with the literature.[17]

*4-Methoxy-N-methyl-N-(trifluoromethyl)aniline (**4**).* It was synthesized according to the general procedure. Colorless liquid (18 mg, 24%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.21 (d, $^{3}J_{H-H}$ = 8.7 Hz, 2H), 6.89−6.83 (m, 2H), 3.80 (s, 3H), 2.97 (q, $^{4}J_{H-F}$ = 1.2 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 158.4 (s), 135.8 (s), 127.5 (q, $^{3}J_{C-F}$ = 1.6 Hz), 124.2 (q, $^{1}J_{C-F}$ = 256.1 Hz), 114.4 (s), 55.6 (s), 36.9 (q, $^{3}J_{C-F}$ = 1.9 Hz). ^{19}F NMR (471 MHz, $CDCl_3$): δ = −61.5 (s). MS (EI): *m*/*z* calcd for $C_9H_{10}F_3NO$ $[M]^+$: 205.1. Found: 205.2. ^{1}H, ^{13}C, and ^{19}F NMR are consistent with the literature.[16]

*N,4-Dimethyl-N-(trifluoromethyl)aniline (**6**).*[70] It was synthesized according to the general procedure. Light yellow liquid (52 mg, 76%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.23−7.07 (m, 4H), 3.00 (q, $^{4}J_{H-F}$ = 1.2 Hz, 3H), 2.34 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 140.4 (s), 136.4 (s), 129.9 (s), 125.4 (q, $^{3}J_{C-F}$ = 1.4 Hz), 123.7 (q, $^{1}J_{C-F}$ = 241.3 Hz), 36.6 (q, $^{3}J_{C-F}$ = 2.3 Hz), 21.1 (s). ^{19}F NMR (471 MHz, $CDCl_3$): δ = −60.9 (s). ^{1}H, ^{13}C, and ^{19}F NMR are consistent with the literature.[16]

*N-Ethyl-N-(trifluoromethyl)aniline (**7**).*[70] It was synthesized according to the general procedure. Light yellow liquid (18 mg, 26%). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.37−7.33 (m, 2H), 7.27−7.23 (m, 3H), 3.42−3.39 (m, 2H), 1.08 (t, $^{3}J_{H-H}$ = 7.2 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ = 140.9 (s), 129.3 (s), 126.9 (s), 126.8 (s), 123.7 (q, $^{1}J_{C-F}$ = 254.7 Hz), 43.9 (s), 13.9 (s). ^{19}F NMR (471 MHz, $CDCl_3$): δ = −57.6 (s). ^{1}H, ^{13}C, and ^{19}F NMR are consistent with the literature.[11]

*1-(Trifluoromethyl)-1,2,3,4-tetrahydroquinoline (**9**).* It was synthesized according to the general procedure. Yellow liquid (42 mg, 21%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.22−7.04 (m, 3H), 7.03−6.89 (m, 1H), 3.55−3.41 (m, 2H), 2.80 (t, $^{3}J_{H-H}$ = 6.5 Hz, 2H), 2.07−1.91 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 137.7 (s), 129.5 (s), 128.3 (s), 126.8 (s), 123.2 (q, $^{1}J_{C-F}$ = 254.1 Hz), 122.9 (s), 119.9 (q, $^{3}J_{C-F}$ = 3.7 Hz), 43.9 (q, $^{3}J_{C-F}$ = 2.1 Hz), 27.3 (s), 22.1 (s). ^{19}F NMR (470 MHz, $CDCl_3$): δ = −56.7 (s). MS (EI): *m*/*z* calcd for $C_{10}H_{10}F_3N$ $[M]^+$: 201.1. Found: 201.2. ^{1}H, ^{13}C, and ^{19}F NMR are consistent with the literature.[16]

*4-(N-Methyl-N-(trifluoromethyl)amino)benzonitrile (**10**).* It was synthesized according to the general procedure. Colorless liquid (48 mg, 67%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.64−7.63 (m, 2H), 7.26−7.25 (m, 2H), 3.24−3.03 (m, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 146.5 (s), 133.3 (s), 122.6 (q, $^{1}J_{C-F}$ = 257.8 Hz), 122.1 (q, $^{3}J_{C-F}$ = 2.3 Hz), 118.7 (s), 107.9 (s), 35.3 (q, $^{3}J_{C-F}$ = 2.3 Hz). ^{19}F NMR (470 MHz, $CDCl_3$): δ = −58.8 (s). MS (EI): *m*/*z* calcd for $C_{10}H_{10}F_3N$ $[M]^+$: 200.1. Found: 200.1. ^{1}H, ^{13}C, and ^{19}F NMR are consistent with the literature.[16]

*4-Fluoro-N-methyl-N-(trifluoromethyl)aniline (**11**).*[70] It was synthesized according to the general procedure. Yellow liquid (29 mg, 42%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.25−7.23 (m, 2H, C<u>H</u>), 7.06−7.02 (m, 2H, C<u>H</u>), 2.99 (q, $^{4}J_{H-F}$ = 1.1 Hz, 3H, C<u>H</u>$_3$). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 161.2 (d, $^{1}J_{C-F}$ = 246.0 Hz, CH<u>C</u>F), 138.9 (d, $^{4}J_{C-F}$ = 3.2 Hz, N<u>C</u>CH), 127.6 (d, $^{3}J_{C-F}$ = 8.6 Hz, FCCH<u>C</u>H), 123.6 (q, $^{1}J_{C-F}$ = 257.4 Hz, <u>C</u>F$_3$), 116.1 (d, $^{2}J_{C-F}$ = 22.6 Hz, FC<u>C</u>H), 36.8 (q, $^{3}J_{C-F}$ = 2.0 Hz, <u>C</u>H$_3$). ^{19}F NMR (471 MHz, $CDCl_3$): δ = −61.3 (s, 3F, C<u>F</u>$_3$), −115.4 (s, 1F, CHC<u>F</u>).

https://dx.doi.org/10.1021/acs.jmedchem.0c01457
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*3-Fluoro-N-methyl-N-(trifluoromethyl)aniline (**12**).*[70] It was synthesized according to the general procedure. Colorless liquid (38 mg, 56%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.33−7.28 (m, 1H, FCCHCH), 7.04−7.01 (m, 1H, NCCHCH), 6.97−6.90 (m, 2H, FCCH, FCCH), 3.05 (q, $^4J_{H-F}$ = 1.3 Hz, 3H, CH_3). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 163.0 (d, $^1J_{C-F}$ = 241.3 Hz, FCCH), 144.4 (d, $^3J_{C-F}$ = 9.5 Hz, NCCH), 130.3 (d, $^3J_{C-F}$ = 9.2 Hz, FCCHCH), 123.2 (q, $^1J_{C-F}$ = 259.6 Hz, CF_3), 119.8 (dq, $^4J_{C-F}$ = 1.8 Hz, $^4J_{C-F}$ = 1.7 Hz, NCCHCH), 112.8 (d, $^2J_{C-F}$ = 21.4 Hz, FCCHCH), 111.7 (dq, $^2J_{C-F}$ = 23.5 Hz, $^4J_{C-F}$ = 1.7 Hz, NCCHCF), 36.2 (q, $^3J_{C-F}$ = 2.1 Hz, CH_3). ^{19}F NMR (471 MHz, $CDCl_3$): δ = −60.3 (s, 3F, CF_3), −111.7 (s, 1F, CHCF).

*N-Methyl-N,4-bis(trifluoromethyl)aniline (**13**).*[70] It was synthesized according to the general procedure. Colorless liquid (32 mg, 36%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.62−7.60 (m, 2H, CF_3CCH), 7.32−7.30 (m, 2H, NCCH), 3.11 (q, $^4J_{H-F}$ = 1.4 Hz, 3H, CH_3). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 145.8 (s, NCCH), 127.4 (q, $^2J_{C-F}$ = 33.0 Hz, CF_3C), 126.4 (q, $^3J_{C-F}$ = 3.6 Hz, CF_3CCH), 124.1 (q, $^1J_{C-F}$ = 271.3 Hz, CF_3), 123.2 (q, $^4J_{C-F}$ = 1.9 Hz, NCCH), 123.0 (q, $^1J_{C-F}$ = 257.4 Hz, CF_3), 35.8 (q, $^3J_{C-F}$ = 2.2 Hz, CH_3). ^{19}F NMR (471 MHz, $CDCl_3$): δ = −59.6 (s, 3F, CF_3), −62.4 (s, 3F, CF_3).

*1-(Trifluoromethyl)-1H-benzo[d]imidazole (**16**).* Dibromodifluoromethane (1.6 mL, 18 mmol, 1.5 equiv) was added under vigorous stirring to a suspension of 1*H*-benzo[*d*]imidazole (1.41 g, 11.9 mmol, 1.0 equiv), sodium hydride (60% dispersion in mineral oil, 480 mg, 12.0 mmol, 1.0 equiv), and tetrabutylammonium bromide (23 mg, 71 μmol, 0.4 mol %) in DMF (2.4 mL) at 0 °C. The reaction mixture was gradually warmed to 25 °C within 2 h and stirred at 25 °C for 2 h. Water (10 mL) was then added dropwise; the product was extracted with diethylether (5 × 10 mL), and the combined organic phases were washed with water (5 × 10 mL), dried over $MgSO_4$, and filtered. The solvent was removed *in vacuo*, and the resultant crude 4-(1-(bromodifluoromethyl)-1*H*-benzo[*d*]imidazole) was dissolved in sulfolane (HPLC grade, 2.8 mL). To this solution was added tetramethylammonium fluoride (201 mg, 2.16 mmol, 0.2 equiv), and the resulting mixture was stirred at 90 °C. After 12 h, the reaction mixture was allowed to cool to room temperature and directly subjected to automated flash column chromatography using a gradient from 0 to 10% ethyl acetate in heptane to give 1-(trifluoromethyl)-1*H*-benzo-[*d*]imidazole (**16**) as a yellow powder (38 mg, 2% over two steps). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.87 (s, 1H), 7.91 (d, $^3J_{H-H}$ = 8.0 Hz, 1H), 7.46 (t, $^3J_{H-H}$ = 7.6 Hz, 1H), 7.40 (t, $^3J_{H-H}$ = 7.7 Hz, 1H), 7.29 (d, $^3J_{H-H}$ = 8.1 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6): δ = 143.7 (s), 141.1 (s), 130.2 (s), 125.4 (s), 124.5 (s), 120.8 (s), 115.0 (q, $^1J_{C-F}$ = 252.3 Hz), 111.2 (s). ^{19}F NMR (471 MHz, DMSO-d_6): δ = −59.1 (s). ^{1}H, ^{13}C, and ^{19}F NMR are consistent with the literature.[71]

*4-(Methyl(trifluoromethyl)amino)-N-(5-methylisoxazol-3-yl)-benzenesulfonamide (**19a**).* A solution of 4-(methylamino)-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide (134 mg, 501 μmol, 1.0 equiv), triphenylphosphine (393 mg, 1.50 mmol, 3.0 equiv), and sodium trifluoromethanesulfinate (117 mg, 750 μmol, 1.5 equiv) in deoxygenated acetonitrile (2.5 mL) was stirred at room temperature. After 165 min, silver(I) fluoride (285 mg, 2.25 mmol, 4.5 equiv) was added and the resulting mixture was stirred at room temperature. After 3 h, the solvent was removed *in vacuo* and the resulting crude product was purified using preparative HPLC-system A with a gradient from 20 to 55% acetonitrile in water/acetonitrile 95:5 (aqueous phase modified with 0.2% formic acid) within 25 min to give 4-(methyl-(trifluoromethyl)amino)-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide (**19a**) as a yellow solid (14 mg, 8%) with a purity of 84%.[72] ^{1}H NMR (500 MHz, DMSO-d_6): δ = 11.39 (s br, 1H, NH), 7.87−7.83 (m, 2H, SCCH), 7.47−7.43 (m, 2H, NCCHCH), 6.14 (q br, $^4J_{H-H}$ = 0.9 Hz, 1H, OCCH), 3.12 (q, $^4J_{H-F}$ = 1.6 Hz, 3H, NCH_3), 2.30 (d, $^4J_{H-H}$ = 0.8 Hz, 3H, CCH_3). ^{13}C NMR (126 MHz, DMSO-d_6): δ = 170.3 (s, CCH_3), 157.6 (s, NHCN), 145.7 (s, NCCHCH), 135.5 (s, CS), 128.1 (s, SCCH), 122.4 (q, $^1J_{C-F}$ = 256.3 Hz, CF_3), 122.3 (q, $^4J_{C-F}$ = 2.1 Hz, NCCHCH), 95.4 (s, OCCH), 35.0 (q, $^3J_{C-F}$ = 2.0 Hz, NCH_3), 12.1 (s, CCH_3). ^{19}F NMR (471 MHz, DMSO-d_6): δ = −57.4 (s). HRMS (ESI): *m*/*z* calcd for $C_{12}H_{12}F_3N_3O_3S$ + H^+ $[M + H]^+$: 336.0625. Found: 336.0637.

*4-(Dimethylamino)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (**19b**).* A mixture of 4-(methylamino)-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide (100 mg, 374 μmol, 1.0 equiv) and paraformaldehyde (22 mg, 0.75 mmol, 2.0 equiv) in methanol (7.5 mL) was stirred for 5 min. Sodium cyanoborohydride (94 mg, 1.5 mmol, 4.0 equiv) was then added, and the resulting mixture was stirred at room temperature for 20 h. The mixture was then heated to 40 °C for 3 h. The reaction mixture was allowed to cool to room temperature and was quenched with an aqueous saturated $NaHCO_3$ solution (30 mL). The product was extracted with DCM (1 × 50 mL); the organic phase was washed with an aqueous saturated $NaHCO_3$ solution (1 × 30 mL), dried over $MgSO_4$, and filtered, and the solvent was removed *in vacuo*. The resulting residue was purified using preparative HPLC-system A with a gradient from 20 to 55% acetonitrile in water/acetonitrile 95:5 (aqueous phase modified with 0.2% formic acid) within 25 min to give 4-(dimethylamino)-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide (**19b**) as a yellow solid (4 mg, 2%). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 11.01 (s, 1H, NH), 7.61−7.58 (m, 2H, SCCH), 6.76−6.73 (m, 2H, NCCHCH), 6.10 (s, 1H, OCCH), 2.98 (s, 6H, NCH_3), 2.28 (s, 3H, CCH_3). ^{13}C NMR (126 MHz, DMSO-d_6): δ = 169.9 (CCH_3), 158.0 (NHCN), 152.8 (NCCHCH), 128.4 (SCCH), 124.3 (CS), 110.9 (NCCHCH), 95.3 (OCCH), 40.4 (NCH_3), 12.1 (CCH_3). HRMS (ESI): *m*/*z* calcd for $C_{12}H_{13}F_3N_3O_3S$ + H^+ $[M + H]^+$: 282.0907. Found: 282.0918.

*2-(Dimethylamino)ethyl 4-(butyl(methyl)amino)benzoate Formate (**20b**).* A solution of 2-(dimethylamino)ethyl 4-(butylamino)-benzoate (100 mg, 378 μmol, 1.0 equiv) and paraformaldehyde (23 mg, 0.76 mmol, 2.0 equiv) in methanol (7.6 mL) was stirred at room temperature for 5 min. Sodium cyanoborohydride (95 mg, 1.5 mmol, 4.0 equiv) was then added, and the reaction mixture was stirred at room temperature for 20 h. The mixture was then heated to 40 °C for an additional 3 h. The reaction mixture was then allowed to cool to room temperature and was quenched by the addition of an aqueous saturated $NaHCO_3$ solution (30 mL). The reaction mixture was extracted with DCM (1 × 50 mL); the organic phase was washed with an aqueous saturated $NaHCO_3$ solution (1 × 30 mL), dried over $MgSO_4$, and filtered, and the solvent removed *in vacuo*. The resultant residue was purified using preparative HPLC-system B with a gradient from 5 to 95% acetonitrile in water (aqueous phase modified with 0.2% formic acid) within 10 min to give 2-(dimethylamino)ethyl 4-(butyl(methyl)-amino)benzoate formate (**20b**) as a pale yellow solid (7 mg, 5%). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.36 (s, 1H), 7.76−7.72 (m, 2H), 6.71−6.68 (m, 2H), 4.25 (t, $^3J_{H-H}$ = 5.8 Hz, 2H), 3.47−3.42 (m, 2H), 3.42−3.36 (m, 2H), 2.96 (s, 3H), 2.21 (s, 6H), 1.53−1.46 (m, 2H), 1.34−1.25 (m, 2H), 0.90 (t, $^3J_{H-H}$ = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6): δ = 165.8, 165.0, 152.3, 130.9, 115.4, 110.6, 61.7, 57.5, 51.1, 45.4, 38.0, 28.4, 19.6, 13.9. HRMS (ESI): *m*/*z* calcd for $C_{16}H_{27}N_2O_2$ + H^+ $[M + H]^+$: 279.2073. Found: 279.2073.

*N-(2-Methyl-5-(1-(trifluoromethyl)-1H-imidazol-4-yl)phenyl)-4-(pyridin-2-ylmethoxy)benzamide (**21a**).* A mixture of (4-methyl-3-(4-(pyridin-2-ylmethoxy)benzamido)phenyl)boronic acid[30] (100 mg, 276 μmol, 1.0 equiv), Cs_2CO_3 (270 mg, 829 μmol, 3.0 equiv), $Pd(PPh_3)_4$ (48 mg, 42 μmol, 15 mol %), and 4-bromo-1-(trifluoromethyl)-1*H*-imidazole (77 mg, 0.36 mmol, 1.3 equiv) in deoxygenated 1,4-dioxane (1.4 mL) and deoxygenated water (460 μL) was stirred at 150 °C. After 5 h, the reaction mixture was allowed to cool to room temperature and extracted with ethyl acetate (3 × 5 mL); the combined organic phases were washed with an aqueous saturated NaCl solution (2 × 5 mL), dried over $MgSO_4$, filtered, and the solvent was removed *in vacuo*. The resulting residue was purified using automated flash column chromatography with a gradient from 5 to 50% ethyl acetate in heptane to give *N*-(2-methyl-5-(1-(trifluoromethyl)-1*H*-imidazol-4-yl)phenyl)-4-(pyridin-2-ylmethoxy)benzamide (**21a**) as a colorless powder (50 mg, 40%). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.83 (s, 1H, NH), 8.60−8.59 (m, 1H, NCHCH), 8.44 (s, 1H, NCHN), 7.98−7.95 (m, 2H, CHCHCO), 7.85 (td, $^3J_{H-H}$ = 7.7 Hz, $^4J_{H-H}$ = 1.7 Hz, 1H, NCHCHCH), 7.54 (d, $^3J_{H-H}$ = 7.8 Hz, 1H, NCHCHCHCH), 7.47 (d, $^4J_{H-H}$ = 1.0 Hz, 1H, CH_3CCCH), 7.38 (d, $^3J_{H-H}$ = 8.2 Hz, 1H,

https://dx.doi.org/10.1021/acs.jmedchem.0c01457
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CH_3CCH), 7.36 (dd, $^3J_{H-H}$ = 7.4 Hz, $^3J_{H-H}$ = 5.0 Hz, 1H, NCHCH), 7.25 (dd, $^3J_{H-H}$ = 7.7 Hz, $^4J_{H-H}$ = 1.0 Hz, 1H, CH_3CCHCH), 7.22 (s, 1H, CF_3NCHC), 7.17−7.14 (m, 2H, OCCH), 5.28 (s, 2H, CH_2), 2.28 (s, 3H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6): δ = 164.8 (s, C(O)), 160.8 (s, $COCH_2$), 156.3 (s, OCH_2C), 149.2 (s, NCHCH), 137.1 (s, NCHCHCH), 136.9 (s, CH_3C), 136.7 (q br, $^3J_{C-F}$ = 2.1 Hz, NCHN), 134.9 (s, CH_3CCNH), 130.7 (s, CH_3CCHCH), 130.7 (q, $^3J_{C-F}$ = 2.1 Hz, CF_3NCHC), 130.4 (s, CF_3NCHC), 129.7 (s, CHCHCO), 126.9 (s, C(O)C or CH_3CCCH), 126.8 (s, C(O)C or CH_3CCH), 126.4 (s, CH_3CCHCH), 125.1 (s, $CH_3CCHCHC$), 123.1 (s, NCHCH), 121.8 (s, NCHCHCHCH), 118.1 (q, $^1J_{C-F}$ = 264.6 Hz, CF_3), 114.5 (s, OCCH), 70.4 (s, CH_2), 17.8 (s, CH_3). ^{19}F NMR (471 MHz, DMSO-d_6): δ = −51.8 (s, CF_3). HRMS (ESI): m/z calcd for $C_{24}H_{19}F_3N_4O_2$ + H^+ $[M + H]^+$: 453.1533. Found: 453.1541.

*4-(1-(Trifluoromethyl)-1H-benzo[d]imidazol-2-yl)thiazole (**22a**).* Dibromodifluoromethane (136 μL, 1.49 mmol, 1.5 equiv) was added under vigorous stirring to a suspension of thiabendazole (200 mg, 994 μmol, 1.0 equiv), sodium hydride (60% dispersion in mineral oil, 44 mg, 1.1 mmol, 1.1 equiv), and tetrabutylammonium bromide (2 mg, 6 μmol, 0.4 mol %) at 0 °C in DMF (3.8 mL). The resulting reaction mixture was gradually warmed to room temperature within 2 h and stirred at room temperature overnight. An aqueous saturated NH_4Cl solution (5 mL) was added dropwise. The product was extracted with diethylether (5 × 4 mL). The combined organic phases were washed with water (5 × 4 mL), dried over $MgSO_4$ and filtered; the solvent was removed *in vacuo*; and the resultant crude product was subjected to automated flash column chromatography with a gradient from 5 to 10% ethyl acetate in heptane. The resultant product was dissolved in sulfolane (HPLC grade, 2.4 mL), tetramethylammonium fluoride (50 mg, 0.54 mmol, 0.5 equiv) was added, and the reaction mixture was stirred at 60 °C. After 12 h, the reaction mixture was allowed to cool to room temperature; the product was extracted with diethylether (3 × 5 mL); the combined organic phases were washed with water (1 × 5 mL), dried over $MgSO_4$, and filtered; and the solvent was removed *in vacuo*. The crude material was purified using preparative HPLC-system C with a gradient from 5 to 95% acetonitrile in water (aqueous phase modified with 0.2% ammonia) within 10 min to give 4-(1-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)thiazole (**22a**) as a colorless solid (25 mg, 9% over two steps). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.34 (d, $^4J_{H-H}$ = 2.0 Hz, 1H, NCHS), 8.55 (d, $^4J_{H-H}$ = 2.0 Hz, 1H, SCHC), 7.89−7.85 (m, 1H, $CF_3NCCHCHCHCH$), 7.78−7.75 (m, 1H, CF_3NCCH), 7.53−7.47 (m, 2H, $CF_3NCCHCHCH$). ^{13}C NMR (126 MHz, DMSO-d_6): δ = 155.4 (s, NCHS), 145.0 (s, NCCHS or NCN), 144.4 (s, NCCHS or NCN), 141.6 (s, CF_3NCCN), 131.9 (s, CF_3NCCN), 125.9 (s, $CF_3NCCHCHCH$ or $CF_3NCCHCHCH$), 125.0 (s, $CF_3NCCHCHCH$ or $CF_3NCCHCHCH$), 124.6 (s, SCHC), 120.5 (s, $CF_3NCCHCHCHCH$), 118.9 (q, $^1J_{C-F}$ = 264.4 Hz, CF_3), 112.6 (q, $^4J_{C-F}$ = 4.4 Hz, CF_3NCCH). ^{19}F NMR (470 MHz, DMSO-d_6): δ = −51.0 (s, CF_3). HRMS (ESI): m/z calcd for $C_{11}H_6F_3N_3S$ + H^+ $[M + H]^+$: 270.0308. Found: 270.0307.

*N^4-((1r,4r)-4-(Dimethylamino)cyclohexyl)-N^2-(1-(trifluoromethyl)-1H-pyrazol-4-yl)pyrrolo[2,1-f][1,2,4]triazine-2,4-diamine Formate (**23a**).* A mixture of 1-(trifluoromethyl)-1*H*-pyrazol-4-amine (51 mg, 0.34 mmol, 2.0 equiv), tripotassium phosphate (181 mg, 853 μmol, 5.0 equiv), (1*r*,4*r*)-N^1-(2-chloropyrrolo[2,1-*f*][1,2,4]triazin-4-yl)-N^4,N^4-dimethylcyclohexane-1,4-diamine[33] (50 mg, 0.17 mmol, 1.0 equiv), and XPhos Pd G3 (29 mg, 34 μmol, 20 mol %) in deoxygenated ^{t}BuOH (5 mL) was stirred at 80 °C. After 15 h, the reaction mixture was allowed to cool to room temperature and the solvent was removed *in vacuo*. The resulting residue was purified using automated flash column chromatography with a gradient from 5 to 30% ethyl acetate in heptane (both mobile phases modified with 5% ammonia in MeOH) followed by purification using preparative HPLC-system D with a gradient from 5 to 95% MeCN in water (aqueous phase modified with 0.1 M formic acid) within 8 min to give N^4-((1*r*,4*r*)-4-(dimethylamino)cyclohexyl)-N^2-(1-(trifluoromethyl)-1*H*-pyrazol-4-yl)pyrrolo[2,1-*f*][1,2,4]triazine-2,4-diamine formate (**23a**) as a colorless solid (20 mg, 29%). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.97 (s, 1H, NHCCH), 8.36 (s, 1H, CF_3NNCH), 8.32 (s, 1H, HCOO of formate), 7.92 (s, 1H, CF_3NCH), 7.88 (d, $^3J_{H-H}$ = 7.9 Hz, 1H, NHCH), 7.49−7.48 (m, 1H, NCHCHCH), 6.82 (dd, $^3J_{H-H}$ = 4.4 Hz, $^4J_{H-H}$ = 1.7 Hz, 1H, NCHCHCH), 6.41 (dd, $^3J_{H-H}$ = 4.4 Hz, $^3J_{H-H}$ = 2.5 Hz, 1H, NCHCHCH), 4.08−3.98 (m, 1H, NHCH), 2.36−2.27 (m, 1H, CH_3NCH), 2.27 (s, 6H, NCH_3), 2.05−2.02 (m, 2H, $NHCHCH_2$), 1.94−1.89 (m, 2H, CH_3NCHCH_2), 1.45−1.37 (m, 2H, $NHCHCH_2$), 1.35−1.27 (m, 2H, CH_3NCHCH_2). ^{13}C NMR (126 MHz, DMSO-d_6): δ = 165.2 (s, HCOO of formate), 153.2 (s, NCNCNH), 152.9 (s, NCNCNH), 137.0 (s, CF_3NCH), 127.0 (s, CF_3NCHC), 118.7 (q, $^1J_{C-F}$ = 260.6 Hz, CF_3), 118.0 (s, NCHCHCH), 115.2 (s, CF_3NNCH), 112.0 (s, NCHCHCHC), 108.5 (s, NCHCHCH), 100.8 (s, NCHCHCH), 62.2 (s, CH_3NCH), 48.5 (s, NHCH), 40.9 (s, NCH_3), 31.0 (s, CH_3NCHCH_2), 26.6 (s, $NHCHCH_2$). ^{19}F NMR (471 MHz, DMSO-d_6): δ = −59.3 (s, CF_3). HRMS (ESI): m/z calcd for $C_{18}H_{23}F_3N_8$ + H^+ $[M + H]^+$: 409.2071. Found: 409.2075.

*N^4-((1r,4r)-4-(Dimethylamino)cyclohexyl)-N^2-(1-(iso-propyl)-1H-pyrazol-4-yl)pyrrolo[2,1-f][1,2,4]triazine-2,4-diamine (**23c**).* A mixture of 1-(*iso*-propyl)-1*H*-pyrazol-4-amine (21 mg, 0.17 mmol, 2.0 equiv), tripotassium phosphate (90 mg, 0.43 mmol, 5.1 equiv), (1*r*,4*r*)-N^1-(2-chloropyrrolo[2,1-*f*][1,2,4]triazin-4-yl)-N^4,N^4-dimethylcyclohexane-1,4-diamine[33] (25 mg, 85 μmol, 1.0 equiv), and XPhos Pd G3 (14 mg, 17 μmol, 20 mol %) in deoxygenated ^{t}BuOH (5 mL) was stirred at 90 °C. After 8 h, additional tripotassium phosphate (90 mg, 0.43 mmol, 5.1 equiv) and XPhos Pd G3 (14 mg, 17 μmol, 20 mol %) were added at room temperature, and stirring at 90 °C was continued for an additional 14 h. The reaction mixture was then allowed to cool to room temperature, and the solvent was removed *in vacuo*. The resulting residue was purified using preparative HPLC-system C with a gradient from 5 to 95% acetonitrile in water (aqueous phase modified with 0.2% ammonia) within 10 min to give N^4-((1*r*,4*r*)-4-(dimethylamino)-cyclohexyl)-N^2-(1-(*iso*-propyl)-1*H*-pyrazol-4-yl)pyrrolo[2,1-*f*][1,2,4]-triazine-2,4-diamine (**23c**) as a brown solid (3 mg, 9%). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.39 (s, 1H, NHCCH), 7.85 (s, 1H, ^{i}PrNNCH), 7.67 (d, $^3J_{H-H}$ = 8.0 Hz, 1H, NHCH), 7.42 (s, 1H, ^{i}PrNCH), 7.35 (dd, $^3J_{H-H}$ = 2.3 Hz, $^4J_{H-H}$ = 1.8 Hz, 1H, NCHCHCH), 6.76 (dd, $^3J_{H-H}$ = 4.4 Hz, $^4J_{H-H}$ = 1.7 Hz, 1H, NCHCHCH), 6.35 (dd, $^3J_{H-H}$ = 4.3 Hz, $^3J_{H-H}$ = 2.4 Hz, 1H, NCHCHCH), 4.42 (sept, $^3J_{H-H}$ = 6.8 Hz, $CHCH_3$), 4.07−3.99 (m, 1H, NHCH), 2.23−2.16 (m, 1H, CH_3NCH), 2.20 (s, 6H, NCH_3), 2.03−2.00 (m, 2H, $NHCHCH_2$), 1.90−1.87 (m, 2H, CH_3NCHCH_2), 1.41−1.23 (m, 4H, $NHCHCH_2$ and CH_3NCHCH_2), 1.40 (d, $^3J_{H-H}$ = 6.7 Hz, 6H, $CHCH_3$). ^{13}C NMR (126 MHz, DMSO-d_6): δ = 153.6 (NCNCNH), 153.0 (NCNCNH), 129.1 (^{i}PrNCH), 123.7 (^{i}PrNCHC), 117.4 (NCHCHCH), 115.3 (^{i}PrNNCH), 112.1 (NCHCHCHC), 108.0 (NCHCHCH), 100.3 (NCHCHCH), 62.3 (CH_3NCH), 57.5 ($CHCH_3$), 48.4 (NHCH), 41.3 (NCH_3), 31.3 (CH_3NCHCH_2), 29.5 ($NHCHCH_2$), 27.0 ($CHCH_3$). HRMS (ESI): m/z calcd for $C_{20}H_{30}N_8$ + H^+ $[M + H]^+$: 383.2667. Found: 383.2642.

*N^2-(1-(tert-Butyl)-1H-pyrazol-4-yl)-N^4-((1r,4r)-4-(dimethylamino)cyclohexyl)pyrrolo[2,1-f][1,2,4]triazine-2,4-diamine (**23d**).* A mixture of 1-(*tert*-butyl)-1*H*-pyrazol-4-amine (47 mg, 0.34 mmol, 2.0 equiv), tripotassium phosphate (181 mg, 853 μmol, 5.0 equiv), (1*r*,4*r*)-N^1-(2-chloropyrrolo[2,1-*f*][1,2,4]triazin-4-yl)-N^4,N^4-dimethylcyclohexane-1,4-diamine[33] (50 mg, 0.17 mmol, 1.0 equiv), and XPhos Pd G3 (29 mg, 34 μmol, 20 mol %) in deoxygenated ^{t}BuOH (5 mL) was stirred at 80 °C. After 6 h, the reaction mixture was allowed to cool to room temperature and the solvent was removed *in vacuo*. The resulting residue was purified using preparative SFC-system A with a gradient from 35 to 40% MeOH/H_2O 97:3 (modified with 50 mM ammonia) within 11 min, followed by a second purification using HPLC-system E with a gradient from 5 to 95% acetonitrile (modified with 0.1 M formic acid) within 10 min, followed by a third purification using preparative SFC-system B with a gradient from 27 to 32% MeOH (modified with 20 mM ammonia) to give N^2-(1-(*tert*-butyl)-1*H*-pyrazol-4-yl)-N^4-((1*r*,4*r*)-4-(dimethylamino)cyclohexyl)pyrrolo[2,1-*f*][1,2,4]triazine-2,4-diamine (**23d**) as a yellow oil (8 mg, 12%). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.36 (s, 1H, NHCCH), 7.91 (d, $^4J_{H-H}$ = 0.8 Hz, 1H, ^{t}BuNNCH), 7.66 (d, $^3J_{H-H}$ = 8.1 Hz, 1H, NHCH), 7.45 (d, $^4J_{H-H}$ = 0.7 Hz, 1H, ^{t}BuNCH), 7.36−7.35 (m, 1H, NCHCHCH), 6.76 (dd, $^3J_{H-H}$ = 4.4 Hz, $^4J_{H-H}$ = 1.7 Hz, 1H, NCHCHCH), 6.35 (dd, $^3J_{H-H}$ = 4.4 Hz,

https://dx.doi.org/10.1021/acs.jmedchem.0c01457
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$^3J_{H-H}$ = 2.4 Hz, 1H, NCHCHCH), 4.08−3.99 (m, 1H, NHCH), 2.21−2.16 (m, 1H, CH_3NCH), 2.19 (s, 6H, NCH_3), 2.02−1.99 (m, 2H, NHCHCH$_2$), 1.89−1.86 (m, 2H, CH_3NCHCH_2), 1.51 (s, 9H, CCH_3), 1.44−1.36 (m, 2H, NHCHCH$_2$), 1.31−1.23 (m, 2H, CH_3NCHCH_2). ^{13}C NMR (126 MHz, DMSO-d_6): δ = 153.6 (NCNCNH), 153.0 (NCNCNH), 129.1 (^{t}BuNCH), 123.7 (^{t}BuNCHC), 117.4 (NCHCHCH), 115.3 (^{t}BuNNCH), 112.1 (NCHCHCHC), 108.0 (NCHCHCH), 100.3 (NCHCHCH), 62.3 (CH_3NCH), 57.5 (CCH_3), 48.4 (NHCH), 41.3 (NCH_3), 31.3 (CH_3NCHCH_2), 29.5 (CCH_3), 27.0 (NHCHCH$_2$). HRMS (ESI): m/z calcd for $C_{21}H_{32}N_8$ + H^+ [M + H]$^+$: 397.2823. Found: 397.2825.

*2-(3,4-Dimethoxyphenyl)-N-(1-(trifluoromethyl)-1H-pyrazol-4-yl)acetamide (**24a**).* A solution of 2-(3,4-dimethoxyphenyl)acetic acid (39 mg, 0.20 mmol, 1.0 equiv), HOBt·xH$_2$O (30 mg), and EDC·HCl (38 mg, 0.20 mmol, 1.0 equiv) in DCM (1.4 mL) was stirred at room temperature. After 30 min, 1-(trifluoromethyl)-*1H*-pyrazol-4-amine (30 mg, 0.20 mmol, 1.0 equiv) was added, and the resulting reaction mixture was stirred at room temperature for additional 14 h. Water (5 mL) was then added, and the product was extracted with ethyl acetate (3 × 10 mL). The combined organic phases were washed with water (1 × 10 mL), dried over $MgSO_4$ and filtered, and the solvent was removed *in vacuo*. The crude product was purified using automated flash column chromatography with a gradient from 5 to 50% ethyl acetate in heptane to give 2-(3,4-dimethoxyphenyl)-*N*-(1-(trifluoromethyl)-1*H*-pyrazol-4-yl)acetamide (**24a**) as a colorless powder (52 mg, 80%). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 10.49 (s, 1H, NH), 8.41−8.40 (m, 1H, CF_3NCH), 7.94−7.93 (m, 1H, CF_3NNCH), 6.91 (d, $^4J_{H-H}$ = 1.9 Hz, 1H, CH_3OCCHC), 6.90 (d, $^3J_{H-H}$ = 8.2 Hz, 1H, $CH_3OCCHCH$), 6.81 (dd, $^3J_{H-H}$ = 8.2 Hz, $^4J_{H-H}$ = 2.0 Hz, 1H, $CH_3OCCHCH$), 3.73 (s, 3H, CH_3O), 3.72 (s, 3H, CH_3O), 3.54 (s, 2H, CH_2). ^{13}C NMR (126 MHz, DMSO-d_6): δ = 168.8 (s, $CH_2C(O)$), 148.5 (s, CH_3OC), 147.7 (s, CH_3OC), 136.7 (s, CF_3NNCH), 127.8 (s, $CCH_2C(O)$), 124.3 (q, $^4J_{C-F}$ = 1.6 Hz, CHCNH), 121.1 (s, $CH_3OCCHCH$), 118.1 (s, CF_3NCH), 118.0 (q, $^1J_{C-F}$ = 261.2 Hz, CF_3), 113.0 (s, CH_3OCCHC), 111.8 (s, $CH_3OCCHCH$), 55.4 (s, OCH_3), 55.5 (s, OCH_3), 41.9 (s, CH_2). ^{19}F NMR (471 MHz, DMSO-d_6): δ = −59.4 (s, CF_3). HRMS (ESI): m/z calcd for $C_{14}H_{14}F_3N_3O_3$ + H^+ [M + H]$^+$: 330.1060. Found: 330.1064.

*2-(3,4-Dimethoxyphenyl)-N-(1-iso-propyl-1H-pyrazol-4-yl)-acetamide (**24c**).* A purple solution of 2-(3,4-dimethoxyphenyl)acetic acid (47 mg, 0.24 mmol, 1.0 equiv), HOBt·xH$_2$O (37 mg), 1-*iso*-propyl-1*H*-pyrazol-4-amine (30 mg, 0.24 mmol, 1.0 equiv), and EDC·HCl (46 mg, 0.24 mmol, 1.0 equiv) in DCM (1.7 mL) was stirred at room temperature overnight. The reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic phases were washed with water (1 × 10 mL), dried over $MgSO_4$ and filtered, and the solvent was removed *in vacuo*. The crude product was purified using preparative SFC-system C with a gradient from 10 to 15% MeOH (modified with 20 mM ammonia) within 11 min to give 2-(3,4-dimethoxyphenyl)-*N*-(1-*iso*-propyl-1*H*-pyrazol-4-yl)acetamide (**24c**) as a colorless oil (20 mg, 27%). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 10.06 (s, 1H, NH), 7.86 (d br, $^4J_{H-H}$ = 0.5 Hz, 1H, ^{i}PrNNCH), 7.39 (d, $^4J_{H-H}$ = 0.7 Hz, 1H, ^{i}PrNCH), 6.91 (d, $^4J_{H-H}$ = 2.0 Hz, 1H, CH_3OCCHC), 6.88 (d, $^3J_{H-H}$ = 8.2 Hz, 1H, $CH_3OCCHCH$), 6.80 (dd, $^3J_{H-H}$ = 8.2 Hz, $^4J_{H-H}$ = 2.0 Hz, 1H, $CH_3OCCHCH$), 4.42 (sept, $^3J_{H-H}$ = 6.7 Hz, 1H, $CHCH_3$), 3.73 (s, 3H, CH_3O), 3.71 (s, 3H, CH_3O), 3.46 (s, 2H, CH_2), 1.35 (d, $^3J_{H-H}$ = 6.7 Hz, 6H, $CHCH_3$). ^{13}C NMR (126 MHz, DMSO-d_6): δ = 167.7 ($CH_2C(O)$), 148.5 (CH_3OC), 147.6 (CH_3OC), 129.1 (^{i}PrNCH), 128.5 ($CCH_2C(O)$), 121.3 (CHCNH), 121.0 ($CH_3OCCHCH$), 118.0 (^{i}PrNNCH), 113.0 (CH_3OCCHC), 111.8 ($CH_3OCCHCH$), 55.6 (OCH_3), 55.5 (OCH_3), 52.9 (CCH_3), 42.1 (CH_2), 22.6 (CCH_3). HRMS (ESI): m/z calcd for $C_{16}H_{21}N_3O_3$ + H^+ [M + H]$^+$: 304.1656. Found: 304.1671.

*N-(1-(tert-Butyl)-1H-pyrazol-4-yl)-2-(3,4-dimethoxyphenyl)-acetamide (**24d**).* A solution of 2-(3,4-dimethoxyphenyl)acetic acid (42 mg, 0.21 mmol, 1.0 equiv), HOBt·xH$_2$O (33 mg), 1-(*tert*-butyl)-1*H*-pyrazol-4-amine (30 mg, 0.22 mmol, 1.0 equiv), and EDC·HCl (41 mg, 0.21 mmol, 1.0 equiv) in DCM (1.5 mL) was stirred at room temperature for 23 h. The solvent was then removed *in vacuo*, and the resulting residue was purified using preparative HPLC-system B with a gradient from 5 to 95% acetonitrile in water (aqueous phase modified with 0.1 M formic acid) within 10 min to give *N*-(1-(*tert*-butyl)-1*H*-pyrazol-4-yl)-2-(3,4-dimethoxyphenyl)acetamide (**24d**) as a brown solid (9 mg, 14%). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 10.06 (s, 1H, NH), 7.89 (d, $^4J_{H-H}$ = 0.7 Hz, 1H, ^{t}BuNNCH), 7.42 (d, $^4J_{H-H}$ = 0.7 Hz, 1H, ^{t}BuNCH), 6.90 (d, $^4J_{H-H}$ = 2.0 Hz, 1H, CH_3OCCHC), 6.88 (d, $^3J_{H-H}$ = 8.2 Hz, 1H, $CH_3OCCHCH$), 6.80 (dd, $^3J_{H-H}$ = 8.2 Hz, $^4J_{H-H}$ = 2.0 Hz, 1H, $CH_3OCCHCH$), 3.73 (s, 3H, CH_3O), 3.71 (s, 3H, CH_3O), 3.46 (s, 2H, CH_2), 1.46 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6): δ = 167.7 ($CH_2C(O)$), 148.5 (CH_3OC), 147.6 (CH_3OC), 129.1 (^{t}BuNCH), 128.5 ($CCH_2C(O)$), 121.3 (CHCNH), 121.0 ($CH_3OCCHCH$), 116.8 (^{t}BuNNCH), 113.0 (CH_3OCCHC), 111.8 ($CH_3OCCHCH$), 57.9 (CCH_3), 55.6 (OCH_3), 55.4 (OCH_3), 42.1 (CH_2), 29.4 (CCH_3). HRMS (ESI): m/z calcd for $C_{17}H_{23}N_3O_3$ + H^+ [M + H]$^+$: 318.1812. Found: 318.1808.

*1-(4-(4-(1-(Trifluoromethyl)-1H-pyrazole-4-carbonyl)piperazin-1-yl)phenyl)ethan-1-one (**25a**).* EDC·HCl (75 mg, 0.39 mmol, 1.0 equiv) and DMAP (48 mg, 0.39 mmol, 1.0 equiv) were added to a solution of 1-(4-(piperazin-1-yl)phenyl)ethan-1-one (95 mg, 0.47 mmol, 1.2 equiv) and 1-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (70 mg, 0.39 mmol, 1.0 equiv) in DMF (2.8 mL). The resulting reaction mixture was stirred overnight at room temperature. The solvent was then removed *in vacuo*, and the product was purified using automated flash column chromatography with a gradient from 5 to 50% ethyl acetate in heptane to give 1-(4-(4-(1-(trifluoromethyl)-1*H*-pyrazole-4-carbonyl)piperazin-1-yl)phenyl)ethan-1-one (**25a**) as a colorless powder (87 mg, 62%, as mixture of two rotamers as evident by two carbon signals for C(O)NCH_2 in ^{13}C NMR). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.89 (s, 1H, CF_3NCH), 8.21 (s, 1H, CF_3NNCH), 7.85−7.82 (m, 2H, C(O)CCH), 6.99−6.96 (m, 2H, NCCH), 3.75−3.73 (m, 4H, C(O)NCH_2), 3.46−3.44 (m, 4H, CHCNCH_2), 2.46 (s, 3H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6): δ = 195.7 (s, $CH_3C(O)$), 161.1 (s, NC(O)), 153.4 (s, NCCH), 144.1 (s, CF_3NNCH), 130.8 (s, CF_3NCH), 130.2 (s, NCCHCH), 126.9 (s, C(O)CCHCH), 119.5 (s, C(O)CCHN), 117.7 (q, $^1J_{C-F}$ = 263.0 Hz, CF_3), 113.2 (s, NCCH), 46.6 (s, C(O)NCH_2 of rotamer 1), 46.3 (s, C(O)NCH_2 of rotamer 2), 41.2 (s, CHCNCH_2), 26.2 (s, CH_3). ^{19}F NMR (471 MHz, DMSO-d_6): δ = −59.2 (s, CF_3). HRMS (ESI): m/z calcd for $C_{17}H_{17}F_3N_4O_2$ + H^+ [M + H]$^+$: 367.1377. Found: 367.1367.

*1-(4-(4-(1-iso-Propyl-1H-pyrazole-4-carbonyl)piperazin-1-yl)-phenyl)ethan-1-one (**25c**).* EDC·HCl (62 mg, 0.32 mmol, 1.0 equiv) and DMAP (40 mg, 0.33 mmol, 1.0 equiv) were added to a solution of 1-(4-(piperazin-1-yl)phenyl)ethan-1-one (79 mg, 0.39 mmol, 1.2 equiv) and 1-(*iso*-propyl)-1*H*-pyrazole-4-carboxylic acid (50 mg, 0.32 mmol, 1.0 equiv) in DMF (1.8 mL), and the yellow solution was stirred at room temperature. After 6 h the solvent was removed *in vacuo* and the resulting residue purified using SFC-system A with a gradient from 12 to 17% MeOH/H_2O 97:3 (modified with 50 mM ammonia) within 11 min, followed by a second purification using preparative HPLC-system F with a gradient from 2 to 94% acetonitrile in water (aqueous phase modified with 0.2% ammonia) within 10 min, to give 1-(4-(4-(1-*iso*-propyl-1*H*-pyrazole-4-carbonyl)piperazin-1-yl)phenyl)-ethan-1-one (**25c**) as a colorless solid (41 mg, 38%). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.15 (d, $^4J_{H-H}$ = 0.8 Hz, 1H, ^{i}PrNCH), 7.85−7.82 (m, 2H, C(O)CCH), 7.72 (d, $^4J_{H-H}$ = 0.8 Hz, 1H, ^{i}PrNNCH), 6.99−6.96 (m, 2H, NCCH), 4.53 (sept, $^3J_{H-H}$ = 6.7 Hz, 1H, CH_3CH), 3.77−3.75 (m, 4H, C(O)NCH_2), 3.44−3.42 (m, 4H, CHCNCH_2), 2.46 (s, 3H, C(O)CH_3), 1.43 (d, $^3J_{H-H}$ = 6.7 Hz, 6H, CH_3CH). ^{13}C NMR (126 MHz, DMSO-d_6): δ = 195.6 ($CH_3C(O)$), 162.9 (NC(O)), 153.5 (NCCH), 138.8 (^{i}PrNNCH), 130.2 (NCCHCH), 129.7 (^{i}PrNCH), 126.8 (C(O)CCHCH), 115.8 (C(O)CCHN), 113.1 (NCCH), 53.3 ($CHCH_3$), 46.5 (2C, C(O)NCH_2 and CHCNCH_2), 26.2 (C(O)CH_3), 22.6 ($CHCH_3$). HRMS (ESI): m/z calcd for $C_{19}H_{24}N_4O_2$ + H^+ [M + H]$^+$: 341.1973. Found: 341.1969.

*1-(4-(4-(1-tert-Butyl-1H-pyrazole-4-carbonyl)piperazin-1-yl)-phenyl)ethan-1-one (**25d**).* EDC·HCl (80 mg, 0.42 mmol, 1.0 equiv) and DMAP (51 mg, 0.42 mmol, 1.0 equiv) were added to a solution of 1-(4-(piperazin-1-yl)phenyl)ethan-1-one (102 mg, 499 μmol, 1.2

https://dx.doi.org/10.1021/acs.jmedchem.0c01457
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equiv) and 1-(*tert*-butyl)-1*H*-pyrazole-4-carboxylic acid (70 mg, 0.42 mmol, 1.0 equiv) in DMF (3.0 mL), and the yellow solution was stirred at room temperature. After 6 h, the solvent was removed *in vacuo* and the resulting residue was purified using preparative SFC-system A with a gradient from 12 to 17% MeOH/H_2O 97:3 (modified with 50 mM ammonia) within 11 min to give 1-(4-(4-(1-*tert*-butyl-1*H*-pyrazole-4-carbonyl)piperazin-1-yl)phenyl)ethan-1-one (**25d**) as a colorless solid (43 mg, 29%). ^{1}H NMR (600 MHz, DMSO-d_6): δ = 8.13 (d, $^4J_{H-H}$ = 0.5 Hz, 1H, ^{t}BuNC<u>H</u>), 7.84−7.82 (m, 2H, C(O)CC<u>H</u>), 7.74 (d br, $^4J_{H-H}$ = 0.5 Hz, 1H, ^{t}BuNNCH), 6.97−6.95 (m, 2H, NCC<u>H</u>), 3.77−3.75 (m, 4H, C(O)NC<u>H$_2$</u>), 3.43−3.41 (m, 4H, CHCNC<u>H$_2$</u>), 2.45 (s, 3H, C(O)C<u>H$_3$</u>), 1.54 (s, 9H, NCC<u>H$_3$</u>). ^{13}C NMR (151 MHz, DMSO-d_6): δ = 195.7 (CH_3<u>C</u>(O)), 163.1 (N<u>C</u>(O)), 153.5 (N<u>C</u>CH), 138.6 (^{t}BuNN<u>C</u>H), 130.2 (NCCH<u>C</u>H), 128.7 (^{t}BuN<u>C</u>H), 126.8 (C(O)<u>C</u>CHCH), 115.8 (C(O)<u>C</u>CHN), 113.1 (NC<u>C</u>H), 58.8 (N<u>C</u>CH$_3$), 46.6 (2C, CHCN<u>C</u>H$_2$ and C(O)N<u>C</u>H$_2$), 29.4 (NC<u>C</u>H$_3$), 26.1 (C(O)<u>C</u>H$_3$). HRMS (ESI): *m*/*z* calcd for $C_{20}H_{26}N_4O_2$ + H^+ $[M + H]^+$: 355.2129. Found: 355.2124.

*Hydrolysis of 4-Methoxy-N-methyl-N-(trifluoromethyl)aniline (**4**) to Form (4-Methoxyphenyl)(methyl)carbamic Fluoride (**17**).* A solution of 4-methoxy-*N*-methyl-*N*-(trifluoromethyl)aniline (**4**, 2 mg, 10 μmol) in DMSO-d_6/D_2O 1:4 (150 μL) was left standing under air at room temperature. After 1 h, the reaction mixture was analyzed by NMR and GCMS. Due to the partial double bond character of the N−C(O) bond, two rotamers in a ratio of 1:1 are observed in NMR. ^{1}H NMR (500 MHz, DMSO-d_6/D_2O 1:4): δ = 7.20 (d, $^3J_{H-H}$ = 8.9 Hz, 2H, C<u>H</u>), 7.18−7.14 (m, 2H, C<u>H</u>), 6.91−6.86 (m, 4H, C<u>H</u>), 3.68 (s, 3H, OC<u>H$_3$</u>), 3.67 (s, 3H, OC<u>H$_3$</u>), 3.15 (d, $^4J_{H-F}$ = 0.8 Hz, 3H, NC<u>H$_3$</u>), 3.12 (d, $^4J_{H-F}$ = 0.7 Hz, 3H, NC<u>H$_3$</u>). ^{13}C NMR (126 MHz, DMSO-d_6/D_2O 1:4): δ = 159.9 (s, CH_3O<u>C</u>), 159.8 (s, CH_3O<u>C</u>), 149.4 (d, $^1J_{C-F}$ = 284.3 Hz, <u>C</u>(O)F), 149.2 (d, $^1J_{C-F}$ = 202.4 Hz, <u>C</u>(O)F), 136.0 (s, N<u>C</u>CH), 135.2 (s, N<u>C</u>CH), 128.8 (s, OCCH<u>C</u>H), 128.5 (s, OCCH<u>C</u>H), 116.5 (s, OCC<u>H</u>), 116.3 (s, OCC<u>H</u>), 57.2 (s, O<u>C</u>H$_3$), 57.2 (s, O<u>C</u>H$_3$), 40.3 (s, N<u>C</u>H$_3$), 39.9 (s, N<u>C</u>H$_3$).^{19}F NMR (471 MHz, DMSO-d_6/D_2O 1:4): δ = −17.9 (s, C(O)<u>F</u>), −20.5 (s, C(O)<u>F</u>). GCMS (EI): *m*/*z* calcd for $C_9H_{10}FNO_2^+$ $[M]^+$: 183.1. Found: 183.1.

*Hydrolysis of 2-(Dimethylamino)ethyl 4-(butyl(trifluoromethyl)-amino)benzoate (**20a**) to Form 2-(Dimethylamino)ethyl 4-(butyl-(fluorocarbonyl)amino)benzoate (**26**).* A solution of 2-(dimethylamino)ethyl 4-(butyl(trifluoromethyl)amino)benzoate (**20a**, 2 mg, 6 μmol) in DMSO-d_6/D_2O 1:4 (3.2 mL) was left standing under air at room temperature. After 24 h, the solvent was removed *in vacuo* and the reaction mixture was analyzed by NMR and LCMS. Due to the partial double bond character of the N−C(O) bond, two rotamers in a ratio of 0.4:1 are observed in the ^{19}F NMR.[73] ^{1}H NMR (500 MHz, CD_2Cl_2): δ = 8.09 (d, $^3J_{H-H}$ = 8.4 Hz, 2H, C(O)CC<u>H</u>), 7.32 (d, $^3J_{H-H}$ = 8.0 Hz, 2H, NCC<u>H</u>), 4.45 (t, $^3J_{H-H}$ = 5.7 Hz, 2H, CH_2C<u>H$_2$</u>O), 3.72 (t, $^3J_{H-H}$ = 7.5 Hz, 2H, C(O)NC<u>H$_2$</u>), 2.77 (t, $^3J_{H-H}$ = 5.6 Hz, 2H, C<u>H$_2$</u>CH_2O), 2.37 (s, 6H, NC<u>H$_3$</u>), 1.62−1.51 (m, 2H, C<u>H$_2$</u>CH_2CH_3), 1.35 (sept, $^3J_{H-H}$ = 7.5 Hz, 2H, CH_2C<u>H$_2$</u>CH_3), 0.91 (t, $^3J_{H-H}$ = 7.4 Hz, 3H, CH_2CH_3). ^{13}C NMR (126 MHz, CD_2Cl_2): δ = 165.9 (s, <u>C</u>(O)O), 146.1 (d, $^1J_{C-F}$ = 298.7 Hz, <u>C</u>(O)F), 144.1 (s, C(O)<u>C</u>), 131.3 (s, C(O)C<u>C</u>H), 130.2 (s, N<u>C</u>CH), 127.2 (s, NC<u>C</u>H), 63.4 (s, CH_2C<u>H$_2$</u>O), 58.1 (s, C<u>H$_2$</u>CH_2O), 51.9 (s, C(O)N<u>C</u>H$_2$), 45.9 (s, N<u>C</u>H$_3$), 30.0 (s, <u>C</u>H$_2$$CH_2CH_3$), 20.2 (s, CH_2<u>C</u>H$_2$$CH_3$), 13.9 (s, CH_2CH_2<u>C</u>H$_3$). ^{19}F NMR (471 MHz, CD_2Cl_2): δ = −14.1 (s, 1F, C(O)<u>F</u>), −17.9 (s, 0.4F, C(O)<u>F</u>). HRMS (ESI): *m*/*z* calcd for $C_{16}H_{23}FN_2O_3$ + H^+ $[M + H]^+$: 311.1766. Found: 311.1775.

Stability Study in DMSO. Solutions (10 mM) of the respective compounds in DMSO were left standing at room temperature in glass vials without any additional precautions against moisture or oxygen. After 1 month, aliquots (10 μL) were taken out and analyzed with LCMS.

Stability Study in H_2O/DMSO 4:1. The respective compounds were dissolved in DMSO and then mixed with water to prepare a 2 mM stock solution in 4:1 H_2O/DMSO. Due to the weak UV absorption of *N*-trifluoromethyl pyrazole, a 10 mM solution of this compound was used. The stock solutions were left standing at room temperature in glass vials without any additional precautions against moisture or oxygen. After 0, 0.5, 6, and 24 h and 1 month, aliquots (10 μL) were taken and analyzed with LCMS. The area under the absorbance curve between 220 and 350 nm of the remaining *N*-trifluoromethyl amine at the respective time point was calculated and divided by the area under the absorbance curve between 220 and 350 nm of the *N*-trifluoromethyl amine at t = 0 h.

Determination of stability at pH 1.0, 7.4, and 10.0. A 25 μM solution of the respective compound in 0.1 M HCl solution (pH 1.0) or 20 mM sodium phosphate buffer (pH 7.4) or 20 mM sodium carbonate buffer (pH 10.0) was incubated at 70 °C at 300 rpm using an Eppendorf Thermomixer Comfort plate shaker. After 0, 2, 4, 8, and 24 h, an aliquot (150 μL) was analyzed using a Waters Acquity UPLC H-Class/QDA equipped with a Waters Xselect HSS T3 C18 column (2.5 μm, 2.1 mm × 50 mm) with a gradient of 5−98% acetonitrile in water (modified with 0.1% formic acid) within 4 min at 40 °C to determine the peak area of the parent compound. The slope *k* is determined by linear regression of the natural logarithm of the peak area of the parent compound vs incubation time using Microsoft Excel. The half-life $t_{1/2}$ is determined using the following equation

$$t_{1/2} = -0.693/k$$

The extrapolated half-life at 25 °C is calculated by taking the measured half-life at 70 °C and assuming a factor of 2 change in reaction rate for each 10 °C reduction in temperature.

Measuring Compound Reactivity against GSH. The reactivity of the compounds against GSH was determined by the DMPK department at Pharmaron. To do so, a 1 μM solution of the respective compound was incubated in the presence of 4.6 mM glutathione in 0.02 M phosphate-buffered saline (pH 7.4) and 1 mM EDTA-Na_2 at 37 °C. Verapamil was used as an internal standard. The loss of the parent was monitored by LCMS using a Waters UPLC ACQ-TQC equipped with an Acquity UPLC system and a Waters Xselect HSS T3 C18 column (2.5 μm, 2.1 mm × 50 mm). The slope k was determined by linear regression of the natural logarithm of the area ratio (remaining parent peak area normalized to verapamil peak area) of the parent drug vs incubation time. The half-life was calculated using the following equation:

$$t_{1/2} = -0.693/k$$

To rule out non-GSH-mediated loss of the parent, a control reaction of the respective compound in buffer alone was investigated in parallel. Additionally, to determining the loss of the parent, the potential formation of the corresponding glutathione adduct was analyzed by LCMS.

Measurements of Shake-Flask log $D_{7.4}$ and Metabolic Stability in Human Liver Microsomes. Log $D_{7.4}$ measurements and measurements of the metabolic stability in human liver microsomes were performed according to Wernevik et al.[74]

Chromlog $D_{7.4}$ Measurements. A 0.5 mM DMSO solution (1 μL) of the compound was analyzed by LCMS using a Waters Acquity with a BEH C18 column (1.7 μm, 50 mm × 2.1 mm) using a gradient from 0 to 100% acetonitrile/H_2O 95:5 in acetonitrile/H_2O 5:95 (adjusted with ammonia to pH 7.4) within 1.76 min with a flow rate of 1.0 mL/min at 40 °C. The retention factor *k*′ was calculated from the measured retention time r_t of the compound using the following formula:

$$k' = (r_t - t_0)/t_0$$

with t_0 being the retention time of DMSO. Metoprolol, warfarin, propranolol, chlorpromazine, and felodipine were used as standards. A calibration curve by plotting the measured *k*′ of the standards vs their literature known log $D_{7.4}$ values was used to transform the determined k′ of the sample into the chromlog $D_{7.4}$.

ePSA measurements. A 1.2 mM DMSO solution of the respective compound was analyzed by SFC using a Phenomenex Chirex 3014 column (4.6 mm × 50 mm) at 40 °C with 20 mM ammonium formate in MeOH as the co-solvent. A gradient from 5 to 60% of the co-solvent within 2 min with a flow rate of 4 mL/min was used. Antipyrene, chlorpromazine, desipramine, pindolol, diclofenac, 3-nitrobenzoic acid, bumetanide, and furosemide were used as calibration standards. A calibration curve by plotting the measured retention times of the

https://dx.doi.org/10.1021/acs.jmedchem.0c01457
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calibration standards vs their known ePSA values was used to transform the measured retention time of the analyte into its ePSA value.

Caco-2 Measurements. Caco-2 measurements were performed by the DMPK department at Pharmaron as described by Fredlund et al.[75]

pK_a measurements. The pK_a was determined spectrophotometrically by Pion using a SiriusT3 following a procedure of Hossain et al.[76]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c01457.

NMR spectra for novel compounds and purity analyses (PDF)

Molecular formula strings and associated data (CSV)

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Author Contributions

S.S. and R.J.C. conceived the project and wrote the majority of the manuscript. S.S. led the project and synthesized some of the investigated compounds. S.S., R.J.C., and W.C. designed the compounds investigated and analyzed data. H.C., J.K., and T.Q. synthesized some of the investigated compounds, supported the analysis of the data, and wrote parts of the manuscript. All authors have given approval to the final version of the manuscript.

Notes

The authors declare the following competing financial interest(s): S.S., J.K., R.J.C, and W.C. are employees and shareholders of AstraZeneca. H.C. and T.Q. declare no conflict of interest.

■ ACKNOWLEDGMENTS

We would like to thank the analytical and separation science teams at AstraZeneca Gothenburg for purification of final compounds and analytical support. We also thank colleagues at AstraZeneca and Pharmaron for measuring the *in vitro* properties. Furthermore, we thank Matthew Perry and Martin Hemmerling (both AstraZeneca Gothenburg) for critically proofreading the manuscript and James Bird (AstraZeneca Gothenburg) for fruitful discussions on the measured *in vitro* properties. H.C. and T.Q. thank the Erasmus+ program for fellowships during their internships at AstraZeneca.

■ ABBREVIATIONS

au, arbitrary units; BCRP, breast cancer resistance protein; BRAF, b-rapidly accelerated fibrosarcoma; chromlog $D_{7.4}$, chromatographically determined logarithm of distribution coefficient at pH 7.4; Caco-2, human epithelial colorectal adenocarcinoma cells; DAST, (diethylamino)sulfur trifluoride; DMAP, 4-(dimethylamino)pyridine; EDC, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide; ePSA, experimental polar surface area; GCMS, gas chromatography-mass spectrometry; GSH, glutathione; HLM, human liver microsome; HOBt, 1-hydroxybenzotriazole; IRAK4, interleukin-1 receptor associated kinase 4; LCMS, liquid chromatography-mass spectrometry; log $D_{7.4}$, logarithm of distribution coefficient in 1-octanol water at pH 7.4; MRP2, multidrug-associated protein 2; TEA, triethylamine; UPLC, ultra-performance liquid chromatography

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