Discovery of New Imidazo[2,1-*b*]thiazole Derivatives as Potent Pan-RAF Inhibitors with Promising *In vitro* and *In vivo* Anti-melanoma Activity

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Abstract- BRAF is an important component of MAPK cascade. Mutation of BRAF, in particular V600E, leads to hyperactivation of MAPK pathway and uncontrolled cellular growth. Resistance to selective inhibitors of mutated BRAF is a major obstacle against treatment of many cancer types. In this work, a series of new imidazo[2,1-*b*]thiazol-5-yl)pyrimidine derivatives possessing terminal sulfonamide moiety were synthesized. Pan-RAF inhibitory effect of the new series was investigated, and structure-activity relationship is discussed. Antiproliferative activity of the target compounds was tested against NCI-60 cell line panel. The most active compounds were further tested to obtain their IC_{50} values against cancer cells. Compound **27c** with terminal open chain sulfonamide and **38a** with cyclic sulfamide moiety showed the highest activity in enzymatic and cellular assay, and both compounds were able to inhibit phosphorylation of MEK and ERK. Compound **38a** was selected for testing its *in vivo* activity against melanoma. Cellular and animal activities are reported.

Key words: Imidazo[2,1-*b*]thiazole; Kinase; Melanoma; Pan-RAF; RAF, V600E-BRAF.

Introduction

The MAPK / ERK pathway is a cascade of events that is considered as a fundamental action for cell growth and survival.[1] Any mutation of one or more members of this pathway leads to hyperactivation and ultimately cancer.[2] RAF kinases are serine/threonine kinases that are closely related to cancer since discovered by Rapp *et al*.[3] Three distinct subtypes of RAF kinases are known,[4,5] in addition to homologues of BRAF which were characterized in *Drosophilla melanogaster* (D-RAF) and *Caenorhabditis elegans*.[6,7] RAF is the first downstream effector of RAS, and both kinases are important regulators of ERK-MAPK.

Identification of BRAF mutation paved the way for a new era of cancer therapy.[8] There are more than thirty identified mutations of BRAF kinase,[9,10] out of which V600E-RAF is the most abundant type of BRAF mutation. BRAF point mutation occurs in 60% of malignant melanoma cases,[11] thyroid cancer,[12] colorectal carcinoma, lung cancer, papillary craniopharyngioma,[13] hairy cell leukemia,[14,15] and metanephric kidney adenoma.[16] C-RAF kinase is over-expressed in renal cell carcinoma,[17] hepatocellular carcinoma,[18] and associated with poor prognosis in ovarian[19] and androgen-insenstive prostate cancer.[20]

Sorafenib (**I**, Figure 1) was initially introduced as CRAF inhibitor to treat RAS-mutated cancer[21] prior to the emerging of BRAF mutation in cancer and it is binding to ATP binding pocket. It is considered as the first RAF inhibitor to be used clinically. Recently, two new entries; vemurafenib (**II**, Figure 1)[22] and dabrafenib (**III**, Figure 1)[23], were introduced for treatment of metastatic melanoma.

Although the breakthrough occurred by the introduction of selective V600E-BRAF in the treatment of melanoma, resistance to selective BRAF arises after eight months of treatment.[24-27]

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The acquired resistance occurs by reactivation of MAPK pathway as a result of either genetic or epigenetic changes.[28-31] Genetic causes exist in 52% of resistant melanoma and include BRAF amplification, MAP2K1 and MAP2K2 mutations, RAS mutation, and to less extent mutations in the PI3K pathway.[31,32] Melanoma cells subjected to selective V600E-BRAF inhibitors can switch to another RAF isoform to maintain MAPK activity. In addition, over-expression of CRAF can also lead to MAPK reactivation.[33] The RAF dimer dilemma is another obstacle against the successfulness of first generation selective V600E-BRAF inhibitors such as vemurafenib. Pan-RAF inhibition is one of the potential approaches toward fixing this issue.[34]

After the discovery of resistance to selective V600E-BRAF inhibitors, a hypothesis was established that the usage of pan-RAF inhibitors could be useful in case of patients having oncogenic V600E-BRAF and NRAS in which wild-type BRAF and CRAF play a critical role in tumor growth. In addition, using pan-RAF inhibitors could overcome resistance to traditional selective V600E-BRAF and could be used as monotherapy for treatment of melanoma instead of combined therapy.[35] Two pan-RAF inhibitors; TAK632 (**IV**, Figure 1)[35,36] and LY3009120 (**V**, Figure 1)[37] were introduced and showed high success to produce minimum paradoxical activation and good activity in both mutated BRAF and RAS cases. Recently, pan-RAF inhibitors exhibited antitumor effect on lung cancer[38] and were able to induce apoptosis in acute myeloid leukemia cells.[39] Despite the clinical significant of pan-RAF inhibitors, the number of candidates and scaffolds that act as pan-RAF inhibitor are lower-than-expected need. Also, the potency of pan-RAF inhibitors is lower when compared to selective V600E-BRAF (IC_{50} of pan-RAF inhibitors are still high when compared to vemurafenib and dabrafenib).

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In our previous work, we reported the antiproliferative and kinase inhibitory effects of imidazo[2,1-*b*]thiazole and imidazo[2,1-*b*]oxazole with different terminal substitutions such as urea, amide, or sulfonamide[40-45] as part of comprehensive program to produce selective mutated BRAF inhibitors. According to the results obtained, we reported compound **VI** that possesses inhibitory effect against both V600E-BRAF and CRAF (IC_{50} values are 40 and 19 nM, respectively) (Figure 1). Although design and synthesis of selective inhibitors of V600E-BRAF is more difficult compared with broad-spectrum kinase inhibitors, obtaining highly selective pan-RAF inhibitor is more challenging. Fine tuning of compound **VI** structure yielded compound **VII** which exhibits higher potency against wild-type BRAF, V600E-BRAF and CRAF with IC_{50} values of 22, 9, and 18 nM, respectively. In order to optimize the obtained structure over RAF kinases, we replaced imidazo[2,1-*b*]thiazole with imidazo[2,1-*b*]oxazole. Imidazo[2,1-*b*]oxazole derivatives showed moderate kinase and cellular activity. So, we retained imidazo[2,1-*b*]thiazole as main scaffold and started molecular modification based on crystal structure of V600E-BRAF. Bioisosteric replacement of *m*-fluoro with *m*-hydroxyl on the phenyl ring attached to position 6 of the imidazothiazole nucleus and testing both open chain sulfonamide and cyclic sulfamide were main structural modifications to be done on compound **VII**. In addition to hydroxyl group, we decided to add methoxy group at position 3 in the phenyl ring at position 6 to investigate the effect of hydrogen bond acceptor and hydrogen bond donor on kinase activity. According to our previous work and molecular modeling study performed at the beginning of our anticancer project, we decided to limit the spacer between pyrimidine ring and sulfonamide terminal side chain to be ethylene or propylene only. Synthetic procedures as well as *in vitro* and *in vivo* results are reported herein in details.

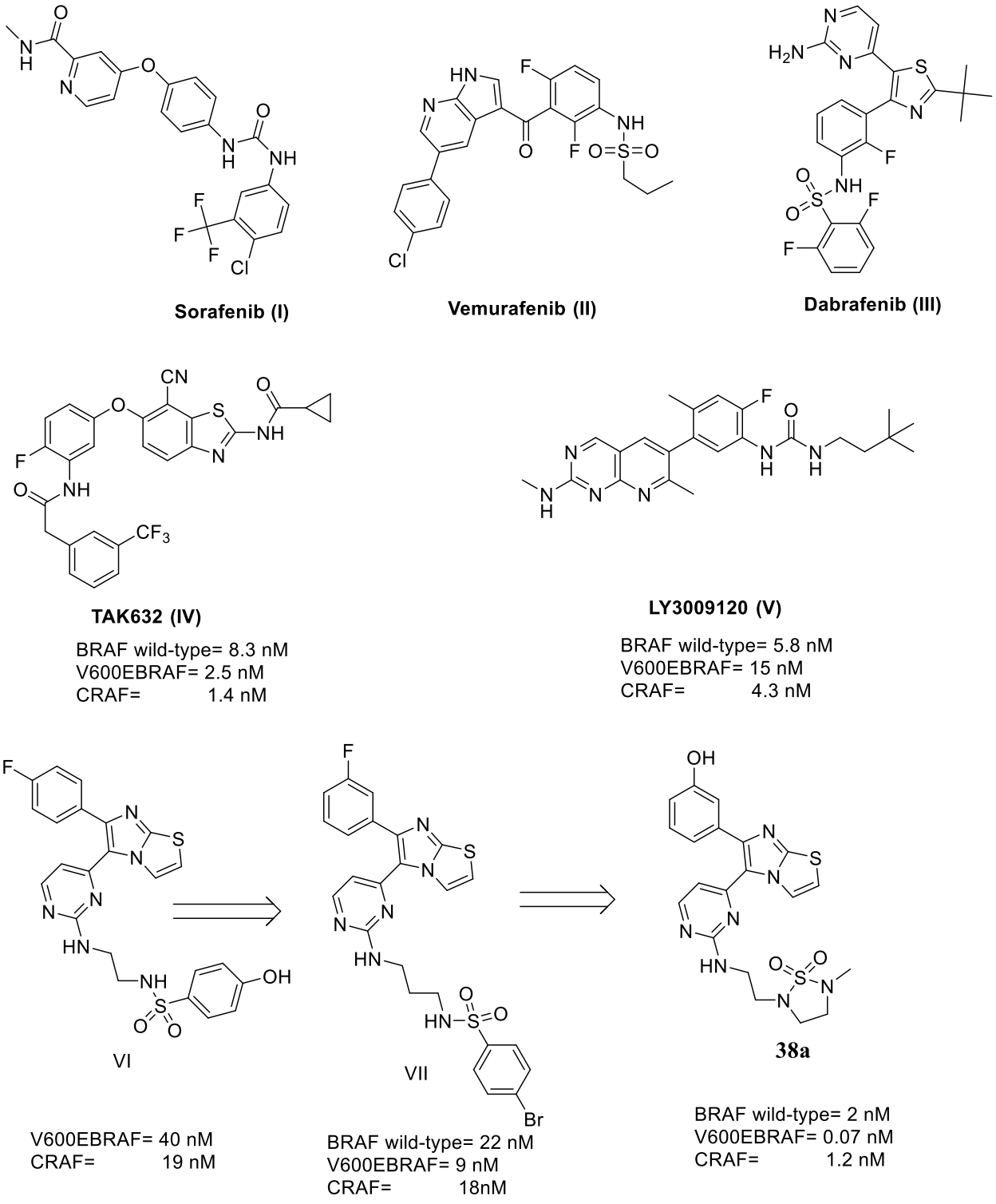

Figure 1. Chemical structures of selective V600E-BRAF and Pan-RAF inhibitors and rational design of final target compounds.

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Results and discussion

Chemistry

Synthesis of the final compounds was accomplished by adopting the synthetic pathways illustrated in Schemes 1-6. Our strategy is divided into two main steps; the first step is synthesis of key methyl sulfonyl intermediates **14-16** while the second step is the synthesis of sulfonamide side chains **22a-g**, **23a-f** and **36a-d** possessing free amino group. The last synthetic step involves reaction of the amines with mesyl intermediates to get the target compounds **24-27** (Scheme 4) in addition to **37** and **38** (Scheme 6).

Synthesis of mesyl intermediates is presented in Scheme 1. Refluxing of 2-aminothiazole (**4**) with appropriate 2-bromo-1-phenylethan-1-one derivative **5-7** in absolute ethanol followed by basification with ammonium hydroxide led to cyclization and formation imidazothiazole intermediates **8-10**. Coupling reaction of compounds **8-10** with 4-chloro-2-(methylthio)pyrimidine using palladium acetate, cesium carbonate, and triphenylphosphine in anhydrous dimethylformamide at 80 °C produced arylated products **11-13**.[46] Oxidation of methylthio group of compounds **11-13** using oxone in mixture of methanol and water gave the corresponding mesyl analogues **14-16**.

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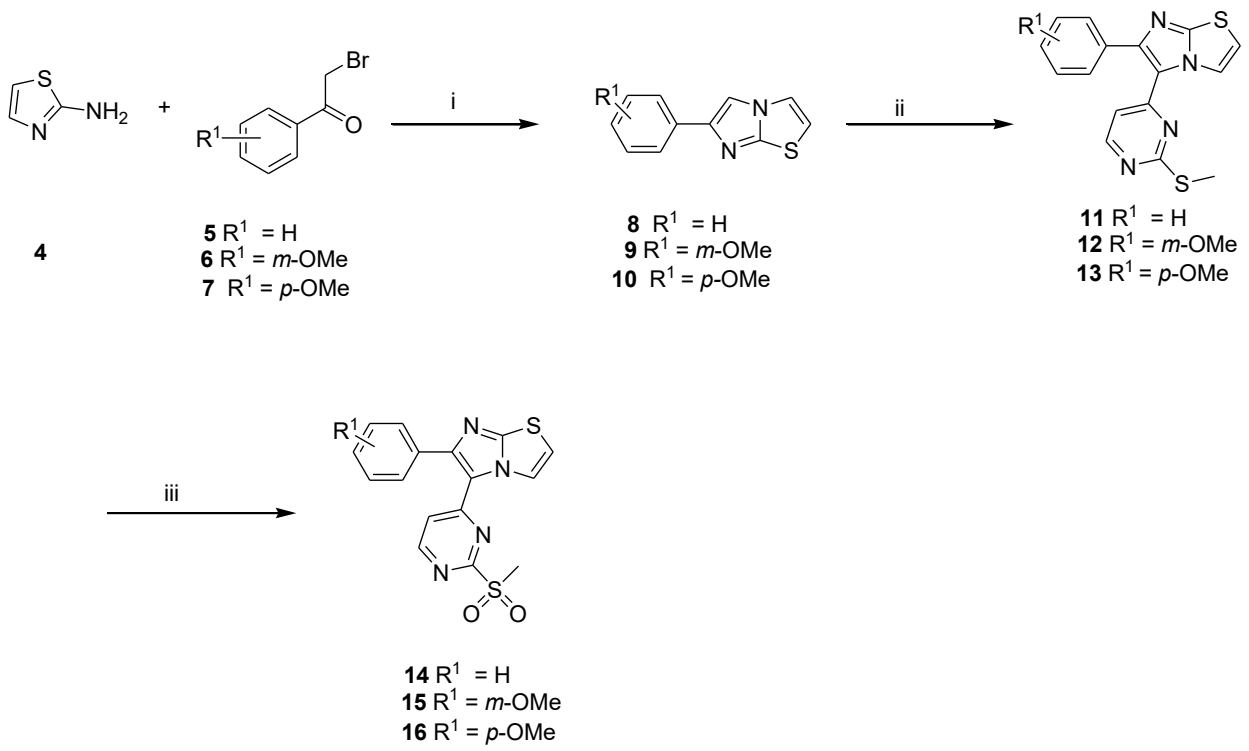

Scheme 1: Synthesis of compounds **14-16**; Reagents and conditions: i) Ethanol, reflux, 16 h, 70-80%; ii) 4-Chloro-2-(methylthio)pyrimidine, $Pd(OAc)_2$, Cs_2CO_3, PPh_3, DMF, 80 °C, 16 h, 9-15%; iii) Oxone, MeOH, H_2O, rt, 16 h, 90-95%.

The low yield of step 2 in Scheme 1 encouraged us to look for an alternative pathway to synthesize compound **12**. Scheme 2 shows the alternative strategy to produce compound **12**. Bromination of 6-(3-methoxyphenyl)imidazo[2,1-*b*]thiazole (**9**) using bromine in dichloromethane formed the corresponding 5-bromo analogue **17** in quantitative yield. Reaction of compound **17** with Bis(pinacolato)diboron in present of catalytic amount of 1,1′-Bis(diphenylphosphino)ferrocene] dichloropalladium(II) and potassium acetate as a base gave 6-(3-methoxyphenyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)imidazo[2,1-*b*]thiazole (**18**). Suzuki coupling of boronic acid

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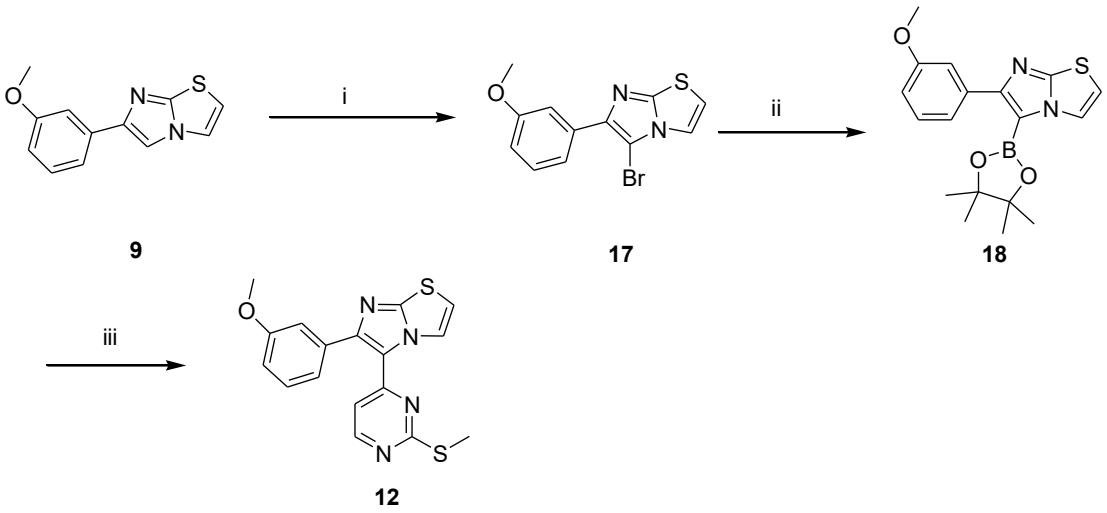
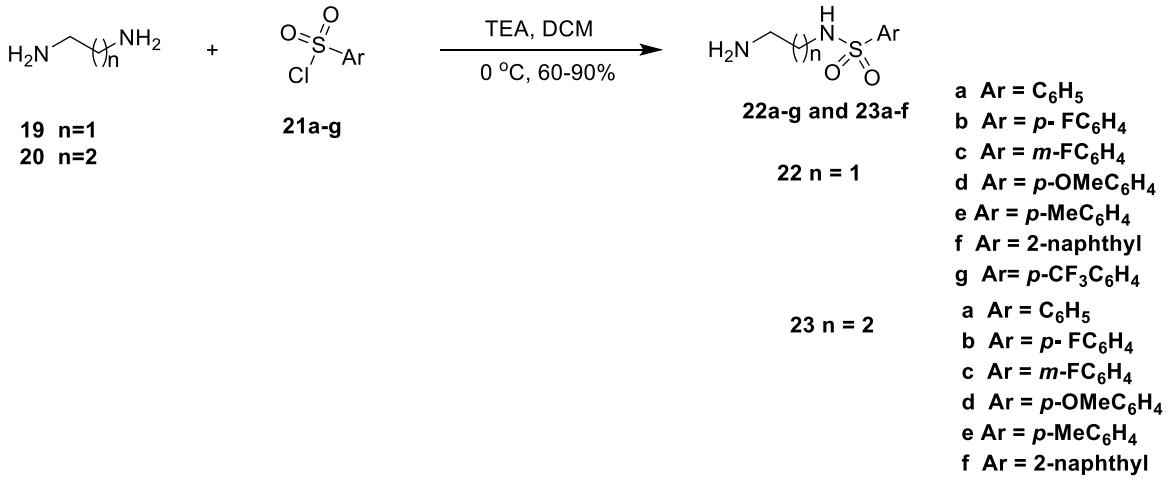
ester (**18)** with 4-chloro-2-(methylthio)pyrimidine using cesium carbonate as strong base and 1,1′-bis(diphenylphosphino)ferrocene dichloropalladium(II) as catalyst produced compound **12**.[45]



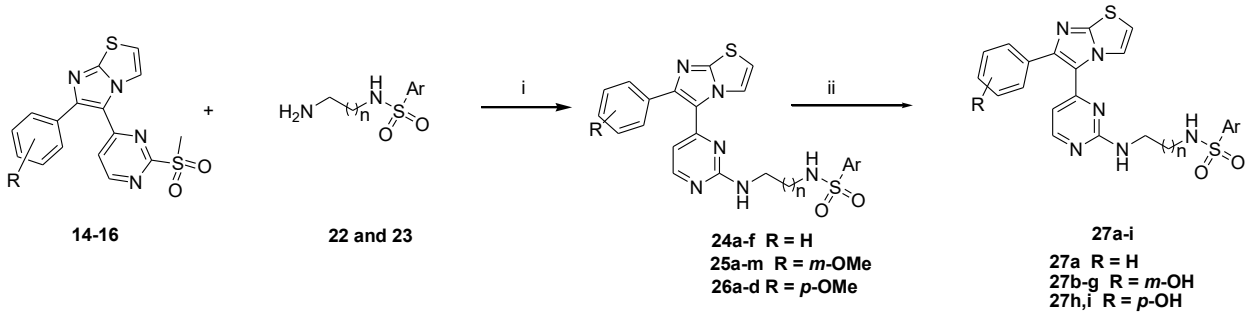
Scheme 2: Alternative pathway for synthesis of compound **12**; Reagents and conditions: i) Br_2, dichloromethane, rt, 24 h, 60%; ii) potassium acetate, bis-(pinacolate)diboron, Pd(dppf)Cl_2 DMSO, 80 °C, 12 h, 70%; iii) 4-chloro-2-(methylthio)pyrimidine, Pd(dppf)Cl_2 , Cs_2CO_3, DME, H_2O, 80 °C, 12 h, 30%.

Synthesis of the open chain sulfonamide side chains was achieved through single step pathway through reaction of ethylenediamine (**19**) or propylenediamine (**20**) with appropriate sulfonyl chloride **21a-g** in presence of triethylamine (TEA) [Scheme 3]. Compounds **19** and **20** were used in large excess (30 equivalents) and the sulfonyl chlorides were diluted with dichloromethane and added drop-wisely at 0 °C.



Scheme 3: Synthesis of sulfonamide side chains **22** and **23**.

The final target compounds **24-27** were synthesized as shown in Scheme 4. Reaction of mesyl intermediates **14-16** with amino reactants **22** or **23** in dimethyl sulfoxide and using *N,N*-diisopropylethylamine as a base at 80 °C led to formation of **24a-f**, **25a-m**, and **26a-d**. Demethylation of methoxy compounds **25** and **26** using boron tribromide solution in dichloromethane at -78 °C produced the target hydroxyl compounds **27a-i**.



Scheme 4: Synthesis of final target compounds **24** and **27;** Reagents and conditions: i) *N,N*-diisopropylethylamine, DMSO, 80 °C, 8 h, 20-62%; ii) BBr_3, dichloromethane, -78 °C, 10-50%.

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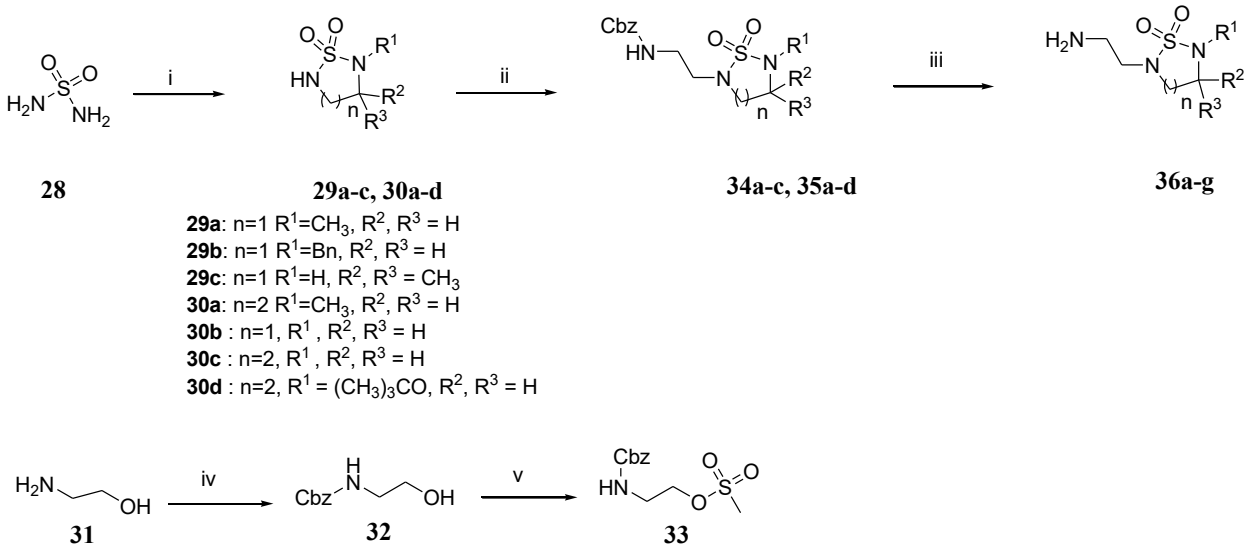
The synthesis of cyclic sulfamide side chains was done as illustrated in Scheme 5. Synthesis of compounds **29a-c** and **30a-d** was done via the condensation of sulfamide (**28**) with appropriate diamine in refluxed pyridine. Moreover, 2-ethanolamine (**31**) was protected with benzyl chloroformate to give benzyl (2-hydroxyethyl)carbamate (**32**). Reaction of compound **32** with methanesulfonyl chloride in presence of triethylamine gave Cbz-protected methanesulfonate intermediate **33**. Coupling of compounds **33** and **29/30** in dimethylformamide and using sodium hydride as a base produced compounds **34a-c** and **35a-d**, which upon deprotection with palladium/C under hydrogen atmosphere resulted in cyclic sulfamide side chain **36a-g**.



Scheme 5: Synthesis of cyclic sulfamide side chain **36a-g**. Reagents and conditions: i) appropriate ethylenediamine, pyridine, reflux, 3 h, 32-52%; ii) **34**, NaH (60% in mineral oil), DMF, 42-76%; iii) H_2/ Pd-C, MeOH, rt, 1 h; iv) benzyl chloroformate, TEA, CH_2Cl_2, 0 °C, 59%; v) methanesulfonyl chloride, TEA, CH_2Cl_2, 0 °C, 53%.

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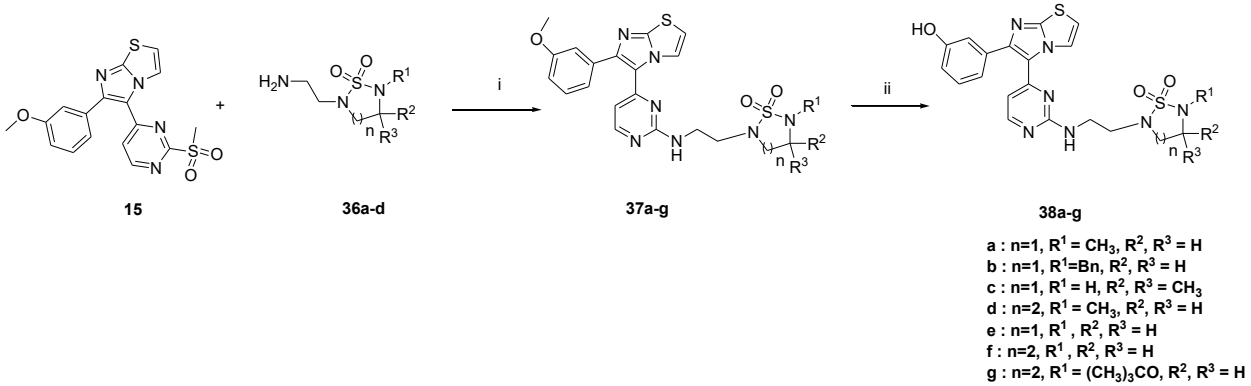
In a similar manner, synthesis of the target compounds **37a-g** was performed through the coupling of compound **15** and cyclic sulfamide side chains **36a-g** in presence of Hunig's base. Demethylation of compounds **37a-g** by boron tribromide in dichloromethane led to formation of corresponding hydroxyl analogues **38a-g**.



Scheme 6: Synthesis of final target compounds **37a-g** and **38a-g**. Reagents and conditions: i) *N,N*-diisopropylethylamine, DMSO, 80 °C, 8h, 29-64%; ii) BBr_3, dichloromethane, -78 °C, 16-27%.

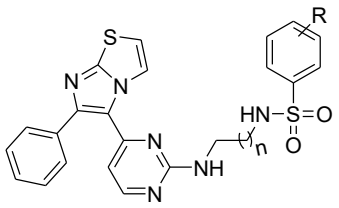
Biological screening

Kinase profiling

The primary biological screening was to test the ability of the final compounds to inhibit wild-type BRAF, V600E-BRAF and CRAF (Tables 1-5). Both mean inhibition percentage and IC_{50} values were determined for each group of final compounds. For *N*-(2-((4-(6-phenylimidazo[2,1-*b*]thiazol-5-yl)pyrimidin-2-yl) derivatives (**24a-f**), compounds with propylene linker between pyrimidine and sulfonamide terminal moiety **24d-f** showed higher activity in terms of both inhibition percentage and IC_{50} compared to ethylene linker **24a-c**. Compound **24d** with *p*-fluoro substituent is the most potent against the 3 isoforms of RAF kinase with an IC_{50} values of 276, 57, and 87 nM

over wild-type BRAF, V600E-BRAF, and CRAF, respectively. The activity over mutated BRAF was higher than CRAF and finally wild-type BRAF. The order of activity are compounds having *p*-F (**24a** and **24d**) followed by *p*-OMe (**24b** and **24e**) and finally compounds possessing *p*-Me group (**24c** and **24f**) [Table 1].

Table 1: *In vitro* enzyme activity of compounds **24a-f**.



Compound No	n	R	BRAF^{wt}		V600E-BRAF		CRAF	
			%[a]	IC_{50}[b]	% [a]	IC_{50}[b]	% [a]	IC_{50}[b]
24a	1	4-F	96.62 ±1.02	375±2.25	97.42±0.64	69.1±2.13	97.01±0.12	112±1.95
24b	1	4-OCH_3	95.41±0.95	459±3.51	96.32±0.46	77.5±1.52	95.67±0.55	172±2.15
24c	1	4-CH_3	92.51±0.81	623±3.28	94.86±0.98	89.3±2.05	93.21±0.37	251±2.71
24d	2	4-F	98.50±0.75	276±1.10	99.21±0.42	57.7±0.41	99.13±0.31	87.8±1.51
24e	2	4-OCH_3	97.26±0.98	299±4.21	98.64±0.54	66.1±1.27	98.92±0.24	98.3±2.34
24f	2	4-CH_3	96.51±1.32	521±4.42	97.72±0.82	85.3±1.72	96.99±0.96	201±5.01

[a]: Mean %inhibition at single dose (10 μM). Data are represented as mean value ± S.D.

[b]: IC_{50} (nM). Values are presented as mean value (duplicate test) ± S.D.

The first modification to optimize the enzyme activity was the addition of methoxy group at the phenyl ring at position 6 of the imidazo[2,1-*b*]thiazole scaffold. Table 2 shows the activity of compounds **25a-m** over wild-type BRAF, V600E-BRAF, and CRAF. Generally, the compounds had higher potency over V600E-BRAF and CRAF compared to wild-type BRAF. Compounds

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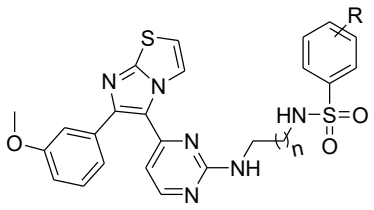
25h-m with propylene linker are more potent over wild-type BRAF and mutated BRAF. On the other hand, compounds **25a-g** with ethylene spacer show insignificant potency difference against CRAF compared to propylene spacer compounds **25h-m**. In case of wild-type BRAF, compounds **25i** with *p*-fluorobenzene sulfonamide and propylene linker and **25j** with *m*- fluorophenyl sulfonamide and propylene linker showed the highest activity against wild-type BRAF with IC_{50} values of 0.43 μM and 0.52 μM, respectively. Regarding the activity against V600E-BRAF, compound **25i** showed an IC_{50} of 0.09 μM followed by **25g** (*p*-CF_3 and ethylene) with IC_{50} 0.098 μM and finally **25j** with an IC_{50} = 0.10 μM. CRAF inhibitory effect of compounds **25a-m** is ranging from 0.095 μM for **25i** to 0.22 μM for **25a**. The order of activity over CRAF started with compound **25b** (*p*-fluorobenzene sulfonamide and ethylene spacer) with IC_{50} 0.10 μM, **25c** (*m*-fluorobenzene sulfonamide and ethylene linker) with IC_{50} 0.106 μM, **25j** with IC_{50} 0.11 μM, and **25h** (unsubstituted benzenesulfonamide & propylene) with IC_{50} = 0.12 μM. Compounds having small electron-withdrawing group **25b, 25c**, **25i**, and **25j** showed no significant difference in potency over both V600E-BRAF and CRAF and only compound **25i** exerted higher potency over wild-type BRAF. (Table 2).

Table 2. *In vitro* enzyme activity of compounds **25a-m**.



Compound No	n	R	$BRAF^{wt}$		V600E-BRAF		CRAF	
			%[a]	IC_{50}[b]	% [a]	IC_{50}[b]	% [a]	IC_{50}[b]
25a	1	H	90.21±0.81	941±3.01	95.61±0.81	120±2.11	92.22±0.31	220±2.56

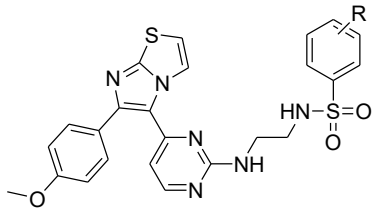
25b	1	4-F	95.25±0.95	522±5.26	96.86±0.71	115±3.12	97.32±1.15	100±1.58
25c	1	3-F	94.92±0.79	561±6.12	96.69±0.52	112±2.85	97.14±0.69	106±3.21
25d	1	4-OCH_3	92.11±0.82	745±4.55	94.33±0.54	160±4.32	95.62±1.06	151±2.28
25e	1	4-CH_3	89.59±0.96	987±8.21	92.67±0.77	177±3.68	94.35±0.44	163±5.21
25f	1	1-naphthyl	90.64±0.88	921±9.32	93.66±0.87	169±4.33	94.65±0.74	190±2.99
25g	1	4-CF_3	93.96±0.97	687±10.51	98.21±1.56	98.1±1.27	97.31±0.44	142±2.11
25h	2	H	92.41±0.69	721±4.21	96.11±2.10	109±5.12	93.55±0.68	129±3.26
25i	2	4-F	97.53±1.12	430±5.64	98.37±0.89	90.3±1.13	98.12±1.01	95.2±1.21
25j	2	3-F	95.22±1.02	526±7.22	97.65±0.78	102±1.58	97.16±0.99	111±1.31
25k	2	4-OCH_3	94.32±0.93	589±6.66	95.55±1.25	156±6.32	95.27±0.33	164±4.21
25l	2	4-CH_3	90.57±0.71	894±5.26	94.71±0.45	168±7.16	92.35±0.54	209±2.55
25m	2	1-naphthyl	91.92±1.01	842±10.35	94.92±0.38	165±5.54	93.95±0.99	172±1.98

[a]: Mean %inhibition at single dose (10 μM). Data are represented as mean value ± S.D.

[b]: IC_{50} (nM). Values are presented as mean value (duplicate test) ± S.D.

The second modification step was to change the position of methoxy group from *meta* to *para* (compounds **26a-d**, Table 3). This modification dramatically reduced the inhibitory effect against all RAF isoform. The IC_{50} values over V600E-BRAF and CRAF are more than 10 μM. The best potency among these derivatives was that of compounds **26a** and **26b** against wild-type B-RAF (IC_{50} = 9.21 and 9.89 μM, respectively).

Table 3. *In vitro* enzyme activity of compounds **26a-d**.



Compound No	R	BRAF^{wt}		V600E-BRAF		CRAF	
		%[a]	IC_{50}[b]	% [a]	IC_{50}[b]	% [a]	IC_{50}[b]
26a	4-F	60.21±0.92	9210±260	45.36±0.48	13250±130	25.52±0.23	32220±550
26b	4-OCH_3	51.65±0.77	9890±650	29.62±1.02	26310±420	32.68±0.54	24320±1210
26c	4-CH_3	35.25±0.33	19250±370	31.32±0.84	26560±630	39.89±0.71	21330±1320
26d	1-Naphthyl	33.25±1.52	20780±450	21.63±0.66	> 35000	26.88±0.92	28920±1010

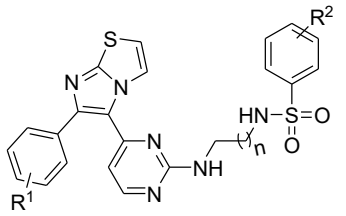
[a]: Mean %inhibition at single dose (10 μM). Data are represented as Mean value ± S.D.

[b]: IC_{50} (nM). Values are presented as mean value (duplicate test) ± S.D.

The next modification was to change hydrogen bond acceptor methoxy group to hydroxyl group which has both hydrogen bond acceptor and donor properties in addition to smaller size and higher hydrophilicity compared to methoxy group (Table 4). The demethylated compounds showed great increase in enzyme inhibitory effect over the 3 enzymes for both unsubstituted phenyl and *meta*-substituted phenyl imidazo[2,1-*b*]thiazole, while the *para*-substituted derivative did not show any improvement toward the investigated enzymes. Compounds **27b-g** possessing 3-hydroxyphenyl moiety at position 6 of imidazo[2,1-*b*]thiazole nucleus have greater activities compared to unsubstituted and 4-hydroxyl analogues. Against wild-type BRAF, compound **27c** (*p*-fluorobenzene sulfonamide and ethylene linker) is the most potent compound with IC_{50} 2.7 nM

followed by **27f** (*m*-fluorobenzene sulfonamide and propylene) with an IC_{50} equals 4.2 nM, **27g** (*p*-toluene sulfonamide and propylene) with an IC_{50} = 18 nM, and compound **27e** (unsubstituted benzene sulfonamide and propylene linker) with an IC_{50} value of 29 nM. Compound **27f** had the lowest IC_{50} of 0.98 nM followed by **27c** with IC_{50} = 1 nM and **27g** with IC_{50} equals 9 nM. Compound **27h** and **27i** with 4-hydroxyphenyl showed the highest IC_{50s}; 25.12 µM and 21.33 µM, respectively. Finally, CRAF activities were ranging from 5 nM for compound **27f** and 6 nM for compound **27c** to 19.36 µM for compound **27h**. Compound **27a** with unsubstituted phenyl at position 6 showed higher IC_{50} compared to 3-hydroxyl derivatives with an IC_{50} of 75 nM.

Table 4. *In vitro* enzyme activity of compounds **27a-i**.



Compd No	n	R^1	R^2	$BRAF^{wt}$		V600E-BRAF		CRAF	
				%[a]	IC_{50}[b]	%[a]	IC_{50}[b]	%[a]	IC_{50}[b]
27a	1	H	4-OH	99.56±0.51	97±1.2	99.25±0.33	65±3	98.59±0.62	75±5.2
27b	1	3-OH	H	97.89±0.26	35±2.5	98.64±0.51	19±1	98.12±0.35	21±2.3
27c	1	3-OH	4-F	100±0.11	2.7±1	100±0.11	1±0.2	99.14±0.87	6±0.5
27d	1	3-OH	4-CH_3	98.21±0.09	64±3	97.89±0.64	55±5	98.32±0.44	62±2.1
27e	2	3-OH	H	98.91±0.15	29±1	99.35±0.69	20±2	98.75±0.74	23±1.5
27f	2	3-OH	3-F	100±0.08	4.2±0.12	100±0.25	**0.98±0.012**	99.87±0.67	5±0.13
27g	2	3-OH	4-CH_3	100±0.23	18±3	100±0.31	9±0.8	99.92±0.51	13±1.6
27h	1	4-OH	4-OH	51±0.62	9710±260	35.32±0.22	25120±1560	39.57±0.27	19360±1350

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27i	1	4-OH	4- CH_3	47±0.44	11320±1020	39.51±0.12	21330±2430	40.29±0.36	16510±2150

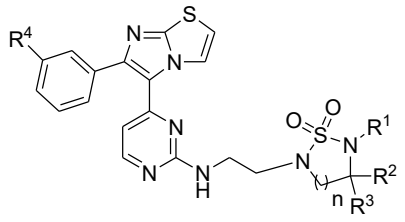
[a]: Mean %inhibition at single dose (10 μM). Data are represented as Mean value ± S.D.

[b]: IC_{50} (nM). Values are presented as mean value (duplicate test) ± S.D.

We finally decided to check the effect of using cyclic sulfamide moiety instead of open chain sulfonamide and keeping the *meta*-hydroxyphenyl moiety at position 6 of imidazo[2,1-*b*]thiazole. Table 5 shows the activity of cyclic sulfamide derivatives **37a-g** and **38a-g** against wild-type BRAF, V600E-BRAF and CRAF. The phenolic compounds **38a-g** are proved to be more active against all RAF isoforms compared to methoxy derivatives **37a-g.** In addition, five-membered ring cyclic sulfamides **37a** and **38a** were more potent than six-membered ring cyclic sulfamides **37d** and **38d**. Compound **38a** with *N*-Me cyclic sulfamide and 3-hydroxyphenyl exhibited the highest activity over wild-type BRAF, V600E-BRAF and CRAF with IC_{50} 2, 0.07, and 1.12 nM, respectively. The methoxy analogue of **38a**, compound **37a**, demonstrated lower potency toward the 3 enzymes with IC_{50} = 6.12 nM over wild-type BRAF, 1.51 nM over V600E-BRAF, and 2.52 nM over CRAF. Replacement of methyl group with bulkier group such as benzyl reduced the activity for both methoxy and hydroxyl compounds. The *N*-benzyl derivative of methoxyphenyl **37b** showed higher IC_{50} against the three RAF isoform with value of 17.32 nM for wild-type BRAF, 8.36 nM for V600E-mutated BRAF and 4.32 nM for CRAF. The hydroxyl analogue **38b** exhibited slightly higher potency over the 3 enzymes with IC_{50} 15.55 nM over wild-type BRAF, 7.25 nM over V600E-BRAF, and 3.91 nM over CRAF. Removal of methyl group decreased the activity compared to methyl-containing compounds but to less degree compared to benzyl derivatives. Replacement of five-membered ring sulfonamide with six-membered ring led to lower activity compared to *N*-methyl five-membered ring, but was higher compared to unsubstituted five-membered ring and *N*-benzyl derivative. Unsubstituted cyclic sulfamides and bulky *tert*-

butylcarbonyl substitutions (**37e, 37f, 37g, 38e, 38f**, and **38g**) are significantly less active compared to derivatives with small alkyl substitution (**37a** and **38a**).

Table 5. *In vitro* enzyme activity of compounds **37a-g** and **38a-g**.



Compound No	R^1	R^2	R^3	R^4	n	$BRAF^{wt}$		V600E-BRAF		CRAF	
						%[a]	IC_{50}[b]	% [a]	IC_{50}[b]	% [a]	IC_{50}[b]
37a	CH_3	H	H	OCH_3	1	96.77±0.71	6.12±0.23	97.62±0.13	1.51±0.02	97.85±0.81	2.52±0.24
37b	Bn	H	H	OCH_3	1	95.33±0.16	17.32±0.53	96.22±0.31	8.36±0.09	96.62±0.26	4.32±0.17
37c	H	CH_3	CH_3	OCH_3	1	98.41±0.18	13.68±0.25	95.41±0.52	8.65±0.07	98.14±0.18	5.63±0.2
37d	CH_3	H	H	OCH_3	2	97.52±0.27	8.26±0.43	97.89±0.33	2.62±0.26	96.51±0.36	2.33±0.16
37e	H	H	H	OCH_3	1	96.52±0.21	20.32±0.52	98.25±0.27	10.51±0.13	95.36±0.16	5.61±0.22
37f	H	H	H	OCH_3	2	97.21±0.34	18.15±0.41	99.51±0.19	9.96±0.27	97.25±0.23	7.22±0.31
37g	$(CH_3)_3CO$	H	H	OCH_3	2	95.96±0.35	23.33±0.44	97.46±0.20	12.57±0.26	94.96±0.29	11.21±0.28
38a	CH_3	H	H	OH	1	100±0.19	**2.00±0.12**	98.40±0.47	**0.07±0.0012**	100±0.56	**1.12±0.021**
38b	Bn	H	H	OH	1	96.68±0.41	15.55±0.35	96.32±0.41	7.25±0.19	98.66±0.45	3.91±0.39
38c	H	CH_3	CH_3	OH	1	98.72±0.57	10.32±0.11	96.55±0.55	6.35±0.17	99.12±0.28	4.26±0.33
38d	CH_3	H	H	OH	2	98.61±0.22	6.33±0.23	99.35±0.83	3.77±0.27	97.25±0.39	1.96±0.092

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38e	H	H	H	OH	1	98.91±0.67	7.25±0.30	99.21±0.49	4.56±0.13	97.84±0.29	6.32±0.24
38f	H	H	H	OH	2	99.01±0.54	9.56±0.09	99.64±0.52	5.21±0.19	96.89±0.32	7.21±0.17
38g	$(CH_3)_3CO$	H	H	OH	2	96.65±0.66	11.21±0.25	95.95±0.61	9.57±0.23	94.57±0.47	9.98±0.22

[a]: Mean %inhibition at single dose (10 μM). Data are represented as Mean value ± S.D.

[b]: IC_{50} (**nM**). Values are presented as mean value (duplicate test) ± S.D.

Compound **38a** with the highest enzyme activity against the three RAF isozymes was tested over a panel of 52 kinases of diversity of families at single dose concentration 10 μM to check the selectivity of the compound. In addition to the three RAF isozymes, compound **38a** showed strong inhibitory activity against FLT3, MAPK8, MAPK14, and MAPK11. In addition, moderate inhibitory effect was observed over ABL1, ERN1, HIPK1, FGFR1, NTRK2, and ZAK (Figure 2). The potency of compound **38a** over the most sensitive enzymes was determined by measuring of its IC_{50} over these sensitive enzymes (Table 6). Compound **38a** showed sub-micromolar activity against MAPK8 and MAPK11 with IC_{50} values equal 910 and 820 nM, respectively. Compound **38a** had one-digit micromole over FLT3, HIPK1, MAPK14, ZAK and NTRK2 with IC_{50} 1.51, 3.78, 1.02, 9.01, and 8.04 μM, respectively. Therefore, it can be concluded that compound **38a** is relatively selective against RAF kinases (V600E-BRAF, CRAF, and wild-type BRAF) compared to the other tested kinases, i.e. pan-RAF potent inhibitor. In addition to compound **38a**, two compounds belong to methoxy derivatives (**25g** and **25j**) and two hydroxyl derivatives (**27c** and **27f**) were tested against the same kinases. Compounds **27c** and **27f** should similar selectivity to compound **38a**. Interestingly, compounds **25g** and **25j** showed high activity over MAPK8, MAPK11 and MAPK14, which was almost equipotent to their RAF isoforms inhibitory activity (Table 6).

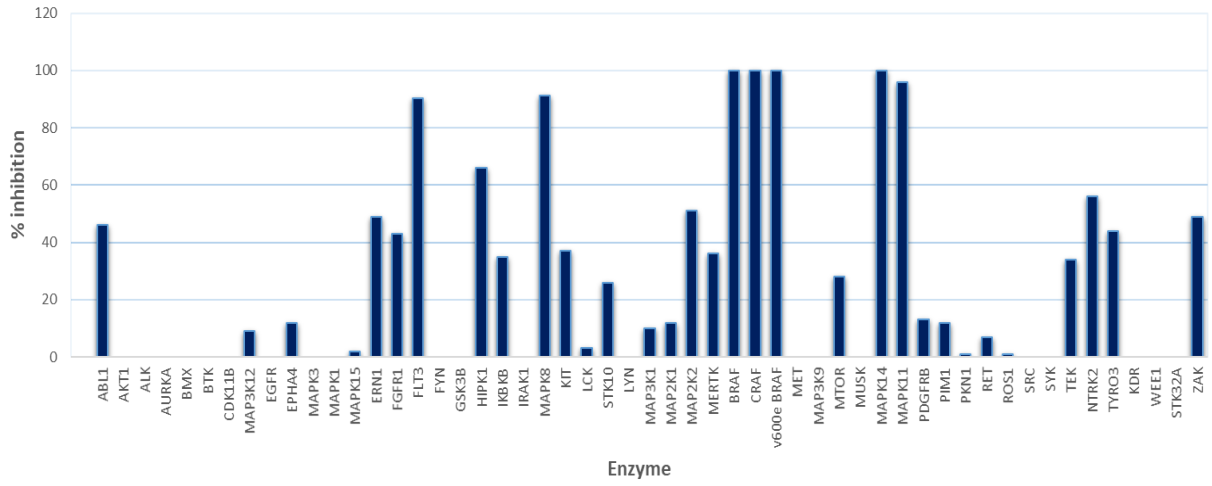

Figure 2. Percent inhibition of **38a** over 52 kinases at 10 μM.

Table 6. IC_{50} values (μM) of compounds **25g**, **25j**, **27c**, **27f**, and **38a** over the most sensitive kinases.

Enzyme	**25g**	**25j**	**27c**	**27f**	**38a**
ABL1	9.85±0.32	7.98±0.48	10.37±0.77	13.58±0.36	12.37 ± 0.67
ERN1	> 20	15.65±0.97	9.54±0.41	10.25±0.29	11.32 ± 0.49
FGFR	15.56±1.01	11.80±76	9.58±0.92	10.61±0.89	13.52 ± 0.76
FLT3	2.17±0.10	2.98±0.45	1.99±0.31	1.48±0.43	1.51 ± 0.06
HIPK1	4.58±0.51	3.84±0.36	5.13±0.24	4.88±0.75	3.78 ± 0.24
MAPK8	0.42±0.019	0.36±0.01	1.35±0.11	1.87±0.17	0.91 ± 0.08
MAPK11	0.12±0.007	0.095±0.005	1.21±0.12	0.98±0.04	0.82 ± 0.09
MAPK14	0.098±0.003	0.088±0.007	2.01±0.19	1.78±0.16	1.02 ± 0.12
ZAK	8.11±0.88	11.25±0.79	7.58±0.69	10.29±0.34	9.01 ± 0.72

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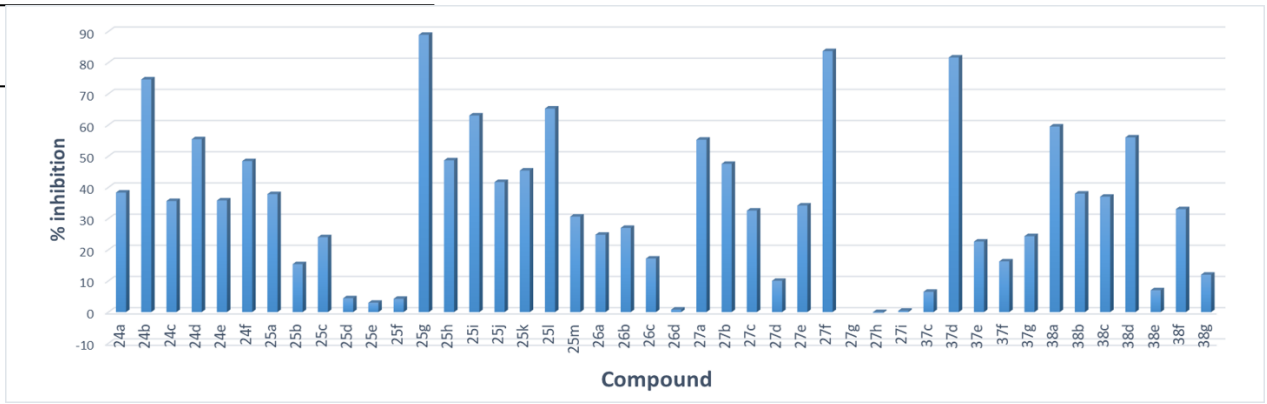
NTRK2	9.25±0.74	7.58±0.65	8.71±0.45	10.27±0.91	8.04 ± 0.97

Antiproliferative activity

The final compounds were submitted to the National Cancer Institute (NCI, USA), and selected for one-dose testing against 60 human cancer cell line assay (breast, CNS, colon, leukemia, melanoma, non-small cell lung, ovarian, prostate, and renal cancer). Compounds **25g, 27f,** and **37d** showed more than 80% mean inhibition percentages over the 60 cell lines. In addition, compounds **24b**, and **25i** have mean inhibition percentages over 60% (Figure 3).

A)



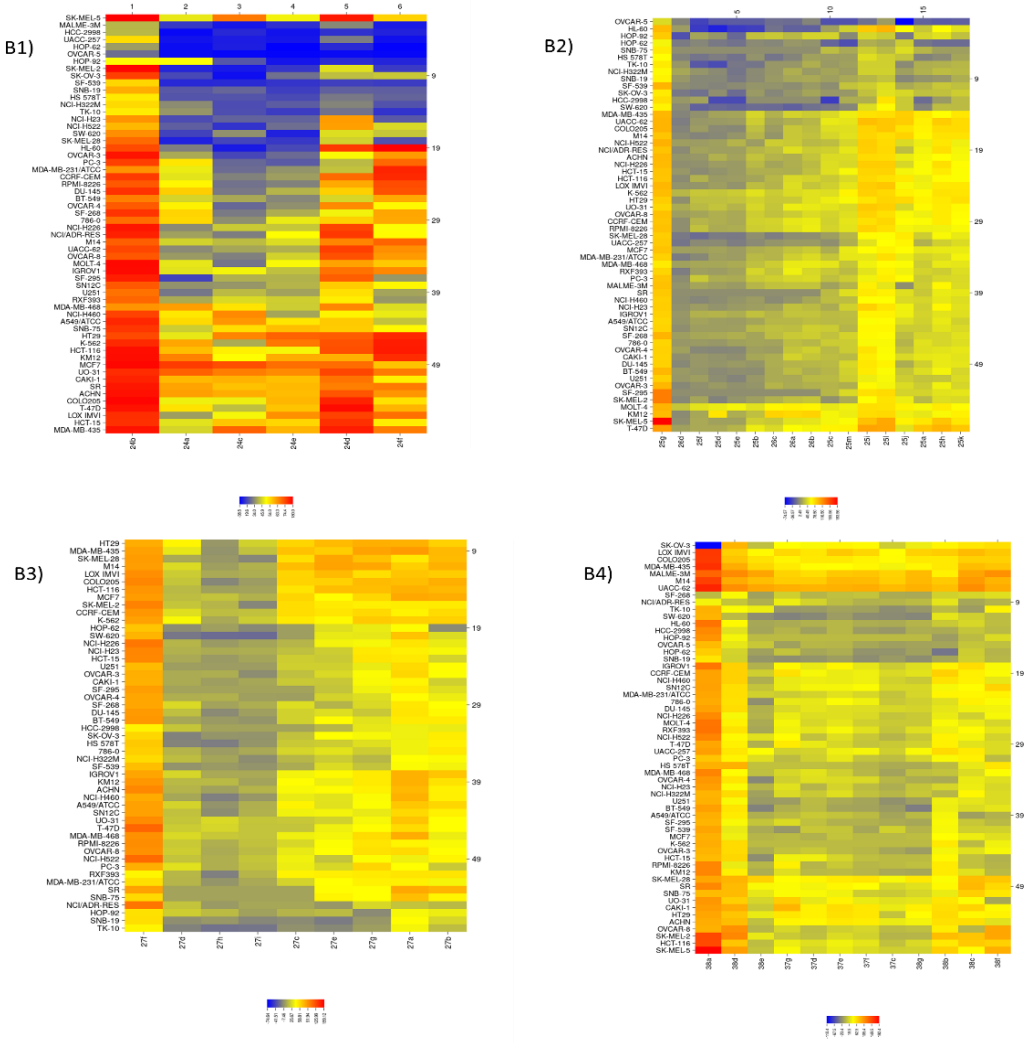


Figure 3. A) Mean inhibition percentage of the target compounds over cancer 60 cell lines at 10 µM concentration., B) Heat map of final target compounds over NCI 60 cell lines B1) Final target compounds **24a-f,** B2) Final target compounds **25a-m** and **26a-d**, B3) Final target compounds **27a-i**, B4) Final target compounds **37a-g**, and **38a-g**.

Profound investigation of each cancer sub-type is presented in Figure 4. In general, compounds **25** and **27** exhibited higher inhibition percentage over most cell lines. In case of leukemia cell lines, compounds **25g**, **25j**, and **27f** had the highest %inhibition with mean values 93.20%, 93.80%, and 93.81%, respectively followed by **24b** and **35i** with mean %inhibition values of 81.30% and 79.50%, respectively. For non-small cell lung cancer cell lines, compounds **25g**, **25j**, and **27f**

showed the highest activity with mean inhibition percentage 81.3%, 83.3%, and 83.1%. In case of colon cancer, compound **25g** had the strongest inhibitory effect with mean inhibition percentage of 86.2% followed by **25f** and **24b** with mean inhibition percentages of 83.09% and 76.7%, respectively. The activity of final target compounds over CNS cancer cell lines was lower compared to other subpanels. The highest mean inhibition percentages are encountered with compounds **25g** and **27f** with values 91.40% and 83.09%, respectively. The inhibitory effect of final compounds over melanoma cell lines was higher than the other cancer types. Compound **24b** was the most active amongst derivatives with unsubstituted phenyl ring at position 6 of the imidazothiazole nucleus with mean percentage inhibition of 83.8% followed by **24d** with percentage inhibition equals 59.7%. Within the 3-methoxyphenyl derivatives, compounds **25g** and **25i** exhibited a lethal effect over melanoma cell lines with mean inhibition percentages of 107.8% and 101.2%, respectively. Compound **27f** is the most potent among the hydroxyl group-containing compounds with mean percentage inhibition 101.29% followed by **27c** with mean percentage inhibition of 75.51%. Compounds **38a, 38d** and **38c** are the most active among cyclic sulfamide-possessing compounds with mean percentage inhibition values of 97.91%, 84.78%, and 79.71%, respectively. Finally, the activity over ovarian cell lines showed that compounds **25g** is the most potent with mean %inhibition = 80% followed by **27f** with mean %inhibition around 75%, and finally **24b** with mean inhibition percentage = 73.2%.

Compounds **24b, 25g, 25j, 27c, 27f, 38a,** and **38d** were selected to be tested at 5-dose mode in the NCI to determine their IC_{50} over NCI-60 cell lines (Table 7). Compound **24b** shows potency ranging from 2.19 μM over colon cancer cell lines, 13.3 μM over breast cancer cell lines, to 4.03 μM against melanoma cell lines. Compound **25g** exhibited the highest activity over leukemia cell lines with mean IC_{50} of 0.51 μM followed by prostate cancer with IC_{50} 0.9 μM. The lowest activity

for **25g** was 3.85 μM against ovarian cell lines. Compounds **27c, 27f, 38a,** and **38d** exhibited the highest potency over melanoma cell lines with mean IC_{50} 2.03, 1.49, 2.03, 0.85 and 1.79 μM, respectively. Compound **27f** is the most potent over leukemia, non-small cell lung cancer, CNS, ovarian, renal, prostate, and breast cancer. On the other hand, compounds **38a** and **38d** possessing cyclic sulfamide terminal moiety exhibited high selectivity toward melanoma cell lines compared to the other cell lines. Compound **38a** is 6.79 times more selective against melanoma subpanel than the second most sensitive subpanel, renal cancer cell lines.

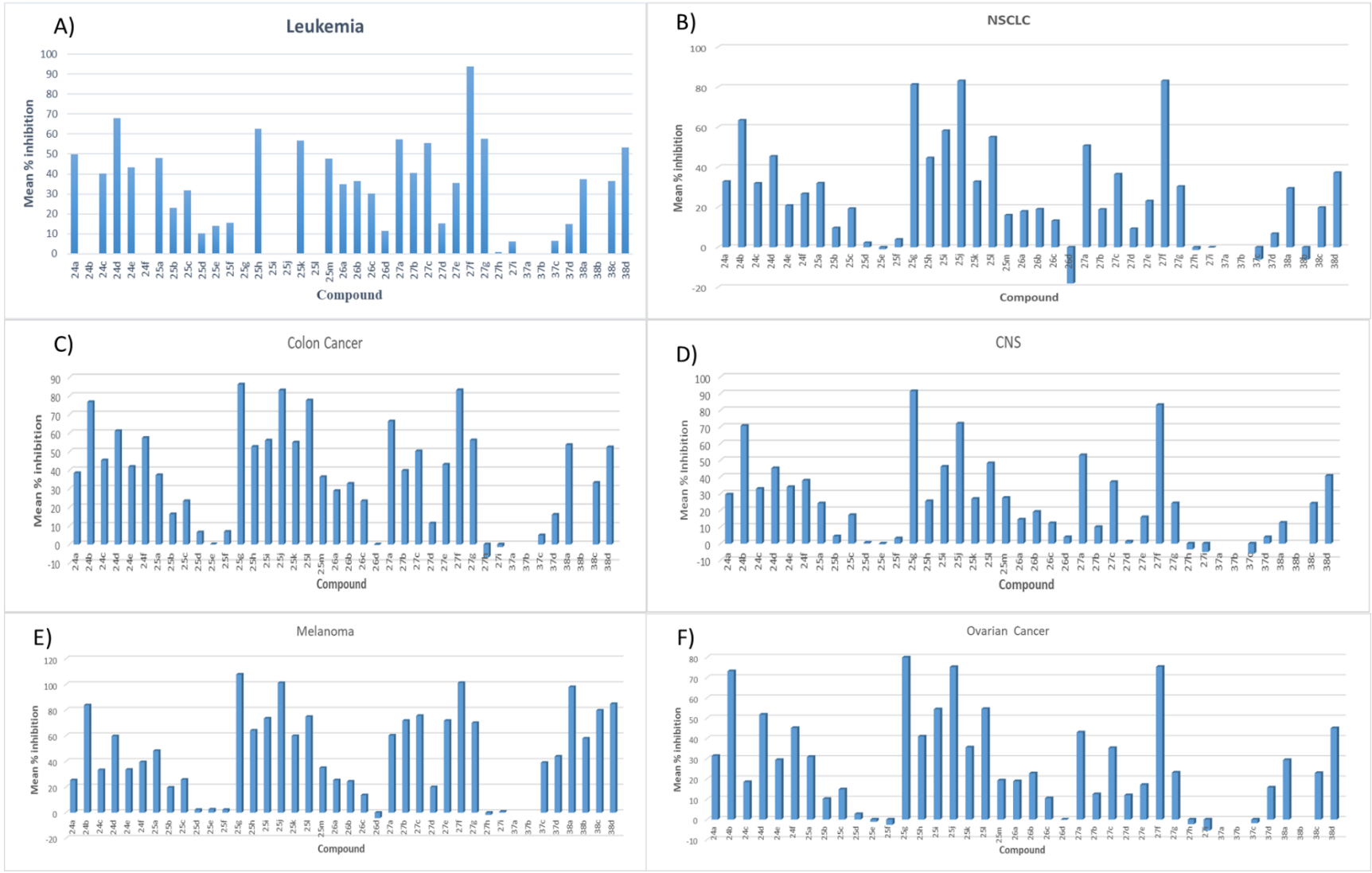


Figure 4. Mean inhibition percentages of the target compounds over different types of cancers.

Table 7. Mean IC_{50} of the test compounds over *in vitro* subpanel cancer cell lines [a]

		Compound No.					
		24b	**25g**	**27c**	**27f**	**38a**	**38d**
Subpanel cancer cell line	Leukemia	2.99	0.51	2.82	2.04	12.17	11.87
	Non-small lung	5.42	2.76	3.26	2.55	14.56	13.55
	Colon	2.19	2.75	5.42	3.02	10.15	8.08
	CNS	6.46	2.73	7.58	3.19	24.50	16.41
	Melanoma	4.03	2.24	1.49	2.03	**0.85**	1.79
	Ovarian	3.57	3.85	14.18	2.97	20.67	12.05
	Renal	3.70	1.76	5.38	2.93	5.77	5.98
	Prostate	2.45	0.90	7.30	2.38	14.30	11.57
	Breast	13.3	1.17	4.10	2.41	11.10	11.93

[a] Mean IC_{50} values were calculated by dividing the summation of IC_{50} values of the compound over cell lines of the same cancer type by the number of cell lines in the subpanel.

The IC_{50} and total growth inhibition (TGI) values of compounds **24b**, **25g**, **27c**, **27f**, **38a**, and **38d** that were tested in 5-dose testing mode against melanoma cell lines are presented in Table 8. The cyclic sulfamide derivatives **38a** and **38d** are obviously the most potent amid this series against melanoma cell lines. Both compounds exerted strong potency with IC_{50} values ranging from 2-digit nanomolar to one-digit micromolar scale. Compound **38a** with *N*-methyl 5-membered cyclic sulfamide is generally more potent than the *N*-methyl-substituted 6-membered analogue **38d**. It

seems that ring expansion in this part of the molecule is not very favorable for activity. The promising results of compound **38a** encouraged us to extend our biological investigations on it.

Table 8. IC_{50} and total growth inhibition (TGI) values (μM) of the most potent compounds over NCI melanoma cancer cell lines

Comp. / Cell line	**24b**		**25g**		**27c**		**27f**		**38a**		**38d**	
	IG_{50}	**TG**	**IG_{50}**	**TG**	**IG_{50}**	**TG**	**IG_{50}**	**TG**	**IG_{50}**	**TG**	**IG_{50}**	**TG**
LOX-IMVI	3.9	>100	3.26	19.2	4.11	20	1.91	5.82	2.15	19.2	4.36	28.2
MALME-3M	8.7	>100	2.35	5.22	0.279	--	2.6	7.86	**0.072**	**0.77**	**0.036**	**0.408**
M14	2.7	>100	0.69	3.25	0.567	15	2.54	10.2	**0.302**	13.3	**0.174**	12.9
MDA-MB-435	1.2	>100	1.44	5.88	0.939	11.6	2.00	6.51	**0.381**	>100	**0.490**	51.6
SK-MEL-2	4.9	>100	3.04	9.26	4.49	20.2	2.32	6.57	2.63	9.21	2.26	7.36
SK-MEL-28	5.7	>100	3.68	55.5	0.66	24	2.29	6.86	**0.176**	>100	**0.240**	52
SK-MEL-5	1.7	>100	1.24	2.52	1.61	3.98	1.06	2.28	1.73	17	0.348	10.3
UACC-257	6.4	>100	4.41	>100	0.568	16.5	2.85	>100	**0.170**	ND	8.21	>100
UACC-62	1.1	>100	0.48	3.40	0.270	10.3	0.75	2.73	**0.059**	5.0	**0.058**	3.14

ND: Not determined.

Immunoblot assay

Compounds **27c** as the most potent open sulfonamide derivative over melanoma cell lines and **38a** as the most potent cyclic sulfamide derivative over melanoma cell lines were chosen to check their ability to inhibit MAPK pathway inside melanoma cells (Figure 5). To assess the effect of **27c** and **38a** on the RAF/MEK/ERK signaling pathway, A375 cells were treated with the tested compounds at three different concentrations (0.1, 0.5, and 1 μM) for 24 h and immunoblotted with antibodies

against phospho-MEK1/2, phospho-ERK1/2, ERK, and MEK, respectively. Both compounds showed remarkable reduction on phosphorylation of both MEK and ERK at all tested concentrations. Dose-dependent inhibition is noticed in western blotting of both compounds against phospho-MEK1/2, phospho-ERK1/2. Compounds **27c** and **38a** showed higher inhibitory activity compared to vemurafenib at same concentration (5 μM) (Figure 5)

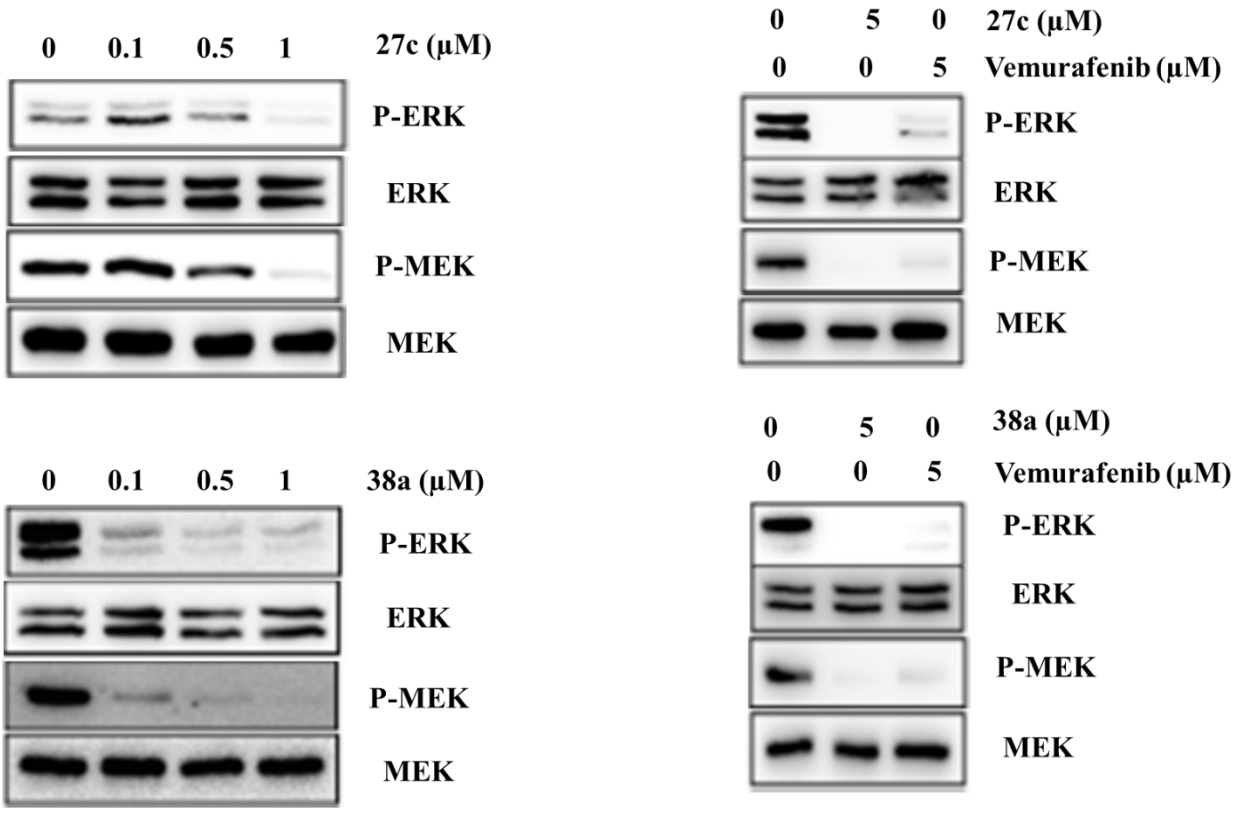


Figure 5. Effect of **27c** and **38a** on phosphorylation of MEK and ERK on different concentrations (0, 0.1, 0.5, and 1 μM) and comparing both compounds phosphorylation activity compared to vemurafenib at 5 μM

MTT assay against L132 normal cell line, normal skin fibroblast cell line (BJ1), and acute in vivo toxicity

In order to move to the next step and check the *in vivo* activity of the promising pan-RAF inhibitor, both normal cell cytotoxicity and acute animal toxicity were tested. The antiproliferative activity of tested compounds over human embryonic pulmonary epithelial cells (L132) and normal skin fibroblast cell line (BJ1) are presented in Table 9. All the tested compounds showed high IC_{50} over L132 and BJ1 cell lines which indicate high therapeutic index for these compounds. Comparison of the activities of compound **38a** over both melanoma cell lines (0.73 μM) and L132 cell line (45.00 μM) showed 61.6-fold higher selectivity toward melanoma than normal cells. On the other hand, compound **38a** is 688-fold more selective to MALME-3M melanoma cell line compared to BJ1 cell line. The acute toxicity of the most potent compound **38a** was adopted by injection of tested compound in male Hsd:Athymic Nude-Foxn1nu (Harlan co. (USA)) mice at five different doses 50 mg/kg, 100 mg/kg, 250 mg/kg, 500 mg/kg, and 1000 mg/kg). At doses 50, 100 and 250 mg/kg, there was no sign of toxicity over a group of 7 animals for each concentration. At 500 mg/kg one animal was lost, while at dose 1000 mg/kg three animal died out of the 7-animal group. It can be concluded that compound **38a** is safe enough up to 250 mg/kg dose.

Table 9. IC_{50} values (μM) of representative compounds over L132 and BJ1.

Compound No.	**L132** **IC_{50}**	**BJ1** **IC_{50}**	**Selectivity** [a]
24b	60.25 ± 3.01	55.64 ± 2.71	6.4
25g	74.51 ± 2.14	62.36 ± 2.33	26.5
25j	49.02 ± 2.35	42.32 ± 1.52	ND

26c	465.85 ± 3.46	125.65 ± 3.11	ND
27f	56.30 ± 1.57	45.22 ± 2.67	17.4
27c	52.37 ± 2.89	50.62 ± 2.96	181.5
38a	45.00 ± 1.36	49.56 ± 3.15	688.33
38d	93.59 ± 4.65	75.64 ± 2.66	2101.11

a: selectivity was determined by dividing IC_{50} value against normal skin fibroblast / IC_{50} value against MALME-3M melanoma cell line

In vivo antitumor activity of compound 38a

The next step was to test the ability of compound **38a** to inhibit tumor growth in animal model. Animal model was performed using A375 melanoma xenograft in male BALB/C nude mice using vemurafenib as standard drug and DMSO as negative control (Figure 6). Our candidate **38a** was injected daily to two groups of mice in two different doses (25 mg/kg and 50 mg/kg) five days following subcutaneous injection of a human-derived malignant melanoma cell line A375 cell line to nude mice ($2x10^6$ in 200 μL). The effect of compound **38a** on tumor volume and weight were measured. At the end of the experiment, the negative control (DMSO group) showed an average tumor volume of 400 mm^3 and average tumor weight of 3.15 g, while vemurafenib-treated group showed reduction of average tumor size to 126 mm^3 and average tumor weight to 1.2 g. The animals that received 25 mg/kg of compound **38a** showed reduction of tumor size by 68.2% (150 mm^3) and reduction of tumor weight by 46.8% (2.7 g). On the other hand, animals received 50 mg/kg exhibited reduction of tumor size by 75% (100 mm^3) and that of tumor weight by 68.75% (1.00 g). Thus compound **38a** at a dose of 50 mg/kg exhibited more promising results than the standard drug, vemurafenib. That dose is well tolerated by the animals as per the *in vivo* toxicity experiment.

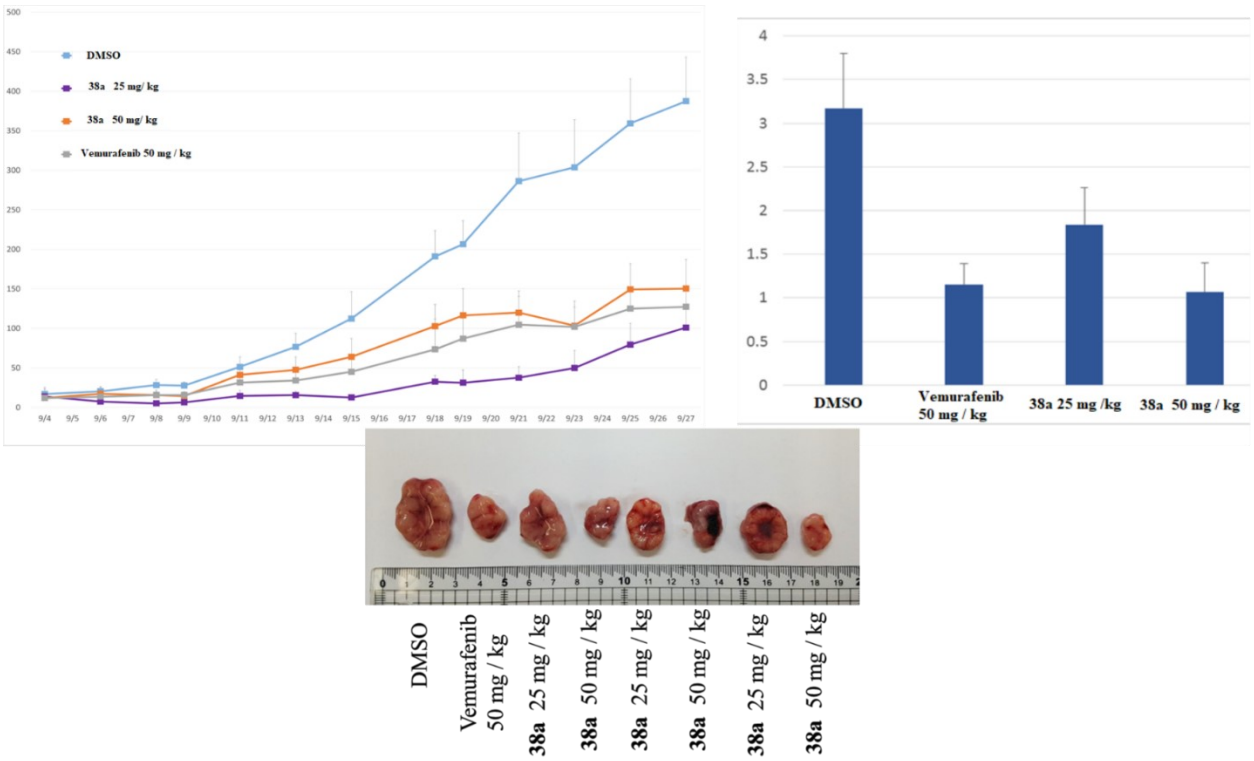


Figure 6. *In vivo* antitumor activity of compound **38a** in mice bearing the A375 xenograft; A) **38a** was administered daily by i.p. injection at the doses indicated (25 or 50 mg/kg). Vemurafenib was was administered at a dose of 50 mg/kg daily by i.p. injection. Tumor volumes were measured each two days. Data are represented as mean ± S.E.M. for each treatment group; B) tumor weights measured after 3 weeks for **38a**-treated group, vehicle, and vemurafenib-treated group. The graph shows the mean ± S.E.M. calculated from tumors in each group. C) Representative tumor images of A375 xenografts.

In vivo pharmacokinetic and Caco-2 cell permeability test of compound 38a

Compound **38a** showed high potency over RAF kinases and cancer cell lines. In addition, it had remarkable *in vivo* anti-melanoma activity. The above-mentioned results encouraged us to investigate the pharmacokinetic of compound **38a** and its permeability and plasma stability.

Pharmacokinetic parameters for compound **38a** were determined in rat and are summarized in Table 10 and Figure 7. The compound was given in dose 5 mg/kg for both IV and PO. Compound **38a** reached its highest concentration after 2.08 h, its half-life is 1.72 h and 3.35 h following IV and oral administration, respectively, and its bioavailability is 6.59%. IV clearance was 2.07 (mL min^{-1} kg^{-1}) with volume of distribution equal to 0.578 L/kg. Caco-2 permeability test was applied to check **38a** human absorption. The obtained data in Table 11 revealed that more than 80% human absorption with permeability value 1.4 x 10^{-5} cm/sec and Efflux ratio (ER) 1.53. The plasma stability test for compound **38a** were performed in both human plasma and rate plasma. Compound **38a** has high stability profile in both human and rat plasma. After 30 min, 97.2 % of compound **38a** remained unchanged after 30 min which decreased to 95.4% after 120 min, while that of procaine was 1.2% after 30 min and 0.2% after 2 h and the percentage for diltiazem was 91.5% after 30 min and 89.3% after 2 h (Table 12).

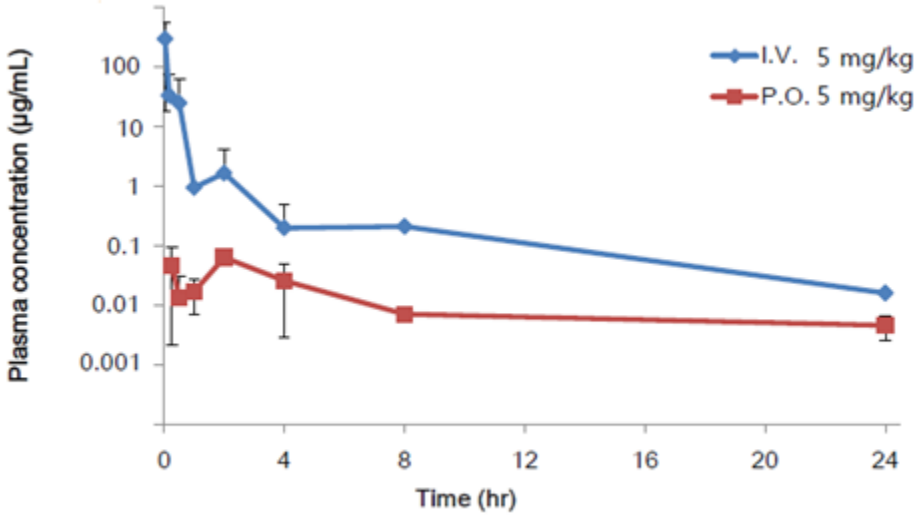


Figure 7. Plasma concentration after intravenous and oral administration at a dose of 5 mg/kg (n=3)

Table 10. Pharmacokinetic parameters of compound **38a** after administration of 5 mg/kg by both IV and PO

Parameter	IV (5 mg/kg)	PO (5 mg/kg)
T_{max} (h)	----------	2.08 ± 0.44
C_{max} (µg/mL)	----------	0.09687 ± 0.0025
$T_{1/2}$ (h)	1.72 ± 0.41	3.35 ± 0.57
AUC $_{0 \rightarrow T}$	6.57 ± 0.81	0.433 ± 0.021
AUC $_{0 \rightarrow \infty}$	6.59 ± 0.82	0.196 ±0.035
CL (mL min^{-1} kg^{-1})	2.07 ±0.25	----------
Vd (L/kg)	0.578 ± 0.05	----------
F (%)	----------	6.59%

Table 11. Permeability and efflux ratio (ER) of compound **38a** (P_{app}, $\times 10^{-5}$ cm/sec).

Compound	A to B	B to A	Efflux ratio (ER)
38a	1.421	2.175	1.53
Caffeine	2.605	2.484	0.95
Ofloxacin	0.584	0.727	1.25
Atenolol	0.013	0.014	1.08

Table 12. Plasma stability of compound **38a** (% remaining)

Compound	Human		Rat	
	30 min	120 min	30 min	120 min
38a	97.2	95.4	90.6	76.6
Procaine	1.2	0.2	84.9	42.7
Diltiazem	91.5	89.3	86.3	45.4

Molecular docking of compound 38a

In order to gain a better understanding of the potency of the synthesized compounds and guide further SAR studies, molecular docking into the domain of V600E-BRAF kinase for compound **38a** was performed. All calculations were done using MOE 2008.10 software installed on 2.0G Core 2 Duo. The crystal structure of V600E-BRAF kinase in complex with vemurafenib (PDB code: 1UWJ) was obtained from protein data bank (PDB) (http://www.rcsb.org/pdb). The automated docking program of MOE 2008.10 was used for docking of **38a** into the domain of V600E-BRAF kinase. The complex was energy-minimized with a MMFF94x force-field till the gradient convergence 0.01 kcal/mol was reached. The docking study has revealed that the ligands have bound in the active site of one of the protomers in the protein dimer through the formation of strong hydrogen bonds between the binding site and the ligand. For compound **38a**, the molecular docking data are illustrated in Figure 8. Compound **38a** showed four hydrogen bonding interactions in the binding site (one hydrogen bond between 3-hydroxyphenyl group at position 6 of imidazothiazole nucleus and Ser 455, two hydrogen bonds between one oxygen from sulfamide group and Asp 594 and Lys 483, and one hydrogen bond between protonated sulfur of the imidazothiazole nucleus and Ser 535). In addition to hydrogen bonding, aromatic interaction between pyrimidine ring and Val 471 was recorded.

This is an author accepted peer-reviewed manuscript of the following research article: Abdel-Maksoud et al., Discovery of New Imidazo[2,1-b]thiazole Derivatives as Potent Pan-RAF Inhibitors with Promising In Vitro and In Vivo Anti-melanoma Activity, *J. Med. Chem.* 2021, 64, 10, 6877–6901

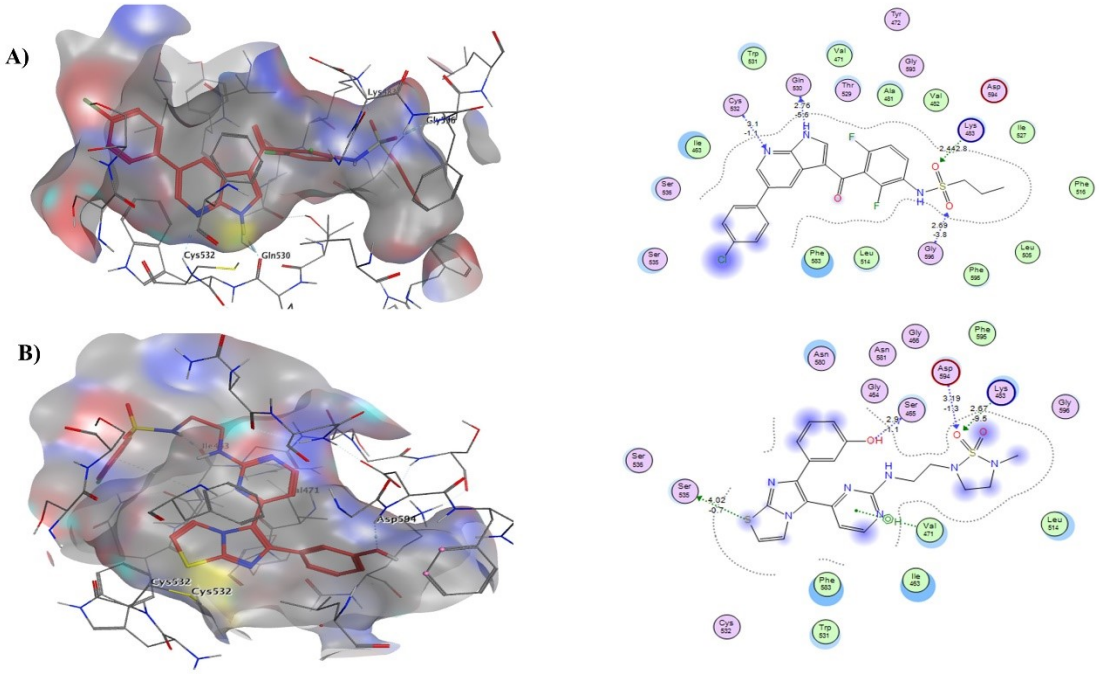


Figure 8. Molecular docking and 2D presentation of binding mode for A) Vemurafenib; B) **38a;** into V600E-BRAF kinase domain (PDB code: 1UWJ).

Conclusion

In this study, a new series of rationally designed imidazo[2,1-*b*]thiazole was synthesized and screened for antiproliferative activity as well as kinase inhibitory effect. The new synthesized compounds were divided into two distinct groups; open chain sulfonamide (**24a-f, 25a-m, 26a-d,** and **27a-i**) and cyclic sulfamide (**37a-g** and **38a-g**).

Open chain sulfonamide final target compounds were divided into four distinct groups. The first group possesses unsubstituted phenyl ring at position 6 of the imidazo[2,1-*b*]thiazole scaffold (compounds **24a-f**). These compounds had lower IC_{50} over V600E-BRAF and CRAF compared to wild type BRAF. Small electron-withdrawing group and propylene spacer between pyrimidine

ring and terminal sulfonamide moiety gave the highest activity (compound **24d**) over the three RAF isoforms with IC_{50} values of 256, 57, and 87 nM over wild-type BRAF, V600E-BRAF and CRAF, respectively.

The second and third groups open chain sulfonamide compounds contain methoxy group on phenyl ring at position 6 of imidazo[2,1-*b*]thiazole nucleus. Compounds **25a-m** have 3-methoxyphenyl and compounds **26a-d** contain 4-methoxyphenyl moiety. Changing the position of methoxy group from *meta* to *para* showed a dramatic decrease in enzyme activity and compounds **26a-d** showed much higher IC_{50} values compared to compounds **25a-m**. Upon designing of *meta*-methoxy compounds and based on our previous work only *para*-substituted aryl sulfonamides and small electron-withdrawing *meta* aryl sulfonamide were synthesized. In these compounds, both ethylene and propylene spacers showed insignificant difference in activity over RAF isoforms. The most potent compound in this group is **25i** that carries 4-fluorobenzenesulfonamide and propylene spacer with IC_{50} of 430, 90, and 95 nM over BRAF, V600E-BRAF, and CRAF, respectively followed by compound **25g** that possesses 4-CF_3 and ethylene spacer with IC_{50} values of 681, 98, and 142 nM over BRAF, V600E-BRAF, and CRAF, respectively.

The last group of open chain sulfonamide derivatives is **27a-i** that carry hydroxyl (either *para* or *meta*) phenyl at position 6 imidazo[2,1-*b*]thiazole scaffold. Compounds with 3-OH showed higher activity compared to 4-OH analogues. In these derivatives, compounds with electron-withdrawing group on terminal sulfonamide terminal exerted the highest activity. Moreover, both ethylene and propylene analogues showed similar potency over RAF isoforms. Compound **27f** (*m*-F and propylene spacer) showed the highest activity with IC_{50} of 4, 0.98, and 5 nM over BRAF, V600E-

BRAF, and CRAF, respectively followed by **27c** (*p*-F & ethylene spacer) with IC_{50} values equal 2.7, 1, and 6 nM over BRAF, V600E-BRAF and CRAF, respectively.

The main structural modification applied to open chain sulfonamide series was to make cyclic sulfamide derivatives **37a-g** and **38 a-g**. Compounds **37a-g** contain *m*-methoxyphenyl at position 6 of the imidazothiazole nucleus and either five or six-membered ring cyclic sulfamide terminal moiety. Cyclic sulfamide moiety was substituted with H, small alkyl, *tert*-butylcarbonyl, or benzyl terminals. Small alkyl-substituted compounds are more potent compared to other substitutes. In addition, *m*-OH derivatives are more active compared to their methoxy congeners. The most potent compound is **38a** with IC_{50} values of 2, 0.07, and 1.2 nM over BRAF, V600E-BRAF, and CRAF, respectively.

The kinase panel screening for compound **38a** showed high activity over ABL1, ERN1, HIPK1, FGFR1, NTRK2, and ZAK in addition to RAF isoforms activity. To check the selectivity of most potent pan-RAF inhibitors, IC_{50} of **25g** and **25j** (as representatives of methoxy-containing derivatives), **27c** and **27f** (as representatives of hydroxyl-containing analogues), and **38a** (the most potent compound and cyclic sulfamide representative) were calculated over the most sensitive kinases. Compounds **27c**, **27f**, and **38a** showed high selectivity for RAF isoforms compared to the other sensitive kinases. On the other hand, methoxy-containing compounds showed high activity over MAPK8, MAPK11, and MAPK 14 beside their RAF inhibitory effect.

The antiproliferative activity over NCI-60 cell lines for final target compounds showed that compound **24b, 25g**, **27c, 27f, 38a**, and **38d** had the highest activity among all final targets. Compounds **38a** and **38d** exhibited the lowest IC_{50} values over melanoma cell lines (72, 302, 381,

176, 170, and 59 nM for **38a** over MALME-3M, M14, MDA-MB-435, SK-MEL-28, UACC-257, and UACC-62, respectively).

Immunoblot assay of both compounds **27c** and **38a** proved that their antiproliferative activities came from their potential inhibitory effect against ERK pathway. Both compounds are able to inhibit phosphorylation of both MEK and ERK at different dose intervals. At 5 μM concentration, both compounds are stronger than vemurafenib regarding inhibition of both MEK and ERK phosphorylation. Normal cell line cytotoxicity revealed high selectivity toward cancer cell lines over normal cell lines with selectivity index reaching 600 for **38a** and 2000 for **38d.** *In vivo* investigations show stronger anticancer activity at 50 mg/kg dose compared to vemurafenib as positive control at the same dose range, and no toxicity was encountered at the same dose. Compound **38a** showed high plasma stability and good permeability and its pharmacokinetic profile showed moderate half-life with oral bioavailability equals 6.59%. Molecular docking of compound **38a** into the binding site of V600E-BRAF showed that 3-hydroxyl group on phenyl ring at position 6 is important for binding and added additional hydrogen bonding. This explains the previously discussed higher potency of hydroxyl derivatives than the methoxy analogues.

This study ends up with a promising pan-RAF drug lead **38a** for treatment of melanoma. It can be further investigated in clinical studies, and further tuning of the structure is currently being considered. The structure modifications include replacement of OH group with NH_2 to improve solubility and microsomal stability, changing the substitution on cycle sulfamide moiety (ethyl, propyl, or direct aryl) to improve both enzyme and cellular activity, and checking the effect of multisubstituents on both phenyl ring at position 6 and terminal sulfamide. Improving the oral bioavailability is also one of our top goals in the next phase of this study.

Experimental

General

All solvents and reagents were commercially available and used without further purification. The target compounds and intermediates were purified by column chromatography using silica gel (0.040–0.063 mm, 230–400 mesh) and technical grade solvents. Analytical thin layer chromatography (TLC) was performed on silica gel 60 F254 plates from Merck. IR spectra (KBr disks) were recorded with a Bruker FT-IR instrument (Bruker Bioscience, Billerica, MA, USA). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker Avance 400 or 300 spectrometers using tetramethylsilane as an internal standard and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), brs (broad singlet), or dd (doublet of doublets). LC-MS analysis was carried out using the following system: Waters 2998 photodiode array detector, Waters 3100 mass detector, Waters SFO system fluidics organizer, Waters 2545 binary gradient module, Waters reagent manager, Waters 2767 sample manager, Sunfire™ C18 column (4.6 × 50 mm, 5 μm particle size); Solvent gradient = 95% A at 0 min, 1% A at 5 min; solvent A: 0.035% trifluoroacetic acid (TFA) in water; solvent B: 0.035% TFA in CH3OH; flow rate = 3.0 mL/min; the AUC was calculated using Waters MassLynx 4.1 software. Solvents and liquid reagents were transferred using hypodermic syringes. Purity % of all the target compounds were determined by HPLC and found to be >95%. All animal experiments were conducted in compliance with institutional guidelines.

General procedure for synthesis of compounds ***8-10***

A mixture of 2-aminothiazole (**4**, 4.74 g, 47.4 mmol) and appropriate 2-bromoacetopenone derivative (**5-7**, 47.4 mmol) in absolute ethanol (60 mL) was refluxed for 16 h with vigorous stirring. The reaction mixture was concentrated to 30 mL under reduced pressure. To the remaining suspension, 50 mL of ice water was added followed by 30% ammonia solution (100 mL). The mixture was stirred for an additional 2 h, and the formed precipitate was filtered off and dried under reduced pressure at 50 ℃ to obtain the titled compounds.

*6-Phenylimidazo[2,1-b]thiazole (**8**)*: Yield 78%; solid; mp. 143-144 ℃; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 8.63 (d, 1H, *J* = 7.2 Hz), 8.29 (t, 1H, *J* = 5.7 Hz), 7.77 (t, 3H, J = 6.7 Hz), 7.49-7.36 (m, 3H); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 148.1, 138.4, 129.9, 129.6, 127.7, 125.7, 121.9, 119.6, 111.5; IR (KBr) cm^{-1}: 3139, 3120, 1955, 1884, 1602; LC-MS: m/z calculated for $C_{11}H_8N_2S$:200.04, found 201.0 $(M+1)^+$.

*6-(3-Methoxyphenyl)imidazo[2,1-b]thiazole (**9**):* Yield 80%; solid; mp 136-137 ℃; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 8.64 (d, 1H, *J* = 1.2 Hz), 8.27 (dd, 1H, *J* = 1.3, 4.2 Hz), 7.70 (dd, 1H, *J* = 1.2, 4.2 Hz), 7.42 (d, 3H, *J* = 6.0 Hz), 7.00 (dd, 1H, *J* = 1.6, 6.00 Hz), 3.83 (s, 3H); ^{13}C-NMR (DMSO-d_6, 75 MHz) *δ* 160.2, 148.3, 139.45, 130.9, 129.9, 121.9, 118.7, 117.9, 115.4, 111.6, 111.2, 55.9; IR (KBr) cm^{-1}: 3131, 3118, 2996, 2940, 1677, 1597; LC-MS: m/z calculated for $C_{12}H_{10}N_2OS$: 230.05, found 231.0 $(M+1)^+$.

*6-(4-Methoxyphenyl)imidazo[2,1-b]thiazole (**10**):* Yield 70%; solid; mp 145-146 ℃; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 8.08 (s, 1H), 7.88 (d, 1H, *J* = 3.0 Hz), 7.74 (d, 2H, *J* = 9.0 Hz), 7.21 (d, 1H, *J* = 6.0 Hz), 6.95 (d, 2H, *J* = 9.0 Hz), 3.76 (s, 3H); ^{13}C-NMR (DMSO-d_6, 75 MHz) *δ* 158.9, 149.4, 146.7, 127.3, 126.5, 120.4, 114.5, 113.0, 108.6, 55.5; IR (KBr) cm^{-1}: 3133, 3109, 2990, 1683, 1603; LC-MS: m/z calculated for $C_{12}H_{10}N_2OS$: 230.05, found 231.0 $(M+1)^+$.

*General procedure for preparation of compounds **11-13***

In a 3-neck flask, 4-chloro-2-(methylthio)pyrimidine (6.5 g, 41.3 mmol), cesium carbonate (13.4 g, 41.3 mmol), palladium acetate (1.22 g, 5.5 mmol), and triphenylphosphine (2.896 g, 11.04 mmol) were mixed with appropriate 6-phenylimidazo[2,1-*b*]thiazole derivative (**8-10**, 41.3 mmol). The system was evacuated from air and replaced by nitrogen twice. Anhydrous DMF (60 mL) was added and the mixture was purged with nitrogen thrice. The reaction mixture was stirred at 80 ℃ for 16 h. the mixture was cooled to room temperature and 300 mL of distilled water was added followed by 200 mL of ethyl acetate. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (2 x 200 mL). The combined organic solvent was dried using anhydrous sodium sulfate and evaporated. The residue was subject to column chromatography using hexane: ethyl acetate (5:1 v/v).

*5-(2-(Methylthio)pyrimidin-4-yl)-6-phenylimidazo[2,1-b]thiazole (**11**)*: Yield 15%; white solid; mp 143-144 ℃; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.62 (dd, 1H, *J* = 0.7, 4.5 Hz), 8.23 (dd, 1H, *J* = 0.7, 5.4 Hz), 7.65-7.62 (m, 2H), 7.47-7.45 (m, 3H), 6.97 (dd, 1H, *J* = 0.7, 4.5 Hz), 6.89 (dd, 1H, *J* = 0.7, 4.8 Hz), 2.64 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) *δ* 172.4, 156.2, 156.2, 152.7, 151.1, 134.6, 129.2, 128.9, 128.8, 122.2, 120.1, 112.7, 112.0, 14.2; IR (KBr) cm^{-1}: 3054, 6103, 1538, 1685; LC-MS: m/z calculated for $C_{16}H_{12}N_4S_2$: 324.05, found 325.0 $(M+1)^+$.

*6-(3-Methoxyphenyl)-5-(2-(methylthio)pyrimidin-4-yl)imidazo[2,1-b]thiazole (**12**)*: Yield 9%; white solid; mp 108-109 ℃; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.61 (d, 1H, *J* = 3.4 Hz), 8.24 (d, 1H, *J* = 4.1 Hz), 7.35 (t, 1H, *J* = 5.9 Hz), 7.18 (d, 2H, *J* = 5.8 Hz), 4.78 (d, 2H, *J* = 3.4 Hz), 6.92(d, 1H, *J* = 4.1 Hz), 3.82 (s, 3H), 2.24 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 172.4, 159.9, 156.3, 156.1, 152.7, 150.9, 135.8, 129.8, 122.2, 121.5, 120.2, 115.2, 114.0, 112.8, 112.2, 55.4, 14.2; IR (KBr)

cm^{-1}: 3153, 3114, 2919, 1602, 670; LC-MS: m/z calculated for $C_{17}H_{14}N_4OS_2$: 354.06, found 355.10 $(M+1)^+$.

6-(4-Methoxyphenyl)-5-(2-(methylthio)pyrimidin-4-yl)imidazo[2,1-b]thiazole ***(13)****:* Yield 15%; white solid; mp 151-152 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.57 (d, 1H, *J* = 2.7 Hz), 8.21 (d, 1H, *J* = 3.2 Hz), 7.53 (dd, 2H, *J* = 2.0, 6.8 Hz), 6.98-6.89 (m, 4H), 3.86 (s, 3H), 2.60 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) *δ* 172.3, 160.1, 156.2, 152.6, 151.1, 130.4, 126.9, 122.3, 119.8, 114.2, 112.5, 111.8, 55.3, 14.2; IR (KBr) cm^{-1}: 1906, 1607, 1556, 630; LC-MS: m/z calculated for $C_{17}H_{14}N_4OS_2$: 354.06, found 355.10 $(M+1)^+$.

General procedure for preparation of compounds ***14-16***

To a solution of compound **11-13** (6 mmol) in methanol (250 mL), a solution of oxone (12.3 g, 18 mmol) in water (50 mL) was added. The mixture was stirred at room temperature for 16 h. The organic solvent was removed under reduced pressure and the remaining aqueous solution was extracted with CH_2Cl_2 (50 mL). The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (3 x 25 mL). The combined organic layer extracts were washed with brine, dried over anhydrous sodium sulfate, and filtered. The organic solvent was evaporated under reduced pressure and the residue was purified by flash column chromatography using hexane: ethyl acetate 5:3 v/v.

5-(2-(Methylsulfonyl)pyrimidin-4-yl)-6-phenylimidazo[2,1-b]thiazole ***(14)****:* Yield 95%; white solid; mp 176-177 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.99 (d, 1H, *J* = 4.4 Hz), 8.58 (d, 1H, *J* = 5.4 Hz), 7.73 (d, 2H, *J* = 2.5 Hz), 7.66-7.47 (m, 3H), 7.45 (d, 1H, *J* = 5.2 Hz), 7.17 (d, 1H, *J* = 4.4 Hz), 3.47 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) *δ* 165.7, 157.5, 156.8, 153.3, 134.1, 133.3, 130.0,

129.5, 129.1, 129.0, 128.3, 119.5, 117.5, 113.8, 39.3; IR (KBr) cm^{-1}: 3137, 3110, 2931, 1979, 1902, 1797, 1444, 1373; LC-MS: m/z calculated for $C_{16}H_{12}N_4O_2S_2$: 356.05, found 357.1 $(M+1)^+$. *6-(3-Methoxyphenyl)-5-(2-(methylsulfonyl)pyrimidin-4-yl)imidazo[2,1-b]thiazole* ***(15)****:* Yield 90%; white solid; mp 125-126 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.86 (d, 1H, *J* = 3.4 Hz), 8.49 (d, 1H, *J* = 4.2 Hz), 7.39 (d, 2H, *J* = 3.3 Hz), 7.17 (d, 2H, *J* = 5.5 Hz), 7.07-7.02 (m, 2H), 3.84 (s, 3H), 3.36 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) *δ* 165.6, 160.1, 157.4, 156.9, 156.7, 154.3, 153.0, 135.3, 130.2, 123.1, 121.2, 119.54, 117.8, 115.6, 114.0, 55.4, 39.3; IR (KBr) cm^{-1}: 3159, 3088, 1601, 1574, 1487, 1317; LC-MS: m/z calculated for $C_{17}H_{14}N_4O_3S_2$: 386.05, found 387.1 $(M+1)^+$. *Compound* ***16****:* Yield 85%; white solid; mp 192-193 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.88 (d, 1H, *J* = 3.0 Hz), 8.51 (d, 1H, *J* = 6.0 Hz), 7.57 (d, 1H, *J* = 9.0 Hz), 7.42 (d, 1H, *J* = 6.0 Hz), 7.04 (d, 4H, *J* = 9.0 Hz), 3.92 (s, 3H), 3.39 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) *δ* 165.7, 160.6, 157.6, 156.7, 154.4, 153.4, 130.4, 126.4, 123.3, 119.3, 117.3, 114.5, 113.5, 55.4, 39.3; IR (KBr) cm^{-1}: 3185, 3113, 1647, 1610, 1567, 1449, 1348; LC-MS: m/z calculated for $C_{17}H_{14}N_4O_3S_2$: 386.05, f ound 387.1 $(M+1)^+$.

Preparation of 5-bromo-6-(3-methoxyphenyl)imidazo[2,1-b]thiazole ***(17)***

A solution of bromine (1.01 g, 6.4 mmol) in dichloromethane 30 mL was added over 1h to a solution of compound **9** (2.00 g, 6.4 mmol) in dry dichloromethane (50 mL) at 0 °C. The reaction mixture was stirred for 24 h at room temperature. Water was added and the organic layer was separated, dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The residue was subjected to column chromatography using hexane: ethyl acetate 10:1 v/v to give 1.60 g (60 %) of the desired product as pale yellow solid. mp 90-91°C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.15 (d, 1H, *J* = 9.0 Hz), 7.12 (d, 1H, *J* = 3.0 Hz), 7.30-7.35 (m, 2H), 6.92 (dd, 1H, *J* = 3.0, 9.0

Hz), 6.86 (d, 1H, *J* = 3.0 Hz), 3.90 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 159.7, 148.7, 143.6, 134.4, 129.4, 119.3, 117.5, 113.9, 113.0, 111.9, 90.3, 55.3; IR (KBr) cm^{-1}: 1911, 1574, 1455, 564; LC-MS: m/z calculated for $C_{12}H_9BrN_2OS$: 307.18, found 308.10 $(M+1)^+$.

*Preparation of 6-(3-methoxyphenyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)imidazo[2,1-b]thiazole (**18**);* To a solution of compound **17** (1 g, 3.2 mmol) in DMSO (6.8 mL), potassium acetate (1.27 g, 12.94 mmol), bis-(pinacolato)diboron (1.62 g, 6.2 mmol), and 1,10-bis(diphenylphosphino)ferrocene palladium dichloride (Pd(dppf)Cl_2, 0.23 g, 0.32 mmol) were added. The mixture was degassed and charged with nitrogen, and stirred at 80 ℃ for 12 h. The reaction was cooled to ambient temperature, diluted with ethyl acetate (100 mL), and filtered through celite. The ethyl acetate extract was washed with water (5 x 100 mL) and dried over anhydrous sodium sulfate. The organic layer was filtrated and concentrated. the residue was purified on a silica gel column eluting with hexane:ethyl acetate 6:1 v/v to provide 800 mg (70%) as a white solid. mp 140-141 ℃; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.71 (s, 1H), 7.44 (s, 1H), 7.39 (t, 2H, *J* = 4.5 Hz), 7.34 (t, 1H, *J* = 6.0 Hz), 6.85 (d, 1H, *J* = 6.0 Hz), 6.79 (d, 2H, *J* = 3.0 Hz), 3.88 (s, 3H), 1.27 (d, 12H, *J* = 12.0 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 159.9, 150.1, 147.5, 135.4, 129.6, 118.5, 117.6, 113.5, 112.6, 110.3, 108.3, 82.6, 75.0, 55.3, 24.8, 24.6; IR (KBr) cm^{-1}: 3133, 3109,1768, 1603, 1467.

*Synthesis of 6-(3-Methoxyphenyl)-5-(2-(methylthio)pyrimidin-4-yl)imidazo[2,1-b]thiazole (**12**);* To a solution of 4-chloro-2-(methylthio)pyrimidine (45 mg, 0.28 mmol) and 6-(4-methoxyphenyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)imidazo[2,1-*b*]thiazole (**18**,100 mg, 0.28 mmol) in DME (3 mL) was added aqueous solution of Cs_2CO_3 (210 mg, 0.65 mmol) in

water (1 mL), followed by the addition of 1,10-bis(diphenylphosphino)ferrocene palladium dichloride (Pd(dppf)Cl_2,16.1 mg, 0.022 mmol). The reaction flask was degassed and charged with nitrogen then heated at 80 °C for 12 h. The reaction mixture was diluted with ethyl acetate, washed with water and the organic layer was separated, dried over anhydrous sodium sulfate and evaporated. The residual oil was purified by column chromatography using hexane:ethyl acetate 5:1 v/v to give 30 mg product (30 %) as white solid.

*General procedure for synthesis of N-(2-aminoethyl)benzene (substituted benzene) sulfonamide (**22a-g**) and N-(2-aminopropyl)benzene (subtituted benzene) sulfonamide (**23a-f**)*

To a solution of ethylenediamine (**19**) or 1,3-diaminopropane (**20**) (75 mmol) and triethylamine (1.07 mL, 7.6 mmol) in anhydrous dichloromethane (5 mL), a solution of appropriate sulfonyl chloride (**21a-g)** (2.5 mmol) in anhydrous dichloromethane (5 mL) was added dropwise at 0 °C. The reaction mixture was stirred overnight. Ethyl acetate and saturated solution of Na_2CO_3 were added and the organic layer was separated, washed with distilled water, dried over Na_2SO_4 and evaporated. The residue was purified by flash column using dichloromethane:methanol 10:1 v/v.

*N-(2-aminoethyl)benzenesulfonamide (**22a**):* Yield 80% as white solid; mp 80-81 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.77 (d, 2H, *J* = 9.0 Hz), 7.46-7.38 (m, 3H), 3.99 (brs, 3H), 3.86 (t, 2H, *J* = 3.0 Hz), 2.67 (t, 2H, *J* = 3.0 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) *δ* 140.0, 132.4, 129.1, 126.8, 45.2, 40.9.

*N-(2-aminoethyl)-4-fluorobenzenesulfonamide **(22b)**:* 72% as white solid; mp. 108-109 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.84-7.79 (m, 2H), 7.11 (t, 2H, *J* = 6.0 Hz), 2.89 (t, 2H, *J* = 6.0 Hz),

2.71(t, 2H, *J* = 6.0 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 166.7, 136.0, 129.7, 116.3, 45.2, 40.9; LC-MS: m/z calculated for $C_8H_{11}FN_2O_2S$: 218.06, found: 219.1 $(M+1)^+$.

N-(2-aminoethyl)-3-fluorobenzenesulfonamide ***(22c)****:* Yield 65% as viscous light brawn oil; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.65-7.44 (m, 3H), 7.24-7.19 (m, 1H), 4.29 (brs, 2H), 2.59 (t, 2H, *J* = 6.0 Hz) , 2.76 (t, 2H, *J* = 6.0 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 163.5, 161.0, 142.1 (*J* = 18 Hz), 131.1, 122.7 (*J* = 39 Hz), 114.3 (*J* = 69 Hz), 45.3, 41.0.

N-(2-aminoethyl)-4-methoxybenzenesulfonamide ***(22d)****:* Yield 82% as white solid; mp 90-91 ℃; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.78 (d, 2H, *J* = 9.0 Hz), 6.96 (d, 2H, *J* = 6.0 Hz), 3.85 (s, 3H), 3.10 (brs, 2H), 2.93 (t, 2H, *J* = 6.0 Hz), 2.76 (t, 2H, *J* = 6.0 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 163.3, 132.2, 129.6, 114.8, 56.2, 46.0, 41.6; LC-MS: m/z calculated for $C_9H_{14}N_2O_3S$: 230.07, found: 231.10 $(M+1)^+$.

N-(2-aminoethyl)-4-methylbenzenesulfonamide ***(22e)****:* Yield 74% as white solid; mp 120-121 ℃; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.75 (d, 2H, *J* = 9.0 Hz), 7.39 (d, 2H, *J* = 9.0 Hz), 3.45 (s, 2H), 2.89 (t, 2H, *J* = 6.0 Hz), 2.66 (t, 1H, *J* = 6.0 Hz), 2.44 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 143.3, 137.0, 129.7, 127.0, 45.5, 41.0, 21.5; LC-MS: m/z calculated for $C_9H14_4N_2O_2S$: 214.08, found: 215.10 $(M+1)^+$.

N-(2-aminoethyl)naphthalene-2-sulfonamide ***(22f)****:* Yield 90% as white solid; mp 132-133 ℃; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.43 (s, 1H), 8.15 (t, 2H, *J* = 6.0 Hz), 8.04 (d, 1H, *J* = 9.0 Hz), 7.82 (d, 1H, *J* = 9.0 Hz), 7.70 (d, 2H, *J* = 6.0 Hz), 3.32 (s, 2H), 2.75 (t, 2H, *J* = 6.0 Hz), 2.50 (t, 2H, *J* = 3.0 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 136.8, 134.7, 132.1, 129.5, 129.2, 128.7, 128.3, 127.8, 127.5, 122.3, 45.5, 41.0; LC-MS: m/z calculated for $C_{12}H_{14}N_2O_2S$: 250.08, found: 251.10 $(M+1)^+$.

N-(2-aminoethyl)-4-(trifluoromethyl)benzenesulfonamide ***(22g)****:* Yield 60% as viscous oil; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.99 (d, 1H, *J* = 6.0 Hz), 7.64 (d, 1H, *J* = 9.0 Hz), 7.52 (t, 2H, *J* = 6.0 Hz), 3.15 (s,1H), 2.91 (s, 2H), 2.67 (s, 2H); ^{13}C-NMR ($CDCl_3$, 75 MHz) *δ* 138.7, 132.6, 130.6, 128.2, 128.1, 44.9, 40.8.

N-(3-aminopropyl)benzenesulfonamide ***(23a)****:* Yield 84% as buff solid; mp 71-72 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.90 (d, 2H, *J* = 6.0 Hz), 7.60-7.51 (m, 3H), 3.11 (t, 2H, *J* = 6.0 Hz), 2.82 (t, 2H, *J* = 6.0 Hz), 1.62 (q, 2H, *J* = 6.0 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) *δ* 139.8, 132.4, 129.1, 126.9, 41.1, 38.7, 30.2.

N-(3-aminopropyl)-4-fluorobenzenesulfonamide ***(23b)****:* Yield 82% as white solid; mp 104-105 °C; ^{1}H-NMR (CD_3OD, 300 MHz) δ 7.90 (dd, 2H, *J* = 5.1, 8.9 Hz), 7.32 (t, 2H, *J* = 8.7 Hz), 2.92 (t, 2H, *J* = 6.0 Hz), 2.67 (t, 2H, *J* = 6.0 Hz), 1.61 (q, 2H, *J* = 6.0 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) *δ* 162.9, 136.2, 129.4 , 116.1, 41.1, 39.2, 31.5.

N-(3-aminopropyl)-3-fluorobenzenesulfonamide ***(23c)****:* Yield 55% as viscous oil; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.52 (brs, 3H), 7.27 (s, 1H), 3.28 (s, 2H), 3.08 (s, 2H), 2.79 (s, 2H), 1.62 (s,2H); ^{13}C-NMR ($CDCl_3$, 75 MHz) *δ* 160.7, 142.3, 130.8 (*J* = 30 Hz), 122.6, 119.5, 114.3, 42.7, 40.5, 31.0.

N-(3-aminopropyl)-4-methoxybenzenesulfonamide ***(23d)****:* Yield 75% as white solid; mp 76-77 °C; ^{1}H-NMR (CD_3OD, 300 MHz) δ 7.79 (d, 2H, *J* = 8.9 Hz), 7.08 (d, 2H, *J* = 8.9 Hz), 3.88 (s, 3H), 2.88 (t, 2H, *J* = 6.8 Hz), 2.66 (t, 2H, *J* = 6.9 Hz), 1.60 (t, 2H, *J* = 6.9 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) *δ* 162.6, 131.6, 129.0, 114.2, 55.6, 41.6, 39.7, 31.1.

N-(3-aminopropyl)-4-methylbenzenesulfonamide ***(23e)****:* Yield 70% as white solid; mp 119-120 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.64 (d, 2H, *J* = 7.6 Hz), 7.19 (d, 2H, *J* = 8.0 Hz), 3.27 (brs, 3H),

2.89 (t, 2H, *J* = 6.00 Hz), 2.64 (t, 2H, *J* = 6.0 Hz), 2.31 (s, 3H), 1.48 (t, 2H, *J* = 6.0 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 143.1, 137.1, 129.6, 127.0, 42.1, 40.1, 31.4, 21.4; LC-MS: m/z calculated for $C_{10}H_{16}N_2O_2S$: 228.07, found: 229.10 $(M+1)^+$.

N-(3-aminopropyl)naphthalene-2-sulfonamide ***(23f)****:* Yield 79% as white solid; mp 114-115 ℃; ^{1}H-NMR ($CDCl_3$,400 MHz) δ 8.41 (s, 1H), 7.94-7.83 (m, 4H), 7.58 (d, 2H, *J* = 8.0 Hz), 3.12 (brs, 1H), 3.06 (t, 2H, *J* = 6.0 Hz), 2.73 (t, 2H, *J* = 6.0 Hz), 1.56 (t, 2H, *J* = 6.4 Hz); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 136.9, 134.7, 132.1, 129.4, 129.1, 128.5, 128.4, 128.1, 127.8, 127.4, 122.3, 42.5, 40.4, 31.2; LC-MS: m/z calculated for $C_{13}H_{16}N_2O_2S$: 264.09, found: 265.10 $(M+1)^+$.

*Synthesis of 4-methoxy-N-(2-((4-(6-phenylimidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino) ethyl)benzene sulfonamide (****24a)***

A mixture of compound **14** (0.32 g, 0.92 mmol), **22b** (0.54 g, 2.48 mmol), and *N,N*-diisopropylethylamine (0.57 mL, 3.3 mmol) in DMSO (10 mL) was stirred at 80 ℃ for 8 h. The mixture was cooled to room temperature, quenched with water (20 mL), and extracted with ethyl acetate (3 x 20 mL). The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography. The title product was obtained as orange solid (0.24 g, 52%). mp 121-122 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, *J* = 4.4 Hz, 1H), 7.93 (d, *J* = 5.2 Hz, 1H), 7.87 (d, *J* = 7.2 Hz, 2H), 7.62 (dd, *J* = 7.2, *J* = 4.0 Hz, 2H), 7.52 (d, *J* = 7.2,Hz , 2H), 7.48 – 7.44 (m, 6H), 6.55 (d, *J* = 4.8 Hz, 1H), 6.39 (s, 1H, NH), 3.64 (s, 2H), 3.25 (d, *J* = 6.0 Hz, 2H),^{13}C-NMR (DMSO-d_6, 75 MHz) δ 164.2, 162.5, 160.9, 158.2, 156.2, 151.6, 148.0, 140.9, 132.7, 131.7 (*J* = 7.5 Hz), 129.6, 126.8, 121.0, 115.9 , 114.5, 105.7, 38.7, 29.5; LC-MS:

m/z calculated for $C_{23}H_{19}FN_6O_2S_2$, 494.10, found 495.10 $(M+1)^+$; HRMS calculated for $C_{23}H_{19}FN_6O_2S_2$, is 494.0994, found 495.1072 $(M+1)^+$.

*4-methoxy-N-(2-((4-(6-phenylimidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl)benzene sulfonamide **(24b)**:* Obtained from the reaction of compounds **14** and **22d** using the same procedure for compound **24a** to yield 65% as white solid. mp 199-200 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.11 (d, *J* = 1.6 Hz, 1H), 7.93 (d, *J* = 5.8 Hz, 1H), 7.73 (d, *J* = 9.2 Hz, 2H), 7.63 – 7.60 (m, 2H), 7.44-7.42 (m, 4H), 7.24 (d, *J* = 6.8 Hz, 2H, Ar-H), 6.54 (d, *J* = 2.8 Hz, 1H, Ar-H), 6.27 (s, 1H, NH), 5.90 (s, 1H, NH), 3.93 (s, 3H), 3.61 (d, *J* = 5.2 Hz, 2H), 3.22 (d, *J* = 5.6 Hz, 2H); ^{13}C-NMR (DMSO-d_6, 100 MHz) *δ* 163.8, 162.5, 161.4, 158.1, 156.2, 151.6, 148.1, 142.9, 138.0, 131.7, 130.1, 127.0, 121.0, 115.9, 114.4, 105.7, 55.3, 41.7, 29.5; LC-MS: m/z calculated for $C_{24}H_{22}N_6O_2S_2$, 506.12, found 507.10 $(M+1)^+$; HRMS calculated for $C_{24}H_{22}N_6O_2S_2$, 506.1194, found 507.1272 $(M+1)^+$.

*4-Methyl-N-(2-((4-(6-phenylimidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl)benzene sulfonamide **(24c)**:* Obtained from the reaction of compounds **14** and **22e** using the same procedure for compound **24a** to yield 60 % as light brawn solid. mp 242-243 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.14 (s, 1H), 7.96 (s, 1H), 7.71-7.62 (m, 4H), 7.49 (s, 4H), 7.44 (s, 2H), 6.54 (s, 1H), 6.18 (s, 1H), 5.77 (s, 1H), 3.47 (s, 2H), 3.04 (s, 2H), 2.35 (s, 3H); ^{13}C-NMR ($CDCl_3$, 75 MHz) *δ* 162.7, 162.2, 157.1, 152.2, 150.2, 134.8, 131.7, 129.3, 129.0, 128.6, 128.6, 122.0, 120.6, 114.2, 112.7, 107.2, 40.2, 38.2, 29.9; LC-MS: m/z calculated for $C_{24}H_{22}N_6O_2S_2$, 490.12, found 491.10 $(M+1)^+$; HRMS calculated for $C_{24}H_{22}N_6O_2S_2$, 490.1245, found 491.1323 $(M+1)^+$.

*4-Fluoro-N-(3-((4-(6-phenylimidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)propyl)benzene sulfonamide **(24d)**:* Obtained from the reaction of compounds **14** and **23b** using the same procedure for compound **24a** to yield 68% as buff solid. mp 188-189 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.06

(d, 1H, *J* = 5.2 Hz), 7.85 (dd, 3H, *J* = 5.6, 8.8 Hz), 7.59 (dd, 2H, *J* = 1.6, 7.6 Hz), 7.48-7.37 (m, 7H), 6.31 (d, 1H, *J* = 5.2 Hz), 2.87 (t, 2H, *J* = 6.8 Hz), 2.51 (t, 2H, *J* = 2.0 Hz), 1.70 (t, 2H, *J* = 6.4 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 166.0, 164.7, 159.6, 158.7, 147.2, 140.1, 139.3, 131.0, 129.7 (*J* = 21 Hz), 128.8, 127.6, 125.1, 122.7, 119.9, 116.0 (*J* = 22.5 Hz), 114.8, 112.2, 106.0, 40.0, 38.2, 30.0; IR (KBr) cm^{-1}: 3252, 3152, 3113, 2957, 1728, 1549, 1491, 1455, 1336; LC-MS: m/z calculated for $C_{23}H_{21}FN_6O_2S_2$: 508.12, found 509.10 $(M+1)^+$; HRMS calculated for $C_{23}H_{21}FN_6O_2S_2$: 508.1151, found 509.1229 $(M+1)^+$.

*4-Methoxy-N-(3-((4-(6-phenylimidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)propyl)benzenes ulfonamide (**24e**)*: Obtained from the reaction of compounds **14** and **23d** using the same procedure for compound **24a** to yield 45% as white solid. mp 88-89 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.55 (d, 1H, *J* = 6.0 Hz), 8.02 (d, 1H, *J* = 6.0 Hz), 7.75 (d, 2H, *J* = 9.0 Hz), 7.67 (dd, 2H, *J* = 3.0, 6.0 Hz), 7.45 (t, 3H, *J* = 3.0 Hz), 6.98 (d, 1H, *J* = 6.0 Hz), 6.92 (d, 2H, *J* = 9.0 Hz), 6.50 (d, 1H, *J* = 9.0 Hz), 5.53 (brs, 1H), 3.83 (s, 3H), 3.57 (q, 2H, *J* = 6.0 Hz), 3.05 (q, 2H, *J* = 6.0 Hz), 1.80 (t, 2H, *J* = 6.0 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 162.7, 162.2, 157.1, 152.2, 150.2, 134.8, 131.7, 129.3, 129.0, 128.6, 128.6, 122.0, 120.6, 114.2, 112.7, 107.2, 55.5, 40.2, 38.2, 29.9; LC-MS: m/z calculated for $C_{25}H_{24}N_6O_3S_2$: 520.14, found: 521.10 $(M+1)^+$.

*4-Methyl-N-(3-((4-(6-phenylimidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)propyl)benzene sulfonamide (**24f**)*: Obtained from the reaction of compounds **14** and **23e** using the same procedure for compound **24a** to yield 30% as viscous oil; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.52 (d, 1H, *J* = 4.0 Hz), 7.99 (s, 1H), 7.72 (d, 2H, *J* = 8.0 Hz), 7.29 (t, 1H, *J* = 8.0 Hz), 7.16 (s, 1H), 6.94-6.88 (m, 5H), 6.51 (d, 1H, *J* = 5.2 Hz), 5.50 (brs, 1H), 3.52 (brs, 2H), 3.00 (brs, 2H), 2.04 (s, 3H), 1.75 (d, 2H, *J* = 4.8 Hz).

*N-(2-((4-(6-(3-methoxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl)benzene sulfonamide (**25a**)*: Obtained from the reaction of compounds **15** and **22a** using the same procedure for compound **24a** to yield 40% as white solid; mp.152-153 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.53 (d, 1H, J = 6.0 Hz), 8.04 (d, 1H, J = 6.0 Hz), 7.87 (d, 2H, J = 6.0 Hz), 7.57-7.49 (m, 4H), 7.36 (t, 1H, J = 7.5 Hz), 7.20 (s, 2H), 6.99 (d, 1H, J = 9.0 Hz), 6.96 (d, 1H, J = 6.0 Hz), 6.60 (d, 1H, J = 6.0 Hz), 5.50 (t, 1H, J = 6.0 Hz), 3.86 (s, 3H), 3.64 (t, 2H, J = 6.0 Hz), 3.28 (t, 2H, J = 6.0 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 162.1, 159.7, 157.1, 152.1, 139.9 136.7, 132.6, 129.6, 129.1, 126.9, 122.1, 121.7, 114.9, 114.2, 112.6, 107.9, 55.4, 41.4, 39.9; IR (KBr) cm^{-1}: 3246, 3126, 3061, 2859, 1603, 1430, 1325; LC-MS: m/z calculated for $C_{24}H_{22}N_6O_3S_2$: 506.12, found 507.10 $(M+1)^+$.

*4-Fluoro-N-(2-((4-(6-(3-methoxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl) benzenesulfonamide (**25b**)*: Obtained from the reaction of compounds **15** and **22b** using the same procedure for compound **24a** to yield 35% as white solid; mp.163-164 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.51 (d, 1H, J = 4.0 Hz), 8.00 (d, 1H, J = 4. Hz), 7.87 (dd, 2H, J = 4.0, 8.0 Hz), 7.34 (d, 1H, J = 8.0 Hz), 7.31 (d, 1H, J = 16.0 Hz), 7.19-7.13 (m, 4H), 7.01-6.95 (m, 2H), 6.58 (d, 1H, J = 8.0 Hz), 5.33 (brs, 1H), 3.85 (s, 3H), 3.64 (dd, 2H, J = 4.0, 12.0 Hz), 3.26 (dd, 2H, J = 4.0, 12.0 Hz); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 166.2, 163.7, 161.6, 159.7, 157.2, 156.3, 152.3, 150.3, 135.0, 129.7 (J = 9.6 Hz), 122.3, 121.7, 120.6, 116.3, 114.6, 107.5. 55.4, 43.1, 41.3; LC-MS: m/z calculated for $C_{24}H_{21}FN_6O_3S_2$: 524.12, found: 525.10 $(M+1)^+$.

*3-Fluoro-N-(2-((4-(6-(3-methoxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl) benzenesulfonamide (**25c**)*: Obtained from the reaction of **15** and **22c** using the same procedure for compound **24a** to yield 30% as white solid; mp 146-147 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.43 (s, 1H), 7.94 (s, 1H), 7.59 (s, 1H), 7.51 (s, 1H), 7.39 (s, 1H), 7.27 (d, 1H, J = 3.6 Hz), 7.13 (brs, 3H), 6.93 (s, 1H), 6.88 (s, 1H), 6.50 (s, 1H). 5.66 (s, 1H), 3.80 (s, 3H), 3.55 (s, 2H), 3.20 (s, 2H),

1.25 (s, 1H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 163.6, 162.0, 161.1, 159.7, 157.0, 152.1, 150.0, 142.2, 135.9, 130.9, 129.7, 122.6, 122.3, 122.0, 121.8, 120.6, 119.7, 114.7, 114.5, 114.2, 114.1, 112.6, 107.7 (J = 12 Hz), 55.3, 43.4, 41.3; IR (KBr) cm^{-1}: 3294, 3085, 2866, 1946, 1574, 1455, 1331; LC-MS: m/z calculated for $C_{24}H_{21}FN_6O_3S_2$.:524.11, found: 525.10 $(M+1)^+$.

4-Methoxy-N-(2-((4-(6-(3-methoxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl)benzenesulfonamide ***(25d)*** *:* Obtained from the reaction of **15** and **22d** using the same procedure for compound **24a** to yield 35% as white solid; mp 88-89 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.55 (d, 1H, J = 4.4 Hz), 8.04 (d, 1H, J = 5.4 Hz), 7.79 (d, 2H, J = 8.8 Hz), 7.37 (s, 1H), 7.30 (s, 1H), 7.21 (s, 1H), 6.94 (d, 4H, J = 9.0 Hz), 6.60 (d, 1H, J = 5.4 Hz), 5.46-5.44 (brs, NH), 3.86 (s, 3H), 3.83 (s, 3H), 3.64 (brs, 2H), 3.25 (brs, 2H); (KBr) cm^{-1}: 3585, 3249, 3082, 2855, 1736, 1455, 1322; LC-MS: m/z calculated for $C_{25}H_{24}N_6O_4S_2$: 526.13, found: 527.10 $(M+1)^+$.

N-(2-((4-(6-(3-methoxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl)-4-methylbenzenesulfonamide ***(25e)****:* Obtained from the reaction of **15** and **22e** using the same procedure for compound **24a** to yield 46% as viscous oil; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.25 (s, 1H), 8.01 (t, 1H, J = 3.0 Hz), 7.73 (dd, 2H, J = 3.0, 6.0 Hz), 7.56 (t, 2H, J = 4.2 Hz), 7.00-6.92 (m, 5H), 6.57 (t, 1H, J = 3.0 Hz), 5.52 (brs, NH), 3.90 (s, 3H), 3.63 (brs, 2H), 3.26 (brs, 2H), 2.39 (s, 3H); LC-MS: m/z calculated for $C_{25}H_{24}N_6O_3S_2$: 520.14, found: 521.10 $(M+1)^+$.

N-(2-((4-(6-(3-methoxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl) naphthalene-2-sulfonamide ***(25f)****:* Obtained from the reaction of **15** and **22f** using the same procedure for compound **24a** to yield 55% as white solid; mp 136-137 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.40 (s, 2H), 7.94 (d, 2H, J = 6.0 Hz), 7.84 (d, 3H, J = 9.0 Hz), 7.77 (d, 1H, J = 9.0 Hz), 7.32 (d, 1H, J = 9.0 Hz), 7.17 (d, 2H, J = 3.0 Hz), 6.97 (d, 1H, J = 9.0 Hz), 6.56 (d, 1H, J = 6.0 Hz), 6.48 (d, 1H, J = 3.0 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 161.9, 159.7, 156.9, 152.0,150.0,

136.7, 136.1, 134.6, 132.0, 129.6, 129.4, 129.0, 128.6, 128.2, 127.7, 127.4, 122.1, 121.7, 114.8, 114.3, 112.5, 107.5, 55.3, 43.5, 41.2.; IR (KBr) cm^{-1}: 3600, 3254, 3129, 3087, 1672, 1601, 1451, 1428, 1322; LC-MS: m/z calculated for $C_{28}H_{24}N_6O_3S_2$: 556.14, found: 557.10 $(M+1)^+$.

*N-(2-((4-(6-(3-methoxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl)-4-(trifluoromethyl)benzenesulfonamide (**25g**)*: Obtained from the reaction of **15** and **22g** using the same procedure for compound **24a** to yield 20% as colorless viscous oil; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 8.05 (d, 1H, J = 5.2 Hz), 7.73 (t, 2H, J =7.2 Hz), 7.59 (dd, 2H, J = 2.0, 8.0 Hz), 7.47 (d, 4H, J = 7.2 Hz), 7.06 (d, 2H, J = 8.8 Hz), 6.31 (d, 1H, J = 5.2 Hz), 3.79 (s, 3H), 3.39 (brs, 2H), 2.94 (brs, 2H); LC-MS: m/z calculated for $C_{25}H_{21}F_3N_6O_3S_2$: 574.1, found: 575.10 $(M+1)^+$; HRMS calculated for $C_{25}H_{21}F_3N_6O_3S_2$: 574.1069 found: 575.1148 $(M+1)^+$.

*N-(3-((4-(6-(3-methoxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)propyl)benzene sulfonamide (**25h**)*: Obtained from the reaction of **15** and **23a** using the same procedure for compound **24a** to yield 55% white solid; mp 136-137; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.55 (d, 1H, J = 6.0 Hz), 8.02 (d, 1H, J = 6.0 Hz), 7.75 (d, 2H, J = 9.0 Hz), 7.62 (q, 2H, J = 3.0 Hz), 7.45 (t, 3H, J = 3.0 Hz), 6.98 (d, 1H, J = 6.0 Hz), 6.92 (d, 2H, J = 9.0 Hz), 6.50 (d, 1H, J = 6.0 Hz), 5.53 (brs, NH), 3.83 (s, 3H), 3.57 (q, 2H, J = 6.0 Hz), 3.05 (q, 2H, J = 6.0 Hz), 1.80 (t, 2H, J = 6.0 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 162.7, 162.2, 157.1, 152.2, 150.2,134.8, 131.7, 129.3, 129.0, 128.6, 128.6, 122.0, 120.6, 114.2, 112.7, 107.2, 55.5, 40.2, 38.2, 29.9; LC-MS: m/z calculated for $C_{25}H_{24}N_6O_3S_2$: 520.14; found: 521.10 $(M+1)^+$.

*4-Fluoro-N-(3-((4-(6-(3-methoxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)propyl)benzenesulfonamide (**25i**)* : Obtained from the reaction of compounds **15** and **23b** using the same procedure for compound **24a** to yield 59% white solid; mp 76-77 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.53 (d, 1H, J = 4.4 Hz), 8.03 (d, 1H, J = 5.6 Hz), 7.82 (dd, 2H, J = 1.6, 5.2

Hz), 7.35 (t, 1H, *J* = 8.0 Hz), 7.18-7.11 (m, 4H), 7.00-6.96 (m, 2H), 6.55 (d, 2H, *J* = 5.2 Hz), 5.53 (brs, NH), 3.84 (s, 3H), 3.56 (q, 2H, *J* = 6.40 Hz), 3.05 (q, 2H, *J* = 6.40 Hz), 1.79 (p, 2H, *J* = 6.00 Hz); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 166.1, 163.6, 162.2, 159.7, 157.2, 157.0, 152.1, 150.0, 136.3, 136.0, 129.6, 129.5, 121.9, 121.7, 120.6, 116.3, 114.7, 114.4, 112.7, 107.4, 55.3, 40.1, 38.1, 30.0; IR (KBr) cm^{-1}: 3390, 3264, 3116, 2933, 1574, 1522, 1430, 1330; LC-MS: m/z calculated for $C_{25}H_{23}FN_6O_3S_2$: 538.13, found: 539.10 $(M+1)^+$.

*3-Fluoro-N-(3-((4-(6-(3-methoxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)propyl)benzenesulfonamide (**25j**):* Obtained from the reaction of compounds **15** and **23c** using the same procedure for compound **24a** to yield 30% white solid; mp 78-79 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.42 (d, *J* = 4.0 Hz, 1H), 8.09 (d, *J* = 8.0 Hz, 1H), 7.76 (d, *J* = 12.0 Hz, 2H), 7.75-7.35 (m, 3H), 7.30 (d, *J* = 4.0 Hz, 1H), 7.18-6.95 (m, 2H), 6.53 (t, *J* = 8.0 Hz, 2H), 5.51 (d, *J* = 8.0 Hz, 1H), 3.81 (s, 3H), 3.56 (q, *J* = 8.0 Hz, 2H), 3.04 (q, J = 8.0 Hz, 2H), 1.84 (t, *J* = 8.0 Hz, 2H); LC-MS: m/z calculated for $C_{25}H_{23}FN_6O_3S_2$: 538.13, found: 539.10 $(M+1)^+$; HRMS calculated for $C_{25}H_{23}FN_6O_3S_2$: 538.1257, found: 539.1335 $(M+1)^+$.

*4-Methoxy-N-(3-((4-(6-(3-methoxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)propyl)benzenesulfonamide (**25k**):* Obtained from the reaction of compounds **15** and **23d** using the same procedure for compound **24a** to yield 56 % white solid; mp 96-97 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.55 (d, 1H, *J* = 6.0 Hz), 6.03 (d, 1H, *J* = 6.0 Hz), 7.74 (d, 2H, *J* = 9.0 Hz), 7.35-7.30 (m, 2H), 7.20-7.18 (m, 3H), 6.94 (dd, 2H, *J* = 6.0, 9.0 Hz), 6.54 (t, 1H, *J* = 3.0 Hz), 5.32 (brs, NH), 3.84 (s, 3H), 3.83 (s, 3H), 3.56 (q, 2H, *J* = 6.0 Hz), 3.04 (d, 2H, *J* = 6.0 Hz), 1.79 (t, 2H, *J* = 6.0 Hz); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 162.7, 162.2, 159.7, 157.1, 152.1, 149.9, 136.0, 131.7, 129.6, 129.0, 122.1, 121.7, 120.7, 114.8, 114.3, 114.2, 112.7, 107.3, 55.5, 55.3, 40.2, 38.2.

29.8; IR (KBr) cm^{-1}: 3382, 3264, 3117, 2938, 1903, 1574, 1455, 1328; LC-MS: m/z calculated for $C_{26}H_{26}N_6O_4S_2$: 550.15, found :551.10 $(M+1)^+$.

*N-(3-((4-(6-(3-methoxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)propyl)-4-methylbenzenesulfonamide **(25l)**:* Obtained from the reaction of compounds **15** and **23e** using the same procedure for compound **24a** to yield 42% as white solid; mp 78-79 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.57 (d, 1H, J = 3.0 Hz), 7.71 (d, 2H, J = 9.0 Hz), 7.36 (d, 3H, J = 9.0 Hz), 7.22 (d, 2H, J = 6.0 Hz), 7.03 (s, 2H), 6.59 (d, 1H, J = 6.0 Hz), 5.56 (brs, NH), 3.87 (s, 3H), 3.63 (t, 2H, J = 6.0 Hz), 1.63 (t, 2H, J = 6.0 Hz); LC-MS: m/z calculated for $C_{26}H_{26}N_6O_3S_2$: 534.15, found: 535.10 $(M+1)^+$.

*N-(3-((4-(6-(3-methoxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)propyl)naphthalene-2-sulfonamide **(25m)**:* Obtained from the reaction of compounds **15** and **23f** using the same procedure for compound **24a** to yield 40% as white solid; mp 98-99 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.38 (s, 1H), 8.06 (s, 1H), 7.97 (d, J = 4.0 Hz,1H), 7.88-7.76 (m, 4 H), 7.60-7.52 (m, 2H), 7.41 (s, 1H), 7.31 (dd, J = 4.0, 8.0 Hz, 1H), 7.18 (d, J = 8.0 Hz, 2H), 6.59 (dd, J = 4.0 Hz, 1H), 6.54 (d, J = 4.0 Hz, 1H), 5.68 (t, J = 8.0 Hz, 1H), 3.81 (s, 3H), 3.52 (q, J = 8.0 Hz, 2H), 3.07 (q, J = 8.0 Hz, 2H), 1.78 (t, J = 8.0 Hz, 2H) ,; ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 162.1, 159.7, 157.0, 152.1, 149.9, 136.9, 136.0, 134.6, 132.1, 129.7, 129.4, 129.4, 129.1, 129.0, 128.7, 128.5, 128.2, 128.0, 127.8, 127.7, 127.4, 122.2, 121.9, 120.7, 114.7, 114.4, 114.2, 107.1, 55.3, 40.3, 38.2, 29.7; IR (KBr) cm^{-1}: 3377, 3259, 3117, 1574, 1450, 1369, 1327; LC-MS: m/z calculated for $C_{29}H_{26}N_6O_3S_2$: 570.15, found :571.10 $(M+1)^+$.

*4-Fluoro-N-(2-((4-(6-(4-methoxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl)benzenesulfonamide **(26a)**:* Obtained from the reaction of compounds **16** and **22b** using the same procedure for compound **24a** to yield 29%; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 8.05

(d, 1H, *J* = 6.0 Hz), 7.67 (d, 4H, *J* = 6.0 Hz), 7.50 (d, 2H, *J* = 9.0 Hz), 7.33 (d, 2H, *J* = 6.0 Hz), 7.02 (d, 2H, *J* = 9.0 Hz), 6.34 (d, 1H, *J* = 3.0 Hz), 3.82 (s, 3H), 2.93 (t, 2H, *J* = 6.0 Hz), 2.38 (brs, 2H); IR (KBr) cm^{-1}: 3364, 3304, 3063, 2924, 2861, 1924, 1597, 1455, 1318; LC-MS: m/z calculated for $C_{24}H_{21}FN_6O_3S_2$: 524.13, found 525.10 $(M+1)^+$.

*4-Methoxy-N-(2-((4-(6-(4-methoxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl)benzenesulfonamide **(26b)**:* Obtained from the reaction of compounds **16** and **22d** using the same procedure for compound **24a** to yield 31%; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 8.04 (d, 1H, *J* = 6.0 Hz), 7.71 (d, 2H, *J* = 9.0 Hz), 7.60 (brs, 1H), 7.49 (d, 2H, *J* = 8.4 Hz), 7.43 (s, 1H), 7.03 (t, 4H, *J* = 9.0 Hz), 6.34 (d, 1H, *J* = 3.0 Hz), 3.81 (s, 3H), 3.77 (s, 3H), 3.30 (brs, 2H), 2.94 (brs, 2H); LC-MS: m/z calculated for $C_{25}H_{24}N_6O_4S_2$: 536.13, found 537.10 $(M+1)^+$.

*N-(2-((4-(6-(4-methoxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl)-4-methylbenzenesulfonamide (**26c**):* Obtained from the reaction of compounds **16** and **22e** using the same procedure for compound **24a** to yield 33% as buff solid; mp 195-196 °C; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 8.53 (d, 1H, *J* = 6.0 Hz), 8.02 (d, 1H, *J* = 6.0 Hz), 7.73 (t, 2H, *J* = 4.5 Hz), 7.57 (t, 2H, *J* = 3.0 Hz), 7.31-7.26 (m, 2H), 6.96 (t, 2H, *J* = 4.5 Hz), 6.58 (t, 1H, *J* = 3.0 Hz), 5.56 (brs, NH), 3.90 (s, 3H), 3.64 (brs, 2H), 3.26 (brs, 2H), 2.39 (s, 3H); IR (KBr) cm^{-1}: 3253, 3078, 2841, 1954, 1736, 1372, 1324.

*N-(2-((4-(6-(4-methoxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl) naphthalene-1-sulfonamide (**26d**):* Obtained from the reaction of compounds **16** and **33** using the same procedure for compound **41** to yield 37% as yellowish white solid; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.40 (brs, 1H), 7.93-7.82 (m, 4H), 7.35 (brs, 3H), 7.34-7.11 (m, 3H), 6.97 (d, 1H, *J* = 9.0 Hz), 6.47 (brs, 1H), 3.83 (s, 3H), 3.58 (brs, 2H), 3.30 (brs, 2H).

*Synthetic procedure for 4-hydroxy-N-(2-((4-(6-phenylimidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl)benzenesulfonamide (**27a**)*

To a solution of compound **24a** (50 mg, 0.1 mmol) in anhydrous dichloromethane (1 mL), BBr_3 (0.02 mL of 1 M solution in dichloromethane, 0.3 mmol) was added dropwise at −78 °C under N_2. The reaction mixture was stirred at the same temperature for 30 min. The mixture was allowed to warm to room temperature and stirred for 1 h. The reaction mixture was quenched with saturated aqueous Na_2CO_3. Ethyl acetate (10 mL) was added and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (3 x 3 mL). The combined organic layer extract was washed with brine and dried over anhydrous Na_2SO_4. After evaporation of the organic solvent, the residue was purified by column chromatography using hexane:ethyl acetate 1:2 v/v to give 25 mg (51.4%) as buff solid. mp 208-209 °C; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 10.33 (s, 1H), 8.83 (s, NH), 8.05 (d, 1H, J = 3.0 Hz), 7.63-7.57 (m, 4H), 7.46 (s, 5H), 6.87 (t, 2H, J = 4.5 Hz), 6.32 (d, 1H, J = 6.0 Hz), 3.18 (s, 2H), 2.90 (brs, 2H); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 163.8, 162.5, 161.4, 158.1, 156.2, 151.6, 148.1, 142.9, 138.0, 131.7, 130.1, 127.0, 121.0, 116.1, 115.9, 115.7, 114.4, 105.7 49.0, 29.5; LC-MS: m/z calculated for $C_{23}H_{20}N_6O_3S_2$:492.10, found 493.10 $(M+1)^+$; HRMS calculated for $C_{23}H_{20}N_6O_3S_2$:492.1038, found 493.1116 $(M+1)^+$.

*N-(2-((4-(6-(3-hydroxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl) benzenesulfonamide (**27b**):* It was prepared by the same demethylation reaction as compound **27a**. Yielding 44 % of dark orange solid; mp 70-71 °C; ^{1}H-NMR (CD_3OD, 400 MHz) δ 9.64 (s, 1H, exchangeable), 8.64 (bs, 1H, exchangeable), 8.19 (s, 1H), 8.10 (d, J = 5.2 Hz, 1H), 7.85 (d, J = 7.0 Hz, 3H), 7.62 (d, J = 7.3 Hz, 3H), 7.31 (t, J = 7.5 Hz, 1H), 7.03 (d, J = 7.6 Hz, 2H), 6.88 (d, J = 7.3 Hz, 1H), 6.50 (d, J = 5.2 Hz, 1H), 3.40 (s, 2H), 2.99 (s, 2H); IR (KBr) cm^{-1}: 3115, 2854, 1639, 1574, 1445, 1328; LC-MS: m/z calculated for $C_{23}H_{20}N_6O_3S_2$: 492.10, found 493.10 $(M+1)^+$.

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*4-Fluoro-N-(2-((4-(6-(3-hydroxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl)benzenesulfonamide **(27c)**:* It was prepared by demethylation of compound **25d** using the same procedure to prepare compound **27a**. Yield 32%; dark orange solid; mp 70-71 °C; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 10.35 (s, 1H), 9.00 (brs, NH), 7.16 (d, 4H, J = 9.0 Hz), 7.45 (brs, 2H), 7.30 (t, 2H, J = 9.0 Hz), 7.86 (d, 2H, J = 9.0 Hz), 6.29 (d, 1H, J = 9.0 Hz), 3.33 (brs, 2H), 2.90 (d, 2H, J = 9.0 Hz); IR (KBr) cm^{-1}: 3389, 3065, 2927, 1731, 1614, 1552, 1499, 1446, 1326, 1157; LC-MS: m/z calculated for $C_{23}H_{19}FN_6O_3S_2$: 510.09, found: 511.10 $(M+1)^+$; HRMS calculated for $C_{23}H_{19}FN_6O_3S_2$: 510.0944 found: 511.1022 $(M+1)^+$.

*N-(2-((4-(6-(3-hydroxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl)-4-methylbenzenesulfonamide **(27d)**:* It was prepared by demethylation of compound **25e** using the same procedure to prepare compound **27a**. Yield 10%; buff solid; mp 115-116 °C; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 8.02 (brs, 1H), 7.69 (brs, 2H), 6.67 (d, 6H, J = 6.0 Hz), 6.40 (d, 5H, J = 9.0 Hz), 3.10 (t, 2H, J = 6.0 Hz), 2.74 (d, 2H, J = 6.0 Hz), 2.38 (s, 3H); LC-MS: m/z calculated for $C_{24}H_{22}N_6O_3S_2$: 506.12, found: 507.10 $(M+1)^+$; HRMS calculated for $C_{24}H_{22}N_6O_3S_2$: 506.1195, found: 507.1273 $(M+1)^+$.

*N-(3-((4-(6-(3-hydroxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)propyl)benzene sulfonamide **(27e)**:* It was prepared by demethylation of compound **25h** using the same procedure to prepare compound **27a**. Yield 33%; brown solid; mp 77-78 °C; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 8.71 (brs, 1H), 7.91 (d, 1H, J = 3.0 Hz), 7.83 (d, 1H, J = 9.0 Hz), 7.52 (d, 4H, J = 6.0 Hz), 7.32 (d, 2H, J = 9.0 Hz), 7.00 (s, 1H), 6.92 (d, 3H, J = 6.0 Hz), 6.46 (d, 1H, J = 3.0 Hz), 3.49 (brs, 2H), 2.98 (d, 2H, J = 6.0 Hz), 1.82 (d, 2H, J = 6.0 Hz); LC-MS: m/z calculated for $C_{24}H_{22}N_6O_3S_2$: 506.12, found 507.10 $(M+1)^+$.

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*3-Fluoro-N-(3-((4-(6-(3-hydroxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)propyl)benzenesulfonamide (**27f**):* It was prepared by demethylation of compound **25j** using the same procedure to prepare compound **27a**. Yield 37%; light yellow solid; mp 111-112 °C; ^{1}H-NMR (CD_3OD, 400 MHz) δ 8.29 (s, 1H), 7.93 (d, 1H, J = 5.5 Hz,), 7.81 (d, 1H, J = 1.5 Hz), 7.66 (d, 1H, J = 7.9 Hz), 7.62-7.51 (m, 2H), 7.45-7.43 (m, 1H), 7.42-7.24 (m, 3H), 7.20-7.16 (m, 1H), 6.38 (d, 1H, J = 5.4 Hz), 3.45 (s, 2H), 2.99 (t, 2H, J = 6.4 Hz), 1.80 (t, 2H, J = 6.7 Hz); ^{13}C-NMR (CD_3OD, 101 MHz) δ 163.8, 162.1, 161.4, 156.4, 155.5, 144.5, 142.6, 139.1, 136.6 , 130.9, 130.8 , 125.1, 122.5, 119.1, 118.9, 117.0, 115.9, 115.1, 113.7 , 104.8 , 40.3, 38.1, 29.2; LC-MS (m/z) calculated for $C_{24}H_{21}FN_6O_3S_2$ (m/z): 524.11, found: 525.0 $(M+1)^+$; HRMS calculated for $C_{24}H_{21}FN_6O_3S_2$ (m/z): 524.1101, found: 525.1179 $(M+1)^+$.

*N-(3-((4-(6-(3-hydroxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)propyl)-4-methylbenzenesulfonamide **(27g)**:* It was prepared by demethylation of compound **25l** using the same procedure to prepare compound **27a**. Yield 40%; mp 171-172 °C; ^{1}H-NMR (400 MHz, MeOD) δ 8.40 (s, 1H), 8.02 (d, 1H, J = 5.4 Hz), 7.89 (d, 1H, J = 1.4 Hz), 7.72 (d, 2H, J = 8.2 Hz), 7.55-7.49 (m, 1H), 7.45 (d, 1H, J = 7.7 Hz), 7.37-7.35 (m, 3H), 7.24 (m, 1H), 6.47 (d, 1H, J = 5.4 Hz), 3.48 (s, 2H), 2.98 (t, 2H, J = 6.7 Hz), 2.39 (s, 3H), 1.81-1.78 (m, 2H); ^{13}C-NMR (CD_3OD, 101 MHz) δ 162.1, 157.1, 155.7, 143.5, 137.3, 130.1, 129.3, 126.5, 125.2, 123.6, 116.1, 115.3, 106.2, 104.7, 40.1, 38.3, 29.8, 19.9; LC-MS (m/z) calculated for $C_{25}H_{24}N_6O3S_2$ (m/z): 520.14, found: 521.10 $(M+1)^+$.

*4-Hydroxy-N-(2-((4-(6-(4-hydroxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl)benzenesulfonamide **(27h)**:* It was prepared by demethylation of compound **26b** using the same procedure to prepare compound **27a**. Yield 15%; light yellow solid; mp 160-161 °C; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 8.04 (d, 1H, J = 5.5 Hz), 7.95 (s, 3H), 7.61 (d, 2H, J =

6.0 Hz), 7.41 (d, 1H, *J* = 3.5 Hz), 7.37 (d, 1H, *J* = 8.6 Hz), 6.85 (t, 4H, *J* = 8.9 Hz), 6.36 (d, 2H, *J* = 5.3 Hz), 2.89 (s, 2H), 2.73 (s, 2H); IR (KBr) cm^{-1}: 3391, 3294, 3089, 2929, 1914, 1452, 1330; LC-MS: m/z calculated for $C_{23}H_{20}F_3N_6O_4S_2$: 508.10, found 509.00 $(M+1)^+$.

*N-(2-((4-(6-(4-hydroxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl)-4-methylbenzenesulfonamide **(27i)**:* It was prepared by demethylation of compound **26c** using the same procedure to prepare compound **27a**. Yield 33%; buff solid; mp 163-164 °C; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 9.73 (s, 1H) , 8.03 (d, 1H, *J* = 6.0 Hz), 7.66 (d, 2H, *J* = 8.1 Hz), 7.38 (s, 1H), 7.34 (d, 3H, *J* = 3.2 Hz), 6.83 (d, 2H, *J* = 6.3 Hz), 6.35 (d, 1H, *J* = 5.3 Hz), 2.92 (d, 4H, *J* = 5.3 Hz), 2.31 (s, 3H); LC-MS: m/z calculated for $C_{24}H_{22}N_6O_3S_2$: 506.12, found 507.10 $(M+1)^+$.

*Synthesis of compounds **29a-c** and **30a-d**.*

They were prepared according to well-known procedure.[47]

*Synthesis of Compound **33***

It was performed according to reported procedures.[48]

*Synthesis of cyclic sulfamide side chains **36a-g.***

They were prepared by adopting the reported procedures.[49]

*General procedure for preparation of the target compounds (**37a-g**).* A mixture of compound **15** (355.52 mg, 0.92 mmol), and selected compound from **36a-g** (2.48 mmol), and diisopropylethylamine (DIPEA, 0.57 mL, 3.3 mmol) in DMSO (10 mL) was stirred at 80 °C for 8 h. The mixture was cooled to room temperature, quenched with water (20 mL), then extracted with ethyl acetate (3 x 20 mL). The combined organic layer extracts were washed with brine, dried over

anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography.

*2-(2-((4-(6-(3-Methoxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl)-5-methyl-1,2,5-thiadiazolidine 1,1-dioxide **(37a)**:* Yield 50%; white solid; mp 144-145 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.58 (d, 1H, J = 4.5 Hz), 8.06 (d, 1H, J = 5.3 Hz), 7.33 (t, 1H, J = 7.8 Hz), 7.23-7.19 (m, 2H), 6.98-6.90 (m, 2H), 6.58 (d, 1H, J = 5.4 Hz), 3.83 (s, 3H), 3.78 (q, 2H, J = 6.1 Hz), 3.43-3.30 (m, 6H), 2.78 (s, 3H); LC-MS: m/z calculated for $C_{29}H_{26}F_3N_7O_3S$: 485.13, found: 486.10 $(M+1)^+$.

*2-Benzyl-5-(2-((4-(6-(3-methoxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl)-1,2,5-thiadiazolidine 1,1-dioxide **(37b)**:* Yield 64%; white solid; mp 151-152 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.59 (d, 1H, J = 4.4 Hz), 8.06 (d, 1H, J = 5.4 Hz), 7.39-7.31 (m, 6H), 7.22-7.19 (m, 2H), 6.98-6.94 (m, 2H), 6.59 (d, 1H, J = 5.4 Hz), 4.22 (s, 2H), 3.83-3.77 (m, 5H), 3.39 (q, 4H, J = 6.7 Hz), 3.20 (t, 2H, J = 6.5 Hz); LC-MS: m/z calculated for $C_{27}H_{27}N_7O_3S_2$: 561.16, found: 562.10 $(M+1)^+$.

*2-(2-((4-(6-(3-Methoxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl)-4,4-dimethyl-1,2,5-thiadiazolidine 1,1-dioxide **(37c)**:* Yield 54%; white solid; mp 186-187 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.57 (d, 1H, J = 4.5 Hz), 8.06 (d, 1H, J = 5.4 Hz), 7.33 (t, 1H, J = 8.0 Hz), 7.26-7.19 (m, 2H), 6.96-6.91 (m, 2H), 6.58 (d, 1H, J = 5.4 Hz), 5.49 (m, 1H), 3.83-3.77 (m, 4H), 3.35 (t, 2H, J = 5.9 Hz), 3.21 (s, 2H), 1.43 (s, 6H); LC-MS: m/z calculated for $C_{22}H_{25}N_7O_3S_2$: 498.15, found: 499.10 $(M+1)^+$.

*2-(2-((4-(6-(3-Methoxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl)-6-methyl-1,2,6-thiadiazinane 1,1-dioxide **(37d)**:* Yield 54 %; white solid; mp 105-106 °C; ^{1}H-NMR ($CDCl_3$,

300 MHz) δ 8.61 (d, 1H, J = 5.3 Hz), 8.10 (d, 1H, J = 5.3 Hz), 7.41-7.34 (m, 1H), 7.26-7.23 (m, 2H), 7.01-6.99 (m, 2H), 6.61 (d, 1H, J = 5.5 Hz), 5.47 (m, 1H), 3.86 (s, 3H), 3.77-3.71 (m, 2H), 3.55 (t, 2H, J = 5.5 Hz), 3.46-3.36 (m, 4H), 2.81 (s, 3H), 1.87-1.85 (m, 2H); LC-MS: m/z calculated for $C_{22}H_{25}N_7O_3S_2$: 499.13, found: 500.00 $(M+1)^+$.

2-(2-((4-(6-(3-Methoxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl)-1,2,5-thiadiazolidine 1,1-dioxide ***(37e)*** : White solid (49%); mp 100-101°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.61 (d, 1H, J = 4.5 Hz), 8.10 (d, 1H, J = 5.4 Hz), 7.37 (t, 1H, J = 7.6 Hz), 7.25-7.22 (m, 2H), 7.01-6.99 (m, 2H), 6.63 (d, 1H, J = 5.4 Hz), 5.55 (t, 1H, J = 6.1 Hz), 4.44 (s, 1H), 3.87 (s, 3H), 3.85-3.80 (q, 2H, J = 6.0 Hz), 3.59-3.53 (m, 4H), 3.40 (t, 2H, J = 6.1 Hz); LC-MS: m/z calculated for $C_{20}H_{21}N_7O_3S_2$: 471 Found: 472 $(M+1)^+$.

2-(2-((4-(6-(3-Methoxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl)-1,2,6-thiadiazinane 1,1-dioxide ***(37f)*** *:* White solid (38%); mp 102-103°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.49 (d, 1H, J = 4.5 Hz), 7.86 (s, 1H), 7.28 (t, 1H, J = 8.2 Hz), 7.12-7.10 (m, 2H), 6.93-6.90 (m, 2H), 6.52 (d, 2H, J = 5.8 Hz), 3.76 (s, 3H), 3.66 (q, 2H, J = 5.9 Hz), 3.51-3.44 (m, 2H), 3.38 (t, 2H, J = 5.5 Hz), 3.3 (t, 2H, J = 6.2 Hz), 1.90-1.82 (m, 2H); LC-MS: m/z calculated for $C_{21}H_{23}N_7O_3S_2$: 485 Found: 486 $(M+1)^+$.

Tert-butyl 6-(2-((4-(6-(3-methoxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl)-1,2,6-thiadiazinane-2-carboxylate 1,1-dioxide ***(37g)*** : White solid (29%); mp 102-103°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.56(d, 1H, J = 3.4 Hz), 8.06 (d, 1H, J = 4.1 Hz), 7.31 (t, 1H, J = 5.7 Hz), 7.24-7.19 (m, 2H), 7.01-6.99 (m, 2H), 6.62 (s, 1H), 5.47 (s, 1H), 3.99 (t, 2H, J = 5.7 Hz),

3.87 (s, 3H), 3.75 (q, 2H, *J* = 5.9 Hz), 3.68 (t, 2H, *J* = 5.8 Hz), 3.51 (t, 2H, *J* = 6.1 Hz), 1.90-1.82 (m, 2H), 1.55 (s, 9H); LC-MS: m/z calculated for $C_{26}H_{31}N_7O_5S_2$: 584 Found: 585 $(M+1)^+$.

Synthetic procedure for 2-(2-((4-(6-(3-hydroxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl)-5-methyl-1,2,5-thiadiazolidine 1,1-dioxide ***(38a)*** **:**

To a solution of compound **37a** (50 mg, 0.1 mmol) in dichloromethane (1 mL), BBr_3 (0.02 mL of a 1 M solution in dichloromethane, 0.3 mmol) was added dropwise at −78 °C under N_2. The reaction mixture was stirred at the same temperature for 30 min. The mixture was allowed to warm to room temperature and stirred for another 1 h. The reaction mixture was quenched with saturated aqueous sodium carbonate. Ethyl acetate (5 mL) was added, and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (3 x 3 mL). The combined organic layer extract was washed with brine and dried over anhydrous sodium sulfate. After evaporation of the organic solvent, the residue was purified by column chromatography using hexane:ethyl acetate 1:2 v/v to give 12.71 mg (27 %) of the pure product as light yellow solid. mp 151-152 °C; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 8.65 (brs, 1H), 7.96 (d, 1H, *J* = 5.3 Hz), 7.29-7.21 (m, 7H), 6.43 (d, 1H, *J* = 5.3 Hz), 3.67 (t, 2H, *J* = 5.6 Hz), 3.44 (t, 2H, *J* = 5.4 Hz) 3.33-3.28 (m, 4H), 2.70 (s, 3H); ^{13}C-NMR (CD_3OD, 101 MHz) δ 159.9, 157.4, 154.4, 152.6, 135.4, 134.5, 114.7, 113.8, 113.6, 104.4, 54.4, 46.6, 45.8, 39.7, 25.5; LC-MS: m/z calculated for $C_{20}H_{21}N_7O_3S_2$: 471.11, found: 472.1 $(M+1)^+$; HRMS calculated for $C_{20}H_{21}N_7O_3S_2$: 471.1147, found: 472.1225 $(M+1)^+$.

Compounds **38b-g** were synthesized by the same procedure utilized for demethylation and synthesis of compound **38a**.

2-Benzyl-5-(2-((4-(6-(3-hydroxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl)-1,2,5-thiadiazolidine 1,1-dioxide ***(38b)****:* Yield 23%; white solid; mp 155-156 °C; ^{1}H-NMR

(DMSO-d_6, 300 MHz) δ 9.56 (s, 1H), 8.17 (d, 1H, J = 5.4 Hz), 7.37-7.25 (m, 9H), 6.98 (d, 2H, J = 6.5 Hz), 6.82 (d, 1H, J = 8.7 Hz), 6.41 (d, 1H, J = 8.7 Hz), 4.12 (s, 2H), 3.57 (s, 4H), 3.21-3.13 (m, 4H); LC-MS: m/z calculated for $C_{26}H_{25}N_7O_3S_2$: 547.15; found: 548.1 $(M+1)^+$.

*2-(2-((4-(6-(3-Hydroxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl)-4,4-dimethyl-1,2,5-thiadiazolidine 1,1-dioxide (**38c**):* Yield 25%; white solid; mp 196-197 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.55 (d, 1H, J = 5.4 Hz), 8.04 (d, 1H, J = 5.4 Hz), 7.30-7.28 (m, 1H), 7.15-7.13 (m, 2H), 6.95 (d, H, J = 8.1 Hz), 3.76 (t, 2H, J = 6.1 Hz), 3.33 (t, 2H, J = 5.9 Hz), 3.25 (s, 2H), 1.45 (s, 6H); LC-MS: m/z calculated for C21H23N7O3S2: 485.13, found: 486.10 $(M+1)^+$.

*2-(2-((4-(6-(3-Hydroxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl)-6-methyl-1,2,6-thiadiazinane 1,1-dioxide **(38d)**:* Yield 21%; white solid; mp 184-185 °C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.57 (d, 1H, J = 4.5 Hz), 8.01 (d, 1H, J = 5.4 Hz), 7.28-7.26 (m, 1H), 7.15-7.11 (m, 2H), 6.88 (d, H, J = 7.7 Hz), 6.59 (d, 1H, J = 5.3 Hz) 3.67 (t, 2H, J = 5.4 Hz), 3.48 (t, 2H, J = 5.2 Hz), 3.37 (t, 2H, J = 6.0 Hz), 2.75 (s, 3H), 1.78-1.72 (m, 2H); LC-MS: m/z calculated for $C_{21}H_{23}N_7O_3S_2$: 485, found: 489 $(M+1)^+$.

*2-(2-((4-(6-(3-Hydroxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl)-1,2,5-thiadiazolidine 1,1-dioxide (**38e**)* : White solid (22%); mp 195-196 °C; ^{1}H NMR (CD_3OD, 300 MHz) δ 8.65 (s, 1H), 7.90 (d, 1H, J = 5.4 Hz), 7.21-7.16 (m, 2H), 6.93-6.89 (m, 2H), 6.78 (s, 1H), 3.6 (t, 2H, J = 5.6 Hz), 3.3 (t, 2H, J = 5.5 Hz), 3.15 (t, 2H, J = 6.3 Hz); LC-MS: m/z calculated for $C_{19}H_{19}N_7O_3S_2$: 457 Found: 458 $(M+1)^+$.

*2-(2-((4-(6-(3-Hydroxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl)-1,2,6-thiadiazinane 1,1-dioxide **(38f)*** : White solid (25%); mp 183-184 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.85 (s, 1H), 8.14 (d, 1H, J = 5.9 Hz), 7.78 (d, 1H, J = 4.1 Hz), 7.47 (t, 1H, J = 7.7 Hz), 7.17-

7.09 (m, 3H), 6.74 (d, H, *J* = 6.7 Hz), 3.88-3.85 (m, 2H), 3.60 (t, 2H, *J* = 7.7 Hz), 3.44-3.41 (m, 4H), 1.78 (m, 2H); LC-MS: m/z calculated for $C_{20}H_{21}N_7O_3S_2$: 471 Found: 472 $(M+1)^+$.

*Tert-butyl 6-(2-((4-(6-(3-hydroxyphenyl)imidazo[2,1-b]thiazol-5-yl)pyrimidin-2-yl)amino)ethyl) -1,2,6-thiadiazinane-2-carboxylate 1,1-dioxide (**38g**)* : White solid (16%); mp 180-181 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.57 (d, 1H, *J* = 4.5 Hz), 8.04 (d, 1H, *J* = 5.6 Hz), 7.26-7.19 (m, 2H), 6.96-6.91 (m, 2H), 6.58 (d, H, *J* = 5.4 Hz), 3.72 (t, 2H, *J* = 5.9 Hz), 3.54 (t, 2H, *J* = 5.8 Hz), 3.45 (t, 2H, *J* = 5.1 Hz), 3.36 (t, 2H, *J* = 5.1 Hz), 1.78 (m, 3H), 1.61 (s, 9H); LC-MS: m/z calculated for $C_{25}H_{29}N_7O_5S_2$: 571 Found: 572 $(M+1)^+$.

***In vitro* enzyme assay**

The enzymatic assays of wild-type BRAF, V600E-BRAF and CRAF were performed in Reaction Biology Corp. (http://www.reactionbiology.com) using the standard protocol and at 1 μM ATP concentration and 3-fold dilution factor. In a final reaction volume of 25 μL, kinase (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 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 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.

***In vitro* kinase panel assay**

Kinase-tagged T7 phage strains were grown in parallel in 24-well blocks in an *E. coli* host derived from the BL21 strain. *E. coli* were grown to log-phase and infected with T7 phage from a frozen stock (multiplicity of infection = 0.4) and incubated with shaking at 32 °C until lysis (90-150

minutes). The lysates were centrifuged (6,000 x g) and filtered (0.2 μm) to remove cell debris. The remaining kinases were produced in HEK-293 cells and subsequently tagged with DNA for qPCR detection. Streptavidin-coated magnetic beads were treated with biotinylated small molecule ligands for 30 minutes at room temperature to generate affinity resins for kinase assays. The liganded beads were blocked with excess biotin and washed with blocking buffer (SeaBlock (Pierce), 1 % BSA, 0.05 % Tween 20, 1 mM DTT) to remove unbound ligand and to reduce non-specific phage binding. Binding reactions were assembled by combining kinases, liganded affinity beads, and test compounds in 1x binding buffer (20 % SeaBlock, 0.17x PBS, 0.05 % Tween 20, 6 mM DTT). Test compounds were prepared as 40x stocks in 100% DMSO and directly diluted into the assay. All reactions were performed in polypropylene 384-well plates in a final volume of 0.04 mL. The assay plates were incubated at room temperature with shaking for 1 hour and the affinity beads were washed with wash buffer (1x PBS, 0.05 % Tween 20). The beads were then re-suspended in elution buffer (1x PBS, 0.05 % Tween 20, 0.5 μM non-biotinylated affinity ligand) and incubated at room temperature with shaking for 30 minutes. The kinase concentration in the eluates was measured by qPCR.

NCI-60 Cancer Cell Line Screening

Screening against a panel of 60 cancer cell lines was applied at the National Cancer Institute (NCI), Bethesda, Maryland, USA (www.dtp.nci.nih.gov), applying their standard protocol (https://dtp.cancer.gov/discovery_development/nci-60/methodology.htm).

MTT assay against L132 (human embryonic pulmonary epithelial cells)

The human lung normal cell line (L132; human embryonic pulmonary epithelial cells) was obtained from the Korean cell line bank (KCLB, Seoul, Korea). Cells were cultured in RPMI 1640

supplemented with 10% heat-deactivated FBS, penicillin (100 units/mL) and streptomycin sulfate (100 μg/mL). Cells were cultured at 37 °C in an atmosphere of 5% CO_2. MTT assay was used to determine sample cytotoxicity. Cells (5 x 10^4) were seeded in each well containing 100 μL of the medium supplemented with 10 % FBS in a 96-well plate. After 24 hours, various concentrations of the tested samples were added. After 48 hours, 20 μL of MTT (5 mg/mL stock solution in phosphate buffered saline (PBS)) was added and the plates were incubated for an additional 4 hours. The medium was discarded and formazan blue, which was formed in the cells, was dissolved with 200 μL DMSO. The optical density was measured at 540 nm using microplate readers (Molecular Devices, CA, U.S.A).

MTT assay against BJ1 cells (normal skin fibroblast cell line)

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay developed by Mosmann was modified by Miura and used to determine the in vitro inhibitory effects of the test compound on cell growth. A medium containing 10×10^3 cells (BJ1 cells) in a fresh complete growth medium was seeded into each well of a 96-well microplate, with the compound solution added simultaneously to triplicate wells, before the final volume was made up to 100 mL. The plate was incubated at 37°C for 72 h in a humidified atmosphere of 5% CO2 using a water- jacketed carbon dioxide incubator (TC2323; Sheldon, Cornelius, OR). The medium was aspirated, fresh medium (without serum) was added, and cells were incubated, with different concentrations of the sample, to give a final concentration of 500, 100, 50, 25, 12.5, 6.25, 3.125, and 0.78 mg/mL. Cells were suspended in DMEM-F12, 1% antibiotic–antimycotic mixture (10,000 U/mL potassium penicillin, 10,000 mg/mL streptomycin sulfate, and 25 mg/mL amphotericin B) and 1% L-glutamine in a 96-well flat-bottom microplate at 37°C under 5% CO2. After 48 h of incubation,

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the medium was aspirated; 200 mL of 10% sodium dodecyl sulfate (SDS) in deionized water was added to each well and further incubated overnight at 37°C under 5% CO2. Then, 200 mL of 10% SDS in deionized water was added to each well to stop the reaction and to solubilize any MTT formazan that had formed, and then it was incubated overnight at 37°C. Also, 100 mL of 0.02 N HCl/50% *N,N*-dimethylformamide, 20% SDS was added to solubilize any MTT formazan that had formed. The optical density of each well was measured at 575 nm (OD575) using a microplate multiwell reader (model 3350; Bio -Rad Laboratories Inc., Hercules, CA), and the inhibition of cell growth (%) was calculated as (1 − T/ C) × 100, where C is the mean OD575 of the control group and T is that of the treated group. The IC_{50} value was determined from the dose–response curve.

Caco-2 cell permeability test of compound 38a

Caco-2 cells were seeded in 12-well transwells with 5×10^5 cells and cultured for 3 weeks. Compound **38a** was diluted with transport buffer and 1% DMSO (Transport buffer: 10 mM glucose, 4 mM sodium bicarbonate, 1 mM HEPES in HBSS (pH7.4)). Caco-2 monolayer was placed between partition A and partition B. Compound **38a** in transport buffer was placed on side A and only transport buffer only on side B (to calculate A to B). Compound **38a** in transport buffer was placed on side B and only transport buffer only on side A (to calculate B to A). For each test, the temperature was kept at 37°C and sample was taken every 15 min for 1h. In the sample obtained from each side, acetonitrile and internal standard used for mass spectrometry were added to reach a final concentration of 5 μM. The concentration of each sample was measured using LC/MS/MS.

Molecular docking of compound 38a

The X-ray crystal structure of V600E-BRAF oncogenic mutant kinase in complex with vemurafenib (PDB ID: 1UWJ) was downloaded from the protein data bank (www.rcsb.org) in PDB format. The 2D structure of compound **38a** was drawn using ChemDraw software. Molecular Operating Environment (MOE, 2014.0901) software was used for the molecular docking operation of the target compound **38a** with V600E-BRAF kinase domain (PDB ID: 1UWJ). The kinase was prepared for the molecular docking procedure by applying 3D protonation of enzyme amino acids and the native ligands. In addition, water of crystallization was removed from V600E-BRAF kinase domains. The active site of the enzyme was isolated. The docking simulation of native ligand with the active site of V600E-BRAF kinase was investigated in order to validate the docking protocol. Both 3D protonation and energy minimization were performed for the target compounds using MOE software.

Immunoblot assay

A375 cells were disrupted in RIPA buffer containing 150 mM NaCl, 50 mM Tris-HCl (pH 7.4), 0.25% sodium deoxycholate, 1 mM EDTA, 1% NP40, 1 mM NaF, 0.2 mM phenylmethyl sulfonyl fluoride, 0.1 mM sodium orthovanadate, and a protease inhibitor cocktail (Roche Life Science, Indianapolis, IN, USA). The proteins were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (Millipore, Burlington, MA, USA) blocked in 5% skim milk and probed with the indicated antibodies. The immunoblots were visualized using a SuperSignal West Femto chemiluminescence substrate (Thermo Fisher Scientific, Waltham, MA, USA) and detected using the LAS 4000-mini biomolecular imaging system (FUJIFILM, Tokyo, Japan).

***In vivo* antitumor activity**

The anticancer activity of compound **38a** was performed by using A375 melanoma xenograft in male Hsd: Athymic Nude-Foxn1nu (Harlan co. (USA)) mice (8-12 weeks old and 25-40g weight). The test animals were divided into four groups, each group composed of seven mice. Group 1: control group which took placebo and did not take any drug; Group 2: each animal received 25 mg/kg/day of compound **38a**; Group 3: each animal received 50 mg/kg/day of compound **38a**; Group 4: each animal received 50 mg/kg/day of vemurafenib. The four groups were monitored for 21 days, and both body weight and tumor size were measured every 3 days. The animals were kept under controlled weather and feeding conditions.

***In vivo* pharmacokinetic of compound 38a**

Compound **38a** was administered intravenously and orally at a dose of 5 mg/kg in rats. Each group composed of 3 animals (7-8 weeks old male SD rat (Core Tech, Hana Corporation). The weight of animals were 250-300g. Animals were kept at fixed temperature 22 ± 2℃, and relative humidity of 50 ± 5%. Animals were kept under 12h light and dark cycle. Animals were allowed to freely reach food and water. Animals were fasted 16 h before oral dose. Compound **38a** was dissolved in 5% DMSO, 40% PEG400, and 55% distilled water. For oral dose 500 μL was used and 250 μL was used for intravenous injection. Serum samples were collected each 30 min. The blood concentration of the test compounds was determined by LC-MS/MS (Agilent 1290 infinity II series equipped with on-line degasser, binary pump, thermostatted well-plate autosampler and column compartment, Waters Atlantis® HSS T3 (2.1 × 100 mm, 1.9 μm) column, and mobile phase linear gradient from 95% A (0.1% formic acid in water) /5% B (0.1% formic acid in acetonitrile) to 5% A/95% B, 6500+ QTRAP LC-MS/MS/MS system, Turbo Spray Ion Drive as ion source, and Carbamazepine as internal standard. Pharmacokinetic parameters were obtained by non-

compartmental analysis of the plasma concentration–time profiles using KineticaTM (Thermo Fisher Scientific, Inc., Woburn, MA, USA).

Plasma stability

About 10 μM of compound **38a** was mixed with human plasma and rat plasma and the mixture was shaked at 37 °C for 30 min and 120 min. After each time interval, acetonitrile was added and the mixture was centrifugated (14,000 rpm, 4 °C). The supernatant was injected into the LC-MS to detect the remaining amount of compound **38a**.

Supporting Information Availability

The supplementary file includes tables summarizing the inhibition percentage values of the target compounds over NCI-60 cell line panel, figures showing heat maps of NCI results of the target compounds, raw NCI results, characterization charts (^{1}H-NMR, ^{13}C-NMR, LC-MS, and HRMS), HPLC traces, and 2D/3D docking files. Molecular Formula Strings file including the target compound SMILES and IC_{50} values against BRAF (wild-type), V600E-BRAF, and CRAF is also provided.

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Abbreviations used

brs, broad singlet; d, doublet; dd, doublet of doublets; DIPEA, *N,N*-diisopropylethylamine; ER, efflux ratio; HRMS, high resolution mass spectrometry; m, multiplet; NCI, National Cancer Institute; q, quartet; s, singlet; t, triplet; TEA, triethylamine; TGI, total growth inhibition.

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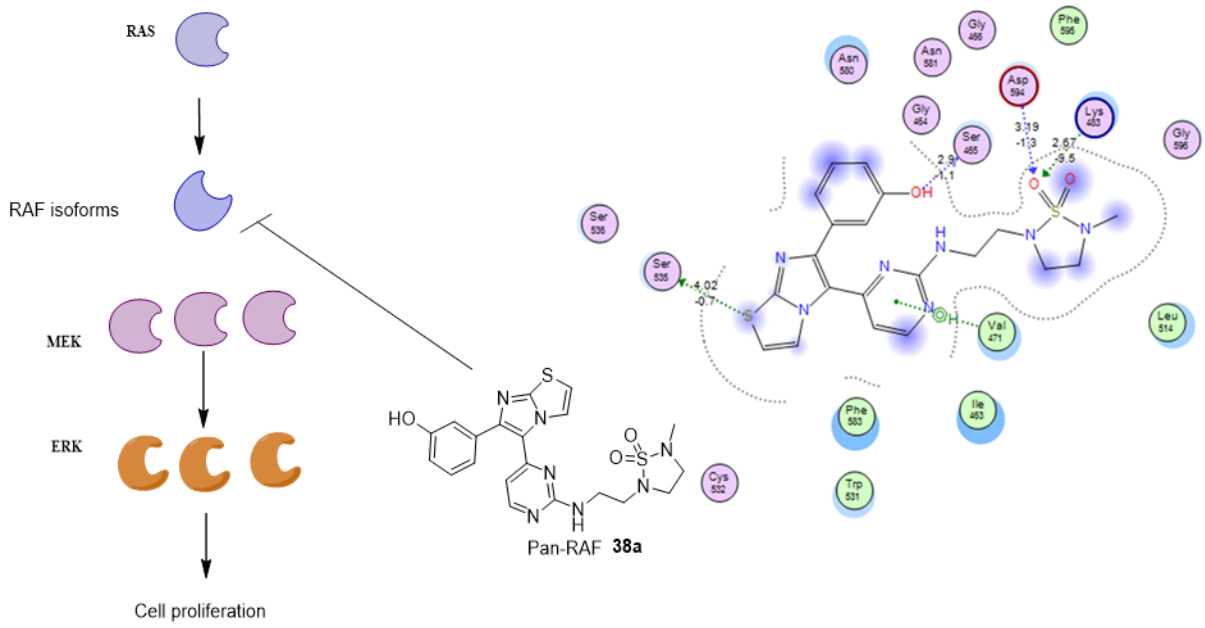
Table of Content graphic



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