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Arylazolyl(azinyl)thioacetanilides. Part 10: Design, synthesis and biological evaluation of novel substituted imidazopyridinylthioacetanilides as potent HIV-1 inhibitors ☆

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ABSTRACT

In continuation of our efforts toward the discovery of potent HIV-1 NNRTIs with novel structures, we have employed a scaffold hopping strategy to explore the chemically diversed space of bioactive compounds. The original arylazolylthioacetanilide platform was replaced with different imidazopyridinylthioacetanilide scaffolds to yield the optimal pharmacophore moieties in order to generate novel NNRTIs with desirable potency. Some of the new compounds proved able to inhibit HIV-1 replication in the low micromolar range. In particular, compound **5b16** displayed the most potent anti-HIV-1 activity (EC_{50} = 0.21 ± 0.06 μM), inhibiting HIV-1 IIIB replication in MT-4 cells more effectively than dideoxycytidine (EC_{50} = 1.4 ± 0.1 μM) and similarly with nevirapine (EC_{50} = 0.20 ± 0.10 μM). Preliminary structure–activity relationship (SAR) of the newly synthesized congeners is discussed, and molecular modeling study is performed to rationalize the SAR conclusions.

1. Introduction

Acquired inmmune deficiency syndrome (AIDS) mainly caused by human immunodeficiency virus type-1 (HIV-1), continues to be a major leading pandemic disease wordwide with approximately 40 million people living with HIV.[1] Despite the meaningful results having been achieved by highly active antiretroviral therapy (HAART), definitive cure of AIDS by chemotherapeutics is still a long way off.[2] Non-nucleoside reverse transcriptase inhibitors (NNRTIs) with their unique antiviral potency and high selectivity, nowadays represent very potent anti-AIDS drugs that specifically target the HIV-1 reverse transcriptase (RT).[3] Nevertheless, the long-term use of NNRTIs in the clinical leads to the emergence of drug-resistant viruses and potentially severe side effects. As a consequence, discovery of novel NNRTIs candidates, especially with potency against the clinically relevant mutant strains of HIV-1, is a continuous goal of drug development.[4]

Recently, arylazolylthioacetanilides, a particular series of five-membered heterocyclic derivatives, have attracted lots of attention to the development of novel NNRTIs because of their high potency against wild-type and resistant HIV-1 strains, favorable bioavailability and low toxicity.[5–11] Among them, 1,2,4-triazole derivatives VRX-480733 and RDEA806[12], were chosen as candidates for further studies. Especially, RDEA806, a potent and promising Ardea Biosciences' NNRTI lead, is currently in phase IIa clinical trials (Fig. 1).

Meanwhile, during the course of our continued effort toward the development of potent NNRTIs, we embarked on a chemical evolution of lead compounds by 'follow-on'-based drug discovery strategies[13], such as the structure-based bioisosterism principle.[14] Herefrom, several series of new arylazolylthioacetanilide derivatives were designed, synthesized and identified as promising NNRTIs with potent anti-HIV activities in cell lines infected with either wild-type or mutant HIV-1 strains.[15–23] Especially, 1,2,3-thiadiazole derivative **ZP7** (Fig. 1) exhibited the most potent anti-HIV-1 activity (EC_{50} = 36.4 nM), inhibiting HIV-1 replication in MT-4 cells with sevenfold and eightfold higher efficiency than nevirapine (NVP) and delavirdine (DLV) respectively.[16] Molecular modeling studies demonstrated that the five-membered heterocycle portion

☆ Parts 1–9 were reported in references 15–23.

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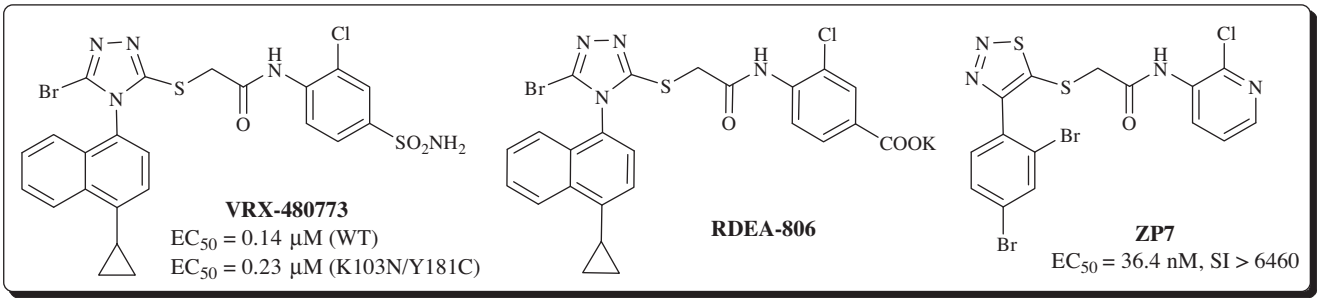


Figure 1. Azolylthioacetanilide-based NNRTIs.

of these inhibitors could be acting as a scaffold which orients the pharmacophores into the proper geometry for binding and being a key structural element to form hydrogen bond with Lys101 in the RT pocket.[16]

Encouraged by these promising results, and in order to further explore the chemically diversed space and the SARs of arylazolylthioacetanilides, we employed the scaffold hopping strategy, a useful method to discover structurally novel compounds by modifying the central core structure of the known active compounds.[24] Using this approach aimed at replacing the five-membered azole ring of arylazolylthioacetanilides with a fused heterocyclic ring, we designed two kinds of scaffolds (i.e. imidazo[4,5-b]pyridine and imidazo[4,5-c]pyridine) for synthesis (Fig. 2), owing to their synthetic accessibility and drug-like properties. Based on our previous SAR results[15–23], the other fragments which were considered to be necessary for conserving anti-HIV-1 activity, such as the "-SCH_2CONH-" linker and the substituted arylamines, were maintained. As a result, two series of novel imidazopyridinylthioacetanilide derivatives were synthesized and evaluated in vitro for their anti-HIV activities.

2. Results and discussion

2.1. Chemistry

The general synthetic route utilized to obtain the desired compounds 2-(3-mesityl-3*H*-imidazo[4,5-b]pyridin-2-ylthio)-*N*-phenylacetamide (**5a** series) and 2-(1-mesityl-1*H*-imidazo[4,5-c]pyridin -2-ylthio)-*N*-phenylacetamide (**5b** series) was straightforward and is outlined in Scheme 1. For the **5a** series, the commercially available 2-chloro-3-nitropyridine **1a** was converted to N-mesityl-3-nitropyridin-2-amine (**2a**) by reaction with 2,4,6-trimethyl-benzenamine in the presence of KF at 120 °C for several hours.[25] For the **5b** series, the commercially available 4-chloro-3-nitropyridine **1b** was reacted with 2,4,6-trimethyl-benzenamine and $NaHCO_3$ in EtOH at ambient temperature to obtain N-mesityl-3-nitropyridin-4-amine (**2b**).[26] Under the condition of Pd/C catalysis, the nitro group of **2a** or **2b** is reducted by hydrogenation into amino group, which achieved the intermediates *N*-mesitylpyridine-diamine **3a** and **3b** correspondingly. The cyclization reaction of **3a** or **3b** with potassium *O*-ethyl carbonodithioate in the presence of $NaHCO_3$ in refluxing EtOH, gave the key compound *N*-mesityl-3-nitropyridin-2-amine **4a** or *N*-mesityl-3-nitropyridin-4-amine **4b** after acidification.[27] The final imidazopyridine thioacetanilides **5a1–5a16** or **5b1–5b16** were obtained by reaction of **4a** or **4b** with substituted anilines in the presence of K_2CO_3 in an easy, rapid and profitable way.[28] Both analytical and spectral data of all the newly synthesized compounds are in full agreement with the proposed structures.

2.2. Biological activity

2.2.1. Anti-HIV activity evaluation

The newly synthesized imidazopyridinylthioacetanilide derivatives were evaluated for anti-HIV activity by determining their ability to inhibit the replication of the HIV-1 III_B strain, HIV-1 mutant strain RES056 (K103 N/Y181C double RT mutant), and HIV-2 ROD strain in MT-4 cells in comparison with nevirapine (NVP), zidovudine (azidothymidine, AZT), dideoxycytidine (DDC), delavir-

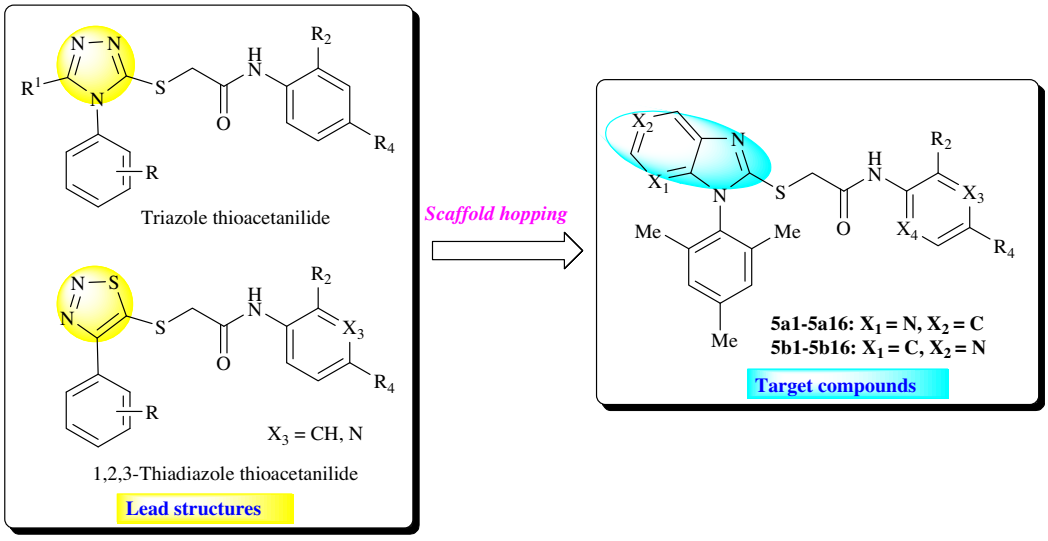


Figure 2. The scaffold hopping replacement of azoles by imidazopyridines.

dine (DLV) and efavirenz (EFV) used as reference drugs. The cytotoxicity of the compounds was determined in parallel. Comparisons of inhibitory concentration (EC_{50}), cytotoxic concentration (CC_{50}), and selective index (SI, given by the CC_{50}/EC_{50} ratio) for different compounds are presented in Table 1 and Table 2.

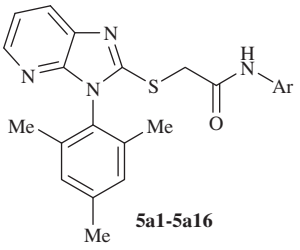
As can be seen from Table 1 and Table 2, one third of the tested compounds inhibited HIV-1 III_B replication at micromolar concentrations. In particular, compounds **5a16** and **5b16** were the most potent inhibitors against HIV-1 (IIIB) replication of these two series (EC_{50} = 0.75 ± 0.14 μM and 0.21 ± 0.06 μM, respectively), which were more effectively than DDC (EC_{50} = 1.39 ± 0.05 μM), and possessed similar anti-HIV-1 inhibitory activity as that of NVP (EC_{50} = 0.20 ± 0.10 μM). Some other compounds, **5a8**, **5b4**, **5b7**, **5b8**, **5b9**, **5b13** and **5b14** also exhibited moderate to good activities against HIV-1 strain III_B with EC_{50} values in the range of 0.99–5.7 μM, which reach the same order of magnitude as that of DDC (1.39 ± 0.05 μM). These results indicated that the imidazopyridine is an appropriate hopping scaffold instead of the five-membered heterocycles in the lead compounds.

Compared with the results shown in Table 1 and Table 2, it was found that substantial difference in antiviral activity against HIV-1 III_B can be observed in the two series of compounds, with the following order of active sequence: imidazo[4,5-c]pyridine series >imidazo[4,5-b]pyridine series. Therefore, the N atom position in the imidazopyridine core is crucial for maintaining the antiviral activity. Probably, a suitable position of the N atom was beneficial for improving the binding affinity (hydrogen bonding interaction) between the active binding site and the inhibitors, and thus enhancing the biological activity. Besides, the results in each series revealed a principle that whenever the *ortho* substitution (such as nitro and halogen) or the *para* substitution (such as nitro, acetyl and sulfonamide) at the phenyl ring of the anilide moiety, the presence of electron-withdrawing groups is essential to improve the potency against HIV-1. For instance, in the case of compounds **5b4** and **5b7** in the imidazo[4,5-c]pyridine series, introduction of a methyl group in the *para* position at the phenyl ring of the anilide moiety led to **5b5** and **5b6** with decreased activity, whereas introduction of 4-acetyl and 4-sulfonamide in **5b4** led to **5b8** and **5b16**, respectively, with markedly improved activity, indicating that the electronic nature or the steric-hindrance of the substituent at the *para* position remarkably influenced the anti-HIV-1 activity. Similar SARs is also present in the imidazo[4,5-b]pyridine series. These SAR conclusions are also grossly in agreement with the previously reported results in the arylazolylthioacetanilide series.[15,16]

It is interesting to note that, compounds **5a16** and **5b16**, with a sulfonamide group at the *para* position of the anilide moiety possessed outstanding inhibitory activity comparing to their counterparts. Practically, the sulfonamide is a common privileged group used in a lot of NNRTI scaffolds because its increased polarity can accommodate the chemical environment in this region of RT, leading to the possibility of increasing affinity by generating hydrogen bonding interactions.[29]

In general, the newly designed imidazopyridinylthioacetanilide compounds had moderate activities against HIV-1 (III_B), but were less active than the previously reported 1,2,3-thiadiazole thioacetanilides[16] and imidazole thioacetanilides.[19] These results would provide valuable information for further modification in the central aromatic moietey of the arylazolylthioacetanilide NNRTI.

Table 1
Anti-HIV activity, cytotoxicity and selectivity indices of 2-(3-mesityl-3H-imidazo[4,5-b]pyridin-2-ylthio)-*N*-phenylacetamide derivatives (**5a1**–**5a16**) and positive drugs

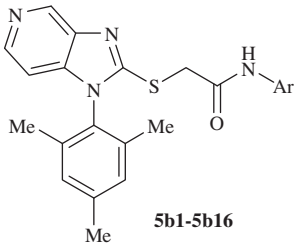


5a1-5a16

Compd	Ar	EC_{50}[a] (μM)			CC_{50}[b] (μM)	SI[c]
		HIV-1 III_B	RES056	HIV-2 ROD		HIV-1 III_B
5a1	Phenyl	>26	—	>26	26 ± 4.4	<1
5a2	2-Chlorophenyl	>3.9	—	>3.9	3.9 ± 1.5	<1
5a3	2-Fluorophenyl	>3.7	—	>3.7	3.7 ± 1.2	<1
5a4	2-Bromophenyl	>5.6	—	>5.6	5.6 ± 0.5	<1
5a5	2-Bromo-4-methylphenyl	>0.99	—	>0.99	0.99 ± 0.1	<1
5a6	4-Methyl-2-nitrophenyl	>144	—	>144	144 ± 35	<1
5a7	2-Nitrophenyl	⩾6.7	>39	>39	39 ± 17	⩽6
5a8	4-Acetyl-2-bromophenyl	3.6 ± 1.0	>46	>46	46 ± 29	13
5a9	2-Chloropyridin-3-yl	>6.0	—	>6.0	6.0 ± 0.8	<1
5a10	2,4-Dichlorophenyl	>3.0	—	>3.0	3.9 ± 0.2	<1
5a11	*o*-tolyl	>15	—	>15	15 ± 6.9	<1
5a12	(Methyl 3-bromobenzoate)-4-yl	8.9 ± 0.29	>80	>80	80 ± 42	9
5a13	(Ethyl 3-bromobenzoate)-4-yl	9.9 ± 4.1	>117	>117	117 ± 46	12
5a14	2-Chloro-4-nitrophenyl	>25	—	>25	25 ± 1.5	<1
5a15	3-Bromo-5-methylpyridin-2-yl	>102	—	>102	102 ± 46	<1
5a16	2-Bromo-4-sulfonamidephenyl	0.75 ± 0.14	>22	>22	22 ± 2.6	30
NVP		200 ± 105	>15	—	>15	>75
AZT		6.0 ± 0.57	0.01 ± 0.009	0.007 ± 0.0008	⩾50	⩾8295
DDC		1394 ± 50	—	1.7 ± 0.25	>95	>68
EFV		6.6 ± 0.87	0.55 ± 0.06	—	>6.3	>964
DLV		35 ± 4.7	>36.19	—	>36	>1034

In bold are the values of active compounds.
[a] EC_{50}: concentration of compound required to achieve 50% protection of MT-4 cells against HIV-1-induced cytotoxicity, as determined by the MTT method.
[b] CC_{50}: concentration required to reduce the viability of mock-infected cells by 50%, as determined by the MTT method.
[c] SI: selectivity index (CC_{50}/EC_{50}). The SI values: X 1 stand for ⩾1or <1.

Table 2
Anti-HIV activity, cytotoxicity and selectivity indices of 2-(1-mesityl-1*H*-imidazo[4,5-c]pyridin-2-ylthio)-*N*-phenylacetamide derivatives (**5b1–5b16**).



5b1-5b16

Compd	Ar	EC_{50} (μM)			CC_{50} (μM)	SI
		HIV-1III_B	RES056	HIV-2 ROD		HIV-1 III_B
5b1	Phenyl	>17	—	>17	17 ± 0.5	<1
5b2	2-Chlorophenyl	>19	—	>19	19 ± 0.6	<1
5b3	2-Fluorophenyl	>18	—	>18	18 ± 0.7	<1
5b4	2-Bromophenyl	5.2 ± 0.49	>22	>22	22 ± 6.2	4
5b5	2-Bromo-4-methylphenyl	>5.9	—	>5.9	5.9 ± 0.5	<1
5b6	4-Methyl-2-nitrophenyl	⩾1.2	>6.2	>6.2	6.2 ± 1.7	⩽5
5b7	2-Nitrophenyl	5.7 ± 5.07	>9.9	>9.9	9.9 ± 1.9	2
5b8	4-Acetyl-2-bromophenyl	0.99 ± 0.26	>61	>61	61 ± 29	61
5b9	2-Chloropyridin-3-yl	1.7 ± 0.16	>22	>22	22 ± 6.3	12
5b10	2,4-Dichlorophenyl	⩾1.1	>140	>140	140 ± 26	⩽127
5b11	*o*-tolyl	>22	—	>22	22 ± 9.6	<1
5b12	(Methyl 3-bromobenzoate)-4-yl	⩾2.5	>27	>27	27 ± 9.1	⩽11
5b13	(Ethyl 3-bromobenzoate)-4-yl	4.7 ± 0.39	>22	>22	22 ± 2.1	5
5b14	2-Chloro-4-nitrophenyl	1.4 ± 0.6	>29	>29	29 ± 2.6	20
5b15	3-Bromo-5-methylpyridin-2-yl	>61	—	>61	61 ± 63	<1
5b16	2-Bromo-4-sulfonamidephenyl	0.21 ± 0.05	>9.7	>9.7	9.7 ± 3.7	46

In addition, all of the title compounds were evaluated for their capability to inhibit HIV-2 (strain ROD) and HIV-1 RES056 mutant strain replication in MT-4 cells, but no one was active against these strains (Table 1 and Table 2). It can be concluded that two new series of imidazopyridinylthioacetanilide derivatives were specific for HIV-1 and thus belonged to the NNRTIs.

2.2.2. HIV-1 RT inhibition assay

With the aim to further confirm the drug target of imidazopyridinylthioacetanilide derivatives, one selected title compound **5b16** was tested in enzymatic assays against highly purified recombinant HIV-1 RT using poly(rC)-oligo(dG) as template primer. Compound **5b16** exhibited moderate inhibition of enzymatic activity with an IC_{50} value (50% of the inhibitory concentration of tested compounds required to inhibit biotin deoxyuridine triphosphate (biotin-dUTP) incorporation into the HIV-1 RT by 50%) of 4.4 μM, which was slightly higher than that of NVP (1.2 μM).

2.3. Molecular modeling analysis

By means of Autodock Vina [http://vina.scripps.edu], molecular modeling of compound **5b16** docked into the NNRTIs binding pocket (NNIBP) of HIV-1 RT was employed to understand the interactions between these inhibitors and the target. X-ray crystal structure of HIV-1 RT with benzophenone taken from PDB (3DLG) was used as the input structure for docking calculations because of the high degree of structural similarity between arylazolylthioacetanilides and benzophenones.[5] Default parameters were used as described in the Autodock Vina manual unless otherwise specified. The theoretical binding mode of **5b16** to the NNIBP is shown in Fig. 3.

Results suggest that this class of compounds shares the similar binding mode with previous arylazolylthioacetanilides.[16] As illustrated in Fig. 3, the trimethylbenzene ring of **5b16** fits into the aromatic-rich binding pocket, surrounded by the aromatic side chains of Tyr188, Phe227, and Trp229. Detailed analysis of the binding mode showed that phenyl ring interacts favorably with the Tyr188 side chain, giving rise to a positive π-π stacking interaction. The inhibitor's amide carbonyl forms a key hydrogen bond with the backbone N-H of Lys103, which is important for the affinity between inhibitor and RT. The 2-bromo-4-sulfonamidephenyl moiety of **5b16** is close to Pro236, and the sulfonamide moiety points toward the solvent exposed region. Therefore, 4-substituent at the phenyl ring of the anilide moiety allows hydrophilic groups as the preferred substituents, which can explain why **5b16** and **5a16** stand out of other congeners.

It is noteworthy to emphasize that, as shown in Fig. 3, although imidazopyridine moiety is close to Lys101, the position of N atom in pyridine did not favor forming the hydrogen binding interaction with backbone of Lys101, which may rationalize the SAR conclusion that anti-HIV activity is strongly dependent on the position of the N atom of the pyridine ring, and also illustrate the reason for their lost potency against the mutant RES056 strain.

According to our molecular modeling investigations on the binding mode of **5b16** to the NNIBP of HIV-1 RT, together with the SAR studies as described above, we postulate that keeping favorable position of the heteroatom in this or alternative structural classes may be beneficial to enhance the interaction between the inhibitors and the RT.

In summary, the results of the AutoDocking analysis seem to support our newly designed and synthesized compounds. Further structural optimization will consider these aspects in future design attempts.

3. Conclusion

In summary, the present work is an extension of our ongoing efforts toward the development and the identification of new molecules with anti-HIV activity. In the present investigation, the "scaffold hopping"-based approach led to the identification of

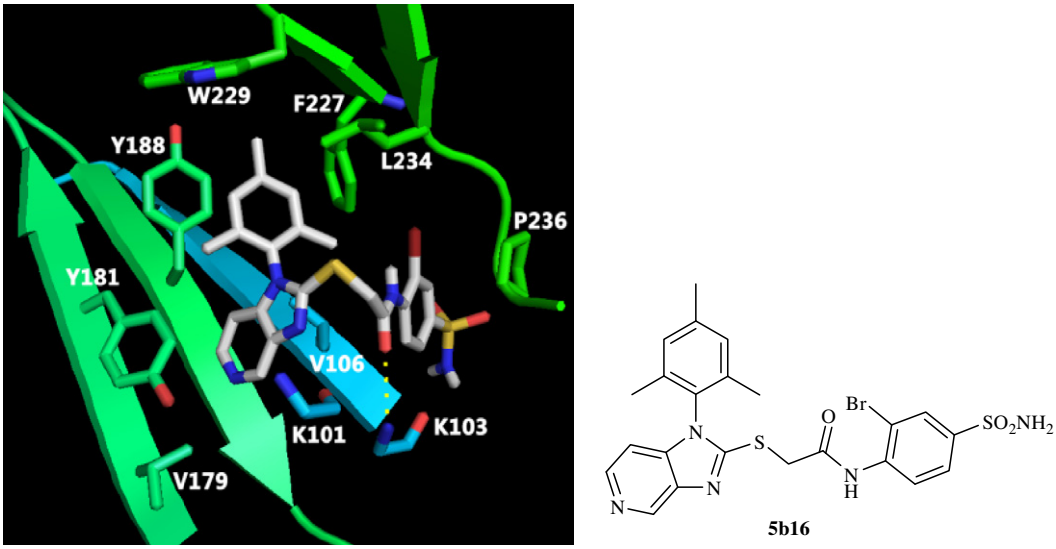


Figure 3. Molecular model of **5b16** in the allosteric site of HIV-1 RT (PDB code: 3DLG). The docking result of **5b16** is showed by PyMOL.

imidazopyridinylthioacetanilide NNRTIs with potency against HIV-1 replication in the low micromolar concentration range. Among them, compound **5b16** was identified as the most promising candidate with favorable inhibitory activity against HIV-1 III_B (EC_{50} = 0.21 ± 0.06 μM), which was more effective than DDC (EC_{50} = 1.4 ± 0.05 μM) and was similar to NVP (EC_{50} = 0.20 ± 0.10 μM). Preliminary SAR results for the newly synthesized congeners and docking studies are presented, providing insights for discovery of more potent NNRTIs with diverse structures. Alternative structural classes derived from arylazolylthioacetanilides have been studied in our laboratories and will be reported in due course.

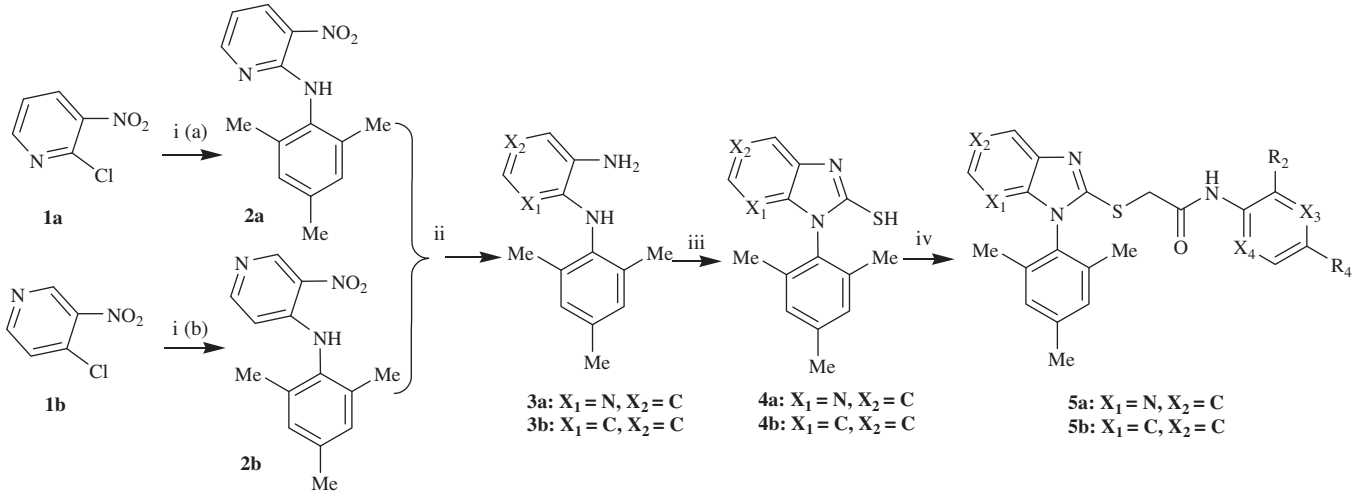
4. Experimental

4.1. Chemistry

All melting points were determined on a micromelting point apparatus and are uncorrected. Infrared spectra (IR) were recorded with a Nexus 470FT-IR Spectrometer (Thermo Nicolet, Company). ^{1}H NMR spectra were recorded on a Bruker Avance 600 or 400 spectrometer at 600 or 400 MHz, using DMSO-d_6 as solvent and tetramethylsilane (TMS) as internal standard. ^{13}C-NMR spectra were run in the same instrument at 150 or 100 MHz.Chemical shifts are expressed in δ units and TMS as internal reference. Mass spectra were taken on a LC Autosampler Device: standard G1313A instrument (Agilent Technologies, Company). TLC was performed on Silica Gel GF254 for TLC (Merck) and spots were visualized by iodine vapors or by irradiation with UV light (254 nm). Flash column chromatography was performed on column packed with Silica Gel 60 (200–300 mesh). Solvents were reagent grade and, when necessary, were purified and dried by standard methods. Concentration of the reaction solutions involved the use of rotary evaporator at reduced pressure.

4.1.1. General procedure for the synthesis of 2-(3-mesityl-3*H*-imidazo[4,5-b]pyridin-2-ylthio)-*N*-phenylacetamide (5a series)

4.1.1.1. General Procedure for the synthesis of N-mesityl-3-nitropyridin-2-amine (2a) [25]. The reaction mixture of 2-chloro-3-nitropyridine (**1a**) (1.58 g, 0.01 mol), 2,4,6-trimethylbenzenamine (5.4 g, 0.04 mol), and KF (0.88 g, 0.015 mol) was slowly heated to reflux temperature (120 °C) with stirring and continued for 10 h (monitored by TLC). The suspension was cooled and then poured into 150 mL water and 20 mL ammonia. The mixture was then extracted with CH_2Cl_2 (30 mL), washed successively with



Scheme 1. Reagentsc and conditions: (i) 2,4,6-trimethylbenzenamine, (a) KF, 120 °C; (b) $NaHCO_3$, EtOH, △; (ii) H_2, Pd/C; (iii) $NaHCO_3$, H_2O, EtOH, $CH_3CH_2OCS_2K$, reflux. acidification; (iv) K_2CO_3, CH_3COCH_3, $ClCH_2CONHAr$.

water (2 × 30 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure to afford crude product **2a** as a brown oil. Then, the pruduct was purified by recrystallization from alcohol-petroleum ether to obtain yellow solid **2a**, Yellow needle crystals, yield: 65%, MS (ESI): *m*/*z* 258.12 (M+1). $C_{14}H_{15}N_3O_2$ (257.12).

4.1.1.2. General procedure for the synthesis of 3-mesityl-3*H*-imidazo[4,5-b]pyridine -2-thiol (4a). The reaction system containing intermediate **2a** (6 g, 0.023 mol), Pd/C (0.2 g) and 50 mL alcohol was vacuumed and fulled with excess hydrogen gas. The reaction mixture was kept for 15 h and then evaporated under reduced pressure. The obtained crude pruduct was washed by petroleum ether and evaporated under reduced pressure again to give purified compound **3a**. Grey powder, yield: 65.6%. mp:181–184 °C. MS (ESI): *m*/*z* 228.6. $C_{14}H_{17}N_3$ (227.14).

Intermediate **3a** (1.2 g, 5.3 mmol), $EtOCS_2K$ (1 g, 6.2 mmol) and $NaHCO_3$ (0.1 g, 1.2 mmol) were dissolved in alcohol (25 mL) and water (5 ml), then the solution flowed back for 5 h with stirring.[27] The reaction fluid was cooled to room temperature and then 50 ml water and 15 mL NaOH solution (2 M) was added. The precipitate that formed was removed by suction filtration and the filtrate was modulated to ph 7 by adding acetic acid. Precipiteted compond **4a** was filtered by suction filtration and air-dried to a yellowish-white powder. Grey powder, yield: 65.6%. mp:247–253 °C. MS (ESI): *m*/*z* 270.7.$C_{15}H_{15}N_3S$ (269.1).

4.1.1.3. General procedure for the synthesis of 2-(3-mesityl-3*H*-imidazo [4,5-b]pyridin- 2-ylthio)-*N*-phenyl-acetamide (5a1–5a16). Compound **4a** (0.15 g, 0.5 mmol), K_2CO_3 (0.14 g, 1.0 mmol), from different 2-chloro-*N*-(substituted aromatic group)acetamides (0.05 mmol), was dissolved in acetone (10 mL).[28] The reaction mixture was stirred at ambient temperature for 2 h and then evaporated under reduced pressure. The residue was dissovled with a small amount CH_2Cl_2 and chromatographed on silica gel using ethyl acetate-petroleum ether system. Pure fractions were collected and concentrated, giving the desired compounds **5a1–5a16**.

4.1.1.4. 2-(3-mesityl-3*H*-imidazo[4,5-b]pyridin-2-ylthio)-N-phenylacetamide (5a1). White crystals, yield: 54.7%. mp: 174–176 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 10.42 (s, 1H, NH), 8.14 (d, 1H, *J* = 4.2 Hz, C_5-imidazo[4,5-b]pyridin-H), 8.00 (d, 1H, *J* = 7.8 Hz, C_7-imidazo[4,5-b]pyridin-H), 7.56 (d, 2H, *J* = 7.8 Hz, C_6-Ph′-H, C_2-Ph′-H), 7.30 (t, 2H, *J* = 7.8 Hz, C_3-Ph′-H, C_5-Ph′-H), 7.27 (dd, 1H, J_1 = 4.2 Hz, J_2 = 7.8 Hz, C_6-imidazo[4,5-b]pyridin-H), 7.15 (s, 2H, $C_{3,5}$-Ph-H), 7.05 (t, 1H, *J* = 7.8 Hz, C_4-Ph′-H), 4.37 (s, 2H, S-CH_2), 2.37 (s, 3H, Me), 1.86 (s, 6H, 2 × Me). ESI-MS: *m*/*z* 403.6 (M+1). $C_{23}H_{22}N_4OS$ (402.15).

4.1.1.5. N-(2-chlorophenyl)-2-(3-mesityl-3*H*-imidazo[4,5-b]pyridin-2-ylthio)acetamide (5a2). White crystals, yield: 64.5%. mp: 176–180 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 9.96 (s, 1H, NH), 8.16 (d, 1H, *J* = 4.8 Hz, C_5-imidazo[4,5-b]pyridin-H), 8.06 (d, 1H, *J* = 7.8 Hz, C_6-Ph′-H), 7.74 (d, 1H, *J* = 7.8 Hz, C_3-Ph′-H), 7.64 (d, 1H, *J* = 7.8 Hz, C_7-imidazo[4,5-b]pyridin-H), 7.37 (t, 1H, *J* = 7.8 Hz, C_5-Ph′-H), 7.30 (dd, 1H, J_1 = 4.8 Hz, J_2 = 7.8 Hz, C_6-imidazo[4,5-b]pyridin-H), 7.14 (s, 2H, $C_{3,5}$-Ph-H), 7.12(t, 1H, *J* = 7.8 Hz, C_4-Ph′-H), 4.37 (s, 2H, S-CH_2), 2.36 (s, 3H, Me), 1.85 (s, 6H, 2×Me). ESI-MS: *m*/*z* 437.6 (M+1), 439.5(M+3). $C_{23}H_{21}ClN_4OS$ (436.11).

4.1.1.6. N-(2-fluorophenyl)-2-(3-mesityl-3*H*-imidazo[4,5-b]pyridin-2-ylthio)acetamide (5a3). White crystals, yield: 55.6%. mp:179–181 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 10.32 (s, 1H, NH), 8.16 (d, 1H, *J* = 4.8 Hz, C_5-imidazo[4,5-b]pyridin-H), 8.02 (d, 1H, *J* = 7.8 Hz, C_6-Ph′-H), 7.90 (d, 1H, *J* = 9.6 Hz, C_7-imidazo[4,5-b]pyridin-H), 7.29 (dd, 1H, J_1 = 4.8 Hz, J_2 = 9.6 Hz, C_6-imidazo[4,5-b]pyridin-H), 7.25 (m, 1H, C_5-Ph′-H), 7.15 (m, 4H, $C_{3,5}$-Ph-H, $C_{3,4}$-Ph′-H), 4.39 (s, 2H, S-CH_2), 2.50 (s, 3H, Me), 1.85 (s, 6H, 2×Me). ESI-MS: *m*/*z* 421.4 (M+1), 423.4 (M+3). $C_{23}H_{23}FN_4OS$ (420.14).

4.1.1.7. N-(2-bromophenyl)-2-(3-mesityl-3*H*-imidazo[4,5-b]pyridin-2-ylthio)acetamide (5a4). White crystals, yield: 44.6%. mp: 188–191 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 9.96 (s, 1H, NH), 8.16 (d, 1H, *J* = 4.8 Hz, C_5-imidazo[4,5-b]pyridin-H), 8.07 (d, 1H, *J* = 7.8 Hz, C_6-Ph′-H), 7.74 (d, 1H, *J* = 7.8 Hz, C_3-Ph′-H), 7.65 (d, 1H, *J* = 7.8 Hz, C_7-imidazo[4,5-b]pyridin-H), 7.38 (t, 1H, *J* = 7.8 Hz, C_5-Ph′-H), 7.29 (dd, 1H, J_1 = 4.8 Hz, J_2 = 7.8 Hz, C_6-imidazo[4,5-b]pyridin-H), 7.14 (s, 2H, $C_{3,5}$-Ph-H), 7.11(t, 1H, *J* = 7.8 Hz, C_4-Ph′-H), 4.37 (s, 2H, S-CH_2), 2.36 (s, 3H, Me), 1.85 (s, 6H, 2×Me). ESI-MS: *m*/*z* 481.4 (M+1), 483.4(M+3), 485.4(M+5). $C_{23}H_{21}ClN_4OS$ (480.06).

4.1.1.8. N-(2-bromo-4-methylphenyl)-2-(3-mesityl-3*H*-imidazo[4,5-b]pyridin-2-ylthio)acetamide (5a5). White crystals, yield: 67.6%. mp: 205-209 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 9.87 (s, 1H, NH), 8.16 (d, 1H, *J* = 5.4 Hz, C_5-imidazo[4,5-b]pyridin-H), 8.06 (d, 1H, *J* = 7.8 Hz, C_6-Ph′-H), 7.57 (d, 1H, *J* = 8.4 Hz C_7-imidazo[4,5-b]pyridin-H), 7.47 (s, 1H, C_3-Ph′-H), 7.31 (dd, 1H, J_1 = 4.8 Hz, J_2 = 7.8 Hz, C_6-imidazo[4,5-b]pyridin-H), 7.17 (d, 1H, *J* = 7.8 Hz, C_4-Ph′-H), 7.14 (m, 2H, $C_{3,5}$-Ph-H), 4.34 (s, 2H, S-CH_2), 2.50 (s, 3H, Me), 2.26 (s, 3H, Me), 1.84 (s, 6H, 2 × Me). ESI-MS: *m*/*z* 495.4 (M+1), 497.5 (M+3). 499.3 (M+5). $C_{24}H_{25}BrN_4OS$ (494.08).

4.1.1.9. 2-(3-mesityl-3*H*-imidazo[4,5-b]pyridin-2-ylthio)-N-(4-methyl-2-nitrophenyl)acetamide (5a6). Yellow crystals, yield: 65.4%. mp: 230–233 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 10.52 (s, 1H, NH), 8.15 (d, 1H, *J* = 4.8 Hz, C_5-imidazo[4,5-b]pyridin-H), 8.02 (d, 1H, *J* = 7.8 Hz, C_6-Ph′-H), 7.81 (d, 1H, *J* = 1.8 Hz, C_3-Ph′-H), 7.71 (d, 1H, *J* = 8.4 Hz C_7-imidazo[4,5-b]pyridin-H), 7.40 (dd, 1H, J_1 = 7.8 Hz, J_2 = 1.8 Hz, C_5-Ph′-H), 7.28 (dd, 1H, J_1 = 8.4 Hz, J_2 = 4.8 Hz, C_6-imidazo[4,5-b]pyridin-H), 7.14 (s, 2H, $C_{3,5}$-Ph-H), 4.34 (s, 2H, S-CH_2), 2.36 (s, 6H, 2 × Me), 1.84 (s, 6H, 2×Me). ESI-MS: *m*/*z* 462.5(M+1). $C_{24}H_{23}N_5O_3S$ (461.15).

4.1.1.10. 2-(3-mesityl-3*H*-imidazo[4,5-b]pyridin-2-ylthio)-N-(2-nitrophenyl)acetamide (5a7). White crystals, yield: 63.6%. mp: 194–197 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 10.82 (s, 1H, NH), 8.15 (d, 1H, *J* = 4.8 Hz, C_5-imidazo[4,5-b]pyridin-H), 8.03 (d, 1H, *J* = 8.4 Hz, C_3-Ph′-H), 7.98 (d, 1H, *J* = 8.4 Hz, C_6-Ph′-H), 7.84 (d, 1H, *J* = 8.4 Hz, C_7-imidazo[4,5-b]pyridin-H), 7.73 (t, 1H, *J* = 8.4 Hz, C_5-Ph′-H), 7.38 (t, 1H, *J* = 8.4 Hz, C_4-Ph′-H), 7.29 (dd, 1H, J_1 = 4.8 Hz, J_2 = 8.4 Hz, C_6-imidazo[4,5-b]pyridin-H), 7.14 (s, 2H, $C_{3,5}$-Ph-H), 4.36 (s, 2H, S-CH_2), 2.36 (s, 3H, Me), 1.84 (s, 6H, 2 × Me). ESI-MS: *m*/*z* 448.5 (M+1), 450.4(M+3). $C_{23}H_{21}N_5O_3$-S(447.14).

4.1.1.11. N-(4-acetyl-2-bromophenyl)-2-(3-mesityl-3*H*-imidazo[4,5-b]pyridin-2-ylthio)acetamide (5a8). White crystals, yield: 67.8%. mp: 210–213 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 10.09 (s, 1H, NH), 8.17 (d, 1H, *J* = 4.8 Hz, C_5-imidazo[4,5-b]pyridin-H), 8.07 (m, 1H, C_3-Ph′-H), 7.95 (d, 1H, *J* = 8.4 Hz, C_6-Ph′-H), 7.63 (d, 1H, *J* = 7.8 Hz, C_7-imidazo[4,5-b]pyridin-H), 7.30 (dd, 1H, J_1 = 4.8 Hz, J_2 = 7.8 Hz, C_6-imidazo[4,5-b]pyridin-H), 7.24 (t, 1H, *J* = 8.4 Hz, C_5-Ph′-H), 7.14 (s, 2H, $C_{3,5}$-Ph-H), 4.37 (s, 2H, S-CH_2), 2.50 (s, 3H, Me), 2.36 (s, 3H, Me), 1.85 (s, 6H, 2×Me). ESI-MS: *m*/*z* 523.4(M+1), 525.5(M+3). $C_{25}H_{23}BrN_4O_2S$ (522.07). ^{13}C-NMR (100 MHz, DMSO-d_6, ppm) δ: 196.30 (Ph′-C=O), 167.33 (N-C=O), 154.28, 149.36, 143.57, 140.49, 140.20, 136.83 (2 × C),

135.37, 134.70, 133.08, 129.74 (2×C), 129.18, 128.79, 125.77, 124.20, 119.00, 115.35, 35.54 (CH_2-S), 27.06 (Me-C=O), 21.19 (CH_3-Ph), 17.61 (2×Me-Ph).

4.1.1.12. N-(2-chloropyridin-3-yl)-2-(3-mesityl-3*H*-imidazo[4,5-b]pyridin-2-ylthio)acetamide (5a9). White crystals, yield: 57.7%. mp: 216–220 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 10.23 (s, 1H, NH), 8.27 (d, 1H, J = 7.8 Hz, C_4-pyridine-H), 8.19 (d, 1H, J = 4.8 Hz, C_6-pyridine-H), 8.16 (d, 1H, J = 4.8 Hz, C_5-imidazo[4,5-b]pyridin-H), 8.04 (d, 1H, J = 8.4 Hz, C_7-imidazo [4,5-b]pyridin-H), 7.44 (dd, 1H, J_1 = 4.8 Hz, J_2 = 8.4 Hz, C_6-imidazo[4,5-b]pyridin-H), 7.30 (dd, 1H, J_1 = 4.8 Hz, J_2 = 7.8 Hz, C_5-pyridine-H), 7.14 (s, 2H, $C_{3,5}$-Ph-H), 4.41 (s, 2H, S-CH_2), 2.36 (s, 3H, Me), 1.85 (s, 6H, 2 × Me). ESI-MS: m/z 438.5 (M+1), 440.5 (M+3). $C_{22}H_{22}ClN_5OS$(437.11).

4.1.1.13. N-(2,4-dichlorophenyl)-2-(3-mesityl-3*H*-imidazo[4,5-b]pyridin-2-ylthio)acetamide (5a10). White crystals, yield: 62.6%. mp: 185–187 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 10.14 (s, 1H, NH), 8.16 (d, 1H, J = 4.8 Hz, C_5-imidazo[4,5-b]pyridin-H), 8.04 (d, 1H, J = 7.8 Hz, C_6-Ph′-H), 7.87 (d, 1H, J = 8.4 Hz, C_7-imidazo[4,5-b]pyridin-H), 7.66 (d, 1H, J = 1.8 Hz, C_3-Ph′-H), 7.42 (dd, 1H, J_1 = 7.8 Hz, J_2 = 1.8 Hz, C_5-Ph′-H), 7.30 (dd, 1H, J_1 = 4.8 Hz, J_2 = 8.4 Hz, C_6-imidazo[4,5-b]pyridin-H), 7.14 (s, 2H, $C_{3,5}$-Ph-H), 4.38 (s, 2H, S-CH_2), 2.36 (s, 3H, Me), 1.84 (s, 6H, 2 × Me). ESI-MS: m/z 471.4(M+1), 474.3(M+3). $C_{23}H_{20}Cl_2N_4OS$ (470.07).

4.1.1.14. 2-(3-mesityl-3*H*-imidazo[4,5-b]pyridin-2-ylthio)-N-o-tolylacetamide (5a11). White crystals, yield: 62.6%. mp: 185–187 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 9.78 (s, 1H, NH), 8.15 (d, 1H, J = 4.8 Hz, C_5-imidazo[4,5-b]pyridin-H), 8.02 (d, 1H, J = 8.4 Hz, C_7-imidazo[4,5-b]pyridin-H), 7.43 (d, 1H, J = 7.8 Hz, C_6-Ph′-H), 7.29 (t, 1H, J = 7.8 Hz, C_5-Ph′-H), 7.19 (m, 2H, C_6-imidazo[4,5-b]pyridin-6H, C_3-Ph′-H), 7.15 (s, 2H, $C_{3,5}$-Ph-H), 7.07(t, 1H, J =7.8 Hz, C_4-Ph′-H), 4.35 (s, 2H, S-CH_2), 2.36 (s, 3H, Me), 1.85 (s, 6H, 2 × Me). ESI- MS: m/z 417.6(M+1). $C_{24}H_{24}N_4OS$(416.17).

4.1.1.15. Methyl 3-bromo-4-(2-(3-mesityl-3*H*-imidazo[4,5-b]pyridin-2-ylthio)acetamido) benzoate (5a12). White crystals, yield: 59.9%. mp: 188–191 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 10.13 (s, 1H, NH), 8.17 (s, 1H, J = 4.2 Hz, C_5-imidazo[4,5-b]pyridin-H), 8.13 (d, 1H, J = 2.4 Hz, C_3-Ph′-H), 8.07 (m, 2H, C_6-Ph′-H, C_5-Ph′-H), 7.95 (d, 1H, J = 8.4 Hz, C_7-imidazo[4,5-b]pyridin-H), 7.30 (dd, 1H, J_1 = 4.8 Hz, J_2 = 7.8 Hz, C_6-imidazo[4,5-b]pyridin-H), 7.14 (s, 2H, $C_{3,5}$-Ph-H), 4.41 (s, 2H, S-CH_2), 3.85(s, 3H, $COCH_3$), 2.36 (s, 3H, Me), 1.84 (s, 6H, 2×Me). ^{13}C-NMR (100 MHz, DMSO-d_6, ppm) δ: 167.36 (N-C=O), 165.03 (O–C=O), 154.29, 149.34, 143.56, 140.72, 140.20, 136.83 (2 × C), 135.35, 133.88, 129.74, 129.64 (2 × C), 129.16, 127.34, 125.78, 124.36, 119.01, 115.06, 52.85 (MeO), 35.50 (CH_2-S), 21.20 (CH_3-Ph), 17.62 (2×Me-Ph). ESI-MS: m/z 539.4(M+1), 543.5(M+5). $C_{25}H_{23}BrN_4O_3S$ (538.07).

4.1.1.16. Ethyl 3-bromo-4-(2-(3-mesityl-3*H*-imidazo[4,5-b]pyridin-2-ylthio)acetamido) benzoate (5a13). White crystals, yield: 63.9%. mp:181–183 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 10.10 (s, 1H, NH), 8.17 (d, 1H, J = 4.8 Hz, C_5-imidazo[4,5-b]pyridin-5H), 8.16 (d, 1H, J = 1.8 Hz, C_3-Ph′-H), 8.09 (m, 2H, C_5-Ph′-H, C_6-Ph′-H), 7.95 (d, 1H, J = 8.4 Hz, C_7-imidazo[4,5-b]pyridin-H), 7.30 (dd, 1H, J_1 = 4.8 Hz, J_2 = 8.4 Hz, C_6-imidazo[4,5-b]pyridin-H), 7.14 (s, 2H, $C_{3,5}$-Ph-H), 4.41 (s, 2H, S-CH_2), 4.30 (q, 2H, J = 7.2 Hz, OCH_2CH_3), 2.36 (s, 3H, Me), 1.85 (s, 6H, 2×Me). 1.31 (t, 3H, J = 7.2 Hz, OCH_2CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, ppm) δ: 167.33 (N–C=O), 164.52 (O–C=O), 154.28, 149.37, 143.56, 140.67, 140.43, 136.83 (2 × C), 135.37, 133.80, 129.74 (2 × C), 129.59, 129.18, 127.68, 125.77, 124.35, 118.99, 115.08, 61.54 (CH_2-O), 35.53 (CH_2-S), 21.19 (CH_3-Ph), 17.60 (2 × Me-Ph), 14.56 (Me). ESI-MS: m/z 555.3 (M+3). $C_{26}H_{25}BrN_4O_3S$ (552.38).

4.1.1.17. N-(2-chloro-4-nitrophenyl)-2-(3-mesityl-3*H*-imidazo[4,5-b]pyridin-2-ylthio)acetamide (5a14). White crystals, yield: 54.9%. mp:268–271 °C. ESI-MS: m/z 482.4 (M+1), 484.6 (M+3). $C_{23}H_{20}ClN_5O_3S$ (481.1).

4.1.1.18. N-(3-bromo-5-methylpyridin-2-yl)-2-(3-mesityl-3*H*-imidazo[4,5-b]pyridin-2-ylthio)acetamide (5a15). White crystals, yield: 71.7%. mp:171–173 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 10.43 (s, 1H, NH), 8.37(d, J = 3.0 Hz, C_5-pyridine-H), 8.22 (d, J = 3.0 Hz, C_3-pyridine-H), 8.17 (d, 1H, J = 4.8 Hz, C_5-imidazo[4,5-b]pyridin-5H), 8.05 (dd, 1H, J_1 = 8.4 Hz, J_2 = 1.2 Hz, C_7-imidazo[4,5-b]pyridin-H) 7.31 (dd, 1H, J_1 = 4.8 Hz, J_2 = 8.4 Hz, C_6-imidazo[4,5-b]pyridin-H), 7.15 (s, 2H, $C_{3,5}$-Ph-H), 4.41 (s, 2H, S-CH_2), 2.36 (s, 3H, Me), 1.85 (s, 6H, 2×Me) 1.27 (s, 3H, Me). ESI-MS: m/z 496.4 (M+1), 498.4 (M+3). $C_{23}H_{22}BrN_5OS$ (495.07).

4.1.1.19. N-(2-bromo-4-sulfonamidephenyl)-2-(1-mesityl-1*H*-imidazo[4,5-c]pyridin-2-ylthio) acetamide (5a16). White solid, yield: 75.8%. mp:173–175 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 10.15 (s, 1H, NH), 8.16 (d, 1H, J = 8.4 Hz, C_5-Ph′-H), 8.08 (d, 1H, J = 4.8 Hz, C_5-imidazo[4,5-b]pyridin-H), 8.03 (s, 1H, C_3-Ph′-H), 8.01(d, 1H, J = 8.4 Hz, C_6-Ph′-H), 7.79 (d, 1H, J = 8.4 Hz, C_7-imidazo[4,5-b]pyridin-H), 7.14 (s, 2H, $C_{3,5}$-Ph-H), 7.30 (dd, 1H, J_1 = 8.4 Hz, J_2 = 4.8 Hz, C_6-imidazo[4,5-b]pyridin-H), 4.41 (s, 2H, S-CH_2), 2.36 (s, 3H, Me), 1.85 (s, 6H, 2×Me). ^{13}C-NMR (100 MHz, DMSO-d_6, ppm) δ: 167.28 (N–C=O), 154.28, 149.37, 143.54, 141.81, 140.19, 139.41, 136.84 (2 × C), 135.39, 130.50, 129.74 (2 × C), 129.20, 126.09, 125.75, 125.28, 118.99, 115.72, 35.46 (CH_2-S), 21.20 (Me-Ph), 17.62 (2 × Me-Ph). ESI-MS: m/z 560.3 (M+1), 562.2 (M+3), 564.3 (M+5). $C_{23}H_{22}BrN_5O_3S_2$ (559.03).

4.1.2. General procedure for the synthesis of 2-(1-mesityl-1*H*-imidazo[4,5-c]pyridin-2-ylthio)-*N*-phenylacetamide (5b series)

4.1.2.1. General Procedure for the synthesis of N-mesityl-3-nitropyridin-4-amine (2b). Compound 4-chloro-3-nitropyridine **(1b)** (15.8 g, 0.1 mol), 2,4,6-trimethyl benzenamine (14.85 g, 0.11 mol), and $NaHCO_3$ (25.2 g, 0.3 mol) was dissolved in alcohol (250 mL) [26]. The reaction mixture was heated to reflux temperature with stirring and kept for 10 h and then continued the reaction at ambient tempreture for 12 h (monitored by TLC). The reaction liquid was evaporated under reduced pressure. Then, the residue was dissovled with a small amount CH_2Cl_2 and chromatographed on silica gel using ethyl acetate-petroleum ether system. Pure fractions were collected and concentrated, giving yellow solid ***2b***, yield: 57.8%, mp:133–139 °C. MS (ESI): m/z 258.12 (M+1). $C_{14}H_{15}N_3O_2$ (257.12).

4.1.2.2. General Procedure for the synthesis of 1-mesityl-1*H*-imidazo[4,5-c]pyridine-2-thiol (4b). With a similar procedure for compound **4a** [27], the 1-mesityl-1*H*-imidazo[4,5-c]pyridine-2-thiol (**4b**) was synthesized starting from *N*-mesityl-3-nitropyridin-4-amine (**2b**).

Compound **3b**: Brown powder, yield: 77.8%, mp:185–192 °C. MS (ESI): m/z 228.6 (M+1). $C_{14}H_{17}N_3$ (227.14). Compound **4b**: White powder, yield: 82.5%.

4.1.2.3. General Procedure for the synthesis of 2-(1-mesityl-1*H*-imidazo[4,5-c]pyridin-2-yl thio)-N-phenylacetamide (5b1-5b16). With a similar procedure for compounds **5a1–5a16** [28], the 2-(1-mesityl-1*H*-imidazo-[4,5-c]pyridin-2-ylthio)-N-phenylacetamides (**5b1–5b16**) were synthesized starting from 1-mesityl-1*H*-imidazo[4,5-c]pyridine-2-thiol (**4b**).

4.1.2.4. 2-(1-mesityl-1*H*-imidazo[4,5-c]pyridin-2-ylthio)-N-phenylacetamide (5b1). White crystals, yield: 59.1%. mp: 175–178 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 10.44 (s, 1H, NH), 8.92 (s, 1H, C_4-imidazo[4,5-c]pyridine-H), 8.27 (d, 1H, J = 5.4 Hz, C_6-imidazo[4,5-c]pyridine-H), 7.57 (d, 2H, J = 7.8 Hz, C_6-Ph′-H, C_2-Ph′-H), 7.31 (t, 2H, J = 7.8 Hz, C_5-Ph′-H, C_3-Ph′-H), 7.19 (s, 2H, $C_{3,5}$-Ph-H), 7.05 (m, 1H, C_4-Ph′-H), 6.98 (d, 1H, J = 5.4 Hz, C_7-imidazo[4,5-c]pyridine-H), 4.41 (s, 2H, S-CH_2), 2.37 (s, 3H, Me), 1.86 (s, 6H, 2 × Me). ESI-MS: m/z 403.5(M+1), $C_{23}H_{22}N_4OS$ (402.15).

4.1.2.5. N-(2-chlorophenyl)-2-(1-mesityl-1*H*-imidazo[4,5-c]pyridin-2-ylthio)acetamide (5b2). White crystals, yield: 63.3%. mp:176–178 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 10.05 (s, 1H, NH), 8.94 (s, 1H, C_4-imidazo[4,5-c]pyridine-H), 8.28 (d, 1H, J = 5.4 Hz, C_6-imidazo[4,5-c]pyridine-H), 7.81 (d, 1H, J = 7.2 Hz, C_6-Ph′-H), 7.48 (d, 1H, J = 8.4 Hz, C_3-Ph′-H), 7.32 (t, 1H, J = 7.2 Hz, C_5-Ph′-H), 7.18 (s, 3H, C_4-Ph′-H, $C_{3,5}$-Ph-H), 7.00 (d, 1H, J = 5.4 Hz, C_7-imidazo[4,5-c]pyridine-H), 4.41 (s, 2H, S-CH_2), 2.37 (s, 3H, Me), 1.85 (s, 6H, 2 × Me). ESI-MS: m/z 437.5(M+1), 439.5(M+3), 441.1(M+5), $C_{23}H_{23}ClN_4OS$ (436.11).

4.1.2.6. N-(2-fluorophenyl)-2-(1-mesityl-1*H*-imidazo[4,5-c]pyridin-2-ylthio)acetamide (5b3). Grey crystals, yield: 70.3%. mp:178–180 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 10.30 (s, 1H, NH), 8.92 (s, 1H, C_4-imidazo[4,5-c]pyridine-H), 8.28 (d, 1H, J = 5.4 Hz, C_6-imidazo[4,5-c]pyridine-H), 7.90 (m, 1H, C_6-Ph′-H), 7.27 (m, 1H, C_5-Ph′-H), 7.15 (m, 2H, C_4-Ph′-H, C_3-Ph′-H), 7.19 (s, 2H, $C_{3,5}$-Ph-H), 6.99 (d, 1H, J = 5.4 Hz, C_7-imidazo[4,5-c]pyridine-H), 4.43 (s, 2H, S-CH_2), 2.37 (s, 3H, Me), 1.85 (s, 6H, 2 × Me). ESI-MS: m/z 421.5 (M+1), 423.5 (M+3). $C_{23}H_{21}FN_4OS$ (420.14).

4.1.2.7. N-(2-bromophenyl)-2-(1-mesityl-1*H*-imidazo[4,5-c]pyridin-2-ylthio)acetamide (5b4). Grey solid, yield: 69,0%. mp:179–181 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 9.96 (s, 1H, NH), 8.96 (s, 1H, C_4-imidazo[4,5-c]pyridine-H), 8.28 (d, 1H, J = 5.4 Hz, C_6-imidazo[4,5-c]pyridine-H), 7.71 (dd, 1H, J_1 = 7.2 Hz, J_2 = 1.2 Hz, C_6-Ph′-H), 7.65 (d, 1H, J = 7.2 Hz, C_3-Ph′-H), 7.37 (t, 1H, J = 7.2 Hz, C_5-Ph′-H), 7.18 (s, 2H, $C_{3,5}$-Ph-H), 7.12 (t, 1H, J = 7.2 Hz, C_4-Ph′-H), 7.00 (d, 1H, J = 5.4 Hz, C_7-imidazo[4,5-c]pyridine-H), 4.40 (s, 2H, S-CH_2), 2.37 (s, 3H, Me), 1.85 (s, 6H, 2 × Me). ESI-MS: m/z 481.4 (M+1), 483.3 (M+3), 485.6 (M+5). $C_{23}H_{21}BrN_4OS$ (480.06).

4.1.2.8. N-(2-bromo-4-methylphenyl)-2-(1-mesityl-1*H*-imidazo[4,5-c]pyridin-2-ylthio)acetamide(5b5). White crystals, yield: 75.5%. mp:191–195 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 9.88 (s, 1H, NH), 8.96 (s, 1H, C_4-imidazo[4,5-c]pyridine-H), 8.31 (d, 1H, J = 5.4 Hz, C_6-imidazo[4,5-c]pyridine-H), 7.55 (d, 1H, J = 7.2 Hz, C_6-Ph′-H), 7.45 (s, 1H, C_3-Ph′-H), 7.17 (s, 3H, C_5-Ph′-H, $C_{3,5}$-Ph-H), 6.98 (d, 1H, J = 5.4 Hz, C_7-imidazo[4,5-c]pyridine-H), 4.37 (s, 2H, S-CH_2), 2.37 (s, 3H, Me), 2.27 (s, 3H, Me), 1.85 (s, 6H, 2 × Me). ESI-MS: m/z 495.3 (M+1), 497.5 (M+3), 599.2 (M+5). $C_{24}H_{25}BrN_4OS$ (494.08).

4.1.2.9. 2-(1-mesityl-1*H*-imidazo[4,5-c]pyridin-2-ylthio)-N-(4-methyl-2-nitrophenyl)acetamide (5b6). Yellow crystals, yield: 71.1%. mp: 221–223 °C. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ: 10.72 (s, 1H, NH), 8.94 (s, 1H, C_4-imidazo[4,5-c]pyridine-H), 8.29 (d, 1H, J = 5.4 Hz, C_6-imidazo[4,5-c]pyridine-H), 7.82 (s, 1H, C_3-Ph′-H), 7.73 (d, 1H, J = 8.4 Hz, C_5-Ph′-H), 7.55 (d, 1H, J = 8.4 Hz, C_6-Ph′-H), 7.18 (s, 2H, $C_{3,5}$-Ph-H), 6.99 (d, 1H, J = 5.4 Hz, C_7-imidazo[4,5-c]pyridine-H), 4.38 (s, 2H, S-CH_2), 2.37 (s, 3H, Me), 2.36 (s, 3H, Me) 1.85 (s, 6H, 2 × Me). ESI-MS: m/z 462.5 (M+1), 464.3 (M+3). $C_{24}H_{25}N_5O_3S$ (461.15).

4.1.2.10. 2-(1-mesityl-1*H*-imidazo[4,5-c]pyridin-2-ylthio)-N-(2-nitrophenyl)acetamide (5b7). Yellow crystals, yield: 67.1%. mp:192–195 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 10.82 (s, 1H, NH), 8.93 (s, 1H, C_4-imidazo[4,5-c]pyridine-H), 8.27 (d, 1H, J = 4.8 Hz, C_6-imidazo[4,5-c]pyridine-H), 7.98 (dd, 1H, J_1 = 7.8 Hz, J_2 = 1.2 Hz, C_3-Ph′-H), 7.82 (dd, 1H, J_1 = 7.8 Hz, J_2 = 0.6 Hz, C_6-Ph′-H), 7.73 (t, 1H, J = 7.8 Hz, C_5-Ph′-H), 7.38 (t, 1H, J = 7.8 Hz, C_4-Ph′-H), 7.18 (s, 2H, $C_{3,5}$-Ph-H), 6.99 (d, 1H, J = 4.8 Hz, C_7-imidazo[4,5-c]pyridine-H), 4.44 (s, 2H, S-CH_2), 2.50 (s, 3H, Me), 1.85 (s, 6H, 2 × Me). ESI-MS: m/z 448.5 (M+1).$C_{23}H_{21}N_5O_3S$ (447.14).

4.1.2.11. N-(4-acetyl-2-bromophenyl)-2-(1-mesityl-1*H*-imidazo[4,5-c]pyridin-2-ylthio)acetamide (5b8). White crystals, yield: 70.7%. mp:242–244 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 10.09 (s, 1H, NH), 8.98 (s, 1H, C_4-imidazo[4,5-c]pyridine-H), 8.28 (d, 1H, J = 4.8 Hz, C_6-imidazo[4,5-c]pyridine-H), 8.17 (s, 1H, C_3-Ph′-H), 7.96 (d, 1H, J = 1.8 Hz, C_6-Ph′-H), 7.95 (d, 1H, J = 1.8 Hz, C_5-Ph′-H), 7.19 (s, 2H, $C_{3,5}$-Ph-H), 7.00 (d, 1H, J = 4.8 Hz, C_7-imidazo[4,5-c]pyridine-H), 4.44 (s, 2H, S-CH_2), 2.50 (s, 3H, $COCH_3$), 2.37 (s, 3H, Me), 1.85 (s, 6H, 2×Me). ^{13}C NMR (100 MHz, DMSO-d_6, ppm) δ: 196.36 (Ph′–C═O), 167.18 (N–C═O), 154.82, 142.74, 141.28, 140.66, 140.58, 140.47, 140.32, 136.64 (2 × C), 134.79, 133.12, 130.14, 128.79 (2 × C), 128.75, 124.48, 115.62, 105.16, 36.18 (CH_2-S), 27.09 (Me-C═O), 21.22 (Me-Ph), 17.39 (2×Me-Ph). ESI-MS: m/z 523.5 (M+1), 525.5 (M+3). $C_{25}H_{23}BrN_4O_2S$ (522.07)

4.1.2.12. N-(2-chloropyridin-3-yl)-2-(1-mesityl-1*H*-imidazo[4,5-c]pyridin-2-ylthio)acetamide (5b9). White crystals, yield: 56.2%. mp:220–223 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 10.22 (s, 1H, NH), 8.94 (s, 1H, C_4-imidazo[4,5-c]pyridine-H), 8.29 (d, 1H, J = 5.4 Hz, C_6-imidazo[4,5-c]pyridine-H), 8.25 (dd, 1H, J_1 = 7.8 Hz, J_2 = 1.8 Hz, C_6-pyridine-H), 8.20 (dd, 1H, J_1 = 4.8 Hz, J_2 = 1.8 Hz, C_4-pyridine-H), 7.44 (dd, 1H, J_1 = 7.8 Hz, J_2 = 4.8 Hz, C_5-pyridine-H), 7.19 (s, 2H, $C_{3,5}$-Ph-H), 7.00 (s, 1H, J = 5.4 Hz, C_7-imidazo[4,5-c]pyridine-H), 4.44 (s, 2H, S-CH_2), 2.50 (s, 3H, Me), 1.85 (s, 6H, 2 × Me). ^{13}C NMR (100 MHz, DMSO-d_6, ppm) δ: 167.34 (N-C=O), 154.88, 145.74, 142.72, 142.66, 141.25, 140.64, 140.59, 140.21, 136.65 (2 × C), 133.43, 132.25, 130.12, 128.80 (2×C), 123.99, 105.14, 36.01 (CH_2-S), 21.21 (Me-Ph), 17.37 (2 × Me-Ph). ESI-MS: m/z 438.5 (M+1). $C_{22}H_{20}ClN_5OS$ (437.11).

4.1.2.13. N-(2,4-dichlorophenyl)-2-(1-mesityl-1*H*-imidazo[4,5-c]pyridin-2-ylthio)acetamide (5b10). White crystals, yield: 63.7%. mp:250–254 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 10.11 (s, 1H, NH), 8.32 (s, 1H, C_4-imidazo[4,5-c]pyridine-H), 8.28 (d, 1H, J = 5.4 Hz, C_6-imidazo[4,5-c]pyridine-H), 7.84 (d, 1H, J = 8.4 Hz, C_6-Ph′-H), 7.67 (d, 1H, J = 1.8 Hz, C_3-Ph′-H), 7.42 (dd, 1H, J_1 = 8.4 Hz, J_2 = 1.8 Hz, C_5-Ph′-H), 7.18 (s, 2H, $C_{3,5}$-Ph-H), 6.99 (d, 1H, J = 5.4 Hz, C_7-imidazo[4,5-c]pyridine-H), 4.41 (s, 2H, S-CH_2), 2.37 (s, 3H, Me), 1.84 (s, 6H, 2 × Me). ESI-MS: m/z 471.8 (M+1). $C_{23}H_{20}Cl_2N_4OS$ (470.07).

4.1.2.14. 2-(1-mesityl-1*H*-imidazo[4,5-c]pyridin-2-ylthio)-N-o-tolylacetamide (5b11). White solid, yield: 68.5%. mp:153–156 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 10.70 (s, 1H, NH), 8.93 (s, 1H, C_4-imidazo[4,5-c]pyridine-H), 8.27 (d, 1H, J = 4.8 Hz, C_6-imidazo[4,5-c]pyridine-H), 7.81 (s, 1H, C_6-Ph′-H), 7.68 (d, 1H, J = 8.4 Hz, C_5-Ph′-H), 7.54 (m, 2H, C_4-Ph′-H, C_3-Ph′-H), 7.18 (s, 2H, $C_{3,5}$-Ph-H), 6.98 (d, 1H, J = 4.8 Hz, C_7-imidazo [4,5-c]pyridine-H), 4.36 (s, 2H, S-CH_2), 2.36 (s, 6H, 2×Me), 1.84 (s, 6H, 2 × Me). ESI-MS: m/z 417.6 (M+1). $C_{24}H_{26}N_4OS$ (416.17).

4.1.2.15. Methyl3-bromo-4-(2-(1-mesityl-1*H*-imidazo[4,5-c]pyridin-2-ylthio)acetamido)benzoate (5b12). White crystals, yield: 69.7%. mp:202–205 °C. ^{1}H NMR (600 MHz, DMSO-d_6,

ppm) δ: 10.10 (s, 1H, NH), 8.97 (s, 1H, C_4-imidazo[4,5-c]pyridine-H), 8.28 (d, 1H, J = 5.4 Hz, C_6-imidazo[4,5-c]pyridine-H), 8.14 (d, 1H, J = 1.8 Hz, C_3-Ph′-H), 8.03 (d, 1H, J = 8.4 Hz, C_6-Ph′-H), 7.94 (dd, 1H, J_1 = 8.4 Hz, J_2 = 1.8 Hz, C_5-Ph′-H), 7.19 (s, 2H, $C_{3,5}$-Ph-H), 6.99 (d, 1H, J = 5.4 Hz, C_7-imidazo[4,5-c]pyridine-H), 4.44 (s, 2H, S-CH_2), 3.34 (s, 3H, OCH_3), 2.37 (s, 3H, Me), 1.85 (s, 6H, 2 × Me). ESI-MS: m/z 539.4 (M+1), 541.4 (M+3), 543.5 (M+5). $C_{25}H_{23}BrN_4O_3S$ (538.07).

4.1.2.16. Ethyl3-bromo-4-(2-(1-mesityl-1*H*-imidazo[4,5-c]pyridin-2-ylthio)acetamido)benzoate (5b13). White crystals, yield: 56.7%. mp:201–203 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 10.09 (s, 1H, NH), 8.97 (s, 1H, C_4-imidazo[4,5-c]pyridine-H), 8.28 (d, 1H, J = 5.4 Hz, C_6-imidazo[4,5-c]pyridine-H), 8.14 (d, 1H, J = 1.8 Hz, C_3-Ph′-H), 8.02 (d, 1H, J = 8.4 Hz, C_6-Ph′-H), 7.94 (dd, 1H, J_1 = 8.4 Hz, J_2 = 1.8 Hz, C_5-Ph′-H), 7.19 (s, 2H, $C_{3,5}$-Ph-H), 6.99 (d, 1H, J = 4.8 Hz, C_7-imidazo[4,5-c]pyridine-7H), 4.44 (s, 2H, S-CH_2), 4.30 (q, 2H, J = 6.6 Hz, $COCH_2$), 2.37 (s, 3H, Me), 1.85 (s, 6H, 2 × Me), 1.31 (t, 3H, J = 6.6 Hz, Me). ESI-MS: m/z 553.4 (M+1), 555.4 (M+3). $C_{26}H_{25}BrN_4O_3S$ (552.08).

4.1.2.17. N-(2-chloro-4-nitrophenyl)-2-(1-mesityl-1*H*-imidazo[4,5-c]pyridin-2-ylthio)acetamide (5b14). Grey crystals, yield: 71.2%. mp:245–247 °C. ^{1}H NMR (400 MHz, DMSO-d_6, ppm) δ: 10.41 (s, 1H, NH), 8.94 (s, 1H, C_4-imidazo[4,5-c]pyridine-H), 8.38 (d, 1H, J = 2.4 Hz, C_6-imidazo[4,5-c]pyridine-H), 8.31(d, 1H, J = 1.2 Hz, C_5-Ph′-H), 8.29 (s, 1H, C_3-Ph′-H), 8.22 (d, 1H, J = 1.2 Hz, C_6-Ph′-H), 7.19 (s, 2H, $C_{3,5}$-Ph-H), 8.24 (d, 1H, J = 2.4 Hz, C_7-imidazo[4,5-c]pyridine-H), 4.50 (s, 2H, S-CH_2), 2.37 (s, 3H, Me) 1.85 (s, 6H, 2 × Me). ^{13}C NMR (100 MHz, DMSO-d_6, ppm) δ: 167.13 (N–C═O), 154.29, 143.17, 142.23, 141.20, 140.73, 140.13, 139.97, 139.66, 136.07 (2 × C), 129.58 (2 × C), 128.20, 124.90, 123.87, 123.28, 122.87, 104.62, 35.69 (CH_2-S), 20.65 (Me-Ph), 16.79 (2×Me-Ph). ESI-MS: m/z 482.2 (M+1), 484.12 (M+3). $C_{23}H_{20}ClN_5O_3S$ (481.1).

4.1.2.18. N-(3-bromo-5-methylpyridin-2-yl)-2-(1-mesityl-1*H*-imidazo[4,5-c]pyridin-2-ylthio)acetamide (5b15). White crystals, yield: 60.0%. mp:155-157 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 10.41 (s, 1H, NH), 8.95 (s, 1H, imidazo[4,5-c]pyridine-4H), 8.38 (d, 1H, J = 5.4 Hz, imidazo[4,5-c]pyridine-6H), 8.29 (m, 2H, pyridine-H), 7.19 (s, 2H, PhH), 7.00 (d, 1H, J = 5.4 Hz, imidazo[4,5-c]pyridine-7H), 4.49 (s, 2H, S-CH_2), 2.37 (s, 3H, Me), 1.85 (s, 6H, 2 × Me). ESI-MS: m/z 496.3 (M+1), 498.4 (M+3), 500.2 (M+5). $C_{23}H_{22}BrN_5OS$ (495.07).

4.1.2.19. N-(4-sulfonamide-2-bromophenyl)-2-(1-mesityl-1*H*-imidazo[4,5-c]pyridin-2-ylthio)acetamide (5b16). White crystals, yield: 62.2%. mp:190–192 °C. ^{1}H NMR (600 MHz, DMSO-d_6, ppm) δ: 10.15 (s, 1H, NH), 8.97 (s, 1H, C_4-imidazo[4,5-c]pyridine-H), 8.28 (d, 1H, J = 5.4 Hz, C_6-imidazo[4,5-c]pyridine-H), 8.04 (s, 1H, C_3-Ph′-H), 7.98 (d, 1H, J = 8.4 Hz, C_5-Ph′-H), 7.79 (d, 1H, J = 8.4 Hz, C_6-Ph′-H), 7.46 (s, 2H, $C_{3,5}$-Ph-H), 7.00 (d, 1H, J = 5.4 Hz, C_7-imidazo[4,5-c]pyridine-H), 4.44 (s, 2H, S-CH_2), 2.50 (s, 3H, Me), 1.85 (s, 6H, 2×Me). ^{13}C-NMR (100 MHz, DMSO-d_6, ppm) δ: 167.14 (N-C=O), 154.82, 142.72, 141.87, 141.27, 140.65, 140.60, 140.30, 139.39, 136.65 (2×C), 130.51, 130.13, 128.79 (2×C), 126.06, 125.54, 115.97, 105.14, 36.09 (CH_2-S), 21.22 (Me-Ph), 17.40 (2 × Me-Ph). ESI-MS: m/z 560.3 (M+1), 562.2 (M+3), 564.3 (M+5). $C_{23}H_{22}BrN_5O_3S_2$ (559.03).

4.2. In vitro anti-HIV assay

The methodology of the anti-HIV assay has been previously described.[30,31] Stock solutions (10 × final concentration) of test compounds were added in 25-μL volumes to two series of triplicate wells to allow simultaneous evaluation of their effects on mock- and HIV-infected cells at the beginning of each experiment. Serial fivefold dilutions of test compounds were made directly in flat-bottomed 96-well microtiter trays using a Biomek 3000 robot (Beckman instruments, Fullerton, CA). Untreated control HIV- and mock-infected cell samples were included for each sample.

HIV-1(III_B)[32] or HIV-2 (ROD)[33] stock (50 μL) at 100–300 $CCID_{50}$ (cell culture infectious dose) or culture medium was added to either the infected or mock-infected wells of the microtiter tray. Mock-infected cells were used to evaluate the effect of test compound on uninfected cells in order to assess the cytotoxicity of the test compound. Exponentially growing MT-4 cells[34] were centrifuged for 5 min at 1000 rpm and the supernatant was discarded. The MT-4 cells were resuspended at 6×10^5 cells/mL, and 50-μL volumes were transferred to the microtiter tray wells. Five days after infection, the viability of mock- and HIV-infected cells was examined spectrophotometrically by the MTT assay.

The MTT assay is based on the reduction of yellow colored 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Acros Organics, Geel, Belgium) by mitochondrial dehydrogenase of metabolically active cells to a blue-purple formazan that can be measured spectrophotometrically. The absorbances were read in an eight-channel computer-controlled photometer (Multiscan Ascent Reader, Labsystems, Helsinki, Finland), at two wavelengths (540 and 690 nm). All data were calculated using the median OD (optical density) value of three wells. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration of the test compound that reduced the absorbance (OD540) of the mock-infected control samples by 50%. The 50% effective concentration (EC_{50}) was defined as the compound concentration required for inhibiting virus-induced syncytium formation by 50%.

4.3. HIV-1 RT inhibition assay

Inhibition of HIV-1 RT was developed using nucleotides linked to microtiter plate with colorimetric detection of incorporated biotin-dUTP into homopolymer template primers.[35] The incorporated quantities of biotin-dUTP into the enzyme represented the activity of HIV-1 RT. IC_{50} values corresponded to the concentration of the imidazopyridinylthioacetanilide derivatives required to inhibit biotin-dUTP incorporation into the HIV-1 RT by 50%.

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