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FULL-LENGTH PAPER

Sustainable synthesis and automated deposition: an accessible discovery screening library of fragment-like purines

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Abstract A sub-library of 88 information-rich lead-like purine derivatives were prepared and deposited in an open access academic screening facility. The rationale for the synthesis of these rigid low complexity structures was the privileged character of the purine heterocycle associated with its inherent probability of interactions with multiple adenine-related targets. Although generally expected to be weak binders in many assays, such fragment-like compounds are estimated to match diverse binding sites. It is suggested that heterocycles with many anchor points for hydrogen bonds can be anticipated to undergo very specific interactions to produce more negative enthalpies and thus provide superior starting points for lead optimization than compounds that owe their activity to entropic effects. The in vitro cytotoxicity of the small compounds on a panel of human cancer cell lines has been investigated and some of them showed marked unselective or selective toxicity. This data may be useful if these fragments are to be incorporated into drug-like structures via metabolically cleavable connections. The sub-library will be implemented as part of the ChemBioNet (www.chembionet.info) library, and it is open to screening campaigns of academic research groups striving for a fragment-based approach in their biological assays.

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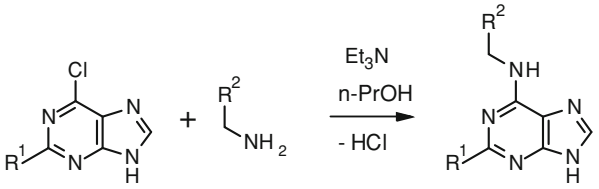
Abbreviations

DMF	N,N dimethyl formamide
IT-TOF HR-MS	Ion-trap time-of-flight high-resolution mass spectrometry
MPLC	Medium pressure liquid chromatography

Introduction

The hydrogen-bonding capacity and versatile coordination ability of adenine toward metal ions makes this fragment a privileged structure [1] in medicinal chemistry. The former constitutes molecular recognition motifs crucial for correct DNA base-pairing and stabilization of double-helical structure, while the latter not only is manifested on charge dissipation but also influences the pK_a of the exocyclic nitrogen. The five-membered imidazole ring harbored in the adenine framework also makes this nucleobase effective in biochemical catalysis, for instance, in the ribosomal peptidyltransferase center. With this in mind, we have exploited additional adenine-derived structures as fragment-like building blocks [2] for bioactive compounds. To gain knowledge of the versatile functional roles of the adenine framework, we generated a series of 88 decorated purines with substituents on the C2 and/or C6 position creating secondary amines or thioethers, leaving the four imino nitrogen atoms unaltered. The purines with lipophilic anchor motif at C6, with and without an amino group at C2, could be useful in the construction of larger drug-like molecules and, thus, could be formed upon decomposition of more complex derivatives by bond-braking metabolic processes. Therefore, we tested the purified congeners of the decorated scaffold for cytotoxic activity in

Scheme 1 Microwave-assisted synthesis of benzylamine adenine analogs **3a–f**, **4a–p**, and **5a–j**. R^1 = H (**1a**), Cl (**1b**), or NH_2 (**1c**), R^2 = various (to be deduced from **3** to **5**). 150 W 20 min



1a-c **2a-o** **3a-f, 4a, c-f, i-p, 5a-c, f-j**

	R^1	R^2
3a	H	2-Methoxyphenyl
3b	H	4-(2-Thienyl)phenyl
3c	H	2,4,6-Trimethylphenyl
3d	H	2,3-Difluorophenyl
3e	H	2-Morpholino-phenyl
3f	H	3-Morpholino-phenyl

	R^1	R^2
4a	NH_2	2-Methoxyphenyl
4c	NH_2	Benzothiophen-4-yl
4d	NH_2	2-Morpholino-phenyl
4e	NH_2	4-Phenyl-phenyl
4f	NH_2	4-(2-Thienyl)phenyl
4i	NH_2	2-Trifluormethyl-phenyl
4j	NH_2	3-Trifluormethyl-phenyl
4k	NH_2	4-Trifluormetyl-phenyl
4l	NH_2	2,3-Difluorophenyl
4m	NH_2	2,4-Difluorophenyl
4n	NH_2	3,5-Difluorophenyl
4o	NH_2	2,4-Dimethoxyphenyl
4p	NH_2	2,4,6-Trimethylphenyl

	R^1	R^2
5a	Cl	Benzothiophen-4-yl
5b	Cl	4-(2-Thienyl)phenyl
5c	Cl	3-Morpholino-phenyl
5f	Cl	2-Trifluormethylphenyl
5g	Cl	3-Trifluormethylphenyl
5h	Cl	4-Trifluormethylphenyl
5i	Cl	2,3-Difluorophenyl
5j	Cl	2,4-Difluorophenyl

a panel of four human cancer cell lines. The main purpose of this study was to contribute to a compound collection designed to be screened against diverse biological targets for primary hit identification.

Chemistry

Purines substituted at C6 (compounds **3a–f**, **4a–p**, **5a–j**, **12a–i**, **13a–i**, **14a–e**) were synthesized by nucleophilic substitution of the C6-chlorine atom of 6-chloropurine [3,4] **1a**, 2-amino-6-chloropurine **1b** or 2,6-dichloropurine **1c** with the appropriate benzylamine and derivatives (Scheme 1) in environmentally benign solvents.

With an excess of triethylamine as base and *n*-propanol as standard solvent or *n*-butanol for the benzyl thioethers (**8a–o** and **9a–i**), the reactions were carried out in closed vessels using microwave irradiation [3] in a single-mode microwave reactor (Scheme 2). In this way, the reactions were driven to completion within minutes instead of hours when conventional heating was used.

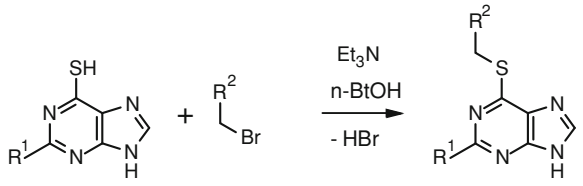
Thus, energy consumption was low and the majority of products crystallized from the reaction mixture spontaneously within 1–2 days upon standing, or immediately upon cooling in the case of the 2-chloro-substituted compounds **5a–j**, **14a–e**. The reaction parameters were optimized by analyzing the reaction mixture by HPLC and NMR in early stage of the experiments. Removal of the solvent was done by means of rotary evaporation. Purification by MPLC was performed to increase purity in the rare cases where spontaneous crystallization failed to occur. With green chemistry in mind, an eluent mixture was selected for MPLC-purification that could easily be recycled by fast distillation at low temperature (dichloromethane/ethanol).

For the synthesis of derivatives **3j–l**, aminopyridines **11a** and **b** and aliphatic amine **11c** were used in a similar manner (Scheme 3).

For the synthesis of 6-phenylthio and 6-pyridylthio derivatives **12–14**, a similar synthetic approach was preferred (Scheme 4), although the 6-thiobenzyl purine derivatives **8a–o** and **9a–i** can be obtained by two different routes. One route consists of the reaction of 6-chloropurine **1a** with the appropriate thiols [3] in an analogous fashion. Alternatively, S-alkylation of mercaptopurine [5] **6a** or 2-aminomercaptorurine **6b** with benzyl bromides **7** leads to the same products. Due to the fact that a broad variety of benzyl bromides are commercially available, the latter route was selected for the synthesis of compounds **8a–o** and **9a–i**.

A small subset of compounds from the three series **3**, **4**, and **5** were synthesized by reacting the appropriate starting material **1a–c** with a methyl-substituted thiophene derivative **15a** and **b** (Scheme 5).

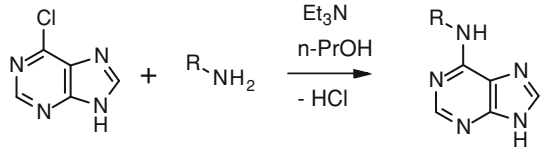
For the synthesis of 7- and 9-alkylated purines **17a–d**, the purine starting material **1a** was reacted with the appropriate alkyl halides in an equimolar mixture as described by Laufer et al. [6] (Scheme 6). Despite several attempts to perform these reactions in environmentally friendly solvents as well, best results were obtained with water-free DMF. Anhydrous potassium carbonate was added in excess and the reactions proceeded at room temperature. Potassium carbonate could be easily filtered off but the mixture had to be extracted with dichloromethane in order to be able to separate the product



6a-b **7a-o** **8, 9**

	R^1	R^2		R^1	R^2
8a	H	3-Bromophenyl	**9a**	NH_2	3-Bromophenyl
8b	H	4-Bromophenyl	**9b**	NH_2	4-Bromophenyl
8c	H	3-Methoxyphenyl	**9c**	NH_2	2-Trifluoromethylphenyl
8d	H	4-Benzoyl-phenyl	**9d**	NH_2	2,3-Difluorophenyl
8e	H	4-Phenyl-phenyl	**9e**	NH_2	2,4-Difluorophenyl
8f	H	2-Trifluoromethylphenyl	**9f**	NH_2	2,5-Difluorophenyl
8g	H	4-Trifluoromethylphenyl	**9g**	NH_2	2,6-Difluorophenyl
8h	H	2,3-Difluorophenyl	**9h**	NH_2	3,4-Difluorophenyl
8i	H	2,4-Difluorophenyl	**9i**	NH_2	3,5-Difluorophenyl
8j	H	2,5-Difluorophenyl			
8k	H	2,6-Difluorophenyl			
8l	H	3,4-Difluorophenyl			
8m	H	3,5-Difluorophenyl			
8n	H	4,5-Difluorophenyl			
8o	H	2,5-Dimethoxyphenyl			

Scheme 2 Microwave-assisted synthesis of benzylthioether adenine analogs **8a–o** and **9a–i**. R^1 = H or NH_2, R^2 = various (to be deduced from **8** and **9**).



1a **11a-c** **3j-l**

	R
3j	4-Pyridyl
3k	3-Carboxy-4-pyridyl
3l	2-(4-Benzylpiperazin-1-yl)ethyl

Scheme 3 Microwave-assisted synthesis of various "non-benzyl" decorated adenine analogs **3j–l**. R = various (to be deduced from **3j–l**)

from the non-volatile solvent DMF. After removal of the dichloromethane under reduced pressure, the crude products had to be purified by MPLC by the conditions described above.

Biology

After preparative chromatographic purification and in-depth spectroscopic characterization by NMR and Ion-trap time-of-flight high-resolution mass spectrometry (IT-TOF HR-MS), 88 purine derivatives were obtained in moderate to good yields and high purities. The compounds were then subjected to screening for antiproliferative activity in a panel of four human tumor cell lines, all at a concentration of 20 μM, by using a microtiter assay [7] based on the staining of cell mass with crystal violet. This panel includes the cell lines 5637 (bladder cancer), A-427 (small cell lung cancer), DAN-G (pancreas cancer), and LCLC (large cell lung cancer). Malignant diseases associated with these cell lines are difficult to treat, especially pancreatic cancer. The results of the biological evaluation are listed in Table 1. Compounds showing greater than 50 % growth inhibition in one or more cell lines are considered active. Most of the compounds were inactive. Compounds that showed activity in more than two of the four cell lines were considered non-selective (e.g., compounds **3b**, **8e**, **8m**, **9e**, **12b**, **12i**, **13b**, **13d**, **13k**, **14b**, and **17d**), while compounds that showed good activity in only one cell line and proved to be inactive in the others were considered to act selectively (e.g., compounds **3a**, **3k**, **4a**, **4e**, **4f**, **4i**, **8b**, **8c**, **8g**, **9d**, **9g**, **12a**, **12b**, and **14c**).

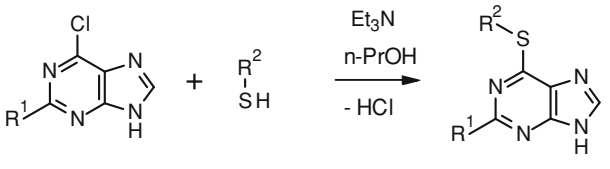
Our experience has shown that compounds that exhibit selective activity against particular cell lines, while being less active against others are more likely to have antitumour activity than compounds that are active against all cell lines with the same potency [7].

Discussion

The most important contributions to protein–ligand interactions are ruled by changes in entropy (ΔS) and enthalpy [8] (ΔH). Recently, it has been proposed that ligands with an enhanced enthalpic contribution (ΔH) compared to the entropic term ($-T\Delta S$) of the free energy equation ($\Delta G = \Delta H - T\Delta S$) provide superior starting points [9–11] for lead optimization in drug research. Thus, ligand efficiency, enthalpic and entropic effects are key to the understanding of ligand–target interaction and have to be taken into account in strategic planning of medicinal chemistry projects and especially library design [12]. Logically, one would expect that rigid compounds decorated with lipophilic side-chains and covalently linked with a minimum number of rotational bonds such as purines would loose fewer degrees of freedom upon binding [9–12] to a molecular target. Thus, increased enthalpic contributions both from N–H-hydrogen-bonding and hydrophobic contacts, should be obtainable with such a scaffold. In accordance with recent insights, entropic effects, such as increasing loss of residual mobility of the bound ligand [12], is not the strategic focus of the compound collection.

Laufer et al. [6] demonstrated that decorated adenines of the type investigated here can be regarded as a valuable tool kit for the evaluation of bonding and selectivity patterns [13] for a wide variety of kinases [14]. In further work, we will modify substituents at the N9 position [15], creating novel adenosine and guanosine derivates as well as prodrug structures and fragments thereof.

Scheme 4 Microwave-assisted synthesis of phenylthioether adenine analogs **12a–i**, **13a–i**, and **14a–e**. R^1 = H, Cl, or NH_2, R^2 = various (to be deduced from **12–14**)



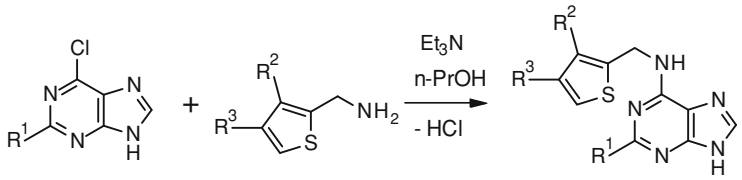
1a-c **10** **12-14**

	R^1	R^2
12a	H	2-Fluorophenyl
12b	H	3-Fluorophenyl
12c	H	2-Bromophenyl
12d	H	3-Bromophenyl
12e	H	4-Bromophenyl
12f	H	4-Hydroxyphenyl
12g	H	2,3-Dichlorophenyl
12h	H	2,4-Dichlorophenyl
12i	H	2,5-Dichlorophenyl
12j	H	2-Pyridyl

	R^1	R^2
13a	NH_2	2-Fluorophenyl
13b	NH_2	3-Fluorophenyl
13c	NH_2	2-Bromophenyl
13d	NH_2	3-Bromophenyl
13e	NH_2	3-Hydroxyphenyl
13f	NH_2	4-Hydroxyphenyl
13g	NH_2	4-Methoxyphenyl
13h	NH_2	2,4-Difluorophenyl
13i	NH_2	2,5-Difluorophenyl
13j	NH_2	2-Pyridyl
13k	NH_2	4-Pyridyl

	R^1	R^2
14a	Cl	2-Fluorophenyl
14b	Cl	3-Fluorophenyl
14c	Cl	2-Bromophenyl
14d	Cl	3-Bromophenyl
14e	Cl	2,4-Difluorophenyl

Scheme 5 Microwave-assisted synthesis of thienyl decorated adenine analogs **3g–h**, **4g–h**, and **5d–e**. R^1 = H, Cl, or NH_2, **15a** R^2 = $-CH_3$, R^3 = –H, **15b** R^2 = –H, R^3 = $-CH_3$ (adenine analogs accordingly)



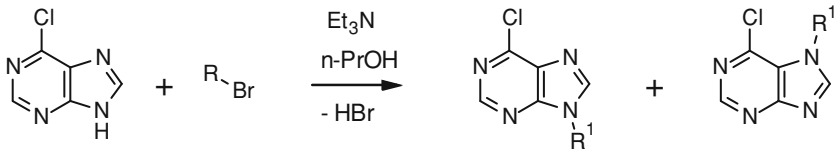
1a-c **15a-b** **3g**, **h**, **4g**, **h**, **5d**,**e**

	R^1	R^2	R^3
3g	H	CH_3	H
3h	H	H	CH_3

	R^1	R^2	R^3
4g	NH_2	CH_3	H
4h	NH_2	H	CH_3

	R^1	R^2	R^3
5d	Cl	CH_3	H
5e	Cl	H	CH_3

Scheme 6 Synthesis of N9-alkyl-substituted 6-chloropurine analogs **17a**, **c** , and **d** and N7-alkylated regioisomer **167**. R = ethyl, 4-phenylbenzyl, or 5-bromopentyl



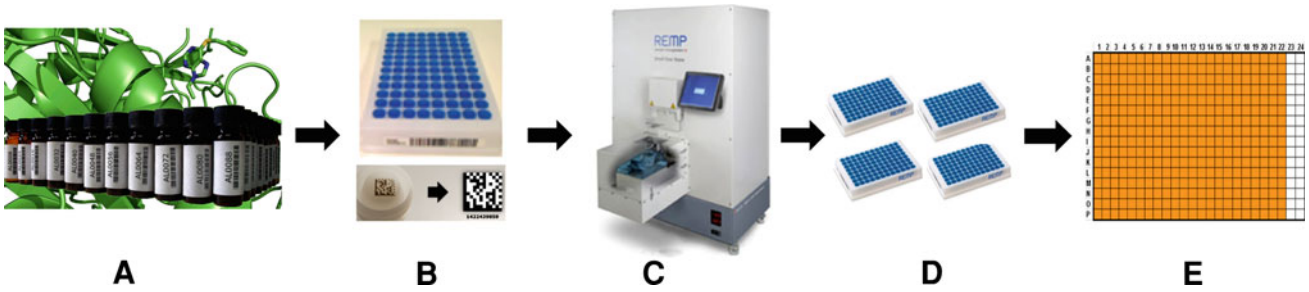
1 **16a-c** **17a**, **c**, **d** **17b**

	R^1
17a	(4-Phenyl)benzyl
17b	(4-Phenyl)benzyl
17c	Ethyl
17d	5-(6-Chloropurin-9-yl)pentyl

Naturally occurring nucleobases permit versatile metal ion coordination, which is invoked in metal ion–nucleic acid interactions in biological systems. These novel derivatives also hold potential as ligands for the construction of new platinum-derived coordination complexes for anticancer therapy. This strategy will be further explored by our group in due course.

The marked unselective (e.g., compounds **3b**, **8e**, **8m**, **9e**, **12b**, **12i**, **13b**, **13d**, **13k**, **14b**, and **19d**) or selective cytotoxicity of compounds (e.g., compounds **3a**, **3k**, **4a**, **4e**, **4f**, **4i**, **8b**, **8c**, **8g**, **9d**, **9g**, **12a**, **12b**, and **14c**) could be directly exploited if the compounds could be selectively delivered to malignant cells, e.g., by incorporation into prodrugs that are actively taken up by transport proteins. This approach is also being followed and reported on in future publications.

Due to the higher hit prospect of privileged structures, chances are that fewer compounds need to be screened to identify starting points for chemical optimization.



Scheme 7 Workflow of automated deposition: weighing of 88 compounds in barcoded glass vials (*A*), transfer of DMSO solubilized compounds in 2D-barcoded Remp tubes (*B*), storage of 96 Remp plates in automated small size store (*C*), usage of 4 × 96 Remp plates with 4 × 88 compounds (*D*) for the preparation of a 384 screening plate (*E*) whose last two columns are reserved for positive and negative controls

Conclusion

The reported syntheses enable the sustainable screening of privileged fragment- or lead-like purines. The synthesis of decorated derivatives of the heterocyclic scaffolds was performed by avoiding toxic catalysts in environmentally benign solvents such as propanol and butanol. For the chromatographic purification, the toxic and less benign halogenated solvent dichloromethane was selected because the used eluent mixture could be readily recycled via distillation at very low temperature, leading to minimal energy consumption for heating purposes.

Most of the compounds showed no cytotoxicity, which is a positive attribute if these fragments are to be used in the design of drugs that need to have low toxicity. However, approximately 28 % of the compounds showed antiproliferative activity on at least one cell line, making them interesting starting points for the design of anticancer drugs.

These structures represent a useful toolkit for the investigation of many protein targets. Thus, the screening collection was not only evaluated for one but was also made publicly available at the Screening Unit of the Leibniz Institut für Molekulare Pharmakologie (FMP), Berlin, Germany. The fully characterized 88 test compounds are delivered in barcoded glass vials and stored in 2D-barcoded 96-Remp plates by usage of an automated compound management system. Using robotic automation, 10 mM DMSO stock solutions of the samples are transferred from 4 × 96 Remp plates filled with 4 × 88 compounds to a 384 screening plate whose last two columns are reserved for positive and negative controls in the screening process (Scheme 7).

The electronic submission of compounds with the corresponding structure data file (.sdf) is easily generated using an interactive website developed and provided by the Screening Unit (.sdf file and information to the website found in supporting information). Screening of the fragment-like purines can be conducted at the Screening Unit and small aliquots of compounds can be obtained in an automated hitpicking process on request. As demonstrated here, small libraries with a nucleobase scaffold can efficiently be prepared and made available. Undoubtedly, much larger libraries of highly diverse compounds can be deposited in a similar fashion for high-throughput screening assays. The combination of efforts of various laboratories, automated compound handling, and broader availability of commercially not available screening compounds with reported toxicity will contribute to highly efficient and thus economically attractive and successful hit identification in academic research settings.

Experimental

NMR spectra were recorded on a Bruker BioSpin UltraShield 400 magnet with Avance III console, or a DPX 200 MHz spectrometer, using tetramethylsilane as internal standard. The purity of all compounds was deduced from NMR data as well as HPLC, using a VWR LaChrom or a Shimadzu 20A-Prominence HPLC-system with SPD-M20A photodiode array and ESD-LT II evaporative light-scattering detector using CC 125/4 Nucleodur100-5 C18 ec columns, supplied by Macherey-Nagel. HR-MS data were obtained offline or online using a Shimadzu LCMS-IT-TOF instrument. TLC reaction control was performed on Macherey-Nagel Polygram Sil G/UV254 precoated microplates, spots were visualized under UV-illumination at 254 nm. IR spectra were recorded on a ThermoFisher Nicolet IR200 FT-IR Spectrometer. Hydrogenations was performed in a HY 1000 apparatus (Hyscho). Microwave reactions were executed using a Discover reactor (CEM).

General procedure for the microwave-assisted preparation of adenine derivatives (3–5) and mercaptopurine analogs (8, 9, 12–14)

The purine starting material **1a**–**c** or **6a**, **b** and an equimolar amount of the appropriate benzylamines **2a**–**o**, alkyl halogenides **7**, phenylthiols **10**, aminopyridine **11a**, **b**, alkylamine **11c**, or thienyl derivate **15** were suspended in *n*-propanol (or in *n*-butanol in case of the alkyl halides **7**). An excess of

Table 1 Percent (%) cell growth relative to an untreated control after a 96 h exposure to substance at 20 μM

Entry	Compound	Tested cell line			
		A427	LCLC-103H	5637	DAN-G
3a	6-(2-Methoxyphenyl)methylamino-9*H*-purine	59 ± 44	57 ± 26	43 ± 28	71 ± 24
3b	6-[4-(2-thienyl)phenyl]methylamino-9*H*-purine	42 ± 12	45 ± 16	0 ± 4	32 ± 18
3c	6-(2,4,6-Trimethylphenyl)methylamino-9*H*-purine	101 ± 15	92 ± 7	115 ± 1	117 ± 3
3d	6-(2,3-Difluorophenyl)methylamino-9*H*-purine	55 ± 13	77 ± 27	61 ± 22	88 ± 15
3e	6-(2-Morpholinophenyl)methylamino-9*H*-purine	92 ± 12	101 ± 21	87 ± 31	152 ± 23
3f	6-(3-Morpholinophenyl)methylamino-9*H*-purine	91 ± 13	108 ± 9	45 ± 35	168 ± 57
3g	6-(3-Methyl-2-thienyl)methylamino-9*H*-purine	90 ± 11	92 ± 14	83 ± 15	96 ± 6
3h	6-(4-Methyl-2-thienyl)methylamino-9*H*-purine	80 ± 18	87 ± 26	76 ± 1	85 ± 5
3i	6-(1-Benzothiophen-5-yl)methylamino-9*H*-purine	64 ± 4	83 ± 13	70 ± 24	89 ± 9
3j	6-(4-Pyridyl)amino-9*H*-purine	84 ± 16	72 ± 10	63 ± 40	119 ± 24
3k	6-[(3-Carboxy-4-pyridyl)amino]-9*H*-purine	51 ± 10	77 ± 53	32 ± 15	87 ± 26
3l	6-[2-(4-Benzylpiperazin-1-yl)ethylamino]-9*H*-purine	81 ± 20	74 ± 14	53 ± 36	87 ± 6
2-Amino-9*H*-adenines					
4a	2-Amino-6-(2-methoxyphenyl)methylamino-9*H*-purine	66 ± 14	87 ± 10	39 ± 25	112 ± 34
4b	2-Amino-6-[(2-pyridyl)methylamino]-9*H*-purine	96 ± 39	87 ± 15	135 ± 63	133 ±42
4c	2-Amino-6-(1-benzothiophen-5-ylmethylamino)-9*H*-purine	85 ± 39	93 ± 1	100 ± 56	121 ± 47
4d	2-Amino-6-(2-morpholinophenyl)methylamino-9*H*-purine	105 ± 20	109 ± 8	120 ± 7	116 ± 4
4e	2-Amino-6-(4-phenylphenyl)methylamino-9*H*-purine	73 ± 23	61 ± 30	33 ± 24	36 ± 14
4f	2-Amino-6-[2-(2-thienyl)phenyl]methylamino-9*H*-purine	62 ± 22	78 ± 24	21 ± 32	137 ± 32
4g	2-Amino-6-(3-methyl-2-thienyl)methyl-9*H*-purine	96 ± 3	100 ± 16	87 ± 24	90 ± 9
4h	2-Amino-6-(4-methyl-2-thienyl)methylamino-9*H*-purine	89 ± 3	94 ± 5	74 ± 33	86 ± 8
4i	2-Amino-6-(2-trifluoromethylphenyl)methylamino-9*H*-purine	79 ± 19	71 ± 33	41 ± 19	77 ± 27
4j	2-Amino-6-(3-trifluoromethylphenyl)methylamino-9*H*-purine	87 ± 20	122 ± 49	91 ± 1	82 ± 3
4k	2-Amino-6-(4-trifluoromethylphenyl)methylamino-9*H*-purine	73 ± 34	68 ± 43	55 ± 37	73 ± 34
4l	2-Amino-6-(2,3-difluorophenyl)methylamino-9*H*-purine	81 ± 13	90 ± 19	71 ± 24	94 ± 11
4m	2-Amino-(2,4-difluorophenyl)methylamino-9*H*-purine	90 ± 18	86 ± 10	107 ± 7	93 ± 13
4n	2-Amino-(3,5-difluorophenyl)methylamino-9*H*-purine	70 ± 24	64 ± 33	127 ± 63	110 ± 33
4o	2-Amino-6-(2,4-dimethoxyphenyl)methylamino-9*H*-purine	90 ± 31	125 ± 50	72 ± 14	114 ± 22
4p	2-Amino-6-(2,4,6-trimethylphenyl)methylamino-9*H*-purine	98 ± 14	99 ± 11	109 ± 10	106 ± 11
2-Chloro-9*H*-adenines					
5a	6-(Benzothiophen-4-ylmethyl)amino-2-chloro-9*H*-purine	63 ± 11	77 ± 3	67 ± 2	81 ± 4
5b	2-Chloro-6-[4-(2-thienyl)phenyl]methylamino-9*H*-purine	137 ± 44	65 ± 46	40 ± 37	81 ± 32
5c	2-Chloro-6-(3-morpholinophenyl)methylamino-9*H*-purine	47 ± 20	77 ± 21	65 ± 4	77 ± 15
5d	2-Chloro-6-(3-methyl-2-thienyl)methylamino-9*H*-purine	71 ± 18	64 ± 6	62 ± 1	81 ± 5
5e	2-Chloro-6-(4-methyl-2-thienyl)methylamino-9*H*-purine	87 ± 43	99 ± 84	64 ± 63	77 ± 31
5f	2-Chloro-6-(2-trifluoromethylphenyl)methylamino-9*H*-purine	94 ± 10	130 ± 44	115 ± 27	104 ± 10
5g	2-Chloro-6-(3-trifluoromethylphenyl)methylamino-9*H*-purine	84 ± 17	134 ± 52	93 ± 15	96 ± 18
5h	2-Chloro-6-(4-trifluoromethylphenyl)methylamino-9*H*-purine	88 ± 18	97 ± 40	88 ± 12	88 ± 9
5i	2-Chloro-6-(2,3-difluorophenyl)methylamino-9*H*-purine	145 ± 50	84 ± 44	78 ± 59	100 ± 20
5j	2-Chloro-6-(2,4-difluorophenyl)methylamino-9*H*-purine	129 ± 47	70 ± 44	59 ± 47	86 ± 26
Benzyl-substituted 9*H*-mercaptopurines					
8a	6-[(3-Bromophenyl)methylsulfanyl]-9*H*-purine	137 ± 53	155 ± 57	164 ± 9	147 ± 29
8b	6-[(4-Bromophenyl)methylsulfanyl]-9*H*-purine	39 ± 1	49 ± 16	6 ± 6	60 ± 24
8c	6-[(4-Methoxyphenyl)methylsulfanyl]-9*H*-purine	82 ± 27	96 ± 14	41 ± 61	143 ± 36
8d	6-(4-Benzoyl-phenyl)methylsulfanyl-9*H*-purine	92 ± 11	85 ± 11	76 ± 22	94 ± 5
8e	6-[(4-Phenylphenyl)methylsulfanyl]-9*H*-purine	2 ± 36	20 ± 14	0 ± 24	38 ± 15

Table 1 continued

8f	6-{[2-(Trifluoromethyl)phenyl]methylsulfanyl}-9*H*-purine	76 ± 35	81 ± 20	82 ± 115	115 ± 52
8g	6-{[4-(Trifluoromethyl)phenyl]methylsulfanyl}-9*H*-purine	25 ± 29	35 ± 15	63 ± 16	66 ± 26
8h	6-[(2,3-Difluorophenyl)methylsulfanyl]-9*H*-purine	83 ± 32	91 ± 14	50 ± 29	165 ± 61
8i	6-[(2,4-Difluorophenyl)methylsulfanyl]-9*H*-purine	126 ± 11	91 ± 9	88 ± 28	102 ± 9
8j	6-[(2,5-Difluorophenyl)methylsulfanyl]-9*H*-purine	151 ± 14	82 ± 115	93 ± 40	64 ± 20
8k	6-[(2,6-Difluorophenyl)methylsulfanyl]-9*H*-purine	105 ± 13	117 ± 9	209 ± 136	208 ± 70
8l	6-[(3,4-Difluorophenyl)methylsulfanyl]-9*H*-purine	108 ± 20	106 ± 1	79 ± 18	121 ± 40
8m	6-[(3,5-Difluorophenyl)methylsulfanyl]-9*H*-purine	4 ± 2	1 ± 3	16 ± 17	0 ± 3
8n	6-[(3,5-Difluorophenyl)methylsulfanyl]-9*H*-purine	123 ± 35	107 ± 12	207 ± 133	80 ± 21
8o	6-[(2,5-Dimethoxyphenyl)methylsulfanyl]-9*H*-purine	95 ± 3	94 ± 19	108 ± 1	95 ± 4
Benzyl-substituted 2-amino-9*H*-mercaptopurines					
9a	2-Amino-6-[(3-bromophenyl)methylsulfanyl]-9*H*-purine	103 ± 25	129 ± 65	117 ± 33	96 ± 4
9b	2-Amino-6-[(4-bromophenyl)methylsulfanyl]-9*H*-purine	60 ± 14	82 ± 33	94 ± 23	92 ± 16
9c	2-Amino-6-(2-trifluoromethylphenyl)methylsulfanyl-9*H*-purine	56 ± 4	64 ± 32	80 ± 29	85 ± 16
9d	2-Amino-6-[(2,3-difluorophenyl)methylsulfanyl]-9H-purine	25 ± 18	67 ± 11	59 ± 10	64 ± 14
9e	2-Amino-6-[(2,4-difluorophenyl)methylsulfanyl]-9*H*-purine	0 ± 29	3 ± 2	25 ± 21	7 ± 36
9f	2-Amino-6-[(2,5-difluorophenyl)methylsulfanyl]-9*H*-purine	158 ± 28	76 ± 17	57 ± 23	39 ± 19
9g	2-Amino-6-[(2,6-difluorophenyl)methylsulfanyl]-9*H*-purine	0 ± 8	52 ± 8	59 ± 10	41 ± 26
9h	2-Amino-6-[(3,4-difluorophenyl)methylsulfanyl]-9*H*-purine	54 ± 13	77 ± 25	51 ± 21	80 ± 20
9i	2-Amino-6-[(3,5-difluorophenyl)methylsulfanyl]-9*H*-purine	64 ± 5	53 ± 17	50 ± 10	59 ± 2
Phenyl-substituted 9*H*-mercaptopurines					
12a	6-(2-Fluorophenyl)sulfanyl-9*H*-purine	70 ± 34	59 ± 43	34 ± 21	67 ± 38
12b	6-(3-Fluorophenyl)sulfanyl-9*H*-purine	52 ± 25	49 ± 38	21 ± 3	51 ± 33
12c	6-(2-Bromophenyl)sulfanyl-9*H*-purine	80 ± 22	95 ± 22	73 ± 38	171 ± 46
12d	6-(3-Bromophenyl)sulfanyl-9*H*-purine	70 ± 34	89 ± 20	99 ± 11	137 ± 57
12e	6-(4-Bromophenyl)sulfanyl-9*H*-purine	99 ± 16	81 ± 4	98 ± 22	94 ± 7
12f	6-(4-Hydroxyphenyl)sulfanyl-9*H*-purine	70 ± 11	111 ± 29	79 ± 33	228 ± 66
12g	6-(2,3-Dichlorophenyl)sulfanyl-9*H*-purine	66 ± 23	94 ± 24	57 ± 51	178 ± 43
12h	6-(2,4-Dichlorophenyl)sulfanyl-9*H*-purine	71 ± 12	85 ± 19	67 ± 36	157 ± 48
12i	6-(2,5-Dichlorophenyl)sulfanyl-9*H*-purine	21 ± 9	28 ± 16	34 ± 15	38 ± 10
12j	6-(4-Pyridyl)sulfanyl-9*H*-purine	51 ± 20	95 ± 21	124 ± 41	53 ± 19
13a	2-Amino-6-(2-fluorophenyl)sulfanyl-9*H*-purine	60 ± 25	50 ± 32	88 ± 56	58 ± 29
13b	2-Amino-6-(3-fluorophenyl)sulfanyl-9*H*-purine	46 ± 26	19 ± 12	35 ± 6	44 ± 29
13c	2-Amino-6-(2-bromophenyl)sulfanyl-9*H*-purine	83 ± 44	68 ± 16	40 ± 17	71 ± 7
13d	2-Amino-6-(3-bromophenyl)sulfanyl-9*H*-purine	59 ± 53	35 ± 23	26 ± 27	31 ± 27
13e	2-Amino-6-(3-hydroxyphenyl)sulfanyl-9*H*-purine	94 ± 4	90 ± 5	88 ± 35	102 ± 2
13f	2-Amino-6-(4-hydroxyphenyl)sulfanyl-9*H*-purine	73 ± 13	83 ± 4	85 ± 18	94 ± 7
13g	2-Amino-6-(4-methoxyphenyl)sulfanyl-9*H*-purine	85 ± 23	73 ± 8	53 ± 10	80 ± 5
13h	2-Amino-6-(2,4-dichlorophenyl)sulfanyl-9*H*-purine	66 ± 26	76 ± 6	44 ± 5	94 ± 11
13i	2-Amino-6-(2,5-dichlorophenyl)sulfanyl-9*H*-purine	107 ± 16	97 ± 13	92 ± 3	106 ± 7
13j	2-Amino-6-(2-pyridyl)sulfanyl-9*H*-purine	76 ± 11	104 ± 47	68 ± 43	103 ± 25
13k	2-Amino-6-(4-pyridyl)sulfanyl-9*H*-purine	0 ± 4	28 ± 6	30 ± 12	19 ± 15
2-Chloro-(substituted-phenyl)-sulfanyl-9*H*-purines					
14a	2-Chloro-6-(2-fluorophenyl)sulfanyl-9*H*-purine	68 ± 15	59 ± 37	55 ± 6	66 ± 17
14b	2-Chloro-6-(2-bromophenyl)sulfanyl-9*H*-purine	41 ± 16	12 ± 11	16 ± 7	33 ± 13
14c	2-Chloro-6-(3-bromophenyl)sulfanyl-9*H*-purine	54 ± 37	20 ± 8	64 ± 15	56 ± 11
14d	2-Chloro-6-(2,4-dichlorophenyl)sulfanyl-9*H*-purine	64 ± 8	74 ± 10	66 ± 7	86 ± 6

Table 1 continued

9-Alkyl-substituted 9*H*-purines					
17a	6-Chloro-9-[(4-phenylphenyl)methyl]-9*H*-purine	0 ± 8	5 ± 3	0 ± 2	0 ± 3
17b	6-Chloro-7-[(4-phenylphenyl)methyl]-7*H*-purine	0 ± 5	1 ± 3	0 ± 2	0 ± 3
17c	6-Chloro-9-ethyl-9*H*-purine	92 ± 25	73 ± 14	55 ± 28	90 ± 8
17d	6-Chloro-7-[5-(6-chloropurin-9-yl)pentyl]purine	30 ± 14	25 ± 20	27 ± 19	42 ± 27
17e	9-Ethyl-6-(2-methoxyphenyl)methylamino-9*H*-purine	57 ± 14	70 ± 28	45 ± 22	84 ± 22

Results are averages ± the standard deviation from three independent experiments

triethylamine was added and the mixture heated in a closed vessel for 20 min at 140 °C in a microwave reactor. A typical procedure used 100 mg purine starting material in a 10 mL microwave reactor vessel. A scale up to a size of 2 g product was successful for the compounds **3a**, **9d**, and **9e**. The majority of products crystallized from the reaction mixture within 1–2 days upon standing or immediately upon cooling in the case of 2-chloro-substituted compounds **5a**–**j**, **14a**–**e**.

General procedure for the preparation of 7-and 9-alkylated purines (17a–d)

6-Chloropurine (**1a**) and an equimolar amount of the appropriate alkyl halides were dissolved in dry DMF. Anhydrous potassium carbonate was added and the mixture was stirred at room temperature for several days. Potassium carbonate was filtered and the mixture was extracted with dichloromethane. After removal of the dichloromethane under reduced pressure, the crude products had to be purified by MPLC using the conditions described above.

6-(2,4,6-Trimethylphenyl)methylamino-9*H*-purine (**3c**)

^{1}H-NMR: 400 MHz, ([D_6]-DMSO) δ (ppm) = 12.49 (s, 1H, H-9); 8.26 (s, 1H, H-2); 8.13 (s, 1H, H-8); 6.85 (s, 2H, $H_{aromatic}$); 4.66 (s, 2H, –CH_2); 2.32 (s, 6H, –CH_3); 2.22 (s, 3H, –CH_3)

^{13}C-NMR: 100 MHz, ([D_6]-DMSO) δ (ppm) = 154.1 (C-4); 152.3 (C-2); 150.4 (C-8); 137.7 (C-6); 137.2 (2C, C-2′ + C-6′, $C_{aromatic}$); 136.4 (C-1′, $C_{aromatic}$); 132.0 (C-5); 128.7 (2C, C-3′ + C-5′); 62.5 (CH_2); 20.6 (1C, C-4′, C_{methyl}); 19.6 (2C, C-2′ + C-6′, $CH_{3\,methyl}$)

IR:$\tilde{\nu}$ (cm^{-1})=1618, 1503, 849, 612.

HR-ESI-MS $[M + H]^+$ calcd 266.1411 found 266.1405

mp: 252 °C (decomposition)

6-(2-Morpholinophenyl)methylamino-9*H*-purine (**3e**)

^{1}H-NMR: 400 MHz, ([D_6]-DMSO) δ (ppm) = 12.50 (s, 1H, H-9); 8.17 (s, 1H, H-8); 7.23–7.09 (m, 4H, $H_{aromatic}$); 4.83 (s, 2H, $CH_{2\,benzyl}$); 3.76 (s, 4H, $CH_{2\,morpho.}$); 2.89 (s, 4H, $CH_{2\,morpho.}$)

^{13}C-NMR: 100 MHz, ([D_6]-DMSO) δ (ppm) = 156.1 (C-4); 154.1 (C-2); 126.2 (C-8); 127.4–119.6 ($C_{aromatic}$); 66.64 ($CH_{2\,morpho.}$); 52.5 ($CH_{2\,morpho.}$); 45.4 ($CH_{2\,benzyl}$)

IR:$\tilde{\nu}$ (cm^{-1}) = 1617, 1591, 1452, 1252, 1111, 931.

HR-ESI-MS $[M + H]^+$ calcd 309.1469 found 309.1485

mp: 228 °C

2-Amino-6-(2-methoxyphenyl)methylamino-9*H*-purine (**4a**)

^{1}H-NMR: 400 MHz, ([D_6]-DMSO) δ (ppm) = 12.11 (s, 1H, H-9); 7.67 (s, 1H, H-8); 7.36 (s, 1H, NH); 7.19–6.83 (4H, $_{aromatic}$); 5.7 (s, 2H, NH_2); 4.61 (s, 2H, $CH_{2\,benzyl}$); 3.83 (s, 3H, $CH_{3\,methoxy}$)

^{13}C-NMR: 100 MHz, ([D_6]-DMSO) δ (ppm) = 160.2 (C-2′$_{aromatic}$); 156.5 (C-2); 139.4 (C-1′$_{aromatic}$); 135.3 (C-6); 127.9 (C-4); 127.5–110.1 (5C $_{aromatic}$); 62.5 ($CH_{2\,benzyl}$); 55.2 ($CH_{3\,methoxy}$)

IR:$\tilde{\nu}$ (cm^{-1}) = 1617, 1591, 1452, 1252, 1111, 931.

HR-ESI-MS $[M + H]^+$ calcd 271.1309 found 271.1300

mp: 173–174 °C

2-Amino-6-(2-morpholinophenyl)methylamino-9*H*-purine (**4d**)

^{1}H-NMR: 400 MHz, ([D_6]-DMSO) δ (ppm) = 12.10 (s, 1H, H-9); 7.66 (s, 1H, H-8); 7.22–7.02 (m, 4H, $H_{aromatic}$); 5.69 (s, 2H, –$CH_{2\,benzyl}$); 4.76 (s, 1H, NH); 4.37 (s, 2H, NH_2); 3.76 (s, 2H, CH_2, H-$2_{morpho.}$); 2.89 (s, 2H, CH_2, H-$3_{morpho.}$)

^{13}C-NMR: 163.09 (C-$2_{aromatic}$); 160.6 (C-8); 151.6 (C-5); 143.9 (C-6); 131.3 (C-$6_{aromatic}$); 130.87 (C-$5_{aromatic}$); 129.9 (C4); 129.1 (C$4_{aromatic}$); 66.6 (CH_2, C-$2_{morpho.}$); 62.5 ($CH_{2\,benzyl}$); 52.5 (CH_2, C-$3_{morpho.}$)

IR:$\tilde{\nu}$ (cm^{-1}) = 1604, 1380, 1105, 768.

HR-ESI-MS $[M + H]^+$ calcd 326.1724 found 326.1728

mp: 154 °C

6-(Benzothiophen-4-ylmethyl)amino-2-chloro-9*H*-purine (**5a**)

1H-NMR: 400 MHz, ([D_6]-DMSO) δ (ppm) = 13.11 (s, 1H, H-9); 8.79 (s, 1H, H-8); 8.48–7.38 (5H, $H_{aromatic}$); 4,78 (s, 2H,$CH_{2\,benzyl}$)

13C-NMR: 100 MHz, ([D_6]-DMSO) δ (ppm) = 161.2 (C-4); 152.9 (C-2); 139.6 (C-8); 137.7 (C-6); 128.5 (C-5); 128.0–122.1 ($C_{aromatic}$); 62.46 ($CH_{2\,benzyl}$)

IR:$\tilde{\nu}$ (cm^{-1})) = 1619, 1342, 1248, 931, 701

HR-ESI-MS $[M + H]^+$ calcd 316.0418 found 316.0426

mp: 219 °C

2-Chloro-6-(2-trifluoromethylphenyl)methylamino-9*H*-purine (**5f**)

^{1}H-NMR: 400 MHz, ([D_6]-DMSO) δ (ppm) = 11.50 (s, 1H, H-9); 8.73 (s, 1H, NH); 8.20 (s, 1H, H-8); 7.75–7.47 (m, 4H, $H_{aromatic}$); 4.84 (s, 2H, CH_2)

^{13}C-NMR: 100 MHz, ([D_6]-DMSO) δ (ppm) = 152.8 (C-4); 148.4 (C-2); 132.8 ($C_{aromatic}$); 13 0.73 (C-6); 128.6 ($C_{aromatic}$); 127.4 ($C_{aromatic}$); 125.9 ($C_{aromatic}$); 124.6 (C-5); 45.7 ($CH_{2\,benzyl}$)

^{19}F-NMR: 377 MHz, ([D_6]-DMSO) δ (ppm) = −56.08; −58.15

IR:$\tilde{\nu}$ (cm^{-1}) = 1630, 1592, 1305, 1092, 933,

HR-ESI-MS $[M + H]^+$ calcd 328.0571 found 328.0583

mp: 203 °C

6-[(3-Bromophenyl)methylsulfanyl]-9*H*-purine (**8a**)

^{1}H-NMR: 400 MHz, ([D_6]-DMSO) δ (ppm) = 13.58 (s, 1H, H-9); 8.75 (s, 1H, H-2); 8.47 (s, 1H, H-8); 7.70 (s, 1H, $H_{aromatic}$); 7.5–7.262 (3H, $H_{aromatic}$); 4.663 (s, 2H, $CH_{2\,benzyl}$)

^{13}C-NMR: 100 MHz, ([D_6]-DMSO) δ (ppm) = 151.4 (C-2); 143.3 (C-4); 141.1 (C-8); 137.2–128.1 ($C_{aromatic}$); 121.5 (C-5); 30.8 ($CH_{2\,benzyl}$)

IR:$\tilde{\nu}$ (cm^{-1}) = 2671, 1472, 1396, 1196, 1033.

HR-ESI-MS $[M + H]^+$ calcd 320.9804 found 320.9639

mp: 252 °C

6-{[4-(Trifluoromethyl)phenyl]methylsulfanyl}-9*H*-purine (**8g**)

^{1}H-NMR: 400 MHz, ([D_6]-DMSO) δ (ppm) = 13.57 (s, 1H, H-9); 8.83 (s, 1H, H-2); 8.46 (s, 1H, H-8); 7.70–7.69 (m, 4H, $_{aromatic}$); 4.75 (s, 2H, $CH_{2\,benzyl}$))

^{13}C-NMR: 100 MHz, ([D_6]-DMSO) δ (ppm) = 157.6 (C-4); 151.4 (C-2); 149.4 (C-8); 143.3 (C-6); 143.2 ($C\text{-}1'_{aromatic}$); 129.7 (2C, C-2′, C-6′, $C_{aromatic}$); 125.3 (2C, C-3′, C-5′, $C_{aromatic}$); 30.8 ($CH_{2\,benzyl}$)

^{19}F-NMR: 377 MHz, ([D_6]-DMSO) δ (ppm) = −60.48 (s)

IR:$\tilde{\nu}$ (cm^{-1}) = 1568, 1319, 1113, 1064, 845.

HR-ESI-MS $[M + H]^+$ calcd 309.0427 found 309.0449

mp: 136 °C

6-[(2,5-Difluorophenyl)methylsulfanyl]-9*H*-purine **8j**

^{1}H-NMR: 400 MHz, ([D_6]-DMSO) δ (ppm) = 8.75 (s, 1H, H-8); 8.47 (s, 1H, H-2); 7.42–7.16 (m, 3H, $_{aromatic}$); 4.66 (s, 2H, $CH_{2\,benzyl}$)

^{13}C-NMR: 100 MHz, ([D_6]-DMSO) δ (ppm) = 159.1 (C-2′, $C_{aromatic}$); 157.9 (C-5′, C $_{aromatic}$); 156.7 (C-4); 155.5 (C-2); 151.5 (C-8); 143.7 (C-6); 127.1 (C-1′, $C_{aromatic}$); 126.8 (C-5); 117.7–115.6 (C-3′, C-4′, C-6′, C $_{aromatic}$); 25.1 ($CH_{2\,benzyl}$)

^{19}F-NMR: 377 MHz, ([D_6]-DMSO) δ (ppm) = −118.1 (s) −121.88 (s)

IR:$\tilde{\nu}$ (cm^{-1}) = 1569, 1492, 1382, 1231, 813.

HR-ESI-MS $[M + H]^+$ calcd 279.0510 found 279.0521

mp: 184 °C

6-[(2,6-Difluorophenyl)methylsulfanyl]-9*H*-purine (**8k**)

^{1}H-NMR: 400 MHz, ([D_6]-DMSO) δ (ppm) = 13.60 (s, 1H, H-9); 8.77 (s, 1H, H-2); 8.47 (s, 1H, H-8); 7.45–7.13 (m, 3H, $_{aromatic}$); 4.74 (s, 2H, $CH_{2\,benzyl}$)

^{13}C-NMR: 100 MHz, ([D_6]-DMSO) δ (ppm) = 162.1 (C-4); 162.0 (C-2); 159.6 (2C, C-2′, C-6′, C $_{aromatic}$); 149.5 (C-8); 130.3 (C-4′, $C_{aromatic}$); 113.2 (C-1′, $C_{aromatic}$); 111.9 (2C, C-3′, C-5′, $C_{aromatic}$); 19.2 ($CH_{2\,benzyl}$)

^{19}F-NMR: 377 MHz, ([D_6]-DMSO) δ (ppm) = −113.06 (s)

IR:$\tilde{\nu}$ (cm^{-1}) = 1567, 1466, 1232, 955, 780.

HR-ESI-MS $[M + H]^+$ calcd 279.0510 found 279.0517

mp: 185 °C

2-Amino-6-[(2,3-difluorophenyl)methylsulfanyl]-9*H*-purine (**9d**)

^{1}H-NMR: 400 MHz, ([D_6]-DMSO) δ (ppm) = 12.20 (s, 1H, H-9); 8.35 (s, 1H, H-2); 7.51–7.31 (3H, $_{aromatic}$); 6.75 (s, 2H, NH_2); 4.62 (s, 2H, $CH_{2\,benzyl}$)

^{13}C-NMR: 100 MHz, ([D_6]-DMSO) δ (ppm) = 159.5 (C-4); 156.8 (C-2); 153.4 (C-3′, $C_{aromatic}$); 152.2 (C-2′, $C_{aromatic}$); 148.3 (C-8); 141.6 (C-6); 128.3 (C-1′, $C_{aromatic}$); 128.1–116.3 (C $_{aromatic}$); 25.6 ($CH_{2\,benzyl}$)

^{19}F-NMR: 377 MHz, ([D_6]-DMSO) δ (ppm) = −138.86 (s), −142.18 (s)

IR:$\tilde{\nu}$ (cm^{-1}) = 1607, 1562, 1475, 913.

HR-ESI-MS $[M + H]^+$ calcd 294.0619 found 294.0601

mp: 204 °C

2-Amino-6-[(2,4-difluorophenyl)methylsulfanyl]-9*H*-purine (**9e**)

^{1}H-NMR: 400 MHz, ([D_6]-DMSO) δ (ppm) = 12.52 (s, 1H, H-9); 8.06 (s, 1H, H-2); 7.75–7.04 (3H, $H_{aromatic}$); 6.88 (s,2H, NH_2); 4.54 (s, 2H,$CH_{2\,benzyl}$)
^{13}C-NMR: 100 MHz, ([D_6]-DMSO) δ (ppm) = 171.3 (C-4); 152.76 (C-2); 153.6 (C-3′$_{aromatic}$); 152.2 (C-2′$_{aromatic}$); 140.9 (C-8); 139.5 (C-6); 121.7–104.0 (C′$_{aromatic}$); 45.9 ($CH_{2\,benzyl}$)
^{19}F-NMR: 377 MHz, ([D_6]-DMSO) δ (ppm) = −110.6 (s), −111.8 (s)
IR: $\tilde{\nu}$ (cm^{-1}) = 1559, 1465, 1033
HR-ESI-MS [M + H]$^+$ calcd 294.0619 found 294.0628
mp: 254 °C

6-(3-Bromophenyl)sulfanyl-9*H*-purine (**12d**)

^{1}H-NMR: 400 MHz, ([D_6]-DMSO) δ (ppm) = 13.53 (s, 1H, H-9); 8.58 (s, 1H, H-2); 8.52 (s, 1H, H-8); 7.87 (s, 1H, $_{aromatic}$); 7.70–7.44 (m, 3H, $_{aromatic}$)
^{13}C-NMR: 100 MHz, ([D_6]-DMSO) δ (ppm) = 151.6 (C-2); 143.7 (C-4); 137.2–129.4 ($C_{aromatic}$); 121.7 (C-5)
IR:$\tilde{\nu}$ (cm^{-1}) = 1558, 1232, 854, 640.
HR-ESI-MS [M + H]$^+$ calcd 308.9575 found 308.962
mp: 202 °C (decomposition)

6-(4-Bromophenyl)sulfanyl-9*H*-purine (**12e**)

^{1}H-NMR: 400 MHz, ([D_6]-DMSO) δ (ppm) = 13.55 (s, 1H, H-9); 8.56 (s, 1H, H-2); 8.52 (s, 1H, H-8); 7.69 (d, 2H, J = 8.7 Hz, $_{aromatic}$); 7.58 (d, 2H,J = 8.7Hz, $_{aromatic}$)
^{13}C-NMR: 100 MHz, ([D_6]-DMSO) δ (ppm) = 160.1; 151.6 (C-2); 151.7 (C-8); 145.7; 137.4 7(2C, C-2′ + C-6′; $C_{arom.}$); 132.3 (2C, C-3′ + C-5′, $C_{aromatic}$); 128.6 (C-5); 126.6 (C-1, $C_{aromatic}$); 123.21 (C-4′, $C_{aromatic}$)
IR:$\tilde{\nu}$ (cm^{-1}) = 1560, 1232, 853, 640.
HR-ESI-MS [M + H]$^+$ calcd 308.9575 found 308.962
mp: 208 °C (decomposition)

6-(4-Hydroxyphenyl)sulfanyl-9*H*-purine (**12f**)

^{1}H-NMR: 400 MHz, ([D_6]-DMSO) δ (ppm) = 13.52 (s, 1H, H-9); 9.93 (s, 1H, OH); 8.50 (s, 1H, H-2); 8.45 (s, 1H, H-8); 7.41 (d, 2H, J = 8.4Hz, $_{aromatic}$); 6.882 (d, 2H, J = 8.4Hz, $_{aromatic}$)
^{13}C-NMR: 100 MHz, ([D_6]-DMSO) δ (ppm) = 159.3 (1C, C-4$_{aromatic}$); 158.91 (1C, C-8); 151.59 (1C, C-4); 143.16 (1C, C-6); 137.51 (2C, C-3 + C-5$_{aromatic}$); 129.53 (1C, C-5); 116.41.51 (2C, C-2 + C-6$_{aromatic.}$)
IR:$\tilde{\nu}$ (cm^{-1}) = 1567, 1381, 1225, 639.
HR-ESI-MS [M + H]$^+$ calcd 245.0492 found 245.0503
mp: 268 °C (decomposition)

6-(2,3-Dichlorophenyl)sulfanyl-9*H*-purine (**12g**)

^{1}H-NMR: 400 MHz, ([D_6]-DMSO) δ (ppm) = 13.65 (s, 1H, H-9); 8.57 (s, 1H, H-2); 8.53 (s, 1H, H-8); 7.83–7.79 (m, 2H, $_{aromatic}$) 7.47 (s, 1H, $_{aromatic}$);
^{13}C-NMR: 100 MHz, ([D_6]-DMSO) δ (ppm) = 151.6 (2C, C-4 + C-8); 136.4 (1C, C-5); 144.1 (1C, C-6); 132.7 (1C, C-3); 132.28 (2C, C-1 + C-3$_{aromatic}$); 129.12 (1C, C-4 $_{aromatic}$); 128.7 (2C, C-5 + C-6$_{aromatic}$)
IR:$\tilde{\nu}$ (cm^{-1}) = 1557, 1232, 853, 641.
HR-ESI-MS [M + H]$^+$ calcd 296.969 found 296.977
mp: 214 °C

6-(2,5-Dichlorophenyl)sulfanyl-9*H*-purine (**12i**)

^{1}H-NMR: 400 MHz, ([D_6]-DMSO) δ (ppm) = 13.05 (s, 1H, H-9); 8.58 (s, 1H, H-2); 8.53 (s, 1H, H-8); 7.94 (d, 1H, J = 2.0Hz, H-6$_{aromatic}$); 7.73–7.62 (m, 2H, H-3, H-6 $_{aromatic}$)
^{13}C-NMR: 100 MHz, ([D_6]-DMSO) δ (ppm) = 159.3 (1C, $C_{aromatic}$); 158.9 (1C, C-8); 151.6 (1C, C-4); 143.2 (1C, C-6); 137.5 (2C, C-3 + C-5, $C_{aromatic}$); 129.5 (1C, C-5); 116.4 (2C, C-2 + C-6, $C_{aromatic}$)
IR:$\tilde{\nu}$ (cm^{-1}) = 1592, 1382, 1229, 637.
HR-ESI-MS [M + H]$^+$ calcd 296.9763 found 296.9773
mp: 208 °C (decomposition)

2-Amino-6-(2-bromophenyl)sulfanyl-9*H*-purine (**13c**)

^{1}H-NMR: 400 MHz, ([D_6]-DMSO) δ (ppm) = 12.60 (s, 1H, H-9); 7.94 (s, 1H, H-8); 7.79–7.38 (4H, $H_{aromatic}$); 6.22 (s, 2H, NH_2)
^{13}C-NMR: 100 MHz, ([D_6]-DMSO) δ (ppm) = 159.7 (C-2′$_{aromatic}$); 156.6 (C-4); 151.4 (C-2); 139.5 (C-8); 137.3 (C-6); 130.9–123.7 (5C′$_{aromatic}$)
IR: $\tilde{\nu}$(cm^{-1}) = 1613, 1558, 1502, 909, 740
HR-ESI-MS [M + H]$^+$ calcd 321.9757 found 321.9752
mp: 230 °C

2-Amino-6-(3-bromophenyl)sulfanyl-9*H*-purine (**13d**)

^{1}H-NMR: 400 MHz, ([D_6]-DMSO) δ (ppm) = 12.63 (s, 1H, H-9); 8.10 (s, 1H, H-8); 7.79–7.61 (4H, $H_{aromatic}$); 6.26 (s, 2H, NH_2)
^{13}C-NMR: 100 MHz, ([D_6]-DMSO) δ (ppm) = 159.8 (C-2′$_{aromatic}$); 156.9 (C-4); 152.5 (C-2); 139.7 (C-8); 133.7 (C-6); 131.7–121.7 (5C′$_{aromatic}$)
IR: $\tilde{\nu}$(cm^{-1}) = 1615, 909, 651
HR-ESI-MS [M + H]$^+$ calcd 321.9757 found 321.9752
mp: 212 °C (decomposition)

2-Amino-6-(4-methoxyphenyl)sulfanyl-9*H*-purine (**13g**)

^{1}H-NMR: 400 MHz, ([D_6]-DMSO) δ (ppm) = 12.53 (s, 1H, H-9); 7.92 (s, 1H, H-8); 7.52–7.49 (2H, H-3′$_{aromatic}$+ H-5′$_{aromatic}$); 7.03–7.00 (2H, H-2′$_{aromatic}$+H-6′$_{aromatic}$); 6.13 (s, 2H, NH_2); 3.81 (s, 3H, $CH_{3\,methoxy}$)

^{13}C-NMR: 100 MHz, ([D_6]-DMSO) δ (ppm) = 160.0 (C-4); 159.7 (C-4′$_{aromatic}$); 155.0 (C-2); 151.7 (C-8); 145.7 (C-4); 136.8 (2C, C-2′$_{aromatic}$, C-6′$_{aromatic}$); 128.6 (C-5); 132.1 (C-1′$_{aromatic}$); 115.0 (2C, C-3′$_{aromatic}$ +C-5′$_{aromatic}$); 55.3 ($CH_{3\,methoxy}$)

IR: $\tilde{\nu}$ (cm^{-1}) = 1614, 1551, 1240, 932, 821

HR-ESI-MS [M + H]$^+$ calcd 274.0757 found 274.0746

mp: 261 °C

2-Chloro-6-(2,4-dichlorophenylyl)sulfanyl-9*H*-adenine (**14d**)

^{1}H-NMR: 400 MHz, ([D_6]-DMSO) δ (ppm) = 13.32 (s, 1H, H-9); 8.53 (s, 1H, H-8); 7.88–7.55 (3H, $H_{aromatic}$)

^{13}C-NMR: 100 MHz, ([D_6]-DMSO) δ (ppm) = 159.9 (C-4′$_{aromatic}$); 159.7 (C-8); 152.9 (C-2); 140.2 (C-6); 136.1–128.3 ($C_{aromatic}$); 124.9 (C-5)

IR: $\tilde{\nu}$ (cm^{-1}) = 1557, 1446, 1344, 1231, 867, 809

HR-ESI-MS [M + H]$^+$ calcd 330.9373 found 330.9376

mp: 111 °C (decomposition)

Cell growth inhibition assay

The compounds were tested for their growth inhibitory activities in a panel of four human tumor cell lines by using a microtiter assay based on the crystal violet staining method for adherent cells [7]. The antiproliferative activity was determined in two lung human cancer cell lines (A 427 and LCLC), one pancreatic human cancer cell line (DAN-G), and one human bladder cancer cell line (5637), which were all obtained from the German Collection of Microbiology and Cell Culture (DSZK, Braunschweig, FRG). The cells were plated out 24 h prior to testing and all compounds were tested at 20 μM by diluting a 20 mM DMSO stock solution into cell culture medium (RPMI medium + 10 % fetal calf serum). Cell cultures were exposed to compound for 96 h, after which time the medium was removed and the cells were fixed with a 1 % glutaraldehyde solution. Plates were stored at 4 °C until being stained with crystal violet as described earlier [7].

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