Structure-activity relationship studies and pharmacological characterization of N5-heteroarylalkyl-substituted-2-(2-furanyl)

Structure-activity relationship studies and pharmacological characterization of N^5-heteroarylalkyl-substituted-2-(2-furanyl)thiazolo[5,4-*d*]pyrimidine-5,7-diamine-based derivatives as inverse agonists at human A_{2A} adenosine receptor

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Abstract

This paper describes the synthesis and characterization of N^5-(hetero)arylalkyl-substituted-thiazolo[5,4-*d*]pyrimidine-5,7-diamine derivatives (**4-19**) as novel human (h) A_{2A} adenosine receptor (AR) inverse agonists. Competition binding and cyclic AMP assays indicate that the examined compounds behave as hA_{2A} AR inverse agonists showing binding affinity values in the nanomolar or subnanomolar range. Notably, compounds **4**, **5**, **6** and **11** showed two affinity values for the hA_{2A} ARs with the highest (KH) falling in the femtomolar range and the lowest (KL) of the nanomolar order. In addition, in cyclic AMP assays, compounds **4**, **5**, **6** and **11** exibited potency (IC_{50}) values in the picomolar range.

This study has confirmed that 2-(2-furanyl)thiazolo[5,4-*d*]pyrimidine-5,7-diamine-based derivatives represent a unique new class of hA_{2A} AR inverse agonists.

KEYWORDS. G protein coupled receptors; A_{2A} adenosine receptors; inverse agonists; thiazolopyrimidine derivatives; bicyclic heteroaromatic system.

ABBREVIATIONS. AR, adenosine receptor; cAMP, 3',5'-cyclic adenosine monophosphate; CHO, chinese hamster ovary; DPCPX, 8-cyclopentyl-1,3-dipropyl-xanthine; I-AB-MECA, 4-[((4-amino-3-iodophenyl)methyl)amino]-5'-N-methylcarboxamidoadenosine; **1**, (4-(2-[7-amino-2-(2-furil)[1,2.4] triazolo[2,3-a][1,3,5]triazin-5-ylamino] ethyl) phenol); **28**, 2-p-(2-carboxyethyl) phenethylamino-5'-N-ethylcarboxamidoadenosine; NECA, 5'-N-ethyl-carboxamidoadenosine; AB-MECA, 4-[((4-aminophenyl)methyl)amino]-5'-N-methylcarboxamidoadenosine; TCA, trichloroacetic acid; MW, microwave.

1. Introduction

The ubiquitous nucleoside adenosine mediates several effects, mainly through interaction with four G-protein coupled receptors, named A_1, A_{2A}, A_{2B} and A_3 adenosine receptors (ARs), expressed in several cells and tissues [1]. ARs have been well characterized from a structural point of view, for their cellular and tissue distribution and for their physiological effects. Cell signaling transduction pathways following ARs activation are primarily linked to cyclic adenosine monophosphate (cAMP) modulation. In particular, A_1 and A_3 ARs are negatively coupled with adenylyl cyclase and exert an inhibitory effect on cAMP production, while A_{2A} and A_{2B} ARs stimulate adenylyl cyclase activity inducing an increase in cAMP levels [1]. Several papers present in the literature have suggested that ARs are implicated in numerous pathological functions, and fundamental in severe human diseases such as neurodegenerative and cardiovascular disorders, inflammatory diseases, and cancer [2, 3]. Moreover, extracellular adenosine concentration is significantly increased under conditions of hypoxia, ischemia or high metabolism that typically occur in pathological or stressful situations thereby enabling to enhance activation of ARs under these pathological conditions [3, 4]. This intriguing scenario has suggested to researchers the development of molecules able to bind ARs as novel potential drugs [5-8].

Several ligands have been developed up to now having a wide spectrum of activity such as agonists/partial agonists, allosteric enhancers, antagonists and/or inverse agonists [8]. However, the complexity of AR signaling, due to the widespread distribution of the receptors in the body and the redundancy of their effects, is responsible for the limited number of AR drugs commercially available. Nowadays, an A_{2A} AR agonist (Regadenoson from CV Therapeutics & Astellas) is approved for computed tomography myocardial and coronary artery perfusion imaging

[9] and an A_{2A} AR antagonist (Istradefylline from Kyowa Hakko Kirin Co) in Japan is used for the treatment of Parkinson's disease [10].

As a part of our efforts to identify novel human (h) AR antagonists [11-17], we recently reported on the development of compounds bearing the thiazolo[5,4-*d*]pyrimidine nucleus [18,19]. In detail, taking the well known hA_{2A} AR selective inverse agonist **1** (ZM 241385) [20] as lead compound, a small library of 2-(2-furanyl)-thiazolo[5,4-*d*]pyrimidine-5,7-diamine derivatives was synthesized and tested, leading to the identification of two compounds, **2** and **3,** which resulted highly selective hA_{2A} AR inverse agonists more potent than **1** (Figure 1) [19].

(Figure 1)

Notably, in [^{3}H]-**1** ([^{3}H]-ZM 241385) competition binding experiments, compounds **2** and **3** were characterized by two different affinity values (KH and KL) for hA_{2A} AR with high affinity (KH) value in the femtomolar range and low affinity (KL) value of nanomolar order. Furthermore, thiazolopyrimidines **2** and **3** were also tested in *in vivo* murine models of acute pain, showing an antinociceptive activity equal to or greater than morphine [19].

Derivatives **2** and **3**, although showing nanomolar affinities for hA_1 and hA_3 ARs and good potencies for the hA_{2B} subtype, due to their hA_{2A} femtomolar affinities, can be considered the most potent and selective hA_{2A} AR inverse agonists reported so far. These results encouraged us to further investigate the structure-activity relationships within this series. Hence, we herein report the design and pharmacological characterization of novel 2-(2-furanyl)-thiazolo[5,4-*d*]pyrimidine-5,7-diamine derivatives (**4-13**) substituted at the N^5 nitrogen atom with a heteroarylalkyl chain (Figure 1). Modifications at this level were performed by varying the nature and position of the heteroatom and the length of the alkyl chain to shed more light on the steric and electronic requirements of the hA_{2A} binding site.

Next, we focused our attention toward replacement of the 2-(2-furanyl) moiety in order to probe the interactions between the substituent at this position and the receptor cleft. Thus, we synthesized compounds **14-19** in which the 2-(2-furanyl) ring of **2**, **3** and **4** was replaced by a 2-(5-methyl-2-furanyl) moiety (**14-16**) or by its isostere 2-(2-thienyl) group (**17-19**) (Figure 1).

2. Chemistry

N^5-(Hetero)arylalkyl-thiazolo[5,4-*d*]pyrimidine-5,7-diamine derivatives **4-19** were obtained by reacting the 7-amino-5-chloro-thiazolo[5,4-*d*]pyrimidine derivatives **20 [**19], **21-22** with the (hetero)arylalkylamines of interest, under microwave irradiation at 200 °C (Scheme 1). All the employed amines were commercial except for the 2-(2-furanyl)ethylamine which was synthesized starting from the furan-2-carboxyaldheyde according to literature data [21].

(Scheme 1)

The 7-amino-5-chloro derivative **20** was synthesized as previously reported [19]. Similarly, compounds **21-22** were obtained starting from the 5-amino-6-sulfanylpyridine-2,4-diol **23** [22] which reacted with acyl chlorides of interest in NMP at 160 °C to afford the bicyclic thiazolo[5,4-*d*]pyrimidine-5,7-diol derivatives **24-25** (Scheme 2). Reaction of these latter with $POCl_3$ afforded the corresponding 5,7-dichloro substituted compounds **26-27** which were treated with a 33% aqueous solution of ammonia to yield the desired 7-amino-5-chloro derivatives **21-22**.

(Scheme 2)

3. Results and discussion

Derivatives **4-19** were pharmacologically evaluated at hA_1, hA_{2A}, hA_{2B}, and hA_3 ARs stably expressed in Chinese hamster ovary (CHO) cells by using competition binding and cyclic AMP assays.

The affinity for hA_1, hA_{2A}, and hA_3 AR (expressed as K_i values) and the potency for the hA_{2B} AR (expressed as IC_{50} values) of the novel compounds **4-19** are displayed in Table 1. The affinity and potency data of the previously reported thiazolopyrimidines **2** and **3** and of the triazolotriazine **1**, as reference compounds are also reported (Table 1).

(Table 1)

Thiazolo[5,4-*d*]pyrimidines **4-13** bearing a (2-furanyl) moiety at position 2 of the bicyclic core have been found to be very potent ligands at the hA_{2A} AR. Most notably, compounds **4**, **5**, **6**, and **11** showed, like references **2** and **3**, two affinity values for the A_{2A} subtype: the highest (KH) in the femtomolar range, the lowest (KL) of nanomolar order. Also the other novel 2-(2-furanyl)-thiazolopyrimidines (**7-10**, **12-13**) proved to be highly potent at the hA_{2A} subtype, showing binding affinities in the subnanomolar (**7**, **8**, **13**) or low nanomolar range (**9**, **10**, **12**).

Most of the 2-(2-furanyl) substituted compounds **4-13** also possessed nanomolar affinity for the hA_1 and hA_3 subtypes, except compounds **9**, **10**, and **13** which bind the hA_3 AR receptor with a K_i > 100 nM. It is worth noting that compounds **4-13** also interact with the hA_{2B} AR, showing potencies in the nanomolar range, **7** and **13** being the most potent with IC_{50} values of 8.96 nM and 4.21 nM, respectively.

Analyzing the binding results in detail, when R_2 = 2-furanyl and R_5 = CH_2-heteroaryl (compounds **4-10**), the presence of a 2-thienyl (**4**), 2-furanyl (**5**), or 3-pyridyl (**6**) group in R_5 affords a very high KH value for the hA_{2A} AR (KH = 10.7 fM (**4**), 39 fM (**5**), and 217 fM (**6**)) and a KL value of the nanomolar order (KL = 3.82 nM (**4**), 1.73 nM (**5**), and 0.68 nM (**6**)). As stated above,

derivatives **4**, **5**, and **6** also bind the other hARs but, due to their hA_{2A} femtomolar affinities, they can be considered extremely selective ligands for this subtype.

Movement of the heteroatom of the appended R_5 substituent of derivatives **4** and **6** resulted in the loss of the double affinity for the hA_{2A} receptor. In fact, derivative **7**, which bears a (3-thienyl) moiety at R_5, shows one affinity value which however falls in the subnanomolar range (K_i = 0.25 nM). The same applies to the N^5-(2-pyridyl)methyl and the N^5-(4-pyridyl)methyl derivatives **8** and **9** which maintain binding affinities in the sub or nanomolar range (K_i =0.42 nM (**8**), and K_i = 12 nM (**9**)). Also the presence of the 2-pyrazinyl ring in R_5 (compound **10**) is profitable for hA_{2A} AR receptor interaction (K_i = 5.14 nM).

In general, derivatives **7-10** also bind the other hAR subtypes well, exhibiting affinities and potencies in the nanomolar range. In particular, the N^5-(3-thienyl)methyl derivative **7** is the most interesting since its high affinities at all the hARs make it a potent and balanced pan-hAR ligand.

Homologation of the methylene linker of the lateral chain of compounds **4**, **5,** and **6** yielded the corresponding N^5-ethyl derivatives **11-13**. The N^5-(2-thienyl)ethyl derivative **11** maintains the double affinity at the hA_{2A} AR being, like its N^5-(2-thienyl)methyl analogue **4**, an extremely selective hA_{2A} AR ligand with a high affinity value in the femtomolar range (KH=10.6 fM). Instead, the N^5-(2-furanyl)ethyl (**12**) and the N^5-(3-pyridyl)ethyl derivatives (**13**), differently from their corresponding inferior homologous **5** and **6**, show one affinity value for the hA_{2A} AR receptor in the nanomolar and subnanomolar range (K_i =2.15 nM (**12**), and K_i = 0.24 nM (**13**)). Moreover, they too bind all hARs with affinities and potencies ranging from low to high nanomolar range, thus behaving as pan-AR ligands.

When the 2-(2-furanyl) group of compounds **2**, **3**, and **4** was substituted with a 2-(5-methyl-2-furanyl) moiety, a significant drop of hAR affinities was obtained. In fact, compounds **14-16** not

only lose the double affinity for the hA_{2A} subtype, but also exhibit a decreased capability to bind all hARs, with respect to the references **2**, **3** and **4**. These data clearly indicate that the presence of a small group on the 2-(2-furanyl) moiety negatively influences the interaction of thiazolo[5,4-*d*]pyrimidine-5,7-diamine-based derivatives with all the hAR subtypes. Instead, replacement of the 2-(2-furanyl) substituent of **2**, **3**, and **4** with the isostere 2-(2-thienyl) group affords compounds **17-19** which, although losing the double hA_{2A} affinity of the references **2**, **3**, and **4** , maintain a hA_{2A} binding affinity in the low nanomolar range.

Figure 2 shows the competition curves of compounds **4**, **5**, **6**, and **11** which displayed biphasic profiles whereas the other thiazolopyrimidines **7-10**, **12-19** presented monophasic inhibition curves (Table 1).

(Figure 2)

The biphasic profile observed for compounds **4**, **5**, **6**, and **11** and for the references **2** and **3** [19], may be interpreted on the basis of the extended ternary complex model of receptor action. According to this model, a receptor can exist as an equilibrium of different conformational states that include the inactive state (R), partly activated forms (R*) and the activated form coupled to G protein (R*G). Certain compounds may be able to differentiate some of these receptor conformations. However, according to this model, neutral antagonists present the same affinity for the different receptor states/conformations and should not recognize different receptor states [23-24]. Therefore, the discrimination of receptor subpopulations by compounds **4**, **5**, **6**, and **11** prompted us to verify their behavior by evaluating their antagonist/inverse agonist potencies at the hA_{2A} AR in functional *in vitro* assays.

Thus, compounds **4-13, 17, 19** were tested to assess their ability to modulate cAMP production in basal conditions or in the presence of the full agonist **28** (CGS 21680) which showed an EC_{50} of

12±2 nM and Emax of 100% [25]. The data are presented in Table 2 together with those of references **2**, **3** and **1**.

(Table 2)

All the examined compounds were able to inhibit basal cAMP accumulation, thus they all behave as inverse agonists. In particular, compounds **4**, **5**, **6**, and **11** show IC_{50} values from 11 to 1.6 pM (Table 2) with efficacy values (Emax) from 61% to 64% (Figure 3, Table 2). It should be noted that compounds **4**, **5**, **6** and **11** are very potent inverse agonists, comparable to **2** and **3**, with potencies 10^3-fold greater than that of **1** (IC_{50} = 1.24 nM). Moreover, according to their subnanomolar hA_{2A} AR affinity, compounds **7**, **8**, and **13** were found to inhibit basal cAMP accumulation with IC_{50} values from 0.29 to 0.68 nM (Table 2), which are slightly greater than that of **1**.

(Figure 3)

Moreover, all the texted compounds were also able to inhibit cAMP production stimulated by the agonist **28** (10 nM) thus revealing, as expected, an antagonist/inverse agonist profile. In particular, these compounds were able to reduce the cAMP accumulation over the basal level as indicated by Emax data (from 115 to 141 %, Table 2). As above, compounds **4**, **5**, **6**, and **11** blocked the effect of the agonist with potency values (IC_{50} values from 36 to 59 pM, Table 2) comparable to those of **2** and **3** (IC_{50} = 51, and 95 pM, respectively) and greater than that of **1** (IC_{50} = 0.83 nM). Notably, they were able not only to block the agonist effect but also to inhibit the constitutive activity of the receptor. In fact, as shown in Figure 4, compounds **4**, **5**, **6**, and **11** achieve negative levels of cAMP accumulation below the basal cAMP production, which in this experiment is set at zero.

(Figure 4)

Finally, it has to be noted that compounds which show monophasic competition curves (**7-10**, **12**, **13**, **17** , **19** and the reference **1**) displayed in the cAMP assays similar IC_{50} values in the presence and absence of the agonist. In contrast, compounds **4**, **5**, **6** and **11** as well as the references **2** and **3**, which show a biphasic binding profile, resulted to be more potent in the absence of the agonist. Furhermore, the inhibition curve of compounds **4**, **5**, **6** and **11** obtained in cAMP assays showed monophasic pattern in contrast to the biphasic curve found in binding assays. This is most likely due to the characteristics of the binding assay that highlight the specific interaction between the examined compounds and the radioligand for the competition with the binding site. On the other hand, from the cAMP assay it is possible to obtain informations on the effects of the compounds that represent a phase following ligand-receptor interaction.

Additional experiments were carried out evaluating compounds **4-13, 17,** and **19** in hA_1, hA_{2B}, or hA_3 CHO cells to verify their effect on cAMP production in basal conditions. As indicated by the data in Table 3, none of the tested compounds have shown an inverse agonist profile for hA_1, hA_{2B}, and hA_3 ARs.

(Table 3)

In order to ensure the druggability potential of compounds **4**, **5**, **6**, and **11**, their chemical and physical properties were determined *in silico* using pkCSM, an online tool for calculating small molecules pharmacokinetic properties [26]. Data reported in Table 4 show that compounds **4**, **5**, **6**, and **11** satisfied the typical drug-like characteristics (Lipinski "Rule of 5") [27].

(Table 4)

Moreover, compounds **4**, **5**, **6** and **11** possess other important predictors of good oral bioavailability such as a reduced molecular flexibility measured by the number of rotatable bonds, and a low polar surface area [28].

4. Conclusions

In summary, by way of a conventional structure-affinity relationship study we discovered new 2-(2-furanyl)thiazolo[5,4-*d*]pyrimidine-5,7-diamine-based derivatives as hA_{2A} AR inverse agonists characterized by binding affinities in the nanomolar or subnanomolar range. This study has confirmed that 2-(2-furanyl)thiazolo[5,4-*d*]pyrimidine-5,7-diamine-based derivatives represent a unique new class of hA_{2A} AR inverse agonists. Notably, compounds **4**, **5**, **6**, and **11**, as well as the references **2** and **3**, emerge as the most potent and selective hA_{2A} AR inverse agonists reported so far with two affinity values for the A_{2A} subtype the highest (KH) falling in the femtomolar range, and with potency (IC_{50}) values in cyclic AMP assays of picomolar order.

Pharmacological advantages of inverse agonism in different pathologies are well known due to their capability to inhibit the constitutive activity of the receptors. As a consequence, the availability of potent hA_{2A} AR inverse agonists may help to clarify the clinical relevance of these compounds for the treatment of pain [19] and important diseases such as neurological disorders [6, 29], dermal fibrosis [30], retinal dysfunctions [31], and cancer [32].

5. Experimental section

5.1. Chemistry

Microwave-assisted synthesis were performed using an Initiator EXP Microwave Biotage instrument (frequency of irradiation: 2.45 GHz). Reactions were routinely monitored by thin-layer chromatography (TLC) on silica gel (0.20 mm, F254, Merck, Germany, aluminum sheets). Silica gel 60 (70-230 mesh, Merck, Germany) was used for column chromatography. Melting points were determined using a Gallenkamp melting point instrument and are uncorrected. Compounds

were named following IUPAC rules as applied by ACD/ChemSketch. Elemental analyses were performed with a Flash E1112 Thermofinnigan elemental analyzer for C, H, N and the results are within ±0.4% of the theoretical values. ^{1}H NMR and ^{13}C NMR experiments were performed with a Bruker Avance 400 instrument (400 MHz for ^{1}H and 100 MHz for ^{13}C NMR). Spectra were recorded at 300 K, using DMSO-d_6 as solvent. Peaks positions are given in parts per million using the residual non-deuterated solvent as the internal standard. Data are reported as follows: chemical shift (ppm), integrated intensity, multiplicity (indicated as: s, singlet; br s, broad singlet; exch, exchangeable proton with D_2O; d, doublet; t, triplet; q, quartet; m, multiplet and combination thereof), coupling costants (J) values in Hertz (Hz). Scanned ^{1}H and ^{13}C NMR spectra of some selected compounds (**4**, **5**, **6**, **11**, **15**, **16**, **17**) are reported in the Supplementary data.

*5.1.1. General procedure for the synthesis of the 2-heteroaryl-N^5-substituted-thiazolo[5,4-d]pyrimidine-5,7-diamine derivatives (**4-19**)*

The proper amine (3 mmol) was added to a solution of the 5-chloro-7-amine derivatives **20** [19] or **21-22** (1 mmol) in n-BuOH (2 ml). The reaction mixture was microwave irradiated at 200 °C for 20 minutes, then cooled at rt and basified with aqueous KOH solution (50%). Addition of water afforded a solid which was collected by filtration and washed with Et_2O. The crude material was purified by crystallization or by chromatography.

5.1.1.1. 2-(Furan-2-yl)-N^5-(thiophen-2-yl-methyl)[1,3]thiazolo[5,4-d]pyrimidine-5,7-diamine **4**. Yield 78%. Mp 192-196 °C (isopropanol). ^{1}H NMR: δ 4.64 (d, 2H, J = 6.2), 6.71-6.73 (m, 1H), 6.93-6.95 (m, 1H), 6.99-7.00 (m, 1H), 7.06-7.07 (m, 1H), 7.23 (br s, exch 2H), 7.32-7.33 (m, 1H), 7.40 (t, exch 1H, J = 6.2), 7.90 (s, 1H). Anal. calcd. for ($C_{14}H_{11}N_5OS_2$): C, 51.05%; H, 3.37%; N, 21.26%. Anal. found: C, 50.90%; H, 3.44%; N, 21.02%.

5.1.1.2. 2-(Furan-2-yl)-N^5-(furan-2-yl-methyl)[1,3]thiazolo[5,4-d]pyrimidine-5,7-diamine **5**.

The crude product was purified by column chromatography (eluting system: cyclohexane/ethyl acetate 3/7), and then by crystallization. Yield 43%. Mp 220-224 °C (ethanol). ^{1}H NMR: δ 4.47 (d, 2H, J = 6.0), 6.24-6.25 (m, 1H), 6.35-6.37 (m, 1H), 6.71-6.72 (m, 1H), 7.05-7.07 (m, 1H), 7.23-7.27 (br m, exch 3H), 7.55 (s, 1H), 7.90 (s, 1H). Anal. calcd. for ($C_{14}H_{11}N_5O_2S$): C, 53.66%; H, 3.54%; N, 22.35%. Anal. found: C, 53.71%; H, 3.68%; N, 22.43%.

5.1.1.3. 2-(Furan-2-yl)-N^5-(pyridin-3-yl-methyl)[1,3]thiazolo[5,4-d]pyrimidine-5,7-diamine **6**.

Yield 81%. Mp 212-215 °C (ethanol) ^{1}H NMR: δ 4.60 (d, 2H, J = 6.1), 6.71-6.72 (m, 1H), 7.04-7.05 (m, 1H), 7.22-7.25 (m, exch 2H + 1H), 7.31 (d, 1H, J = 7.9), 7.37 (br s, exch 1H), 7.73 (t, 1H, J = 7.6), 7.89 (s, 1H), 8.50-8.51 (m, 1H). Anal. calcd. for ($C_{15}H_{12}N_6OS$): C, 55.54%; H, 3.73%; N, 25.91%. Anal. found: C, 55.73%; H, 3.94%; N, 26.07%.

5.1.1.4. 2-(Furan-2-yl)-N^5-(thiophen-3-yl-methyl)[1,3]thiazolo[5,4-d]pyrimidine-5,7-diamine **7**.

The crude product was purified by column chromatography (eluting system: ethyl acetate, cyclohexane/methanol 5/5/2) and then by crystallization. Yield 53%. Mp 209-212°C (ethyl acetate). ^{1}H NMR: δ 4.47 (d, 2H, J= 6.1), 6.72 (dd, 1H, J = 3.3, 1.7), 7.05-7.06 (m, 1H), 7.10 (br s, exch 1H), 7.22 (br s, exch 2H), 7.29-7.32 (m, 2H), 7.45 (dd, 1H, J = 4.8, 3.0), 7.89-7.90 (m, 1H). Anal. calcd. for ($C_{14}H_{11}N_5OS_2$): C, 51.05%; H, 3.37%; N, 21.26%. Anal. found: C, 51.30%; H, 3.45%; N, 21.59%.

5.1.1.5. 2-(Furan-2-yl)-N^5-(pyridin-2-yl-methyl)[1,3]thiazolo[5,4-d]pyrimidine-5,7-diamine **8**.

Yield 74%. Mp 213-217 °C (ethanol) ^{1}H NMR: δ 4.62 (d, 2H, J=5.9), 6.71-6.74 (m, 1H), 7.05-7.09 (m, 1H), 7.25-7.40 (m, exch 3H + 2H), 7.77-7.81 (m, 1H), 7.87-7.90 (m, 1H), 8.50-8.52 (m,

1H). Anal. calcd. for ($C_{15}H_{12}N_6OS$): C, 55.54%; H, 3.73%; N, 25.91%. Anal. found: C, 55.61%; H, 3.65%; N, 25.82%.

5.1.1.6. 2-(Furan-2-yl)-N^5-(pyridin-4-yl-methyl)[1,3]thiazolo[5,4-d]pyrimidine-5,7-diamine **9**. The crude product was purified by column chromatography (eluting system: ethyl acetate/methanol 9/2.5). Yield 60%. Mp: 223-225 °C. ^{1}H NMR: δ 4.51 (d, 2H, J= 6.0) 6.71-6.72 (m, 1H), 7.04-7.05 (m, 1H), 7.23 (s, exch 2H), 7.30-7.31 (m, 2H), 7.47 (br s, exch 1H), 7.89-7.90 (m, 1H), 8.47-8.48 (m, 2H). Anal. calcd. for ($C_{15}H_{12}N_6OS$): C, 55.54%; H, 3.73%; N, 25.91%. Anal. found: C, 55.63%; H, 3.86%; N, 25.75%.

5.1.1.7. 2-(Furan-2-yl)-N^5-(pyrazin-2-yl-methyl)[1,3]thiazolo[5,4-d]pyrimidine-5,7-diamine **10**. Yield 65%. Mp: 236-238 °C (acetic acid/ethanol). ^{1}H NMR: δ 4.63 (d, 2H, J= 6.1), 6.72 (dd, 1H, J = 3.4, 1.7), 7.05-7.06 (m, 1H), 7.27 (s, exch 2H), 7.48 (br s, exch 1H), 7.89-7.90 (m, 1H), 8.50-8.51 (m, 1H), 8.57-8.58 (m, 1H), 8.62 (s, 1H). Anal. calcd. for ($C_{14}H_{11}N_7OS$): C, 51.68%; H, 3.41%; N, 30.14%. Anal. found: C, 51.77%; H, 3.50%; N, 30.20%.

5.1.1.8. 2-(Furan-2-yl)-N^5-[2-(thiophen-2-yl)ethyl][1,3]thiazolo[5,4-d]pyrimidine-5,7-diamine **11**.

Yield 78%. Mp 216-218 °C (nitromethane) ^{1}H NMR: δ 3.06 (t, 2H, J = 7.2), 3.51(dd, 2H, J =13.4, 7.0), 6.71-6.72 (m, 1H), 6.92-6.96 (m, 3H), 7.04-7.05 (m, 1H), 7.18 (br s, exch 2H),7.32-7.34 (m, exch 1H), 7.89-7.90 (m, 1H). Anal. calcd. for ($C_{15}H_{13}N_5OS_2$): C, 52.46%; H, 3.82%; N, 20.39%. Anal. found: C, 52.74%; H, 3.93%; N, 20.65%.

5.1.1.9. 2-(Furan-2-yl)-N^5-[2-(furan-2-yl)ethyl][1,3]thiazolo[5,4-d]pyrimidine-5,7-diamine **12**. The crude product was purified by column chromatography (eluting system: ethyl acetate/cyclohexane 1/1). Yield 82%. Mp 198-200 °C. ^{1}H NMR: δ 2.87 (t, 2H, J= 7.0), 3.51-3.53

(m, 2H), 6.17-6.18 (m, 1H), 6.35-6.36 (m, 1H), 6.71-6.72 (m, 1H), 6.90 (br s, exch 1H), 7.04-7.05 (m, 1H), 7.17 (br s, exch 2H), 7.51-7.52 (m, 1H), 7.88-7.89 (m, 1H). Anal. calcd. for ($C_{15}H_{13}N_5O_2S$): C, 55.03%; H, 4.00%; N, 21.39%. Anal. found: C, 55.22%; H, 4.13%; N, 21.42%.

5.1.1.10. 2-(Furan-2-yl)-N^5-[2-(pyridin-3-yl)ethyl][1,3]thiazolo[5,4-d]pyrimidine-5,7-diamine **13**.

The crude product was purified by column chromatography (eluting system: ethyl acetate/cyclohexane/methanol 8/1/1). Yield 50%. Mp 176-178 °C. ^{1}H NMR: δ 2.87 (t, 2H, J = 7.0), 3.50 (dd, 2H, J = 12.9, 6.7), 6.72-6.73 (m, 1H), 6.95 (br s, exch 1H), 7.04-7.05 (m, 1H,), 7.18 (br s, exch 2H), 7.30 (dd, 1H, J = 7.6, 4.7), 7.68 (d, 1H, J = 6.6), 7.89-7.90 (m, 1H), 8.40 (d, 1H, J = 4.7), 8.47 (s, 1H). Anal. calcd. for ($C_{16}H_{14}N_6OS$): C, 56.79%; H, 4.17%; N, 24.84%. Anal. found: C, 56.45%; H, 4.01%; N, 24.99%.

5.1.1.11. N^5-(2-Methoxybenzyl)-2-(5-methylfuran-2-yl)[1,3]thiazolo[5,4-d]pyrimidine-5,7-diamine **14**.

Yield 70%. Mp 215-217 °C (acetic acid/ethyl acetate). ^{1}H NMR: δ 2.37 (s, 3H), 3.82 (s, 3H), 4.47 (d, 2H, J = 6.4), 6.32-6.34 (m, 1H), 6.87 (t, 1H, J = 7.5), 6.93-6.98 (m, 2H), 7.05 (br s, exch 1H), 7.13 (br s, exch 2H), 7.18-7.22 (m, 2H). Anal. calcd. for ($C_{18}H_{17}N_5O_2S$): C, 58.84%; H, 4.66%; N, 19.06%. Anal. found: C, 58.89%; H, 4.44%; N, 19.21%.

5.1.1.12. N^5-(3-Methoxybenzyl)-2-(5-methylfuran-2-yl)[1,3]thiazolo[5,4-d]pyrimidine-5,7-diamine **15**.

Yield 40%. Mp 196-198 °C (ethyl acetate/ ethylene glycol monomethyl ether). ^{1}H NMR: δ 2.37 (s, 3H), 3.72 (s, 3H), 4.46 (d, 2H, J= 5.6), 6.32-6.34 (m, 1H), 6.76-6.78 (m, 1H), 6.88-6.89 (m,

2H), 6.92-6.93 (m, 1H), 7.16 (broad s, exch 2H), 7.19-7.22 (m, 1H), 7.32 (broad s, exch 1H). ^{13}C NMR: δ 13.92, 44.57, 55.39, 109.46, 111.45, 112.23, 113.18, 119.68, 129.64, 142.87, 147.10, 154.57, 157.36, 159.68, 160.31. Anal. calcd. for ($C_{18}H_{17}N_5O_2S$): C, 58.84%; H, 4.66%; N, 19.06%. Anal. found: C, 58.96%; H, 4.78%; N, 18.79%.

5.1.1.13. 2-(5-Methylfuran-2-yl)-N^5-(thiophen-2-yl-methyl)[1,3]thiazolo[5,4-d]pyrimidine-5,7-diamine **16**.

The crude product was purified by column chromatography (eluting system: ethyl acetate/cyclohexane 1/1).Yield 80%. Mp 200-203 °C. ^{1}H NMR: δ 2.38 (s, 3H), 4.63 (d, 2H, J = 6), 6.33-6.34 (m, 1H), 6.94-6.95 (m, 2H), 6.98-6.99 (m, 1H), 7.19 (s, exch 2H), 7.32-7.35 (m, 1H), 7.36 (broad s, exch 1H). ^{13}C NMR: δ 13.91, 109.46, 111.55, 124.90, 125.31, 126.96, 144.45, 145.99, 147.09, 154.62, 157.39, 159.91, 164.78. Anal. calcd. for ($C_{15}H_{13}N_5OS_2$): C, 52.46%; H, 3.82%; N, 20.39%. Anal. found: C, 52.33%; H, 4.10%; N, 20.24%.

5.1.1.14. N^5-(2-Methoxybenzyl)-2-(thiophen-2-yl)[1,3]thiazolo[5,4-d]pyrimidine-5,7-diamine **17**. Yield 72%. Mp 186-188 °C (acetic acid). ^{1}H NMR: δ 3.82 (s, 3H), 4.47-4.48 (d, 2H, J = 6.2), 6.88 (t, 1H, J = 7.4), 6.97 (d, 1H, J=7.9), 7.12-7.22 (m, exch 3H + 3H), 7.57-7.59 (m, 1H), 7.72-7.74 (m, 1H). ^{13}C NMR: δ 55.70, 110.68, 120.50, 127.48, 127.93, 128.00, 128.43, 128.75, 129.33, 137.50, 157.05, 157.25, 160.58. Anal. calcd. for ($C_{17}H_{15}N_5OS_2$): C, 55.26%; H, 4.09%; N, 18.96%. Anal. found: C, 55.48%; H, 4.24%; N, 19.05%.

5.1.1.15. N^5-(3-Methoxybenzyl)-2-(thiophen-2-yl)[1,3]thiazolo[5,4-d]pyrimidine-5,7-diamine **18**. Yield 40%. Mp 148-150 °C (acetic acid). ^{1}H NMR: δ 3.72 (s, 3H), 4.47 (d, 2H, J= 6.3), 6.76-6.78 (m, 1H), 6.89-6.90 (m, 2H), 7.13-7.23 (m, exch 2H + 2H), 7.36 (br s, exch 1H), 7.58-7.59 (m, 1H),

7.73-7.74 (m, 1H). Anal. calcd. for ($C_{17}H_{15}N_5OS_2$): C, 55.26%; H, 4.09%; N, 18.96%. Anal. found: C, 55.38%; H, 4.15%; N, 19.12%.

5.1.1.16. 2-(Thiophen-2-yl)-N^5-(thiophen-2-yl-methyl)[1,3]thiazolo[5,4-d]pyrimidine-5,7-diamine **19**.

Yield 70%. Mp 208-210 °C (acetic acid). ^{1}H NMR: δ 4.64 (d, 2H, J = 5.1), 6.93-6.98 (m, 1H), 7.00-7.01 (m, 1H), 7.17-7.23 (m, 3H, exch 2H + 1H), 7.32-7.34 (m, 1H), 7.45 (br s, exch 1H), 7.61-7.62 (m, 1H) 7.73-7.75 (m, 1H). Anal. calcd. for ($C_{14}H_{11}N_5S_3$): C, 48.64%; H, 3.21%; N, 20.27%. Anal. found: C, 48.57%; H, 3.39%; N, 20.41%.

*5.1.2. General procedure for obtaining 5-chloro-thiazolo[5,4-d]pyrimidin-7-amine derivatives (**21-22**).*

A suspension of the 5,7-dichloro-thiazolo[5,4-*d*]pyrimidine derivatives **26-27** (3 mmol) in a mixture of 33% aqueous ammonia solution (15 mL) and ethanol (15 mL) was refluxed for 6h. The mixture was cooled at rt, and the solid was collected by filtration and recrystallized.

5.1.2.1. 5-Chloro-2-(5-methylfuran-2-yl)[1,3]thiazolo[5,4-d]pyrimidin-7-amine **21**.

Yield 75%. Mp >300 °C (dimethylformamide/water). ^{1}H NMR: δ 2.39 (s, 3H), 6.40-6.41 (m, 1H), 7.17-7.18 (m, 1H), 8.20 (br s, exch 2H). Anal. calcd. for ($C_{10}H_7ClN_4OS$): C, 45.03%; H, 2.65%; N, 21.01%. Anal. found: C, 45.31%; H, 2.99%; N, 21.23%.

5.1.2.2. 5-Chloro-2-(thiophen-2-yl)[1,3]thiazolo[5,4-d]pyrimidin-7-amine **22**.

Yield 70%. Mp >300 °C (2-methoxyethanol). ^{1}H NMR: δ 7.23-7.25 (m, 1H), 7.82-7.83 (m, 1H),7.88-7.89 (m, 1H) 8.13 (br s, exch 1H), 8.28 (br s, exch 1H) Anal. calcd. for ($C_9H_5N_4ClS_2$): C, 40.22%; H, 1.88%; N, 20.85%. Anal. found: C, 40.51%; H, 2.03%; N, 20.97%.

*5.1.3. General procedure for obtaining thiazolo[5,4-d]pyrimidine-5,7-diol derivatives (**24-25**).*

To a suspension of the amino-6-sulfanylpyrimidine-2,4-diol **23 [**22] (8 mmol) in NMP dry, the proper carbonyl choride (8 mmol) was slowly added. The mixture was heated at 150 °C under N_2 atmosphere for 14 h, and then cooled to rt. Addition of water (100 mL) afforded a precipitate, which was collected by filtration and purified by crystallization.

5.1.3.1. 2-(5-Methylfuran-2-yl)[1,3]thiazolo[5,4-d]pyrimidine-5,7-diol **24**.

Yield 90%. Mp > 300 °C (dimethylformamide/water). ^{1}H NMR: δ 2.37 (s, 3H), 6.35-6.37 (m, 1H), 7.06-7.08 (m, 1H), 11.38 (s, exch 1H), 12.06 (br s, exch 1H). Anal. calcd. for ($C_{10}H_7N_4O_3S$): C, 48.19%; H, 2.83%; N, 16.86%. Anal. found: C, 48.33%; H, 2.99%; N, 17.05%.

5.1.3.2. 2-(Thiophen-2-yl)[1,3]thiazolo[5,4-d]pyrimidine-5,7-diol **25**.

Yield 75%. Mp >300 °C (dimethylformamide). ^{1}H NMR: δ 7.17-7.19 (m, 1H), 7.70-7.71 (m, 1H), 7-77-7.78 (m, 1H), 11.37 (s, exch 1H), 12.08 (br s, exch 1H) Anal. calcd. for ($C_9H_5N_4O_2S_2$): C, 43.02%; H, 2.01%; N, 16.72%. Anal. found: C, 42.87%; H, 2.36%; N, 16.88%.

*5.1.4. General procedure for obtaining 5,7-dichloro-thiazolo[5,4-d]pyrimidine derivatives (**26-27**).*

A suspension of the proper 5,7-dihydroxy derivative **24-25** (6 mmol) in $POCl_3$ (20 mL) was microwave irradiated at 170 °C for 40 min. The organic phase was concentrated under vacuum, the material was taken up twice with cyclohexane (20 mL) and the organics evaporated. The residue was added with ice-water (100 g) affording a precipitate, which was collected by filtration and used in the next step without further purification.

5.1.4.1. 5,7-Dichloro-2-(5-methylfuran-2-yl)[1,3]thiazolo[5,4-d]pyrimidine **26**.

Yield 65%. ^{1}H NMR: δ 2.46 (s, 3H), 6.55-6.57 (m, 1H), 7.56-7-57 (m, 1H).

5.1.4.2. 5,7-Dichloro-2-(thiophen-2-yl)[1,3]thiazolo[5,4-d]pyrimidine **27**.

Yield 75%. ^{1}H NMR: δ 7.32-7.33 (m, 1H), 8.07-8.10 (m, 2H).

5.2. *In vitro pharmacology.*

5.2.1. Materials.

[^{3}H]-DPCPX ([^{3}H]1,3-dipropyl-8-cyclopentyl-xanthine; specific activity, 120 Ci/mmol), [^{125}I]-ABMECA ([^{125}I]4-aminobenzyl-5'-N-methyl-carboxamidoadenosine; specific activity, 2200 Ci/mmol), and [^{3}H]cyclic AMP ([^{3}H]cyclic adenosine monophosphate; specific activity, 22 Ci/mmol) were obtained from Perkin Elmer Research Products (Boston, MA); [^{3}H]-**1** ([^{3}H](4-(2-[7-amino-2-(2-furil)[1,2.4] triazolo[2,3-a][1,3,5]triazin-5-ylamino] ethyl) phenol); specific activity, 17 Ci/mmol) was obtained from Biotrend (Cologne, Germany). DPCPX, NECA (N-ethylcarboxamido adenosine), AB-MECA, **28** and **1** were obtained from Sigma Aldrich (St. Louis, MO).

5.2.2. Cell culture and membrane preparation.

Cell culture and membrane preparation from Chinese Hamster Ovary (CHO) cells transfected with hA_1, hA_{2A}, hA_{2B} and hA_3 ARs were previously described [19]. Briefly, the cells were cultured in Dulbecco's modified Eagle's medium F12 until membrane preparation or the utilization in cAMP experiments.

5.2.3. Competition binding experiments.

All synthesized compounds were tested for their affinity to hA_1, hA_{2A} and hA_3 ARs and competition binding experiments were carried out as previously described [19]. In particular, inhibition experiments to A_{2A} ARs were performed incubating the membrane suspension (50 μg of protein/100 μl) with the radioligand [^{3}H]-**1** (1 nM) in the presence of different concentrations of the tested compounds for 60 min at 4°C in 50 mM TrisHCl (pH 7.4), 10 mM $MgCl_2$. Non-specific binding was defined as binding in the presence of **1** (1 μM) and was about 20% of the total binding [33].

5.2.4. Cyclic AMP assays.

In CHO cells transfected with hA_{2B} ARs, cAMP assays were performed in order to evaluate the potency of the novel compounds in the inhibition of cAMP levels stimulated by NECA (100 nM). The potency and efficacy of the examined compounds were also evaluated for hA_{2A}ARs investigating their capability to inhibit cAMP levels in both basal conditions and stimulated by compound **28** at the 10 nM concentration. Additional experiments were carried out evaluating the tested compounds at the 10 μM concentration in hA_1, hA_{2B} or hA_3CHO cells to verify their effect on cAMP production in basal condition. The measurement of cAMP levels was performed by using a competition protein binding assay with [^{3}H]-cAMP as previously described in details [19].

5.2.5. Statistical analysis.

The protein concentration was determined according to a Bio-Rad method with bovine albumin as a standard reference [34]. The data are expressed as the mean ± SEM of n = 4 independent experiments. Statistical analysis of the data was performed using one way ANOVA followed by Dunnett's post hoc test. Inhibitory binding constants, K_i, will be calculated from the IC_{50} values according to the Cheng and Prusoff equation: $K_i = IC_{50}/(1 + [C^*]/K_D^*)$, where [C*] is the

concentration of the radioligand and K_D* its dissociation constant [36]. KH and KL were obtained by fitting binding data to a two sites binding model by using Graph PAD Prism (San Diego, CA, USA). IC_{50} values obtained in cAMP assays were calculated by non-linear regression analysis using the equation for a sigmoid concentration-response curve [35].

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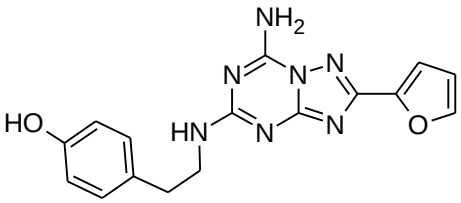
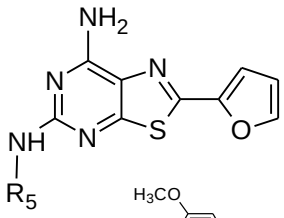
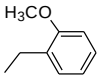
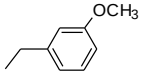
Figure 1. ZM 241385 **1**, previously reported thiazolo[5,4-d]pyrimidine **2** and **3**, and the newly synthesized thiazolo[5,4-*d*]pyrimidine-5,7-diamine-based derivatives **4-19**.

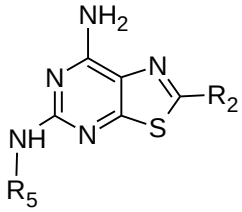


1 ZM 241385



2 R_5 = 

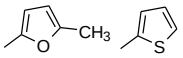
3 R_5 = 



R_5 = (hetero)arylalkyl chain

R_2 = 

4-13

R_2 = 

14-19

Figure 2. Competition curves of specific [^{3}H]-**1** binding to hA_{2A} ARs of compounds **4** (A), **5** (B), **6** (C) and **11** (D) characterized by biphasic curves. Data represent means ± SEM of four experiments each performed in triplicate.

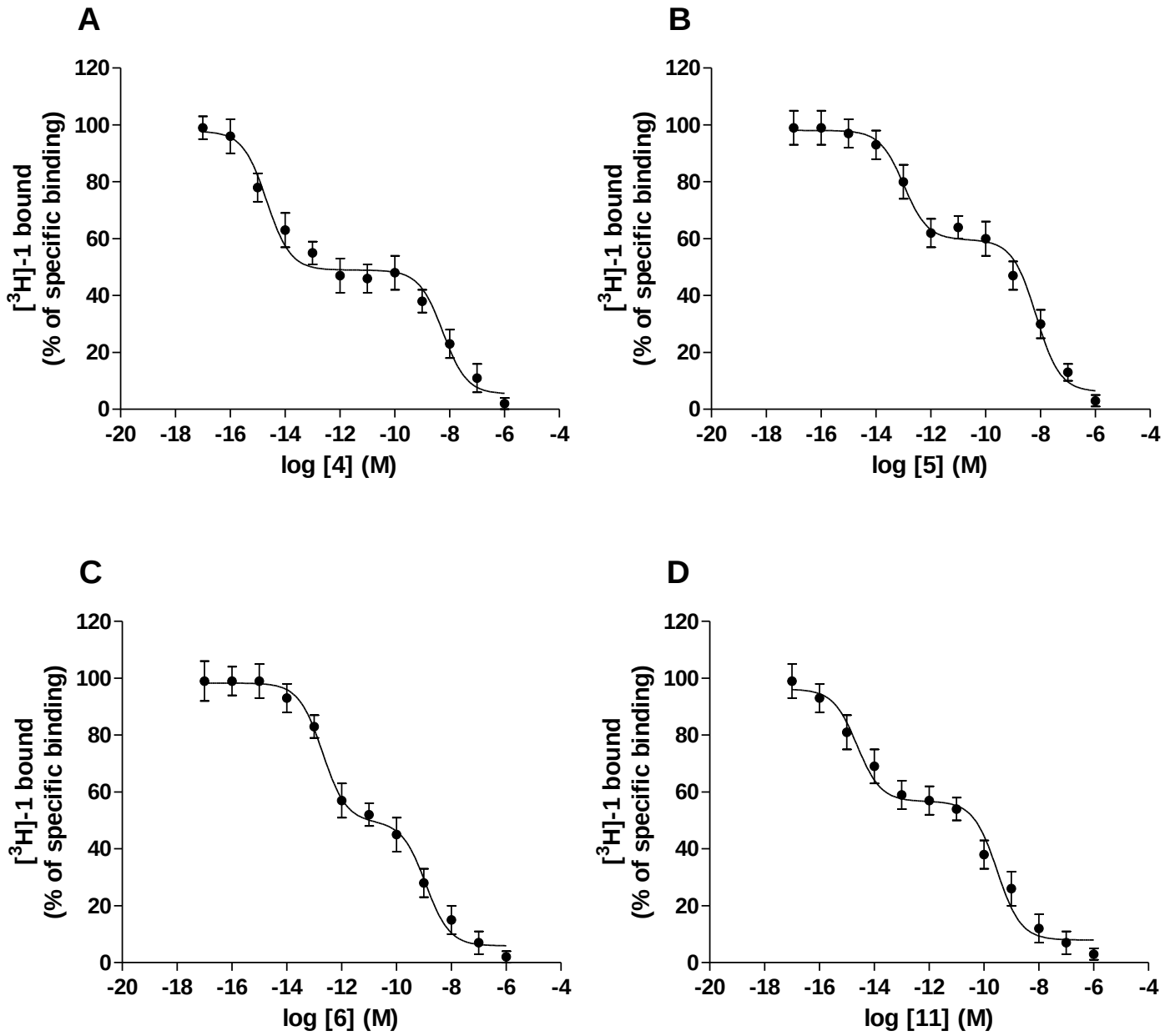


Figure 3. Inhibition of cAMP levels in hA_{2A} CHO cells by compounds **4** (A), **5** (B), **6** (C) and **11** (D). Drug effects are expressed as a percentage of cAMP production in basal conditions. Data represent means ± SEM of four experiments each performed in triplicate.

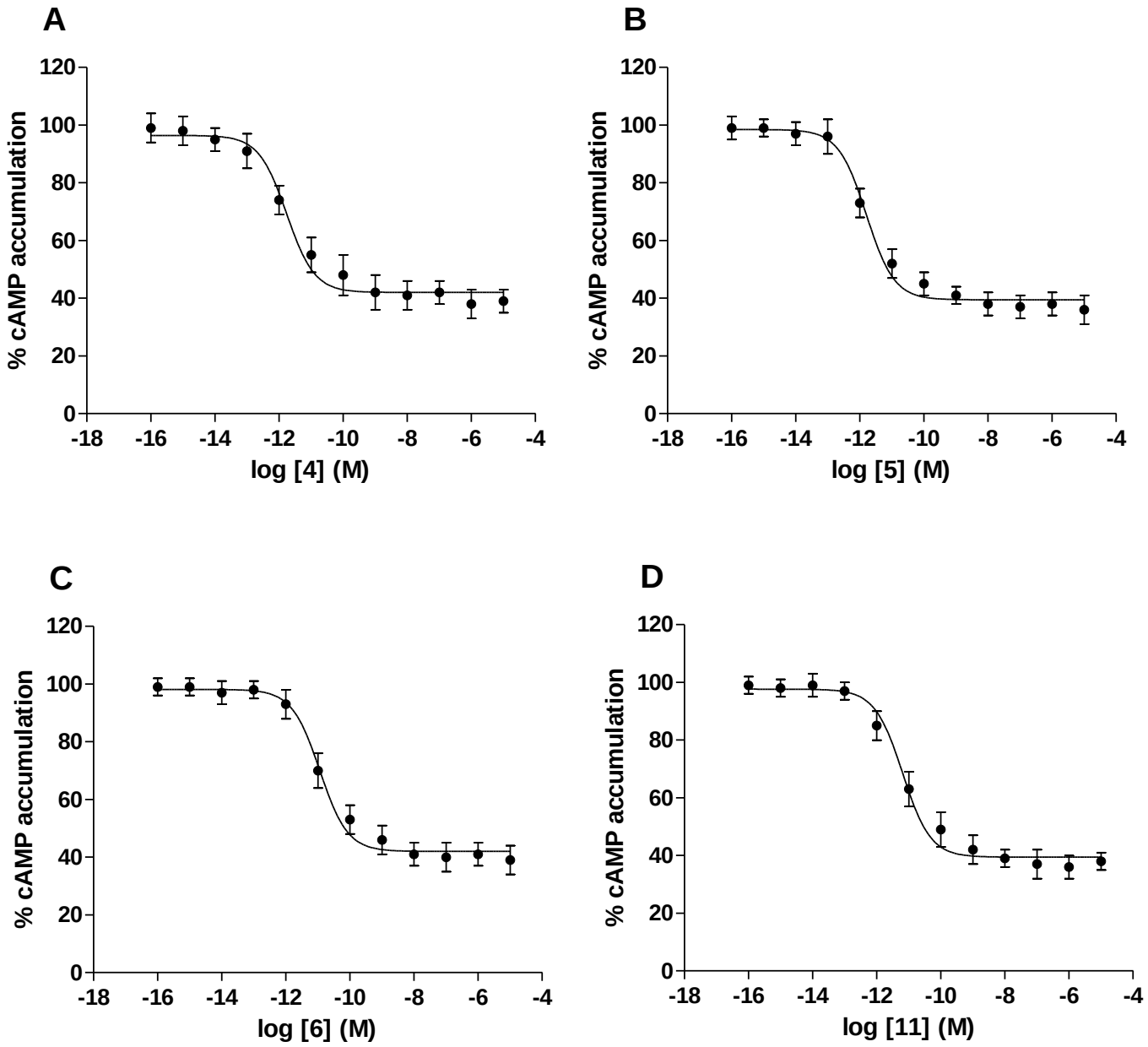
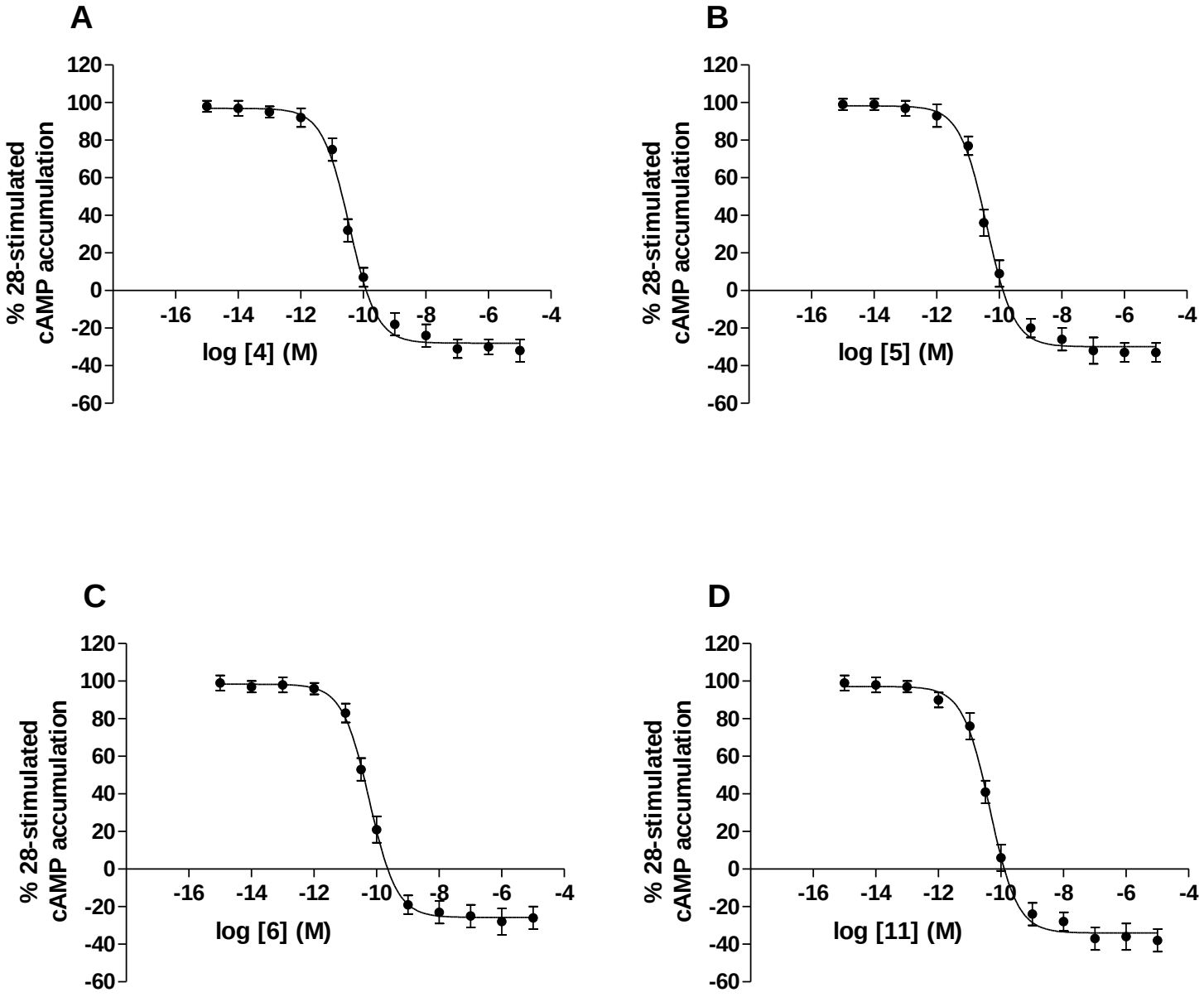
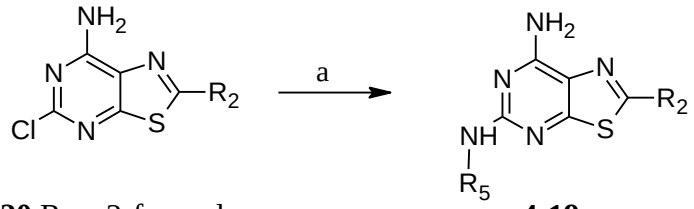


Figure 4. Inhibition of cAMP levels in h A_{2A} CHO cells by compounds **4** (A), **5** (B), **6** (C) and **11** (D). Drug effects are expressed as a percentage of cAMP production in the presence agonist **28** (10 nM). Data represent means ± SEM of four experiments each performed in triplicate.



Scheme 1[a]

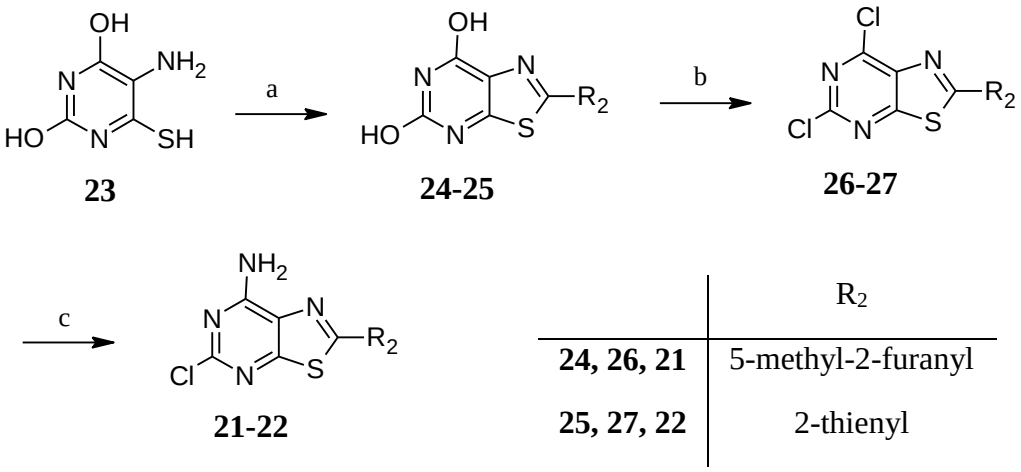


20 R_2 = 2-furanyl
21 R_2 = 5-methyl-2-furanyl
22 R_2 = 2-thienyl

4-19
for R_2 and R_5 see Table1

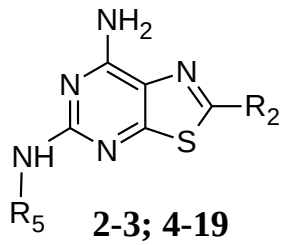
[a] Reagents and conditions: a) R_5NH_2, nBuOH, MW, 200 °C, 20 min, (40-80 %).

Scheme 2[a]

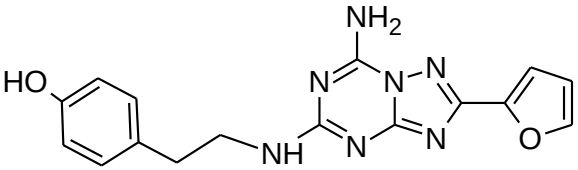


[a] Reagents and conditions: a) R_2COCl, NMP, 160 °C, (70-90 %); b) $POCl_3$, MW, 170 °C, 40 min, (65-75%); c) NH_3/H_2O, 85 °C, (~ 70%).

Table 1. Affinity (K_i or KH, KL) and potency (IC_{50}) of novel compounds in comparison with previously reported **2** and **3** and the reference compound **1** on ARs.



2-3; 4-19



1

compd	R_2	R_5	hA_1AR[a] K_i (nM)	$hA_{2A}AR$[b] K_i (nM) or KH* (fM) and KL** (nM)	hA_3AR[c] K_i (nM)	$hA_{2B}AR$[d] IC_{50} (nM)
4	2-furanyl	CH_2(2-thienyl)	12.5±1.1	10.7±1.0 * 3.82±0.31 **	6.43±0.58	75 ±8
5	2-furanyl	CH_2(2-furanyl)	38±4	39±4 * 1.73±0.15 **	4.72±0.38	82±9
6	2-furanyl	CH_2(3-pyridyl)	7.12±0.65	217±19 * 0.68±0.05 **	18.2±1.7	109±11
7	2-furanyl	CH_2(3-thienyl)	8.12±0.71	0.25±0.02	3.14±0.29	8.96±0.82
8	2-furanyl	CH_2(2-pyridyl)	28±3	0.42±0.04	59±5	95±9
9	2-furanyl	CH_2(4-pyridyl)	47±4	12±1	827±48	33±2
10	2-furanyl	CH_2(2-pyrazinyl)	25±4	5.14±0.48	157±14	13.2±1.2
11	2-furanyl	CH_2CH_2(2-thienyl)	4.92±0.37	10.6±0.9 * 18±2 **	65±6	112±11
12	2-furanyl	CH_2CH_2(2-furanyl)	5.24±0.46	2.15±0.19	23±2	14±1

13	2-furanyl	CH_2CH_2(3-pyridyl)	2.61±0.22	0.24±0.01	174±11	4.21±0.32
14	5-methyl-2-furanyl	CH_2(2-methoxyphenyl)	29±2	20±2	141±12	389±34
15	5-methyl-2furanyl	CH_2(3-methoxyphenyl)	70±6	144±12	160±13	120±11
16	5-methyl-2-furanyl	CH_2(2-thienyl)	71±6	34±3	15.3±1.4	91±9
17	2-thienyl	CH_2(2-methoxyphenyl)	17.3±1.5	2.24±0.21	275±22	4571±328
18	2-thienyl	CH_2(3-methoxyphenyl)	37±4	22±2	430±36	2773±214
19	2-thienyl	CH_2(2-thienyl)	73±6	10.1±1.1	38±4	547±47
2[e]	2-furanyl	CH_2(2-methoxyphenyl)	3.54±0.32	3.55±0.42* 6.45±0.57**	36±3	313±29
3[e]	2-furanyl	CH_2(3-methoxyphenyl)	8.16±0.72	5.31±0.52* 26±2**	92±8	452±42
1			178±13	0.87±0.06	674±57	51±5

Affinity values obtained from displacement of specific [^{3}H]DPCPX [a], [^{3}H]-**1**[b] or [^{125}I]AB-MECA [c] binding to hA_1ARs, $hA_{2A}ARs$ or A_3ARs, respectively (n = 3-6). Percentage of inhibition (I%) is determined at 10 μM concentration of the tested compounds. [d] Potency (IC_{50}) in cAMP assays to $hA_{2B}ARs$. Data are expressed as means ± SEM. [e] Reference 19.

Table 2. Potency (IC_{50}) and Efficacy (Emax) of the tested compounds in comparison with reference compounds **2**, and **3** on cyclic AMP assays in $hA_{2A}CHO$ cells.

compound	IC_{50} (nM)[a]	Emax (%)[b]	IC_{50} (nM)[c]	Emax (%)[d]
4	0.0017±0.0002	61±6	0.036±0.003	132±12
5	0.0016±0.0002	64±6	0.040±0.004	133±13
6	0.011±0.001	61±5	0.059±0.006	126±12
7	0.36±0.04	67±6	0.41±0.03	137±13
8	0.68±0.06	54±5	1.3±0.1	128±11
9	15.3±1.2	43±4	18.7±1.6	122±10
10	8.27±0.72	48±4	11.3±0.96	117±10
11	0.0064±0.0005	62±6	0.045±0.004	138±13
12	2.93±0.22	56±6	4.26±0.37	121±11
13	0.29±0.03	71±7	0.35±0.04	141±14
17	3.36±0.27	62±6	4.41±0.39	129±11
19	13.2±1.2	44±4	17.6±1.5	115±9
2[e]	0.0019±0.0002	63±5	0.051±0.004	138±12
3[e]	0.0083±0.0007	41±3	0.095±0.008	136±11
1	1.24±0.29	43±3	0.83±0.075	121±10

Potency (IC_{50}) [a,c] and Efficacy (Emax) [b,d] of the tested compounds in the absence [a,b] or in the presence [c,d] of **28** (10 nM), respectively. Data are expressed as mean ± SEM. [e] Reference 19.

Table 3. Capability of the tested compounds in comparison with reference compounds **2**, and **3** to modulate cyclic AMP production in hA_1CHO cells, hA_{2B}CHO cells and hA_3CHO cells.

compound	% cAMP		
	hA_1CHO cells	hA_{2B}CHO cells	hA_3CHO cells
4	98±7	99±6	96±6
5	101±9	101±8	104±7
6	96±6	97±6	100±8
7	98±6	99±7	105±7
8	103±8	98±8	101±7
9	103±7	103±9	102±7
10	102±7	102±9	98±8
11	100±9	99±8	94±7
12	95±7	103±6	97±6
13	102±8	97±6	102±7
17	99±7	106±9	103±9
19	97±8	96±8	99±8
2[a]	106±8	91±8	94±8
3[a]	104±8	106±8	99±8
1	101±7	99±8	105±8

The data, expressed as mean ± SEM, indicate the percentage of cAMP modulation over basal level (100%) by the tested compounds at the 10 μM concentration. [a] Reference 19.

Table 4. Physical and chemical properties of compounds **4**, **5**, **6** and **11** using pkCSM [26].

Property	Preferred values	Calculated values			
		4	**5**	**6**	**11**
MW	< 500	329.41	313.342	324.369	343.437
LogP	<5	3.6021	3.13.36	2.9356	3.6446
H donors	≤ 5	2	2	2	2
H acceptors	≤ 10	8	8	8	8
Rotable bonds	≤ 10	4	4	4	5
Surface area	< 140	133.216	128.37	134.795	139.581