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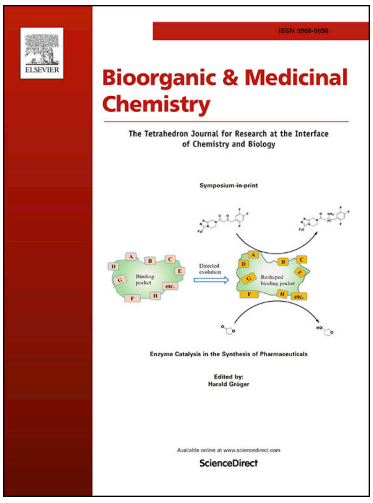

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PII: S0968-0896(20)30314-X
DOI: https://doi.org/10.1016/j.bmc.2020.115493
Reference: BMC 115493

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 4 February 2020
Revised Date: 4 April 2020
Accepted Date: 5 April 2020

Please cite this article as: M.S. Abdel-Maksoud, E.M.H. Ali, U.M. Ammar, K.I. Mersal, K. Ho Yoo, C-H. Oh, Design and synthesis of novel pyrrolo[2,3-*b*]pyridine derivatives targeting ^{V600E}BRAF, *Bioorganic & Medicinal Chemistry* (2020), doi: https://doi.org/10.1016/j.bmc.2020.115493

Design and synthesis of novel pyrrolo[2,3-*b*]pyridine derivatives targeting ^{V600E}BRAF

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Abstract

Several pyrrolo[2,3-*b*]pyridine-based B-RAF inhibitors are well known and some of them are currently FDA approved as anticancer agents. Based on the structure of these FDA approved ^{V600E}B-RAF inhibitors, two series of pyrrolo[2,3-*b*]pyridine scaffold were designed and synthesized in attempt to develop new potent ^{V600E}B-RAF inhibitors. The 38 synthesized compounds were biologically evaluated for their ^{V600E}B-RAF inhibitory effect at single dose (10uM). Compounds with high percent inhibition were tested to determine their IC_{50} over ^{V600E}B-RAF. Compounds **34e** and **35** showed the highest inhibitory effect with IC_{50} values of 0.085 µM and 0.080 µM, respectively. Headed for excessive biological evaluation, the synthesized derivatives were tested over sixty diverse human cancer cell lines. Only compound **35** emerged as a potent cytotoxic agent against different panel of human cancer cell lines.

Keywords

B-RAF inhibitors; Kinase inhibitor; Anticancer; pyrrolo[2,3-*b*]pyridine; SAR.

1. Introduction

BRAF is a serine/threonine kinase that plays an important role in MAPK singling pathway. The activation of MAPK pathway is vital for normal cellular functions such as cell differentiation and growth[1, 2]. Oncogenic activation of MAPK pathway as a result of BRAF mutation occurs in 50-70% of melanomas[3, 4], up to 70% of thyroid carcinoma[5-7] and 10% of colorectal cancers[8]. B-RAF kinase can be activated by oncogenic mutations, such as B-RAF V600E mutation (in which valine amino acid in position 600 is replaced by glutamic acid). ^{V600E}BRAF is prevalent in melanomas 63%[9-13] and papillary thyroid carcinomas up to 50%[3].

Targeting ^{V600E}BRAF by small molecules is a good strategy for treatment of MAPK pathway hyper-activation in case of melanoma[14, 15]. In addition, the combination of ^{V600E}BRAF and MEK inhibitors showed great success in preclinical study for treatment of advanced solid tumors[16-18].

Due to the widespread of different forms of RAF kinase in different types of cancer, it has been considered as a very important anticancer drug target. RAF kinase inhibitors have been

extensively developed. Sorafenib (Nexavar®, BAY- 43-9006) is a diarylurea inhibitor for B-RAF that has been approved for the treatment of advanced renal cell carcinoma (RCC) and Hepatocellular carcinoma (HCC). Sorafenib is a small molecule inhibitor of several protein kinases, among which is B-RAF[19, 20]. Vemurafenib (PLX4032, Zelboraf) and dabrafenib (GSK2118436) were approved by the U.S. Food and Drug Administration (FDA) for treatment of late-stage melanoma. Both are selective inhibitors of V600E mutated B-RAF kinase and cause programmed cell death in melanoma cell lines, and hence high potency against melanoma cell lines. Melanoma cells without this mutation are not inhibited by vemurafenib and dabrafenib[20-22]. Mono-therapy treatment with V600EBRAF produced significant improve in both survival rate and patient life style. Despite of the successful of selective V600EBRAF inhibitors, this success is short-lived and resistance to these inhibitors appear from 5 to 8.8 months.16-19 The acquired resistant to selective V600EBRAF makes the development of new candidates to be a new therapy for V600EBRAF embedded cancers an essential issue.

Pyrrolo[2,3-*b*]pyridine scaffold is present in a variety of B-RAF approved and preclinical investigated inhibitors (Fig. 1)[14, 23-25]. Starting from our previous work on pyrrolopyridine scaffold and based on our BRAF related researches [26-29] and the high therapeutic applications of pyrrolo[2,3-*b*]pyridine scaffold we developed a new pyrrolo[2,3-*b*]pyridine candidates in order to check their ^{V600E}BRAF inhibitory effect. In the current work, pyrrolo[2,3-*b*]pyridine scaffold was used as a building block in attempt to produce potent ^{V600E}B-RAF inhibitors. Two synthetic pathways were used to produce two sets of new pyrrolo[2,3-*b*]pyridine-based derivatives **24**, **25**, **26**, **27**, **34**, and **35** (Fig. 2). The new compounds were evaluated *in vitro* against ^{V600E}BRAF kinase enzyme. Moreover, all the synthesized derivatives were subjected to NCI sixty human cancer cell lines assay. The preliminary biological data of the first group of compounds **24**, **25**, **26**, **27, 34 and 35** showed satisfied enzyme inhibitory activity. Compounds **34a-g** and **35** exhibited high enzymatic and cellular activities upon tested against ^{V600E}BRAF kinase enzyme and NCI sixty human cancer cell lines. The biological evaluation data indicated that the structural modification in the second group of compounds (**34** and **35**) has a tangible effect on enhancing the activity against both the enzyme and human cancer cell lines.

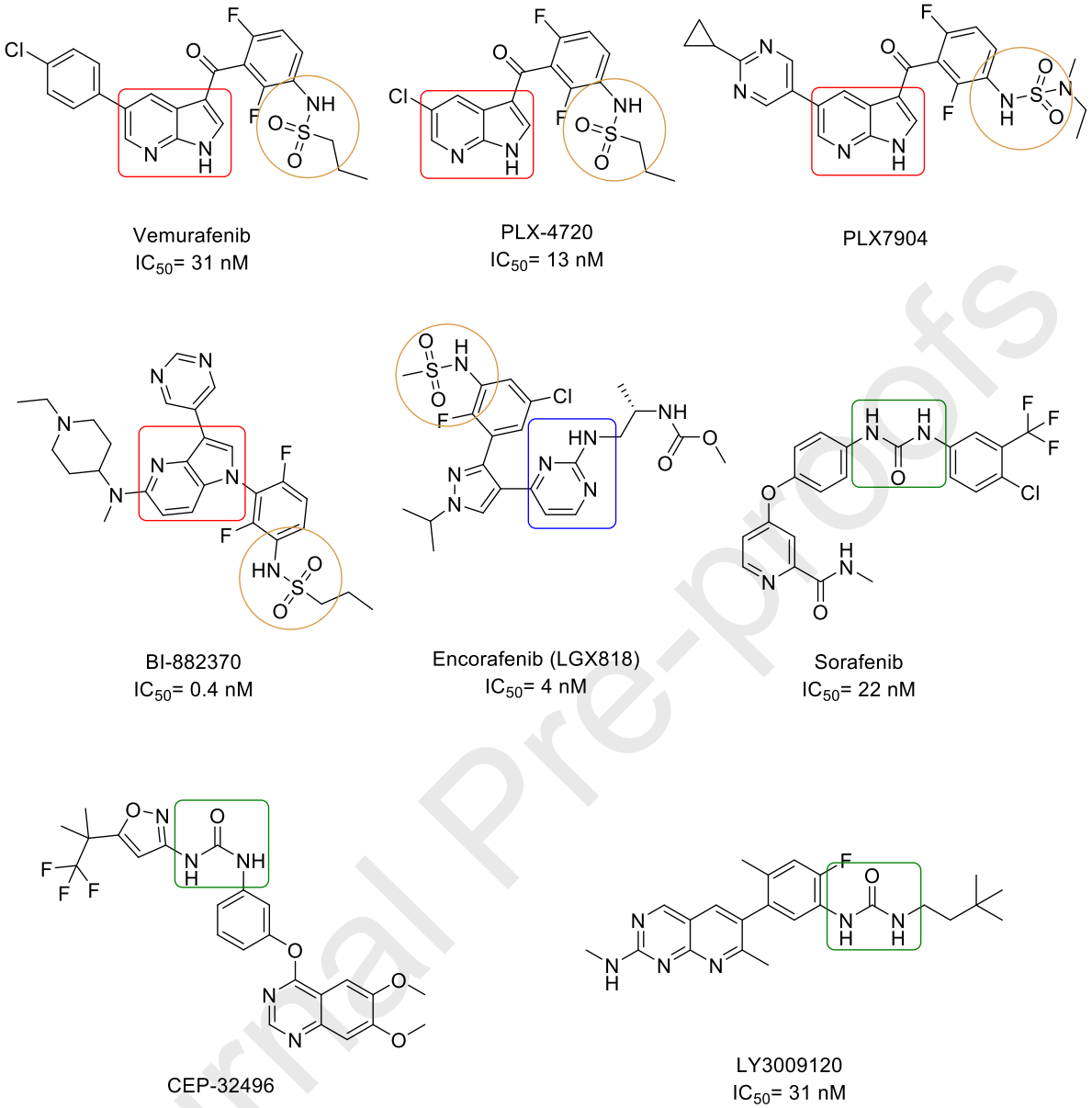
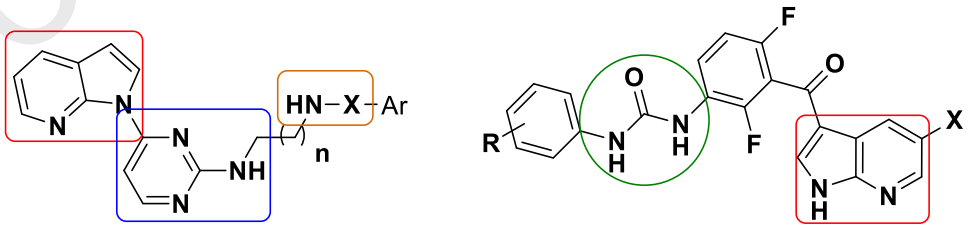


Figure 1: Structures of reported B-RAF inhibitors.



24a-h, **25a-h**, **26a-g**, and **27a-g**

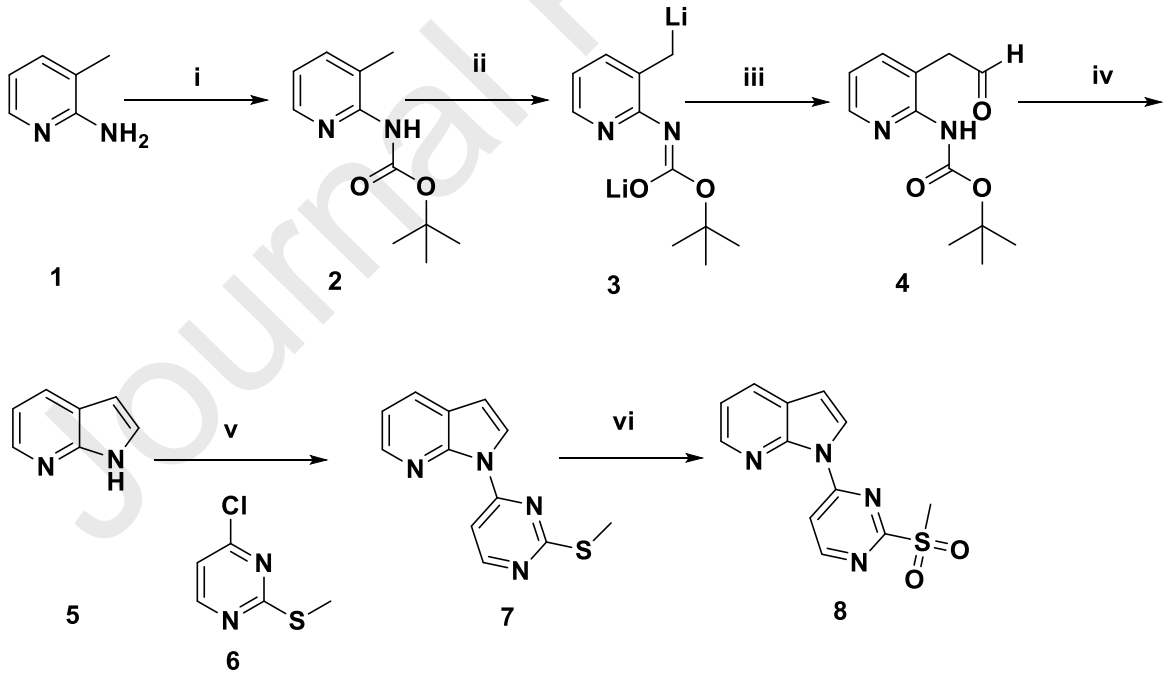
34a-g and **35**

Figure 2: General Structures of the final targeted compounds **24a-h**, **25a-h**, **26a-g**, **27a-g**, **34a-g**, and **35**.

2. Results & discussion

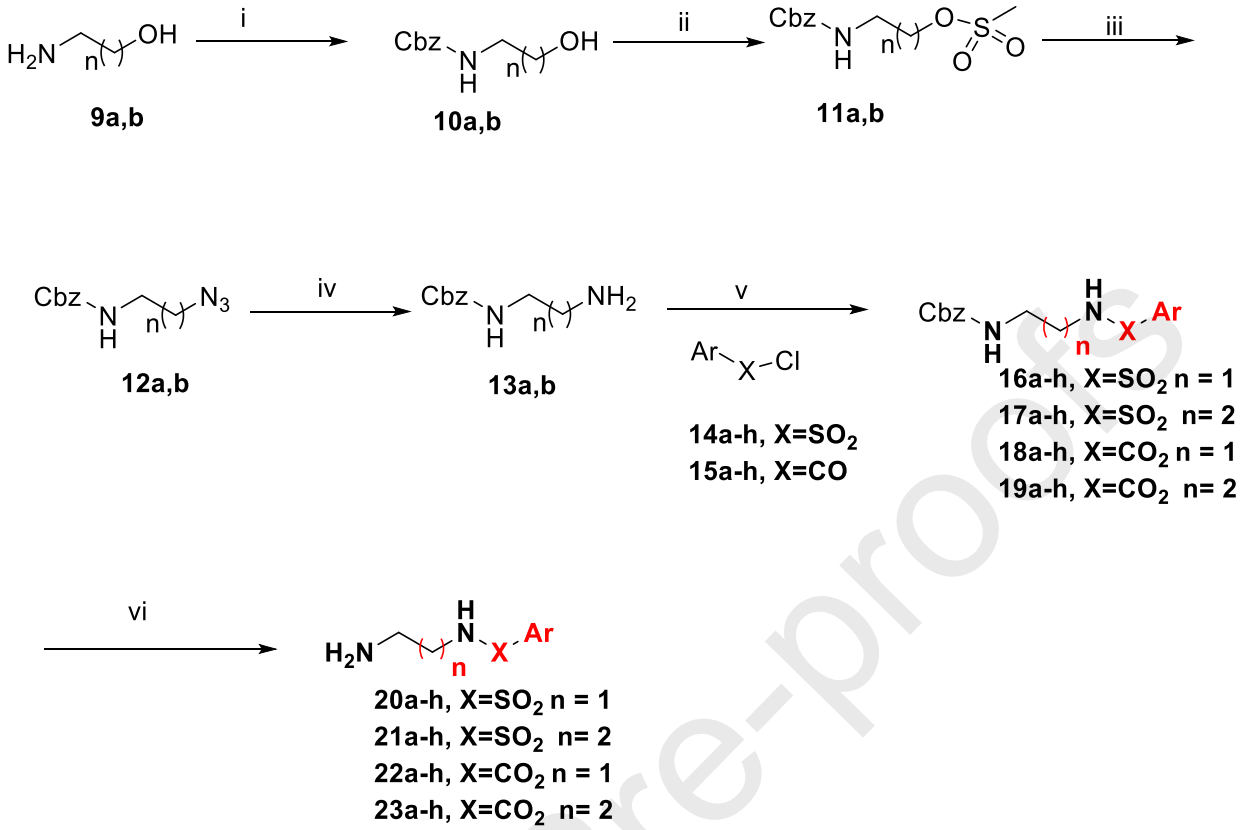
2.1. Chemistry

The synthesis of the N1 substituted ethyl and propyl- 1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin targeted compounds **24**, **25**, **26**, and **27** (Table 1) was performed as described in **Scheme 1, Scheme 2, and Scheme 3**. Starting by protecting 3-methylpyridin-2-amine (**1)** using di-tert-butyl dicarbonate to obtain tert-butyl (3-methylpyridin-2-yl)carbamate (**2**). Compound **2** reacted with butyl lithium to give lithium (E)-((2-((tert-butoxyoxidomethylene)amino)pyridin-3-yl)methyl)lithium (**3**). The dilithio compound **3** was condensed with DMF to give tert-butyl (3-(2-oxoethyl)pyridin-2-yl)carbamate (**4)** from which 7-azaindole (**5)** was obtained by acidic hydrolysis, cyclisation, and dehydration. 7-azaindole (**5)** was -arylated with 4-chloro-2-(methylthio)pyrimidine (**6**) using sodium hydride to give 1-(2-(methylthio)pyrimidin-4-yl)-1H-pyrrolo[2,3-b]pyridine (**7**). Oxidation of methylthio group using oxone produced the key intermediate 1-(2-(methylsulfonyl)pyrimidin-4-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (**8)**.



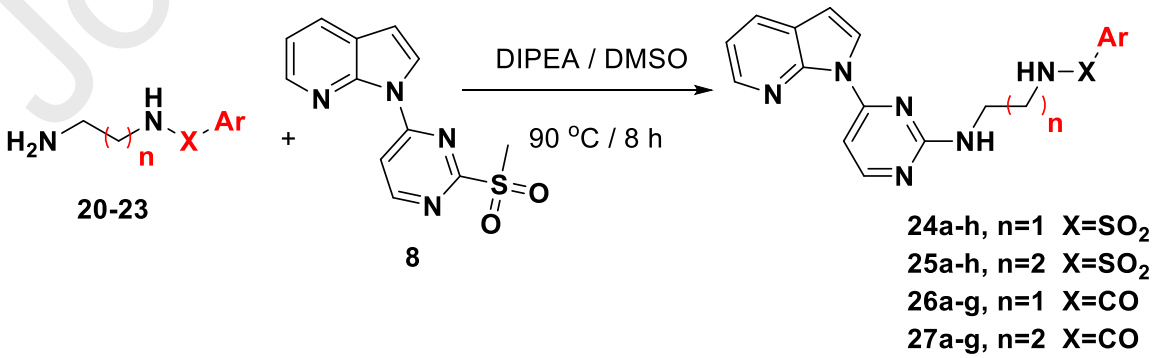
Scheme 1: Synthesis of intermediate **8**; **Reagents and conditions: i)** Boc anh., DMAP, DIPEA, THF, rt, 12 h.; **ii)** n-BuLi, THF, 0 °C, 4 h.; **iii)** DMF, HCl, 0 °C; **iv)** HCl, reflux, 8 h.; **v)** NaH, DMF, 80 °C, 12 h.; **vi)** Oxone, MeOH/H_2O, rt, 18 h.

The preparation of N^1-sustituted ethane-1,2 diamines **20**, and **22** and N^1-sustituted propane-1,3-diamines **21** and **23** was carried out by reaction of 2-aminoethan-1-ol (**9a)** and 3-aminopropan-1-ol (**9b)** with benzyl chloroformate to give benzyl (2-hydroxyethyl)carbamate (**10a**) and benzyl (3-hydroxyethyl)carbamate (**10b**), followed by conversion of OH to mesylate group with methane sulfonyl chloride to obtain 2-(((benzyloxy)carbonyl)amino)ethyl methanesulfonate (**11a**) and 3-(((benzyloxy)carbonyl)amino)propyl methanesulfonate (**11b**). The mesylates, **11**, were then reacted with sodium azide in dry dimethylformamide to afford benzyl (2-azidoethyl)carbamate (**12a)**, and benzyl (3-azidopropyl)carbamate (**12b**) which were converted to the corresponding amines **13** by reduction with triphenylphosphine in methanol. Coupling of the produced amines **13** with appropriate sulfonyl chlorides **14** or benzoyl chlorides **15** in dichloromethane gave compounds **16**, **17**, **18**, and **19**. The protected amino linkers **16**-**19** were then subjected to Pd/C in methanol to afford N-(2-aminoethyl)substituted amides(sulfonamides) and N-(3-aminopropyl)substituted amides(sulfonamides) side chains **20**-**23**. (**Scheme 2**).



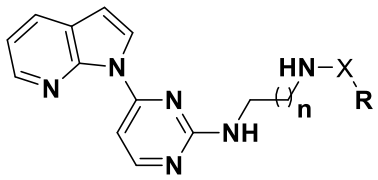
Scheme 2: Synthesis of side chains **20a-h**, **21a-h**, **22a-h**, and **23 a-h**; **Reagents and conditions: i)** Benzyl chloroformate, TEA, MC, 0 °C 12 h.; **ii)** Methane sulfonyl chloride, TEA, MC, 0 °C, 4 h.; **iii)** NaN_3, DMSO, 70 °C, 2 h; **iv)** Triphenyl phosphine, MeOH, reflux, 2 h.; **v)** Appropriate arylsufonyl chloride or acid chloride, DIPEA, MC, 0 °C .; **vi)** H_2/Pd-C, MeOH, rt, 2 h .

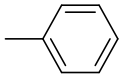
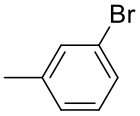
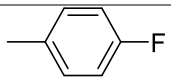
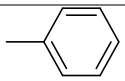
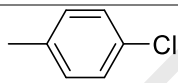
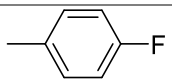
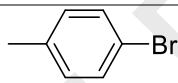
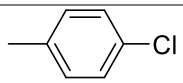
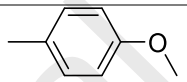
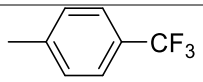
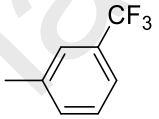
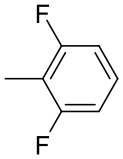
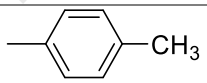
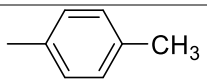
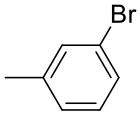
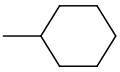
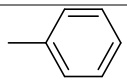
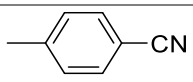
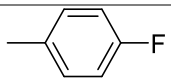
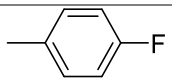
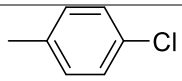
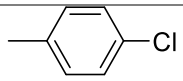
Nucleophilic substitution reaction of the different aliphatic amines **20**-**23** on the key intermediate **8** in presence of N,N-diisopropylethyl amine produced the final target compounds **24**, **25**, **26**, and **27**. (**Scheme 3**).

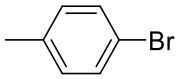
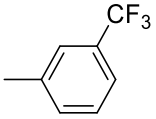
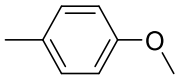
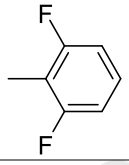
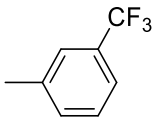
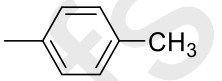
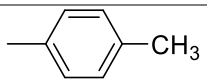


Scheme 3: Synthesis of the final target compounds **24a-h**, **25a-h**, **26a-g**, and **27a-g**.

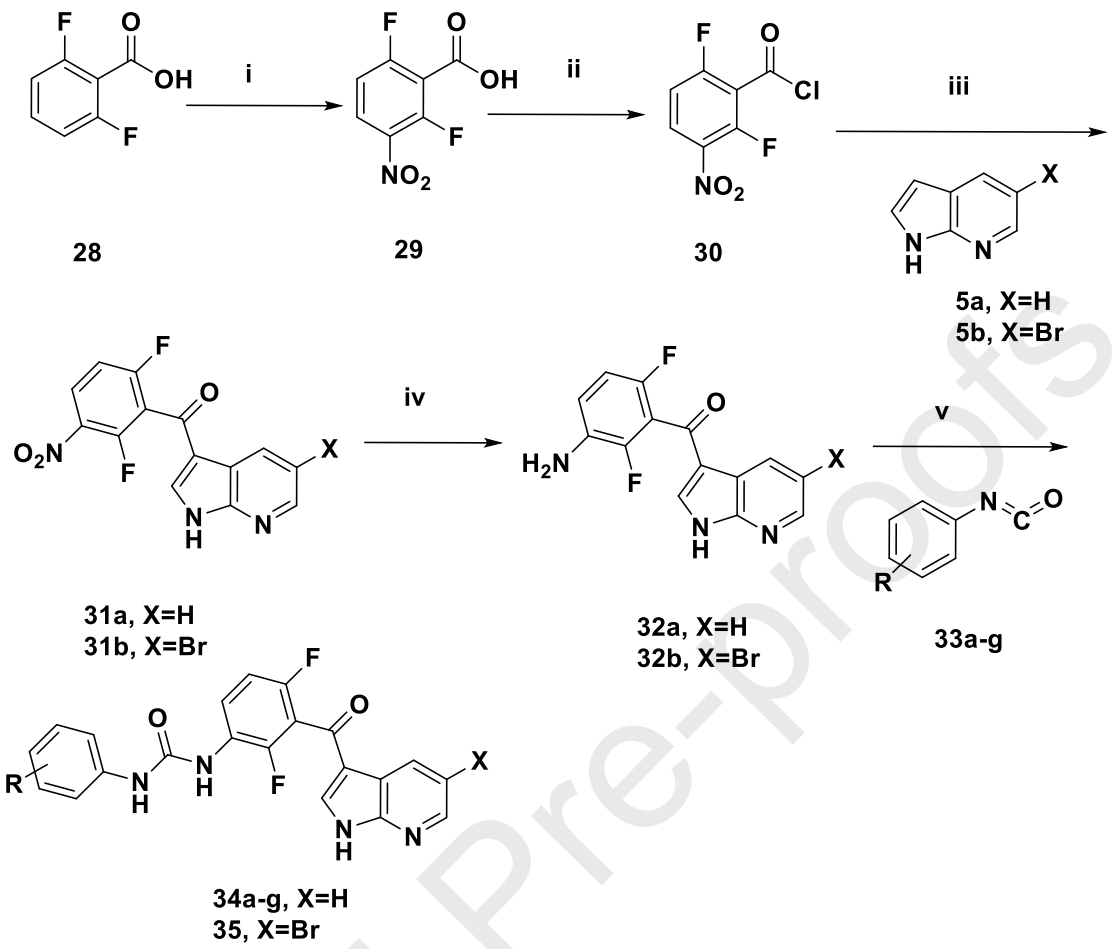
Table 1: Structure of the final target compounds **24a-h**, **25a-h**, **26a-g**, and **27a-g**:



Comp.	n	x	R	Comp.	n	x	R
24a	1	SO_2		**25h**	2	SO_2	
24b	1	SO_2		**26a**	1	CO	
24c	1	SO_2		**26b**	1	CO	
24d	1	SO_2		**26c**	1	CO	
24e	1	SO_2		**26d**	1	CO	
24f	1	SO_2		**26e**	1	CO	
24g	1	SO_2		**26f**	1	CO	
24h	1	SO_2		**26g**	1	CO	
25a	2	SO_2		**27a**	2	CO	
25b	2	SO_2		**27b**	2	CO	
25c	2	SO_2		**27c**	2	CO	

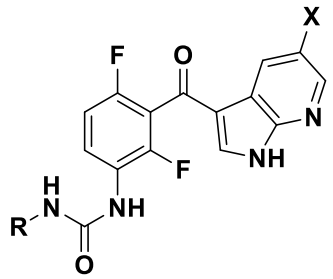
25d	2	SO_2		**27d**	2	CO	
25e	2	SO_2		**27e**	2	CO	
25f	2	SO_2		**27f**	2	CO	
25g	2	SO_2		**27g**	2	CO	

For biological evaluation expansion, a new series of the Azaindole derivatives were prepared with different substitutional pattern owing to enhance the biological inhibitory activity. 1-(3-(5-subs-1H-pyrrolo[2,3-*b*]pyridine-3-carbonyl)-2,4-difluorophenyl)-3-(4-substituted-phenyl)urea **34a-g** and **35** (Table 2) were synthesized starting from the nitration of 2,6-difluorobenzoic acid **28** using conc. H_2SO_4 and conc. HNO_3 producing the nitrated compound **29**. Reaction of carboxylic acid derivative **29** with thionyl chloride producing the acid chloride form **30**. Friedel–Crafts acylation was carried out between the acid chloride derivative **30** and Azaindole derivatives **5a,b** using aluminium chloride as lewis acid reagent producing the corresponding (2,6-difluoro-3-nitrophenyl)(1H-pyrrolo[2,3-b]pyridin-3-yl)methanone derivatives **31a,b**. Reduction of the nitro derivatives **31a,b** by pd/C under H_2 giving the amino derivatives **32a,b**. The targeted urea compounds **34a-g** and **35** were prepared by the reaction of the amino compounds **32a,b** with different substituted phenyl isocyanates **33a-g**. (**Scheme 4**).

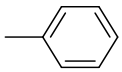
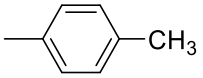
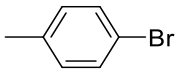
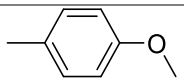
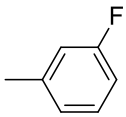
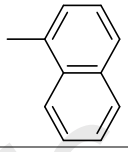
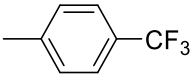
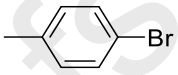


Scheme 4 : Synthesis of final target compounds **34a-g** and **35**; **Reagents and conditions: i)** Conc. HNO_3, Conc. H_2SO_4, 0 °C, 2 h.; **ii)** Thionyl chloride, DMF, 70 °C, 5 h.; **iii)** Aluminium chloride, DCM, 50°C, 12h; **iv)** $SnCl_2.2H_2O$, EtOAc, 80 °C, 4 h.; **v)** THF, 0 °C .

Table 2: Structure of the final target compounds **34a-g** and **35**:



Comp.	X	R	Comp.	X	R

34a	H		**34e**	H	
34b	H		**34f**	H	
34c	H		**34g**	H	
34d	H		**35**	Br	

2.2. Biological Evaluation

2.2.1. V600E-B-RAF and wild type BRAF Kinases Inhibition

The Azaindole-based compounds were reported as BRAF kinase inhibitors,[30-33] The synthesized Azaindole derivatives **24a-h**, **25a-h**, **26a-g**, **27a-g**, **34a-g** and **35** were tested for their inhibitory effect over V600E-B-RAF using an enzyme-linked immunosorbent assay (ELISA). As reported in table 3 and table 4, tested compounds exhibited a wide range of inhibition activity against V600E-B-RAF. The preliminary % inhibition data of first Azaindole-based series **24a-h**, **25a-h**, **26a-g**, **27a-g** revealed that most of sulfonamide moiety containing derivatives **24** and **25** have higher inhibition activity than the ones with amide moiety **26** and **27**. Moreover, the propyl linker of the sulfonamide possessing derivatives **25a-h** is bearing to have great effect on the inhibitory activity with % inhibition mean of 97%. Among the highest active inhibitors **25a-h**, the IC_{50} of seven compounds **25a,b,d,e,f,g,h** were determined. The obtained IC_{50} values are ranging from 0.35 µM to 1.1 µM. The para-substitution with electron withdrawing groups on the terminal phenyl sulfonamide in compounds **25b**, and **25d** enhanced the activity compared to the electron donating substituted derivative **25g**. However, compound **25e** with electron donating methoxy group revealed quite similar inhibitory activity like compounds **25b**, and **25d** with electron withdrawing groups. Nevertheless, the meta-substitution with electron withdrawing groups on the terminal phenyl sulfonamide in compounds **25f** and **25h** showed slight drop in inhibitory activity compared to para substituted derivatives **25b**, **25d**, and **25e**. Compound **25d** with para bromo group is the highest active inhibitor (IC_{50} =0.35 µM) among the first Azaindole-based set. In our attempts to produce more potent V600E-B-RAF inhibitors, a

second set of Azaindole-based derivatives bearing urea moiety **34a-g** and **35** were synthesized and subjected to V600E-B-RAF enzyme assay evaluation. In similar manner, the inhibition of wild type BRAF was determined to the final target compounds. Generally, the final compounds have lower inhibitory effect over wild type BRAF compared to mutated BRAF. Only compounds **26f** and **34c** produced slightly higher activity over wild type BRAF compared to V600EBRAF. The % inhibition values are depicted in Table 3.

Table 3: Enzyme % Inhibition Activity of the final target compounds **24a-h**, **25a-h**, **26a-g**, **27a-g**, **34a-g** and **35** over BRAF (V600E) and wild type BRAF at 10 uM .

Comp.	BRAF (V600E) % Inhibition at (10 µM)	BRAF (WT) % Inhibition at (10 µM)	Comp.	BRAF (V600E) % Inhibition at (10 µM)	BRAF (WT) % Inhibition at (10 µM)
24a	66.45	52.31	**26d**	32.90	12.05
24b	89.51	55.87	**26e**	-1.90	0.1
24c	63.66	49.21	**26f**	81.81	53.84
24d	65.90	44.64	**26g**	73.66	43.44
24e	56.70	39.25	**27a**	69.37	60.91
24f	18.15	10.21	**27b**	67.50	58.74
24g	41.12	26.32	**27c**	80.69	72.14
24h	65.35	55.20	**27d**	15.33	9.45
25a	95.54	81.41	**27e**	72.58	67.24
25b	95.19	79.65	**27f**	61.65	49.99
25c	96.83	66.46	**27g**	49.63	41.25
25d	97.73	75.21	**34a**	94.5	86.46
25e	94.48	82.14	**34b**	92	80.11
25f	96.10	86.32	**34c**	49	51.22
25g	92.96	83.29	**34d**	98.7	79.26
25h	96.70	89.38	**34e**	98.4	77.57
26a	86.27	86.34	**34f**	81.3	69.63
26b	69.99	56.77	**34g**	85.6	68.94
26c	0.16	-1.1	**35**	92.3	72.54

From the revealed inhibitory activity data of the tested compounds, we found that the para-substitution on the terminal phenyl urea derivatives **34d** and **34e** has a slight increase in the activity rather than the unsubstituted derivative **34a**. Conversely, the meta- substitution on the

terminal phenyl urea derivative **34c** showed a huge drop in the inhibitory activity. 5-Bromo substitution on the Azaindole ring in compound **35** showed to have no effect in the inhibitory activity. The IC_{50} of the most potent inhibitors **34d**, **34e**, and **35** with para-substitution with triflouromethyl group and methyl group, respectively were determined Table 4. The three tested compounds showed Nano molar IC_{50} and compound **35** showed the most potential inhibitory effect against ^{V600E}BRAF (80 nM).

The data reported in Table 3 and Table 4 revealed that the structural modification on the second set of the Azaindole-based series **34a-g** and **35** has a tangible effect for activity improvement over the first set of Azaindole-based series **24a-h**, **25a-h**, **26a-g**, and **27a-g**.

Table 4: IC_{50} values of most active ^{V600E}BRAF inhibitors compounds against ^{V600E}BRAF kinases.

Comp.	**IC_{50} (μM)** BRAF (V600E)
25a	0.75 ± 0.012
25b	0.88 ± 0.009
25d	0.35 ± 0.016
25e	0.79 ± 0.010
25f	1.1 ± 0.0210
25g	1 ± 0.0113
25h	0.5 ± 0.002
34d	0.114 ± 0.002
34e	0.085 ± 0.001
35	0.080 ± 0.003

2.2.2. NCI 60 cell lines one dose test

All the synthesized derivatives were submitted to the NCI, USA for in vitro cytotoxicity assay against a 60 cell lines panel belonging to nine cancer types (leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and

breast cancer). The first Azaindole-based series **24a-h**, **25a-h**, **26a-g**, and **27a-g** exhibited no remarkable activity against the cell growth according to 60 cell line results (supporting information and table 1s).

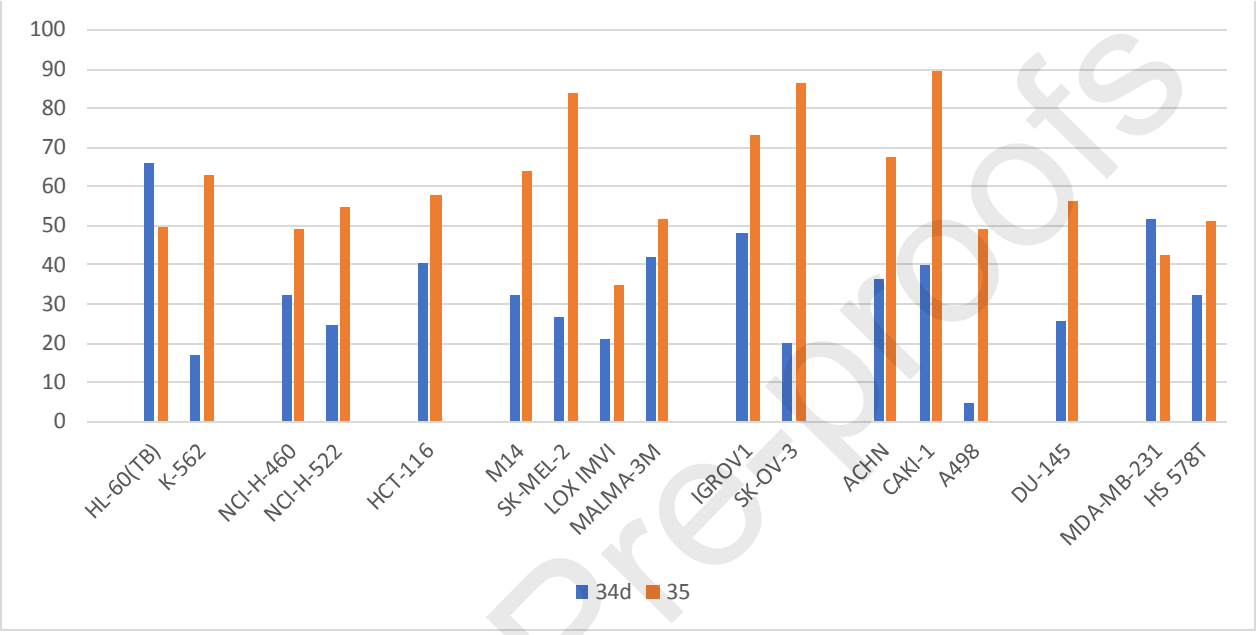


Figure 3: % inhibition of **34d**, and **35** on most sensitive cancer cell lines at 10 uM**.**

Among the second set derivatives **34a-g** and **35**, only compound **35** with Bromo substitution at C^5 of the Azaindole ring showed moderate inhibitory activity against different cell lines (Figure 3) such as Leukemia K-562 (65%), Melanoma M14 (65%), Melanoma SK-MEL-2 (85 %), Ovarian cancer IGROV1 (75 %), Ovarian cancer SK-OV-3 (87 %), Renal cancer ACHN (70%), and Renal cancer CAKI-1 (90%). Moreover, compound **34d** with CF_3 terminal substituted urea, exhibited a moderate inhibitory activity against Leukemia HL-60(TB) (67 %) and Leukemia K-562 (83 %) (Fig.3).

Table 5: IC_{50} of compounds 34d and 35 over most sensitive cell lines

Cell line	Compound IC_{50}(uM)	
	34d	**35**
Leukemia		
HL-60(TB)	8.12 ± 0.09	10.91 ± 0.12
K-562	25.31 ± 0.22	7.21 ± 0.14
Non-Small Cell Lung cancer		
NCI-H-460	26.42 ± 0.31	10.53 ± 0.21
NCI-H-522	29.11 ± 0.24	9.01 ± 0.15
Colon		
HCT-116	15.53 ± 0.27	8.71 ± 0.18
Melanoma		
M14	24.32 ± 0.21	7.92 ± 0.22
SK-MEL-2	21.12 ± 0.32	6.02 ± 0.16
LOX IMVI	27.02 ± 0.35	26.53 ± 0.13
MALMA-3M	12.35 ± 0.62	9.26 ± 0.55
Ovarian cancer		
IGROV1	9.66 ± 0.42	5.06 ± 0.33
SK-OV-3	18.78 ± 0.31	4.52 ± 0.27
Renal cancer		
ACHN	21.33 ± 29	7.62 ± 0.18
CAKl-1	14.01 ± 0.37	3.04 ± 0.16
A498	32.33 ± 0.52	11.24 ± 0.11
Prostate cancer		
DU-145	27.56 ± 0.26	9.62 ± 0.32
Breast cancer		
MDA-MB-231	9.72 ± 0.25	12.24 ± 0.10
HS 578T	21.35 ± 0.41	9.36 ± 0.42

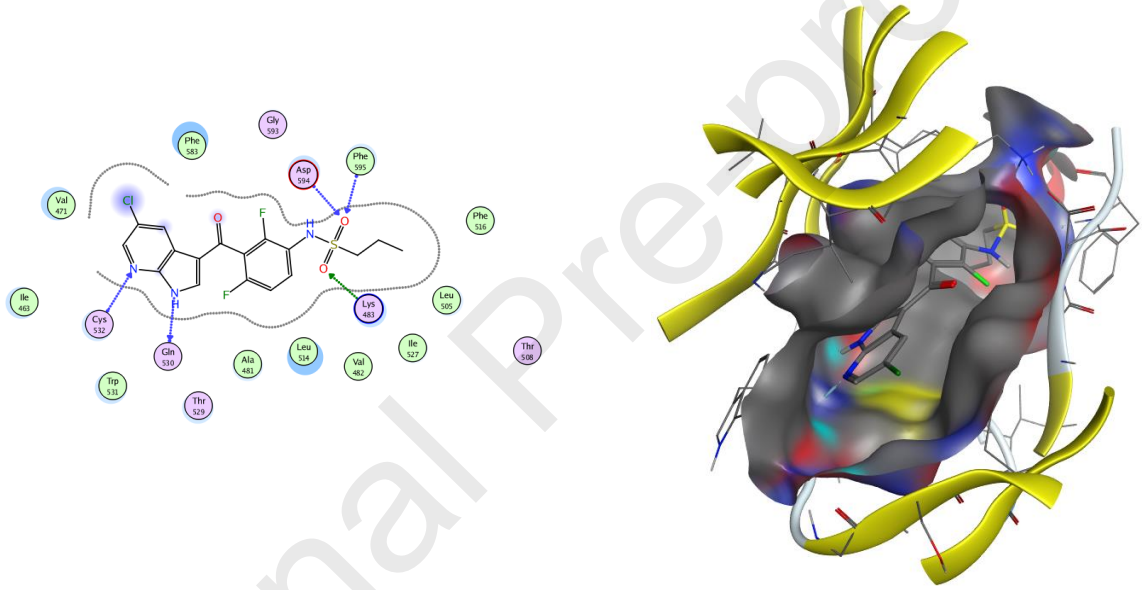
In order to compare the potencies of compounds **34d** and **35** over the most sensitive cell lines, the concentration required to reduce cellular growth to 50% after treatment of the cells with tested compound compared to untreated cell (IC_{50}) was determined for both tested compounds (Table 5). For leukemia cell lines, compound **35** exhibited the highest activity with IC_{50} 7.21 uM over K-562 while compound **34d** was more potent on HL-60(TB) with IC_{50} 8.12 uM. Regarding non-small cell lung cancer, compound **35** was more potent on both NCI-H-460 and NCI-H-522 with IC_{50}s 10.53 and 9.01 uM, respectively. Compound **35** was also stronger over colon cancer cell line HCT-116 , melanoma cell lines M14, SK-MEL-2, LOX IMVI and MALMA-3M , ovarian cancer cell lines IGROV1 and SK-OV-3, renal cancer cell lines ACHN and CAKl-1, prostate cancer cell line DU-145 and breast cancer cell line HS 578T. Compound **34d** showed higher activity against only MDA-MB-231 breast cancer cell line with IC_{50} 9.72 uM.

2.3. Molecular docking

Molecular docking study was conducted to demonstrate binding modes prospects of the second synthetic series **34a-g** and **35** into BRAF kinase binding site. In this study, we used the known crystal structure of PLX4720-BRAF inhibitor complex (PDB: 3C4C). The low root mean square deviation, RMSD (0.6346) of PLX4720-BRAF kinase domain complex with dock score (-12.12 kcal/mol, Table 6) proved the validity of the used docking protocol. The resulted docking poses of the native ligand (PLX4720) showed H-bonding interaction between the N-pryridine and NH-Pyrole of the Azaindole moiety and Cys 532 and Gln 530 amino acids of the hinge binder backbone. In addition, the sulphonamide moiety formed three hydrogen bonds between the SO_2 group and Lys 483, Asp 594 and Phe 595 amino acids. The binding affinity of the ligand was evaluated with energy score (S, Kcal/mol) (Table 6). Applying the same docking protocol into the synthetic hits **34a-g** and **35** showed a similar binding behaviour like the native ligand. Compounds **34f**, **34g**, and **35** preserved the H-bonding interaction with Cys 532 in the hinge binding region (Fig. 4). Moreover, the urea moiety in all compounds played the same role of

sulphonamide moiety in the native ligand PLX4720 by formation of H-bonding interaction with a variety of amino acids such as Asp 594, Phe 595, and Lys 483. In addition, arene-arene and arene-H interactions were displayed between Pyridine ring and Pyrrole ring, and Phe 583 and Gly 464. Unlike the native ligand PLX4720, the terminal substituted phenyl group in compounds **34b**, **34d**, and **35** showed variable interaction modes with Thr 508, Lys 483, and Leu 505.

A



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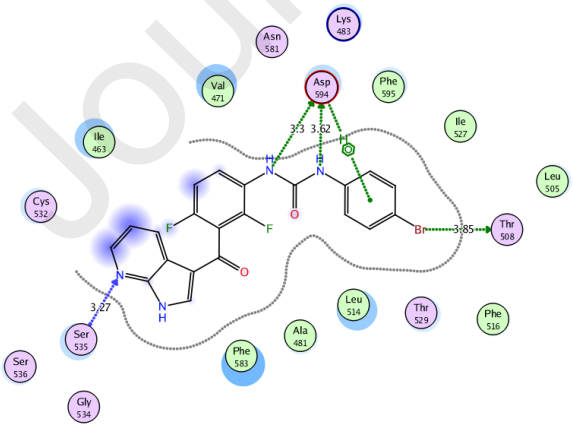

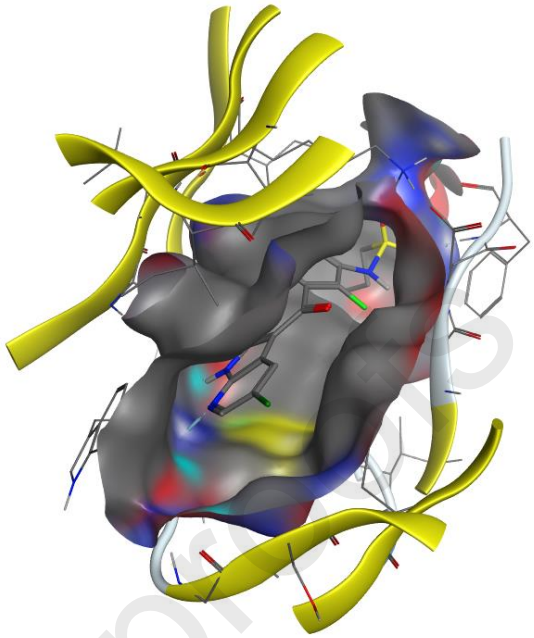

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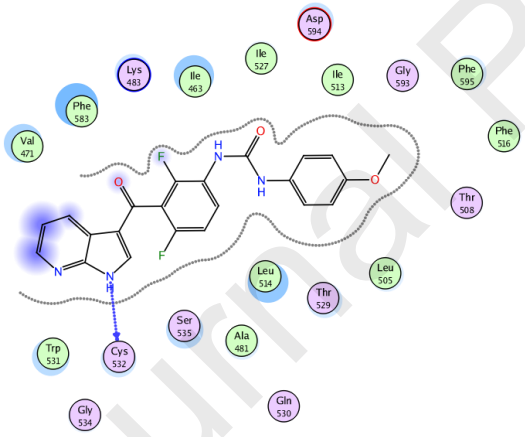

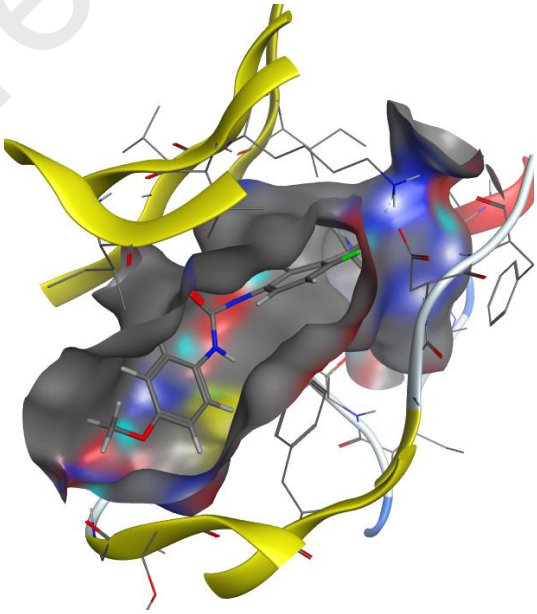

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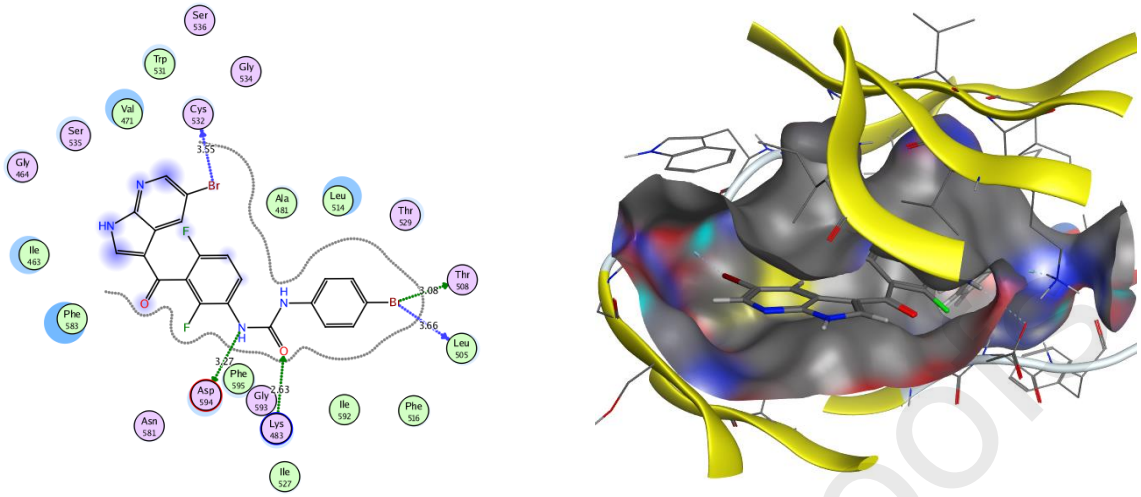

Figure 4. 2D and 3D interaction diagram of docking study output (PDB ID: 3C4C) **A)** 2D and 3D interaction of **PLX4720** (native ligand) with BRAF kinase enzyme domain**; B)** 2D and 3D interaction of **34b** with BRAF kinase enzyme domain **C)** 2D and 3D interaction of **34f** with BRAF kinase enzyme domain**; D)** 2D and 3D interaction of **35** with BRAF kinase enzyme domain.

Table 6: Docking results of target compounds **34a-g** and **35** with BRAF kinase enzyme domain (PDB ID: 3C4C).

Compound	Energy score (Kcal/mol)	Amino acid	Binding group	Interaction	Hydrogen bond length (Å)
PLX4720	-12.12	Cys 532	N pyridine	H-bond	2.96
		Gln 530	NH pyrrole	H-bond	2.96
		Asp 594	SO_2	H-bond	2.65
		Phe 595	SO_2	H-bond	2.72
		Lys 483	SO_2	H-bond	2.56
34a	-12.24	Gly 464	Pyridine ring	Arene-H	
		Phe 583	Pyrrole ring	Arene-H	
		Asp 594	CO	H-bond	3.19
		Phe 595	CO	H-bond	3.45
		Lys 483	CO	H-bond	3.35
34b	-12.38	Ser 535	N pyridine	H-bond	3.27
		Asp 594	NH	H-bond	3.3
		Asp 594	NH	H-bond	3.62
		Asp 594	Phenyl	Arene-H	
		Thr 508	Br	H-bond	3.85

34c	-13.20	Phe 583 Asp 594 Phe 595	Pyrrole ring CO CO	Arene-Arene H-bond H-bond	 3.23 2.84
34d	-13.81	Gly 464 Lys 483	Pyridine ring CF_3	Arene-H H-bond	 2.82
34e	-12.51	Asp 594 Phe 583 Phe 583	CO NH NH	H-bond Arene-H Arene-H	 2.99
34f	-12.48	Cys 532	NH pyrrole	H-bond	3.46
34g	-12.40	Cys 532	N pyridine	H-bond	2.88
35	-12.09	Cys 532 Asp 594 Lys 483 Leu 505 Thr 508	Br-Azaindole NH CO Br-Phenyl Br-Phenyl	H-bond H-bond H-bond H-bond H-bond	3.55 3.27 2.63 3.66 3.08

3. Conclusion

Two series of Azaindole-based derivatives were synthesized and tested against ^{V600E}B-RAF kinase to investigate their potency. The biological evaluation data revealed that the substitution arrangement modification in the second set of azaindole-based derivatives has a remarkable effect in enhancing the activity. In the first set of compound, SAR studies revealed that most of sulfonamide moiety containing derivatives **24** and **25** have higher inhibition activity over the amide bearing derivatives **26** and **27**. Additionally, the terminal propyl linker containing derivatives **25a-h** showed higher potency than the one with ethyl linker **24a-h**. Compound **25d** with para bromo group is the highest active ^{V600E}BRAF inhibitor (IC_{50} = 0.35 µM) among the first Azaindole-based set. The substitution diversity on the urea moiety containing second set showed variable inhibitory activities. Compound **35** showed that most potential inhibitory effect against ^{V600E}BRAF (IC_{50} = 0.080 µM). Among the synthesised compounds of the two series, compound **35** exhibited cytotoxicity activity against different panel of human cancer cell lines.

Overall, the second set of the Azaindole derivatives represent a therapeutically promising scaffold for future structural optimization to develop a new anticancer agent.

4. Experimental

4.1. Chemistry

General

The intermediate compounds **8** and **20-23** as well as the target compounds **24a–h**, **25a–h**, **26a–g** and **27a–g** were purified by flash column chromatography using silica gel 60 (0.040‑0.063 mm, 230‑400 mesh ASTM) and technical grade solvents. ^{1}H NMR and ^{13}C NMR analyses were carried out on a Bruker Avance 400 spectrometer using tetramethylsilane (TMS) as an internal standard. Melting points were measured on a Walden Precision Apparatus Electrothermal 9300 apparatus and were uncorrected. LC-MS analysis was conducted 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% TFA in water; solvent B: 0.035% TFA in MeOH; flow rate=3.0 ml/min; the AUC was calculated using Waters MassLynx 4.1 software. The solvents and liquid reagents were transferred using hypodermic syringes. All the solvents and reagents were purchased from commercial companies, and used as such.

Synthesis of 1*H*-pyrrolo[2,3-*b*]pyridine (**5**)

It was carried out utilizing the 4-step procedure previously reported in the literature[34].

Synthesis of 1-(2-(methylthio)pyrimidin-4-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (**7**)

To a solution of compound **5** (10 g, 84.74 mmol) in DMF (40 ml) under N_2 is added sodium hydride (3.55 g of a 60% suspension in mineral oil, 88.8 mmol) and stirred for 1 h. 4-chloro-2-(methylthio)pyrimidine **6** (15 g, 93.21 mmol, 1.1 eq) in DMF is slowly added and the reaction stirred for 12 h at 80 °C. The reaction is quenched with a $NaHCO_3$ solution before extracting with DCM. The combined extracts were washed with water and brine then dried over anhydrous Na_2SO_4. The solvent is removed in vacuo to give the title intermediate **7**.

Yield: 85%. m.p.: 137 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.74 (d, J = 5.6 Hz, 1H, Ar-H), 8.7 (d, J = 5.6 Hz, 1H, Ar-H), 8.43 (m, 2H, Ar-H), 8.14 (dd, J = 1.6 Hz, J = 8 Hz, 1H, Ar-H), 7.34 (q, J = 3.2 Hz, 1H, Ar-H), 6.86 (d, J = 4 Hz, 1H, Ar-H), 2.6 (s, 3H, S$\underline{CH_3}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.74

(Ar-C), 159.61 (Ar-C), 156.74 (Ar-C), 148.12 (Ar-C), 144.36 (Ar-C), 130.66 (Ar-C), 125.54 (Ar-C), 124.14 (Ar-C), 119.95 (Ar-C), 104.27 (Ar-C), 105.63 (Ar-C), 14.1 (SCH_3).

Synthesis of 1-(2-(methylsulfonyl)pyrimidin-4-yl)-1*H*-pyrrolo[2,3-*b*]pyridine (**8**)

To a solution of compound **7** (10 g, 41.32 mmol) in methanol (100 ml) is added a solution of oxone (84.5 g, 124 mmol, 3 eq.) in water (100 ml) dropwise at rt and stirred at rt for 48 h. The reaction mixture was concentrated under vacuo. The reaction mass was extracted with dichloromethane (20 ml) and the organic layer was separated. The aqueous layer was extracted with dichloromehane. The combined extracts were dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuo. The crude residue was purified by column chromatography (hexane:ethyl acetate; 2:1) to give the titled product **8**.

Yield: 80%. m.p.: 197 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.51 (t, *J* = 4.4 Hz, 2H, Ar-H), 8.34 (d, *J* = 4 Hz, 1H, Ar-H), 8.26 (d, *J* = 6.8 Hz, 1H, Ar-H), 8.14 (q, *J* = 1.6 Hz, 1H, Ar-H), 7.34 (t, *J* = 3.2 Hz, 1H, Ar-H), 6.85 (d, *J* = 4 Hz, 1H, Ar-H), 3.53 (s, 3H, SO_2CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.79 (Ar-C), 160.84 (Ar-C), 151.97 (Ar-C), 144.08 (Ar-C), 142.65 (Ar-C), 130.56 (Ar-C), 125.86 (Ar-C), 119.17 (Ar-C), 115.99 (Ar-C), 104.09 (Ar-C), 103.98 (Ar-C), 100.68 (Ar-C), 95.64 (Ar-C), 46.07 (SO_2CH_3).

Synthesis of N^1-sustituted ethane(propane)-1,2(3)diamine (**20a-h**, **21a-h**, **22a-g**, and **23a-g**)

Synthesis of side chains (**20a-h**, **21a-h**, **22a-g**, and **23a-g**) was carried out through a previously reported pathway[35, 36].

Synthesis of N-(2-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)ethyl(propyl))substituted sulfonamide(amide) (**24a-h**, **25a-h**, **26a-g**, and **27a-g**)

To a solution of compound **8** (0.36 mmol) in DMSO (4 ml), compounds **20a-h**, **21a-h**, **22a-g**, and **23a-g** (0.54 mmol) and DIPEA (500 mg, 3 mmol) were added. The reaction mixture was stirred at 90 °C for 8 h. The reaction mixture was cooled and extracted between Ethyl acetate and water. The organic extract was dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuo. The crude residue was purified by column chromatography (hexane:ethyl acetate; 2:1) to give the titled products **24a-h**, **25a-h**, **26a-g**, and **27a-g**.

N-(2-((4-(1H-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)ethyl)benzenesulfonamide (**24a**)

Yield: 70%. m.p.: 221 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.43 (dd, *J* = 1.6 Hz, *J* = 8.2 Hz, 2H, Ar-H), 8.38 (d, *J* = 5.6 Hz, 1H, Ar-H), 8.21 (d, *J* = 5.6 Hz, 1H, Ar-H), 8.13 (dd, *J* = 1.6 Hz, *J* = 8 Hz, 1H, Ar-H), 7.83 (d, *J* = 6.8 Hz, 2H, Ar-H), 7.6 (m, 3H, Ar-H), 7.31 (t, *J* = 4.8 Hz, 2H, Ar-H), 6.83 (d, *J* = 3.6 Hz, 1H, Ar-H), 3.42 (t, *J* = 6.8 Hz, 2H, NH$\underline{CH_2}$-), 3 (d, *J* = 6.4 Hz, 2H, -$\underline{CH_2}$-$NHSO_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.28 (Ar-C), 160.54 (Ar-C), 156.43 (Ar-C), 146.09 (Ar-C), 143.94 (Ar-C), 140.96 (Ar-C), 132.82 (Ar-C), 130.17 (Ar-C), 129.66 (Ar-C), 126.86 (Ar-C), 123.44 (Ar-C), 118.58 (Ar-C), 104.51 (Ar-C), 40.6 (NH$\underline{CH_2}$-), 39.35 ($\underline{CH_2}$-$NHSO_2$). LC/MS calculated for $C_{19}H_{18}N_6O_2S$ is 394.12, Found: 394.45 $(M+1)^+$.

N-(2-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)ethyl)-4-fluorobenzenesulfonamide (**24b**)

Yield: 65%. m.p.: 185-187 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (m, 4H, Ar-H), 8.27 (d, *J* = 4 Hz, 1H, Ar-H), 7.9 (q, *J* = 4 Hz, 2H, Ar-H), 7.52 (q, *J* = 7.2 Hz, 1H, Ar-H), 7.21 (q, *J* = 12.2 Hz, 1H, Ar-H), 7.12 (t, *J* = 8.4 Hz, 1H, Ar-H), 6.65 (d, *J* = 4 Hz, 1H, Ar-H), 6.5 (brs, 1H, Ar-H), 6.14 (brs, 1H, NHCH2), 5.50 (s, 1H, NHSO2), 3.64 (d, *J* = 5.2 Hz, 2H, C**H**$_2$-CH_2-$NHSO_2$), 3.31 (s, 2H, CH_2-C**H**$_2$-$NHSO_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.97 (Ar-C), 159.32 (Ar-C), 156.95 (Ar-C), 148.27 (Ar-C), 142.5 (Ar-C), 129.69 (Ar-C), 129.6 (Ar-C), 129.28 (Ar-C), 129.04 (Ar-C), 126.92 (Ar-C), 125.14 (Ar-C), 122.96 (Ar-C), 117.68 (Ar-C), 116.11 (Ar-C), 104.26 (Ar-C), 41.24 (-$\underline{CH_2}$-$NHSO_2$). LC/MS calculated for $C_{19}H_{17}FN_6O_2S$ is 412.11, Found: 413.1 $(M+1)^+$.

N-(2-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)ethyl)-4-chlorobenzenesulfonamide (**24c**)

Yield: 67%. m.p.: 181-183 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (d, *J* = 9.6 Hz, 2H, Ar-H), 8.29 (s, 1H, Ar-H), 7.96 (d, *J* = 6.4 Hz, 1H, Ar-H), 7.85 (d, *J* = 5.2 Hz, 2H, Ar-H), 7.48 (t, *J* = 10 Hz, 3H, Ar-H), 7.29 (s, 1H, Ar-H), 7.22 (s, 1H, Ar-H), 6.66 (s, 1H, Ar-H), 5.85 (s, 1H, $\underline{NH}CH_2$), 5.38 (s, 1H, $\underline{NH}SO_2$), 3.64 (s, 2H, NH$\underline{CH_2}$-), 3.32 (s, 2H, $\underline{CH_2}$-$NHSO_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.37 (Ar-C), 159.21(Ar-C), 156.83(Ar-C), 148.04 (Ar-C), 143.94 (Ar-C), 140.99 (Ar-C), 132.02 (Ar-C), 130.16 (Ar-C), 129.64 (Ar-C), 126.87 (Ar-C), 123.94 (Ar-C), 118.52 (Ar-C), 104.5 (Ar-C), 42.18 (NH$\underline{CH_2}$-), 39.36 ($\underline{CH_2}$-$NHSO_2$). LC/MS calculated for $C_{19}H_{17}ClN_6O_2S$ is 428.08, Found: 429.1 $(M+1)^+$.

N-(2-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)ethyl)-4-bromobenzenesulfonamide (**24d**)

Yield: 72%. m.p.: 189-191 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42 (dd, *J* = 1.44 Hz, *J* = 7.4 Hz, 1H, Ar-H), 8.38 (d, *J* = 5.48 Hz, 1H, Ar-H), 8.22 (d, *J* = 5.48 Hz, 1H, Ar-H), 8.13 (dd, *J* = 5.48 Hz, *J* = 8.4 Hz, 1H, Ar-H), 7.93 (s, 1H, Ar-H), 7.75 (q, *J* = 5.6 Hz, 3H, Ar-H), 7.31 (q, *J* = 3.04 Hz, 2H, Ar-H), 6.82 (d, *J* = 3.92 Hz, 1H, Ar-H), 3.4 (d, *J* = 6.44 Hz, 2H, NH$\underline{CH_2}$), 3.02 (t, *J* = 6.44 Hz, 2H, -$\underline{CH_2}$-$NHSO_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.12 (Ar-C), 148.06 (Ar-C), 143.93 (Ar-C), 140.29 (Ar-C), 132.71 (Ar-C), 130.15 (Ar-C), 128.93 (Ar-C), 126.58 (Ar-C), 123.94 (Ar-C), 118.51 (Ar-C), 104.51 (Ar-C), 42.05 (NH$\underline{CH_2}$-), 39.36 (-$\underline{CH_2}$-$NHSO_2$). LC/MS calculated for $C_{19}H_{17}BrN_6O_2S$ is 473.3, Found: 474.6 $(M+1)^+$.

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N-(2-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)ethyl)-4-methoxybenzenesulfonamide (**24e**)

Yield: 55%. m.p.: 195-197 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (t, *J* = 1.64 Hz, 2H, Ar-H), 8.36 (d, *J* = 5.64 Hz, 1H, Ar-H), 8.3 (d, *J* = 3.96 Hz, 1H, Ar-H), 7.96 (q, *J* = 1.56 Hz, 1H, Ar-H), 7.78 (s, 1H, Ar-H), 7.76 (s, 1H, Ar-H), 7.29 (s, 2H, Ar-H), 7.22 (d, *J* = 4.8 Hz, 1H, Ar-H), 6.92 (s, 1H, Ar-H), 6.89 (s, 1H, Ar-H), 6.66 (d, *J* = 3.96 Hz, 1H, Ar-H), 3.78 (s, 3H, OCH_3), 3.63 (q, *J* = 5.8 Hz, 2H, NH$\underline{CH_2}$-), 3.29 (q, *J* = 5.6 Hz, 2H, CH_2-$\underline{CH_2}$-$NHSO_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.49 (Ar-C), 162.14 (Ar-C), 156.38 (Ar-C), 148.04 (Ar-C), 143.91 (Ar-C), 132.56 (Ar-C), 130.13 (Ar-C), 129.05 (Ar-C), 123.093 (Ar-C), 118.5 (Ar-C), 114.72 (Ar-C), 104.46 (Ar-C), 55.96 (OCH_3), 42.09 (NH$\underline{CH_2}$-), 39.35 ($\underline{CH_2}$-$NHSO_2$). LC/MS calculated for $C_{20}H_{20}N_6O_3S$ is 424.4, Found: 425.1 $(M+1)^+$.

N-(2-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)ethyl)-3-(trifluoromethyl)benzenesulfonamide (**24f**)

Yield: 63%. m.p.: 199-201 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (s, 1H, Ar-H), 8.36 (d, *J* = 5.6 Hz, 2H, Ar-H), 8.26 (d, *J* = 3.96 Hz, 1H, Ar-H), 8.11 (s, 1H, Ar-H), 7.97 (d, *J* = 1.6 Hz, 1H, Ar-H), 7.76 (d, *J* = 7.84 Hz, 1H, Ar-H), 7.58 (t, *J* = 7.84 Hz, 1H, Ar-H), 7.29 (s, 1H, Ar-H), 7.23 (q, *J* = 3.04 Hz, 1H, Ar-H), 6.67 (d, *J* = 3.96 Hz, 1H, Ar-H), 6.45 (brs, 1H, $\underline{NH}CH_2$), 5.34 (brs, 1H, $\underline{NH}$SO2), 3.65 (q, *J* =

3.64 Hz, 2H, NH-$\underline{CH_2}$-), 3.35 (q, *J* = 5.28 Hz, 2H, $\underline{CH_2}$-$NHSO_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.16 (Ar-C), 148.04 (Ar-C), 143.94 (Ar-C), 142.28 (Ar-C), 131.31 (Ar-C), 130.97 (Ar-C), 130.44 (Ar-C), 130.16 (Ar-C), 129.58 (Ar-C), 123.93 (Ar-C), 123.4 (Ar-C), 118.52 (Ar-C), 104.48 (Ar-C), 41.05 (NH$\underline{CH_2}$-), 39.36 (-$\underline{CH_2}$-$NHSO_2$). LC/MS calculated for $C_{20}H_{17}F_3N_6O_2S$ is 462.45, Found: 463.1 $(M+1)^+$.

N-(2-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)ethyl)-4-methylbenzenesulfonamide (**24g**)

Yield: 67%. m.p.: 221-223 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42 (q, *J* = 3.24 Hz, 2H, Ar-H), 8.37 (d, *J* = 5.48 Hz, 1H, Ar-H), 8.21 (d, *J* = 5.52 Hz, 1H, Ar-H), 8.13 (d, *J* = 1.44 Hz, 1H, Ar-H), 7.69 (d, *J* = 8.12 Hz, 3H, Ar-H), 7.35 (s, 1H, Ar-H), 7.32 (s, 1H, Ar-H), 7.3 (t, *J* = 4.76 Hz, 1H, Ar-H), 6.82 (d, *J* = 3.96 Hz, 1H, Ar-H), 3.4 (t, *J* = 6.52 Hz, 2H, NH$\underline{CH_2}$-), 2.97 (s, 2H, $\underline{CH_2}$-$NHSO_2$), 2.3 (s, 3H, $\underline{CH_3}$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.04 (Ar-C), 143.93 (Ar-C), 142.99 (Ar-C), 130.16 (Ar-C), 130.03 (Ar-C), 126.93 (Ar-C), 123.93 (Ar-C), 118.51 (Ar-C), 104.48 (Ar-C), 41.01 (CH_2-$\underline{CH_2}$-$NHSO_2$), 39.35 (NH$\underline{CH_2}$-), 21.33 (CH_3). LC/MS calculated for $C_{20}H_{20}N_6O_2S$ is 408.48, Found: 409.1 $(M+1)^+$.

N-(2-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)ethyl)-3-bromobenzenesulfonamide (**24h**)

Yield: 59%. m.p.: 185-187 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (q, *J* = 4 Hz, 2H, Ar-H), 8.34 (d, *J* = 5.4 Hz, 1H, Ar-H), 8.29 (d, *J* = 3.48 Hz, 1H, Ar-H), 7.96 (d, *J* = 7.56 Hz, 1H, Ar-H), 7.85 (d, *J* = 7.44 Hz, 2H, Ar-H), 7.46 (t, *J* = 7.44 Hz, 2H, Ar-H), 7.29 (s, 1H, Ar-H), 7.22 (d, *J* = 4.88 Hz, 1H, Ar-H), 6.66 (d, *J* = 3.6 Hz, 1H, Ar-H), 5.88 (s, 1H, $\underline{NH}CH_2$), 5.41 (s, 1H, $\underline{NH}SO_2$), 3.64 (d, *J* = 5.36 Hz, 2H, NH$\underline{CH_2}$-), 3.32 (d, *J* = 5.08 Hz, 2H, $\underline{CH_2}$-$NHSO_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.95 (Ar-C), 159.32 (Ar-C), 156.93 (Ar-C), 148.29 (Ar-C), 143.47 (Ar-C), 139.94 (Ar-C), 132.52 (Ar-C), 129.25 (Ar-C), 129.03 (Ar-C), 126.93 (Ar-C), 125.22 (Ar-C), 123.97 (Ar-C), 117.95 (Ar-C), 104.2 (Ar-C), 101.56 (Ar-C), 41.28 (NH-$\underline{CH_2}$-), 29.7 (-$\underline{CH_2}$-$NHSO_2$). LC/MS calculated for $C_{19}H_{17}BrN_6O_2S$ is 473.3, Found: 474.3 $(M+1)^+$.

N-(3-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)propyl)benzenesulfonamide (**25a**)

Yield: 72%. m.p.: 165-167 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (dd, *J* = 1.28 Hz, *J* = 7.2 Hz,1H, Ar-H), 8.34 (s, 2H, Ar-H), 8.31 (d, *J* = 3.92 Hz, 1H, Ar-H), 7.95 (dd, *J* = 1.28 Hz, *J* = 7.2 Hz, 1H, Ar-H), 7.79 (d, *J* = 6.92 Hz, 2H, Ar-H), 7.49 (t, *J* = 7.24 Hz, 1H, Ar-H), 7.42 (t, *J* = 7.44 Hz, 2H, Ar-H), 7.21 (q, J = 2.96 Hz, 1H, Ar-H), 6.66 (d, *J* = 3.88 Hz, 1H, Ar-H), 5.36 (brs, 1H, $\underline{NH}SO_2$), 3.58 (q, *J* = 6.04 Hz, 2H, NH-$\underline{CH_2}$-CH_2-), 3.08 (q, *J* = 6.12 Hz, 2H, $\underline{CH_2}$-$NHSO_2$), 1.82 (t, *J* = 5.92 Hz, 2H, CH_2-$\underline{CH_2}$-CH_2-). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.14 (Ar-C), 159.25 (Ar-C), 156.93 (Ar-C), 148.26 (Ar-C), 143.45 (Ar-C), 140.19 (Ar-C), 132.4 (Ar-C), 129.6 (Ar-C), 129.01 (Ar-C), 126.81 (Ar-C), 125.21 (Ar-C), 123.95 (Ar-C), 117.91 (Ar-C), 101.19 (Ar-C), 101.11 (Ar-C), 41.07 (NH-$\underline{CH_2}$-), 37.86 ($\underline{CH_2}$-$NHSO_2$), 29.7 (CH_2-$\underline{CH_2}$-CH_2-). LC/MS calculated for $C_{20}H_{20}N_6O_2S$ is 408.48, Found: 409.1(M+1)$^{+}$.

N-(3-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)propyl)-4-fluorobenzenesulfonamide (**25b**)

Yield: 70%. m.p.: 141- 143°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (d, *J* = 4.04 Hz, 1H, Ar-H), 8.38 (d, *J* = 5.36 Hz, 1H, Ar-H), 8.33 (d, *J* = 5.56 Hz, 1H, Ar-H), 8.27 (t, *J* = 4.08 Hz, 1H, Ar-H), 7.95 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.85 (d, *J* = 3.96 Hz, 2H, Ar-H), 7.49 (m, 1H, Ar-H), 7.21 (d, *J* = 2.52 Hz, 1H, Ar-H), 7.12 (t, *J* = 8.48 Hz, 1H, Ar-H), 6.65 (d, *J* = 3.72 Hz, 1H, Ar-H), 5.5 (brs, 1H, $\underline{NH}SO_2$), 3.63 (d, *J* = 5.16 Hz, 2H,NH- $\underline{CH_2}$-CH_2- 3.31 (s, 2H, -$\underline{CH_2}$-$NHSO_2$), 1.82 (t, *J* = 6 Hz, 2H, CH_2-$\underline{CH_2}$-CH_2-). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.11 (Ar-C), 163.59 (Ar-C), 162.11 (Ar-C), 159.16 (Ar-C), 156.96 (Ar-C), 148.24 (Ar-C), 143.49 (Ar-C), 129.73 (Ar-C), 129.64 (Ar-C), 129.57 (Ar-C), 129.29 (Ar-C), 125.15 (Ar-C), 123.93 (Ar-C), 117.96 (Ar-C), 116.53 (Ar-C), 116.11 (Ar-C), 104.27 (Ar-C), 40.1 (NH-$\underline{CH_2}$-CH_2), 37.89 (-$\underline{CH_2}$-$NHSO_2$), 29.69 (CH_2-$\underline{CH_2}$-CH_2-). LC/MS calculated for $C_{20}H_{19}FN_6O_2S$ is 426.47, Found: .426.1(M)$^{+}$.

N-(3-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)propyl)-4-chlorobenzenesulfonamide (**25c**)

Yield: 72%. m.p.: 161-163 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (dd, *J* = 1.4 Hz, *J* = 7.6 Hz,1H, Ar-H), 8.33 (s, 2H, Ar-H), 8.31 (d, *J* = 3.92 Hz, 1H, Ar-H), 7.94 (dd, *J* = 1.44 Hz, *J* = 7.8 Hz, 1H, Ar-H), 7.79 (d, *J* = 7.08 Hz, 2H, Ar-H), 7.49 (t, *J* = 7.28 Hz, 1H, Ar-H), 7.42 (t, *J* = 7.48 Hz, 2H, Ar-H), 7.2 (q, *J* = 3.04 Hz, 1H, Ar-H), 6.66 (d, *J* = 3.92 Hz, 1H, Ar-H), 5.41 (brs, 1H, $\underline{NH}SO_2$), 3.48 (q, *J* = 6.32

Hz, 2H, NH-$\underline{CH_2}$-CH_2-), 3.08 (q, *J* = 6.28 Hz, 2H, CH_2-$\underline{CH_2}$-$NHSO_2$), 1.81 (t, *J* = 5.92 Hz, 2H, CH_2-$\underline{CH_2}$-CH_2-). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.11 (Ar-C), 159.21 (Ar-C), 156.93 (Ar-C), 148.26 (Ar-C), 143.45 (Ar-C), 140.2 (Ar-C), 132.39 (Ar-C), 129.25 (Ar-C), 129.01 (Ar-C), 126.81 (Ar-C), 125.21 (Ar-C), 123.95 (Ar-C), 117.91 (Ar-C), 104.19 (Ar-C), 101.06 (Ar-C), 40.09 (NH-$\underline{CH_2}$-CH_2-), 37.88 (-CH_2-$\underline{CH_2}$-$NHSO_2$), 29.7 (CH_2-$\underline{CH_2}$-CH_2-). LC/MS calculated for $C_{20}H_{19}ClN_6O_2S$ is 442.97, Found: .426.1$(M)^+$.

N-(3-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)propyl)-4-bromobenzenesulfonamide (**25d**)

Yield: 63%. m.p.: 160-162 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.4 (dd, *J* = 1.53 Hz, *J* = 8.2 Hz,1H, Ar-H), 8.3 (t, *J* = 3.96 Hz, 2H, Ar-H), 7.93 (dd, *J* = 1.56 Hz, *J* = 8.4 Hz, 1H, Ar-H), 7.79 (d, *J* = 7.25 Hz, 2H, Ar-H), 7.48 (t, *J* = 7.28 Hz, 1H, Ar-H), 7.41 (t, *J* = 7.56 Hz, 2H, Ar-H), 7.19 (q, *J* = 3.04 Hz, 1H, Ar-H), 6.64 (d, *J* = 3.96 Hz, 1H, Ar-H), 5.49 (brs, 1H, $\underline{NH}SO_2$), 3.57 (q, *J* = 6.32 Hz, 2H,NH-$\underline{CH_2}$-CH_2), 3.07 (q, *J* = 6.28 Hz, 2H, CH_2-$\underline{CH_2}$-$NHSO_2$), 1.81 (t, *J* = 5.96 Hz, 2H, CH_2-$\underline{CH_2}$-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.07 (Ar-C), 159.16 (Ar-C), 156.92 (Ar-C), 148.24 (Ar-C), 143.43 (Ar-C), 140.16 (Ar-C), 132.4 (Ar-C), 129.24 (Ar-C), 129.02 (Ar-C), 126.82 (Ar-C), 125.22 (Ar-C), 123.94 (Ar-C), 117.9 (Ar-C), 101.17 (Ar-C), 100.99 (Ar-C), 40.15 (NH-$\underline{CH_2}$-CH_2-), 37.93 (-$\underline{CH_2}$-$NHSO_2$), 29.7 (CH_2-$\underline{CH_2}$-CH_2-). LC/MS calculated for $C_{20}H_{19}BrN_6O_2S$ is 487.38, Found: 488.1$(M+1)^+$.

N-(3-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)propyl)-4-methoxybenzenesulfonamide (**25e**)

Yield: 65%. m.p.: 157-159 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (dd, *J* = 1.52 Hz, *J* = 8.4 Hz, 1H, Ar-H), 8.34 (s, 2H, Ar-H), 8.32 (d, *J* = 3.92 Hz, 1H, Ar-H), 7.95 (dd, *J* = 1.56 Hz, *J* = 8.4 Hz, 1H, Ar-H), 7.72 (d, *J* = 8.32 Hz, 2H, Ar-H), 7.21 (q, *J* = 3 Hz, 1H, Ar-H), 6.87 (d, *J* = 8.64 Hz, 2H, Ar-H), 6.66 (d, *J* = 3.92 Hz, 1H, Ar-H), 5.4 (s, 1H, Ar-H), 3.79 (s, 3H, OCH_3), 3.58 (q, *J* = 6.08 Hz, 2H, NH-$\underline{CH_2}$-CH_2-CH_2), 3.05 (q, *J* = 6.2 Hz, 2H, -$\underline{CH_2}$-$NHSO_2$), 1.81 (t, *J* = 6.04 Hz, 2H, CH_2-$\underline{CH_2}$-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.65 (Ar-C), 162.11 (Ar-C), 159.25 (Ar-C), 156.92 (Ar-C), 148.26 (Ar-C), 143.43 (Ar-C), 131.75 (Ar-C), 129.25 (Ar-C), 129.11 (Ar-C), 128.97 (Ar-C), 125.24 (Ar-C), 123.95 (Ar-C), 117.9 (Ar-C), 114.15 (Ar-C), 104.15 (Ar-C), 101.05 (Ar-C), 55.49 (OCH_3), 40.07 (NH-$\underline{CH_2}$-), 37.92

($\underline{CH_2}$-$NHSO_2$), 30.03 (CH_2-$\underline{CH_2}$-CH_2). LC/MS calculated for $C_{21}H_{22}N_6O_3S$ is 438.51, Found: 439.1$(M+1)^+$.

N-(3-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)propyl)-3-(trifluoromethyl)benzenesulfonamide (**25f**)

Yield: 75%. m.p.: 139-141 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (t, *J* = 0.94 Hz, 1H, Ar-H), 8.29 (s, 2H, Ar-H), 8.27 (d, *J* = 3.96 Hz, 1H, Ar-H), 8.11 (s, 1H, Ar-H), 7.97 (d, *J* = 7.28 Hz, 1H, Ar-H), 7.9 (dd, *J* = 1.4 Hz, *J* = 7.8 Hz,1H, Ar-H), 7.74 (d, *J* = 7.36 Hz, 1H, Ar-H), 7.54 (t, *J* = 6.4 Hz, 1H, Ar-H), 7.16 (q, *J* = 2.96 Hz, 1H, Ar-H), 6.61 (d, *J* = 3.92 Hz, 1H, Ar-H), 5.62 (brs, 1H, $\underline{NH}$-SO_2), 3.57 (q, *J* = 6.12 Hz, 2H, NH-$\underline{CH_2}$-CH_2), 3.1 (d, *J* = 5.72 Hz, 2H, CH_2-$\underline{CH_2}$-$NHSO_2$), 1.83 (t, *J* = 5.44 Hz, 2H, CH_2-$\underline{CH_2}$-CH_2-). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.79 (Ar-C), 165.04 (Ar-C), 158.97 (Ar-C), 156.94 (Ar-C), 148.2 (Ar-C), 143.42 (Ar-C), 141.65 (Ar-C), 132.11 (Ar-C), 130.94 (Ar-C), 129.85 (Ar-C), 128.99 (Ar-C), 127.27 (Ar-C), 124.55 (Ar-C), 117.9 (Ar-C), 104.21 (Ar-C), 101.01 (Ar-C), 40.22 (NH-$\underline{CH_2}$-CH_2), 37.97 (-$\underline{CH_2}$-$NHSO_2$), 29.69 (CH_2-$\underline{CH_2}$-CH_2-). LC/MS calculated for $C_{21}H_{19}F_3N_6O_2S$ is 476.48, Found: 477.1$(M+1)^+$.

N-(3-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)propyl)-4-methylbenzenesulfonamide (**25g**)

Yield: 65%. m.p.: 181-183 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (dd, *J* = 1.48 Hz, *J* = 7.4 Hz, 1H, Ar-H), 8.36 (s, 2H, Ar-H), 8.32 (d, *J* = 4 Hz, 1H, Ar-H), 7.96 (dd, *J* = 1.52 Hz, *J* = 8.6 Hz,1H, Ar-H), 7.66 (d, *J* = 7.48 Hz, 2H, Ar-H), 7.29 (s, 1H, Ar-H), 7.21 (m, 3H, Ar-H), 6.67 (d, *J* = 3.96 Hz, 1H, Ar-H), 5.24 (s, 1H, $\underline{NHSO2}$), 3.6 (q, *J* = 6.44 Hz, 2H, NH-$\underline{CH_2}$-CH_2-), 3.06 (q, *J* = 6.36 Hz, 2H,-$\underline{CH_2}$-SO_2), 2.35 (s, 3H, $\underline{CH_3}$), 1.81 (t, *J* = 6.04 Hz, 2H, CH_2-$\underline{CH_2}$-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.19 (Ar-C), 148.29 (Ar-C), 143.46 (Ar-C), 143.13 (Ar-C), 129.61 (Ar-C), 129.26 (Ar-C), 126.87 (Ar-C), 125.21 (Ar-C), 123.95 (Ar-C), 117.91 (Ar-C), 101.17 (Ar-C), 40.02 (NH-$\underline{CH_2}$-), 37.83 (-$\underline{CH_2}$-$NHSO_2$), 30.22 (CH_2-$\underline{CH_2}$-CH_2-), 21.41 ($\underline{CH_3}$). LC/MS calculated for $C_{21}H_{22}N_6O_2S$ is 422.51, Found: 423.0$(M+1)^+$.

N-(3-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)propyl)-3-bromobenzenesulfonamide (**25h**)

Yield: 75%. m.p.: 165-167 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (t, *J* = 0.96 Hz, 1H, Ar-H), 8.33 (s, 2H, Ar-H), 8.31 (d, *J* = 3.92 Hz, 1H, Ar-H), 7.94 (dd, *J* = 1.12 Hz, *J* = 7.2 Hz, 1H, Ar-H), 7.79 (d, *J* = 6.92 Hz, 2H, Ar-H), 7.49 (t, *J* = 7.2 Hz, 1H, Ar-H), 7.42 (t, *J* = 7.36 Hz, 2H, Ar-H), 7.2 (q, *J* = 2.96 Hz, 1H, Ar-H), 6.65 (d, *J* = 3.84 Hz, 1H, Ar-H), 5.42 (brs, 1H, NHSO2), 3.58 (q, *J* = 6.24 Hz, 2H, NH-CH_2), 3.07 (q, *J* = 6.2 Hz, 2H, CH_2-$NHSO_2$), 1.81 (t, *J* = 5.84 Hz, 2H, CH_2-CH_2-CH_2-$NHSO_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.61 (Ar-C), 162.1 (Ar-C), 159.18 (Ar-C), 156.93 (Ar-C), 148.25 (Ar-C), 143.44 (Ar-C), 140.18 (Ar-C), 132.39 (Ar-C), 129.25 (Ar-C), 129.01 (Ar-C), 126.81 (Ar-C), 125.22 (Ar-C), 123.94 (Ar-C), 117.91 (Ar-C), 104.18 (Ar-C), 101.05 (Ar-C), 40.1 (NH-CH_2-CH_2), 37.89 (**CH_2**-$NHSO_2$), 30.1 (CH_2-CH_2-CH_2-$NHSO_2$). LC/MS calculated for $C_{20}H_{19}BrN_6O_2S$ is 487.38, Found: 488.1(M+1)$^{+}$.

N-(2-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)ethyl)benzamide (**26a**)

Yield: 74%. m.p.: 199-201 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (t, *J* = 5.6 Hz, 2H, Ar-H), 8.34 (d, *J* = 3.48 Hz, 2H, Ar-H), 7.94 (d, *J* = 7.64 Hz, 1H, Ar-H), 7.76 (d, *J* = 6.84 Hz, 2H, Ar-H), 7.4 (s, 1H, Ar-H), 7.29 (s, 2H, Ar-H), 7.25 (q, *J* = 4.84 Hz, 1H, Ar-H), 6.63 (d, *J* = 3.76 Hz, 1H, Ar-H), 3.79 (d, *J* = 14.2 Hz, 4H, CH_2-CH_2-NHCO). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.95 (C=O), 157.19 (Ar-C), 148.3 (Ar-C), 143.47 (Ar-C), 131.32 (Ar-C), 129.32 (Ar-C), 128.36 (Ar-C), 126.94 (Ar-C), 125.3 (Ar-C), 124.05 (Ar-C), 118.09 (Ar-C), 104.51 (Ar-C), 101.28 (Ar-C), 41.12 (NH**CH_2**-), 29.7 (CH_2-NHCO). LC/MS calculated for $C_{20}H_{18}N_6O$ is 358.41, Found: 459.1(M+1)$^{+}$.

N-(2-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)ethyl)-4-fluorobenzamide (**26b**)

Yield: 78%. m.p.: 205-207 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (t, *J* = 4.84 Hz, 2H, Ar-H), 8.32 (t, *J* = 4 Hz, 2H, Ar-H), 7.95 (q, *J* = 1.36 Hz, 1H, Ar-H), 7.76 (s, 2H, Ar-H), 7.23 (q, *J* = 4.8 Hz, 1H, Ar-H), 6.94 (s, 2H, Ar-H), 6.64 (d, *J* = 4 Hz, 1H, Ar-H), 3.81 (s, 2H, NHCH_2-), 3.75 (s, 2H, CH_2-NHCO). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.86 (C=O), 157.21 (Ar-C), 148.27 (Ar-C), 143.54 (Ar-C), 129.38 (Ar-C), 129.3 (Ar-C), 129.21 (Ar-C), 125.2 (Ar-C), 124.04 (Ar-C), 118.18 (Ar-C), 115.43 (Ar-C), 115.21 (Ar-C), 104.62 (Ar-C), 41.04 (2xCH_2-). LC/MS calculated for $C_{20}H_{17}FN_6O$ is 376.4., Found: 376.9(M)$^{+}$.

N-(2-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)ethyl)-4-chlorobenzamide (**26c**)

Yield: 55%. m.p.: 191-193 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (d, *J* = 5.84 Hz, 1H, Ar-H), 8.42 (dd, *J* = 1.56 Hz, *J* = 8 Hz,1H, Ar-H), 8.35 (d, *J* = 4 Hz, 2H, Ar-H), 7.95 (dd, *J* = 1.6 Hz, *J* = 8.2 Hz, 1H, Ar-H), 7.77 (d, *J* = 7.52 Hz, 2H, Ar-H), 7.3 (d, *J* = 9.28 Hz, 2H, Ar-H), 7.23 (q, *J* = 4.77 Hz, 1H, Ar-H), 6.65 (d, *J* = 4 Hz, 1H, Ar-H), 3.82 (q, *J* = 2.68 Hz, 2H, NH-CH_2-), 3.78 (t, *J* = 4.88 Hz, 2H, CH_2-NHCO). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.94 (C=O), 157.33 (Ar-C), 148.33 (Ar-C), 143.51 (Ar-C), 134.28 (Ar-C), 131.34 (Ar-C), 129.38 (Ar-C), 128.37 (Ar-C), 126.95 (Ar-C), 125.3 (Ar-C), 124.1 (Ar-C), 118.2 (Ar-C), 41.13 (2 x -CH_2-). LC/MS calculated for $C_{20}H_{17}ClN_6O$ is 392.85, Found: 393.1$(M+1)^+$.

N-(2-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)ethyl)-4-(trifluoromethyl)benzamide (**26d**)

Yield: 60%. m.p.: 201-203 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (t, *J* = 25 Hz, 4H, Ar-H), 7.94 (d, *J* = 6.76 Hz, 2H, Ar-H), 7.83 (s, 1H, Ar-H), 7.71 (t, *J* = 6.92 Hz, 1H, Ar-H), 7.53 (d, *J* = 25 Hz, 2H, Ar-H), 6.61 (s, 1H, Ar-H), 3.83-3.69 (m, 4H, CH_2-CH_2-). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.58 (C=O), 157.17 (Ar-C), 148.23 (Ar-C), 143.58 (Ar-C), 132.77 (Ar-C), 130.9 (Ar-C), 129.41 (Ar-C), 127.56 (Ar-C), 127.39 (Ar-C), 125.3 (Ar-C), 125.11 (Ar-C), 123.98 (Ar-C), 118.19 (Ar-C), 104.61 (Ar-C), 40.89 (NH-CH_2-), 29.7 (-CH_2-NHCO). LC/MS calculated for $C_{21}H_{17}F_3N_6O$ is 426.4, Found: 427.0$(M+1)^+$.

N-(2-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)ethyl)-2,6-difluorobenzamide (**26e**)

Yield: 60%. m.p.: 209-211 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, *J* = 5.84 Hz, 1H, Ar-H), 8.41 (dd, *J* = 1.24 Hz, *J* = 7.2 Hz, 1H, Ar-H), 8.35 (d, *J* = 3.96 Hz, 1H, Ar-H), 8.29 (d, *J* = 5.8 Hz, 1H, Ar-H), 7.94 (dd, *J* = 1.4 Hz, *J* = 7.8 Hz, 1H, Ar-H), 7.29 (s, 1H, Ar-H), 7.22 (q, *J* = 2.96 Hz, 1H, Ar-H), 6.86 (t, *J* = 8.04 Hz, 2H, Ar-H), 6.65 (d, *J* = 3.96 Hz, 1H, Ar-H), 3.83-3.76 (m, 4H, CH_2-CH_2-). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.87 (C=O), 143.48 (Ar-C), 131.51 (Ar-C), 129.36 (Ar-C), 125.31 (Ar-C), 124.12 (Ar-C), 118.2 (Ar-C), 111.99 (Ar-C), 111.74 (Ar-C), 40.83 (NH-CH_2-), 29.69 (CH_2-NHCO). LC/MS calculated for $C_{20}H_{16}F_2N_6O$ is 394.14, Found: 395.1$(M+1)^+$.

N-(2-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)ethyl)-4-methylbenzamide (**26f**)

Yield: 65%. m.p.: 143-145 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (d, *J* = 1.96 Hz, 1H, Ar-H), 8.3 (s, 2H, Ar-H), 7.89 (d, *J* = 7.52 Hz, 1H, Ar-H), 7.63 (d, *J* = 6.4 Hz, 3H, Ar-H), 7.16 (t, *J* = 4.68 Hz, 1H, Ar-H), 7 (d, *J* = 7.04 Hz, 2H, Ar-H), 6.57 (d, *J* = 3.32 Hz, 1H, Ar-H), 3.72 (s, 4H, $\underline{CH_2}$-$\underline{CH_2}$-), 2.61 (s, 3H, $\underline{CH_3}$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.97 (C=O), 162.19 (Ar-C), 158.96 (Ar-C), 156.86 (Ar-C), 151.5 (Ar-C), 148.21 (Ar-C), 143.33 (Ar-C), 141.53 (Ar-C), 135.89 (Ar-C), 131.5 (Ar-C), 129.14 (Ar-C), 128.89 (Ar-C), 127.15 (Ar-C), 126.98 (Ar-C), 125.49 (Ar-C), 117.84 (Ar-C), 103.99 (Ar-C), 41.17 (NH-$\underline{CH_2}$-), 29.67 ($\underline{CH_2}$-NHCO), 21.24 ($\underline{CH_3}$). LC/MS calculated for $C_{21}H_{20}N_6O$ is 372.4, Found: 372$(M)^+$.

N-(2-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)ethyl)cyclohexanecarboxamide (**26g**)

Yield: 74%. m.p.: 213-215 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (d, *J* = 1.6 Hz, 1H, Ar-H), 8.21 (d, *J* = 5.6 Hz, 1H, Ar-H), 8.12 (dd, *J* = 1.6 Hz, *J* = 8 Hz, 1H, Ar-H), 7.81 (t, *J* = 5.2 Hz, 1H, Ar-H), 7.31 (q, *J* = 4.8 Hz, 2H, Ar-H), 6.81 (d, *J* = 4 Hz, 1H, Ar-H), 3.41 (t, *J* = 6.4 Hz, 2H, NH-$\underline{CH_2}$-), 3.23 (t, *J* = 5.6 Hz, 2H, $\underline{CH_2}$-NHCO), 2.07 (q, *J* = 11.6 Hz, 1H, Aliph-H), 1.67 (d, *J* = 10.8 Hz, 4H, Aliph-H), 1.59 (d, *J* = 8.4 Hz, 1H, Aliph-H), 1.33 (t, *J* = 12 Hz, 2H, Aliph-H), 1.19 (m, 3H, Aliph-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.8 (C=O), 171.14 (Ar-C), 157.3 (Ar-C), 148.35 (Ar-C), 148.35 (Ar-C), 143.51 (Ar-C), 129.36 (Ar-C), 125.32 (Ar-C), 124.09 (Ar-C), 118.16 (Ar-C), 45.44 (NH-$\underline{CH_2}$-), 40.1 ($\underline{CH_2}$-NHCO), 34.5 (Aliph-C), 29.3 (Aliph-C), 29.04 (Aliph-C), 25.68 (Aliph-C), 25.26 (Aliph-C), 22.64 (Aliph-C). LC/MS calculated for $C_{20}H_{24}N_6O$ is 364.4, Found: 364.9$(M)^+$.

N-(3-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)propyl)-4-cyanobenzamide (**27a**)

Yield: 70%. m.p.: 179-181 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (m, 5H, Ar-H), 7.93 (t, *J* = 9.8 Hz, 1H, Ar-H), 7.75 (d, *J* = 7.32 Hz, 1H, Ar-H), 7.29 (s, 1H, Ar-H), 7.21 (m, *J* = 9.08 Hz, 2H, Ar-H), 6.61 (q, *J* = 3.96 Hz, 1H, Ar-H), 5.69 (s, 1H, NH), 3.65 (m, 4H, $\underline{CH_2}$-CH_2-$\underline{CH_2}$-NHCO), 2.04 (t, *J* = 7.6 Hz, 2H, CH_2-$\underline{CH_2}$-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.37 (Ar-C), 159.12 (Ar-C), 143.41 (Ar-C), 143.32 (Ar-C), 129.17 (Ar-C), 129.1 (Ar-C), 126.91 (Ar-C), 125.33 (Ar-C), 117.85 (Ar-C), 117.73 (Ar-C), 103.97 (Ar-C), 103.77 (Ar-C), 38.67 (NH-$\underline{CH_2}$-), 29.99 (-$\underline{CH_2}$-NHCO), 29.7 (CH_2-$\underline{CH_2}$-CH_2-NHCO).

N-(3-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)propyl)-4-fluorobenzamide (**27b**)

Yield: 72%. m.p.: 167-169 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (t, *J* = 3.75 Hz, 2H, Ar-H), 8.32 (d, *J* = 5.52 Hz, 2H, Ar-H), 7.92 (dd, *J* = 7.48 Hz, *J* = 14.6 Hz, 3H, Ar-H), 7.54 (s, 1H, Ar-H), 7.18 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.08 (d, *J* = 7.32 Hz, 2H, Ar-H), 6.6 (d, *J* = 2.8 Hz, 1H, Ar-H), 5.84 (s, 1H, NH), 3.64 (d, *J* = 5.32 Hz, 2H, NH-$\underline{CH_2}$-), 3.58 (d, *J* = 5.32 Hz, 2H, $\underline{CH_2}$-NHCO), 1.92 (s, 2H, CH_2-$\underline{CH_2}$-CH_2-NHCO). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.62 (C=O), 163.35 (Ar-C), 158.55 (Ar-C), 157.13 (Ar-C), 148.27 (Ar-C), 143.44 (Ar-C), 143.33 (Ar-C), 130.93 (Ar-C), 129.32 (Ar-C), 129.24 (Ar-C), 128.81 (Ar-C), 125.33 (Ar-C), 123.95 (Ar-C), 117.95 (Ar-C), 115.66 (Ar-C), 104.17 (Ar-C), 100.95 (Ar-C), 38.32 (NH-$\underline{CH_2}$-), 29.69 (-**CH_2**-NHCO), 29.31 (CH_2-$\underline{CH_2}$-CH_2-NHCO). LC/MS calculated for $C_{21}H_{19}FN_6O$ is 390.4, Found: 391.8$(M+1)^+$.

N-(3-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)propyl)-4-chlorobenzamide (**27c**)

Yield: 78%. m.p.: 191-193 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (d, *J* = 4.64 Hz, 1H, Ar-H), 8.38 (d, *J* = 5.56 Hz, 1H, Ar-H), 8.34 (t, *J* = 3.92 Hz, 2H, Ar-H), 7.89 (m, 3H, Ar-H), 7.5 (t, *J* = 7.12 Hz, 1H, Ar-H), 7.43 (t, *J* = 7.32 Hz, 2H, Ar-H), 7.19 (m, 1H, Ar-H), 6.6 (t, *J* = 3.84 Hz, 1H, Ar-H), 5.79 (s, 1H, NH), 3.66 (t, *J* = 5.96 Hz, 2H, NH$\underline{CH_2}$-), 3.6 (t, *J* = 6.08 Hz, 2H, $\underline{CH_2}$-NHCO), 1.94 (t, *J* = 5.6 Hz, 2H, CH_2-$\underline{CH_2}$-CH_2-). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.69 (C=O), 158.71 (Ar-C), 157.11 (Ar-C), 148.3 (Ar-C), 143.41 (Ar-C), 143.35 (Ar-C), 134.8 (Ar-C), 131.32 (Ar-C), 129.22 (Ar-C), 128.51 (Ar-C), 126.95 (Ar-C), 125.29 (Ar-C), 123.97 (Ar-C), 117.91 (Ar-C), 117.81 (Ar-C), 104.11 (Ar-C), 100.97 (Ar-C), 38.66 (NH-$\underline{CH_2}$-), 29.91 (-$\underline{CH_2}$-NHCO), 29.7 (CH_2-$\underline{CH_2}$-CH_2-). LC/MS calculated for $C_{21}H_{19}ClN_6O$ is 407.83, Found: 408.9$(M+1)^+$.

N-(3-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)propyl)-4-(trifluoromethyl)benzamide (**27d**)

Yield: 70%. m.p.: 189-191 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (t, *J* = 4.24 Hz, 2H, Ar-H), 8.33 (t, *J* = 4.44 Hz, 2H, Ar-H), 7.93 (t, *J* = 8.64 Hz, 3H, Ar-H), 7.68 (d, *J* = 6.2 Hz, 2H, Ar-H), 7.21 (t, *J* = 6.72 Hz, 1H, Ar-H), 6.61 (s, 1H, Ar-H), 5.86 (s, 1H, NH), 3.68 (d, *J* = 5.1 Hz, 2H, NH-$\underline{CH_2}$-CH_2), 3.62 (d, *J* = 5.04 Hz, 2H, -$\underline{CH_2}$- NHCO), 1.96 (s, 2H, CH_2-$\underline{CH_2}$-CH_2-).^{13}C NMR (100 MHz, $CDCl_3$) δ 166.27 (C=O), 157.39 (Ar-C), 148.32 (Ar-C), 143.56 (Ar-C), 143.42 (Ar-C), 129.38 (Ar-C), 129.20 (Ar-C), 127.47 (Ar-C), 125.53 (Ar-C), 125.50 (Ar-C), 125.14 (Ar-C), 124.02 (Ar-C), 118.06 (Ar-C), 104.41

(Ar-C), 101.12 (Ar-C), 38.27 ($\underline{CH_2}$-CH_2-$\underline{CH_2}$- NHCO), 29.69 (CH_2-$\underline{CH_2}$-CH_2- NHCO). LC/MS calculated for $C_{22}H_{19}F_3N_6O$ is 440.4, Found: 441.8$(M+1)^+$.

N-(3-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)propyl)-2,6-difluorobenzamide (**27e**)

Yield: 65%. m.p.: 169-171 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (t, *J* = 13.12 Hz, 3H, Ar-H), 7.92 (d, *J* = 6.16 Hz, 1H, Ar-H), 7.73 (s, 2H, Ar-H), 7.55 (s, 2H, Ar-H), 7.32 (d, *J* = 20.2 Hz, 1H, Ar-H), 7.19 (s, 1H, Ar-H), 6.93 (s, 1H, Ar-H), 6.58 (s, 1H, Ar-H), 3.64 (d, *J* = 21.08 Hz, 4H, $\underline{CH_2}$-CH_2-$\underline{CH_2}$-NHCO), 2.01-1.97 (m, *J* = 28.08 Hz, 2H, CH_2-$\underline{CH_2}$-CH_2- NHCO). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.71 (Ar-C), 160.62 (Ar-C), 143.42 (Ar-C), 132.32 (Ar-C), 131.38 (Ar-C), 130.9 (Ar-C), 129.22 (Ar-C), 128.82 (Ar-C), 125.2 (Ar-C), 123.99 (Ar-C), 117.95 (Ar-C), 112.01 (Ar-C), 111.76 (Ar-C), 104.21 (Ar-C), 66.2 (Ar-C), 38.26 (NH-$\underline{CH_2}$-CH_2-), 29.69 (-$\underline{CH_2}$- NHCO), 29.24 (CH_2-$\underline{CH_2}$-CH_2-NHCO). LC/MS calculated for $C_{21}H_{18}F_2N_6O$ is 408.4, Found: 409.2$(M+1)^+$.

N-(3-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)propyl)-4-methylbenzamide (**27f**)

Yield: 62%. m.p.: 171-173 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (d, *J* = 3.84 Hz, 1H, Ar-H), 8.35 (d, *J* = 4.36 Hz, 1H, Ar-H), 8.32 (d, *J* = 3.92 Hz, 1H, Ar-H), 7.91 (d, *J* = 7.56 Hz, 1H, Ar-H), 7.77 (q, *J* = 7.16 Hz, 2H, Ar-H), 7.19 (m, 3H, Ar-H), 6.59 (d, *J* = 3.68 Hz, 1H, Ar-H), 5.84 (s, 1H, Ar-H), 3.6 (m, 4H, NH-$\underline{CH_2}$-CH_2-$\underline{CH_2}$-NHCO), 2.37 (s, 3H, $\underline{CH_3}$), 1.91 (s, 2H, CH_2-$\underline{CH_2}$-CH_2-). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.7 (C=O), 157.19 (Ar-C), 148.3 (Ar-C), 143.42 (Ar-C), 141.67 (Ar-C), 131.91 (Ar-C), 131.49 (Ar-C), 129.22 (Ar-C), 129.15 (Ar-C), 127.04 (Ar-C), 126.96 (Ar-C), 125.31 (Ar-C), 123.99 (Ar-C), 117.96 (Ar-C), 104.21 (Ar-C), 100.88 (Ar-C), 38.39 (NH-$\underline{CH_2}$-), 29.84 (-$\underline{CH_2}$-NHCO), 29.7 (CH_2-$\underline{CH_2}$-CH_2-NHCO), 21.42 ($\underline{CH_3}$). LC/MS calculated for $C_{22}H_{22}N_6O$ is 386.4, Found: 389.0$(M+1)^+$.

N-(3-((4-(1*H*-pyrrolo[2,3-*b*]pyridin-1-yl)pyrimidin-2-yl)amino)propyl)cyclohexanecarboxamide (**27g**)

Yield: 65%. m.p.: 193-195 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (dd, *J* = 1.36 Hz, *J* = 7.4 Hz, 1H, Ar-H), 8.36 (t, *J* = 4.96 Hz, 3H, Ar-H), 7.94 (dd, *J* = 1.44 Hz, *J* = 7.8 Hz, 1H, Ar-H), 7.19 (m, 1H, Ar-H), 6.64 (d, *J* = 3.92 Hz, 1H, Ar-H), 5.62 (s, 1H, NH), 3.57 (q, *J* = 6.36 Hz, 2H, NH-$\underline{CH_2}$-), 3.39 (q, *J* =

6.16 Hz, 2H, CH_2-NHCO), 1.89 (d, *J* = 12.28 Hz, 2H, CH_2-CH_2-CH_2-NHCO), 1.81 (q, *J* = 5.72 Hz, 4H, Aliph-H), 1.46 (q, *J* = 11.84 Hz, 2H, Aliph-H), 1.27 (m, 6H, Aliph-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.41 (C=O), 162.19 (Ar-C), 159.36 (Ar-C), 156.94 (Ar-C), 148.27 (Ar-C), 143.38 (Ar-C), 143.31 (Ar-C), 129.17 (Ar-C), 129.08 (Ar-C), 125.39 (Ar-C), 125.33 (Ar-C), 123.91 (Ar-C), 117.79 (Ar-C), 103.85 (Ar-C), 103.72 (Ar-C), 45.67 (Aliph-C), 38.24 (NH-CH_2-), 36.14 (Aliph-C), 29.77 (CH_2-NHCO), 25.77 (Aliph-C). LC/MS calculated for $C_{21}H_{26}N_6O$ is 378.4, Found: 378.9$(M)^+$.

Synthesis of 2,6-difluoro-3-nitrobenzoic acid (**29**)

To a solution of 2,6-difluorobenzoic acid **28** (10 g, 63.3 mmol) in Conc. H_2SO_4 (20 ml), Conc. HNO_3 (10 ml) is added dropwise with caution at 0 °C and stirred for 4 h. The reaction mixture was poured to Iced water. The produced white precipitate was filtered, washed with water, and dried to give the titled product **29**.

Yield: 85%. m.p.: 98-100 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9 (s, 1H, OH), 8.33 (m, 1H, A-H), 7.21 (m, 1H, A-H) ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.64 (C=O), 162.16 (Ar-C), 156.47 (Ar-C), 130.38 (Ar-C), 113.08 (Ar-C), 112.81 (Ar-C), 112.19 (Ar-C).

Synthesis of 2,6-difluoro-3-nitrobenzoyl chloride (**30**)

A solution of 2,6-difluoro-3-nitrobenzoic acid **29** (10 g, 50 mmol) in thionyl chloride was refluxed at 60 °C for 4 h. The reaction mixture was concentrated under vacuo producing a yellowish brown residue which was subjected to the next step without purification.

Synthesis of (2,6-difluoro-3-nitrophenyl)(5-substituted-1H-pyrrolo[2,3-*b*]pyridin-3-yl)methanone (**31a,b**)

A mixture of the azaindole **5a,b** (2 mmol) and $AlCl_3$ (10 mmol, 5 eq.) was dissolved in DCM anhydrous (20 ml). the mixture was stirred at 0 °C for 1 hour. To the reaction mixture a solution of compound **30** (2 mmol, 1eq.) was added dropwise under N_2 at 0 °C. the reaction mixture was stirred at RT for 12 h. After reaction completion, the reaction mixture was neutralized by $NaHCO_3$ solution, extracted by Ethyl acetate, evaporated under vacuo producing a yellowish white solid of compound **31a,b**.

(2,6-difluoro-3-nitrophenyl)(1H-pyrrolo[2,3-*b*]pyridin-3-yl)methanone (**31a**)

Yield: 70%. m.p.: 192-194 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13 (s, 1H, NH), 8.53 (d, J = 7.2 Hz, 1H, Ar-H), 8.46 (m, 2H, Ar-H), 8.43 (t, J = 3.2 Hz, 1H, Ar-H), 7.55 (t, J = 8 Hz, 1H, Ar-H), 7.38 (q, J = 3.2 Hz, 1H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.27 (Ar-C), 149.98 (Ar-C), 145.74 (Ar-C), 139.64 (Ar-C), 129.94 (Ar-C), 119.53 (Ar-C), 117.79 (Ar-C), 115.75 (Ar-C).

(5-bromo-1H-pyrrolo[2,3-*b*]pyridin-3-yl)(2,6-difluoro-3-nitrophenyl)methanone (**31b**):

Yield: 55%. m.p.: 220-222 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.24 (s, 1H, NH), 8.67 (s, 1H, Ar-H), 8.66 (s, 1H, Ar-H), 8.47 (m, 2H, Ar-H), 7.56 (t, *J* = 8 Hz, 1H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.37 (Ar-C), 148.4 (Ar-C), 146.05 (Ar-C), 140.81 (Ar-C), 132.71 (Ar-C), 129.54 (Ar-C), 119.42 (Ar-C), 114.94 (Ar-C), 113.93 (Ar-C), 113.67 (Ar-C).

Synthesis of (3-amino-2,6-difluorophenyl)(5-substituted-1H-pyrrolo[2,3-*b*]pyridin-3-yl)methanone (**32a,b**)

To a solution of compound **31** in MeOH, Pd/C (10%) was added portion wise with continuous shaking. The reaction mixture was stirred under H_2 at RT for 12 h. After reaction completion, the solution was filtered over celite, evaporated under vacuo. The resulted residue **32a,b** were used in the next step without further purification.

Synthesis of 1-(substituted-phenyl)-3-(2,4-difluoro-3-(1H-pyrrolo[2,3-*b*]pyridine-3-carbonyl)phenyl)urea **34a-g**

To a solution of **32a** (1 mmol) in anhydrous THF, different phenyl isocyanates **33a-g** (1.1 mmol, 1.1eq.) were added dropwise at 0 °C under N2.the reaction mixture was stirred at RT for 12 hours. The resulted precipitate was filtered, washed with THF, and dried producing the corresponding products **34a-g**.

1-(2,4-difluoro-3-(1H-pyrrolo[2,3-*b*]pyridine-3-carbonyl)phenyl)-3-phenylurea (**34a**):

Yield: 70%. m.p.: 285-287 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.87 (s, 1H, NH), 9.07 (s, 1H, Ar-H), 8.63 (d, *J* = 20.4 Hz, 1H, Ar-H), 8.49 (d, *J* = 7.6 Hz, 1H, Ar-H), 8.42 (d, *J* = 4.4 Hz, 1H, Ar-H), 8.27 (q, *J* = 6 Hz, 1H, Ar-H), 8.19 (s, 1H, Ar-H), 7.45 (d, *J* = 1.6 Hz, 2H, Ar-H), 7.29 (d, *J* = 8.4 Hz, 2H, Ar-H), 6.98 (m, 2H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.45 (C=O), 153 (Ar-C), 152.77 (Ar-C), 149.84 (Ar-C), 145.48 (Ar-C), 140.18 (Ar-C), 139.80 (Ar-C), 138.51 (Ar-C), 129.85 (Ar-C),

129.36 (Ar-C), 129.25 (Ar-C), 122.64 (Ar-C), 122.26 (Ar-C), 119.3 (Ar-C), 118.68 (Ar-C), 118.65 (Ar-C), 117.91 (Ar-C), 116.08 (Ar-C), 114.32 (Ar-C). LC/MS calculated for $C_{21}H_{14}F_2N_4O_2$ is 392.37, Found: 394.1(M+1)+.

1-(4-bromophenyl)-3-(2,4-difluoro-3-(1H-pyrrolo[2,3-*b*]pyridine-3-carbonyl)phenyl)urea (**34b**): Yield: 55%. m.p.: 284-286 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.87 (s, 1H, NH), 8.64 (s, 1H, Ar-H), 8.49 (d, *J* = 7.8 Hz, 1H, Ar-H), 8.42 (dd, *J* = 4.1 Hz, *J* = 10.4 Hz, 1H, Ar-H), 8.19 (s, 1H, Ar-H), 7.46 (q, *J* = 5.36 Hz, 2H, Ar-H), 7.36 (q, *J* = 4.72 Hz, 1H, Ar-H), 7.24 (t, *J* = 8.52 Hz, 1H, Ar-H), 7.13 (d, J = 8.64 Hz, 1H, Ar-H), 6.51 (d, *J* = 8.68 Hz, 1H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.91(C=O),152.68 (Ar-C), 150.03 (Ar-C), 148.54 (Ar-C), 143.42 (Ar-C), 139.24 (Ar-C), 132.98 (Ar-C), 131.77 (Ar-C), 129.99 (Ar-C), 129.26(Ar-C), 120.72 (Ar-C), 120.62 (Ar-C), 119.35 (Ar-C), 118.52(Ar-C), 116.23 (Ar-C), 113.92(Ar-C). LC/MS calculated for $C_{21}H_{13}BrF_2N_4O_2$ is 470.26, Found: 471.1(M+1)$^+$.

1-(2,4-difluoro-3-(1H-pyrrolo[2,3-*b*]pyridine-3-carbonyl)phenyl)-3-(3-fluorophenyl)urea (**34c**):

Yield: 75%. m.p.: 287-289 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.87 (s, 1H, NH), 9.29 (s, 1H, Ar-H), 8.66 (s, 1H, Ar-H), 8.49 (d, *J* = 7.6 Hz, 1H, Ar-H), 8.42 (t, *J* = 1.2 Hz, 1H, Ar-H), 7.52 (t, *J* = 12 Hz, 1H, Ar-H), 7.35 (m, 2H, Ar-H), 7.23 (t, *J* = 8.8 Hz, 1H, Ar-H), 7.12 (t, *J* = 7.6 Hz, 1H, Ar-H), 6.81 (d, *J* = 8.4 Hz, 1H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.39 (C=O), 152.67 (Ar-C), 149.83 (Ar-C), 145.49 (Ar-C), 141.72 (Ar-C), 141.6 (Ar-C), 138.5 (Ar-C), 130.96 (Ar-C), 130.76 (Ar-C), 129.86 (Ar-C), 119.31 (Ar-C), 117.9 (Ar-C), 116.07 (Ar-C), 114.55 (Ar-C), 110.32 (Ar-C), 108.88 (Ar-C), 105.63 (Ar-C), 105.25 (Ar-C), 101.92 (Ar-C), 100.27 (Ar-C). LC/MS calculated for $C_{21}H_{13}F_3N_4O_2$ is 410.36, Found: 410.4(M)$^+$.

1-(2,4-difluoro-3-(1H-pyrrolo[2,3-*b*]pyridine-3-carbonyl)phenyl)-3-(4-(trifluoromethyl)phenyl)urea (**34d**):

Yield: 78%. m.p.: 290-292 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.88 (s, 1H, NH), 9.48 (s, 1H, Ar-H), 8.73 (d, *J* = 1.68 Hz, 1H, Ar-H), 8.5 (d, *J* = 7.4 Hz, 1H, Ar-H), 8.43 (dd, *J* = 1.6 Hz, *J* = 8.2 Hz, 1H, Ar-H), 8.25 (m, 1H, Ar-H), 8.2 (s, 1H, Ar-H), 7.36 (q, *J* = 3.16 Hz, 1H, Ar-H), 7.31 (d, *J* = 8.48 Hz,

1H, Ar-H), 7.24 (m, 1H, Ar-H), 6.65 (d, J = 8.32 Hz, 1H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.34 (C=O), 152.59 (Ar-C), 149.84 (Ar-C), 145.48 (Ar-C), 143.54 (Ar-C), 129.85 (Ar-C), 126.66 (Ar-C), 119.29 (Ar-C), 118.59 (Ar-C), 118.37 (Ar-C), 117.89 (Ar-C), 116.05 (Ar-C), 113.43 (Ar-C). LC/MS calculated for $C_{22}H_{13}F_3N_4O_2$ is 460.36, Found: 461.4(M+1)$^+$.

1-(2,4-difluoro-3-(1H-pyrrolo[2,3-*b*]pyridine-3-carbonyl)phenyl)-3-(p-tolyl)urea (**34e**):

Yield: 80%. m.p.: 273-275 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.81 (s, 1H, NH), 8.98 (s, 1H, Ar-H), 8.73 (d, *J* = 7.8 Hz, 1H, Ar-H), 8.68 (d, *J* = 4.08 Hz, 1H, Ar-H), 8.5 (s, 1H, Ar-H), 7.65 (m, 1H, Ar-H), 7.58 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.35 (d, *J* = 8.24 Hz, 2H, Ar-H), 7.12 (d, *J* = 8.12 Hz, 2H, Ar-H), 2.25 (s, 3H, C**H**$_3$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.46 (C=O), 153.08 (Ar-C), 152.77 (Ar-C), 149.83 (Ar-C), 145.46 (Ar-C), 138.48 (Ar-C), 137.21 (Ar-C), 137.21 (Ar-C), 131.49 (Ar-C), 130.95 (Ar-C), 129.84 (Ar-C), 129.73 (Ar-C), 129.6 (Ar-C), 119.28 (Ar-C), 118.76 (Ar-C), 118.67 (Ar-C), 117.9 (Ar-C), 116.08 (Ar-C), 20.8 (CH3). LC/MS calculated for $C_{22}H_{16}F_2N_4O_2$ is 406.39, Found: 406.5(M)$^+$.

1-(2,4-difluoro-3-(1H-pyrrolo[2,3-*b*]pyridine-3-carbonyl)phenyl)-3-(4-methoxyphenyl)urea (**34f**):

Yield: 75%. m.p.: 266-268 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.9 (d, *J* = 4.48 Hz, 1H, Ar-H), 8.51 (q, *J* = 1.96 Hz, 1H, Ar-H), 8.19 (s, 1H, Ar-H), 7.38 (t, *J* = 2.4 Hz, 1H, Ar-H), 7.36 (t, *J* = 2.28 Hz, 2H, Ar-H), 7.34 (d, *J* = 3.24 Hz, 1H, Ar-H), 6.9 (s, 1H, Ar-H), 6.88 (d, *J* = 3.92 Hz, 3H, Ar-H), 3.73 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 181.4876 (C=O), 155.1657 (Ar-C), 153.4122 (Ar-C), 149.8172 (Ar-C), 145.4657 (Ar-C), 138.4368 (Ar-C), 133.4072 (Ar-C), 129.8419 (Ar-C), 122.4524 (Ar-C), 120.5333 (Ar-C), 117.8981 (Ar-C), 116.0889 (Ar-C), 115.408 (Ar-C), 114.9573 (Ar-C), 114.5529 (Ar-C), 114.4264 (Ar-C), 112.1104 (Ar-C), 55.6537 (OCH_3). LC/MS calculated for $C_{22}H_{16}F_2N_4O_3$ is 422.39, Found: 423.1(M+1)$^+$.

1-(2,4-difluoro-3-(1H-pyrrolo[2,3-*b*]pyridine-3-carbonyl)phenyl)-3-(naphthalen-1-yl)urea (**34g**):

Yield: 65%. m.p.: 235-237 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.33 (s, 1H, NH), 9.18 (t, *J* = 9.08 Hz, 3H, Ar-H), 8.8 (t, *J* = 2.52 Hz, 2H, Ar-H), 8.6 (s, 1H, Ar-H), 8.44 (d, *J* = 5.28 Hz, 1H, Ar-H), 8.35 (d, *J* = 8.44 Hz, 1H, Ar-H), 8.17 (d, *J* = 8.02 Hz, 2H, Ar-H), 8.07 (d, *J* = 8.02 Hz, 2H, Ar-H), 7.94 (d, *J*

= 7.88 Hz, 2H, Ar-H), 7.83 (d, *J* = 8.32 Hz, 1H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 182.62 (C=O), 153.22 (Ar-C), 146.98 (Ar-C), 145.14 (Ar-C), 138.54 (Ar-C), 136.09 (Ar-C), 132.53 (Ar-C), 129.12 (Ar-C), 128.24 (Ar-C), 126.88 (Ar-C), 125.55 (Ar-C), 123.74 (Ar-C), 120.33 (Ar-C), 119.29 (Ar-C), 118.07 (Ar-C), 116.12 (Ar-C), 109.43 (Ar-C), 105.79 (Ar-C). LC/MS calculated for $C_{25}H_{16}F_2N_4O_2$ is 442.43, Found: 443.1$(M+1)^+$.

Synthesis of 1-(3-(5-bromo-1H-pyrrolo[2,3-*b*]pyridine-3-carbonyl)-2,4-difluorophenyl)-3-(4-bromophenyl)urea **35**

To a solution of **32b** (1 mmol) in anhydrous THF, 4-bromophenyl isocyanate (1.1 mmol, 1.1eq.) was added dropwise at 0 °C under N2.the reaction mixture was stirred at RT for 12 hours. The resulted precipitate was filtered, washed with THF, and dried producing the corresponding product **35**.

Yield: 50%. m.p.: 260 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.1 (s, 1H, NH), 8.85 (s, 1H, Ar-H), 8.63 (t, *J* = 0.036 Hz, 2H, Ar-H), 8.51 (d, *J* = 2.4 Hz, 1H, Ar-H), 8.29 (s, 1H, Ar-H), 7.46 (s, 1H, Ar-H), 7.45 (d, *J* = 2.8 Hz, 2H, Ar-H), 7.24 (t, *J* = 7.6 Hz, 1H, Ar-H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.21 (C=O), 152.64 (Ar-C), 148.35 (Ar-C), 145.7 (Ar-C), 139.95 (Ar-C), 139.43 (Ar-C), 139.22 (Ar-C), 131.62 (Ar-C), 119.58 (Ar-C), 115.47 (Ar-C), 114.71 (Ar-C), 114.07 (Ar-C), 113.86 (Ar-C), 112.06 (Ar-C). LC/MS calculated for $C_{21}H_{12}Br_2F_2N_4O_2$ is 550.16, Found: 550.4$(M)^+$.

4.2. Biological evaluation

4.2.1. In vitro kinase assay

Reaction Biology Corp. Kinase HotSpot™ service was used for screening of compounds **24a-h, 25a-h, 26a-g, 27a-g, 34a-g and 35**. Assay protocol: as reported on Reaction Biology Corp..

4.2.2. Antitumor screening

Screening against the cancer cell lines was carried out for compounds **24a-h, 25a-h, 26a-g, 27a-g, 34a-g and 35** at the National Cancer Institute (NCI), Bethesda, Maryland, USA, applying the standard protocol of the NCI [37].

4.3. Molecular docking

The X-ray crystal structure of PLX4720-BRAF kinase enzyme complex with (PDB ID: 3C4C)[31] was downloaded from the protein data bank (www.rcsb.org) in PDB format. The 2D structure of the target compounds were assembled using ChemDraw software.

Molecular Operating Environment (MOE) software was used for the molecular docking operation of the target compounds **34a-g** and **35** with BRAF kinase enzyme domain (PDB ID: 3C4C). The enzyme was prepared for the molecular docking procedure by applying 3D protonation of both enzyme amino acids and the native ligand (dabrafenib). In addition, water of crystallization was removed from both BRAF kinase enzyme domains. Moreover, the BRAF kinase enzyme active site was isolated.

The docking simulation of native ligand (PLX4720) with the BRAF kinase enzyme active site (PDB ID: 3C4C) was investigated in order to validate the docking protocol. Both 3D protonation and energy minimization were performed for the target compounds using MOE software.

Acknowledgments

This work was supported by Korea Institute of Science and Technology (KIST), and KIST Project (2E29340). We are grateful to the National Cancer Institute (NCI), Bethesda, Maryland, USA, for testing the antiproliferative activity of the target compounds against 60 cancer cell lines of nine different cancer types.

Conflict of interest

The authors declare no conflict of interest.

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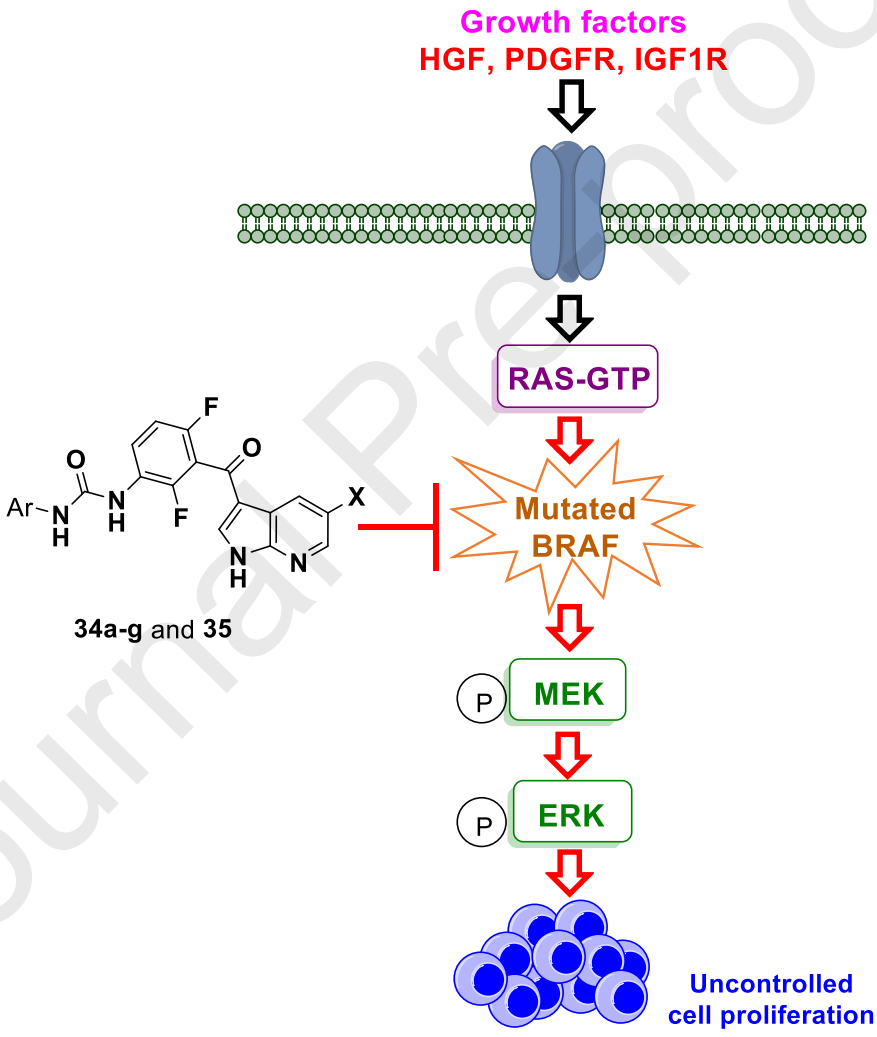
Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Design and synthesis of novel pyrrolo[2,3-*b*]pyridine derivatives targeting ^{V600E}BRAF

Graphical abstract



Design and synthesis of novel pyrrolo[2,3-*b*]pyridine derivatives targeting ^{V600E}BRAF

Highlights

- Two sets of pyrrolo[2,3-*b*]pyridine- based derivatives were designed and synthesized.
- The synthesized compounds were evaluated for their ^{V600E}BRAF Enzyme inhibitory activity.
- Compounds **34d** and **34e** emerged as the most potent enzyme inhibitors.
- The cytotoxicity assay was performed against NCI-60 cell line panel.
- Compound **35** exhibited the highest cytotoxic agent.