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8-Benzyltetrahydropyrazino[2,1-*f*]purinediones: Water-Soluble Tricyclic Xanthine Derivatives as Multitarget Drugs for Neurodegenerative Diseases

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8-Benzyl-substituted tetrahydropyrazino[2,1-*f*]purinediones were designed as tricyclic xanthine derivatives containing a basic nitrogen atom in the tetrahydropyrazine ring to improve water solubility. A library of 69 derivatives was prepared and evaluated in radioligand binding studies at adenosine receptor (AR) subtypes and for their ability to inhibit monoamine oxidases (MAO). Potent dual-target-directed A_1/A_{2A} adenosine receptor antagonists were identified. Several compounds showed triple-target inhibition; one of the best compounds was 8-(2,4-dichloro-5-fluorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (**72**) (human AR: K_i A_1 217 nM, A_{2A} 233 nM; IC_{50} MAO-B: 508 nM). Dichlorinated compound **36** [8-(3,4-dichlorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione] was found to be the best triple-target drug in rat (K_i A_1 351 nM, A_{2A} 322 nM; IC_{50} MAO-B: 260 nM), and may serve as a useful tool for preclinical proof-of-principle studies. Compounds that act at multiple targets relevant for symptomatic as well as disease-modifying treatment of neurodegenerative diseases are expected to show advantages over single-target therapeutics.

Introduction

Adenosine is a modulator of many physiological and pathophysiological processes exhibiting central nervous system (CNS) depressant, cardiodepressant, antidiuretic, and immunomodulatory effects.[1] The nucleoside exerts its effects through activation of specific G protein-coupled cell membrane receptors termed A_1, A_{2A}, A_{2B}, and A_3 adenosine receptors (ARs).[2] The different AR subtypes show distinct tissue and cell distribution. The dominant subtypes in the central nervous system (CNS) are A_1 and A_{2A}, whereas A_{2B} and A_3ARs typically display low expression levels in the brain. A_1ARs are highly expressed in many regions of the brain including cortex, hippocampus, and caudate–putamen, but also in several peripheral organs and tissues, such as the heart, lung, kidney, and fat cells.[2] Potential therapeutic applications of selective A_1AR antagonists include renal and cardiac failure, and the treatment of cognitive dysfunction, as observed in Alzheimer's disease (AD), due to their CNS stimulatory effects.[3,4] A_{2A}ARs show a restricted expression in the brain with high levels in the basal ganglia, where they are co-expressed and form heteromers with dopamine D_2 receptors.[5–7] Blockade of A_{2A}ARs has shown beneficial effects in animal models and in clinical studies of Parkinson's disease (PD).[8–10] In addition, A_{2A}AR antagonists were found to exhibit neuroprotective effects in preclinical studies and may therefore exert disease-modifying properties in neurodegenerative disorders such as PD and AD.[11–14]

Monoamine oxidases A and B (MAO-A and MAO-B) are flavine adenine dinucleotide (FAD)-dependent mitochondrial enzymes. They catalyze the oxidative deamination of various amine neurotransmitters, such as (nor)epinephrine, dopamine, and serotonin, and of xenobiotic arylalkylamines, including 2-phenylethylamine and tryptamine.[15] Nonselective inhibitors of MAO, for example, tranylcypromine, and selective MAO-A inhibitors, for example, moclobemide, are used for the treatment of depression. Selective MAO-B inhibitors are applied as adjunctive therapeutics for PD in combination with the prodrug levodopa to increase its bioavailability and that of its active metabolite dopamine. Besides the irreversible MAO-B inhibitors selegiline (**1a**) and rasagiline, reversible inhibitors such as lazabemide (**1b**) and safinamide (**1c**, Figure 1) have been devel-

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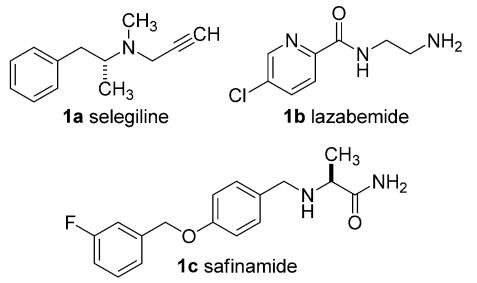


Figure 1. Structures of standard MAO-B inhibitors.

oped for the treatment of PD.[16–20] MAO inhibitors decrease the production of hydrogen peroxide, a product of the MAO reaction, and may therefore protect the brains of PD patients that are treated with levodopa from oxidative stress.[15,20] MAO-B inhibitors have also been proposed as novel, neuroprotective therapeutics for AD.[21]

Therapies that act at multiple targets and provide both symptomatic and neuroprotective effects may be more effective in treating complex neurodegenerative diseases such as AD or PD than drugs interacting with a single target.[22–27] Recently, two dual-target approaches for the treatment of PD have been suggested and both include A_{2A}AR blockade. Dual antagonism of A_1 and A_{2A}ARs has been found to be highly effective in different animal models of PD: A_1/A_{2A}AR antagonists improve motor disabilities and may be neuroprotective by A_{2A}AR blockade and may improve cognitive function by A_1AR antagonism.[28–32]

Examples of AR antagonists are shown in Figure 2. The xanthine derivatives caffeine (**2a**) and theophylline (**2b**) are about equally active at all human AR subtypes.[33,34] In epidemiological studies caffeine intake has been associated with a lower incidence of PD and AD.[35,36] The aminopyrazine ASP-5854 (**3**), a dual-target A_1/A_{2A}AR antagonist has been extensively characterized in several models of PD and cognition.[28] In monkeys, ASP-5854 reversed haloperidol-induced catalepsy with an ED_{50} value of 0.1 mg kg^{-1} p.o.[37] ASP-5854 produced positive results in the rat passive avoidance test, a model of cognition,[31] whereas the A_{2A}-selective antagonist istradefylline (KW-6002, **4a**) failed to show cognitive-enhancing effects when tested under the same conditions. These results support the hypothesis that a dual A_{2A}/A_2AR antagonist may provide additional benefit to PD patients relative to selective A_{2A}ARs antagonists, because of their positive effects on cognitive impairment associated with the disease. Such drugs may also be useful for AD therapy.

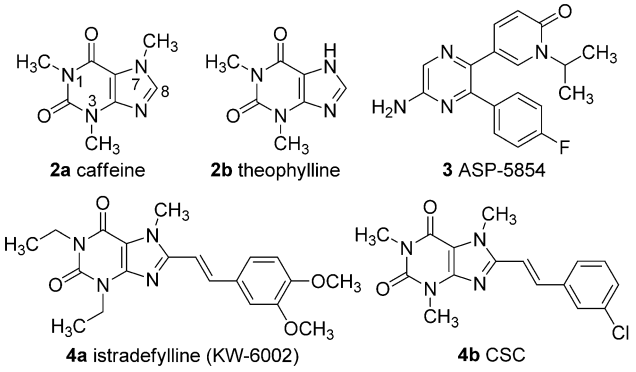


Figure 2. Structures of adenosine receptor antagonists including dual- and multitarget drugs.

Another dual-target approach was reported by Chen et al. who evaluated the A_{2A}AR antagonist (*E*)-8-(3-chlorostyryl)caffeine (CSC, **4b**) for inhibition of MAO-B.[38] The authors suggested that the neuroprotective properties of compound **4b** in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD may be partly due to MAO-B inhibition in synergy with its A_{2A}AR antagonistic activity.[38,39] Recently, Rivara et al.[40] reported a new series of 9-deazaxanthines based on previous studies by our group.[41,42] They showed that the 9-deaza analogue of CSC also acted as a relatively potent dual target A_{2A}AR/MAO-B inhibitor (K_i human A_{2A}AR: 260 nM; IC_{50} human MAO-B: 200 nM).

Thus, the approach to design compounds that inhibit MAO-B in addition to blocking A_{2A}ARs (dual-target approach), and ancillary A_1AR antagonistic activity (triple-target approach) may result in synergistic or additive effects in the treatment of PD. The same concept may be extended to the treatment of AD, for which several multitarget drug approaches including MAO-B inhibition have recently been developed.[43,44] The resulting drugs will exhibit a broad spectrum of activities showing effects on several symptoms of these complex neurodegenerative diseases.[39]

Some of us—in collaboration with the group of K. Kieć-Kononowocz (Jagiellonian University, Kraków, Poland)—have previously reported the development of tetrahydropyrimido[2,1-*f*]purinediones **5** (Figure 3), a class of compounds that can be envisaged as tricyclic caffeine derivatives.[45–50] They represent structural analogues of 8-styrylxanthine derivatives, which are sterically constrained by annulation of a tetrahydropyrimidine ring to the 7,8-position of caffeine mimicking the 8-*E*-styryl substructure of A_{2A}AR antagonists **4a** and **4b**. So far, this approach has led to relatively potent A_{2A}AR antagonists, some of which showed good selectivity over A_1ARs.[46] However, a drawback of this class of compounds is their low water solubility. In continuing efforts to develop improved, more water-soluble A_{2A}AR antagonists, structure **6** has been designed. In **6**, the nitrogen atom at position 9 of the tricyclic compounds **5** was formally shifted to position 8. The nitrogen atom in position 8 of compounds **6** is expected to exhibit increased basicity as it

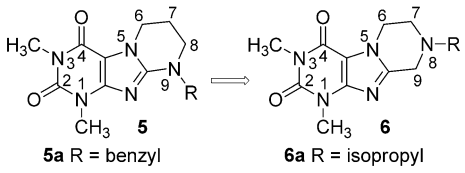


Figure 3. Tetrahydropyrimido[2,1-*f*]purinediones **5** and related tetrahydropyrazino[2,1-*f*]purinedione **6**.

is not connected to an aromatic ring, provided the substituent R is not aromatic. Consequently, compounds **6** should exhibit better water solubility than the related tricyclic xanthine derivatives **5** at physiological pH values.

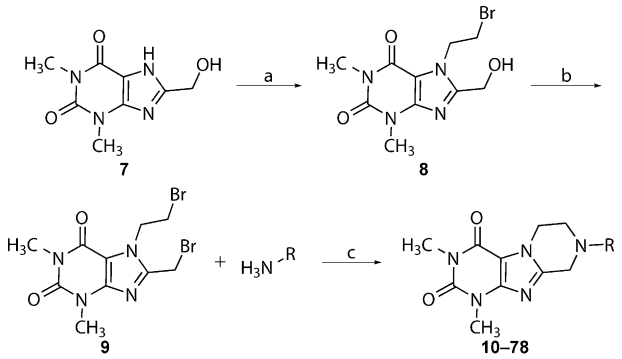
Recently, we reported the synthesis and biological evaluation of a first representative of this novel class of AR antagonists, namely 1,3-dimethyl-8-isopropyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (**6a**).[51] This class of compounds has been poorly investigated so far, and only very few derivatives have been described.[52,53]

Herein, we report on the synthesis of a large series of novel tetrahydropyrazino[2,1-*f*]purinedione derivatives **6** and their evaluation as antagonists at ARs and inhibitors of MAO isoenzymes, MAO-B and MAO-A. Benzyl residues bearing a variety of substituents were introduced at position 8 of the tetrahydropyrazino[2,1-*f*]purinedione scaffold **6** to modulate the compounds' biological activities while keeping the basicity of the nitrogen atom N8 to allow protonation.

Results and Discussion

Chemistry

Starting from 1,3-dimethyl-8-hydroxymethylxanthine (**7**),[54] the synthesis of a library of tetrahydropyrazino[2,1-*f*]purinediones **10**–**78** was carried out in a three-step procedure (Scheme 1).



Scheme 1. Synthesis of 8-substituted 1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-diones **10**–**78**. *Reagents and conditions*: a) 1,2-dibromoethane, DMF, NEt_3, 80 °C, 6 h; b) PBr_3, CH_2Cl_2, 0 °C→RT, 1 h; c) dimethoxyethane, *N*,*N*-diisopropylethylamine, RT, 16 h.

Xanthine **7** was alkylated at position 7 by treatment with 1,2-dibromoethane in the presence of triethylamine. The hydroxy function of **8** was subsequently converted into the corresponding bromide by treatment with phosphorus tribromide. The resulting 7-(2-bromoethyl)-8-(bromomethyl)-1,3-dimethylpurine-2,4-dione (**9**) was not isolated, but directly reacted in a parallel manner with a large number of mono-, di-, or trisubstituted benzylamines under basic conditions to afford a library of tetrahydropyrazino[2,1-*f*]purinediones **10**–**78** in moderate to very good yields (24–81%, calculated over two steps from **8**). The structures of all products were confirmed by NMR and MS analyses. Melting points were determined for all new compounds. The purity of the tested compounds was confirmed by HPLC coupled to electrospray ionization mass spectrometry (ESI-MS) using two different methods (see the Experimental Section below for details) and shown to be generally >96%.

Biological evaluation and determination of water solubility

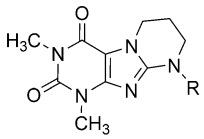
The synthesized tetrahydropyrazino[2,1-*f*]purinediones were initially evaluated in radioligand binding assays for their affinity to A_1ARs in rat brain cortical membrane and to A_{2A}ARs in rat brain striatal membrane preparations. Selected compounds were further investigated for their affinity to human A_1 and A_{2A}ARs recombinantly expressed in Chinese hamster ovary (CHO) cells. All compounds were additionally investigated for their affinity to human A_{2B} and A_3ARs recombinantly expressed in CHO cells, to determine their AR subtype selectivity. [^{3}H]2-Chloro-N^6-cyclopentyladenosine ([^{3}H]CCPA),[55] [^{3}H]3-(3-hydroxypropyl)-8-(*m*-methoxystyryl)-7-methyl-1-propargylxanthine ([^{3}H]MSX-2),[56] [^{3}H]8-(4-(4-chlorophenyl)piperazine-1-sulfonyl)phenyl)-1-propylxanthine ([^{3}H]PSB-603),[57] and [^{3}H]2-phenyl-8-ethyl-4-methyl-(8*R*)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]purine-5-one ([^{3}H]PSB-11)[58] were used as radioligands in A_1, A_{2A}, A_{2B}, and A_3AR binding studies, respectively. Functional studies were not performed as it is well known that all xanthine derivatives lacking a ribose moiety, including tricyclic compounds, can only act as antagonists, but never as agonists, at ARs.

All compounds were tested for inhibition of human MAO-B at a concentration of 10 μM. For compounds that showed inhibition >70%, full concentration–inhibition curves were recorded and IC_{50} values were determined. Two select compounds, **32** and **72**, were also tested for inhibition of rat MAO-B to investigate their suitability for animal studies in rodents. Potent inhibitors were additionally investigated for inhibition of human MAO-A to assess their selectivity. Results are presented in Tables 1 and 2. Data of standard ligands are included for comparison. Water solubility of selected compounds at different pH values was determined by thermodynamic solubility measurements (Supporting Information Table S1).

SAR at adenosine receptors

Initial studies showed that the unsubstituted parent compound of this new class of AR antagonists, 8-benzyl-1,3-dimethyltetrahydropyrazino[2,1-*f*]purinedione (**10**), was more potent than its isomer, 9-benzyl-1,3-dimethyltetrahydropyrimido[2,1-*f*]purinedione (**5a**)[44] especially at the A_1AR (see Table 1). Compound **10** was found to be a potent, selective A_1 antagonist in rat (K_i rat A_1: 79.3 nM), but somewhat less potent at human A_1 receptors (K_i human A_1: 265 nM). It was also able to block A_{2A}ARs (K_i human A_{2A}: 1060 nM, rat A_{2A}: 598 nM), but was inactive at human A_{2B} and A_3ARs. Structure–activity relationships (SAR) were subsequently explored by systematic substitution of the benzyl residue: 1) through introduction of one, two, or three substituents into the phenyl ring, 2) by bioisosteric replacement of the phenyl ring, and 3) by substitution

Table 1. Adenosine receptor affinities of 1,3-dimethyltetrahydropyrazino[2,1-*f*]purinediones and standard compounds.



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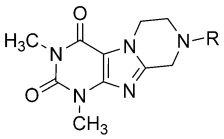


10–78

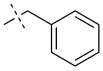
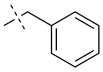
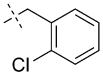
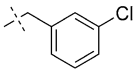
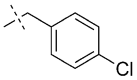
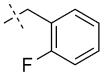
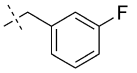
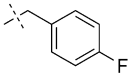
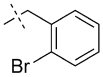
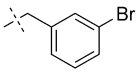
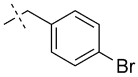
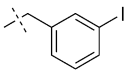
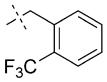
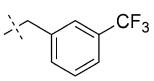
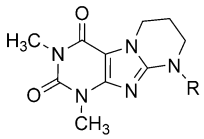
Compd	R	$K_i \pm$ SEM [nM] (h, human; r, rat)			
		A_1 vs. [^{3}H]CCPA[a]	A_{2A} vs. [^{3}H]MSX-2[a]	A_{2B} vs. [^{3}H]PSB-603[b]	A_3 vs. [^{3}H]PSB-11[b]
Standard compounds[c]					
caffeine (**1a**)		44900 (h) 41000 (r)	23400 (h) 32500 (r)	33800 (h) 30000 (r)	13300 (h) > 10000 (r)
istradefylline (**4a**)		841 (h) 230 (r)	12 (h) 4.46 (r)	> 10000 (h)	4470 (h)
CSC (**4b**)		> 10000 (14%)[d] (h) 28000 (r)	38.0 ± 11.0 (h) 54 (r)	8200 (h)	> 10000 (h)
9-Benzyl-1,3-dimethyltetrahydropyrimido[2,1-*f*]purinedione[e]					
5a		3580 (r)	1090 (r)	nd[f]	nd[f]
8-Benzyl-1,3-dimethyltetrahydropyrazino[2,1-*f*]purinedione					
10		265 ± 68 (h) 79.3 ± 12.0 (r)	1060 ± 300 (h) 598 ± 102 (r)	> 1000 (h) (15%)[d]	> 10000 (h) (21%)[d]
Monosubstituted 8-benzyl-1,3-dimethyltetrahydropyrazino[2,1-*f*]purinediones					
11		152 ± 21 (h) 40.5 ± 5.1 (r)	1260 ± 370 (r)	> 1000 (h) (5%)[d]	> 1000 (h) (31%)[d]
12		80.6 ± 20.0 (h) 42.0 ± 6.5 (r)	1330 ± 110 (r)	> 1000 (h) (5%)[d]	> 10000 (h) (11%)[d]
13		427 ± 25 (h) 54.2 ± 2.9 (r)	1250 ± 60 (r)	> 300 (h) (3%)[d]	> 10000 (h) (39%)[d]
14		218 ± 74 (h) 15.5 ± 2.8 (r)	> 1000 (r) (29%)[d]	> 1000 (h) (1%)[d]	> 1000 (h) (18%)[d]
15		215 ± 24 (h) 61.9 ± 19.7 (r)	962 ± 156 (h) > 1000 (r) (34%)[d]	> 300 (h) (6%)[d]	> 10000 (h) (30%)[d]
16		> 1500 (r) (22%)[d]	> 1000 (r) (36%)[d]	> 1000 (h) (1%)[d]	> 10000 (h) (13%)[d]
17		85.7 ± 13.6 (h) 40.1 ± 18.8 (r)	291 ± 59 (h) 257 ± 36 (r)	> 1000 (h) (11%)[d]	> 1000 (h) (4%)[d]
18		64.4 ± 15.1 (h) 52.8 ± 4.4 (r)	769 ± 27 (h) 621 ± 123 (r)	> 1000 (h) (7%)	> 10000 (h) (29%)[d]
19		> 1500 (r) (29%)[d]	3860 ± 220 (h) 1060 ± 10 (r)	> 1000 (h) (20%)[d]	> 1000 (h) (21%)[d]
20		87.7 ± 28.6 (h) 25.5 ± 4.4 (r)	834 ± 155 (h) 384 ± 80 (r)	> 1000 (h) (9%)[d]	> 1000 (h) (24%)[d]
21		> 1500 (r) (38%)[d]	> 1000 (r) (35%)[d]	> 1000 (h) (−7%)[d]	> 1000 (h) (14%)[d]
22		127 ± 20 (h) 26.0 ± 1.8 (r)	533 ± 199 (h) 476 ± 51 (r)	> 300 (h) (9%)[d]	> 10000 (h) (25%)[d]

Table 1. (Continued)



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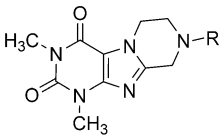


10–78

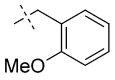
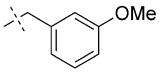
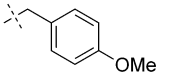
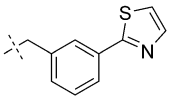
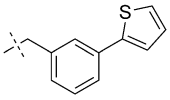
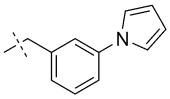
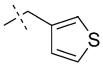
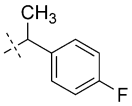
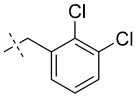
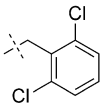
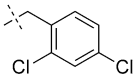
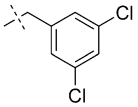
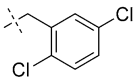
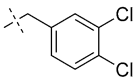
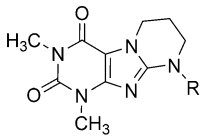
Compd	R	$K_i \pm$ SEM [nM] (h, human; r, rat)			
		A_1 vs. [^{3}H]CCPA[a]	A_{2A} vs. [^{3}H]MSX-2[a]	A_{2B} vs. [^{3}H]PSB-603[b]	A_3 vs. [^{3}H]PSB-11[b]
23		216 ± 18 (h) 74.4 ± 4.4 (r)	653 ± 177 (h) 387 ± 127 (r)	> 1000 (h) (20%)[d]	> 1000 (h) (13%)[d]
24		392 ± 123 (h) 51.1 ± 7.3 (r)	1300 ± 80 (r)	> 1000 (h) (−2%)[d]	> 10000 (h) (28%)[d]
25		> 1500 (r) (21%)[d]	> 1000 (r) (14%)[d]	> 1000 (h) (6%)[d]	> 10000 (h) (12%)[d]
26		76.2 ± 9.2 (h) 98.8 ± 5.6 (r)	1020 ± 140 (h) 558 ± 134 (r)	> 1000 (h) (17%)[d]	> 10000 (h) (25%)[d]
27		21.8 ± 3.1 (h) 10.1 ± 1.7 (r)	426 ± 83 (h) 375 ± 89 (r)	> 1000 (h) (9%)[d]	6490 ± 1380 (h)[a]
28		59.1 ± 18.9 (h) 21.4 ± 3.7 (r)	437 ± 30 (h) 184 ± 50 (r)	> 1000 (h) (20%)[d]	> 10000 (h) (23%)[d]
29		61.8 ± 17.4 (h) 32.9 ± 7.4 (r)	569 ± 87 (h) 335 ± 41 (r)	> 1000 (h) (4%)[d]	> 10000 (h) (16%)[d]
30		> 1500 (r) (4%)[d]	1210 ± 40 (r)	> 1000 (h) (9%)[d]	> 10000 (h) (10%)[d]
Disubstituted 8-benzyl-1,3-dimethyltetrahydropyrazino[2,1-*f*]purinediones					
31		116 ± 15 (h) 86.8 ± 19.8 (r)	94.3 ± 10.4 (h) 993 ± 81 (r)	> 1000 (h) (−4%)[d]	> 1000 (h) (27%)[d]
32		227 ± 34 (h) 25.5 ± 3.0 (r)	782 ± 129 (h) 402 ± 53 (r)	> 300 (h) (1%)[d]	> 1000 (h) (25%)[d]
33		296 ± 34 (h) 33.5 ± 4.0 (r)	662 ± 154 (h) 174 ± 13 (r)	> 300 (h) (12%)[d]	7840 ± 1850 (h)[a]
34		257 ± 71 (h) 44.4 ± 7.5 (r)	2440 ± 290 (h) 927 ± 280 (r)	> 300 (h) (20%)[d]	> 1000 (h) (37%)[d]
35		89.8 ± 18.3 (h) 14.1 ± 0.8 (r)	320 ± 82 (h) 484 ± 73 (r)	> 300 (h) (8%)[d]	> 1000 (h) (10%)[d]
36		791 ± 110 (h) 351 ± 57 (r)	1510 ± 031 (h) 322 ± 129 (r)	> 1000 (h) (15%)[d]	> 1000 (h) (13%)[d]

Table 1. (Continued)



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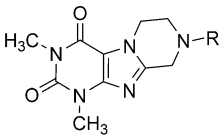


10–78

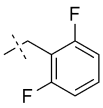
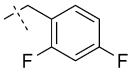
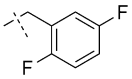
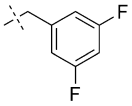
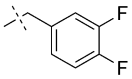
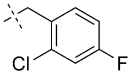
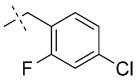
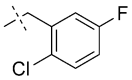
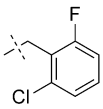
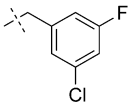
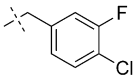
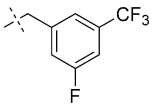
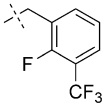
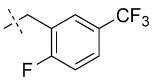
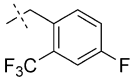
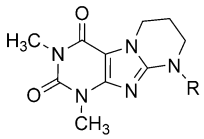
Compd	R	$K_i \pm$ SEM [nM] (h, human; r, rat) A_1 vs. [^{3}H]CCPA[a]	A_{2A} vs. [^{3}H]MSX-2[a]	A_{2B} vs. [^{3}H]PSB-603[b]	A_3 vs. [^{3}H]PSB-11[b]
37		376 ± 20 (h) 238 ± 50 (r)	3660 ± 660 (r)	> 1000 (h) (−7%)[d]	> 1000 (h) (23%)[d]
38		> 1500 (r) (20%)[d]	> 1000 (r) (34%)[d]	> 1000 (h) (10%)[d]	> 1000 (h) (28%)[d]
39		687 ± 172 (h) 77.1 ± 18.6 (r)	> 1000 (r) (32%)[d]	> 1000 (h) (15%)[d]	> 1000 (h) (14%)[d]
40		794 ± 174 (h) 85.5 ± 15.5 (r)	1220 ± 220 (r)	> 1000 (h) (4%)[d]	> 1000 (h) (13%)[d]
41		> 1500 (r) (17%)[d]	> 1000 (r) (29%)[d]	> 1000 (h) (16%)[d]	> 1000 (h) (26%)[d]
42		231 ± 70 (h) 107 ± 14 (r)	1270 ± 220 (h) 518 ± 78 (r)	> 1000 (h) (16%)[d]	> 1000 (h) (−4%)[d]
43		> 1500 (r) (35%)[d]	> 1000 (r) (41%)[d]	> 1000 (h) (11%)[d]	> 1000 (h) (15%)[d]
44		243 ± 5 (h) 54.4 ± 8.8 (r)	812 ± 151 (h) 624 ± 79 (r)	> 1000 (h) (11%)[d]	> 1000 (h) (18%)[d]
45		186 ± 36 (h) 99.9 ± 23.0 (r)	1000 ± 120 (h) 758 ± 161 (r)	> 1000 (h) (3%)[d]	> 1000 (h) (15%)[d]
46		384 ± 95 (h) 95.5 ± 23.0 (r)	1070 ± 200 (r)	> 1000 (h) (−3%)[d]	> 10000 (h) (23%)[d]
47		1500 (r) (21%)[d]	1330 ± 50 (r)	> 1000 (h) (7%)[d]	> 10000 (h) (21%)[d]
48		150 ± 31 (h) 28.9 ± 9.4 (r)	1150 ± 200 (h) 681 ± 170 (r)	> 1000 (h) (4%)[d]	> 1000 (h) (12%)[d]
49		247 ± 54 (h) 144 ± 29 (r)	1420 ± 430 (h) 652 ± 127 (r)	> 1000 (h) (4%)[d]	> 10000 (h) (17%)[d]
50		84.3 ± 17.6 (h) 151 ± 43 (r)	1020 ± 280 (r)	> 1000 (h) (2%)[d]	> 1000 (h) (3%)[d]
51		> 1500 (r) (26%)[d]	2570 ± 660 (r)	> 1000 (h) (9%)[d]	> 1000 (h) (−3%)[d]

Table 1. (Continued)



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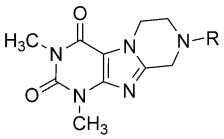


10–78

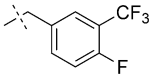
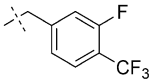
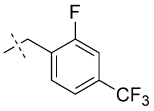
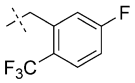
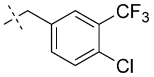
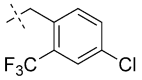
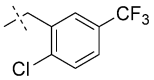
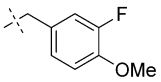
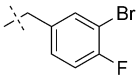
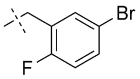
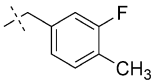
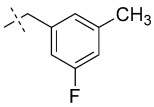
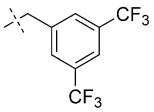
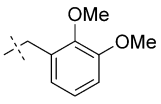
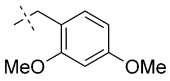
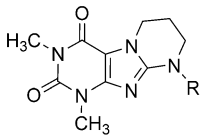
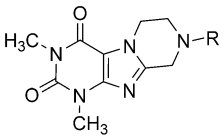
Compd	R	$K_i \pm$ SEM [nM] (h, human; r, rat)			
		A_1 vs. [^{3}H]CCPA[a]	A_{2A} vs. [^{3}H]MSX-2[a]	A_{2B} vs. [^{3}H]PSB-603[b]	A_3 vs. [^{3}H]PSB-11[b]
52		> 1500 (r) (14%)[d]	1870 ± 464 (r)	> 1000 (h) (29%)[d]	> 10000 (h) (22%)[d]
53		> 1500 (r) (5%)[d]	> 1000 (r) (35%)[d]	> 1000 (h) (17%)[d]	> 100 (h) (3%)[d]
54		> 1500 (r) (10%)[d]	> 1000 (r) (33%)[d]	> 1000 (h) (14%)[d]	> 10000 (h) (30%)[d]
55		678 ± 99 (h) 611 ± 123 (r)	> 1000 (r) (28%)[d]	> 1000 (h) (13%)[d]	> 1000 (h) (17%)[d]
56		> 1500 (r) (9%)[d]	1160 ± 210 (r)	> 1000 (h) (10%)[d]	> 10000 (h) (18%)[d]
57		> 1500 (r) (31%)[d]	> 1000 (r) (36%)[d]	> 1000 (h) (12%)[d]	> 1000 (h) (17%)[d]
58		67.5 ± 10.6 (h) 23 ± 2 (r)[b]	261 ± 47 (h) 358 ± 114 (r)	> 1000 (h) (10%)[d]	> 1000 (h) (−6%)[d]
59		> 1500 (r) (26%)[d]	> 1000 (r) (35%)[d]	> 1000 (h) (8%)[d]	> 300 (h) (−4%)[d]
60		> 1500 (r) (21%)[d]	> 1000 (r) (43%)[d]	> 1000 (h) (12%)[d]	> 1000 (h) (16%)[d]
61		76.5 ± 3.0 (h) 36 ± 8 (r)[b]	4730 ± 1790 (h) 991 ± 14 (r)	> 1000 (h) (1%)[d]	> 1000 (h) (12%)[d]
62		> 1500 (r) (35%)[d]	1910 ± 560 (h) 729 ± 125 (r)	> 1000 (h) (13%)[d]	> 1000 (h) (17%)[d]
63		51.9 ± 12.4 (h) 46 ± 6 (r)[b]	1100 ± 100 (r)	> 1000 (h) (24%)[d]	> 1000 (h) (14%)[d]
64		> 1500 (r) (−2%)[d]	> 1000 (r) (13%)[d]	> 1000 (h) (4%)[d]	> 1000 (h) (6%)[d]
65		217 ± 49 (h) 134 ± 40 (r)	1120 ± 310 (h) 236 ± 24 (r)	> 1000 (h) (−3%)[d]	> 10000 (h) (25%)[d]
66		> 1500 (r) (28%)[d]	> 1000 (r) (10%)[d]	> 100 (h) (7%)[d]	> 10000 (h) (29%)[d]

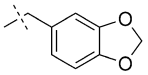
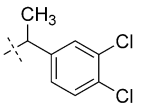
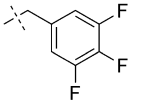
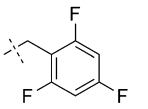
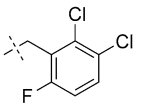
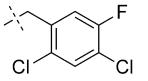
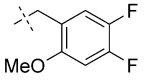
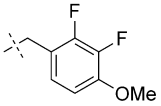
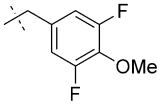
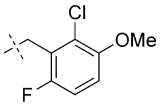
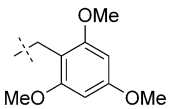
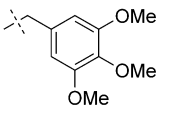
Table 1. (Continued)



5



10–78

Compd	R	$K_i \pm$ SEM [nM] (h, human; r, rat)			
		A_1 vs. [^{3}H]CCPA[a]	A_{2A} vs. [^{3}H]MSX-2[a]	A_{2B} vs. [^{3}H]PSB-603[b]	A_3 vs. [^{3}H]PSB-11[b]
67		>1500 (r) (24%)[d]	>1000 (r) (10%)[d]	>300 (h) (17%)[d]	>1000 (h) (12%)[d]
68		1530 ± 240 (r)	692 ± 171 (h) 688 ± 33 (r)	>1000 (h) (8%)[d]	>1000 (h) (−5%)[d]
Trisubstituted 8-benzyl-1,3-dimethyltetrahydropyrazino[2,1-*f*]purinediones					
69		>1500 (r) (10%)[d]	1670 ± 380 (r)	>1000 (h) (7%)[d]	>100 (h) (−10%)[d]
70		>1500 (r) (19%)[d]	1880 ± 890 (r)	>1000 (h) (2%)[d]	>1000 (h) (2%)[d]
71		91.4 ± 14.8 (h) 73 ± 2 (r)[b]	1080 ± 30 (h) 897 ± 266 (r)	>1000 (h) (9%)[d]	>100 (h) (−10%)[d]
72		217 ± 64 (h) 111 ± 24 (r)	268 ± 75 (h) 603 ± 54 (r)	>1000 (h) (5%)[d]	>300 (h) (8%)[d]
73		>1500 (r) (22%)[d]	1390 ± 300 (r)	>1000 (h) (2%)[d]	>300 (h) (−5%)[d]
74		>1500 (r) (17%)[d]	>1000 (r) (28%)[d]	>1000 (h) (21%)[d]	>300 (h) (14%)[d]
75		>1500 (r) (−3%)[d]	2670 ± 630 (r)	>1000 (h) (14%)[d]	>10000 (h) (19%)[d]
76		752 ± 86 (h) 170 ± 16 (r)	3810 ± 280 (h) 823 ± 142 (r)	>1000 (h) (9%)[d]	>100 (h) (−3%)[d]
77		3040 ± 80 (h) 343 ± 108 (r)	1700 ± 210 (r)	>1000 (h) (5%)[d]	>1000 (h) (21%)[d]
78		>1500 (r) (3%)[d]	>1000 (r) (20%)[d]	>1000 (h) (5%)[d]	>1000 (h) (5%)[d]

[a] $n = 3$. [b] $n = 2$. [c] Data are from Müller and Jacobson[33] or are unpublished data from our research group. [d] Percent inhibition of radioligand binding at the indicated concentration. [e] Data are from Drabczyńska et al.[47] [f] nd: not determined.

leading to 1-methylbenzyl residues. Our initial goal was to increase the A_{2A}AR affinity of the parent compound **10**.

All investigated derivatives **10**–**78** showed no or only negligible affinity for the A_{2B} and A_3AR subtypes. Within the monosubstituted series (**11**–**28**), *ortho* or *meta* substituents (halogen, methoxy, trifluoromethyl) were, in most cases, better tolerated than *para* substitution (for example, *p*-F **16**, *p*-Br **19**, and *p*-OMe **25**). Exceptions from this rule were the *p*-chlorobenzyl derivative **13**, which was similarly potent to its *o*- and *m*-chlorobenzyl isomers **11** and **12**, and the *o*-trifluoromethyl-substituted compound **21**, which showed decreased affinity. Many of the compounds of this series retained high A_1AR affinity, and some of them showed the desired increase in A_{2A} affinity. In particular, *o*-bromo substitution (compound **17**) led to a large increase in A_{2A} affinity combined with a moderate increase in A_1 affinity. Derivative **17** was the best balanced dual-target A_1/A_{2A} antagonist of the present series with similar potency at human and rat ARs (K_i human, A_1: 85.7 nM, A_{2A}: 291 nM; K_i rat, A_1: 40.1 nM, A_{2A}: 257 nM). The *m*-trifluoromethyl derivative **22** showed a similar profile.

As a next step, heterocyclic substituents (thiazolyl **26**, 2-thienyl **27**, and 1-pyrrolyl **28**) were introduced into the *m*-position of the benzyl ring. These modifications were also well tolerated by A_1 and A_{2A}ARs, and **27** and **28** led to increases in affinities at both receptor subtypes. The most potent compound was the *m*-(2-thienyl)benzyl derivative **27**, the most potent A_1 antagonist of the present series (K_i human A_1: 21.8 nM; rat A_1: 10.1 nM) which also showed good, although ~30-fold lower A_{2A} affinity (K_i human A_{2A}: 426 nM; rat A_{2A}: 375 nM).

Bioisosteric replacement of the benzyl moiety of the parent benzyl derivative **10** by a 2-thienylmethyl moiety (compound **29**) was well tolerated. Affinity for both A_1 and A_{2A} were even improved (two- to fourfold, compare **10** and **29**).

Within the series displaying a disubstituted 8-benzyl moiety (**31**–**68**), *para*-substituents at the benzyl ring (**38**, **41**, **43**, **47**, **51**–**54**, **56**, **57**, **59**, **60**, **62**, **66**, and **67**) largely decreased affinity for both A_1 and A_{2A}ARs in line with the SARs obtained for the monosubstituted pyrazinopurinediones. However, a 2,4-dichloro and a 3,4-dichloro substitution pattern, as displayed by compounds **33** and **36**, was tolerated by both rat AR subtypes. Compound **33** proved to be a dual A_1 and A_{2A} antagonist (K_i human A_1: 296 nM, A_{2A}: 662 nM; K_i rat A_1: 33.5 nM, A_{2A}: 174 nM). Among the compounds with 2,3-, 2,5-, 2,6-, or 3,5-disubstitution several derivatives showed relatively high affinity for both, A_1 and A_{2A}ARs, mostly with a certain preference for the A_1AR. The affinity for the human receptors was in many cases lower than for rat ARs. The best compounds of this series were the 2,3-dichlorobenzyl derivative **31** (K_i human A_1: 116 nM, A_{2A}: 94.3 nM), the 2,5-dichlorobenzyl derivative **35** (K_i human A_1: 89.9 nM, A_{2A}: 320 nM; K_i rat A_1: 14.1 nM, rat A_{2A}: 484 nM), and the 2-chloro-5-trifluoromethylbenzyl derivative **58** (K_i human 67.5 nM, A_{2A}: 261 nM; K_i rat A_1: 23 nM, A_{2A}: 358 nM).

The 3,4-dichlorobenzyl derivative **36** was found to be a balanced A_1/A_{2A} antagonist with moderate potency at human, but higher potency at rat ARs (K_i human A_1: 791 nM, A_{2A}: 1510 nM; A_1 rat: 351 nM, A_{2A}: 322 nM). The introduction of a methyl group attached to the methylene group of the benzyl residue of compound **36**, resulting in compound **68**, which was predicted to display an increased basicity of the N8 atom, was tolerated by A_1 and even much better by A_{2A}ARs.

A series of 10 trisubstituted derivatives **69**–**78** was studied, most of which contained *para*-substituents in addition to two additional residues. The SARs were in line with those observed in the other series. All compounds displaying a *para*-substituent other than chloro showed low AR affinity. The best A_1/A_{2A} antagonist was compound **72** bearing a 2,4-dichloro-5-fluorobenzyl substituent (K_i human A_1: 217 nM, A_{2A}: 268 nM; K_i rat A_1: 111 nM, A_{2A}: 603 nM).

Moderate to large species differences have previously been described for different classes of AR antagonists.[50,59,60] As preclinical in vivo evaluation is typically performed in rodents, mainly rats or mice, we compared affinities of the investigated 1,3-dimethyltetrahydropyrazino[2,1-*f*]purinediones at rat and human A_1 and A_{2A}ARs. Correlation plots of pK_i values for both species are shown in the Supporting Information (Figures S1 and S2). For many compounds large species differences were observed at the A_1AR. Except for compounds **26** and **50** (bearing a 3-(thiazol-2-yl)benzyl or a 2-fluoro-5-(trifluoromethyl)benzyl residue) all compounds of this series investigated in both species displayed a preference for the rat over the human A_1AR. In particular, compounds **13**, **14**, **24**, **32**, **33**, **34**, **39**, and **40** were 5.8–14.1-fold more potent at the rat than at the human A_1AR. The Pearson product–moment correlation coefficient *r* was calculated to be 0.617.

Species differences of the investigated tetrahydropyrazino-[2,1-*f*]purinediones at A_{2A}ARs were less pronounced. Although most of the tested compounds showed somewhat higher affinity for the rat than the human A_{2A}AR, for a few derivatives the opposite was true (Supporting Information Figure S2). For example, the most potent antagonist at the human A_{2A}AR of this series, compound **31**, displayed a tenfold lower affinity at the rat A_{2A}AR. For correlation of rat and human A_{2A}AR affinity the *r* coefficient was calculated to be 0.382.

SAR at monoamine oxidases

Test results of 1,3-dimethyltetrahydropyrazino[2,1-*f*]purinediones at MAO-A and MAO-B are collected in Table 2. All of the tested derivatives were found to be inactive at MAO-A. Compounds from the monosubstituted series (**11**–**28**) bearing a halogen atom in the *meta* or *para* position (**12**, **13**, **15**, **16**, **18**, **19**, and **20**) or either a trifluoromethyl group (**22**) or a thienyl moiety in the *meta* position (**27**) inhibited the enzyme with IC_{50} values in the low micromolar range. In contrast, substitution of the *ortho*-position (**11**, **14**, **17**, and **21**) or a methoxy substituent in any position (**23**–**25**) abrogated MAO-B inhibitory activity.

Consistent observations were made in the disubstituted series of 8-benzyl-1,3-dimethyltetrahydropyrazino[2,1-*f*]purinediones (**31**–**68**). Compounds with a 2,6-substitution pattern (**32**, **37**, and **45**) or a large *ortho* substituent, such as a trifluoromethyl group (**51**, **55**, and **57**), or a methoxy residue in any po-

Table 2. Human (h) MAO-A and human MAO-B inhibitory potencies of 1,3-dimethyltetrahydropyrazino[2,1-*f*]purinediones and standard compounds.

Compd	$IC_{50} \pm SEM$ [nM][a] hMAO-A	hMAO-B
Standard compounds		
selegiline (**1a**)	nd[b]	6.13 ± 0.85
lazabemide (**1b**)	nd	17.6 ± 4.2
safinamide (**1c**)	nd	7.67 ± 1.81
caffeine (**2a**)	> 500 000 (33%)[c]	> 500 000 (16%)[c]
istradefylline (**4a**)	> 10 000 (18%)[c]	> 10 000 (20%)[c]
CSC (**4b**)	> 10 000 (23%)[c]	18.1 ± 3.3
1,3-Dimethyltetrahydropyrazino[2,1-*f*]purinediones		
10	nd	> 10 000 (36%)[c]
11	> 10 000 (5%)[c]	~ 10 000 (58%)[c]
12	> 10 000 (4%)[c]	1270 ± 270
13	> 10 000 (8%)[c]	1690 ± 130
14	nd	> 10 000 (43%)[c]
15	> 10 000 (5%)[c]	4840 ± 340
16	> 10 000 (−10%)[c]	2660 ± 660
17	> 10 000 (4%)[c]	~ 10 000 (58%)[c]
18	> 10 000 (1%)[c]	2140 ± 270
19	> 10 000 (2%)[c]	4390 ± 950
20	> 10 000 (1%)[c]	2460 ± 130
21	nd	> 10 000 (40%)[c]
22	> 10 000 (3%)[c]	2670 ± 530
23	nd	> 10 000 (8%)[c]
24	nd	> 10 000 (29%)[c]
25	nd	> 10 000 (39%)[c]
26	nd	~ 10 000 (45%)[c]
27	nd	4730 ± 690
28	nd	~ 10 000 (60%)[c]
29	nd	> 10 000 (28%)[c]
30	> 10 000 (7%)[c]	1440 ± 180
31	> 10 000 (7%)[c]	3350 ± 620
32	nd	> 10 000 (42%)[c]
33	> 10 000 (12%)[c]	3810 ± 560
34	> 10 000 (7%)[c]	250 ± 6
35	> 10 000 (8%)[c]	1060 ± 30
36	> 10 000 (5%)[c]	197 ± 25 (rat: 260 ± 38)
37	nd	> 10 000 (13%)[c]
38	> 10 000 (3%)[c]	838 ± 57
39	> 10 000 (0%)[c]	802 ± 30
40	> 10 000 (1%)[c]	840 ± 133
41	> 10 000 (−4%)[c]	663 ± 80
42	> 10 000 (5%)[c]	685 ± 106
43	> 10 000 (−10%)[c]	711 ± 75
44	> 10 000 (2%)[c]	932 ± 33
45	nd	> 10 000 (22%)[c]
46	> 10 000 (−3%)[c]	652 ± 103
47	> 10 000 (3%)[c]	548 ± 65
48	> 10 000 (5%)[c]	3560 ± 340
49	> 10 000 (7%)[c]	2050 ± 300
50	> 10 000 (2%)[c]	3350 ± 1160
51	nd	> 10 000 (25%)[c]
52	> 10 000 (1%)[c]	1190 ± 90
53	> 10 000 (1%)[c]	1530 ± 15
54	> 10 000 (−4%)[c]	2650 ± 880
55	nd	> 10 000 (33%)[c]
56	> 10 000 (4%)[c]	1060 ± 60
57	> 10 000 (10%)[c]	~ 10 000 (48%)[c]
58	> 10 000 (5%)[c]	1330 ± 230
59	> 10 000 (4%)[c]	~ 10 000 (50%)[c]
60	> 10 000 (3%)[c]	306 ± 4
61	> 10 000 (8%)[c]	512 ± 65
62	> 10 000 (3%)[c]	1020 ± 130
63	> 10 000 (−1%)[c]	687 ± 89
64	> 10 000 (3%)[c]	2060 ± 600
65	nd	> 10 000 (18%)[c]
66	nd	> 10 000 (24%)[c]
67	nd	> 10 000 (22%)[c]
68	> 10 000 (5%)[c]	371 ± 76
69	> 10 000 (4%)[c]	223 ± 37
70	nd	> 10 000 (30%)[c]
71	> 10 000 (7%)[c]	> 10 000 (41%)[c]
72	> 10 000 (6%)[c]	508 ± 23 (rat: 3000 ± 60)
73	> 10 000 (2%)[c]	3370 ± 450
74	nd	> 10 000 (14%)[c]
75	> 10 000 (4%)[c]	~ 10 000 (48%)[c]
76	> 10 000 (5%)[c]	~ 10 000 (45%)[c]
77	nd	> 10 000 (28%)[c]
78	nd	> 10 000 (0%)[c]

[a] $n = 3$. [b] nd: not determined. [c] Percent inhibition at the indicated concentration.

sition (**59**, **65**, and **66**), proved to be inactive at MAO-B. Tetrahydropyrazino[2,1-*f*]purinediones with 3,4- or 3,5-dihalogen substitution at the benzyl moiety yielded MAO-B inhibitors with nanomolar to low micromolar IC_{50} values.

Among these compounds, the 3,4- and 3,5-dichlorobenzyl-substituted derivatives **34** and **36** were the most potent MAO-B inhibitors of the present series (**34**: $IC_{50} = 250$ nM; **36**: $IC_{50} = 197$ nM). The introduction of a methyl group at the methylene spacer of the benzyl moiety was well tolerated by MAO-B (compare **68** and **36**). Among the trisubstituted derivatives only compound **69** bearing a 3,4,5-trifluorobenzyl moiety at position 8 ($IC_{50} = 223$ nM) and compound **72** with a 2,4-dichloro-5-fluorobenzyl residue ($IC_{50} = 508$ nM) showed notable MAO-B inhibition.

Dual- and triple-target drugs

The goal of the present study was to obtain dual- and triple-target drugs for the potential treatment of neurodegenerative diseases including PD and AD. We initially investigated the SARs of a set of 8-benzyltetrahydropyrazino[2,1-*f*]purinediones at A_{2A}ARs and at MAO-B to study the requirements of each of the main targets (see Figure 4, top). As expected, the SARs at the very different target structures were quite different. However, there was a common chemical space accepted by both targets (see Figure 4, bottom), which allowed us to obtain dual-target drugs. Some of those compounds showed additional blockade of A_1ARs by chance. Dual A_1/A_{2A} antagonists were identified including compound **17** (*o*-bromobenzyl derivative; K_i human A_1: 85.7 nM, rat A_1: 40.1 nM; human A_{2A}: 291 nM, rat A_{2A}: 257 nM). We discovered adenosine A_1 and A_{2A}AR antagonists with ancillary MAO-B inhibitory activity. For example, compound **34** was found to be an equipotent A_1 antagonist and inhibitor of MAO-B (*m*,*m*-dichlorobenzyl derivative; K_i human A_1: 257 nM, IC_{50} human MAO-B: 250 nM), whereas **61** was more potent at A_1 (*o*-F,*m*-Br-benzyl derivative; K_i

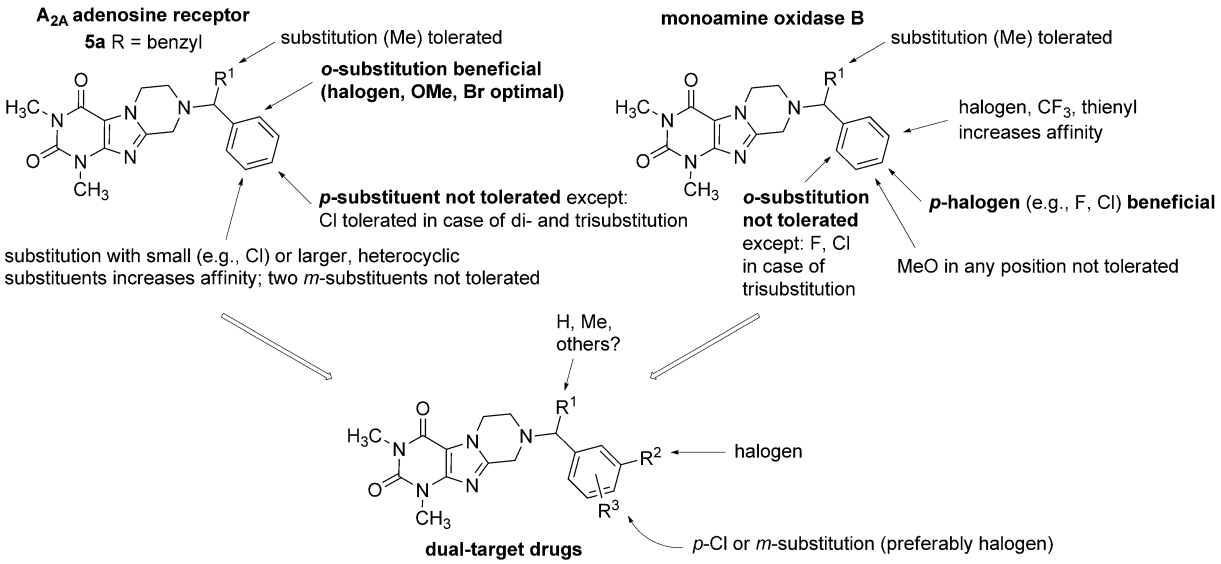


Figure 4. SAR of 8-benzyltetrahydropyrazino[2,1-*f*]purinediones at individual targets, A_{2A}AR, and MAO-B (main differences are indicated), and deduction of common SARs at both targets.

human A_1: 76.5 nM) than at MAO-B (IC_{50} human MAO-B: 512 nM). Compound **72** (*o*-Cl,*m*-F,*p*-Cl-benzyl derivative) was developed as a potent triple-target drug blocking human A_1 (K_i 217 nM) and A_{2A}ARs (K_i 268 nM), and inhibiting human MAO-B (IC_{50} 508 nM) in the same concentration range. Compound **36** (*m*,*p*-dichlorobenzyl derivative) was nearly equipotent at rat A_1AR (K_i 351 nM), rat A_{2A}AR (K_i 322 nM) and rat MAO-B (IC_{50} 260 nM), and will therefore be a useful tool for proof-of-principle studies to confirm this multitarget approach in rodent models of neurodegenerative diseases.

Water solubility

The water solubility of selected compounds was determined by thermodynamic solubility measurements at physiologically relevant pH values, namely pH 1, pH 4, and pH 7.4 (Supporting Information Table S1). All tested compounds showed good solubility at pH 1. Especially certain substituents at the *ortho*-position of the benzyl ring had an impact on solubility. Introduction of a trifluoromethyl group at that position resulted in less soluble compounds (**21** and **55**). In contrast, compounds **65** and **73**, both bearing a methoxy group in the *ortho*-position of the benzyl ring, displayed high solubility ($>1.5\ mg\,mL^{-1}$) and were also soluble at a higher pH value of 4 (0.04 to $0.05\ mg\,mL^{-1}$). Excellent solubility was also measured for the *o*-fluorobenzyl derivative **14**, which showed good solubility at all three pH values ($>1.5\ mg\,mL^{-1}$ at pH 1, $0.006\ mg\,mL^{-1}$ at pH 4, $0.002\ mg\,mL^{-1}$ at pH 7.4). The 3,4-dichlorobenzyl derivative **36** displayed higher solubility than the 2,6-dichlorobenzyl derivative **32** and the 2,5-dichlorobenzyl derivative **35**. The lowest solubility in the series of dichlorobenzyl derivatives was observed for the 3,5-dichlorobenzyl derivative **34**. For compound **36** a log*D* (octanol–buffer pH 7.4, RT) was determined to be 3.1.

Our data indicate that the nitrogen atom N8 of the 8-benzyltetrahydropyrazino[2,1-*f*]purinediones can be protonated under physiological conditions in the stomach. This property may be important for oral bioavailability of this new class of AR antagonists and MAO-B inhibitors. The basicity of N8 can be fine-tuned by substitution of the benzyl residue. Appropriate substitution can lead to compounds which are sufficiently soluble even at pH 7.4.

Pharmacokinetic studies

For the best triple-target compound **36**, which showed drug-like physicochemical properties, preliminary pharmacokinetic evaluation was performed (Supporting Information Table S2). It was found to show oral bioavailability in rat (50%), CNS penetration with a brain–plasma ratio of 0.8, and a half-life in rat of 1.5 h. Clearance in rat and human liver microsomes was in an acceptable range (see Table S2). Measurements of plasma–protein binding showed a free fraction of 2–6% in the plasma of mouse, rat, and human, and in mouse brain.

Conclusions

A library of 69 novel 8-benzyl-substituted 1,3-dimethyltetrahydropyrazino[2,1-*f*]purinediones has been synthesized and optimized as multitarget drugs for the potential treatment of neurodegenerative diseases such as Parkinson's and Alzheimer's disease. We identified compounds that act on two or three different targets which are thought to be relevant for the treatment of neurodegenerative diseases. Such compounds are expected to be superior to single-target drugs for the therapy of complex diseases. Compounds potently blocking A_1 and A_{2A}ARs and inhibiting MAO-B, all at sub-micromolar concentrations, were discovered. The thus far poorly investigated class of tricyclic xanthine derivatives has the advantage

of improved water solubility in comparison with the well-investigated isomeric tricyclic 1,3-dimethyltetrahydropyrimido[2,1-*f*]purinediones due to increased basicity. Preliminary pharmacokinetic studies of one of the most potent compounds have been promising indicating oral bioavailability and brain penetration. The present work represents the first extensive SAR study of this new class of AR antagonists and MAO-B inhibitors. Further modification, for example, at positions 1 and 3, and more variations at N8 of the tetrahydropyrazino[2,1-*f*]purinedione core are currently being explored.

Experimental Section

Chemistry

All commercially available reagents and solvents were used without further purification. The reactions were monitored by thin layer chromatography (TLC) using aluminum sheets coated with silica gel 60 F_{254} (Merck). Melting points were determined on a Büchi 530 melting point apparatus and are uncorrected. Column chromatography was performed on silica gel 0.040–0.063 mm (Fluka) using a Sepacore flash chromatography system (Büchi). ^{1}H NMR and ^{13}C NMR data were recorded on a Bruker Advance spectrometer at 500 MHz for proton and 125 MHz for carbon at ambient temperature. Shifts are given in ppm relative to the remaining protons of the deuterated solvents. Mass spectra were recorded on an API 2000 mass spectrometer (electron spray ion source, Applied Biosystems, Darmstadt, Germany) coupled with an Agilent 1100 HPLC system using a Phenomenex Luna HPLC C_{18} column (50×2.00 mm, particle size 3 μm). The purity of the tested compounds was determined by HPLC–UV obtained on an LC–MS instrument (Applied Biosystems API 2000 LC–MS/MS, HPLC Agilent 1100) using the following procedure: the compounds at a concentration of 1.0 mg mL^{-1} were dissolved in MeOH, and if necessary, sonication was used to complete dissolution. Then, 10 μL of the substance solution was injected into a Phenomenex Luna C_{18} HPLC column (50×2.00 mm, particle size 3 μm) and elution was performed for 30 min at a flow rate of 250 μL min^{-1} with a gradient of H_2O/MeOH either containing 2 mM ammonium acetate from 90:10 up to 0:100, starting the gradient after 10 min (system A) or containing 2 mM ammonium acetate and 0.1% formic acid from 90:10 up to 0:100, starting the gradient after 10 min (system B) or containing 2 mM ammonium acetate from 60:40 up to 0:100 for 30 min, starting the gradient after 0 min and ending after 20 min (system C). UV absorption was detected from λ 220 to 400 nm using a diode array detector.

7-(2-Bromoethyl)-8-(hydroxymethyl)-1,3-dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione (8): 8-(Hydroxymethyl)-1,3-dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione (**7**) (1.05 g, 5.0 mmol) was dissolved in DMF (10 mL), and NEt_3 (2.1 mL, 15.0 mmol) and 1,2-dibromoethane (2.82 g, 15.0 mmol) were added. The solution was stirred for 6 h at 80 °C. Then, the volatiles were removed in vacuo and the residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH 40:1 to 20:1). Yield: 0.90 g, 55%; mp: 203 °C; ^{1}H NMR (MeOD): δ = 4.81 (t, ^3J = 6.6 Hz, 2H, N7-CH_2), 4.74 (s, 2H, CH_2), 3.88 (t, ^3J = 6.7 Hz, 2H, N7-CH_2-CH_2), 3.59 (s, 3H, N3-CH_3), 3.40 ppm (s, 3H, N1-CH_3); ^{13}C NMR (MeOD): δ = 156.6 (C9a), 155.7 (C4), 153.7 (C2), 150.0 (C10a), 108.6 (C4a), 58.8 (CH_2), 50.7 (N7-CH_2), 31.7 (N7-CH_2-CH_2), 30.7 (N1-CH_3), 28.7 ppm (N3-CH_3); ESI-MS: negative mode 315.2, 317.2 $[M-H]^-$, positive mode 317.0, 319.0 $[M+H]^+$.

General procedure for the synthesis of tetrahydropyrazino[2,1-*f*]purinediones 10–78: 7-(2-Bromoethyl)-8-(hydroxymethyl)-1,3-dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione (**8**) (1000 mg, 3.2 mmol) was dissolved in dry CH_2Cl_2 (30 mL) and cooled to 0 °C. A solution of PBr_3 (900 μL, 9.4 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise. The solution was allowed to warm to RT and stirred for 1 h. Then it was cooled to 0 °C and excess PBr_3 was carefully hydrolyzed by slow addition of cold saturated aqueous $NaHCO_3$ (~10 mL), setting the solution to pH 8. The organic layer was then separated, and the aqueous layer extracted with CH_2Cl_2 (2×50 mL). The combined organic extracts were dried (Na_2SO_4), and the solvent was removed in vacuo. The crude 7-(2-bromoethyl)-8-bromo-1,3-dimethylpurine-2,4-dione (**9**) was used directly in the next step. The residue was dissolved in a mixture of dimethoxyethane (100 mL) and DIPEA (5 mL). The solution was split into ten vessels. Then, the appropriate amine (0.64 mmol) was added and the reactions were stirred overnight at RT. The volatiles were removed in vacuo and tetrahydropyrazino[2,1-*f*]purinediones **10**–**78** precipitated upon addition of H_2O (20 mL). For purification, the compounds were either filtered off and washed with H_2O (3×5 mL) and Et_2O (3×10 mL), or subjected to flash chromatography (silica gel, gradient of CH_2Cl_2/MeOH 100:0 to 40:1). The yields of compounds **10**–**78** were calculated over two steps from **8**.

8-Benzyl-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (10): Yield: 53%; mp: 153 °C; ^{1}H NMR ($CDCl_3$): δ = 7.33–7.28 (m, 5H, phe), 4.32 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.73–3.71 (m, 4H, N8-CH_2, C9-H_2), 3.50 (s, 3H, N1-CH_3), 3.35 (s, 3H, N3-CH_3), 2.93 ppm (t, ^3J = 5.4 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.9 (C10a), 136.5 (C1, phe), 129.1 (C2/C6, phe), 128.6 (C3/C5, phe), 127.9 (C4, phe), 106.5 (C4a), 61.9 (N8-CH_2), 51.2 (C7), 48.7 (C9), 44.2 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 326.3 $[M+H]^+$; HPLC: 99.3% (C).

8-(2-Chlorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (11): Yield: 70%; mp: 233 °C; ^{1}H NMR ($CDCl_3$): δ = 7.46–7.45 (m, 1H, C3-H, phe), 7.40–7.38 (m, 1H, C6-H, phe), 7.27–7.23 (m, 2H, C4-/C5-H, phe), 4.37 (t, ^3J = 5.3 Hz, 2H, C6-H_2), 3.89 (s, 2H, N8-CH_2), 3.84 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.38 (s, 3H, N3-CH_3), 3.04 ppm (t, ^3J = 5.2 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 154.9 (C9a), 151.7 (C4), 148.4 (C2), 147.7 (C10a), 134.5 (C1, phe), 133.7 (C2, phe), 130.9 (C3, phe), 129.8 (C5, phe), 129.1 (C6, phe), 127.0 (C4, phe), 106.6 (C4a), 58.3 (N8-CH_2), 51.1 (C7), 48.9 (C9), 44.2 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 360.1 $[M+H]^+$; HPLC: 97.4% (A) and 97.7% (B).

8-(3-Chlorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (12): Yield: 68%; mp: 214 °C; ^{1}H NMR ($CDCl_3$): δ = 7.35 (s, 1H, C2-H, phe), 7.28–7.26 (m, 2H, C4-/C5-H, phe), 7.23–7.22 (m, 1H, C6-H, phe), 4.35 (t, ^3J = 5.5 Hz, 2H, C6-H_2), 3.74 (s, 2H, N8-CH_2), 3.72 (s, 2H, C9-H_2), 3.52 (s, 3H, N1-CH_3), 3.38 (s, 3H, N3-CH_3), 2.94 ppm (t, ^3J = 5.5 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 154.9 (C9a), 151.7 (C4), 148.4 (C2), 147.8 (C10a), 138.7 (C1, phe), 134.6 (C3, phe), 129.9 (C5, phe), 128.9 (C2, phe), 128.0 (C4, phe), 127.0 (C6, phe), 106.6 (C4a), 61.3 (N8-CH_2), 51.2 (C7), 48.8 (C9), 44.3 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 360.3 $[M+H]^+$; HPLC: 98.1% (A) and 98.0% (B).

8-(4-Chlorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (13): Purification by column chromatography. Yield: 50%; mp: 191 °C; ^{1}H NMR ($CDCl_3$): δ = 7.31 (d, ^3J = 8.5 Hz, 2H, C3-/C5-H, phe), 7.28 (d, ^3J = 8.5 Hz, 2H, C2-/C6-H, phe), 4.35 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.73 (s, 1H, N8-CH_2), 3.71 (s, 2H, C9-H_2), 3.51 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 2.94 ppm (t, ^3J = 5.3 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 155.0 (C9a), 151.7 (C4),

148.5 (C2), 147.6 (C10a), 133.7 (C1, phe), 130.3 (C4, phe), 130.3 (C3/C5, phe), 128.9 (C2/C6, phe), 106.6 (C4a), 61.2 (N8-CH_2), 51.2 (C7), 48.8 (C9), 44.2 (C6), 29.7 (N1-CH_3), 27.9 ppm (N3-CH_3); ESI-MS: positive mode 360.1 $[M+H]^+$; HPLC: 99.9% (A) and 99.8% (B).

8-(2-Fluorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (14): Yield: 55%; mp: 198 °C; ^{1}H NMR ($CDCl_3$): $\delta=7.40$–7.36 (m, 1H, C4-H, phe), 7.32–7.27 (m, C3-H, phe), 7.16–7.13 (dd, $^3J=6.3$ Hz, $^4J=1.3$ Hz, 1H, C6-H, phe), 7.10–7.06 (m, 2H, C5-H, phe), 4.36 (t, $^3J=5.4$ Hz, 2H, C6-H_2), 3.84 (s, 2H, N8-CH_2), 3.80 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.39 (s, 3H, N3-CH_3), 3.00 ppm (t, $^3J=5.4$ Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): $\delta=161.4$ (d, $^1J_{C,F}=245.4$ Hz, C2, phe), 155.0 (C9a), 151.7 (C4), 148.5 (C2), 148.0 (C10a), 131.4 (d, $^3J_{C,F}=4.0$ Hz, C6, phe), 129.7 (d, $^3J_{C,F}=8.2$ Hz, C4, phe), 124.3 (d, $^4J_{C,F}=3.5$ Hz, C5, phe), 123.1 (d, $^2J_{C,F}=14.4$ Hz, C1, phe), 115.6 (d, $^2J_{C,F}=22.0$ Hz, C3, phe), 106.6 (C4a), 54.4 (d, $J_{C,F}=1.5$ Hz, N8-CH_2), 51.0 (C7), 48.8 (C9), 44.3 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 344.3 $[M+H]^+$; HPLC: 99.9% (A) and 99.9% (B).

8-(3-Fluorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (15): Purification by column chromatography. Yield: 41%; mp: 207 °C; ^{1}H NMR ($CDCl_3$): $\delta=7.32$–7.29 (m, 1H, C5-H, phe), 7.12–7.10 (m, 1H, C4-H, phe), 7.09–7.07 (m, 1H, C6-H, phe), 7.00–6.97 (m, 1H, C2-H, phe), 4.35 (t, $^3J=5.4$ Hz, 2H, C6-H_2), 3.75 (s, 2H, N8-CH_2), 3.74 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.38 (s, 3H, N3-CH_3), 2.96 ppm (t, $^3J=5.5$ Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): $\delta=160.0$ (d, $^1J_{C,F}=246.5$ Hz, C3, phe), 155.0 (C9a), 151.7 (C4), 148.4 (C2), 147.8 (C10a), 139.2 (d, $^3J_{C,F}=7.0$ Hz, C1, phe), 130.1 (d, $^3J_{C,F}=8.2$ Hz, C5, phe), 124.4 (d, $^4J_{C,F}=2.6$ Hz, C6, phe), 115.7 (d, $^2J_{C,F}=21.5$ Hz, C2, phe), 114.8 (d, $^2J_{C,F}=21.1$ Hz, C4, phe), 106.5 (C4a), 61.3 (N8-CH_2), 51.3 (C7), 48.8 (C9), 44.3 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 344.3 $[M+H]^+$; HPLC: 97.7% (A) and 96.8% (B).

8-(4-Fluorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (16): Purification by column chromatography. Yield: 31%; mp: 188 °C; ^{1}H NMR ($CDCl_3$): $\delta=7.31$–7.29 (m, 2H, C2-/C6-H, phe), 7.03–7.00 (m, 2H, C3-/C5-H, phe), 4.34 (t, $^3J=5.1$ Hz, 2H, C6-H_2), 3.72 (s, 2H, N8-CH_2), 3.71 (s, 2H, C9-H_2), 3.50 (s, 3H, N1-CH_3), 3.36 (s, 3H, N3-CH_3), 2.94 ppm (t, $^3J=4.8$ Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): $\delta=162.4$ (d, $^1J_{C,F}=246.5$ Hz, C4, phe), 155.0 (C9a), 151.7 (C4), 148.4 (C2), 147.7 (C10a), 132.0 (C1, phe), 130.6 (d, $^3J_{C,F}=7.8$ Hz, C2/C6, phe), 115.4 (d, $^2J_{C,F}=21.4$ Hz, C3/C5, phe), 106.6 (C4a), 61.1 (N8-CH_2), 51.1 (C7), 48.7 (C9), 44.2 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 344.3 $[M+H]^+$; HPLC: 97.5% (A) and 97.4% (B).

8-(2-Bromobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (17): Yield: 61%; mp: 219 °C; ^{1}H NMR ($CDCl_3$): $\delta=7.55$ (dd, $^3J=8.2$ Hz, $^4J=1.3$ Hz, 1H, C3-H), 7.44 (d, $^3J=7.3$ Hz, 1H, C6-H, phe), 7.30–7.27 (m, 1H, C4-H, phe), 7.14 (dd, $^3J=7.6$ Hz, $^3J=7.6$ Hz, 1H, C5-H, phe), 4.35 (t, $^3J=5.4$ Hz, 2H, C6-H_2), 3.86 (s, 2H, N8-CH_2), 3.83 (s, 2H, C9-H_2), 3.51 (s, 3H, N1-CH_3), 3.36 (s, 3H, N3-CH_3), 3.02 ppm (t, $^3J=5.4$ Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): $\delta=154.9$ (C9a), 151.7 (C4), 148.4 (C2), 147.7 (C10a), 135.6 (C1, phe), 133.1 (C3, phe), 131.0 (C6, phe), 129.4 (C4, phe), 127.6 (C5, phe), 124.8 (C2, phe), 106.6 (C4a), 60.8 (N8-CH_2), 51.0 (C7), 48.8 (C9), 44.2 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 404.0 and 406.1 $[M+H]^+$; HPLC: 97.5% (A) and 97.3% (B).

8-(3-Bromobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (18): Yield: 74%; mp: 204 °C; ^{1}H NMR ($CDCl_3$): $\delta=7.58$ (s, 1H, C2-H, phe), 7.51 (dd, $^3J=8.2$ Hz, $^4J=1.2$ Hz, 1H, C4-H, phe), 7.45 (d, $^3J=6.9$ Hz, 1H, C6-H, phe), 7.30–7.27 (m, 1H, C5-H, phe), 7.14 (dd, $^3J=7.6$ Hz, $^3J=7.6$ Hz, 1H, C5-H, phe), 4.55 (t, $^3J=5.4$ Hz, 2H, C6-H_2), 3.93 (br s, 4H, N8-CH_2, C9-H_2), 3.52 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 3.18 ppm (t, $^3J=5.1$ Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): $\delta=154.9$ (C9a), 151.7 (C4), 148.4 (C2), 147.7 (C10a), 132.7 (C1, phe), 132.2 (C2, phe), 130.7 (C4, phe), 128.5 (C5, phe), 128.4 (C6, phe), 123.1 (C3, phe), 106.8 (C4a), 60.7 (N8-CH_2), 50.1 (C7), 48.5 (C9), 42.9 (C6), 29.8 (N1-CH_3), 27.9 ppm (N3-CH_3); ESI-MS: positive mode 404.0 and 406.1 $[M+H]^+$; HPLC: 97.5% (A) and 97.3% (B).

8-(4-Bromobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (19): Purification by column chromatography. Yield: 44%; mp: 231 °C; ^{1}H NMR ($CDCl_3$): $\delta=7.45$ (d, $^3J=8.2$ Hz, C3-/C5-H, 2H, phe), 7.44 (d, $^3J=8.2$ Hz, 2H, C2/C6-H, phe), 4.32 (t, $^3J=5.4$ Hz, 2H, C6-H_2), 3.72 (s, 2H, N8-CH_2), 3.67 (s, 2H, C9-H_2), 3.51 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 2.92 ppm (t, $^3J=5.4$ Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): $\delta=154.9$ (C9a), 151.7 (C4), 148.4 (C2), 147.7 (C10a), 135.6 (C1, phe), 131.0 (C3/C5, phe), 130.6 (C2/C6, phe), 121.7 (C4, phe), 106.6 (C4a), 61.2 (N8-CH_2), 51.0 (C7), 48.8 (C9), 44.2 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 404.0 and 406.1 $[M+H]^+$; HPLC: 96.4% (A) and 98.3% (B).

8-(3-Iodobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (20): Purification by column chromatography. Yield: 38%; mp: 218 °C; ^{1}H NMR ($CDCl_3$): $\delta=7.79$ (s, 1H, C2-H, phe), 7.73 (d, $^3J=7.9$ Hz, 1H, C4-H, phe), 7.55 (d, $^3J=6.7$ Hz, 1H, C6-H, phe), 7.17–7.13 (m, C5-H, phe), 4.60 (t, $^3J=5.4$ Hz, 2H, C6-H_2), 3.99 (s, 2H, N8-CH_2), 3.98 (s, 2H, C9-H_2), 3.52 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 3.25 ppm (t, $^3J=5.1$ Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): $\delta=154.9$ (C9a), 151.7 (C4), 148.4 (C2), 147.7 (C10a), 139.1 (C2, phe), 138.7 (C1, phe), 130.9 (C4, phe), 129.3 (C^5, phe), 128.7 (C^6, phe), 106.8 (C4a), 94.0 (C^3, phe), 60.7 (N8-CH_2), 50.1 (C7), 48.5 (C9), 42.9 (C6), 29.8 (N1-CH_3), 27.9 ppm (N3-CH_3); ESI-MS: positive mode 404.0 and 406.1 $[M+H]^+$; HPLC: 97.5% (A) and 97.3% (B).

8-(2-(Trifluoromethyl)benzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (21): Purification by column chromatography. Yield: 60%; mp: 236 °C; ^{1}H NMR ($CDCl_3$): $\delta=7.75$ (d, $^3J=7.5$ Hz, 1H, C6-H, phe), 7.67 (d, $^3J=7.8$ Hz, 1H, C2-H, phe), 7.54 (dd, $^3J=7.5$ Hz, $^3J=7.5$ Hz, 1H, C5-H, phe), 7.39 (dd, $^3J=7.6$ Hz, $^3J=7.6$, 1H, C4-H, phe), 4.37 (t, $^3J=5.4$ Hz, 2H, C6-H_2), 3.92 (s, 2H, N8-CH_2), 3.80 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.38 (s, 3H, N3-CH_3), 2.99 ppm (t, $^3J=5.4$ Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): $\delta=155.0$ (C9a), 151.7 (C4), 148.4 (C2), 147.7 (C10a), 135.6 (C1, phe), 132.1 (C^5, phe), 130.5 (C^6, phe), 128.9 (q, $^2J_{C,F}=30.3$ Hz, C2, phe), 127.7 (C4, phe), 126.1 (q, $^3J_{C,F}=5.7$ Hz, C3, phe), 124.2 (q, $^1J_{C,F}=273.9$ Hz, CF_3), 106.6 (C4a), 57.4 (N8-CH_2), 51.3 (C7), 49.0 (C9), 44.3 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 394.4 $[M+H]^+$; HPLC: 98.7% (A) and 99.0% (B).

8-(3-(Trifluoromethyl)benzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (22): Yield: 48%; mp: 165 °C; ^{1}H NMR ($CDCl_3$): $\delta=7.67$–7.64 (m, 2H, C2-/C6-H, phe), 7.60–7.59 (m, 1H, C5-H, phe), 7.52–7.49 (m, 1H, C4-H, phe), 4.46 (t, $^3J=5.4$ Hz, 2H, C6-H_2), 3.91 (s, 2H, N8-CH_2), 3.85 (s, 2H, C9-H_2), 3.52 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 3.09 ppm (t, $^3J=5.3$ Hz, 2H, C7-H_2); ESI-MS: positive mode 394.4 $[M+H]^+$; HPLC: 97.5% (A) and 97.2% (B).

8-(2-Methoxybenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino-[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (23): Yield: 65%; mp: 181 °C; ^{1}H NMR ($CDCl_3$): $\delta=7.29$ (1H, d, $^3J=7.2$ Hz, C6-H, phe), 7.14–7.11 (m, 1H, C4-H, phe), 6.89–6.86 (m, 1H, C5-H, phe), 6.83 (d, $^3J=8.2$ Hz, 1H, C3-H, phe), 4.60 (t, $^3J=5.4$ Hz, 2H, C6-H_2), 3.99 (s, 2H, N8-CH_2), 3.85 (br s, 5H, C9-H_2, OCH_3), 3.52 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 3.25 ppm (t, $^3J=5.1$ Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$):

δ = 165.8 (C2, phe), 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.6 (C10a), 130.2 (C4 and C^6, phe), 127.5 (C1, phe), 121.2 (C^5, phe), 111.0 (C^3, phe), 106.5 (C4a), 55.5 (N8-CH_2), 55.6 (OCH_3), 50.1 (C7), 48.5 (C9), 42.9 (C6), 29.8 (N1-CH_3), 27.9 ppm (N3-CH_3); ESI-MS: positive mode 355.9 $[M+H]^+$; HPLC: 99.6% (A) and 99.7% (B).

8-(3-Methoxybenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino-[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (24): Yield: 73%; mp: 141 °C; ^{1}H NMR ($CDCl_3$): δ = 7.22 (dd, ^3J = 7.2 Hz, ^3J = 7.6 Hz, 1H, C5-H, phe), 6.91 (s, 1H, C2-H, phe), 6.90 (d, ^3J = 6.8 Hz, 1H, C4-H, phe), 6.82 (dd, ^3J = 7.3 Hz, ^4J = 1.6 Hz, 1H, C6-H, phe), 4.35 (br s, 2H, C6-H_2), 3.78–3.74 (m, 7H, N8-CH_2, C9-H_2, OCH_3), 3.52 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 2.96 ppm (br s, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 159.9 (C^3, phe), 155.0 (C9a), 151.7 (C4), 148.4 (C2), 147.7 (C10a), 136.1 (C1, phe), 129.7 (C^5, phe), 121.4 (C^6, phe), 114.6 (C4, phe), 113.4 (C2, phe), 106.6 (C4a), 61.8 (N8-CH_2), 55.2 (OCH_3), 51.1 (C7), 48.7 (C9), 44.0 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 355.9 $[M+H]^+$; HPLC: 99.0% (A) and 99.0% (B).

8-(4-Methoxybenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino-[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (25): Purification by column chromatography. Yield: 33%; mp: 164 °C; ^{1}H NMR ($CDCl_3$): δ = 7.30 (d, ^3J = 8.5 Hz, 2H, C2-/C6-H, phe), 6.87 (d, ^3J = 8.5 Hz, 2H, C3-/C5-H, phe), 4.35 (br s, 2H, C6-H_2), 3.78–3.74 (br s, 7H, N8-CH_2, C9-H_2, OCH_3), 3.52 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 2.96 ppm (br s, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 159.9 (C4, phe), 154.9 (C9a), 151.7 (C4), 148.4 (C2), 147.7 (C10a), 130.9 (C2/C6, phe), 128.9 (C1, phe), 114.3 (C3/C5, phe), 105.9 (C4a), 61.8 (N8-CH_2), 55.2 (OCH_3), 51.1 (C7), 48.7 (C9), 44.0 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 355.9 $[M+H]^+$; HPLC: 99.0% (A) and 99.0% (B).

1,3-Dimethyl-8-(3-(thiazol-2-yl)benzyl)-6,7,8,9-tetrahydro-pyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (26): Yield: 61%; mp: 171 °C; ^{1}H NMR ($CDCl_3$): δ = 7.98 (s, 1H, C2-H, phe), 7.89–7.87 m, 2H, C4-H, thiazol, C4-H, phe), 7.45–7.40 (m, 2H, C5-H, Phe, C5-H, thiazol), 7.35 (d, ^3J = 3.3 Hz, 1H, C6-H, phe), 4.37 (t, ^3J = 5.5 Hz, 2H, C6-H_2), 3.81 (s, 2H, N8-CH_2), 3.79 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.39 (s, 3H, N3-CH_3), 3.00 ppm (t, ^3J = 5.5 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 168.0 (C2, thiazol), 155.0 (C9a), 151.8 (C4), 148.5 (C2), 148.0 (C10a), 143.7 (C4, thiazol), 137.7 (C1, phe), 134.0 (C^3, phe), 130.5 (C^6, phe), 129.3 (C^5, phe), 126.9 (C2, phe), 126.2 (C4, phe), 119.0 (C^5, thiazol), 106.8 (C4a), 61.6 (N8-CH_2), 50.3 (C7), 48.5 (C9), 44.4 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 409.4 $[M+H]^+$; HPLC: 99.0% (A) and 97.3% (B).

1,3-Dimethyl-8-(3-(thiophen-2-yl)benzyl)-6,7,8,9-tetrahydro-pyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (27): Purification by column chromatography. Yield: 66%; mp: 195 °C; ^{1}H NMR ($CDCl_3$): δ = 7.59 (s, 1H, C2-H, phe), 7.57–7.55 (m, 1H, C4-H, phe), 7.36 (dd, ^3J = 7.7 Hz, ^3J = 7.7 Hz, 1H, C5-H, phe), 7.32 (dd, ^3J = 3.6 Hz, ^4J = 1.1 Hz, 1H, C3-H, thienyl), 7.27–7.26 (m, 1H, C5-H, thienyl), 7.25 (br s, 1H, C6-H, phe), 7.08 (dd, ^3J = 5.1 Hz, ^3J = 3.6 Hz, 1H, C4-H, thienyl), 4.35 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.77 (s, 2H, N8-CH_2), 3.76 (s, 2H, C9-H_2), 3.52 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 2.97 ppm (t, ^3J = 5.1 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 155.0 (C9a), 151.8 (C4), 148.5 (C2), 148.0 (C10a), 144.0 (C1, thienyl), 137.3 (C1, phe), 134.8 (C^3, phe), 129.2 (C2, phe), 128.0 (C5/C6, phe), 126.4 (C^5, thienyl), 125.4 (C4, thienyl), 125.0 (C^3, thienyl), 123.3 (C4, phe), 106.6 (C4a), 61.7 (N8-CH_2), 50.1 (C7), 48.5 (C9), 42.9 (C6), 29.8 (N1-CH_3), 27.9 ppm (N3-CH_3); ESI-MS: positive mode 408.4 $[M+H]^+$; HPLC: 99.9% (A) and 99.1% (B).

8-(3-(1*H*-Pyrrol-1-yl)benzyl)-1,3-dimethyl-6,7,8,9-tetrahydro-pyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (28): Yield: 58%; mp: 188 °C; ^{1}H NMR ($CDCl_3$): δ = 7.42–7.41 (m, 1H, C2-H, phe), 7.40 (dd, ^3J = 7.7 Hz, ^3J = 7.7 Hz, 1H, C5-H, phe), 7.34 (ddd, ^3J = 8.0 Hz, ^4J = 2.2 Hz, ^4J = 1.3 Hz, 1H, C4-H, phe), 7.22 (ddd, ^3J = 7.6 Hz, ^4J = 1.3 Hz, ^4J = 1.3 Hz, 1H, C6-H, phe), 7.09–7.08 (m, 2H, C2-/C5-H, pyrrolyl), 6.35–6.34 (m, 2H, C3-/C4-H, pyrrolyl), 4.37 (t, ^3J = 5.5 Hz, 2H, C6-H_2), 3.79 (s, 2H, N8-CH_2), 3.78 (s, 2H, C9-H_2), 3.54 (s, 3H, N1-CH_3), 3.40 (s, 3H, N3-CH_3), 3.00 ppm (t, ^3J = 5.5 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 155.0 (C9a), 151.8 (C4), 148.5 (C2), 148.0 (C10a), 141.1 (C^3, phe), 138.4 (C1, phe), 129.8 (C^5, phe), 126.0 (C^6, phe), 120.8 (C4, phe), 119.9 (C2, phe), 119.3 (C2/C5, pyrrolyl), 110.6 (C3/C4, pyrrolyl), 106.6 (C4a), 61.7 (N8-CH_2), 51.3 (C7), 48.9 (C9), 44.3 (C6), 29.7 (N1-CH_3), 27.9 ppm (N3-CH_3); ESI-MS: positive mode 391.4 $[M+H]^+$; HPLC: 97.7% (A) and 97.5% (B).

1,3-Dimethyl-8-(thiophen-3-yl)-6,7,8,9-tetrahydropyrazino[2,1-*f*]-purine-2,4(1*H*,3*H*)-dione (29): Yield: 66%; mp: 212 °C; ^{1}H NMR ($CDCl_3$): δ = 7.30 (d, ^3J = 4.8 Hz, 1H, C5-H, thienyl), 7.16 (s, 1H, C2-H, thienyl), 7.06 (d, ^3J = 4.8 Hz, 1H, C4-H, thienyl), 4.33 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.75 (s, 2H, N8-CH_2), 3.72 (s, 2H, C9-H_2), 3.52 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 2.93 ppm (t, ^3J = 5.4 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 155.0 (C9a), 151.8 (C4), 148.5 (C2), 148.0 (C10a), 137.4 (C^3, thienyl), 128.0 (C4, thienyl), 126.3 (C^5, thienyl), 123.5 (C2, thienyl), 106.8 (C4a), 61.7 (N8-CH_2), 50.1 (C7), 48.5 (C9), 42.9 (C6), 29.8 (N1-CH_3), 27.9 ppm (N3-CH_3); ESI-MS: positive mode 408.4 $[M+H]^+$; HPLC: 99.9% (A) and 99.1% (B).

(*R*,*S*)-8-(1-(4-Fluorophenyl)ethyl)-1,3-dimethyl-6,7,8,9-tetrahydro-pyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (30): Purification by column chromatography. Yield: 50%; mp: 208 °C; ^{1}H NMR ($CDCl_3$): δ = 7.33–7.30 (m, 2H, C2-/C6-H, phe), 7.06–7.03 (m, 2H, C3-/C5-H, phe), 4.32 (br s, 2H, C6-H_2), 3.92–3.90 (m, 1H, N8-CH), 3.73–3.65 (m, 2H, C9-H_2), 3.54 (s, 3H, N1-CH_3), 3.39 (s, 3H, N3-CH_3), 2.93 (br s, 2H, C7-H_2), 1.54 ppm (br s, 3H, CH_3); ^{13}C NMR ($CDCl_3$): δ = 162.3 (d, $^1J_{C,F}$ = 246.6 Hz, C4, phe), 154.9 (C9a), 151.7 (C4), 148.5 (C2), 148.0 (C10a), 130.9 (C1, phe) 129.0 (d, $^3J_{C,F}$ = 7.6 Hz, C^2/C6, phe), 115.6 (d, $^4J_{C,F}$ = 21.2 Hz, C3/C5, phe), 106.5 (C4a), 63.4 (N8-CH), 48.9 (C7), 46.5 (C9), 44.3 (C6), 29.7 (N1-CH_3), 27.8 (N3-CH_3), 19.5 ppm (CH_3); ESI-MS: positive mode 358.3 $[M+H]^+$; HPLC: 97.0% (A) and 97.7% (B).

8-(2,3-Dichlorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino-[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (31): Yield: 59%; mp: 221 °C; ^{1}H NMR ($CDCl_3$): δ = 7.44–7.40 (m, 2H, C4-/C6-H, phe), 7.20–7.18 (m, 1H, C5-H, phe), 4.38 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.91 (s, 2H, N8-CH_2), 3.83 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.36 (s, 3H, N3-CH_3), 3.02 ppm (t, ^3J = 5.4 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.6 (C10a), 136.3 (C1, phe), 133.6 (C2, phe), 132.7 (C^3, phe), 130.0 (C4, phe), 128.8 (C^6, phe), 127.4 (C^5, phe), 106.5 (C4a), 58.9 (N8-CH_2), 51.1 (C7), 49.0 (C9), 44.1 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 394.1 $[M+H]^+$; HPLC: 97.3% (A) and 97.2% (B).

8-(2,6-Dichlorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino-[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (32): Yield: 55%; mp: 257 °C; ^{1}H NMR ($CDCl_3$): δ = 7.33 (d, ^3J = 8.4 Hz, 2H, C3-H/C5-H, phe), 7.18 (dd, ^3J = 8.4 Hz, 1H, C4-H, phe), 4.38 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.91 (s, 2H, N8-CH_2), 3.83 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.36 (s, 3H, N3-CH_3), 3.02 ppm (t, ^3J = 5.4 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.6 (C10a), 137.0 (C2/C6, phe), 133.4 (C1, phe), 129.7 (C4, phe), 128.6 (C3/C5, phe), 106.5 (C4a), 55.4 (N8-CH_2), 51.1 (C7), 49.0 (C9), 44.1 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 394.1 $[M+H]^+$; HPLC: 97.3% (A) and 97.2% (B).

8-(2,4-Dichlorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino-[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (33): Yield: 58%; mp: 149 °C; ^{1}H NMR ($CDCl_3$): δ = 7.58 (d, ^3J = 7.9 Hz, 1H, C6-H, phe), 7.43 (d, ^4J = 1.9 Hz, 1H, C3-H, phe), 7.28 (dd, ^3J = 8.2 Hz, ^4J = 1.9 Hz, 1H, C5-H, phe), 4.33 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.99 (s, 2H, N8-CH_2), 3.93 (s,

2H, C9-H_2), 3.51 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 3.15 ppm (t, ^3J = 5.7 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.6 (C10a), 137.7 (C2, phe), 135.3 (C1, phe), 132.5 (C4, phe), 129.8 (C3/C6, phe), 127.8 (C^5, phe), 106.5 (C4a), 57.2 (N8-CH_2), 51.2 (C7), 48.8 (C9), 44.3 (C6), 29.8 (N1-CH_3), 27.9 ppm (N3-CH_3); ESI-MS: positive mode 394.3 $[M+H]^+$; HPLC: 98.1% (A) and 98.6% (B).

8-(3,5-Dichlorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino-[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (34): Yield: 58%; mp: 240 °C; ^{1}H NMR ($CDCl_3$): δ = 7.28 (d, ^4J = 1.9 Hz, 2H, C2-/C6-H, phe), 7.24 (s, 1H, C4-H, phe), 4.36 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.73 (s, 2H, N8-CH_2), 3.69 (s, 2H, C9-H_2), 3.51 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 2.96 ppm (t, ^3J = 5.4 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.6 (C10a), 140.0 (C1, phe), 135.3 (C3/C5, phe), 128.1 (C4, phe), 127.2 (C2/C6, phe), 106.6 (C4a), 60.8 (N8-CH_2), 51.2 (C7), 48.9 (C9), 44.1 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 394.3 $[M+H]^+$; HPLC: 99.7% (A) and 99.6% (B).

8-(2,5-Dichlorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino-[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (35): Yield: 74%; mp: 208 °C; ^{1}H NMR ($CDCl_3$): δ = 7.49 (d, ^4J = 2.5 Hz, 1H, C6-H, phe), 7.30 (d, ^3J = 8.5 Hz, 1H, C3-H, phe), 7.21 (dd, ^3J = 8.5 Hz, ^4J = 2.5 Hz, 1H, C4-H, phe), 4.36 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.86 (s, 2H, N8-CH_2), 3.84 (s, 2H, C9-H_2), 3.52 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 3.04 ppm (t, ^3J = 5.4 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.6 (C10a), 135.6 (C1, phe), 133.1 (C2, phe), 132.5 (C^5, phe), 130.9 (C^6, phe), 130.6 (C^3, phe), 129.2 (C4, phe), 106.6 (C4a), 57.9 (N8-CH_2), 51.0 (C7), 48.9 (C9), 44.1 (C6), 29.7 (N1-CH_3), 27.9 ppm (N3-CH_3); ESI-MS: positive mode 394.3 $[M+H]^+$; HPLC: 98.6% (A) and 99.1% (B).

8-(3,4-Dichlorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino-[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (36): Yield: 81%; mp: 201 °C; ^{1}H NMR ($CDCl_3$): δ = 7.44 (d, ^4J = 1.9 Hz, 1H, C2-H, phe), 7.40 (d, ^3J = 8.2 Hz, 1H, C5-H, phe), 7.17 (dd, ^3J = 8.2 Hz, ^4J = 2.4 Hz, 1H, C6-H, phe), 4.33 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.72 (s, 2H, N8-CH_2), 3.68 (s, 2H, C9-H_2), 3.51 (s, 3H, N1-CH_3), 3.36 (s, 3H, N3-CH_3), 2.93 ppm (t, ^3J = 5.4 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 155.0 (C9a), 151.7 (C4), 149.5 (C2), 147.6 (C10a), 137.0 (C1, phe), 132.8 (C^3, phe), 131.9 (C4, phe), 130.7 and 130.6 (C2 and C^5, phe), 128.1 (C^6, phe), 106.5 (C4a), 60.7 (N8-CH_2), 51.3 (C7), 48.8 (C9), 44.3 (C6), 29.7 (N1-CH_3), 27.9 ppm (N3-CH_3); ESI-MS: positive mode 394.3 $[M+H]^+$; HPLC: 98.0% (A) and 97.9% (B).

8-(2,6-Difluorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino-[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (37): Yield: 45%; mp: 220 °C; ^{1}H NMR ($CDCl_3$): δ = 7.35–7.29 (m, 1H, C4-H, phe), 6.99–6.93 (m, 2H, C3-/C5-H, phe), 4.40 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.98 (s, 2H, N8-CH_2), 3.86 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.38 (s, 3H, N3-CH_3), 3.04 ppm (t, ^3J = 5.4 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 162.0 (d, $^1J_{C,F}$ = 249.2 Hz, phe), 161.9 (d, $^1J_{C,F}$ = 249.3 Hz, phe), 154.9 (C9a), 151.7 (C4), 148.4 (C2), 147.4 (C10a), 130.4 (dd, $^3J_{C,F}$ = 10.5 Hz, $^3J_{C,F}$ = 10.5 Hz, C4, phe), 111.5 (d, $^2J_{C,F}$ = 25.9 Hz, C3/C5, phe), 111.5 (d, $^2J_{C,F}$ = 15.7 Hz, C1, phe), 106.5 (C4a), 50.1 (N8-CH_2), 48.4 (C7), 47.9 (C9), 44.0 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 362.8 $[M+H]^+$; HPLC: 97.4% (A) and 97.8% (B).

8-(2,4-Difluorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino-[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (38): Purification by column chromatography. Yield: 43%; mp: 241 °C; ^{1}H NMR ($CDCl_3$): δ = 7.41–7.37 (m, 1H, C3-H, phe), 6.91–6.82 (m, 2H, C5-/C6-H, phe), 4.38 (t, ^3J = 5.1 Hz, 2H, C6-H_2), 3.82 (s, 2H, N8-CH_2), 3.80 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.38 (s, 3H, N3-CH_3), 3.02 ppm (t, ^3J = 4.9 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 162.7 (dd, $^1J_{C,F}$ = 249.8 Hz, $^3J_{C,F}$ = 12.0 Hz, C2, phe), 161.4 (dd, $^1J_{C,F}$ = 249.6 Hz, $^3J_{C,F}$ = 11.9 Hz, C4, phe), 154.9 (C9a), 151.7 (C4), 148.4 (C2), 147.3 (C10a), 132.4 (dd, $^3J_{C,F}$ = 7.8 Hz, $^3J_{C,F}$ = 5.7 Hz, C6, phe), 118.6 (C1, phe), 111.6 (dd, $^2J_{C,F}$ = 21.2 Hz, $^4J_{C,F}$ = 2.9 Hz, C5, phe), 106.6 (C4a), 104.4 (dd, $^2J_{C,F}$ = 25.7 Hz, $^2J_{C,F}$ = 25.7 Hz, C3, phe), 53.8 (N8-CH_2), 50.7 (C7), 48.6 (C9), 44.1 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 362.1 $[M+H]^+$; HPLC: 99.9% (A) and 99.9% (B).

8-(2,5-Difluorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino-[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (39): Yield: 31%; mp: 216 °C; ^{1}H NMR ($CDCl_3$): δ = 7.24–7.20 (m, 1H, C3-H, phe), 7.08–6.98 (m, 2H, C4-/C6-H, phe), 4.45 (t, ^3J = 5.3 Hz, 2H, C6-H_2), 3.90 (s, 2H, N8-CH_2), 3.88 (s, 2H, C9-H_2), 3.54 (s, 3H, N1-CH_3), 3.39 (s, 3H, N3-CH_3), 3.10 ppm (t, ^3J = 5.1 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 158.8 (dd, $^1J_{C,F}$ = 243.7 Hz, $^4J_{C,F}$ = 1.6 Hz, C2, phe), 157.2 (dd, $^1J_{C,F}$ = 242.5 Hz, $^4J_{C,F}$ = 2.3 Hz, C5, phe), 154.9 (C9a), 151.7 (C4), 148.5 (C2), 147.5 (C10a), 123.8–123.7 (m, C1, phe)117.7 (d, $^2J_{C,F}$ = 26.2 Hz, C6, phe), 116.8 (dd, $^2J_{C,F}$ = 25.2 Hz, $^3J_{C,F}$ = 8.4 Hz, C3/C4, phe), 106.7 (C4a), 53.8 (N8-CH_2), 50.6 (C7), 48.7 (C9), 43.8 (C6), 29.8 (N1-CH_3), 27.9 ppm (N3-CH_3); ESI-MS: positive mode 362.1 $[M+H]^+$; HPLC: 99.9% (A) and 99.9% (B).

8-(3,5-Difluorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino-[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (40): Yield: 71%; mp: 209 °C; ^{1}H NMR ($CDCl_3$): δ = 6.92–6.89 (m, 1H, C4-H, phe), 6.77–6.73 (m, 2H, C2-/C6-H, phe), 4.38 (t, ^3J = 5.5 Hz, 2H, C6-H_2), 3.76 (s, 2H, N8-CH_2), 3.73 (s, 2H, C9-H_2), 3.54 (s, 3H, N1-CH_3), 3.39 (s, 3H, N3-CH_3), 2.98 ppm (t, ^3J = 5.4 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 163.2 (dd, $^1J_{C,F}$ = 249.3 Hz, $^1J_{C,F}$ = 12.7 Hz, C3/C5, phe), 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.6 (C10a), 140.7 (dd, $^3J_{C,F}$ = 8.9 Hz, $^3J_{C,F}$ = 8.9 Hz, C1, phe), 111.4 (dd, $^2J_{C,F}$ = 19.5 Hz, $^3J_{C,F}$ = 5.9 Hz, C^2/C6, phe), 106.5 (C4a), 103.3 (dd, $^2J_{C,F}$ = 25.3 Hz, $^2J_{C,F}$ = 25.3 Hz, C4, phe), 61.0 (N8-CH_2), 51.3 (C7), 48.9 (C9), 44.3 (C6), 29.7 (N1-CH_3), 27.9 ppm (N3-CH_3); ESI-MS: positive mode 362.1 $[M+H]^+$; HPLC: 99.9% (A) and 99.9% (B).

8-(3,4-Difluorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino-[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (41): Yield: 59%; mp: 220 °C; ^{1}H NMR ($CDCl_3$): δ = 7.23–7.19 (m, 1H, C6-H, phe), 7.16–7.11 (m, C5-H, phe), 7.08–7.05 (m, 1H, C2-H, phe), 4.36 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.74 (s, 2H, N8-CH_2), 3.71 (s, 2H, C9-H_2), 3.54 (s, 3H, N1-CH_3), 3.39 (s, 3H, N3-CH_3), 2.96 ppm (t, ^3J = 5.4 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 155.0 (C9a), 151.7 (C4), 150.5 (dd, $^1J_{C,F}$ = 248.9 Hz, $^2J_{C,F}$ = 12.6 Hz, C3, phe), 149.4 (dd, $^1J_{C,F}$ = 248.5 Hz, $^2J_{C,F}$ = 12.7 Hz, C4, phe), 148.5 (C2), 147.6 (C10a), 133.6 (d, $^3J_{C,F}$ = 4.5 Hz, C1, phe), 124.7 (dd, $^3J_{C,F}$ = 5.9 Hz, $^4J_{C,F}$ = 3.6 Hz, C6, phe), 117.6 (d, $^2J_{C,F}$ = 17.4 Hz, C2, phe), 117.4 (d, $^2J_{C,F}$ = 17.2 Hz, C5, phe), 106.6 (C4a), 60.8 (N8-CH_2), 51.2 (C7), 48.8 (C9), 44.2 (C6), 29.7 (N1-CH_3), 27.9 ppm (N3-CH_3); ESI-MS: negative mode 360.0 $[M-H]^-$, positive mode 362.1 $[M+H]^+$; HPLC: 99.9% (A) and 99.9% (B).

8-(2-Chloro-4-fluorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (42): Yield: 70%; mp: 223 °C; ^{1}H NMR ($CDCl_3$): δ = 7.46–7.43 (m, 1H, C3-H, phe), 7.16–7.14 (m, 1H, C6-H, phe), 7.01–6.98 (m, 1H, C5-H, phe), 4.37 (t, ^3J = 5.3 Hz, 2H, C6-H_2), 3.85 (s, 2H, N8-CH_2), 3.82 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.38 (s, 3H, N3-CH_3), 3.03 ppm (t, ^3J = 5.3 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 161.9 (d, $^1J_{C,F}$ = 250.4 Hz, C4, phe), 155.0 (C9a), 151.7 (C4), 148.4 (C2), 147.6 (C10a), 135.2 (d, $^3J_{C,F}$ = 10.2 Hz, C2, phe), 132.0 (d, $^4J_{C,F}$ = 8.2 Hz, C6, phe), 130.0 (C1, phe), 117.1 (d, $^3J_{C,F}$ = 24.6 Hz, C3, phe), 114.3 (d, $^3J_{C,F}$ = 21.0 Hz, C5, phe), 106.6 (C4a), 57.7 (N8-CH_2), 51.0 (C7), 48.8 (C9), 44.2 (C6), 29.7 (N1-CH_3), 27.9 ppm (N3-CH_3); ESI-MS: positive mode 378.1 $[M+H]^+$; HPLC: 99.9% (A) and 99.9% (B).

8-(4-Chloro-2-fluorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (43): Yield: 53%; mp:

197 °C; ^{1}H NMR ($CDCl_3$): δ = 7.44–7.41 (m, 1H, C3-H, phe), 7.18–7.12 (m, 2H, C5-/C6-H, phe), 4.42 (t, ^3J = 4.8 Hz, 2H, C6-H$_2$), 3.88 (s, 2H, N8-CH$_2$), 3.85 (s, 2H, C9-H$_2$), 3.53 (s, 3H, N1-CH$_3$), 3.38 (s, 3H, N3-CH$_3$), 3.04 ppm (brs, 2H, C7-H$_2$); ^{13}C NMR ($CDCl_3$): δ = 161.1 (d, $^1J_{C,F}$ = 250.6 Hz, C2, phe), 154.9 (C9a), 151.7 (C4), 148.5 (C2), 146.7 (C10a), 135.1 (d, $^3J_{C,F}$ = 6.2 Hz, C4, phe), 132.4 (d, $^3J_{C,F}$ = 2.3 Hz, C6, phe), 125.0 (d, $^4J_{C,F}$ = 2.9 Hz, C5, phe), 120.7 (d, $^2J_{C,F}$ = 15.4 Hz, C1, phe), 116.5 (d, $^2J_{C,F}$ = 25.6 Hz, C3, phe), 106.6 (C4a), 53.7 (N8-CH$_2$), 50.6 (C7), 48.6 (C9), 43.8 (C6), 29.8 (N1-CH$_3$), 27.9 ppm (N3-CH$_3$); ESI-MS: positive mode 378.0 $[M+H]^+$; HPLC: 99.9% (A) and 99.9% (B).

8-(2-Chloro-5-fluorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (44): Purification by column chromatography. Yield: 40%; mp: 242 °C; ^{1}H NMR ($CDCl_3$): δ = 7.36–7.34 (m, C3-H, phe), 7.27–7.24 (m, 1H, C4-H, phe), 6.99–6.95 (m, 1H, C6-H, phe), 4.40 (t, ^3J = 5.4 Hz, 2H, C6-H$_2$), 3.88 (s, 2H, N8-CH$_2$), 3.86 (s, 2H, C9-H$_2$), 3.54 (s, 3H, N1-CH$_3$), 3.39 (s, 3H, N3-CH$_3$), 3.06 ppm (t, ^3J = 5.4 Hz, 2H, C7-H$_2$); ^{13}C NMR ($CDCl_3$): δ = 161.5 (d, $^1J_{C,F}$ = 247.2 Hz, C5, phe), 155.0 (C9a), 151.7 (C4), 148.4 (C2), 147.3 (C10a), 136.1 (d, $^3J_{C,F}$ = 6.0 Hz, C1, phe), 131.0 (d, $^3J_{C,F}$ = 8.1 Hz, C3, phe), 129.0 (d, $^4J_{C,F}$ = 3.0 Hz, C2, phe), 117.5 (d, $^2J_{C,F}$ = 23.7 Hz, C6, phe), 116.1 (d, $^2J_{C,F}$ = 22.9 Hz, C4, phe), 106.6 (C4a), 58.0 (N8-CH$_2$), 51.1 (C7), 49.0 (C9), 44.2 (C6), 29.8 (N1-CH$_3$), 27.9 ppm (N3-CH$_3$); ESI-MS: positive mode 378.6 $[M+H]^+$; HPLC: 99.9% (A) and 99.9% (B).

8-(2-Chloro-6-fluorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (45): Yield: 60%; mp: 240 °C; ^{1}H NMR ($CDCl_3$): δ = 7.28–7.22 (m, 2H, C3-/C5-H, phe), 7.05–7.01 (m, 1H, C4-H, phe), 4.35 (t, ^3J = 5.4 Hz, 2H, C6-H$_2$), 3.96 (d, ^4J = 2.0 Hz, 2H, N8-CH$_2$), 3.87 (s, 2H, C9-H$_2$), 3.52 (s, 3H, N1-CH$_3$), 3.37 (s, 3H, N3-CH$_3$), 3.07 ppm (t, ^3J = 5.4 Hz, 2H, C7-H$_2$); ^{13}C NMR ($CDCl_3$): δ = 162.0 (d, $^1J_{C,F}$ = 249.8 Hz, C6, phe), 154.9 (C9a), 151.7 (C4), 148.4 (C2), 147.7 (C10a), 136.5 (d, $^3J_{C,F}$ = 5.3 Hz, C2, phe), 130.1 (d, $^3J_{C,F}$ = 9.7 Hz, C4, phe), 125.7 (d, $^4J_{C,F}$ = 3.2 Hz, C3, phe), 122.2 (d, $^1J_{C,F}$ = 17.4 Hz, C1, phe), 114.2 (d, $^2J_{C,F}$ = 23.2 Hz, C5, phe), 106.5 (C4a), 51.5 (N8-CH$_2$), 50.7 (C7), 48.8 (C9), 44.3 (C6), 29.7 (N1-CH$_3$), 27.8 ppm (N3-CH$_3$); ESI-MS: positive mode 378.6 $[M+H]^+$; HPLC: 99.9% (A) and 99.9% (B).

8-(3-Chloro-5-fluorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (46): Yield: 74%; mp: 194 °C; ^{1}H NMR ($CDCl_3$): δ = 7.17 (s, 1H, C2-H, phe), 7.05–7.02 (m, C4-/C6-H, phe), 4.40 (t, ^3J = 5.3 Hz, 2H, C6-H$_2$), 3.78 (s, 2H, N8-CH$_2$), 3.75 (s, 2H, C9-H$_2$), 3.54 (s, 3H, N1-CH$_3$), 3.39 (s, 3H, N3-CH$_3$), 3.00 ppm (t, ^3J = 5.4 Hz, 2H, C7-H$_2$); ^{13}C NMR ($CDCl_3$): δ = 162.8 (d, $^1J_{C,F}$ = 250.4 Hz, C5, phe), 155.0 (C9a), 151.7 (C4), 148.4 (C2), 147.2 (C10a), 140.1 (d, $^3J_{C,F}$ = 5.2 Hz, C1, phe), 135.3 (d, $^3J_{C,F}$ = 10.6 Hz, C3, phe), 124.8 (d, $^4J_{C,F}$ = 2.0 Hz, C2, phe), 115.8 (d, $^2J_{C,F}$ = 24.8 Hz, C6, phe), 114.2 (d, $^2J_{C,F}$ = 21.7 Hz, C4, phe), 106.6 (C4a), 60.8 (N8-CH$_2$), 51.1 (C7), 48.9 (C9), 44.1 (C6), 29.8 (N1-CH$_3$), 27.9 ppm (N3-CH$_3$); ESI-MS: positive mode 378.6 $[M+H]^+$; HPLC: 99.9% (A) and 99.9% (B).

8-(4-Chloro-3-fluorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (47): Yield: 71%; mp: 201 °C; ^{1}H NMR ($CDCl_3$): δ = 7.36–7.32 (m, 1H, C5-H, phe), 7.15 (d, $^3J_{H,F}$ = 9.2 Hz, 1H, C2-H, phe), 7.05 (d, ^3J = 8.0 Hz, 1H, C6-H, phe), 4.33 (t, ^3J = 5.2 Hz, 2H, C6-H$_2$), 3.72 (s, 2H, N8-CH$_2$), 3.70 (s, 2H, C9-H$_2$), 3.51 (s, 3H, N1-CH$_3$), 3.36 (s, 3H, N3-CH$_3$), 2.93 ppm (t, ^3J = 5.3 Hz, 2H, C7-H$_2$); ^{13}C NMR ($CDCl_3$): δ = 158.2 (d, $^1J_{C,F}$ = 249.6 Hz, C3, phe), 154.9 (C9a), 151.7 (C4), 148.4 (C2), 147.6 (C10a), 137.7 (d, $^3J_{C,F}$ = 6.2 Hz, C1, phe), 130.7 (C^6, phe), 125.0 (d, $^3J_{C,F}$ = 3.3 Hz, C5, phe), 120.3 (d, $^2J_{C,F}$ = 17.7 Hz, C4, phe), 116.8 (d, $^2J_{C,F}$ = 21.3 Hz, C2, phe), 106.6 (C4a), 60.8 (N8-CH$_2$), 51.3 (C7), 48.8 (C9), 44.2 (C6), 29.7 (N1-CH$_3$), 27.8 ppm (N3-CH$_3$); ESI-MS: positive mode 378.6 $[M+H]^+$; HPLC: 99.9% (A) and 99.9% (B).

8-(3-Fluoro-5-(trifluoromethyl)benzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (48): Yield: 55%; mp: 226 °C; ^{1}H NMR ($CDCl_3$): δ = 7.43 (s, 1H, C6-H, phe), 7.33 (d, $^3J_{H,F}$ = 8.4 Hz, 1H, C4-H, phe), 7.29 (d, $^3J_{H,F}$ = 8.3 Hz, 1H, C2-H, phe), 4.41 (t, ^3J = 5.4 Hz, 2H, C6-H$_2$), 3.83 (s, 2H, N8-CH$_2$), 3.79 (s, 2H, C9-H$_2$), 3.54 (s, 3H, N1-CH$_3$), 3.40 (s, 3H, N3-CH$_3$), 3.02 ppm (t, ^3J = 5.4 Hz, 2H, C7-H$_2$); ^{13}C NMR ($CDCl_3$): δ = 162.7 (d, $^1J_{C,F}$ = 249.8 Hz, C3, phe), 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.1 (C10a), 140.5 (d, $^3J_{C,F}$ = 7.3 Hz, C1, phe), 132.9 (dq, $^2J_{C,F}$ = 33.2 Hz, $^3J_{C,F}$ = 8.2 Hz, C5, phe), 123.1 (q, $^1J_{C,F}$ = 272.7 Hz, CF$_3$), 121.3–121.2 (m, C6, phe), 119.1 (d, $^2J_{C,F}$ = 21.7 Hz, C2, phe), 112.5 (dq, $^2J_{C,F}$ = 24.5 Hz, $^3J_{C,F}$ = 3.5 Hz, C4, phe), 106.7 (C4a), 60.9 (N8-CH$_2$), 51.2 (C7), 48.9 (C9), 44.1 (C6), 29.8 (N1-CH$_3$), 27.9 ppm (N3-CH$_3$); ESI-MS: positive mode 412.4 $[M+H]^+$; HPLC: 99.9% (A) and 99.8% (B).

8-(2-Fluoro-3-(trifluoromethyl)benzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (49): Yield: 60%; mp: 179 °C; ^{1}H NMR ($CDCl_3$): δ = 7.78–7.75 (m, 1H, C4-H, phe), 7.63–7.61 (m, 1H, C5-H, phe), 7.31–7.28 (m, 1H, C6-H, phe), 4.47 (t, ^3J = 5.2 Hz, 2H, C6-H$_2$), 3.99 (s, 2H, N8-CH$_2$), 3.90 (s, 2H, C9-H$_2$), 3.54 (s, 3H, N1-CH$_3$), 3.39 (s, 3H, N3-CH$_3$), 3.13 ppm (t, ^3J = 5.2 Hz, 2H, C7-H$_2$); ^{13}C NMR ($CDCl_3$): δ = 158.3 (d, $^1J_{C,F}$ = 256.6 Hz, C2, phe), 154.9 (C9a), 151.7 (C4), 148.5 (C2), 146.2 (C10a), 135.6 (C6, phe), 127.5–127.4 (m, C4, phe), 124.4 (d, $^4J_{C,F}$ = 4.1 Hz, C5, phe), 122.4 (q, $^1J_{C,F}$ = 272.8 Hz, CF$_3$), 123.3 (d, $^2J_{C,F}$ = 14.8 Hz, C1, phe), 112.4–111.8 (m, C3, phe), 106.5 (C4a), 53.5 (N8-CH$_2$), 50.5 (C7), 48.8 (C9), 43.7 (C6), 29.8 (N1-CH$_3$), 27.9 ppm (N3-CH$_3$); ESI-MS: positive mode 413.0 $[M+H]^+$; HPLC: 98.4% (A) and 98.4% (B).

8-(2-Fluoro-5-(trifluoromethyl)benzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (50): Yield: 48%; mp: 185 °C; ^{1}H NMR ($CDCl_3$): δ = 7.75–7.73 (m, C6-H, phe), 7.61–7.58 (m, C4-H, phe), 7.22–7.19 (m, 1H, C3-H, phe), 4.40 (t, ^3J = 5.4 Hz, 2H, C6-H$_2$), 3.89 (s, 2H, N8-CH$_2$), 3.83 (s, 2H, C9-H$_2$), 3.54 (s, 3H, N1-CH$_3$), 3.39 (s, 3H, N3-CH$_3$), 3.05 ppm (t, ^3J = 5.4 Hz, 2H, C7-H$_2$); ^{13}C NMR ($CDCl_3$): δ = 163.0 (d, $^1J_{C,F}$ = 252.9 Hz, C2, phe), 155.0 (C9a), 151.7 (C4), 148.4 (C2), 147.1 (C10a), 128.7–128.6 (m, C6, phe), 127.5–126.8 (m, C4/C5, phe), 123.6 (q, $^1J_{C,F}$ = 272.1 Hz, CF$_3$), 124.2 (d, $^2J_{C,F}$ = 15.4 Hz, C1, phe), 116.3 (d, $^2J_{C,F}$ = 23.5 Hz, C3, phe), 106.5 (C4a), 53.9 (N8-CH$_2$), 50.9 (C7), 48.6 (C9), 44.2 (C6), 29.7 (N1-CH$_3$), 27.8 ppm (N3-CH$_3$); ESI-MS: positive mode 412.0 $[M+H]^+$; HPLC: 99.7% (A) and 98.9% (B).

8-(4-Fluoro-2-(trifluoromethyl)benzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (51): Yield: 73%; mp: 248 °C; ^{1}H NMR ($CDCl_3$): δ = 7.95–7.92 (m, 1H, C6-H, phe), 7.42–7.40 (m, 1H, C5-H, phe), 7.32–7.28 (m, C3-H, phe), 4.47 (t, ^3J = 5.4 Hz, 2H, C6-H$_2$), 4.00 (s, 2H, N8-CH$_2$), 3.89 (s, 2H, C9-H$_2$), 3.54 (s, 3H, N1-CH$_3$), 3.40 (s, 3H, N3-CH$_3$), 3.10 ppm (t, ^3J = 5.4 Hz, 2H, C7-H$_2$); ESI-MS: positive mode 412.0 $[M+H]^+$; HPLC: 98.8% (A) and 98.9% (B).

8-(4-Fluoro-3-(trifluoromethyl)benzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (52): Yield: 66%; mp: 180 °C; ^{1}H NMR ($CDCl_3$): δ = 7.61–7.59 (m, C6-H, phe), 7.56–7.53 (m, C2-H, phe), 7.21–7.17 (m, 1H, C5-H, phe), 4.36 (t, ^3J = 5.5 Hz, 2H, C6-H$_2$), 3.76 (s, 2H, N8-CH$_2$), 3.74 (s, 2H, C9-H$_2$), 3.53 (s, 3H, N1-CH$_3$), 3.38 (s, 3H, N3-CH$_3$), 2.97 ppm (t, ^3J = 5.5 Hz, 2H, C7-H$_2$); ^{13}C NMR ($CDCl_3$): δ = 159.3 (dd, $^1J_{C,F}$ = 256.7 Hz, $^3J_{C,F}$ = 1.5 Hz, C4, phe), 155.0 (C9a), 151.7 (C4), 148.4 (C2), 147.5 (C10a), 134.1 (d, $^3J_{C,F}$ = 8.3 Hz, C6, phe), 133.0 (d, $^4J_{C,F}$ = 3.7 Hz, C1, phe), 127.4–127.3 (m, C2, phe), 122.4 (q, $^1J_{C,F}$ = 272.5 Hz, CF$_3$), 118.6 (qd, $^2J_{C,F}$ = 33.1 Hz,

$^{2}J_{C,F}$ = 12.8 Hz, C3, phe), 117.2 (d, $^{2}J_{C,F}$ = 20.7 Hz, C5, phe), 106.5 (C4a), 60.7 (N8-CH_2), 51.2 (C7), 48.9 (C9), 44.1 (C6), 29.8 (N1-CH_3), 27.9 ppm (N3-CH_3); ESI-MS: positive mode 412.0 $[M+H]^+$; HPLC: 99.9% (A) and 99.8% (B).

8-(3-Fluoro-4-(trifluoromethyl)benzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (53): Yield: 69%; mp: 211 °C; ^{1}H NMR ($CDCl_3$): δ = 7.59–7.56 (m, 1H, C5-H, phe), 7.26–7.23 (m, C2-/C6-H, phe), 4.37 (t, ^{3}J = 5.4 Hz, 2H, C6-H_2), 3.79 (s, 2H, N8-CH_2), 3.76 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.38 (s, 3H, N3-CH_3), 2.98 ppm (t, ^{3}J = 5.4 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 159.0 (dd, $^{1}J_{C,F}$ = 256.9 Hz, $^{3}J_{C,F}$ = 1.5 Hz, C3, phe), 155.0 (C9a), 151.7 (C4), 148.4 (C2), 147.4 (C10a), 143.1 (d, $^{3}J_{C,F}$ = 7.4 Hz, C1, phe), 126.5–126.4 (m, C^5 Phe), 123.1 (d, $^{1}J_{C,F}$ = 3.3 Hz, C6, phe), 121.5 (q, $^{1}J_{C,F}$ = 272.1 Hz, CF_3), 116.9 (qd, $^{1}J_{C,F}$ = 33.1 Hz $^{1}J_{C,F}$ = 12.5 Hz, C4, phe), 115.9 (d, $^{2}J_{C,F}$ = 21.0 Hz, C2, phe), 105.6 (C4a), 60.8 (N8-CH_2), 51.3 (C7), 48.9 (C9), 44.2 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 411.9 $[M+H]^+$; HPLC: 99.9% (A) and 99.9% (B).

8-(2-Fluoro-4-(trifluoromethyl)benzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (54): Purification by column chromatography. Yield: 43%; mp: 185 °C; ^{1}H NMR ($CDCl_3$): δ = 7.18–7.14 (m, 2H, C5-/C6-H, phe), 6.99–6.96 (m, 1H, C3-H, phe), 4.40 (t, ^{3}J = 5.1 Hz, 2H, C6-H_2), 3.86 (s, 2H, N8-CH_2), 3.83 (s, 2H, C9-H_2), 3.51 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 3.04 ppm (t, ^{3}J = 5.1 Hz, 2H, C7-H_2); ESI-MS: positive mode 412.0 $[M+H]^+$; HPLC: 99.1% (A) and 98.8% (B).

8-(5-Fluoro-2-(trifluoromethyl)benzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (55): Yield: 78%; mp: 220 °C; ^{1}H NMR ($CDCl_3$): δ = 7.67 (dd, ^{3}J = 8.7 Hz, $^{4}J_{H,F}$ = 5.3 Hz, 1H, C3-H, phe), 7.49 (dd, ^{4}J = 2.4 Hz, $^{3}J_{H,F}$ = 9.6 Hz, 1H, C6-H, phe), 7.09–7.06 (m, 1H, C4-H, phe), 4.39 (t, ^{3}J = 5.5 Hz, 2H, C6-H_2), 3.91 (s, 2H, N8-CH_2), 3.81 (s, 2H, C9-H_2), 3.55 (s, 3H, N1-CH_3), 3.40 (s, 3H, N3-CH_3), 3.00 ppm (t, ^{3}J = 5.5 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 164.9 (d, $^{1}J_{C,F}$ = 253.3 Hz, C5, phe), 155.0 (C9a), 151.8 (C4), 148.5 (C2), 147.5 (C10a), 139.5 (d, $^{3}J_{C,F}$ = 7.9 Hz, C1, phe), 128.6 (dq, $^{3}J_{C,F}$ = 11.6 Hz, $^{3}J_{C,F}$ = 5.6 Hz, C3, phe), 124.8 (qd, $^{2}J_{C,F}$ = 30.9 Hz, $^{4}J_{C,F}$ = 5.1 Hz, C2, phe), 124.0 (q, $^{1}J_{C,F}$ = 273.1 Hz, CF_3), 117.2 (d, $^{2}J_{C,F}$ = 23.4 Hz, C4, phe), 114.6 (d, $^{2}J_{C,F}$ = 22.0 Hz, C6, phe), 106.7 (C4a), 57.0 (N8-CH_2), 51.4 (C7), 49.1 (C9), 44.4 (C6), 29.7 (N1-CH_3), 27.9 ppm (N3-CH_3); ESI-MS: positive mode 412.0 $[M+H]^+$; HPLC: 99.9% (A) and 99.9% (B).

8-(4-Chloro-3-(trifluoromethyl)benzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (56): Yield: 65%; mp: 193 °C; ^{1}H NMR ($CDCl_3$): δ = 7.67 (s, 1H, C2-H, phe), 7.50–7.49 (m, 2H, C^5-/C6-H, phe), 4.37 (t, ^{3}J = 5.3 Hz, 2H, C6-H_2), 3.78 (s, 2H, N8-CH_2), 3.75 (s, 2H, C9-H_2), 3.51 (s, 3H, N1-CH_3), 3.38 (s, 3H, N3-CH_3), 2.98 ppm (t, ^{3}J = 5.3 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 155.0 (C9a), 151.7 (C4), 148.4 (C2), 147.3 (C10a), 135.9 (C1, phe), 133.1 (C4/C6, phe), 131.8 (C5, phe), 128.7 (q, $^{2}J_{C,F}$ = 31.3 Hz, C3, phe), 127.8 (q, $^{3}J_{C,F}$ = 4.9 Hz, C2, phe), 122.7 (q, $^{1}J_{C,F}$ = 273.4 Hz, CF_3), 106.6 (C4a), 60.7 (N8-CH_2), 51.2 (C7), 48.9 (C9), 44.2 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 428.0 $[M+H]^+$; HPLC: 98.9% (A) and 99.2% (B).

8-(4-Chloro-2-(trifluoromethyl)benzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (57): Yield: 62%; mp: 174 °C; ^{1}H NMR ($CDCl_3$): δ = 7.71 (d, ^{3}J = 8.4 Hz, 1H, C6-H, phe), 7.66 (d, ^{4}J = 2.1 Hz, 1H, C3-H, phe), 7.52 (dd, ^{3}J = 8.4 Hz, ^{4}J = 2.1 Hz, 1H, C5-H, phe), 4.36 (t, ^{3}J = 5.4 Hz, 2H, C6-H_2), 3.88 (s, 2H, N8-CH_2), 3.79 (s, 2H, C9-H_2), 3.54 (s, 3H, N1-CH_3), 3.39 (s, 3H, N3-CH_3), 2.98 ppm (t, ^{3}J = 5.4 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.6 (C10a), 134.4 (C4, phe), 133.7 (C1, phe), 132.2 (C^5, phe), 131.9 (C^6, phe), 130.3 (q, $^{2}J_{C,F}$ = 31.1 Hz, C2, phe), 126.4 (q, $^{3}J_{C,F}$ = 9.4 Hz, C3, phe), 123.4 (q, $^{1}J_{C,F}$ = 274.4 Hz, CF_3), 106.6 (C4a), 56.8 (N8-CH_2), 51.4 (C7), 49.0 (C9), 44.4 (C6), 29.7 (N1-CH_3), 27.9 ppm (N3-CH_3); ESI-MS: positive mode 428.0 $[M+H]^+$; HPLC: 98.9% (A) and 99.4% (B).

8-(2-Chloro-5-(trifluoromethyl)benzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (58): Yield: 79%; mp: 202 °C; ^{1}H NMR ($CDCl_3$): δ = 7.75 (s, 1H, C6-H, phe), 7.53–7.49 (m, 2H, C3-/C4-H, phe), 4.39 (t, ^{3}J = 5.4 Hz, 2H, C6-H_2), 3.90 (s, 2H, N8-CH_2), 3.84 (s, 2H, C9-H_2), 3.54 (s, 3H, N1-CH_3), 3.39 (s, 3H, N3-CH_3), 3.04 ppm (t, ^{3}J = 5.4 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 155.0 (C9a), 151.7 (C4), 148.4 (C2), 147.5 (C10a), 138.1 (C2, phe), 135.6 (C1, phe), 130.3 (C^3, phe), 129.6 (q, $^{2}J_{C,F}$ = 33.0 Hz, C5, phe), 127.3 (q, $^{3}J_{C,F}$ = 3.4 Hz, C6, phe), 125.7 (q, $^{3}J_{C,F}$ = 3.5 Hz, C4, phe), 123.6 (q, $^{1}J_{C,F}$ = 272.3 Hz, CF_3), 106.6 (C4a), 58.0 (N8-CH_2), 51.2 (C7), 49.0 (C9), 44.2 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 427.9 $[M+H]^+$; HPLC: 99.3% (A) and 99.5% (B).

8-(3-Fluoro-4-methoxybenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (59): Purification by column chromatography. Yield: 43%; mp: 184 °C; ^{1}H NMR ($CDCl_3$): δ = 7.10 (dd, ^{4}J = 2.0 Hz and $^{3}J_{H,F}$ = 11.9 Hz, 1H, C2-H, phe), 7.04–7.02 (m, 1H, C6-H, phe), 6.93–6.90 (m, 1H, C5-H, phe), 4.35 (t, ^{3}J = 5.5 Hz, 2H, C6-H_2), 3.89 (s, 3H, OCH_3), 3.73 (s, 2H, N8-CH_2), 3.67 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.38 (s, 3H, N3-CH_3), 2.94 ppm (t, ^{3}J = 5.5 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 155.0 (C9a), 152.5 (d, $^{1}J_{C,F}$ = 246.5 Hz, C3, phe), 151.7 (C4), 148.5 (C2), 147.6 (C10a), 147.3 (d, $^{2}J_{C,F}$ = 10.7 Hz, C4, phe), 129.6 (d, $^{3}J_{C,F}$ = 5.9 Hz, C1, phe), 124.6 (d, $^{4}J_{C,F}$ = 3.3 Hz, C6, phe), 116.6 (d, $^{2}J_{C,F}$ = 18.4 Hz, C2, phe), 113.3 (d, $^{4}J_{C,F}$ = 1.5 Hz, C5, phe), 106.6 (C4a), 61.0 (N8-CH_2), 56.3 (OCH_3), 51.2 (C7), 48.7 (C9), 44.3 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 374.0 $[M+H]^+$; HPLC: 99.7% (A) and 98.8% (B).

8-(3-Bromo-4-fluorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (60): Purification by column chromatography. Yield: 45%; mp: 248 °C; ^{1}H NMR ($CDCl_3$): δ = 7.57 (dd, ^{4}J = 2.1 Hz, $^{4}J_{H,F}$ = 6.6 Hz, 1H, C2-H, phe), 7.30–7.27 (m, 1H, C6-H, phe), 7.11–7.08 (m, 1H, C5-H, phe), 4.38 (t, ^{3}J = 5.4 Hz, 2H, C6-H_2), 3.76 (s, 2H, N8-CH_2), 3.72 (s, 2H, C9-H_2), 3.52 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 2.99 ppm (t, ^{3}J = 5.4 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 158.8 (d, $^{1}J_{C,F}$ = 248.2 Hz, C2, phe), 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.2 (C10a), 134.0 (C2, phe), 133.5 (d, $^{4}J_{C,F}$ = 3.4 Hz, C1, phe), 129.5 (d, $^{3}J_{C,F}$ = 6.7 Hz, C6, phe), 116.7 (d, $^{2}J_{C,F}$ = 22.4 Hz, C5, phe), 109.4 (d, $^{1}J_{C,F}$ = 21.0 Hz, C3, phe), 106.5 (C4a), 60.5 (N8-CH_2), 51.0 (C7), 48.8 (C9), 44.0 (C6), 29.8 (N1-CH_3), 27.9 ppm (N3-CH_3); ESI-MS: positive mode 422.0 and 424.0 $[M+H]^+$; HPLC: 99.9% (A) and 99.9% (B).

8-(5-Bromo-2-fluorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (61): Yield: 71%; mp: 209 °C; ^{1}H NMR ($CDCl_3$): δ = 7.54 (dd, ^{4}J = 2.5 Hz, $^{4}J_{H,F}$ = 6.3 Hz, 1H, C6-H, phe), 7.42–7.39 (m, 1H, C4-H, phe), 7.00–6.96 (m, 1H, C3-H, phe), 4.38 (t, ^{3}J = 5.5 Hz, 2H, C6-H_2), 3.79 (br s, 4H, N8-CH_2, C9-H_2), 3.54 (s, 3H, N1-CH_3), 3.39 (s, 3H, N3-CH_3), 3.00 ppm (t, ^{3}J = 5.5 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 160.3 (d, $^{1}J_{C,F}$ = 247.4 Hz, C2, phe), 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.6 (C10a), 133.8 (C^6, phe), 132.5 (d, $^{3}J_{C,F}$ = 8.4 Hz, C4, phe), 125.6 (d, $^{2}J_{C,F}$ = 15.6 Hz, C1, phe), 117.4 (d, $^{2}J_{C,F}$ = 23.8 Hz, C3, phe), 116.9 (d, $^{4}J_{C,F}$ = 3.6 Hz, C5, phe), 106.5 (C4a), 53.9 (N8-CH_2), 51.0 (C7), 48.9 (C9), 44.3 (C6), 29.7 (N1-CH_3), 27.9 ppm (N3-CH_3); ESI-MS: positive mode 422.0 and 424.0 $[M+H]^+$; HPLC: 99.9% (A) and 99.9% (B).

8-(3-Fluoro-4-methylbenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (62): Yield: 70%; mp: 212 °C; ^{1}H NMR ($CDCl_3$): δ = 7.16–7.12 (m,1H, C5-H, phe), 7.02–6.99

(m, C2-/C6-H, 2H, phe), 4.34 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.74 (s, 2H, N8-CH_2), 3.70 (s, 2H, C9-H_2), 3.54 (s, 3H, N1-CH_3), 3.39 (s, 3H, N3-CH_3), 2.95 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 2.24 ppm (C4-H_3, phe); ^{13}C NMR ($CDCl_3$): δ = 161.4 (d, $^1J_{C,F}$ = 245.3 Hz, C3, phe), 155.0 (C9a), 151.8 (C4), 148.5 (C2), 148.0 (C10a), 136.3 (d, $^3J_{C,F}$ = 7.1 Hz, C1, phe), 131.5 (d, $^3J_{C,F}$ = 5.4 Hz, C5, phe), 124.4 (d, $^2J_{C,F}$ = 17.2 Hz, C4, phe), 124.2 (d, $^4J_{C,F}$ = 3.1 Hz, C6, phe), 115.4 (d, $^2J_{C,F}$ = 22.5 Hz, C2, phe), 106.6 (C4a), 54.0 (N8-CH_2), 51.2 (C7), 48.8 (C9), 44.3 (C6), 29.7 (N1-CH_3), 27.8 (N3-CH_3), 14.3 ppm (d, $^4J_{C,F}$ = 3.4 Hz, CH_3); ESI-MS: positive mode 358.0 $[M+H]^+$; HPLC: 99.9% (A) and 99.9% (B).

8-(3-Fluoro-5-methylbenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (63): Yield: 75%; mp: 162 °C; ^{1}H NMR ($CDCl_3$): δ = 6.92 (s, 1H, C6-H, phe), 6.89 (d, $^3J_{H,F}$ = 9.3 Hz, 1H, C4-H, phe), 6.81 (d, $^3J_{H,F}$ = 9.5 Hz, 1H, C2-H, phe), 4.36 (t, ^3J = 5.5 Hz, 2H, C6-H_2), 3.74 (s, 2H, N8-CH_2), 3.69 (s, 2H, C9-H_2), 3.54 (s, 3H, N1-CH_3), 3.39 (s, 3H, N3-CH_3), 2.96 (t, ^3J = 5.5 Hz, 2H, C7-H_2), 2.32 ppm (C5-H_3, phe); ^{13}C NMR ($CDCl_3$): δ = 163.0 (d, $^1J_{C,F}$ = 245.8 Hz, C3, phe), 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.9 (C10a), 140.6 (d, $^3J_{C,F}$ = 8.0 Hz, C5, phe), 138.8 (d, $^3J_{C,F}$ = 7.7 Hz, C1, phe), 125.2 (d, $^4J_{C,F}$ = 2.2 Hz, C6, phe), 115.4 (d, $^2J_{C,F}$ = 21.0 Hz, C2, phe), 112.7 (d, $^2J_{C,F}$ = 21.6 Hz, C4, phe), 106.5 (C4a), 61.5 (N8-CH_2), 51.3 (C7), 48.9 (C9), 44.3 (C6), 29.7 (N1-CH_3), 27.9 (N3-CH_3), 21.3 ppm (CH_3); ESI-MS: positive mode 412.0 $[M+H]^+$; HPLC: 99.9% (A) and 99.8% (B).

8-(3,5-bis(Trifluoromethyl)benzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (64): Yield: 60%; mp: 228 °C; ^{1}H NMR ($CDCl_3$): δ = 7.84–7.83 (m, 3H, C2-/C4-/C6-H, phe), 4.39 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.87 (s, 2H, N8-CH_2), 3.78 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.39 (s, 3H, N3-CH_3), 3.01 ppm (t, ^3J = 5.4 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 155.0 (C9a), 151.7 (C4), 148.5 (C2), 147.2 (C10a), 139.6 (C1, phe), 132.1 (q, $^2J_{C,F}$ = 33.4 Hz, C3/C5, phe), 128.8 (q, $^3J_{C,F}$ = 2.0 Hz, C^2/C6, phe), 123.2 (q, $^1J_{C,F}$ = 272.8 Hz, CF_3), 122.0–121.9 (m, C4, phe), 106.6 (C4a), 60.8 (N8-CH_2), 51.3 (C7), 49.0 (C9), 44.1 (C6), 29.7 (N1-CH_3), 27.9 ppm (N3-CH_3); ESI-MS: positive mode 462.0 $[M+H]^+$; HPLC: 99.7% (A) and 99.6% (B).

8-(2,3-Dimethoxybenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (65): Purification by column chromatography. Yield: 34%; mp: 172 °C; ^{1}H NMR ($CDCl_3$): δ = 6.87 (dd, ^3J = 7.9 Hz, ^3J = 7.9 Hz, 1H, C5-H, phe), 6.92 (dd, ^3J = 7.6 Hz, ^4J = 1.0 Hz, 1H, C4-H, phe), 6.86 (dd, ^3J = 8.2 Hz, ^4J = 1.3 Hz, 1H, C6-H, phe), 4.30 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.85 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.77 (s, 4H, N8-CH_2, C9-H_2), 3.51 (s, 3H, N1-CH_3), 3.36 (s, 3H, N3-CH_3), 2.95 ppm (t, ^3J = 5.4 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 155.0 (C9a), 152.9 (C3, phe), 151.7 (C4), 148.5 (C2), 148.3 (C2, phe), 147.9 (C10a), 130.2 (C1, phe), 124.0 (C^6, phe), 122.4 (C5, phe), 111.9 (C4, phe), 106.5 (C4a), 61.0 (N8-CH_2), 55.7 (2× OCH_3), 55.6 (N8-CH_2), 51.3 (C7), 48.9 (C9), 44.3 (C6), 29.7 (N1-CH_3), 27.9 ppm (N3-CH_3); ESI-MS: positive mode 485.8 $[M+H]^+$; HPLC: 99.9% (A) and 99.9% (B).

8-(2,4-Dimethoxybenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (66): Purification by column chromatography. Yield: 38%; mp: 162 °C; ^{1}H NMR ($CDCl_3$): δ = 7.21–7.19 (m, 1H, C6-H, phe), 7.43 (d, ^4J = 1.9 Hz, 1H, C3-H, phe), 6.49–6.47 (m, 1H, C5-H, phe), 4.34 (t, ^3J = 5.0 Hz, 2H, C6-H_2), 3.82 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.79 (brs, 2H, N8-CH_2), 3.75 (brs, 2H, C9-H_2), 3.54 (s, 3H, N1-CH_3), 3.39 (s, 3H, N3-CH_3), 2.98 ppm (t, ^3J = 5.0 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 160.6 (C4, phe), 159.0 (C4, phe), 155.1 (C9a), 151.7 (C4), 149.8 (C2), 148.4 (C10a), 131.7 (C^6, phe), 116.5 (C1, phe), 106.5 (C4a), 104.2 (C5, phe), 98.6 (C3, phe), 55.5 (OCH_3), 55.4 (OCH_3), 54.9 (N8-CH_2), 50.9 (C7), 48.6 (C9), 44.3 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 394.3 $[M+H]^+$; HPLC: 98.1% (A) and 98.6% (B).

8-(Benzo[*d*][1,3]dioxol-5-ylmethyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (67): Yield: 71%; mp: 200 °C; ^{1}H NMR ($CDCl_3$): δ = 6.85 (s, 1H, C3-H, phe), 6.77–6.76 (m, 2H, C5-/C6-H, phe), 5.95 (s, 2H, O-CH_2-O, phe), 4.34 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.72 (s, 2H, N8-CH_2), 3.66 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.39 (s, 3H, N3-CH_3), 2.94 ppm (t, ^3J = 5.5 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 155.0 (C9a), 151.7 (C4), 148.5 (C2), 148.0 (C^3, phe), 147.9 (C10a), 147.2 (C4, phe), 130.3 (C1, phe), 122.3 (C^6, phe), 109.2 (C^5, phe), 108.1 (C2, phe), 106.6 (C4a), 101.0 (O-CH_2-O), 61.7 (N8-CH_2), 51.2 (C7), 48.7 (C9), 44.4 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 384.3 $[M+H]^+$; HPLC: 96.5% (A) and 96.7% (B).

(*R*,*S*)-8-(1-(3,4-Dichlorophenyl)ethyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (68): Yield: 62%; mp: 208 °C; ^{1}H NMR ($CDCl_3$): δ = 7.44 (d, ^4J = 1.9 Hz, 1H, C2-H, phe), 7.40 (d, ^3J = 8.2 Hz, 1H, C5-H, phe), 7.17 (dd, ^3J = 8.2 Hz, ^4J = 2.4 Hz, 1H, C6-H, phe), 4.33 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.88 (brs, 1H, N8-CH), 3.68 (s, 2H, C9-H_2), 3.51 (s, 3H, N1-CH_3), 3.36 (s, 3H, N3-CH_3), 2.93 (t, ^3J = 5.4 Hz, 2H, C7-H_2), 1.58 ppm (brs, 3H, CH_3); ESI-MS: positive mode 408.1 $[M+H]^+$; HPLC: 98.7% (A) and 99.3% (B).

8-(3,4,5-Trifluorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (69): Yield: 61%; mp: 220 °C; ^{1}H NMR ($CDCl_3$): δ = 7.03–7.00 (m, 2H, C2-/C6-H, phe), 4.37 (t, ^3J = 5.5 Hz, 2H, C6-H_2), 3.72 (s, 2H, N8-CH_2), 3.69 (s, 2H, C9-H_2), 3.54 (s, 3H, N1-CH_3), 3.40 (s, 3H, N3-CH_3), 2.97 ppm (t, ^3J = 5.5 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 155.0 (C9a), 151.7 (C4), 151.4 (ddd, $^1J_{C,F}$ = 248.1 Hz, $^2J_{C,F}$ = 10.1 Hz, $^3J_{C,F}$ = 3.7 Hz, C3/C5, phe), 148.5 (C2), 147.4 (C10a), 139.5 (dd, $^1J_{C,F}$ = 252.1 Hz, $^2J_{C,F}$ = 15.4 Hz, C4, phe), 133.2–133.0 (m, C1, phe), 112.2 (dd, $^2J_{C,F}$ = 16.3 Hz, $^3J_{C,F}$ = 5.0 Hz, C2/C6, phe), 106.6 (C4a), 60.6 (N8-CH_2), 51.2 (C7), 48.8 (C9), 44.2 (C6), 29.8 (N1-CH_3), 27.9 ppm (N3-CH_3); ESI-MS: positive mode 380.0 $[M+H]^+$; HPLC: 99.6% (A) and 99.3% (B).

8-(2,4,6-Trifluorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (70): Yield: 65%; mp: 268 °C; ^{1}H NMR ($CDCl_3$): δ = 6.73–6.70 (m, 2H, C2-/C6-H, phe), 4.34 (t, ^3J = 5.5 Hz, 2H, C6-H_2), 3.87 (s, 2H, N8-CH_2), 3.79 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.39 (s, 3H, N3-CH_3), 3.00 ppm (t, ^3J = 5.4 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 162.6, (ddd, $^1J_{C,F}$ = 250.6 Hz, $^3J_{C,F}$ = 15.7 Hz, $^3J_{C,F}$ = 15.7 Hz, C4, phe), 162.2 (ddd, $^1J_{C,F}$ = 249.9 Hz, $^3J_{C,F}$ = 14.8 Hz, $^3J_{C,F}$ = 11.1 Hz, C2/C6, phe), 154.9 (C9a), 151.7 (C4), 148.4 (C2), 147.1 (C10a), 107.9 (ddd, $^2J_{C,F}$ = 20.2 Hz, $^2J_{C,F}$ = 20.0 Hz, $^4J_{C,F}$ = 4.4 Hz, C1, phe), 106.5 (C4a), 100.4 (ddd, $^2J_{C,F}$ = 25.4 Hz, $^2J_{C,F}$ = 23.3 Hz, $^4J_{C,F}$ = 2.6 Hz, C3/C5, phe), 50.3 (N8-CH_2), 48.4 (C7), 47.7 (C9), 44.2 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 380.0 $[M+H]^+$; HPLC: 99.9% (A) and 98.9% (B).

8-(2,3-Dichloro-6-fluorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (71): Yield: 53%; mp: 254 °C; ^{1}H NMR ($CDCl_3$): δ = 7.43 (dd, ^3J = 8.9 Hz, $^4J_{H,F}$ = 5.4 Hz, 1H, C4-H, phe), 7.03–6.99 (m, 1H, C5-H, phe), 4.33 (t, ^3J = 5.5 Hz, 2H, C6-H_2), 3.96 (d, $^4J_{H,F}$ = 2.4 Hz, 2H, N8-CH_2), 3.85 (s, 2H, C9-H_2), 3.52 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 3.06 ppm (t, ^3J = 5.4 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 160.1 (d, $^1J_{C,F}$ = 249.5 Hz, C6, phe), 154.9 (C9a), 151.7 (C4), 148.4 (C2), 147.7 (C10a), 134.9 (d, $^3J_{C,F}$ = 5.6 Hz, C2, phe), 130.5 (d, $^3J_{C,F}$ = 9.4 Hz, C4, phe), 129.1 (d, $^4J_{C,F}$ = 3.7 Hz, C3, phe), 124.4 (d, $^2J_{C,F}$ = 18.3 Hz, C1, phe), 114.9 (d, $^2J_{C,F}$ = 24.7 Hz, C5, phe), 106.5 (C4a), 52.4 (N8-CH_2), 50.8 (C7), 48.9 (C9), 44.3 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 412.0 $[M+H]^+$; HPLC: 99.9% (A) and 99.9% (B).

8-(2,4-Dichloro-5-fluorobenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (72): Yield: 54%; mp: 230 °C; ^{1}H NMR ($CDCl_3$): δ = 7.45 (d, $^4J_{H,F}$ = 6.6 Hz, 1H, C3-H, phe), 7.32 (d, $^3J_{H,F}$ = 9.4 Hz, 1H, C6-H, phe), 4.38 (t, ^3J = 5.5 Hz, 2H, C6-H_2), 3.83 (s, 2H, N8-CH_2), 3.82 (s, 2H, C9-H_2), 3.55 (s, 3H, N1-CH_3), 3.39 (s, 3H, N3-CH_3), 3.02 ppm (t, ^3J = 5.5 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 157.0 (d, $^1J_{C,F}$ = 249.8 Hz, C5, phe), 155.0 (C9a), 151.7 (C4), 148.4 (C2), 147.4 (C10a), 135.1 (d, $^3J_{C,F}$ = 6.2 Hz, C1, phe), 131.1 (C^3, phe), 129.1 (d, $^4J_{C,F}$ = 3.6 Hz, C2, phe), 121.0 (d, $^2J_{C,F}$ = 19.2 Hz, C6, phe), 117.9 (d, $^2J_{C,F}$ = 23.2 Hz, C4, phe), 106.6 (C4a), 57.6 (N8-CH_2), 51.2 (C7), 49.0 (C9), 44.2 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 412.0 $[M+H]^+$; HPLC: 99.9% (A) and 99.5% (B).

8-(4,5-Difluoro-2-methoxybenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (73): Yield: 45%; mp: 208 °C; ^{1}H NMR ($CDCl_3$): δ = 7.21–7.17 (dd, $^3J_{H,F}$ = 10.7 Hz, $^4J_{H,F}$ = 9.1 Hz 1H, C3-H, phe), 6.71 (dd, $^3J_{H,F}$ = 12.0 Hz, $^4J_{H,F}$ = 6.5 Hz, 1H, C6-H, phe), 4.36 (t, ^3J = 5.5 Hz, 2H, C6-H_2), 3.80 (s, 3H, OCH_3), 3.78 (s, 2H, N8-CH_2), 3.72 (s, 2H, C9-H_2), 3.54 (s, 3H, N1-CH_3), 3.39 (s, 3H, N3-CH_3), 2.98 ppm (t, ^3J = 5.5 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 155.0 (C9a), 153.8 (dd, $^3J_{C,F}$ = 7.2 Hz, $^4J_{C,F}$ = 1.6 Hz, C2, phe), 151.7 (C4), 149.7 (d, $^1J_{C,F}$ = 247.8 Hz, $^2J_{C,F}$ = 13.6 Hz, C4, phe), 148.4 (C2), 148.0 (C10a), 144.4 (dd, $^1J_{C,F}$ = 240.8 Hz, $^2J_{C,F}$ = 12.5 Hz, C5, phe), 120.7 (dd, $^3J_{C,F}$ = 4.4 Hz, $^4J_{C,F}$ = 4.1 Hz, C1, phe), 118.5 (d, $^3J_{C,F}$ = 18.6 Hz, C6, phe), 106.6 (C4a), 100.7 (d, $^3J_{C,F}$ = 21.1 Hz, C3, phe), 56.2 (N8-CH_2), 54.2 (OCH_3), 51.0 (C7), 48.9 (C9), 44.3 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 392.0 $[M+H]^+$; HPLC: 99.9% (A) and 99.9% (B).

8-(2,3-Difluoro-4-methoxybenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (74): Purification by column chromatography. Yield: 36%; mp: 208 °C; ^{1}H NMR ($CDCl_3$): δ = 7.06–7.02 (m, 1H, C5-H, phe), 6.76–6.73 (m, 1H, C6-H, phe), 4.35 (t, ^3J = 5.4 Hz, 2H, C6-H_2), 3.91 (s, 3H, OCH_3), 3.79 (s, 2H, N8-CH_2), 3.78 (s, 2H, C9-H_2), 3.54 (s, 3H, N1-CH_3), 3.39 (s, 3H, N3-CH_3), 2.98 ppm (t, ^3J = 5.4 Hz, 2H, C7-H_2); ESI-MS: positive mode 392.0 $[M+H]^+$; HPLC: 99.9% (A) and 99.9% (B).

8-(3,5-Difluoro-4-methoxybenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (75): Yield: 24%; mp: 199 °C; ^{1}H NMR ($CDCl_3$): δ = 6.91 (d, $^3J_{H,F}$ = 8.4 Hz, 2H, C2-/C6-H, phe), 4.36 (t, ^3J = 5.1 Hz, 2H, C6-H_2), 3.98 (s, 3H, OCH_3), 3.73 (s, 2H, N8-CH_2), 3.65 (s, 2H, C9-H_2), 3.53 (s, 3H, N1-CH_3), 3.38 (s, 3H, N3-CH_3), 2.95 ppm (t, ^3J = 5.2 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 155.7 (dd, $^1J_{C,F}$ = 247.3 Hz, $^3J_{C,F}$ = 6.0 Hz, C3/C5, phe), 155.0 (C9a), 151.7 (C4), 148.4 (C2), 147.6 (C10a), 135.8 (dd, $^2J_{C,F}$ = 14.2 Hz, $^2J_{C,F}$ = 14.2 Hz, C4, phe), 132.0 (dd, $^3J_{C,F}$ = 8.1 Hz, $^3J_{C,F}$ = 8.1 Hz, C1, phe), 112.3 (dd, $^2J_{C,F}$ = 17.4 Hz, $^4J_{C,F}$ = 5.7 Hz, C2/C6, phe), 106.6 (C4a), 61.8 (N8-CH_2), 60.7 (OCH_3), 51.2 (C7), 48.8 (C9), 44.2 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 392.0 $[M+H]^+$; HPLC: 99.9% (A) and 99.9% (B).

8-(2-Chloro-6-fluoro-3-methoxybenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (76): Yield: 55%; mp: 238 °C; ^{1}H NMR ($CDCl_3$): δ = 7.03–6.99 (m, 1H, C5-H, phe), 6.90–6.87 (m, 1H, C4-H, phe), 4.34 (t, ^3J = 5.5 Hz, 2H, C6-H_2), 3.97 (d, $^4J_{H,F}$ = 244.4 Hz 2H, N8-CH_2), 3.89 (s, 2H, C9-H_2), 3.87 (s, 3H, OCH_3), 3.53 (s, 3H, N1-CH_3), 3.38 (s, 3H, N3-CH_3), 3.06 ppm (t, ^3J = 5.5 Hz, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 155.9 (d, $^1J_{C,F}$ = 241.8 Hz, C6, phe), 155.0 (C9a), 152.0 (d, $^4J_{C,F}$ = 2.5 Hz, C3, phe), 151.7 (C4), 148.4 (C2), 148.0 (C10a), 124.7 (d, $^3J_{C,F}$ = 5.5 Hz, C2, phe), 123.5 (d, $^2J_{C,F}$ = 18.7 Hz, C1, phe), 113.6 (d, $^2J_{C,F}$ = 24.6 Hz, C4, phe), 112.0 (d, $^1J_{C,F}$ = 9.2 Hz, C4, phe), 106.6 (C4a), 56.7 (N8-CH_2), 51.9 (OCH_3), 50.8 (C7), 48.8 (C9), 44.4 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 392.0 $[M+H]^+$; HPLC: 99.9% (A) and 99.7% (B).

8-(2,4,6-Trimethoxybenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (77): Purification by column chromatography. Yield: 27%; mp: 171 °C; ^{1}H NMR ($CDCl_3$): δ = 6.13 (s, 2H, C3-/C5-H, phe), 4.35 (brs, 2H, C6-H_2), 3.78–3.74 (m, 13H, N8-CH_2, C9-H_2, 3×OCH_3), 3.52 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 2.96 ppm (brs, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 160.1 (C2/C6, phe), 159.9 (C4, phe), 154.9 (C9a), 151.7 (C4), 148.4 (C2), 147.7 (C10a), 105.9 (C4a), 93.8 (C1, phe), 90.6 (C3/C5, phe), 56.0 (OCH_3), 55.5 (OCH_3), 55.2 (OCH_3), 54.2 (N8-CH_2), 51.1 (C7), 48.7 (C9), 44.0 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 416.0 $[M+H]^+$; HPLC: 99.9% (A) and 99.9% (B).

8-(3,4,5-Trimethoxybenzyl)-1,3-dimethyl-6,7,8,9-tetrahydropyrazino[2,1-*f*]purine-2,4(1*H*,3*H*)-dione (78): Purification by column chromatography. Yield: 31%; mp: 172 °C; ^{1}H NMR ($CDCl_3$): δ = 6.57 (s, 2H, C2-/C6-H, phe), 4.36 (brs, 2H, C6-H_2), 3.84 (s, 9H, 3×OCH_3), 3.76 (brs, 2H, N8-CH_2), 3.69 (brs, 2H, C9-H_2), 3.52 (s, 3H, N1-CH_3), 3.37 (s, 3H, N3-CH_3), 2.97 ppm (brs, 2H, C7-H_2); ^{13}C NMR ($CDCl_3$): δ = 154.9 (C9a), 153.4 (C3/C5, phe), 151.7 (C4), 148.4 (C2), 147.7 (C10a), 137.1 (C4, phe), 133.5 (C1, phe), 105.9 (C4a), 93.8 (C1, phe), 90.6 (C-^{3}/C5, phe), 62.1 (N8-CH_2), 60.8 (OCH_3), 56.2 (2×OCH_3), 51.1 (C7), 48.7 (C9), 44.2 (C6), 29.7 (N1-CH_3), 27.8 ppm (N3-CH_3); ESI-MS: positive mode 416.0 $[M+H]^+$; HPLC: 99.9% (A) and 99.9% (B).

Biological evaluation

Radioligand binding assays at adenosine receptors: The radioligands were obtained from the following sources: [^{3}H]CCPA from Amersham Biosciences (58 Ci mmol^{-1}), [^{3}H]MSX-2 from Amersham Biosciences (84 Ci mmol^{-1}), [^{3}H]PSB-603 from Amersham Biosciences (73 Ci mmol^{-1}), and [^{3}H]PSB-11 (53 Ci mmol^{-1}) from Quotient Bioresearch. The nonradioactive precursors of [^{3}H]MSX-2,[56] [^{3}H]PSB-603,[57] and [^{3}H]PSB-11[58] were synthesized in our laboratory. Membrane preparations and radioligand binding assays at rat A_1 (rat brain cortex) and rat A_{2A}ARs (rat brain striatum) were performed as previously described.[61,62] For assays at human A_1, A_{2A}, A_{2B}, and A_3ARs, CHO cell membranes expressing one of the human AR subtypes were used as previously reported.[57]

Monoamine oxidase assays: The determination of MAO-A and MAO-B inhibition was performed using commercially available recombinant human MAO-A and MAO-B enzymes expressed in baculovirus-infected insect cells (Sigma–Aldrich, M7316 and M7441) applying the commercially available Amplex Red monoamine oxidase assay kit (Invitrogen A12214). The assays were performed as previously described.[59] The determination of rat MAO-B inhibition was performed using mitochondrial-enriched fractions from male Sprague–Dawley rat livers. The assays were conducted as previously described.[56]

Determination of water solubility

Water solubility (in mg mL^{-1}) was determined using the following procedure: at RT, 500 μL of buffer was added to 1 mg of precisely weighed compound and sonicated for 30 sec. The pH value was checked and adjusted if deviating more than 0.2 pH units from the target value. The solution was left for 1 h at RT and then filtered through a 0.45 μm filter. A reference solution of precisely weighed 1 mg of the same compound dissolved in methanol was prepared. Both solutions were injected into a Chromatographic Acquity UPLC

System (from Waters) equipped with a BEH RP18 column (2.1× 50 mm 1.7 μM) and coupled to a photodiode array detector (200–400 nm). The elution was performed for 3.33 min at a flow rate of 750 μL min^{-1} at a column temperature of 65 °C with a gradient of eluent A [H_2O/acetonitrile/trifluoroacetic acid (95/5/0.05)] and eluent B [acetonitrile] from 90:10 to 10:90, starting the gradient after 0.33 min and ending after 2.00 min.

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