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7-Amino-2-aryl/heteroaryl-5-oxo-5,8-dihydro[1,2,4]triazolo[1,5-*a*]pyridine-6-carbonitriles: Synthesis and Adenosine Receptor Binding Studies

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ABSTRACT

A series of novel 7-amino-5-oxo-2-substituted-aryl/heteroaryl-5,8-dihydro[1,2,4]triazolo[1,5-*a*]-pyridine-6-carbonitriles (**4a-4t**) was synthesized, characterized and evaluated for their binding affinity and selectivity towards hA_1, hA_{2A}, hA_{2B} and hA_3 adenosine receptors (ARs). Compound **4a** with a phenyl ring at 2-position of the triazolo moiety of the scaffold showed high affinity and selectivity for hA_1 AR (K_i hA_1 = 0.076 μM, hA_{2A} = 25.6 μM and hA_3 > 100 μM). Introduction of various electron donating and withdrawing groups at different positions of the phenyl ring resulted in drastic reduction in affinity and selectivity towards all the ARs, except compound **4b** with a phenol group at 2-position. Interestingly, the replacement of the phenyl ring with a smaller heterocyclic thiophene ring (π excessive system) resulted in further improvement of affinity for hA_1 AR of compound **4t** (K_i hA_1 = 0.051 μM, hA_{2A} = 9.01 μM and hA_3 > 13.9 μM) while retaining the significant selectivity against all other AR subtypes similar to compound **4a**. The encouraging results for compounds **4a** and **4t** indicate that substitution at 2-position of the scaffold with π-excessive systems other than thiophene may lead to even more potent and selective hA_1 AR antagonists.

Keywords: A_1 adenosine receptors, adenosine receptor antagonists, cyanoacetic hydrazides, triazolopyridines

1. INTRODUCTION

The physiological effects of endogenous adenosine on various organ systems are myriad and complex which are generally elicited upon activation of any of the four G protein coupled receptors (GPCRs) denoted as A_1, A_{2A}, A_{2B} and A_3 adenosine receptors (ARs) (Borea, Varani, Gessi, Merighi, & Vincenzi, 2018; Fredholm, IJzerman, Jacobson, Klotz, & Linden, 2001). Receptor-mediated actions of A_1 and A_3 ARs are mainly linked to inhibition of adenylyl cyclase (AC) activity and a consequent decrease in the intracellular level of cyclic AMP (cAMP), whereas A_{2A} and A_{2B} ARs stimulate the AC activity leading to an increase of cAMP (Merighi, Gessi, & Borea, 2018). The A_1 AR is prominently expressed in the central nervous system (CNS), spinal cord, heart and kidney. Agonists of A_1 AR could be useful as neuro- and cardio-protective agents as well as for the treatment of cardiac arrhythmias, pain, diabetes type-2, reduction of lipolysis in adipose tissue and intraocular pressure in glaucoma (Cosimelli et al., 2016; Gao et al., 2018). In contrast, A_1 AR antagonists might be beneficial

for the treatment of acute heart failure, asthma and chronic obstructive pulmonary disease (COPD) (Brown, Spina, & Page, 2008; Gao et al., 2018; Kiesman, Elzein, & Zablocki, 2009). The A_{2A} receptor antagonists are considered to be a potential non-dopaminergic therapeutic approach for the treatment of neurodegenerative movement disorders such as Parkinson's disease (PD) and Huntington's disease, which resulted in the discovery and development of the xanthine derivative istradefylline as the first marketed selective A_{2A} AR antagonist as an adjunctive therapy of PD in Japan in 2013 (Pinna, 2014; Preti, Baraldi, Moorman, Borea, & Varani, 2015). The A_{2B} AR antagonists on the other hand are being investigated for the treatment of type 2 diabetes, asthma and gastrointestinal disorders (Joseph et al., 2008; Kalla & Zablocki, 2009; Sun & Huang, 2016). Similarly, agonists selective for A_3 AR are currently under investigation for the treatment of autoimmune inflammatory disorders, liver cancer, rheumatoid arthritis, psoriasis, dry eye disease and cardioprotection, respectively, whereas A_3 selective antagonists are developed for the treatment of asthma, inflammatory bowel disease, glaucoma as well as cerebroprotective agent (Brown et al., 2008; Cruz□Monteagudo, Cordeiro, Teijeira, González, & Borges, 2010; Jacobson et al., 2018).

Theophylline **(i)** and caffeine **(ii)**, the archetypal antagonists, have no selectivity for the A_1 AR. Thus, a lot of efforts have been made to develop xanthine and non-xanthine analogues with higher selectivity and affinity towards specific AR subtypes. Despite the continuous efforts made by various researchers only few analogues with promising A_1 affinity have been identified so far (Borghini, Pietra, Leonardi, Giorgi, & Bianucci, 2013; Cosimelli et al., 2016; Gao et al., 2018; Kiesman et al., 2009; Romagnoli, Baraldi, Moorman, Borea, & Varani, 2015). In particular, 8-amino-2-aryl-[1,2,4]triazolo[1,5-a]pyridine-6-carboxyl amides **(ii)** and isomeric 5-amino-2-aryl-[1,2,4]triazolo[1,5-a]pyridine-7-carboxyl amide derivatives **(iv)** showed good affinity and selectivity towards hA_{2A} versus hA_1 ARs (Guba, Nettekoven, Püllmann, Riemer, & Schmitt, 2004). Thus, it was our thought of interest to further investigate any changes in affinity and selectivity towards ARs for a novel series of triazolopyridine derivatives (**4a-4t**) with 7-amino (NH_2) and 6-carbonitrile (CN) group, while varying the substitution pattern at the 2-position of the scaffold with various aryl/heteroaryl moieties (Figure 1). In continuation of our interest in the search of novel ligands for adenosine receptors (Balakumar et al., 2012; Chandrasekaran et al., 2018; Deb et al., 2018; Kaur et al., 2011; Kishore, Balakumar, Rao, Roy, & Roy, 2011; Prasad et al., 2008; Sirisha et al., 2010; Veeraswamy et al., 2013) and in the light of the above mentioned literature reports, some novel 5-oxo[1,2,4]triazolo[1,5-*a*]pyridine-6-carbonitrile derivatives (**4a-4t**) have been synthesized as potential selective ligands for hA_1 AR.

2. RESULTS AND DISCUSSION

2.1. Chemistry

The synthetic route of the target compounds (**4a-4t**) is outlined in Figure 2. The 2-cyanoacetohydrazide (**1**) was treated with various aryl/heteroaryl aldehydes (**2**) under mild acidic conditions during which hydrazide reacts with the carbonyl group of the aldehyde and undergoes dehydration to give rise to key intermediates, cyanoacetic acid substituted benzylidene/hetero-arylidene hydrazides (**3a-3t**) (Gorobets, Yousefi, Belaj, & Kappe, 2004; Metwally & Abdelrazek, 2005).

The synthesized cyanoacetic acid substituted benzylidene/hetero-arylidene hydrazides (**3a-3t**) were treated with malononitrile in the presence of an amount of a catalytic base (piperidine) using ethanol as solvent to obtain the final products (**4a-4t**). The products thus obtained were purified and characterized as 7-amino-5-oxo-2-phenyl/substitutedphenyl/hetero-aryl-5,8-dihydro-[1,2,4]triazolo[1,5-α]pyridine-6-carbonitriles (**4a-4t**) based on their spectrometric analysis. Compounds 4a, 4b, 4f, 4p preparation were reported earlier by one pot synthetic procedure (El-Hamid Ismail, 1996; Ismail, 1996) and their reported melting points were well corroborated with our melting points of these compounds. However, this method was not applicable for the synthesis of other derivatives. Hence a two pot synthetic procedure was developed. It is worth mentioning that LCMS data of all the target compounds indicated a purity of > 95 %.

Details of materials and methods for the synthesis and characterization data (IR, NMR and Mass spectra) of compounds along with physical properties of intermediate compounds (**3a-3t)** are presented in Appendix S1 and Table S1 respectively, under supporting information.

2.2. Biological activity

All the synthesized target compounds (**4a-4t**) were evaluated for their affinity and selectivity towards human A_1, A_{2A}, A_{2B} and A_3 ARs, expressed in CHO cells (Table 1) by following the previously reported procedures. The radioligands [^{3}H]-2-chloro-N^6-cyclopentyl adenosine ([^{3}H]CCPA), [^{3}H]-5'-*N*-ethylcarboxamido adenosine ([^{3}H]NECA) and [^{3}H]-2-(1-hexynyl)-N^6-methyl adenosine ([^{3}H]HEMADO) were used in binding assays for hA_1, hA_{2A}, and hA_3 ARs, respectively. Due to the lack of a useful radioligand for A_{2B} receptor a possible

interaction of the new compounds with the A_{2B} AR was tested in functional experiments. A possible agonistic activity at hA_{2B} AR was determined by stimulation of adenylyl cyclase activity, while inhibition of NECA-stimulated adenylyl cyclase activity was used as a measurement of an antagonist interaction. Data for hA_{2B} AR are not shown as none of the compounds showed measurable interaction with the hA_{2B} AR in an agonistic or antagonistic fashion (EC_{50} or IC_{50} values > 60 μM). Materials and methods required for the evaluation of binding affinity and selectivity of compounds (**4a-4t**) towards human A_1, $A_{2A,}$ A_{2B} and A_3 ARs are described in Appendix S1.

In this study, five-membered thiazole and six-membered benzene rings with diverse functional groups were substituted at the 2-position of the triazolo ring to get the targeted analogues (**4a-4t**), while retaining a nitrile and a free amino group at 6- and 7-position of the scaffold. The AR binding data shows that the affinity and selectivity of the target compounds (**4a-4t**) varied with a change in the aryl/heteroaryl substitution at the 2-position of the scaffold. Among the 2-aryl analogues (**4a-4r**), compound **4a** with an un-substituted phenyl ring at the 2-position of the triazolo moiety of the scaffold was found to possess the highest affinity and selectivity for hA_1 AR (K_i hA_1 = 0.076 μM, hA_{2A} = 25.6 μM and hA_3 > 100 μM). Substitution of the phenyl ring with hydroxyl (OH) and methyl (CH_3) group at the *para* position decreased the affinity for hA_1 AR of compounds **4b** and **4c** respectively (**4a > 4b > 4c**). Further introduction of a ring activating *N,N*-dimethyl amino group at *para* position of the phenyl ring (compound **4d**) and methoxy (OCH_3) substitution at various positions of the phenyl ring (compounds **4e-4i**) drastically reduced the affinity for hA_1 AR. Compounds with a combination of both hydroxyl (OH) and methoxy (OCH_3) substitutions at various positions of the phenyl ring (**4j** and **4k**) showed little improvement in affinity for hA_1 AR as compared to compounds **4e-4i**, in particular, compound **4j** with hydroxyl and methoxy group at *ortho* and *meta* positions of the phenyl ring exhibited moderate affinity with high selectivity for hA_1 AR (K_i hA_1 = 0.875 μM, hA_{2A} = > 100 μM and hA_3 > 47.60 μM).

Introduction of an electron withdrawing nitro group (NO_2) at the *ortho* or *para* position of the phenyl ring resulted in compounds **4l** and **4m** with insignificant affinity for all the ARs. Interestingly, the compounds **4n-4r** with a halide (F/Cl/Br) substituted phenyl ring at the 2-position of the scaffold showed moderate to good affinity for hA_1 AR with significant selectivity against the other AR subtypes. In particular, compound **4o** with a chloro (Cl)

substitution at 2-position of the phenyl ring showed significant affinity and selectivity towards hA_1 AR (K_i hA_1 = 0.294 μM, hA_{2A} > 100 μM and hA_3 > 100 μM).

The replacement of the phenyl/substituted phenyl group (compounds **4a-4r**) with a more reactive smaller heterocyclic thiophene moiety (π excessive system) resulted in further improvement for compound **4t** displaying the highest affinity and significant selectivity towards hA_1 AR (K_i hA_1 = 0.051 μM, hA_{2A} = 9.01 μM and hA_3 > 13.9 μM), whereas replacement of the phenyl group with a pyridine ring (π-deficient system) showed detrimental effect with reduced affinity for hA_1 AR in case of compound **4s** (K_i hA_1 = 0.859 μM, hA_{2A} = 6.43 μM and hA_3 > 60 μM). This wide variation in affinity might be attributed to the higher electron density of thiophene over pyridine and phenyl rings. The electron pairs on sulfur are delocalized in the π-electron system to a significant extent and are readily available for bond formation which might facilitate crucial interactions with the amino acid residues at the binding site of the hA_1 AR leading to greater binding affinity. Moreover, a six-membered ring as a substituent could be too big in size to fit into the receptor binding site for proper interaction with target amino acid residues. However, it is well known that pyridine because of the negative inductive effect of nitrogen suffers from weaker resonance stabilization as compared to a phenyl group and this further explains the differences in affinities of the thiophene, phenyl and pyridyl derivatives.

3. CONCLUSIONS

In conclusion, a series of novel 7-amino-5-oxo-2-substituted phenyl/heteroaryl-5,8-dihydro-[1,2,4]triazolo[1,5-*α*]pyridine-6-carbonitrile derivatives (**4a-4t**) was synthesized, characterized and evaluated for A_1 affinity and selectivity. Structure activity relationship (SAR) studies revealed that among the 2-aryl analogues (**4a-4r**), the compound **4a** with unsubstituted phenyl ring at the 2-position of the scaffold possesses the highest affinity and selectivity for hA_1 AR (K_i hA_1 = 0.076 μM, hA_{2A} = 25.6 μM and hA_3 > 100 μM). Introduction of various electron donating and withdrawing group/s at different positions of the phenyl ring showed detrimental effect in affinity and selectivity towards all the ARs. For example, compound **4i** with 3,4,5-trimethoxyphenyl group did not show any affinity for ARs (K_i hA_1 > 60 μM, hA_{2A} > 60 μM and hA_3 > 100 μM), whereas compound **4l** with 3-nitrophenyl group showed insignificant affinity and selectivity for ARs. Interestingly, the compounds (**4n-4r**) with a halide (F/Cl/Br) substituted phenyl ring at the 2-position of the

scaffold showed good affinity with significant selectivity for hA_1 AR. In particular, compound **4o** with 2-chlorophenyl group showed maximum affinity and selectivity for hA_1 AR (K_i hA_1 = 0.294 μM, hA_{2A} > 100 μM and hA_3 > 340 μM) as compared to other halide substituted compounds (**4n, 4p-4r**). The replacement of the phenyl group with a more reactive smaller heterocyclic thiophene moiety (π excessive system) resulted in further improvement of affinity as seen in the case of compound **4t** displaying the highest affinity and significant selectivity for hA_1 AR (K_i hA_1 = 0.051 μM, hA_{2A} = 9.01 μM and hA_3 > 13.9 μM). Thus, further optimization of this scaffold with various substituted five-membered aromatic ring systems like thiophene may lead to the discovery and development of highly potent A_1 AR ligands with improved affinity and selectivity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Appendix S1: Methods and materials (details) for synthesis and characterization data of compounds, and biological activity studies.

Table S1: Physical properties of intermediate compounds **3a-3t.**

FIGURE LEGENDS

Figure 1: Chemical structures of xanthine analogues theophylline **(i),** caffeine **(ii),** isomeric triazolopyridine-7-carboxylic acid derivatives **(iii, iv)** and newly designed compounds **(4a-4t).**

Figure 2: Synthesis of 7-amino-2-aryl/heteroaryl-5-oxo-5,8-dihydro[1,2,4]triazolo[1,5-a]pyridine-6-carbonitriles (**4a-4t**). Reagents and conditions: (i) acetic acid, EtOH, reflux; (ii) $CNCH_2CN$, piperidine, EtOH, reflux.

TABLE

Table 1: Binding affinity (K_i) of target compounds (**4a-4t**) at hA_1, hA_{2A} and hA_3 ARs and selectivity against hA_{2A} and hA_3 ARs.

REFERENCES

Balakumar, C., Kishore, D. P., Rao, K. V., Narayana, B. L., Rajwinder, K., Rajkumar, V., & Rao, A. R. (2012). Design, microwave-assisted synthesis and in silico docking studies of new 4*H*-pyrimido[2,1-b]benzothiazole-2-arylamino-3-cyano-4-ones as possible adenosine A_{2B} receptor antagonists.

Borea, P. A., Varani, K., Gessi, S., Merighi, S., & Vincenzi, F. (2018). *The Adenosine Receptors* (Vol. 34): Springer.

Borghini, A., Pietra, D., Leonardi, M., Giorgi, I., & Bianucci, A. M. (2013). N□[9□(ortho□Fluorobenzyl)□2□Phenyl□8□Azapurin□6□yl]□Amides as Potent and Selective Ligands for A_1 Adenosine Receptors. *Chemical biology & drug design, 82*(1), 22-38.

Brown, R., Spina, D., & Page, C. (2008). Adenosine receptors and asthma. *British journal of pharmacology, 153*(S1), S446-S456.

Chandrasekaran, B., Deb, P. K., Kachler, S., Akkinepalli, R. R., Mailavaram, R., & Klotz, K.-N. (2018). Synthesis and adenosine receptors binding studies of new fluorinated analogues of pyrido[2,3-d]pyrimidines and quinazolines. *Medicinal Chemistry Research, 27*(3), 756-767.

Cosimelli, B., Greco, G., Laneri, S., Novellino, E., Sacchi, A., Trincavelli, M. L., . . . Martini, C. (2016). 4□amino□6□alkyloxy□2□alkylthiopyrimidine derivatives as novel

non□nucleoside agonists for the adenosine A_1 receptor. *Chemical biology & drug design, 88*(5), 724-729.

Cruz□Monteagudo, M., Cordeiro, M. N. D., Teijeira, M., González, M. P., & Borges, F. (2010). Multidimensional Drug Design: Simultaneous Analysis of Binding and Relative Efficacy Profiles of N^6□substituted□4′□thioadenosines A_3 Adenosine Receptor Agonists. *Chemical biology & drug design, 75*(6), 607-618.

Deb, P. K., Mailavaram, R., Chandrasekaran, B., Kaki, V. R., Kaur, R., Kachler, S., . . . Akkinepally, R. R. (2018). Synthesis, adenosine receptor binding and molecular modelling studies of novel thieno[2,3□d]pyrimidine derivatives. *Chemical biology & drug design, 91*(4), 962-969.

El-Hamid Ismail, A. (1996). Efficient one step synthesis of [1,2,4]triazolo[1,5-a]pyridines. *Pharmazie, 51*(12), 982-983.

Fredholm, B. B., IJzerman, A. P., Jacobson, K. A., Klotz, K.-N., & Linden, J. (2001). International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacological reviews, 53*(4), 527-552.

Gao, Z.-G., Tosh, D. K., Jain, S., Yu, J., Suresh, R. R., & Jacobson, K. A. (2018). A_1 Adenosine Receptor Agonists, Antagonists, and Allosteric Modulators *The Adenosine Receptors* (pp. 59-89): Springer.

Gorobets, N. Y., Yousefi, B. H., Belaj, F., & Kappe, C. O. (2004). Rapid microwave-assisted solution phase synthesis of substituted 2-pyridone libraries. *Tetrahedron, 60*(39), 8633-8644.

Guba, W., Nettekoven, M., Püllmann, B., Riemer, C., & Schmitt, S. (2004). Comparison of inhibitory activity of isomeric triazolopyridine derivatives towards adenosine receptor subtypes or do similar structures reveal similar bioactivities? *Bioorganic & medicinal chemistry letters, 14*(12), 3307-3312.

Ismail, A. (1996). One step efficient synthesis of [1,2,4]triazolo[1,5-a]pyridines. *Afinidad, 53*(466), 410-412.

Jacobson, K. A., Tosh, D. K., Gao, Z.-G., Yu, J., Suresh, R. R., Rao, H., . . . Tabrizi, M. A. (2018). Medicinal Chemistry of the A_3 Adenosine Receptor *The Adenosine Receptors* (pp. 169-198): Springer.

Joseph, T. B., Suneel Kumar, B., Santhosh, B., Kriti, S., Pramod, A., Ravikumar, M., & Kishore, M. (2008). Quantitative structure activity relationship and pharmacophore studies of adenosine receptor A_{2B} inhibitors. *Chemical biology & drug design, 72*(5), 395-408.

Kalla, R. V., & Zablocki, J. (2009). Progress in the discovery of selective, high affinity A_{2B} adenosine receptor antagonists as clinical candidates. *Purinergic signalling, 5*(1), 21-29.

Kaur, R., D PRAN, K., Narayana, B. L., Rao, K. V., Balakumar, C., Rajkumar, V., & Rao, A. R. (2011). A facile microwave-assisted synthesis of 8,9-cycloalkathieno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidin-5-(6H)-ones. *Journal of Chemical Sciences, 123*(1), 69-73.

Kiesman, W. F., Elzein, E., & Zablocki, J. (2009). A_1 adenosine receptor antagonists, agonists, and allosteric enhancers *Adenosine receptors in health and disease* (pp. 25-58): Springer.

Kishore, D. P., Balakumar, C., Rao, A. R., Roy, P. P., & Roy, K. (2011). QSAR of adenosine receptor antagonists: exploring physicochemical requirements for binding of pyrazolo[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine derivatives with human adenosine A_3 receptor subtype. *Bioorganic & medicinal chemistry letters, 21*(2), 818-823.

Merighi, S., Gessi, S., & Borea, P. A. (2018). Adenosine Receptors: Structure, Distribution, and Signal Transduction *The Adenosine Receptors* (pp. 33-57): Springer.

Metwally, N. H., & Abdelrazek, F. M. (2005). Reaction of anthranilonitrile with some active methylene reagents: Synthesis of some new quinoline and quinazoline derivatives. *Synthetic communications, 35*(18), 2481-2487.

Pinna, A. (2014). Adenosine A_{2A} receptor antagonists in Parkinson's disease: progress in clinical trials from the newly approved istradefylline to drugs in early development and those already discontinued. *CNS drugs, 28*(5), 455-474.

Prasad, M. R., Rao, A. R., Rao, P. S., Rajan, K. S., Meena, S., & Madhavi, K. (2008). Synthesis and adenosine receptor binding studies of some novel triazolothienopyrimidines. *European Journal of Medicinal Chemistry, 43*(3), 614-620.

Preti, D., Baraldi, P. G., Moorman, A. R., Borea, P. A., & Varani, K. (2015). History and perspectives of A_{2A} adenosine receptor antagonists as potential therapeutic agents. *Medicinal research reviews, 35*(4), 790-848.

Romagnoli, R., Baraldi, P. G., Moorman, A. R., Borea, P. A., & Varani, K. (2015). Current status of A_1 adenosine receptor allosteric enhancers. *Future medicinal chemistry, 7*(10), 1247-1259.

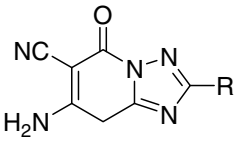
Sirisha, B., Narsaiah, B., Yakaiah, T., Gayatri, G., Sastry, G. N., Prasad, M. R., & Rao, A. R. (2010). Synthesis and theoretical studies on energetics of novel N-and O-

perfluoroalkyl triazole tagged thienopyrimidines–Their potential as adenosine receptor ligands. *European Journal of Medicinal Chemistry, 45*(5), 1739-1745.

Sun, Y., & Huang, P. (2016). Adenosine A_{2B} receptor: from cell biology to human diseases. *Frontiers in chemistry, 4*, 37.

Veeraswamy, B., Balakumar, C., Meryem, K., Christin, V., Christa, E., Kurumurthy, C., . . . Raghuramarao, A. (2013). Synthesis of novel pyrido[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine derivatives: potent and selective adenosine A_3 receptor antagonists. *Arch Pharm, 346*, 699-707.

Table 1 Binding affinity (K_i) of target compounds (**4a-4t**) at hA_1, hA_{2A} and hA_3 ARs and selectivity against hA_{2A} and hA_3 ARs.



(4a-4t)

Comp. No.	R	K_i, μM*			Selectivity	
		hA_1[a]	hA_{2A}[b]	hA_3[c]	hA_{2A} / hA_1	hA_3 / hA_1
4a	phenyl	0.076 (0.0694-0.0831)	25.6 (17.7-36.9)	> 100	340	1300
4b	4-hydroxy-phenyl	0.115 (0.0826-0.160)	3.64 (3.47-3.83)	> 30	32	> 260
4c	*p*-tolyl	0.525 (0.499-0.552)	> 100	> 60	> 190	> 110
4d	4-*N*,*N*-dimethyl-phenyl	5.50 (4.99-6.05)	> 100	> 60	> 18	> 11
4e	2-methoxy-phenyl	5.73 (4.81-6.83)	> 100	> 100	> 17	> 17
4f	4-methoxy-phenyl	4.25 (4.12-4.39)	> 100	> 100	> 24	> 24
4g	3,4-dimethoxy-phenyl	8.13 (7.13-9.28)	> 60	> 60	> 7	> 7
4h	2,4-dimethoxy-phenyl	1.00 (0.859-11.8)	> 30	19.5 (11.5-32.9)	> 30	20
4i	3,4,5-trimethoxy-phenyl	> 60	> 60	> 100	-	-
4j	2-hydroxy-3-methoxy-phenyl	0.875 (0.83-0.922)	> 100	47.6 (42.4-53.5)	> 110	54
4k	3,5-dimethoxy-4-hydroxy-phenyl	1.76 (1.54-2.01)	1.37 (1.01-1.86)	> 100	0.8	> 57
4l	3-nitro-phenyl	2.45 (2.28-2.62)	52.2 (40.9-66.7)	5.49 (4.86-6.2)	21	2
4m	4-nitro-phenyl	7.29 (5.9-9.0)	> 100	> 100	> 14	> 14
4n	4-fluoro-phenyl	0.413 (0.326-0.523)	24.4 (15.9-37.4)	> 100	59	> 240
4o	2-chloro-phenyl	0.294 (0.273-0.315)	> 100	> 100	> 340	> 340
4p	4-chloro-phenyl	0.832 (0.723-0.958)	35.4 (30.5-40.9)	> 30	> 43	> 36
4q	2,4-dichloro-phenyl	0.732 (0.663-0.808)	> 100	> 60	> 140	> 82
4r	4-bromo-phenyl	0.589 (0.481-0.721)	> 60	24.6 (21.6-27.9)	> 100	42
4s	2-pyridyl	0.859 (0.755-0.976)	6.43 (3.25-12.7)	> 60	7	> 70
4t	thiophene	0.0510 (0.0451-0.0577)	9.01 (6.37-12.7)	13.9 (12.6-15.3)	176	> 270

[*] *K_i values are given as geometric means with 95% confidence intervals in parentheses.*

[a] *Displacement of specific [^{3}H]CCPA binding at human A_1 AR expressed in CHO cells (n = 3).*

[b] *Displacement of specific [^{3}H]NECA binding at human A_{2A} AR expressed in CHO cells (n = 3).*

[c] *Displacement of specific [^{3}H]HEMADO binding at human A_3 AR expressed in CHO cells (n = 3).*

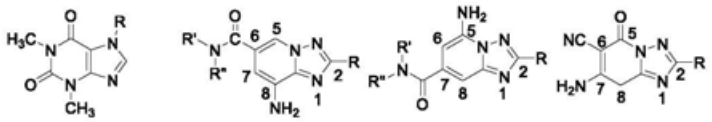
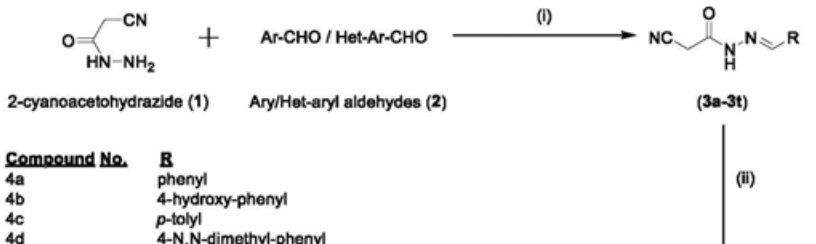


Figure 1. Chemical structures of xanthine analogues theophylline (i), caffeine (ii), isomeric triazolopyridine-7-carboxylic acid derivatives (III, Iv) and newly designed compounds **(4a-4t)**.



Compound No.	R
4a	phenyl
4b	4-hydroxy-phenyl
4c	*p*-tolyl
4d	4-N,N-dimethyl-phenyl
4e	2-methoxy-phenyl
4f	4-methoxy-phenyl
4g	3,4-dimethoxy-phenyl
4h	2,4-dimethoxy-phenyl
4i	3,4,5-trimethoxy-phenyl
4j	2-hydroxy-3methoxy-phenyl
4k	3,5-dimethoxy-4-hydroxy-phenyl
4l	3-nitro-phenyl
4m	4-nitro-phenyl
4n	4-fluoro-phenyl
4o	2-chloro-phenyl
4p	4-chloro-phenyl
4q	2,4-dichloro-phenyl
4r	4-bromo-phenyl
4s	pyridyl
4t	thiophene

Figure 2 Synthesis of 7-amino-2-aryl/heteroaryl-5-oxo-5,8-dihydro[1,2,4]triazolo[1,5-a]pyridine-6-carbonitriles (**4a-4t**). Reagents and conditions: (i) acetic acid, EtOH, reflux; (ii) $CNCH_2CN$, piperidine, EtOH, reflux.