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Structure based design and syntheses of amino-1*H*-pyrazole amide derivatives as selective Raf kinase inhibitors in melanoma cells

Mi-hyun Kim [a], Minjung Kim [a], Hana Yu [a], Hwan Kim [b], Kyung Ho Yoo [b], Taebo Sim [b], Jung-Mi Hah [a,*]

[a] Department of Pharmacy, College of Pharmacy, Hanyang University, 1271 Sa 3-Dong, Sangnok-gu, Ansan-si, Gyunggi-do 426-791, South Korea
[b] Life Sciences Research Division, Korea Institute of Science and Technology, PO Box 131, Cheongryang, Seoul 130-650, South Korea

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ABSTRACT

The synthesis of a novel series of *N*-(5-amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl amide derivatives **6a–o**, **7a–s** and their antiproliferative activities against A375P melanoma cell line were described. Most compounds showed competitive antiproliferative activities to sorafenib, the reference standard. Among them, *N*-(5-amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-5-(3-(4-chloro-3-(trifluoromethyl)phenyl) ureido)-2-methylbenzamide **7c** exhibited potent activities (GI_{50} = 0.27 μM). Especially, **7c** was found to be a potent and selective B-Raf V600E and C-Raf inhibitor (IC_{50} = 0.26 μM, IC_{50} = 0.11 μM, respectively), showing a possibility as melanoma therapeutics.

1. Introduction

The 'Ras/Raf/Mek/Erk pathway' is a well-known cell signaling network for cell survival, growth, and proliferation.[1] The Ras proteins are membrane-bound small G-protein, whereas Raf, Mek, and Erk are cytosolic protein kinases that compose a sequential signaling cascade. Out of these complicated cascade, Raf kinase has been the most studied drug target since mutations of the Raf protein were found in approximately 7% of human cancers[2,3] with particularly high frequency in melanoma (50–70%), ovarian (35%), thyroid (30%), and colorectal (10%) cancers.

The discovery of the most frequent V600E (>85%) B-Raf mutations in 50% of melanoma have raised the expectation for targeted therapy.[2] The V600E B-Raf mutations show a 500-fold increase in catalytic activity, providing cancer cells with both proliferation and survival signals.[4] Therefore, B-Raf V600E is a high-interest therapeutic target for the treatment of human cancers.

In our melanoma program, we were intrigued by the well characterized Raf inhibitor sorafenib, the bi-aryl urea compound from Bayer. Sorafenib is a potent inhibitor of preactivated C-Raf, B-Raf and oncogenically activated B-Raf kinases (V600E B-Raf: IC_{50} = 43 nM), as well as it holds a unique binding mode for Raf protein. Crystal structure of V600E B-Raf kinase domains in complex with sorafenib showed that the inhibitor held the activation segment in an inactive conformation[5], namely type II inhibition.

The primary part of pharmacophore was still considered to be hinge binder in designing a new type II inhibitor for B-Raf V600E, and we recently reported the imidazolopyrazole bicyclic ring[6] as a novel scaffold for Raf inhibitor. Although the hinge hydrogen bonding in B-Raf active site is known less critical compared with that in other kinase inhibitors, we concluded that the imidazolopyrazole bicyclic ring was not the best scaffold based on SAR study. Furthermore, the docking analysis showed that the length of the imidazolopyrazole bicycle was short for an effective hydrogen bonding with Glu_{500} and Asp_{593} and hydrophobic interaction with secondary pocket.

In an attempt to find a better hinge binding scaffold with optimal length for effective hydrogen bonding and hydrophobic interactions (Fig. 1), we tried to crack the imidazole ring in pyrazoleimidazole scaffold[6], and relieve the strain; therefore, we came up with novel amino-1*H*-pyrazole amide derivatives. In docking experiment (Fig. 2), this new scaffold showed not only proper hinge binding, but also plausible hydrogen bonding and effective hydrophobic interactions.

The structure of this series comprises *N*-(5-amino-1-alkyl-1*H*-pyrazol-4-yl amide part, the middle phenyl ring moiety and aromatic tail part connected by amide or urea linkage. Specifically, we modified the structures by introducing two different direction of connectivity (4-, 5-nitro) in middle phenyl group, changing the spacer (amide or urea) and various aromatic tails to understand the relative interaction of hydrophobic tail groups.

* Corresponding author. Tel.: +82 31 400 5803; fax: +82 31 400 5958.
E-mail address: jhah@hanyang.ac.kr (J.-M. Hah).

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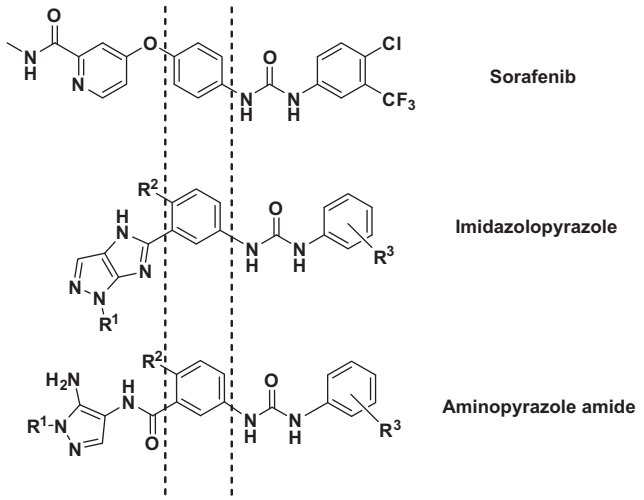


Figure 1. Anatomy of sorafenib and imidazolopyrazole derivatives, amino-1*H*-pyrazole amide derivatives.

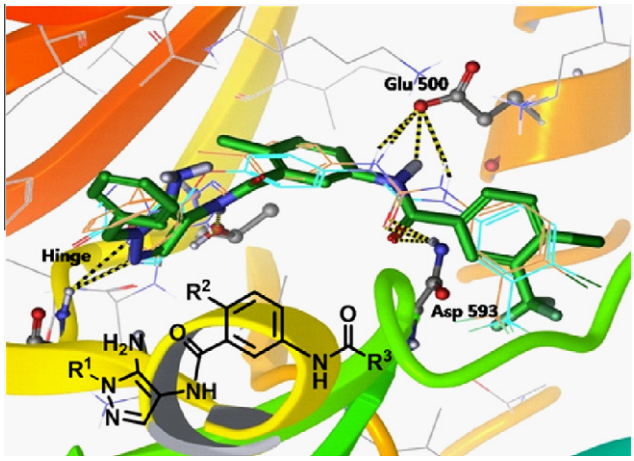


Figure 2. Docking structures of designed amino-1*H*-pyrazole amide scaffold (bold, green) overlayed with sorafenib (thin, cyan) and imidazolopyrazole derivatives (thin, orange) in B-Raf V600E.

2. Chemistry

The general synthesis of amino-1*H*-pyrazole amide derivatives is shown in Scheme 1. The 1-(4-methoxybenzyl)-4-nitroso-1*H*-pyrazol-5-amine (**2**) was made with *p*-methoxybenzyl hydrazine and 3-methoxyacrylonitrile in one pot process as reported in reference.[7] Then, the nitroso group was reduced to give diamine (**3**) using $SnCl_2$.[8] The pyrazolediamine **3** was then reacted with 2-methyl 5- (or 4-) nitro benzoic acid to provide single amide product **4**. The selective amidation at 4-amino group was considered to be due to the steric effect of PMB and stronger nucleophilicity by resonance. Then the nitro group in **4** was reduced to amino group and linked with various aromatic acids under EDCI/HOBt conditions to give amide (**6a**–**6n**) or directly aromatic isocynate to give urea (**7a**–**7p**) analogues.

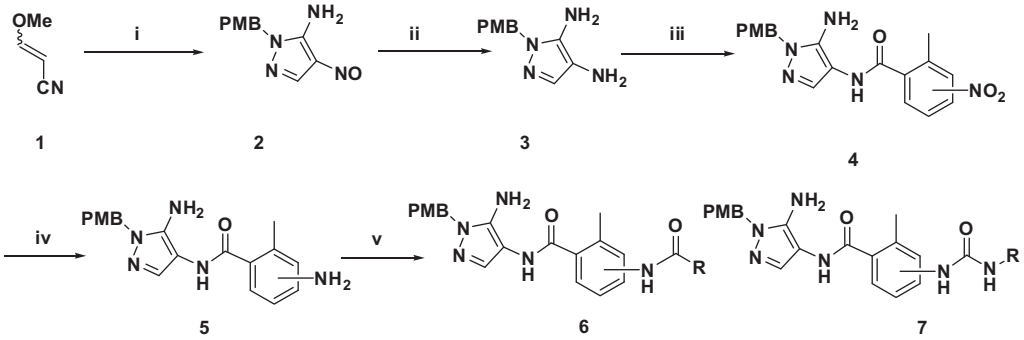
3. Results and discussion

The synthesized amino-1*H*-pyrazole amide derivatives **6a**–**6o** were evaluated for antiproliferative activities against human melanoma cell line, and Table 1 shows the antiproliferative activities[9] of amino-1*H*-pyrazole amide derivatives linked with various aromatic tail groups by amide bond. The activities are compared with sorafenib as a reference (GI_{50} values) against A375P[10] human melanoma cell line and also normal fibroblast cell line HS27. In general, the amide derivatives **6** showed poor antiproliferative activities against both cell lines, while 5-amino substituted **6a**, **6b**, **6g** obtained medieval potency against A375P. With the same hydrophobic tail group, the substitution on 5-position seems better in hydrophobic interaction (**6g** > **6h**).

Continuously, we determined antiproliferative activity of amino-1*H*-pyrazole ureido amide derivatives and **7a**–**7s** with various aromatic tail groups by urea linkage (Table 2). Interestingly, when we replaced the PMB-group of 1-*N* in pyrazole moiety with benzyl group, the antiproliferative potency was dropped dramatically (**8c** vs **7c**), and it was surprising since we found very different results in our old SAR data of pyrazoloimidazole scaffold.[6] Therefore, we kept the PMB group in our scaffold and continued variation in substitution of middle phenyl ring and hydrophobic tails. Urea derivatives were more potent than amide derivatives in general, having preference of 2-methyl 5-aminopheurea linked middle phenyl ring (**7c** > **7b**; 7i > **7h**; 7k > **7j**) like in amide series **6**. Also, the mono-aromatic tails (**7b, 7c, 7d, 7h, 7i**) were preferred as a hydrophobic tail rather than bulky bi-aryl tails.

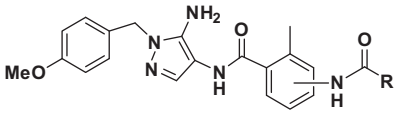
We further investigated enzymatic activities (B-Raf V600E, C-Raf) of several selected compounds. Indeed, we found amide derivatives **6a**, **6b** were only C-Raf inhibitors, but urea **7b** is inhibitors of both Raf kinases (Table 3).

Considering that C-Raf is also associated significantly with disease progression and cell proliferation in a subset of melanoma,[11–13] the finding of dual Raf-kinase inhibitor **7c** was valuable, and it was selected as a lead compound for further study. Shown in Table 4, the representative compound **7c** were screened

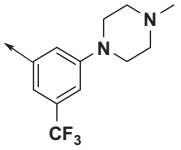
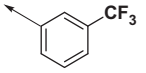
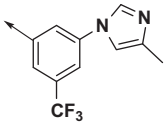
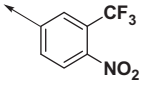
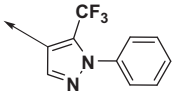
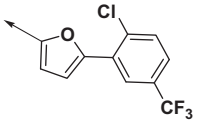
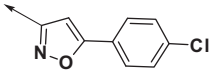
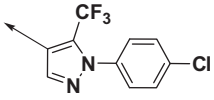
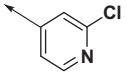
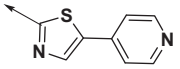
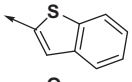
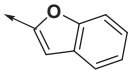


Scheme 1. Reagents and reaction conditions: (i) $PMBHNNH_2$, $NaNO_2$, EtOH, 50 °C; (ii) $SnCl_2$ $2H_2O$, EtOH, 80 °C; (iii) 2-CH_3-5-NO_2-C_6H_3-CO_2H, HOBt, EDCI, TEA, DMF, 80 °C, 4 h; (iv) HCl, $SnCl_2{\cdot}2H_2O$, EtOH, 80 °C, 1.5 h; (v) RCO_2H, EDCI, TEA, HOBt, DMF or RNCO, THF.

Table 1
Antiproliferative activity of amino-1*H*-pyrazole amide derivatives amide derivatives **6a**–**6n**



6

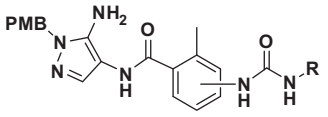
Compd	Substitution	R	A375P (GI_{50}, μM)	HS27 (GI_{50}, μM)
6a	5-		4.51	>30
6b	5-		5.53	>30
6c	5-		11.3	>30
6d	5-		27.9	>30
6e	4-		NA	>30
6f	5-		NA	>30
6g	5-		1.39	>30
6h	4-		24.9	>30
6i	5-		>30	>30
6j	4-		NA	>30
6k	4-		NA	>30
6l	5-		NA	>30
6m	5-		NA	>30
6n	4-		>30	>30
6o	4-		>30	>30
Sorafenib		5.58		7.85

on selected 30 different kinases panel at a single dose concentration of 10 μM and it was revealed that the compound has an excellent selectivity profile. While this compound has completely inhibited C-Raf and more than 97% at this concentration, the inhibition exerted in most other kinases tested activity was below 20%.

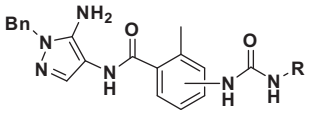
4. Conclusions

A series of amino-1*H*-pyrazole amide derivatives **6a**–**6o** and **7a**–**7s**, **8c** based on the structural features of sorafenib has been synthesized and evaluated their antiproliferative activities against

Table 2
Antiproliferative activity of amino-1*H*-pyrazole amide derivatives with urea linkage **7a**–**7s**



7



8

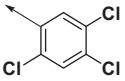
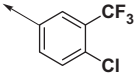
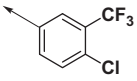
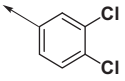
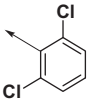
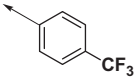
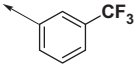
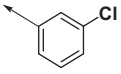
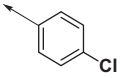
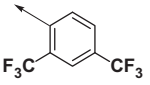
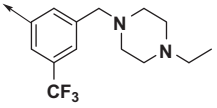
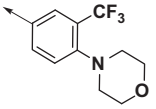
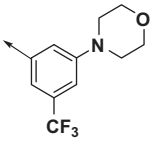
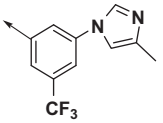
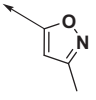
Compd	Substitution	R	A375P (GI_{50}, μM)	HS27 (GI_{50}, μM)
7a	5-		11.4	>30
7b	4-		33.2	>30
7c	5-		0.27	>30
8c	5-		15.9	>30
7d	4-		4.12	>30
7e	5-		NA	>30
7f	5-		>30	>30
7g	4-		>30	>30
7h	4-		36.5	>30
7i	5-		0.21	>30
7j	4-		NA	>30
7k	5-		8.73	>30
7l	5-		NA	>30
7m	5-		NA	>30
7n	4-		98.3	>30
7o	4-		>100	>30
7p	4-		51.5	>30
7q	4-		5.71	>30
7r	5-		NA	>30

Table 2 (*continued*)

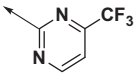
Compd	Substitution	R	A375P (GI_{50}, μM)	HS27 (GI_{50}, μM)
7s	4-		NA	>30
Sorafenib		5.58		7.85

Table 3
Enzymatic activities of selected compounds

	IC_{50} (nM) B-Raf V600E	IC_{50} (nM) C-Raf
6a	NA	240.3
6b	NA	22.41
7b	NA	8319
7c	264	107.1
GW5074	3.86	2.87

Table 4
Percentages of enzymatic inhibitions by compound **7c** (10 μM) on selected protein kinases

Kinase	% Inhibition	Staurosporine IC_{50} (nM)
AKT1 (dPH, S473D)	3.7	4.72
ALK	7.7	2.71
Aurora A	0	<1.0
b-RAF (V599E)	97	3.32[a]
c-MET	13	193.5
c-Src	16	4.79
CDK1/cyclin B	0	3.20
CDK2/cyclinE	0	2.13
EGFR/ERBB1	16	101.40
ERK2/MAPK1/P42MAPK	0	11380.0
FAK/PTK2	1.5	4.13
FGFR3	0.1	21.50
FLT3	13	<1.0
GSK3β	1.4	2.73
IGF-1R	0.7	51.62
JAK3	0	<1.0
JNK1a1	7.1	1592
JNK3/MAPK10	13	>20000
KDR/VEGFR2	0	9.42
MEK1	0	18.19
mTOR/FRAP1	1.2	3018[b]
p70S6K	0	<1.0
PKA	0	<1.0
PLK1	6.3	167.40
RAF1	100	2.87[a]
RON/MST1R	0	277.10
ROS/ROS1	0	<1.0
SYK	0	<1.0
TRKB/NTRK2	6.6	<1.0

[a] Data of GW5074.[14]
[b] Data of LY294002.[15]

A375P human cell line, together with normal cell lines in control. Several compounds in this scaffold showed potent antiproliferative activities, and furthermore, one of the best compound **7c** has been confirmed as a potent and selective Raf kinases inhibitor (IC_{50} = 264 nM on V600E B-Raf, 107 nM on C-Raf), suggesting that the amino-1*H*-pyrazole amide derivatives could serve as a promising scaffold for new therapeutics of melanoma, having absolutely impressive kinase profiling.

5. Materials and methods

5.1. Chemistry general

All chemicals (reagent grade) used 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 gel 60 GF254, E. Merck, Germany) and visualized in ultraviolet (UV) light (254 nm). ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian unity Plus 300 MHz and Brucker Avance 400 MHz spectrometer at 25 °C, using tetramethylsilane (TMS) as the internal standard. High-resolution MS (HR/MS) experiments were conducted with a Finnigan LTQ Orbitrap mass spectrometry (Thermo Fisher Scientific Inc., MA, USA) operated in positive-ion electrospray mode.

5.1.1. Synthesis of 1-(4-methoxybenzyl)-4-nitroso-1*H*-pyrazol-5-amine (2)

In round bottom flask, 35% HCl (6 mL, 71.6 mmol) was stirred at −15 °C, and a mixture of 3-methoxyacrylonitrile **1** (1.3 g, 15.9 mmol) and 30% $NaNO_2$ solution (3.6 g, 15.9 mmol) in MeOH (6 mL) was added dropwise at −15 to −5 °C. Then the mixture was stirred at same temperature for 1 h. To remove excess amount of nitrosyl chloride, the reaction mixture was flushed with N_2 for 5 min. Then a mixture of 4-methoxybenzylhydrazine hydrochloride (3 g, 15.9 mmol), water (4 mL) and MeOH (4 mL) was added, stirred at 50 °C for 2 h. After cooling the reaction mixture to 10 °C, more water (4 mL) was added, and neutralized with NH_4OH. The reaction mixture was cooled to 5 °C, stirred for 30 min, filtered off and washed with cold water and MeOH to give desired compound **2** (3 g, 81%) as red solid. ^{1}H NMR (300 MHz, $CDCl_3$) 8.58 (1H, s), 7.13 (2H, d, *J* = 16.75 Hz), 6.93 (2H, d, *J* = 13.36 Hz), 6.23 (2H, br s), 5.06 (2H, s), 3.80 (3H, s).

5.1.2. Synthesis of 4,5-diamino-1-(4-methoxy-benzyl)pyrazole (3)

A suspension of **2** (100 mg, 0.43 mmol) in EtOH (0.86 mL) was acidified with 35% HCl (0.22 mL, 2.15 mmol), then it was stirred at 80 °C. A solution of $SnCl_2{\cdot}2H_2O$ (213.4 mg, 0.95 mmol) in EtOH (0.25 mL) was added to dissolved adduct over 15 min. Stirring was continued for 30 min and the clear solution was cooled to room temperature, then was poured into ice. The pH was made slightly basic (pH 7–8) by addition of saturated aqueous sodium bicarbonate before being extracted with organic solvent. Because compound **2** is very polar, IPA/$CHCl_3$ = 4:1 solution was used for extraction. The organic phase is thoroughly washed with brine, dried over sodium sulfate. Evaporation of the solvent leaves crude 100 mg of 4,5-diamino-1-(4-methoxy-benzyl)pyrazole **3**, which gives one spot on TLC. Purification was not necessary at this step. (^{1}H NMR (400 MHz, $CDCl_3$) 7.15 (1H, s), 7.11 (2H, d, *J* = 8.55 Hz), 6.85 (2H, d, *J* = 8.64 Hz), 5.11 (2H, s), 3.76 (3H, s).

5.1.3. Synthesis of *N*-(5-amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-2-methyl-5-nitrobenzamide (4)

A solution of **3** compound (348 mg, 1.59 mmol), 2-methyl-5-nitrobenzoic acid (289 mg, 1.59 mmol), HOBt (323 mg, 2.39 mmol), EDCI (397 mg, 2.07 mmol) and TEA (0.26 mL, 1.91 mmol) in DMF (5 mL) was heated at 80 °C for overnight. The reaction mixture diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate. The organic layer dried over Na_2SO_4. Purification of column chromatography with hexane/ethyl acetate = 1:1. Giving

12 compound (520 mg, 85.7%), white solid. ^{1}H NMR (400 MHz, DMSO-d_6) 9.56 (1H, s), 8.16 (1H, s), 7.45 (1H, d, J = 7.43 Hz), 7.346 (1H, s), 7.33 (1H, d, J = 9.748 Hz), 7.31 (2H, d, J = 7.11 Hz), 6.88 (2H, d, J = 17.96 Hz), 4.78 (2H, s), 3.66 (3H, s), 2.26 (3H, s).

5.1.4. Synthesis of 5-amino-*N*-(5-amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-2-methylbenzamide (5)

To a suspension of **12** (1.36 mmol) in EtOH (5 mL) a solution of $SnCl_2{\cdot}2H_2O$ (0.95 mmol) in EtOH (0.25 mL) was added over 15 min. Stirring continued for 30 min then the clear solution was cooled room temperature. The pH is made slightly basic (pH 7–8) by addition of saturated aqueous sodium bicarbonate before being extracted with ethyl acetate. The organic phase is thoroughly washes with brine, dried over sodium sulfate. Evaporation of the solvent leaves crude of 4,5-diamino-1-(4-methoxy-benzyl)pyrazole **13**, which gives one spot on TLC. ^{1}H NMR (400 MHz, DMSO-d_6) 9.75 (1H, s), 7.14 (1H, d, J = 2.5 Hz), 7.45 (1H, d, J = 7.432 Hz), 7.346 (1H, s), 7.33 (1H, d, J = 9.748 Hz), 7.31 (2H, d, J = 7.11 Hz), 6.88 (2H, d, J = 17.96 Hz), 4.78 (2H, s), 3.66 (3H, s), 2.26 (3H, s).

5.1.5. General synthses of *N*-(5-amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-2-methyl-benzamido benzamide (6a–6o)

A solution of **5** compound (10 mg, 0.028 mmol), substituted benzoic acid (0.028 mmol), HOBt (6.8 mg, 0.05 mmol), EDCI (8.05 mg, 0.04 mmol) and TEA (10 μL, 0.07 mmol) in DMF (0.5 mL) was stirred at room temperature for overnight. The reaction mixture was dilluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate. The organic layer dried over Na_2SO_4. Purification of column chromatography with Hexane/ethyl acetate = 1:1 to afford compound **6** as a white solid.

5.1.5.1. *N*-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-2-methyl-5-(3-(4-methylpiperazin-1-yl)-5-(trifluoromethyl)benzamido)benzamide (6a).

^{1}H NMR (400 MHz, DMSO-d_6) 10.30 (1H, s), 9.57 (1H, s), 8.22 (1H, s), 7.92 (2H, m), 7.82 (1H, s), 7.79 (1H, s), 7.74 (1H, s), 7.62 (1H, s), 7.44 (1H, s) 7.37 (1H, s), 7.27 (1H, d, J = 8.51 Hz), 7.15 (2H, d, J = 8.83 Hz), 6.88 (2H, d, J = 8.46 Hz), 5.13 (2H, s), 5.05 (2H, s), 3.72 (3H, s), 2.34 (3H, s); HRMS calcd for $C_{32}H_{34}O_3N_7F_3$ 622.2754. Found 621.2759.

5.1.5.2. *N*-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-2-methyl-5-(3-(trifluoromethyl)benzamido) benzamide (6b).

^{1}H NMR (400 MHz, DMSO-d_6) 9.54 (1H, s), 9.14 (1H, s), 8.92 (1H, s), 8.01 (1H, s), 7.55 (2H, s), 7.52 (1H, s), 7.44 (1H, s) 7.30 (1H, d, J = 8.05 Hz), 7.18 (1H, d, J = 8.06 Hz), 7.15 (2H, d, J = 8.22Hz), 6.88 (2H, d, J = 8.588 Hz), 5.13 (2H, s), 5.05 (2H, s), 3.72 (3H, s), 2.34 (3H, s); HRMS calcd for $C_{27}H_{24}O_3N_5F_3$ 524.1910. Found 524.1902.

5.1.5.3. *N*-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-2-methyl-5-(3-(4-methyl-1*H*-imidazol-1-yl)-5-(trifluoromethyl)-benzamido)benzamide (6c).

^{1}H NMR (400 MHz, DMSO-d_6) 10.33 (1H, s), 9.52 (1H, s), 8.1 (1H, s), 7.90 (1H, s), 7.81 (1H, s), 7.79 (1H, s), 7.77 (1H, s), 7.64 (1H, s), 7.44 (1H, s), 7.27 (1H, d, J = 8.12 Hz), 7.15 (2H, d, J = 8.83 Hz), 6.88 (2H, d, J = 8.50Hz), 5.13 (2H, s), 5.05 (2H, s), 3.72 (3H, s),, 2.34 (3H, s), 2.27 (3H, s) HRMS calcd for $C_{31}H_{28}O_3N_7F_3$ 604.2285. Found 604.2288.

5.1.5.4. *N*-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-2-methyl-5-(4-nitro-3-(trifluoromethyl) benzamido) benzamide (6d).

^{1}H NMR (400 MHz, DMSO-d_6) 10.47 (1H, s), 9.54 (1H, s), 8.32 (2H, m), 8.16 (1H, d, J = 9.01 Hz), 8.01 (1H, s), 7.55 (2H, s), 7.52 (1H, s), 7.44 (1H, s), 7.15 (2H, d, J = 8.22 Hz), 6.88 (2H, d, J = 8.588 Hz), 5.13 (2H, s), 5.05 (2H, s), 3.72 (3H, s), 2.34 (3H, s); HRMS calcd for C_{27} H_{23} O_5 N_6F_3 569.1819. Found 569.1758.

5.1.5.5. *N*-(3-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-ylcarbamoyl)-4-methylphenyl)-1-phenyl-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (6e).

^{1}H NMR (400 MHz, DMSO-d_6) 10.54 (1H, s), 9.55 (1H, s), 8.23 (1H, s), 7.76 (1H, d), 7.43 (2H, d, J = 6.3 Hz), 7.29 (1H, d, J = 8.11 Hz), 7.14–7.05 (4H, m), 6.86 (2H, d, J = 8.57 Hz), 5.14 (2H, s), 5.05 (2H, s), 3.83 (3H, S), 3.72 (3H, d, J = 1.62 Hz), 2.40 (3H, s); HRMS calcd for $C_{30}H_{26}O_3N_7F_3$ 590.2128. Found 590.2123.

5.1.5.6. *N*-(3-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-ylcarbamoyl)-4-methylphenyl)-5-(2-chloro-5-(trifluoromethyl)phenyl)furan-2-carboxamide (6f).

^{1}H NMR (400 MHz, DMSO-d_6) 10.46 (1H, s), 9.48 (1H, s), 8.47 (1H, s), 7.88 (1H, d, J = 8.5 Hz), 7.80 (1H, d, J = 8.5 Hz), 7.70 (1H, d, J = 8.5 Hz), 7.65 (1H, s), 7.54–7.49 (2H, m), 7.42 (1H, s), 7.15 (2H, d, J = 8.5 Hz), 6.88 (2H, d, J = 8.5 Hz), 5.15 (2H, s), 5.05 (2H, s), 3.72 (3H, s), 2.41 (3H, s); HRMS calcd for $C_{31}H_{25}O_4$ N_5ClF_3 624.1626. Found 624.1624.

5.1.5.7. *N*-(3-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-ylcarbamoyl)-4-methylphenyl)-5-(4-chlorophenyl)isoxazole-3-carboxamide (6g).

^{1}H NMR (400 MHz, DMSO-d_6) 10.80 (1H, s), 9.58 (1H, s), 8.09 (2H, d, J = 7.98 Hz), 7.93 (1H, d, J = 9.93 Hz) 7.71 (2H, s) 7.66 (2H, d, J = 8.58 Hz), 7.48 (2H, d, J = 8.11 Hz), 7.29 (1H, d, J = 8.11 Hz), 7.15 (2H, d, J = 8.57 Hz), 6.88 (2H, d, J = 8.57 Hz), 5.14 (2H, s), 5.05 (2H, s), 3.72 (3H, d, J = 1.62 Hz), 2.40 (3H, s); HRMS calcd for $C_{29}H_{25}O_4N_6Cl$ 557.1705. Found 557.1696.

5.1.5.8. *N*-(4-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-ylcarbamoyl)-4-methylphenyl)-5-(4-chlorophenyl)isoxazole-3-carboxamide (6h).

^{1}H NMR (400 MHz, DMSO-d_6) 10.86 (1H, s), 9.50 (1H, s), 8.01 (2H, d, J = 8.28 Hz), 7.71 (2H, s), 7.66 (2H, d, J = 8.58 Hz), 7.56 (1H, s), 7.49 (1H, d, J = 8.07 Hz), 7.42 (1H, s), 7.15 (2H, d, J = 8.57 Hz), 6.88 (2H, d, J = 8.57 Hz), 5.14 (2H, s), 5.05 (2H, s), 3.72 (3H, d, J = 1.62 Hz), 2.40 (3H, s); HRMS calcd for C_{29} $H_{25}O_4N_6Cl$ 557.1705. Found 557.1696.

5.1.5.9. *N*-(3-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-ylcarbamoyl)-4-methylphenyl)-1-phenyl-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (6i).

^{1}H NMR (400 MHz, DMSO-d_6) 10.86 (1H, s), 9.50 (1H, s), 8.01 (2H, d, J = 8.28 Hz), 7.71 (2H, s), 7.66 (2H, d, J = 8.58 Hz), 7.56 (1H, s), 7.49 (1H, d, J = 8.07 Hz), 7.42 (1H, s), 7.15 (2H, d, J = 8.57 Hz), 6.88 (2H, d, J = 8.57 Hz), 5.14 (2H, s), 5.05 (2H, s), 3.72 (3H, d, J = 1.62 Hz), 2.40 (3H, s); HRMS calcd for $C_{31}H_{28}O_4N_7F_3$ 620.2234. Found 620.2233.

5.1.5.10. *N*-(4-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-ylcarbamoyl)-3-methylphenyl)-5-methylisoxazole-3-carboxamide (6j).

^{1}H NMR1H NMR (400 MHz, DMSO-d_6) 10.71 (1H, s), 7.68 (1H, s), 7.67 (1H, d, J = 7.83 Hz), 7.46 (1H, d, J = 8.07 Hz), 7.41 (1H, s), 7.15 (2H, d, J = 8.5 Hz), 6.88 (2H, d, J = 8.5 Hz), 6.66 (1H, s), 5.14 (2H, s), 5.05 (2H, s), 3.72 (3H, s), 2.37 (3H, s); HRMS calcd for $C_{24}H_{24}O_4N_6$ 461.1938. Found 461.1931.

5.1.5.11. *N*-(4-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-ylcarbamoyl)-3-methylphenyl)benzo[b]thiophene-2-carboxamide (6k).

^{1}H NMR (400 MHz, DMSO-d_6) 10.41 (1H, s), 9.47 (1H, s), 8.87 (1H, s), 8.77 (1H, s), 8.66 (1H, s), 7.87–7.80 (2H, m), 7.51 (1H, d, J = 8.12 Hz), 7.48 (1H, s), 7.15 (2H, d, J = 8.54 Hz), 6.88 (2H, d, J = 8.59 Hz), 5.14 (2H, s), 5.05 (2H, s), 3.72 (3H, s), 2.37 (3H, s); HRMS calcd for $C_{24}H_{23}O_3N_7$ 458.1941. Found 458.1937.

5.1.5.12. *N*-(4-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-ylcarbamoyl)-3-methylphenyl)-2-chloroisonicotinamide (6l).

^{1}H NMR (400 MHz, DMSO-d_6) 10.63 (1H, s), 9.47 (1H, s), 8.63 (1H, d, J = 5.08 Hz), 8.01 (1H, s), 7.87 (1H, dd), 7.68–7.65 (2H, m), 7.50

(1H, d, *J* = 8.32 Hz), 7.41 (1H, s), 7.15 (2H, d, *J* = 8.57 Hz), 6.88 (2H, d, *J* = 8.57 Hz), 5.14 (2H, s), 5.05 (2H, s), 3.72 (3H, d, *J* = 1.62 Hz), 2.40 (3H, s); HRMS calcd for $C_{25}H_{23}O_3N_6Cl$ 491.1599. Found 491.1593.

5.1.5.13. *N*-(4-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-ylcarbamoyl)-3-methylphenyl)-2-(pyridin-4-yl)thiazole-4-carboxamide (6m). ^{1}H NMR (400 MHz, DMSO-d_6) 10.35 (1H, s), 9.47 (1H, s), 8.80–8.78 (2H, m), 8.66 (1H, s), 8.13 (2H, m), 7.84–7.72 (2H, m), 7.51 (1H, d, *J* = 8.41 Hz), 7.42 (1H, s), 7.15 (2H, d, *J* = 8.5 Hz), 6.88 (2H, d, *J* = 8.5 Hz), 6.66 (1H, s), 5.14 (2H, s), 5.05 (2H, s), 3.72 (3H, s), 2.37 (3H, s); HRMS calcd for $C_{28}H_{25}O_3N_7S$ 540.1819. Found 540.1817.

5.1.5.14. *N*-(4-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-ylcarbamoyl)-3-methylphenyl)pyrazine-2-carboxamide (6n). ^{1}H NMR (400 MHz, DMSO-d_6) 10.59 (1H, s), 9.47 (1H, s), 8.39 (1H, s), 8.07 (1H, d, *J* = 8.09 Hz), 8.02 (1H, d, *J* = 8.09 Hz), 7.7 (1H, m), 7.67 (2H, m), 7.50 (2H, d, *J* = 7.23 Hz), 7.42 (1H, s), 7.15 (2H, d, *J* = 8.57 Hz), 6.88 (2H, d, *J* = 8.57 Hz), 5.14 (2H, s), 5.05 (2H, s), 3.72 (3H, d, *J* = 1.62 Hz), 2.41 (3H, s); HRMS calcd for $C_{28}H_{25}O_3N_5S$ 512.1757. Found 512.1748.

5.1.5.15. *N*-(4-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-ylcarbamoyl)-3-methylphenyl)benzofuran-2-carboxamide (6o). ^{1}H NMR (400 MHz, DMSO-d_6) 10.60 (1H, s), 9.46 (1H, s), 7.84 (1H, d, *J* = 7.66 Hz), 7.79 (1H, d, *J* = 0.65 Hz) 7.74 (1H, s), 7.72 (2H, s), 7.54–7.48 (2H, m), 7.42 (1H, s), 7.39–7.36 (1H, m) 7.15 (2H, d, *J* = 8.57 Hz), 6.88 (2H, d, *J* = 8.57 Hz), 5.14 (2H, s), 5.05 (2H, s), 3.72 (3H, d, *J* = 1.62 Hz), 2.40 (3H, s); HRMS calcd for $C_{28}H_{25}O_4N_5$ 496.1986. Found 496.1978.

5.1.6. General synthses of *N*-(5-amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-2-methyl-ureido benzamide (7a–7s)

A mixture of 5-amino-*N*-(5-amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-2-methylbenzamide **5** and substituted isocyanate in THF was stirred at room temperature overnight. The reaction mixture was cooled, extracted with CH_2Cl_2. The organic layer was washed with water and brine. After drying over anhydrous $MgSO_4$, and the mixture was evaporated under reduced pressure to give a residue. The residue was purified by silica gel chromatography.

5.1.6.1. *N*-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-2-methyl-5-(3-(2,4,5-trichlorophenyl)ureido) benzamide (7a). ^{1}H NMR (400 MHz, DMSO-d_6) 9.61 (1H, s), 9.55 (1H, s), 8.53 (1H, s), 7.90 (1H, s), 7.77 (1H,s), 7.68–7.62 (3H, m), 7.54 (1H, s), 7.43 (1H, s), 7.35 (1H, d, *J* = 5.89 Hz), 7.21 (2H, d, *J* = 8.86 Hz), 7.15 (2H, d, *J* = 8.42 Hz), 7.10 (1H, s), 6.88 (2H, d, *J* = 8.7 Hz), 5.13 (2H, s), 5.05 (2H, s), 3.72 (3H, s), 2.44 (3H, s); HRMS calcd for $C_{26}H_{23}O_3N_6Cl_3$ 573.0976. Found 573.0970.

5.1.6.2. *N*-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-4-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)-2-methylbenzamide (7b). ^{1}H NMR (400 MHz, DMSO-d_6) 9.10 (1H, s), 7.40 (1H, m), 7.21 (1H, d, *J* = 8.688 Hz), 7.14 (2H, d, *J* = 8.44 Hz), 6.89 (2H, s), 6.39 (2H, s), 5.37 (2H, s), 5.15 (2H, s), 5.05 (2H, s), 3.72 (3H, s), 2.32 (3H, s); HRMS calcd for $C_{27}H_{24}O_3N_6ClF_3$ 573.1629. Found 573.1623.

5.1.6.3. *N*-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-5-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)-2-methylbenzamide (7c). ^{1}H NMR (400 MHz, DMSO-d_6) 9.53 (1H, s), 9.19 (1H, s), 8.89 (1H, s), 8.10 (1H, d, *J* = 2.38 Hz), 7.65–7.59 (3H, m), 7.54 (1H, d, *J* = 2.26 Hz), 7.44 (1H, s), 7.41 (1H, d, *J* = 2.132 Hz), 7.19 (2H, d, *J* = 8.492 Hz), 7.15 (2H, d, *J* = 8.67 Hz), 6.87 (2H, d, *J* = 8.67 Hz), 5.12 (2H, s), 5.05 (2H, s), 3.72 (3H, s), 2.32 (3H, s); HRMS calcd for $C_{27}H_{24}O_3N_6ClF_3$ 573.1629. Found 573.1622.

5.1.6.4. *N*-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-4-(3-(3,4-dichlorophenyl)ureido)-2-methylbenzamide (7d). ^{1}H NMR (400 MHz, DMSO-d_6) 9.39 (1H, s), 9.05 (1H, s), 8.93 (1H, s), 7.89 (1H, d, *J* = 2.51 Hz), 7.50 (2H, d, *J* = 5.7 Hz), 7.40 (2H, s), 7.35–7.31 (2H, m), 7.14 (2H, d, *J* = 8.62 Hz), 6.87 (2H, d, *J* = 8.62 Hz), 5.12 (2H, s), 5.04 (2H, s), 3.72 (3H, s), 2.36 (3H, s); HRMS calcd for $C_{26}H_{24}O_3N_6Cl_2$ 539.1366. Found 539.1361.

5.1.6.5. *N*-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-5-(3-(2,6-dichlorophenyl)ureido)-2-methyl benzamide (7e). ^{1}H NMR (400 MHz, DMSO-d_6) 9.58 (1H, s), 9.55 (1H, s), 8.53 (1H, s), 7.77 (1H, s), 7.53 (2H, d, *J* = 5.69Hz), 7.43 (1H, s), 7.35 (1H, d, *J* = 5.89 Hz), 7.22 (1H, s), 7.15 (2H, d, *J* = 8.42 Hz), 7.11 (1H, s), 6.88 (2H, d, *J* = 8.7 Hz), 5.13 (2H, s), 5.05 (2H, s), 3.72 (3H, s), 2.44 (3H, s); HRMS calcd for $C_{26}H_{24}O_3N_6Cl_2$ 539.1366 Found 539.1359

5.1.6.6. N-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-2-methyl-4-(3-(3-(trifluoromethyl)phenyl)ureido) benzamide (7f). ^{1}H NMR (400 MHz, DMSO-d_6) 9.54 (1H, s), 9.14 (1H, s), 8.92 (1H, s), 8.01 (1H, s), 7.55 (2H, s), 7.52 (1H, s), 7.44 (1H, s) 7.30 (1H, d, *J* = 8.05 Hz), 7.18 (1H, d, *J* = 8.06 Hz), 7.15 (2H, d, *J* = 8.22 Hz), 6.88 (2H, d, *J* = 8.588 Hz), 5.13 (2H, s), 5.05 (2H, s), 3.72 (3H, s), 2.34 (3H, s); HRMS calcd for $C_{27}H_{25}O_3N_6F_3$ 539.2019. Found 539.2012.

5.1.6.7. N-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-2-methyl-5-(3-(4-(trifluoromethyl)phenyl)ureido) benzamide (7g). ^{1}H NMR (400 MHz, DMSO-d_6) 9.14 (1H, s), 7.55 (2H, m), 7.38 (1H, s), 7.21 (1H, d, *J* = 8.55Hz), 7.14 (2H, d, *J* = 8.44 Hz), 6.89 (2H, s), 6.39 (2H, s), 5.37 (2H, s), 5.15 (2H, s), 5.05 (2H, s), 3.72 (3H, s), 2.32 (3H, s); HRMS calcd for $C_{27}H_{25}O_3N_6F_3$ 539.2019. Found 539.2011.

5.1.6.8. *N*-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-4-(3-(3-chlorophenyl)ureido)-2-methylbenzamide (7h). ^{1}H NMR (400 MHz, DMSO-d_6) 9.92 (1H, s), 9.45 (1H, s), 8.55 (1H, s), 7.77 (1H, d, *J* = 3.34 Hz), 7.48 (2H, d, *J* = 4.88 Hz), 7.31 (2H, m), 7.14 (2H, d, *J* = 8.62 Hz), 6.87 (2H, d, *J* = 8.62 Hz), 5.12 (2H, s), 5.04 (2H, s), 3.72 (3H, s), 2.36 (3H, s); HRMS calcd for $C_{26}H_{25}O_3N_6Cl_3$ 505.1756. Found. 505.1748.

5.1.6.9. *N*-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-5-(3-(3-chlorophenyl)ureido)-2-methylbenzamide (7i). ^{1}H NMR (400 MHz, DMSO-d_6) 9.68 (1H, s), 9.54 (1H, s), 8.45 (1H, s), 7.65–7.55 (2H, m), 7.39 (1H, m), 7.35 (1H, d, *J* = 2.6 Hz), 7.34 (1H, s), 7.31 (1H, s), 7.29–7.27 (1H, m), 7.22 (1H, s), 7.14 (2H, d, *J* = 8.52 Hz), 6.87 (2H, d, *J* = 8.50 Hz), 5.10 (2H, s), 5.05 (2H, s), 3.72 (3H, s), 2.32 (3H, s); HRMS calcd for $C_{26}H_{25}O_3N_6Cl_3$ 505.1756. Found 505.1747.

5.1.6.10. *N*-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-4-(3-(4-chlorophenyl)ureido)-2-methylbenzamide (7j). ^{1}H NMR (400 MHz, DMSO-d_6) 10.21 (1H, s), 10.04 (1H, s), 8.89 (1H, s), 8.10 (1H, d, *J* = 2.38 Hz), 7.54 (1H, d, *J* = 2.26 Hz), 7.44 (1H, s), 7.41 (1H, d, *J* = 2.132 Hz), 7.17 (2H, d, *J* = 9.21 Hz), 7.15 (2H, d, *J* = 8.67 Hz), 6.87 (2H, d, *J* = 8.67 Hz), 6.44 (2H, d, *J* = 9.24 Hz), 5.12 (2H, s), 5.05 (2H, s), 3.72 (3H, s), 2.32 (3H, s); HRMS calcd for $C_{26}H_{25}O_3N_6Cl_3$ 505.1756. Found 505.1749.

5.1.6.11. *N*-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-5-(3-(4-chlorophenyl)ureido)-2-methylbenzamide (7k). ^{1}H NMR (400 MHz, DMSO-d_6) 9.68 (1H, s), 9.54 (1H, s), 8.45 (1H, s), 7.65–7.55 (2H, m), 7.39 (1H, m), 7.35 (1H, d, *J* = 2.6 Hz), 7.34 (1H, s), 7.31 (1H, s), 7.29–7.27 (1H, m), 7.22 (1H, s), 7.14 (2H, d, *J* = 8.52 Hz), 6.87 (2H, d, *J* = 8.50 Hz), 5.10 (2H, s), 5.05 (2H, s),

3.72 (3H, s), 2.32 (3H, s); HRMS calcd for $C_{26}H_{25}O_3N_6Cl_3$ 505.1756. Found 505.1748.

5.1.6.12. *N*-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-5-(3-cyclohexylureido)-2-methylbenzamide (7l). ^{1}H NMR (400 MHz, DMSO-d_6) 9.51 (1H, s), 8.47 (1H, s), 7.39 (1H, s), 7.35 (1H, d, J = 2.6 Hz), 7.34 (1H, s), 7.29–7.27 (1H, m), 7.22 (1H, s), 7.14 (2H, d, J = 8.57 Hz), 6.87 (2H, d, J = 8.56 Hz), 5.10 (2H, s), 5.05 (2H, s), 3.72 (3H, s), 2.32 (3H, s), 1.81–1.64 (4H, m), 1.28–1.14 (6 H, m); HRMS calcd for $C_{26}H_{32}O_3N_6$ 477.2615. Found 477.2607.

5.1.6.13. *N*-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-5-(3-(2,4-bis(trifluoromethyl)phenyl)ureido)-2-methylbenzamide (7m). ^{1}H NMR (400 MHz, DMSO-d_6) 9.50 (1H, s), 9.17 (1H, s), 8.89 (1H, s), 8.10 (1H, d, J = 2.38 Hz), 7.69–7.61 (4H, m), 7.54 (1H, d, J = 2.26 Hz), 7.47 (1H, s), 7.43 (1H, d, J = 2.56 Hz), 7.31 (1H, d, J = 9.23 Hz), 7.15 (2H, d, J = 8.67 Hz), 6.87 (2H, d, J = 8.67 Hz), 5.12 (2H, s), 5.05 (2H, s), 3.72 (3H, s), 2.32 (3H, s); HRMS calcd for $C_{28}H_{24}O_3N_6F_6$ 607.1893. Found 607.1885.

5.1.6.14. *N*-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-5-(3-(4-((4-ethylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)ureido)-2-methylbenzamide (7n). ^{1}H NMR (400 MHz, DMSO-d_6) 9.58 (1H, s), 9.26 (1H, s), 9.02 (1H, s), 7.65–7.56 (4H, m), 7.40 (1H, dd, J = 2.33 Hz, 8.32 Hz) 7.22 (1H, d, J = 6.6 Hz), 7.16 (2H, d, J = 8.75 Hz), 6.89 (2H, d, J = 8.67 Hz), 5.13 (2H, s), 5.08 (2H, s), 3.72 (3H, s), 3.16 (2H, s), 3.14 (3H, m), 2.93 (4H, t, J = 12.4 Hz), 2.40 (4H, t, J = 12.3 Hz), 2.34 (3H, s), 2.29 (3H, s), 1.18 (3H, t, J = 7.24 Hz); HRMS calcd for C_{34} $H_{39}O_3N_8F_3$ 665.3176. Found 665.3187.

5.1.6.15. *N*-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-2-methyl-4-(3-(2-morpholino-5-(trifluoromethyl)phenyl)ureido)-benzamide (7o). ^{1}H NMR (400 MHz, DMSO-d_6) 9.91 (1H, s), 9.43 (1H, s), 8.47 (1H, s), 8.35 (1H, s), 7.49–7.30 (6 H, m), 7.15 (2H, d, J = 8.5 Hz), 6.88 (2H, d, J = 8.5 Hz), 5.15 (2H, s), 5.05 (2H, s), 3.72 (3H, s), 3.88 (4H, s), 2.86 (4H, s), 2.41 (3H, s); HRMS calcd for $C_{31}H_{32}O_4N_7F_3$ 624.2547. Found 624.2540.

5.1.6.16. *N*-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-2-methyl-4-(3-(3-morpholino-5-(trifluoromethyl)phenyl)ureido)-benzamide (7p). ^{1}H NMR (400 MHz, DMSO-d_6) 9.39 (1H, s), 9.11 (1H, d, J = 9.24 Hz), 8.95 (1H, s), 8.87 (1H, s), 7.40 (1H, s), 7.35 (2H, s), 7.32 (1H, s), 7.20 (1H, m), 7.16–7.13 (2H, m), 6.88–6.84 (2H, m), 6.39 (1H, s), 5.42 (1H, s), 5.12 (1H, s), 5.05 (2H, t, J = 7.88 Hz), 3.74 (4H, t, J = 4.78 Hz) 3.72 (3H, d, J = 1.62 Hz), 3.16 (4H, t, J = 4.78 Hz), 2.36 (3H, s); HRMS calcd for $C_{31}H_{32}O_4N_7F_3$ 624.2547. Found 624.2545.

5.1.6.17. *N*-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-2-methyl-4-(3-(3-(4-methyl-1*H*-imidazol-1-yl)-5-(trifluoromethyl)phenyl)ureido)benzamide (7q). ^{1}H NMR (400 MHz, DMSO-d_6) 9.40 (1H, s), 9.26 (1H, s), 9.12 (1H, s), 8.95 (1H, s), 8.87 (1H, s), 7.40 (1H, s), 7.35 (2H, s), 7.32 (1H, s), 7.20 (1H, m), 7.16–7.13 (2H, m), 6.88–6.84 (2H, m), 6.39 (1H, s), 5.42 (1H, s), 5.12 (1H, s), 5.05 (2H, t, J = 7.88 Hz), 3.74 (4H, t, J = 4.78 Hz) 3.72 (3H, d, J = 1.62 Hz), 3.16 (4H, t, J = 4.78 Hz), 2.36 (3H, s); HRMS calcd for $C_{31}H_{29}O_3N_8F_3$ 619.2394. Found 619.2398.

5.1.6.18. *N*-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-2-methyl-5-(3-(3-methylisoxazol-5-yl)ureido)benzamide (7r). ^{1}H NMR (400 MHz, DMSO-d_6) 9.58 (1H, s), 9.26 (1H, s), 7.65–7.56 (3H, m), 7.40 (1H, s) 7.22 (1H, d, J = 6.6 Hz), 7.16 (2H, d, J = 8.75 Hz), 6.89 (2H, d, J = 8.64 Hz), 6.61 (1H, s), 5.13 (2H, s), 5.07 (2H, s), 3.72 (3H, s), 2.35 (3H, d, J = 5.58Hz), 2.34 (3H, s); HRMS calcd for $C_{24}H_{25}O_4N_7$ 476.2047. Found 476.2041.

5.1.6.19. *N*-(5-Amino-1-(4-methoxybenzyl)-1*H*-pyrazol-4-yl)-2-methyl-4-(3-(4-(trifluoromethyl)pyrimidin-2-yl) ureido)benzamide (7s). ^{1}H NMR (400 MHz, DMSO-d_6) 9.84 (1H, s), 9.11 (1H, s), 8.12 (1H, d, J = 4.56 Hz), 7.21 (1H, d, J = 8.12 Hz), 7.14 (2H, d, J = 8.44 Hz), 6.89 (2H, d, J = 8.50 Hz), 6.39 (1H, d), 5.37 (2H, s), 5.15 (2H, s), 5.05 (2H, s), 3.72 (3H, s), 2.32 (3H, s); HRMS calcd for $C_{25}H_{23}O_3N_8F_3$ 541.1924. Found 541.1919.

5.1.6.20. *N*-(5-Amino-1-benzyl-1*H*-pyrazol-4-yl)-5-(3-(4-chloro-3-(trifluoromethyl)phenyl)ureido)-2-methylbenzamide (8c). ^{1}H NMR (400 MHz, DMSO-d_6 9.58 (1H, s), 9.22 (1H, s), 8.93 (1H, s), 8.10 (1H, d, J = 2.31 Hz), 7.63–7.61 (2H, m), 7.55 (1H, d, J = 2.26 Hz), 7.47 (1H, s), 7.43 (1H, dd, J = 2.22 Hz, 9.8 Hz), 7.31 (2H, d, J = 7.74 Hz), 7.26 (1H, d, J = 6.88 Hz), 7.20 (1H, s), 7.17 (2H, d, J = 6.8 Hz), 5.16 (2H, s), 5.12 (2H, s), 2.32 (3H, s); HRMS calcd for $C_{26}H_{22}O_2N_6ClF_3$ 543.1524. Found 543.1517.

5.2. 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 hours 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-diphenyltetrazolium 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.

5.3. Docking simulations

Molecular docking of compound **7c** into 3D X-ray structure of V600E B-Raf (PDB code: 1uwj) was carried out using the Glide (SCHRODINGER software package Version 9.1).

5.4. Selected kinase profiling

We used Reaction Biology Corp. Kinase HotSpot^{SM} service (http://www.reactionbiology.com) for screening of **7b**, and IC_{50} Profiler Express for IC_{50} measurement. Assay protocol: In a final reaction volume of 25 μL, C-RAF (h) (5–10 mU) is incubated with 25 mM Tris pH 7.5, 0.02 mM EGTA, 0.66 mg/mL myelin basic protein, 10 mM Mg 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 is stopped by the addition of 5 μL of a 3% phosphoric acid solution. 10 μL of the reaction is 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.

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