Monatsh Chem (2017) 148:1767–1780
DOI 10.1007/s00706-017-1943-7

ORIGINAL PAPER

Design, synthesis, and evaluation of novel hydrazide hydrochlorides of 6-aminopyrazolo[1,5-*a*]pyrimidine-3-carboxamides as potent Aurora kinase inhibitors

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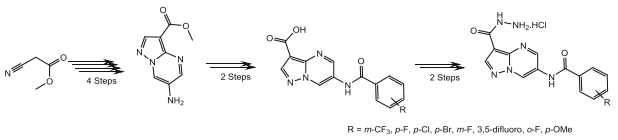
Received: 14 October 2016 / Accepted: 19 February 2017 / Published online: 30 June 2017

Abstract The Aurora kinases play a key role in mitosis and are overexpressed in multiple human tumor types; there has been considerable interest in developing Aurora kinase inhibitors as antitumor agents, particularly Aurora A and Aurora B kinases. A series of novel hydrazide hydrochlorides of pyrazolo[1,5-*a*]pyrimidine carboxamides were designed and synthesized and their inhibitory activities against Aurora kinases were evaluated. Some of the tested compounds exhibited low micromolar to nanomolar activity with respect to the inhibition of Aurora A kinase. The most potent compound in this series was found to be a potent inhibitor of Aurora A in an HTRF enzymatic assay with an IC_{50} as low as 23 nM. A structure–activity relationship study indicated that halogen substitution in the benzene ring of amide plays an important role in kinase inhibitory potency.

Graphical abstract



Electronic supplementary material The online version of this article (doi:10.1007/s00706-017-1943-7) contains supplementary material, which is available to authorized users.

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Keywords Carboxamides · Hydrazide hydrochloride · Pyrazolo[1,5-*a*]pyrimidine · Anticancer activity · Aurora A · Aurora B

Introduction

Aurora kinases are the members of serine/threonine kinases and they are strongly associated with human cancer [1, 2]. Humans feature a family of Aurora kinases with three members: isoforms A, B, and C. Both Aurora A and Aurora B are significantly overexpressed in a wide range of human cancer cell lines. The overexpression of Aurora A causes aberrant phosphorylation of normal cell cycle targets and cytoplasmic targets, leading to chromosomal instability, oncogenic transformation, tumor progression, and development of chemoresistance [3]. Similarly, the overexpression of Aurora B increases the phosphorylation of histone H3, forming more aggressive tumors in transgenic mouse models [4, 5]. Mechanistically, Aurora kinases (A, B, and C) are regulatory proteins and play key roles in the mitotic events of cell division [6, 7].

Over the past decade, extensive research has been carried out towards the discovery of Aurora-selective small-molecule inhibitors. As a result, a handful of small-molecule Aurora inhibitors have been identified. Among them, VX-680 [8] and SNS-314 [9, 10] have entered human clinical trials as pan-Aurora kinase inhibitors; MLN8237 [11] and MK-5108 [12] are undergoing clinical assessment as Aurora A-specific inhibitors as shown in Fig. 1. Recently, selective Aurora B inhibitor barasertib (AZD1152) [13] and the pan-aurora kinase inhibitor danusertib (PHA-739358) [14] have been utilized in clinical trials for the treatment of leukemia, myeloma, and other solid tumors [15, 16].

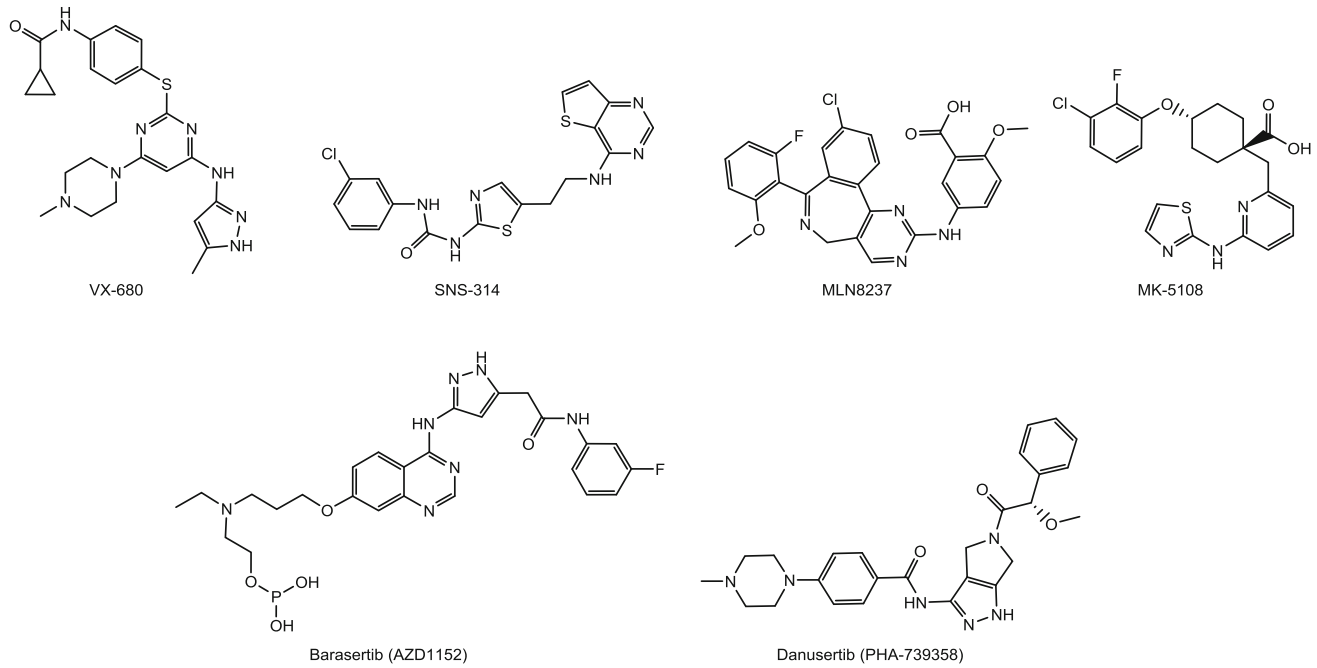


Fig. 1 The structures of Aurora kinase inhibitors

In addition, a number of several heterocyclic amide derivatives have been detailed as potent Aurora kinase inhibitors. We envisioned that there are less reports for amides from pyrazolo[1,5-*a*]pyrimidine pharmacophore in this category and it was thought it would be worthwhile to synthesize some new pyrazolo[1,5-*a*]pyrimidine-bearing amides. In this present article, we describe the design, synthesis, and biochemical evaluation of a novel series of pyrazolo[1,5-*a*]pyrimidine analogs with representative example as shown in Fig. 2 showing good antiproliferative activity against both Aurora A and Aurora B.

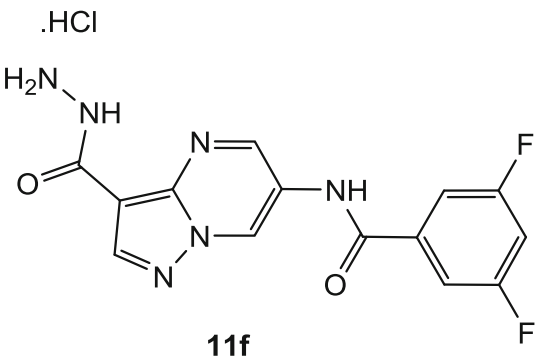


Fig. 2 Chemical structure of compound **11f** which showed good aurora kinase activity

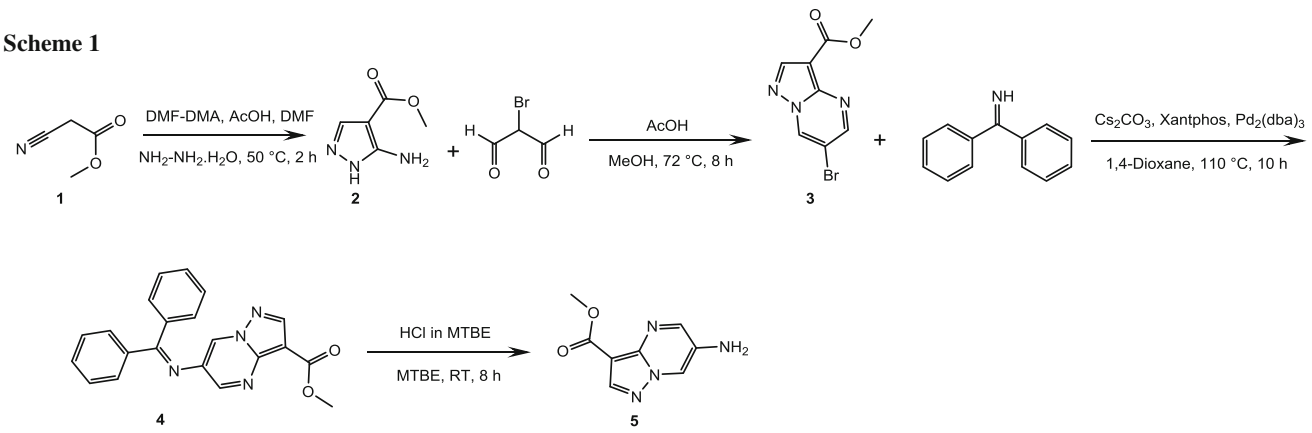
Results and discussion

Chemistry

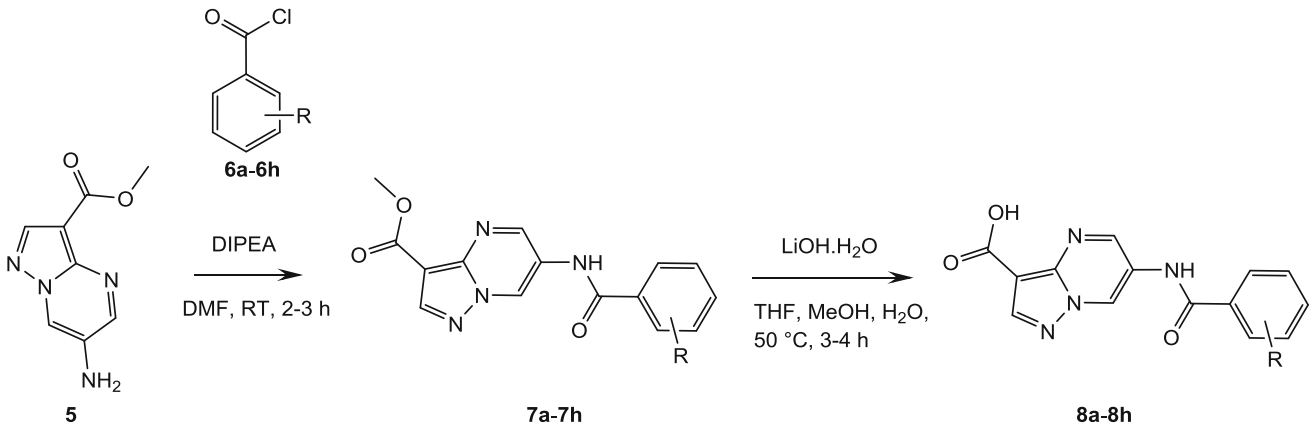
The novel hydrazide hydrochloride derivatives of 6-aminopyrazolo[1,5-*a*]pyrimidine-3-carboxamides described herein were synthesized over eight consecutive steps. The first four steps involved the synthesis of novel compound 6-aminopyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid methyl ester **5** as shown in Scheme 1. Previously described methods were used for the synthesis of both 5-amino-1*H*-pyrazole-4-carboxylic acid methyl ester (**2**) [17–19] and 6-bromopyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid methyl ester **3** [20]. The treatment of ester **3** with benzophenone imine in presence of cesium carbonate, xantphos, and $Pd_2(dba)_3$ in 1,4-dioxane at 110 °C for 10 h gave methyl 6-[(diphenylmethylidine)amino] pyrazolo[1,5-*a*]pyrimidine-3-carboxylate **4** [21–23]. The treatment of imine with HCl in MTBE gave 6-aminopyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid methyl ester **5**.

The further design involved synthesis of substituted benzoylamino-pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acids **8a**–**8h** in two steps, as shown in Scheme 2. Step 5 involved the synthesis of carboxylic acid methyl esters **7a**–**7h** which were obtained by the treatment of 6-aminopyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid methyl ester **5** with the corresponding acid chlorides **6a**–**6h** in the presence of DIPEA at room temperature. The above carboxylic acid methyl esters **7a**–**7h** were subsequently treated with lithium hydroxide monohydrate at 50 °C in THF, MeOH,

Scheme 1



Scheme 2



R= *m*-CF_3, *p*-F, *p*-Cl, *p*-Br, *m*-F, 3,5-difluoro, *o*-F, *p*-OMe

and H_2O for 2 h to give the corresponding benzoyl-aminopyrazolo[1,5-*a*]pyrimidine-3-carboxylic acids **8a**–**8h** via step 6.

The final step involved the synthesis of the target compounds which involved the synthesis of corresponding hydrochloride salts of hydrazides **11a**–**11h** in two steps, as shown in Scheme 3. Step 7 involved the synthesis of respective hydrazine carboxylic acid *tert*-butyl esters **10a**–**10h** from **8a**–**8h** using *tert*-butyl carbazate (**9**) in the presence of DIPEA, HOAT, EDC.HCl in DMF at room temperature. The above hydrazine carboxylic acid *tert*-butyl esters **10a**–**10h** were reacted with HCl in 1,4-dioxane to obtain the target molecules **11a**–**11h** via step 8.

Biological screening

An in vitro kinase assay was performed at a single-dose concentration of 10 μM over Aurora A and Aurora B to evaluate the Aurora kinase inhibitory activity of the synthesized pyrazolo[1,5-*a*]pyrimidine carboxamide compounds **10a**–**10h** and **11a**–**11h**. Table 1 shows the percentage of inhibition of kinase activity as an average of duplicate assay compared to the known non-selective kinase inhibitor, staurosporine, as a positive control. Out of these 16 derivatives, 4 analogs (**11b**, **11e**, **11f**, and **11g**) showed high potency over 90% inhibitions against Aurora A, while the mean inhibitions were moderate in the rest of the compounds. Interestingly, most of these analogs were relatively less inhibitive against Aurora B (20.3–80.1% inhibition at 10 μM). Meanwhile, it is observed that analog **11f** showed good highest inhibition against both Aurora A and Aurora B, while, analog **11a** showed almost a lack of the mean inhibition against both Aurora A and Aurora B.

After a single-dose preliminary screening, compounds **11a**–**11h** were tested for further activity study (Table 2). These compounds were tested against Aurora A and Aurora

Scheme 3

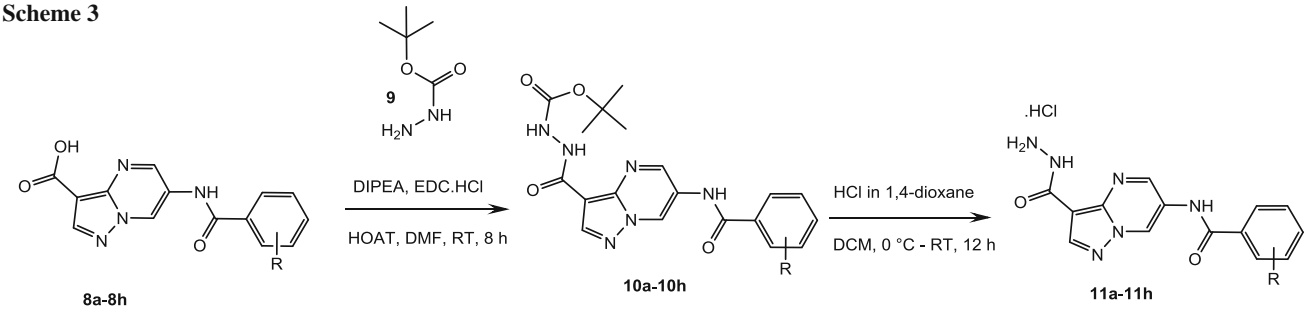


Table 1 Inhibitory activity of compounds **10a–10h** and **11a–11h** against Aur A and Aur B

Compound	R^1	% Inhibition[a]	
		Aur A	Aur B
10a	*m*-CF_3	64.1	20.3
10b	*p*-F	74.4	36.2
10c	*p*-Cl	50.2	51.9
10d	*p*-Br	41.6	49.8
10e	*m*-F	67.2	58.2
10f	3,5-Difluoro	79.3	53.8
10g	*o*-F	71.2	46.3
10h	*p*-OMe	42.8	35.4
11a	*m*-CF_3	81.6	42.1
11b	*p*-F	90.4	56.7
11c	*p*-Cl	86.5	37.9
11d	*p*-Br	82.8	32.8
11e	*m*-F	92.4	55.8
11f	3,5-Difluoro	94.5	80.1
11g	*o*-F	93.3	65.8
11h	*p*-OMe	80.2	34.3

[a] Values are expressed as the means of three independent determinations and are within plus or minus 10%

Table 2 IC_{50} of compounds **11a–11h** over Aur A and Aur B

Compound	R^1	Aur A[a] Enzymatic, IC_{50}/μM	Aur B[a] Enzymatic, IC_{50}/μM
11a	*m*-CF_3	2.83 ± 0.21	58.21 ± 5.11
11b	p-F	0.47 ± 0.036	38.13 ± 3.71
11c	*p*-Cl	2.67 ± 0.24	43.26 ± 4.10
11d	*p*-Br	1.76 ± 0.015	49.63 ± 4.84
11e	*m*-F	0.071 ± 0.006	30.56 ± 3.73
11f	3,5-Difluoro	0.023 ± 0.002	9.63 ± 0.92
11g	*o*-F	0.048 ± 0.004	22.82 ± 2.67
11h	*p*-OMe	2.65 ± 0.231	66.39 ± 6.10

[a] IC_{50} values were determined from logarithmic concentration-inhibition curves and are represented as means of at least two separate experiments

B by measuring the ability to inhibit phosphorylation of a biotinylated-polypeptide substrate (p-GAT, CIS Bio International) in a homogeneous time-resolved fluorescence (HTRF) assay at an ATP concentration of 30 μM. The results were reported as a 50% inhibition concentration value (IC_{50}). The IC_{50} values for these selected compounds were determined in a 10-dose IC_{50} mode with threefold serial dilution starting at 30 μM as listed in Table 2. Most of the compounds inhibited Aurora A with IC_{50} values in the nanomolar to low micromolar range. Especially, compounds **11e**, **11f**, and **11g** revealed nanomolar IC_{50} values. These results were encouraging with respect to the internal standard staurosporine which showed a potency of $IC_{50} = 1.5$ nM in the internal enzymatic Aurora A assay. Once again, these analogs were relatively less active against Aurora B. Only the most potent compound **11f** showed the highest Aurora B activity with IC_{50} value reaching 9.63 μM in the enzymatic assay, whereas the internal standard staurosporine which showed a potency of $IC_{50} = 9$ nM in the internal enzymatic Aurora assay.

Structure–activity relationship

As can be seen from Table 1, a number of compounds exhibited a robust inhibitory activity against isolated Aurora A enzyme. With varied halogen, methoxy, and trihalomethyl substituents in benzamide position, it was observed that 3,5-difluoro, *o*-fluoro, *m*-fluoro, and *p*-fluoro substituted benzamide hydrazine hydrochlorides (**10c**, **10f**, **10i**, and **10m**) gave better enzymatic activity. Amongst these derivatives **11f** gave the highest activity ($IC_{50} = 23$ nM) against isolated Aurora A enzyme, which contains 3,5-difluoro in the benzamide group as shown in Table 2. The activity order came in the following sequence 3,5-difluoro > *o*-F > *m*-F > *p*-F > *m*-CF_3. More or less the same activity sequence was observed against Aurora B enzyme. Compound **11f** showed highest Aurora B activity ($IC_{50} = 9.63$ μM) in the enzymatic assay.

In general, the structure–activity relationship study indicated that halogen substitution plays important roles in kinase inhibitory potency. It is worth noting that compound **11a** showed almost a lack of the mean inhibition against both Aurora A and Aurora B, suggesting that the—CF_3 group of the benzamide played a key role in the binding of inhibitors to Aurora kinase. Interestingly, compound **11f** showed good inhibition against both Aurora A and Aurora B, due to the presence of 3,5-difluoro group in benzamide.

Conclusion

A new 6-aminopyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid scaffold, useful for kinase inhibition, has been designed and synthesized. We have disclosed the synthesis of a novel series of carboxamide containing hydrazide hydrochloride derivatives of the above scaffold as potent inhibitors of Aurora kinase. The compounds also provide an opportunity of laying the foundation for promising molecules of anticancer potency.

Experimental

Chemicals were of analytical grade and obtained from Sigma-Aldrich Co. The purification of all intermediates and final compounds was carried out by column chromatography using Merck silica gel with 230–400 mesh size. The purity of synthesized compounds was determined by thin layer chromatography experiments, performed on alumina-backed silica gel 40 F254 plates (Merck, Darmstadt, Germany). TLC observation of these plates were carried out by illuminating under UV (254 nm) lamp and $KMnO_4$ stain solution. Melting points were determined using Büchi B-540 instrument. All ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AM-300 (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR) and Bruker AM-400 (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR), Bruker BioSpin Corp., Germany. The chemical shifts are reported in ppm (δ) with reference to TMS as an internal standard. The signals are designed as follows: s, singlet; d, doublet; t, triplet; m, multiplet; brs, broad singlet. IR spectra for all the compounds were recorded using a Bruker Alpha FTIR spectrometer using a diamond ATR single reflectance module (24 scans). Molecular weights for all the synthesized compounds were checked by LC–MS 6200 series Agilent Technology. Elemental analysis was carried out with a Perkin-Elmer model 240-C apparatus. The results of elemental analysis (C, H, and N) were within ±0.4% of the calculated amounts.

5-Amino-1*H*-pyrazole-4-carboxylic acid methyl ester (**2**) [17–19] and 6-bromopyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid methyl ester **3** [20] were synthesized according to previously described methods.

Methyl 6-[(Diphenylmethylidene)amino]pyrazolo[1,5-a]pyrimidine-3-carboxylic acid methyl ester (**4**, $C_{21}H_{16}N_4O_2$)

To a degassed suspension of 12.00 g of 6-bromopyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid methyl ester (**3**, 46.86 mmol) and 21.37 g of cesium carbonate (65.60 mmol) in 180 cm^3 of 1,4-dioxane was added 2.44 g of 4,5-bis(diphenylphosphino)-9,9-dimethylanthene (4.21 mmol) and 1.40 g of tris(dibenzylideneacetone)dipalladium(0) dichloromethane complex (1.40 mmol), followed by the drop wise addition of 12.73 g of benzophenone imine (70.29 mmol). The resultant reaction mixture was heated to 110 °C for 10 h under argon atmosphere. The completion of reaction was monitored by TLC. The reaction mixture was filtered through Celite bed and was washed with 50 cm^3 of ethyl acetate; the filtrate was concentrated to dryness under reduced pressure to afford the crude product. The crude product was purified by column chromatography over silica gel with chloroform/methanol (100:2, v/v) to afford methyl 6-[(diphenylmethylidene)amino]pyrazolo[1,5-*a*]pyrimidine-3-carboxylate (**4**) as a pale yellow solid (12.52 g, 75%). M.p.: 211.6–213.1 °C; TLC: $R_f = 0.22$ (EtOAc-hexane 2:8); IR (ATR): $\bar{\nu} = 1240$ (C–N stretch), 1704 (C=N stretch), 1751 (C=O stretch) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 3.77$ (3H, s, ester CH_3), 7.30–7.32 (3H, m, ArH), 7.37–7.47 (2H, m, ArH), 7.52 (2H, dd, $J_1 = 7.6$ Hz, $J_2 = 1.6$ Hz, ArH), 7.59–7.63 (1H, m, ArH), 7.73 (2H, dd, $J_1 = 7.2$ Hz, $J_2 = 1.6$ Hz, ArH), 8.38 (1H, d, $J = 2.4$ Hz, ArH), 8.51 (1H, s, ArH), 8.84 (1H, d, $J = 2.0$ Hz, ArH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 51.5$, 101.8, 127.4,129.0 (3 peaks), 129.4 (2 peaks),129.6 (2 peaks), 129.8 (2 peaks), 132.4, 135.2, 136.4, 138.3, 144.2, 147.5, 149.5, 162.4, 173.4 ppm; LC–MS (ESI): $m/z = 357.2$ ([M+H]$^+$).

6-Aminopyrazolo[1,5-a]pyrimidine-3-carboxylic acid methyl ester (**5**, $C_8H_8N_4O_2$)

To a suspension of 12.00 g of methyl 6-[(diphenylmethylidene)amino]pyrazolo[1,5-*a*]pyrimidine-3-carboxylate (**4**, 133.67 mmol) in 240 cm^3 of methyl *tert*-butyl ether was added 30 cm^3 of 4.5 M hydrochloric acid in methyl *tert*-butyl ether maintaining the temperature between 10 and 15 °C over a period of 20 min under nitrogen atmosphere. The resultant reaction mixture was stirred at room temperature (28 °C) for 8 h. The completion of reaction was monitored by TLC. The precipitate from the reaction mixture was filtered, washed with 40 cm^3 of methyl *tert*-butyl ether and dried under vacuum at room temperature (28 °C) for 6 h to afford hydrochloric salt of 6-

aminopyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid methyl ester. The suspension of hydrochloric salt of amine in 60 cm^3 of water was basified (pH ~10–12) with 10% aqueous potassium carbonate and the product was extracted to 2 × 60 cm^3 of *n*-butanol. The combined *n*-butanol layer was dried over anhydrous sodium sulfate and concentrated to dryness under reduced pressure to afford 6-aminopyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid methyl ester (**5**) as a pale yellow solid (5.95 g, 93%). M.p.: 197.2–198.6 °C; TLC: $R_f = 0.31$ (MeOH–$CHCl_3$ 1:9); IR (ATR): $\bar{\nu} = 1255$ (C–N stretch), 1550 (N–H bend), 3232 and 3399 (N–H stretch) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 3.76$ (3H, s, ester CH_3), 5.52 (2H, s, amine NH_2), 8.28 (1H, d, $J = 2.4$ Hz, ArH), 8.33 (1H s, ArH), 8.48 (1H, d, $J = 2.4$ Hz, ArH) ppm; ^{13}C NMR (400 MHz, DMSO-d_6): $\delta = 51.2$, 101.0, 117.0, 135.5, 141.7, 145.6, 147.2, 162.7 ppm; LC–MS (ESI): $m/z = 192.9$ ($[M+H]^+$).

General procedure for the synthesis of substituted carboxylic acid methyl esters ***7a–7h***

6-Aminopyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid methyl ester (**5**, 0.55 g, 2.86 mmol) was dissolved in 5.50 cm^3 of *N*,*N*-dimethylformamide and 1.10 g of *N*,*N*-diisopropylethylamine (8.58 mmol) and corresponding acid chlorides **6a–6h** (3.14 mmol) were added drop wise under nitrogen atmosphere. The reaction mixture was gradually allowed to attain room temperature (28 °C) and stirred for 2–3 h. The completion of reaction was monitored by TLC. The reaction mixture was poured to 60 cm^3 of ice cold water, stirred for 1 h and the precipitate formed was filtered, washed with 5 cm^3 of water and dried under vacuum at room temperature (28 °C) for 12 h to afford the crude product. The crude product was crystallized by digesting in 5 cm^3 of ethyl acetate and 25 cm^3 of *n*-heptane at room temperature (28 °C), stirred for 1 h, filtered, washed with 5 cm^3 of *n*-heptane and dried under vacuum at room temperature (28 °C) to afford the corresponding substituted carboxylic acid methyl esters **7a–7h**.

6-[3-(Trifluoromethyl)benzoylamino]pyrazolo[1,5-a]pyrimidine-3-carboxylic acid methyl ester (**7a**, $C_{16}H_{11}F_3N_4O_3$)

This compound was prepared by the reaction of 6-aminopyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid methyl ester (**5**) with 0.65 g of 3-(trifluoromethyl)benzoyl chloride (**6a**, 3.14 mmol) in the presence of *N*,*N*-diisopropylethylamine in *N*,*N*-dimethylformamide. The crude product was obtained as a yellow solid, which on crystallization afforded **7a** as a pale yellow solid (0.88 g, 85%). M.p.: 223.1–224.9 °C; TLC: $R_f = 0.41$ (EtOAc-hexane 4:6); IR (ATR): $\bar{\nu} = 1572$ (N–H bend), 1660 (amidic C=O), 1701 (ester C=O), 3311 (N–H stretch) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 3.82$ (3H, s, ester CH_3), 7.84 (1H, t, $J = 7.0$ Hz, ArH), 8.03 (1H, d, $J = 7.6$ Hz, ArH), 8.31–8.36 (2H, m, ArH), 8.61 (1H, s, ArH), 9.04 (1H, d, $J = 2.4$ Hz, ArH), 9.72 (1H, d, $J = 2.0$ Hz, ArH), 11.07 (1H, s, amide NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 51.6$, 102.2, 120.3, 123.0, 125.7, 128.4 (q, $^1J_{C\text{-}F} = 271.0$ Hz), 124.7, 124.8, 125.2, 127.4, 129.3, 129.3 (d, $^3J_{C\text{-}F} = 3.0$ Hz), 129.6, 129.9, 130.3 (q, $^2J_{C\text{-}F} = 31.0$ Hz), 130.4, 132.4, 134.7, 144.4, 147.7, 149.0, 162.5, 165.1 ppm; LC–MS (ESI): $m/z = 363.0$ ($[M-H]^-$).

6-(4-Fluorobenzoylamino)pyrazolo[1,5-a]pyrimidine-3-carboxylic acid methyl ester (**7b**, $C_{15}H_{11}FN_4O_3$)

This compound was prepared by the reaction of 6-aminopyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid methyl ester (**5**) with 0.50 g of 4-fluorobenzoyl chloride (**6b**, 3.14 mmol) in the presence of *N*,*N*-diisopropylethylamine in *N*,*N*-dimethylformamide. The crude product was obtained as a yellow solid, which on crystallization afforded **7b** as a pale yellow solid (0.77 g, 86%). M.p.: 232.5–234.4 °C; TLC: $R_f = 0.41$ (EtOAc-hexane 4:6); IR (ATR): $\bar{\nu} = 1560$ (N–H bend), 1659 (amidic C=O), 1708 (ester C=O), 3306 (N–H stretch) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 3.82$ (3H, s, ester CH_3), 7.44 (2H, t, $J = 8.8$ Hz, ArH), 8.11 (2H, dd, $J_1 = 5.4$ Hz, $J_2 = 9.2$ Hz, ArH), 8.60 (1H, s, ArH), 9.05 (1H, d, $J = 2.4$ Hz, ArH), 9.72 (1H, d, $J = 2.4$ Hz, ArH), 10.89 (1H, s, amide NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 51.6$, 102.1, 116.0, 116.2 (d, $^2J_{C\text{-}F} = 22.0$ Hz), 125.5, 127.1, 130.3, 130.3 (d, $^4J_{C\text{-}F} = 2.0$ Hz), 131.0, 131.1 (d, $^3J_{C\text{-}F} = 10.0$ Hz), 144.4, 147.7, 149.1, 162.5, 163.7, 166.2 (d, $^1J_{C\text{-}F} = 249.0$ Hz), 165.5 ppm; LC–MS (ESI): $m/z = 315.2$ ($[M+H]^+$).

6-(4-Chlorobenzoylamino)pyrazolo[1,5-a]pyrimidine-3-carboxylic acid methyl ester (**7c**, $C_{15}H_{11}ClN_4O_3$)

This compound was prepared by the reaction of 6-aminopyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid methyl ester **5** with 0.55 g of 4-chlorobenzoyl chloride (**6c**, 3.14 mmol) in the presence of *N*,*N*-diisopropylethylamine in *N*,*N*-dimethylformamide. The crude product was obtained as a yellow solid, which on crystallization afforded **7c** as a pale yellow solid (0.78 g, 83%). M.p.: 231.4–233.1 °C; TLC: $R_f = 0.41$ (EtOAc-hexane 4:6); IR (ATR): $\bar{\nu} = 1563$ (N–H bend), 1661 (amidic C=O), 1710 (ester C=O), 3307 (N–H stretch) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 3.82$ (3H, s, ester CH_3), 7.68 (2H,d, $J = 8.4$ Hz, ArH), 8.05 (2H, d, $J = 8.8$ Hz, ArH), 8.61 (1H, s, ArH), 9.04 (1H, d, $J = 2.4$ Hz, ArH), 9.73 (1H, d, $J = 2.4$ Hz, ArH), 10.93 (1H, s, amide NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 51.6$, 102.1, 125.4, 127.2, 129.2 (2 peaks), 130.2 (2 peaks), 132.5, 137.7, 144.4, 147.7, 149.1, 162.5, 165.5 ppm; LC–MS (ESI): $m/z = 329.6$ ($[M-H]^-$).

6-(4-Bromobenzoylamino)pyrazolo[1,5-a]pyrimidine-3-carboxylic acid methyl ester (**7d**, $C_{15}H_{11}BrN_4O_3$)

This compound was prepared by the reaction of 6-aminopyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid methyl ester (**5**) with 0.69 g of 4-bromobenzoyl chloride (**6d**, 3.14 mmol) in the presence of *N*,*N*-diisopropylethylamine in *N*,*N*-dimethylformamide. The crude product was obtained as a yellow solid, which on crystallization afforded **7d** as a pale yellow solid (0.85 g, 80%). M.p.: 229.4–232.0 °C; TLC: $R_f = 0.41$ (EtOAc-hexane 4:6); IR (ATR): $\bar{\nu} = 1560$ (N–H bend), 1657 (amidic C=O), 1709 (ester C=O), 3305 (N–H stretch) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 3.82$ (3H, s, ester CH_3), 7.82 (2H, dd, $J_1 = 2.0$ Hz, $J_2 = 6.8$ Hz, ArH), 7.97 (2H, dd, $J_1 = 1.8$ Hz, $J_2 = 6.6$ Hz, ArH), 8.62 (1H, s, ArH), 9.04 (1H, d, $J = 2.4$ Hz, ArH), 9.72 (1H, d, $J = 2.4$ Hz, ArH), 10.93 (1H, s, amide NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 51.6$, 102.1, 125.4, 126.7, 127.2, 130.3 (2 peaks), 132.1 (2 peaks), 132.9, 144.4, 147.7, 149.1, 162.5, 165.6 ppm; LC–MS (ESI): $m/z = 376.7$ ($[M+H]^+$).

6-(3-Fluorobenzoylamino)pyrazolo[1,5-a]pyrimidine-3-carboxylic acid methyl ester (**7e**, $C_{15}H_{11}FN_4O_3$)

This compound was prepared by the reaction of 6-aminopyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid methyl ester **5** with 0.50 g of 3-fluorobenzoyl chloride (**6e**, 3.14 mmol) in the presence of *N*,*N*-diisopropylethylamine in *N*,*N*-dimethylformamide. The crude product was obtained as a yellow solid, which on crystallization afforded **7e** as a pale yellow solid (0.77 g, 86%). M.p.: 243.4–244.9 °C; TLC: $R_f = 0.41$ (EtOAc-hexane 4:6); IR (ATR): $\bar{\nu} = 1560$ (N–H bend), 1658 (amidic C=O), 1709 (ester C=O), 3305 (N–H stretch) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6); $\delta = 3.82$ (3H, s, ester CH_3), 7.52 (1H, dt, $J_1 = 6.4$ Hz, $J_2 = 10.8$ Hz, ArH), 7.64 (1H, td, $J_1 = 5.6$ Hz, $J_2 = 8.0$ Hz, ArH), 7.81–7.88 (2H, m, ArH), 8.60 (1H, s, ArH), 9.04 (1H, d, $J = 2.4$ Hz, ArH), 9.71 (1H, d, $J = 2.4$ Hz, ArH), 10.92 (1H, s, amide NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 51.6$, 102.1, 114.9, 115.2 (d, $^2J_{C\text{-}F} = 23.0$ Hz), 119.6, 119.8 (d, $^2J_{C\text{-}F} = 21.0$ Hz), 124.5, 124.5 (d, $^4J_{C\text{-}F} = 2.0$ Hz), 125.3, 127.2, 131.3, 131.4 (d, $^3J_{C\text{-}F} = 8.0$ Hz), 136.0, 136.1 (d, $^3J_{C\text{-}F} = 7.0$ Hz), 144.4, 147.7, 149.0, 161.2, 163.6 (d, $^1J_{C\text{-}F} = 243.0$ Hz), 162.5, 165.2, 165.2 ppm; LC–MS (ESI): $m/z = 313.1$ ($[M-H]^-$).

6-(3,5-Difluorobenzoylamino)pyrazolo[1,5-a]pyrimidine-3-carboxylic acid methyl ester (**7f**, $C_{15}H_{10}F_2N_4O_3$)

This compound was prepared by the reaction of 6-aminopyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid methyl ester **5** with 0.55 g of 3,5-difluorobenzoyl chloride (**6f**, 3.14 mmol) in the presence of *N*,*N*-diisopropylethylamine in *N*,*N*-dimethylformamide. The crude product was obtained as a yellow solid, which on crystallization afforded **7f** as a pale yellow solid (0.79 g, 83%). M.p.: 228.9–230.8 °C; TLC: $R_f = 0.41$ (EtOAc-hexane 4:6); IR (ATR): $\bar{\nu} = 1562$ (N–H bend), 1680 (amidic C=O), 1697 (ester C=O), 3360 (N–H stretch) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 3.82$ (3H, s, ester CH_3), 7.61 (1H, dt, $J_1 = 6.4$ Hz, $J_2 = 11.6$ Hz, ArH), 7.74 (2H, dd, $J_1 = 2.0$ Hz, $J_2 = 8.0$ Hz, ArH), 8.63 (1H, s, ArH), 9.03 (1H, d, $J = 2.0$ Hz, ArH), 9.71 (1H, d, $J = 2.4$ Hz, ArH), 10.99 (1H, s, amide NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 51.6$, 102.2, 108.0, 108.3, 108.5 (t, $^2J_{C\text{-}F} = 26.0$ Hz), 111.6, 111.8 (d, $^2J_{C\text{-}F} = 19.0$ Hz), 111.7, 111.9 (d, $^2J_{C\text{-}F} = 20.0$ Hz), 125.1, 127.5, 137.1, 137.2, 137.3 (t, $^3J_{C\text{-}F} = 9.0$ Hz), 144.5, 147.8, 149.0, 161.4, 163.9 (d, $^1J_{C\text{-}F} = 246.0$ Hz), 161.6, 164.0 (d, $^1J_{C\text{-}F} = 246.0$ Hz), 162.5, 164.2 ppm; LC–MS (ESI): $m/z = 331.1$ ($[M-H]^-$).

6-(2-Fluorobenzoylamino)pyrazolo[1,5-a]pyrimidine-3-carboxylic acid methyl ester (**7g**, $C_{15}H_{11}FN_4O_3$)

This compound was prepared by the reaction of 6-aminopyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid methyl ester **5** with 0.50 g of 2-fluorobenzoyl chloride (**6g**, 3.14 mmol) in the presence of *N*,*N*-diisopropylethylamine in *N*,*N*-dimethylformamide. The crude product was obtained as a yellow solid, which on crystallization afforded **7g** as a pale yellow solid (0.78 g, 87%). M.p.: 236.7–238.5 °C; TLC: $R_f = 0.41$ (EtOAc-hexane 4:6); IR (ATR): $\bar{\nu} = 1560$ (N–H bend), 1658 (amidic C=O), 1709 (ester C=O), 3305 (N–H stretch) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 3.82$ (3H, s, ester CH_3), 7.37–7.44 (2H, m, ArH), 7.62–7.68 (1H, m, ArH), 7.77 (1H, dt, $J_1 = 6.0$ Hz, $J_2 = 13.6$ Hz, ArH), 8.61 (1H, s, ArH), 8.94 (1H, d, $J = 2.0$ Hz, ArH), 9.71 (1H, d, $J = 2.4$ Hz, ArH), 11.05 (1H, s, amide NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 51.6$, 102.2, 116.7, 117.0 (d, $^2J_{C\text{-}F} = 22.0$ Hz), 123.6, 123.8 (d, $^2J_{C\text{-}F} = 14.0$ Hz), 125.2, 125.2 (d, $^3J_{C\text{-}F} = 7.0$ Hz), 125.2, 126.7, 130.6, 130.6 (d, $^4J_{C\text{-}F} = 3.0$ Hz), 133.9, 134.0 (d, $^3J_{C\text{-}F} = 9.0$ Hz), 144.4, 147.7, 148.5, 158.3, 160.8 (d, $^1J_{C\text{-}F} = 249.0$ Hz), 162.5, 163.9 ppm; LC–MS (ESI): $m/z = 313.0$ ($[M-H]^-$).

6-(4-Methoxybenzoylamino)pyrazolo[1,5-a]pyrimidine-3-carboxylic acid methyl ester (**7h**, $C_{16}H_{14}N_4O4$)

This compound was prepared by the reaction of 6-aminopyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid methyl ester **5** with 0.53 g of 4-methoxybenzoyl chloride (**6h**, 3.14 mmol) in the presence of *N*,*N*-diisopropylethylamine in *N*,*N*-dimethylformamide. The crude product was obtained as a yellow solid, which on crystallization afforded **7h** as a pale yellow solid (0.82 g, 88%). M.p.: 241.6–243.5 °C; TLC: $R_f = 0.41$ (EtOAc-hexane 4:6); IR (ATR): $\bar{\nu} = 1560$ (N–H bend), 1658 (amidic C=O), 1709 (ester C=O), 3305 (N–H stretch) cm^{-1}; ^1H NMR

(400 MHz, DMSO-d_6): $\delta = 3.82$ (3H, s, ester CH_3), 3.85 (3H, s, methoxy CH_3), 7.12 (2H, d, $J = 8.8$ Hz, ArH), 8.03 (2H, d, $J = 8.8$ Hz, ArH), 8.59 (1H, s, ArH), 9.06 (1H, d, $J = 2.4$ Hz, ArH), 9.73 (1H, d, $J = 2.4$ Hz, ArH), 10.70 (1H, s, amide NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 51.5$, 53.9, 102.0, 114.3 (2 peaks), 125.8, 125.8, 126.8, 130.3 (2 peaks), 144.3, 147.59, 149.2, 162.5, 162.9, 165.9 ppm; LC–MS (ESI): $m/z = 325.2$ ($[M-H]^-$).

*General procedure for the synthesis of substituted carboxylic acids **8a–8h***

To a suspension of substituted carboxylic acid methyl ester **7a–7h** (1.00 mmol) in 2.5 cm^3 of tetrahydrofuran, 2.5 cm^3 of methanol, and 2.2 cm^3 of water was added lithium hydroxide monohydrate (3.00 mmol) and heated to 72 °C under nitrogen atmosphere for 3–4 h. The completion of reaction was monitored by TLC. The reaction mixture was gradually allowed to attain room temperature (28 °C) and concentrated to dryness under reduced pressure. The residue was diluted with 10 cm^3 of ice cold water, acidified (pH ~5–6) with concentrated hydrochloric acid and stirred for 1 h. The precipitate formed was filtered, washed with 2 cm^3 of water and dried at room temperature (28 °C) for 12 h to afford the crude product. The crude product was crystallized by digesting in 10 cm^3 of methanol for 1 h at room temperature (28 °C), filtered, washed with 2 cm^3 of methanol, and dried under vacuum at room temperature (28 °C) for 4 h to afford the corresponding substituted carboxylic acids **8a–8h**.

6-[3-(Trifluoromethyl)benzoylamino]pyrazolo[1,5-a]pyrimidine-3-carboxylic acid (**8a**, $C_{15}H_9F_3N_4O_3$)

This compound was prepared by the reaction of 0.75 g of 6-[3-(trifluoromethyl)benzoylamino]pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid methyl ester (**7a**, 2.04 mmol) with 0.26 g of lithium hydroxide monohydrate (6.17 mmol) in tetrahydrofuran, methanol, and water. The crude product was obtained as a yellow solid, which on crystallization afforded **8a** as a pale yellow solid (0.54 g, 76%). M.p.: 262.3–264.1 °C; TLC: $R_f = 0.10$ (MeOH–$CHCl_3$ 1:9); IR (ATR): $\bar{\nu} = 1561$ (N–H bend), 1633 (amidic C=O), 1671 (acid C=O), 3105 (N–H stretch), 3435 (O–H) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 7.85$ (1H, t, $J = 8.0$ Hz, ArH), 8.04 (1H, d, $J = 7.6$ Hz, ArH), 8.26–8.37 (1H, m, ArH), 8.45 (1H, d, $J = 2.8$ Hz, ArH), 8.56 (1H, s, ArH), 9.02 (1H, d, $J = 2.4$ Hz, ArH), 9.71 (1H, d, $J = 2.0$ Hz, ArH), 11.07 (1H, s, amide NH), 12.44 (1H, s, acid-OH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 103.3$, 120., 123.0, 125.7, 128.4 (q, $^1J_{C\text{-}F} = 271.0$ Hz), 124.7, 124.8, 125.0, 127.3, 129.2, 129.3 (d, $^3J_{C\text{-}F} = 4.0$ Hz), 129.3, 129.6, 129.9, 130.3 (q, $^2J_{C\text{-}F} = 32.0$ Hz), 130.5, 132.4, 134.8, 144.6, 147.9, 148.7, 163.5, 163.7, 165.1 ppm; LC–MS (ESI): $m/z = 348.9$ ($[M-H]^-$).

6-(4-Fluorobenzoylamino)pyrazolo[1,5-a]pyrimidine-3-carboxylic acid (**8b**, $C_{14}H_9FN_4O_3$)

This compound was prepared by the reaction of 0.75 g of 6-(4-fluorobenzoylamino)pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid methyl ester (**7b**, 2.38 mmol) with 0.30 g of lithium hydroxide monohydrate (7.16 mmol) in tetrahydrofuran, methanol, and water. The crude product was obtained as a yellow solid, which on crystallization afforded **8b** as a pale yellow solid (0.56 g, 78%). M.p.: 274.8–276.9 °C; TLC: $R_f = 0.10$ (MeOH–$CHCl_3$ 1:9); IR (ATR): $\bar{\nu} = 1562$ (N–H bend), 1633 (amidic C=O), 1674 (acid C=O), 3104 (N–H stretch), 3436 (O–H) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 7.44$ (2H, t, $J = 8.6$ Hz, ArH), 8.11 (2H, dd, $J_1 = 5.4$ Hz, $J_2 = 8.6$ Hz, ArH), 8.51 (1H, s, ArH), 9.01 (1H, d, $J = 2.0$ Hz, ArH), 9.70 (1H, d, $J = 2.4$ Hz, ArH), 10.85 (1H, s, amide NH), 12.43 (1H, s, acid-OH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 103.1$, 116.0, 116.2 (d, $^2J_{C\text{-}F} = 22.0$ Hz), 125.3, 126.9, 130.3, 130.3 (d, $^4J_{C\text{-}F} = 2.0$ Hz), 131.0, 131.1 (d, $^3J_{C\text{-}F} = 9.0$ Hz), 144.5, 147.8, 148.7, 163.6, 166.1 (d, $^1J_{C\text{-}F} = 249.0$ Hz), 163.5, 165.4 ppm; LC–MS (ESI): $m/z = 300.9$ ($[M+H]^+$).

6-(4-Chlorobenzoylamino)pyrazolo[1,5-a]pyrimidine-3-carboxylic acid (**8c**, $C_{14}H_9ClN_4O_3$)

This compound was prepared by the reaction of 0.75 g of 6-(4-chlorobenzoylamino)pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid methyl ester (**7c**, 2.26 mmol) with 0.28 g of lithium hydroxide monohydrate (6.80 mmol) in tetrahydrofuran, methanol, and water. The crude product was obtained as a yellow solid, which on crystallization afforded **8c** as a pale yellow solid (0.55 g, 77%). M.p.: 270.3–272.5 °C; TLC: $R_f = 0.10$ (MeOH–$CHCl_3$ 1:9); IR (ATR): $\bar{\nu} = 1632$ (amidic C=O), 1677 (acid C=O), 3120 (N–H stretch), 3322 (O–H) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 7.64$ (2H, d, $J = 6.0$ Hz, ArH), 8.08 (2H, d, $J = 8.4$ Hz, ArH), 8.45 (1H, s, ArH), 8.99 (1H, s, ArH), 9.69 (1H, s, amide NH), 11.13 (1H, s, acid-OH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 105.9$, 124.8, 127.0, 129.1 (2 peaks), 130.2 (2 peaks), 132.6, 137.5, 144.2, 147.6, 148.1, 164.4, 165.4 ppm; LC–MS (ESI): $m/z = 316.0$ ($[M-H]^-$).

6-(4-Bromobenzoylamino)pyrazolo[1,5-a]pyrimidine-3-carboxylic acid (**8d**, $C_{14}H_9BrN_4O_3$)

This compound was prepared by the reaction of 0.75 g of 6-(4-bromobenzoylamino)pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid methyl ester (**7d**, 2.00 mmol) with 0.25 g of lithium hydroxide monohydrate (6.00 mmol) in tetrahydrofuran, methanol, and water. The crude product was obtained as a yellow solid, which on crystallization afforded **8d** as a pale yellow solid (0.57 g, 79%). M.p.: 268.2–269.9 °C; TLC: $R_f = 0.10$ (MeOH–$CHCl_3$ 1:9); IR (ATR): $\bar{\nu} = 1566$ (N–H bend), 1633 (amidic C=O), 1678

(acid C=O), 3119 (N–H stretch), 3323 (O–H) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 7.81$ (2H, d, $J = 8.4$ Hz, ArH), 8.00 (2H, d, $J = 8.4$ Hz, ArH), 8.47 (1H, s, A–H), 9.00 (1H, d, $J = 2.0$ Hz, ArH), 9.69 (1H, s, amide NH), 11.07 (1H, s, acid-OH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 105.4$, 124.9, 126.5, 127.0, 130.4 (2 peaks), 132.1 (2 peaks), 133.0, 144.3, 147.7, 148.2, 164.1, 165.6 ppm; LC–MS (ESI): $m/z = 359.0$ ($[M-2H]^-$).

6-(3-Fluorobenzoylamino)pyrazolo[1,5-a]pyrimidine-3-carboxylic acid (**8e**, $C_{14}H9FN_4O_3$)

This compound was prepared by the reaction of 0.75 g of 6-(3-fluorobenzoylamino)pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid methyl ester (**7e**, 2.38 mmol) with 0.30 g of lithium hydroxide monohydrate (7.15 mmol) in tetrahydrofuran, methanol, and water. The crude product was obtained as a yellow solid, which on crystallization afforded **8e** as a pale yellow solid (0.54 g, 76%). M.p.: 268.3–270.1 °C; TLC: $R_f = 0.10$ (MeOH–$CHCl_3$ 1:9); IR (ATR): $\bar{\nu} = 1562$ (N–H bend), 1635 (amidic C=O), 1673 (acid C=O), 3104 (N–H stretch), 3436 (O–H) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 7.51$ (1H, dt, $J_1 = 5.6$ Hz, $J_2 = 13.6$ Hz, ArH), 7.65 (1H, td, $J_1 = 6.0$ Hz, $J_2 = 8.0$ Hz, ArH), 7.82-7.89 (2H, m, ArH), 8.55 (1H, s, ArH), 9.02 (1H, d, $J = 2.4$ Hz, ArH), 9.71 (1H, d, $J = 2.4$ Hz, ArH), 10.89 (1H, s, amide NH), 12.44 (1H, s, acid-OH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 103.2$, 114.9, 115.2 (d, $^2J_{C\text{-}F} = 23.0$ Hz), 119.5, 119.8 (d, $^2J_{C\text{-}F} = 21.0$ Hz), 124.4, 124.4 (d, $^4J_{C\text{-}F} = 3.0$ Hz), 125.1, 127.0, 131.3, 131.3 (d, $^3J_{C\text{-}F} = 7.0$ Hz), 136.0, 136.1 (d, $^3J_{C\text{-}F} = 7.0$ Hz), 144.5, 147.8, 148.6, 161.2, 163.6 (d, $^1J_{C\text{-}F} = 243.0$ Hz), 163.5, 165.1, 165.1 ppm; LC–MS (ESI): $m/z = 299.1$ ($[M-H]^-$).

6-(3,5-Difluorobenzoylamino)pyrazolo[1,5-a]pyrimidine-3-carboxylic acid (**8f**, $C_{14}H_8F_2N_4O_3$)

This compound was prepared by the reaction of 0.75 g of 6-(3,5-difluorobenzoylamino)pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid methyl ester (**7f**, 72.25 mmol) with 0.28 g lithium hydroxide monohydrate (6.77 mmol) in tetrahydrofuran, methanol, and water. The crude product was obtained as a yellow solid, which on crystallization afforded **8f** as a pale yellow solid (0.54 g, 76%). M.p.: 259.6–261.4 °C; TLC: $R_f = 0.10$ (MeOH–$CHCl_3$ 1:9); IR (ATR): $\bar{\nu} = 1556$ (N–H bend), 1631 (amidic C=O), 1668 (acid C=O), 3128 (N–H stretch), 3363 (O–H) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 7.60$ (1H, dt, $J_1 = 7.2$ Hz, $J_2 = 16.0$ Hz, ArH), 7.74 (2H, t, $J = 6.4$ Hz, ArH), 8.55 (1H, s, ArH), 8.99 (1H, d, $J = 2.4$ Hz, ArH), 9.68 (1H, d, $J = 2.4$ Hz, ArH), 10.93 (1H, s, amide NH), 12.45 (1H, s, acid-OH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 103.3$, 108.0, 108.2, 108.5 (t, $^2J_{C\text{-}F} = 26.0$ Hz), 116.6, 111.8 (d, $^2J_{C\text{-}F} = 19.0$ Hz), 111.6, 111.8 (d, $^2J_{C\text{-}F} = 20.0$ Hz), 124.8, 127.2, 137.1, 137.2, 137.3 (t, $^3J_{C\text{-}F} = 8.0$ Hz), 144.6, 147.9, 148.6, 161.4, 163.9 (d, $^1J_{C\text{-}F} = 248.0$ Hz), 161.5, 164.0 ($^1J_{C\text{-}F} = 246.0$ Hz), 163.4, 163.9 ppm; LC–MS (ESI): $m/z = 317.1$ ($[M-H]^-$).

6-(2-Fluorobenzoylamino)pyrazolo[1,5-a]pyrimidine-3-carboxylic acid (**8g**, $C_{14}H_9FN_4O_3$)

This compound was prepared by the reaction of 0.75 g of 6-(2-fluorobenzoylamino)pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid methyl ester (**7g**, 2.38 mmol) with 0.30 g of lithium hydroxide monohydrate (7.16 mmol) in tetrahydrofuran, methanol, and water. The crude product was obtained as a yellow solid, which on crystallization afforded **8g** as a pale yellow solid (0.55 g, 77%). M.p.: 269.9–271.6 °C; TLC: $R_f = 0.10$ (MeOH–$CHCl_3$ 1:9); IR (ATR): $\bar{\nu} = 1563$ (N–H bend), 1635 (amidic C=O), 1673 (acid C=O), 3104 (N–H stretch), 3436 (O–H) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 7.37$–7.44 (2H, m, ArH), 7.62–7.68 (1H, m, ArH), 7.78 (1H, dt, $J_1 = 6.0$, $J_2 = 13.2$ Hz, ArH), 8.55 (1H, s, ArH), 8.92 (1H, d, $J = 2.4$ Hz, ArH), 9.69 (1H, d, $J = 2.4$ Hz, ArH), 11.02 (1H, s, amide NH), 12.43 (1H, s, acid-OH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 103.2$, 116.7, 117.0 (d, $^2J_{C\text{-}F} = 21.0$ Hz), 123.7, 123.8 (d, $^3J_{C\text{-}F} = 14.0$ Hz), 125.0, 125.2 (d, $^2J_{C\text{-}F} = 21.0$ Hz), 126.6, 130.6, 130.64 (d, $^4J_{C\text{-}F} = 2.0$ Hz), 133.9, 134.0 (d, $^3J_{C\text{-}F} = 9.0$ Hz), 144.6, 147.9, 148.2, 158.3, 160.8 (d, $^1J_{C\text{-}F} = 249.0$ Hz), 163.52, 163.9 ppm; LC–MS (ESI): $m/z = 299.2$ ($[M-H]^-$).

6-(4-Methoxybenzoylamino)pyrazolo[1,5-a]pyrimidine-3-carboxylic acid (**8h**, $C_{15}H_{12}N_4O_3$)

This compound was prepared by the reaction of 0.75 g of 6-(4-methoxybenzoylamino)pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid methyl ester (**7h**, 2.29 mmol) with 0.29 g of lithium hydroxide monohydrate (6.89 mmol) in tetrahydrofuran, methanol, and water. The crude product was obtained as a yellow solid, which on crystallization afforded **8h** as a pale yellow solid (0.56 g, 78%). M.p.: 263.4–265.3 °C; TLC: $R_f = 0.10$ (MeOH–$CHCl_3$ 1:9); IR (ATR): $\bar{\nu} = 1563$ (N–H bend), 1635 (amidic C=O), 1673 (acid C=O), 3104 (N–H stretch), 3436 (O–H) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 3.85$ (3H, s, methoxy CH_3), 7.11 (2H, d, $J = 8.8$ Hz, ArH), 8.03 (2H, d, $J = 9.2$ Hz, ArH), 8.52 (1H, s, Ar–H), 9.03 (1H, d, $J = 2.4$ Hz, ArH), 9.70 (1H, d, $J = 2.0$ Hz, ArH), 10.65 (1H, s, amide NH), 12.41 (1H, s, acid-OH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 55.9$, 103.0, 114.3, 125.6, 125.8, 126.6, 130.2, 144.4, 147.7, 148.7, 162.9, 163.5, 165.9 ppm; LC–MS (ESI): $m/z = 311.1$ ($[M-H]^-$).

*General procedure for the synthesis of compounds **10a–10h***

To a suspension of substituted carboxylic acid **8a–8h** (1.00 mmol) in 2.5 cm^3 of *N*,*N*-dimethylformamide was added *tert*-butyl carbazate (**9**, 1.10 mmol), ethyl carbodiimide hydrochloride (1.50 mmol), 1-hydroxy-7-azabenzotriazole (1.20 mmol), followed by the drop wise addition of *N*,*N*-diisopropylethylamine (3.00 mmol) and stirred at room temperature (28 °C) under nitrogen atmosphere for 12 h. The completion of reaction was monitored by TLC. The reaction mixture was poured into 25 cm^3 of ice cold water, stirred for 1 h, filtered, washed with 5 cm^3 of water, and dried under vacuum at room temperature (28 °C) for 8 h to afford the crude product. The crude product was purified by column chromatography over silica gel with chloroform/methanol (100:2 v/v) to afford the compounds **10a–10h**.

N′-[6-[3-(Trifluoromethyl)benzoylamino]pyrazolo[1,5-a]pyrimidine-3-carbonyl]hydrazinecarboxylic acid tert-butyl ester (**10a**, $C_{20}H_{19}F_3N_6O_4$)

This compound was prepared by the reaction of 0.50 g of 6-[3-(trifluoromethyl)benzoylamino]pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (**8a**, 1.42 mmol) with 0.20 g of *tert*-butyl carbazate (**9**, 1.57 mmol) in presence of 0.33 g of ethyl carbodiimide hydrochloride (2.14 mmol), 0.40 g of 1-hydroxy-7-azabenzotriazole (1.71 mmol), and 0.55 g of *N*,*N*-diisopropylethylamine (4.28 mmol) in *N*,*N*-dimethylformamide. The crude product was obtained as a brown solid, which on column chromatography purification afforded **10a** as a beige solid (0.55 g, 83%). M.p.: 198.5–200.4 °C; TLC: $R_f = 0.71$ (MeOH–$CHCl_3$ 0.5:9.5); IR (ATR): $\bar{\nu} = 1161$ (alkoxy C–O), 1535, 1556, 1618 (N–H bend), 1666, 1687, 1722 (C=O), 3097, 3215, 3284 (N–H stretch) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 1.43$ (9H, s, boc CH_3), 7.85 (1H, t, $J = 8.0$ Hz, ArH), 8.05 (1H, d, $J = 7.6$ Hz, ArH), 8.33 (1H, d, $J = 7.6$ Hz, ArH), 8.37 (1H, s, ArH), 8.61 (1H, s, ArH), 9.05 (1H, d, $J = 5.6$ Hz, amide NH), 9.04 (1H, s, ArH), 9.33 (1H, s, ArH), 9.75 (1H, d, $J = 1.6$ Hz, amide NH), 11.07 (1H, s, amide NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 28.5$ (3 peaks), 79.8, 104.1, 120.3, 123.0, 125.7, 128.4 (q, $^1J_{C\text{-}F} = 271.0$ Hz), 124.7, 124.8, 124.9 (t, $^3J_{C\text{-}F} = 4.0$ Hz), 127.7, 127.8, 127.9 (t, $^3J_{C\text{-}F} = 4.0$ Hz), 129.3, 129.6, 130.0, 130.3 (q, $^2J_{C\text{-}F} = 32.0$ Hz), 130.5, 132.4, 134.8, 143.3, 146.2, 148.5, 155.9, 161.4, 165.1 ppm; LC–MS (ESI): $m/z = 463.2$ ($[M-H]^-$).

N′-[6-(4-Fluorobenzoylamino)pyrazolo[1,5-a]pyrimidine-3-carbonyl]hydrazinecarboxylic acid tert-butyl ester (**10b**, $C_{19}H_{19}FN6O_4$)

This compound was prepared by the reaction of 0.5 g of 6-(4-fluorobenzoylamino)pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (**8b**, 1.66 mmol) with 0.24 g of *tert*-butyl carbazate (**9**, 1.83 mmol) in presence of 0.38 g of ethyl carbodiimide hydrochloride (2.49 mmol), 0.27 g of 1-hydroxy-7-azabenzotriazole (2.00 mmol), and 0.64 g of *N*,*N*-diisopropylethylamine (5.00 mmol) in *N*,*N*-dimethylformamide. The crude product was obtained as a brown solid, which on column chromatography purification afforded **10b** as a beige solid (0.58 g, 84%). M.p.: 208.5–210.2 °C; TLC: $R_f = 0.71$ (MeOH–$CHCl_3$ 0.5:9.5); IR (ATR): $\bar{\nu} = 1161$ (alkoxy C–O), 1510, 1564, 1602 (N–H bend), 1666, 1687, 1708 (C=O), 3078, 3221, 3311 (N–H stretch) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 1.43$ (9H, s, boc CH_3), 7.44 (2H, t, $J = 8.8$ Hz, ArH), 8.11 (2H, dt, $J_1 = 3.6$ Hz, $J_2 = 10.4$ Hz, ArH), 8.60 (1H, s, ArH), 9.02 (1H, d, $J = 6.4$ Hz, amide NH), 9.04 (1H, s, ArH), 9.32 (1H, s, ArH), 9.75 (1H, d, $J = 2.0$ Hz, amide NH), 10.87 (1H, s, amide NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 28.5$ (3 peaks), 79.8, 104.0, 116.0, 116.2 (d, $^2J_{C\text{-}F} = 22.0$ Hz), 125.2, 127.4, 130.3, 130.3 (d, $^4J_{C\text{-}F} = 3.0$ Hz), 131.0, 131.1 (d, $^3J_{C\text{-}F} = 9.0$ Hz), 143.2, 146.1, 148.5, 155.9, 161.4, 163.7, 166.2 (d, $^1J_{C\text{-}F} = 249.0$ Hz), 165.5 ppm; LC–MS (ESI): $m/z = 413.2$ ($[M-H]^-$).

N′-[6-(4-Chlorobenzoylamino)pyrazolo[1,5-a]pyrimidine-3-carbonyl]hydrazinecarboxylic acid tert-butyl ester (**10c**, $C_{19}H_{19}ClN_6O_4$)

This compound was prepared by the reaction of 0.5 g of 6-(4-chlorobenzoylamino)pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (**8c**, 1.57 mmol) with 0.23 g of *tert*-butyl carbazate (**9**, 1.73 mmol) in presence of 0.36 g of ethyl carbodiimide hydrochloride (2.36 mmol), 0.25 g of 1-hydroxy-7-azabenzotriazole (1.89 mmol), and 0.61 g of *N*,*N*-diisopropylethylamine (4.73 mmol) in *N*,*N*-dimethylformamide. The crude product was obtained as a brown solid, which on column chromatography purification afforded **10c** as a beige solid (0.60 g, 89%). M.p.: 212.8–214.1 °C; TLC: $R_f = 0.71$ (MeOH–$CHCl_3$ 0.5:9.5); IR (ATR): $\bar{\nu} = 1157$ (alkoxy C–O), 1521, 1560, 1591 (N–H bend), 1666, 1687, 1708 (C=O), 3103, 3221, 3294 (N–H stretch) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 1.43$ (9H, s, boc CH_3), 7.68 (2H, d, $J = 8.4$ Hz, ArH), 8.06 (2H, d, $J = 8.4$ Hz, ArH), 8.60 (1H, s, ArH), 9.02 (1H, d, $J = 6.4$ Hz, amide NH), 9.03 (1H, s, ArH), 9.32 (1H, s, ArH), 9.75 (1H, d, $J = 2.0$ Hz, amide NH), 10.92 (1H, s, amide NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 28.5$ (3 peaks), 79.8, 104.0, 125.1, 127.4, 129.1 (2 peaks), 130.1 (2 peaks), 132.5, 137.6, 143.2, 146.1, 148.4, 155.9, 161.4, 165.4 ppm; LC–MS (ESI): $m/z = 429.3$ ($[M-H]^-$).

N′-[6-(4-Bromobenzoylamino)pyrazolo[1,5-a]pyrimidine-3-carbonyl]hydrazinecarboxylic acid tert-butyl ester (**10d**, $C_{19}H_{19}BrN_6O_4$)

This compound was prepared by the reaction of 0.50 g of 6-(4-bromobenzoylamino)pyrazolo[1,5-*a*]pyrimidine-

3-carboxylic acid (**8d**, 1.38 mmol) with 0.20 g of *tert*-butyl carbazate (**9**, 1.52 mmol) in presence of 0.32 g of ethyl carbodiimide hydrochloride (2.07 mmol), 0.22 g of 1-hydroxy-7-azabenzotriazole (1.66 mmol), and 0.53 g of *N*,*N*-diisopropylethylamine (4.14 mmol) in *N*,*N*-dimethylformamide. The crude product was obtained as a brown solid, which on column chromatography purification afforded **10d** as a beige solid 0.56 g, 86%). M.p.: 215.6–217.7 °C; TLC: $R_f = 0.71$ (MeOH–$CHCl_3$ 0.5:9.5); IR (ATR): $\bar{\nu} = 1157$ (alkoxy C–O), 1525, 1562, 1589 (N–H bend), 1666, 1687, 1708 (C=O), 3099, 3221, 3254 (N–H stretch) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 1.43$ (9H, s, boc CH_3), 7.82 (2H, d, $J = 8.4$ Hz, ArH), 7.98 (2H, d, $J = 8.4$ Hz, ArH), 8.60 (1H, s, ArH), 9.02 (1H, d, $J = 6.8$ Hz, amide NH), 9.03 (1H, s, ArH), 9.32 (1H, s, ArH), 9.74 (1H, d, $J = 2.0$ Hz, amide NH), 10.92 (1H, s, amide NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 28.5$ (3 peaks), 79.8, 104.0, 125.1, 126.6, 127.4, 130.3 (2 peaks), 132.1 (2 peaks), 132.8, 143.1, 146.1, 148.4, 155.9, 161.4, 165.6 ppm; LC–MS (ESI): $m/z = 474.9$ ($[M-H]^-$).

N′-[3-(2-Fluorobenzoylamino)pyrazolo[1,5-a]pyrimidine-3-carbonyl]hydrazinecarboxylic acid tert-butyl ester (**10e**, $C_{19}H_{19}FN_6O_4$)

This compound was prepared by the reaction of 0.50 g of 6-(3-fluorobenzoylamino)pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (**8e**, 1.66 mmol) with 0.24 g of *tert*-butyl carbazate (**9**, 1.83 mmol) in presence of 0.38 g of ethyl carbodiimide hydrochloride (2.49 mmol), 0.27 g of 1-hydroxy-7-azabenzotriazole (2.00 mmol), and 0.64 g of *N*,*N*-diisopropylethylamine (5.00 mmol) in *N*,*N*-dimethylformamide. The crude product was obtained as a brown solid, which on column chromatography purification afforded **10e** as a beige solid (0.60 g, 87%). M.p.: 206.1–207.9 °C; TLC: $R_f = 0.71$ (MeOH–$CHCl_3$ 0.5:9.5); IR (ATR): $\bar{\nu} = 1160$ (alkoxy C–O), 1512, 1563, 1601 (N–H bend), 1666, 1687, 1705 (C=O), 3078, 3221, 3311 (N–H stretch) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 1.43$ (9H, s, boc CH_3), 7.52 (1H, dt, $J_1 = 6.4$ Hz, $J_2 = 15.2$ Hz, ArH), 7.65 (1H, dt, $J_1 = 6.0$ Hz, $J_2 = 10.0$ Hz, ArH), 7.83-7.90 (2H, m, ArH), 8.61 (1H, s, ArH), 9.03 (1H, d, $J = 6.8$ Hz, amide NH), 9.04 (1H, s, ArH), 9.32 (1H, s, ArH), 9.75 (1H, d, $J = 2.0$ Hz, amide NH), 10.93 (1H, s, amide NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 28.5$ (3 peaks), 79.8, 104.1, 115.0, 115.2 (d, $^2J_{C\text{-}F} = 22.0$ Hz), 119.6, 119.8 (d, $^2J_{C\text{-}F} = 21.0$ Hz), 124.5, 124.5 (d, $^4J_{C\text{-}F} = 2.0$ Hz), 125.0, 127.5, 131.3, 131.4 (d, $^3J_{C\text{-}F} = 8.0$ Hz), 136.1, 136.1 (d, $^3J_{C\text{-}F} = 7.0$ Hz), 143.2, 146.1, 148.5, 155.9, 161.2, 161.4, 163.6, 165.2 (d, $^1J_{C\text{-}F} = 258.0$ Hz) ppm; LC–MS (ESI): $m/z = 413.2$ ($[M-H]^-$).

N′-[6-(3,5-Difluorobenzoylamino)pyrazolo[1,5-a]pyrimidine-3-carbonyl]hydrazinecarboxylic acid tert-butyl ester (**10f**, $C_{19}H_{18}F_2N_6O_4$)

This compound was prepared by the reaction of 0.50 g of 6-(3,5-difluorobenzoylamino)pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (**8f**, 1.57 mmol) with 0.22 g of *tert*-butyl carbazate (**9**, 1.72 mmol) in presence of 0.36 g of ethyl carbodiimide hydrochloride (2.35 mmol), 0.25 g of 1-hydroxy-7-azabenzotriazole (1.88 mmol), and 0.60 g of *N*,*N*-diisopropylethylamine (4.71 mmol) in *N*,*N*-dimethylformamide. The crude product was obtained as a brown solid, which on column chromatography purification afforded **10f** as a beige solid (0.58 g, 86%). M.p.: 203.8–205.7 °C; TLC: $R_f = 0.71$ (MeOH–$CHCl_3$ 0.5:9.5); IR (ATR): $\bar{\nu} = 1159$ (alkoxy C–O), 1511, 1565, 1609 (N–H bend), 1667, 1667, 1709 (C=O), 3077, 3220, 3311 (N–H stretch) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 1.43$ (9H, s, boc CH_3), 7.62 (1H, dt, $J_1 = 4.8$ Hz, $J_2 = 13.6$ Hz, ArH), 7.75 (2H, dt, $J_1 = 4.8$ Hz, $J_2 = 6.4$ Hz, ArH), 8.61 (1H, s, ArH), 9.01 (2H, d, $J = 2.4$ Hz, amide NH, ArH), 9.33 (1H, s, ArH), 9.74 (1H, d, $J = 2.0$ Hz, amide NH), 10.98 (1H, s, amide NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 28.5$ (3 peaks), 79.8, 104.1, 108.0, 108.2, 108.5 (t, $^2J_{C\text{-}F} = 26.0$ Hz), 111.6, 111.8 (d, $^2J_{C\text{-}F} = 19.0$ Hz), 111.7, 111.8 (d, $^2J_{C\text{-}F} = 19.0$ Hz), 124.7, 127.6, 137.1, 137.2, 137.3 (t, $^3J_{C\text{-}F} = 8.0$ Hz), 143.2, 146.2, 148.3, 155.9, 161.4, 163.9 (d, $^1J_{C\text{-}F} = 252.0$ Hz) 161.5, 164.0 (d, $^1J_{C\text{-}F} = 252.0$ Hz), 161.5, 163.9, 164.0 ppm; LC–MS (ESI): $m/z = 432.1$ ($[M-H]^-$).

N′-[6-(2-Fluorobenzoylamino)pyrazolo[1,5-a]pyrimidine-3-carbonyl]hydrazinecarboxylic acid tert-butyl ester (**10g**, $C_{19}H_{19}FN_6O_4$)

This compound was prepared by the reaction of 0.50 g of 6-(2-fluorobenzoylamino)pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (**8g**, 1.66 mmol) with 0.024 g of *tert*-butyl carbazate (**9**, 1.83 mmol) in presence of 0.38 g of ethyl carbodiimide hydrochloride (2.49 mmol), 0.27 g of 1-hydroxy-7-azabenzotriazole (2.00 mmol), and 0.64 g of *N*,*N*-diisopropylethylamine (5.00 mmol) in *N*,*N*-dimethylformamide. The crude product was obtained as a brown solid, which on column chromatography purification afforded **10g** as a beige solid (0.61 g, 89%). M.p.: 200.5–202.6 °C; TLC: $R_f = 0.71$ (MeOH–$CHCl_3$ 0.5:9.5); IR (ATR): $\bar{\nu} = 1159$ (alkoxy C–O), 1511, 1565, 1601 (N–H bend), 1666, 1687, 1708 (C=O), 3075, 3220, 3310 (N–H stretch) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 1.43$ (9H, s, boc CH_3), 7.37–7.44 (2H, m, ArH), 7.63–7.80 (1H, m, ArH), 7.78 (1H, t, $J = 6.8$ Hz, ArH), 8.61 (1H, s, ArH), 8.94 (1H, d, $J = 2.0$ Hz, amide NH), 9.01 (1H, s, ArH), 9.32 (1H, s, ArH), 9.74 (1H, d, $J = 1.6$ Hz, amide NH), 11.04 (1H, s, amide NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 27.4$ (3 peaks), 78.7, 103.1, 115.7, 115.9 (d, $^2J_{C\text{-}}$

$_F$ = 22.0 Hz), 122.6, 122.7 (d, $^3J_{C\text{-}F}$ = 14.0 Hz), 123.8, 124.1 (d, $^2J_{C\text{-}F}$ = 26.0 Hz), 124.1, 125.9, 129.5, 129.5 (d, $^4J_{C\text{-}F}$ = 2.0 Hz), 132.8, 132.9 (d, $^3J_{C\text{-}F}$ = 8.0 Hz), 142.1, 145.1,146.9, 154.8, 157.2, 159.7 (d, $^1J_{C\text{-}F}$ = 249.0 Hz), 160.3, 162.8 ppm; LC–MS (ESI): *m/z* = 413.2 ([M−H]$^-$).

N′-[6-(4-Methoxybenzoylamino)pyrazolo[1,5-a]pyrimidine-3-carbonyl]hydrazinecarboxylic acid tert-butyl ester (**10h**, $C_{20}H_{22}N_6O_5$)

This compound was prepared by the reaction of 0.50 g of 6-(4-methoxybenzoylamino)pyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid (**8h**, 1.60 mmol) with 0.23 g of *tert*-butyl carbazate (**9**, 1.76 mmol) in presence of 0.37 g of ethyl carbodiimide hydrochloride (2.40 mmol), 0.26 g of 1-hydroxy-7-azabenzotriazole (1.92 mmol), and 0.62 g of *N*,*N*-diisopropylethylamine (4.80 mmol) in *N*,*N*-dimethylformamide. The crude product was obtained as a brown solid, which on column chromatography purification afforded **10h** as a beige solid (0.56 g, 83%). M.p.: 213.8–215.5 °C; TLC: R_f = 0.71 (MeOH–$CHCl_3$ 0.5:9.5); IR (ATR): $\bar{\nu}$ = 1157 (alkoxy C–O), 1523, 1563, 1595 (N–H bend), 1666, 1687, 1708 (C=O), 3100, 3221, 3260 (N–H stretch) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.43 (9H, s, boc CH_3), 3.85 (3H, s, methoxy CH_3), 7.11 (2H, d, *J* = 8.88 Hz, ArH), 8.03 (2H, d, *J* = 8.8 Hz, ArH), 8.58 (1H, s, ArH), 9.01 (1H, s, ArH), 9.05 (1H, d, *J* = 2.4 Hz, amide NH), 9.31 (1H, s, ArH), 9.74 (1H, d, *J* = 2.0 Hz, amide NH), 10.68 (1H, s, amide NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 27.4 (3 peaks), 54.9, 78.7, 102.9, 113.2 (2 peaks), 124.4, 124.7, 126.0, 129.2 (2 peaks), 142.0, 144.9, 147.45 154.9, 160.4, 161.8, 164.8 ppm; LC–MS (ESI): *m/z* = 425.2 ([M−H]$^-$).

*General procedure for the synthesis of compounds **11a–11h***

To a suspension of compounds **10a–10h** (1.00 mmol) in 4 cm^3 of dichloromethane was added 1 cm^3 of 4.5 M hydrochloric acid in 1,4-dioxane at 0 °C. The temperature was gradually allowed to attain room temperature (28 °C) and stirred for 12 h under nitrogen atmosphere. The completion of reaction was monitored by TLC. The precipitate from the reaction mixture was filtered, washed with 1 cm^3 of dichloromethane and dried under vacuum at room temperature (28 °C) for 4 h to afford the compounds **11a–11h**.

N-[3-(Hydrazinocarbonyl)pyrazolo[1,5-a]pyrimidin-6-yl]-3-(trifluoromethyl)benzamide hydrochloride (**11a**, $C_{15}H_{12}ClF_3N_6O_2$)

From the reaction of 0.40 g of **10a** (0.86 mmol) with 4.5 M hydrochloric acid in 1,4-dioxane in dichloromethane medium was obtained **11a** as a pale yellow solid (0.29 g, 85%). M.p.: 236.7–238.8 °C; TLC: R_f = 0.10 (MeOH–CHCl3 1:9); IR (ATR): $\bar{\nu}$ = 1521, 1598 (N–H bend), 1657, 1689 (C=O), 3050, 3147 (N–H stretch), 3235, 3412 (1° amine, N–H stretch) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.82 (1H, t, *J* = 8.0 Hz, ArH), 8.01 (1H, d, *J* = 7.6 Hz, ArH), 8.45 (2H, d, *J* = 6.4 Hz, ArH), 8.72 (1H, s, ArH), 9.31 (1H, d, *J* = 2.4 Hz, ArH), 9.80 (1H, d, *J* = 2.0 Hz, ArH), 10.48 (2H, s, amide NH), 11.53 (1H, s, amide NH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 102.2, 118.8, 122.5, 126.1, 129.7 (q, $^1J_{C\text{-}F}$ = 270.9 Hz), 125.0, 125.1, 125.2 (t, $^3J_{C\text{-}F}$ = 4.0 Hz), 125.5, 127.5, 127.6, 127.7 (t, $^3J_{C\text{-}F}$ = 4.0 Hz), 129.2, 129.5, 129.9, 130.2 (q, $^2J_{C\text{-}F}$ = 23.5 Hz), 132.5, 134.4, 143.4, 146.3, 149.0, 160.7, 165.0 ppm; LC–MS (ESI): *m/z* = 365.1 ([M+H−HCl]).

4-Fluoro-N-[3-(hydrazinocarbonyl)pyrazolo[1,5-a]pyrimidin-6-yl]benzamide hydrochloride (**11b**, $C_{14}H_{12}ClFN_6O_2$)

From the reaction of 0.40 g of **10b** (0.96 mmol) with 4.5 M hydrochloric acid in 1,4-dioxane in dichloromethane medium was obtained **11b** as a pale yellow solid (0.29 g, 87%). M.p.: 229.6–231.4 °C; TLC: R_f = 0.10 (MeOH–$CHCl_3$ 1:9); IR (ATR): $\bar{\nu}$ = 1522, 1596 (N–H bend), 1655, 1687 (C=O), 3053, 3154 (2° N–H stretch), 3238, 3417 (1° N–H_2 stretch) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.42 (2H, dt, J_1 = 6.8 Hz, J_2 = 15.6 Hz, ArH), 8.22 (2H, dt, J_1 = 3.2 Hz, J_2 = 10.0 Hz, ArH), 8.71 (1H, s, ArH), 9.30 (1H, d, *J* = 2.0 Hz, ArH), 9.80 (1H, d, *J* = 2.4 Hz, ArH), 10.48 (1H, s, amide NH), 11.28 (1H, s, amide NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 102.2, 115.9, 116.1 (d, $^2J_{C\text{-}F}$ = 22.0 Hz), 125.8, 127.4, 130.1, 130.1 (d, $^4J_{C\text{-}F}$ = 3.0 Hz), 131.3, 131.3 (d, $3J_{C\text{-}F}$ = 9.0 Hz), 143.4, 146.3, 149.1, 160.8, 163.7, 166.2 (d, $^1J_{C\text{-}F}$ = 249.0 Hz), 165.4 ppm; LC–MS (ESI): *m/z* = 315.1 ([M+H−HCl]).

4-Chloro-N-[3-(hydrazinocarbonyl)pyrazolo[1,5-a]pyrimidin-6-yl]benzamide hydrochloride (**11c**, $C_{14}H_{12}Cl_2N_6O_2$)

From the reaction of 0.40 g of **10c** (0.92 mmol) with 4.5 M hydrochloric acid in 1,4-dioxane in dichloromethane medium was obtained **11c** as a pale yellow solid (0.31 g, 91%). M.p.: 238.1–239.9 °C; TLC: R_f = 0.10 (MeOH–$CHCl_3$ 1:9); IR (ATR): $\bar{\nu}$ = 1523, 1597 (N–H bend), 1656, 1687 (C=O), 3053, 3155 (2° N–H stretch), 3238, 3417 (1° N–H_2 stretch) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.95 (2H, d, *J* = 8.4 Hz, ArH), 8.16 (2H, d, *J* = 8.4 Hz, ArH), 8.72 (1H, s, ArH), 9.30 (1H, d, *J* = 2.4 Hz, ArH), 9.80 (1H, d, *J* = 2.4 Hz, ArH), 10.48 (1H, s, amide NH), 11.36 (1H, s, amide NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 102.2, 125.7, 127.4, 129.1 (2 peaks), 130.4 (2 peaks), 132.3, 137.6, 143.4, 146.3, 149.0, 160.8, 165.5 ppm; LC–MS (ESI): *m/z* = 329 ([M–H−HCl]).

4-Bromo-N-[3-(hydrazinocarbonyl)pyrazolo[1,5-a]pyrimidin-6-yl]benzamide hydrochloride
(**11d**, $C_{14}H_{12}ClBrN_6O_2$)
From the reaction of 0.40 g of **10d** (0.84 mmol) with 4.5 M hydrochloric acid in 1,4-dioxane in dichloromethane medium was obtained **11d** as a pale yellow solid (0.30 g, 89%). M.p.: 239.7–242.0 °C; TLC: $R_f = 0.10$ (MeOH–$CHCl_3$ 1:9); IR (ATR): $\bar{\nu} = 1520$, 1595 (N–H bend), 1656, 1687 (C=O), 3054, 3157 (2° N–H stretch), 3238, 3415 (1° N-H_2 stretch) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 7.78$ (2H, d, $J = 8.4$ Hz, ArH), 8.08 (2H, d, $J = 8.4$ Hz, ArH), 8.71 (1H, s, ArH), 9.29 (1H, d, $J = 2.4$ Hz, ArH), 9.79 (1H, d, $J = 2.0$ Hz, ArH), 10.48 (1H, s, amide NH), 11.34 (1H, s, amide NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 102.3$, 125.7, 126.7, 127.5, 130.5 (2 peaks), 132.0 (2 peaks), 132.7, 143.4, 146.3, 149.0, 160.8, 165.6 ppm; LC–MS (ESI): $m/z = 377.3$ ([M+2H−HCl]).

3-Fluoro-N-[3-(hydrazinocarbonyl)pyrazolo[1,5-a]pyrimidin-6-yl]benzamide hydrochloride
(**11e**, $C_{14}H_{12}ClFN_6O_2$)
From the reaction of 0.40 g of **10e** (0.96 mmol) with 4.5 M hydrochloric acid in 1,4-dioxane in dichloromethane medium was obtained **11e** as a pale yellow solid (0.29 g, 88%). M.p.: 234.5–236.4 °C; TLC: $R_f = 0.10$ (MeOH–$CHCl_3$ 1:9); IR (ATR): $\bar{\nu} = 1523$, 1597 (N–H bend), 1656, 1687 (C=O), 3053, 3155 (2° N–H stretch), 3238, 3417 (1° N–H_2 stretch) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 7.48$ (1H, dt, $J_1 = 6.4$ Hz, $J_2 = 14.8$ Hz, ArH), 7.61 (1H, dt, $J_1 = 5.6$ Hz, $J_2 = 10.0$ Hz, ArH), 7.97–8.01 (2H, m, ArH), 8.71 (1H, s, ArH), 9.34 (1H, d, $J = 2.4$ Hz, ArH), 9.80 (1H, d, $J = 2.4$ Hz, ArH), 10.49 (1H, s, amide NH), 11.44 (1H, s, amide NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 102.3$, 115.2, 115.4 (d, $^2J_{C\text{-}F} = 23.0$ Hz), 119.6, 119.8 (d, $^2J_{C\text{-}F} = 21.0$ Hz), 124.7, 124.7 (d, $^4J_{C\text{-}F} = 2.0$ Hz), 125.7, 127.6, 131.2, 131.3 (d, $^3J_{C\text{-}F} = 8.0$ Hz), 135.8, 135.9 (d, $^3J_{C\text{-}F} = 7.0$ Hz), 143.5, 146.3, 149.1, 160.8, 161.2, 163.6 (d, $^1J_{C\text{-}F} = 243.0$ Hz), 165.2, 165.2 ppm; LC–MS (ESI): $m/z = 313.0$ ([M−H−HCl]).

3,5-Difluoro-N-[3-(hydrazinocarbonyl)pyrazolo[1,5-a]pyrimidin-6-yl]benzamide hydrochloride
(**11f**, $C_{14}H_{11}ClF_2N_6O_2$)
From the reaction of 0.40 g of **10f** (0.92 mmol) with 4.5 M hydrochloric acid in 1,4-dioxane in dichloromethane medium was obtained **11f** as a pale yellow solid (0.31 g, 93%). M.p.: 228.8–230.5 °C; TLC: $R_f = 0.10$ (MeOH–$CHCl_3$ 1:9); IR (ATR): $\bar{\nu} = 1568$, 1597 (N–H bend), 1622, 1678 (C=O), 3088, 3190 (2° N–H stretch), 3273, 3457 (1° N-H_2 stretch) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 7.60$ (1H, d, $J = 6.0$ Hz, ArH), 7.87 (2H, s, ArH), 8.83 (1H, s, ArH), 9.29–9.33 (1H, m, ArH), 10.55 (1H, s, amide NH), 11.45 (1H, s, amide NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 102.3$, 108.0, 108.3, 108.5 (t, $^2J_{C\text{-}F} = 26.0$ Hz), 111.8, 112.0 (d, $^2J_{C\text{-}F} = 20.0$ Hz), 111.9, 112.1 (d, $^2J_{C\text{-}F} = 19.0$ Hz), 125.4, 127.7, 136.9, 137.0, 137.1 (t, $^3J_{C\text{-}F} = 9.0$ Hz), 143.5, 146.4, 149.0, 160.8, 161.4, 163.8 (d, $^1J_{C\text{-}F} = 245.0$ Hz), 161.5, 163.9, 164.0 ppm; LC–MS (ESI): $m/z = 331.1$ ([M−H−HCl]).

2-Fluoro-N-[3-(hydrazinocarbonyl)pyrazolo[1,5-a]pyrimidin-6-yl]benzamide hydrochloride
(**11g**, $C_{14}H_{12}ClFN_6O_2$)
From the reaction of 0.40 g of **10g** (0.96 mmol) with 4.5 M hydrochloric acid in 1,4-dioxane in dichloromethane medium was obtained **11g** as a pale yellow solid (0.29 g, 87%). M.p.: 233.5–235.6 °C; TLC: $R_f = 0.10$ (MeOH–$CHCl_3$ 1:9); IR (ATR): $\bar{\nu} = 1556$, 1579 (N–H bend), 1629, 1685 (C=O), 3050, 3152 (2° N–H stretch), 3235, 3414 (1° N-H_2 stretch) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 7.38$-7.45 (2H, m, ArH), 7.64–7.69 (1H, m, ArH), 7.70 (1H, dt, $J_1 = 6.0$ Hz, $J_2 = 13.2$ Hz, ArH), 8.72 (1H, s, ArH), 9.03 (1H, d, $J = 2.0$ Hz, ArH), 9.75 (1H, d, $J = 2.4$ Hz, ArH), 10.37 (1H, s, amide NH), 11.09 (1H, s, amide NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 102.4$, 116.8, 117.0 (d, $^2J_{C\text{-}F} = 21.0$ Hz), 123.5, 123.7 (d, $^3J_{C\text{-}F} = 13.0$ Hz), 125.1, 125.2 (d, $^2J_{C\text{-}F} = 21.0$ Hz), 125.4, 127.1, 130.6, 130.6 (d, $^4J_{C\text{-}F} = 2.0$ Hz), 133.9, 134.0 (d, $^3J_{C\text{-}F} = 9.0$ Hz), 143.5, 146.3, 148.5, 158.3, 160.8 (d, $^1J_{C\text{-}F} = 243.0$ Hz), 160.8, 163.9 ppm; LC–MS (ESI): $m/z = 313.0$ ([M–H−HCl]).

N-[3-(Hydrazinocarbonyl)pyrazolo[1,5-a]pyrimidin-6-yl]-4-methoxybenzamide hydrochloride
(**11h**, $C_{15}H_{15}ClN_6O_3$)
From the reaction of 0.40 g of **10h** (0.93 mmol) with 4.5 M hydrochloric acid in 1,4-dioxane in dichloromethane medium was obtained **11h** as a pale yellow solid (0.30 g, 90%). M.p.: 248.5–250.3 °C; TLC: $R_f = 0.10$ (MeOH–$CHCl_3$ 1:9); IR (ATR): $\bar{\nu} = 1120$ (alkoxy C–O), 1558, 1606 (N–H bend), 1653, 1670 (C=O), 3202, 3304 (2° N–H stretch), 3335, 3400 (1° N–H_2 stretch) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): $\delta = 3.95$ (3H, s, methoxy CH_3), 7.13 (2H, d, $J = 8.8$ Hz, ArH), 8.8 (2H, d, $J = 8.8$ Hz, ArH), 8.70 (1H, d, $J = 1.2$ Hz, ArH), 9.17 (1H, s, ArH), 9.77 (1H, d, $J = 2.0$ Hz, ArH), 10.39 (1H, s, amide NH), 10.80 (1H, s, amide NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 55.9$, 102.1, 114.2 (2 peaks), 125.7, 126.1, 127.2, 130.4 (2 peaks), 143.3, 146.2, 149.1, 160.8, 162.9, 166.0 ppm; LC–MS (ESI): $m/z = 327.3$ ([M+H−HCl]).

The kinase assay

The kinase assays were conducted by hotspot kinase assay platform. The following base reaction buffer was used for the assay: 20 mM Hepes (pH 7.5), 10 mM $MgCl_2$, 1 mM EGTA, 0.02 mg/cm^3 BSA, 0.02% Brij35, 2 mM DTT, 0.1 mM Na_3VO_4, and 1% DMSO.

The reaction procedure is used as follows: the required cofactor for the enzymatic reaction was added to a freshly prepared buffer solution, followed by the addition of the Aurora kinase at a concentration of 20 μM. The contents were mixed gently and the compound dissolved in DMSO was added to the reaction mixture at 10 μM concentration. Compounds were evaluated in a 10-dose IC_{50} mode with threefold serial dilution starting at 30 μM for IC_{50} determination. ^{33}P-ATP was added to the mixture to initiate the reaction, and the mixture was incubated at room temperature for 2–3 h. Staurosporine was used as the control compound in a five-dose IC_{50} mode with tenfold serial dilutions starting at 20 μM, and the reaction was carried out at 10 μM ATP concentration.

Acknowledgements We are thankful to Anthem Biosciences management, Anthem Biosciences, Bangalore, India, for their invaluable support and allocation of resources for this work. We would like to thank Analytical chemistry team, Department of Analytical Chemistry, Anthem Biosciences, Bangalore, India, for having carried out all the analytical work. Also, we would like to thank molecular biology team, Department of Molecular Biology, Anthem Biosciences, Bangalore, India, for executing the Aurora Kinase activity studies.

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