Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters 45 (2004) 2791–2795
Tetrahedron Letters

Preparation of 5-amino-6-oxo-1,6-dihydro[1,2,4]triazine-3-carboxylic acid derivatives and synthesis of compound libraries thereof

Romain Gambert, Christoph Kuratli and Rainer E. Martin*

F. Hoffmann-La Roche Ltd, Pharmaceuticals Division, CH-4070 Basel, Switzerland

Received 20 January 2004; accepted 6 February 2004

Abstract—Treatment of [1,3,5]triazine-2,4,6-tricarboxylic acid triethyl ester (**4**) with arylhydrazines provided 5-amino-6-oxo-1,6-dihydro[1,2,4]triazine-3-carboxylic acid ethyl esters **5a**–**g** in moderate to good yields. Hydrolysis under basic conditions gave the corresponding free carboxylic acids **6a**–**d**. Despite the relatively high number of heteroatoms present the amido *as*-triazine compounds **6a**–**d** showed good solubility in phosphate buffer as determined by a lyophilization solubility assay. Building block **5a** served as starting point for the syntheses of two discrete exocyclic 5-amido and 3-amido compound libraries **7** and **8**, respectively.

As part of our continuing efforts to construct novel compound libraries with interesting structural motifs for high-throughput biological profiling of various medicinal targets, our attention was recently drawn to the structural class of *as*-triazines **1** and **2** as depicted in Figure 1. Surprisingly, upon checking the literature, only two publications have appeared dealing with the synthesis of parent molecules **5a** and **6a** (Table 1).[1,2] However, no further derivatization of these interesting heterocyclic template molecules has been described, which allow three different side chains to be attached easily in a nearly triangular fashion. Therefore, we decided to investigate this potentially interesting class of compounds.

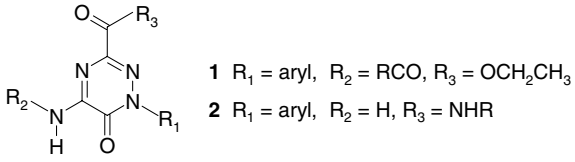


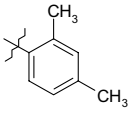
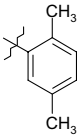
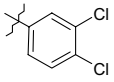
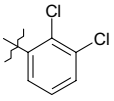
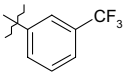
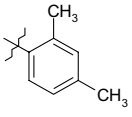
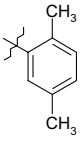
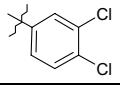
Figure 1. Target structures *as*-triazine ethyl esters **1** and corresponding amides **2**.

The synthesis of building blocks **5a**–**g** and **6a**–**d**, which may serve as starting points for library syntheses are outlined in Scheme 1. The preparation of the key intermediates commenced with commercially available ethyl cyano-formate (**3**), which under dry HCl gas catalysis undergoes in a single step reaction at rt within 2 d trimerization to 2,4,6-tris(ethoxycarbonyl)[1,3,5]triazine (**4**) in 71% yield.[3–5] Addition of arylhydrazines to a solution of the *sym*-triazine **4** in EtOH at rt afforded the corresponding *as*-triazines **5a**–**g** after intramolecular rearrangement in moderate to good yields (Table 1, entries 1–7).[1,2] Alternatively, the reaction can also be conducted under reflux conditions, which reduces reaction times typically down to 2–3 h. In all cases investigated the reaction products precipitated out of solution and could be easily isolated by filtration. Further purification by recrystallization from EtOH provided compounds **5a**–**g** in analytically pure quality.[6] All arylhydrazines applied in this study are commercially available, except 2,3-dichlorophenylhydrazine, which was prepared from 2,3-dichloroaniline by reduction of the corresponding diazonium salt employing $SnCl_2$/HCl.[7,8] Interestingly, conducting the condensation reaction using methyl- or benzylhydrazine did not provide the anticipated products. Saponification of the ethyl ester groups in **5a**–**d** was achieved with 1 M NaOH using either EtOH and H_2O or a mixture of EtOH/THF (3:1)

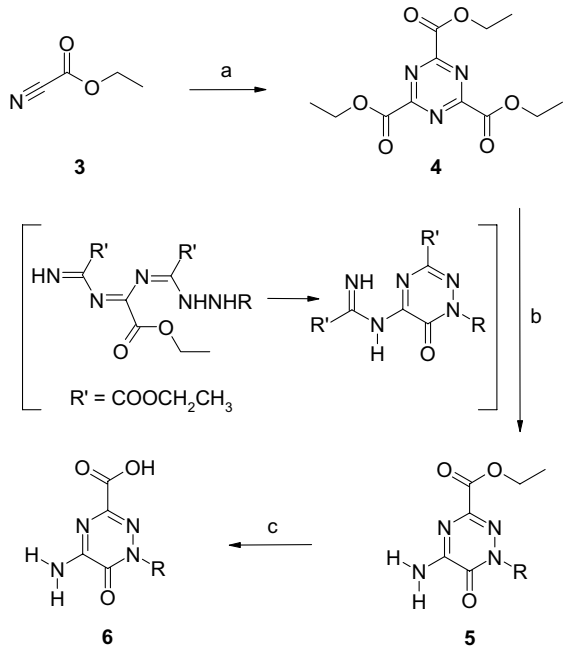
Keywords: Triazine; Synthesis; Combinatorial chemistry; Medicinal chemistry.
* Corresponding author. Tel.: +41-61-6887350; fax: +41-61-6888714; e-mail: rainer_e.martin@roche.com

0040-4039/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2004.02.025

Table 1. Synthesis of *as*-triazine ethyl esters **5a–g** and free acids **6a–d**

Entry	R	Product	Reaction time (h)	Yield (%)[c]
1		**5a**	24[a]	66
2		**5b**	24[a]	59
3		**5c**	48[a]	53
4		**5d**	48[a]	92
5		**5e**	48[a]	61
6		**5f**	48[a]	82
7		**5g**	24[a]	30
8		**6a**	24[b]	70
9		**6b**	24[b]	49
10		**6c**	24[b]	58
11		**6d**	24[b]	36

[a] Conditions: EtOH, rt.
[b] Conditions: 1 M NaOH, EtOH/THF (3:1), H_2O, rt.
[c] Isolated yields. Yields were not optimized.

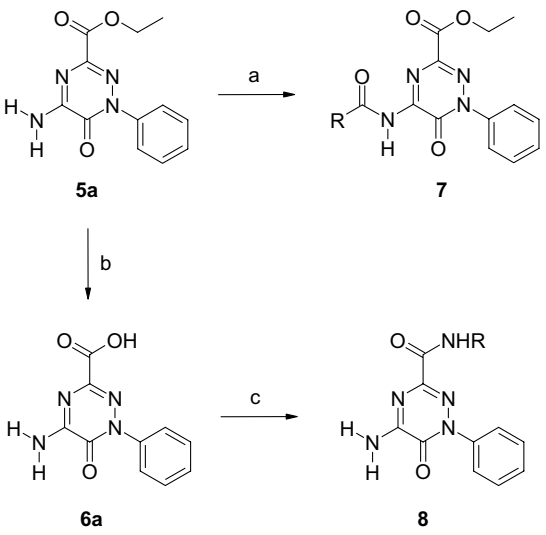


Scheme 1. Reagents and conditions: (a) dry HCl gas, rt, 2 d, 71%; (b) $RNHNH_2$, EtOH, rt; (c) 1 M NaOH, EtOH/THF (3:1), H_2O, rt; isolated yields (see Table 1). Yields were not optimized.

and H_2O depending on the solubility of starting materials (Scheme 1). After stirring for 24 h at rt the solutions were neutralized with 1 M HCl and compounds **6a–d** precipitated out of solution after concentration of the reaction mixture by evaporation of the solvents under reduced pressure (Table 1, entries 8–11). Further purification was accomplished by recrystallization from mixtures of ethanol/H_2O.[9] However, in the case of **6d** preparative HPLC was used to obtain an analytically pure sample.[10] Running the hydrolysis under less dilute conditions resulted after addition of the base in the formation of a gel-like, high viscosity solution, which significantly hampered efficient stirring of the reaction mixture.

The analytical data recorded for **5a** and **6a** are in accordance with those published for these two compounds.[1] The spectroscopic data of all new compounds **5b–g** and **6b–d**, which were fully characterized by ^1H NMR, ^{13}C NMR, FTIR and high-resolution mass spectrometry (HRMS) were consistent with the proposed structures. Solubilities in phosphate buffer of both the ethyl esters and the free acids were assessed using a lyophilization assay.[11] For instance, depending on the aryl substitution pattern solubilities varied for the ethyl ester compounds from 12 μg/mL for **5e** to >348 μg/mL for **5g**. Similarly, lipophilicity changed from $\log D = 2.6$ for **5e** to $\log D = -0.3$ for **5g**, respectively.[12] In the case of the free acids solubilities were typically high as exemplified by >309 μg/mL for **5a** or >347 μg/mL in the case of both **6b** and **6c** whereas $\log D$ values were measured to be lower than −1. The free acids **6a–d** showed two ionization constants typically at pH < 2 and around 2.8, respectively.[13]

With the 5-amino-*as*-triazine **5a** as a central building block the synthesis of exocylic 5-amido **7** and 3-amido **8** compound libraries was examined (Scheme 2). Library **7** containing an exocylic 5-amido group was generated by treatment of 5-amino[1,2,4]triazine-3-carboxylic acid ethyl ester **5a** (reaction scale 0.1 mmol) dissolved in 1 mL DMF with 1.5 equiv of the corresponding acid chlorides in the presence of diisopropylethylamine (DIEA) as a base and shaking at 60 °C for 24 h. Evaporation of DMF and DIEA under reduced pressure and purification of the crude reaction mixture dissolved in DMSO

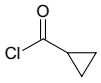
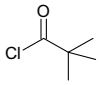
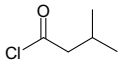
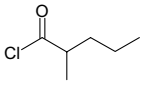
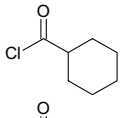
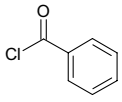
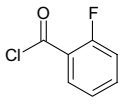
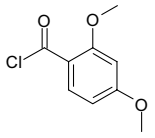
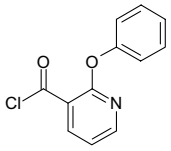
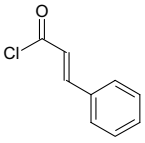
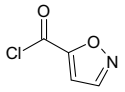
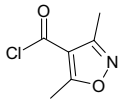


Scheme 2. Reagents and conditions: (a) RCOCl, DIEA, DMF, 60 °C, 24 h; (b) 1 M NaOH, EtOH/THF (3:1), H_2O, rt, 24 h; then (c) RNH_2, CDI or HATU, DIEA, DMF, 70 °C, 24 h.

by preparative HPLC gave compounds **7** in moderate to good yields and typically >95% purity (Table 2).[10] All 12 discrete members of the library were characterized by LC/MS and flow injection ^{1}H NMR analysis. Solubilities of individual members of library **7** varied from values as low as 3 μg/mL for phenoxypyridine **7i** to 361 μg/mL for dimethyloxazole **7l**. Similarly, lipophilicities were strongly affected by the nature of the side chains attached. For instance, the lipophilicities of 2-fluorophenyl **7g** or 2-phenoxypyridine **7i** were too high to be measured due to precipitation, whereas for compounds **7e** and **7h**, medium lipophilicities with $\log D = 2.4$ and 2.0, respectively, were measured and for the oxazole derivative **7k** a very low lipophilicity of only −1.0 was obtained.

The preparation of *as*-triazine library **8** is outlined in Scheme 2. The free carboxylic acid **6a** (reaction scale 0.1 mmol) dissolved in 1 mL DMF was preactivated with 1,1′-carbonyldiimidazole (CDI) for 1 h. Subsequently, DIEA and the corresponding amines (1.5 equiv) were added and the reaction mixture heated to 70 °C for 24 h. Evaporation of solvent and purification by preparative HPLC provided compounds **8** in moderate to good yields (Table 3). Similar results for the amide bond formation were obtained when 7-aza-3-[(dimethyliminium)(dimethylamino)methyl]-1,2,3-benzotriazol-1-ium-1-olate hexafluorophosphate (HATU) was employed as a coupling reagent. The chemical integrity of all compounds of library **8** was examined by LC/MS and flow injection ^{1}H NMR analysis. Again, solubilities and lipophilicities were strongly influenced by the nature of the side chains attached. For instance, compounds **8i** and **8l** displayed a solubility of only 5 μg/mL, whereas for most other compounds in this series such as **8a**,**b**,**f** or **8g** solubilities between 30 and 40 μg/mL were obtained. The highest value was observed for compound **8c** with >434 μg/mL. Unfortunately, $\log D$ values could only be assessed for few compounds in this series as most of them precipitated out from phosphate buffer solution during measurement. The bis(trifluoromethyl) derivative **8i** showed a high lipophilicity with $\log D = 3.2$, whereas medium values of 1.9 and 1.3 were obtained for **8b** and **8a**, respectively. The lowest value in this series was measured for compound **8c** with $\log D = 0.4$.

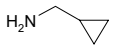
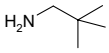
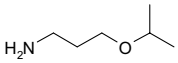
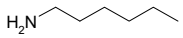
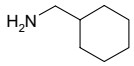
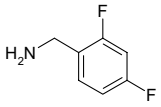
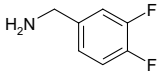
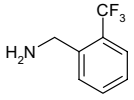
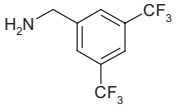
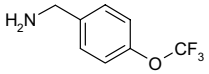
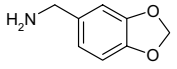
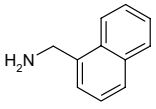
Table 2. Parallel synthesis of 5-amido-*as*-triazine ethyl esters **7**

Entry	Starting material	Acid chloride	Product	Yield (%)[a]
1	**5a**		**7a**	51
2	**5a**		**7b**	13
3	**5a**		**7c**	41
4	**5a**		**7d**	60
5	**5a**		**7e**	55
6	**5a**		**7f**	13
7	**5a**		**7g**	50
8	**5a**		**7h**	78
9	**5a**		**7i**	50
10	**5a**		**7j**	71
11	**5a**		**7k**	29
12	**5a**		**7l**	24

[a] Isolated yields after purification by preparative HPLC.[10]

In conclusion, we have described a convenient approach for the solution phase synthesis of 5-amino[1,2,4]triazine-3-carboxylic acid ethyl esters **5** and the corresponding free carboxylic acids **6**. The rapid assembly of exocylic 5-amido **7** and 3-amido **8** compound libraries

Table 3. Parallel synthesis of 3-amido-5-amino-*as*-triazines **8**

Entry	Starting material	Primary amine	Product	Yield (%)[a]
1	**5a**		**8a**	24
2	**5a**		**8b**	25
3	**5a**		**8c**	18
4	**5a**		**8d**	18
5	**5a**		**8e**	31
6	**5a**		**8f**	23
7	**5a**		**8g**	16
8	**5a**		**8h**	41
9	**5a**		**8i**	63
10	**5a**		**8j**	61
11	**5a**		**8k**	7
12	**5a**		**8l**	56

[a] Isolated yields after purification by preparative HPLC.[10]

was exemplified with building block **5a**. The 5-amino-[1,2,4]triazine-3-carboxylic acid core resembles an interesting and promising template structure that allows numerous further modifications, which might open an avenue to the generation of biologically active molecules.

General procedure: Compound **5d**: To a stirred suspension of *sym*-triazine **4** (3.0 g, 10.0 mmol) in dry EtOH (50 mL) at rt was added 3,4-dichlorophenylhydrazine (5.32 g, 30.0 mmol, 3 equiv). After 48 h the slightly orange precipitate was filtered off and recrystallized from EtOH to give compound **5d** in 92% yield (3.0 g).

Compound **6d**: To a stirred solution of **5d** (0.73 g, 2.2 mmol) in EtOH/THF (300 mL, 3:1) was added H_2O (3 mL) and 1 M NaOH (2.2 mL, 2.2 mmol, 1.0 equiv). After stirring at rt for 24 h, 1 M HCl (6.6 mL, 6.6 mmol, 3.0 equiv) was added and the solution volume reduced to ca. 100 mL by evaporation under reduced pressure. Satd NaCl solution (10 mL) was added and the reaction mixture extracted with ethyl acetate. The collected organic phases were dried over $MgSO_4$ and the solvent evaporated under reduced pressure yielding **6d** in 36% yield (0.24 g) and >90% purity, which is sufficiently high for library synthesis. Analytically pure samples were obtained by purification with preparative HPLC.

Compound library **7a–l**: To a stirred solution of building block **5a** (0.026 g, 0.1 mmol) in 1 mL DMF was added DIEA (0.3 mmol, 3 equiv) and the corresponding acid chloride (0.2 mmol, 2 equiv). After shaking at 60 °C for 24 h the solvent was removed under reduced pressure, the residues dissolved in DMSO, filtered and purified by preparative HPLC.

Compound library **8a–l**: To a stirred solution of building block **6a** (0.026 g, 0.1 mmol) in 1 mL DMF was added DIEA (0.3 mmol, 3 equiv), HATU (0.12 mmol, 1.2 equiv) and the corresponding primary amine (0.18 mmol, 1.8 equiv). After shaking the vials at 70 °C for 24 h the solvent was removed under reduced pressure, the residues dissolved in DMSO, filtered and purified by preparative HPLC.

Acknowledgements

We would like to thank Dr. K. Bleicher, Dr. L. Green, Dr. M. Rogers-Evans and Prof. Klaus Müller for their continuous interest in this work and helpful comments during the preparation of this manuscript. We are also grateful to the analytical services at Roche Basel for providing spectroscopic data.

References and notes

1. Alekseeva, N. V.; Turchin, K. F.; Anisimova, O. S.; Sheinker, Y. N.; Yakhontov, L. N. *Khim. Geterotsik. Soedine* **1989**, *11*, 1529–1538.
2. Alekseeva, N. V.; Turchin, K. F.; Anisimova, O. S.; Sheinker, Y. N.; Yakhontov, L. N. *Khim. Geterotsik. Soedine* **1990**, *12*, 1655–1664.
3. Ott, E. *Chem. Ber.* **1919**, *52*, 656–665.
4. Boger, D. L.; Dang, Q. *Tetrahedron* **1988**, *44*, 3379–3390.
5. Sugiyama, Y.; Sasaki, T.; Nagato, N. *J. Org. Chem.* **1978**, *43*, 4485–4487.
6. Spectroscopic data: Compound **5a**: ^{1}H NMR (400 MHz, DMSO): δ 1.27 (t, $J = 7.2$ Hz, 3H), 4.27 (q, $J = 7.2$ Hz, 2H), 7.36–7.64 (m, 5H), 8.22 (br s, 1H), 8.66 (br s, 1H). ^{13}C NMR (100 MHz, DMSO): δ 14.06, 61.32, 125.31, 128.30, 128.73, 140.31, 141.96, 149.70, 157.68, 162.04. FTIR (ATR, cm^{-1}): 3456 (s), 3088 (br m), 1724 (s), 1668 (s), 1631 (s), 1595 (s), 1560 (m), 1493 (s), 1377 (s), 1246 (s), 1197 (s). HRMS (ESI) m/z calculated for $C_{12}H_{13}N_4O_3$ $(M+H)^+$: 261.0988, found: 261.0990. These data are in accordance with those published in Ref. 1. Compound **5b**: ^{1}H NMR (400 MHz, DMSO): δ 1.25 (t, $J = 7.0$ Hz, 3H), 2.05 (s, 3H), 2.34 (s, 3H), 4.25 (q, $J = 7.0$ Hz, 2H), 7.13–7.25 (m, 3H), 8.20 (br s, 1H), 8.65 (br s, 1H). ^{13}C NMR (100 MHz, DMSO): δ 14.04, 16.94, 20.64, 61.31, 127.00, 127.17, 131.06, 133.86, 136.98, 138.69, 141.90, 149.59, 157.46, 161.97. FTIR (ATR, cm^{-1}): 3460 (s), 3110 (br m), 1736 (s), 1673 (s), 1635 (s), 1504 (m), 1372 (m), 1242 (s), 1194 (s), 1168 (s). HRMS (ESI) m/z calculated for

$C_{14}H_{17}N_4O_3$ $(M+H)^+$: 289.1301, found: 289.1300. Compound **5c**: ^{1}H NMR (400 MHz, DMSO): δ 1.25 (t, $J=7.2$ Hz, 3H), 2.03 (s, 3H), 2.32 (s, 3H), 4.25 (q, $J=7.2$ Hz, 2H), 7.18–7.27 (m, 3H), 8.21 (br s, 1H), 8.67 (br s, 1H). ^{13}C NMR (100 MHz, DMSO): δ 14.05, 16.55, 20.25, 61.33, 127.49, 129.82, 130.42, 131.00, 136.11, 139.22, 141.94, 149.47, 157.46, 161.95. FTIR (ATR, cm^{-1}): 3459 (s), 3122 (br m), 1738 (s), 1669 (s), 1634 (s), 1510 (m), 1369 (m), 1241 (s), 1219 (s), 1176 (s). HRMS (ESI) m/z calculated for $C_{14}H_{17}N_4O_3$ $(M+H)^+$: 289.1301, found: 289.1300. Compound **5d**: ^{1}H NMR (400 MHz, DMSO): δ 1.27 (t, $J=7.2$ Hz, 3H), 4.28 (q, $J=7.2$ Hz, 2H), 7.71 (dd, $J=8.2$, 2.4 Hz, 1H), 7.81 (d, $J=8.2$ Hz, 1H), 8.00 (d, $J=2.4$ Hz, 1H), 8.31 (br s, 1H), 8.74 (br s, 1H). ^{13}C NMR (100 MHz, DMSO): δ 14.05, 61.42, 125.44, 127.07, 130.71, 130.76, 130.93, 139.89, 142.23, 149.74, 157.67, 161.93. FTIR (ATR, cm^{-1}): 3447 (s), 3107 (br m), 1745 (s), 1671 (s), 1645 (s), 1519 (w), 1471 (m), 1376 (m), 1349 (m), 1242 (s), 1196 (s), 1151 (s). HRMS (ESI) m/z calculated for $C_{12}H_{11}N_4O_3Cl_2$ $(M+H)^+$: 329.0208, found: 329.0209. Compound **5e**: ^{1}H NMR (400 MHz, DMSO): δ 1.25 (t, $J=7.2$ Hz, 3H), 4.26 (q, $J=7.2$ Hz, 2H), 7.58 (t, $J=8.0$ Hz, 1H), 7.68 (d, 6.8 Hz, 1H), 7.84 (d, 8.0 Hz, 1H), 8.42 (br s, 1H), 8.82 (br s, 1H). ^{13}C NMR (100 MHz, DMSO): δ 14.03, 61.50, 128.43, 129.06, 129.23, 131.58, 132.37, 139.37, 142.36, 149.32, 157.34, 161.73. FTIR (ATR, cm^{-1}): 3432 (s), 3133 (br m), 1734 (s), 1681 (s), 1638 (s), 1571 (m), 1456 (m), 1424 (m), 1377 (m), 1359 (w), 1237 (s), 1208 (s), 1190 (s), 1150 (s). HRMS (ESI) m/z calculated for $C_{12}H_{11}N_4O_3Cl$ $(M+H)^+$: 329.0208, found: 329.0206. Compound **5f**: ^{1}H NMR (400 MHz, DMSO): δ 1.27 (t, $J=7.0$ Hz, 3H), 4.28 (q, $J=7.0$ Hz, 2H), 7.76–8.07 (m, 4H), 8.30 (br s, 1H), 8.73 (br s, 1H). ^{13}C NMR (100 MHz, DMSO): δ 14.47, 61.83, 122.50, 124.18 (q, $J=271$ Hz), 125.36, 129.70, 129.80 (q, $J=32$ Hz), 130.57, 141.27, 142.69, 150.32, 158.16, 162.41. ^{19}F NMR (282 MHz, DMSO): δ 61.63. FTIR (ATR, cm^{-1}): 3450 (s), 3102 (br m), 1726 (s), 1670 (s), 1651 (s), 1596 (w), 1458 (m), 1379 (m), 1348 (m), 1324 (s), 1313 (s), 1293 (s), 1244 (s), 1176 (s), 1167 (s). HRMS (ESI) m/z calculated for $C_{13}H_{12}N_4O_3F_3$ $(M+H)^+$: 329.0861, found: 329.0861. Compound **5g**: ^{1}H NMR (400 MHz, DMSO): δ 1.26 (t, $J=7.2$ Hz, 3H), 4.27 (q, $J=7.2$ Hz, 2H), 7.54–8.63 (m, 4H), 8.29 (br s, 1H), 8.72 (br s, 1H). ^{13}C NMR (100 MHz, DMSO): δ 14.05, 61.37, 121.56, 124.66, 138.84, 141.83, 141.83, 149.10, 149.67, 151.96, 157.68, 161.95. FTIR (ATR, cm^{-1}): 3461 (s), 3110 (br m), 1729 (s), 1633 (s), 1589 (s), 1571 (s), 1466 (s), 1436 (s), 1376 (s), 1252 (s), 1241 (s), 1201 (s), 1244 (s), 1157 (w). HRMS (ESI) m/z calculated for $C_{11}H_{12}N_5O_3$ $(M+H)^+$: 262.0940, found: 262.0944.

7. Boido, A.; Novelli, F.; Savelli, F.; Sparatore, F.; Russo, S. *Il Farmaco* **1989**, *44*, 279–302.
8. Cox, R. J.; Williams, R. M. *Tetrahedron Lett.* **2002**, *43*, 2149–2152.
9. Spectroscopic data: Compound **6a**: ^{1}H NMR (400 MHz, DMSO): δ 7.42–7.66 (m, 4H), 8.16 (br s, 1H), 8.56 (br s, 1H), 13.10 (br s, 1H). ^{13}C NMR (100 MHz, DMSO): δ 125.21, 128.21, 128.64, 140.34, 142.66, 149.64, 157.66, 163.42. FTIR (ATR, cm^{-1}): 3342 (s), 3155 (br m), 1749 (s), 1718 (s), 1632 (s), 1594 (s), 1494 (s), 1416 (s), 1287 (s), 1242 (s), 1198 (s), 1173 (s), 1135 (s). HRMS (ESI) m/z calculated for $C_{10}H_9N_4O_3$ $(M+H)^+$: 233.0675, found: 233.0677. These data are in accordance with those published in Ref. 1. Compound **6b**: ^{1}H NMR (400 MHz, DMSO): δ 2.01 (s, 1H), 2.32 (s, 1H), 7.12 (br s, 1H), 7.18 (br d, $J=8.0$ Hz, 1H), 7.24 (d, $J=8.0$ Hz, 1H), 7.78 (br s, 1H), 8.40 (br s, 1H), 13.08 (br s, 1H). ^{13}C NMR (100 MHz, DMSO): δ 16.66, 20.27, 127.46, 129.33, 130.20, 130.83, 135.90, 139.82, 148.74, 149.34, 157.06, 164.57. FTIR (ATR, cm^{-1}): 3433 (w), 3148 (br w), 2922 (s), 2853 (m), 1680 (m), 1613 (br s), 1505 (s), 1452 (m), 1400 (s), 1281 (s), 1233 (m), 1183 (m), 1150 (w). HRMS (ESI) m/z calculated for $C_{12}H_{13}N_4O_3$ $(M+H)^+$: 261.0988, found: 261.0988. Compound **6c**: ^{1}H NMR (400 MHz, DMSO): δ 2.04 (s, 1H), 2.32 (s, 1H), 7.18 (br s, 1H), 7.21 (br d, $J=7.8$ Hz, 1H), 7.26 (d, $J=7.8$ Hz, 1H), 8.14 (br s, 1H), 8.56 (br s, 1H), 13.10 (br s, 1H). ^{13}C NMR (100 MHz, DMSO): δ 17.03, 20.65, 126.92, 127.05, 130.90, 133.74, 137.58, 138.12, 148.78, 149.50, 157.09, 164.64. FTIR (ATR, cm^{-1}): 3431 (m), 3171 (br s), 2921 (br s), 1680 (s), 1622 (br s), 1519 (s), 1456 (s), 1420 (s), 1377 (s), 1287 (s), 1241 (w), 1208 (s), 1166 (m). HRMS (ESI) m/z calculated for $C_{12}H_{13}N_4O_3$ $(M+H)^+$: 261.0988, found: 261.0990. Compound **6d**: ^{1}H NMR (400 MHz, DMSO): δ 7.74–8.04 (m, 3H), 8.25 (br s, 1H), 8.65 (br s, 1H), 13.20 (br s, 1H). ^{13}C NMR (100 MHz, DMSO): δ 126.53, 128.15, 131.83, 131.86, 132.16, 141.20, 144.21, 150.95, 158.93, 164.54. FTIR (ATR, cm^{-1}): 3365 (m), 3167 (br m), 1746 (s), 1727 (w), 1663 (s), 1638 (s), 1590 (w), 1473 (s), 1410 (s), 1299 (w), 1234 (s), 1189 (s), 1148 (s), 1037 (s). HRMS (ESI) m/z calculated for $C_{10}H_7N_4O_3Cl_2$ $(M+H)^+$: 300.9895, found: 300.9900.
10. Compounds were purified by preparative HPLC on a Phenomenex Aqua 5 μm C18 125A 60×21.20 mm column equipped with a Gilson Liquid Handler 215 autosampler, two Varian Prep Star Model SD-1 pumps, a Sedex ELSD 75 lightscatter and a Dionex UVD 340S UV detector.
11. Solubility was measured from lyophilized DMSO stock solutions spectrophotometrically at pH = 6.5 in a 0.05 M phosphate buffer.
12. log D values were measured spectrophotometrically at pH = 7.4 in a 1-octanol/50 mM TAPSO buffer system containing 5% (v/v) DMSO.
13. pK_a values were determined spectrophotometrically on a ProfilerSGA instrument in a SGA buffer system containing 10% (v/v) methanol at an ionic strength of 150 mM.