Synthesis of a Novel Series of Imidazo[1,2-α]pyridines as Acyl-CoA: Cholesterol Acyltransferase (ACAT) Inhibitors

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A novel series of imidazo[1,2-α]pyridines was designed, synthesized, and tested for their ability to inhibit acyl-CoA:cholesterol acyltransferase. Preliminary lead optimization efforts resulted in the identification of ACAT inhibitors represented by analogues **5b**, **5c**, **6a**, **6c**, **7b**, and **7c**. The ACAT inhibitory activity of these compounds was further established by potent inhibition of cholesteryl ester formation in HepG2 cells by a representative analogue **7b**.

Key Words: Imidazo[1,2-α]pyridines, Acyl CoA: cholestrol acyl transferase (ACAT), HepG2 cells, Structure-activity relationship

Introduction

Acyl-CoA:cholesterol acyltransferase (ACAT) is a microsomal enzyme that catalyzes biotransformation of free cholesterol to cholesterol esters.[1] Accumulation of cholesterol ester brings about the formation of foam cells from macrophages in the arterial walls, which is a hallmark of atherosclerosis lesions.[2,3] Inhibition of ACAT enzyme activity would therefore reduce plasma cholesterol levels by blocking intestinal cholesterol absorption.[4] Thus, ACAT represents an attractive target for therapeutics designed to have potent hypocholesterolemic and antiarteriosclerotic properties. As a result, considerable efforts have been devoted in recent years to the discovery and development of structurally diversed compounds showing potent ACAT inhibitory activity.[5,6]

Imidazo[1,2-a]pyridines, a novel class of pharmaceutical compounds exhibit a broad range of biological activities.[7] Besides, imidazo[1,2-a]pyridine scaffold is found in a number of marketed drug formulations, such as zolimidine (an antiulcer drug), zolpidem (ahypnotic drug), and alpidem (a nonsedative anxiolytic)(Figure 1).[8] As a result, numerous reports have described the structural modifications of this scaffold with the aim of developing novel therapeutic agents. In view of these findings and with the objective to develop a potent ACAT inhibitor, we performed the synthesis of a new series of imidazo[1,2-a] pyridines and evaluation of their ACAT inhibitory activity. Herein, we describe our preliminary lead optimization efforts culminating in the identification of a novel series of imidazo [1,2-a]pyridines as potent ACAT inhibitors.

Various 2 and 6-substituted imidazo[1,2-a]pyridines, **5a-k**, **6a-i** and **7a-c** were synthesized as outlined in Schemes 1-3. The most common approach for the synthesis of imidazo-[1,2-a]pyridines involves the condensation of α-haloketones with 2-aminopyridines.[9] Accordingly, the synthesis of initial

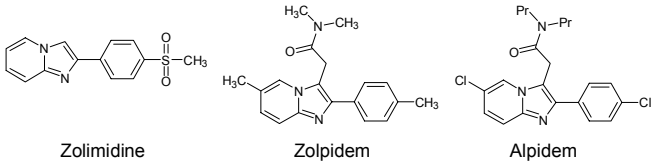


Figure 1. Pharmaceutical compounds with imidazo[1,2-a]pyridine scaffold.

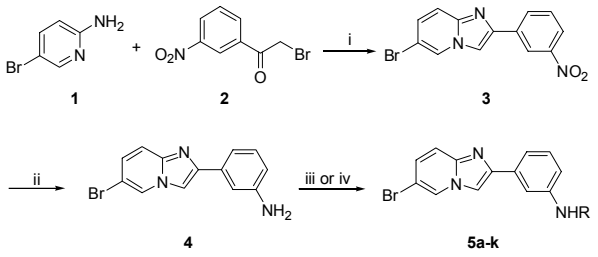
series of imidazo[1,2-a]pyridine analogues **5a-k** were obtained starting from 2-amino-5-bromo pyridine and 2-bromo-1-(3-nitrophenyl)ethanone (Scheme 1). Thus, coupling of **1** with **2** under reflux conditions in a mixture of acetone and ethanol gave **3** in good yield. Subsequent reduction of the nitro group with tin chloride provided the corresponding amino derivative **4** in 82% yield.

For the purpose of preliminary structure activity relationship studies, it was chosen to derivatize the amino group whilst keeping the other end group halogen intact. Consequently, coupling of **4** with suitable benzoic acids using appropriate coupling agents such as PyBOP, HATU or EDC in the presence of Hunig's base furnished the corresponding amide analogues **5a-i** in moderate to excellent yields. Further reaction of **4** with appropriate sulfonyl chlorides in presence of Hunig's base afforded the remaining amide derivatives **5j** and **5k**.

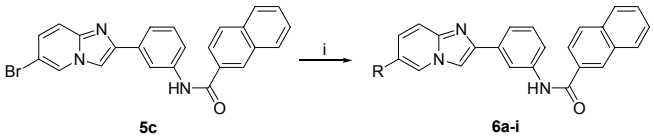
After elaboration of the amino group, we set out to explore the derivatization of halogen group. Thus, compound **5c**, moderate ACAT inhibitor amongst **5a-k** series was subjected to Suzuki cross coupling with appropriate boronic acids to yield the desired cross-coupled products **6a-i** (Scheme 2) in modest to high yields.

As shown in Scheme 3, further reaction of phenol **6i** with trichloroacetyl isocyanate and ethyl chloroacetate furnished corresponding amide **7a** and ester **7b** derivatives, respectively. Subsequent alkaline hydrolysis of **7b** afforded the respective acid analogue **7c** in good yield (Scheme 3).

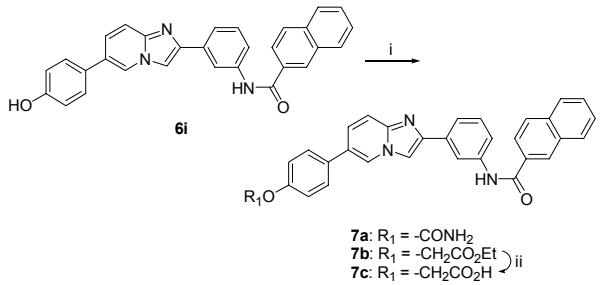
[a]These authors equally contributed to this work.



Scheme 1. [a]Reagents and conditions: (i) acetone: absolute ethanol (1:1), reflux, 12 h; (ii) $SnCl_2$, MeOH, reflux, 12 h; (iii) RCO_2H, PyBOP, DIPEA, DMF, rt, 12 h for **5a**, **5h** and **5i**; HATU, DIPEA, DMF, rt, 12 h for **5b** and **5e**; HBTU, DIPEA, DMF, rt, 12 h for **5c**; EDC, HOAt, DIPEA, DMF, rt, 12 h for **5d**; EDC, HOBt, DIPEA, DMF, rt, 12 h for **5f** and **5g**; (iv) RSO_2Cl, TEA, CH_2Cl_2, 0 °C, 2 h for **5j** and **5k**.



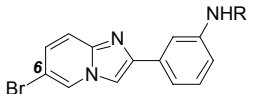
Scheme 2. [a]Reagents and conditions: (i) $RB(OH)_2$, $Pd(PPh_3)_4$, $NaHCO_3$, DME, H_2O, reflux, 12 h.



Scheme 3. [a]Reagents and conditions: (i) trichloroacetyl isocyanate, CH_2Cl_2 , 0 to 25 °C, 2 h for **7a**; ethyl chloroacetate, K_2CO_3, DMF, 25 °C, 12 h for **7b**; (ii) $LiOH{\cdot}H_2O$, THF, H_2O, 25 °C, 2 h.

The newly synthesized compounds were evaluated in vitro for their potential to inhibit human macrophage ACAT activity using a cell-based reporter assay in human HepG2 cell lines and the results are tabulated as IC_{50} values in Table 1 and 3. All the assays were performed under standard assay conditions by employing the previously described assay protocol.[10] Pipercide, a known ACAT inhibitor, was used as a reference standard for comparison, which displayed potent ACAT inhibitory activity with an IC_{50} of 3.7 μM.[10] In order to establish the preliminary structure activity relationship studies, compound **4** which is amenable for easy derivatization at both the ends was chosen as a starting template for the generation of a new series of ACAT inhibitors. At first, we explored the derivatization of amino group of **4** whilst maintaining halogen substituent on 6-poisition of pyridine ring. Thus, compounds **5a-k** were obtained by the reaction of **4** with appropriate benzoic acids/sulfonyl chlorides and the *in vitro* ACAT inhibitory potencies of these compounds are presented in Table 1. Of these, naphthamide analogues **5b** and **5c** exhibited signi-

Table 1. *In-vitro* ACAT inhibitory activities of 6-bromo-imidazopyridine derivatives **5a-k**.



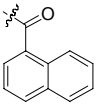
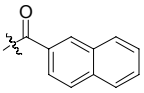
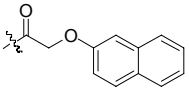
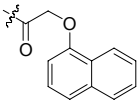
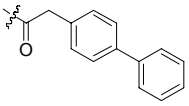
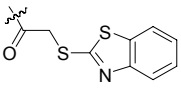
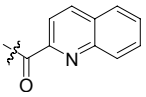
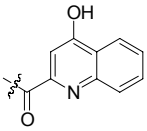
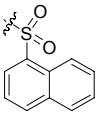
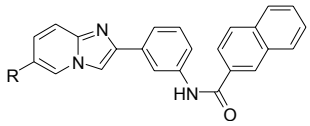
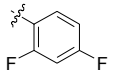
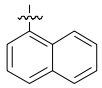
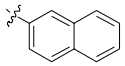
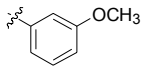
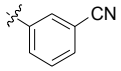
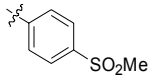
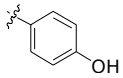
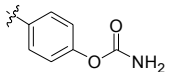
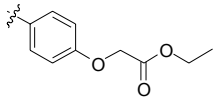
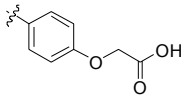
Compound	R	ACAT Inhibition % at 25 μg/mL
5a		< 30
5b		48
5c		56
5d		< 30
5e		< 30
5f		< 30
5g		< 30
5h		< 30
5i		< 30
5j		< 30
5k		< 30

Table 2. Dose dependent in-vitro ACAT inhibitory activity of compound **5c**.

Concentration (μM)	ACAT inhibition (%)
100	76.34
30	50.33
10	26.60
3	15.45
1	15.17

Table 3. *In-vitro* ACAT inhibitory activities of 6-substituted-imidazopyridine analogues **6a-i** and **7a-c**.



Compound	R	ACAT Inhibition IC_{50} (μM)
6a		23.6
6b		> 30
6c		7.1
6d		> 30
6e		> 30
6f		> 30
6g		> 30
6h		> 30
6i		> 30
7a		> 30
7b		8.7
7c		15
Pipercide[10]		3.7

ficant ACAT inhibitory activity of 48% and 56% at the concentration of 25 μg/mL, respectively. As shown in Table 2, compound **5c** inhibited the ACAT activity in a dose dependent manner. On the other hand, all of the other derivatives displayed weak inhibitory activity.

Table 4. Dose dependent ACAT inhibitory activity of compound **7b**.

Compound **7b** (μM)	ACAT inhibition (%)
100	86.13
30	74.95
10	54.29
3	31.98
1	19.23

In view of the significant potency of compound **5c**, we prepared more analogues of **5c** by introducing various aryl groups at 6-position of pyridine ring while retaining the 2-naphthamide moiety on 3-phenyl ring. Thus, compounds **6a-i** and **7a-c** were obtained as described in Schemes 2 and 3, respectively, and in vitro inhibitory potencies of these compounds are tabulated in Table 3. Interestingly, this modification provided potent inhibitors represented by analogues **6a** and **6c**. In general, introduction of nonpolar aromatic ring at 6-position of pyridine ring such as 2,4-difluorobenzene, and 2-naphthalene showed inhibitory activity of ACAT. However, polar residues such as phenol, *p*-methylsulfonylbenzene, *m*-cyanobenzene did not exhibited the inhibitory activity. Likewise, absence of the inhibitory activity of phenolic analogue may be reasoned due to polar hydroxyl group. Therefore, the inhibitory activity was induced by masking of the hydroxyl group of **6i** as shown in the case of **7b**, which displayed significant ACAT inhibitory activity with IC_{50} value of 8.7 μM. Compound **7b** inhibited ACAT activity in a dose dependent manner and 86% inhibition was observed at 100 μM. The corresponding acid **7c** showed two fold decreased inhibitory activity in comparison with **7b** and carbamate **7a** lost the inhibitory activity.

To confirm the ACAT inhibitory potency of imidazo[1,2-a]pyridines, **7b** was chosen for further evaluation. Accordingly, this analogue was evaluated by Western blot analysis for its potential to inhibit ACAT activity in HepG2 cells. As shown in Figure 2, compound **7b** exhibited complete inhibition at 30 μM with an IC_{50} value of 2.02 μM in a dose dependent manner confirming the ACAT inhibitory property of this compound.

Several studies have demonstrated that ACAT inhibitors reduce the plasma cholesterol levels by blocking cholesterol absorption in animal models. Among the current series, compound **7b** was therefore investigated for its inhibitory potency of cholesterol ester formation in HepG2 cells. As shown in Table 5, compound **7b** significantly reduced the cholesterol ester formation in HepG2 cells in a dose-dependent manner. This data further proves the hypothesis reported in the earlier reports that ACAT inhibitors reduce the plasma cholesterol levels. However, more detailed studies are required to establish the mechanism of action of these inhibitors.

In conclusion, a novel series of various 2 and 6-substituted imidazo[1,2-a]pyridines were prepared and evaluated for their ability to inhibit ACAT activity. Preliminary lead optimization efforts resulted in the identification of potent ACAT inhibitors represented by analogues **6a**, **6c**, **7b** and **7c**. The ACAT inhibitory activity of these compounds was further

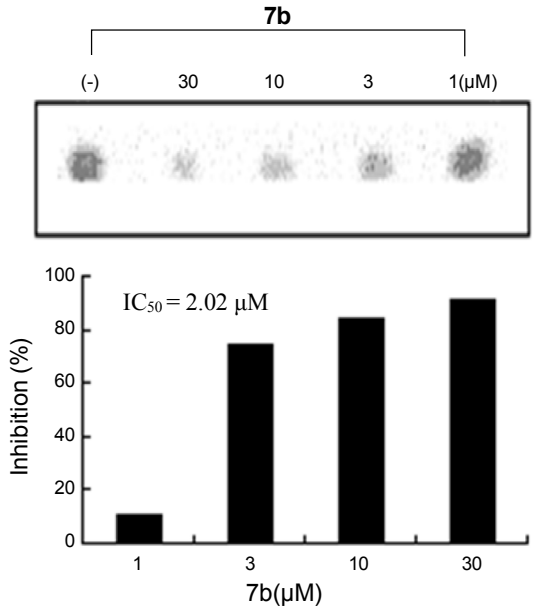


Figure 2. Analysis for ACAT inhibitory activity of compound **7b** in hepG2 cells.

established by potent inhibition of ACAT activity in HepG2 cell line by a representative analogue **7b** which exhibited ACAT inhibition in a dose dependent manner. Based on these results, this analogue was further investigated for its ability to reduce cholesterol ester formation in HepG2 cells. Interestingly, compound **7b** significantly reduced the cholesterol ester formation in a dose-dependent manner and further investigations are necessary to know the mechanism of action of these inhibitors.

Experimental Section

All of the commercial chemicals and solvents are of reagent grade and were used without further purification. All reactions were carried out under an atmosphere of dried argon, in flame-dried glassware. Proton nuclear magnetic resonance (^{1}H NMR) spectra were determined on a Varian (300 MHz) spectrometer. Chemical shifts are provided in parts per million (ppm) downfield from tetramethylsilane (internal standard) with coupling constants in hertz (Hz). Multiplicity is indicated by the following abbreviations: singlet (s), doublet (d), doublet of doublet (dd), triplet (t), pseudo triplet (ps-t), quartet (q), multiplet (m), broad (br). Mass spectra were recorded on a Finnigan ESI mass spectrometer and HRMS (EI-MS) was obtained on a JMS-700 (Jeol, Japan). Products from all reactions were purified to a minimum purity of 96% as determined by HPLC, either by flash column chromatography using silica gel 60 (230-400 mesh Kieselgel 60) or by preparative thin layer chromatography using glass-backed silica gel plates (1 mm thickness) unless otherwise indicated. Additionally, thin-layer chromatography on 0.25 mm silica plates (E. Merck, silica gel 60 F254) was used to monitor reactions. The chromatograms were visualized using ultraviolet illumination, exposure to iodine vapors, dipping in PMA or Hanessian's solution. The purity of the products was checked by reversed phase high-pressure liquid chromatography (RP-HPLC), which was performed either on Dionex Corp. HPLC system or on Waters Corp. HPLC system equipped with a UV detector set at 254 nm. The mobile phases used were A: H2O containing 0.05% TFA, and B: CH_3CN. The HPLC employed an YMC Hydrosphere C18 (HS-302) column (5 μ particle size, 12 nM pore size), 4.6 mm dia. × 150 mm with a flow rate of 1.0 mL/min. Compound purity was assessed using one of the following methods, Method A: gradient 20% B to 100% B in 20 min (Waters Corp. HPLC system); Method B: gradient 20% B to 100% B in 30 min (Dionex Corp. HPLC system).

6-Bromo-2-(3-nitro-phenyl)-imidazo[1,2-a]pyridine (3): A solution of 5-bromo-pyridin-2-ylamine (**1**) (779 mg, 4.50 mmol) and 2-bromo-1-(3-nitro-phenyl)-ethanone (**2**) (732 mg, 3.00 mmol) in acetone and ethanol (20 mL, 1:1) was refluxed overnight. The reaction mixture was concentrated at reduce pressure and then partitioned between ethyl acetate and brine. The organic phase was dried ($MgSO_4$), and concentrated. Purification by silica gel column chromatography (CH_2Cl_2: MeOH = 30:1) gave 6-bromo-2-(3-nitro-phenyl)-imidazo[1,2-a]pyridine as a yellow solid (719 mg, 75% yield): R_f = 0.58 (hexanes:EtOAc:MeOH = 6:3:1); ^{1}H-NMR (DMSO-d_6, 300 Hz) δ 8.91 (1H, s, aromatic), 8.76 (1H, m, aromatic), 8.58 (1H, d, J = 2.4 Hz, aromatic), 8.39 (1H, d, J = 7.5 Hz, aromatic), 8.18 (1H, d, J = 7.8 Hz, aromatic), 7.75 (1H, m, aromatic), 7.62 (1H, d, J = 9.9 Hz, aromatic), 7.43(1H, m, aromatic); MS (ESI) *m/z* 318 (M^++H), 316 (M-H); Purity > 99% (as determined by reverse phase HPLC, method A, t_R= 9.2 min).

3-(6-Bromo-imidazo[1,2-a]pyridin-2-yl)-phenylamine (4): A solution of 6-bromo-2-(3-nitro-phenyl)-imidazo[1,2-a] pyridine (**3**) (335 mg, 1.05 mmol) and $SnCl_2$ (1.19 g, 5.27 mmol) in MeOH (13 mL) was refluxed overnight. After solvent removal *in vacuo* and digestion in ethylacetate, saturated aqueous $NaHCO_3$ was added and the mixture was stirred overnight at room temperature. The mixture was filtered through Celite and organic layer was separated. The combined organic layer was washed with brine, dried ($MgSO_4$) and concentrated. Purification by silica gel column chromatography (hexanes: EtOAc: MeOH = 6:3:1) gave 3-(6-bromo-imidazo[1,2-a]pyridin-2-yl)-phenylamine as a yellow solid (248 mg, 82% yield): R_f = 0.20 (hexanes:EtOAc:MeOH = 6:3:1); ^{1}H-NMR (DMSO-d_6, 300 Hz) δ 8.86 (1H, d, J = 1.2 Hz, aromatic), 8.19 (1H, s, aromatic), 7.53 (1H, d, J = 9.0 Hz, aromatic), 7.33 (1H, dd, J = 9.3 & 1.8 Hz, aromatic), 7.22 (1H, s, aromatic), 7.07 (2H, m, aromatic), 6.53 (1H, m, aromatic), 5.16 (2H, brs, NH_2); MS (ESI) *m/z* 288 (M^++H); Purity > 96% (as determined by reverse

Table 5. Inhibitory Effects of compound **7b** on the cholesterol ester formation in HepG2 cells.[a]

Compd **7b** (μM)	Formation of cholesterol ester (μM/μg protein)
30	0.007
10	0.012
3	0.019
1	0.067
(-)	0.074

[a]HepG2 cells (1×10^5 cells/12 well plate); [1-^{14}C]oleic acid: 17.9 μM; Protein concentration: 0.04 ± 0.02 μg/μL; Incubation time: 6 h

phase HPLC, method A, t_R = 2.8 min).

General procedure for the preparation of 5a-i. To a solution of the 3-(6-bromo-imidazo[1,2-a]pyridin-2-yl)-phenylamine (**4**) (1 equiv) and appropriate carboxylic acids (1.5-2 equiv) in DMF was added benzotriazol-1-yl-*N*-oxy-tris(pyrrolidino)-phosphonium hexafluorophosphate (PyBOP) (2 equiv) and *N*,*N*-diisopropylethylamine (DIPEA) (2 equiv) for **5a**, **5h**, and **5i**, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*,*N*-tetramethyluronium hexafluorophosphate (HATU) (2 equiv) and *N*,*N*-diisopropylethylamine (DIPEA) (2 equiv) for **5b** and **5e**, *O*-benzotriazol-1-yl-*N*,*N*,*N*,*N*-tetramethyluronium hexafluorophosphate (HBTU) (3 equiv) and *N*,*N*-diisopropylethylamine (DIPEA) (3 equiv) for **5c**, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) (1.5 equiv), 1-hydroxy-7-azabenzotriazole hydrate (HOAt) (1.5 equiv), and *N*,*N*-diisopropylethylamine (DIPEA) (1.5 equiv) for **5d**, and 1-[3-(dimethyamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) (1.5 equiv), 1-hydroxylbenzotriazole hydrate (HOBt) (1.5 equiv), and *N*,*N*-diisopropylethylamine (DIPEA) (1.5 equiv) for **5f** and **5g**, respectively. The reaction mixture was stirred at room temperature overnight, and then partitioned between ethyl acetate and brine. The organic phase was dried ($MgSO_4$) and concentrated. Purification by silica gel column chromatography or recrystallization gave the desired products.

Thiophene-2-carboxylic acid [3-(6-bromo-imidazo[1,2-a]pyridin-2-yl)-phenyl]-amide (5a): Obtained by silica gel column chromatography (hexanes:EtOAc:MeOH = 15:3:1) as a white solid (63.5 mg, 69% yield): R_f = 0.39 (hexanes:EtOAc:MeOH = 6:3:1); ^{1}H-NMR (CD_3OD, 300 Hz) δ 8.43 (1H, s, aromatic), 7.98 (2H, m, aromatic), 7.86 (1H, m, aromatic), 7.77 (1H, d, J = 7.8 Hz, aromatic), 7.57 (2H, m, aromatic), 7.27-7.47 (3H, m, aromatic), 7.12 (1H, m, aromatic); MS(ESI) *m/z* 398 (M^++H), 396 (M-H); HRMS (EI) *m/z* calcd for $C_{18}H_{12}BrN_3OS$ [M^+] 396.9884, found: 396.9882; Purity > 99% (as determined by reverse phase HPLC, method A, t_R= 8.4 min).

Naphthalene-1-carboxylic acid [3-(6-bromo-imidazo[1,2-a]pyridin-2-yl)-phenyl]-amide (5b): Obtained by preparative TLC (CH_2Cl_2:MeOH = 20:1) as a yellow solid (212 mg, 62% yield): R_f = 0.45 (hexanes:EtOAc:MeOH = 6:3:1); ^{1}H-NMR (DMSO-d_6, 300 Hz) δ 10.7 (1H, s, NH), 8.91 (1H, d, J = 1.8 Hz, aromatic), 8.55 (1H, s, aromatic), 8.35 (1H, s, aromatic), 8.24 (1H, m, aromatic), 8.09 (1H, d, J = 8.7 Hz, aromatic), 8.03 (1H, m, aromatic), 7.79 (1H, m, aromatic), 7.73 (2H, m, aromatic), 7.58-7.66 (4H, m, aromatic), 7.45 (1H, ps-t, J = 7.8 Hz, aromatic), 7.38 (1H, dd, J = 9.6 & 1.8 Hz, aromatic); MS (ESI) *m/z* 442 (M^++H), 440 (M-H); HRMS (EI) *m/z* calcd for $C_{24}H_{16}BrN_3O$ [M^+] 441.0477, found: 441.0473; Purity > 99% (as determined by HPLC, method A, t_R= 10.1 min).

Naphthalene-2-carboxylic acid [3-(6-bromo-imidazo[1,2-a]pyridin-2-yl)-phenyl]-amide (5c): Obtained by recrystallization (CH_2Cl_2/MeOH) as a white solid (86.3 mg, 48% yield): R_f = 0.47 (hexanes:EtOAc:MeOH = 6:3:1); ^{1}H-NMR (DMSO-d_6, 300 Hz) δ 10.5 (1H, s, NH), 8.92 (1H, d, J = 1.8 Hz, aromatic), 8.64 (1H, s, aromatic), 8.51 (1H, s, aromatic), 8.35 (1H, s, aromatic), 8.01-8.12 (4H, m, aromatic), 7.83 (1H, m, aromatic), 7.58-7.72 (4H, m, aromatic), 7.45 (1H, ps-t, J = 7.8 Hz, aromatic), 7.39 (1H, dd, J = 9.9 Hz & 1.8 Hz, aromatic); MS (ESI) *m/z* 440 (M-H); HRMS (EI) *m/z* calcd for $C_{24}H_{16}BrN_3O$ [M^+] 441.0477, found: 441.0483; Purity > 99% (as determined by HPLC, method A, t_R = 10.7 min).

***N*-[3-(6-Bromo-imidazo[1,2-a]pyridin-2-yl)-phenyl]-2-(naphthalen-2-yloxy)-acetamide (5d):** Obtained by preparative TLC (CH_2Cl_2:MeOH = 15:1) as a yellow solid (33.0 mg, 18% yield): R_f = 0.48 (hexanes:EtOAc:MeOH = 6:3:1); ^{1}H-NMR (DMSO-d_6, 300 Hz) δ 10.2 (1H, s, NH), 8.89 (1H, d, J = 1.5 Hz, aromatic), 8.33 (2H, m, aromatic), 7.80-7.90 (4H, m, aromatic), 7.33-7.68 (9H, m, aromatic), 4.86 (2H, $COCH_2O$); MS (ESI) *m/z* 472 (M^++H), 470 (M-H); HRMS (EI) *m/z* calcd for $C_{25}H_{18}BrN_3O_2$ [M^+] 471.0582, found: 471.0585; Purity > 99% (as determined by HPLC, method A, t_R = 11.1 min).

***N*-[3-(6-Bromo-imidazo[1,2-a]pyridin-2-yl)-phenyl]-2-(naphthalen-1-yloxy)-acetamide (5e):** Obtained by silica gel column chromatography (hexanes:EtOAc:MeOH = 9:3:1) as a white solid (117 mg, 95% yield): R_f = 0.53 (hexanes:EtOAc:MeOH = 6 3:1); ^{1}H-NMR (DMSO-d_6, 300 Hz) δ 10.3 (1H, s, NH), 8.88 (1H, s, aromatic), 8.32-8.37 (3H, m, aromatic), 7.89 (1H, m, aromatic), 7.51-7.68 (6H, m, aromatic), 7.35-7.46 (3H, m, aromatic), 6.96 (1H, d, J = 7.2 Hz, aromatic), 4.95 (2H, s, $COCH_2O$); MS (ESI) *m/z* 472 (M^++H), 470 (M-H), HRMS (EI) *m/z* calcd for $C_{25}H_{18}BrN_3O_2$ [M^+] 471.0582, found: 471.0585; Purity > 99% (as determined by HPLC, method A, t_R = 11.3 min).

2-Biphenyl-4-yl-*N*-[3-(6-bromo-imidazo[1,2-a]pyridin-2-yl)-phenyl]-acetamide (5f): Obtained by recrystallization (CH_2Cl_2/MeOH) as a yellow solid (117 mg, 90% yield): R_f = 0.51 (hexanes:EtOAc:MeOH = 6:3:1); ^{1}H-NMR (DMSO-d_6, 300 Hz) δ 10.3 (1H, s, NH), 8.88 (1H, s, aromatic), 8.30 (2H, m, aromatic), 7.32-7.66 (14H, m, aromatic), 3.71 (2H, s, $COCH_2$); MS (ESI) *m/z* 482 (M^++H); HRMS (EI) *m/z* calcd for $C_{27}H_{20}BrN_3O$ [M^+] 481.0790, found: 481.0795; Purity > 99% (as determined by HPLC, method A, t_R = 11.5 min).

2-(Benzothiazol-2-ylsulfanyl)-*N*-[3-(6-bromo-imidazo[1,2-a]pyridin-2-yl)-phenyl]-acetamide (5g): Obtained by recrystallization (CH_2Cl_2/MeOH) as a white solid (50.0 mg, 39% yield): R_f = 0.35 (hexanes:EtOAc:MeOH = 6:3:1); ^{1}H-NMR (DMSO-d_6, 300 Hz) δ 10.5 (1H, s, NH), 8.87 (1H, d, J = 1.5 Hz, aromatic), 8.31 (2H, m, aromatic), 8.02 (1H, d, J = 8.1 Hz, aromatic), 7.84 (1H, d, J = 8.1 Hz, aromatic), 7.65 (1H, d, J = 7.2 Hz, aromatic), 7.35-7.58 (6H, m, aromatic), 4.42 (2H, s, $COCH_2O$); MS (ESI) *m/z* 495 (M^++H); HRMS (EI) *m/z* calcd for $C_{22}H_{15}BrN_4OS_2$ [M^+] 493.9871, found: 493.9873; Purity > 99% (as determined by HPLC, method A, t_R = 10.5 min).

Quinoline-2-carboxylic acid [3-(6-bromo-imidazo[1,2-a]pyridin-2-yl)-phenyl]-amide (5h): Obtained by recrystallization (hexanes/MeOH) as a white solid (86.1 mg, 47.5% yield): R_f = 0.61 (hexanes:EtOAc:MeOH = 6:3:1); ^{1}H-NMR (DMSO-d_6, 300 Hz) δ 10.9 (1H, s, NH), 8.93 (1H, m, aromatic), 8.63 - 8.67 (2H, m, aromatic), 8.38 (1H, s, aromatic), 8.26-8.31 (2H, m, aromatic), 8.14 (1H, d, J = 7.8 Hz, aromatic), 7.91-7.96 (2H, m, aromatic), 7.73-7.80 (2H, m, aromatic), 7.60 (1H, d, J = 9.6 Hz, aromatic), 7.48 (1H, t, J = 7.8 Hz, aromatic), 7.37-7.41 (1H, m, aromatic); MS (ESI) *m/z* 465 (M^++Na), 441 (M-H); HRMS (EI) *m/z* calcd for $C_{23}H_{15}BrN_4O$ [M^+]

442.0429, found: 442.0429; Purity > 96% (as determined by HPLC, method B, t_R = 15.5 min).

4-Hydroxy-quinoline-2-carboxylic acid [3-(6-bromo-imidazo[1,2-a]pyridin-2-yl)-phenyl]-amide (5i): Obtained by recrystallization (CH_2Cl_2/MeOH) as a yellow solid (12.5 mg, 6.3% yield): R_f = 0.28 (hexanes:EtOAc:MeOH = 6:3:1); ^{1}H-NMR (DMSO-d_6, 300 Hz) δ 10.9 (1H, s, NH), 9.06 (1H, s, aromatic), 8.63 (1H, d, J = 7.8 Hz, aromatic), 8.55 (1H, s, aromatic), 8.42 (1H, d, J = 8.4 Hz, aromatic), 8.34 (1H, d, J = 8.4 Hz, aromatic), 8.17-8.12 (1H, m, aromatic), 8.01 (1H, t, J = 7.2 Hz, aromatic), 7.93 (1H, d, J = 8.1 Hz, aromatic), 7.78-7.63 (4H, m, aromatic), 7.51 (1H, t, J = 8.1 Hz, aromatic); MS (ESI) *m/z* 457 (M-H); HRMS (EI) *m/z* calcd for $C_{23}H_{15}BrN_4O_2$ [M^+] 458.0378, found: 458.0380; Purity > 99% (as determined by HPLC, method B, t_R = 12.9 min).

Thiophene-2-sulfonic acid [3-(6-bromo-imidazo[1,2-a]pyridin-2-yl)-phenyl]-amide (5j): To a solution of 3-(6-bromo-imidazo[1,2-a]pyridin-2-yl)-phenylamine (**4**) (61.3 mg, 0.21 mmol) in CH_2Cl_2 (3.0 mL) was added a solution of triethylamine (0.03 mL, 0.21 mmol) and thiophene-2-sulfonyl chloride (77.7 mg, 0.43 mmol) in CH_2Cl_2 (3 mL) portionwise at 0 °C. After stirring for 2 h, the solution was washed with aqueous 3% HCl, water and saturated aqueous $NaHCO_3$. The organic layer was dried ($MgSO_4$) and concentrated. Purification by preparative TLC (CH_2Cl_2:MeOH = 20:1) gave thiophene-2-sulfonic acid [3-(6-bromo-imidazo[1,2,-a]pyridin-2-yl)-phenyl]-amide as a yellow foam (18.6 mg, 20% yield): R_f = 0.45 (CH_2Cl_2:MeOH = 15:1); ^{1}H-NMR ($CDCl_3$, 300 Hz) δ 8.24 (1H, s, NH), 7.77 (1H, s, aromatic), 7.69 (2H, m, aromatic), 7.47-7.51 (3H, m, aromatic), 7.19-7.36 (4H, m, aromatic), 6.95 (1H, m, aromatic); MS (ESI) *m/z* 434 (M^++H), 432 (M-H); HRMS (EI) *m/z* calcd for $C_{17}H_{12}BrN_3O_2S_2$ [M^+] 432.9554, found: 432.9561; Purity > 96% (as determined by reverse phase HPLC, method A, t_R = 8.6 min).

Naphthalene-1-sulfonic acid [3-(6-bromo-imidazo[1,2-a]pyridin-2-yl)-phenyl]-amide (5k): To a solution of 3-(6-bromo-imidazo[1,2-a]pyridin-2-yl)-phenylamine (**4**) (62.7 mg, 0.22 mmol) and naphthalene-1-sulfonyl chloride (148 mg, 0.65 mmol) in CH_2Cl_2 (4 mL) was added triethylamine (0.03 mL, 0.22 mmol) portionwise at 0 °C. After stirring for 2 h, the solution was washed with aqueous 3% HCl, water and saturated aqueous $NaHCO_3$. The organic layer was dried ($MgSO_4$) and concentrated. Purification by preparative TLC (hexanes: EtOAc:MeOH = 6:3:1) gave naphthalene-1-sulfonic acid [3-(6-iodo-imidazo[1,2-a]pyridin-2-yl)-phenyl]-amide as a yellow foam (21.5 mg, 20% yield): R_f = 0.56 (CH_2Cl_2:MeOH = 15:1); ^{1}H-NMR ($CDCl_3$, 300 Hz) δ 8.69 (1H, d, J = 9.0 Hz, aromatic), 8.22 (1H, s, aromatic), 8.01 (1H, d, J = 8.7 Hz, aromatic), 7.91 (1H, d, J = 8.1 Hz, aromatic), 7.41-7.69 (6H, m, aromatic), 7.17-7.24 (2H, m, aromatic), 6.91-6.97 (2H, m, aromatic), 4.88 (1H, s, NH); MS (ESI) *m/z* 478 (M^++H), 476 (M-H); HRMS (EI) *m/z* calcd for $C_{23}H_{16}BrN_3O_2S$ [M^+] 477.0147, found: 477.0149; Purity > 97% (as determined by reverse phase HPLC, method A, t_R = 10.4 min).

General procedure for the preparation of 6a-i. The napthalene-2-carboxylic acid [3-(6-bromo-imidazo[1,2-a]pyridin-2-yl)-phenyl]-amide (**5c**) (1 equiv) was added to a suspension of $Pd(PPh_3)_4$ (0.02 equiv) in degassed 1,2-dimethoxyethane (DME) at ambient temperature under nitrogen. The mixture was slowly heated to reflux with vigorous stirring overnight. The solution was cooled to ambient temperature and the appropriate boronic acid (1.2 equiv), sodium hydrogen carbonate (4 equiv) and H_2O were added. The mixture was reheated reflux with vigorous stirring for 2 h, then cooled and extracted with ethyl acetate. The combined extracts were concentrated to afford a crude solid, which was purified by silica gel column chromatography or recrystallization.

Naphthalene-2-carboxylic acid {3-[6-(2,4-difluoro-phenyl)-imidazo[1,2-a]pyridin-2-yl]-phenyl}-amide (6a): Obtained as a gray solid (32.9 mg, 43% yield) from 2,4-difluorophenyl boronic acid. R_f = 0.47 (hexanes:EtOAc:MeOH = 6:3:1); ^{1}H-NMR (DMSO-d_6, 300 Hz) δ 10.5 (1H, s, NH), 8.81 (1H, s, aromatic), 8.65 (1H, s, aromatic), 8.53 (1H, s, aromatic), 8.44 (1H, s, aromatic), 8.02-8.12 (4H, m, aromatic), 7.84 (1H, m, aromatic), 7.62-7.76 (5H, m, aromatic), 7.41-7.49 (3H, m, aromatic), 7.26 (1H, m, aromatic); MS (ESI) *m/z* 474 (M-H); HRMS (EI) *m/z* calcd for $C_{30}H_{19}F_2N_3O$ [M^+] 475.1496, found: 475.1501; Purity > 99% (as determined by HPLC, method A, t_R = 11.9 min).

Naphthalene-2-carboxylic acid [3-(6-naphthalen-1-yl-imidazo[1,2-a]pyridin-2-yl)-phenyl]-amide(6b): Obtained as a white solid (59.2 mg, 86% yield) from 1-naphthalene boronic acid: R_f = 0.47 (hexanes:EtOAc:MeOH = 6:3:1); ^{1}H-NMR ($CDCl_3$, 300 Hz) δ 8.40 (2H, m), 8.27 (1H, s), 8.17 (1H, s), 7.85-7.99 (9H, m, aromatic), 7.72 (2H, m, aromatic), 7.45-7.61 (7H, m, aromatic), 7.34 (1H, m, aromatic); MS (ESI) *m/z* 490 (M^++H), 488 (M-H); HRMS (EI) *m/z* calcd for $C_{34}H_{23}N_3O$ [M^+] 489.1841, found: 489.1842; Purity > 99% (as determined by HRMS (EI) *m/z* calcd for $C_{34}H_{23}N_3O$ [M^+] 489.1841, found: 489.1843; HPLC, method A, t_R = 12.6 min).

Naphthalene-2-carboxylic acid [3-(6-naphtalen-2-yl-imidazo-[1,2-a]pyridin-2-yl)-phenyl]-amide(6c): Obtained as a pink solid (35.1 mg, 45% yield) from 2-naphthalene boronic acid: R_f = 0.32 (hexanes:EtOAc:MeOH = 6:3:1); ^{1}H-NMR (CD_3OD, 300 Hz) δ 8.97 (1H, s, aromatic), 8.59 (1H, s, aromatic), 8.47 (1H, m, aromatic), 8.36 (1H, s, aromatic), 8.23 (1H, s, aromatic), 7.84-8.06 (8H, m, aromatic), 7.41-7.80 (9H, m, aromatic); MS (ESI) *m/z* 488 (M-H); Purity > 96% (as determined by HPLC, method A, t_R = 12.9 min).

Naphthalene-2-carboxylic acid [3-(6-thiophen-2-yl-imidazo-[1,2-a]pyridin-2-yl)-phenyl]-amide(6d): Obtained as a yellow solid (16.9 mg, 4.6% yield) from 2-thiophene boronic acid: R_f = 0.37 (hexanes:EtOAc:MeOH = 6:3:1); ^{1}H-NMR (CD_3OD, 300 Hz) δ 8.63 (1H, s, aromatic), 8.51 (1H, s, aromatic), 8.15 (2H, m, aromatic), 7.82-8.02 (4H, m, aromatic), 7.37-7.71 (9H, m, aromatic), 7.11 (1H, ps-t, J = 4.5 Hz, aromatic); MS (ESI) *m/z* 446 (M^++H), 444 (M-H); HRMS (EI) *m/z* calcd for $C_{28}H_{19}N_3OS$ [M^+] 445.1249, found: 445.1246; Purity > 96% (as determined by HPLC, method A, t_R = 11.4 min).

Naphthalene-2-carboxylic acid {3-[6-(3-methoxy-phenyl)-imidazo[1,2-a]pyridin-2-yl]-phenyl}-amide (6e): Obtained as a yellow solid (51.9 mg, 69.1% yield) from 3-methoxyl phenyl boronic acid: R_f = 0.23 (hexanes:EtOAc:MeOH = 6:3:1); ^{1}H-NMR ($CDCl_3$, 300 Hz) δ 8.70 (1H, s, NH), 8.41 (1H, s, aromatic), 8.22 (2H, d, J = 11.1 Hz, aromatic), 7.81-7.95 (6H, m, aromatic), 7.63 (2H, d, J = 9.3 Hz, aromatic), 7.42-7.57 (2H, m,

aromatic), 7.35-7.42 (3H, m, aromatic), 7.06-7.12 (2H, m, aromatic), 6.91-6.95 (1H, m, aromatic), 3.87 (3H, s, CH_3); MS (ESI) *m/z* 470 (M^++H), 468 (M-H); HRMS (EI) *m/z* calcd for $C_{31}H_{23}N_3O_2$ [M^+] 469.1790, found: 469.1799; Purity > 99% (as determined by HPLC, method A, t_R = 11.7 min).

Naphthalene-2-carboxylic acid {3-[6-(3-cyano-phenyl)-imidazo[1,2-a]pyridin-2-yl]-phenyl}-amide (6f): Obtained as a yellow solid (42.7 mg, 65.7% yield from 3-cyano phenyl boronic acid: R_f = 0.22 (CH_2Cl_2:MeOH = 15:1); ^{1}H-NMR (DMSO-d_6, 300 Hz) δ 11.34 (1H, s, NH), 9.06 (1H, s, aromatic), 8.66 (1H, s, aromatic), 8.54 (1H, s, aromatic), 8.38 (1H, s, aromatic), 8.26 (1H, s, aromatic), 8.02-8.12 (5H, m, aromatic), 7.88 (2H, d, *J* = 7.2 Hz, aromatic),7.64-7.76 (6H, m, aromatic), 7.46 (1H, ps-t, *J* = 7.8 Hz, aromatic); MS (ESI) *m/z* 465 (M^++H), 463 (M-H); HRMS (EI) *m/z* calcd for $C_{31}H_{20}N_4O$ [M^+] 464.1637, found: 464.1641; Purity > 99% (as determined by HPLC, method A, t_R = 10.4 min).

Naphthalene-2-carboxylic acid {3-[6-(4-methanesulfonyl-phenyl)-imidazo[1,2-a]pyridin-2-yl]-phenyl}-amide (6g): Obtained as a white solid (20.2 mg, 23.0% yield) from 4-methanesulfonyl-phenyl boronic acid: R_f = 0.27 (CH_2Cl_2:MeOH = 30:1); ^{1}H-NMR (DMSO-d_6, 300 Hz) δ 10.6 (1H, s, NH), 9.09 (1H, s, aromatic), 8.66 (1H, s, aromatic), 8.55 (1H, s, aromatic), 8.43 (1H, s, aromatic), 8.01-8.13 (8H, m, aromatic), 7.86 (1H, m, aromatic), 7.64-7.76 (5H, m, aromatic), 7.47 (1H, d, *J* = 8.1 Hz, aromatic), 3.28 (3H, s, CH_3); MS (ESI) *m/z* 518 (M^++H), 516 (M-H); HRMS (EI) *m/z* calcd for $C_{31}H_{23}N_3O_3S$ [M^+] 517.1460, found: 517.1451; Purity > 99% (as determined by HPLC, method A, t_R = 10.4 min).

Naphthalene-2-carboxylic acid [3-(6-pyridin-3-yl-imidazo [1,2-a]pyridin-2-yl)-phenyl]-amide (6h): Obtained as a white solid (18.0 mg, 23% yield) from 3-pyridine boronic acid: R_f = 0.40 (CH_2Cl_2:MeOH = 10:1); ^{1}H-NMR (CD_3OD, 300 Hz) δ 8.86 (2H, m, aromatic), 8.55 (2H, m, aromatic), 8.29 (2H, m, aromatic), 8.16 (1H, m, aromatic), 7.94-8.06 (4H, m, aromatic), 7.55-7.80 (7H, m, aromatic), 7.48 (1H, ps-t, *J* = 7.8 Hz, aromatic); MS (ESI) *m/z* 441 (M^++H), 439 (M-H); HRMS (EI) *m/z* calcd for $C_{29}H_{20}N_4O$ [M^+] 440.1637, found: 440.1640; Purity > 96% (as determined by HPLC, method A, t_R = 7.9 min).

Naphthalene-2-carboxylic acid {3-[6-(4-hydroxy-phenyl)-imidazo[1,2-a]pyridin-2-yl]-phenyl}-amide (6i): Obtained as a gray solid (75.7 mg, 98% yield) from 4-hydroxyphenyl boronic acid: R_f = 0.21 (hexanes:EtOAc:MeOH = 6:3:1); ^{1}H-NMR (DMSO-d_6, 300 Hz) δ 10.5 (1H, s, NH), 9.67 (1H, brs, OH), 8.79 (1H, s, aromatic), 8.66 (1H, s, aromatic), 8.51 (1H, s, aromatic), 8.35 (1H, s, aromatic), 8.02-8.12 (4H, m, aromatic), 7.85 (1H, m, aromatic), 7.62-7.73 (4H, m, aromatic), 7.53-7.56 (3H, m, aromatic), 7.45 (1H, ps-t, *J* = 8.1 Hz, aromatic), 6.89 (2H, m, aromatic); MS (ESI) *m/z* 456 (M^++H), 454 (M-H); HRMS (EI) *m/z* calcd for $C_{30}H_{21}N_3O_2$ [M^+] 455.1634, found: 455.1626; Purity > 99% (as determined by HPLC, method A, t_R = 10.3 min).

Carbamic acid 4-(2-{3-[(naphthalene-2-carbonyl)-amino]-phenyl}-imidazo[1,2-a]pyridin-6-yl)-phenyl ester (7a): To a stirred solution of naphthalene-2-carboxylic acid {3-[6-(4-hydroxy-phenyl)-imidazo[1,2-a]pyridin-2-yl]-phenyl}-amide (**6i**) (100 mg, 0.22 mmol) in CH_2Cl_2 (2 mL) was added trichloroacetyl isocyanate (0.065 mL, 0.55 mmol) at 0 °C and the solution was stirred at rt for 1 h. To the reaction mixture was added CH_2Cl_2 and appropriate Al_2O_3. The mixture was stirred for 0.5 h and filtered. The filtrate was concentrated. Purification by preparative TLC (CH_2Cl_2:MeOH = 20:1) gave carbamic acid 4-(2-{3-[(naphthalene-2-carbonyl)-amino]-phenyl}-imidazo[1,2-a]pyridin-6-yl)-phenyl ester as a white solid (22.8 mg, 21 % yield): R_f = 0.21 (hexanes:EtOAc:MeOH = 6:3:1); ^{1}H-NMR (DMSO-d_6, 300 Hz) δ 10.6 (1H, s, NH), 9.18 (1H, s, aromatic), 8.64 (1H, s, aromatic), 8.37 (1H, s, aromatic), 7.96-8.12 (5H, m, aromatic), 7.47-7.84 (9H, m, aromatic), 7.26 (2H, m, aromatic); MS (ESI) *m/z* 521 (M^++Na), 497 (M-H); HRMS (EI) *m/z* calcd for $C_{31}H_{22}N_4O_3$ [M^+] 498.1692, found: 498.1681; Purity > 96% (as determined by reverse phase HPLC, method B, t_R= 8.3 min).

[4-(2-{3-[(Naphthalene-2-carbonyl)-amino]-phenyl}-imidazo[1,2-a]pyridin-6-yl)-phenoxy]-acetic acid ethyl ester (7b): To a mixture of the naphthalene-2-carboxylic acid {3-[6-(4-hydroxy-phenyl)-imidazo[1,2-a]pyridin-2-yl]-phenyl}-amide (**6i**) (57.3 mg, 0.13 mmol) and potassium carbonate (52.2 mg, 0.19 mmol) in DMF (4 mL) was added ethyl chloroacetate (0.020 mL, 0.38 mmol). The reaction mixture was stirred at room temperature overnight, and then partitioned between ethyl acetate and brine. The organic phase was dried ($MgSO_4$) and concentrated. Purification by preparative TLC (hexanes: EtOAc:MeOH = 9:3:1) gave [4-(2-{3-[(naphthalene-2-carbonyl)-amino]-phenyl}-imidazo[1,2-a]pyridin-6-yl)-phenoxy]acetic acid ethyl ester as a solid (48.9 mg, 70% yield): R_f = 0.37 (hexanes:EtOAc:MeOH = 6:3:1); ^{1}H-NMR ($CDCl_3$, 300 Hz) δ 8.39 (1H, s, NH), 8.19-8.28 (3H, m, aromatic), 7.88-7.96 (6H, m, aromatic), 7.73 (1H, d, *J* = 7.8 Hz, aromatic), 7.57-7.66 (3H, m, aromatic), 7.45-7.50 (3H, m, aromatic), 7.38 (1H, d, *J* = 9.6 Hz, aromatic), 7.02 (2H, m, aromatic), 4.68 (2H, s, OCH_2), 4.30 (2H, q, *J* = 6.9 Hz, $COOCH_2$), 1.33 (3H, t, *J* = 6.9 Hz, CH_3); MS (ESI) *m/z* 542 (M^++H), 540 (M-H); HRMS (EI) *m/z* calcd for $C_{34}H_{27}N_3O_4$ [M^+] 541.2007, found: 541.2007; Purity > 99% (as determined by reverse phase HPLC, method A, t_R = 11.9 min).

[4-(2-{3-[(Naphthalene-2-carbonyl)-amino]-phenyl}-imidazo[1,2-a]pyridin-6-yl)-phenoxy]-acetic acid (7c): To a solution of [4-(2-{3-[(Naphthalene-2-carbonyl)-amino]-phenyl}-imidazo[1,2-a]pyridin-6-yl)-phenoxy]-acetic acid ethyl ester (**7b**) (36.3 mg, 0.067 mmol) in THF/H_2O (1:1, 8 mL) was added LiOH·H_2O (5.45 mg, 0.13 mmol) at room temperature. The resulting mixture was stirred overnight, and then acidified with 10% HCl to pH 2. The reaction mixture was filtered. The white solid was washed ethyl acetate and gave [4-(2-{3[(naphthalene-2-carbonyl)-amino]-phenyl}-imidazo[1,2-a] pyridin-6-yl)-phenoxy]-acetic acid as a white solid (28.5 mg, 83% yield): R_f = 0.14 (CH_2Cl_2:MeOH = 6:1); ^{1}H-NMR (CD_3OD, 300 Hz) δ 8.97 (1H, s, aromatic), 8.48-8.55 (3H, m, aromatic), 8.18 (1H, dd, *J* = 9.3 Hz & 1.2 Hz, aromatic), 7.89-8.03 (5H, m, aromatic), 7.78 (1H, m, aromatic), 7.55-7.66 (6H, m, aromatic), 7.09 (2H, m, aromatic), 4.70 (2H, s, OCH_2); MS (ESI) *m/z* 514 (M^++H), 512 (M-H); HRMS (EI) *m/z* calcd for $C_{32}H_{23}N_3O_4$ [M^+] 513.1689, found: 513.1687; Purity > 98% (as determined by HPLC, method A, t_R = 10.4 min).

Assay for ACAT and cholesterol ester formation in HepG2

cell. ACAT activity and cholesterol ester formation in HepG2 cells was assayed as described previously.[10] HepG2 cells were seeded in a 6 well plate at the density of 1×10^6 cells/mL/well and cultured in the medium containing 10% FBS for 2 days and then cultured overnight in the medium containing 5% LPDS(or 1% BSA). The medium was replaced and cells were incubated with 2.5 μL of sample or 0.1% DMSO, a vehicle of sample, and [l-^{14}C]oleic acid (0.5 μCi) for 6 hr in 6 well plate. Then, the medium was removed, and the cells were washed three times with PBS. The intracellular lipids of the cells were extracted by hexane/isopropanol (3:2) and the organic phase was evaporated under nitrogen. Total lipid was separated by silica gel TLC plate in petroleum ether/diethyl ether/acetic acid (90:10:1) and the amount of radioactivity was analyzed with a bioimagina analyzer (BAS-1500, FUJIFILM).

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