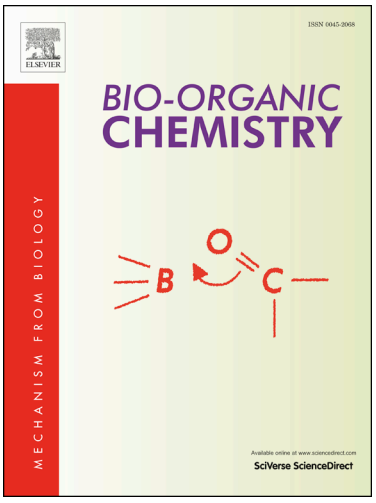

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Design, Synthesis and Docking Study of Pyridine and Thieno[2,3-b] pyridine Derivatives as Anticancer PIM-1 Kinase inhibitors

Marwa E. Abdelaziz*, Mostafa M. M. El-Miligy, Salwa M. Fahmy, Mona A. Mahran, Aly A. Hazzaa
Pharmaceutical Chemistry Department, Faculty of Pharmacy, Alexandria University, Alexandria 21521, Egypt

*Author for correspondence:
Tel: +20 1285838955
Fax: +20 34873273
marwaehab22@yahoo.com

Abstract

A series of pyridine and thieno[2,3-*b*]pyridine derivatives have been designed and synthesized as anticancer PIM-1 kinase inhibitors. Thirty-seven compounds were selected by NCI to be tested initially at a single dose (10 µM) in the full NCI 60 cell line panel. Compound **5b** showed potent anticancer activity and was tested twice in the five-dose assay which confirmed its potent antitumor activity (GI_{50} values 0.302 to 3.57 µM) against all tested tumor cell lines except six cell lines where they showed moderate sensitivity. This compound was sent to NCI biological evaluation committee and still under consideration for further testing. In addition, the most active anticancer compounds in each series, **5b**, **8d**, **10c**, **13h**, and **15e**, were evaluated for their PIM-1 kinase inhibitory activity. Compound **8d** was the most potent one with IC_{50} = 0.019 µM followed by **5b**, **15e**, **10c** and **13h** with IC_{50} values 0.044, 0.083, 0.128 and 0.479 µM respectively. Moreover, docking study of the most active compounds inPIM-1 kinase active site was consistent with the *in vitro* activity.

Keywords:
Pyridine
Thieno[2,3-*b*]pyridine
PIM-1 Kinase inhibitors
Anticancer

1. Introduction

PIM kinases are serine/threonine kinases and include PIM-1, PIM-2, and PIM-3 subtypes. PIM kinases have a special hinge region sequence [1-3]. PIM-1 kinase is an important drug target against different malignancies as it participates in the progression and initiation of lymphomas, leukemia and solid tumors such as prostate, pancreas and colon [4]. Also, it is associated with several cellular functions such as survival, apoptosis, differentiation and proliferation. Dysregulation of PIM-1 kinase promotes carcinogenesis of different types of tumors. Thus, PIM-1 kinase inhibition is an important strategy for treatment of different types of tumor. It was reported that, 4,6-diaryl-3-cyano-2-pyridone derivatives showed potent anticancer activity through inhibition of PIM-1 kinase. For example, compound **I** was declared as a potent PIM-1 kinase inhibitor with IC_{50} = 0.050 µM [5]. In addition, compound **II** showed potent anticancer and PIM-1 kinase inhibitory activity with IC_{50} = 0.94 µM [6]. On the other hand, bio isosteric replacement of 2-hydroxy group to 2-amino as in compounds **III** and **IV** retained potent PIM-1 inhibitory activities with IC_{50} =0.058 µM and 0.0111 µM respectively [7]. Furthermore, the fusion of pyridine with five or six membered heterocyclic moieties like pyrazole in compound **V** or pyridine in compound **VI** revealed potent PIM-1 inhibitors with IC_{50} = 0.00003 µM and 0.001 µM respectively [8] (figure 1). This indicates the potentiality of 4,6-diaryl-3-cyano-2-substitutedpyridine skeleton as a template for further optimization to get more potent PIM-1 kinase inhibitor.

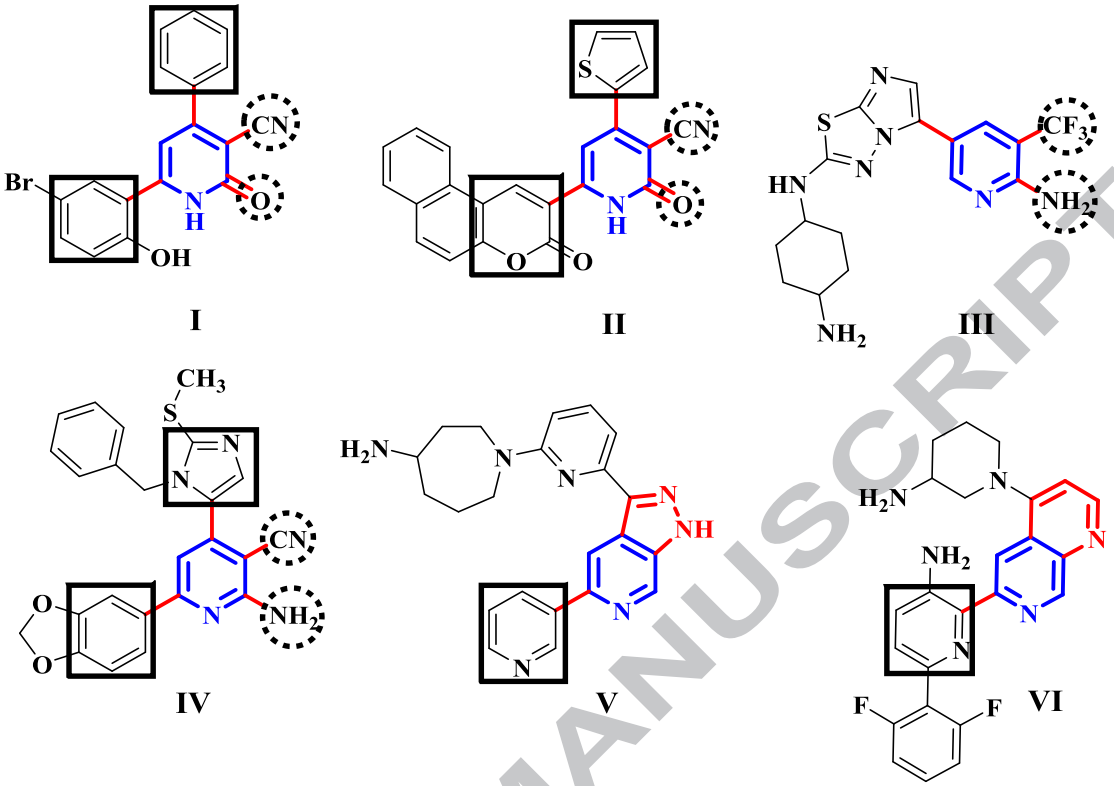
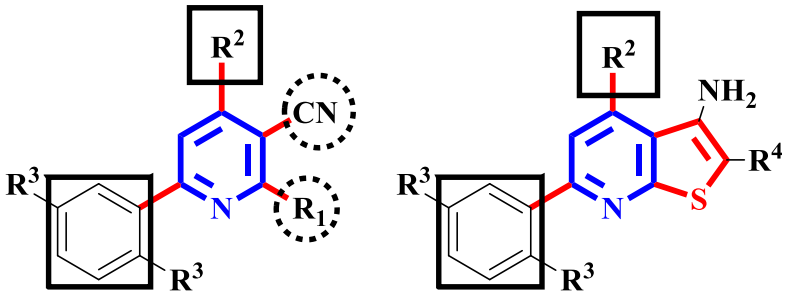


Figure 1: Reported pyridine derivatives as PIM-1 kinase inhibitors

In the present investigation, new pyridine derivatives were designed to keep the structural features for PIM-1 kinase inhibition. Pyridine ring was substituted at position 4 by aromatic moiety which was substituted with either hydrophilic or lipophilic groups. Also, pyridine ring was substituted at position 6 with 2,5-dihydroxyphenyl, 2-hydroxy-5-methoxyphenyl or 2,5-dimethoxyphenyl. Moreover, the oxygen at position 2 of pyridine was bioisosterically replaced by either sulfur or nitrogen. In addition, the pyridine ring was fused with five membered heterocyclic thiophene moiety to increase the rigidity of the molecule in order to study the effect of these structural variations on the interaction with the PIM-1 kinase hence the anticancer activity (figure 2).



R^1 = OH, NH_2, SH, SR

R^2 = C_6H_5, 4-ClC_6H_4, 4-BrC_6H_4, 4-$CH_3OC_6H_4$, thien-2-yl, pyridin-3-yl

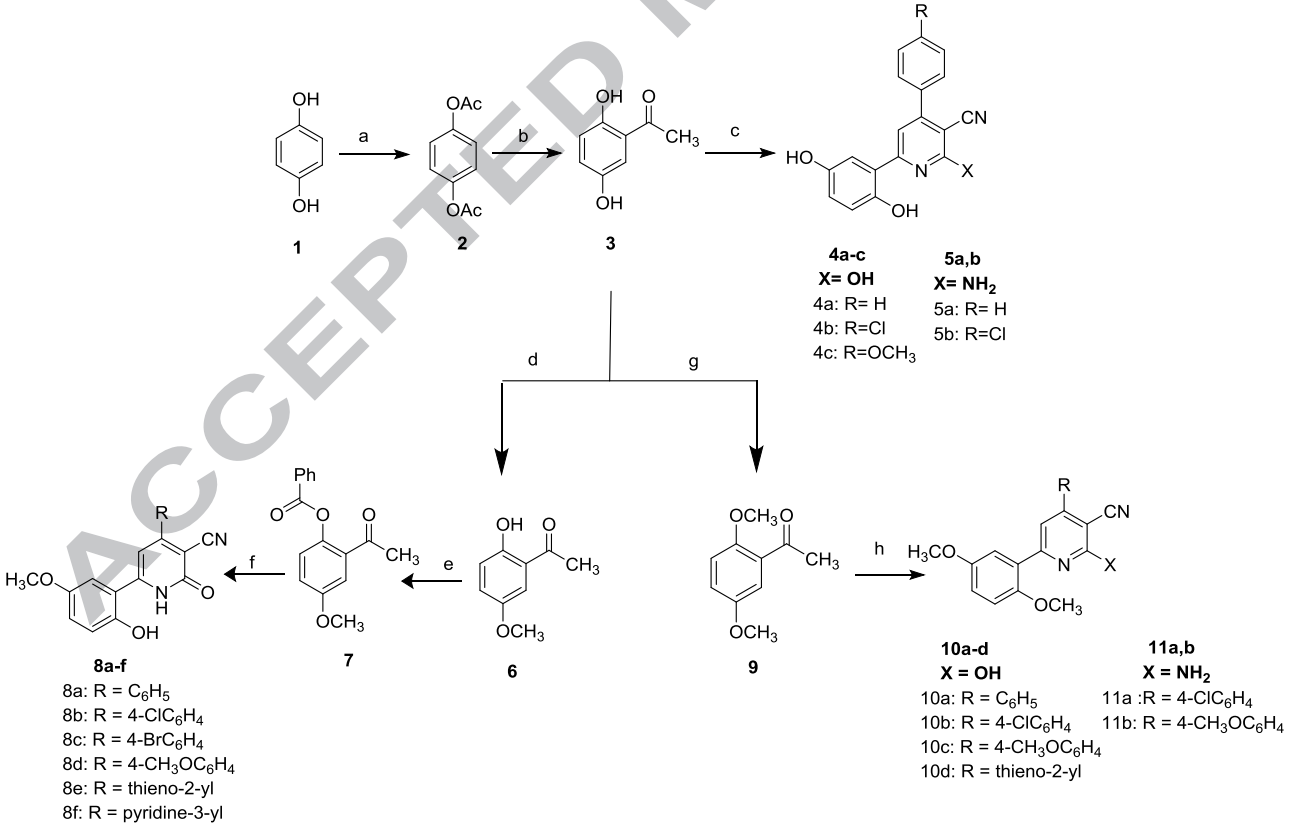
R^3 = OH, OCH_3

R^4 = CH_3, C_6H_5, 4-ClC_6H_4, 4-$CH_3OC_6H_4$

Figure 2: Design of pyridine and thieno[2,3-*b*]pyridine derivatives as anticancer PIM-1 kinase inhibitors.

2. Results and discussion

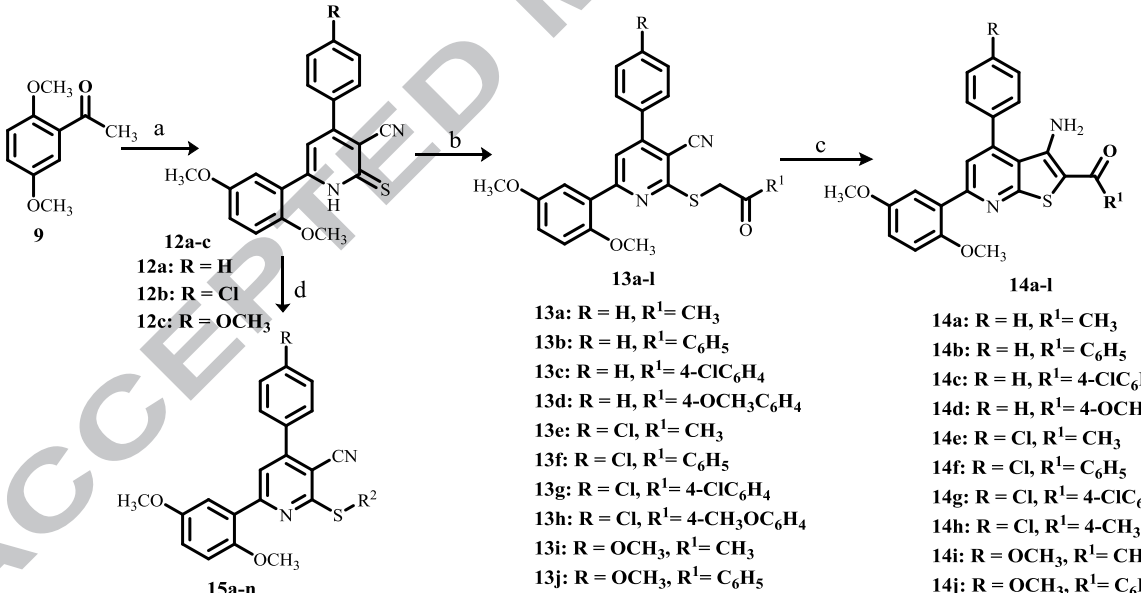
2.1. Chemistry



scheme 1. (a) Ac_2O / $C.H_2SO_4$; (b) anhy. $AlCl_3$; (c) 4-RC_6H_4CHO/ $CNCH_2COOC_2H_5$ or CH_2CNCH_2/ amm. acetate, reflux; (d) K_2CO_3/ CH_3I/ Dry acetone; (e) benzoyl chloride/ pyridine; (f) RCHO/ $CNCH_2COOC_2H_5$/ amm. acetate, reflux; (g) $(CH_3)_2SO_4$/ K_2CO_3; (h) RCHO/ $CNCH_2COOC_2H_5$ or CH_2CNCH_2/ amm. acetate, reflux.

The synthetic strategies used for the preparation of the target compounds were outlined in two schemes. Scheme 1 described the synthesis of 2,5-dihydroxy acetophenone **3** through Fries rearrangement of hydroquinone diacetate **2** [9]. 2,5-dihydroxy acetophenone **3** was then subjected to one pot reaction with the appropriate aromatic aldehyde and ethyl cyanoacetate or malononitrile in the presence of excess ammonium acetate to give the corresponding 3-cyanopyridones **4a-c** or 2-amino-3-cyanopyridines **5a,b,** respectively, but failed to react with cyan thioacetamide to give the corresponding thiopyridine derivative. Structures of the prepared compounds **4a-c**, **5a,b** were confirmed by elemental microanalysis, IR, ^{1}H-NMR for all compounds, beside EL/MS and ^{13}C-NMR spectrum for compound **4c**. Furthermore, scheme 1 explained the synthesis of 2-hydroxy-5-methoxyacetophenone **6** using reported procedure [10]. Trials to prepare the 2-thioxopyridine derivatives through one pot reaction of compound **6** with the appropriate aldehyde and cyan thioacetamide or through chalcone were unsuccessful. Moreover, the Gewald reaction of chalcone with sulfur and malononitrile or ethyl cyanoacetate to prepare pyridine-2-thione[11]or pyridine-2-one [12] respectively was fruitless. Another strategy to prepare pyridine-2-thione derivatives was followed by protecting the hydroxyl group of 2-hydroxy-5-methoxyacetophenone **6** with an easily hydrolyzed benzoyl group. Such benzoyl ester **7** was prepared by esterification of **6** with benzoyl chloride in the presence of pyridine according to the reported reaction conditions [13-15]. Then one pot reaction of 2-benzoyloxy-5-methoxyacetophenone **7** with aldehyde and ethyl cyanoacetate or cyan thioacetamide in the presence of ammonium acetate gave the desired pyridine-2-one derivatives **8a-f** but failed to prepare pyridine-2-thione derivatives. Fortunately, the benzoyl substitution was hydrolyzed during the reaction conditions and gave the required free hydroxyl derivatives **8a-f**. Structures of the synthesized compounds **8a-f** were confirmed by elemental microanalysis, IR, ^{1}H-NMR for all compounds, beside EL/MS and ^{13}C-NMR spectrum for compound **8d.** Moreover, scheme 1 illustrated the synthesis of the key intermediate 2,5-dimethoxyacetophenone **9** by methylation of **3** according to the reported method [16]. One pot reactions of the dimethoxy acetophenone **9** with different aldehydes and ethyl cyanoacetate or malononitrile in the presence of catalytic amount of ammonium acetate produced the target 2-pyridone **10a-d** and 2-aminopyridine **11a,b** derivatives, respectively. Structures of compounds **10a-d**, **11a,b** were confirmed by elemental microanalysis, IR, ^{1}H-NMR for all compounds, also EL/MS and ^{13}C-NMR spectrum for compounds **10b** and **11b**.

Scheme 2 outlined the preparation of pyridine-2-thione derivatives **12a-c** through one pot reaction of dimethoxy acetophenone **9** with different aldehydes and cyan thioacetamide in the presence of catalytic amount of ammonium acetate. Also the preparation of the required compounds **13a-l** by refluxing **12a-c** with chloroacetone or phenacyl bromide derivatives and anhydrous potassium carbonate. Structures of compounds **12a-c**, **13a-l** were confirmed by elemental microanalysis, IR, ^{1}H-NMR for all compounds, beside EL/MS and ^{13}C-NMR spectrum for compound **13k**. Compounds **14a-l** were prepared through cyclization of compounds **13a-l** in ethanolic sodium ethoxide solution. Compounds **15a-l** were prepared through S-alkylation of **12a-c** with different alkyl halides and anhydrous potassium carbonate in acetone. Finally, the target compounds **15m,n** were prepared through reaction of **12a,b**, ethyl bromo acetate and anhydrous potassium carbonate in dry acetone. Structures of the prepared compounds **14a-l** and **15a-n** were confirmed by elemental microanalysis, IR, ^{1}H-NMR for all compounds, beside EL/MS and ^{13}C-NMR spectrum for compounds **14k** and **15a,m.**



Scheme 2. (a) 4-RC_6H_4CHO / $CNCH_2CSNH_2$ / amm. acetate, reflux(b) XCH_2COR^1 / K_2CO_3; (c) EtONa; (d) $BrCH_2COOC_2H_5$ or R^2X / K_2CO_3

2.2. Biological evaluation

2.2.1. Anticancer screening

All synthesized compounds were submitted to the NCI and thirty seven compounds, **5a,b, 8a,b,d, 10c, 11a,b, 13c,f-l, 14a,b,d-l, 15a,c-f,h-j,l** and **15n**, were selected to be tested initially at a single dose (10 μM) in the full NCI 60 cell line panel. The obtained results (supplementary data) represented percentage growth of the tumor cell lines treated with compounds under investigation relative to control cell experiments. One-dose screen results of the 2-aminopyridine derivatives **5a,b** and **11a,b** showed that the compound **5b** exhibited significantly high growth inhibition percentages (GI%). Furthermore, it showed selectivity against CNS, breast, prostate, non-small cell lung, renal cancer, melanoma, leukemia, ovarian and colon cancer, respectively (table 1). Accordingly, compound **5b** was passed in the five-dose assay where it showed potent activity against most of the tested tumor cell lines as illustrated from its median growth inhibitory concentration (GI_{50}), total growth inhibitory concentration (TGI) and median lethal concentration (LC_{50}) vales (supplementary data). Consequently, NCI decided to repeat the five-dose assay and the result was nearly equivalent to the first dose results. Therefore, it was referred to Biological Evaluation Committee of NCI. As it exhibited remarkable good antitumor activity (GI_{50} values 0.302 to 3.57 μM) against all tested tumor cell lines except six cell lines where they showed moderate sensitivity. Concerning pyridone derivatives **8a,b,d** and **10c**, compound **8d** was the most active among this series. On the other hand Compound **8a** exhibited lower anticancer activity. As a result, it can be concluded that 4-substituted phenyl at 4-position of pyridone moiety (**8d**, **8b**) increased anticancer activity than the unsubstituted one (**8a**). The hydrophilic (methoxy substitution **8d**) was more effective than the lipophilic (chloro derivative **8b**). Replacement of the OH group in compound **8d** by the methoxy group as in compound **10c** retained the activity but still less active than **8d**. Furthermore, compound **10c** showed considerable anticancer activity. For S-phenacyl derivatives **13c,f-h,j-l**, compounds **13f,l** displayed mild activity. While compound **13h** exerted high anticancer activity. These results supported the previous finding that the presence of the lipophilic chloro substitution at p-position of 4-phenyl pyridine moiety increased the anticancer activity. Compounds with p-chloro substitution as **13f** and **13h** showed better anticancer activity than either p-OCH_3 substitution as **13j-l** or unsubstitution as in **13c**. Despite the presence of bulky S-phenacyl

moieties, the 4- methoxy phenacyl derivative **13h** showed a remarkable increase in anticancer activity over the unsubstituted **13f,j** and 4-chloro phenacyl derivative **13c,g,k**. Compound **13i** as S-acetylated derivatives exerted good anticancer activity. Therefore, S-substituted by 2-propanone-1-yl resulted in retaining anticancer activity. This could be due to the presence of polar carbonyl moiety which could increase interaction with the hydrophilic active site. Concerning thieno pyridine derivatives **14a,b,d-l**, cyclization of the S-phenacyl or S-acetylated derivatives as in compounds **14a,e,h,i** dramatically decreased the anticancer activity. This finding supported that the presence of the cyano group is important for the activity. For the S-alkyl derivatives **15a,c-f,h-j,l**, compound **15e** was the most active among this series. While compounds **15a,i** showed mild activity. Concerning S-ethyl derivatives, compound **15f** exhibited good activity. Compound **15j** in turn, showed very mild activity. For S-benzyl derivatives, compound **15d** exhibited good activity. However, compound **15h** showed mild activity. Unfortunately, compound **15l** did not have any significant activity. According to the above results, it can be concluded that, the presence of both lipophilic 4-chlorophenyl at 4-position of pyridine and small alkyl group on sulfur resulted in better anticancer activity as shown in the S-methyl derivative **15e**, and S-ethyl derivative **15f** while increasing the bulkiness and lipophilicity of the substituent at sulfur **15h** or changing the substituent at 4-position of phenyl pyridine moiety from the lipophilic Cl to the hydrophilic OCH_3 (**15i,j,l**) or H (**15a,c**) resulted in abolishment of anticancer activity.

Table 1: Median growth inhibitory concentrations (GI_{50}, µM) of *in vitro* subpanel tumor cell lines and GI_{50} (µM) full panel mean graph-midpoints (MG-MID) for compound 5b.

Subpanel tumor cell lines [a]	GI_{50} (MG-MID[b])	Selectivity ratio
I	2.68	0.82
II	1.15	1.92
III	6.02	0.36
IV	0.89	2.48
V	2.30	0.96
VI	2.82	0.78

VII	1.90	1.16
VIII	1.13	1.95
IX	1.05	2.10
Full panel	2.21	-

[a] **I**, leukemia; **II**, non-small cell lung cancer; **III**, colon cancer; **IV**, CNS cancer; **V**, melanoma; **VI**, ovarian cancer; **VII**, renal cancer; **VIII**, prostate cancer; **IX**, breast cancer.
[b] GI_{50} (µM) subpanel mean graph-midpoints (MG-MID) = The average sensitivity of subpanel cell lines toward the test agent.

2.2.2. PIM-1 kinase inhibitory activity

PIM-1 kinase inhibitory activity was evaluated for the most active compounds in each series and the results revealed excellent PIM-1 kinase inhibitory activity with high percentage inhibition. Amazingly, among them, compound **8d** showed the highest inhibitory activity comparable to the reference drug with IC_{50} = 0.019 µM and 0.013 µM respectively. Moreover, compounds **5b**, **15e** and **10c** showed remarkable inhibitory activity with IC_{50} values 0.044, 0.083 and 0.1128 µM respectively. While compound **13h** showed moderate inhibitory activity with IC_{50} 0.479 µM as shown in table **2**.

Table 2: PIM-1 kinase inhibitory activities of selected compounds

Compound number	**IC_{50}[a] (µM)**
8d	0.019
5b	0.044
10c	0.128
15e	0.083
13h	0.479
Staurosporin (reference drug)	0.013
Compound I (reference)[b]	0.050[5]

a: IC_{50} value is the compound concentration required to produce 50% inhibition of PIM-1 kinase.
*b: compound **I** is the lead structure used to design a new series. Its structure has been mentioned in fig.1*

2.3. Structure activity correlation:

Several pyridine containing compounds were reported in the literature to have anticancer activity[17]. Specially, 3-cyano-2-pyridones (the core scaffold)

were declared to have anticancer activity through inhibition of PIM-1 kinase[5]. PIM-1 kinase inhibition activity was merged by the addition of highly hydrophilic substitution at the 4-aryl moiety[18]. Based on these data, we designed and synthesized several new pyridone derivatives having 2,5-dihydroxy **4a-c**, 2-hydroxy-5-methoxy **8a-f** and 2,5-dimethoxy- 6-phenyl-3-cyanopyridones **10a-d** with different substitutions on the phenyl ring at position 4. Among these series, the 2-hydroxy-5-methoxy derivatives showed the highest anticancer activity in the following order; 4-methoxy **8d**, 4-chloro **8b**, followed by the unsubstituted 4-phenyl derivative **8a**. Two other series (2-amino and 2-thiopyridine) a bioisostere of the core structure were designed and synthesized in order to study the effect of such structural variation on the anticancer activity. Interestingly, 2-Amino-4-(4-chlorophenyl)-6-(2,5-dihydroxyphenyl) pyridine-3-carbonitrile **5b** exhibited the highest anticancer activity of the all synthesized compounds (GI_{50} values 0.302 to 3.57 μM). This compound was chosen by National Cancer Institute (NCI) for extent evaluation to the five-dose study. This interesting result revealed the importance of NH_2 rather than the oxygen atom at the 2-position of the pyridine ring. Alkylation of the 2-thiopyridine to obtain S-substituted derivatives were also performed. 4-(4-Chlorophenyl)-6-(2,5-dimethoxyphenyl)-2-(methylthio)pyridine-3-carbonitrile **15e** was the most active among this series. Hence, the presence of both lipophilic 4-chlorophenyl at 4-position of pyridine and a small alkyl group on sulfur resulted in better anticancer activity, while increasing the bulkiness and lipophilicity of the substituent at sulfur as e.g. benzyl ring or changing the substituent at 4-position of phenyl pyridine moiety from the lipophilic Cl to the hydrophilic OCH_3 or H resulted in the abolishment of anticancer activity. Tailing the phenacyl moiety to the thiopyridine nucleus was conducted in order to study the effect of position isostere of carbonyl group on the anticancer activity. In general, the anticancer evaluation of this series showed decrease in activity if compared to S-alkyl derivatives except for compound 4-(4-Chlorophenyl)-6-(2,5-dimethoxyphenyl)-2-((2-(4-methoxyphenyl)-2-oxoethyl) pyridine-3-carbonitrile **13h** that showed reasonable anticancer activity**.** Finally, cyclization of the S-phenacyl or S-acetylated derivatives **14a,e,h,i** dramatically decrease the anticancer activity. This finding supported that the presence of the cyano group is important for the activity. From PIM-1 kinase inhibitory data, pyridone derivative with hydrophilic substitution at 4-position and 2-hydroxy-5-methoxyphenyl at 6-position **8d** possess higher activity than that bearing 2,5-dimethoxyphenyl **10c** which revealed the importance of the hydroxyl group. In comparison of the

compounds bearing 2,5-dimethoxyphenyl at position 6 (**10c, 15e, 13h**), the presence of small substitution at position 2 with either polar OH or nonpolar S-CH_3 in compounds **10c** and **15e**, respectively retained potent PIM-1 kinase inhibitory activity. While substitution by bulky 4-methoxy phenacyl thio derivative **13h** decreased the activity. Finally, we can conclude that the polarity of substitution at 2-position didn't affect PIM-1kinase inhibitory activity. While the size affects the activity. Substitution with either polar OCH_3 or lipophilic Cl also didn't affect the activity.

2.4. Molecular modeling

2.4.1. *In silico* prediction of physicochemical properties for compounds 5b, 8d, 10c, 15e, 13h the most active anticancer.

Table 3: *In silico* physicochemical properties and drug-likeness data of active compounds.

Molinspiration					
	5b	**8d**	**10c**	**13h**	**15e**
Log P [a]	3.75	3.96	4.24	6.88	5.96
M.W [b]	337.77	348.36	362.38	531.03	396.90
HBA [c]	5	6	6	6	4
HBD [d]	4	2	1	0	0
Lipinski's violation	0	0	0	2	1
TPSA [e] **(A^2)**	103.16	95.61	84.61	81.46	55.15
%ABS [f]	73.40	76.02	79.81	80.9	90
Volume (A^3)	280.42	306,69	324.22	455.04	338.88
NROTB [g]	2	4	5	9	5
Molsoft					
S [h] **(mg/L)**	0.24	1.40	0.61	0.00	0.01
Drug likeness model score	0.13	0.26	0.09	0.69	0.29

[a] **LogP**, logarithm of compound partition coefficient between n-octanol and water; [b] **M.W.**, molecular weight; [c] **HBA**, number of hydrogen bond acceptors; [d] **HBD**, number of hydrogen bond donors; [e] **TPSA**, topological polar surface area; [f] **%ABS**, percentage of absorption; [g] **NROTB**, number of rotatable bonds; [h] **S**, solubility

Hydrogen-bonding capacity is an important parameter for describing drug permeability [19]. A poor absorption is more likely when H-bond donors are

more than 5 and H-bond acceptors are more than 10. All compounds were shown to possess accepted H-bond donors and H-bond acceptors as shown in table **3**. Also, for good membrane permeability logP value should be ≤ 5. Compounds **5b, 8d, 10c** had low logP values. Moreover, all compounds obeyed Lipinski's rule of five except compound **13h** as its molecular weigh more than 500 and logP more than 5. All Compounds**,** indeed**,** demonstrated Topological polar surface area (TPSA) value within the acceptable range. Additionally, they displayed the high percentage of absorption (%ABS) which indicates its good bioavailability by oral administration. Furthermore, they were found to fulfill the requirements of solubility of more than 0.0001 mg/L. Therefore, it could be considered as drug candidates for oral absorption. Computed drug-likeness score for all compounds are presented in table **3**. Compounds having positive values should be considered as drug-like. All compounds showed positive values which indicated that all compounds considered as drug like.

2.4.2. Docking with PIM-1 kinase

The most active candidates **5b, 8d, 10c**, **13h**, and **15e were docked** to PIM1 kinase active site(PDB ID code 2OBJ). Figures 5-14 , respectively.

Table 4: Docking results of the active compounds in PIM-1 kinase active site.

Compound number	Score in kcal/mol	H- bonding interaction with amino acids	Lipophilic and hydrophilic interactions with amino acids
Reference Fig. 3,4	-6.43	Hydrogen bond between N of CN and Phe 49 and arene H bonding between 6-phenyl ring and lle 185.	Lipophilic with Val 52, lle 185, Leu 120, lle 104, leu 93 and Phe 49 Hydrophilic with Ser 46, Ser 51 Gly 48, Gly 50, Gly 47, Asn 172.
5b Fig. 5,6	-6.33	Hydrogen bond between N of CN and Lys 67 and arene H bonding between pyridine ring and Val 52.	Lipophilic with **Val 52**, **Phe 49**, **lle 185**, Leu 174, Val 126, Leu 44, **Leu 120, lle 104.** Hydrophilic with Asn **172.**
8d Fig. 7,8	-6.59	Hydrogen bond between N of CN and Lys 67 and arene H bonding between 6-phenyl ring and leu 174.	Lipophilic with **Val 52**, **Phe 49**, Leu 174, Leu 44, **Leu 120**, Val 126, Ala 65, **lle 104** Hydrophilic with Gln 127

10c Fig. 9,10	-7.24	Arene H bonding between pyridine ring and leu 174.	Lipophilic with **Val 52, lle 104**, **Leu 120**, Ala 65**, lle 185, Leu 93,** Leu 44, Pro 123, Val 126 Hydrophilic with **Ser 48**, Gly 47
13h Fig, 11,12	-7.74	Arene H bonding between pyridine ring and Val 52.	Lipophilic **with Val 52, lle 185, Leu 120, lle 104, leu 93 and Phe 49,** Leu 174, Val 126, Ala 65, Leu 44. Hydrophilic with **Ser 46, Gly 47, Gly 50, Gly 45,** Gln 127.
15e Fig. 13,14	-7.49	Two arene H bonding between pyridine ring and Val 52, and between 6-phenyl ring and Leu 174	Lipophilic with **Val 52**, **lle 104**, **lle 185**, Leu 174, Val 126, Pro 123, Leu 44, Ala 65, **Leu 120.** Hydrophilic with **Ser 46**, Gly 45**, Gly 47**, **Asn 172**.

*** Bold amino acids indicated common amino acids involved in binding interaction of the reference and new synthesized compounds with the enzyme active site**

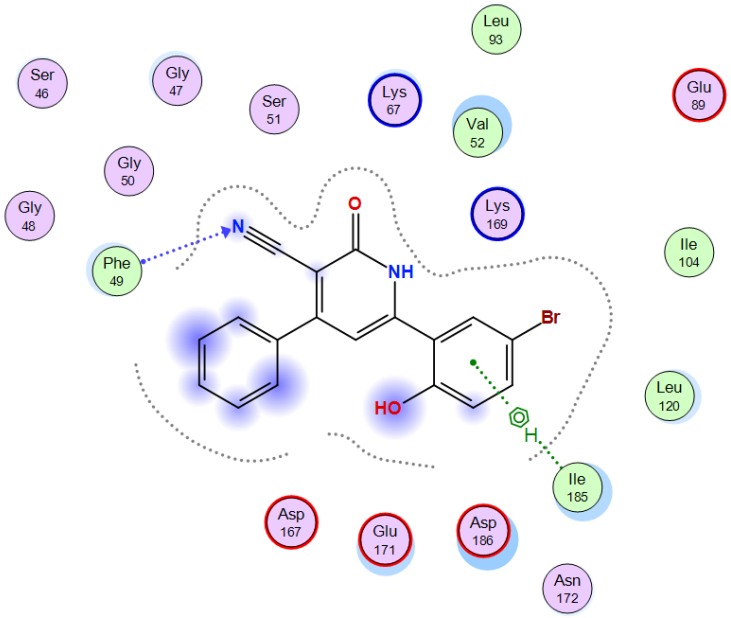


Figure. 3 Docking of VRV **(reference)** with PIM-1 kinase active site (PDB ID code 2OBJ)

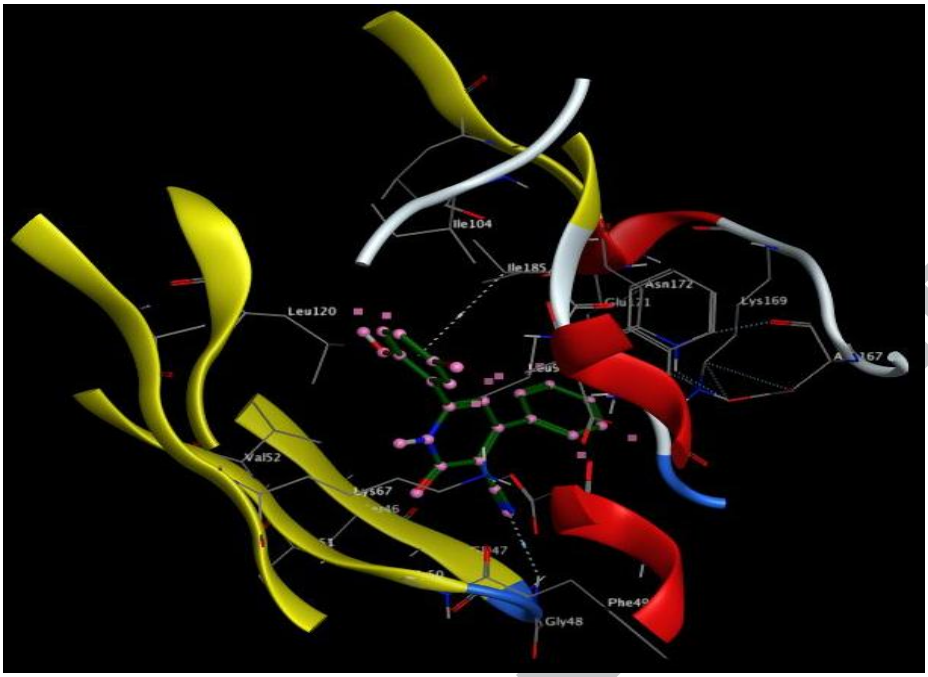


Figure 4. 3D structure of docking of **VRV (reference)** with PIM-1 kinase active site

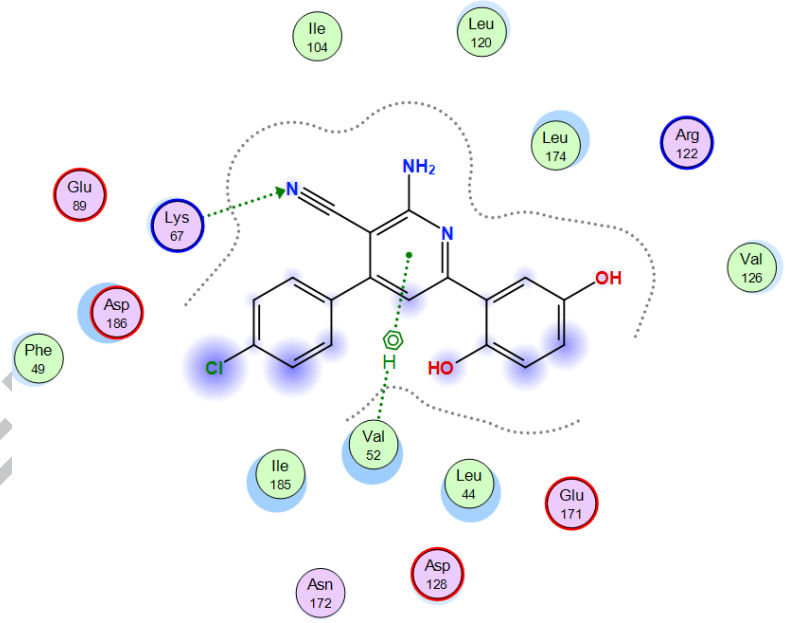


Figure 5. Mode of binding of **5b** inside PIM-1 kinase active site

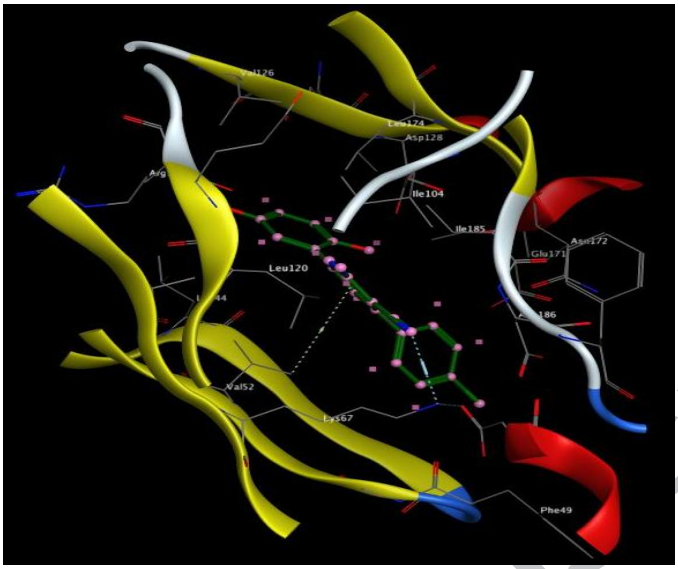


Figure 6. 3D structure for docking of mode of binding **5b** inside PIM-1 kinase active site

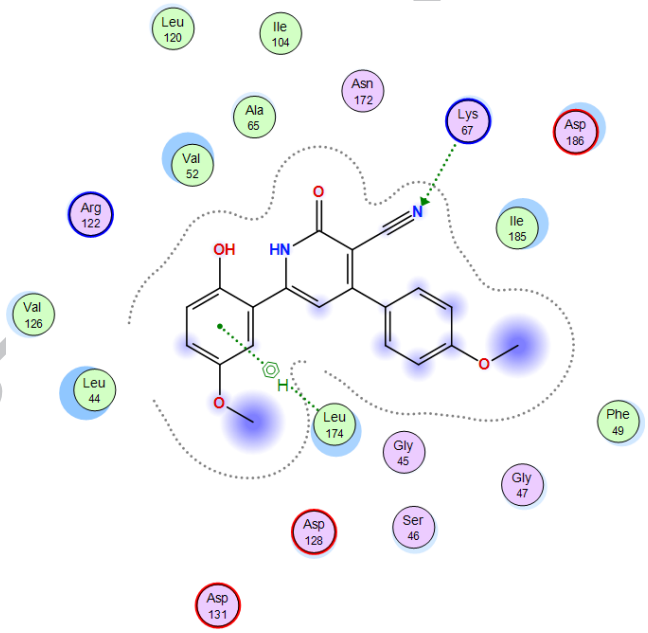


Figure 7. Mode of binding of **8d** inside PIM-1 kinase active site

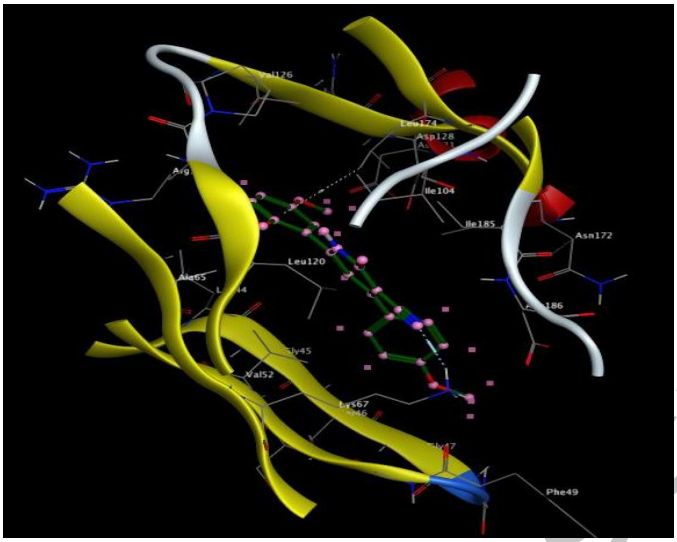


Figure 8. 3D structure for docking of mode of binding **8d** inside PIM-1 kinase active site

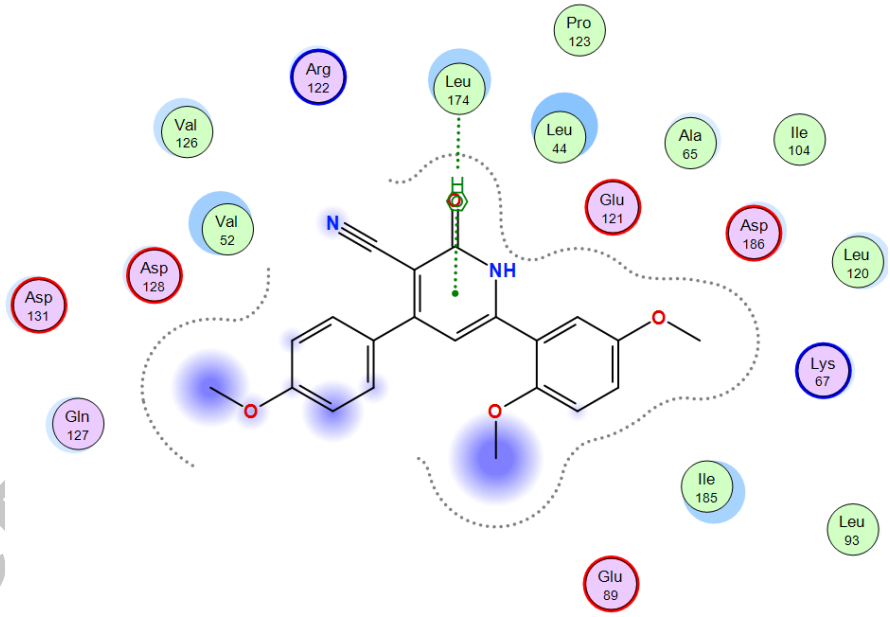


Figure 9. Mode of binding of **10c** inside PIM-1 kinase active site

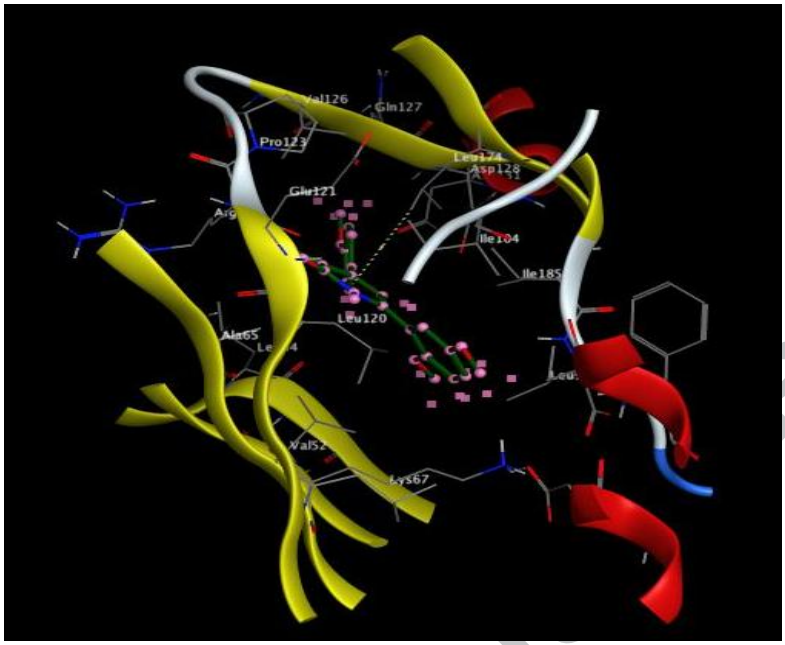


Figure 10. 3D structure for docking of mode of binding **10c** inside PIM-1 kinase active site

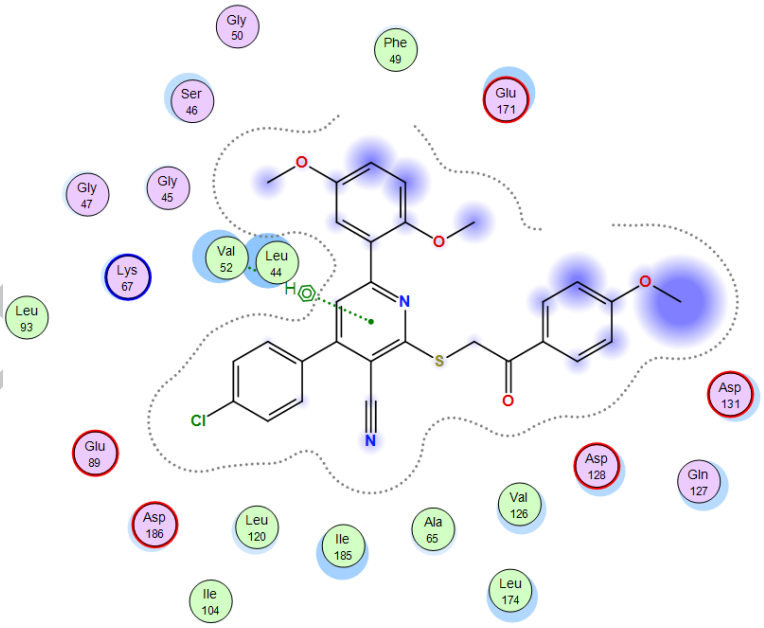


Figure 11. Mode of binding of **13h** inside PIM-1 kinase active site

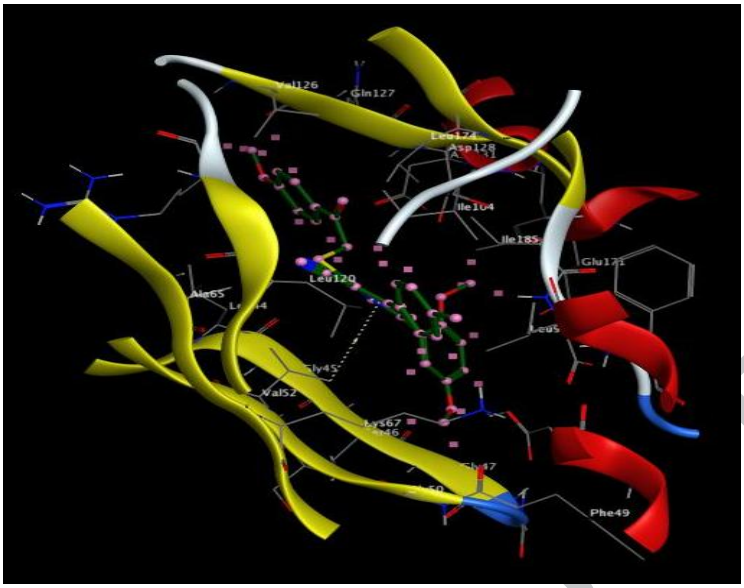


Figure 12. 3D structure for docking of mode of binding **13h** inside PIM-1 kinase active site

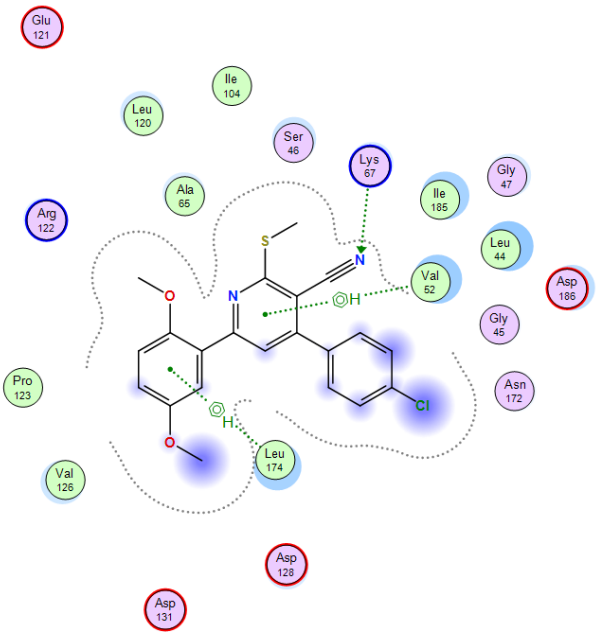


Figure 13. Mode of binding of **15e** inside PIM-1 kinase active site

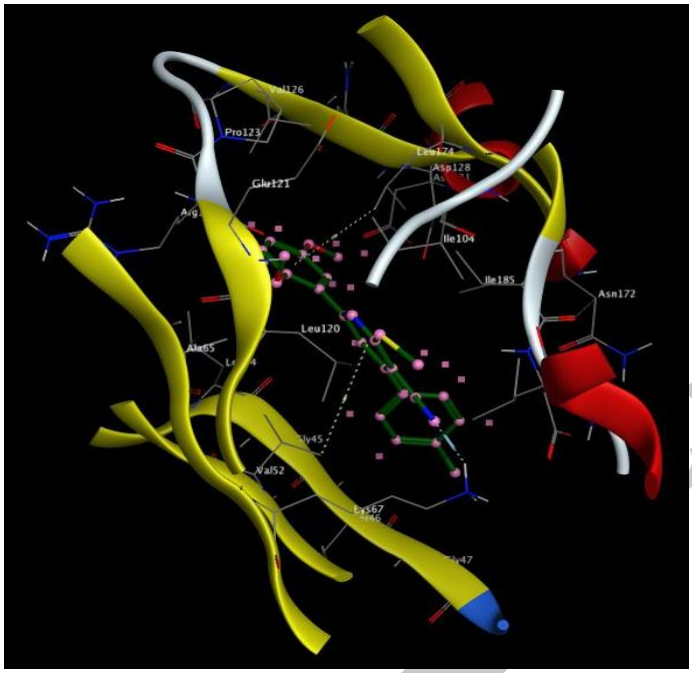


Figure 14. 3D structure for docking of mode of binding **15e** inside PIM-1 kinase active site

Docking studies of the most active compounds inside the PIM-1 kinase active site showed binding score higher than the reference compound. It is worth mentioning that, five compounds and the reference interacted with almost the same amino acids in polar and hydrophobic interactions (table **4**).

3. Conclusion

A series of pyridine and thieno[2,3-b]pyridine derivatives have been synthesized as anticancer PIM-1 kinase inhibitors. Thirty-seven compounds were selected by NCI to be tested initially at a single dose (10µM) in the full NCI 60 cell line panel. Compound **5b** showed potent anticancer activity and was tested twice in the five-dose assay and was sent to NCI biological evaluation committee and still under consideration for further testing. In addition, the most active anticancer compounds in each series, **5b**, **8d**, **10c**, **13h**, and **15e**, were evaluated for their PIM-1 kinase inhibitory activity. Compound **8d** was the most potent one with IC50 = 0.019 µM followed by **5b**, **15e**, **10c** and **13h** with IC50 values 0.044, 0.083, 0.128 and 0.479 µM respectively. Moreover, docking study of the most active compounds in PIM-1 kinase active site was consistent with the *in vitro* activity. Compounds **8d** and **10c** showed a high binding score, which confirm the importance of the presence of the hydrophilic substitution at

position 4 of the phenyl at position 4 of pyridone ring beside the hydrophilic OH on phenyl at position 6 of pyridone ring in interaction with PIM-1 kinase active site. In addition, compounds **15e** and **13h** showed high binding score which confirm the importance of the presence of the lipophilic substitution at position 4 of the phenyl at position 4 of pyridine beside the presence of flexible S-substitution at position 2 of pyridine in the interaction with PIM-1 kinase active site. Finally, the contradiction that compound **8d** showed the highest PIM-1 kinase inhibitory activity, while cell growth was strongly inhibited by compound **5b** could be explained due to the differences in physiochemical properties between 8d and 5b which could affect the bioavailability at the active site. Further study should be performed to clarify such point.

4. Experimental

4.1. Chemistry

All chemicals and solvents were purchased from commercial suppliers. Melting points were determined in open-glass capillaries using a Griffin melting point apparatus and were uncorrected. The progress of the reactions was monitored by thin-layer chromatography (TLC) on commercially available precoated silica gel aluminum-backed plates and the spots were visualized by exposure to iodine vapors or UV-lamp at λ 254 nm for few seconds. Infrared spectra (IR) were recorded, for KBr discs, on a PerkinElmer RXIFT-IR. Nuclear magnetic resonance spectra, ^{1}H-NMR and ^{13}C-NMR, were recorded on Jeol spectrometer (500 MHz) and on Varian Mercury VX (300 MHz) spectrometer using deuterated dimethyl sulfoxide or chloroform as solvents. The data were reported as chemical shifts or δ values (ppm) relative to tetramethyl silane (TMS) as internal standard. Signals were indicated by the following abbreviations: *s* = singlet, *d* = doublet, *t* = triplet, *q* = quartet and *m* = multiplet, dd = doublet of doublet and br. = broad. Electron impact mass spectra (EIMS) were run on a gas chromatograph/mass spectrophotometer Shimadzu GCMS/QP-2010 plus (70 eV). Relative intensity % corresponding to the most characteristic fragments is recorded. Compounds **2**[20], **3**[9], **8**[10], **12**[15], **15**[21], **16**[16]were prepared according to reported procedures

4.1.1. General experimental procedure for the synthesis of 6-(2,5-Dihydroxyphenyl)-2-oxo-4-(4-substitutedphenyl)-1,2-dihydropyridine-3-carbonitriles **4a-c**

A mixture of 2,5-dihydroxyacetophenone **3** (2 g, 13.15 mmol), substituted aldehyde (13.15 mmol), ethyl cyanoacetate (1.4 ml, 13.15 mmol) and ammonium acetate (8 g, 105.26 mmol) in 20 ml absolute ethanol was heated under reflux for 10 hours during which the product was separated out. Then the reaction mixture was cooled and the yellow precipitate was filtered, washed with cold ethanol, air dried and crystallized from ethanol.

4.1.1.2 6-(2,5-Dihydroxyphenyl)-2-oxo-4-phenyl-1,2-dihydropyridine-3-carbonitrile (4a)

Yield, 30%; m.p>300°C; IR (KBr, cm^{-1}): 3324 (OH), 3136 (NH), 2206 (C≡N), 1598 (C=O), 1538 (C=C). ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 6.59 (brs, 1H, pyridone-C_5-H);6.78-6.84 (m, 2H, Ar-$C_{3,4}$-H); 6.92 (brs, 1H, Ar-C_6-H); 7.55-7.57 (m, 3H, Ar–$C_{3',4',5'}$-H); 7.67-7.69 (m, 2H, Ar-$C_{2',6'}$-H); 9.01, 9.83, 12.35 (3s, each 1H, 2OH and NH, D_2O-exchangeable). Elemental analysis Calcd for $C_{18}H_{12}N_2O_3$ (304.31): C 71.05, H 3.97, N 9.21. Found C 71.32, H 4.02, N 9.37.

4.1.1.3. 4-(4-Chlorophenyl)-6-(2,5-dihydroxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (4b)

Yield, 25%; m.p>300°C; IR (KBr, cm^{-1}): 3331 (OH), 3141(NH), 2233(C≡N), 1642 (C=O), 1606 (C=C). ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 6.58 (brs, 1H, pyridone-C_5-H); 6.76-6.80 (m, 2H, Ar-$C_{3,4}$-H); 6.89 (brs, 1H, Ar-C_6-H); 7.60 (d, *J*=8.4 Hz, 2H, Ar–$C_{2',6'}$-H); 7.69 (d, *J*=8.4Hz, 2H, Ar-$C_{3',5'}$-H); 9.10, 9.89, 12.38 (3s, each 1H, 2OH and NH, D_2O-exchangeable). Elemental analysis Calcd for $C_{18}H_{11}ClN_2O_3$ (338.75): C 63.82, H 3.27, N 8.27. Found: C 64.09, H 3.24, N 8.41.

4.1.1.4. 6-(2,5-Dihydroxyphenyl)-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (4c)

Yield, 30%; m.p>300°C; IR (KBr, cm^{-1}): 3314 (OH), 3148 (NH), 2230 (C≡N), 1642 (C=O), 1609 (C=C). ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 3.84 (s, 3H, OCH_3); 6.65 (brs, 1H, pyridone-C_5-H); 6.78-6.85 (m, 2H, Ar-$C_{3,4}$-H); 6.95 (brs, 1H, Ar-C_6-H); 7.10 (d, *J*=8.1 Hz, 2H, Ar–$C_{3',5'}$-H); 7.68 (d, *J*=8.1Hz, 2H, Ar-$C_{2',6'}$-H); 9.05, 9.90, 12.21 (3s, each 1H, 2OH and NH, D_2O-exchangeable).^{13}C-NMR (300 MHz, DMSO-d_6, δ ppm): 55.36 (OCH_3); 114.19 (Ar-$C_{3',5'}$); 115.12, 115.20, 115.22 (pyridine-C_5 and Ar-$C_{1,6}$); 116.84 (pyridine-C_3); 117.57 (C≡N); 119.0, 119.41 (Ar-$C_{3,4}$); 128.17 (Ar-$C_{1'}$); 129.82 (Ar-$C_{2',6'}$); 149.91,149.97 (Ar-$C_{2,5}$); 150.02, 150.04 (pyridine-$C_{4,6}$); 160.92,160.95 (Ar-$C_{4'}$and C=O). EI-MS m/z (% relative abundance): 334 [$M^{+\bullet}$] (100); 149 (35.77);

120 (38.47); 77 (41.60); 64 (53.76). Elemental analysis Calcd for $C_{19}H_{14}N_2O_4$ (334.33): C 68.26, H 4.22, N 8.38. Found: C 68.40, H 4.31, N, 8.49.

4.1.2. General experimental procedure for the synthesis of 2-Amino-6-(2,5-dihydroxyphenyl)-4-(4-substitutedphenyl) pyridine-3-carbonitriles 5a,b

A mixture of 2,5-dihydroxyacetophenone **3** (1g, 6.57 mmol), substituted aldehyde (6.57 mmol), malononitrile (0.43g, 6.57 mmol) and ammonium acetate (4g, 52.63 mmol) in 20 ml absolute ethanol was heated under reflux for 10 hours during which the product was separated out. Then the reaction mixture was cooled and the precipitate was filtered, washed with cold ethanol, air dried and crystallized from mixture of dimethyl formamide and triturate with petroleum ether.

4.1.2.1 2-Amino-6-(2,5-dihydroxyphenyl)-4-(phenyl) pyridine-3-carbonitrile (5a)

Yield, 30%; m.p>300°C; IR (KBr, ν cm^{-1}): 3475(OH), 3297, 3169 (NH_2), 2205(C≡N), 1662 (NH, bending), 1595 (mixed C=N, C=C). ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7.15 (dd, *J*=9, 3 Hz, 1H, Ar-C_4-H);7.38 (d, *J*=8.7 Hz, 1H, Ar-C_3-H); 7.42 (brs, 2H, NH_2, D_2O-exchangeable); 7.57-7.61 (m, 3H, Ar–$C_{3',4',5'}$-H); 7.88 (d, *J*=3 Hz, 1H, Ar-C_6-H); 8.22 (s, 1H, pyridine-C_5-H); 8.41-8.44 (m, 2H, Ar-$C_{2',6'}$-H); 9.84 (s, 1H, OH, D_2O-exchangeable). Elemental analysis Calcd for $C_{18}H_{13}N_3O_2$ (303.32): C 71.28, H 4.32, N 13.85. Found C 71.45, H 4.41, N 14.01.

4.1.2.2. 2-Amino-4-(4-chlorophenyl)-6-(2,5-dihydroxyphenyl) pyridine-3-carbonitrile (5b)

Yield, 30%; m.p>300°C; IR (KBr, νcm^{-1}): 3449(OH), 3316, 3180 (NH_2), 2209(C≡N), 1651 (NH, bending), 1591 (mixed C=N, C=C). ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 7.13 (dd, *J*=8.7, 2.4 Hz, 1H, Ar-C_4-H);7.35 (d, *J*=9 Hz, 1H, Ar-C_3-H); 7.41 (brs, 2H, NH_2, D_2O-exchangeable); 7.63 (d, *J*=8.4 Hz, 2H, Ar- $C_{2',6'}$-H); 7.84 (d, *J*=2.4 Hz, 1H, Ar-C_6-H); 8.19 (s, 1H, pyridine-C_5-H); 8.43 (d, *J*=8.4 Hz, 2H, Ar-$C_{3',5'}$-H); 9.83 (s, 1H, OH, D_2O-exchangeable). Elemental analysis Calcd for $C_{18}H_{12}ClN_3O_2$ (337.76): C 64.01, H 3.58, N 12.44. Found: C 64.18, H 3.56, N 12.62.

4.1.3. General experimental procedure for synthesis of 6-(2-Hydroxy-5-methoxyphenyl)-2-oxo-4-substituted-1,2-dihydropyridine-3-carbonitriles 8a-f

A mixture of 2-benzoyloxy-5-methoxyacetophenone**11** (2 g, 7.40 mmol), substituted aldehyde (7.40 mmol), ethyl cyanoacetate (0.78 ml, 7.40 mmol) and ammonium acetate (4.56 g, 59.25 mmol) were refluxed in ethanol (10 ml) with

stirring for 3 hours during which the product was separated out. The reaction mixture was cooled and the yellow precipitate was filtered, washed with cold ethanol, air dried and crystallized from acetone.

4.1.3.1. 6-(2-Hydroxy-5-methoxyphenyl)-2-oxo-4-phenyl-1,2-dihydropyridine-3-carbonitrile (8a)

Yield, 35%; m.p. 290°C (shrinkage); IR (KBr, ν cm^{-1}): 3394(OH), 3154 (NH); 2217 (C≡N); 1651 (C=O); 1608 (C=C); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 3.74 (s, 3H, OCH_3); 6.78 (brs, 1H,pyridone-C_5-H); 6.91 (d, *J*=9 Hz, 1H, Ar-C_3-H); 6.98 (dd, *J*=9, 3 Hz, 1H, Ar-C_4-H); 7.15 (d, *J*=3 Hz, 1H, Ar-C_6-H); 7.54-7.58 (m, 3H, Ar-H); 7.67-7.71 (m, 2H, Ar-H); 10.2, 12.5 (2brs, 2H, OH and NH, D_2O-exchangeable). Elemental analysis Calcd for $C_{19}H_{14}N_2O_3$ (318.33): C 71.69, H 4.43, N 8.80. Found: C 71.82, H 4.48, N 8.94.

4.1.3.2. 4-(4-Chlorophenyl)-6-(2-hydroxy-5-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (8b)

Yield, 30%; m.p. >300°C; IR (KBr, ν cm^{-1}): 3400 (OH), 3146 (NH); 2216 (C≡N); 1657 (C=O); 1604 (C=C); ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 3.69 (s, 3H, OCH_3) ; 6.79 (s, 1H, pyridone-C_5-H); 6.83 (d, *J*=9 Hz, 1H, Ar-C_3-H); 6.90 (dd, , *J*=9, 3 Hz, 1H, Ar-C_4-H); 7.16 (d, *J*=3 Hz, 1H, Ar-C_6-H); 7.58 (d, *J*=8.4 Hz, 2H, p-chlorophenyl $C_{2,6}$-H); 7.67 (d, *J*=8.4 Hz, 2H, p-chlorophenyl $C_{3,5}$-H); 12.09, 13.79 (2brs, 2H, OH and NH, D_2O-exchangeable). EI-MS m/z (% relative abundance): 354 [$M^{+\bullet}$+2] (22.31); 352 [$M^{+\bullet}$] (61.17); 339 (34.97); 337 (100); 176 (19.42). Elemental analysis Calcd for $C_{19}H_{13}ClN_2O_3$ (352.77): C 64.69, H 3.71, N 7.94. Found: C 64.87, H 3.77, N 8.03.

4.1.3.3. 4-(4-Bromophenyl)-6-(2-hydroxy-5-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (8c)

Yield, 30%; m.p. >300°C; IR (KBr, ν cm^{-1}): 3399 (OH), 3154 (NH); 2221 (C≡N); 1643 (C=O); 1613 (C=C); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 3.73 (s, 3H, OCH_3); 6.77 (brs, 1H, pyridone-C_5-H); 6.92 (d, *J* =9 Hz, 1H, Ar-C_3-H); 6.97 (dd, *J*=9, 3 Hz, 1H, Ar-C_4-H); 7.10 (d, *J*=3 Hz, 1H, Ar-C_6-H); 7.61 (d, *J*=9 Hz, 2H, p-bromophenyl $C_{2,6}$-H); 7.74 (d, *J*=9 Hz, 2H, p-bromophenyl $C_{3,5}$-H); 10.2, 12.5 (2brs, 2H, OH and NH, D_2O-exchangeable). Elemental analysis Calcd for $C_{19}H_{13}BrN_2O_3$ (397.23): C 57.45, H 3.30, N 7.05. Found: C 57.68, H 3.27, N 7.18.

4.1.3.4. 6-(2-Hydroxy-5-methoxyphenyl)-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (8d)

Yield, 25%; m.p. 289°C (shrinkage); IR (KBr, νcm^{-1}): 3336 (OH), 3170 (NH); 2219 (C≡N); 1639 (C=O); 1607 (C=C); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 3.75, 3.85 (2s, 6H, 2OCH_3); 6.75 (brs, 1H,pyridone-C_5-H); 6.91 (d, *J*=9 Hz, 1H, Ar-C_3-H); 6.96 (dd, *J*=8.7, 2.7 Hz, 1H, Ar-C_4-H); 7.11-7.13 (m, 3H, Ar-C_6 and p-methoxyphenyl $C_{3,5}$-H); 7.69 (d, *J*=8.7 Hz, 2H, p-methoxyphenyl $C_{2,6}$-H); 10.2, 12.4 (2brs, 2H, OH and NH, D_2O-exchangeable). ^{13}C-NMR (300 MHz, DMSO-d_6, δ ppm): 55.37, 55.61 (2OCH_3); 107.33 (pyridine-C_5); 113.60, 116.93 (Ar-$C_{4,6}$); 114.19 (p-methoxyphenyl-$C_{3,5}$); 117.72 (pyridine- C_3); 118.84 (Ar-C_3); 118.92 (C≡N); 120.61 (p-methoxyphenyl-C_1); 128.26 (Ar-C_1); 129.86 (p-methoxyphenyl-$C_{2,6}$); 133.46 (pyridine-C_6); 149.87 (pyridine-C_4); 152.05 (Ar-C_2); 158.76 (Ar-C_5); 160.94 (p-methoxyphenyl-C_4); 161.72 (C=O). Elemental analysis Calcd for $C_{20}H_{16}N_2O_4$ (348.36):C 68.96, H 4.63, N 8.04. Found: C 69.12, H 4.72, N 8.11.

4.1.3.5. 6-(2-Hydroxy-5-methoxyphenyl)-2-oxo-4-(thiophen-2-yl)-1,2-dihydropyridine-3-carbonitrile (8e)

Yield, 35%; m.p. >300°C; IR (KBr, νcm^{-1}):3410(OH), 3193 (NH); 2213 (C≡N); 1638 (C=O); 1607 (C=C); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 3.75 (s, 3H, OCH_3); 6.88 (s, 1H,pyridone C_5-H); 6.92 (d, *J*=9 Hz, 1H, Ar-C_3-H); 6.97 (dd, *J*=9, 3 Hz, 1H, Ar-C_4-H); 7.11 (d, *J*=3 Hz, 1H, Ar-C_6-H); 7.32 (dt, *J*=3.9,1.2 Hz, 1H, thiophene C_3-H); 7.89 (dd, *J*=5.1, 1.2 Hz, 1H, thiophene C_2-H); 7.93 (dd, *J*=3.9, 1.2 Hz, 1H, thiophene C_4-H); 10.05, 12.3 (2brs, 2H, OH and NH, D_2O-exchangeable). Elemental analysis Calcd for $C_{17}H_{12}N_2O_3S$ (324.35): C 62.95, H 3.73, N 8.64. Found: C 63.04, H 3.79, N 8.82.

4.1.3.6. 6'-(2-Hydroxy-5-methoxyphenyl)-2'-oxo-1',2'-dihydro-[3,4'-bipyridine]-3'-carbonitrile (8f)

Yield, 35%; m.p. >300°C; IR (KBr, νcm^{-1}):3436(OH); 3122 (NH); 2219 (C≡N); 1651 (C=O); 1604 (C=C); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm: 3.75 (s, 3H, OCH_3); 6.89 (s, 1H,pyridine C_5-H); 6.92 (d, *J*=9 Hz, 1H, Ar-C_3-H); 6.99 (dd, *J*=3 Hz, *J*=9 Hz, 1H, Ar-C_4-H); 7.19 (d, *J*=3 Hz, 1H, Ar-C_6-H); 7.60 (t, *J*=6 Hz, 1H, pyridine-C_5'-H); 8.14 (dd, *J*=3 Hz, *J*=6 Hz, 1H, pyridine-C_6'-H); 8.76 (d, *J*=6 Hz, 1H, pyridine-C_4'-H); 8.90 (s, 1H, pyridine-C_2'-H); 10.2, 12.6 (2brs, 12H, OH and NH, D_2O-exchangeable). Elemental analysis Calcd for $C_{18}H_{13}N_3O_3$ (319.32): C 67.71, H 4.10, N 13.16. Found: C 67.87, H 4.17, N 13.31.

4.1.4. General experimental procedure for synthesis of 6-(2,5-Dimethoxyphenyl)-2-oxo-4-substituted-1,2-dihydropyridine-3-carbonitriles 10a-d

A mixture of 2,5-dimethoxyacetophenone **9** (1 g, 5.55 mmol), substituted aldehyde (5.55 mmol), ethyl cyanoacetate (0.59 ml, 5.55 mmol) and ammonium acetate (3.42 g, 44.44 mmol) were refluxed in ethanol (10 ml) with stirring for 3 hours during which the product was separated out. The reaction mixture was cooled and the yellow precipitate was filtered, washed with cold ethanol, air dried and crystallized from dimethyl formamide.

4.1.4.1. 6-(2,5-Dimethoxyphenyl)-2-oxo-4-phenyl-1,2-dihydropyridine-3-carbonitrile (10a)

Yield, 32%; m.p. 247-249°C; IR (KBr, ν cm^{-1}): 3348 (NH); 2221 (C≡N); 1635 (C=O); 1588 (C=C); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 3.76, 3.78 (2s, each 3H, 2OCH_3); 6.59 (s, 1H, pyridone-C_5-H); 7.05-7.09 (m, 2H, Ar -$C_{3,4}$-H); 7.13 (d, *J*=2.7 Hz, 1H, Ar-C_6-H); 7.55-7.57 (m, 3H, phenyl $C_{3,4,5}$–H); 7.67-7.72 (m, 2H, phenyl $C_{2,6}$–H); 12.55 (brs, 1H, NH, D_2O-exchangeable). Elemental analysis Calcd for $C_{20}H_{16}N_2O_3$ (332.36):C 72.28, H 4.85, N 8.43. Found: C 72.49, H 4.94, N 8.62.

4.1.4.2. 4-(4-Chlorophenyl)-6-(2,5-dimethoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (10b)

Yield, 35%; m.p. 263-265 °C; IR (KBr, ν cm^{-1}): 3436 (NH); 2219 (C≡N); 1647 (C=O); 1595 (C=C); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 3.76, 3.77 (2s, each 3H, 2OCH_3); 6.59 (s, 1H, pyridone-C_5-H); 7.05-7.09 (m, 2H, Ar -$C_{3,4}$-H); 7.13 (d, *J*=2.4 Hz, 1H, Ar-C_6-H); 7.63 (d, *J*=8.7 Hz, 2H, p-chlorophenyl $C_{2,6}$-H); 7.73 (d, *J*=8.7 Hz, 2H, p-chlorophenyl $C_{3,5}$-H); 12.59 (brs, 1H, NH, D_2O-exchangeable). ^{13}C-NMR (300 MHz, DMSO-d_6, δ ppm): 55.63, 56.05 (2OCH_3); 107.62 (pyridine-C_5); 113.00, 113.10, 115.37, 116.27 (Ar-$C_{1,3,4,6}$); 117.64 (C≡N); 121.72 (pyridine-C_3); 128.80 (p-chlorophenyl-$C_{3,5}$); 130.04 (p-chlorophenyl-$C_{2,6}$); 134.76 (p-chlorophenyl-C_1); 135.24 (p-chlorophenyl-C_4); 149.38 (pyridine-C_6); 150.71 (pyridine-C_4); 152.90, 158.38 (Ar-$C_{2,5}$); 161.00 (C=O). EI-MS m/z (% relative abundance): 368 [$M^{+\bullet}$+2] (21.27); 366 [$M^{+\bullet}$] (63.63); 348 (34.05); 231 (100); 135 (49.51); 82 (41.06). Elemental analysis Calcd for $C_{20}H_{15}ClN_2O_3$ (366.80): C 65.49, H 4.12, N 7.64. Found: C 65.61, H 4.17, N 7.76.

4.1.4.3. 6-(2,5-Dimethoxyphenyl)-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (10c)

Yield, 37%; m.p 261-262 °C; IR (KBr, ν cm^{-1}): 3434 (NH); 2220 (C≡N); 1629 (C=O); 1597 (C=C); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 3.76, 3.77, 3.84 (3s, each 3H, 3OCH_3); 6.56 (s, 1H, pyridone-C_5-H); 7.08-7.12 (m, 5H, Ar -$C_{3,4,6}$ and p-methoxyphenyl $C_{3,5}$-H); 7.70 (d, *J*=9 Hz, 2H, p-methoxyphenyl $C_{2,6}$–H); 12.44 (brs, 1H, NH, D_2O-exchangeable). Elemental analysis Calcd for $C_{21}H_{18}N_2O_4$ (362.39): C 69.60, H 5.01, N 7.73. Found: C 69.82, H 5.09, N 7.86.

4.1.4.4. 6-(2,5-Dimethoxyphenyl)-2-oxo-4-(thiophen-2-yl)-1,2-dihydropyridine-3-carbonitrile (10d)

Yield, 32%; m.p. 243-245 °C; IR (KBr, ν cm^{-1}): 3438 (NH); 2219 (C≡N); 1646 (C=O);1595 (C=C); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 3.77, 3.78 (2s, each 3H, 2OCH_3); 6.74 (s, 1H, pyridone-C_5-H); 7.06-7.13 (m, 3H, Ar -$C_{3,4,6}$-H); 7.31 (dt, *J*=5.1, 1.2 Hz, 1H, thiophene C_4-H); 7.97-8.00 (m, 2H, thiophene $C_{3,5}$–H); 12.47 (brs, 1H, NH, D_2O-exchangeable). Elemental analysis Calcd for $C_{18}H_{14}N_2O_3S$ (338.38): C 63.89, H 4.17, N 8.28, S 9.47. Found: C 64.04, H 4.23, N 8.37, S 9.56.

4.1.5. General experimental procedure for synthesis of 2-Amino-6-(2,5-dimethoxyphenyl)-4-(4-substituted phenyl)pyridine-3-carbonitriles11a,b

A mixture of 2,5-dimethoxyacetophenone **9** (1 g, 5.55 mmol), 4-substituted benzaldehyde (5.55 mmol), malononitrile (0.36 g, 5.55 mmol) and ammonium acetate (3.42 g, 44.44 mmol) in 20 ml absolute ethanol was heated under reflux for 10 hours during which the product was separated out. Then the reaction mixture was cooled and the precipitate was filtered, washed with cold ethanol, air dried and crystallized from ethanol.

4.1.5.1. 2-Amino-4-(4-chlorophenyl)-6-(2,5-dimethoxyphenyl) pyridine-3-carbonitrile (11a)

Yield, 30%; m.p. 245-246°C; IR (KBr, ν cm^{-1}): 3418, 3226 (NH_2); 2204 (C≡N); 1647 (N-H bending), 1571 (mixed C=N, C=C); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 3.82, 3.84 (2s, each 3H, 2OCH_3); 5.35 (s, 2H, NH_2, D_2O-exchangeable); 6.95-6.96 (m, 2H, Ar-$C_{3,4}$-H); 7.38 (s, 1H, pyridine-C_5-H); 7.40 (d, *J*=2.7 Hz, 1H, Ar-C_6-H); 7.50 (d, *J*=9 Hz, 2H, Ar- $C_{2',6'}$-H); 7.58 (d, *J*=9 Hz, 2H, Ar-$C_{3',5'}$-H). Elemental analysis Calcd for $C_{20}H_{16}ClN_3O_2$ (365.82): C 65.67, H 4.41, N 11.49. Found: C 65.84, H 4.45, N 11.68.

4.1.5.2. 2-Amino-6-(2,5-dimethoxyphenyl)-4-(4-methoxyphenyl) pyridine-3-carbonitrile (11b)

Yield, 30%; m.p. 190-191°C; IR (KBr, ν cm^{-1}): 3417, 3326 (NH_2); 2204 (C≡N); 1648 (N-H, bending), 1569 (mixed C=N, C=C); ^{1}H-NMR (300 MHz,

DMSO-d_6, δ ppm): 3.75, 3.76, 3.83 (3s, each 3H, 3OCH_3); 6.85 (s, 2H, NH_2, D_2O-exchangeable); 7.01 (dd, *J*=9, 3 Hz, 1H, Ar-C_4-H); 7.05-7.11 (m, 3H, Ar-$C_{3,3',5'}$-H); 7.19 (s, 1H, pyridine-C_5-H); 7.36 (d, *J*=3 Hz, 1H, Ar-C_6-H); 7.57 (d, *J*=7.8 Hz, 2H, Ar- $C_{2',6'}$-H); ^{13}C-NMR (300 MHz, DMSO-d_6, δ ppm): 55.28, 55.50, 56.22 (3OCH_3); 85.96 (pyridine-C_3); 113.51 (pyridine-C_5); 114.24 (Ar-$C_{3',5'}$); 115.83 (Ar-$C_{3,4}$), 115.87 (Ar-C_6); 117.22 (C≡N); 128.01 (Ar-C_1); 129.14 (Ar-$C_{1'}$); 129.60 (Ar-$C_{2',6'}$); 151.39 (pyridine-C_4); 153.08 (pyridine-C_6); 153.18 (Ar-C_2); 157.42 (Ar-C_5); 160.32 (Ar-$C_{4'}$); 160.75 (pyridine-C_2). EI-MS m/z (% relative abundance): 361 [$M^{+\bullet}$] (29.24); 226 (100); 121 (15.20). Elemental analysis Calcd for $C_{21}H_{19}N_3O_3$ (361.40): C 69.79, H 5.30, N 11.63. Found: C 69.94, H 5.36, N 11.81

4.1.6. General experimental procedure for synthesis of 6-(2,5-Dimethoxyphenyl)-4-(substitutedphenyl)-2-thioxo-1,2-dihydropyridine-3-carbonitriles 12a-c

A mixture of 2,5-dimethoxyacetophenone **9** (3 g, 16.66 mmol), 4-substituted benzaldehyde (16.66 mmol), cyanthioacetamide (1.66 g, 16.66 mmol) and ammonium acetate (10.26 g, 133.33 mmol) in absolute ethanol (10 ml) was refluxed with stirring for 3 hours during which the product was separated out. Then the reaction mixture was cooled and the yellow precipitate was filtered, washed with cold ethanol, air dried and crystallized from acetone.

4.1.6.1. 6-(2,5-Dimethoxyphenyl)-4-phenyl-2-thioxo-1,2-dihydropyridine-3-carbonitrile (12a)

Yield, 40%; m.p. 230°C; IR (KBr, ν cm^{-1}): 3401 (NH); 2217 (C≡N); 1591 (C=C); 1545, 1387, 1125, 1088 (N-C=S); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 3.77, 3.80 (2s, each 3H, 2OCH_3); 6.99 (s, 1H, pyridine-C_5-H); 7.06-7.11 (m, 2H, Ar-$C_{3,4}$-H); 7.15 (s, 1H, Ar-C_6-H); 7.56-7.65 (m, 3H, Ar-$C_{3',4',5'}$-H); 7.70-7.74 (m, 2H, Ar-$C_{2',6'}$-H); 13.99 (brs, 1H, NH, D_2O-exchangeable). Elemental analysis Calcd for $C_{20}H_{16}N_2O_2S$ (348.42): C 68.95, H 4.63, N 8.04, S 9.20. Found: C 69.12, H 4.72, N 8.17, S 9.28.

4.1.6.2. 4-(4-Chlorophenyl)-6-(2,5-dimethoxyphenyl)-2-thioxo-1,2-dihydropyridine-3-carbonitrile (12b)

Yield, 40%; m.p. 235-238°C; IR (KBr, ν cm^{-1}): 3423 (NH); 2222 (C≡N); 1589 (C=C); 1546, 1389, 1132, 1087 (N-C=S); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 3.77, 3.79 (2s, each 3H, 2OCH_3); 7.00 (s, 1H, pyridine-C_5-H); 7.11 (d, *J*=1.5 Hz, 2H, Ar-$C_{3,6}$-H); 7.15 (dd, *J*=6, 1.5 Hz, 1H, Ar-C_4-H); 7.64 (d, *J*=8.7 Hz, 2H, Ar-$C_{2',6'}$-H); 7.75 (d, *J*=8.7 Hz, 2H, Ar-$C_{3',5'}$-H); 14.02 (brs, 1H, NH,

D_2O-exchangeable). Elemental analysis Calcd for $C_{20}H_{15}ClN_2O_2S$ (382.86): C 62.74, H 3.95, N 7.32, S 8.37. Found: C 62.89, H 3.93, N 7.41, S 8.44.

4.1.6.3. 6-(2,5-Dimethoxyphenyl)-4-(4-methoxyphenyl)-2-thioxo-1,2-dihydropyridine-3-carbonitrile (12c)

Yield, 38%; m.p. 199-200°C; IR (KBr, ν cm^{-1}): 3448 (NH); 2216 (C≡N); 1584 (C=C); 1545,1312, 1181, 1031 (N-C=S); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 3.77, 3.79, 3.84 (3s, each 3H, $3OCH_3$); 6.97 (s, 1H, pyridine- C_5-H); 7.09-7.15 (m, 5H, Ar-$C_{3,4,6}$ and Ar-$C_{3',5'}$-H); 7.72 (d, *J*=8.7 Hz, 2H, Ar-$C_{2',6'}$-H); 13.88 (brs, 1H, NH, D_2O-exchangeable). Elemental analysis Calcd for $C_{21}H_{18}N_2O_3S$ (378.45): C 66.65, H 4.79, N 7.40, S 8.47. Found: C 66.74, H 4.83, N 7.52, S 8.56.

4.1.7. General procedure for synthesis of 6-(2,5-Dimethoxyphenyl)-4-(4-substitutedphenyl)-2-(2-substituted-2-oxoethylthio)pyridine-3-carbonitriles 13a-l

A mixture of 6-(2,5-dimethoxyphenyl)-4-(4-substitutedphenyl)-2-thioxo-1,2-dihydropyridine-3-carbonitrile **12a-c** (1 mmol), the appropriate halo-carbonyl derivatives (1 mmol) and anhydrous potassium carbonate (0.20 g, 1.5 mmol) in dry acetone (5 ml) was refluxed for 5 hours. The reaction mixture was then cooled and triturated with water till complete precipitation. The formed yellow precipitate was filtered, washed with water, air dried and crystallized from toluene.

4.1.7.1. 6-(2,5-Dimethoxyphenyl)-2-((2-oxopropyl)thio)-4-(phenyl)pyridine-3-carbonitrile (13a)

Yield, 94.8%; m.p. 159-160°C; IR (KBr, ν cm^{-1}): 2217 (C≡N); 1708 (C=O); 1558 (mixed C=C, C=N); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 2.35 (s, 3H, $COCH_3$); 3.84, 3.88 (2s, each 3H, $2OCH_3$); 4.08 (s, 2H, CH_2CO); 6.95 (d, *J*=9 Hz, 1H, Ar-C_3-H); 7.00 (dd, *J*=9, 3 Hz, 1H, Ar-C_4-H); 7.42 (d, *J*=2.4 Hz, 1H, Ar-C_6-H); 7.52-7.54 (m, 3H, Ar-$C_{3',4',5'}$-H); 7.63 (dd, *J*=8.1, 4.5 Hz, 2H, Ar-$C_{2',6'}$-H); 7.85 (s, 1H, pyridine-C_5-H).Elemental analysis Calcd for $C_{23}H_{20}N_2O_3S$ (404.48): C 68.30, H 4.98, N 6.93, S 7.93. Found: C 68.44, H 4.96, N 7.14, S 8.12.

4.1.7.2. 6-(2,5-Dimethoxyphenyl)-2-((2-oxo-2-phenylethyl)thio)-4-(phenyl)pyridine-3-carbonitrile (13b)

Yield, 97%; m.p. 159-160°C; IR (KBr, ν cm^{-1}): 2216 (C≡N); 1685 (C=O); 1559 (mixed C=C, C=N); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 3.50, 3.79 (2s, each 3H, $2OCH_3$); 4.82 (s, 2H, S-CH_2); 6.90 (d, *J*=1.8 Hz, 2H, Ar-$C_{3,4}$-H); 7.25 (m, 1H, Ar-C_6-H, under $CDCl_3$); 7.45-7.48 (t, *J*=7.8 Hz, 2H, Ar-$C_{2',6'}$-H); 7.51-

7.54 (m, 3H, Ar-$C_{3',4',5'}$-H); 7.61-7.62 (m, 3H, benzoyl $C_{3,4,5}$-H); 7.81 (s, 1H, pyridine-C_5-H); 8.03 (d, *J*=8.4 Hz, 2H, benzoyl-$C_{2,6}$-H).Elemental analysis Calcd for $C_{28}H_{22}N_2O_3S$ (466.55): C 72.08, H 4.75, N 6.00, S 6.87. Found: C 72.26, H 4.84, N 6.07, S 6.94.

4.1.7.3. 2-((2-(4-Chlorophenyl)-2-oxoethyl)thio)-6-(2,5-dimethoxyphenyl)-4-(phenyl)pyridine-3-carbonitrile (13c)

Yield, 94.5%; m.p. 205-207°C; IR (KBr, ν cm^{-1}):2217 (C≡N); 1686 (C=O); 1559 (mixed C=N, C=C);^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 3.60, 3.78 (2s, each 3H, $2OCH_3$); 4.73 (s, 2H, S-CH_2); 6.89 (d, *J*=1.8 Hz, 2H, Ar-$C_{3,4}$-H); 7.15 (t, *J*=1.8 Hz, 1H, Ar-C_6-H); 7.40 (d, *J*=8.4 Hz, 2H, p-chlorophenyl-$C_{2,6}$-H); 7.51-7.53 (m, 3H, Ar- $C_{3',4',5'}$-H); 7.60-7.62 (m, 2H, Ar-$C_{2',6'}$-H); 7.78 (s, 1H, pyridine-C_5-H); 7.96 (d, *J*=8.4 Hz,2H, p-chlorophenyl-$C_{3,5}$-H).Elemental analysis Calcd for $C_{28}H_{21}ClN_2O_3S$ (501.00): C 67.13, H 4.22, N 5.59, S 6.40. Found: C 67.26, H 4.21, N 5.69, S 6.51.

4.1.7.4. 6-(2,5-Dimethoxyphenyl)-2-((2-(4-methoxyphenyl)-2-oxoethyl)thio)-4-(phenyl)pyridine-3-carbonitrile (13d)

Yield, 90%; m.p. 173-174°C; IR (KBr, ν cm^{-1}):2212 (C≡N); 1685 (C=O); 1595 (mixed C=C, C=N); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 3.54, 3.80, 3.88 (3s, each 3H, $3OCH_3$); 4.79 (s, 2H, S-CH_2); 6.90 (d, *J*=1.8 Hz, 2H, Ar-$C_{3,4}$-H); 6.92 (d, *J*=9 Hz, 2H, p-methoxyphenyl $C_{3,5}$-H); 7.28 (t, *J*=1.8 Hz, 1H, Ar-C_6-H); 7.51-7.55 (m, 3H, Ar-$C_{3',4',5'}$-H); 7.61-7.64 (m, 2H, Ar-$C_{2',6'}$-H); 7.81 (s, 1H, pyridine-C_5-H); 8.04 (d, *J*=9 Hz, 2H, p-methoxyphenyl $C_{2,6}$-H). Elemental analysis Calcd for $C_{29}H_{24}N_2O_4S$ (496.58): C 70.14, H 4.87, N 5.64, S 6.46. Found: C 70.31, H 4.94, N 5.75, S 6.54.

4.1.7.5. 4-(4-Chlorophenyl)-6-(2,5-dimethoxyphenyl)-2-((2-oxopropyl)thio)pyridine-3-carbonitrile (13e)

Yield, 93%; m.p. 169-170°C; IR (KBr, ν cm^{-1}): 2217 (C≡N); 1708 (C=O); 1561 (mixed C=C, C=N); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.25 (s, 3H, $COCH_3$); 3.79, 3.80 (2s, each 3H, $2OCH_3$); 4.29 (s, 2H, S-CH_2); 7.08 (dd, *J*=9, 3 Hz, 1H, Ar-C_4-H); 7.14 (d, *J*=9 Hz, 1H, Ar-C_3-H); 7.36 (d, *J*=3 Hz, 1H, Ar-C_6-H); 7.66 (d, *J*=8.4 Hz, 2H, Ar–$C_{2',6'}$-H); 7.73 (d, *J*=8.4 Hz, 2H, Ar-$C_{3',5'}$-H); 7.80 (s, 1H, pyridine-C_5-H).Elemental analysis Calcd for $C_{23}H_{19}ClN_2O_3S$ (438.93): C 62.94, H 4.36, N 6.38, S 7.30. Found: C 63.07, H 4.41, N 6.50, S 7.36.

4.1.7.6. 4-(4-Chlorophenyl)-6-(2,5-dimethoxyphenyl)-2-((2-oxo-2-phenylethyl)thio)pyridine-3-carbonitrile (13f)

Yield, 89%; m.p. 186-190°C; IR (KBr, ν cm^{-1}): 2211 (C≡N); 1681 (C=O); 1558 (mixed C=C, C=N); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 3.41, 3.74 (2s, each 3H, 2OCH_3); 5.07 (s, 2H, S-CH_2); 6.94 (dd, *J*=9, 3 Hz, 1H, Ar-C_4-H); 7.05 (d, *J*=9 Hz, 1H, Ar-C_3-H); 7.18 (d, *J*=3 Hz, 1H, Ar-C_6-H); 7.52 (t, *J*=7.8 Hz, 2H, benzoyl $C_{3,5}$-H); 7.65-7.69 (m, 3H, benzoylC_4- and Ar–$C_{2',6'}$-H); 7.74 (d, *J*=8.7 Hz, 2H, Ar-$C_{3',5'}$-H); 7.80 (s, 1H, pyridine-C_5-H); 8.05 (d, *J*=8.4 Hz, 2H, benzoyl $C_{2,6}$-H).Elemental analysis Calcd for $C_{28}H_{21}ClN_2O_3S$ (501.00): C 67.13, H 4.23, N 5.59, S 6.40. Found: C 67.22, H 4.31, N 5.64, S 6.48.

4.1.7.7. 4-(4-Chlorophenyl)-2-((2-(4-chlorophenyl)-2-oxoethyl)thio)-6-(2,5-dimethoxyphenyl) pyridine-3-carbonitrile (13g)

Yield, 87%; m.p. 218-220°C; IR (KBr, ν cm^{-1}): 2217 (C≡N); 1687 (C=O); 1560 (mixed C=C, C=N); ^{1}H-NMR (500 MHz, $CDCl_3$, δ ppm): 3.56, 3.77 (2s, each 3H, 2OCH_3); 4.71 (s, 2H, S-CH_2); 6.87 (s, 2H, Ar-$C_{3,4}$-H); 7.09 (s, 1H, Ar-C_6-H); 7.39 (d, *J*=8.4 Hz, 2H, p-chloro phenyl $C_{2,6}$-H); 7.49 (d, *J*=8.4 Hz, 2H, Ar–$C_{2',6'}$-H); 7.55 (d, *J*=8.4 Hz, 2H, Ar–$C_{3',5'}$-H); 7.73 (s, 1H, pyridine-C_5-H); 7.95 (d, *J*=8.4 Hz, 2H, p-chlorophenyl-$C_{3,5}$-H). Elemental analysis Calcd for $C_{28}H_{20}Cl_2N_2O_3S$ (535.44): C 62.81, H 3.77, N 5.23, S 5.99. Found: C 62.97, H 3.74, N 5.37, S 6.06.

4.1.7.8. 4-(4-Chlorophenyl)-6-(2,5-dimethoxyphenyl)-2-((2-(4-methoxyphenyl)-2-oxoethyl) pyridine-3-carbonitrile (13h)

Yield, 81%; m.p. 210-213°C; IR (KBr, ν cm^{-1}): 2210 (C≡N); 1669 (C=O); 1598 (mixed C=C,C=N); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 3.53, 3.79, 3.89 (3s, each 3H, 3OCH_3); 4.78 (s, 2H, S-CH_2); 6.89-6.94 (m, 4H, Ar-$C_{3,4}$ and p-methoxyphenyl $C_{3,5}$-H); 7.28 (t, *J*=1.8 Hz, 1H, Ar-C_6-H, under $CDCl_3$); 7.50 (d, *J*=8.4 Hz, 2H, Ar-$C_{2',6'}$-H); 7.56 (d, *J*=8.7 Hz, 2H, Ar-$C_{3',5'}$-H);7.77 (s, 1H, pyridine-C_5-H); 8.03 (d, *J*=9 Hz, 2H, p-methoxyphenyl-$C_{2,6}$-H). Elemental analysis Calcd for $C_{29}H_{23}ClN_2O_4S$ (531.02): C 65.59, H 4.37, N 5.28, S 6.04. Found: C 65.73, H 4.44, N 5.37, S 6.11.

4.1.7.9. 6-(2,5-Dimethoxyphenyl)-4-(4-methoxyphenyl)-2-((2-oxopropyl)thio)pyridine-3-carbonitrile (13i)

Yield, 78%; m.p. 151-152°C; IR (KBr, ν cm^{-1}): 2216 (C≡N); 1708 (C=O); 1613 (mixed C=C, C=N);^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.25 (s, 3H, $COCH_3$); 3.80, 3.81.3.85 (3s, each 3H, 3OCH_3); 4.27 (s, 2H, S-CH_2); 7.07 (dd, *J*=9, 3 Hz, 2H, Ar-$C_{3,4}$-H); 7.14 (d, *J*=9 Hz, 2H, Ar–$C_{3',5'}$-H); 7.35 (d, *J*=3 Hz, 1H, Ar-C_6-H); 7.67 (d, *J*=9 Hz, 2H, Ar-$C_{2',6'}$-H); 7.77 (s, 1H, pyridine-C_5-

H).Elemental analysis Calcd for $C_{24}H_{22}N_2O_4S$ (434.51): C 66.34, H 5.10, N 6.45, S 7.38. Found: C 66.51, 5.08, N 6.56, S 7.43.

4.1.7.10. 6-(2,5-Dimethoxyphenyl)-4-(4-methoxyphenyl)-2-((2-oxo-2-phenylethyl)thio)pyridine-3-carbonitrile (13j)

Yield, 92%; m.p. 161-162°C; IR (KBr, ν cm^{-1}): 2213 (C≡N); 1693 (C=O); 1558 (mixed C=C,C=N); ^{1}H-NMR (500 MHz, $CDCl_3$, δ ppm): 3.45, 3.77, 3.82 (3s, each 3H, $3OCH_3$); 4.81 (s, 2H, S-CH_2); 6.87 (s, 2H, Ar-$C_{3,4}$-H); 7.03 (d, *J*=6.9 Hz, 2H, Ar-$C_{3',5'}$-H); 7.20 (s, 1H, Ar-C_6-H); 7.44 (t, 2H, phenyl $C_{3,5}$-H); 7.56-7.60 (m, 3H, phenylC_4- and Ar–$C_{2',6'}$-H); 7.76 (s, 1H, pyridine-C_5-H); 8.03 (d, *J*=7.6 Hz,2H, phenyl $C_{2,6}$-H).Elemental analysis Calcd for $C_{29}H_{24}N_2O_4S$ (496.58): C 70.14, H 4.87, N 5.64, S 6.46. Found: C 70.39, H 4.93, N 5.69, S 6.57.

4.1.7.11. 2-((2-(4-Chlorophenyl)-2-oxoethyl)thio)-6-(2,5-dimethoxyphenyl)-4-(4-methoxyphenyl)pyridine-3-carbonitrile (13k)

Yield, 90%; m.p. 202-206°C; IR (KBr, ν cm^{-1}): 2214 (C≡N); 1687 (C=O); 1561 (mixed C=C,C=N); ^{1}H-NMR (500 MHz, $CDCl_3$, δ ppm): 3.01, 3.77, 3.87 (3s, each 3H, $3OCH_3$); 4.70 (s, 2H, S-CH_2); 6.87 (s, 2H, Ar-$C_{3,4}$-H); 7.04 (d, *J*=8.4 Hz, 2H, Ar–$C_{3',5'}$-H); 7.09 (s, 1H, Ar-C_6-H); 7.38 (d, *J*=8.4 Hz, 2H, p-chlorophenyl $C_{3,5}$-H); 7.58 (d, *J* = 9Hz, Ar–$C_{2',6'}$-H); 7.73 (s, 1H, pyridine-C_5-H); 7.94 (d, *J*=8.4 Hz, 2H, p-chlorophenyl-$C_{2,6}$-H).^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 3.48, 3.74, 3.85 (3s, each 3H, $3OCH_3$); 5.00 (s, 2H, S-CH_2); 6.93 (dd, *J*=9, 3.3 Hz, 1H, Ar-C_4-H); 7.03 (d, *J*=9 Hz, 1H, Ar-C_3-H); 7.06 (d, *J*=3.3 Hz, 1H, Ar-C_6-H); 7.14 (d, *J*=8.7 Hz, 2H, Ar–$C_{3',5'}$-H); 7.56 (d, *J*=9 Hz, 2H, p-chlorophenyl-$C_{3,5}$-H); 7.66 (d, *J*=8.7 Hz, 2H, Ar–$C_{2',6'}$-H); 7.75 (s, 1H, pyridine-C_5-H); 8.04 (d, *J*=9 Hz, 2H, p-chlorophenyl-$C_{2,6}$-H). ^{13}C-NMR (500 MHz, $CDCl_3$, δ ppm): 37.48 (S-CH_2); 55.53, 55.60, 56.29 ($3OCH_3$); 112.74 (pyridine-C_3); 114.55 (Ar- $C_{3',5'}$); 116.17 (C≡N); 121.25 (pyridine-C_5), 116.59, 127.50, 128.50 (Ar-$C_{3,4,6}$); 129.07 (p-chlorophenyl-$C_{3,5}$); 129.35 (Ar-C_1); 129.96 (Ar-$C_{2',6'}$); 130.10 (p-chlorophenyl-$C_{2,6}$); 130.80 (Ar-$C_{1'}$); 134.64 (p-chlorophenyl-C_1); 139.90 (p-chlorophenyl-C_4); 151.65 (pyridine-C_4); 153.28, 153.68 (Ar-C_2 and pyridine-C_6); 157.17 (Ar-C_5); 161.12 (Ar-$C_{4'}$); 161.17 (pyridine-C_2); 192.42 (C=O). EI-MS m/z (% relative abundance): 532 [$M^{+\bullet}$+2] (1.15); 530 [$M^{+\bullet}$] (2.76); 391(30.72); 139 (100); 111 (54.49). Elemental analysis Calcd for $C_{29}H_{23}ClN_2O_4S$ (531.02): C 65.59, H 4.37, N 5.28, S 6.04. Found: C 65.72, H 4.42, N 5.35, S 6.11.

4.1.7.12. 6-(2,5-Dimethoxyphenyl)-4-(4-methoxyphenyl)-2-((2-(4-methoxyphenyl)-2-oxoethyl)thio)pyridine-3-carbonitrile (13l)

Yield, 93%; m.p. 181-184°C; IR (KBr, ν cm^{-1}): 2212 (C≡N); 1674 (C=O); 1599 (mixed C=C,C=N); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 3.55, 3.80, 3.88, 3.89 (4s, each 3H, $4OCH_3$); 4.78 (s, 2H, S-CH_2); 6.89-6.93 (m, 4H, Ar-$C_{3,4}$ and p-methoxy phenyl $C_{3,5}$-H); 7.04 (d, *J*=8.4 Hz, 2H, Ar-$C_{3',5'}$-H); 7.28 (t, *J*=1.5 Hz, 1H, Ar-C_6-H, under $CDCl_3$);7.59 (d, *J*=8.4 Hz, 2H, $C_{2',6'}$-H); 7.78 (s, 1H, pyridine-C_5-H); 8.03 (d, *J*=8.4 Hz, 2H, p-methoxy phenyl $C_{2,6}$-H). Elemental analysis Calcd for $C_{30}H_{26}N_2O_5S$ (526.61): C 68.42, H 4.98, N 5.32, S 6.09. Found: C 68.71, H 5.09, N 5.41, S 6.17.

4.1.8. General experimental procedure for synthesis of [3-Amino-6-(2,5-dimethoxyphenyl)-4-substitutedphenylthieno[2,3-b]pyridin-2 yl](substituted)methanones 14a-l

A solution of 6-(2,5-dimethoxyphenyl)-4-(4-ubstitutedphenyl)-2-(2-substituted-2-oxoethylthio)pyridine-3-carbonitriles **13** (1 mmol) in (5 ml) ethanolic sodium ethoxide [sodium metal, 0.07g (3 mmol), in absolute ethanol (5 ml) till no evolution of hydrogen gas] was heated under reflux for two hours. Then the reaction mixture was cooled and the formed precipitate was filtered, washed with ethanol, air dried and crystallized from toluene.

4.1.8.1. 1-[3-Amino-6-(2,5-dimethoxyphenyl)-4-phenylthieno[2,3-b]pyridin-2-yl]ethan-1-one (14a)

Yield, 74%; m.p.142-143°C; IR (KBr, ν cm^{-1}): 3463, 3321 (NH_2); 1621 (C=O); 1589 (mixed C=C, C=N);^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 2.48 (s, 3H, $COCH_3$); 3.81, 3.87 (2s, each 3H, $2OCH_3$); 6.97 (d,*J*=2.4 Hz, 2H, Ar-$C_{3,4}$-H); 7.50-755 (m, 6H, Ar-$C_{6,2',3',4',5',6'}$-H); 7.73 (s, 1H, pyridine-C_5-H).Elemental analysis Calcd for $C_{23}H_{20}N_2O_3S$ (404.48): C 68.30, H 4.98, N 6.93, S 7.93. Found: C 68.37, H 5.04, N 7.02, S 8.08.

4.1.8.2. [3-Amino-6-(2,5-dimethoxyphenyl)-4-phenylthieno[2,3-b]pyridin-2-yl](phenyl) methanone(14b)

Yield, 90%; m.p.168-169°C; IR (KBr, ν cm^{-1}): 3464, 3284 (NH_2); 1584 (C=O); 1532 (mixed C=C,C=N); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 3.81, 3.86 (2s, each 3H, $2OCH_3$); 6.97 (d, *J*=2.4 Hz, 2H, Ar-$C_{3,4}$-H); 7.48-7.57 (m, 9H, Ar-$C_{6,2',3',4',5',6'}$ and benzoyl $C_{3,4,5}$-H); 7.77 (s, 1H, pyridine-C_5-H); 7.88 (dd, , *J*=7.2, 1.5Hz, 2H, benzoyl $C_{2,6}$-H). Elemental analysis Calcd for$C_{28}H_{22}N_2O_3S$ (466.56): C 72.08, H 4.75, N 6.00, S 6.87. Found: C 72.19, H 4.81, N 6.13, S 7.08.

4.1.8.3. [3-Amino-6-(2,5-dimethoxyphenyl)-4-phenylthieno[2,3-b]pyridin-2-yl](4-chlorophenyl)methanone (14c)

Yield, 80%; m.p.184-186°C; IR (KBr, ν cm^{-1}): 3467, 3293 (NH_2); 1588 (C=O); 1530 (mixed C=C, C=N); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 3.81, 3.86 (2s, each 3H, $2OCH_3$); 6.97 (d, *J*=3 Hz, 2H, Ar-$C_{3,4}$-H); 7.46 (d, *J*=8.4 Hz, 2H, p-chlorophenyl $C_{3,5}$-H); 7.54-7.57 (m, 6H, Ar-$C_{6,2',3',4',5',6'}$-H); 7.78 (s, 1H, pyridine-C_5-H); 7.83 (d, *J*=8.4 Hz, 2H, p-chlorophenyl $C_{2,6}$-H). Elemental analysis Calcd for $C_{28}H_{21}ClN_2O_3S$ (501.00): C 67.13, H 4.23, N 5.59, S 6.40. Found: C 67.29, H 4.26, N 5.64, S 6.48

4.1.8.4. [3-Amino-6-(2,5-dimethoxyphenyl)-4-phenylthieno[2,3-b]pyridin-2-yl](4-methoxyphenyl)methanone (14d)

Yield, 70%; m.p.181-182 °C; IR (KBr, ν cm^{-1}): 3472, 3301 (NH_2); 1586 (C=O); 1528 (mixed C=C, C=N); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 3.81, 3.86, 3.89 (3s, each 3H, $3OCH_3$); 6.81 (brs, 2H, NH_2, D_2O-exchangeable); 6.96-7.00 (m, 4H, Ar-$C_{3,4}$ and p-methoxyphenyl $C_{3,5}$-H); 7.54-7.57 (m, 6H, Ar-$C_{6,2',3',4',5',6'}$-H); 7.76 (s, 1H, pyridine-C_5-H); 7.92 (d, *J*=9 Hz, 2H, p-methoxyphenyl $C_{2,6}$-H). Elemental analysis Calcd for $C_{29}H_{24}N_2O_4S$ (496.58): C 70.14, H 4.87, N 5.64, S 6.46. Found: C 70.29, H 4.91, N 5.79, S 6.56.

4.1.8.5. 1-[3-Amino-4-(4-chlorophenyl)-6-(2,5-dimethoxyphenyl)thieno[2,3-b]pyridin-2-yl]ethan-1-one (14e)

Yield, 86%; m.p.191-192°C; IR (KBr, ν cm^{-1}): 3484, 3328 (NH_2); 1617 (C=O); 1580 (mixed C=C, C=N); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.39 (s, 3H, $COCH_3$); 3.77, 3.78 (2s, each 3H, $2OCH_3$); 6.59 (brs, 2H, NH_2, D_2O-exchangeable); 7.05 (dd, *J*=9, 3 Hz, 1H, Ar-C_4-H); 7.12 (d, *J*=9 Hz, 1H, Ar-C_3-H); 7.42 (d, *J*=3 Hz, 1H, Ar-C_6-H); 7.58 (d, *J*=8.7 Hz, 2H, Ar-$C_{2',6'}$-H); 7.64 (d, *J*=8.7 Hz, 2H, Ar-$C_{3',5'}$-H); 7.70 (s,1H, pyridine-C_5-H).Elemental analysis Calcd for $C_{23}H_{19}ClN_2O_3S$ (438.93):C 62.94, H 4.36, N 6.38, S 7.30. Found: C 63.05, H 4.43, N 6.47, S 7.37.

4.1.8.6. [3-Amino-4-(4-chlorophenyl)-6-(2,5-dimethoxyphenyl)thieno[2,3-b]pyridin-2-yl](phenyl) methanone(14f)

Yield, 87%; m.p.180-183°C; IR (KBr, ν cm^{-1}): 3473, 3254 (NH_2); 1582 (C=O); 1529 (mixed C=C, C=N); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 3.76, 3.78 (2s, each 3H, $2OCH_3$); 7.01 (brs, 2H, NH_2, D_2O-exchangeable); 7.05 (dd, *J*=9, 3 Hz, 1H, Ar-C_4-H); 7.13 (d, *J*=9 Hz, 1H, Ar-C_3-H); 7.41 (d, *J*=3 Hz, 1H, Ar-C_6-H); 7.52-7.59 (m, 4H, Ar- $C_{2',3',5',6'}$-H); 7.62-7.69 (m, 3H, benzoyl $C_{3,4,5}$-H); 7.74 (s, 1H, pyridine-C_5-H); 7.79 (dd, *J*=7.8, 1.5Hz, 2H, benzoyl $C_{2,6}$-H). Elemental analysis Calcd for $C_{28}H_{21}ClN_2O_3S$ (501.00): C 67.13, H 4.23, N 5.59, S 6.40. Found: C 67.19, H 4.28, N 5.67, S 6.43.

4.1.8.7. [3-Amino-4-(4-chlorophenyl)-6-(2,5-dimethoxyphenyl)thieno[2,3-b]pyridin-2-yl](4-chlorophenyl)methanone (14g)

Yield, 93%; m.p.207-210°C; IR (KBr, ν cm^{-1}): 3490, 3313 (NH_2), 1586 (C=O), 1528 (mixed C=C, C=N), ^{1}H-NMR (500 MHz, $CDCl_3$, δ ppm): 3.81, 3.82 (2s, each 3H, $2OCH_3$); 6.86 (brs, 2H, NH_2, D_2O-exchangeable); 6.95 (d, *J*=9 Hz, 1H, Ar-C_3-H); 6.99 (dd, *J*=9, 3 Hz, 1H, Ar-C_4-H); 7.46 (d, *J*=8.4 Hz, 2H, Ar-$C_{2',6'}$-H); 7.49 (d, *J*=8.4 Hz, 2H, Ar-$C_{3',5'}$-H); 7.53 (d, *J*=3 Hz, 1H, Ar-C_6-H); 7.57 (d, *J*=8.4 Hz, 2H, p-chlorophenyl $C_{3,5}$-H); 7.76 (s, 1H, pyridine-C_5-H); 7.81 (d, *J*=8.4 Hz, 2H, p-chlorophenyl-$C_{2,6}$-H). Elemental analysis Calcd for $C_{28}H_{20}Cl_2N_2O_3S$ (535.44): C 62.81, H 3.77, N 5.23, S 5.99. Found: C 62.89, H 3.78, N 5.34, S 6.08.

4.1.8.8. [3-Amino-4-(4-chlorophenyl)-6-(2,5-dimethoxyphenyl)thieno[2,3-b]pyridin-2-yl](4-methoxyphenyl)methanone (14h)

Yield, 90%; m.p.189-192°C; IR (KBr, ν cm^{-1}): 3482, 3293 (NH_2), 1599 (C=O), 1589 (mixed C=C, C=N); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 3.82, 3.86, 3.90 (3s, each 3H, $3OCH_3$); 6.76 (brs, 2H, NH_2, D_2O-exchangeable); 6.97-7.00 (m, 4H, Ar-$C_{3,4}$ and p-methoxy phenyl $C_{3,5}$-H); 7.49 (d, *J*=8.7 Hz, 2H, Ar-$C_{2',6'}$-H); 7.55-7.58 (m, 3H, Ar-$C_{6,3',5'}$-H); 7.74 (s, 1H, pyridine- C_5-H); 7.92 (d, *J*=8.7 Hz, 2H, p-methoxyphenyl-$C_{2,6}$-H). Elemental analysis Calcd for$C_{29}H_{23}ClN_2O_4S$ (531.02): C 65.59, H 4.37, N 5.28, S 6.04. Found: C 65.71, H 4.42, N 5.39, S 6.12.

4.1.8.9. 1-[3-Amino-6-(2,5-dimethoxyphenyl)-4-(4-methoxyphenyl)thieno[2,3-b]pyridin-2-yl]ethan-1-one (14i)

Yield, 75%; m.p.176-177°C; IR (KBr, ν cm^{-1}): 3478, 3324 (NH_2); 1612 (C=O); 1577 (mixed C=C, C=N); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.39 (s, 3H, $COCH_3$); 3.77, 3.78, 3.85 (3s, each 3H, $3OCH_3$); 6.62 (brs, 2H, NH_2, D_2O-exchangeable); 7.05 (dd, *J*=9, 3 Hz, 1H, Ar-C_4-H); 7.12-7.16 (m, 3H, Ar-$C_{3,3',5'}$-H); 7.41 (d, *J*=3 Hz, 1H, Ar-C_6-H); 7.31 (d, *J*=8.7 Hz, 2H, Ar-$C_{2',6'}$-H); 7.68 (s, 1H, pyridine-C_5-H).Elemental analysis Calcd for $C_{24}H_{22}N_2O_4S$ (434.51):C 66.34, H 5.10, N 6.45, S 7.38. Found: C 66.48, H 5.13, N 6.52, S 7.45.

4.1.8.10. [3-Amino-6-(2,5-dimethoxyphenyl)-4-(4-methoxyphenyl)thieno[2,3-b]pyridin-2-yl](phenyl)methanone (14j)

Yield, 90%; m.p.162-164°C; IR (KBr, ν cm^{-1}): 3478, 3319 (NH_2), 1605 (C=O), 1587 (mixed C=C, C=N); ^{1}H-NMR (500 MHz, $CDCl_3$, δ ppm): 3.80,

3.85, 3.91 (3s, each 3H, $3OCH_3$); 6.79 (brs, 2H, NH_2, D_2O-exchangeable); 6.93-6.98 (m, 2H, Ar-$C_{3,4}$-H); 7.09 (d, *J* = 8.4Hz, 2H, Ar-$C_{3',5'}$-H); 7.46-7.51 (m, 6H, Ar-$C_{6,2',6'}$ and benzoyl $C_{3,4,5}$-H); 7.74 (s, 1H, pyridine- C_5-H); 7.85 (d, *J*=8.4 Hz, 2H, benzoyl $C_{2,6}$-H). Elemental analysis Calcd for $C_{29}H_{24}N_2O_4S$ (496.58): C 70.14, H 4.87, N 5.64, S 6.46. Found: C 70.34, H 4.90, N 5.73, S 6.53.

4.1.8.11. [3-Amino-6-(2,5-dimethoxyphenyl)-4-(4-methoxyphenyl)thieno[2,3-b]pyridin-2-yl](4-chlorophenyl)methanone (14k)

Yield, 87%; m.p.195-196°C; IR (KBr, ν cm^{-1}): 3475, 3325 (NH_2), 1589 (C=O), 1532 (mixed C=C, C=N); ^{1}H-NMR (500 MHz, $CDCl_3$, δ ppm): 3.81, 3.86, 3.91 (3s, each 3H, $3OCH_3$); 6.86 (brs, 2H, NH_2, D_2O-exchangeable); 6.94 (d, *J*=8.4 Hz, 1H, Ar-C_3-H); 6.98 (dd, *J*=8.4, 3 Hz, 1H, Ar-C_4-H); 7.09 (d, *J*=7.6 Hz, 2H, Ar-$C_{3',5'}$-H); 7.44-7.48 (m, 4H, Ar-$C_{2',6'}$ and p-chlorophenyl-$C_{3,5}$-H); 7.51 (d, *J*=3 Hz,1H, Ar-C_6-H); 7.75 (s, 1H, pyridine- C_5-H); 7.81 (d, *J*=7.6 Hz, 2H, p-chlorophenyl-$C_{2,6}$-H). ^{13}C-NMR (500 MHz, $CDCl_3$, δ ppm): 55.58, 56.10, 56.52 ($3OCH_3$); 105.36 (thiophene-C_2); 113.35, 115.94, 117.72 (Ar-$C_{3,4,6}$); 114.70 (Ar-$C_{3',5'}$); 120.67 (pyridine-C_5); 123.48 (Ar-$C_{1'}$); 125.45 (Ar-C_1); 126.20 (thiophene-C_3); 128.78 (p-chlorophenyl-$C_{3,5}$); 129.43 (Ar-$C_{2',6'}$); 130.00 (p-chlorophenyl-$C_{2,6}$); 131.22 (p-chlorophenyl-C_1); 132.88 (pyridine-C_3); 137.48 (p-chlorophenyl-C_4); 139.27 (pyridine-C_4); 148.62 (pyridine-C_6); 150.85 (Ar-C_2); 151.75 (Ar-C_5); 154.10 (pyridine-C_2); 160.74 (Ar-$C_{4'}$); 189.20 (C=O). EI-MS m/z (% relative abundance): 532 [$M^{+\bullet}$+2] (28.45); 531 (32.55); 530 [$M^{+\bullet}$] (62.42); 139(96.28); 111 (70.27); 91 (100). Elemental analysis Calcd for $C_{29}H_{23}ClN_2O_4S$ (531.02): C 65.59, H 4.37, N 5.28, S 6.04. Found: C 65.73, H 4.41, N 5.39, S 6.11.

4.1.8.12. [3-Amino-6-(2,5-dimethoxyphenyl)-4-(4-methoxyphenyl)thieno[2,3-b]pyridin-2-yl](4-methoxyphenyl)methanone (14l)

Yield, 63%; m.p. 197-199 °C; IR (KBr, ν cm^{-1}): 3471, 3292 (NH_2), 1590 (C=O), 1537 (mixed C=C, C=N); ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm): 3.81, 3.86, 3.89, 3.92 (4s, each 3H, $4OCH_3$); 6.89 (brs, 2H, NH_2, D_2O-exchangeable); 6.96 (d, *J*=1.2 Hz, 2H, Ar-$C_{3,4}$-H); 6.99 (d, *J*=8.7 Hz, 2H, Ar-$C_{3',5'}$-H); 7.10 (d, *J*=8.4 Hz, 2H, p-methoxyphenyl-$C_{3,5}$-H); 7.47 (d, *J*=8.7 Hz, 2H, Ar-$C_{2',6'}$-H); 7.54 (t, *J*=1.2 Hz, 1H, Ar-C_6-H); 7.73 (s, 1H, pyridine-C_5-H); 7.92 (d, *J*=8.4 Hz, 2H, p-methoxyphenyl-$C_{2,6}$-H). Elemental analysis Calcd for $C_{30}H_{26}N_2O_5S$ (526.61): C 68.42, H 4.98, N 5.32, S 6.09. Found: C 68.69, H 5.04, N 5.39, S 6.18.

4.1.9. General experimental procedure for synthesis of 2-(Alkylthio)-6-(2,5-dimethoxyphenyl)-4-(4-substituted phenyl)pyridine-3-carbonitriles 15a-l

A mixture of 6-(2,5-dimethoxyphenyl)-4-(4-substitutedphenyl)-2-thioxo-1,2-dihydropyridine-3-carbonitrile **12a-c** (1 mmol), alkyl halides (1 mmol) and anhydrous potassium carbonate(0.20 g, 1.5 mmol) in dry acetone (10 ml) was heated under reflux for 4 hours then cooled and triturated with water till complete precipitation of the product. The green precipitate was filtered, washed with water, air dried and crystallized from toluene.

4.1.9.1. 6-(2,5-Dimethoxyphenyl)-2-(methylthio)-4-(phenyl)pyridine-3-carbonitrile (15a)

Yield, 90%; m.p. 175-176°C; IR (KBr, ν cm^{-1}): 2209 (C≡N); 1560 (mixed C=C, C=N); ^{1}H-NMR (500 MHz, $CDCl_3$, δ ppm): 2.73 (s, 3H, S-CH_3); 3.84 (s, 6H, 2OCH_3); 6.96 (d, *J*=9 Hz, 1H, Ar-C_3-H); 7.00 (dd, *J*=9, 3 Hz, 1H, Ar-C_4-H); 7.48-7.54 (m, 3H, Ar-$C_{3',4',5'}$-H); 7.62 (dd, *J*=6, 1.5 Hz, 2H, Ar–$C_{2',6'}$-H); 7.66 (d, *J*=3 Hz, 1H, Ar-C_6-H); 7.87 (s, 1H, pyridine-C_5-H). ^{13}C-NMR (500 MHz, $CDCl_3$, δ ppm): 13.81 (S-CH_3); 55.89, 56.40 (2OCH_3); 113.14, 116.17, 116.40 (Ar-$C_{3,4,6}$); 116.80 (pyridine-C_3); 120.66 (pyridine-C_5); 118.02 (C≡N); 127.64 (Ar-C_1); 128.63 (Ar-$C_{2',6'}$); 129.03 (Ar-$C_{3',5'}$); 129.91 (Ar-$C_{4'}$); 136.60 (Ar-$C_{1'}$); 152.19 (pyridine-C_4); 153.45 (pyridine-C_6); 153.87 (Ar-C_2); 156.96 (Ar-C_5); 163.65 (pyridine-C_2). EI-MS m/z (% relative abundance): 362 [$M^{+\bullet}$] (62.39); 361 [$M^{+\bullet}$-1] (72.74); 331(40.68); 227 (100); 173 (38.90); 166 (44.49); 138 (49.38).Elemental analysis Calcd for $C_{21}H_{18}N_2O_2S$ (362.45): C 69.59, H 5.01, N 7.73, S 8.85. Found: C 69.74, H 5.14, N 7.89, S 9.04.

4.1.9.2. 2-(Ethylthio)-6-(2,5-dimethoxyphenyl)-4-(phenyl)pyridine-3-carbonitrile (15b)

Yield, 90%; m.p. 157-159°C; IR (KBr, ν cm^{-1}):2218 (C≡N); 1560 (mixed C=N, C=C); ^{1}H-NMR (500 MHz, $CDCl_3$, δ ppm): 1.48 (t, *J*=7.6 Hz, 3H, S-$CH_2C\underline{H_3}$); 3.37 (q, *J*=7.6 Hz, 2H, S-$C\underline{H_2}CH_3$); 3.84 (s, 6H, 2OCH_3); 6.96 (d, *J*=8.4 Hz, 1H, Ar-C_3-H); 6.99 (dd, *J*=9, 3 Hz, 1H, Ar-C_4-H); 7.48-7.54 (m, 3H, Ar-$C_{3',4',5'}$-H); 7.61-7.63 (m, 2H, Ar-$C_{2',6'}$-H); 7.64 (d, *J*=3 Hz, 1H, Ar-C_6); 7.87 (s, 1H, pyridine-C_5-H).Elemental analysis Calcd for $C_{22}H_{20}N_2O_2S$ (376.47): C 70.19, H 5.35, N 7.44, S 8.52. Found: C 70.28, H 5.41, N 7.53, S 8.66.

4.1.9.3. 2-(Allylthio)-6-(2,5-dimethoxyphenyl)-4-(phenyl)pyridine-3-carbonitrile(15c)

Yield, 83.7%; m.p. 159-161°C; IR (KBr, ν cm^{-1}):2217 (C≡N); 1557 (mixed C=N, C=C); ^{1}H-NMR(500 MHz, $CDCl_3$, δ ppm): 3.83, 3.84 (2s, each

3H, 2OCH_3); 4.03 (d, *J*=6.1 Hz, 2H, S-CH_2); 5.16 (d, *J*=9.9 Hz, 1H, S-CH_2-CH=C$\underline{H}_{cis}$); 5.36 (dd, *J*=16.8, 1.5 Hz 1H, S-CH_2CH=C$\underline{H}_{trans}$); 6.03-6.09 (m, 1H, S-CH_2-C$\underline{H}$=CH_2); 6.96 (d, *J*=9 Hz, 1H, Ar-C_3-H); 6.99 (dd, *J*=9, 3 Hz, 1H, Ar-C_4-H); 7.48-7.54 (m, 3H, Ar-$C_{3',4',5'}$-H); 7.61-7.63 (m, 3H, Ar-$C_{6,2',6'}$-H); 7.88 (s, 1H, pyridine-C_5-H). Elemental analysis Calcd for $C_{23}H_{20}N_2O_2S$ (388.49): C 71.11, H 5.19, N 7.21, S 8.25. Found: C 71.37, H 5.22, N 7.39, S 8.29.

4.1.9.4. 2-(Benzylthio)-6-(2,5-dimethoxyphenyl)-4-(phenyl)pyridine-3-carbonitrile (15d)

Yield, 92.6%; m.p. 162-163°C; IR (KBr, ν cm^{-1}):2213 (C≡N); 1558 (mixed C=C,C=N); 1497 (C-S-C); ^{1}H-NMR(500 MHz, $CDCl_3$, δ ppm): 3.74, 3.84 (2s, each 3H, 2OCH_3); 4.64 (s, 2H, S-CH_2); 6.96 (d, *J*=9 Hz, 1H, Ar-C_3-H); 6.99 (dd, *J*=9, 3 Hz, 1H, Ar-C_4-H); 7.25-7.26 (m, 1H, phenyl-C_4-H); 7.29-7.30 (m, 2H, phenyl-$C_{3,5}$-H); 7.45 (d, *J*=7.6 Hz, 2H, phenyl-$C_{2,6}$-H); 7.50-7.53 (m, 3H, Ar-$C_{3',4',5'}$-H); 7.57 (d, *J*=2.3 Hz, 1H, Ar-C_6-H); 7.62 (dd, *J*=7.6, 1.5 Hz, 2H, Ar-$C_{2',6'}$-H); 7.87 (s, 1H, pyridine-C_5-H). Elemental analysis Calcd for $C_{27}H_{22}N_2O_2S$ (438.55): C 73.95, H 5.06, N 6.39, S 7.31. Found: C 74.13, H 5.11, N 6.48, S 7.39.

4.1.9.5. 4-(4-Chlorophenyl)-6-(2,5-dimethoxyphenyl)-2-(methylthio)pyridine-3-carbonitrile (15e)

Yield, 87%; m.p.189-191°C; IR (KBr, ν cm^{-1}): 2213 (C≡N); 1556 (mixed C=C,C=N); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.71 (s, 3H, S-CH_3); 3.78, 3.81 (2s, each 3H, 2OCH_3); 7.09 (dd, *J*=9, 3 Hz, 1H, Ar-C_4-H); 7.16 (d, *J*=9 Hz, 1H, Ar-C_3-H); 7.59 (d, *J*=3 Hz, 1H, Ar-C_6-H); 7.66 (d, *J*=8.7 Hz, 2H, Ar–$C_{2',6'}$-H); 7.72 (d, *J*=8.7 Hz, 2H, Ar-$C_{3',5'}$-H); 7.85 (s, 1H, pyridine-C_5-H). Elemental analysis Calcd for $C_{21}H_{17}ClN_2O_2S$ (396.89): C 63.55, H 4.32, N 7.06, S 8.08. Found: C 63.64, H 4.37, N 7.18, S 8.19.

4.1.9.6. 4-(4-Chlorophenyl)-6-(2,5-dimethoxyphenyl)-2-(ethylthio)pyridine-3-carbonitrile (15f)

Yield, 93%; m.p. 154-155°C; IR (KBr, ν cm^{-1}): 2218 (C≡N); 1563 (mixed C=C,C=N); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 1.41 (t, *J*=7.2 Hz, 3H, S-CH_2C$\underline{H}_3$); 3.35 (q, *J*=7.2 Hz, 2H, S-C$\underline{H}_2$$CH_3$); 3.78, 3.81 (2s, each 3H, 2OCH_3); 7.09 (dd, *J*=9, 3 Hz, 1H, Ar-C_4-H); 7.16 (d, *J*=9 Hz, 1H, Ar-C_3-H); 7.55 (d, *J*=3 Hz, 1H, Ar-C_6-H); 7.66 (d, *J*=8.7 Hz, 2H, Ar–$C_{2',6'}$-H); 7.72 (d, *J*=8.4Hz, 2H, Ar-$C_{3',5'}$-H); 7.85 (s, 1H, pyridine-C_5-H). Elemental analysis Calcd for $C_{22}H_{19}ClN_2O_2S$ (410.92): C 64.31, H 4.66, N 6.82, S 7.80. Found: C 64.41, H 4.73, N 6.89, S 7.87.

4.1.9.7. 2-(Allylthio)-4-(4-chlorophenyl)-6-(2,5-dimethoxyphenyl)pyridine-3-carbonitrile (15g)

Yield, 82%; m.p.163-165°C; IR (KBr, ν cm^{-1}): 2216 (C≡N); 1561 (mixed C=C,C=N); ^{1}H-NMR(300 MHz, DMSO-d_6, δ ppm): 3.78, 3.81 (2s, each 3H, 2OCH_3); 4.05 (d, *J*=6.6 Hz, 2H, S-CH_2-); 5.16 (d, *J*=9.9 Hz, 1H, S-CH_2-CH=C$\underline{H}_{cis}$); 5.36 (d, *J*=17.1 Hz, 1H, S-CH_2CH=C$\underline{H}_{trans}$); 6.00-6.06 (m, 1H, S-CH_2-C$\underline{H}$=CH_2); 7.09 (dd, *J*=9, 3 Hz, 1H, Ar-C_4-H); 7.16 (d, *J*=9.3 Hz, 1H, Ar-C_3-H); 7.56 (d, *J* = 3Hz, 1H, Ar-C_6-H); 7.66 (d, *J*=8.7Hz, 2H, Ar–$C_{2',6'}$-H); 7.72 (d, *J*=8.4 Hz, 2H, Ar-$C_{3',5'}$-H); 7.86 (s, 1H, pyridine-C_5-H). Elemental analysis Calcd for $C_{23}H_{19}ClN_2O_2S$ (422.93): C 65.32, H 4.53, N 6.62, S 7.58. Found: C 65.47, H 4.50, N 6.71, S 7.55.

4.1.9.8. 2-(Benzylthio)-4-(4-chlorophenyl)-6-(2,5-dimethoxyphenyl)pyridine-3-carbonitrile (15h)

Yield, 91.7%; m.p.178-179°C; IR (KBr, ν cm^{-1}): 2213 (C≡N); 1560 (mixed C=C,C=N); ^{1}H-NMR(300 MHz, DMSO-d_6, δ ppm): 3.71, 3.81 (2s, each 3H, 2OCH_3); 4.67 (s, 2H, S-CH_2); 7.09 (dd, *J*=9, 3 Hz, 1H, Ar-C_4-H); 7.16 (d, *J*=9 Hz, 1H, Ar-C_3-H); 7.26-7.35 (m, 3H, benzyl $C_{3,4,5}$-H); 7.46 (d, *J*=6.6 Hz, 2H, benzyl $C_{2,6}$-H); 7.52 (d, *J*=3 Hz, 1H, Ar-C_6-H); 7.65 (d, *J*=8.7 Hz, 2H, Ar–$C_{2',6'}$-H); 7.72 (d, *J*=8.4 Hz, 2H, Ar-$C_{3',5'}$-H); 7.86 (s, 1H, pyridine-C_5-H). Elemental analysis Calcd for $C_{27}H_{21}ClN_2O_2S$ (472.99): C 68.56, H 4.48, N 5.92, S 6.78. Found: C 68.72, H 4.46, N 5.97, S 6.86.

4.1.9.9. 6-(2,5-Dimethoxyphenyl)-4-(4-methoxyphenyl)-2-(methylthio)pyridine-3-carbonitrile (15i)

Yield, 67%; m.p. 172-173°C; IR (KBr, ν cm^{-1}):2209 (C≡N); 1558 (mixed C=C,C=N); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 2.70 (s, 3H, S-CH_3); 3.78, 3.82, 3.85 (3s, each 3H, 3OCH_3); 7.08 (dd, *J*=9, 3 Hz, 1H, Ar-C_4-H); 7.12-7.17 (m, 3H, Ar–$C_{3,3',5'}$-H); 7.59 (d, *J*=3 Hz, 1H, Ar-C_6-H); 7.66 (d, *J*=8.7 Hz, 2H, Ar–$C_{2',6'}$-H); 7.83 (s, 1H, pyridine-C_5-H). Elemental analysis Calcd for$C_{22}H_{20}N_2O_3S$ (392.47): C 67.33, H 5.14, N 7.14, S 8.17. Found: C 67.46, H 5.21, N 7.22, S 8.24.

4.1.9.10. 2-(Ethylthio)-6-(2,5-dimethoxyphenyl)-4-(4-methoxyphenyl)pyridine-3-carbonitrile (15j)

Yield, 83.7%; m.p.129-130°C; IR (KBr, ν cm^{-1}):2216 (C≡N); 1560 (mixed C=C,C=N); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 1.41 (t, *J*=7.2 Hz, 3H, S-CH_2CH_3); 3.33 (q, *J*=7.2 Hz, 2H, S-CH_2CH_3); 3.78, 3.82, 3.85 (3s, each 3H, 3OCH_3); 7.08 (dd, *J*=9, 3 Hz, 1H, Ar-C_4-H); 7.11-7.17 (m, 3H, Ar–$C_{3,3',5'}$-

H); 7.55 (d, *J*=3.3 Hz, 1H, Ar-C_6-H); 7.65 (d, *J*=8.7 Hz, 2H, Ar–$C_{2',6'}$-H); 7.82 (s, 1H, pyridine-C_5-H). Elemental analysis Calcd for $C_{23}H_{22}N_2O_3S$ (406.50): C 67.96, H 5.46, N 6.89, S 7.89. Found: C 68.12, H 5.52, N 6.96, S 7.92.

4.1.9.11. 2-(Allylthio)-6-(2,5-dimethoxyphenyl)-4-(4-methoxyphenyl)pyridine-3-carbonitrile (15k)

Yield, 66% ; m.p.150-152°C; IR (KBr, ν cm^{-1}): 2214 (C≡N); 1559 (mixed C=C,C=N); ^{1}H-NMR(300 MHz, DMSO-d_6, δ ppm): 3.77, 3.82, 3.85 (3s, each 3H, 3OCH_3); 4.04 (d, *J*=6.6 Hz, 2H, S-CH_2); 5.16 (d, *J*=9.9 Hz, 1H, S-CH_2-CH=C$\underline{H}_{cis}$); 5.38 (d, *J*=16.8 Hz, 1H, S-CH_2CH=C$\underline{H}_{trans}$); 5.98-6.15 (m, 1H, S-CH_2-C$\underline{H}$=CH_2); 7.08 (dd, *J*=9, 3 Hz, 1H, Ar-C_4-H); 7.13-7.17 (m, 3H, Ar–$C_{3,3',5'}$-H); 7.55 (d, *J*=3.3 Hz, 1H, Ar-C_6-H); 7.66 (d, *J*=8.7 Hz, 2H, Ar-$C_{2',6'}$-H); 7.84 (s, 1H, pyridine-C_5-H). Elemental analysis Calcd for $C_{24}H_{22}N_2O_3S$ (418.51): C 68.88, H 5.30, N 6.69, S 7.66. Found: C 69.04, H 5.34, N 6.07, S 7.74.

4.1.9.12. 2-(Benzylthio)-6-(2,5-dimethoxyphenyl)-4-(4-methoxyphenyl)pyridine-3-carbonitrile (15l)

Yield, 81%; m.p. 177-178°C; IR (KBr, ν cm^{-1}): 2210 (C≡N); 1558 (mixed C=C,C=N); ^{1}H-NMR(300 MHz, DMSO-d_6, δ ppm): 3.71, 3.81, 3.84 (3s, each 3H, 3OCH_3); 4.65 (s, 2H, S-C$\underline{H}_2$); 7.06-7.17 (m, 4H, Ar-$C_{3,4,3',5'}$-H); 7.26-7.34 (m, 3H, benzyl $C_{3,4,5}$-H); 7.46 (d, *J*= 7.8 Hz, 2H, benzyl $C_{2,6}$-H); 7.51 (d, *J*=3 Hz, 1H, Ar-C_6-H); 7.66 (d, *J*=8.7 Hz, 2H, Ar-$C_{2',6'}$-H); 7.83 (s, 1H, pyridine-C_5-H). Elemental analysis Calcd for $C_{28}H_{24}N_2O_3S$ (468.57): C 71.77, H 5.16, N 5.98, S 6.84. Found: C 71.89, H 5.19, N 6.53, S 7.43.

4.1.10. General experimental procedure for synthesis of Ethyl 2-{[3-cyano-6-(2,5-dimethoxyphenyl)-4-(4-substitutedphenyl)- pyridine-2-yl]thio}acetate 15m,n

A mixture of 6-(2,5-dimethoxyphenyl)-4-(4-substitutedphenyl)-2-thioxo-1,2-dihydro pyridine-3-carbonitrile **12b,c** (1 mmol), ethyl bromoacetate (0.11 ml, 1 mmol) and anhydrous potassium carbonate (0.20 g, 1.5 mmol) in dry acetone (5 ml) was refluxed for 5 hours. The reaction mixture was then cooled and triturated with water till complete precipitation. The green precipitate was filtered, washed with water, air dried and crystallized from ethanol.

4.1.10.1. Ethyl 2-{[4-(4-chlorophenyl)-3-cyano-6-(2,5-dimethoxyphenyl)pyridin-2-yl]thio}acetate (15m)

Yield, 90%; m.p.138-139°C; IR (KBr, ν cm^{-1}): 2215 (C≡N), 1742 (C=O), 1562 (mixed C=C, C=N); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 1.08 (t, *J*=7.2 Hz, 3H, CH_2-C$\underline{H}_3$); 3.80 (s, 6H, 2OCH_3); 4.06 (q, *J*=7.2 Hz, 2H, C$\underline{H}_2$-

CH_3); 4.23 (s, 2H, S-CH_2); 7.09 (dd, *J*=9, 3 Hz, 1H, Ar-C_4-H); 7.14 (d, *J*=9 Hz, 1H, Ar-C_3-H); 7.50 (d, *J*=3 Hz, 1H, Ar-C_6-H); 7.66 (d, *J*=8.7 Hz, 2H, Ar-$C_{2',6'}$-H); 7.73 (d, *J*=8.7 Hz, 2H, Ar-$C_{3',5'}$-H); 7.89 (s, 1H, pyridine-C_5-H).^{13}C-NMR (300 MHz, DMSO, δ ppm): 13.62 (CH_3); 32.48 (S-CH_2); 55.42, 56.17 (2OCH_3); 61.08 ($C\underline{H}_2$-CH_3); 102.03 (pyridine-C_3); 113.50, 115.29, 116.08 (Ar-$C_{3,4,6}$); 116.87 (C≡N); 120.44 (pyridine-C_5); 126.08 (Ar-C_1); 129.02 (Ar-$C_{2',6'}$); 130.29 (Ar-$C_{3',5'}$); 134.46 (Ar-C_4'); 135.12 (Ar-C_1'); 151.71 (Ar-C_2); 151.79 (Ar-C_5); 153.22 (pyridine-C_4); 156.71 (pyridine-C_6); 161.02 (pyridine-C_2); 168.42 (C=O). Elemental analysis Calcd for $C_{24}H_{21}ClN_2O_4S$ (468.95): C 61.47, H 4.51, N 5.97, S 6.84. Found: C 61.62, H 4.58, N 6.04, S 6.76.

4.1.10.2. Ethyl 2-{[3-cyano-6-(2,5-dimethoxyphenyl)-4-(4-methoxyphenyl)pyridin-2-yl]thio} (acetate (15n)

Yield, 85%; m.p.120°C; IR (KBr, ν cm^{-1}): 2206 (C≡N), 1747 (C=O), 1459 (mixed C=C, C=N); ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 1.07 (t, *J*=7.2 Hz, 3H, CH_2-$C\underline{H}_3$); 3.79, 3.80, 3.84 (3s, each3H, 3OCH_3); 4.05 (q, *J*=7.5 Hz, 2H, $C\underline{H}_2$-CH_3); 4.21 (s, 2H, S-CH_2); 7.02-7.15 (m, 4H, Ar-$C_{3,4,}$ $C_{3',5'}$-H); 7.49 (brs, 1H, Ar-C_6-H); 7.67 (d, *J*=7.8 Hz, 2H, Ar-$C_{2',6'}$-H); 7.86 (s, 1H, pyridine-C_5-H).Elemental analysis Calcd for$C_{25}H_{24}N_2O_5S$ (464.54): C 64.64, H 5.21, N 6.03, S 6.90. Found: C 64.78, H 5.29, N 6.11, S 6.95

4.2. Biological Evaluation

4.2.1. In-vitro anticancer screening procedure (supplementary data page 11)

Thirty-seven compounds were selected by NCI to be tested initially at a single dose (10μM) in the full NCI 60 cell line panel according to reported procedure [22-24]. Compound **5b** showed potent anticancer activity and was tested twice in the five-dose assay and was sent to NCI biological evaluation committee and still under consideration for further testing.

4.2.2 PIM-1 kinase inhibitory Assays (supplementary data page 12)

The most active five compounds were evaluated *in vitro* for their ability to inhibit PIM-1 kinase enzyme using . HTS scan PIM -1 kinase assay Kit # 7573. The test compounds were dissolved in DMSO and four concentrations were prepared (25, 5, 1, 0.2 μM) according to reported method [25].

4.3. Molecular modeling

4.3.1. *In silico* prediction of physicochemical properties and pharmacokinetic profile procedure

In the present investigation, compound **5b** was subjected to molecular properties and bioactivity prediction by Molinspirationonline property calculation toolkit,[26] drug-likeness and solubility parameter calculation by Mol-Soft software,[27] ADME profiling by PreADMET calculator[28] and toxicity-risk assessment by Osiris property explorer [29] to filter and analyze their overall potential to qualify for a drug.

4.3.2. Molecular docking study

The molecular modeling study was performed using the Molecular Operating Environment (**MOE 2016.08**) software[30]. the three dimensional structures and conformations of the enzymes were acquired from the Protein Data Bank (PDB) website. PIM-1 kinase (PDB ID code 2obj). The ligand molecules were constructed in MOE using the builder module, and collected in a database. The database was prepared by using the option "Protonate 3D" to add hydrogens, calculate partial charges and minimize energy (using Force Field MMFF94x).In addition, the protein structure was prepared by deleting the repeated chains, water molecules and any surfactants, hydrogens were also added to the atom of the receptor and the partial charges were calculated. MOE was used to calculate the best score between the ligands and the enzymes' binding sites. Scoring was determined using alpha HB as scoring function. The resulted database contained the score between the ligands' conformers and the enzyme binding sites in kcal/mol. To confirm the credibility of docking results, self-docking was used to validate the adopted docking protocol in which co-crystallized ligand (VRV) was drawn in MOE, prepared as the targeted compounds (hydrogens addition, partial charges calculation and energy minimization), and then docked into the active site of the protein using the same protocol. The top ranked pose exhibited Root mean square deviation (RMSD) value of less than 1.5 Å from the experimental crystal structure. This result indicated that Molecular Operating Environment (MOE) docking can reliably predict docking pose for the studied compounds to enzyme. It was reported that values less than 1.5 or 2 Å were a sign of a successful and reliable docking protocol.

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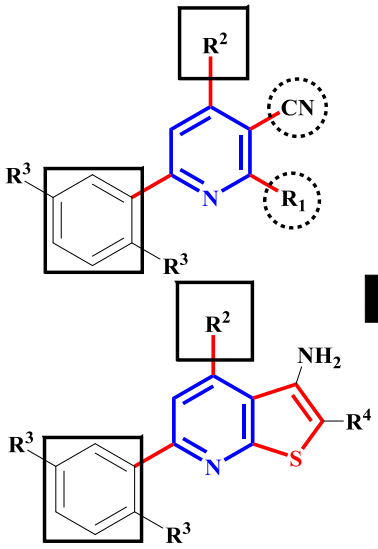
Highlights

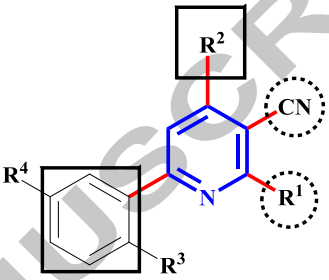
- Pyridine and thieno[2,3-*b*]pyridine derivatives as PIM-1 kinase inhibitors.
- Five compounds showed *in vitro* anticancer and PIM-1 kinase inhibitory activities.
- Compound **5b** was tested twice in NCI five-dose assay and proved its activity.
- Compound **5b** is under consideration of NCI biological evaluation committee.

Graphical abstract



Design of PIM -1 kinase inhibitors

R^1 = OH, NH_2, SH, SR
R^2 = C_6H_5, 4-ClC_6H_4, 4-BrC_6H_4, 4-$CH_3OC_6H_4$, thien-2-yl, pyridin-3-yl
R^3 = OH, OCH_3
R^4 = CH_3, C_6H_5, 4-ClC_6H_4, 4-$CH_3OC_6H_4$



Potent PIM -1 kinase inhibitors

5b: R^1= NH_2, R^2= 4-ClC_6H_4, R^3,R^4= OH
8d: R^1= OH, R^2= 4-$OCH_3C_6H_4$, R^3= OH, R^4= OCH_3
10c: R^1= OH, R^2= 4-$OCH_3C_6H_4$, R^3, R^4= OCH_3
13h: R^1= $SCH_2COC_6H_4$-4-OCH_3, R^2= 4-ClC_6H_4, R^3, R^4= OCH_3
15e: R^1= SCH_3, R^2= 4-ClC_6H_4, R^3, R^4= OCH_3