European Journal of Medicinal Chemistry 108 (2016) 529–541

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

5,7-Disubstituted-[1,2,4]triazolo[1,5-*a*][1,3,5]triazines as pharmacological tools to explore the antagonist selectivity profiles toward adenosine receptors

Stephanie Federico [a], Antonella Ciancetta [b], Nicola Porta [b], Sara Redenti [a], Giorgia Pastorin [c], Barbara Cacciari [d], Karl Norbert Klotz [e], Stefano Moro [b, **], Giampiero Spalluto [a, *]

[a] Dipartimento di Scienze Chimiche e Farmaceutiche, Università di Trieste, Piazzale Europa 1, 34127 Trieste, Italy
[b] Molecular Modeling Section (MMS), Dipartimento di Scienze del Farmaco, Università di Padova, Via Marzolo 5, 35131 Padova, Italy
[c] Department of Pharmacy, National University of Singapore, 3 Science Drive 2, 117543 Singapore
[d] Dipartimento di Scienze Chimiche e Farmaceutiche, Università degli Studi di Ferrara, Via Fossato di Mortara 17-19, 44100 Ferrara, Italy
[e] Institut für Pharmakologie und Toxicologie, Universität of Würzburg, Versbacher Strasse 9, 97078 Würzburg, Germany

ARTICLE INFO

Article history:
Received 2 September 2015
Received in revised form
3 December 2015
Accepted 10 December 2015
Available online 15 December 2015

Keywords:
G protein-coupled receptor
Antagonists
Molecular modeling
Structure activity relationship
Triazolo-triazine
Adenosine receptors

ABSTRACT

The structure–activity relationship of new 5,7-disubstituted-[1,2,4]triazolo[1,5-*a*][1,3,5]triazines as adenosine receptors (ARs) antagonists has been explored. The introduction of a benzylamino group at C5 with a free amino group at C7 increases the affinity toward all the ARs subtypes (**10**: $K_ihA_1 = 94.6$ nM; $K_ihA_{2A} = 1.11$ nM; $IC_{50}hA_{2B} = 2214$ nM; $K_ihA_3 = 30.8$ nM). Replacing the free amino group at C7 with a phenylureido moiety yields a potent and quite selective hA_{2A} AR antagonist (**14**: hA_{2A} AR $K_i = 1.44$ nM; $hA_1/hA_{2A} = 216.0$; $hA_3/hA_{2A} = 20.6$). This trend diverges from the analysis on the pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine series previously reported. With the help of an *in silico* receptor-driven approach, we have rationalized these observations and elucidated from a molecular point of view the role of the benzylamino group at C5 in determining affinity toward the hA_{2A} AR.

1. Introduction

Adenosine receptors (ARs) are members of the G protein-coupled receptors (GPCRs) superfamily. To date, four ARs subtypes – the A_1 AR, A_{2A} AR, A_{2B} AR, and A_3 AR – are currently known [1], that exert their physiological functions through the activation or inhibition of various second messenger systems. In particular, the modulation of adenylate cyclase activity is considered the principal intracellular signal transduction mediated by these receptors [2,3]. Activation or blockade of ARs is responsible for a wide range of effects in numerous organ systems suggesting potential therapeutic applications of ARs modulators. In particular, the cardioprotective [4,5] and neuroprotective [6,7] effects associated with ARs activation have been clearly demonstrated during periods of cardiac and cerebral ischemia, respectively. Moreover, the use of antagonists of distinct AR subtypes could be useful in the treatment of asthma [8,9] or neurological diseases such as Parkinson's disease [10].

In recent years, the synthesis of a large variety of ARs agonists and antagonists for the pharmacological characterization of this GPCRs family has been reported [11]. Diverse classes of heterocyclic derivatives have been proposed as ARs antagonists, exhibiting high levels of both affinity and selectivity. Within this framework, our

Abbreviations: AR, adenosine receptor; CCPA, 2-chloro-N6-cyclopentyladenosine; CHO, Chinese hamster ovary; IEele, per residue electrostatic contributions to the interaction energy; IEFs, Interaction Energy Fingerprints; IEhyd, per residue hydrophobic contributions to the interaction energy; NECA, 5′-N-ethylcarboxamidoadenosine; PTP, pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine; R-PIA, R-(-)-N6-(2-phenylpropyl)adenosine; TEA, triethylamine; ZM 241385, 4-[2-[7-amino-2-(2-furyl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-yl-amino]ethyl-phenol; TM, transmembrane; TT, [1,2,4]triazolo[1,5-*a*][1,3,5]triazine; EL2, second extracellular loop.

* Corresponding author.
** Corresponding author.
E-mail addresses: stefano.moro@unipd.it (S. Moro), spalluto@units.it (G. Spalluto).

http://dx.doi.org/10.1016/j.ejmech.2015.12.019
0223-5234/

research groups have extensively investigated the pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (PTP) nucleus as basis for the design of ARs antagonists [12–23]. Through the modulation of the substitution pattern at the C5 ($C5^{PTP}$) and N7 ($N7^{PTP}$) positions, potent and selective human (h) A_{2A} AR and A_3 AR antagonists (compounds **A** [15] and **B** [18], Chart 1) were reported. Nevertheless, these derivatives, likewise to other tricyclic structures, suffer from limited aqueous solubility and require complicated synthetic routes. To overcome these limitations, we explored in recent years the synthesis of simplified bicyclic systems, such as [1,2,4]triazolo[1,5-*c*]pyrimidines [24], and [1,2,4]triazolo[1,5-*a*][1,3,5]triazines (TT) [25,22]. The TT nucleus, in particular, represents one of the most appealing bicyclic cores. In fact, one of the most potent and selective A_{2A} AR antagonists yet reported – 4-[2-[7-amino-2-(2-furyl)-[1,2,4]triazolo[1,5-*a*][1,3,5]triazin-5-yl-amino]ethylphenol (ZM241385, Chart 2) is based on this scaffold [26,27]. This compound also binds with good affinity to the hA_{2B} AR (28 nM), and its tritiated form is used as radioligand for this receptor subtype [28].

In recent years [22], we explored the structure-activity-relationship (SAR) of the introduction at C5 position of the TT scaffold of solubilizing groups aimed at enhancing both the aqueous solubility and the physicochemical properties. The resulting compounds maintained potency at the hA_{2A} AR and, in some cases, subtype selectivity. In another study [25], we investigated the effect of the substitutions at both the C5 and C7 position: compounds bearing a free amino group in C7 showed good affinity at the rat (r) A_{2A} AR, whereas the introduction of a phenylureido moiety slightly increased the affinity at the hA_3 AR with respect to the unsubstituted derivatives. In the present study, we further explore both the C5 and C7 positions. The newly synthesized compounds have been assessed at all four hARs, and the results rationalized from a molecular point of view with the help of computational methodologies.

2. Results and discussion

2.1. Chemistry

All the compounds were synthesized according to the procedure reported in Schemes 1–3. 5,7-Diphenoxy-2-furoyl-[1,2,4]triazolo[1,5-*a*][1,3,5]triazine (**5**) and 7-amino-5-phenoxy-2-furoyl-[1,2,4]triazolo[1,5-*a*][1,3,5]triazine (**6**) were obtained following the procedure reported in literature by Caulkett et al. as depicted in Scheme 1 [29].

By reacting compound **6** with the appropriate amines in ethanol, in a sealed tube at 100 °C, the 7-amino-5-aminosubstituted-2-furoyl-[1,2,4]triazolo[1,5-*a*][1,3,5]triazine derivatives (**7**–**10**) were obtained. Compounds **8**–**10** were already reported by Caulkett & coll [30]. Reaction of derivatives **7**–**10** with phenylisocyanate gives the corresponding 7-phenylureas (**11**–**14**) while reaction with benzoyl chloride or phenylacetyl chloride affords the corresponding 7-amido derivatives (**15**–**22**, Scheme 2).

The synthetic pathway to obtain the 5,7-diaminosubstituted compounds (**26**–**37**) is reported in Scheme 3. Firstly, the 5,7-diphenoxy-2-furoyl-[1,2,4]triazolo[1,5-*a*][1,3,5]triazine (**5**) was substituted at the C5 position with different amines in ethanol, in a

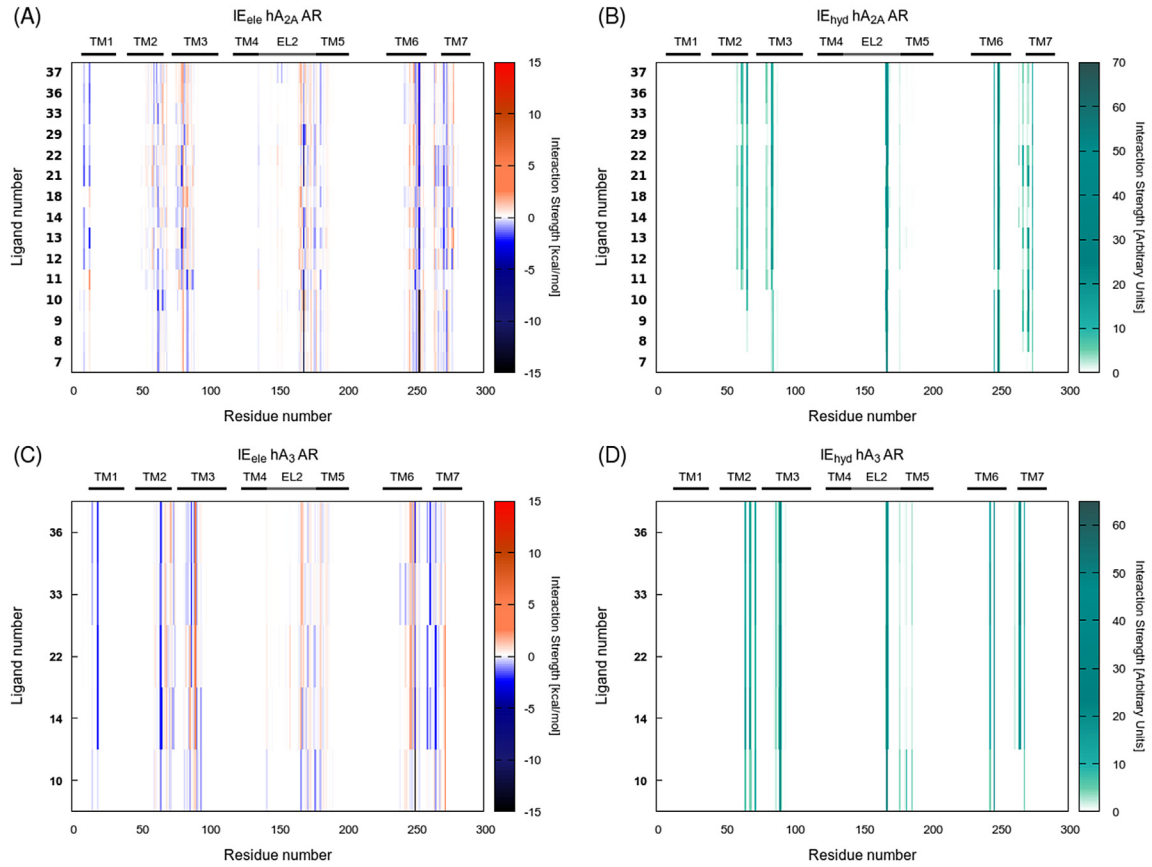

Fig. 1. Per residue IEele and IEhyd maps for the most energetically favorable docking poses of compounds with Ki < 100 nM. The maps have been computed for selected poses of the considered compound inside the orthosteric binding site of hA_{2A} AR (panels A and B) and the putative binding site of hA_3 AR (panels C and D). Electrostatic energy contributions are expressed in kcal/mol, whereas hydrophobic contributions are in arbitrary units. Ranges reported in the x-axes represent the length of protein sequences (truncated at 300 residues).