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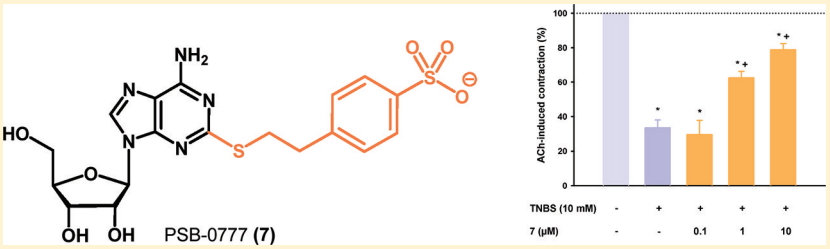
Development of Polar Adenosine A_{2A} Receptor Agonists for Inflammatory Bowel Disease: Synergism with A_{2B} Antagonists

Ali El-Tayeb,[†,§] Sebastian Michael,[‡] Aliaa Abdelrahman,[†] Andrea Behrenswerth,[†] Sabrina Gollos,[†] Karen Nieber,[‡] and Christa E. Müller*[,†]

[†]PharmaCenter Bonn, Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, An der Immenburg 4, D-53121 Bonn, Germany

[‡]University of Leipzig, Institute of Pharmacy, Talstrasse 33, D-04109 Leipzig, Germany

Supporting Information



ABSTRACT: Adenosine A_{2A} receptor agonists for the local treatment of inflammatory bowel disease (IBS) were designed and synthesized. Polar groups were introduced to prevent peroral absorption and subsequent systemic, e.g., hypotensive, side effects. 4-(2-{6-Amino-9-[(2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl]-9*H*-purin-2-ylthio}ethyl)-benzenesulfonic acid (**7**, PSB-0777) was selected for further evaluation in rat ileum/jejunum preparations in ex vivo experiments. Compound **7** significantly improved impaired acetylcholine-induced contractions induced by 2,4,6-trinitrobenzenesulfonic acid and showed synergism with an A_{2B}-selective antagonist. Thus, nonabsorbable, locally active A_{2A} agonists, as a monotherapy or in combination with an A_{2B} antagonist, may be an efficient novel treatment for IBS, preventing the severe systemic side effects of known A_{2A} agonists.

KEYWORDS: *A_{2A} receptor agonist, inflammatory bowel disease, anti-inflammatory drug, nonabsorbable A_{2A} receptor agonist, A_{2B} receptor antagonist*

Adenosine receptors (AR) are G protein-coupled receptors. Four subtypes have been cloned and characterized, A_1, A_{2A}, A_{2B}, and A_3.[1] In brain, A_{2A}AR are expressed in high density in the striatum, while in the periphery, A_{2A}AR are highly expressed in the intestinal mucosa, enteric neurons, hepatocytes, and a variety of immune cells. In the intestine, A_{2A}AR are expressed in the jejunum, ileum, and cecum. They are coupled to G_s proteins and thereby activate adenylate cyclase.[1,2] Crystal structures of the human A_{2A} receptor in complex with agonists, including adenosine (**1**) and NECA (**2**),[3,4] and also with an antagonist[5] have recently been published. A_{2A}-selective AR agonists [for example, **3** and **4** (Figure 1)] typically possess a large substituent at position 2 of the adenosine core.

Activation of the A_{2A}AR on a variety of inflammatory cell types leads to anti-inflammatory effects that can attenuate injury as a result of mucosal inflammation, ischemia, or sepsis. Adenosine, being part of the innate immune system, is one of the strongest endogenous immunosuppressive agents. Therefore, A_{2A}AR agonists have been suggested as novel anti-inflammatory drugs.[2,6,7] Inflammatory bowel disease (IBD) is an inflammatory condition in the gastrointestinal tract. A_{2A}AR agonists have had beneficial effects on the development of intestinal inflammation in a variety of animal models of IBD,

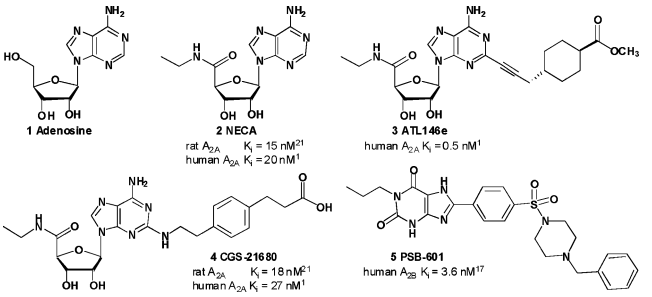


Figure 1. Structures of selected A_{2A} adenosine receptor agonists and a selective A_{2B} antagonist.

including Crohn's disease, colitis, and irritable bowel disease, as well as other inflammatory conditions.[8−11]

However, the systemic use of A_{2A} adenosine receptor agonists as anti-inflammatory drugs is limited by their potent hypotensive activity caused by the activation of A_{2A}AR expressed in heart and blood vessels.[1,7]

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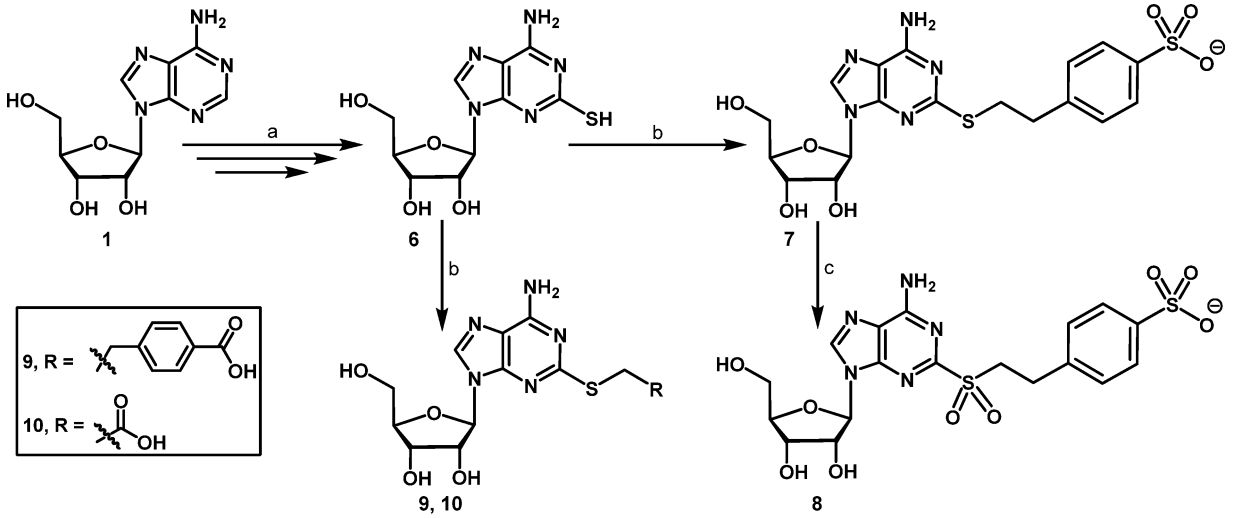
Scheme 1. Synthesis of the Target Adenosine Derivatives[a]



[a]Reagents and conditions: (a) three steps, (1) H_2O_2, CH_3COOH; (2) 5 N aq. NaOH; (3) CS_2, MeOH, H_2O, 120 °C autoclave, 5 h; (b) 4-(2-bromoethyl)benzenesulfonic acid for compound 7, 4-(2-bromoethyl)benzoic acid for compound **9**, and bromoacetic acid for compound **10**, H_2O/NaOH, rt, 4−9 h; (c) *m*-chloroperbenzoic acid, ethanol, 0−5 °C → room temperature, 12 h.

A recent strategy for achieving a certain drug targeting effect has been the preparation of phosphorylated adenosine-derived A_{2A}AR agonists (AMP derivatives), which would be preferably dephosphorylated in inflamed tissues with a high level of expression of ecto-5′-nucleotidase.[12] This is thought to allow a separation of anti-inflammatory and hypotensive effects because the concentration of the active drug, the A_{2A}AR agonist, would be achieved only locally at the sites of inflammation with high ecto-5′-nucleotidase activity.[12] An additional approach to preventing side effects has been local inhalation therapy for the treatment of chronic obstructive pulmonary disease.[13]

In this study, we pursued yet another approach, the development of highly polar, perorally nonabsorbable A_{2A}-selective agonists. Such compounds would serve as a local therapy, e.g., of inflammatory bowel disease, avoiding the hypotensive effects of centrally acting anti-inflammatory A_{2A} agonists. Besides, such polar, highly water-soluble A_{2A}AR agonists would be suitable for parenteral application (e.g., inhalation and injection), as well. Therefore, we introduced an acidic function such as a sulfonate or carboxylate moiety into 2-(ar)alkylthio-substituted adenosine derivatives and evaluated their A_{2A} agonistic activity as well as the ability of a selected compound to reduce the inflammation in an inflamed rat ileum/jejunum preparation. Because of the low pK_a value of free sulfonic acid groups ($pK_a < 1$), the A_{2A}AR agonists bearing a sulfonate function will be deprotonated under physiological conditions and expected to be nonabsorbable;[14] therefore, they would constitute a local therapy for IBD. In contrast to A_{2A} receptors, A_{2B}AR have been shown to exhibit proinflammatory effects in several organs, including lung and the intestine.[1,2,6,7,15] A_{2B}AR are predominantly expressed in colonic epithelial cells. They are upregulated in models of intestinal inflammatory disease,[15,16] and A_{2B}AR antagonists have been suggested as anti-inflammatory drugs.[2,7,15] In this study, we therefore wanted to investigate the possibility of synergism with regard to the anti-inflammatory effects of a highly polar, not perorally absorbable A_{2A}-selective agonist in combination with A_{2B}AR antagonist PSB-601 [**5** (Figure 1); K_i(hA_{2B}AR) = 3.6 nM][17] and to determine their ability to reduce inflammation in ex vivo experiments in an inflamed rat ileum/jejunum preparation.

To obtain our target compounds, we started from 2-thioadenosine (**6**), which was synthesized from adenosine (**1**) according to published procedures (Scheme 1; for details, see the Supporting Information).[12,18] 2-Alkylated derivatives **7**, **9**, and **10** were obtained by reaction of **6** with the corresponding (ar)alkyl bromide in water in the presence of NaOH as a base (Scheme 1).[12,19] Sulfone derivative **8** was prepared by oxidation of compound **7** using *m*-chloroperoxybenzoic acid in ethanol.[20]

The synthesized 2-substituted (ar)alkylthioadenosine derivatives were investigated in radioligand binding studies of human and rat A_{2A}AR using the agonist radioligand [^{3}H]CGS21680.[21] Selectivity versus other adenosine receptor subtypes was assessed by performing radioligand binding studies with human and rat A_1AR using [^{3}H]CCPA,[22] human A_{2B}AR using [^{3}H]PSB-603,[16] and human A_3AR using [^{3}H]PSB-11[23] and/or [^{3}H]NECA[24] as radioligands (see Table 1).

Analysis of the structure−activity relationships of the synthesized 2-substituted adenosine derivatives showed that introduction of a carboxymethyl moiety directly linked to 2-thioadenosine in compound **10** led to a compound with only low affinity for A_{2A}AR. The introduction of an acidic moiety into the para position of the phenyl ring of the previously described 2-(phenylethylthio)adenosine [K_i(rat A_{2A}) = 18.7 nM][12,25] decreased A_{2A} affinity by only 2-fold in the case of introduction of a sulfonate moiety [compound **7**; K_i(rat A_{2A}) = 44.4 nM] and by 8-fold in case of introduction of a carboxylate function (compound **9**; K_i = 152 nM). The selectivity of both compounds versus A_1AR was markedly increased by the acidic function [K_i(rat A_1) values of ≥10000 nM for **7** and >1000 nM for **9**] in comparison to that of 2-(phenylethylthio)adenosine [K_i(rat A_1) = 180 nM].[12]

Species differences exist as compound **7** exhibited a lower affinity for human A_{2A} receptors (K_i = 360 nM) compared with that for rat brain striatal membrane A_{2A} receptors (K_i = 44.4 nM). Surprisingly, compound **9**, which was active at rat A_{2A}AR (K_i = 152 nM), lost its affinity for human A_{2A} receptors (Table 1). The results were reversed in the case of A_1 receptors. Compound **7**, which showed no activity with rat brain cortical membrane AR ($K_i \geq 10000$ nM), was well tolerated at human

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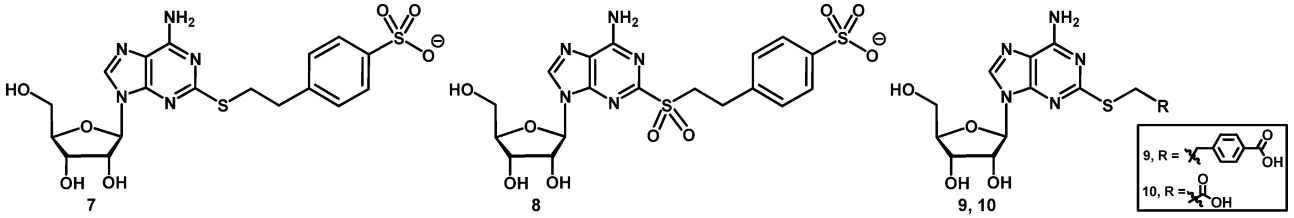
Table 1. Adenosine Receptor Affinities of Adenosine Derivatives Bearing Acidic Functions at the 2-Substituent



	$K_i \pm$ SEM (nM) (n = 3)						
	A_1 receptor [^{3}H]CCPA		A_{2A} receptor [^{3}H]CGS21680		A_{2B} receptor [^{3}H]PSB-603[a]	A_3 receptor [^{3}H]PSB-11	
nucleoside	rat brain cortex	human recombinant	rat brain striatum	human recombinant	human recombinant	human recombinant	pK_a, log P, and log D (pH 7.4)[g]
2 (NECA)	5.1[1]	13.6[22]	15[21]	20[1]	1890[16]	6.2[22]	log P = −2.00 log D = −2.00
4 (CGS21680)	1800[1]	289[1]	18[21]	27[1]	>10000[16]	114[26,b]	pK_a = 4.72 log P = 0.25 log D = −2.15
7 (PSB-0777)	≥10000	541 ± 167	44.4 ± 2.4	360 ± 30	>10000	≫10000, >1000[d]	pK_a = −2.35 log P = 0.31 log D = −2.07
8	1800 ± 300	545 ± 183	>10000	>10000[c]	nd[e]	>10000	pK_a = −2.60 log P = 1.23 log D = −3.60
9	1010 ± 180	404 ± 197	152 ± 10	≥10000[c]	>10000	>10000, >1000[d]	pK_a = 4.23 log P = 0.78 log D = −2.16
10	1930 ± 612	nd[e]	>10000[f]	>10000[c]	nd[e]	>10000	pK_a = 3.30 log P = 1.62 log D = −4.88

[a]Antagonist radioligand because an agonist radioligand for A_{2B}AR does not exist. [b][^{125}I]I-AB-MECA used as a radioligand. [c]n = 2. [d]Versus agonist radioligand [^{3}H]NECA. [e]Not determined. [f]Versus antagonist radioligand [^{3}H]MSX-2. [g]Calculated by the MarvinSketch program from ChemAxon, online version; log P was calculated for the nonionic species of the compounds.

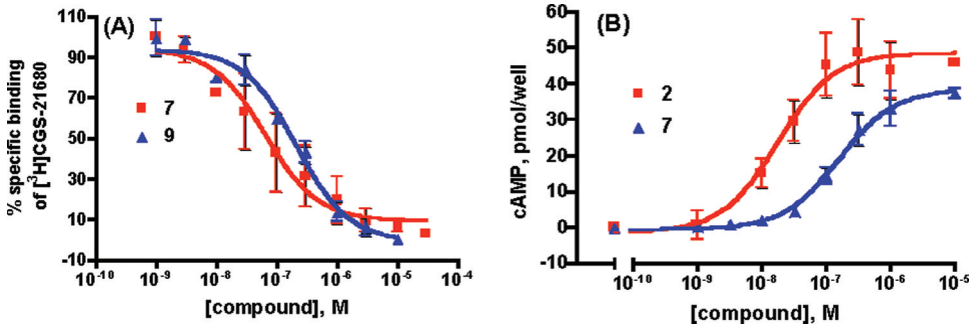


Figure 2. (A) Competition curves of compounds **7** and **9** vs 10 nM [^{3}H]CGS-21680 in rat brain striatal membranes. K_i values of 44.4 ± 2.4 nM (**7**) and 152 ± 10 nM (**9**) were determined. (B) Concentration−response curves of **2** and **7** in cAMP accumulation assays using CHO-K1 cells expressing the human A_{2A}AR (n = 3). EC_{50} values of 17.6 ± 14 nM (**2**) and 117 ± 10 nM (**7**) were determined. All data represent means ± SEM of three separate experiments performed in triplicate.

A_1 receptors with a K_i value of 541 nM. Likewise, the affinity of compound **9** for human A_1 receptors was enhanced (K_i = 404 nM) compared with that for rat brain A_1AR (K_i = 1010 nM). Oxidation of the sulfur atom in the thioether linkage of compound **7** to form the corresponding sulfone derivative **8** led to a loss of affinity for the A_{2A}AR, while the affinity for human A_1AR was retained (K_i = 545 nM). All tested compounds showed no or negligible affinity for human A_{2B} and A_3AR.

As shown in Table 1, the best results were obtained with compound **7** (PSB-0777), which showed high affinity for the A_{2A}AR with a K_i value of 44.4 nM and high selectivity (>225-fold) versus the other AR subtypes. It was superior to the corresponding carboxylate **9**. Selected concentration−inhibition curves are shown in Figure 2A. Compound **7** showed affinity for both human and rat A_{2A}AR. Therefore, **7** was selected as a pharmacological tool for further evaluation to perform proof-of-principle studies in a model of inflammation. Functional properties of the new ligand **7** were assessed by adenylate cyclase assays measuring accumulation of cAMP in CHO cells stably expressing the human A_{2A}AR. For comparison, the full

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agonist NECA (**2**) was investigated under the same condition. Concentration−response curves of **2** and **7** were obtained showing that **7** acted as an agonist at A_{2A}AR. For NECA, an EC_{50} value of 17.6 nM was determined, while **7** showed an EC_{50} value of 117 nM (Figure 2B). Thus, **7** exhibited an ∼6-fold lower potency for human A_{2A}AR expressed in CHO cells compared with that of NECA. The efficacy of **7** was not significantly different from that of NECA, indicating that **7** is a full agonist of A_{2A}AR.

Compound **7** (PSB-0777) was further evaluated in untreated and inflamed rat ileum/jejunum preparations in ex vivo experiments.[15,27,28] Acetylcholine (Ach, 1 mM)-induced contractions were assessed in the absence of A_{2A}AR agonist **7** (set at 100%) and in its presence. Agonist **7** increased concentration-dependently Ach contractions (see the Supporting Information). A statistically significant increase of 17.5 ± 5.7% compared to the control ($P < 0.05$; $n = 12$) was found at a concentration of **7** of 10 μM. The increase was prevented by the A_{2A} antagonist 1,3,7-trimethyl-8-(3-chlorostyryl)xanthine (CSC, 0.2 μM; 89.6 ± 5.2% of control; $P > 0.05$; $n = 12$). Thereafter, **7** (0.1−10 μM) was preincubated simultaneously with 2,4,6-trinitrobenzenesulfonic acid (TNBS, 10 mM), which induced acute inflammation. The A_{2A} agonist restored concentration-dependently the TNBS-induced inhibition (41.6 ± 3.7%) of the Ach contractions; the effects were significant at concentrations of 1 and 10 μM [62.7 ± 3.8 and 78.9 ± 3.5% of control, respectively; $n = 9$ (Figure 3A)].

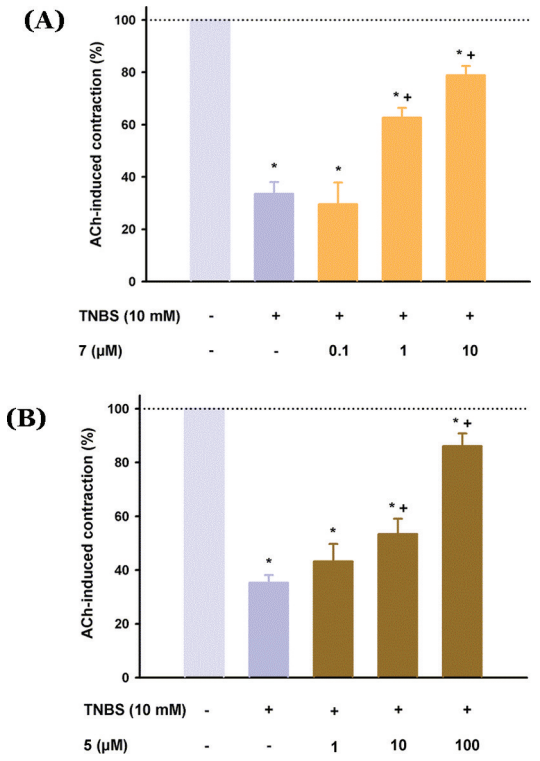

Figure 3. Effects of A_{2A}AR agonist **7** and A_{2B}AR antagonist **5** on the TNBS-induced reduction of the 1 mM Ach-induced contractions in rat ileum/jejunum segments. (A) Concentration-dependent effect of **7** (0.1−10 μM) on the TNBS-induced attenuation of the 1 mM Ach-induced contractions. (B) Concentration-dependent effect of **5** (1.0−100 μM) on the TNBS-induced attenuation of the 1 mM Ach-induced contractions. Means ± SEM of nine or seven experiments. *$P < 0.05$ vs control; $^{+}P < 0.05$ vs TNBS-reduced Ach contraction.

Comparable experiments were performed with A_{2B} antagonist **5** (Figure 3B). Compound **5** (1−100 μM) was without effect on the Ach contractions in untreated preparations. However, it reversed concentration-dependently the TNBS-induced reduction (35.2 ± 2.9%) of the Ach-induced contractions to 53.3 ± 5.7% (10 μM) and 86.1 ± 4.7% (100 μM) of the control, and this effect was statistically significant. The combination of **7** (0.1 μM) and **5** (1 μM) each at a concentration without a significant effect alone was tested in further experiments. It significantly reduced the TNBS-induced impairment of Ach contractions (43.6 ± 8.3%) to 65.7 ± 3.8% of the control (Figure 4; $P < 0.05$; $n = 9$).

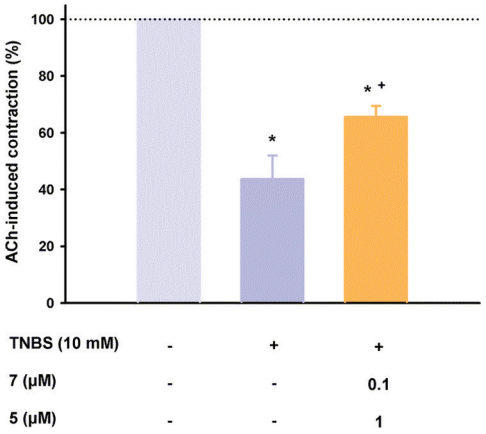

Figure 4. Effect of the combined preincubation of **7** (0.01 μM) and **5** (1.0 μM) on the TNBS-induced decrease in the 1 mM Ach-induced contractions in rat ileum/jejunum segments. Means ± SEM of 12 experiments. *$P < 0.05$ vs control.

In conclusion, we have successfully developed polar A_{2A}AR agonists. They have been proven to be promising drugs for the local treatment of inflammatory intestinal diseases and can be expected to be devoid of hypotensive side effects. Furthermore, additivity and even potential synergism between the A_{2A} agonist and A_{2B} antagonist were observed in an ex vivo model.

EXPERIMENTAL PROCEDURES

For syntheses, the synthesized 2-thioadenosine[12,18] (**6**, 1 mmol) was dissolved in 20 mL of water, and 5 mL of sodium hydroxide (0.5 N) was added to the reaction mixture followed by the addition of 4-(2-bromoethyl)benzenesulfonic acid for compound **7**, 4-(2-bromoethyl)-benzoic acid for compound **9**, or bromoacetic acid for compound **10** (1.2 mmol) 10 min later. The reaction mixture was stirred for 4−9 h at room temperature, and the completion of the reaction was assessed by TLC (2:1:1 *n*-butanol/CH_3COOH/H_2O). The reaction mixture was evaporated to dryness under reduced pressure, and the crude product was crystallized first several times from methanol and then from ethanol to afford after drying the pure products as a white powder.
For the synthesis of sulfone derivative **8**, compound **7** (1 mmol) was dissolved in ethanol (10 mL) and cooled in an ice bath (0−5 °C), to which a solution of *m*-chloroperoxybenzoic acid (4 mmol) was slowly added. The reaction mixture was then stirred for 12 h at room temperature. After the completion of the reaction, the solvent was evaporated under reduced pressure and the residue was recrystallized several times from methanol to afford the pure product, compound **8**.

Purity was confirmed by ESI-LC/MS spectra and found to be >98% for all final products.

4-(2-{6-Amino-9-[(2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)-tetrahydrofuran-2-yl]-9*H*-purin-2-ylthio}ethyl)benzenesulfonic acid (**7**): ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.93−2.95 (m, 2H), 3.20−3.27 (m, 2H), 3.51−3.64 (m, 2H), 3.92−3.94 (q, 1H, $J = 3.67$ Hz),

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4.12−4.13 (q, 1H, J = 2.41 Hz), 4.58−4.60 (t, 1H, J = 5.51 Hz), 5.0 (m, 1H), 5.20 (m, 1H), 5.39 (m, 1H), 5.86−5.88 (d, 1H, J = 5.99 Hz), 7.25−7.54 (m, 6H), 8.22 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 31.87, 35.40, 61.67, 70.58, 73.51, 85.60, 87.29, 117.05, 125.79, 128.0, 138.69, 140.91, 146.67, 150.40, 155.73, 163.62; LC/ESI-MS negative mode 482 ($[M - H]^-$), positive mode 484 ($[M + H]^+$).

Biological evaluation of rat intestinal preparations was performed as described previously[15,27,28] (for details, see the Supporting Information).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthetic procedures, ^{1}H and ^{13}C NMR spectral data, HPLC−MS purity data, and a description of pharmacological experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Pharmazeutisches Institut, An der Immenburg 4, D-53121 Bonn, Germany. Phone: +49-228-73-2301. Fax: +49-228-73-2567. E-mail: christa.mueller@uni-bonn.de.

Notes

§On leave from the University of Al-Azhar, Assiut, Egypt.

■ ABBREVIATIONS

A_{2A}AR, A_{2A} adenosine receptors; A_{2B}AR, A_{2B} adenosine receptors; Ach, acetylcholine; CSC, 1,3,7-trimethyl-8-(3-chlorostyryl)-xanthine; IBD, inflammatory bowel disease; PSB-601, 8-[4-(4-benzylpiperazide-1-sulfonyl)phenyl]-1-propylxanthine; PSB-0777, 4-(2-{6-amino-9-[(2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl]-9*H*-purin-2-ylthio}ethyl)-benzenesulfonic acid; SEM, standard error of the mean; TNBS, 2,4,6-trinitrobenzenesulfonic acid

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