[1,2,4]Triazolo[1,5-*c*]quinazolin-2-amines as novel adenosine receptor antagonists

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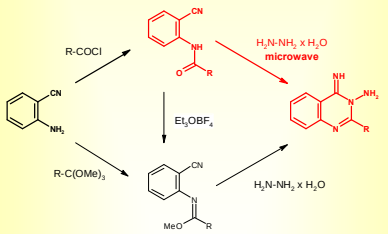
Microwave-assisted Synthesis of 4-Imino-quinazolin-3-amines

4-Imino-quinazolin-3-amines are versatile intermediates in the synthesis of [1,2,4]triazolo[1,5-c]quinazoline derivatives. The common methods for the generation of 4-imino-quinazolin-3-amines involve the reaction of imino-esters with hydrazine hydrate. These imino-esters can be envisaged as activated amides and are either generated by the reaction of 2-amino-aryl-nitriles with appropriate orthoesters[1] or by reacting N-acylated 2-amino-aryl-nitriles with triethyloxonium tetrafluoroborate (Meerwein´s salt).[2] Both methods have severe drawbacks: The first is limited by the poor availability of complex orthoesters, while the second is unreliable and involves the use of a highly cancerogenic reagent.



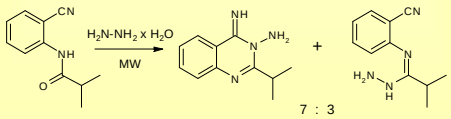
Our approach was to react hydrazine hydrate with non-activated *N*-acylated 2-amino-aryl-nitriles under microwave irradiation. The latter reagents are easily accessible by reacting 2-aminobenzonitrile with the corresponding acyl halogenides.

First experiments with benzoic acid derivatives (R = Ph, p-Br-Ph, p-F-Ph, and o-F-Ph) gave very encouraging results: After 10 minutes at 120°C under microwave irradiation with an 8-fold excess of hydrazine hydrate, the desired 4-imino-quinazolin-3-amines could be isolated in 47-62% yield. If nitro groups were present, they were reduced to the corresponding amines, which could be isolated in low yields. Heteroaromatically substituted 2-carboxamidobenzonitriles gave yields from 33 to 51%, except for R = 3-furyl, which was decomposed.

R	Yield [%]	Annotations
phenyl	60	
4-bromophenyl	54	
3-fluorophenyl	47	
4-fluorophenyl	62	
3-nitrophenyl	20	reduction to amine
4-nitrophenyl	4	reduction to amine
2-thienyl	35	
3-thienyl	51	
2-furyl	33	
3-furyl	-	decomposition
benzyl	37	77 : 23[1]
2-phenethyl	96	38 : 62[1]
3,4,5-trimethoxyphenethyl	33	70 : 30[1]
isopropyl	38	70 : 30[1]

Conditions: 8 eq. of hydrazine hydrate, MW: 10 min /120°C / max. 40 W. 1: ratio of 4-imino-quinazolin-3-amine to N-(2-cyanophenyl)-alkylimidoacid hydrazide (as determined by NMR)

N-Acylated 2-aminobenzonitriles derived from alkyl acyl halogenides usually gave a mixture of 4-imino-quinazolin-3-amines and their precursor N-(2-cyanophenyl)-alkyl-imidoacid hydrazides. These mixtures did not need to be separated as these two substances give the same product in the subsequent reactions.



Summary

✓ A new microwave-assisted synthesis for 4-imino-quinazolin-3-amines has been developed and applied

✓ Several 2- and 5-substituted [1,2,4]triazolo[1,5-c]quinazoline derivatives have been synthesized and 5-phenyl[1,2,4]triazolo[1,5-*c*]quinazolin-2-amine was identified as new lead structure for the development of adenosine receptor antagonists

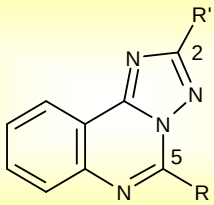
✓ A small library of related compounds was produced and tested for A_1 and A_{2A} affinity

✓ Several of these compound possessed K_i values in the low nanomolar range

Introduction

Selective adenosine A_{2A} receptor antagonists are currently in clinical development for the treatment of Parkinson's disease. Furthermore, antidepressive, neuroprotective, and analgetic properties have been demonstrated in animal models.[3,4]

Screening of compounds synthesized by our collaboration partners[5] indicated that [1,2,4]triazolo[1,5-c]quinazoline derivatives could be of interest as adenosine A_{2A} receptor antagonists, so we set out to explore the SARs of this class of compounds. We did so in two stages, first modifying the 2-position in the five-membered ring, and then producing derivatives with different substitutents at the 5-position.



[1,2,4]Triazolo[1,5-*c*]quinazolines with Different Substitutents at the 2-Position

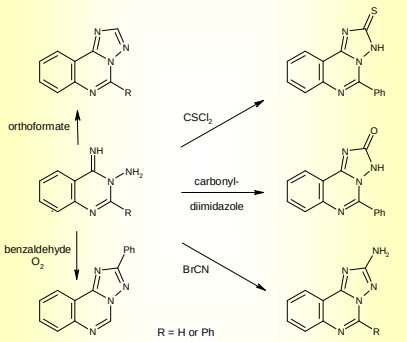
4-Imino-quinazolin-3-amine and 2-phenyl-4-imino-quinazolin-3-amine were chosen as model precursors and treated with a variety of reagents:

Treatment with triethylorthoformate led to 2-unsubstituted [1,2,4]triazolo[1,5-*c*]quinazolines (R' = H)

Treatment with cyanogen bromide led to [1,2,4]triazolo[1,5-*c*]quinazolin-2-amines (R' = NH_2)

2-Phenyl-4-imino-quinazolin-3-amine was further treated with carbonyl diimidazole and thiophosgene to yield 5-phenyl[1,2,4]triazolo[1,5-*c*]quinazolin-2(3*H*)-one and –thione, respectively

4-Imino-quinazolin-3-amine was converted to 2-phenyl[1,2,4]triazolo[1,5-*c*]quinazoline (R' = Ph) by heating with benzaldehyde (oxidation probably by atmosperic oxygen)



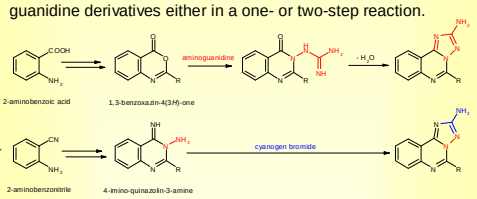
Testing of the synthesized compounds for affinity to rat A_1 and A_{2A} receptors revealed that only 5-phenyl[1,2,4]triazolo[1,5-*c*]quinazolin-2-amine had high affinity in the lower nano-molar range. The amino group at the 2-position seems to be critical for this effect, as other substituents lowered the affinity drastically. The comparison of the values for different substituents at the 5-position indicated that the large lipophilic phenyl group was indeed beneficial here.

R'	R	A_1 (rat cortex)	A_{2A} (rat striatum)
(2-position)	(5-position)	K_i [nM]	K_i [nM]
H	H	> 10000	> 10000
H	Ph	1232	> 10000
Ph	H	630	2661
NH_2	H	4694	2171
NH_2	Ph	280	**31.5**
OH	Ph	1345	1647
SH	Ph	> 10000	> 10000

5-Substituted [1,2,4]Triazolo[1,5-*c*]quinazolin-2-amines

As [1,2,4]triazolo[1,5-c]quinazolin-2-amines had shown the highest affinity for adenosine receptors, a set of structurally diverse compounds was synthesized and tested. The compounds can be divided into two groups: those with an alkyl or (hetero)aromatic substitutent, and those with substituted amino groups at the 5-position.

Two strategies were employed to synthesize alkyl- and (hetero)aromatically substituted derivatives: (i) reaction of 4-imino-quinazolin-3-amines with cyanogen bromide in ethanol at 95°C (under pressure), and (ii) conversion of 1,3-benzoxazin-4(3*H*)-ones with aminoguanidine and cyclization of the resulting guanidine derivatives either in a one- or two-step reaction.

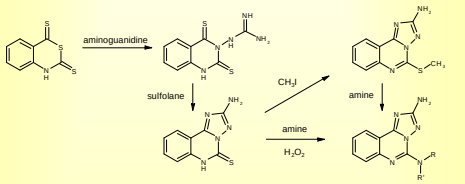


The products were tested for affinity at rat A_1 and A_{2A} receptors. Aromatic substitutents with the size of a phenyl ring showed high affinity to adenosine receptors. A fluorine atom at the 3-position of the phenyl ring was well tolerated, while a fluorine atom at the 4-position led to a 10-fold decrease in affinity. Bromine in the 4-postion led to an even more dramatic loss of affinity. All heteroaromatic substituents conferred high affinity: The thienyl derivatives had even higher A_{2A} affinity than the lead compound 5-phenyl[1,2,4]triazolo[1,5-c]quinazolin-2-amine, 2-furyl was well tolerated, pyridine residues were less favorable. Receptor subtype selectivity was low. The lead compound, 5-phenyl[1,2,4]triazolo[1,5-c]quinazolin-2-amine, showed 9-fold selectivity for A_{2A} over A_1 (3-fold at human receptors), the 3-thienyl-derivative 3-fold (7-fold at human receptors). Selectivity of the other tested (hetero)aromatically substituted derivatives was even lower. This trend was also present in 5-aryl-substituted [1,2,4]triazolo[1,5-c]quinazolin-2-amines, which generally possessed lower affinity for adenosine receptors. In one case, namely the isopropyl derivative, there was even an 11-fold selectivity for A_1 over A_{2A}.

R	Synthesis		A_1 (rat cortex)	A_{2A} (rat striat.)	
(5-position)	Meth	Yield	K_i [nM]	K_i [nM]	K_i A_1/A_{2A}
phenyl	A	46	280	31.5	8.9
phenyl (human)	B	78	223	79.2	2.8
3-fluorophenyl	B	85	324	117	2.8
4-fluorophenyl	B	48	3085	1713	1.8
4-bromophenyl	B	90	> 10000	> 10000	-
2-thienyl	B	70	22	16	1.4
3-thienyl	B	82	87	27	3.2
3-thienyl (human)	-	-	353	51.2	6.9
2-furyl	B	84	87	69	1.3
3-pyridyl	A	36	1120	1360	0.8
4-pyridyl	A	42	1090	420	2.6
H	B	39	4694	2171	2.2
methyl	A	32	> 10000	4450	-
isopropyl	B	71	168	1921	0.09
benzyl	B	93	> 10000	1870	-
1-phenethyl	B	78	1236	872	1.4
2-phenethyl	B	28	4098	5057	0.8
trimethoxy-2-phenethyl	B	60	> 10000	13300	-

n.d. : not determined / Method A: from the benzoxazinone / Method B: from the iminoquinazolinamine

The 5-amino-substituted [1,2,4]triazolo[1,5-c]quinazolin-2-amines were synthesized from trithioisatoic anhydride. After reaction with aminoguanidine, cyclisation was performed by heating in sulfolane to yield 2-amino[1,2,4]triazolo[1,5-c]quinazolin-5(6*H*)-thione.[6] This compound could either be reacted with the corresponding amine, either alone or under oxidative conditions (H_2O_2), or be methylated to give 5-methyl-sulfanyl[1,2,4]triazolo[1,5-c]quinazolin-2-amine as an activated intermediate, which could be reacted with an amine.



The affinity of the products was generally lower than for the compound described above, with small, lipophilic residues being most potent. Selectivity was generally low.

R	Synthesis		A_1 (rat cortex)	A_{2A} (rat striat.)	
(5-position)	Meth	Yield	K_i [nM]	K_i [nM]	K_i A_1/A_{2A}
SH	-	60	> 10000	> 10000	-
S-Me	-	100	962	303	3.2
$NH\text{-}NH_2$	A	99	> 10000	> 10000	-
pyrrolidine	A	40	864	274	3.2
pyrrolidine (human)	-	-	708	338	2.1
piperidine	B	51	2652	845	3.1
morpholine	B	67	> 10000	5447	-
aminobutyl	B	16	555	208	2.7
aminoethanol	C	84	> 10000	2536	-
aminobenzyl	B	73	1726	2077	0.8
aminophenethyl	C	22	> 10000	7232	-

n.d. : not determined / Method A: thione & amine in ethanol under reflux
Method B thione & amine & aqueous H_2O_2 at 60°C / Method C: methylsulfanyl & neat amine at 120°C

Literature:

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