Organic & Biomolecular Chemistry

RSC Publishing

PAPER

View Article Online
View Journal | View Issue

Cite this: Org. Biomol. Chem., 2013, 11, 3706

Structural modifications of (*Z*)-3-(2-aminoethyl)-5-(4-ethoxybenzylidene)thiazolidine-2,4-dione that improve selectivity for inhibiting the proliferation of melanoma cells containing active ERK signaling

Kwan-Young Jung,†[a] Ramin Samadani,†[a] Jay Chauhan,[a] Kerrick Nevels,[a] Jeremy L. Yap,[a] Jun Zhang,[a] Shilpa Worlikar,[a] Maryanna E. Lanning,[a] Lijia Chen,[a] Mary Ensey,[b] Sagar Shukla,[a] Rosene Salmo,[c] Geoffrey Heinzl,[a] Caryn Gordon,[d] Troy Dukes,[e] Alexander D. MacKerell, Jr.,[a,f] Paul Shapiro*[a,f] and Steven Fletcher*[a,f]

Received 30th January 2013,
Accepted 9th April 2013

DOI: 10.1039/c3ob40199e

www.rsc.org/obc

We herein report on the pharmacophore determination of the ERK docking domain inhibitor (*Z*)-3-(2-aminoethyl)-5-(4-ethoxybenzylidene)thiazolidine-2,4-dione, which has led to the discovery of compounds with greater selectivities for inhibiting the proliferation of melanoma cells containing active ERK signaling.

Introduction

The extracellular signal-regulated kinases-1 and 2 (ERK1/2) are ubiquitous mediators of intracellular signaling events and are regulated in response to a variety of membrane receptors including receptor tyrosine kinases (RTK), G-protein coupled receptors, and cytokine receptors.[1] Activated receptors promote the sequential activation of the Ras G-proteins, Raf kinases, MAP or ERK kinases 1 and 2 (MEK1/2), and finally ERK1/2. MEK1/2 are thought to be the primary kinases that directly activate ERK1/2 through phosphorylation of a threonine and a tyrosine located in a TXY motif found in MAP kinase family members.[1b] The ERK proteins may phosphorylate and regulate the activity of hundreds of different substrate proteins found in both the cytoplasm and nucleus.[1b,2] As such, many extracellular signals use ERK1/2 to transmit information to regulate a variety of cellular functions including proliferation, survival, differentiation, and migration.

Significant efforts in developing anti-cancer therapies have focused on targeted inhibition of the ERK1/2 pathway in the context of cancers containing activating mutations in upstream RTK, Ras and B-Raf proteins.[3] The utility of many of these targeted compounds has been limited due to issues with lack of efficacy, toxicity, and the development of drug resistance through mutations or activation of alternative survival pathways.[3l,4] Promising new drugs that target the mutated and active form of B-Raf in melanoma cells have clinical limitations due to the unanticipated activation of the ERK1/2 pathway through alternative mechanisms and the activation of compensatory signaling pathways that lead to drug resistance.[5]

While constitutive activation of the ERK1/2 proteins function in sustaining cancer cell proliferation and survival, regulated activity of ERK1/2 serves integral roles in normal cell processes. Thus, in the absence of discriminating between cancer and normal cells, inhibitors that completely block ERK1/2 signaling are destined to have off-target toxicity to normal cells. Given that ERK proteins may have nearly 300 interacting partners with over half of these being phosphorylated substrates,[2] the identification of molecules that disrupt ERK1/2 interactions with substrates relevant to cancer cell proliferation and survival may have a significant impact on the development of novel inhibitors that prevent the phosphorylation and regulation of ERK substrates involved in disease processes while preserving ERK functions in normal cells. In the current studies, we describe the optimization of compounds that are predicted to target ERK substrate docking sites and their selective inhibition of melanoma cancer cells containing constitutively activated ERK signaling. Moreover, we show that

[a]*Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy, 20 N Pine St., Baltimore, MD 21201, USA. E-mail: sfletche@rx.umaryland.edu, pshapiro@rx.umaryland.edu; Fax: +1 410 706 5017; Tel: +1 410 706 6361*
[b]*Notre Dame of Maryland University, 4701 N Charles Street, Baltimore, MD 21212, USA*
[c]*Indiana University Purdue University Indianapolis, 420 University Boulevard Indianapolis, IN 46202, USA*
[d]*University of Maryland, College Park, MD 20742, USA*
[e]*Antioch Diploma Plus High School, 2555 Harford Road, Baltimore, MD 21218, USA*
[f]*University of Maryland Greenebaum Cancer Center, Baltimore, MD 21201, USA*
†These authors contributed equally to this work.

optimized compounds are effective at inhibiting melanoma cells that have become resistant to B-Raf targeted therapies.

Chemistry

We previously reported the successful use of computer-aided drug design (CADD) to search for low-molecular-weight docking domain inhibitors of ERK.[6] One of the compounds, identified as **76**, binds ERK2 with a K_d value of 5 μM and blocks phosphorylation of the downstream ERK targets RSK-1 and Elk-1. Moreover, **76** inhibit2 growth of HeLa cervical carcinoma and A549 lung carcinoma cells as assessed by colony formation assay in a dose dependent manner with IC_{50} values of ~20 μM.[6b] Compound **76** was predicted by CADD to target a polar cleft within the D-recruitment site (DRS), which facilitates ERK interactions with substrates containing D domains (also referred to as the DEJL motif or Docking site for ERK or JNK, LXL).[7] The DRS consists of aspartate residues that form the common docking (CD) and threonine residues in ED docking sites.[8] It was proposed that the primary amino group of **76** was engaged in salt bridge interactions with Asp316 and Asp319 of the CD site and was within 5–7 Å of Thr157 and Thr158 of the ED site.[6b] In addition, commercially available analogues of **76** obtained from a structure-based similarity search showed activity in preliminary experiments (not shown), indicating the compound to be a suitable lead for additional optimization.[9] To determine the pharmacophore of **76**, which would help direct future optimization efforts, we embarked on a structure–activity relationship (SAR) study. Comprised of three distinct components – a 4-ethoxybenzylidene group, a thiazolidine-2,4-dione (TZD) core and an ethylamine tail (Fig. 1) – compound **76** was re-synthesized in just three linear steps (Scheme 1). Briefly, a Knoevenagel condensation of thiazolidine-2,4-dione (**1**) with 4-ethoxybenzaldehyde afforded benzylidene **2**. Alkylation of the acidic imide NH of **2** was then accomplished *via* Mitsunobu conditions (diisopropyl azodicarboxylate (DIAD) and triphenylphosphine (PPh_3)) with *N*-trityl-ethanolamine to give **3**, which, upon brief treatment with TFA, furnished the lead compound **76**.

Recently, Li *et al.* conducted an SAR analysis of the 4-ethoxyphenyl moiety of **76**.[10] It was found that shifting the 4-ethoxy group to the 2-position of the phenyl ring led to increased inhibition of human leukemia U937 cell proliferation. In a subsequent study, removal of the 4-ethoxy group and extension of the benzylidene moiety to a 3-phenylpropylidene moiety

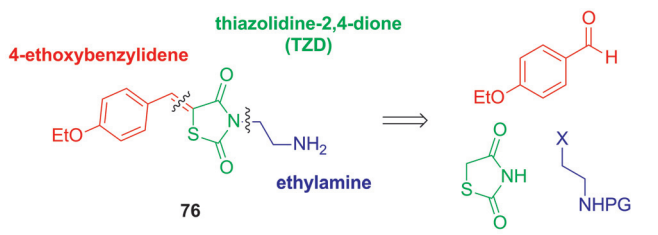
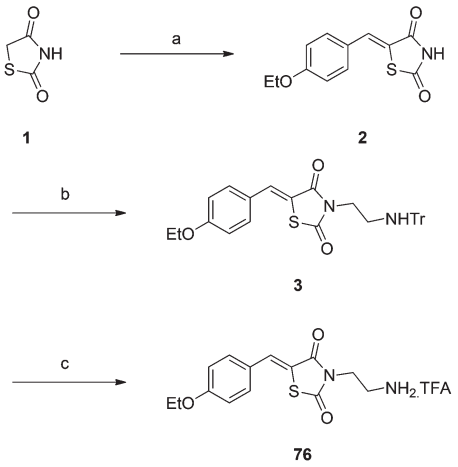


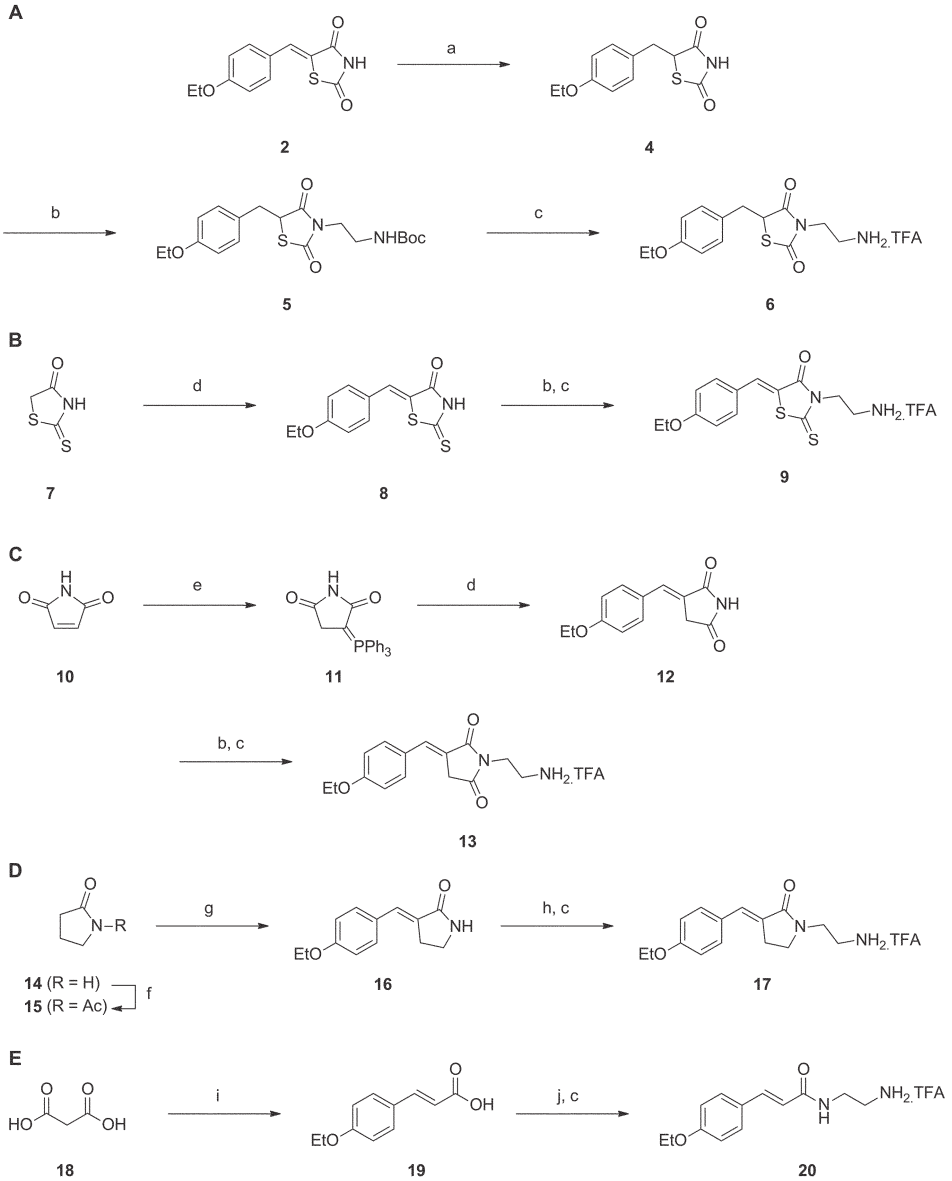
Fig. 1 A retrosynthetic analysis reveals the structural components of lead compound **76**.



Scheme 1 (a) 4-Ethoxybenzaldehyde, cat. piperidine, EtOH, reflux, 16 h, 77%; (b) $HOCH_2CH_2NHTr$, PPh_3, DIAD, THF, rt, 16 h, 75%; (c) TFA–CH_2Cl_2, 1 : 1, rt, 30 min, 99%.

furnished a dual inhibitor of the Raf/MEK/ERK and PI3K/Akt signaling pathways.[11] In the present work, a cell-based SAR analysis of **76** focused on the TZD component and ethylamine moiety, in which cancer cells with activated ERK signaling were employed. Additionally, further novel analogs that explored the SAR of the 4-ethoxyphenyl group were also prepared, directly complementing the aforementioned studies.[10]

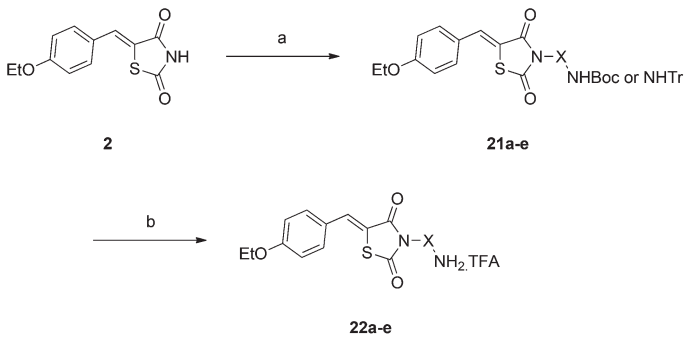
Thiazolidine-2,4-dione (TZD) SAR. In order to determine the contribution of the TZD portion of **76** to the inhibition of ERK, we prepared all of the TZD analogues (**6**, **9**, **13**, **17** and **20**) depicted in Scheme 2. First, to examine its role, the benzylidene C=C double bond of **76** was chemoselectively reduced with $LiBH_4$ as shown in Scheme 2A to deliver compound **4**, the imide nitrogen of which was alkylated with *N*-Boc-2-bromoethylamine to give **5**, and then TFA-mediated removal of the Boc group furnished target molecule **6**. In order to generate the rhodanine analogue (**9**) of **76**, the chemistry carried out was the same as that set forth in Scheme 1, substituting **1**for rhodanine (**7**). For the cyclic imide derivative **13**, reaction of maleimide (**10**) with PPh_3 afforded the stabilized phosphorane **11**, which underwent a Wittig reaction with 4-ethoxybenzaldehyde to furnish benzylidene **12**. *N*-Alkylation of **12** with *N*-Boc-2-bromoethylamine, and subsequent removal of the Boc group gave the imide-functionalized product **13**. Acetylation of pyrrolidin-2-one (**14**), followed by a modified Knoevenagel condensation reaction with 4-ethoxybenzaldehyde yielded benzylidene **16**. In this case, the amide NH of **16** was insufficiently acidic to undergo the Mitsunobu reaction with *N*-trityl-ethanolamine (**5**); instead, *N*-trityl-ethanolamine was first activated as its *O*-mesylate and then conjugated to the amide anion of **16**. Deprotection of the Tr group by treatment with TFA afforded the target molecule **17**. Finally, to investigate the importance of the cyclic nature of the TZD ring of **76**, we constructed the acyclic analog **20**. Once more, a Knoevenagel condensation played a key role in the synthesis, conjugating malonic acid (**18**) to 4-ethoxybenzaldehyde to furnish the cinnaminic acid derivative **19**, which



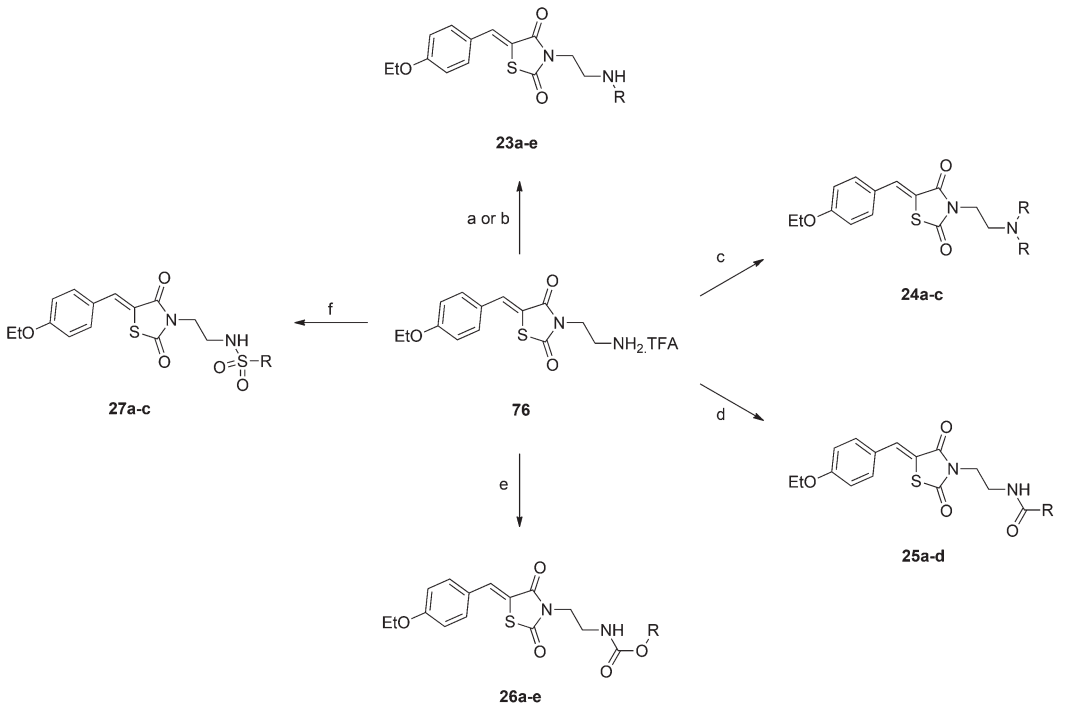
Scheme 2 (a) $LiBH_4$, THF–pyridine, 80 °C, 12 h, 77%; (b) $BrCH_2CH_2NHBoc$, K_2CO_3, DMF, rt, 16 h, 43–53%; (c) TFA–CH_2Cl_2, 1 : 1, rt, 1 h, 43–99%; (d) 4-ethoxybenzaldehyde, cat. piperidine, EtOH, reflux, 1–16 h, 77–93%; (e) PPh_3, acetone, reflux, 1 h, 83%; (f) Ac_2O, THF, reflux, 2 h, 77%; (g) 4-ethoxybenzaldehyde, NaO^tBu, 0 °C to 55 °C, THF, 1 h, 45%; (h) $MsOCH_2CH_2NHTr$, NaH, rt, DMF, 16 h, 58%; (i) 4-ethoxybenzaldehyde, piperidine, pyridine, 120 °C, 4 h, 99%; (j) $BocNHCH_2CH_2NH_2$, HBTU, DIPEA, DMF, rt, 3 h, 99%.

was then coupled to *N*-Boc-ethylenediamine and subsequently deprotected to give acrylamide derivative **20**.

Ethylamine SAR. The ethylamine group of **76** was modulated to a variety of alternative groups; these can be divided into three categories: alkylamino substitutions, *N*-functionalized ethylamino substitutions, which may be further subdivided into basic and non-basic substitutions, and, finally, alkyl and benzylic substitutions. For the alkylamino substitutions, which were designed to investigate the optimal spacer between the TZD ring and the basic amino group, as well as the identity of the basic group itself, benzylidene **2** was prepared as earlier described. The imide NH of **2** was next alkylated *via* classical conditions or the Mitsunobu reaction with the appropriate bromide or alcohol, respectively, to afford *N*-Boc or *N*-Tr intermediates **21**, as shown in Scheme 3, which were then deprotected under acidic conditions to deliver the final products **22** as their TFA salts. For the basic *N*-functionalized ethylamino substitutions (Scheme 4), **76** was converted to secondary amines **23a–e** either through mono-reductive amination conditions or *de novo* syntheses, and tertiary amines **24a–c** through one-pot, double-reductive aminations. In the course of our work, we discovered that the neutral *N*-Boc-protected derivative of **76** exhibited some activity in the cellular assays. Presumably, this molecule exhibits a different binding mode to the parent compound that carries a basic, primary amine. Nonetheless, we elected to investigate further



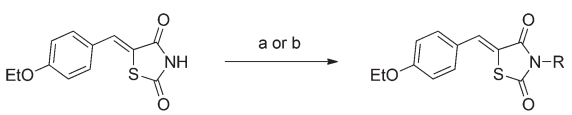
Scheme 3 (a) Br-X-NHBoc, K_2CO_3, DMF, rt, 3 h, 16–79% or HO-X-NHTr, PPh_3, DIAD, THF, 45 °C, 16 h, 99%; (b) TFA–CH_2Cl_2, 1 : 1, rt, 1 h, 36–97%.



Scheme 4 (a) R^2CHO, $NaBH(OAc)_3$, CH_2ClCH_2Cl, rt, 16 h, 38–69%; (b) 1. $HOCH_2CH_2N(R)Tr$, PPh_3, DIAD, THF, rt, 16 h; 2. TFA–CH_2Cl_2, rt, 30 min, 28–37% (two steps); (c) excess R^2CHO, $NaBH(OAc)_3$, CH_2ClCH_2Cl, rt, 16 h, 69–93%; (d) $(RCO)_2O$, CH_2Cl_2, DIPEA, rt, 16 h, 81–92% or 4-cyanobenzoic acid, HBTU, DIPEA, DMF, rt, 16 h, 92%; (e) ROCOCl, DIPEA, CH_2Cl_2, rt, 16 h, 96–98%; (f) RSO_2Cl, DIPEA, CH_2Cl_2, rt, 16 h, 92–98%.

substitutions of this ethylamino group that included additional non-basic derivatives. Compound **76** was thus subjected to a variety of acylations, alkoxycarbonylations and sulfonylations to furnish the corresponding amides (**25a–d**), carbamates (**26a–e**) and sulfonamides (**27a–c**), respectively, according to standard chemistry outlined in Scheme 4. Finally, for the non-amino-based alkyl and benzylic substitutions, the imide NH of **3** was functionalized using classical conditions with K_2CO_3 and the appropriate alkyl or benzylic bromide or chloride at room temperature, 60 °C or 100 °C, depending on the reactivity of the halide, to afford compounds **28a–s** (Scheme 5).

4-Ethoxyphenyl SAR. Variation of the 4-ethoxyphenyl component of **76** was accomplished *via* the chemistry described in Scheme 6. Briefly, after a Mitsunobu reaction of **1** with *N*-trityl-ethanolamine, resulting compound **29** was subjected to

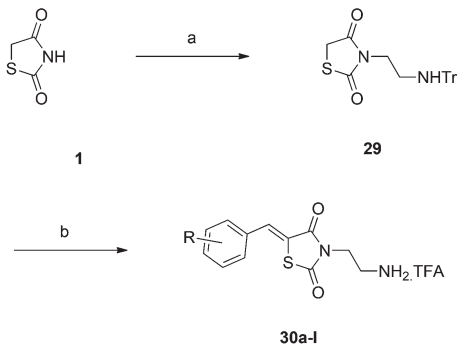


Scheme 5 (a) HO-X-NHTr, PPh_3, DIAD, THF, 45 °C, 12 h, 61–95%; (b) RBr, K_2CO_3, DMF, rt or 100 °C, 3 h, 78–99% or RCl, K_2CO_3, DMF, 60 °C, 3 h, 93–96%.

Knoevenagel condensations with a variety of aldehydes, and then subsequently deprotected under acidic conditions to furnish the final molecules **30a–l**.

Biology

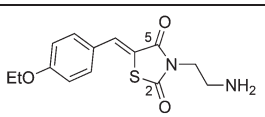
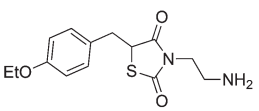
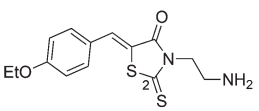
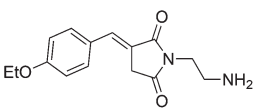
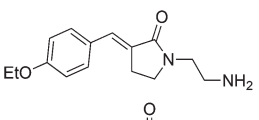
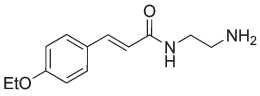
For initial phenotypic screening, we evaluated the activity of **76** analogues on proliferation of A375 and SK-MEL-28 melanoma



Scheme 6 (a) $HOCH_2CH_2NHTr$, PPh_3, DIAD, THF, rt, 16 h, 75%; (b) 1. ArCHO, cat. piperidine, EtOH, reflux, 16 h; 2. RBr or RI, K_2CO_3, DMF, 70 °C, 12 h; 3. TFA–CH_2Cl_2, 1 : 1, rt, 30 min, 20–73% (two or three steps).

cell lines, which have constitutively active ERK1/2 due to a homozygous mutation in B-Raf that drives proliferation and survival.[12] In addition, compounds were tested in RPMI-7951 cells, which also contain mutated B-Raf and are resistant to B-Raf inhibitors due to the overexpression of MAP3K8 (the gene encoding COT/Tpl2) that provides an alternative mechanism to indirectly or directly activate ERK proteins.[5c,13] We also studied the compounds' effects on HL-60 leukemic cells that are p53 defective and have an activating mutation in N-Ras, and, therefore, also demonstrate upregulation of ERK1/2. For comparison, HeLa cervical carcinoma and Jurkat T-cell leukemia cells, which are p53 defective cancer cell lines but contain no known activating mutations in the ERK pathway, were studied.[14] All compounds were initially tested at 100 μM for their abilities to inhibit cell proliferation, and data are presented as a percentage of the vehicle-treated control (100%). Table 1 shows the cell data for analogues of **76** in which the TZD core was varied. As compared to the parent compound **76**, which showed selective inhibition of melanoma cells, reduction of the benzylidene double bond (compound **6**) led to almost complete loss of growth inhibition activity in all cell lines, indicating this double bond plays either a structural role to maintain **76** in a β-strand-like structure[15] and/or is functionally significant as a Michael acceptor. That is, the biological activity of **76** may derive, in part, from its ability to act as an irreversible inhibitor through covalent alkylation of amino acid side chains, particularly cysteines, on the surface of the ERK1/2 proteins. The isosteric replacement of the 2-carbonyl oxygen with a sulfur atom (compound **9**) resulted both in a reduction of inhibitory activity and a reduction in selectivity for melanoma cells. The endocyclic sulfur appears to be important, since its replacement with an isosteric methylene group (compound **13**) resulted in a less potent and selective inhibitor of cells containing B-Raf or N-Ras mutations compared to the parent compound **76**, indicating the sulfur atom may function as a hydrogen bond acceptor. Removal of the carbonyl group of **13** that is juxtaposed to the endocyclic sulfur in **76** resulted in compound **17** that was almost completely bereft of inhibitory activity (Table 1). Furthermore, deletion of the two endocyclic methylene groups of **17** afforded the non-cyclic compound **20**, which exhibited little inhibition of any of the cells. Taken together, these data confirm the importance of

Table 1 Cell viability SAR of thiazolidine-2,5-dione (TZD) ring of **76** at 100 μM

		Cell viability (% of vehicle)					
Compound number	Structure	SK-MEL-28	A375	RPMI-7951	HL-60	HeLa	Jurkat
76		11 ± 7	2.1 ± 2	0.3 ± 0.02	4.4 ± 1	53 ± 3	59 ± 5
6		88 ± 3	92 ± 6	70 ± 5	41 ± 17	90 ± 4	108 ± 10
9		45 ± 7	21 ± 10	3.3 ± 2	7.9 ± 3	26 ± 6	52 ± 4
13		65 ± 4	29 ± 3	19 ± 5	58 ± 28	75 ± 2	100 ± 10
17		79 ± 6	95 ± 2	96 ± 3	73 ± 9	89 ± 4	92 ± 2
20		83 ± 4	81 ± 5	93 ± 9	59 ± 25	94 ± 5	89 ± 7

the benzylidene double bond and the TZD core towards effective inhibition of cell lines harboring constitutive ERK activation.

Analysis of the results in Table 2 reveals that, with the exception of the longer 3-propylamine derivative **22a**, modification of the ethyl portion of the ethylamine tail of **76** led to either a reduction in activity and/or selectivity against melanoma cells (compare data for **76** with that for **22a–e**). Alkylation of the primary amino group of **76** to afford secondary amines resulted in a drop in inhibitory activity with small groups (*e.g.* methyl, **23a**) that could be recovered with bulkier groups (*e.g.* isobutyl, **23c**; benzyl, **23d**). However, selective inhibition of cell lines containing activating B-Raf or N-Ras mutations was largely lost. The corresponding tertiary amines **24a–c** exhibited no inhibition of any of the cell lines. Collectively, these findings demonstrate the significance of the primary amino group of **76**, suggesting it may engage in multiple hydrogen bonds. The corresponding tertiary amines **24a–c** exhibited no inhibition of any of the cell lines. Similarly, little inhibition of cell proliferation was observed upon replacement of the ethylamine group ($pK_a \sim 10$) with the less basic imidazole derivative **28a** ($pK_a \sim 7$) or the 2-aminopyridine derivative **28b** ($pK_a \sim 6$), the latter of which could be envisaged to engage in hydrogen-bonded chelates with Asp316 or Asp319 through its tautomeric pyridin-2(1*H*)-imine. However, growth inhibitory activity was observed with the considerably more basic ($pK_a \sim 12$) guanidine derivative **28c**, although there was no apparent selectivity for the melanoma cells over HL-60, HeLa or Jurkat cells. A finely-tuned basic group, therefore, is required at the terminus of the ethyl group of **76** to confer both potency and selectivity against cells with constitutive ERK activation.

In general, data in Table 3 demonstrate that blockade of the amino group of **76** as neutral amides, carbamates and sulfonamides (**25a–27c**) afforded compounds with little to no inhibitory activity, underscoring the importance of the basic character of the primary amino group of **76**, which is consistent with binding acidic residues, such as Asp316 and Asp319, in salt bridge interactions, as originally proposed.[6b] An exception is the Fmoc derivative **26e**, which demonstrated selective inhibition of melanoma cells.

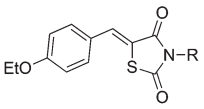
As shown in Table 4, removal of (**3**) and replacement of the ethylamine moiety with non-basic groups (**28d–s**) for the most part eliminated selectivity and potency in inhibiting melanoma cell proliferation, reinforcing the significance of this component of lead compound **76** that was established above (Tables 2 and 3). In sharp contrast, *para*-substituted benzoic acid derivative **28q** proved highly selective for inhibiting A375 cells but not the other melanoma cell lines with mutated B-Raf suggesting this compound may have other cellular targets. Furthermore, we discovered that the location of the carboxylic acid function of **28q** is significant, as evidenced by the *meta* (**28r**) and *ortho* (**28s**) isomers, which had reduced inhibition of, and selectivity towards, A375 cells. Given its lack of basic character, **28q** may also target a different binding site than the lead compound **76**.

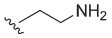
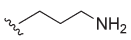
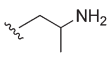
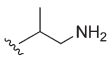
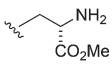
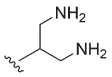
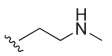
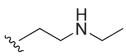
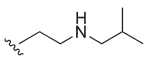
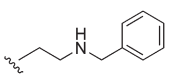
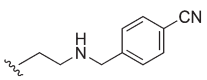
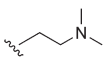
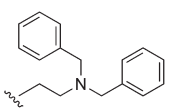
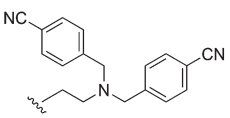
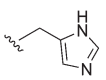
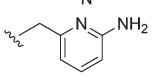
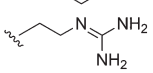
Some of the greatest variation in inhibitory potency and selectivity was seen upon changes in the 4-ethoxyphenyl group of **76** (Table 5). First, the importance of the 4-ethoxy moiety was established by synthesizing and testing unsubstituted compound **30a**, which showed some inhibition only in the HL-60 cell line. Moreover, the location of the ethoxy group proved significant, since shifting it from the *para* (compound **76**) to the *meta* or *ortho* positions (compounds **30b** and **30c**, respectively) resulted in reduced growth inhibition and selectivity for melanoma cells with activating mutations in the ERK pathway, although inhibition of HL-60 cells was maintained. Comparison of the data for the *para*-substituted isosteres **30d** and **30f** reveal that a polar group at this position is preferred to deliver both potency and, particularly, selectivity for inhibiting melanoma and HL-60 cells. This is supported by comparing the data for isosteres **76** and **30e**, wherein the latter, although a potent inhibitor of the melanoma cell proliferation, exhibited 10 fold less selectivity for HL-60 cells, which must be attributed to the replacement of the *para*-oxygen with a *para*-methylene group. The addition of further hydrophobicity to the *para*-hydroxyl of **30f** also generated potent and selective inhibitors with *para*-benzyloxy and *para*-isobutoxy derivatives **30g** and **30h**, respectively, completely inhibiting proliferation of melanoma and HL-60 cells and demonstrating excellent selectivity over HeLa and Jurkat cell lines. Given that Li *et al.* had discovered substitution at the *ortho* position of the phenyl ring of **76** resulted in compounds with potent activity against the proliferation of human leukemia U937 cells,[10] we prepared the focused set of *ortho*-functionalized congeners **30j–l**. Indeed, this led to a series of highly cytotoxic compounds. However, at the same time, selectivity at 100 μM for the ERK-dependent melanoma cell lines appeared to be lost.

In summary, our findings indicate that the pharmacophore of **76** requires a 4-alkoxyphenyl group connected to a TZD ring by virtue of a *Z*-double bond, along with an ethyl linker connecting a primary amine to the imide nitrogen of the TZD ring. Whilst the endocyclic sulfur appears to be important, the *Z*-double bond is not. Functionalization of the primary amino group of **76** was detrimental to its biological activity, suggesting its basicity and capacity to engage in several hydrogen bonding interactions are important for inhibitory activity.

The initial screening of compounds suggested several new compounds that were selective inhibitors of cancer cell lines with activated ERK signaling. To further examine the effects of potential lead compounds, we performed dose-response assays to determine GI_{50} values on a select number of compounds that showed selectivity for inhibiting proliferation of cells with activated ERK signaling. An example of the dose response curve for compound **30h** and the B-Raf inhibitor PLX4032 for inhibiting the growth of melanoma cell lines is shown in Fig. 2. Importantly, these data demonstrate that **30h** is equally effective at inhibiting melanoma cells that have become resistant to PLX4032 as it is against PLX4032-sensitive cells. A summary of GI_{50} values for select lead compounds along with comparisons to known ERK pathway inhibitors in various cell

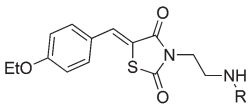
Table 2 Cell viability SAR of basic replacements of ethylamine tail of **76** at 100 μM

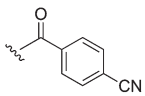
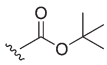
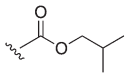
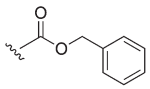
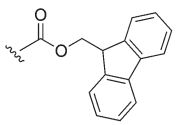
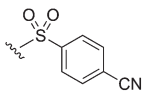
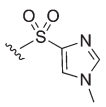


Compound number	R	SK-MEL-28	Cell viability (% of vehicle) A375	RPMI-7951	HL-60	HeLa	Jurkat
76		11 ± 7	2.1 ± 2	0.3 ± 0.02	4.4 ± 1	53 ± 3	59 ± 5
22a		0.5 ± 0.01	0.7 ± 0.1	0.6 ± 0.1	8.0 ± 72	12 ± 2	9.0 ± 2
22b		32 ± 4	25 ± 4	16 ± 1	7.3 ± 3	49 ± 7	58 ± 10
22c		28 ± 4	13 ± 2	22 ± 3	8.1 ± 3	45 ± 7	54 ± 10
22d		33 ± 9	41 ± 11	1.8 ± 0.1	25 ± 5	87 ± 4	100 ± 5
22e		32 ± 2	19 ± 4	25 ± 3	11 ± 4	62 ± 2	81 ± 9
23a		91 ± 1	95 ± 2	54 ± 7	17 ± 11	85 ± 4	83 ± 6
23b		63 ± 10	51 ± 4	60 ± 4	14 ± 4	81 ± 2	69 ± 10
23c		1.3 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	10 ± 4	3.7 ± 0.4	2.5 ± 0.1
23d		25 ± 6	13 ± 1	2.5 ± 0.3	5.0 ± 2	37 ± 1	89 ± 4
23e		96 ± 6	89 ± 3	96 ± 5	75 ± 25	88 ± 2	98 ± 5
24a		101 ± 0.3	115 ± 2	130 ± 4	110 ± 14	90 ± 5	95 ± 1
24b		104 ± 4	122 ± 7	120 ± 3	109 ± 24	92 ± 9	104 ± 2
24c		107 ± 3	115 ± 1	108 ± 9	108 ± 16	97 ± 8	100 ± 2
28a		74 ± 6	101 ± 2	70 ± 2	123 ± 20	80 ± 4	106 ± 6
28b		86 ± 3	111 ± 1	101 ± 4	107 ± 26	87 ± 6	105 ± 5
28c		55 ± 6	31 ± 4	42 ± 6	20 ± 6	17 ± 2	6.5 ± 0.5

lines is shown in Table 6. These data highlight several key points of these SAR studies. First, TZD-containing compounds have been identified as selective inhibitors of cancer cell lines containing activated ERK signaling. Second, changes in the 4-ethoxyphenyl component of the parent compound **76** improve selectivity and potency for inhibiting melanoma cell growth. Third, compound **30h** is a potent inhibitor of melanoma cell lines that have become resistant to the clinically relevant B-Raf and MEK inhibitors. As shown in Table 6, **30h** is about three times as potent as the MEK inhibitor AZD6244 in preventing growth of the drug-resistant RPMI7951 melanoma cell line. Lastly, **30h** is as potent as the ATP-competitive pyrazolylpyrrole ERK inhibitor (*N*-(1-(3-chloro-4-fluorophenyl)-2-hydroxyethyl)-4-(4-(3-chlorophenyl)-1*H*-pyrazol-5-yl)-1*H*-pyrrole-2-carboxamide, "ERK Inhibitor")[16] in preventing melanoma cell proliferation and provides an alternative non-ATP-

Table 3 Cell viability SAR of *N*-functionalization of ethylamine tail of **76** at 100 μM



			Cell viability (% of vehicle)				
Compound number	R	SK-MEL-28	A375	RPMI-7951	HL-60	HeLa	Jurkat
25a		105 ± 2	121 ± 4	124 ± 5	106 ± 23	100 ± 11	105 ± 8
25b		105 ± 2	118 ± 4	127 ± 3	101 ± 23	98 ± 11	103 ± 6
25c		73 ± 1	84 ± 2	62 ± 7	31 ± 5	81 ± ± 1	92 ± 7
25d		103 ± 2	118 ± 3	122 ± 10	101 ± 12	99 ± 9	102 ± 7
26a		88 ± 3	96 ± 4	102 ± 4	104 ± 31	84 ± 4	97 ± 7
26b		83 ± 5	88 ± 3	67 ± 4	95 ± 21	88 ± 5	111 ± 9
26c		94 ± 1	102 ± 4	75 ± 12	80 ± 28	95 ± 3	110 ± 4
26d		97 ± 1	103 ± 3	54 ± 6	85 ± 27	98 ± 6	112 ± 5
26e		29 ± 7	43 ± 3	27 ± 3	103 ± 13	85 ± 4	139 ± 14
27a		87 ± 2	94 ± 2	68 ± 3	82 ± 44	82 ± 1	85 ± 3
27b		95 ± 3	109 ± 6	99 ± 4	101 ± 52	90 ± 4	103 ± 3
27c		92 ± 5	107 ± 2	118 ± 6	51 ± 31	91 ± 5	97 ± 4

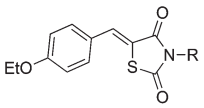
dependent approach to target cancer cells that are dependent on active ERK signaling.

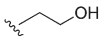
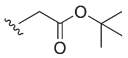
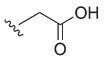
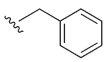
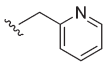
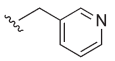
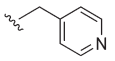
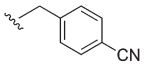
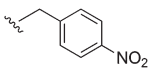
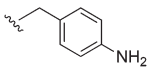
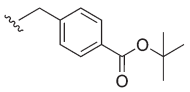
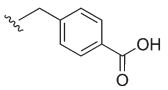
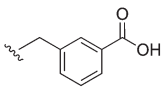
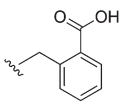
To further demonstrate that the test compounds were targeting the ERK signaling pathway, we examined the activity of the activator protein-1 (AP-1) promoter and the serum response element (SRE), which are regulated by the ERK substrates c-Fos and Elk-1, respectively.[17] Compounds **76** and **30g** were both potent inhibitors of AP-1 and SRE promoter activity with estimated IC_{50} values around 5 μM or less (Fig. 3A and B). Interestingly, **30h** also inhibited ERK-mediated AP-1 and SRE promoter activity but was less potent than compounds **76** and **30g** at lower concentrations. This suggests differences in how these compounds inhibit ERK signaling functions, which is further supported by the selective inhibition of the SRE promoter activity by compound **22a** (Fig. 3B).

Conclusions

The current study provides a comprehensive structure–function analysis of TZD-based compounds and their ability to inhibit the proliferation of cancer cells containing activated ERK signaling. These compounds support the utility of developing novel TZD compounds that have been recognized to have potential applications for treating cancers such as melanoma.[18] We have identified several chemical features (Fig. 4)

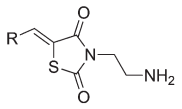
Table 4 Cell viability SAR of non-basic ethylamine replacements of **76** at 100 μM

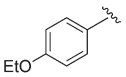
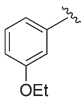
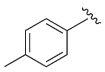
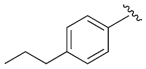
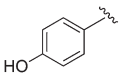
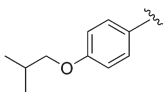
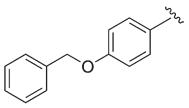
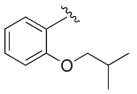
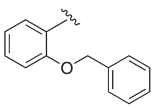
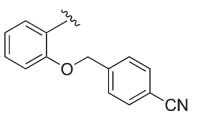


Compound number	R	SK-MEL-28	Cell viability (% of vehicle) A375	RPMI-7951	HL-60	HeLa	Jurkat
2		103 ± 1	110 ± 10	144 ± 2	103 ± 16	98 ± 13	104 ± 4
28d		101 ± 3	119 ± 5	116 ± 10	80 ± 26	96 ± 4	101 ± 5
28e		91 ± 1	116 ± 4	137 ± 8	91 ± 19	91 ± 2	101 ± 4
28f		97 ± 3	113 ± 9	128 ± 3	99 ± 14	87 ± 4	99 ± 5
28g		101 ± 2	117 ± 7	110 ± 3	95 ± 18	88 ± 7	108 ± 3
28h		106 ± 1	122 ± 6	133 ± 9	97 ± 27	104 ± 3	108 ± 3
28i		90 ± 3	110 ± 4	114 ± 20	65 ± 1	95 ± 4	105 ± 4
28j		95 ± 3	124 ± 6	114 ± 5	89 ± 31	89 ± 5	101 ± 7
28k		79 ± 4	105 ± 4	102 ± 2	65 ± 12	80 ± 2	98 ± 2
28l		79 ± 3	105 ± 12	102 ± 3	82 ± 54	81 ± 5	96 ± 11
28m		97 ± 7	108 ± 3	106 ± 8	86 ± 37	95 ± 3	103 ± 11
28n		104 ± 3	117 ± 3	119 ± 13	97 ± 23	97 ± 9	101 ± 9
28o		94 ± 5	119 ± 6	102 ± 23	86 ± 21	89 ± 9	97 ± 7
28p		105 ± 3	119 ± 1	122 ± 1	98 ± 24	100 ± 6	101 ± 2
28q		59 ± 13	2.0 ± 0.5	34 ± 3	84 ± 37	64 ± 2	85 ± 3
28r		83 ± 7	59 ± 1	83 ± 6	41 ± 22	75 ± 4	80 ± 3
28s		49 ± 8	39 ± 1	45 ± 8	31 ± 15	61 ± 4	46 ± 1

in compounds such as **30h** that promote selective and potent inhibition of cancer cells lines containing active ERK signaling as a result of B-Raf or N-Ras mutations. Essential for activity is a TZD core, the imide nitrogen of which should be functionalized with an ethyl moiety terminating in a primary amino group. Furthermore, the acidic methylene of the TZD ring must be linked to a *para*-alkoxy-substituted phenyl ring through a *Z*-double bond. Given that other transformed cell lines are not affected by the compounds, our findings suggests that their mechanisms of action involves inhibition of ERK signaling and not general toxicity. Moreover, these compounds are potent inhibitors of ERK-regulated transcription and further support ERK as the molecular target. Of particular importance is the ability for several compounds to inhibit melanoma cell lines that have become resistant to clinically relevant B-Raf inhibitors. Recent studies support the use of ERK-targeted compounds to overcome alternative signaling pathways that contribute to drug resistance.[19] Future studies

Table 5 Cell viability SAR of 4-ethoxyphenyl moiety of **76** at 100 μM



			Cell viability (% of vehicle)				
Compound number	R	SK-MEL-28	A375	RPMI-7951	HL-60	HeLa	Jurkat
30a		88 ± 7	82 ± 9	89 ± 17	28 ± 20	82 ± 10	88 ± 4
76		11 ± 7	2.1 ± 2	0.3 ± 0.02	4.4 ± 1	53 ± 3	59 ± 5
30b		36 ± 6	19 ± 3	30 ± 2	4.8 ± 2	40 ± 1	54 ± 5
30c		52 ± 11	22 ± 9	45 ± 2	8.9 ± 4	39 ± 3	66 ± 5
30d		31 ± 7	23 ± 6	23 ± 1	4.8 ± 2	54 ± 3	71 ± 6
30e		0.6 ± 0.4	0.6 ± 0.1	0.7 ± 0.1	5.9 ± 2	11 ± 2	16 ± 8
30f		38 ± 3	8.8 ± 2	24 ± 5	17 ± 12	63 ± 10	96 ± 3
30g		0.8 ± 0.2	1.8 ± 0.5	0.3 ± 0.04	5.0 ± 2	48 ± 3	91 ± 8
30h		1.0 ± 1	0.6 ± 0.4	0.2 ± 0.04	5.2 ± 2	59 ± 3	87 ± 5
30i		85 ± 9	78 ± 3	102 ± 10	24 ± 8	68 ± 3	99 ± 5
30j		1.0 ± 0.1	0.8 ± 0.02	1.0 ± 0.04	9.5 ± 3.4	0.8 ± 0.1	1.8 ± 0.1
30k		0.6 ± 0.04	0.6 ± 0.03	0.9 ± 0.1	8.3 ± 3.4	0.6 ± 0.1	1.5 ± 0.1
30l		0.5 ± 0.01	0.5 ± 0.04	0.6 ± 0.03	5.7 ± 2	8.0 ± 2	6.7 ± 1

will be important to provide a more comprehensive biological characterization of the lead compounds identified in these studies and the ERK substrates they affect.

Experimental

Unless otherwise stated, all reactions were performed under an inert (N_2) atmosphere. Reagents and solvents were reagent grade and purchased from Sigma-Aldrich, Alfa Aesar, Oakwood Chemicals and TCI America. ^{1}H and ^{13}C NMR spectra were recorded on Varian INOVA 400 MHz and Varian INOVA 500 MHz NMR spectrometers at 25 °C. Chemical shifts are reported in parts per million (ppm). The residual solvent peak was used as an internal reference. The mass spectra were obtained on an Electrospray TOF (ESI-TOF) mass spectrometer (Bruker amaZon X). Prior to biological testing, final compounds were confirmed to be >95% pure by HPLC

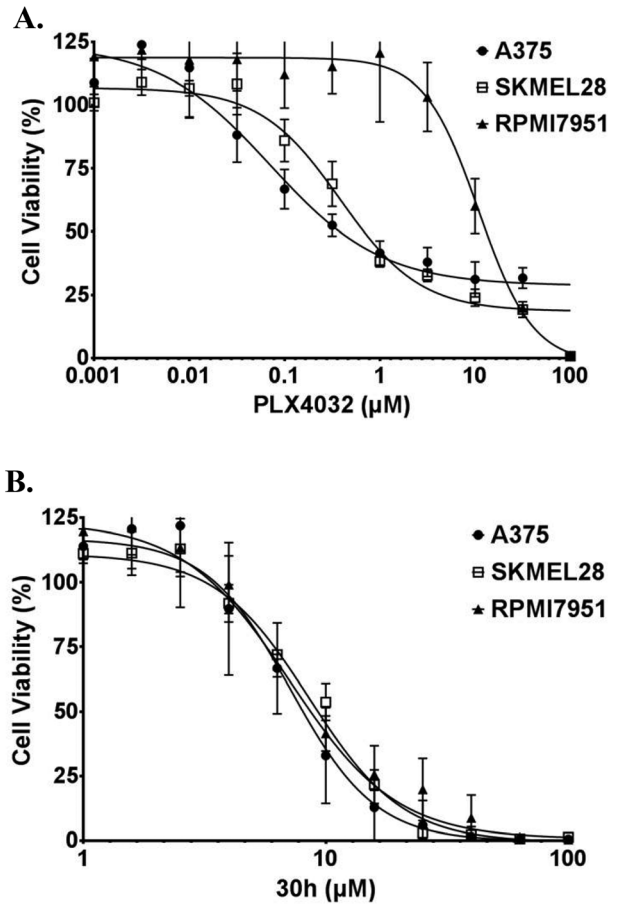


Fig. 2 Melanoma cell growth inhibition dose response curves for PLX4032 and lead compound **30h**. Melanoma cells (A375, SK-MEL-28, and RPMI7951) were seeded at a density of 5000 per well in 96-well plate. After overnight incubation, cells were treated with varying doses of PLX4032 (A) or 31 h (B) for two days. Cell viability was determined by the addition of Cell Titer-Blue Reagent (20 μL per well) followed by 2 hours of incubation at 37 °C after which fluorescence was recorded (555/585 nm). Cell viability was expressed as percentage of vehicle control and data represent the average of three individual experiments.

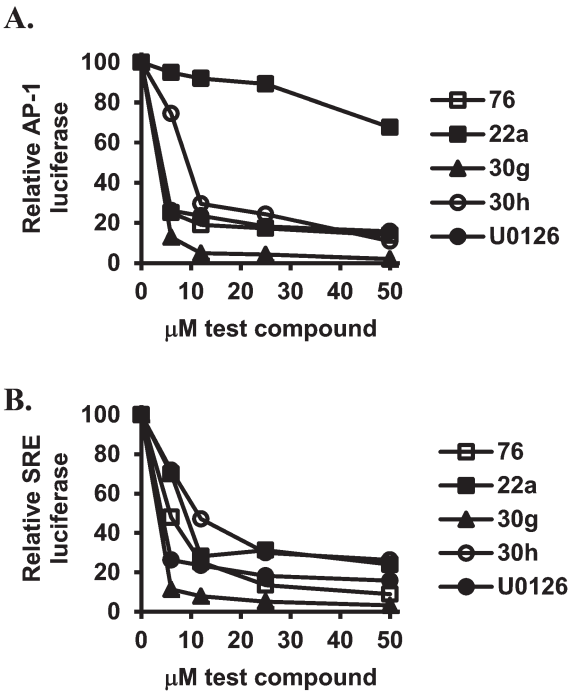


Fig. 3 Effects of test compounds on AP-1 and SRE promoter activity. ERK-mediated promoter activity was tested in HeLa cells transfected with AP-1 (A) or SRE (B) promoter constructs driving luciferase expression. The ERK pathway was stimulated with EGF (25 ng mL^{-1}) in the presence or absence of the indicated concentrations of each test compound and luciferase activity was determined after 6 hours. The MEK inhibitor, U0126, was used as a positive control.

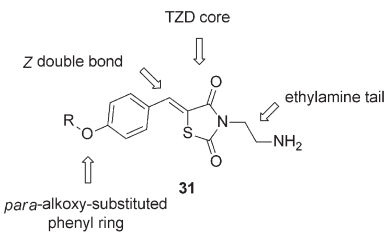


Fig. 4 Pharmacophore of **76** that elicits cytotoxicity towards melanoma cells harboring constitutive ERK activation.

Table 6 GI_{50} values (μM) of select lead compounds. ND = not determined

Compound	A375	SK-MEL-28	RPMI7951	HeLa	Jurkat
76	29	34	46	>50	>100
22a	24	46	17	ND	ND
30g	22	36	11	~100	>100
30h	7.0	9.0	7.0	>100	>100
PLX4032	0.07	0.38	11	>10	>10
AZD6244	0.03	0.17	20	>10	>10
ERK Inhibitor	5.0	4.0	11	>10	>10

chromatography using a Waters 1525 analytical/preparative HPLC fitted with a C18 reversed-phase column (Atlantis T3: 4.6 mm × 150 mm; Symmetry: 4.6 mm × 150 mm; X-Bridge: 4.6 mm × 150 mm) according to the following conditions with solvents (A) H_2O/0.1% TFA, (B) CH_3CN–H_2O, 9 : 1 with 0.1% TFA, (C) H_2O, (D) CH_3CN–H_2O, 9 : 1, (E) H_2O/0.1% NH_4OH, (F) CH_3CN–H_2O, 9 : 1 with 0.1% NH_4OH at 1 ml min^{-1}: (I) a gradient of 75% A to 100% B over 32 min, Atlantis; (II) a gradient of 50% A to 100% B over 22 min, Symmetry; (III) a gradient of 75% A to 100% B over 62 min, Atlantis; (IV) a gradient of 100% A to 100% B over 30 min, Atlantis; (V) a gradient of 75% A to 100% B over 52 min, Atlantis; (VI) a gradient of 50% A to 100% B over 30 min, Atlantis; (VII) a gradient of 100% A to 100% B over 52 min, Atlantis; (VIII) a gradient of 50% A to 100% B over 52 min, Atlantis; (IX) a gradient of 100% A to 100% B over 22 min, Symmetry; (X) a gradient of 50% C to 100% D, X-Bridge; (XI) a gradient of 100% E to 100% F over 22 min, X-Bridge; (XII) a gradient of 25% E to 100% F over 22 min, X-Bridge; (XIII) an isocratic gradient of 100% F for 5 min followed by a gradient of 100% E to 100% F over 22 min, X-Bridge.

(*Z*)-5-(4-Ethoxybenzylidene)thiazolidine-2,4-dione (2)

4-Ethoxybenzaldehyde (2.78 mL, 20.0 mmol) and 2,4-thiazolidinedione **1** (3.75 g, 32.0 mmol) were dissolved in 80 mL of anhydrous ethanol at room temperature and piperidine (0.60 mL, 6.0 mmol) was added to the reaction flask. The reaction mixture was stirred at 75 °C for 1 day and then allowed to cool down to room temperature until a yellow crystalline precipitate formed, collected by filtration, washed with water and dried to afford compound **3** in 77.0% (3.84 g) yield as a yellow solid. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.83 (1H, s, C*H*), 7.46 (2H, d, J = 9.0 Hz, *Ar*), 6.98 (2H, d, J = 9.0 Hz, *Ar*), 4.10 (2H, q, J = 6.5 Hz, OCH_2CH_3), 1.43 (3H, d, J = 6.5 Hz, OCH_2CH_3); MS (ESI) m/z Calcd for $C_{12}H_{11}NO_3S$ (M^+): 249.0. Found: 250.0 ($M + H^+$); HPLC purity (condition I), 99.2% (t_R, 14.60 min), HPLC purity (condition V), 98.5% (t_R, 6.49 min).

(*Z*)-3-(2-Aminoethyl)-5-(4-ethoxybenzylidene)thiazolidine-2,4-dione (3)

Compound **2** (1.26 g, 5.08 mmol), 2-(tritylamino)ethanol (2.00 g, 6.60 mmol) and triphenylphosphine (1.80 g, 6.86 mmol) were dissolved in 51 mL of anhydrous THF, and stirred at room temperature for 5 min. DIAD (1.3 mL, 6.60 mmol) was added to the reaction mixture and then stirred overnight at 45 °C. The reaction mixture was diluted with ethyl acetate and washed with sat'd NH_4Cl aqueous solution. The organic layer was collected, dried over anhydrous Na_2SO_4, filtered, concentrated and purified by silica column chromatography (30% ethyl acetate in hexanes) to afford **3** (75.0%, 2.71 g) as a white solid.

(*Z*)-3-(2-Aminoethyl)-5-(4-ethoxybenzylidene)thiazolidine-2,4-dione (76)

The title compound **76** was prepared from **3** (1.43 g, 2.68 mmol) in a manner to that described for **6** in 72.0% (1.09 g) as a white solid. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 8.06 (2H, bs, NH_2), 7.85 (1H, s, C*H*), 7.56 (2H, d, J = 8.4 Hz, *Ar*), 7.07 (2H, d, J = 8.4 Hz, *Ar*), 4.08 (2H, q, J = 6.4 Hz, OCH_2CH_3), 3.87 (2H, m, CH_2), 3.05 (2H, m, CH_2), 1.31 (3H, t, J = 6.4 Hz, OCH_2CH_3); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 167.9, 166.0, 160.5, 132.8, 132.2, 132.1, 125.2, 118.2, 115.4, 115.3, 63.5, 36.9, 14.5.

5-(4-Ethoxybenzyl)thiazolidine-2,4-dione (4)

To a suspension of lithium borohydride (0.22 g, 10.04 mmol) in 40 mL of THF–pyridine (ratio 1 : 1), compound **3** (1.00 g, 4.02 mmol) was added at 0 °C. The reaction mixture was stirred at 80 °C for 12 h and then cooled to 0 °C. 21 mL of 4 N HCl aqueous solution was added carefully and the resulting suspension was stirred at 0 °C for 10 min, and refluxed for 1 h. After cooling down at room temperature, the solvent was removed by evaporation. The residue was dissolved in ethyl acetate and washed with water. The organic layer was collected, dried over anhydrous Na_2SO_4, filtered, concentrated and purified by silica column chromatography (50% ethyl acetate in hexanes) to afford compound **4** in 76.6% (0.77 g) yield as a colorless oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 9.07 (1H, bs, N*H*), 7.17 (2H, d, J = 8.5 Hz, *Ar*), 6.88 (2H, d, J = 8.5 Hz, *Ar*), 4.53 (1H, dd, J = 3.5 Hz, J = 10.0 Hz, C*H*S), 4.06 (2H, q, J = 8.0 Hz, OCH_2CH_3), 3.52 (1H, m, CH_2), 3.12 (1H, dd, J = 4.5 Hz, J = 10.0 Hz, CH_2), 1.44 (3H, t, J = 8.0 Hz, OCH_2CH_3).

***tert*-Butyl (2-(5-(4-ethoxybenzyl)-2,4-dioxothiazolidin-3-yl)ethyl)-carbamate (5)**

Potassium carbonate (1.40 g, 9.68 mmol) was added to a solution of compound **4** (303 mg, 1.21 mmol) in 4 mL of anhydrous DMF at 0 °C. After stirring the reaction mixture for 20 min, a solution of *tert*-butyl (2-bromoethyl)carbamate (268 mg, 1.21 mmol) in anhydrous DMF (2 mL) was added and then the reaction mixture was stirred at room temperature for 3 h. The reaction was quenched by adding water and then extracted with ethyl acetate. The ethyl acetate layer was washed with 10% NH_4Cl aqueous solution followed by water and dried over anhydrous Na_2SO_4, filtered, concentrated and purified by silica column chromatography (20% ethyl acetate in hexanes) to afford compound **5** in 53.1% (253 mg) as a colorless oil. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.14 (2H, d, J = 7.5 Hz, *Ar*), 6.85 (2H, d, J = 7.5 Hz, *Ar*), 4.51 (1H, m, CH_2CHS), 4.43 (1H, m, N*H*), 4.02 (2H, q, J = 6.5 Hz, OCH_2CH_3), 3.68 (2H, t, J = 5.5 Hz, NCH_2), 3.43 (1H, m, CH_2CH), 3.26 (2H, m, CH_2NH), 3.12 (1H, m, CH_2CH), 1.43 (9H, s, $C(CH_3)_3$), 1.40 (3H, t, J = 8.0 Hz, OCH_2CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 173.8, 171.0, 157.6, 155.7, 130.2, 130.1, 128.5, 114.4, 114.3, 77.8, 62.9, 51.1, 51.0, 41.5, 37.0, 36.4, 28.2, 28.1, 14.6, 14.5.

3-(2-Aminoethyl)-5-(4-ethoxybenzyl)thiazolidine-2,4-dione (6)

Compound **5** (80 mg, 0.20 mmol) was dissolved in 50% TFA in dichloromethane (2 mL) and then stirred at room temperature for 1 h. The solvent was removed by evaporation and the residue was dissolved in dioxane (2 mL), and evaporated again. The solid was collected by filtration, washed with ethyl ether, and dried to afford compound **6** in 58.5% (48 mg) yield as a white solid. ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.17 (2H, d, J = 9.0 Hz, *Ar*), 6.86 (2H, d, J = 9.0 Hz, *Ar*), 4.72 (1H, dd, J = 3.5 Hz, J = 8.5 Hz, CH_2CHS), 4.01 (2H, q, J = 6.5 Hz, OCH_2CH_3), 3.83 (2H, t, J = 5.5 Hz, NCH_2), 3.45 (1H, dd, J = 3.5 Hz, J = 14.5 Hz, CH_2CH), 3.13–3.06 (3H, m, CH_2CH + CH_2NH_2), 1.37 (3H, t, J = 6.5 Hz, OCH_2CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 174.0, 171.4, 157.6, 130.2, 130.1, 128.4, 114.4, 114.3, 62.9, 51.4, 38.7, 36.6, 36.2, 14.6; MS (ESI) m/z Calcd for $C_{14}H_{18}N_2O_3S$ (M^+): 294.1, Found: 295.0 ($M + H^+$); HPLC purity (condition IX), 96.2% (t_R, 3.31 min), HPLC purity (condition XI), 94.6% (t_R, 3.97 min).

(*Z*)-5-(4-Ethoxybenzylidene)-2-thioxothiazolidin-4-one (8)

4-Ethoxybenzaldehyde (2.78 mL, 20.00 mmol) and rhodanine **7** (3.75 g, 32.0 mmol) were dissolved in 80 mL of anhydrous ethanol at room temperature, and piperidine (0.60 mL, 6.0 mmol) was added to the reaction flask. The reaction mixture was stirred at 75 °C for 1 day and then allowed to cool down to room temperature until a yellow crystalline precipitate

formed, and the solid was collected by filtration, washed with water and ethyl acetate, and dried to afford compound **8** in 80.0% (2.99 g) yield as a yellow solid. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 13.74 (1H, bs, N*H*), 7.59 (1H, s, C*H*), 7.54 (2H, d, J = 8.4, *Ar*), 7.08 (2H, d, J = 8.4, *Ar*), 4.10 (2H, q, J = 7.2, CH_2CH$_3$), 1.34 (3H, t, J = 7.2, CH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 169.3, 160.3, 160.2, 139.4, 127.9, 126.6, 123.8, 122.6, 87.0, 32.8.

(*Z*)-3-(2-Aminoethyl)-5-(4-ethoxybenzylidene)-2-thioxothiazolidin-4-one (9)

To compound **8** (50 mg, 0.18 mmol), HOCH$_2$CH$_2$NHTr (54 mg, 0.18 mmol) and triphenylphosphine (73 mg, 0.28 mmol) dissolved in 15 mL of anhydrous THF was added DIAD (55 μL, 0.28 mmol) and stirred for 12 h. Reaction was reduced *in vacuo* and purified by column chromatography (10% ethyl acetate in hexanes) to give 67 mg (67.7%) of a yellow oil. Deprotection following the procedure of compound **6** gave the title compound **9** as a yellow solid (53%, 40 mg). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 7.90 (2H, bs, NH_2), 7.80 (1H, s, C*H*), 7.63 (2H, d, J = 8.0, *Ar*), 7.13 (2H, d, J = 8.0, *Ar*), 4.29 (2H, t, J = 6.0, CH_2), 4.14 (2H, q, J = 7.0, CH_2CH$_3$), 3.21–3.13 (2H, m, CH_2), 1.36 (3H, t, J = 7.0, CH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 193.8, 167.5, 160.8, 133.1, 132.8, 125.2, 119.1, 115.5, 63.6, 41.7, 36.4, 14.4; MS (ESI) m/z Calcd for $C_{14}H_{16}N_2O_2S_2$ (M^+): 308.1, Found: 309.0 (M + H^+); HPLC purity (condition IX), 99.0% (t_R, 3.62 min), HPLC purity (condition X), 95.2% (t_R, 5.94 min).

3-(Triphenylphosphoranylidene)pyrrolidine-2,5-dione (11)

Maleimide (0.60 g, 6.05 mmol) and triphenylphosphine (1.57 g, 5.97 mmol) were dissolved in 40 mL of acetone and the reaction mixture was stirred at 65 °C for 1 h. After cooling down to room temperature, the solid was collected by filtration, washed with acetone, and dried to afford compound **11** in 82.5% (1.77 g) yield as a white solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 9.74 (1H, s, N*H*), 7.73–7.62 (15H, m, 3× *Ar*), 2.97 (2H, s, CH_2).

(*E*)-3-(4-Ethoxybenzylidene)pyrrolidine-2,5-dione (12)

The title compound **12** was prepared from 4-ethoxybenzaldehyde (0.22 mL, 1.60 mmol) and compound **11** (1.03 g, 2.88 mmol) in a manner similar to that described for **3** in 92.5% (342 mg) yield as a yellow solid. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.53 (1H, s, C*H*), 7.44 (2H, d, J = 8.5 Hz, *Ar*), 6.96 (2H, d, J = 8.5 Hz, *Ar*), 4.08 (2H, q, J = 6.5 Hz, OCH_2CH$_3$), 1.44 (3H, t, J = 6.5 Hz, OCH$_2$CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 174.8, 171.8, 160.9, 135.0, 132.3, 126.6, 121.4, 115.3, 63.9, 35.3, 14.8.

(*E*)-1-(2-Aminoethyl)-3-(4-ethoxybenzylidene)pyrrolidine-2,5-dione (13)

Step 1. Potassium carbonate (180 mg, 1.30 mmol) was added to a solution of compound **12** (100 mg, 0.43 mmol) in 3 mL of anhydrous DMF at 0 °C. After stirring the reaction mixture for 20 min, a solution of *tert*-butyl (2-bromoethyl)carbamate (96 mg, 0.43 mmol) in anhydrous DMF (1 mL) was added and then the reaction mixture was stirred at room temperature for 3 h. The reaction was quenched by adding water and extracted with ethyl acetate. The ethyl acetate layer was washed with 10% NH_4Cl aqueous solution followed by water and dried over anhydrous Na_2SO_4, filtered, concentrated and purified by silica column chromatography (20% ethyl acetate in hexanes) to afford 70 mg (43.2%) of *Boc-protected* **13** as a white solid. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.56 (1H, s, C*H*), 7.44 (2H, d, J = 8.0 Hz, *Ar*), 6.96 (2H, d, J = 8.0 Hz, *Ar*), 4.87 (1H, bs, N*H*), 4.08 (2H, q, J = 6.5 Hz, OCH_2CH$_3$), 3.77 (2H, m, CH_2), 3.54 (2H, s, CH_2), 3.40 (2H, m, CH_2), 1.44 (3H, t, J = 6.5 Hz, OCH$_2$CH_3), 1.38 (9H, s, C(CH_3)$_3$); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 174.6, 171.6, 160.7, 156.2, 134.5, 132.2, 126.8, 120.5, 115.2, 79.6, 63.8, 39.3, 39.0, 34.2, 28.4, 14.8.

Step 2. The title compound **13** was prepared from *Boc-protected* **13** (47 mg, 0.13 mmol) in a manner similar to that described for **6** in 92.0% (45 mg) yield as a white solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 7.94 (2H, s, NH_2), 7.62 (2H, d, J = 9.0 Hz, *Ar*), 7.44 (1H, s, C*H*), 7.03 (2H, d, J = 9.0 Hz, *Ar*), 4.10 (2H, q, J = 6.0 Hz, OCH_2CH$_3$), 3.76 (2H, m, CH_2), 3.61 (2H, m, CH_2), 3.04 (2H, m, CH_2), 1.34 (3H, t, J = 6.0 Hz, OCH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 174.6, 170.9, 159.9, 132.1, 132.0, 126.5, 122.2, 114.9, 63.3, 36.9, 35.8, 33.9, 14.5; MS (ESI) m/z Calcd for $C_{15}H_{18}N_2O_3$ (M^+): 274.1, Found: 275.0 (M + H^+); HPLC purity (condition IX), 97.8% (t_R, 3.24 min), HPLC purity (condition X), 98.1% (t_R, 3.54 min).

(*E*)-3-(4-Ethoxybenzylidene)pyrrolidin-2-one (16)

4-Ethoxybenzaldehyde (633 mg, 4.22 mmol) and **15** (596 mg, 4.69 mmol) were dissolved in 6 mL of anhydrous THF, and potassium *tert*-butoxide (631 mg, 5.62 mmol) was added to the reaction flask. The reaction mixture was stirred at 55 °C for 1 h and then allowed to cool down to room temperature until a yellow crystalline precipitate formed, and the solid was collected by filtration, washed with water and ethyl acetate, and dried to afford compound **16** in 45.0% (411 mg) as a yellow solid. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 8.03 (1H, bs, N*H*), 7.46 (2H, d, J = 8.8, *Ar*), 7.03 (1H, s, C*H*), 6.97 (2H, d, J = 8.8, *Ar*), 4.05 (2H, q, J = 6.8, CH_2CH$_3$), 3.39–3.32 (2H, m, CH_2), 3.39–2.98 (2H, m, CH_2), 1.33 (3H, t, J = 6.8, CH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 100 MHz) 170.9, 158.5, 130.9, 129.7, 128.1, 127.5, 114.6, 63.1, 38.7, 25.7, 14.6.

(*E*)-1-(2-Aminoethyl)-3-(4-ethoxybenzylidene)pyrrolidin-2-one (17)

To compound **16** (50 mg, 0.23 mmol) in 7 mL of anhydrous DMF was added sodium hydride (10 mg, 0.23 mmol) and stirred for 1 h. MsOCH$_2$CH$_2$NHTr (174 mg, 0.46 mmol) was added and stirred overnight. Reaction was reduced *in vacuo* and the product was purified by column chromatography (10% ethyl acetate in hexanes) to give 67 mg (58%) of clear oil. Deprotection following the procedure of compound **6** gave the title compound **17** as a yellow solid (45%, 33 mg). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 7.82 (2H, bs, NH_2), 7.50 (2H, d, J = 7.8, *Ar*), 7.10 (1H, s, C*H*), 6.99 (2H, d, J = 7.8, *Ar*), 4.06 (2H, q, J = 7.0,

CH_2CH_3), 3.57 (2H, t, J = 6.0, CH_2), 3.49 (2H, t, J = 6.0, CH_2), 3.09–2.95 (4H, m, 2× CH_2), 1.36 (3H, t, J = 7.0, CH_2CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 169.3, 158.7, 131.0, 129.3, 128.2, 127.9, 114.7, 63.2, 44.0, 40.5, 36.7, 24.1, 14.7; MS (ESI) m/z Calcd for $C_{15}H_{20}N_2O_2$ (M^+): 260.2, Found: 261.1 ($M + H^+$); HPLC purity (condition IX), 97.8% (t_R, 3.00 min), HPLC purity (condition X), 99.1% (t_R, 3.97 min).

(*E*)-3-(4-Ethoxyphenyl)acrylic acid (19)

A solution of 4-ethoxybenzaldehyde (1.00 mL, 7.20 mmol) and piperidine (0.11 mL, 1.08 mmol) in 40 mL of pyridine was heated to 120 °C. A solution of malonic acid (1.50 g, 14.40 mmol) in 40 mL of pyridine was added dropwise over 30 min and the reaction solution was stirred at 120 °C for 4 h. After cooling down to 0 °C, excess amount of concentrated HCl was added carefully to make ~pH 1. A white crystalline precipitate formed, and the solid was collected by filtration, washed with 0.1 N HCl aqueous solution, and dried to afford compound **19** in 99.5% (1.40 g) of pure **19** as a white solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 12.14 (1H, s, CO_2H), 7.62 (2H, d, J = 9.0 Hz, *Ar*), 7.56 (1H, d, J = 15.5 Hz, C*H*), 6.95 (2H, d, J = 9.0 Hz, *Ar*), 6.38 (1H, d, J = 15.5 Hz, C*H*), 4.07 (2H, q, J = 6.5 Hz, OCH_2CH_3), 1.33 (3H, t, J = 6.5 Hz, OCH_2CH_3).

(*E*)-*N*-(2-Aminoethyl)-3-(4-ethoxyphenyl)acrylamide (20)

Step 1. To a mixture of compound **19** (120 mg, 0.63 mmol), HBTU (360 mg, 0.94 mmol), *tert*-butyl (2-aminoethyl)carbamate (100 mg, 0.63 mmol) and DIPEA (163 μL, 0.94 mmol) was added 6 mL of DMF at room temperature. The reaction mixture was stirred for 3 h at room temperature and then the reaction was quenched by adding water. The white solid was collected by filtration, washed with water and ethyl ether, and dried to afford 208 mg (99.5%) of Boc-protected **20** as a white solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 8.04 (1H, m, N*H*), 7.51 (2H, d, J = 8.0 Hz, *Ar*), 7.39 (1H, d, J = 15.5 Hz, C*H*), 6.97 (2H, d, J = 8.0 Hz, *Ar*), 6.85 (1H, m, N*H*), 6.47 (1H, d, J = 15.5 Hz, C*H*), 4.07 (2H, q, J = 6.5 Hz, OCH_2CH_3), 3.21 (2H, q, J = 6.0 Hz, CH_2NH), 3.04 (2H, q, J = 6.0 Hz, $NHCH_2$), 1.39 (9H, s, $C(CH_3)_3$), 1.34 (3H, t, J = 6.5 Hz, OCH_2CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 165.4, 159.5, 155.6, 138.3, 129.0, 127.3, 119.6, 114.7, 77.6, 63.1, 38.8, 28.2, 14.5.

Step 2. The title compound **20** was prepared from *Boc-protected* **20** (60 mg, 0.18 mmol) in a manner similar to that described for **6** in 75.0% (47 mg) yield as a white solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 8.30 (1H, m, N*H*), 7.88 (2H, m, NH_2), 7.53 (2H, d, J = 8.0 Hz, *Ar*), 7.44 (1H, d, J = 15.5 Hz, C*H*), 6.98 (2H, d, J = 8.0 Hz, *Ar*), 6.50 (1H, d, J = 15.5 Hz, C*H*), 4.08 (2H, q, J = 7.0 Hz, OCH_2CH_3), 3.43 (2H, m, CH_2NH_2), 2.94 (2H, m, $NHCH_2$), 1.35 (3H, t, J = 7.0 Hz, OCH_2CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 166.1, 159.7, 138.9, 129.1, 127.1, 119.1, 114.8, 66.3, 63.2, 38.8, 36.7, 14.5; MS (ESI) m/z Calcd for $C_{13}H_{18}N_2O_2$ (M^+): 234.1, Found: 235.0 ($M + H^+$); HPLC purity (condition IX), 99.0% (t_R, 3.10 min), HPLC purity (condition XIII), 99.8% (t_R, 3.29 min).

(*Z*)-*tert*-Butyl (3-(5-(4-ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)-propyl)carbamate (21a)

Potassium carbonate (350 mg, 2.53 mmol) was added to a solution of compound **2** (210 mg, 0.84 mmol) in 4 mL of anhydrous DMF at 0 °C. After stirring of reaction mixture for 20 min, a solution of *tert*-butyl (3-bromopropyl)carbamate (200 mg, 0.84 mmol) in anhydrous DMF (3 mL) was added and then the reaction mixture was stirred at room temperature for 3 h. The reaction was quenched by adding water and extracted with ethyl acetate. The ethyl acetate layer was washed with 10% NH_4Cl aqueous solution followed by water and dried over anhydrous Na_2SO_4, filtered, concentrated and purified by silica column chromatography (20% ethyl acetate in hexanes) to afford compound **21a** (78.8%, 270 mg) as a white solid. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.85 (1H, s, C*H*), 7.46 (2H, d, J = 9.0 Hz, *Ar*), 6.98 (2H, d, J = 9.0 Hz, *Ar*), 5.02 (1H, m, N*H*), 4.11 (2H, q, J = 6.5 Hz, OCH_2CH_3), 3.84 (2H, t, J = 6.5 Hz, CH_2), 3.12 (2H, m, CH_2), 1.85 (2H, t, J = 6.5 Hz, CH_2), 1.44 (9H, s, $C(CH_3)_3$), 1.23 (3H, d, J = 6.5 Hz, OCH_2CH_3).

(*Z*)-*tert*-Butyl (1-(5-(4-ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)-propan-2-yl)carbamate (21b)

The title compound **21b** was prepared from compound **2** (260 mg, 1.04 mmol) and *tert*-butyl (1-bromopropan-2-yl)carbamate (247 mg, 1.04 mmol) in a manner similar to that described for **21a**. Yield = 22.5%, 95.0 mg; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.85 (1H, s, C*H*), 7.46 (2H, d, J = 9.0 Hz, *Ar*), 6.98 (2H, d, J = 9.0 Hz, *Ar*), 4.60 (1H, m, N*H*), 4.10 (3H, q, J = 7.0 Hz, OCH_2CH_3 + $CHCH_3$), 3.73 (1H, m, NCH_2), 1.45 (3H, t, J = 8.0 Hz, OCH_2CH_3), 1.36 (9H, s, $C(CH_3)_3$), 1.21 (3H, d, J = 6.5 Hz, $CHCH_3$); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 168.7, 167.0, 161.0, 155.5, 134.0, 132.4, 125.9, 118.4, 115.4, 79.6, 63.9, 47.1, 45.2, 28.4, 18.7, 14.8.

(*Z*)-*tert*-Butyl (2-(5-(4-ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)propyl)carbamate (21c)

The title compound **21c** was prepared from compound **2** (300 mg, 1.20 mmol) and *tert*-butyl (2-bromopropyl)carbamate (286 mg, 1.20 mg) in a manner similar to that described for **21a**. Yield = 15.4%, 75 mg; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.84 (1H, s, C*H*), 7.45 (2H, d, J = 8.5 Hz, *Ar*), 6.97 (2H, d, J = 8.5 Hz, *Ar*), 4.67 (1H, m, N*H*), 4.11 (1H, m, $CHCH_3$), 4.09 (2H, q, J = 7.0 Hz, OCH_2CH_3), 3.75 (2H, m, $CHCH_2$), 1.45 (3H, d, J = 6.5 Hz, CH_3CH), 1.35 (9H, s, $C(CH_3)_3$), 1.20 (3H, t, J = 6.5 Hz, OCH_2CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 168.4, 167.0, 161.0, 156.0, 134.0, 133.7, 132.4, 125.9, 115.4, 79.7, 63.9, 51.4, 47.1, 45.2, 42.7, 28.4, 18.7, 15.2, 14.8.

(*S*,*Z*)-Methyl 2-((*tert*-butoxycarbonyl)amino)-3-(5-(4-ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)propanoate (21d)

Compound **2** (285 mg, 1.15 mmol), (*S*)-methyl 2-((*tert*-butoxycarbonyl)amino)-3-hydroxypropanoate (251 mg, 1.15 mmol) and triphenylphosphine (452 mg, 1.72 mmol) were dissolved in 11 mL of anhydrous THF, and stirred at room temperature for 5 min. DIAD (338 μL, 1.72 mmol) was added to the reaction

mixture and then stirred overnight at 45 °C. The reaction mixture was diluted with ethyl acetate and washed with sat'd NH_4Cl aqueous solution. The organic layer was collected, dried over anhydrous Na_2SO_4, filtered, concentrated and purified by silica column chromatography (30% ethyl acetate in hexanes) to afford compound **21d** (99.5%, 517 mg) as a white solid. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.86 (1H, s, C*H*), 7.46 (2H, d, J = 9.0 Hz, *Ar*), 6.98 (2H, d, J = 9.0 Hz, *Ar*), 5.31 (1H, d, J = 8.5 Hz, N*H*), 4.69 (1H, m, C*H*NH), 4.17–4.03 (4H, m, NCH_2 + OCH_2CH_3), 3.79 (3H, s, OCH_3), 1.44 (3H, t, J = 8.0 Hz, OCH_2CH_3), 1.39 (9H, s, $C(CH_3)_3$).

(*Z*)-Di-*tert*-butyl (2-(5-(4-ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)propane-1,3-diyl)dicarbamate (21e)

The title compound **21e** was prepared from compound **2** (292 mg, 1.17 mmol) and di-*tert*-butyl (2-bromopropane-1,3-diyl)dicarbamate (413 mg, 1.17 mg) in a manner similar to that described for **21a**. Yield = 23.9%, 130 mg; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.85 (1H, s, C*H*), 7.46 (2H, d, J = 9.0 Hz, *Ar*), 6.98 (2H, d, J = 9.0 Hz, *Ar*), 5.07 (1H, m, N*H*), 4.90 (1H, m, N*H*), 4.10 (2H, q, J = 7.0 Hz, OCH_2CH_3), 4.01 (1H, m, $CH(CH_2)_2$), 3.87 (1H, m, CH_2NH), 3.80 (1H, m, CH_2NH), 3.28 (2H, m, CH_2NH), 1.56 (3H, t, J = 7.0 Hz, OCH_2CH_3), 1.45 (9H, s, $C(CH_3)_3$), 1.38 (9H, s, $C(CH_3)_3$); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 168.7, 167.0, 161.2, 156.7, 155.9, 134.4, 132.5, 125.8, 118.1, 115.4, 94.9, 80.0, 79.9, 64.0, 50.5, 43.4, 42.1, 28.5, 28.4, 14.8.

(*Z*)-3-(3-Aminopropyl)-5-(4-ethoxybenzylidene)thiazolidine-2,4-dione (22a)

The title compound **22a** was prepared from compound **21a** (157 mg, 0.39 mmol) in a manner similar to that described for **6** in 90.3% (148 mg) yield as a white solid. ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.79 (1H, s, C*H*), 7.45 (2H, d, J = 8.5 Hz, *Ar*), 6.97 (2H, d, J = 8.0 Hz, *Ar*), 4.04 (2H, q, J = 7.5 Hz, OCH_2CH_3), 3.79 (2H, t, J = 7.0 Hz, CH_2), 2.90 (2H, t, J = 7.5 Hz, CH_2), 1.94 (2H, m, CH_2), 1.35 (3H, t, J = 7.5 Hz, OCH_2CH_3); ^{13}C-NMR (CD_3OD, 125 MHz) δ 167.6, 165.9, 160.5, 132.9, 132.2, 125.2, 117.9, 115.3, 63.5, 38.7, 36.6, 25.6, 14.4; MS (ESI) m/z Calcd for $C_{15}H_{18}N_2O_3S$ (M^+): 306.1, Found: 307.0 (M + H^+); HPLC purity (condition IX), 99.8% (t_R, 3.75 min), HPLC purity (condition X), 98.5% (t_R, 4.71 min).

(*Z*)-3-(2-Aminopropyl)-5-(4-ethoxybenzylidene)thiazolidine-2,4-dione (22b)

The title compound **22b** was prepared from compound **21b** (57 mg, 0.14 mmol) in a manner similar to that described for **6** in 76.5% (45 mg) yield as a white solid. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.88 (1H, s, C*H*), 7.45 (2H, d, J = 8.0 Hz, *Ar*), 6.99 (2H, d, J = 8.0 Hz, *Ar*), 4.11 (2H, m, OCH_2CH_3), 3.98 (1H, m, NH_2), 3.90 (1H, m, N*H*$_2$), 3.69 (1H, m, C*H*NH_2), 3.48 (2H, m, CH_2CH), 1.46 (3H, d, J = 6.5 Hz, $CHCH_3$), 1.21 (3H, t, J = 6.5 Hz, OCH_2CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 168.1, 166.2, 160.55, 132.9, 132.2, 125.2, 118.1, 115.4, 66.3, 63.5, 45.4, 44.3, 16.2, 14.5; MS (ESI) m/z Calcd for $C_{15}H_{18}N_2O_3S$ (M^+): 306.1, Found: 307.0 (M + H^+); HPLC purity (condition IX), 94.8% (t_R, 3.71 min), HPLC purity (condition X), 94.8% (t_R, 4.94 min).

(*Z*)-3-(1-Aminopropan-2-yl)-5-(4-ethoxybenzylidene)-thiazolidine-2,4-dione (22c)

The title compound **22c** was prepared from compound **21c** (64 mg, 0.16 mmol) in a manner similar to that described for **6** in 71.4% (48 mg) yield as a white solid. ^{1}H-NMR ($CDCl_3$ + CD_3OD, 500 MHz) δ 7.85 (1H, s, C*H*), 7.44 (2H, d, J = 7.5 Hz, *Ar*), 6.97 (2H, d, J = 7.5 Hz, *Ar*), 4.08 (2H, q, J = 6.5 Hz, OCH_2CH_3), 4.00 (1H, m, NC*H*), 3.88 (1H, m, CH_2NH_2), 3.67 (1H, m, CH_2NH_2), 2.70 (2H, s, NH_2), 1.44 (3H, t, J = 6.5 Hz, OCH_2CH_3), 1.37 (3H, d, J = 8.0 Hz, $CHCH_3$); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 167.8, 165.9, 160.4, 132.9, 132.6, 132.2, 125.3, 118.1, 115.4, 63.5, 48.4, 45.4, 44.3, 16.2, 15.4, 14.5; MS (ESI) m/z Calcd for $C_{15}H_{18}N_2O_3S$ (M^+): 306.1, Found: 307.0 (M + H^+); HPLC purity (condition IX), 98.5% (t_R, 3.59 min).

(*S*,*Z*)-Methyl 2-amino-3-(5-(4-ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)propanoate (22d)

The title compound **22d** was prepared from compound **21d** (120 mg, 0.27 mmol) in a manner similar to that described for **6** in 35.5% (44 mg) yield as a light-yellow solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 8.62 (2H, s, NH_2), 7.91 (1H, s, C*H*), 7.61 (2H, d, J = 9.0 Hz, *Ar*), 7.12 (2H, d, J = 9.0 Hz, *Ar*), 4.33 (1H, t, J = 7.0 Hz, C*H*NH_2), 4.14–4.06 (4H, m, NCH_2 + OCH_2CH_3), 3.75 (3H, s, OCH_3), 1.35 (3H, t, J = 8.0 Hz, OCH_2CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 167.7, 167.6, 165.8, 160.6, 133.4, 132.3, 125.1, 117.6, 115.5, 115.4, 94.3, 63.6, 53.2, 50.0, 40.6, 14.5, 14.4; MS (ESI) m/z Calcd for $C_{16}H_{18}N_2O_5S$ (M^+): 350.1, Found: 351.0 (M + H^+); HPLC purity (condition IX), 94.6% (t_R, 3.45 min), HPLC purity (condition XI), 99.2% (t_R, 5.13 min).

(*Z*)-3-(1,3-Diaminopropan-2-yl)-5-(4-ethoxybenzylidene)-thiazolidine-2,4-dione (22e)

The title compound **22e** was prepared from compound **21e** (70 mg, 0.13 mmol) in a manner similar to that described for **6** in 96.7% (55 mg) yield as a light-yellow solid. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.84 (1H, s, C*H*), 7.40 (2H, d, J = 9.0 Hz, *Ar*), 6.91 (2H, d, J = 9.0 Hz, *Ar*), 4.11 (1H, m, NC*H*), 4.09 (2H, q, J = 6.5 Hz, OCH_2CH_3), 3.47 (2H, m, CH_2), 3.43 (2H, m, CH_2), 3.21–2.65 (4H, bs, 2× NH_2), 1.19 (3H, t, J = 6.5 Hz, OCH_2CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 168.3, 166.2, 160.6, 133.1, 133.0, 132.2, 125.2, 118.2, 118.0, 115.9, 115.5, 115.3, 63.5, 47.7, 47.6, 41.2, 14.5, 14.4; MS (ESI) m/z Calcd for $C_{15}H_{19}N_3O_3S$ (M^+): 321.1, Found: 322.0 (M + H^+); HPLC purity (condition IX), 95.3% (t_R, 3.14 min), HPLC purity (condition X), 91.0% (t_R, 4.33 min).

(*Z*)-5-(4-Ethoxybenzylidene)-3-(2-(methylamino)ethyl)-thiazolidine-2,4-dione (23a)

To a solution of compound **76** (122 mg, 0.30 mmol) in 4 mL of 1,2-dichloroethane was added triethylamine (84 μL, 0.60 mmol), aqueous formaldehyde (37%, 24 μL, 0.30 mmol) and sodium triacetoxyborohydride (254 mg, 1.20 mmol). The reaction mixture was allowed to stir overnight at room temperature. The reaction was quenched by adding sat'd aqueous $NaHCO_3$ and stirred at room temperature for 20 min. The

reaction mixture was partitioned between ethyl acetate and sat'd aqueous $NaHCO_3$, the organic layer was washed with brine, collected, dried over anhydrous Na_2SO_4, filtered, concentrated and purified by silica column chromatography using dichloromethane–methanol–NH_4OH (ratio: 92/7/1) to afford compound **23a** (38.1%, 35 mg) as a yellow solid. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 9.88 (1H, bs, N*H*), 7.86 (1H, s, C*H*), 7.57 (2H, d, J = 8.4 Hz, *Ar*), 7.08 (2H, d, J = 8.4 Hz, *Ar*), 4.08 (2H, q, J = 6.8 Hz, OCH_2CH$_3$), 3.97 (2H, m, CH_2), 3.53 (2H, m, CH_2), 2.82 (3H, m, CH_3), 1.31 (3H, t, J = 6.8 Hz, OCH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 168.3, 166.4, 160.9, 133.5, 132.7, 125.6, 118.4, 115.8, 63.9, 54.2, 42.7, 37.0, 14.9; MS (ESI) m/z Calcd for $C_{15}H_{18}N_2O_3S$ (M^+): 306.1, Found: 307.0 (M + H^+); HPLC purity (condition IX), 94.8% (t_R, 4.25 min), HPLC purity (condition II), 90.7% (t_R, 7.39 min).

(*Z*)-5-(4-Ethoxybenzylidene)-3-(2-(ethylamino)ethyl)-thiazolidine-2,4-dione (23b)

To compound **3** (225 mg, 0.91 mmol), HOCH$_2$CH$_2$N(Et)Tr (300 mg, 0.91 mmol) and triphenylphosphine (357 mg, 1.36 mmol) dissolved in anhydrous THF (20 mL) was added DIAD (268 μL, 1.36 mmol) and stirred for 12 h. Reaction was reduced *in vacuo* and column chromatography to give 276 mg of a yellow solid. Deprotection following the procedure of compound **6** gave the title compound **23b** as a yellow solid (28.0%, 83 mg). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 8.61 (1H, bs, N*H*), 7.91 (1H, s, C*H*), 7.61 (2H, d, J = 7.5, *Ar*), 7.11 (2H, d, J = 7.5, *Ar*), 4.12 (2H, q, J = 7.0, OCH_2CH$_3$), 3.94 (2H, t, J = 6.0, CH_2), 3.26–3.19 (2H, m, CH_2), 3.00 (2H, q, J = 6.5, NCH_2CH$_3$), 1.35 (3H, t, J = 7.0, OCH$_2$CH_3), 1.17 (3H, t, J = 6.5, NCH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 167.8, 166.0, 165.6, 133.1, 132.3, 125.2, 118.0, 115.4, 63.6, 44.0, 42.1, 38.0, 14.5, 10.8; MS (ESI) m/z Calcd for $C_{16}H_{20}N_2O_3S$ (M^+): 320.1, Found: 321.1 (M + H^+); HPLC purity (condition IX), 99.4% (t_R, 3.66 min), HPLC purity (condition X), 98.8% (t_R, 5.07 min).

(*Z*)-5-(4-Ethoxybenzylidene)-3-(2-(isobutylamino)ethyl)-thiazolidine-2,4-dione (23c)

To compound **3** (90 mg, 0.36 mmol), HOCH$_2$CH$_2$N(iBu)Tr (130 mg, 0.36 mmol) and triphenylphosphine (142 mg, 0.54 mmol) dissolved in anhydrous THF (20 mL) was added DIAD (107 μL, 0.54 mmol) and stirred for 12 h. Reaction was reduced *in vacuo* and column chromatography to give 130 mg of a yellow foam. Deprotection following the procedure of compound **6** gave the title compound **23c** as a yellow solid (37.1%, 46 mg). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 8.49 (1H, bs, N*H*), 7.92 (1H, s, C*H*), 7.61 (2H, d, J = 8.0, *Ar*), 7.11 (2H, d, J = 8.0, *Ar*), 4.12 (2H, q, J = 7.0, CH_2CH$_3$), 3.98 (2H, t, J = 5.5, CH_2), 3.26–3.17 (2H, m, CH_2), 2.88–2.79 (2H, m, CH_2), 1.97–1.88 (1H, m, CH(CH$_3$)$_2$), 1.35 (3H, t, J = 7.0, CH$_2$CH_3), 0.93 (6H, d, J = 7.0, 2× CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 167.8, 166.0, 160.6, 133.1, 132.3, 125.2, 118.0, 115.4, 63.6, 53.8, 44.8, 37.5, 25.5, 19.9, 14.5; MS (ESI) m/z Calcd for $C_{18}H_{24}N_2O_3S$ (M^+): 348.2, Found: 349.1 (M + H^+); HPLC purity (condition IX), 99.2% (t_R, 3.75 min), HPLC purity (condition X), 98.7% (t_R, 6.71 min).

(*Z*)-3-(2-(Benzylamino)ethyl)-5-(4-ethoxybenzylidene)-thiazolidine-2,4-dione (23d)

The title compound **23d** was prepared from compound **76** (204 mg, 0.70 mmol), benzaldehyde (71 μL, 0.70 mmol) and sodium triacetoxyborohydride (445 mg, 2.10 mmol) in a manner similar to that described for **23a** in 46.0% (123 mg) yield as a light-yellow solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 7.86 (1H, s, C*H*), 7.58 (2H, d, J = 8.0 Hz, *Ar*), 7.28–7.19 (5H, m, *Ar*), 7.09 (2H, d, J = 8.0 Hz, *Ar*), 4.11 (2H, q, J = 7.0 Hz, OCH_2CH$_3$), 3.75 (2H, t, J = 6.0, CH_2), 3.67 (2H, m, CH_2), 2.72 (2H, t, J = 6.0 Hz, CH_2), 1.34 (3H, t, J = 7.0 Hz, OCH$_2$CH_3).

(*Z*)-4-(((2-(5-(4-Ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)-ethyl)amino)methyl)benzonitrile (23e)

The title compound **23e** was prepared from compound **76** (146 mg, 0.50 mmol), 4-cyanobenzaldehyde (37 μL, 0.50 mmol) and sodium triacetoxyborohydride (318 mg, 1.50 mmol) in a manner similar to that described for **23a** in 69.0% (140 mg) yield as a light-yellow solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 9.07 (1H, s, N*H*), 7.95 (2H, d, J = 8.0 Hz, *Ar*), 7.90 (1H, s, C*H*), 7.67 (2H, d, J = 8.0 Hz, *Ar*), 7.60 (2H, d, J = 8.0 Hz, *Ar*), 7.10 (2H, d, J = 8.0 Hz, *Ar*), 4.30 (2H, m, CH_2), 4.11 (2H, q, J = 7.0 Hz, OCH_2CH$_3$), 3.98 (2H, m, CH_2), 3.27 (2H, m, CH_2), 1.34 (3H, t, J = 7.0 Hz, OCH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 168.3, 166.4, 161.0, 133.5, 133.1, 132.7, 131.2, 125.5, 118.8, 118.4, 115.8, 112.2, 64.0, 49.9, 44.8, 38.2, 14.9.

(*Z*)-3-(2-(Dimethylamino)ethyl)-5-(4-ethoxybenzylidene)-thiazolidine-2,4-dione (24a)

To a solution of compound **76** (122 mg, 0.30 mmol) in 4 mL of 1,2-dichloroethane was added triethylamine (84 μL, 0.60 mmol), aqueous formaldehyde (37%, 24 μL, 0.30 mmol) and sodium triacetoxyborohydride (254 mg, 1.20 mmol). The reaction mixture was allowed to stir overnight at room temperature. The reaction was quenched by adding sat'd aqueous $NaHCO_3$ and stirred at room temperature for 20 min. The reaction mixture was partitioned between ethyl acetate and sat'd aqueous $NaHCO_3$, the organic layer was washed with brine, collected, dried over anhydrous Na_2SO_4, filtered, concentrated and purified by silica column chromatography using dichloromethane–methanol–NH_4OH (ratio: 92/7/1) to afford compound **24a** (76.2%, 73 mg) as a yellow solid. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 7.83 (1H, s, C*H*), 7.56 (2H, d, J = 8.4 Hz, *Ar*), 7.05 (2H, d, J = 8.4 Hz, *Ar*), 4.08 (2H, q, J = 6.4 Hz, OCH_2CH$_3$), 3.64 (2H, m, CH_2), 3.31 (6H, s, 2 × CH_3), 3.23 (2H, m, CH_2), 1.31 (3H, t, J = 6.4 Hz, OCH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 170.0, 168.0, 166.3, 160.8, 132.9, 132.6, 125.7, 118.5, 115.7, 63.9, 41.9, 36.5, 22.9, 14.9; MS (ESI) m/z Calcd for $C_{16}H_{20}N_2O_3S$ (M^+): 320.1, Found: 321.0 (M + H^+); HPLC purity (condition IX), 98.5% (t_R, 5.72 min), HPLC purity (condition II), 96.0% (t_R, 8.71 min).

(*Z*)-3-(2-(Dibenzylamino)ethyl)-5-(4-ethoxybenzylidene)-thiazolidine-2,4-dione (24b)

The title compound **24b** was prepared from compound **76** (117 mg, 0.40 mmol), benzaldehyde (82 μL, 0.80 mmol) and sodium triacetoxyborohydride (254 mg, 1.20 mmol) in a manner similar to that described for **23a** in 93.1% (176 mg) yield as a light-yellow solid. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 7.72 (1H, s, C*H*), 7.59 (2H, d, J = 8.4 Hz, *Ar*), 7.20–7.16 (8H, m, *Ar*), 7.12–7.07 (4H, m, *Ar*), 4.08 (2H, q, J = 6.8 Hz, OCH_2CH$_3$), 3.75 (2H, m, CH_2), 3.32 (4H, s, 2× CH$_2$), 2.54 (2H, m, CH_2), 1.32 (3H, t, J = 6.8 Hz, OCH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 167.6, 165.9, 160.8, 139.4, 133.1, 132.6, 128.8, 128.5, 127.2, 125.8, 118.3, 115.8, 63.9, 57.7, 50.1, 14.9; MS (ESI) m/z Calcd for $C_{28}H_{28}N_2O_3S$ (M^+): 472.2, Found: 473.1 ($M + H^+$).

(*Z*)-3-(((4-Cyanobenzyl)(2-(5-(4-ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)ethyl)amino)methyl)benzonitrile (24c)

The title compound **24c** was prepared from compound **76** (88 mg, 0.30 mmol), 4-cyanobenzaldehyde (79 mg, 0.60 mmol) and sodium triacetoxyborohydride (190 mg, 0.90 mmol) in a manner similar to that described for **23a** in 68.5% (110 mg) yield as a yellow solid. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.72 (1H, s, C*H*), 7.52–7.50 (6H, m, *Ar*), 7.53–7.34 (4H, m, *Ar*), 7.02 (2H, d, J = 8.0 Hz, *Ar*), 4.11 (2H, q, J = 6.8 Hz, OCH_2CH$_3$), 3.84 (2H, m, CH_2), 3.58 (4H, s, 2× CH_2), 2.69 (2H, m, CH_2), 1.44 (3H, t, J = 6.8 Hz, OCH$_2$CH_3); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 167.9, 166.1, 161.2, 144.2, 134.0, 132.4, 132.2, 129.3, 125.3, 118.7, 117.6, 115.4, 111.1, 63.8, 58.0, 50.7, 39.2, 29.6, 14.6; MS (ESI) m/z Calcd for $C_{30}H_{26}N_4O_3S$ (M^+): 522.2, Found: 523.1 ($M + H^+$).

(*Z*)-*N*-(2-(5-(4-Ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)-ethyl)acetamide (25a)

To a solution of compound **76** (101 mg, 0.25 mmol) in 5 mL of dichloromethane was added triethylamine (105 μL, 0.75 mmol) and acetic anhydride (26.0 μL, 0.28 mmol). After being stirred at room temperature overnight, the reaction solution was diluted with 50 mL of dichloromethane, washed with water, collected the organic layer, dried over Na_2SO_4, filtered, concentrated and purified by silica column chromatography (33% dichloromethane in ethyl acetate) to afford 74 mg (88.6%) of the title compound **25a** as a light-yellow solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 7.99 (1H, t, J = 6.0 Hz, N*H*), 7.86 (1H, s, C*H*), 7.59 (2H, d, J = 9.0 Hz, *Ar*), 7.10 (2H, d, J = 9.0 Hz, *Ar*), 4.11 (2H, q, J = 7.0 Hz, OCH_2CH$_3$), 3.68 (2H, t, J = 6.0 Hz, CH_2), 3.27 (2H, m, CH_2), 1.72 (3H, s, CH_3), 1.34 (3H, t, J = 7.0 Hz, OCH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 169.5, 167.5, 165.9, 160.4, 132.5, 132.2, 125.3, 118.1, 115.3, 63.5, 41.4, 36.0, 22.4, 14.5; MS (ESI) m/z Calcd for $C_{16}H_{18}N_2O_4S$ (M^+): 334.1, Found: 335.0 ($M + H^+$); HPLC purity (condition I), 97.5% (t_R, 3.38 min), HPLC purity (condition III), 99.5% (t_R, 4.82 min).

(*Z*)-*N*-(2-(5-(4-Ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)-ethyl)-2,2,2-trifluoroacetamide (25b)

To a solution of compound **76** (81 mg, 0.20 mmol) in 4 mL of dichloromethane was added potassium carbonate (83 mg, 0.60 mmol) followed by trifluoroacetic anhydride (31 μL, 0.22 mmol). After being stirred at room temperature for 3 h, the reaction solution was diluted with 50 mL of dichloromethane, washed with water, collected the organic layer, dried over Na_2SO_4, filtered, concentrated and purified by silica column chromatography (6% ethyl acetate in dichloromethane) to afford 62 mg (81.0%) of the title compound **25b** as a light-yellow solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 9.56 (1H, m, N*H*), 7.88 (1H, s, C*H*), 7.60 (2H, d, J = 8.5 Hz, *Ar*), 7.10 (2H, d, J = 8.5 Hz, *Ar*), 4.11 (2H, q, J = 7.0 Hz, OCH_2CH$_3$), 3.79 (2H, t, J = 6.0 Hz, CH_2), 3.45 (2H, m, CH_2), 1.34 (3H, t, J = 7.0 Hz, OCH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 167.6, 165.9, 160.4, 156.8, 132.7, 132.2, 125.2, 118.0, 116.9, 115.4, 114.6, 63.5, 40.5, 37.0, 14.5; MS (ESI) m/z Calcd for $C_{16}H_{15}F_3N_2O_4S$ (M^+): 388.1, Found: 389.0 ($M + H^+$); HPLC purity (condition I), 97.1% (t_R, 21.01 min), HPLC purity (condition III), 98.3% (t_R, 21.70 min).

(*Z*)-*tert*-Butyl (2-(5-(4-ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)ethyl)carbamate (25c)

To a solution of compound **76** (101 mg, 0.25 mmol) in 3 mL of dichloromethane was added DIPEA (131 μL, 0.75 mmol) followed by pivaloyl chloride (34 μL, 0.28 mmol). After being stirred at room temperature overnight, the reaction solution was diluted with 50 mL of dichloromethane, washed with water, collected the organic layer, dried over Na_2SO_4, filtered, concentrated and purified by silica column chromatography (10% ethyl acetate in hexanes) to afford 89 mg (90.8%) of the title compound **25c** as a yellow solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 7.84 (1H, s, C*H*), 7.64 (1H, t, J = 5.5 Hz, N*H*), 7.58 (2H, d, J = 8.0 Hz, *Ar*), 7.09 (2H, d, J = 8.0 Hz, *Ar*), 4.11 (2H, q, J = 7.0 Hz, OCH_2CH$_3$), 3.69 (2H, m, CH_2), 3.30 (2H, m, CH_2), 1.34 (3H, t, J = 7.0 Hz, OCH$_2$CH_3), 1.01 (9H, s, C(CH_3)$_3$); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 177.6, 167.5, 165.9, 160.3, 132.2, 132.1, 125.3, 118.3, 115.3, 63.5, 41.4, 37.9, 36.3, 27.3, 14.5; MS (ESI) m/z Calcd for $C_{19}H_{24}N_2O_5S$ (M^+): 392.1, Found: 393.1 ($M + H^+$); HPLC purity (condition I), 98.7% (t_R, 20.59 min), HPLC purity (condition VIII), 99.2% (t_R, 11.43 min).

(*Z*)-4-Cyano-*N*-(2-(5-(4-ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)ethyl)benzamide (25d)

To a solution of 4-cyanobenzoic acid (46 mg, 0.31 mmol) in 3 mL of DMF was added DIPEA (131 μL, 0.75 mmol) followed by HBTU (114 mg, 0.30 mmol). After being stirred at room temperature for 1 h, compound **76** (101 mg, 0.25 mmol) was added and then the reaction mixture was stirred at room temperature overnight. The reaction solution was diluted with 50 mL of ethyl acetate and washed with water (30 mL × 5), collected the organic layer, dried over Na_2SO_4, filtered, concentrated and purified by silica column chromatography (15%

ethyl acetate in hexanes) to afford 97 mg (92.1%) of the title compound **25d** as a light-yellow solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 8.89 (1H, t, J = 6.0 Hz, N*H*), 7.96 (2H, d, J = 9.0 Hz, *Ar*), 7.87 (1H, s, C*H*), 7.86 (2H, d, J = 9.0 Hz, *Ar*), 7.59 (2H, d, J = 9.0 Hz, *Ar*), 7.10 (2H, d, J = 9.0 Hz, *Ar*), 4.11 (2H, q, J = 7.0 Hz, OC*H*$_2$CH$_3$), 3.84 (2H, m, C*H*$_2$), 3.54 (2H, m, C*H*$_2$), 1.34 (3H, t, J = 7.0 Hz, OCH$_2$C*H*$_3$); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 167.7, 166.0, 165.4, 160.4, 138.4, 132.6, 132.4, 132.2, 127.9, 125.3, 118.3, 118.1, 115.3, 113.5, 63.5, 41.3, 37.0, 14.5; MS (ESI) m/z Calcd for $C_{22}H_{19}N_3O_4S$ (M^+): 421.1, Found: 422.1 (M + H^+); HPLC purity (condition I), 99.1% (t_R, 20.25 min), HPLC purity (condition III), 98.3% (t_R, 6.85 min).

(*Z*)-Methyl (2-(5-(4-ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)ethyl)carbamate (26a)

The title compound **26a** was prepared from compound **76** (101 mg, 0.25 mmol), methyl chloroformate (21 μL, 0.28 mmol) and DIPEA (131 μL, 0.75 mmol) in a manner similar to that described for **25a** in 98.2% (86 mg) yield as a yellow solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 7.87 (1H, s, C*H*), 7.59 (2H, d, J = 8.0 Hz, *Ar*), 7.27 (1H, m, N*H*), 7.10 (2H, d, J = 8.0 Hz, *Ar*), 4.10 (2H, q, J = 7.0 Hz, OC*H*$_2$CH$_3$), 3.70 (2H, m, C*H*$_2$), 3.50 (3H, s, C*H*$_3$), 3.22 (2H, m, C*H*$_2$), 1.35 (3H, t, J = 7.0 Hz, OCH$_2$C*H*$_3$); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 167.5, 165.9, 160.4, 156.8, 132.5, 132.2, 125.3, 118.2, 115.3, 63.5, 51.3, 41.5, 37.8, 14.5; MS (ESI) m/z Calcd for $C_{16}H_{18}N_2O_5S$ (M^+): 350.1, Found: 351.0 (M + H^+); HPLC purity (condition I), 100% (t_R, 16.80 min), HPLC purity (condition V), 94.6% (t_R, 5.75 min).

(*Z*)-*tert*-Butyl (2-(5-(4-ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)ethyl)carbamate (26b)

The title compound **26b** was prepared from compound **76** (405 mg, 0.50 mmol), di-*tert*-butyl dicarbonate (153 mg, 0.70 mmol) and DIPEA (261 μL, 1.50 mmol) in a manner similar to that described for **25a** in 97.9% (192 mg) yield as a light-yellow solid. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.83 (1H, s, C*H*), 7.44 (2H, d, J = 7.6 Hz, *Ar*), 6.96 (2H, d, J = 7.6 Hz, *Ar*), 4.78 (1H, m, N*H*), 4.08 (2H, q, J = 6.4 Hz, OC*H*$_2$CH$_3$), 3.87 (2H, m, C*H*$_2$), 3.40 (2H, m, C*H*$_2$), 1.44 (3H, t, J = 6.4 Hz, OCH$_2$C*H*$_3$), 1.37 (9H, s, C(C*H*$_3$)$_3$); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 168.3, 166.7, 160.9, 155.9, 133.9, 132.2, 125.6, 118.0, 115.1, 79.6, 63.7, 41.6, 38.8, 20.2, 14.6; MS (ESI) m/z Calcd for $C_{19}H_{24}N_2O_5S$ (M^+): 392.1, Found: 393.1 (M + H^+); HPLC purity (condition I), 98.3% (t_R, 23.74 min), HPLC purity (condition V), 98.1% (t_R, 36.56 min).

(*Z*)-Isobutyl (2-(5-(4-ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)ethyl)carbamate (26c)

The title compound **26c** was prepared from compound **76** (70 mg, 0.17 mmol), isobutyl chloroformate (25 μL, 0.19 mmol) and DIPEA (75 μL, 0.43 mmol) in a manner similar to that described for **25a** in 96.4% (65 mg) yield as a light-yellow solid. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.84 (1H, s, C*H*), 7.45 (2H, d, J = 9.0 Hz, *Ar*), 6.97 (2H, d, J = 9.0 Hz, *Ar*), 4.97 (1H, s, N*H*), 4.09 (2H, q, J = 7.5 Hz, OC*H*$_2$CH$_3$), 3.91 (2H, t, J = 5.5 Hz, NC*H*$_2$), 3.81 (2H, d, J = 6.5 Hz, C(=O)OC*H*$_2$), 3.49 (2H, m, C*H*$_2$N), 1.87 (1H, m, C*H*(CH$_3$)$_2$), 1.43 (3H, t, J = 7.5 Hz, OCH$_2$C*H*$_3$), 0.88 (6H, d, J = 6.5 Hz, CH(C*H*$_3$)$_2$); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 168.6, 166.9, 161.1, 157.0, 134.3, 132.5, 125.8, 118.1, 115.4, 71.4, 64.0, 41.6, 39.6, 28.1, 19.1, 14.8; MS (ESI) m/z Calcd for $C_{19}H_{24}N_2O_5S$ (M^+): 392.1, Found: 393.0 (M + H^+); HPLC purity (condition I), 98.2% (t_R, 23.71 min), HPLC purity (condition III), 97.7% (t_R, 36.64 min).

(*Z*)-Benzyl (2-(5-(4-ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)ethyl)carbamate (26d)

The title compound **26d** was prepared from compound **76** (70 mg, 0.17 mmol), benzyl chloroformate (27 μL, 0.19 mmol) and DIPEA (75 μL, 0.43 mmol) in a manner similar to that described for **25a** in 95.5% (70 mg) yield as a light-yellow solid. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.83 (1H, s, C*H*), 7.44 (2H, d, J = 8.5 Hz, *Ar*), 7.32–7.25 (5H, m, *Ar*), 6.97 (2H, d, J = 8.5 Hz, *Ar*), 5.07 (3H, s, C(=O)OC*H*$_2$ + N*H*), 4.10 (2H, q, J = 7.5 Hz, OC*H*$_2$CH$_3$), 3.92 (2H, m, NC*H*$_2$), 3.51 (2H, m, C*H*$_2$N), 1.44 (3H, t, J = 7.5 Hz, OCH$_2$C*H*$_3$); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 168.6, 166.9, 161.2, 156.6, 136.6, 134.4, 132.5, 128.6, 128.2, 125.8, 118.0, 115.4, 67.0, 64.0, 41.5, 39.7, 14.8; MS (ESI) m/z Calcd for $C_{22}H_{22}N_2O_5S$ (M^+): 426.1, Found: 427.0 (M + H^+); HPLC purity (condition I), 97.3% (t_R, 23.84 min), HPLC purity (condition III), 92.3% (t_R, 38.28 min).

(*Z*)-(9*H*-Fluoren-9-yl)methyl (2-(5-(4-ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)ethyl)carbamate (26e)

The title compound **26e** was prepared from compound **76** (101 mg, 0.25 mmol), Fmoc chloride (71 mg, 0.28 mmol) and DIPEA (131 μL, 0.75 mmol) in a manner similar to that described for **25a** in 96.4% (124 mg) yield as a yellow solid. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.79 (1H, s, C*H*), 7.73 (2H, d, J = 7.2 Hz, *Fmoc*), 7.56 (2H, d, J = 8.0 Hz, *Ar*), 7.37 (4H, m, *Fmoc*), 7.27 (2H, m, *Fmoc*), 6.92 (2H, d, J = 8.0 Hz, *Ar*), 5.17 (1H, m, N*H*), 4.32 (2H, d, J = 6.8 Hz, C*H*$_2$-*Fomc*), 4.18 (1H, m, C*H*-*Fomc*), 4.06 (2H, q, J = 6.8 Hz, OC*H*$_2$CH$_3$), 3.93 (2H, m, C*H*$_2$), 3.52 (2H, m, C*H*$_2$), 1.42 (3H, t, J = 6.8 Hz, OCH$_2$C*H*$_3$); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 168.4, 166.7, 160.9, 156.4, 143.9, 141.2, 134.3, 132.3, 127.5, 127.0, 125.4, 125.1, 119.8, 117.7, 115.1, 67.0, 63.7, 47.1, 41.3, 39.5, 14.6; MS (ESI) m/z Calcd for $C_{29}H_{26}N_2O_5S$ (M^+): 514.2, Found: 515.1 (M + H^+); HPLC purity (condition I), 97.0% (t_R, 28.04 min), HPLC purity (condition V), 96.0% (t_R, 44.48 min).

(*Z*)-*N*-(2-(5-(4-Ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)-ethyl)methanesulfonamide (27a)

The title compound **27a** was prepared from compound **76** (101 mg, 0.25 mmol), methylsulfonyl chloride (21 μL, 028 mmol) and DIPEA (131 μL, 0.75 mmol) in a manner similar to that described for **25a** in 96.2% (89 mg) yield as a yellow solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 7.86 (1H, s, C*H*), 7.59 (2H, d, J = 9.0 Hz, *Ar*), 7.28 (1H, t, J = 6.0 Hz, N*H*), 7.10 (2H, d, J = 9.0 Hz, *Ar*), 4.11 (2H, q, J = 7.0 Hz, OC*H*$_2$CH$_3$), 3.74 (2H, t, J = 6.0 Hz, C*H*$_2$), 3.22 (2H, m, C*H*$_2$), 2.88 (3H, s, C*H*$_3$), 1.34 (3H, t, J = 7.0 Hz, OCH$_2$C*H*$_3$); ^{13}C-NMR (DMSO-d_6,

125 MHz) δ 167.5, 165.8, 160.4, 132.6, 132.2, 125.3, 118.1, 115.3, 63.5, 41.8, 14.5; MS (ESI) *m*/*z* Calcd for $C_{15}H_{18}N_2O_5S_2$ (M^+): 370.1, Found: 371.0 (M + H^+); HPLC purity (condition II), 95.2% (t_R, 14.41 min), HPLC purity (condition III), 99.5% (t_R, 5.31 min).

(*Z*)-4-Cyano-*N*-(2-(5-(4-ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)ethyl)benzenesulfonamide (27b)

The title compound **27b** was prepared from compound **76** (101 mg, 0.25 mmol), 4-cyano-benzenesulfonyl chloride (55 mg, 0.28 mmol) and DIPEA (131 μL, 0.75 mmol) in a manner similar to that described for **25a** in 92.0% (105 mg) yield as a light-yellow solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 8.24 (1H, t, *J* = 6.0 Hz, N*H*), 8.07 (2H, d, *J* = 9.0 Hz, *Ar*), 7.91 (2H, d, *J* = 9.0 Hz, *Ar*), 7.84 (1H, s, C*H*), 7.58 (2H, d, *J* = 9.0 Hz, *Ar*), 7.10 (2H, d, *J* = 9.0 Hz, *Ar*), 4.10 (2H, q, *J* = 7.0 Hz, OCH_2CH$_3$), 3.68 (2H, t, *J* = 6.0 Hz, CH_2), 3.10 (2H, m, CH_2), 1.34 (3H, t, *J* = 7.0 Hz, OCH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 167.4, 165.7, 160.4, 144.6, 133.5, 132.7, 132.2, 127.1, 125.2, 117.9, 117.6, 115.3, 114.9, 63.5, 41.4, 14.5; MS (ESI) *m*/*z* Calcd for $C_{21}H_{19}N_3O_5S_2$ (M^+): 457.1, Found: 458.0 (M + H^+); HPLC purity (condition I), 97.7% (t_R, 21.40 min), HPLC purity (condition III), 97.9% (t_R, 24.19 min).

(*Z*)-*N*-(2-(5-(4-Ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)-ethyl)-1-methyl-1*H*-imidazole-4-sulfonamide (27c)

The title compound **27c** was prepared from compound **76** (101 mg, 0.25 mmol), 1-methylimidazole-4-sulfonyl chloride (50 mg, 0.28 mmol) and DIPEA (131 μL, 0.75 mmol) in a manner similar to that described for **25a** in 98.0% (108 mg) yield as a light-yellow solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 7.83 (1H, s, C*H*), 7.74 (1H, m, N*H*), 7.68 (1H, s, *Im*), 7.66 (1H, s, *Im*), 7.58 (2H, d, *J* = 9.0 Hz, *Ar*), 7.10 (2H, d, *J* = 9.0 Hz, *Ar*), 4.10 (2H, q, *J* = 7.0 Hz, OCH_2CH$_3$), 3.69 (2H, t, *J* = 6.0 Hz, CH_2), 3.67 (3H, s, *Im*-CH_3), 3.10 (2H, m, CH_2), 1.33 (3H, t, *J* = 7.0 Hz, OCH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 167.4, 165.7, 160.4, 139.5, 132.4, 132.2, 125.3, 123.9, 118.2, 115.3, 63.5, 41.6, 33.4, 14.5; MS (ESI) *m*/*z* Calcd for $C_{18}H_{20}N_4O_5S_2$ (M^+): 436.1, Found: 437.0 (M + H^+); HPLC purity (condition IV), 100% (t_R, 5.74 min), HPLC purity (condition II), 100% (t_R, 8.67 min).

(*Z*)-3-((1*H*-Imidazol-5-yl)methyl)-5-(4-ethoxybenzylidene)-thiazolidine-2,4-dione (28a)

Step 1. To a solution of compound **2** (150 mg, 0.60 mmol) in 20 mL of anhydrous THF was added (1-trityl-1*H*-imidazol-5-yl)-methanol (205 mg, 0.60 mmol), triphenylphosphine (237 mg, 0.90 mmol) and DIAD (178 μL, 0.90 mmol). The reaction mixture was stirred at 45 °C overnight. The solvent was removed by evaporation and the residue was dissolved in ethyl acetate, and washed with sat'd aqueous NH_4Cl. The organic layer was collected, dried over anhydrous Na_2SO4, filtered, concentrated and purified by silica column chromatography (30% ethyl acetate in hexanes) to afford trityl-protected **28a** in 61.1% (210 mg) yield as a white solid.

Step 2 (cleavage of trityl group). The trityl-protected **28a** (90 mg, 0.16 mmol) was dissolved in 20% TFA solution in dichloromethane and the reaction mixture was stirred at room temperature for 1 h. The solvent was removed by evaporation and the precipitate was collected by filtration, washed with ethyl ether, and dried to afford 61 mg (84.8%) of pure **28a** as a light-yellow solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 8.76 (1H, s, *Im*), 7.79 (1H, s, C*H*), 7.50 (1H, s, *Im*), 7.47 (2H, d, *J* = 7.5 Hz, *Ar*), 6.98 (2H, d, *J* = 7.5 Hz, *Ar*), 4.77 (2H, s, NCH_2), 3.99 (2H, q, *J* = 6.0 Hz, OCH_2CH$_3$), 1.22 (3H, t, *J* = 6.0 Hz, OCH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 167.1, 165.2, 160.6, 134.6, 133.4, 132.3, 127.9, 125.1, 117.7, 115.4, 63.5, 35.6, 14.4; MS (ESI) *m*/*z* Calcd for $C_{16}H_{15}N_3O_3S$ (M^+): 329.1, Found: 330.0 (M + H^+); HPLC purity (condition IX), 97.5% (t_R, 3.57 min), HPLC purity (condition X), 95.4% (t_R, 6.74 min).

(*Z*)-3-((6-Aminopyridin-2-yl)methyl)-5-(4-ethoxybenzylidene)-thiazolidine-2,4-dione (28b)

Step 1. Potassium carbonate (131 mg, 0.95 mmol) was added to a solution of compound **2** (78 mg, 0.32 mmol) in 3 mL of anhydrous DMF at 0 °C. After stirring of the reaction mixture for 20 min, a solution of *tert*-butyl (6-(bromomethyl)pyridin-2-yl)carbamate (90 mg, 0.32 mmol) in 1 mL of anhydrous DMF was added and then the reaction mixture was stirred at room temperature for 3 h. The reaction was quenched by adding water and extracted with ethyl acetate. The ethyl acetate layer was washed with 10% NH_4Cl aqueous solution followed by water and dried over anhydrous Na_2SO_4, filtered, concentrated and purified by silica column chromatography (20% ethyl acetate in hexanes) to afford 112 mg (78.1%) of Boc-protected **28b** as a yellow solid. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.88 (1H, s, C*H*), 7.86 (1H, d, *J* = 8.0 Hz, *Py*), 7.64 (1H, d, *J* = 8.0 Hz, *Py*), 7.48 (2H, d, *J* = 9.0 Hz, *Ar*), 6.99 (2H, d, *J* = 8.0 Hz, *Ar*), 6.94 (1H, d, *J* = 8.0 Hz, *Py*), 4.94 (2H, s, NCH_2), 4.10 (2H, q, *J* = 7.0 Hz, OCH_2CH$_3$), 1.50 (9H, s, C(CH_3)$_3$), 1.44 (3H, t, *J* = 7.0 Hz, OCH$_2$CH_3).

Step 2 (cleavage of Boc group). The title compound **28b** was prepared from Boc-protected **28b** (60 mg, 0.13 mmol) in a manner similar to that described for **6** in 78.0% (48 mg) yield as a yellow solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 7.93 (1H, s, C*H*), 7.68 (1H, t, *J* = 8.0 Hz, *Py*), 7.62 (2H, d, *J* = 9.0 Hz, *Ar*), 7.12 (2H, d, *J* = 8.0 Hz, *Ar*), 6.73 (1H, d, *J* = 8.0 Hz, *Py*), 6.64 (1H, d, *J* = 8.0 Hz, *Py*), 4.84 (2H, s, NCH_2), 4.12 (2H, q, *J* = 7.0 Hz, OCH_2CH$_3$), 1.35 (3H, t, *J* = 7.0 Hz, OCH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 167.4, 165.4, 160.6, 155.9, 133.6, 132.4, 125.2, 117.6, 115.4, 115.3, 110.9, 109.5, 63.5, 42.6, 14.4; MS (ESI) *m*/*z* Calcd for $C_{18}H_{17}N_3O_3S$ (M^+): 355.1, Found: 356.0 (M + H^+); HPLC purity (condition IX), 99.1% (t_R, 3.88 min), HPLC purity (condition X), 97.4% (t_R, 11.28 min).

(*Z*)-1-(2-(5-(4-Ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)-ethyl)guanidine (28c)

The title compound **28c** was prepared from compound **2** (101 mg, 0.25 mmol), *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine (85 mg, 0.27 mmol) and DIPEA (65 μL, 0.38 mmol) in a manner similar to that described for **28b** in 81.1% (108 mg) yield as a yellow solid. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 7.84 (1H, s, C*H*), 7.70 (1H, m, N*H*), 7.56 (2H, d, *J* = 8.4 Hz, *Ar*),

7.34–7.28 (3H, bs, N*H* + N*H*$_2$), 7.07 (2H, d, *J* = 8.4 Hz, *Ar*), 4.06 (2H, q, *J* = 6.8 Hz, OC*H*$_2$CH$_3$), 3.72 (2H, m, C*H*$_2$), 3.38 (2H, m, C*H*$_2$), 1.31 (3H, t, *J* = 6.8 Hz, OCH$_2$C*H*$_3$); ^{13}C-NMR (DMSO-d$_6$, 100 MHz) *δ* 168.0, 166.2, 160.9, 157.3, 133.2, 132.6, 125.6, 118.4, 115.8, 63.9, 41.1, 38.7, 14.9; MS (ESI) *m*/*z* Calcd for $C_{15}H_{18}N_4O_3S$ (M^+): 334.1, Found: 335.1 (M + H^+); HPLC purity (condition IX), 100% (t_R, 4.14 min), HPLC purity (condition II), 98.5% (t_R, 6.38 min).

(*Z*)-5-(4-Ethoxybenzylidene)-3-propylthiazolidine-2,4-dione (28d)

Potassium carbonate (93 mg, 0.60 mmol) was added to a solution of compound **2** (75 mg, 0.30 mmol) in 3 mL of anhydrous DMF at 0 °C. After stirring of the reaction mixture for 20 min, a solution of 1-bromopropane (30 μL, 0.33 mmol) in 1 mL of anhydrous DMF was added and then the reaction mixture was stirred at room temperature overnight. The reaction was quenched by adding water and extracted with ethyl acetate. The ethyl acetate layer was washed with water (4 × 20 mL) and dried over anhydrous Na_2SO_4, filtered, concentrated and purified by silica column chromatography (15% ethyl acetate in hexanes) to afford 82 mg (93.9%) as a light-yellow solid. ^{1}H-NMR (DMSO-d$_6$, 500 MHz) *δ* 7.86 (1H, s, C*H*), 7.57 (2H, d, *J* = 8.0 Hz, *Ar*), 7.09 (2H, d, *J* = 8.0 Hz, *Ar*), 4.11 (2H, q, *J* = 7.0 Hz, OC*H*$_2$CH$_3$), 3.60 (2H, NC*H*$_2$), 1.59 (2H, m, C*H*$_2$), 1.34 (3H, t, *J* = 7.0 Hz, OCH$_2$C*H*$_3$), 0.85 (3H, t, *J* = 8.0 Hz, CH$_2$C*H*$_3$); ^{13}C-NMR (DMSO-d$_6$, 125 MHz) *δ* 167.4, 165.8, 160.4, 132.9, 132.2, 125.3, 117.8, 115.3, 63.5, 43.0, 20.5, 14.4, 11.0; MS (ESI) *m*/*z* Calcd for $C_{15}H_{17}NO_3S$ (M^+): 291.1, Found: 292.0 (M + H^+); HPLC purity (condition I), 99.5% (t_R, 26.10 min), HPLC purity (condition III), 98.6% (t_R, 44.36 min).

(*Z*)-5-(4-Ethoxybenzylidene)-3-(2-hydroxyethyl)thiazolidine-2,4-dione (28e)

The title compound **28e** was prepared from compound **2** (249 mg, 1.00 mmol), ethane-1,2-diol (111 μL, 2.00 mmol), triphenylphosphine (524 mg, 2.00 mmol) and DIAD (394 μL, 2.00 mmol) in a manner similar to that described for **9** in 95.2% (279 mg) yield as a white solid. ^{1}H-NMR (DMSO-d$_6$, 400 MHz) *δ* 7.83 (1H, s, C*H*), 7.55 (2H, d, *J* = 8.0 Hz, *Ar*), 7.06 (2H, d, *J* = 8.0 Hz, *Ar*), 4.07 (2H, q, *J* = 6.4 Hz, OC*H*$_2$CH$_3$), 3.68 (2H, m, C*H*$_2$), 3.54 (2H, m, C*H*$_2$), 1.32 (3H, t, *J* = 6.4 Hz, OCH$_2$C*H*$_3$); ^{13}C-NMR (DMSO-d$_6$, 100 MHz) *δ* 167.4, 165.9, 160.4, 132.6, 132.3, 125.4, 117.5, 115.2, 63.5, 44.1, 39.9, 14.5; MS (ESI) *m*/*z* Calcd for $C_{14}H_{15}NO_4S$ (M^+): 293.1, Found: 294.0 (M + H^+); HPLC purity (condition I), 98.8% (t_R, 3.55 min), HPLC purity (condition VIII), 96.3% (t_R, 6.54 min).

(*Z*)-3-(2-Chloroethyl)-5-(4-ethoxybenzylidene)thiazolidine-2,4-dione (28f)

The title compound **28f** was prepared from compound **2** (249 mg, 1.00 mmol), 2-chloroethanol (100 μL, 1.50 mmol), triphenylphosphine (393 mg, 1.50 mmol) and DIAD (295 μL, 1.50 mmol) in a manner similar to that described for **9** in 88.5% (276 mg) yield as a light-yellow solid. ^{1}H-NMR ($CDCl_3$, 400 MHz) *δ* 7.85 (1H, s, C*H*), 7.42 (2H, d, *J* = 8.4 Hz, *Ar*), 6.96 (2H, d, *J* = 8.4 Hz, *Ar*), 4.08 (2H, q, *J* = 6.8 Hz, OC*H*$_2$CH$_3$), 4.06 (2H, m, C*H*$_2$), 3.74 (2H, t, *J* = 5.2 Hz, C*H*$_2$), 1.42 (3H, t, *J* = 6.8 Hz, OCH$_2$C*H*$_3$); ^{13}C-NMR ($CDCl_3$, 100 MHz) *δ* 167.9, 166.1, 161.0, 134.4, 132.3, 125.4, 117.5, 115.2, 63.8, 42.6, 39.8, 14.6; MS (ESI) *m*/*z* Calcd for $C_{14}H_{14}ClNO_3S$ (M^+): 311.0, Found: 312.0 (M + H^+); HPLC purity (condition I), 98.9% (t_R, 24.50 min), HPLC purity (condition III), 96.8% (t_R, 38.45 min).

(*Z*)-*tert*-Butyl 2-(5-(4-ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)acetate (28g)

The title compound **28g** was prepared from compound **2** (249 mg, 1.00 mmol), *tert*-butyl bromoacetate (215 mg, 1.10 mmol) and potassium carbonate (276 mg, 2.00 mmol) in a manner similar to that described for **28d** in 99.0% (360 mg) yield as a pale-yellow solid. ^{1}H-NMR (DMSO-d$_6$, 500 MHz) *δ* 7.95 (1H, s, C*H*), 7.62 (2H, d, *J* = 9.0 Hz, *Ar*), 7.11 (2H, d, *J* = 9.0 Hz, *Ar*), 4.37 (2H, s, NC*H*$_2$), 4.12 (2H, q, *J* = 7.0 Hz, OC*H*$_2$CH$_3$), 1.42 (9H, s, C(C*H*$_3$)$_3$), 1.35 (3H, t, *J* = 7.0 Hz, OCH$_2$C*H*$_3$); ^{13}C-NMR (DMSO-d$_6$, 125 MHz) *δ* 166.9, 165.6, 165.1, 160.7, 134.0, 132.5, 125.0, 117.0, 115.4, 115.3, 94.2, 82.4, 63.6, 42.8, 27.6, 27.5, 14.5; MS (ESI) *m*/*z* Calcd for $C_{18}H_{21}NO_5S$ (M^+): 363.1, Found: 364.0 (M + H^+); HPLC purity (condition I), 97.3% (t_R, 25.73 min), HPLC purity (condition III), 98.9% (t_R, 47.53 min).

(*Z*)-2-(5-(4-Ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)acetic acid (28h)

The title compound **28h** was prepared by deprotection of *tert*-butyl group of compound **28g** (300 mg, 0.82 mmol). Deprotection following the procedure of compound **6** gave the title compound **28h** as a yellow solid (93.4%, 235 mg). ^{1}H-NMR (DMSO-d$_6$, 500 MHz) *δ* 13.40 (1H, bs, CO$_2$*H*), 7.94 (1H, s, C*H*), 7.61 (2H, d, *J* = 8.0 Hz, *Ar*), 7.11 (2H, d, *J* = 8.0 Hz, *Ar*), 4.37 (2H, s, NC*H*$_2$), 4.12 (2H, q, *J* = 7.0 Hz, OC*H*$_2$CH$_3$), 1.34 (3H, t, *J* = 7.0 Hz, OCH$_2$C*H*$_3$); ^{13}C-NMR (DMSO-d$_6$, 125 MHz) *δ* 168.0, 167.0, 165.1, 160.7, 133.9, 132.4, 125.0, 117.2, 115.4, 63.5, 42.2, 14.4; MS (ESI) *m*/*z* Calcd for $C_{14}H_{13}NO_5S$ (M^+): 307.1, Found: 308.0 (M + H^+); HPLC purity (condition I), 100% (t_R, 16.91 min), HPLC purity (condition III), 98.3% (t_R, 5.25 min).

(*Z*)-3-Benzyl-5-(4-ethoxybenzylidene)thiazolidine-2,4-dione (28i)

The title compound **28i** was prepared from compound **2** (75 mg, 0.30 mmol), benzyl bromide (39 μL, 0.33 mmol) and potassium carbonate (83 mg, 0.60 mmol) in a manner similar to that described for **28d** in 99.6% (101 mg) yield as a white solid. ^{1}H-NMR (DMSO-d$_6$, 500 MHz) *δ* 7.92 (1H, s, C*H*), 7.59 (2H, d, *J* = 9.0 Hz, *Ar*), 7.35 (2H, d, *J* = 7.0 Hz, *Ar*), 7.31 (3H, m, *Ar*), 7.10 (2H, d, *J* = 9.0 Hz, *Ar*), 4.83 (2H, s, NC*H*$_2$), 4.11 (2H, q, *J* = 7.0 Hz, OC*H*$_2$CH$_3$), 1.34 (3H, t, *J* = 7.0 Hz, OCH$_2$C*H*$_3$); ^{13}C-NMR (DMSO-d$_6$, 125 MHz) *δ* 167.3, 165.6, 160.5, 135.5, 133.5, 132.3, 128.6, 127.7, 127.6, 125.2, 117.6, 115.3, 63.5, 44.5, 14.4; MS (ESI) *m*/*z* Calcd for $C_{19}H_{17}NO_3S$ (M^+): 339.1, Found: 340.1 (M + H^+); HPLC purity (condition I),

100% (t_R, 25.50 min), HPLC purity (condition III), 99.7% (t_R, 48.88 min).

(*Z*)-5-(4-Ethoxybenzylidene)-3-(pyridin-2-ylmethyl)thiazolidine-2,4-dione (28j)

The title compound **28j** was prepared from compound **2** (75 mg, 0.30 mmol), 2-picolyl chloride·HCl (54 mg, 0.33 mmol), sodium iodide (45 mg, 0.30 mmol) and potassium carbonate (104 mg, 0.75 mmol) in a manner similar to that described for **28d** in 97.5% (99 mg) yield as a white solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 8.50 (1H, d, J = 4.0 Hz, *Py*), 7.92 (1H, s, C*H*), 7.84 (1H, t, J = 8.0 Hz, *Py*), 7.61 (2H, d, J = 8.0 Hz, *Ar*), 7.46 (1H, d, J = 8.0 Hz, *Py*), 7.34 (1H, t, J = 6.0 Hz, *Py*), 7.11 (2H, d, J = 8.0 Hz, *Ar*), 4.99 (2H, s, NCH_2), 4.12 (2H, q, J = 7.0 Hz, OCH_2CH$_3$), 1.35 (3H, t, J = 7.0 Hz, OCH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 167.3, 165.6, 160.5, 153.7, 148.7, 137.5, 133.4, 132.3, 125.2, 122.9, 121.7, 117.8, 115.3, 94.3, 63.5, 45.3, 14.4; MS (ESI) m/z Calcd for $C_{18}H_{16}N_2O_3S$ (M^+): 340.1, Found: 341.0 (M + H^+); HPLC purity (condition IV), 99.0% (t_R, 6.02 min), HPLC purity (condition II), 99.2% (t_R, 13.35 min).

(*Z*)-5-(4-Ethoxybenzylidene)-3-(pyridin-3-ylmethyl)thiazolidine-2,4-dione (28k)

The title compound **28k** was prepared from compound **2** (75 mg, 0.30 mmol), 3-picolyl chloride·HCl (54 mg, 0.33 mmol), sodium iodide (45 mg, 0.30 mmol) and potassium carbonate (124 mg, 0.90 mmol) in a manner similar to that described for **28d** in 93.1% (95 mg) yield as a white solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 8.73 (1H, s, *Py*), 8.67 (1H, d, J = 3.5 Hz, *Py*), 8.07 (1H, d, J = 8.0 Hz, *Py*), 7.92 (1H, s, C*H*), 7.65 (1H, d, J = 8.0 Hz, *Py*), 7.59 (2H, d, J = 9.0 Hz, *Ar*), 7.10 (2H, d, J = 9.0 Hz, *Ar*), 4.95 (2H, s, CH_2), 4.11 (2H, q, J = 7.0 Hz, OCH_2CH$_3$), 1.34 (3H, t, J = 7.0 Hz, OCH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 167.5, 165.6, 160.6, 146.1, 146.0, 139.4, 133.5, 132.8, 132.3, 125.2, 125.0, 117.7, 115.4, 94.3, 63.5, 42.0, 14.4; MS (ESI) m/z Calcd for $C_{18}H_{16}N_2O_3S$ (M^+): 340.1, Found: 341.0 (M + H^+); HPLC purity (condition IV), 100% (t_R, 4.47 min), HPLC purity (condition II), 97.1% (t_R, 11.96 min).

(*Z*)-5-(4-Ethoxybenzylidene)-3-(pyridin-4-ylmethyl)thiazolidine-2,4-dione (28l)

The title compound **28l** was prepared from compound **2** (75 mg, 0.30 mmol), 4-picolyl chloride·HCl (54 mg, 0.33 mmol), sodium iodide (45 mg, 0.30 mmol) and potassium carbonate (124 mg, 0.90 mmol) in a manner similar to that described for **28d** in 96.1% (98 mg) yield as a white solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 8.72 (2H, d, J = 8.0 Hz, *Py*), 7.95 (1H, s, C*H*), 7.68 (2H, d, J = 8.0 Hz, *Py*), 7.61 (2H, d, J = 9.0 Hz, *Ar*), 7.12 (2H, d, J = 9.0 Hz, *Ar*), 5.02 (2H, s, CH_2), 4.13 (2H, q, J = 7.0 Hz, OCH_2CH$_3$), 1.35 (3H, t, J = 7.0 Hz, OCH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 167.5, 165.5, 160.6, 149.7, 146.1, 133.7, 132.4, 125.1, 123.5, 117.6, 115.4, 63.5, 43.6, 14.4; MS (ESI) m/z Calcd for $C_{18}H_{16}N_2O_3S$ (M^+): 340.1, Found: 341.0 (M + H^+); HPLC purity (condition IV), 100% (t_R, 4.62 min), HPLC purity (condition II), 98.1% (t_R, 11.57 min).

(*Z*)-4-((5-(4-Ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)-methyl)benzonitrile (28m)

The title compound **28m** was prepared from compound **2** (75 mg, 0.30 mmol), *p*-cyanobenzyl bromide (65 mg, 0.33 mmol) and potassium carbonate (83 mg, 0.60 mmol) in a manner similar to that described for **28d** in 81.5% (89 mg) yield as a light-yellow solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 7.93 (1H, s, C*H*), 7.84 (2H, d, J = 9.0 Hz, *Ar*), 7.61 (2H, d, J = 9.0 Hz, *Ar*), 7.51 (2H, d, J = 9.0 Hz, *Ar*), 7.11 (2H, d, J = 9.0 Hz, *Ar*), 4.92 (2H, s, NCH_2), 4.12 (2H, q, J = 7.0 Hz, OCH_2CH$_3$), 1.35 (3H, t, J = 7.0 Hz, OCH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 167.4, 165.6, 160.6, 141.0, 133.7, 132.6, 132.4, 128.3, 125.2, 118.6, 117.6, 115.4, 110.5, 94.3, 63.5, 44.2, 14.5; MS (ESI) m/z Calcd for $C_{20}H_{16}N_2O_3S$ (M^+): 364.1, Found: 365.1 (M + H^+); HPLC purity (condition I), 99.7% (t_R, 26.08 min), HPLC purity (condition III), 98.9% (t_R, 42.43 min).

(*Z*)-5-(4-Ethoxybenzylidene)-3-(4-nitrobenzyl)thiazolidine-2,4-dione (28n)

The title compound **28n** was prepared from compound **2** (150 mg, 0.60 mmol), *p*-nitrobenzyl bromide (143 mg, 0.66 mmol) and potassium carbonate (166 mg, 1.20 mmol) in a manner similar to that described for **28d** in 99.5% (229 mg) yield as a white solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 8.22 (2H, d, J = 9.0 Hz, *Ar*), 7.94 (1H, s, C*H*), 7.61 (2H, d, J = 9.0 Hz, *Ar*), 7.58 (2H, d, J = 9.0 Hz, *Ar*), 7.11 (2H, d, J = 9.0 Hz, *Ar*), 4.97 (2H, s, NCH_2), 4.12 (2H, q, J = 7.0 Hz, OCH_2CH$_3$), 1.36 (3H, t, J = 7.0 Hz, OCH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 167.9, 166.0, 161.0, 147.4, 143.5, 134.1, 132.8, 131.3, 129.2, 129.0, 125.6, 124.4, 124.2, 118.0, 115.8, 64.0, 44.4, 14.9; MS (ESI) m/z Calcd for $C_{19}H_{16}N_2O_5S$ (M^+): 384.1, Found: 385.0 (M + H^+); HPLC purity (condition I), 97.4% (t_R, 26.75 min), HPLC purity (condition III), 94.7% (t_R, 45.74 min).

(*Z*)-3-(4-Aminobenzyl)-5-(4-ethoxybenzylidene)thiazolidine-2,4-dione (28o)

To a solution of compound **28n** (100 mg, 0.26 mol) in 10 mL of chloroform–ethanol co-solvent (ratio, 4 : 1) was added tin(II) chloride·dihydrate (293 mg, 1.30 mmol). The reaction mixture was stirred at 45 °C overnight. The reaction mixture was diluted with dichloromethane and washed with sat'd aqueous $NaHCO_3$, collected the organic layer, dried over anhydrous Na_2SO_4, filtered, concentrated and purified by silica column chromatography (10% ethyl acetate in dichloromethane) to afford 88 mg (97.0%) of pure **28o** as a yellow-orange solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 7.91 (1H, s, C*H*), 7.59 (2H, d, J = 8.0 Hz, *Ar*), 7.27 (2H, d, J = 8.0 Hz, *Ar*), 7.10 (2H, d, J = 8.0 Hz, *Ar*), 7.02 (2H, d, J = 8.0 Hz, *Ar*), 4.77 (2H, s, NCH_2), 4.11 (2H, q, J = 7.0 Hz, OCH_2CH$_3$), 1.34 (3H, t, J = 7.0 Hz, OCH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 167.3, 165.6, 160.5, 138.3, 133.4, 132.3, 130.2, 129.0, 128.8, 128.5, 125.2, 119.5, 118.2, 117.6, 115.3, 63.5, 44.2, 14.4; MS (ESI) m/z Calcd for $C_{19}H_{18}N_2O_3S$ (M^+): 354.1, Found: 355.1 (M + H^+); HPLC

purity (condition IV), 100% (t_R, 4.94 min), HPLC purity (condition II), 97.3% (t_R, 14.30 min).

(*Z*)-*tert*-Butyl 4-((5-(4-ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)methyl)benzoate (28p)

The title compound **28p** was prepared from compound **2** (75 mg, 0.30 mmol), *tert*-butyl 4-(bromomethyl)benzoate (106 mg, 0.39 mmol) and potassium carbonate (83 mg, 0.60 mmol) in a manner similar to that described for **28d** in 96.4% (127 mg) yield as a white solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 7.93 (1H, s, C*H*), 7.88 (2H, d, *J* = 8.0 Hz, *Ar*), 7.60 (2H, d, *J* = 8.0 Hz, *Ar*), 7.42 (2H, d, *J* = 8.0 Hz, *Ar*), 7.10 (2H, d, *J* = 8.0 Hz, *Ar*), 4.89 (2H, s, CH_2), 4.11 (2H, q, *J* = 7.0 Hz, OCH_2CH$_3$), 1.52 (9H, s, C(CH_3)$_3$), 1.34 (3H, t, *J* = 7.0 Hz, OCH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 167.3, 165.5, 164.6, 160.6, 140.4, 133.6, 132.4, 130.7, 129.3, 127.6, 125.2, 117.5, 115.4, 80.7, 63.5, 44.3, 27.7, 14.4; MS (ESI) *m*/*z* Calcd for $C_{24}H_{25}NO_5S$ (M^+): 439.1, Found: 440.0 (M + H^+); HPLC purity (condition VI), 100% (t_R, 28.77 min), HPLC purity (condition III), 98.6% (t_R, 51.92 min).

(*Z*)-4-((5-(4-Ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)-methyl)benzoic acid (28q)

The title compound **28q** was prepared by deprotection of *tert*-butyl group of compound **28p** (100 mg, 0.23 mmol). Deprotection following the procedure of compound **6** gave the title compound **28q** as a white solid (85 mg, 97.3%). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 12.92 (1H, bs, CO$_2$*H*), 7.94 (1H, s, C*H*), 7.91 (2H, d, *J* = 8.0 Hz, *Ar*), 7.60 (2H, d, *J* = 8.0 Hz, *Ar*), 7.42 (2H, d, *J* = 8.0 Hz, *Ar*), 7.10 (2H, d, *J* = 8.0 Hz, *Ar*), 4.90 (2H, s, CH_2), 4.11 (2H, q, *J* = 7.0 Hz, OCH_2CH$_3$), 1.34 (3H, t, *J* = 7.0 Hz, OCH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 167.4, 166.9, 165.6, 160.6, 140.3, 133.6, 132.4, 130.2, 129.6, 127.5, 125.2, 117.5, 115.3, 63.5, 44.3, 14.4; MS (ESI) *m*/*z* Calcd for $C_{20}H_{17}NO_5S$ (M^+): 383.1, Found: 384.0 (M + H^+); HPLC purity (condition I), 99.1% (t_R, 22.22 min), HPLC purity (condition VII), 96.9% (t_R, 38.40 min).

(*Z*)-3-((5-(4-Ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)-methyl)benzoic acid (28r)

Step 1. Potassium carbonate (166 mg, 1.20 mmol) was added to a solution of compound **2** (100 mg, 0.40 mmol) in 4 mL of anhydrous DMF at 0 °C. After stirring of the reaction mixture for 20 min, a solution of *tert*-butyl 3-(bromomethyl)benzoate (110 mg, 0.40 mmol) in 1 mL of anhydrous DMF was added and then the reaction mixture was stirred at room temperature for 3 h. The reaction was quenched by adding water and extracted with ethyl acetate. The ethyl acetate layer was washed with 10% NH_4Cl aqueous solution followed by water and dried over anhydrous Na_2SO_4, filtered, concentrated and purified by silica column chromatography (10% ethyl acetate in hexanes) to afford 116 mg (66.0%) of *tert*-butyl-**28r** as a light-yellow solid. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 8.02 (1H, s, C*H*), 7.92 (1H, d, *J* = 7.5 Hz, *Ar*), 7.86 (1H, s, *Ar*), 7.57 (1H, d, *J* = 7.5 Hz, *Ar*), 7.45 (2H, d, *J* = 9.0 Hz, *Ar*), 7.38 (1H, d, *J* = 7.5 Hz, *Ar*), 6.97 (2H, d, *J* = 9.0 Hz, *Ar*), 4.93 (2H, s, NCH_2), 4.08 (2H, q, *J* = 6.5 Hz, OCH_2CH$_3$), 1.58 (9H, s, C(CH_3)$_3$), 1.44 (3H, t, *J* = 6.5 Hz, OCH$_2$CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 168.1, 166.4, 165.5, 161.2, 135.6, 134.4, 132.7, 132.5, 129.8, 129.4, 128.8, 125.8, 118.2, 115.4, 81.4, 64.0, 45.0, 28.3, 14.8.

Step 2 (cleavage of tert-butyl group). The title compound **28r** was prepared from *tert*-butyl-protected-**28r** (60 mg, 0.14 mmol) in a manner similar to that described for **6** in 62.0% (42 mg) yield as a white solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 13.07 (1H, bs, CO$_2$*H*), 7.93 (1H, s, C*H*), 7.89 (2H, d, *J* = 8.0 Hz, *Ar*), 7.59 (2H, d, *J* = 8.0 Hz, *Ar*), 7.58 (1H, s, *Ar*), 7.49 (1H, t, *J* = 8.0 Hz, *Ar*), 7.09 (2H, d, *J* = 8.0 Hz, *Ar*), 4.90 (2H, s, CH_2), 4.11 (2H, q, *J* = 7.0 Hz, OCH_2CH$_3$), 1.34 (3H, t, *J* = 7.0 Hz, OCH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 167.4, 166.9, 165.6, 160.6, 136.0, 133.7, 132.4, 132.2, 131.1, 129.0, 128.7, 128.5, 125.1, 117.5, 115.3, 63.5, 44.3, 14.4; MS (ESI) *m*/*z* Calcd for $C_{20}H_{17}NO_5S$ (M^+): 383.1, Found: 384.0 (M + H^+); HPLC purity (condition I), 97.4% (t_R, 22.14 min), HPLC purity (condition III), 94.9% (t_R, 32.36 min).

(*Z*)-2-((5-(4-Ethoxybenzylidene)-2,4-dioxothiazolidin-3-yl)-methyl)benzoic acid (28s)

Step 1. The compound *tert*-butyl-protected-**28s** was prepared from compound **2** (290 mg, 1.16 mmol), *tert*-butyl 2-(bromomethyl)benzoate (314 mg, 1.16 mmol) and potassium carbonate (480 mg, 3.48 mmol) in a manner similar to that described for **28r** (*step 1*) in 61.8% (315 mg) yield as a white-yellow solid. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.93 (1H, d, *J* = 7.5 Hz, *Ar*), 7.89 (1H, s, C*H*), 7.48 (2H, d, *J* = 8.5 Hz, *Ar*), 7.41 (1H, t, *J* = 7.5 Hz, *Ar*), 7.30 (1H, t, *J* = 7.5 Hz, *Ar*), 7.09 (1H, d, *J* = 7.5 Hz, *Ar*), 6.98 (2H, d, *J* = 8.5 Hz, *Ar*), 5.40 (2H, s, CH_2), 4.10 (2H, q, *J* = 8.0 Hz, OCH_2CH$_3$), 1.63 (9H, s, C(CH_3)$_3$), 1.44 (3H, t, *J* = 8.0 Hz, OCH$_2$CH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 168.1, 166.6, 166.4, 161.1, 136.3, 134.4, 132.5, 132.2, 131.2, 127.5, 126.3, 125.8, 118.2, 115.4, 82.0, 64.0, 43.5, 28.4, 14.8.

Step 2 (cleavage of tert-butyl group). The title compound **28s** was prepared from *tert*-butyl-protected-**28s** (100 mg, 0.23 mmol) in a manner similar to that described for **6** in 67.1% (76 mg) yield as a white solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 13.21 (1H, s, CO$_2$*H*), 7.95 (1H, d, *J* = 8.0 Hz, *Ar*), 7.93 (1H, s, C*H*), 7.62 (2H, d, *J* = 9.0 Hz, *Ar*), 7.53 (1H, t, *J* = 8.0 Hz, *Ar*), 7.40 (1H, t, *J* = 8.0 Hz, *Ar*), 7.29 (1H, t, *J* = 7.5 Hz, Ar), 7.11 (2H, d, *J* = 9.0 Hz, *Ar*), 5.22 (2H, s, CH_2), 4.12 (2H, q, *J* = 7.0 Hz, OCH_2CH$_3$), 1.34 (3H, t, *J* = 7.0 Hz, OCH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 168.1, 167.5, 165.8, 160.5, 136.2, 133.5, 132.5, 132.3, 130.8, 129.2, 127.4, 125.7, 125.2, 117.8, 115.4, 63.5, 43.2, 14.5; MS (ESI) *m*/*z* Calcd for $C_{20}H_{17}NO_5S$ (M^+): 383.1, Found: 384.0 (M + H^+); HPLC purity (condition I), 98.6% (t_R, 22.48 min), HPLC purity (condition XI), 98.7% (t_R, 18.85 min).

3-(2-(Tritylamino)ethyl)thiazolidine-2,4-dione (29)

To a solution of compound **1** (900 mg, 7.69 mmol) in 80 mL of anhydrous THF was 2-(tritylamino)ethanol (3.03 g, 10.00 mmol), triphenylphosphine (2.72 g, 10.40 mmol) and DIAD (1.97 mL, 10.00 mmol). The reaction mixture was stirred at room temperature overnight. The solvent was removed by

evaporation and the residue was dissolved in ethyl acetate, and washed with sat'd aqueous NH_4Cl. The organic layer was collected, dried over anhydrous Na_2SO_4, filtered, concentrated and purified by silica column chromatography (20% ethyl acetate in hexanes) to afford **29** in 75.0% (2.31 g) yield as a white solid. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.43–7.21 (15H, m, *Trt*), 4.08 (2H, s, thiazolidine-CH_2), 3.52 (2H, t, J = 7.0 Hz, NCH_2), 2.61 (2H, m, CH_2NH).

(*Z*)-3-(2-Aminoethyl)-5-benzylidenethiazolidine-2,4-dione (30a)

Benzaldehyde (63 μL, 0.62 mmol) and compound **29** (250 mg, 0.62 mmol) were dissolved in 10 mL of anhydrous ethanol at room temperature, and piperidine (7 μL, 0.062 mmol) was added to the reaction solution. The reaction mixture was stirred at 75 °C for 1 day and then allowed to cool down to room temperature until a yellow crystalline precipitate formed, and the solid was collected by filtration, washed with water and ethyl acetate, and dried to afford the trityl-protected form of the title compound ("trityl-**30a**") as a white solid. Deprotection of the trityl group with TFA according to the procedure for compound **6** gave the title compound **30a** as a white solid (48.1%, 74 mg). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 7.91 (1H, s, C*H*), 7.87 (2H, bs, NH_2), 7.62 (2H, d, J = 9.0 Hz, *Ar*), 7.52–7.47 (3H, m, *Ar*), 3.88 (2H, t, J = 5.6, CH_2), 3.09 (2H, t, J = 5.6, CH_2); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 168.2, 166.3, 133.3, 133.1, 131.1, 130.4, 129.9, 122.0, 39.6, 37.3; MS (ESI) m/z Calcd for $C_{12}H_{12}N_2O_2S$ (M^+): 248.1, Found: 249.0 (M + H^+). HPLC purity (condition IX), 99.8% (t_R, 3.56 min), HPLC purity (condition II), 98.0% (t_R, 4.54 min).

(*Z*)-3-(2-Aminoethyl)-5-(3-ethoxybenzylidene)thiazolidine-2,4-dione (30b)

The title compound **30b** was prepared from 3-ethoxybenzaldehyde (87 μL, 0.62 mmol) and compound **29** (250 mg, 0.62 mmol) and piperidine (7.0 μL, 0.062 mmol) in a manner similar to that described for **30a** in 20.4% (50 mg) yield as a white solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 7.96 (2H, bs, NH_2), 7.91 (1H, s, C*H*), 7.46 (1H, t, J = 8.4, *Ar*), 7.22 (2H, m, *Ar*), 7.08 (1H, d, J = 8.4, *Ar*), 4.08 (2H, q, J = 6.8, CH_2CH$_3$), 3.91 (2H, t, J = 5.6, CH_2), 3.09 (2H, t, J = 5.6, CH_2), 1.35 (3H, t, J = 6.8, CH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 167.7, 165.9, 159.0, 134.3, 132.8, 130.6, 121.9, 121.7, 116.9, 115.9, 63.3, 36.9, 14.6; MS (ESI) m/z Calcd for $C_{14}H_{16}N_2O_3S$ (M^+): 292.1, Found: 293.0 (M + H^+); HPLC purity (condition IX), 91.4% (t_R, 3.51 min), HPLC purity (condition X), 97.3% (t_R, 4.78 min).

(*Z*)-3-(2-Aminoethyl)-5-(2-ethoxybenzylidene)thiazolidine-2,4-dione (30c)

The title compound **30c** was prepared from 2-ethoxybenzaldehyde (87 μL, 0.62 mmol) and compound **29** (250 mg, 0.62 mmol) and piperidine (7.0 μL, 0.062 mmol) in a manner similar to that described for **30a** in 35.4% (64 mg) yield as a white solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 8.16 (1H, s, C*H*), 7.96 (2H, bs, NH_2), 7.48 (1H, t, J = 6.5 Hz, *Ar*), 7.42 (1H, t, J = 6.5 Hz, *Ar*), 7.17 (1H, t, J = 6.5 Hz, *Ar*), 7.10 (1H, t, J = 6.5 Hz, *Ar*), 4.17 (2H, q, J = 7.0 Hz, OCH_2CH$_3$), 3.89 (2H, m, CH_2), 3.08 (2H, m, CH_2), 1.38 (3H, t, J = 7.0 Hz, OCH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 168.0, 166.0, 157.4, 132.6, 128.6, 127.6, 121.5, 121.4, 120.9, 112.8, 94.2, 64.0, 36.9, 14.5; MS (ESI) m/z Calcd for $C_{14}H_{16}N_2O_3S$ (M^+): 292.1, Found: 293.0 (M + H^+); HPLC purity (condition IX), 98.3% (t_R, 3.69 min), HPLC purity (condition X), 99.2% (t_R, 4.57 min).

(*Z*)-3-(2-Aminoethyl)-5-(4-methylbenzylidene)thiazolidine-2,4-dione (30d)

The title compound **30d** was prepared from 4-methylbenzaldehyde (73 μL, 0.62 mmol), compound **29** (250 mg, 0.62 mmol) and piperidine (7.0 μL, 0.062 mmol) in a manner similar to that described for **30a** in 36.0% (83 mg) yield as a cream solid. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 8.02 (2H, bs, NH_2), 7.90 (1H, s, C*H*), 7.54 (2H, d, J = 8.0, *Ar*), 7.37 (2H, d, J = 8.0, *Ar*), 3.95–3.87 (2H, m, CH_2), 3.14–3.03 (2H, m, CH_2), 2.37 (3H, s, CH_3); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 167.8, 166.0, 141.0, 132.8, 130.2, 130.2, 130.1, 120.4, 39.2, 36.9, 21.1; MS (ESI) m/z Calcd for $C_{13}H_{14}N_2O_2S$ (M^+): 262.1, Found: 263.0 (M + H^+); HPLC purity (condition IX), 95.4% (t_R, 3.32 min), HPLC purity (condition X), 97.6% (t_R, 4.59 min).

(*Z*)-3-(2-Aminoethyl)-5-(4-propylbenzylidene)thiazolidine-2,4-dione (30e)

The title compound **30e** was prepared from 4-propylbenzaldehyde (92 mg, 0.62 mmol), compound **29** (250 mg, 0.62 mmol) and piperidine (7.0 μL, 0.062 mmol) in a manner similar to that described for **30a** in 38.0% (95 mg) yield as a white solid. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 8.03 (2H, bs, NH_2), 7.91 (1H, s, C*H*), 7.56 (2H, d, J = 8.4, *Ar*), 7.38 (2H, d, J = 8.4, *Ar*), 3.91 (2H, t, J = 5.6, CH_2), 3.14–3.05 (2H, m, CH_2), 2.61 (2H, t, J = 7.2, CH_2CH$_2$CH$_3$), 1.61 (2H, sept, J = 7.2, CH$_2$CH_2CH$_3$), 0.90 (3H, t, J = 7.2, CH$_2$CH$_2$CH_3); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 167.9, 166.0, 145.6, 132.8, 130.5, 130.2, 129.5, 120.5, 39.2, 37.1, 36.9, 23.8, 13.6; MS (ESI) m/z Calcd for $C_{15}H_{18}N_2O_2S$ (M^+): 290.1, Found: 291.1 (M + H^+); HPLC purity (condition IX), 99.3% (t_R, 3.78 min), HPLC purity (condition X), 99.6% (t_R, 6.24 min).

(*Z*)-3-(2-Aminoethyl)-5-(4-hydroxybenzylidene)thiazolidine-2,4-dione (30f)

The title compound **30f** was prepared from 4-hydroxybenzaldehyde (76 mg, 0.62 mmol), compound **29** (250 mg, 0.62 mmol) and piperidine (20.0 μL, 0.19 mmol) in a manner similar to that described for **30a** in 43.0% (102 mg) yield as a yellow solid ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 10.4 (1H, bs, O*H*), 7.91–7.78 (3H, bs, NH_2 + C*H*), 7.51 (2H, d, J = 8.8, *Ar*), 6.94 (2H, d, J = 8.8, *Ar*), 3.89 (2H, t, J = 6.0, CH_2), 3.07 (2H, t, J = 6.0, CH_2); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 168.0, 166.1, 160.2, 133.3, 132.6, 123.8, 116.9, 116.5, 37.0, 22.4; MS (ESI) m/z Calcd for $C_{12}H_{12}N_2O_3S$ (M^+): 264.1, Found: 265.0 (M + H^+); HPLC purity (condition IX), 97.4% (t_R, 2.88 min).

(*Z*)-3-(2-Aminoethyl)-5-(4-isobutoxybenzylidene)thiazolidine-2,4-dione (30g)

To tritylated-**30f** (100 mg, 0.20 mmol) and potassium carbonate (82 mg, 0.60 mmol) in 3 mL of anhydrous DMF was added

isobutyl iodide (54 μL, 0.40 mmol). The reaction was allowed to stir at 70 °C for 12 h. The reaction solution was diluted with ethyl acetate and washed with water, brine, collected the organic phase, dried over anhydrous Na_2SO_4, filtered, concentrated and purified by silica column chromatography. Deprotection following the procedure for compound **6** gave the title compound **30g** as a yellow solid (65.2%, 56 mg). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 7.92 (2H, bs, NH_2), 7.90 (1H, s, CH), 7.60 (2H, d, J = 8.4, *Ar*), 7.12 (2H, d, J = 8.4, *Ar*), 3.90 (2H, t, J = 5.6, CH_2), 3.83 (2H, t, J = 6.8, CH_2i*Pr*), 3.13–3.03 (2H, m, CH_2), 2.03 (1H, sept, J = 6.8, CHMe$_2$), 0.98 (6H, t, J = 6.8, 2 × CH_3); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 167.9, 166.1, 160.8, 132.9, 132.3, 125.3, 118.2, 115.5, 74.0, 36.9, 27.6, 19.0; MS (ESI) m/z Calcd for $C_{16}H_{20}N_2O_3S$ (M^+): 320.1, Found: 321.1 (M + H^+); HPLC purity (condition IX), 97.6% (t_R, 3.97 min), HPLC purity (condition X), 99.8% (t_R, 6.47 min).

(*Z*)-3-(2-Aminoethyl)-5-(4-(benzyloxy)benzylidene)thiazolidine-2,4-dione (30h)

To tritylated-**30f** (100 mg, 0.20 mmol) and potassium carbonate (82 mg, 0.60 mmol) in 3 mL of anhydrous DMF was added benzyl bromide (50 μL, 0.40 mmol). The reaction was allowed to stir at room temperature for 3 h. The reaction solution was diluted with ethyl acetate and washed with water, brine, collected the organic phase, dried over anhydrous Na_2SO_4, filtered, concentrated and purified by silica column chromatography. Deprotection following the procedure for compound **6** gave the title compound **30h** as a yellow solid (97.0%, 91 mg). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 8.29 (2H, bs, NH_2), 7.88 (1H, s, CH) 7.61 (2H, d, J = 9.2, *Ar*), 7.46 (2H, d, J = 7.2, *Ar*), 7.40 (2H, d, J = 7.2, *Ar*), 7.34 (1H, d, J = 7.2, *Ar*), 7.19 (2H, d, J = 9.2, *Ar*), 5.19 (2H, s, CH_2), 3.91 (2H, t, J = 6.0, CH_2), 3.09–2.99 (2H, m, CH_2); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 167.9, 166.0, 160.2, 136.5, 132.6, 132.2, 128.5, 128.1, 127.9, 125.6, 118.6, 115.9, 69.5, 36.5; MS (ESI) m/z Calcd for $C_{19}H_{18}N_2O_3S$ (M^+): 354.1, Found: 355.1 (M + H^+); HPLC purity (condition IX), 98.6% (t_R, 3.91 min), HPLC purity (condition X), 97.1% (t_R, 6.40 min).

(*Z*)-3-(2-Aminoethyl)-5-(2-hydroxybenzylidene)thiazolidine-2,4-dione (30i)

The title compound **30i** was prepared from 2-hydroxybenzaldehyde (76 mg, 0.62 mmol), compound **29** (250 mg, 0.62 mmol) and piperidine (20.0 μL, 0.19 mmol) in a manner similar to that described for **30a** in 50.0% (117 mg) yield as a yellow solid. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 10.75 (1H, s, OH), 8.13 (1H, s, CH), 7.98 (2H, s, NH_2), 7.36 (1H, d, J = 8.0 Hz, *Ar*), 7.32 (1H, d, J = 8.0 Hz, *Ar*), 7.00 (1H, d, J = 8.0 Hz, *Ar*), 6.94 (1H, d, J = 8.0 Hz, *Ar*), 3.89 (2H, m, CH_2), 3.08 (2H, m, CH_2); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 168.1, 166.1, 157.4, 132.5, 128.4, 128.2, 119.9, 119.8, 119.7, 116.2, 39.0, 36.9; MS (ESI) m/z Calcd for $C_{12}H_{12}N_2O_3S$ (M^+): 264.1, Found: 265.0; MS (ESI) m/z Calcd for $C_{12}H_{12}N_2O_3S$ (M^+): 264.1, Found: 265.1 (M + H^+); HPLC purity (condition IX), 97.9% (t_R, 3.19 min), HPLC purity (condition X), 96.8% (t_R, 3.86 min).

(*Z*)-3-(2-Aminoethyl)-5-(2-isobutoxybenzylidene)thiazolidine-2,4-dione (30j)

To tritylated-**30i** (100 mg, 0.20 mmol) and potassium carbonate (82 mg, 0.60 mmol) in 3 mL of anhydrous DMF was added isobutyl iodide (54 μL, 0.40 mmol). The reaction was allowed to stir at 70 °C for 12 h. The reaction solution was diluted with ethyl acetate and washed with water, brine, collected the organic phase, dried over anhydrous Na_2SO_4, filtered, concentrated and purified by silica column chromatography. Deprotection following the procedure for compound **6** gave the title compound **30j** as a yellow solid (73.7%, 64 mg). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 8.18 (1H, s, CH), 7.97 (2H, s, NH_2), 7.49 (1H, t, J = 8.0 Hz, *Ar*), 7.42 (1H, d, J = 8.0 Hz, *Ar*), 7.17 (1H, d, J = 8.0 Hz, *Ar*), 7.10 (1H, t, J = 8.0 Hz, *Ar*), 3.91 (2H, m, OCH_2), 3.87 (2H, m, NCH_2), 3.09 (2H, m, CH_2), 2.08 (1H, m, CH(CH_3)$_2$), 1.02 (6H, d, J = 7.0 Hz, CH(CH_3)$_2$); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 167.9, 166.0, 157.7, 132.7, 128.2, 127.0, 121.5, 121.3, 121.0, 113.0, 74.3, 36.9, 27.7, 18.9; MS (ESI) m/z Calcd for $C_{16}H_{20}N_2O_3S$ (M^+): 320.1, Found: 321.0 (M + H^+); HPLC purity (condition IX), 99.4% (t_R, 3.81 min), HPLC purity (condition II), 99.2% (t_R, 5.29 min).

(*Z*)-3-(2-Aminoethyl)-5-(2-(benzyloxy)benzylidene)thiazolidine-2,4-dione (30k)

To tritylated-**30i** (100 mg, 0.20 mmol) and potassium carbonate (82 mg, 0.60 mmol) in 3 mL of anhydrous DMF was added benzyl bromide (50 μL, 0.40 mmol). The reaction was allowed to stir at room temperature for 3 h. The reaction solution was diluted with ethyl acetate and washed with water, brine, collected the organic phase, dried over anhydrous Na_2SO_4, filtered, concentrated and purified by silica column chromatography. Deprotection following the procedure for compound **6** gave the title compound **30k** as a pale-yellow solid (95.1%, 89 mg). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 8.15 (1H, s, CH), 7.92 (2H, s, NH_2), 7.47 (5H, m, *Ar*), 7.42 (1H, t, J = 8.0 Hz, *Ar*), 7.37 (1H, t, J = 8.0 Hz, *Ar*), 7.28 (1H, t, J = 8.0 Hz, *Ar*), 7.13 (1H, t, J = 8.0 Hz, *Ar*), 5.26 (2H, s, CH_2), 3.88 (2H, m, CH_2), 3.07 (2H, m, CH_2); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 167.9, 166.0, 157.2, 136.5, 132.6, 128.5, 128.4, 128.1, 127.7, 127.3, 121.7, 121.6, 121.3, 113.4, 69.9, 36.9; MS (ESI) m/z Calcd for $C_{19}H_{18}N_2O_3S$ (M^+): 354.1, Found: 355.0 (M + H^+); HPLC purity (condition IX), 99.3% (t_R, 4.13 min), HPLC purity (condition X), 99.6% (t_R, 5.87 min).

(*Z*)-4-((2-((3-(2-Aminoethyl)-2,4-dioxothiazolidin-5-ylidene)-methyl)phenoxy)methyl)benzonitrile (30l)

To tritylated-**30i** (100 mg, 0.20 mmol) and potassium carbonate (82 mg, 0.60 mmol) in 3 mL of anhydrous DMF was added 4-cyanobenzyl bromide (78 mg, 0.40 mmol). The reaction was allowed to stir at room temperature overnight. The reaction solution was diluted with ethyl acetate and washed with water, brine, collected the organic phase, dried over anhydrous Na_2SO_4, filtered, concentrated and purified by silica column chromatography. Deprotection following the procedure for compound **6** gave the title compound **30l** as a light-yellow

solid (79.1%, 78 mg). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 8.15 (1H, s, C*H*), 7.91 (2H, s, NH_2), 7.89 (2H, d, J = 8.0 Hz, *Ar*), 7.66 (2H, d, J = 8.0 Hz, *Ar*), 7.48 (2H, t, J = 8.0 Hz, *Ar*), 7.23 (1H, d, J = 8.0 Hz, *Ar*), 7.15 (1H, t, J = 8.0 Hz, *Ar*), 5.38 (2H, s, CH_2), 3.89 (2H, m, CH_2), 3.08 (2H, m, CH_2); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 167.9, 165.9, 156.8, 142.2, 132.5, 128.6, 128.1, 127.1, 122.0, 121.8, 121.6, 118.6, 113.3, 110.8, 69.0, 36.9; MS (ESI) m/z Calcd for $C_{20}H_{17}N_3O_3S$ (M^+): 379.1, Found: 380.1 ($M + H^+$); HPLC purity (condition IX), 96.1% (t_R, 3.80 min), HPLC purity (condition X), 98.4% (t_R, 5.43 min).

Biology

Cell proliferation assay. Cell proliferation was evaluated by water soluble tetrazolium-1 (WST-1) assay as previously described.[20] Briefly, cells (~5000 per well) were seeded in 96 well plates, allowed to recover for 16–20 hours, and then treated with the indicated test compound for 48 hours. After incubation, WST-1 reagent was added and absorbance was read at 450 nm with background subtraction taken at 650 nm. Values were normalized to the control (DMSO only treated) cells.

Luciferase assay. HeLa cells were seeded in 24-well plates (4×10^4 cells per well) and incubated 18 hours prior to achieve ~60–70% confluent. Cells were transfected with activator protein-1 (pAP1(PMA)-TA-Luc; Clontech) or the serum response element (pGL4.33-SRE; Promega) luciferase reporter plasmids (250 ng per well) using Lipofectamine™ (Invitrogen). After 16 hours, cells were treated with increasing amount of compounds as indicated for 20 minutes, followed by stimulation with EGF (25 ng mL^{-1}) for 4.5 h. The luciferase activity in the cell extracts was determined with a Dual Luciferase Assay System (Promega) according to the manufacturer's instructions. Luciferase activities were monitored with a Lumat LB 9507 luminometer (Berthold Technology) and data were normalized to the amount of protein in each sample.

Acknowledgements

We thank the National Institutes of Health (CA120215 to P. S. and A. D. M., GM100693 to R. S.), the University of Maryland Computer-Aided Drug Design Center and the University of Maryland School of Pharmacy (S. F.) for financial support of this work.

Notes and references

1 (*a*) K. Burkhard, S. Smith, R. Deshmukh, A. D. MacKerell, Jr. and P. Shapiro, Development of extracellular signal-regulated kinase inhibitors, *Curr. Top. Med. Chem.*, 2009, **9**(8), 678–689; (*b*) T. S. Lewis, P. S. Shapiro and N. G. Ahn, Signal transduction through MAP kinase cascades, *Adv. Cancer Res.*, 1998, **74**, 49–139.

2 A. von Kriegsheim, D. Baiocchi, M. Birtwistle, D. Sumpton, W. Bienvenut, N. Morrice, K. Yamada, A. Lamond, G. Kalna, R. Orton, D. Gilbert and W. Kolch, Cell fate decisions are specified by the dynamic ERK interactome, *Nat. Cell Biol.*, 2009, **11**(12), 1458–1464.

3 (*a*) C. W. Reuter, M. A. Morgan and L. Bergmann, Targeting the Ras signaling pathway: a rational, mechanism-based treatment for hematologic malignancies?, *Blood*, 2000, **96**(5), 1655–1669; (*b*) M. S. Brose, P. Volpe, M. Feldman, M. Kumar, I. Rishi, R. Gerrero, E. Einhorn, M. Herlyn, J. Minna, A. Nicholson, J. A. Roth, S. M. Albelda, H. Davies, C. Cox, G. Brignell, P. Stephens, P. A. Futreal, R. Wooster, M. R. Stratton and B. L. Weber, BRAF and RAS mutations in human lung cancer and melanoma, *Cancer Res.*, 2002, **62**(23), 6997–7000; (*c*) J. L. Bos, ras Oncogenes in human cancer: a review, *Cancer Res.*, 1989, **49**(17), 4682–4689; (*d*) H. Davies, G. R. Bignell, C. Cox, P. Stephens, S. Edkins, S. Clegg, J. Teague, H. Woffendin, M. J. Garnett, W. Bottomley, N. Davis, E. Dicks, R. Ewing, Y. Floyd, K. Gray, S. Hall, R. Hawes, J. Hughes, V. Kosmidou, A. Menzies, C. Mould, A. Parker, C. Stevens, S. Watt, S. Hooperd, R. Wilson, H. Jayatilake, B. A. Gusterson, C. Cooper, J. Shipley, D. Hargrave, K. Pritchard-Jones, N. Maitland, G. Chenevix-Trench, G. J. Riggins, D. D. Bigner, G. Palmieri, A. Cossu, A. Flanagan, A. Nicholson, J. W. Ho, S. Y. Leung, S. T. Yuen, B. L. Weber, H. F. Seigler, T. L. Darrow, H. Paterson, R. Marais, C. J. Marshall, R. Wooster, M. R. Stratton and P. A. Futreal, Mutations of the BRAF gene in human cancer, *Nature*, 2002, **417**(6892), 949–954; (*e*) J. Mendelsohn and J. Baselga, Epidermal growth factor receptor targeting in cancer, *Semin. Oncol.*, 2006, **33**(4), 369–385; (*f*) W. Kolch, A. Kotwaliwale, K. Vass and P. Janosch, The role of Raf kinases in malignant transformation, *Expert Rev. Mol. Med.*, 2002, **4**(8), 1–18; (*g*) L. O. Murphy, S. Smith, R. H. Chen, D. C. Fingar and J. Blenis, Molecular interpretation of ERK signal duration by immediate early gene products, *Nat. Cell Biol.*, 2002, **4**(8), 556–564; (*h*) S. M. Wilhelm, C. Carter, L. Tang, D. Wilkie, A. McNabola, H. Rong, C. Chen, X. Zhang, P. Vincent, M. McHugh, Y. Cao, J. Shujath, S. Gawlak, D. Eveleigh, B. Rowley, L. Liu, L. Adnane, M. Lynch, D. Auclair, I. Taylor, R. Gedrich, A. Voznesensky, B. Riedl, L. E. Post, G. Bollag and P. A. Trail, BAY 43–9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis, *Cancer Res.*, 2004, **64**(19), 7099–7109; (*i*) T. B. Lowinger, B. Riedl, J. Dumas and R. A. Smith, Design and discovery of small molecules targeting raf-1 kinase, *Curr. Pharm. Des.*, 2002, **8**(25), 2269–2278; (*j*) N. M. Appels, J. H. Beijnen and J. H. Schellens, Development of farnesyl transferase inhibitors: a review, *Oncologist*, 2005, **10**(8), 565–578; (*k*) A. Willems, K. Gauger, C. Henrichs and N. Harbeck, Antibody therapy for breast cancer, *Anticancer Res.*, 2005, **25**(3A), 1483–1489; (*l*) B. B. Friday and A. A. Adjei, Advances in targeting the Ras/Raf/MEK/Erk mitogen-activated protein kinase cascade with MEK inhibitors for cancer therapy, *Clin. Cancer Res.*, 2008, **14**(2), 342–346;

(*m*) A. Arora and E. M. Scholar, Role of tyrosine kinase inhibitors in cancer therapy, *J. Pharmacol. Exp. Ther.*, 2005, **315**(3), 971–979; (*n*) J. L. Yap, S. Worlikar, A. D. MacKerell, Jr., P. Shapiro and S. Fletcher, Small-molecule inhibitors of the ERK signaling pathway: towards novel anticancer therapeutics, *ChemMedChem*, 2011, **6**(1), 38–48.

4 (*a*) Y. N. Gopal, W. Deng, S. E. Woodman, K. Komurov, P. Ram, P. D. Smith and M. A. Davies, Basal and treatment-induced activation of AKT mediates resistance to cell death by AZD6244 (ARRY-142886) in Braf-mutant human cutaneous melanoma cells, *Cancer Res.*, 2010, **70**(21), 8736–8747; (*b*) D. E. Goggin, K. J. Steadman, R. J. Emery, S. C. Farrow, R. L. Benech-Arnold and S. B. Powles, ABA inhibits germination but not dormancy release in mature imbibed seeds of Lolium rigidum Gaud, *J. Exp. Bot.*, 2009, **60**(12), 3387–3396; (*c*) J. Meng, H. Peng, B. Dai, W. Guo, L. Wange, L. Ji, J. D. Minna, C. M. Chresta, P. D. Smith, B. Fang and J. A. Roth, High level of AKT activity is associated with resistance to MEK inhibitor AZD6244 (ARRY-142886), *Cancer Biol. Ther.*, 2009, **8**(21), 2073–2080; (*d*) J. A. McCubrey, L. S. Steelman, W. H. Chappell, S. L. Abrams, E. W. Wong, F. Chang, B. Lehmann, D. M. Terrian, M. Milella, A. Tafuri, F. Stivala, M. Libra, J. Basecke, C. Evangelisti, A. M. Martelli and R. A. Franklin, Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance, *Biochim. Biophys. Acta*, 2007, **1773**(8), 1263–1284; (*e*) B. N. Rexer, J. A. Engelman and C. L. Arteaga, Overcoming resistance to tyrosine kinase inhibitors: lessons learned from cancer cells treated with EGFR antagonists, *Cell Cycle*, 2009, **8**(1), 18–22; (*f*) F. Morgillo, F. Cantile, M. Fasano, T. Troiani, E. Martinelli and F. Ciardiello, Resistance mechanisms of tumour cells to EGFR inhibitors, *Clin. Transl. Oncol.*, 2009, **11**(5), 270–275.

5 (*a*) P. I. Poulikakos, C. Zhang, G. Bollag, K. M. Shokat and N. Rosen, RAF inhibitors transactivateRAF dimers and ERK signalling in cells with wild-type BRAF, *Nature*, 2010, **464**(7287), 427–430; (*b*) J. Tsai, J. T. Lee, W. Wang, J. Zhang, H. Chod, S. Mamo, R. Bremer, S. Gillette, J. Kong, N. K. Haass, K. Sproesser, L. Li, K. S. Smalley, D. Fong, Y. L. Zhu, A. Marimuthu, H. Nguyen, B. Lam, J. Liu, I. Cheung, J. Rice, Y. Suzuki, C. Luu, C. Settachatgul, R. Shellooe, J. Cantwell, S. H. Kim, J. Schlessinger, K. Y. Zhang, B. L. West, B. Powell, G. Habets, C. Zhang, P. N. Ibrahim, P. Hirth, D. R. Artis, M. Herlyn and G. Bollag, Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**(8), 3041–3046; (*c*) C. M. Johannessen, J. S. Boehm, S. Y. Kim, S. R. Thomas, L. Wardwell, L. A. Johnson, C. M. Emery, N. Stransky, A. P. Cogdill, J. Barretina, G. Caponigro, H. Hieronymus, R. R. Murray, K. Salehi-Ashtiani, D. E. Hill, M. Vidal, J. J. Zhao, X. Yang, O. Alkan, S. Kim, J. L. Harris, C. J. Wilson, V. E. Myer, P. M. Finan, D. E. Root, T. M. Roberts, T. Golub, K. T. Flaherty, R. Dummer, B. L. Weber, W. R. Sellers, R. Schlegel, J. A. Wargo, W. C. Hahn and L. A. Garraway, COT drives resistance to RAF inhibition through MAP kinase pathway reactivation, *Nature*, 2010, **468**(7326), 968–972.

6 (*a*) F. Chen, C. N. Hancock, A. T. Macias, J. Joh, K. Still, S. Zhong, A. D. MacKerell, Jr. and P. Shapiro, Characterization of ATP-independent ERK inhibitors identified through *in silico* analysis of the active ERK2 structure, *Bioorg. Med. Chem. Lett.*, 2006, **16**(24), 6281–6287; (*b*) C. N. Hancock, A. Macias, E. K. Lee, S. Y. Yu, A. D. Mackerell, Jr. and P. Shapiro, Identification of novel extracellular signal-regulated kinase docking domain inhibitors, *J. Med. Chem.*, 2005, **48**(14), 4586–4595.

7 (*a*) A. D. Sharrocks, S. H. Yang and A. Galanis, Docking domains and substrate-specificity determination for MAP kinases, *Trends Biochem. Sci.*, 2000, **25**(9), 448–453; (*b*) T. Tanoue, M. Adachi, T. Moriguchi and E. Nishida, A conserved docking motif in MAP kinases common to substrates, activators and regulators, *Nat. Cell Biol.*, 2000, **2**(2), 110–116.

8 T. Tanoue, R. Maeda, M. Adachi and E. Nishida, Identification of a docking groove on ERK and p38 MAP kinases that regulates the specificity of docking interactions, *EMBO J.*, 2001, **20**(3), 466–479.

9 A. T. Macias, M. Y. Mia, G. Xia, J. Hayashi and A. D. MacKerell, Jr., Lead validation and SAR development via chemical similarity searching, application to compounds targeting the pY+3 site of the SH2 domain of p56lck, *J. Chem. Inf. Model.*, 2005, **45**(6), 1759–1766.

10 Q. Li, A. Al-Ayoubi, T. Guo, H. Zheng, A. Sarkar, T. Nguyen, S. T. Eblen, S. Grant, G. E. Kellogg and S. Zhang, Structure-activity relationship(SAR) studies of 3-(2-amino-ethyl)-5-(4-ethoxy-benzylidene)-thiazolidine-2,4-dione: development of potential substrate-specific ERK1/2 inhibitors, *Bioorg. Med. Chem. Lett.*, 2009, **19**(21), 6042–6046.

11 Q. Li, J. Wu, H. Zheng, K. Liu, T. L. Guo, Y. Liu, S. T. Eblen, S. Grant and S. Zhang, Discovery of 3-(2-aminoethyl)-5-(3-phenyl-propylidene)-thiazolidine-2,4-dione as a dual inhibitor of the Raf/MEK/ERK and the PI3K/Akt signaling pathways, *Bioorg. Med. Chem. Lett.*, 2010, **20**(15), 4526–4530.

12 Y. N. Gopal, W. Deng, S. E. Woodman, K. Komurov, P. Ram, P. D. Smith and M. A. Davies, Basal and treatment-induced activation of AKT mediates resistance to cell death by AZD6244 (ARRY-142886) in Braf-mutant human cutaneous melanoma cells, *Cancer Res.*, 2010, **70**(21), 8736–8747.

13 P. I. Poulikakos and N. Rosen, Mutant BRAF melanomas-dependence and resistance, *Cancer Cell*, 2011, **19**(1), 11–15.

14 (*a*) J. Wesierska-Gadek, M. Gueorguieva, O. Komina, G. Schmid and M. P. Kramer, Signaling of DNA damage is not sufficient to induce p53 response: (re)activation of wt p53 protein strongly depends on cellular context, *J. Cell. Biochem.*, 2008, **103**(5), 1607–1620; (*b*) K. S. Iwamoto, T. Mizuno, T. Ito, N. Tsuyama, S. Kyoizumi and T. Seyama, Gain-of-function p53 mutations enhance alteration of the T-cell receptor following X-irradiation, independently of the cell cycle and cell survival, *Cancer Res.*, 1996, **56**(17),

3862–3865; (*c*) D. Wolf and V. Rotter, Major deletions in the gene encoding the p53 tumor antigen cause lack of p53 expression in HL-60 cells, *Proc. Natl. Acad. Sci. U. S. A.*, 1985, **82**(3), 790–794; (*d*) O. N. Ikediobi, H. Davies, G. Bignell, S. Edkins, C. Stevens, S. O'Meara, T. Santarius, T. Avis, S. Barthorpe, L. Brackenbury, G. Buck, A. Butler, J. Clements, J. Cole, E. Dicks, S. Forbes, K. Gray, K. Halliday, R. Harrison, K. Hills, J. Hinton, C. Hunter, A. Jenkinson, D. Jones, V. Kosmidou, R. Lugg, A. Menzies, T. Mironenko, A. Parker, J. Perry, K. Raine, D. Richardson, R. Shepherd, A. Small, R. Smith, H. Solomon, P. Stephens, J. Teague, C. Tofts, J. Varian, T. Webb, S. West, S. Widaa, A. Yates, W. Reinhold, J. N. Weinstein, M. R. Stratton, P. A. Futreal and R. Wooster, Mutation analysis of 24 known cancer genes in the NCI-60 cell line set, *Mol. Cancer Ther.*, 2006, **5**(11), 2606–2612.

15 W. A. Loughlin, J. D. Tyndall, M. P. Glenn, T. A. Hill and D. P. Fairlie, Update 1 of: beta-strand mimetics, *Chem. Rev.*, 2010, **110**(6), PR32–PR69.

16 A. M. Aronov, C. Baker and G. W. Bemis, *et al.*, Flipped out: structure-guided of selective pyrazolylpyrrole ERK inhibitors, *J. Med. Chem.*, 2007, **50**, 1280–1287.

17 (*a*) R. A. Hipskind, D. Buscher, A. Nordheim and M. Baccarini, Ras/MAP kinase-dependent and -independent signaling pathways target distinct ternary complex factors, *Genes Dev.*, 1994, **8**(15), 1803–1816; (*b*) R. H. Chen, P. C. Juo, T. Curran and J. Blenis, Phosphorylation of c-Fos at the C-terminus enhances its transforming activity, *Oncogene*, 1996, **12**(7), 1493–1502; (*c*) J. A. Frost, H. Steen, P. Shapiro, T. Lewis, N. Ahn, P. E. Shaw and M. H. Cobb, Cross-cascade activation of ERKs and ternary complex factors by Rho family proteins, *EMBO J.*, 1997, **16**(21), 6426–6438.

18 D. Schadendorf, Peroxisome proliferator-activating receptors: a new way to treat melanoma?, *J. Invest. Dermatol.*, 2009, **129**(5), 1061–1063.

19 G. Hatzivassiliou, B. Liu, C. O'Brien, J. M. Spoerke, K. P. Hoeflich, P. M. Haverty, R. Soriano, W. F. Forrest, S. Heldens, H. Chen, K. Toy, C. Ha, W. Zhou, K. Song, L. S. Friedman, L. C. Amler, G. M. Hampton, J. Moffat, M. Belvin and M. R. Lackner, ERK inhibition overcomes acquired resistance to MEK inhibitors, *Mol. Cancer Ther.*, 2012, **11**(5), 1143–1154.

20 S. R. Boston, R. Deshmukh, S. Strome, U. D. Priyakumar, A. D. MacKerell, Jr. and P. Shapiro, Characterization of ERK docking domain inhibitors that induce apoptosis by targeting Rsk-1 and caspase-9, *BMC Cancer*, 2011, **11**, 7.

