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Multigram-Scale Syntheses, Stability, and Photoreactions of A_{2A} Adenosine Receptor Antagonists with 8-Styrylxanthine Structure: Potential Drugs for Parkinson's Disease

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The improved multigram-scale syntheses of the important 8-styrylxanthine A_{2A} adenosine receptor antagonist MSX-2 (**8**), its water-soluble prodrug MXS-3 (**9**), and KW-6002 (**16**) are described. N-Alkylation reactions at different positions of uracil derivatives were optimized. Two different methods for xanthine formation from 6-amino-5-cinnamoylaminouracil precursors were investigated, (a) the elimination of water by alkaline catalysis and (b) hexamethyldisilazane as a condensing agent; the latter was found to be superior. The photosensitivity of 8-styrylxanthines was studied. The (*E*)-configurated stryrylxanthine MSX-2 (**8**) isomerized in diluted solution, and the resulting (*Z*)-isomer (**10a**) was isolated and characterized. Furthermore, we describe for the first time that solid 8-styrylxanthines can dimerize upon exposition to daylight or irradiation with UV light. The resulting cyclobutane derivatives with head-to-tail (*syn*) configuration exhibited a considerably lower A_{2A} adenosine receptor affinity than their parent compounds. The dimerization product of MSX-2 was a moderately potent nonselective A_1 and A_{2A} antagonist ($K_i(A_1) = 273$ nM, $K_i(A_{2A}) = 175$ nM) while the dimer of the related compound KW-6002 was inactive at A_1 and only weakly active at A_{2A} adenosine receptors ($K_i = 1.57$ μM). The light sensitivity of 8-styrylxanthine derivatives, not only in solution, but also in the solid state, has to be considered when using those compounds as pharmacological tools or drugs.

Introduction

In addition to its intracellular physiological functions, the nucleoside adenosine is also an important extracellular signaling molecule.[1] Adenosine activates specific G protein-coupled receptors in cell membranes, whereas the well-known xanthine alkaloid caffeine mediates most of its effects by a blockade of adenosine receptors.[2] The fact that four subtypes of adenosine receptors have been identified opens up the possibility of evoking differential pharmacological effects by designing subtype-specific agonists or antagonists. The A_{2A} subtype of adenosine receptors, one of the most dominant adenosine receptors in the brain, is characterized by a restricted distribution, with high levels found in the caudate-putamen, nucleus accumbens, and olfactory tubercle.[3]

Adenosine receptor antagonists, in particular those with selectivity for the A_{2A} subtype, have been proposed as novel therapeutics for Parkinson's disease. This neurodegenerative disorder is characterized by a chronically proceeding degeneration of certain brain areas, including dopaminergic neurons of the basal ganglia.[3] A_{2A} antagonists ameliorate symptoms of Parkinson's disease, and in addition, they have been shown to exhibit neuroprotective properties in contrast to most currently used anti-Parkinsonian drugs, thus opening up new opportunities in therapy.[3−6] The breakthrough in the discovery of truly A_{2A}-selective antagonists was achieved with the synthesis of 7-methyl-8-styrylxanthine derivatives.[7−9] In the past years, our group has contributed to the design of potent and selective A_{2A} adenosine receptor antagonists resulting in the development of (*E*)-3-(3-hydroxypropyl)-8-[2-(3-methoxyphenyl)vinyl]-7-methyl-1-prop-2-ynyl-3,7-dihydropurine-2,6-dione (MSX-2, **8**)[10] and its water-soluble prodrug (*E*)-phosphoric acid mono-[3-[8-[2-(3-methoxyphenyl)vinyl]-7-methyl-2,6-dioxo-1-prop-2-ynyl-1,2,6,7-tetrahydropurin-3-yl]propyl] ester disodium salt (MSX-3).[10] MSX-2 is used in its tritiated form for radioligand binding studies in tissues expressing A_{2A} receptors in

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vitro.[11] It has also been labeled by carbon-11, a positron-emitting nuclide, and was shown to exhibit a high degree of brain penetration in mice.[12] The phosphate prodrug of MSX-2, MSX-3, exhibits high water solubility (9 mg/mL)[10] and therefore has found broad application as a pharmacological tool for in vivo studies. It has shown activity in a number of animal models of Parkinson's disease, both after systemic and local application. Due to its water-solubility, MSX-3 can be used for intracerebral application by injecting small volumes of drug solution directly into specific brain areas in order to study the mechanism of the anti-Parkinsonian action of A_{2A} antagonists.[13] A related 8-styrylxanthine derivate (*E*)-8-[2-(3,4-dimethoxyphenyl)vinyl]-1,3-diethyl-7-methyl-3,7-dihydropurine-2,6-dione (KW-6002, **16**),[14] which has been developed by Kyowa Hakko Co., Japan, is a promising new drug currently undergoing phase IIb clinical trials for Parkinson's disease and depression in the United States and Japan.[15] MSX-2, MSX-3, and KW-6002 have become standard A_{2A} antagonists and are frequently used in in vitro and in vivo studies.[14,16a−e]

However, photoinduced *E*/*Z*-isomerization of various bioactive 8-styrylxanthines, including CSC (8-(*m*-chlorostyryl)caffeine),[17] CS-DMPX (8-[2-(3-chlorophenyl)vinyl]-3,7-dimethyl-1-prop-2-ynyl-3,7-dihydropurine-2,6-dione),[18] and KW-6002,[19,20] has been described to occur in diluted solutions.[17−19,21] Mixtures containing (*Z*)-configurated 8-styrylxanthine derivatives have been shown to possess much lower A_{2A} adenosine receptor affinity in comparison with their corresponding (*E*)-configurated isomers.[18,19] On the other hand, it had been observed that animal studies with MSX-3 afforded variable results when the solid compound had been exposed to light prior to its application.[22] The resulting compounds of the solid-state photochemical reaction have now been characterized as [2 + 2]-cycloaddition products. To the best of our knowledge, this kind of reaction has not been described for styrylxanthines so far. The cyclobutane derivatives formed showed significantly lower adenosine receptor affinities than their monomeric precursors.

The goals of the present study were to improve and upscale the syntheses of the potent and selective A_{2A} antagonists MSX-2, MSX-3, and KW-6002 in order to make these compounds available as pharmacological tools on a multigram level for extended in vitro and especially for in vivo studies. Furthermore, the photochemical stability and reactivity of 8-styrylxanthines was thoroughly investigated.

Chemistry

The synthesis of MSX-2 ((*E*)-3-(3-hydroxypropyl)-8-[2-(3-methoxyphenyl)vinyl]-7-methyl-1-prop-2-ynyl-3,7-dihydropurine-2,6-dione, **8**), which is substituted at the N1-position by a 3-hydroxypropyl group and at the N3-position by a propargyl group, is more complex than the synthesis of KW-6002 ((*E*)-8-[2-(3,4-dimethoxyphenyl)vinyl]-1,3-diethyl-7-methyl-3,7-dihydro-purine-2,6-dione, **16**), which is substituted by two ethyl groups in the corresponding positions of the xanthine nucleus.

Optimized, Large-Scale Synthesis of MSX-3

Commercially available 6-aminouracil (6-amino-1*H*-pyrimidine-2,4-dione, **1**) was used as a starting material for the synthesis of **9** (MSX-3).[10] Regioselective 3-alkylation of **1** with 3-bromopropyne was based on the method developed by Müller via a silylated intermediate, which was formed by ammonium sulfate-catalyzed reaction of **1** with HMDS (1,1,1,3,3,3-hexamethyldisilazane) under reflux conditions.[23] It was observed that a larger excess (1.7 equiv) of alkylation reagent led to a more complete conversion to 6-amino-3-prop-2-ynyl-1*H*-pyrimidine-2,4-dione **2**. The formation of a small amount of the 3,5-dialkylated byproduct 6-amino-3,5-diprop-2-ynyl-1*H*-pyrimidine-2,4-dione **2a**, which was isolated by column chromatography and completely characterized, does not impede the process in its subsequent stages. Since the C5-position was blocked by an additional propargyl group, **2a** was not nitrosated in the following step, and in contrast to the 5-nitroso derivate 6-amino-5-nitroso-3-prop-2-ynyl-1*H*-pyrimidine-2,4-dione **3**,[24] it remained in solution under the applied reaction conditions (sodium nitrite in aqueous acetic acid). If the nitrosation was carried out at 50−60 °C, a lower temperature than cited in the original literature,[24] nitrosation of **2** resulted in a better yield of **3**, with smaller amounts of darker colored byproducts. The poorly soluble **3** was precipitated from the reaction solution and isolated by filtration.

Reduction of **3** with sodium dithionite in aqueous ammonia yielded 5,6-diamino-3-prop-2-ynyl-1*H*-pyrimidine-2,4-dione **4**,[24,25] a compound considered to be sensitive to oxygen, humidity, and light. After removal of reactive byproducts by recrystallization from water, and subsequent drying under vacuum, pure **4** was found to remain stable for several weeks at room temperature.

Coupling of **4** with (*E*)-3-methoxycinnamic acid, resulting in the formation of an amide bond, has previously been performed by using the water-soluble (3-dimethyl-

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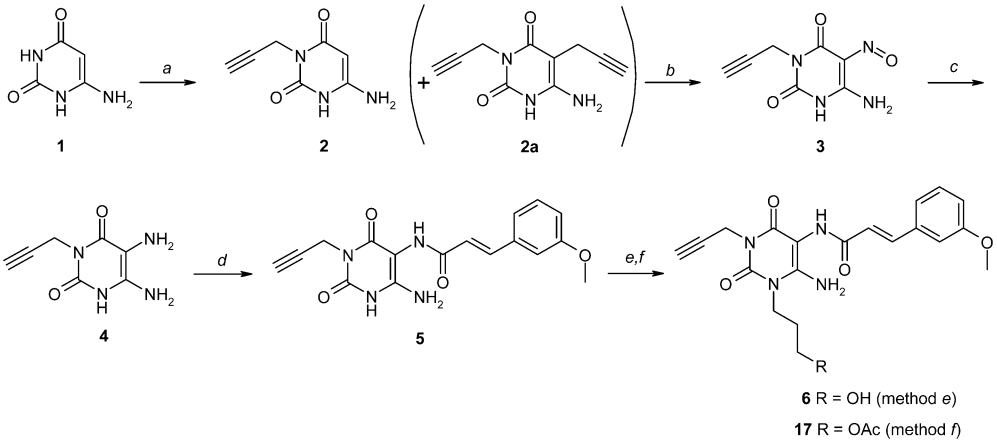
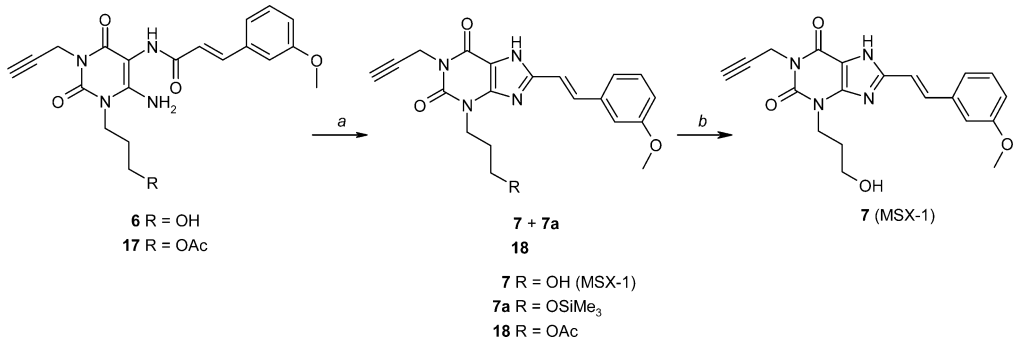
SCHEME 1. Synthesis of (*E*)-N-[6-Amino-1-(3-hydroxypropyl)-2,4-dioxo-3-prop-2-ynyl-1,2,3,4-tetrahydropyrimidin-5-yl]-3-(3-methoxyphenyl)acrylamide (6) and Its Acetic Acid Ester (17)[a]



[a] Reagents, conditions and yields: (a) 2.1 equiv of HMDS, reflux, 60−70 °C, 1.7 equiv of 3-bromopropyne, 120 °C, 2 h, H_2O (75%); (b) aq AcOH, HNO_2, 50−60 °C (72%); (c) sodium dithionite/NH_3/H_2O, 70 °C (67%); (d) (*E*)-3-methoxycinnamic acid chloride, pyridine, ethyl acetate, rt (80%); (e) 1.8 equiv 3-iodopropan-1-ol, DMF, K_2CO_3, rt (90%); (f) 1.7 equiv of acetic acid 3-iodopropyl ester, DMF, K_2CO_3.

SCHEME 2. Ring Closure of Uracil Cinnamoyl Amide Derivatives to the Corresponding Xanthines[a]



[a] Reagents, conditions and yields: (a) HMDS, reflux, methanol/H_2O (85%, **18**); (b) KOH/methanol/H_2O, H^+ (max 92%).

aminopropyl)ethylcarbodiimide hydrochloride (EDC) as a condensing agent.[10] In view of the high costs of the reagent EDC, we used (*E*)-3-methoxycinnamic acid chloride and pyridine as an acid acceptor in ethyl acetate suspension to obtain (*E*)-*N*-(6-amino-2,4-dioxo-3-prop-2-ynyl-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(3-methoxyphenyl)acrylamide **5** resulting in a simplified isolation procedure and an improved yield (80%). Compound **5** is poorly soluble in common solvents but could be recrystallized from hot DMF by the addition of water.

The 3-hydroxypropyl group could be introduced directly at the N1-atom of the uracil derivate **5** either with 3-bromopropan-1-ol at elevated temperature (60 °C)[10] or with 3-iodopropan-1-ol at room temperature, in DMF in the presence of a base.

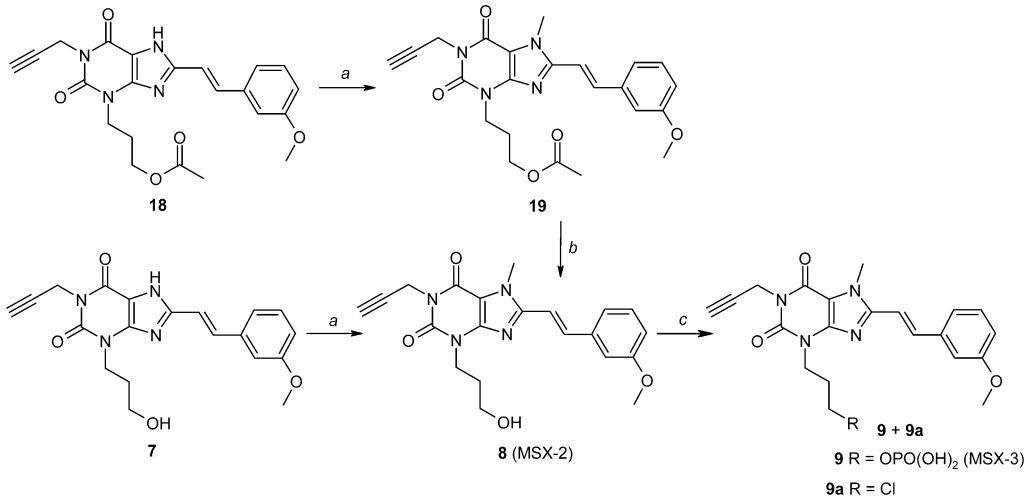
The drawback of direct preparation of the *N*-hydroxypropyl-substituted uracil derivative **6** was that the required large excess of alkylation reagent impeded the subsequent crystallization. Due to the low solubility of **6**, classical extractive isolation of the compound could only be achieved with large quantities of solvent. The subsequent ring-closure reaction of **6** with an excess of HMDS produced xanthine derivate **7** (MSX-1)[10] as well as compound **7a**, which was silylated at the free hydroxypropyl group, in roughly equal proportions. However, alkaline hydrolysis of this mixture led directly to the formation of pure **7**.

A simple solution to the problems mentioned above was to convert the hydroxyl group of 3-iodopropan-1-ol to its acetyl ester. Having thus protected it, a small excess of alkylation reagent was brought to reaction with **5** in DMF in the presence of potassium carbonate. After 24 h at room temperature, an excellent yield of the resulting **17**, (*E*)-acetic acid 3-[6-amino-5-[3-(3-methoxyphenyl)acryloylamino]-2,4-dioxo-3-prop-2-ynyl-3,4-dihydro-2*H*-pyrimidin-1-yl]propyl ester, was obtained and isolated by precipitating the product with water. Further purification was obtained by subsequent recrystallization from methanol/water (Scheme 1).

Ring-closure reaction of **6** and **17** under elimination of water to yield the xanthine ring system could be performed in alkaline aqueous solution or with HMDS, respectively.[10] The disadvantage of the former method was that hydrolysis of the amide bond as a side reaction is not only inevitable but increases drastically with the rise of temperature in the presence of alkali and water.

Reaction of **17** with an excess of HMDS in the presence of ammonium sulfate under reflux conditions produced exclusively the desired xanthine derivative **18** (Scheme 2). Treatment of the reaction mixture with methanol/water under neutral conditions allowed the isolation of the compound. Splitting off the ester group by hydrolysis of the same mixture with alkali in methanol at increased temperature and subsequent precipitation of the product

SCHEME 3. Synthesis of 7-Methylxanthine Derivatives[a]



[a] Reagents, conditions, and yields: (a) MeI, DMF, K_2CO_3 (93%, **8**; 98%, **19**); (b) KOH/methanol, rt, 0.5 h (93%); (c) $PO(OMe)_3/POCl_3$, rt, 1 h, H_2O (82%, **9**).

with water after acidification with hydrochloric acid (to pH 2) proved to be a straightforward method of yielding **7** (Scheme 2).

Methylation in the N7-position of **7** and **18** was achieved almost quantitatively using methyl iodide in DMF and potassium carbonate as a base following standard procedures.[10]

After a reaction time of 2 h at room temperature the methylation of **18** was completed and the acetic acid (*E*)-3-[8-[2-(3-methoxyphenyl)vinyl]-7-methyl-2,6-dioxo-1-prop-2-ynyl-1,2,6,7-tetrahydropurin-3-yl]propyl ester **19** could be precipitated by adding water to the reaction mixture (Scheme 3).

To split off the acetyl protecting group and produce MSX-2 (**8**),[10] **19** was treated for 0.5 h with a methanolic solution of potassium hydroxide. Once the reaction was concluded, pure **8** could be isolated in a high yield after the addition of water and filtering off the pale yellow precipitate.

The varying solubility of the different xanthine derivatives and their corresponding acrylamides is particularly striking. The xanthine precursor **17** was better soluble in halogenated hydrocarbons than **6**, but both compounds could easily be recrystallized from methanol/water.

In contrast to their precursors **6** and **17**, the N7-unsubstituted xanthine **7** was scarcely soluble, while **18** was poorly soluble in common organic solvents. NMR spectra of these compounds could therefore only be measured in DMSO-d_6. However, methylation of the N7-position of the xanthine system led to a high solubility of the derivatives **8** and **19** in halogenated hydrocarbons, an effect which is well-known for caffeine (1,3,7-trimethylxanthine) versus theophylline (1,3-dimethylxanthine).

The phosphoric acid ester **9** (MSX-3 free acid)[10] was obtained by direct phosphorylation of **8** using phosphoryl chloride in phosphoric acid trimethyl ester as a phosphorylating agent.[26] Although the reaction of aliphatic alcohols with phosphoryl chloride generally yields phosphoric acid esters, a possible side reaction is the conversion to the corresponding alkyl chloride. Indeed, the reaction of **8** with phosphoryl chloride in phosphoric acid trimethyl ester as a solvent at room temperature led to (*E*)-phosphoric acid mono[3-[8-[2-(3-methoxyphenyl)-vinyl]-7-methyl-2,6-dioxo-1-prop-2-ynyl-1,2,6,7-tetrahydropurin-3-yl]propyl] ester **9**, contaminated with variable amounts of the chlorinated product **9a**, depending on the reaction conditions (Scheme 3). Side product **9a** could easily be separated by column chromatography and was completely characterized. To minimize its formation, a large excess of the solvent phosphoric acid trimethyl ester was used, in addition to a small excess of phosphoryl chloride (molar ratio **8**/phosphoryl chloride = 1:4). The reaction time was reduced to 1 h, and a temperature of 0 °C was maintained. Subsequently, **9** was precipitated by adding a ca. −20 °C mixture of ice, sodium chloride, and water. After filtration and drying at 70 °C, no further purification steps were required for obtaining a purity of at least 95%. Removal of **9a** and other nonpolar byproducts was achieved by dissolving crude **9** in diluted aqueous sodium hydroxide solution, followed by filtration and reprecipitation of **9** by acidification with hydrochloric acid. The disodium salt of **9** was prepared by dissolving the crude product in a minimal volume of an aqueous solution containing 2 equiv of NaOH. After precipitation by adding brine, the yellow disodium salt (MSX-3) was isolated by filtration and subsequently dried at 70 °C.

In view of the photosensitivity of **8** as well as its xanthine precursors and derivatives, it is recommended that compounds should not be exposed to light. To minimize photolytic degradation reactions, the disodium salt (MSX-3) should be prepared in situ from the free acid **9** just before application. The preparation of a 5 mM aqueous solution of MSX-3 is described in the Experimental Section.

Optimized, Large-Scale Synthesis of KW-6002

The starting material for the synthesis of **16** (KW-6002),[14,20,28] 6-aminouracil-1,3-diethyl-1*H*-pyrimidine-2,4-

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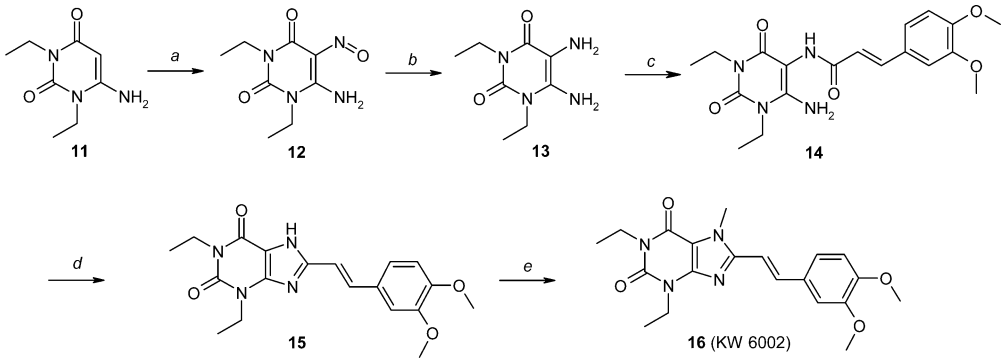
SCHEME 4. Synthesis of KW-6002[a]



[a] Reagents, conditions, and yields: (a) aq AcOH, HNO_2, 50−60 °C (74%); (b) sodium dithionite/NH_3/H_2O, 60 °C (80%); (c) (*E*)-3,4-dimethoxycinnamic acid chloride, pyridine, CH_2Cl_2, 16 h, rt (65%); (d) HMDS, ammonium sulfate, glass pressure tube, 170−180 °C, 4 h (92%); (e) 1.4 equiv of MeI, DMF, potassium carbonate (95%).

dione **11**, was obtained by direct reaction of the dialkyl urea with cyanoacetic acid following the protocol of Papesch and Schröder.[29] Subsequent introduction of the nitroso group in the 5-position to yield 6-amino-1,3-diethyl-5-nitroso-1*H*-pyrimidine-2,4-dione **12** was achieved in high yields analogously to the synthesis of **3**.[30,31]

The reaction of **12** to the 5,6-diamino-1,3-diethyl-1*H*-pyrimidine-2,4-dione **13**[30,31] was achieved, as described above, in aqueous ammonia with sodium dithionite as a reducing agent. Due to its high solubility, **13** could not be isolated by simple concentration of the reaction mixture and subsequent precipitation of the compound in a satisfactory yield. A better result was obtained by extraction of the aqueous reaction mixture with dichloromethane, classical separation, and recrystallization from a mixture of dichloromethane/diethyl ether.

To avoid expensive water-soluble carbodiimide condensing agents,[28,32,33] coupling of the diaminouracil **13** with (*E*)-3,4-dimethoxycinnamic acid chloride was performed in dichloromethane solution in the presence of pyridine, leading to (*E*)-*N*-(6-amino-1,3-diethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(3,4-dimethoxyphenyl)acrylamide **14**.[35] After aqueous extraction of byproducts and concentration of the organic phase, recrystallization of the oily residue from methanol/water afforded compound **14** as colorless crystals.

The subsequent ring-closure reaction of the amide precursor **14** to the corresponding xanthine has been described by Shimada et al. without giving the exact experimental proportions and product isolation protocol.[28] An investigation of the conditions for related reactions described by Suzuki et al.[34] showed that a mixture of equivalent volumes of dioxane and a 1 M aqueous solution of sodium hydroxide yielded (*E*)-8-[2-(3,4-dimethoxyphenyl)vinyl]-1,3-diethyl-3,7-dihydropurine-2,6-dione **15** after a reaction time of 10 min under reflux conditions. After dilution of the reaction mixture with water, the product was precipitated by acidification with hydrochloric acid, filtered, and washed with water. The described procedure led to a very pure product with a maximal yield of 62% only. This observation, confirmed by detection of (*E*)-3,4-dimethoxycinnamic acid in the crude reaction mixture by TLC analysis, pointed again to alkaline hydrolysis of the amide bond as a side reaction.

To avoid this problem, an alternative ring-closure reaction was performed using HMDS as a condensing agent. In fact, elimination of water could be achieved at 170−180 °C in the presence of ammonium sulfate under elevated pressure. After hydrolysis with methanol and filtration, **15** was obtained in a yield of 92%.

An alternative synthesis of compound **15** starting from **13** using the conventional method[34] has recently been described.[20] The reported yield of 35% over two steps is significantly lower as compared to the yields which were obtained by the method described above.

The up-scaled methylation of the N7-position of **15** to **16** was achieved in analogy to the preparation of **8** in DMF using methyl iodide in the presence of potassium carbonate.[10] After a reaction time of less than 5 h at room temperature, KW-6002 (**16**) could be precipitated by adding water and a high yield of the pure, light-yellow product was isolated (Scheme 4).[20,28,32,33,35]

Photochemical Reactions

The preparation of compounds **8** and **9** must be carried out under strict exclusion of light. When stored in normal glass vials and exposed to daylight, within a few days the pale yellow crystals were observed to turn into a colorless solid. TLC investigation of the obtained material indicated a decrease in **8** and the appearance of an additional spot, which could subsequently be identified as a photoreaction product.

The ^{1}H NMR spectrum of the colorless solid, isolated by column chromatography, gave a first indication of the formation of a cyclobutane ring system by a [2 + 2]-

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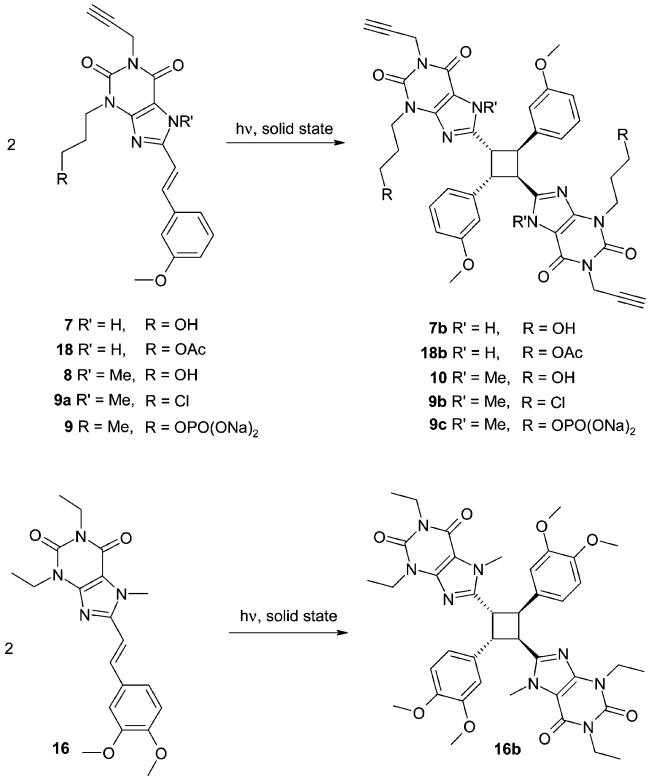
SCHEME 5. Solid-State Photoreactions of 8-Styrylxanthine Derivatives



cycloaddition reaction of two ethylenic bonds. A symmetrical AA′BB′-spin system, typical of 1,2,3,4-tetrasubstituted cyclobutanes, appeared around 4.69 ppm. Correspondingly, the signals for the ethylenic (*E*)-hydrogen atoms of **8** had disappeared.

The mass spectrum of **10** not only confirmed the suggested [2 + 2]-cycloaddition of **8** with a molecular ion M^+ 788 (EI, 35) for the product but also gave some clues to elucidate the possible constitution of the cyclobutane derivative that had been formed. The base peak at *m*/*z* 394 $M^+/2$ (EI, 100) indicated a symmetrical [2 + 2]-reversion of the dimerization product and hence the formation of a h(ead)-to-t(ail) cyclobutane isomer (Scheme 5). Since fragments characteristic of asymmetrical reversion were present neither in the mass spectra of **10** nor in those of all cyclobutanes described in this paper, the formation of hh dimers could be excluded.[36,37]

With regard to *E*/*Z*-isomerization of the starting compound **8**, which represents an asymmetrically substituted ethylene, and the hypothetical existence of both isomers in the reaction mixture, theoretically one can expect eleven stereoisomers formed by *trans-trans*, *trans-cis*, and *cis-cis* dimerization in an hh and ht modus. According to the mass spectral data, the number of possible structures could be reduced to five, because six of the stereoisomers correspond with an hh dimerization and could therefore be excluded.

Three of the remaining five ht isomers are formed theoretically including (*Z*)-configurated ethylene derivatives, which do not dimerize, since the half-life of their first excited singlet state is too short.[38] Apart from that, solid-state photoreaction of (*E*)-configurated ethylenes forces *trans-trans* dimerization, as the fixed positions in the crystal-lattice do not permit conversion to the (*Z*)-isomer.

Having excluded all other possibilities, only two ht isomers had to be considered in the case of compound **10**: the *cis-trans-cis* (*syn*) and the *all-trans* (*anti*) configurations.

With heterostilbenes, which exhibit a strong asymmetry of charge, ht orientations are to be expected and, additionally, along with dipole components perpendicular to the main axis, even *syn*-arrangements.[36] Further differentiation within the two ht isomers should be obtained from specific NMR spectral features. All described ht cyclobutanes with *syn*-configuration derived from asymmetrically substituted (*E*)-stilbenes or from heterocyclic analogues exhibited a symmetric AA′BB′-system for cyclobutane ring protons, consisting of two half-spectra with a lack of fine structure, so that coupling constants could not be easily extracted.[36,39,40] Each half-spectrum generally exhibits four lines. We found similar multiplets for the cyclobutane proton AA′BB′ systems in all of the NMR spectra of cyclic dimers described in this paper. Only a few examples for ht cyclobutanes with *anti*-configuration are described in the literature. On the basis of molecular symmetry (C_{2v}), a theoretical A_2B_2 system with a half-spectrum of seven lines would be expected for the aliphatic cyclobutane hydrogen atoms of these isomers.[40−42] However, superimposition of lines makes it impossible to distinguish the spin systems clearly.

An additional reference to the *syn*-configuration of the isolated cyclobutanes gave the chemical shift of the four protons directly bonded to the cyclobutane ring. The so-called midpoint method, developed by Ben-Efraim and Green,[43] is useful for the rapid and straightforward assignment of isomer structures. It is reported that the center of the cyclobutane proton signals of aryl-substituted derivatives in general appears around 4.5 ppm for the *cis-trans-cis* (*syn*), and around 3.7 ppm for the *all-trans* (*anti*) cyclobutanes.[40,42−44] The center of the cyclobutane multiplets presented for **10** as well as for the cyclobutane derivatives **7b**, **9b**, and **16b**, described below as photoreaction products of 8-styrylxanthines **7**, **9a**, and **16**, appeared between 4.56 and 4.73 ppm. In accordance with theory, ht *syn*-configuration for these compounds is proposed. With a cyclobutane multiplet centered at 4.15 ppm derivative **18b**, described below as photoreaction product of **18**, constituted an exception. After splitting off the acetyl groups by alkaline hydrolysis, the resulting product showed exactly the same ^{1}H NMR spectrum as that obtained for **7b**, which also indicated the likelihood of a ht *syn*-configuration for **18b** (Scheme 5).

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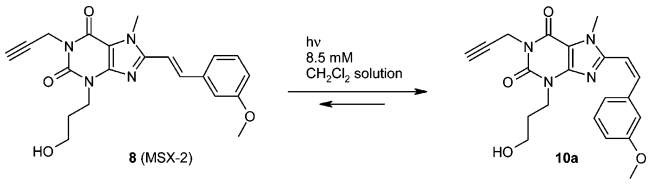
SCHEME 6. Photoreaction of MSX-2 in Diluted Dichloromethane Solution



The photochemical properties of compound **8**, as here described, should apply—in varying degrees—to all xanthine derivates substituted in the 8-position by a styryl group. It proved extremely difficult to predict how easily each of the xanthines described in this study would undergo solid-state photodimerization. In the case of the less sensitive xanthines, it took several weeks to observe evidence of photolysis caused by daylight. In contrast, the light-yellow compounds **8** and **9a** behaved quite differently. When exposed to daylight, in the form of a thin crystalline film in a normal glass vial, they decolorized completely within ca. 8 h and were quantitatively transformed into their corresponding photodimers **10** and **9b**, respectively. The 8-styrylxanthine **16** (KW-6002) proved considerably more stable: Even after 6 months of storage under diffuse daylight, the compound exhibited virtually no sign of photolytic reaction. However, brief irradiation of solid **16** with ultraviolet light led almost quantitatively to the formation of photodimer **16b** (Scheme 5). The colorless xanthine derivatives **7** and **18** are characterized by a free NH-function in the 7-position. They showed a moderate degree of photosensitivity. Exposed to daylight, a thin crystal film of the compounds photolyzed almost completely within ca. 4 weeks to form the corresponding cyclobutanes **7b** and **18b**.

Since it was not possible to prepare an appropriate crystal film of **7**, due to its poor solubility in volatile organic solvents, the results of UV irradiation were unsatisfactory. A brief period of irradiation produced very poorly soluble, obviously polymeric material. This could point to a competing radical reaction in addition to the [2 + 2]-cyclodimerization.

Several photolytic reactions of 8-styrylxanthines in diluted solutions have been described, and it was considered that irradiation leads to the rapid establishment of a photostationary state, an equilibrium of (*E*) and (*Z*) isomers.[17−19,21] On no occasion so far has photodimerization been observed. To support these findings and distinguish them from solid-state photolysis, an 8.5 mM solution of MSX-2 in dichloromethane was irradiated. After 10 min reaction time, analysis by TLC indicated the formation of a reaction product close to the spot of the starting material. The irradiation was stopped after 1.5 h, and the concentrated oily residue was separated by column chromatography. Two main fractions were obtained in this way and analyzed by NMR and mass spectroscopy. The ^{1}H NMR spectrum of the first fraction indicated an enrichment of the starting compound **8**. Its (*Z*)-isomer **10a**, which was slightly contaminated with **8**, could be detected in the second fraction (Scheme 6).

An important evidence of the (*Z*)-isomer was the coupling constant for the ethylenic protons, which was found to be ca. 3 Hz lower for **10a** (12.4 Hz) as compared with the (*E*)-isomer **8** (15.8 Hz), hence, in the expected region for *cis*-arranged protons. The extreme high field shift of ca. 0.4 ppm for the signal of the 7-*N*-methyl group was further evidence of a reaction to the (*Z*)-isomer **10a**. The ^{1}H NMR spectra of all obtained fractions showed no typical signals of cyclobutane protons; hence, when MSX-2 was photolyzed in diluted solution there was no evidence of [2 + 2]-cycloaddition products. The ^{13}C NMR experiment served to confirm these observations: Between 40 and 45 ppm, no typical signals of cyclobutane C-atoms were observed. The mass spectrum of **10a** did not differ from that of the starting compound. These observations do not exclude the possibility that the irradiation of more concentrated solutions of 8-styrylxanthines could also lead to the formation of cyclobutanes.

TABLE 1. Adenosine Receptor Affinities of Dimerization Products of Potent, Selective A_{2A}-Adenosine Receptor Antagonists with 8-Styrylxanthine Structure

	$K_i \pm$ SEM (nM), $n = 3$		
compd	A_1 affinity rat brain cortical membranes vs [^{3}H]CCPA	A_{2A} affinity rat brain striatal membranes vs [^{3}H]MSX-2	A_{2A} selectivity vs A_1 (K_i A_1/K_i A_{2A})
10	273 ± 34	175 ± 15	1.6
16b	>10 000 (7%[a])	1570 ± 33	>7

[a] Displacement at 10 μM, $n = 2$.

Biological Activity

The new cyclobutane derivatives **10** and **16b**, dimerization products of the potent A_{2A}-selective adenosine receptor antagonists **8** and **16**, were investigated in radioligand binding studies at A_1 adenosine receptors of rat brain cortical membranes using the A_1-selective radioligand [^{3}H]-2-chloro-N^6-cyclopentyladenosine ([^{3}H]-CCPA) and at A_{2A} adenosine receptors of rat brain striatal membranes using the A_{2A}-selective radioligand [^{3}H]MSX-2. Data of dimerization products were compared with those of the monomeric compounds. The A_{2A}-selective adenosine receptor antagonists MSX-2 and KW-6002 were similarly potent A_{2A} antagonists with K_i values in the low nanomolar range.[10,28] Both compounds were considerably less active at A_1 receptors. Dimerization reduced the A_{2A} affinity of both compounds dramatically, but the decrease was more pronounced for the KW-6002 dimer (**16b**) as compared to the MSX-2 dimer (**10**, see Table 1).

At A_1 adenosine receptors, dimerization of KW-6002 resulted in a complete loss of affinity. In contrast, the dimerization product of MSX-2 retained some A_1 affinity and was a moderately potent, nonselective adenosine receptor antagonist (**10**, $K_i(A_1) = 273$ nM, $K_i(A_{2A}) = 175$ nM). It is quite surprising that the receptors can accommodate such a bulky dimeric molecule. However, subtle structural differences in the dimers, which did not play a noteworthy role in the monomers, resulted in large differences in A_{2A} (9-fold) and particularly in A_1 affinity (>37-fold) between the dimeric cyclobutane derivatives **10** and **16b**. These structure−activity relationships will be useful for molecular modeling studies of adenosine receptors and their complexes with ligands and may be helpful in future drug design studies.

Conclusions

8-Styrylxanthine derivatives are an important class of A_{2A} adenosine receptor antagonists. For two of the most frequently used derivatives of this structural class of compounds, MSX-3 (**9**) and KW-6002 (**16**), improved, upscaled syntheses have been developed. The overall yield of **9** has been increased (ca. 20%, starting from the uracil derivative **1**), and multigram quantities could be obtained. In the case of **16**, an overall yield of about 30% (starting from **11**) has been reached. For a better understanding of the reactions, byproducts were isolated and characterized, ultimately leading to subtle but considerable improvements in reaction strategies.

The photosensitivity of the 8-styrylxanthines was investigated. In addition to the well-known *E*/*Z*-isomerization that occurs in diluted solutions, it was found that in the solid state some derivatives are even more light-sensitive. Instead of *E*/*Z*-isomerization, [2 + 2]-cycloaddition was observed when the crystals were irradiated by normal daylight or by a mercury lamp at 200−600 nm. No simple correlation could be found between the substitution pattern and the degree of photosensitivity. Generally, N7-unsubstituted 8-styrylxanthines appeared to be less photoreactive than their N7-methylated derivatives. The amide precursors of 8-styrylxanthines did not show any photosensitivity at all. It was proven by NMR sprectroscopy that all solid-state photoreactions described led to head-to-tail *cis-trans-cis* (*syn*) cyclobutane derivatives.

Experimental Section

Compounds **2**−**5**[23−25] and **8**[10] as well as compounds **12**, **13**,[25,30,31] and **16**[20,28,32,33,35] were synthesized according to described procedures with only slight modifications; for details also see the Supporting Information.

6-Amino-3,5-diprop-2-ynyl-1*H*-pyrimidine-2,4-dione (2a). Compound **2a** was separated from crude **2** by column chromatography on silica gel (eluent: CH_2Cl_2/methanol, 3:1). Subsequent recrystallization from ethanol/diethyl ether (1:2) yielded **2a** as slightly reddish crystals in varying proportions, depending on its content in the crude **2**. Data for **2a**: mp 220.5 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.61 (t, J = 2.6 Hz, 1H), 2.98 (t, J = 2.6 Hz, 1H), 3.12 (d, J = 2.6 Hz, 2H), 4.41 (d, J = 2.6 Hz, 2H), 6.27 (s, 2H), 10.45 (s, 1H); ^{13}C NMR (500 MHz, DMSO-d_6) δ 12.4, 28.9, 69.5, 72.4, 80.3, 80.6, 83.2, 149.5, 150.7, 161.4; EIMS (m/z) 203 (M^+, 100).

(*E*)-*N*-[6-Amino-1-(3-hydroxypropyl)-2,4-dioxo-3-prop-2-ynyl-1,2,3,4-tetrahydropyrimidin-5-yl]-3-(3-methoxyphenyl)acrylamide (6).[10] A mixture of 10 g (29.4 mmol) of **5**, 4.1 g of anhydrous K_2CO_3 (30 mmol), and 10 g (54 mmol) of 3-iodopropan-1-ol in DMF (75 mL) was stirred for 16 h at rt. The reaction mixture was diluted with 1000 mL of water and extracted four times with 250 mL of CH_2Cl_2 each. The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated under vacuum, particularly in order to remove traces of DMF. The oily residue was dissolved in a small amount of methanol and then treated with a mixture of diethyl ether and petroleum ether (1:1) to crystallize the product as colorless needles. After filtration and washing with a mixture of diethyl ether and petroleum ether (1:1), the solid was dried at 70 °C (90% yield): mp 123 °C; ^{13}C NMR (125 MHz, DMSO-d_6) δ 30.0, 30.9, 55.3, 58.1, 72.6, 80.1, 87.5, 112.9, 115.3, 119.9, 123.1, 130.2, 136.6, 138.9, 149.8, 152.1, 158.2, 159.8, 165.5; EIMS (m/z) 398 (M^+, 100).

(*E*)-Acetic Acid 3-[6-Amino-5-[3-(3-methoxyphenyl)-acryloylamino]-2,4-dioxo-3-prop-2-ynyl-3,4-dihydro-2*H*-pyrimidin-1-yl]propyl Ester (17). A mixture of 10 g (29.4 mmol) of **5**, anhydrous K_2CO_3 (4.1 g, 30 mmol), and 11.4 g (50 mmol) of acetic acid 3-iodopropyl ester in DMF (75 mL) was stirred for at least 24 h at rt until a clear solution was obtained and no further starting material could be detected by TLC (eluent: CH_2Cl_2/methanol, 7:1). The product was precipitated by the addition of water (75 mL), filtered off, and washed with water (150 mL). The colorless needles, obtained by recrystallization with methanol/water (1:1), were filtered off under reduced pressure and washed with diethyl ether (100 mL). After drying at 70 °C, a pale yellow solid was obtained. Compound **17** could also be isolated by extraction with CH_2Cl_2 as described for compound **7** (92% yield): mp 129.4 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.01 (m, 2H), 2.06 (s, 3H), 2.19 (t, J = 2.2 Hz, 1H), 3.81 (s, 3H), 3.96 (m, 2H), 4.11 (m, 2H), 4.63 (d, J = 2.2 Hz, 2H), 5.98 (s, 2H), 6.67 (d, J = 15.4 Hz, 1H), 6.89−7.27 (m, 4H), 7.56 (d, J = 15.4 Hz, 1H), 7.82 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.9, 27.2, 30.8, 40.6, 55.3, 61.6, 70.9, 78.4, 91.6, 113.1, 115.9, 120.0, 120.6, 135.7, 142.3, 135.7, 149.0, 149.5, 159.1, 159.9, 165.9, 170.8; EIMS (m/z) 440 (M^+, 100).

(*E*)-Acetic Acid 3-[8-[2-(3-Methoxyphenyl)vinyl]-2,6-dioxo-1-prop-2-ynyl-1,2,6,7-tetrahydropurin-3-yl]propyl Ester (18). A mixture of 3 g (6.8 mmol) of **17**, $(NH_4)_2SO_4$ (0.25 g), and HMDS (30 mL) was heated under vigorous reflux and vigorous stirring for 16 h. The reaction was monitored by TLC (eluent: CH_2Cl_2/methanol, 9:1). The end of the reaction was reached when the molten, poorly soluble starting material had been transformed to a fine suspension of colorless product in a clear yellow solution. After hydrolysis by adding methanol and cooling to rt, the colorless solid was filtered off under reduced pressure, washed with methanol (100 mL) and subsequently with diethyl ether (75 mL), and dried at 70 °C (85% yield): mp 219.6 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.91 (s, 3H), 2.02 (m, 2H), 3.04 (t, J = 2,2 Hz, 1H), 3.73 (s, 3H), 4.01 (m, 2H), 4.09 (m, 2H), 4.58 (d, J = 2.2 Hz), 7.01 (d, J = 16.4 Hz, 1H), 6.89−7.31 (m, 4H), 7.58 (d, J = 16.4 Hz, 1H), 13.62 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.1, 27.2, 30.7, 41.1, 55.7, 62.3, 73.3, 80.2, 112.5, 115.7, 116.5, 120.2, 130.5, 136.0, 137.3, 170.7, 107.5, 149.1, 150.4, 150.6, 153.4, 160.2, 170.7; EIMS (m/z) 422 (M^+, 100).

(*E*)-3-(3-Hydroxypropyl)-8-[2-(3-methoxyphenyl)vinyl]-1-prop-2-ynyl-3,7-dihydropurine-2,6-dione (7, MSX-1).[10] A suspension of 5 g of **17** (11.4 mmol) or alternatively 5 g (12.6 mmol) of **6**, 0.25 g of $(NH_4)_2SO_4$, and HMDS (50 mL) was heated under vigorous reflux and stirred for 16 h until the molten, poorly soluble starting compound had been transformed to a fine suspension of the colorless product in a clear yellow solution.

To remove the acetyl group of the intermediate **18** and the silyl group of the O-silylated byproduct **7a**, the reaction mixture was treated with a solution of KOH (2 g) in methanol (50 mL). After being heated at reflux for 0.5 h, the mixture was concentrated under vacuum, and the oily residue was diluted with a mixture of methanol (50 mL) and water 150 mL). The colorless product was precipitated by adding concd HCl dropwise (→ pH ≅ 2), filtered off, washed with water (250 mL), and dried at 70 °C (92% yield): mp 250 °C; ^{13}C NMR (125 MHz, DMSO-d_6) δ 30.3, 31.1, 41.1, 55.3, 58.6, 72.9, 79.9, 112.1, 115.3, 116.1, 119.8, 130.1, 135.3, 136.1, 107.5, 148.8, 150.2, 150.2, 153.2, 159.8; EIMS (m/z) 380 (M^+, 100).

(*E*)-8-[2-(3-Methoxyphenyl)vinyl]-1-prop-2-ynyl-3-(3-trimethylsilanyloxypropyl)-3,7-dihydropurine-2,6-dione (7a). For separation of compound **7a**, the reaction mixture which was obtained from the reaction of **6** with HMDS was hydrolyzed under almost neutral conditions with an excess of methanol. After evaporation of the solvents, the residue was purified by column chromatography on silica gel (eluent: CH_2Cl_2/methanol, 7:1), yielding **7** and **7a** as colorless solids in varying proportions. Data for **7a**: mp > 205 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.09 (s, 9H), 2.07 (m, 2H), 2.17 (t, J = 2.6 Hz, 1H), 3.72 (m, 2H), 3.83 (s, 3H), 4.29 (m, 2H), 4.88 (d, J = 2.6 Hz, 1H), 7.11 (d, J = 16.1 Hz, 1H), 6.87−7.30 (m, 4H), 7.77 (d,

J = 16.1 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 0.1, 30.8, 31.0, 41.8, 55.3, 60.3, 71.0, 78.9, 112.4, 115.2, 115.4, 120.2, 129.8, 136.9, 137.5, 107.1, 150.3, 150.3, 151.7, 154.9, 160.0; EIMS (m/z) 452 (M^+, 30).

(*E*)-Acetic Acid 3-[8-[2-(3-Methoxyphenyl)vinyl]-7-methyl-2,6-dioxo-1-prop-2-ynyl-1,2,6,7-tetrahydropurin-3-yl]-propyl Ester (19). A suspension of 0.2 g (0.47 mmol) of **18**, K_2CO_3 (0.14 g, 1 mmol), and 0.14 g (1 mmol) of methyl iodide in DMF (5 mL) was stirred at rt for 5 h under exclusion of light (TLC control, eluent: CH_2Cl_2/methanol, 9.5:0.5). The pale yellow, light-sensitive product was precipitated by addition of water (30 mL), filtered off under reduced pressure, washed with water (70 mL), and dried at 70 °C (98% yield): mp 158.4 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.03 (s, 3H), 2.16 (t, J = 2.2 Hz, 1H), 2.17 (m, 2H), 3.84 (s, 3H), 4.03 (s, 3H), 4.15 (m, 2H), 4.26 (m, 2H), 4.77 (d, J = 2.2 Hz, 2H), 6.86 (d, J = 15.5 Hz, 1H) 6.89−7.32 (m, 4H), 7.73 (d, J = 15.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.6, 27.1, 30.4, 31.5, 40.6, 55.3, 62.0, 70.4, 78.8, 111.4, 112.7, 115.1, 120.0, 129.9, 136.7, 138.6, 107.8, 148.6, 150.3, 150.5, 154.1, 160.0, 171.0; EIMS (m/z) 436 (M^+, 100).

Hydrolysis of 19. To a solution of 0.5 g KOH in methanol (30 mL), 3 g (7.1 mmol) of **19** were added. The suspension was stirred for 0.5 h at rt. After the complete disappearance of **19** (TLC control, eluent: CH_2Cl_2/methanol, 9.5:0.5), **8** (MSX-2) was precipitated by dilution with water (70 mL), filtered off under reduced pressure, washed with water (200 mL), and dried at 70 °C (93% yield).

(*E*)-Phosphoric Acid Mono[3-[8-[2-(3-methoxyphenyl)-vinyl]-7-methyl-2,6-dioxo-1-prop-2-ynyl-1,2,6,7-tetrahydropurin-3-yl]propyl] Ester (9, MSX-3 Free Acid).[10] A solution of 1.0 g (2.5 mmol) of **8** in phosphoric acid trimethyl ester (10 mL) was prepared by gentle heating. After cooling to 0 °C, 1.0 mL (10 mmol) of phosphoryl chloride was added to the stirred solution. The mixture was stirred at rt under exclusion of light for 0.5 h and subsequently hydrolyzed with a mixture of ice and NaCl at about −20 °C. The formed yellow, light-sensitive precipitate was filtered off, washed with water (350 mL), and dried at 70 °C (≥95% yield): mp 193−195 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.03 (m, 2H), 3.06 (t, J = 2.2 Hz, 1H), 3.81 (s, 3H), 3.93 (m, 2H), 4.03 (s, 3H), 4.11 (m, 2H), 4.59 (d, J = 2.2 Hz, 2H), 6.92−7.31 (m, 5H), 7.66 (d, J = 15.8 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 28.86 (d, J = 7 Hz), 30.11, 31.68, 40.28, 55.39, 63.39 (d, J = 5 Hz), 72.9, 79.8, 112.7, 113.1, 115.4, 120.4, 129.9, 137.1, 137.3, 107.4, 148.0, 150.0, 150.2, 153.4, 159.8; ^{31}P NMR (202 MHz, DMSO-d_6) δ −0.29; ESI negQ1 m/z 473 ($M - H^+$). TLC control: (methanol, R_f = 0.71, spraying reagent for phosphate derivatives: 0.1% $FeCl_3$ and 7% 5′-sulfosalicylic acid in 75% aq ethanol).

Isolation of 9a. When the reaction of **8** to **9** was performed using a larger excess of phosphoryl chloride (molar ratio **8**/$POCl_3$, 1:10) combined with hydrolysis at higher temperature (rt), chlorination of the hydroxyl group became predominant. The byproduct (*E*)-3-(3-chloropropyl)-8-[2-(3-methoxyphenyl)-vinyl]-7-methyl-1-prop-2-ynyl-3,7-dihydropurine-2,6-dione **9a** was isolated by extracting the reaction mixture with CH_2Cl_2 after hydrolysis. The organic extract was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was crystallized from CH_2Cl_2/petroleum ether (1:1) to yield **9a** as a pale yellow solid: mp 223.2 °C dec; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.16 (t, J = 2.2 Hz, 1H), 2.30 (m, 2H), 3.63 (m, 2H), 3.84 (s, 3H), 4.04 (s, 3H), 4.30 (m, 2H), 4.77 (d, J = 2.2 Hz, 2H), 6.86 (d, J = 15.8 Hz, 1H), 6.91−7.32 (m, 4H), 7.76 (d, J = 15.8 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 30.5, 31.2, 31.6, 41.3, 55.4, 42.3, 70.4, 78.7, 111.3, 112.8, 115.2, 120.0, 129.9, 136.8, 138.8, 107.9, 148.5, 150.3, 150.6, 154.1, 160.0; EIMS (m/z) 412 (M^+, 100).

(*E*)-Phosphoric Acid Mono[3-[8-[2-(3-methoxyphenyl)-vinyl]-7-methyl-2,6-dioxo-1-prop-2-ynyl-1,2,6,7-tetrahydropurin-3-yl]propyl] Ester Disodium Salt (MSX-3).[10] In a solution of 90 mg (2.3 mmol) of NaOH in water (ca. 80 mL) was dissolved 500 mg (1.05 mmol) of crude **9** under stirring and/or sonication. The yellow solution was filtered, and the product was precipitated by addiion of brine (ca. 50 mL). After filtration and drying at 70 °C, a light-sensitive, pale yellow solid was obtained (75% yield): ^{13}C NMR (125 MHz, D_2O) δ 31.7, 31.8, 31.8, 33.4, 37.8, 58.2, 64.5 (d, J = 2.7 Hz), 110.4, 114.0, 116.8, 122.1, 132.3, 138.4, 139.8, 150.4, 153.2, 153.5, 156.3, 161.6.

Preparation of an Isotonic Solution of (*E*)-Phosphoric Acid Mono[3-[8-[2-(3-methoxyphenyl)vinyl]-7-methyl-2,6-dioxo-1-prop-2-ynyl-1,2,6,7-tetrahydropurin-3-yl]-propyl] Ester Disodium Salt (MSX-3) from 9. In a solution of 4.5 mg (0.11 mmol) of NaOH in 10 mL of 0.9% saline was dissolved 25 mg (ca. 0.053 mmol) of crude **9** under exclusion of light. After stirring and/or sonication for about 5 min, the slightly turbid solution was filtered off through a 0.45 μm membrane filter. A clear yellow 5 mM solution of MSX-3 disodium salt was obtained.[10]

Photoreactions

Solid-State Photoreaction of 8 (MSX-2) to (1α,2α,3*β*,4*β*)-1,3-Bis[3-(3-hydroxypropyl)-7-methyl-1-prop-2-ynyl-3,7-dihydropurine-2,6-dione-8-yl]-2,4-bis(3-methoxy)phenylcyclobutane (10). A crystal film of 100 mg (0.25 mmol) of **8** was prepared by dissolving the yellowish solid in a minimum of CH_2Cl_2 and subsequently leaving the solvent to evaporate on the surface of a photoreactor. After 90 min of irradiation, the essentially decolorized product was dissolved in CH_2Cl_2, filtered to remove oligomeric material, and recrystallized by the addition of diethyl ether to yield colorless crystals (63 mg): mp 252 °C dec; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.96 (m, 4H), 2.14 (t, J = 2.2 Hz, 2H), 3.51 (m, 4H), 3.64 (s, 6H), 3.71 (s, 6H), 4.24 (m, 4H), 4.44−4.47 and 4.90−4.93 (AA′BB′, 4H), 4.70 (m, 4H), 6.70−7.15 (m, 8H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 30.5, 31.0, 31.8, 39.7, 40.7, 44.5, 55.2, 57.6, 70.5, 78.5, 112.5, 114.2, 119.7, 130.0, 138.9, 107.6, 147.8, 150.6, 152.0, 153.9, 159.8; EIMS (m/z) 788 (M^+, 38). TLC (eluent: ethyl acetate, R_f = 0.45).

Photolysis of 8 in Solution. A 0.85 mM solution of MSX-2, prepared by dissolving 250 mg (0.63 mmol) of **8** in CH_2Cl_2 (850 mL), was transferred into a photoreactor and irradiated with a medium-pressure mercury lamp. The reaction was monitored by TLC. After ca. 10 min, an additional spot was detected by TLC, indicating a photoreaction product (eluent: ethyl acetate, **8**, R_f = 0.68; **10a**, R_f = 0.38). After 1.5 h the reaction was stopped, and the solvent was evaporated. The residue was separated by column chromatography (eluent: CH_2Cl_2/ethanol, 9.5:0.5). Two main fractions were obtained: one enriched with the starting material (*E*)-MSX-2, the other enriched with the (*Z*)-isomer of MSX-2. After evaporation of the solvents, the crude residues were analyzed by mass and NMR spectroscopy.

(*Z*)-3-(3-Hydroxypropyl)-8-[-2-(3-methoxyphenyl)-vinyl]-7-methyl-1-prop-2-ynyl-3,7-dihydropurine-2,6-dione (10a): ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.88 (m, 2H), 2.15 (t, J = 2.5 Hz, 1H), 3.44 (m, 2H), 3.63 (s, 3H), 3.69 (s, 3H), 4.17 (m, 2H), 4.73 (d, J = 2.5 Hz, 2H), 6.31 (d, J = 12.4 Hz, 1H), 6.75−7.91 (m, 4H.), 6.99 (d, J = 12.4 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 30.4, 30.9, 32.4, 39.8, 55.1, 57.9, 70.5, 78.5, 114.2, 114.2, 114.6, 121.0, 129.6, 136.4, 139.8, 107.1, 148.3, 149.2, 150.7, 154.0, 159.5; EIMS (m/z) 394 (M^+, 100).

Solid-State Photoreaction of (*E*)-Phosphoric Acid Mono[3-[8-[2-(3-methoxyphenyl)vinyl]-7-methyl-2,6-dioxo-1-prop-2-ynyl-1,2,6,7-tetrahydropurin-3-yl]propyl] Ester Disodium Salt (9). An analytical sample of **9** disodium salt was exposed to daylight for 4 days. The crude **9c** was analyzed by mass spectrometry: ESI negQ1MS m/z 947 ($M^{4-} + 3H^+$), 969 ($M^{4-} + 2H^+ + Na^+$).

Solid-State Photoreaction of 9a to (1α,2α,3*β*,4*β*)-1,3-Bis[3-(3-chloropropyl)-7-methyl-1-prop-2-ynyl-3,7-dihydropurine-2,6-dion-8-yl]-2,4-bis(3-methoxy)phenylcyclobutane (9b). A thin crystal film of 50 mg (0.12 mmol) of **9a**

was prepared by dissolving the yellow solid in a minimum of CH_2Cl_2 and subsequently leaving the solvent to evaporate on the inner surface of a round-bottomed flask. After 8 h under daylight conditions, the formed colorless product was rerystallized from diethyl ether (30 mg yield): mp 243.8 °C dec; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.14 (t, J = 2.5 Hz, 2H), 2.23 (m, 4H), 3.58 (m, 4H), 3.65 (s, 6H), 3.70 (s, 6H), 4.28 (m, 4H), 4.43−4.46 and 5.00−5.04 (AA′BB′, 4H), 4.69 (m, 4H), 6.68−7.13 (m, 8H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 30.4, 31.3, 31.6, 40.9, 42.5, 40.7, 44.6, 55.2, 70.3, 78.7, 112.0, 114.1, 119.8, 129.7, 139.8, 107.7, 147.6, 150.5, 152.6, 154.0, 159.7; EIMS (*m*/*z*) 825 (M^+, 22).

Solid-State Photoreaction of 16 to (1α, 2α, 3*β*, 4*β*)-1,3-Bis[1,3-diethyl-7-methyl-3,7-dihydropurine-2,6-dion-8-yl]-2,4-bis(3,4-dimethoxy)phenylcyclobutane (16b). A crystal film of 40 mg (0.1 mmol) of **16** was prepared by dissolving the yellow solid in a minimum of CH_2Cl_2 and subsequently leaving the solvent to evaporate on the inner surface of a photoreactor. After 100 min of irradiation, the essentially decolorized product was dissolved in CH_2Cl_2, filtered to remove oligomeric material, and recrystallized by addition of diethyl ether to yield colorless crystals (12 mg): mp 259 °C dec; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.18 (t, 6H, J = 7.3 Hz), 1.33 (t, 6H, J = 7.3 Hz), 3.57 (s, 6H), 3.68 (s, 6H), 3.78 (s, 6H), 3.99 (q, J = 7.3 Hz, 4H), 4.18 (q, J = 7.3 Hz, 4H), 4.26−4.29 and 5.01−5.05 (AA′BB′, 4H), 6.63 (d, J = 1.9 Hz, 2H), 6.68 (d, J = 8.2 Hz, 2H), 6.83 (dd, J = 8.2 (1.9) Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.3, 13.5, 31.4, 36.3, 38.3, 42.0, 43.9, 55.8, 55.8, 108.1, 110.5, 110.9, 120.5, 131.1, 147.1, 148.5, 148.9, 150.6, 152.1, 154.9; EIMS (*m*/*z*) 769 (M^+, 58).

Solid-State Photoreaction of 7 (MSX-1) and 18. A crystal film of **7** or **18**, respectively, was prepared by dissolving ca. 50 mg of the compound in a minimum of THF and subsequently leaving the solvent to evaporate on the inner surface of a round-bottomed flask. After 4 weeks under daylight conditions the crude colorless products were investigated by NMR and mass spectroscopy.

(1α, 2α, 3*β*, 4*β*)-1,3-Bis[3-(3-hydroxypropyl)-1-prop-2-ynyl-3,7-dihydropurine-2,6-dione-8-yl]-2,4-bis(3-methoxy)phenylcyclobutane (7b): ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.72 (m, 4H), 3.02 (t, 2H, J = 2.2 Hz), 3.40 (m, 4H), 3.63 (s, 6H), 4.00 (m, 4H), 4.47 (br s, 2H), 4.60−4.64 and 4.69−4.72 (AA′BB′, 4H), 4.53 (d, J = 2.2 Hz, 4H), 6.66−7.17 (m, 8H), 13.5 (br s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 30.2, 31.0, 40.8, 41.0, 44.7, 55.0, 58.5, 72.8, 78.8, 112.3, 113.3, 119.7, 129.2, 140.3, 107.3, 148.8, 150.0, 150.1, 152.9, 159.1; EIMS (*m*/*z*) 760 (M^+, 25).

(1α, 2α, 3*β*, 4*β*)-1,3-Bis[3-(3-acetoxypropyl)-1-prop-2-ynyl-3,7-dihydropurine-2,6-dion-8-yl]-2,4-bis(3-methoxy)-phenylcyclobutane (18b): ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.00 (m, 4H), 2.04 (s, 6H), 2.27 (t, J = 2.2 Hz, 2H), 3.59 (s, 6H), 4.13 (m, 4H), 4.05−4.10 and 4.18−4.24 (AA′BB′, 4H), 4.76 (m, 4H), 4.79 (s, 4H), 6.55−7.03 (m, 8H), 12.16 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.0, 27.2, 31.0, 41.0, 41.3, 45.4, 55.1, 62.1, 71.2, 78.7, 106.5, 111.5, 113.9, 119.7, 129.3, 140.0, 149.2, 150.2, 154.5, 154.7, 159.5, 171.2; EIMS (*m*/*z*) 844 (M^+, 18).

(*E*)-*N*-(6-Amino-1,3-diethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-3-(3,4-dimethoxyphenyl)-acrylamide (14).[20,28,32,33,35] To a solution of 3 g (15.1 mmol) of **13** and pyridine (6 mL) in CH_2Cl_2 (150 mL) was added 5 g (22 mmol) of (*E*)-3,4-dimethoxycinnamic acid chloride[45] at 20 °C under vigorous stirring. After 16 h of stirring at rt, the mixture was diluted with additional CH_2Cl_2 (100 mL) and washed three times with 100 mL of 5% $NaHCO_3$ solution each. The organic phase was dried over $MgSO_4$, filtered over silica gel, and concentrated in vacuo. The oily residue was recrystallized from methanol/water (1:5) to form colorless needles. After removal of crystal water at 120 °C, a yellowish solid was obtained (65% yield): mp 135−140 °C (sublimation); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.16 (t, J = 7.25 Hz, 3H), 1.28 (t, J = 7.25 Hz, 3H), 3.86 (s, 3H), 3.87 (s, 3H), 3.94 (m, 4H), 5.72 (s, 2H), 6.54 (d, J = 15.4 Hz, 1H), 6.79 (d, J = 8.5 Hz, 1H), 6.97 (d, J = 1.6 Hz, 1H), 7.02 (dd, J = 8.5 (1.6) Hz, 1H), 7.51 (d, J = 15.4 Hz, 1H), 7.81 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.1, 13.3, 36.9, 38.3, 55.8, 55.9, 92.4, 111.0, 117.6, 122.2, 127.5, 142.0, 149.1, 149.7, 150.8, 159.8, 165.8; EIMS (*m*/*z*) 388 (M^+, 100).

(*E*)-8-[2-(3,4-Dimethoxyphenyl)vinyl]-1,3-diethyl-3,7-dihydropurine-2,6-dione (15).[20,28,32,33,35] Method A: To a solution of 1 g (2.6 mmol) of **14** in dioxane (30 mL) was added 30 mL of a 1 M solution of NaOH. The clear yellowish solution was heated under reflux for 10 min, diluted with 250 mL of water, and acidified by dropwise addition of concd HCl (→ pH ≅ 2). The precipitate was filtered off, washed with 300 mL of water, and dried at 70 °C (62% yield).

Method B: A mixture of 1.18 g (3 mmol)of **14**, $(NH_4)_2SO_4$ (0.2 g) and HMDS (10 mL) was heated in a glass pressure tube at 170−180 °C for 4 h. The reaction was monitored by TLC (ethyl acetate/methanol, 1:1). The end of the reaction was reached when the molten, poorly soluble starting material had been transformed to a fine suspension of colorless product in a clear yellow solution. After hydrolysis by addition of methanol (20 mL), the mixture was concentrated under reduced pressure with cooling, and the obtained residue was treated with ethyl acetate (40 mL) to dissolve unconverted starting material. The colorless product was filtered under reduced pressure, washed with ethyl acetate (40 mL), and dried at 70 °C (92% yield): mp 264.7 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.30 (t, J = 7.3 Hz, 3H), 1.39 (t, J = 7.3 Hz, 3H), 3.91 (s, 6H), 4.22 (m, 4H), 6.94 (d, J = 16.2 Hz, 1H) 6.86−7.12 (m, 3H), 7.73 (d, J = 16.2 Hz, 1H), 13.00 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.4, 13.5, 36.9, 39.0, 55.8, 56.0, 107.3, 109.1, 111.2, 113.4, 121.2, 128.6, 136.8, 149.3, 149.7, 150.4, 150.5, 151.7, 155.6; EIMS (*m*/*z*) 370 (M^+, 100).

Receptor Binding Studies. Radioligand competition experiments were performed as previously described using rat brain cortical membrane preparations as a source of A_1, and a rat brain striatal membrane preparation for A_{2A} adenosine receptor assays.[46] [^{3}H]-2-Chloro-N^6-cyclopentyladenosine ([^{3}H]-CCPA) was used as A_1 radioligand and [^{3}H]-(*E*)-3-(3-hydroxypropyl)-8-[2-(3-methoxyphenyl)vinyl]-7-methyl-1-prop-2-ynyl-3,7-dihydropurine-2,6-dione ([^{3}H]MSX-2) as A_{2A} ligand.

The frontal cortex of rat brains was dissected as A_1 receptor source, and right and left striata were dissected for A_{2A} adenosine receptor studies. Tissues were homogenized in 50 mM Tris−HCl buffer (pH 7.4) which was used as buffer in all following steps, unless otherwise noted. Membrane fractions were purified by a series of centrifugation steps as described.[47−49] For each assay, 1 mL of membrane suspension containing about 70 μg/mL protein was preincubated with adenosine deaminase (ADA) to convert endogenous adenosine into inactive inosine. To obtain inhibition curves, six to seven different concentrations of the tested compound in DMSO were prepared, spanning 3 orders of magnitude. At least three separate experiments were performed, each in triplicate.

For radioligand binding to rat brain cortical membranes, the solution of the test compound was diluted with buffer. Subsequently a diluted stock solution of the radioligand was added in order to obtain a final concentration of 0.5 nM of [^{3}H]-CCPA. Finally, the cortical membrane suspension was added and the samples were incubated on a shaking water bath at 23 °C for 90 min.

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Incubation was terminated by rapid filtration using a cell harvester through GF/B glass fiber filters, which had been presoaked with buffer. Filters were rinsed three times with 2 mL each of the buffer. Nonspecific binding was defined using 10 μM of 2-chloroadenosine and amounted to less than 5% of total binding.

For radioligand binding to rat brain striatal membranes the solution of the test compound was diluted with buffer and treated with a diluted stock solution of the radioligand in order to obtain a 1 nM final concentration of [^{3}H]MSX-2. After addition of the striatal membrane suspension, incubation (30 min) and termination by rapid filtration were performed as described above. The filters were presoaked with 0.5% aqueous polyethylenimine solution for 45 min prior to filtration. Nonspecific binding was defined using 50 μM 5′-(*N*-ethylcarboxamido)adenosine (NECA) and amounted to less than 25% of total binding.

Radioactivity of the punched-out wet filters was counted after 9 h of preincubation with 3 mL of scintillation cocktail in a liquid scintillation counter. Data were analyzed using Graph Pad PRISM version 3.0 (San Diego, CA).

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Supporting Information Available: A detailed Experimental Section is given, including improved syntheses, spectroscopic data of all intermediates, and final products, together with assignments of NMR data. Copies of ^{1}H NMR spectra and microanalytical data of all new compounds reported in this manuscript are available. This material is available free of charge via the Internet at http://pubs.acs.org.

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