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Comparison of inhibitory activity of isomeric triazolopyridine derivatives towards adenosine receptor subtypes or do similar structures reveal similar bioactivities?

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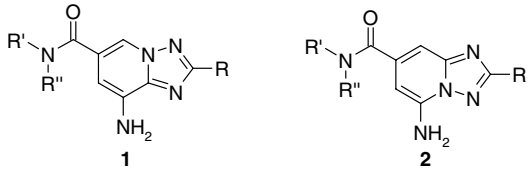
Abstract—The synthesis of an array of 8-amino-2-aryl-[1,2,4]triazolo[1,5-*a*]pyridine-6-carboxyl amide derivatives is described for the first time. A subset of 20 derivatives were compared to their isomeric 5-amino-2-aryl-[1,2,4]triazolo[1,5-*a*]pyridine-7-carboxyl amide counterparts with regard to their potential to inhibit the human adenosine 2a (hA2a) receptor and their selectivity against the human adenosine 1 (hA1) receptor. Based on the analysis of H-bond donor/acceptor capabilities of the isomeric triazolopyridine pairs it can be concluded that the H-bond donor strength of the free amino functionality is the main determinant for hA2a inhibitory activity and hA1 selectivity.

The human adenosine 2a (A2a) receptor can potentially act as modulating site towards the treatment of neurodegenerative diseases.[1] A2a receptor antagonists inhibit the motor depressant effects of dopamine antagonists, like haloperidol, which makes them of particular interest for the treatment of neurodegenerative disorders, such as Parkinson's disease.[2] In the course of a medicinal chemistry project directed towards the identification of new heterocyclic compounds with inhibitory activity towards the hA2a receptor it was previously established that triazolopyridine derivatives can act as potent and selective (vs human adenosine A1) antagonists.[3] In order to further investigate the potential of triazolopyridine derivatives with different substitution pattern to those previously described 8-amino-2-aryl-[1,2,4]triazolo[1,5-*a*]pyridine-6-carboxyl amide derivatives **1** were considered of potential interest. The isomeric 5-amino-2-aryl-[1,2,4]triazolo[1,5-*a*]pyridine-7-carboxyl amide derivatives **2** have been previously investigated (Scheme 1).[3b]



Scheme 1. Isomeric [1,2,4]triazolo[1,5-*a*]pyridine-7-carboxylic acid amide derivatives **1** and **2**.

In this context it appeared particularly interesting to observe any changes in activity/selectivity towards the adenosine receptors for triazolopyridine derivatives **1** in comparison to **2** as all main vectors stretch out from a structurally very similar main scaffold. By comparison of binding affinities/selectivities of a set of triazolopyridines **1** versus the isomeric structures **2** electronic properties of the main scaffold should mainly contribute towards any adenosine antagonism.

The synthesis of the desired triazolopyridine derivatives **1** followed a general synthetic sequence previously described and well established in our laboratory.[3b] 5,6-Diamino-nicotinic acid methyl ester **3**[4] can be synthesised in a three-step procedure from commercially available chloro-nicotinic acid and subsequently reacted in a three-step one-pot procedure to furnish triazolopyridine

Keywords: Adenosine; Amination; Combinatorial chemistry; H-bond donor/acceptor strength triazolopyridine derivatives.
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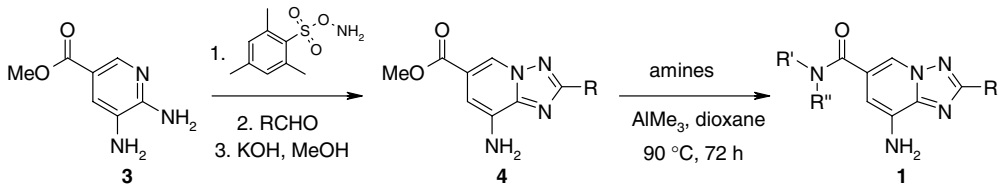
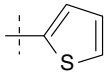
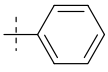
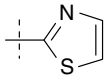
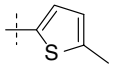
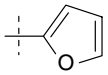
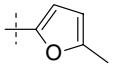
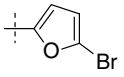
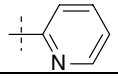


Scheme 2. Reaction sequence towards triazolopyridine derivatives **4** and **1**.

carboxylic acid ester derivatives **4**. The sequence commences with the regiospecific N-amination of **3** with *O*-mesitylenesulfonyl hydroxylamine[5] producing the 1,5,6-triamino-nicotinic acid methyl ester derivative, which can be condensed with various aldehydes and subsequently oxidatively aromatised under basic conditions. Eight different aldehydes were used for the production of a small array of **4** in yields up to 52%.[6] The structural identity was corroborated by ^{1}H NMR, MS and the purity determined by reversed phase analytical HPLC at 230 nm (Scheme 2, Table 1).

The transformation of triazolopyridine ester **4** to the desired amides **1** was conveniently achieved by reacting the respective amine with $AlMe_3$ for 1 h and subsequent reaction with the ester **4** in dioxane at elevated temperatures for a prolonged period of time.[7] However, the yield of the final products **1** are mainly influenced by the reactivity of the starting materials (**4**/amines), which are dependent on their respective steric and electronic properties. In total 118 final amides were obtained in yields up to 44%.[8] A total of 20 triazolopyridine derivatives **1** were obtained, which had an isomeric triazolopyridine derivative counterpart **2**.[3b] All of those compounds were tested against the human A2a and A1 receptor. The results of these isomeric pairs are displayed in Table 2 and reveal that in general triazolopyridine derivatives **2** have a higher binding affinity towards the hA2a receptor and overall have an improved selectivity profile versus hA1 (Table 2).

Table 1. Triazolopyridine ester derivatives **4**

No.	R	Yield (%)[a,b] MH^+_{found}
4a		48 275.3
4b		26 256.1
4c		37 266.1
4d		15 289.2
4e		52 259.0
4f		13 273.2
4g		72 336.1
4h		33 270.0

[a] Isolated yields.
[b] Purity was determined by analytical HPLC–MS at 230 nm.

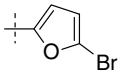
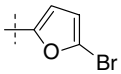
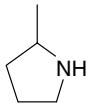
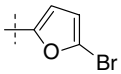
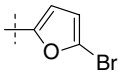
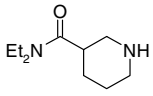
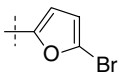
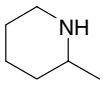
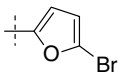
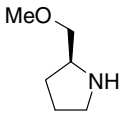
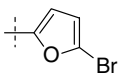
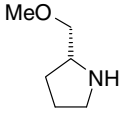
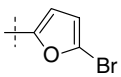
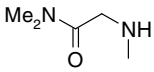
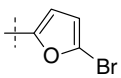
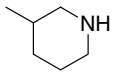
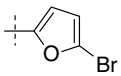
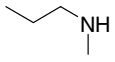
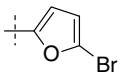
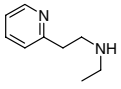
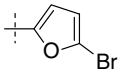
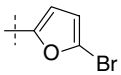
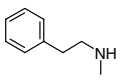
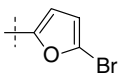
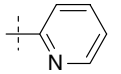
Assuming that the vectors stretching out from the respective triazolopyridine scaffold bind in a comparative fashion it can be assumed that the hydrogen bonding properties of the main scaffold do indeed contribute significantly to the binding affinity towards the hA2a and hA1 receptor.

Therefore, a comparative analysis of the hydrogen bond donor and acceptor strengths of both the 8-amino **1** and 5-amino **2** scaffolds was carried out. The molecular modeling software package Moloc[9] provides for quantitative descriptors of hydrogen bond donor and acceptors strengths for selected atoms. The calculation of these descriptors is based on a calibration with experimentally determined Gibbs free energies of hydration and is superior to other published models.[10] The reference value for both H-bond donor and acceptor strength is water with a value of 1.748. Compounds with a value <1.748 are weaker donors/acceptors than water and vice versa.

When the donor and acceptor strengths of the three nitrogen atoms with hydrogen bond donor/acceptor capabilities in the two scaffold series were compared it became apparent that donor and acceptor strengths were markedly changed for the amino group and the triazolo nitrogen atom, which is located on the same side as the amino moiety. The H-bond acceptor strength of the other triazolo nitrogen did not vary significantly (Fig. 1). Whereas the H-bond acceptor strength of N1 of the 8-amino derivative **1a** was increased with respect to N3 of the 5-amino derivative **2a**, the H-bond donor strength of the amino group in **1a** was reduced in comparison to the donor strength of the amino group in **2a**.

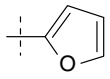
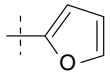
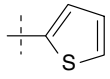
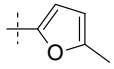
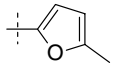
The increase of the H-bond donor strength of the amino group in the 5-amino series **2** is markedly larger (approx. 0.5 units) than the decrease of the H-bond acceptor

Table 2. Isomeric triazolopyridine derivatives **1** and **2**

K_i hA2a (nM) Sel. versus hA1	Yield (%)[a,b] MH^+_{found}	No.	Amine	R	No.	K_i hA2a (nM) Sel. versus hA1
277 8	6 379.7	**1a**			**2a**	3 68
35 36	22 375.6	**1b**			**2b**	9 62
27 42	17 389.7	**1c**			**2c**	4 65
27 36	29 389.7	**1d**			**2d**	6 48
40 66	33 490.6	**1e**			**2e**	8 125
62 31	4 405.6	**1f**			**2f**	2 29
16 64	25 421.4	**1g**			**2g**	12 53
61 20	7 421.8	**1h**			**2h**	2 122
23 129	14 422.8	**1i**			**2i**	3 224
15 52	27 405.7	**1j**			**2j**	3 45
44 26	44 379.8	**1k**			**2k**	6 47
76 19	7 454.8	**1l**			**2l**	4 36
73 13	42 409.7	**1m**			**2m**	9 28
50 12	11 441.8	**1n**			**2n**	5 18
192 19	5 308.8	**1o**			**2o**	57 26

(*continued on next page*)

Table 2 (*continued*)

K_i hA2a (nM) Sel. versus hA1	Yield (%)[a,b] MH^+_{found}	No.	Amine	R	No.	K_i hA2a (nM) Sel. versus hA1
57 74	26 297.7	**1p**			**2p**	11 151
190 18	26 311.8	**1q**			**2q**	27 101
197 4	27 313.7	**1r**			**2r**	104 14
76 22	6 311.8	**1s**			**2s**	17 56
57 22	17 326.0	**1t**			**2t**	14 49

[a] Isolated yields.
[b] Purity was determined by analytical HPLC–MS at 230 nm >90%.

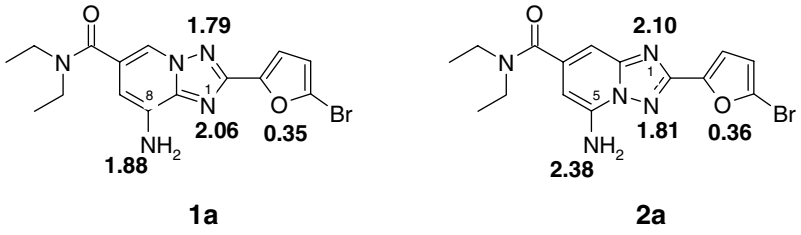


Figure 1. Exemplified H-bond donor and H-bond acceptor strengths for selected atoms of **1a** in comparison to **2a**.

strength (approx. 0.3 units) with respect to the 8-amino series **1**.

This is summarised in Figure 2, where the hydrogen bond and acceptor strengths of all isomeric triazolopyridines have been averaged over both series. For series **2** the averaged H-bond *donor* strength of the amino group is 2.381 ± 0.001 versus 1.884 ± 0.001 for series **1**. The averaged H-bond *acceptor* strength of the adjacent triazolo nitrogen has been determined as 1.807 ± 0.005 for series **2**, whereas for series **1** an average of 2.060 ± 0.003 was obtained. Using the donor/acceptor strength of 1.748 for water as a reference it becomes obvious that the markedly increased donor strength of the amino group is the primary driving force for enhanced affinity and selectivity. The corresponding 3D structures of **1a** and **2a** are displayed with a contouring of the molecular surface and the energetically favourable interaction sites with a GRID[11] water probe (Fig. 2).

Both scaffolds are very similar with respect to topology, shape and H-bond donor/acceptor regions, but subtle differences in the H-bond donor strength of the amino group dramatically influence the affinity towards the hA2a receptor. From this observation it can be concluded that the increase of the H-bond donor strength of the amino group is the main determinant for the increased affinity and selectivity of the 5-amino scaffold series **2**.

This structure–activity relationship is supported by respective analysis of the highly potent and selective competitors' compounds **SCH58261** and **ZM241385**[12,13] (Fig. 3).

Both molecules have an even more pronounced hydrogen bond donor strength of the amino group (2.68 and 2.86, respectively) and a comparable level of acceptor strength of the topologically equivalent nitrogen atoms (1.79 and 1.78, respectively). This clearly validates the predictivity of the SAR model and suggests to design and synthesise novel triazolopyridine derivatives or related heterocyclic systems with increased donor strength of the amino group but with a reduced decrease of the H-bond acceptor strength of the triazolo nitrogen.

In conclusion we devised a synthetic sequence towards previously nondescribed 8-amino-[1,2,4]triazolo[1,5-*a*]pyridine-6-carboxyl amide derivatives **1** in two steps starting from 5,6-diamino-nicotinic acid methyl ester **3**, which was converted by a one-pot three-step procedure to the respective triazolopyridine methyl esters **4**. The

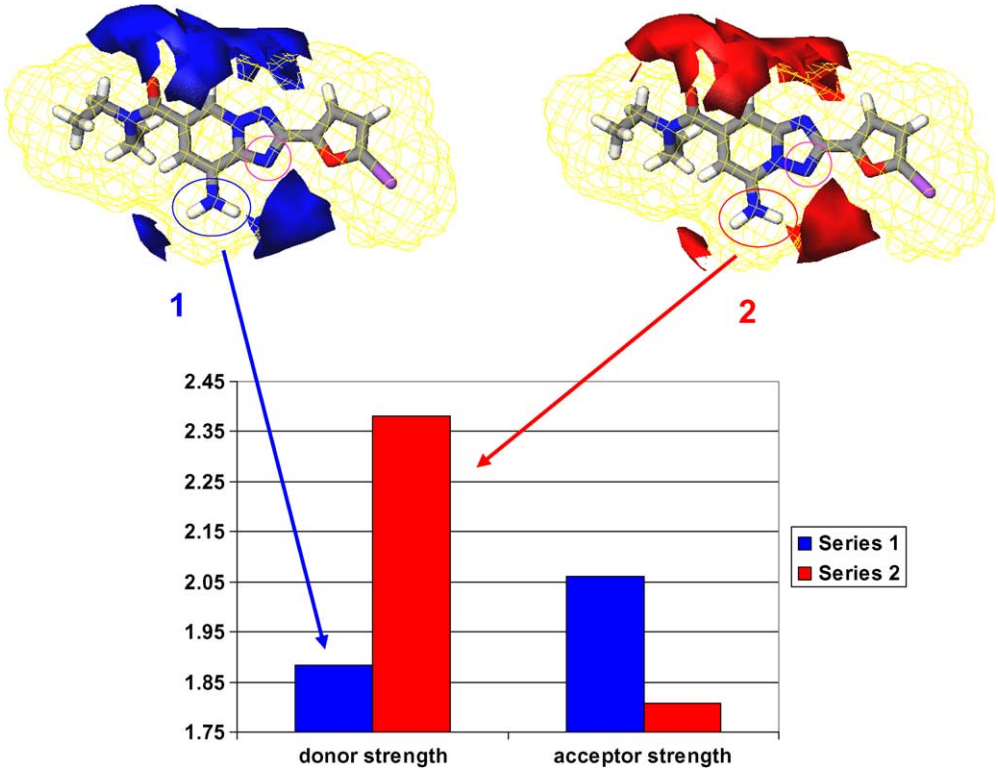


Figure 2. Comparison of the averaged H-bond *donor* and *acceptor* strengths of the amino group and the adjacent triazolo nitrogen in the 8-amino scaffold series **1** and the 5-amino scaffold series **2**. The donor/acceptor strength of water is used as the origin of the *y*-axis. The 3D molecular shape and H-bond interaction sites have been generated with a GRID[11] water probe. The amino H-bond donor groups have been marked with arrows, whereas the triazolo H-bond acceptors have only been highlighted with magenta circles for the sake of clarity.

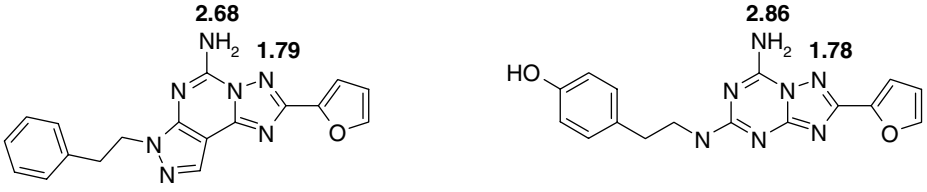


Figure 3. Hydrogen bond donor and acceptors strengths of **SCH58261** and **ZM241385**. The biological values were taken from Ref. 14.

derivatives conveniently underwent the concluding amidation to provide novel compounds, which were compared to their isomeric counterparts obtained through a previously described synthetic campaign from our laboratory. From the correlation of hydrogen bond donor and acceptor strengths of the nitrogen atoms in both scaffold series it could be concluded that the increased donor strength of the amino group in the 5-amino series **2** is the main determinant for increased affinity and hA2a/hA1 selectivity in this series thus favouring in general triazolopyridine derivatives **2**. Although both scaffold series are structurally highly similar, subtle changes in the electron density greatly impact the molecular recognition event and cause a dramatic difference in biological activity. Thus, the molecular similarity principle ('similar structures, similar bioactivities')[15] should be more precisely formulated as 'similar recognition motifs, similar activities'. Based on those results chemistry efforts towards novel triazolopyridine derivatives with improved biological in vitro activities/selectivities and pharmacological profiles are currently undertaken and will be reported in full in due course.

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References and notes

1. (a) Dunwiddie, T. V.; Masino, S. A. *Annu. Rev. Neurosci.* **2001**, *24*, 31–55; (b) Klinger, M.; Freissmuth, M.; Nanoff, C. *Cell. Sign.* **2002**, *14*, 99–108.
2. (a) Muller, C. E.; Scior, T. *Pharm. Acta Helv.* **1993**, *68*, 77–111; (b) Johansson, B.; Georgiev, V.; Fredholm, B. B. *Neuroscience* **1997**, *80*, 1187–1207; (c) Svenningsson, P.; Le Moine, C.; Fisone, G.; Fredholm, B. B. *Prog. Neurobiol.* **1999**, *59*, 355–396.
3. (a) Nettekoven, M. *Synlett* **2001**, *12*, 1917–1920; (b) Brodbeck, B.; Püllmann, B.; Schmitt, S.; Nettekoven, M. *Tetrahedron Lett.* **2003**, *44*, 1675–1678; (c) Nettekoven, M.; Schneider, G. *J. Comb. Chem.* **2003**, *5*, 233–237.
4. Amin, K., Dahlstrom, M., Nordberg, P., Starke, I. PCT Int. Appl. WO9955706A1. CAN 131:322617, 1999.
5. Tamura, Y.; Minamikawa, J.; Miki, Y.; Matsugashita, S.; Ikeda, M. *Tetrahedron Lett.* **1972**, *13*, 4133–4135.
6. General procedure for the synthesis of **4**: A mixture of 1.114 g (6.66 mmol) 5,6-diamino-nicotinic acid methyl ester **3** in 40 mL dioxane was treated with 1.578 g (7.33 mmol) *O*-mesitylenesulfonyl hydroxylamine for 1 h at room temperature. Aldehyde (8 mmol) and molecular sieves 4 Å was added and heated to 100 °C for 4 h before addition of 1.6 mL 5 M KOH in methanol. The mixture was heated to 70 °C for 2 h and stirred at room temperature for 14 h. The volatiles were removed under reduced pressure and the residue was purified by flash column chromatography on silica eluting with a mixture of ethyl acetate and *n*-hexane to yield **4** after evaporation of the volatiles. Compound **4a**: 48%. ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 8.65$ (d, $J = 1.4$ Hz, 1H, H-5), 7.81 (dd, $J_1 = 3.5$ Hz, $J_2 = 1$ Hz, 1H, thiophene H-2), 7.76 (dd, $J_1 = 4.8$ Hz, $J_2 = 1$ Hz, 1H, thiophene H-4), 7.24 (dd, $J_1 = 4.8$ Hz, $J_2 = 3.5$ Hz, 1H, thiophene H-3), 7.09 (d, $J = 1.4$ Hz, 1H, H-7), 6.31 (s, br, 2H, NH_2), 3.88 (s, 3H, OCH_3); MS *m*/*e* (%): 275.3 (MH^+, 100%).
7. General procedure for the synthesis of **1**: A solution of 0.24 mmol amine in 1 mL dioxane was treated with 0.24 mmol trimethylaluminium in toluene and stirred for 1 h at room temperature. 8-Amino-2-(aryl)-[1,2,4]triazolo[1,5-*a*]pyridine-6-carboxylic acid methyl ester **4** (20 mg, 0.06 mmol) in 1 mL dioxane was added and the mixture was heated to 90 °C for 72 h. HCl aq (0.5 mL, 1 N) was added and the volatiles were removed. The residue was taken up in 1.5 mL formic acid and 0.5 mL methanol and purified by reversed phase preparative HPLC eluting with a gradient of acetonitrile and water. The elution solvents were evaporated to obtain the title compound **1**. The NMR samples for **1** were processed with a stop-flow method and the Bruker efficient sample transfer (BEST) procedure. Compound **1d**: 29%. ^{1}H NMR (500 MHz, DMSO): $\delta = 8.21$ (s, 1H, H-5), 7.14 (d, $J = 3.5$ Hz, 1H, furan H-4), 6.82 (d, $J = 3.5$ Hz, 1H, furan H-3), 6.56 (s, 1H, H-7), 6.26 (s, br, 2H, NH_2), 1.62 (m, 4H, NCH_2), 1.52 (m, 6H, CH_2). MS *m*/*e* (%): 389.7 (MH^+, 100%).
8. For a more detailed description of workflow procedures utilised in the preparation of compound arrays please refer to: Nettekoven, M.; Thomas, A. W. *Curr. Med. Chem.* **2002**, *9*, 2179–2190.
9. Gerber Molecular Design; http://www.moloc.ch.
10. Gerber, P. R. *J. Comput.-Aid. Mol. Des.* **1998**, *12*, 37–51.
11. GRID v. 21; http://www.moldiscovery.com.
12. Poucher, S. M.; Keddie, J. R.; Singh, P.; Stoggall, S. M.; Caulkett, P. W. R.; Jones, G.; Collis, M. G. *Br. J. Pharmacol.* **1995**, *115*, 1096–1102.
13. Baraldi, P. G.; Cacciari, B.; Spalluto, G.; Pineda de las Infantas y Villatoro, M. J.; Zocchi, C.; Dionisotti, S.; Ongini, E. *J. Med. Chem.* **1996**, *39*, 1164–1171.
14. Dionisotti, S.; Ongini, E.; Gessi, S.; Irenius, E.; Fredholm, B. B. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1999**, *359*, 7–10.
15. *Concepts and Applications of Molecular Similarity*; Johnson, M. A., Maggiora, G. M., Eds.; Wiley: New York, 1990.