Bioorganic & Medicinal Chemistry xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Discovery of novel 4-aryl-thieno[1,4]diazepin-2-one derivatives targeting multiple protein kinases as anticancer agents

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ARTICLE INFO

Article history:
Received 15 January 2018
Revised 6 February 2018
Accepted 7 February 2018
Available online xxxx

Keywords:
4-Aryl-thieno[1,4]diazepin-2-one
Antiproliferative activity
Hematopoietic cell line
Kinase inhibitor
Multiple protein kinase inhibitor

ABSTRACT

A series of 4-aryl-thieno[1,4]diazepin-2-one were synthesized and evaluated for their antiproliferative activities against the A375P melanoma and U937 hematopoietic cell lines. Several compounds showed very potent antiproliferative activities toward both cell lines and the activities were better than that of sorafenib, the reference standard. Derivatives were made as amide (**8a**–**8i**, **9a**–**9m**) and urea (**10a**–**10d**, **11a**–**11d**) with diverse hydrophobic moieties. One of the most potent inhibitor **10d**, 1-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-3-(4-(2-oxo-2,3-dihydro-*1H*-thieno [3,4-*b*][1,4]diazepin-4-yl)phenyl)urea was found to be very potent inhibitor of multi-protein kinases including FMS kinase (IC_{50} = 3.73 nM) and is a promising candidate for further development in therapeutics for cancer.

1. Introduction

Protein kinases play an essential role in most cell signaling and metabolism pathways. Therefore, the development of protein kinase inhibitors is becoming increasingly important in the development of drugs for peripheral diseases, particularly cancer, and have great utility in signaling pathway characterization due to the importance of protein phosphorylation as a regulatory process.[1–3] As of 2015, there were 28 cancer-targeted protein kinase inhibitors approved by the FDA, including multiple protein kinases inhibitors (Fig. 1).[4]

Multiple protein kinase inhibitors with a potent but non-specific activity profile provide an effective answer to the need for cancer treatment, some of which have been widely used in clinical trials.[5–7] Multi-targeted protein kinase inhibitors are particularly advantageous because they target peripheral supporting cells in addition to the tumor itself, and are less prone to develop resistance. In addition, taking one multi-targeted drug is more beneficial than taking many single targeted inhibitors, since it reduces drug-drug interactions and toxicity.[8,9] In the present study, we focused on designing hinge binders as novel structures functioning as type II protein kinase inhibitors. Specifically, we have been interested in [5.5], [5.7] ring systems with hydrogen bonding donor/acceptors located in two aromatic cycles and have used this approach previously to develop effective protein kinase inhibitors. As shown in Fig. 2, we began with an amino-pyrazole amide scaffold based on the binding mode of a potent inhibitor of inactivated B/C-Raf that possesses a unique binding mode, namely, the type II inhibitor.[10,11] In a previous study from our laboratory, aminopyrazolamide (A) was shown to generate two hydrogen bonds in the hinge region and was a selective and potent inhibitor of both B-Raf V600E and c-Raf kinase.[10] We subsequently designed a seven-membered ring mimicking the intramolecular hydrogen bond at the side of the pyrazole ring, which was introduced as a 'conformation restricted analogue' 6,8-dihydropyrazolo[3,4-*b*][1,4]diazepin-7(*1H*)-one (B).[11] While this molecule (B) also inhibits B-Raf V600E and c-Raf kinases, it shows an unexpected decrease in potency compared to the parent molecule. When we analyzed the docking mode of the inhibitor B, we found that it was capable of forming hydrogen bonds between the hydrogen of the 5-amino group and the carbonyl oxygen of the 4-amide group in the conjugated form and thus had a dual binding mode. As a result, we reasoned that it would be better to replace the pyrazole ring with a non-hydrogen bonding five-membered ring such as a thiophene ring[12] to afford only one possible pair of hydrogen bond in the scaffold (C, C′).

The scaffold evolution of the inhibitors is shown in Fig. 2. Herein, we report the synthesis of 4-aryl-thieno[1,4]diazepin-2-

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https://doi.org/10.1016/j.bmc.2018.02.009
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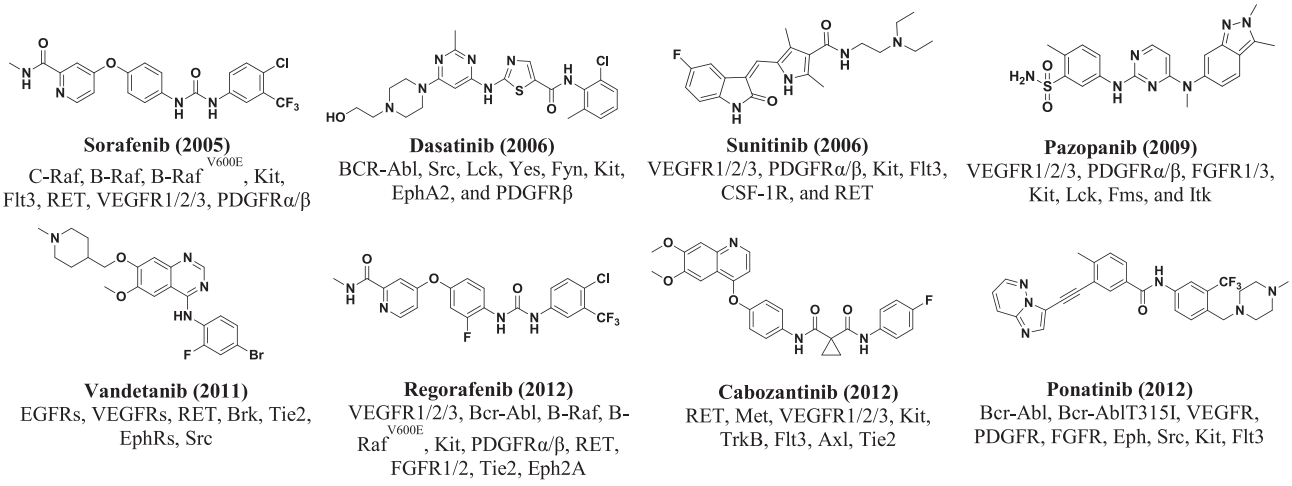


Fig. 1. FDA-approved promising protein kinase inhibitors.

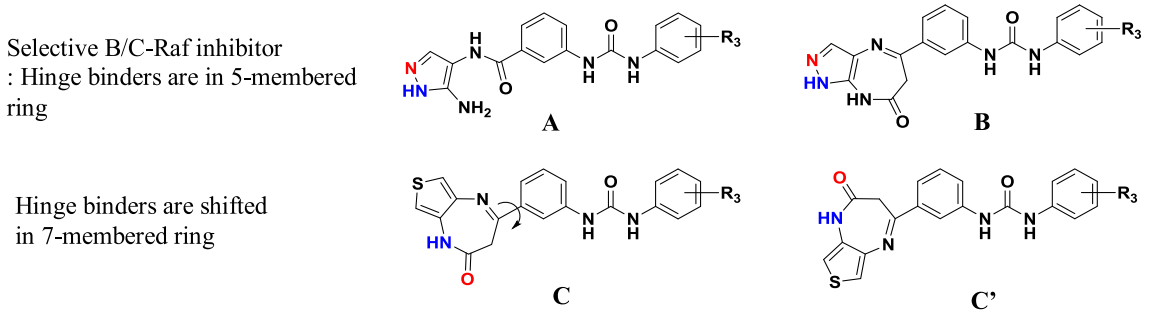


Fig. 2. Evolution of Hinge binder (H-bond donor and acceptor are shown as blue and red) in Type II PKI.

one derivatives and an in vitro evaluation of their anti-proliferative activities in cancer cell lines. The structure-activity relationship (SAR) of the 4-aryl-thieno[1,4]diazepin-2-one derivatives showed excellent improvement in anti-proliferative activity toward the A375P melanoma cell line.

2. Results and discussion

2.1. Chemistry

For the synthesis of 4-aryl-thieno[1,4]diazepin-2-one derivatives, synthetic routes were optimized as outlined in Scheme 1. The synthesis of these 4-aryl-thieno[1,4]diazepin-2-one began from commercially available 3- or /4-benzoic acid chloride (**1**), which was coupled with Meldrum's acid. Acylated Meldrum's acid was followed by ethanolysis and decarboxylation to afford β-keto nitrophenylpropanoic esters **3a**–**3b**.[13] To produce the [5+7] ring system, we prepared thiophene-3,4-diamine dihydrochloride from 2,5-dibromo-3,4-dinitrothiophene (**4**) by reduction using tin power under acidic conditions. Thiophene-3,4-diamine dihydrochloride[14] was neutralized (pH 7–8) using an aqueous KOH solution. Next, the β-keto nitrophenylpropanoic esters **3a**–**3b** were reacted with thiophene-3,4-diamine (**5**) under microwave conditions. The nitro group in compounds **6a**–**6b** was selectively reduced to an amino group using iron and ammonium chloride, followed by coupling with various aromatic acids using HATU in DMF or various acyl chlorides to give amide analogues (**8a**–**8i**, **9a**–**9m**). Urea analogues (**10a**–**10d**, **11a**–**11d**) were synthesized by direct reaction with various aromatic isocyanates.

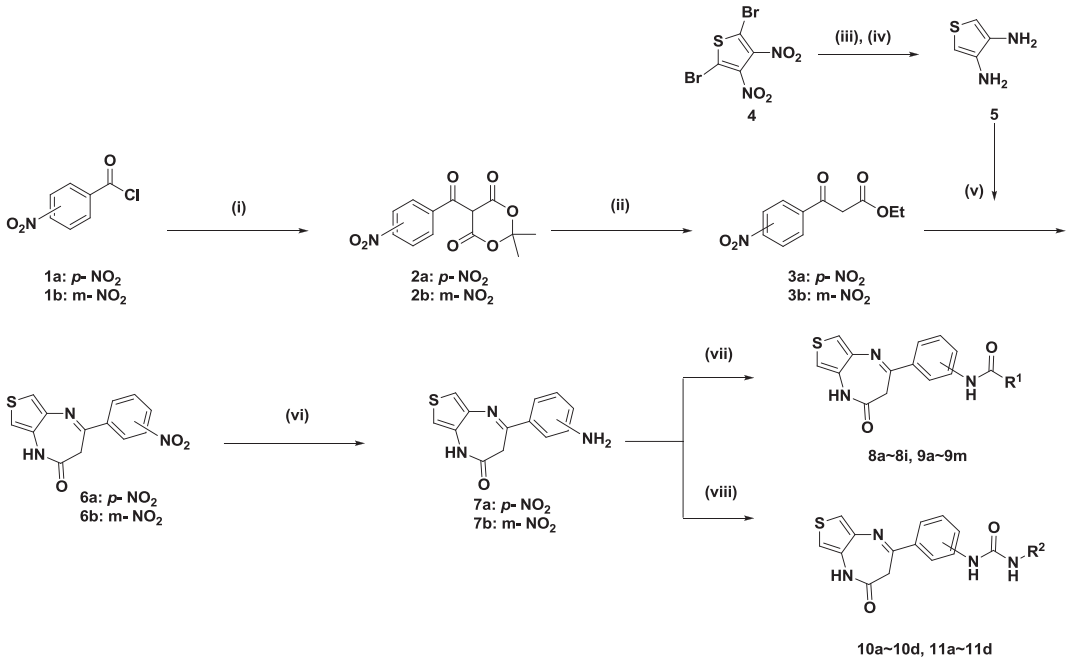
2.2. Biological evaluation

2.2.1. Antiproliferation assays on cancer cell lines

The synthesized compounds **8a**–**8i**, **9a**–**9m**, **10a**–**10d**, and **11a**–**11d** were evaluated for their anticancer activity against A375P[15] (human melanoma) and U937[16] (human histiocytic lymphoma) cell lines by MTT assay. The results are shown in Table 1. The results of this assay showed that all tested compounds had selective potency against the A375P cell line compared to the U937 cell line.

Structure activity relationships (SARs) were inferred from the cell proliferation data reported in Table 1. First, urea link analogues were more potent against the A375P cell line compared to amide link analogues. In general, the amide analogues (**8a**–**8i**, **9a**–**9m**) were not as active toward A375P cells for either the 3- or 4-substituted patterns of the middle phenyl ring. Among the compounds tested, the urea link analogue **10d** had the most potent activity against A375P cells, with an IC_{50} value of 0.32 μM, which was much better than that of the positive control sorafenib (IC_{50} = 3.40 μM).

Secondly, with respect to the substituent position of the middle phenyl ring chain, the 3-substituent amide analogues (**9a**–**9c**) were generally more potent than 4-substituent amide analogues (**8a**–**8c**). Conversely, the 4-substituted urea (**10a**–**10d**) analogues were more potent than the 3-substituted urea analogues (**11a**–**11d**). The inhibitory activities of the urea analogues (**10a**–**10d**, **11a**–**11d**)



Scheme 1. Reagents and conditions; (i) Meldrum's acid, DMAP, MC, −10 °C, 4 h, 2a:40%, **2b**: 42%; (ii) EtOH, reflux, overnight; **3a**: 83%, **3b**: 57%; (iii) c-HCl, Sn power, 0 °C to rt, 2 h, 90%; (iv) KOH (aq), 0 °C, 30 min, pH 7–8, 29%; (v) 5, 500 W microwave, xylene, 10 min, **6a**:56%, **6b**: 65%; (vi) Fe, NH_4Cl, THF:H_2O (1:1), 60 °C, 3 h, **7a**: 87%, **7b**: 77%; (vii) R^1—CO_2H, HATU, TEA, DMF, 45 °C, 12 h, 18–93%; (viii) R^2—N=C=O, THF, r.t, 23–98%.

against the A375P cell line could be grouped as follows: compounds with 1,3,4-substituted benzene tails (**10a**, **10c** and **10d**) showed potent inhibitory activities with IC_{50} values of 1.7, 2.06, 0.32 μM, respectively, while compounds with a simple mono-substitution compound (**10b**) displayed weaker activities. In addition, the inhibitory activities of amide analogues (**8a**–**8i**, **9a**–**9m**) against the A375P cell line was as follows: as with the urea analogues, compounds with 1,3,4,5-benzoic acid tails (**8a**, **8b**, **9a**, **9b** and **9g**) exhibited relatively weak inhibitory activities with IC_{50} values of 7.8, 3.45, 4.99, 1.98, 3.04 μM, respectively, while compounds with either simple mono-substitution or bulky substituents did not exhibit inhibitory activities.

Taken together, the results described above indicate that introduction of aromatic substituents *via* direct urea linkage increased the activity of 3,5-disubstituted derivatives, in particular when one of the substituents was a halide or alkylpiperazine. Indeed, upon comparing the activities of compounds **10a**, **10c**, and **10d** against A375P cells, we found that compound **10d** containing both $-CF_3$ and ethylpiperazinyl substituents at the 3 and 4 positions, respectively, exhibited the most potent activity (IC_{50} = 0.32 μM).

2.2.2. Protein kinase profiling assay

Fig. 3 shows the results of a screen of the representative compound **10d** against a panel of 30 different kinases at a single concentration of 10 μM. The results of this profiling assay showed that the compound was active against multiple protein kinases.[18] Specifically, **10d** effectively inhibited the activity of FLT3, FMS, FLT4/VEGFR3, PDGFRb, and RET and exhibited the greatest inhibitory effects toward FMS. We next evaluated the in vitro enzymatic inhibition of compound **10d** toward c-Kit, FLT3, FMS, LYN, RAF1, FLT4/VEGFR3, PDGFRα PDGFRβ, and RET. As shown in Fig. 4, compound **10d** demonstrated superb activity against FMS kinase in vitro (3.73 nM), but only good potency toward FLT3, PDGFRβ, and RET (27.9 nM, 63.4 nM, 71.6 nM).

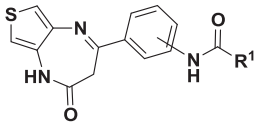
2.3. Molecular docking studies

To better understand the interactions between the synthesized compounds and FMS kinase, molecular docking of the potent compound **10d** into the ATP binding site of FMS kinase (PDB: 3LCO) was conducted with Glide Docking (Schrödinger Maestro software package Version 14.2). The ligand was prepared with protonation conditions at a pH range of 6.8–7.2. The binding mode is displayed in Fig. 4. In this binding mode, compound **10d** was properly bound to the ATP-binding site of FMS via four hydrogen bonds and two π-cation interactions. Specifically, the amino hydrogen atom of the thienodiazepine ring formed a hydrogen bond (1.98 Å) with the oxygen atom of the Cys666 backbone and a π-cation interaction (5.53 Å) with the phenyl ring of Tyr665. In addition, the oxygen atom of the thienodiazepine ring formed a hydrogen bond (1.90 Å) with the amino hydrogen atom of Cys666. This result suggests that the thienodiazepine ring plays a critical role as a hinge binder in ligand-protein binding. In addition, we found that the amino hydrogen of the middle amide bond formed a hydrogen bond (2.35 Å) with the oxygen atom of the Asp796 backbone. The protonated nitrogen of the piperazine formed a π-cation interaction (5.40 Å) with the imidazole ring of His776 and a weak hydrogen bond (2.49 Å) with the side chain of Asp796. Together, these interactions appeared to increase the binding affinity of the synthesized compounds toward FMS kinase.

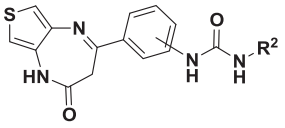
3. Conclusion

In conclusion, a new series of 4-aryl-thieno[1,4]diazepin-2-one derivatives were designed and synthesized based on our previous research. We also performed structure–activity relationship studies of the 4-aryl-thieno[1,4]diazepin-2-one derivatives. Among the derivatives, compound **10d**, 1-(4-((4-ethylpiperazin-1-yl) methyl)-3-(trifluoromethyl)phenyl)-3-(4-(2-oxo-2,3-dihydro-1*H*-thieno[3,4-*b*][1,4]diazepin-4-yl)phenyl)urea, showed the most potent anti-proliferative activity against A375P cell lines, but had

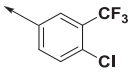
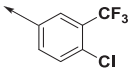
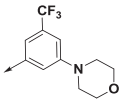
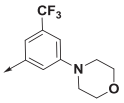
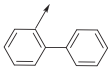
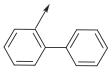
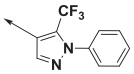
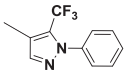
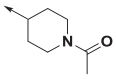
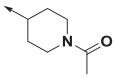
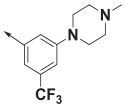
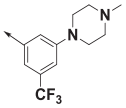
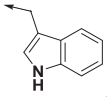
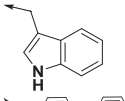
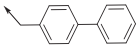
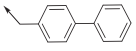
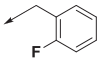
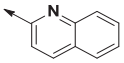
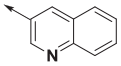
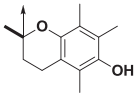
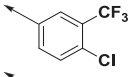
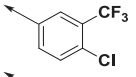
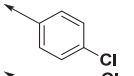
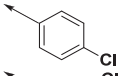
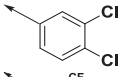
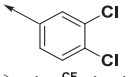
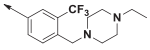
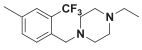
Table 1
Anti-proliferative activities of 4-aryl-thieno[1,4]diazepin-2-one derivatives.



8a~8i, 9a-9m



10a~10d, 11a~11d

Compd	Substitution	R	A375P (GI_{50}, μM)	U937	HS	Compd	Substitution	R	A375P (GI_{50}, μM)	U937	HS
8a	*4-*		7.86 ± 0.25	NA	>30	**9a**	*3-*		4.99 ± 1.12	NA	>30
8b	*4-*		3.45 ± 0.75	NA	>30	**9b**	*3-*		1.98 ± 0.76	NA	>30
8c	*4-*		>30	3.77 ± 0.59	>30	**9c**	*3-*		3.25 ± 0.75	1.03 ± 0.65	>30
8d	*4-*		6.85 ± 1.02	NA	>30	**9d**	*3-*		NA	9.64 ± 1.78	>30
8e	*4-*		>30	NA	>30	**9e**	*3-*		NA	NA	>30
8f	*4-*		NA	NA	>30	**9f**	*3-*		NA	NA	>30
8g	*4-*		>30	>30	>30	**9g**	*3-*		3.04 ± 0.24	NA	>30
8h	*4-*		>30	>30	>30	**9h**	*3-*		>30	>30	>30
8i	*4-*		8.7 ± 1.8	NA	>30	**9i**	*3-*		NA	NA	>30
9j	*3-*		NA	NA	>30	**9k**	*3-*		NA	NA	>30
9l	*3-*		6.87 ± 0.63	NA	>30	**9m**	*3-*		>30	NA	>30
10a	*4-*		1.7 ± 0.36	4.08 ± 0.87	>30	**11a**	*3-*		8.25 ± 1.68	14.27 ± 1.37	>30
10b	*4-*		3.12 ± 0.41	0.964 ± 0.07	>30	**11b**	*3-*		5.04 ± 0.89	4.55 ± 1.22	>30
10c	*4-*		2.06 ± 0.43	NA	>30	**11c**	*3-*		11.0 ± 1.78	3.2 ± 0.97	>30
10d	*4-*		0.32 ± 0.05	NA	>30	**11d**	*3-*		1.82 ± 0.22	2.75 ± 0.52	>30
Sorafenib			3.40 ± 0.31	2.34 ± 0.23	>30						

*Maximum potency showed less than 60% of total growth.

almost no cytotoxic activity against a normal human cell line or the U937 tumor cell line. Protein kinase profiling showed that compound **10d** is a potent inhibitor of FMS kinase (3.73 nM) and also protein kinases such as c-Kit, FLT3, FMS, LYN, RAF1, FLT4/VEGFR3, PDGFRα and PDGFR β, a so-called multi-targeted protein kinase inhibitor. In particular, it exhibits excellent activity against A375, a typical malignant tumor cell line, and shows its potential as an anti-cancer agent. Further modification and mechanistic studies of these compounds in order to improve their potency is currently in progress.

4. Materials and methods

4.1. General chemistry

All chemicals were of reagent grade and were purchased from Aldrich (USA). Separation of the compounds by column chromatography was carried out with silica gel 60 (200–300 mesh ASTM, E. Merck, Germany). The quantity of silica gel used was 50–100 times the weight charged on the column. Thin layer chromatography (TLC) was run on the silica gel-coated aluminum sheets (silica

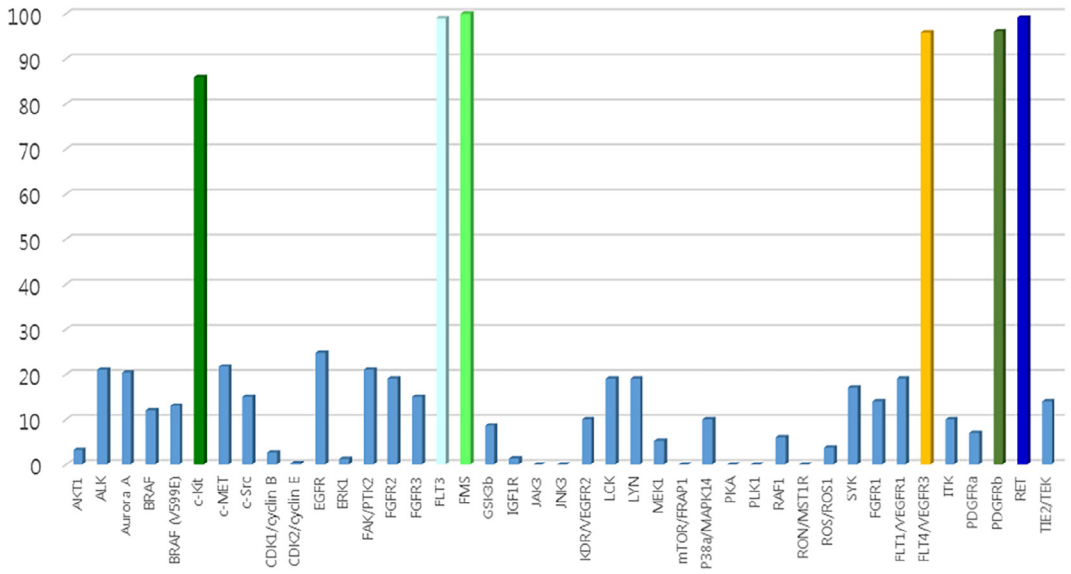


Fig. 3. Percentages of enzymatic inhibitions by **10d** (10 μM) on selected 30 Protein Kinases.[17]

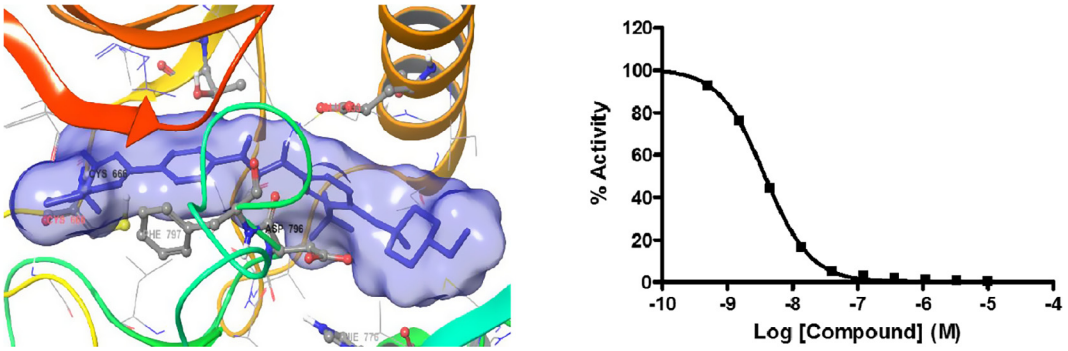


Fig. 4. Docking structures of designed 4-aryl-thieno[1,4]diazepin-2-one derivatives scaffold amide derivatives (tube, blue) in FMS (PDB: 3LCO)[19] and determination of IC_{50} measurement of **10d** on FMS.

gel 60 GF254, E. Merck, Germany) and visualized under ultraviolet (UV) light (254 nm). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Brucker model digital AVANCE III 400 MHz spectrometer at 25 °C using tetramethylsilane (TMS) as an internal standard. High-resolution MS (HR/MS) experiments were conducted with a Finnigan LTQ Orbitrap mass spectrometer (Thermo Fisher Scientific Inc, MA, USA) operated in positive-ion electrospray mode.

*4.1.1. Syntheses of 5 ethyl 3-(nitrophenyl)-3-oxopropanoate (**3a–b**)*

To a solution of Meldrum's acid (3 g, 20.8 mmol) and *N,N*-dimethylamino pyridine (4.8 g, 26 mmol) in methylene chloride (70 ml) nitrobenzoyl chloride (5 g, 42 mmol) was added dropwise at −10 °C. The mixture was then stirred at the same temperature for 4 h. Methylene chloride (50 ml) was then added to the mixture, which was washed with brine, dried over Na_2SO_4, and evaporated to dryness. The concentrated crude product was purified by flash column chromatography with methylene chloride/methanol = (10:1) as the eluent to give the product 2,2-dimethyl-5-(4-nitrobenzoyl)-1,3-dioxane-4,6-dione (**2a**) as a brown solid (40%); 2,2-dimethyl-5-(3-nitrobenzoyl)-1,3-dioxane-4,6-dione (**2b**) as a yellow solid (42%). Without further purification **2a–2b** (2.3 g, 7.8 mmol) was, then dissolved in absolute ethanol (18.4 ml) and heated to reflux (100 °C) for 15 h. After cooling down to ambient temperature, the solvent was removed *in vacuo*. The concentrated crude product was purified by flash column chromatography with methylene chloride as the eluent to give product

*4.1.1.1. Ethyl 3-(4-nitrophenyl)-3-oxopropanoate (**3a**).* The title compound was isolated as a white solid (83%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (2H, d, J = 9.2 Hz), 7.93 (2H, d, J = 9.2 Hz), 5.76 (1H, s), 4.30 (2H, q, J = 7.2 Hz), 1.35 (3H, t, J = 7.2 Hz); ^{13}C NMR (100 MHz, DMSO d_6) δ 192.9, 167.4, 167.3, 150.2, 140.3, 129.8, 127.4, 123.9, 123.8, 60.8, 45.9, 14.0, 13.9; HRMS (ESI) calcd. for $C_{11}H_{11}NNaO_5$ $[M+Na]^+$: 260.2008, found: 260.1555; m.p. 68–69 °C; IR (ATR) ν max/cm^{-1}: 2990, 1732, 1617, 1183, 795.

*4.1.1.2. Ethyl 3-(3-nitrophenyl)-3-oxopropanoate (**3b**).* The title compound was isolated as a yellow solid (57%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.66 (1H, t, J = 1.9 Hz), 8.51 (1H, ddd, J = 8.2, 2.3, 1.0 Hz), 8.38 (1H, ddd, J = 7.8, 1.7, 1.1 Hz), 7.86 (1H, dd, J = 12.0, 4.2 Hz), 4.35 (2H, s), 4.13 (2H, q, J = 7.1 Hz), 1.18 (3H, t, J = 7.1 Hz); ^{13}C NMR (100 MHz, DMSO d_6) δ 202.7, 177.8, 158.6, 147.4, 145.1, 141.2, 138.5, 133.3, 71.3, 71.1, 56.2, 24.6, 24.5; HRMS (ESI) calcd. for $C_{11}H_{11}NNaO_5$ $[M+Na]^+$: 260.2008, found: 260.1642; m.p. 80–82 °C; IR(ATR) ν max/cm^{-1}: 3095, 1620, 1527, 1346, 803.

*4.1.2. Thiophene-3,4-diamine (**5**)*

5-Dibromo-3,4-dinitrothiophene (1.8 g, 5.4 mmol) was added to concentrated hydrochloric acid (23 ml), followed by the addition

of metal tin (4.5 g, 37.8 mmol) at 25 °C. The resulting mixture was stirred at room temperature for 12 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The precipitated crystals were collected by filtration and washed with diethyl ether. The crystals were suspended in ethyl acetate, followed by the addition of a 25% aqueous solution of potassium hydroxide. The resulting mixture was filtered through Celite, and the filtrate was extracted with ethyl acetate. The organic layer was washed with brine and then dried over anhydrous sodium sulfate. The solvent was distilled to yield 3, 4-diaminothiophene **5** (172.8 mg, 29%). ^{1}H NMR (400 MHz, DMSO d_6) δ 5.82 (2H, s), 4.45 (4H, s); ^{13}C NMR (100 MHz, DMSO d_6) δ 126.4, 117.1; HRMS (ESI) calcd. for $C_4H_6N_2S$ $[M+2H]^+$: 116.0252, found: 116.1718; IR(ATR) ν max/cm^{-1}: 2786, 2561, 1522, 808.

*4.1.3. Syntheses of 4-(nitrophenyl)-1H-thieno[3,4-b][1,4]diazepin-2 (3H)-one (**6a**–**b**)*

A mixture of **3a**–**b** (239 mg, 1.0 mmol) and thiophene-3,4-diamine **5** (172.8 mg, 1.5 mmol) in xylene (10 ml) was prepared and heated in a microwave oven at 500 W and 120 °C for 10 min. The reaction mixture was then diluted with ethyl acetate and washed with brine. The organic layer was dried over Na_2SO_4, and the concentrated crude product was purified by flash column chromatography with hexane/ethyl acetate = (1:1) as the eluent to give the indicated product.

*4.1.3.1. 4-(4-Nitrophenyl)-1H-thieno[3,4-b][1,4]diazepin-2(3H)-one (**6a**).* The title compound was isolated as a yellow solid (56%); ^{1}H NMR (400 MHz, DMSO d_6) δ 10.59 (1H, s), 8.36 (2H, d, J = 8.8 Hz), 8.24 (2H, d, J = 8.8 Hz), 7.65 (1H, d, J = 3.6 Hz), 7.06 (1H, d, J = 3.6 Hz), 3.67 (2H, s); ^{13}C NMR (100 MHz, DMSO d_6) δ 165.5, 156.3, 143.7, 141.4, 130.2, 129.5, 128.6, 124.0, 120.7, 110.6, 40.0; HRMS (ESI) calcd. for $C_{13}H_{10}N_3O_3S$ $[M+H]^+$: 288.3010, found: 288.1442; m.p.: 197–202 °C; IR(ATR) ν max/cm^{-1}: 3080, 2959, 1679, 1516, 1342, 708.

*4.1.3.2. 4-(3-Nitrophenyl)-1H-thieno[3,4-b][1,4]diazepin-2(3H)-one (**6b**).* The title compound was isolated as a yellow solid (65%); ^{1}H NMR (400 MHz, DMSO d_6) δ 8.94 (1H, t, J = 1.6 Hz), 8.37–8.32 (2H, m), 8.23 (1H, s), 7.66 (1H, t, J = 8.0 Hz), 7.45 (1H, d, J = 3.6 Hz), 6.91 (1H, d, J = 3.6 Hz), 3.73 (2H, s); ^{13}C NMR (100 MHz, DMSO) δ 165.4, 149.1 133.4, 130.2, 130.0, 125.6, 122.8, 120.6, 110.7, 39.8; HRMS (ESI) calcd. for $C_{13}H_{10}N_3O_3S$ $[M+H]^+$: 288.3010, found: 288.3330; m.p.: 240–247 °C; IR(ATR) ν max/cm^{-1}:2921, 2852, 1731, 1567, 1345, 1079.

*4.1.4. Syntheses of aminophenyl-1H-thieno[3,4-b][1,4]diazepin-2 (3H)-one (**7a**–**7b**)*

To a suspension of 6a-6b (224 mg, 0.8 mmol) in THF/H_2O = 1:1 solution (4 ml), Fe (173.1 mg, 3.1 mmol) and NH_4Cl (172 mg, 3.2 mmol) were added and heated to 60 °C. Stirring was continued for 4 h, and the resulting clear solution was cooled to room temperature. The pH was made slightly basic (pH 7–8) by adding saturated aqueous sodium bicarbonate before extraction with ethyl acetate. The organic phase was thoroughly washed with brine and then dried over Na_2SO_4. Evaporation of the solvent resulted in an oil, and the concentrated crude product was purified by flash column chromatography with methylene chloride/methanol = (10:1) as the eluent to give the indicated product.

*4.1.4.1. 4-(4-Aminophenyl)-1H-thieno[3,4-b][1,4]diazepin-2(3H)-one (**7a**).* The title compound was isolated as a brown solid (87%) ^{1}H NMR (400 MHz, DMSO d_6) δ 10.34 (1H, s), 7.45 (2H, d, J = 8.4 Hz), 7.31 (1H, d, J = 3.6 Hz), 6.95 (1H, d, J = 4.0 Hz), 6.61 (2H, d, J = 8.8 Hz), 5.79 (2H, s),3.50 (2H, s); ^{13}C NMR (100 MHz, DMSO) δ 172.49, 164.34, 159.26, 151.26, 149.16, 137.04, 136.79, 136.58, 133.97, 131.48, 131.38, 126.82, 122.80, 122.41, 118.45, 117.33, 113.50, 111.71, 54.27, 47.31, 45.58, 18.14, 12.07; ^{13}C NMR (100 MHz, DMSO d_6) δ 165.4, 157.7, 151.8, 142.4, 131.6, 129.3, 124.4, 116.6, 113.2, 109.1; HRMS (ESI) calcd. for $C_{13}H_{12}N_3OS$ $[M+H]^+$: 258.3190, found: 258.1784, 258.3904; m.p.: 248–252 °C; IR(ATR) ν max/cm^{-1}: 3066, 1666, 1623, 1555, 1296, 791.

*4.1.4.2. 4-(3-Aminophenyl)-1H-thieno[3,4-b][1,4]diazepin-2(3H)-one (**7b**).* The title compound was isolated as a white solid (77%) ^{1}H NMR (400 MHz, DMSO d_6) δ 10.50 (1H, s), 7.45 (1H, d, J = 3.6 Hz), 7.26 (1H, t, J = 4.0 Hz), 7.14–7.12 (2H, m), 6.99 (1H, d, J = 3.6 Hz), 5.30 (2H, s), 3.50 (2H, s); ^{13}C NMR (100 MHz, DMSO) δ 165.4, 157.7, 149.6, 142.4, 134.3, 133.2, 129.3, 116.6, 114.6, 109.1; HRMS (ESI) calcd. for $C_{13}H_{12}N_3OS$ $[M+H]^+$: 258.3190, found: 258.2698; IR (ATR) ν max/cm^{-1}: 3253, 2922, 2844, 1648, 1604, 1507, 1452, 1375 1251, 1166, 1120.

*4.1.5. General syntheses of N-(3 or 4-(2-oxo-2,3-dihydro-1H-thieno [3,4-b][1,4]diazepin-4-yl)phenyl)amide (**8a**–**8k**), (**9a**–**9o**)*

Aminophenyl-1H-thieno[3,4-*b*][1,4]diazepin-2(3H)-one (**7a**–**7b**) (25.7 mg, 0.1 mmol), aryl acid (1 eq., 0.1 mmol), HATU (1.5 eq., 0.15 mmol), and TEA (1.5 eq., 0.15 mmol) in DMF (0.5 ml) were heated at 45 °C overnight. The reaction mixture was then diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate. The organic layer was dried over Na_2SO_4. The concentrated crude product was purified by flash column chromatography with hexane/ethyl acetate = (1:1) as the eluent to give the indicated product.

*4.1.5.1. 4-Chloro-N-(4-(2-oxo-2,3-dihydro-1H-thieno[3,4-b][1,4]diazepin-4-yl)phenyl)-3-(trifluoromethyl)-benzamide (**8a**).* The title compound was isolated as an ivory solid (92%); ^{1}H NMR (400 MHz, DMSO d_6) δ10.79 (1H, s), 10.52 (1H, s), 8.42 (1H, d, J = 2.0 Hz), 8.29 (1H, dd, J = 8.2 Hz), 8.07 (2H, d, J = 8.8 Hz), 7.94 (3H, m), 7.51 (1H, d, J = 3.6 Hz), 7.02 (1H, d, J = 3.6 Hz), 3.61 (2H, s); ^{13}C NMR (400 MHz, DMSO d_6) δ165.0, 163.3, 157.4, 141.8, 141.1, 133.9, 133.5, 132.9, 131.9, 131.5, 131.4, 128.2, 127.5, 127.1, 126.5, 120.1, 118.4, 109.5; HRMS (ESI) calcd. for $C_{21}H_{14}ClF_3N_3O_2S$ $[M+H]^+$: 464.0442, found: 464.0431; IR (ATR) ν max/cm^{-1}: 3170, 2921, 1671, 1571, 1307, 1135, 1034.

*4.1.5.2. 3-Morpholino-N-(4-(2-oxo-2,3-dihydro-1H-thieno[3,4-b][1,4]diazepin-4-yl)-phenyl)-5-(trifluoromethyl)benzamide (**8b**).* The title compound was isolated as a brown solid (51%); ^{1}H NMR (400 MHz, DMSO d_6) δ10.59 (1H, s), 10.48 (1H, s), 8.06 (2H, d, J = 8.8 Hz), 7.93 (2H, d, J = 8.8 Hz), 7.74 (1H, s), 7.68 (1H, s), 7.50 (1H, d, J = 8.8 Hz), 7.41 (1H, s), 7.02 (1H, d, J = 8.8 Hz), 3.78 (4H, t, J = 4.8 Hz), 3.61 (2H, s); ^{13}C NMR (400 MHz, DMSO d_6) δ165.1, 164.1, 157.5, 154.6, 141.9, 141.5, 133.1, 132.7, 131.4, 130.3, 128.2, 127.5, 127.2, 125.3, 123.8, 120.0, 118.4, 109.6, 66.4, 53.1; HRMS (ESI) calcd. for $C_{25}H_{22}F_3N_4O_3S$ $[M+H]^+$: 515.1359, found: 515.1342.

*4.1.5.3. N-(4-(2-Oxo-2,3-dihydro-1H-thieno[3,4-b][1,4]diazepin-4-yl) phenyl)biphenyl-2-carboxamide (**8c**).* The title compound was isolated as a yellow solid (48%); ^{1}H NMR (400 MHz, DMSO d_6) δ10.51 (1H, s), 10.47 (1H, s), 7.94 (2H, d, J = 8.8 Hz), 7.66 (2H, d, J = 8.8 Hz), 7.62–7.57 (2H, m), 7.53–7.47 (3H, m), 7.45–7.43 (2H, m), 7.37 (2H, t, J = 7.6 Hz), 7.31–7.27 (1H, m), 7.00 (1H, d, J = 3.6 Hz), 3.56 (2H, s); ^{13}C NMR (400 MHz, DMSO d_6) δ168.1, 165.0, 157.5, 151.9, 141.8, 141.5, 139.9, 139.3, 136.8, 132.3, 131.4, 130.0, 128.3, 128.3, 128.2, 127.8, 127.3, 127.2, 119.1, 118.3, 109.5; HRMS (ESI) calcd. for $C_{26}H_{20}N_3O_2S$ $[M+H]^+$: 438.1271, found: 438.1254.

Please cite this article in press as: Lee J., et al. Bioorg. Med. Chem. (2018), https://doi.org/10.1016/j.bmc.2018.02.009

*4.1.5.4. N-(4-(2-Oxo-2,3-dihydro-1H-thieno[3,4-b][1,4]diazepin-4-yl) phenyl)-1-phenyl-5-(trifluoromethyl)-1H-pyrazole -4-carboxamide (**8d**).* The title compound was isolated as a yellow solid (48%); ^{1}H NMR (400 MHz, DMSO d_6) δ10.78 (1H, s), 10.47 (1H, s), 8.36 (1H, s), 8.05 (2H, d, J = 8.8 Hz), 7.87 (2H, d, J = 8.8 Hz), 7.63–7.61 (3H, m), 7.56–7.55 (2H, m), 7.50 (1H, d, J = 4.0 Hz), 7.02 (1H, d, J = 3.6 Hz); ^{13}C NMR (400 MHz, DMSO d_6) δ165.0, 159.2, 157.4, 141.8, 141.1, 139.9, 138.7, 132.8, 131.4, 130.1, 129.7, 129.4, 128.4, 126.0, 121.2, 120.6, 119.4, 118.4, 109.7; HRMS (ESI) calcd. for: $C_{24}H_{17}F_3N_5O_2S$ [M+H]+: 496.1050, found: 496.1016.

*4.1.5.5. 1-Acetyl-N-(4-(2-oxo-2,3-dihydro-1H-thieno[3,4-b][1,4]diazepin-4-yl)phenyl)piperidine-4-carboxamide (**8e**).* The title compound was isolated as a brown solid (72%); ^{1}H NMR (400 MHz, DMSO d_6) δ10.53 (1H, s), 9.97 (1H, s), 7.98 (1H, d, J = 8.0 Hz), 7.74 (1H, d, J = 8.0 Hz), 7.47 (1H, d, J = 3.6 Hz), 7.00 (1H, d, J = 3.6 Hz), 4.43–4.39 (1H, m), 3.89–3.86 (1H, m), 3.57 (2H, s), 3.10–3.04 (1H, m), 2.61–2.59 (2H, m), 2.01 (3H, s), 1.85–1.80 (2H, m), 1.64–1.54 (1H, m), 1.49–1.39 (1H, m); ^{13}C NMR (400 MHz, DMSO d_6) δ173.4, 168.1, 165.0, 157.4, 141.9, 141.8, 132.0, 131.4, 128.3, 118.8, 118.2, 109.5, 45.2, 42.7, 40.3, 28.7, 28.1, 21.3; HRMS (ESI) calcd. for: $C_{21}H_{23}N_4O_3S$ [M+H]$^+$: 411.1485, found: 411.1467.

*4.1.5.6. N-(4-(2-Oxo-2,3-dihydro-1H-thieno[3,4-b][1,4]diazepin-4-yl) phenyl)pyrazine-2-carboxamide (**8f**).* The title compound was isolated as a yellow solid (28%) ^{1}H NMR (400 MHz, DMSO d_6) δ 11.00 (1H, s), 10.50 (1H, s), 9.33 (1H, s), 8.96 (1H, d, J = 2.4 Hz), 8.84 (1H, dd, J = 2.5, 1.5 Hz), 8.10–8.03 (4H, m), 7.51 (1H, d, J = 3.7 Hz), 7.02 (1H, d, J = 3.7 Hz), 3.61 (2H, s); ^{13}C NMR (400 MHz, DMSO d_6) δ165.0, 162.0, 157.4, 147.8, 144.9, 144.2, 143.3, 141.8, 140.6, 133.1, 131.4, 128.2, 120.2, 118.4, 109.5; HRMS (ESI) calcd. for: $C_{18}H_{14}N_5O_2S$ [M+H]$^+$: 364.0863, found: 364.0864.

*4.1.5.7. 3-(4-Methylpiperazin-1-yl)-N-(4-(2-oxo-2,3-dihydro-1H-thieno[3,4-b][1,4]diazepin-4-yl)phenyl)-5-(trifluoromethyl) benzamide (**8g**).* The title compound was isolated as a yellow solid (10%); ^{1}H NMR (400 MHz, DMSO d_6) δ 10.587 (s, 1H), 10.496 (s, 1H), 8.03 (d, J = 9.2 Hz, 2H), 7.91 (d, J = 9.2 Hz, 2H), 7.73 (s, 1H), 7.63 (s, 1H), 7.25 (d, J = 3.6 Hz, 1H), 7.39 (s, 1H), 7.01 (d, J = 3.6 Hz, 1H), 3.60 (s, 2H), 2.55–2.44 (m, 8H), 2.24 (s, 3H); ^{13}C NMR (400 MHz, DMSO d_6) δ165.0, 164.8, 157.4, 151.3, 141.8, 141.4, 138.7, 136.4, 132.8, 131.4, 128.2, 127.5, 120.2, 118.4, 117.4, 114.6, 113.7, 109.6, 54.3, 47.3, 45.6; HRMS (ESI) calcd. for: $C_{26}H_{25}F_3N_5O_2S$ [M+H]$^+$: 528.1676, found: 528.1689; IR(ATR) ν max/cm^{-1}: 3070, 1673, 1596, 1523, 1248, 1115, 837.

*4.1.5.8. 2-(1H-Indol-3-yl)-N-(4-(2-oxo-2,3-dihydro-1H-thieno[3,4-b][1,4]diazepin-4-yl)phenyl)acetamide (**8h**).* The title compound was isolated as a yellow solid (53%); ^{1}H NMR (400 MHz, DMSO d_6) δ 10.94 (1H, s), 10.46 (1H, s), 10.39 (1H, s), 7.97 (2H, d, J = 8.9 Hz), 7.75 (2H, d, J = 8.9 Hz), 7.61 (1H, d, J = 7.9 Hz), 7.47 (1H, d, J = 3.7 Hz), 7.35 (1H, d, J = 8.0 Hz), 7.27 (1H, d, J = 2.3 Hz), 7.09–7.05 (1H, m), 7.01–6.98 (2H, m), 3.77 (2H, s), 3.56 (2H, s); ^{13}C NMR (400 MHz, DMSO d_6) δ170.2, 165.0, 159.4, 157.5, 141.9, 136.1, 132.9, 131.9, 131.4, 128.4, 127.2, 124.0, 121.0, 118.7, 118.5, 118.2, 111.4, 109.5, 108.3, 38.3, 33.9; HRMS (ESI) calcd. for: $C_{23}H_{19}N_4O_2S$ [M+H]$^+$: 415.1223, found: 415.1224.

*4.1.5.9. 2-(Biphenyl-4-yl)-N-(4-(2-oxo-2,3-dihydro-1H-thieno[3,4-b][1,4]diazepin-4-yl)phenyl)acetamide (**8i**).* The title compound was isolated as a brown solid (74%); ^{1}H NMR (400 MHz, DMSO d_6) δ 10.48 (2H, d, J = 7.3 Hz), 7.99 (1H, d, J = 8.9 Hz), 7.75 (1H, d, J = 8.9 Hz), 7.68–7.60 (4H, m), 7.59 (1H, t, J = 2.0 Hz), 7.51 (1H, t, J = 2.2 Hz), 7.48 (1H, d, J = 3.6 Hz), 7.47–7.41 (3H, m), 7.38–7.30 (2H, m), 7.00 (1H, d, J = 3.7 Hz), 3.72 (2H, dd, J = 14.4, 6.7 Hz), 3.57 (2H, s); ^{13}C NMR (400 MHz, DMSO d_6) δ169.5, 165.0, 157.4, 141.8, 141.7, 139.9, 138.6, 135.0, 131.4, 129.7, 129.6, 128.9, 128.4, 127.3, 126.7, 126.6, 126.5, 118.7, 118.3, 109.5, 38.2; HRMS (ESI) calcd. for: $C_{27}H_{22}N_3O_2S$ [M+H]$^+$: 452.1427, found: 452.1432; IR (ATR) ν max/cm^{-1}: 2927, 1669, 1593, 1523, 1254, 1024, 837, 755.

*4.1.5.10. 4-Chloro-N-(3-(2-oxo-2,3-dihydro-1H-thieno[3,4-b][1,4]diazepin-4-yl)phenyl)-3-(trifluoromethyl)-benzamide (**9a**).* The title compound was isolated as a yellow solid (74%); ^{1}H NMR (400 MHz, DMSO d_6) δ10.73 (1H, s), 10.53 (1H, s), 8.44 (1H, d, J = 2.0 Hz), 8.41 (1H, t, J = 1.8 Hz), 8.30 (1H, dd, J = 8.4, 2.0 Hz), 8.03 (1H, dd, J = 7.2, 1.2 Hz), 7.94 (1H, d, J = 8.4 Hz), 7.78 (1H, d, J = 8.4 Hz), 7.56–7.52 (2H, m), 7.03 (1H, d, J = 3.6 Hz), 3.61 (2H, s); ^{13}C NMR (400 MHz, DMSO d_6) δ164.9, 163.3, 157.9, 141.6, 139.1, 138.2, 134.1, 133.9, 133.5, 132.0, 131.4, 129.2, 127.2, 126.5, 124.0, 123.3, 122.9, 119.3, 118.9, 109.7; HRMS (ESI) calcd. for: $C_{21}H_{14}ClF_3N_3O_2S$ [M+H]$^+$: 464.0442, found: 464.0418.

*4.1.5.11. 3-Morpholino-N-(3-(2-oxo-2,3-dihydro-1H-thieno[3,4b][1,4]diazepin-4-yl)phenyl)-5-(trifluoromethyl)-benzamide (**9b**).* The title compound was isolated as a brown solid (62%); ^{1}H NMR (400 MHz, DMSO d_6) δ10.56 (1H, s), 10.53 (1H, s), 8.40 (1H, s), 8.02 (1H, d, J = 9.2 Hz), 7.77–7.75 (2H, m), 7.70 (1H, s), 7.55–7.51 (2H, s), 7.41 (1H, s), 7.03 (1H, d, J = 3.6 Hz), 3.78 (4H, t, J = 4.6 Hz), 3.61 (2H, s); ^{13}C NMR (400 MHz, DMSO d_6) δ165.0, 164.6, 157.9, 151.5, 141.6, 139.3, 138.1, 136.4, 131.3, 130.3, 129.1, 125.5, 123.2, 123.1, 119.4, 118.8, 117.3, 114.0, 113.5, 109.7, 65.9, 47.7, 38.2; HRMS (ESI) calcd. for: $C_{25}H_{22}F_3N_4O_3S$ [M+H]$^+$: 515.1359, found: 515.1331; IR(ATR) ν max/cm^{-1}: 2922, 1671, 1602, 1544, 1120, 1055, 1026, 1002, 835, 759.

*4.1.5.12. N-(3-(2-Oxo-2,3-dihydro-1H-thieno[3,4-b][1,4]diazepin-4-yl)phenyl)biphenyl-2-carboxamide (**9c**).* The title compound was isolated as a yellow solid (55%); ^{1}H NMR (400 MHz, DMSO d_6) δ10.49 (1H, s), 10.43 (1H, s), 8.25 (1H, s), 7.68 (2H, d, J = 8.0 Hz), 7.61–7.57 (2H, m), 7.52 (1H, d, J = 3.6 Hz), 7.49–7.41 (4H, m), 7.40–7.36 (3H, m), 7.30 (1H, t, J = 7.2 Hz), 7.01 (1H, d, J = 3.6 Hz),3.55 (2H, s); ^{13}C NMR (400 MHz, DMSO d_6) δ168.0, 164.9, 157.9, 141.6, 140.0, 139.5, 139.2, 138.1, 136.9, 131.3, 130.0, 129.8, 129.0, 128.3, 128.3, 127.8, 127.3, 127.2, 122.7, 122.0, 118.8, 118.3, 109.6; HRMS (ESI) calcd. for $C_{26}H_{20}N_3O_2S$ [M+H]$^+$: 438.1271, found: 438.1258.

*4.1.5.13. N-(3-(2-Oxo-2,3-dihydro-1H-thieno[3,4-b][1,4]diazepin-4-yl)phenyl)-1-phenyl-5-(trifluoromethyl)-1H-pyrazole-4-carboxamide (**9d**).* The title compound was isolated as a yellow solid (86%); ^{1}H NMR (400 MHz, DMSO d_6) δ10.74 (1H, s), 10.54 (1H, s), 8.38 (1H, s), 8.36 (1H, s), 7.94 (1H, d, J = 8.4 Hz), 7.76 (1H, d, J = 8.4 Hz), 7.63–7.61 (4H, m), 7.56–7.50 (3H, m), 7.04 (1H, d, J = 4.0 Hz), 3.61 (2H, s); ^{13}C NMR (400 MHz, DMSO d_6) δ164.9, 159.1, 157.8, 153.3, 141.6, 139.9, 139.1, 138.7, 138.2, 137.8, 131.3, 130.1, 129.4, 129.2, 126.0, 123.2, 122.2, 121.3, 118.9, 118.5, 109.7; HRMS (ESI) calcd. for $C_{24}H_{17}F_3N_5O_2S$ [M+H]$^+$: 496.1050, found: 496.1020.

*4.1.5.14. 1-Acetyl-N-(3-(2-oxo-2,3-dihydro-1H-thieno[3,4-b][1,4]diazepin-4-yl)phenyl)piperidine-4-carbox-amide (**9e**).* The title compound was isolated as a yellow solid (76%); ^{1}H NMR (400 MHz, DMSO d_6) δ10.52 (1H, s), 10.15 (1H, s), 8.26 (1H, t, J = 1.6 Hz), 7,83 (1H, d, J = 8.0 Hz), 7.67 (1H, d, J = 8.4 Hz), 7.53 (1H, d, J = 3.6 Hz), 7.44 (1H, t, J = 8.0 Hz), 7.02 (1H, d, J = 3.6 Hz), 3.89–3.86 (1H, m), 3.55 (2H, s), 3.11–3.04 (1H, m), 2.63–2.56 (3H, m), 2.04 (3H, s), 1.64–1.54 (2H, m), 1.46–1.2 (2H, m); ^{13}C NMR (400 MHz, DMSO d_6) δ173.2, 168.1, 165.0, 157.9, 141.6, 139.7, 138.1, 131.3, 129.1, 122.3, 121.6, 118.8, 117.9, 109.7, 45.2, 42.7, 40.3, 28.7, 28.1, 21.3; HRMS (ESI) calcd. for $C_{21}H_{23}N_4O_3S$ [M+H]$^+$: 411.1485, found: 411.1473.

*4.1.5.15. N-(3-(2-Oxo-2,3-dihydro-1H-thieno[3,4-b][1,4]diazepin-4-yl)phenyl)pyrazine-2-carboxamide (**9f**).* The title compound was isolated as a yellow solid (99%); ^{1}H NMR (400 MHz, DMSO d_6) δ 10.97 (1H, s), 10.53 (1H, s), 9.33 (1H, d, J = 1.5 Hz), 8.95 (1H, d, J = 2.5 Hz), 8.84 (1H, dd, J = 2.5, 1.5 Hz), 8.63 (1H, s), 8.07 (1H, dd, J = 8.1, 1.3 Hz), 7.80 (1H, d, J = 8.5 Hz), 7.57–0.50 (2H, m), 7.03 (1H, d, J = 3.7 Hz), 3.62 (2H, s); ^{13}C NMR (100 MHz, DMSO d_6) δ165.5, 162.4, 158.3, 148.3, 145.4, 144.6, 143.8, 142.1, 139.1, 138.6, 131.8, 129.6, 123.8, 123.4, 120.1, 119.4, 110.2; HRMS (ESI) calcd. for $C_{18}H_{14}N_5O_2S$ $[M+H]^+$: 364.0863, found: 364.0863.

*4.1.5.16. 3-(4-Methylpiperazin-1-yl)-N-(3-(2-oxo-2,3-dihydro-1H-thieno[3,4-b][1,4]diazepin-4-yl)phenyl)-5-(trifluoromethyl) benzamide (**9g**).* The title compound was isolated as a brown solid (63%); ^{1}H NMR (400 MHz, DMSO d_6) δ 10.55 (s, 1H), 10.53 (s, 1H), 8.40 (t, J = 3.6 Hz, 1H), 8.00 (d, d, J = 1.2, 1.2 Hz, 1H), 7.73 (d, J = 2.4 Hz, 2H), 7.65 (s, 1H), 7.54–7.50 (t, s, J = 7.6 Hz, 2H), 7.38 (s, 1H), 7.01 (d, J = 4 Hz, 1H), 3.61 (s, 2H), 2.55–2.45 (m, 8H), 2.23 (s, 3H); ^{13}C NMR (100 MHz, DMSO d_6) δ165.0, 164.6, 157.9, 151.3, 141.6, 139.3, 138.1, 136.4, 133.9, 131.4, 130.8, 130.3, 130.0, 129.1, 125.5, 123.2, 123.1, 119.4, 118.8, 117.4, 113.6, 109.7, 54.2, 47.3, 45.5; HRMS (ESI) calcd. for $C_{26}H_{25}F_3N_5O_2S$ $[M+H]^+$: 528.1676, found: 528.1685; IR (ATR) ν max/cm^{-1}: 2805, 1666, 1545, 1113, 998, 787.

*4.1.5.17. 2-(1H-Indol-3-yl)-N-(3-(2-oxo-2,3-dihydro-1H-thieno[3,4-b][1,4]diazepin-4-yl)phenyl)acetamide (**9h**).* The title compound was isolated as a yellow solid (93%); ^{1}H NMR (400 MHz, DMSO d_6) δ 10.93 (1H, s), 10.51 (1H, s), 10.33 (1H, s), 8.25 (1H, t, J = 1.8 Hz), 7.88–0.82 (1H, m), 7.63 (1H, dd, J = 16.6, 8.1 Hz), 7.52 (1H, d, J = 3.7 Hz), 7.43 (1H, t, J = 8.0 Hz), 7.35 (1H, dt, J = 8.1, 0.9 Hz), 7.27 (1H, d, J = 2.3 Hz), 7.07 (1H,ddd,J = 8.1, 7.0, 1.2 Hz), 7.01 (1H, dd, J = 5.1, 2.4 Hz), 6.99–0.96 (1H, m), 3.75 (2H,s), 3.56 (2H,s); ^{13}C NMR (100 MHz, DMSO d_6) δ 170.5, 165.4, 158.4, 142.1, 140.3, 138.6, 136.6, 131.8, 129.6, 127.7, 124.4, 122.7, 121.9, 121.5, 119.3, 119.1, 118.9, 118.2, 111.9, 110.1, 108.9; HRMS (ESI) calcd. for $C_{23}H_{19}N_4O_2S$ $[M+H]^+$: 415.1223, found: 415.1224.

*4.1.5.18. 2-(Biphenyl-4-yl)-N-(3-(2-oxo-2,3-dihydro-1H-thieno[3,4-b][1,4]diazepin-4-yl)phenyl)acetamide (**9i**).* The title compound was isolated as a brown solid (89%); ^{1}H NMR (400 MHz, DMSO d_6) δ 10.52 (1H, s), 10.44 (1H, s), 8.26 (1H, t, J = 1.8 Hz), 7.85 (1H, dd, J = 8.1, 1.2 Hz), 7.66 (1H, d, J = 1.4 Hz), 7.64 (2H,s), 7.62 (1H, d, J = 1.8 Hz), 7.52 (1H, d, J = 3.7 Hz), 7.47 (1H, d, J = 1.7 Hz), 7.46 (2H, d, J = 1.3 Hz), 7.43 (2H, d, J = 2.0 Hz), 7.36–7.38 (1H, m), 7.35 (1H, d, J = 1.8 Hz), 7.02 (1H, d, J = 3.7 Hz), 3.71 (2H, d, J = 4.4 Hz), 3.57 (2H, s); ^{13}C NMR (100 MHz, DMSO d_6) δ169.4, 165.0, 157.9, 141.6, 140.00, 139.6, 138.6, 138.2, 135.2, 131.4, 129.8, 129.2, 128.9, 127.3, 126.7, 126.6, 122.4, 121.5, 118.8, 117.8, 109.7, 43.0, 38.3; HRMS (ESI) calcd. for $C_{27}H_{22}N_3O_2S$ $[M+H]^+$: 452.1427, found: 452.1439.

*4.1.5.19. 2-(2-Fluorophenyl)-N-(3-(2-oxo-2,3-dihydro-1H-thieno[3,4-b][1,4]diazepin-4-yl)phenyl)acetamide (**9j**).* The title compound was isolated as a brown solid (79%); ^{1}H NMR (400 MHz, DMSO d_6) δ 10.52 (1H, s), 10.45 (1H, s), 8.29–8.26 (1H, m), 7.81 (1H, ddd, J = 7.8, 1.8, 0.7 Hz), 7.68 (1H, ddd, J = 7.9, 1.8, 1.1 Hz), 7.52 (1H, d, J = 3.7 Hz), 7.46 (1H, d, J = 8.0 Hz), 7.41–7.39 (1H, m), 7.32–7.29 (1H, m), 7.19–7.17 (2H, m), 7.02 (1H, d, J = 3.7 Hz), 3.76 (2H, s), 3.57 (2H, s); ^{13}C NMR (100 MHz, DMSO d_6) δ 168.4, 168.1, 165.0, 159.5, 157.9, 141.6, 139.6, 132.1, 131.9, 131.4, 128.9, 128.5, 124.3, 124.1, 118.8, 115.2, 115.0, 114.8, 109.7, 38.3, 36.4; HRMS (ESI) calcd. for $C_{21}H_{17}FN_3O_2S$ $[M+H]^+$: 394.1020, found: 394.1028.

*4.1.5.20. N-(3-(2-Oxo-2,3-dihydro-1H-thieno[3,4-b][1,4]diazepin-4-yl)phenyl)quinoline-2-carboxamide (**9k**).* The title compound was isolated as a brown solid (66%); ^{1}H NMR (400 MHz, DMSO d_6) δ 10.98 (1H, s), 10.55 (1H, s), 8.64 (1H, t, J = 8.3 Hz), 8.55 (1H, s), 8.48 (1H, d, J = 8.5 Hz), 8.27 (1H, dd, J = 11.3, 2.8 Hz), 8.14 (1H, dd, J = 15.4, 6.7 Hz), 8.04 (1H, d, J = 8.4 Hz), 7.97–7.91 (1H, m), 7.85–7.74 (1H, m), 7.71–7.65 (1H, m), 7.63 (1H, d, J = 8.5 Hz), 7.60–7.53 (1H, m), 7.04 (1H, d, J = 3.7 Hz), 3.66 (2H, s); ^{13}C NMR (100 MHz, DMSO d_6) δ 170.4, 165.0, 150.0, 149.9, 146.0, 145.9, 138.3, 137.3, 130.7, 130.3, 129.4, 129.0, 128.5, 128.2, 128.0, 127.5, 127.4, 121.2, 120.0, 119.5, 118.8, 42.7; HRMS (ESI) calcd. for $C_{23}H_{17}N_4O_2S$ $[M+H]^+$: 413.1067, found: 413.1087.

*4.1.5.21. N-(3-(2-Oxo-2,3-dihydro-1H-thieno[3,4-b][1,4]diazepin-4-yl)phenyl)quinoline-3-carboxamide (**9l**).* The title compound was isolated as a yellow solid (88%); ^{1}H NMR (400 MHz, DMSO d_6) δ 10.85 (1H, s), 10.56 (1H, s), 9.39 (1H, dd, J = 6.2, 2.2 Hz), 9.02 (1H, d, J = 2.0 Hz), 8.87 (1H, d, J = 2.2 Hz), 8.48 (1H, t, J = 2.0 Hz), 8.43 (1H, d, J = 2.1 Hz), 8.19–8.05 (1H, m), 7.96–7.89 (1H, m), 7.84 (1H, ddd, J = 8.5, 6.9, 1.4 Hz), 7.77–7.72 (1H, m), 7.71–7.66 (1H, m), 7.57–7.55 (1H, m), 7.04 (1H, d, J = 3.7 Hz), 3.63 (2H, s); ^{13}C NMR (100 MHz, DMSO d_6) δ 164.6, 162.3, 149.0, 148.1, 147.3, 141.8, 134.6, 133.4, 131.6, 130.5, 130.2, 129.2, 128.8, 128.8, 128.6, 127.6, 127.3, 126.7, 126.4, 119.0, 109.8, 38.2; HRMS (ESI) calcd. for $C_{23}H_{17}N_4O_2S$ $[M+H]^+$: 413.1067, found: 413.1091.

*4.1.5.22. (R)-6-Hydroxy-2,5,7,8-tetramethyl-N-(3-(2-oxo-2,3-dihydro-1H-thieno[3,4-b][1,4]diazepin-4-yl)phenyl)chroman-2-carboxamide (**9m**).* The title compound was isolated as a brown solid (90%); ^{1}H NMR (400 MHz, DMSO d_6) δ 10.51 (1H, s), 9.50 (1H, s), 8.31 (1H, t, J = 1.9 Hz), 7.77 (1H, ddd, J = 8.2, 2.0, 0.8 Hz), 7.69 (1H, ddd, J = 7.8, 1.6, 1.0 Hz), 7.56–7.50 (3H, m), 7.43 (1H, t, J = 8.0 Hz), 7.03 (1H, dd, J = 9.0, 3.5 Hz), 3.57 (2H, s), 2.19 (3H, d, J = 3.0 Hz), 2.09–2.04 (7H, m), 1.54–1.49 (5H, m); ^{13}C NMR (100 MHz, DMSO d_6) δ 172.5, 165.0, 158.0, 146.1, 144.0, 142.0, 138.8, 138.1, 131.4, 129.0, 122.8, 122.6, 121.5, 120.4, 119.0, 118.9, 117.1, 109.7, 77.5, 38.3, 29.4, 24.7, 23.7, 20.8, 12.8, 12.2, 11.8; HRMS (ESI) calcd. for $C_{27}H_{28}N_3O_4S$ $[M+H]^+$: 490.1795, found: 490.1814.

*4.1.6. General syntheses of N-(3 or 4-(2-oxo-2,3-dihydro-1H-thieno[3,4-b][1,4]diazepin-4-yl)phenyl)urea (**10a**–**10d**), (**11a**–**11d**)*

Aminophenyl-1*H*-thieno[3,4-*b*][1,4]diazepin-2(3*H*)-one (**7a**–**7b**) (25.7 mg, 0.1 mmol) and substituted isocyanate (0.12 mmol) in THF were stirred at room temperature overnight. The reaction mixture was diluted with ethyl acetate and washed with brine. The organic layer was dried over Na_2SO_4. The concentrated crude product was purified by flash column chromatography with hexane/ethyl acetate = (1:1) as the eluent to give the indicated product.

*4.1.6.1. 1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(4-(2-oxo-2,3-dihydro-1H-thieno[3,4-b][1,4]diazepin-4-yl)-phenyl)urea (**10a**).* The title compound was isolated as a yellow solid (93%); ^{1}H NMR (400 MHz, DMSO d_6) δ10.46 (1H, s), 9.25 (1H, s), 9.19 (1H, s), 8.13 (1H, d, J = 2.0 Hz), 7.98 (2H, d, J = 8.8 Hz), 7.68–7.60 (4H, m), 7.47 (1H, d, J = 3.6 Hz), 7.00 (1H, d, J = 3.6 Hz), 3.57 (2H, s); ^{13}C NMR (100 MHz, DMSO d_6) δ165.0, 157.5, 152.2, 141.9, 139.1, 132.0, 131.4, 131.1, 128.5, 123.3, 122.6, 118.1, 118.0, 109.5; HRMS (ESI) calcd. for $C_{21}H_{15}ClF_3N_4O_2S$ $[M+H]^+$: 479.0551, found: 479.0538.

*4.1.6.2. 1-(4-Chlorophenyl)-3-(4-(2-oxo-2,3-dihydro-1H-thieno[3,4-b][1,4]diazepin-4-yl)phenyl)urea (**10b**).* The title compound was isolated as a yellow solid (51%); ^{1}H NMR (400 MHz, DMSO d_6) δ10.45 (1H, s), 9.05 (1H, s), 8.91 (1H, s), 7.97 (2H, d, J = 8.8 Hz), 7.59 (2H, d, J = 8.8 Hz), 7.51–7.49 (2H, m), 7.46 (1H, d, J = 3.6 Hz), 7.35–7.33 (2H, m), 7.00 (1H, d, J = 3.6 Hz), 3.57 (2H, s); ^{13}C NMR (100 MHz, DMSO d_6) δ165.1, 157.5, 152.2, 142.3, 142.0, 138.4, 131.4, 130.8, 128.7, 128.5, 125.6, 119.9, 118.0, 117.7, 109.5; HRMS (ESI) calcd. for $C_{20}H_{16}ClN_4O_2S$ $[M+H]^+$: 411.0677, found: 411.0660.

*4.1.6.3. 1-(3,4-Dichlorophenyl)-3-(4-(2-oxo-2,3-dihydro-1H-thieno [3,4-b][1,4]diazepin-4-yl)phenyl)urea (**10c**).* The title compound was isolated as a yellow solid (32%); ^{1}H NMR (400 MHz, DMSO d_6) δ10.45 (1H, s), 9.16 (1H, s), 9.09 (1H, s), 7.98 (2H, d, J = 8.8 Hz), 7.90 (1H, d, J = 2.8 Hz), 7.60 (2H, d, J = 8.8 Hz), 7.53 (1H, d, J = 8.8 Hz), 7.47 (1H, d, J = 4.0 Hz), 7.35 (1H, dd, J = 8.8, 2.4 Hz), 7.00 (1H, d, J = 3.6 Hz), 3.57 (2H, s); ^{13}C NMR (100 MHz, DMSO d_6) δ 165.0, 157.5, 152.1, 142.0, 141.9, 139.7, 131.4, 131.1, 130.6, 128.5, 123.4, 122.6, 119.5, 118.5, 118.1, 117.9, 109.5; HRMS (ESI) calcd. for $C_{20}H_{15}Cl_2N_4O_2S$ [M+H]$^+$: 445.0287, found: 446.0302.

*4.1.6.4. 1-(4-((4-Ethylpiperazin-1-yl)methyl)-3-(trifluoro methyl)phenyl)-3-(4-(2-oxo-2,3-dihydro-1H-thieno-[3,4-b][1,4]diazepin-4-yl) phenyl)urea (**10d**).* The title compound was isolated as a yellow solid (23%); ^{1}H NMR (400 MHz, DMSO d_6) δ10.45 (1H, s), 9.20 (2H, s), 7.99–7.97 (3H, m), 7.63–7.59 (4H, m), 7.47 (1H, d, J = 3.6 Hz), 7.00 (1H, d, J = 3.6 Hz), 3.57 (2H, s), 3.53 (2H, s), 2.39–2.28 (10*H*, m), 0.98 (3H, t, J = 7.2 Hz); ^{13}C NMR (100 MHz, DMSO d_6) δ 165.1, 157.7, 152.4, 142.2, 141.9, 138.7, 138.1, 137.4, 131.4, 131.3, 130.9, 130.2, 128.5, 121.7, 118.0, 117.8, 115.2, 109.5, 57.4, 52.7, 52.3, 51.5, 11.9; HRMS (ESI) calcd. for $C_{28}H_{30}F_3N_6O_2S$ [M+H]$^+$: 571.2098, found: 571.2081; IR (ATR) ν max/cm^{-1}: 3104, 1691, 1586, 1525, 1475, 1300, 1180, 1131.

*4.1.6.5. 1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(3-(2-oxo-2,3-dihydro-1H-thieno[3,4-b][1,4]diazepin-4-yl)-phenyl)urea (**11a**).* The title compound was isolated as a brown solid (87%); ^{1}H NMR (400 MHz, DMSO d_6) δ10.51 (1H, s), 9.17 (1H, s), 9.12 (1H, s), 8.14 (1H, t, J = 1.8 Hz), 8.12 (1H, d, J = 2.4 Hz), 7.70–7.61 (4H, s), 7.54 (1H, d, J = 3.6 Hz), 7.44 (1H, t, J = 7.8 Hz), 7.03 (1H, d, J = 3.6 Hz), 3.58 (2H, s); ^{13}C NMR (100 MHz, DMSO d_6) δ165.0, 158.0, 152.5, 141.6, 139.7, 139.3, 138.3, 132.0, 131.4, 129.2, 126.6, 124.2, 123.2, 122.4, 121.5, 121.1, 118.8, 117.3, 116.9, 109.7; HRMS (ESI) calcd. for $C_{21}H_{15}ClF_3N_4O_2S$ [M+H]$^+$: 479.0551, found: 479.0530.

*4.1.6.6. 1-(4-Chlorophenyl)-3-(3-(2-oxo-2,3-dihydro-1H-thieno[3,4-b][1,4]diazepin-4-yl)phenyl)urea (**11b**).* The title compound was isolated as a brown solid (82%); ^{1}H NMR (400 MHz, DMSO d_6) δ10.51 (1H, s), 8.96 (1H, s), 8.81 (1H, s), 8.13 (1H, t, J = 1.8 Hz), 7.64–7.61 (2H, m), 7.53 (1H, d, J = 3.6 Hz), 7.52–7.49 (2H, m), 7.43 (1H, t, J = 8.0 Hz), 7.35–7.31 (2H, m), 7.02 (1H, d, J = 4.0), 3.58 (2H, s); ^{13}C NMR (100 MHz, DMSO d_6) δ 165.0, 158.0, 152.4, 141.6, 139.9, 138.6, 138.2, 131.3, 129.2, 128.6, 125.4, 121.1, 120.7, 119.8, 118.7, 116.9, 109.6; HRMS (ESI) calcd. for $C_{20}H_{16}ClN_4O_2S$ [M+H]$^+$: 411.0677, found: 411.0654.

*4.1.6.7. 1-(3,4-Dichlorophenyl)-3-(3-(2-oxo-2,3-dihydro-1H-thieno [3,4-b][1,4]diazepin-4-yl)phenyl)urea (**11c**).* The title compound was isolated as a brown solid (35%); ^{1}H NMR (400 MHz, DMSO d_6) δ10.53 (1H, s), 9.08 (1H, s), 9.01 (1H, s), 8.15 (1H, t, J = 2.0 Hz), 7.90 (1H, t, J = 2.4 Hz), 7.64–7.61 (2H, m), 7.55–7.52 (2H, s), 7.44 (1H, t, J = 8.0 Hz), 7.35 (1H, dd, J = 8.0, 2.4 Hz), 7.03 (1H, d, J = 3.6 Hz), 3.58 (2H, s); ^{13}C NMR (100 MHz, DMSO d_6) δ 165.0, 158.0, 152.4, 141.7, 139.9, 139.7, 138.3, 131.4, 131.1, 130.6, 129.2, 123.2, 121.4, 121.0, 119.4, 118.8, 118.5, 117.2, 109.7; HRMS (ESI) calcd. for $C_{20}H_{15}Cl_2N_4O_2S$ [M+H]$^+$: 445.0287, found: 446.0292.

*4.1.6.8. 1-(4-((4-Ethylpiperazin-1-yl)methyl)-3-(trifluoro methyl)phenyl)-3-(3-(2-oxo-2,3-dihydro-1H-thieno-[3,4-b][1,4]diazepin-4-yl) phenyl)urea (**11d**).* The title compound was isolated as a brown solid (28%); ^{1}H NMR (400 MHz, DMSO d_6) δ 10.54 (1H, s), 9.19 (1H, s), 9.18 (1H, s), 8.15 (1H, t, J = 1.6 Hz), 7.93 (1H, d, J = 2.0 Hz), 7.65–7.58 (4H, m), 7.54 (1H, d, J = 3.6 Hz), 7.43 (1H, t, J = 7.8 Hz), 7.03 (1H, d, J = 3.6 Hz), 3.58 (2H, s), 3.52 (2H, s), 2.38–2.28 (10H, m), 0.98 (3H, t, J = 7.2 Hz); ^{13}C NMR (100 MHz, DMSO d_6) δ165.0, 158.1, 152.6, 147.5, 141.6, 139.8, 138.3, 131.4, 130.2, 129.2, 122.3, 121.8, 121.3, 121.0, 118.8, 117.1, 109.7, 107.2, 57.5, 52.8, 52.4, 51.6, 12.0; HRMS (ESI) calcd. for $C_{28}H_{30}F_3N_6O_2S$ [M+H]$^+$: 571.2098, found: 571.2061.

4.2. Antiproliferative activity

A375P cells were purchased from American Type Culture Collection (ATCC, Rockville, MD, US) and maintained in DMEM medium (Welgene, Daegu, Korea) supplemented with 10% FBS (Welgene) and 1% penicillin/streptomycin (Welgene) in a humidified atmosphere with 5% CO_2 at 37 °C. A375P cells were taken from culture substrate with 0.05% trypsin–0.02% EDTA and plated at a density of 5×10^3 cells/well in 96 well plates and then incubated at 37 °C for 24 h in a humidified atmosphere with 5% CO_2 prior to treatment of various concentration (3-fold serial dilution, 12 points) of test compounds. The A357P cell viability was assessed by the conventional 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reduction assay. MTT assays were carried out with CellTiter 96® (Promega) according to the manufacturer's instructions. The absorbance at 590 nm was recorded using EnVision 2103 (Perkin Elmer; Boston, MA, US). The IC_{50} was calculated using GraphPad Prism 4.0 software.

U937 cells were purchased from American Type Culture Collection (ATCC, Rockville, MD, US) and maintained in RPMI 1640 medium (Welgene, Daegu, Korea) supplemented with 10% FBS (Welgene), 1% penicillin/streptomycin (Welgene) and 25 mM HEPES (Welgene) in a humidified atmosphere with 5% CO_2 at 37 °C. U937 cells were taken from culture substrate and plated at a density of 5×10^3 cells/well in 96 well plates and then incubated at 37 °C for 24 h in a humidified atmosphere with 5% CO_2 prior to treatment of various concentration (3-fold serial dilution, 12 points) of test compounds. The U937 cell viability was assessed by the conventional 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reduction assay. MTT assays were carried out with Thiazolyl Blue Tetrazolium Bromide (SIGMA) according to the manufacturer's instructions. The absorbance at 570 nm was recorded using Multiskan EX (Thermo; Waltham, MA, US). The IC50 was calculated using GraphPad Prism 4.0 software

4.3. Docking simulations

Molecular docking of compound **10d** into the 3D X-ray structure of FMS (PDB code: 3LCO) was carried out using Glide (Schrodinger software package Version 14.1). The 3D X-ray protein structure of FMS as a complex with ligand was obtained from the PDB and prepared using Protein Preparation Wizard of the Schrodinger Maestro program. The structures of new designed inhibitors were drawn using Chemdraw, and their 3D conformations were generated using the Schrodinger LigPrep program with the OPLS 2005 force field. When making an optimal grid file, a grid box was manually adjusted from a cube into a cuboid, constraints (hydrogen bonding and hydrophobic cube) were added, and unwanted pockets were treated as excluded volume. Molecular docking of compound **10d** into the structure of FMS produced predictive docking pose, (1) SP (standard precision) and XP (extra precision) docking of compound **10d**, (2) revision of the docking poses through substructure energy minimization using Schrodinger Macro Model, and, (3) scoring of revised the docking pose.

4.4. Selected kinase profiling and IC50 measurement

We used Reaction Biology Corp. Kinase HotSpotSM service (www.reactionbiology.com) for screening of **10d**, and IC50 ProfilerExpress for IC_{50} measurement. Their assay protocol was as follows: in a final reaction volume of 25 μL FMS (h) (5–10 mU) was incubated with 25 mM Tris pH 7.5, 0.02 mM EGTA, 0.66 mg/mL

myelin basic protein, 10 mM magnesium acetate and [γ-^{33}P-ATP] (specific activity approx. 500 cpm/pmol, concentration as required). The reaction is initiated by the addition of the Mg-ATP mix. After incubation for 40 min at room temperature, the reaction was stopped by the addition of 5 μL of a 3% phosphoric acid solution. 10 μL of the reaction was then spotted onto a P30 filtermat and washed three times for 5 min in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting.

Acknowledgments

This work was financially supported by the National Research Foundation of South Korea (Grant No. NRF-2016R1D1A1B0 3931847: J.-M. Hah) and (Grant No. NRF-2017R1A2B4006447; J.-M. Hah). We also express our gratitude to Professor Hyehyun Yoo (Hanyang University) for HR-MS analysis and Dr. Jamie Planck (RBC, Pennsylvania, USA) for helpful support in biological evaluation.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmc.2018.02.009.

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