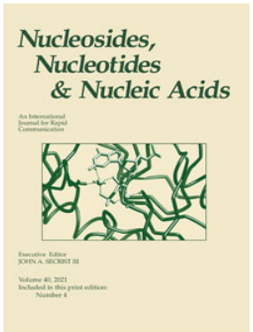


Nucleosides, Nucleotides & Nucleic Acids

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/lncn20

6-Morpholino- and 6-amino-9-sulfonylpurine derivatives. Synthesis, computational analysis, and biological activity

Josipa Matić, Marijana Jukić, Hamit Ismaili, Dijana Saftić, Željka Ban, Tana Tandarić, Robert Vianello, Teuta Opačak-Bernardi, Ljubica Glavaš-Obrovac & Biserka Žinić

To cite this article: Josipa Matić, Marijana Jukić, Hamit Ismaili, Dijana Saftić, Željka Ban, Tana Tandarić, Robert Vianello, Teuta Opačak-Bernardi, Ljubica Glavaš-Obrovac & Biserka Žinić (2021) 6-Morpholino- and 6-amino-9-sulfonylpurine derivatives. Synthesis, computational analysis, and biological activity, Nucleosides, Nucleotides & Nucleic Acids, 40:4, 470-503, DOI: 10.1080/15257770.2021.1896001

To link to this article: https://doi.org/10.1080/15257770.2021.1896001

Published online: 12 Mar 2021.

NUCLEOSIDES, NUCLEOTIDES AND NUCLEIC ACIDS
2021, VOL. 40, NO. 4, 470–503
https://doi.org/10.1080/15257770.2021.1896001

6-Morpholino- and 6-amino-9-sulfonylpurine derivatives. Synthesis, computational analysis, and biological activity

Josipa Matić[a], Marijana Jukić[b], Hamit Ismaili[a,c], Dijana Saftić[a], Željka Ban[a], Tana Tandarić[a], Robert Vianello[a], Teuta Opačak-Bernardi[b], Ljubica Glavaš-Obrovac[b], and Biserka Žinić[a]

[a]Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Zagreb, Croatia; [b]Department of Chemistry, Biochemistry and Clinical Chemistry, Faculty of Medicine, Osijek, Croatia; [c]Faculty of Mathematical and Natural Sciences, University of Prishtina, Prishtina, Kosovo

ABSTRACT

The synthesis of novel 6-chloro/morpholino/amino/-9-sulfonylpurine derivatives was accomplished in two ways, either (i) involving the condensation reaction of 6-chloropurine with commercially available arylsulfonyl chlorides in acetone and the presence of aqueous KOH at 0 °C, followed by the substitution of C6-chlorine with morpholine, or (ii) employing a reversed synthetic approach where 6-morpholinopurine and commercially available adenine bases were reacted with the corresponding alkyl, 2-arylethene and arylsulfonyl chlorides giving the N9 sulfonylated products, the latter particularly used where prior nonselective sulfonylation was observed. In both approaches, the sulfonylation reaction occurred regioselectively at the purine N9 position lacking any concurrent N7 derivatives, except in the case of a smaller methyl substituent on SO_2 and the free amino group at C6 of the purine ring. The tautomeric features of initial N9 unsubstituted purines, as well as stability trends among the prepared *N*-9-sulfonylpurine derivates, were investigated using DFT calculations with an important conclusion that electron-donating C6 substituents are beneficial for the synthesis as they both promote the predominance of the desired N9 tautomers and help to assure the stability of the final products. The newly synthesized 6-morpholino and 6-amino-9-sulfonylpurine derivatives showed antiproliferative activity on human carcinoma, lymphoma, and leukemia cells. Among the tested compounds, 6-morpholino **17** and 6-amino **22** derivatives, with *trans*-*β*-styrenesulfonyl group attached at the N9 position of purine, proved to be the most effective antiproliferative agents, causing accumulation of leukemia cells in subG0 cell cycle phase.

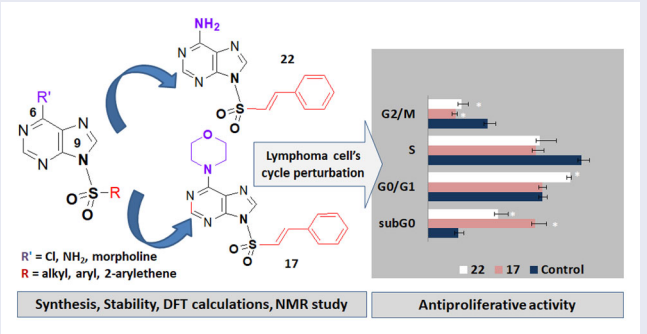
ARTICLE HISTORY
Received 28 October 2020
Accepted 23 February 2021

KEYWORDS
6-Chloropurine; 6-morpholinopurine; adenine; regioselective N-9-sulfonylation; DFT calculations; biological activity

CONTACTS Robert Vianello robert.vianello@irb.hr Computational Organic Chemistry and Biochemistry Group, Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Bijenička cesta 54, HR-10000 Zagreb, Croatia; Ljubica Glavaš-Obrovac lgobrovac@mefos.hr Department of Medicinal Chemistry, Biochemistry and Laboratory Medicine, Faculty of Medicine Osijek, Josip Juraj Strossmayer University of Osijek, Huttlerova 4, HR-31000 Osijek, Croatia; Biserka Žinić bzinic@irb.hr Laboratory for Biomolecular Interactions and Spectroscopy, Division of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, Bijenička cesta 54, HR-10000 Zagreb, Croatia.

Supplemental data for this article is available online at https://doi.org/10.1080/15257770.2021.1896001.

GRAPHICAL ABSTRACT



1. Introduction

Sulfonamides represent an important class of molecules possessing diverse biological activities. They are powerful antimicrobial drugs,[1,2] carbonic anhydrase inhibitors,[3–5] antithyroid agents,[6] antitumor drugs,[7–9] and protease[10,11] inhibitors. Previously, we have synthesized novel pyrimidine nucleobase derivatives containing a sulfonamide pharmacophore by attaching the sulfonyl fragment onto the N1 of pyrimidine bases.[12–14] Obtained *N*-1-sulfonylpyrimidine derivatives showed *in vitro*[15–17] and *in vivo*[18–20] potent antitumor activity. These compounds inhibit DNA, RNA, and protein synthesis and some of them showed the ability to induce apoptosis in treated tumor cells.[15,21] Inspired by interesting biological results with *N*-1-sulfonylpirimidine derivatives, we decided to expand our investigation to *N*-9-sulfonylpurine derivatives.

Purines are an important class of biologically active compounds and many of the clinically used antitumor drugs consist of a modified purine core. Some of the most prominent ones are 6-mercaptopurine and 6-tioguanine,[22] azathioprine,[23] fludarabine,[24] pentostatin,[25] and cladribine.[26] Guanosine analogs, like acyclovir, ganciclovir, and penciclovir, are one of the most well-known antiviral drugs.[27–29] Since purine analogs make one of the key components in various cellular processes[30–32] it is reasonable to assume that modified purines present an exciting target for biological evaluations.

N-9-Sulfonylpurine derivatives have received minimal attention in the literature. Martirosyan *et al.*[33] reported the preparation of *N*-9-tosyladenine and Zemlicka *et al.*[34] obtained the same compound in the reaction of adenallene with *p*-toluenesulfonyl chloride as an unexpected product. We also investigated tosylation and mesylation reactions of adenine.[35] It was found that at room temperature sulfonylation can be performed

regioselectively at the N9 position of adenine using 2 equivalents of sulfonyl chloride in pyridine. Supuran *et al.*[36] synthesized a series of 9-sulfonylated/sulfenylated-6-mercaptopurines by the reaction of 6-mercaptopurine with sulfonyl/sulfenyl halides in acetone and aqueous KOH, as potent antimycobacterial agents possessing MIC values against Mycobacterium tuberculosis. Inspired by these results Gundersen and co-workers[37] reported the synthesis of 6-furyl-9-sulfonylpurine derivatives applying the same set of reaction conditions as reported for the sulfonylation of 6-mercaptopurine. In both cases, the sulfonylation reaction occurred only at the N9 atom, without the concurrent N7 sulfonylation. 6-Phenyl-9-sulfonylpurine analogs,[38] *N*-6-(4-trifluoromethylphenyl)-piperazine derivative and its 9-(*p*-toluenesulfonyl)/9-cyclopentyl analogs[39] showed promising cytotoxic activities.

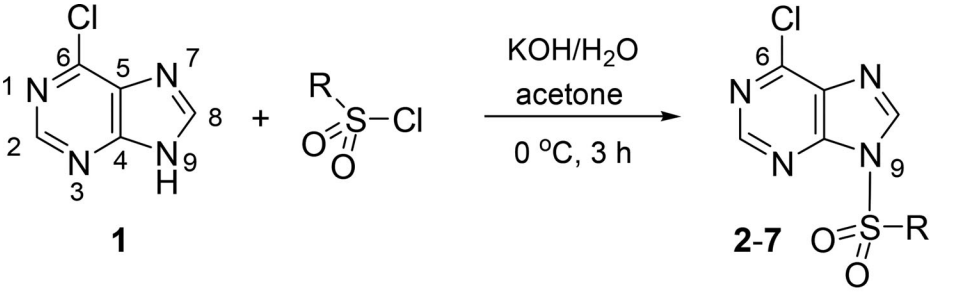
In this paper, we report on the development of a novel class of potentially active compounds, combining the chemistry of purine nucleobases and sulfonamides. The compounds were synthesized by the transformation of new 6-chloro-9-sulfonyl purine derivatives into the appropriate 6-morpholino derivatives or by sulfonylation of adenine and 6-morpholinopurine with sulfonyl chlorides. Further on, the computational study was performed to determine the influence of different C6 substituents on the tautomeric features of the starting N9 unsubstituted systems as well as on the stability of the final *N*-9-sulfonylpurine products. The prepared 6-morpholino and 6-amino-9-sulfonylpurine derivatives were tested on their biological activity on normal human fibroblast cell line and a panel of human tumor cell lines.

2. Results and discussion

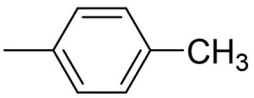
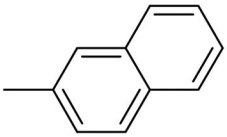
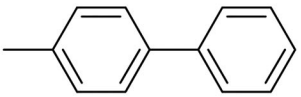
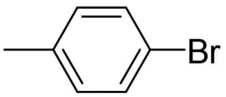
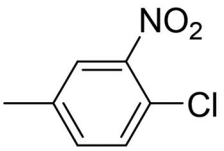
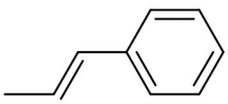
2.1. Synthesis

It is known that N9 substituted derivatives of 6-chloropurine exhibit cytotoxic[40] and antiviral[41] properties, and can also be transformed into different C6 substituted[42] derivatives. In order to have an easy access to varying substituents at the C6 position of the purine base, we decided to investigate the condensation reactions of 6-chloropurine **1** with commercially available alkyl, 2-arylethene and arylsulfonyl chlorides (Table 1) that were previously associated with the biological activity, especially antitumor activity.[14,15,43–47] It is also well known that the alkylation of purine nucleobase under basic conditions in most cases result in a mixture of regioisomeric *N*-7- and *N*-9-alkylpurines.[48,49] The desired N9 substituted compound is usually the major product, but significant amounts of N7 isomers are often observed as well as other alkylation products. Therefore, when investigating the most advantageous method of obtaining *N*-9-

Table 1. Condensation of 6-chloropurine **1** with sulfonyl chlorides and synthesis of 6-chloro-*N*-9-sulfonypurines **2**-**7**.



1 **2-7**

Entry	R	Product	Yield / %[a]
1		**2**	64
2		**3**	68
3		**4**	77
4		**5**	50
5		**6**	66
6		**7**	73
7		-	-[b]

[a]Yields of analytically pure products.
[b]The product decomposes during isolation.

sulfunylpurine, special attention is paid to the identification of possible N7 isomer. The rationalization of the tautomeric properties of the derivatives studied here is performed using computational DFT methods (see later).

Initially, we attempted the sulfonylation of 6-chloropurine **1** with tosyl chloride, following our procedure for regioselective tosylation and mesylation of adenine.[35] The condensation of 6-chloropurine **1** with tosyl chloride in the presence of pyridine at room temperature was not successful and only the starting material was isolated. The same result was obtained by using copper oxide which proved to be a very efficient catalyst for the synthesis of sulfonamides and sulfonic esters using different substrates such as amines, alcohols, phenols.[50] Wong *et al.*[51] reported the successful N9 alkylation of purine ring with the assistance of tetrabutylammonium fluoride (TBAF). With this method, the sulfonylated product **2** was obtained in a poor (5%) yield. Subsequently, we investigated the use of KOH as the strong base, which might lead to the deprotonated purine at the N9 position, and then to allow the condensation with tosyl chloride. 6-Chloro-9-tosyl-9*H*-purine **2** was obtained by Supuran method[36] for the *N*-sulfonylation of 6-mercaptopurine (Table 1, entry 1). Suspension of 6-chloropurine **1** in acetone was treated with aqueous KOH, and the resulting solution was cooled to 0 °C and treated with tosyl chloride. The *N*-9-tosyl product **2** was obtained in 64% yield, as the single product according to the ^{1}H NMR spectrum of the crude product. The ^{1}H NMR spectrum of 9-tosyl product **2** displayed two important signals at δ 9.13 and 8.90 ppm assignable to H-2 and H-8 of the purine base, respectively. The signals are shifted downfield ($\Delta\delta_{\text{H-2}} = 0.41$ and $\Delta\delta_{\text{H-8}} = 0.23$ ppm), compared to chemical shifts of the corresponding protons in the free 6-chloropurine **1** (Supplemental material Fig. S1). The H-8 and H-2 assignments were confirmed by carbon-proton connectivity in the 2 D HMQC spectra (Supplemental material Fig. S4). Additionally, in the NOESY spectrum of **2**, the NOE interaction between the phenyl *ortho* (Ts-b) protons and H-8 supported the assignment of H-8 and also *anti*-orientation of the tosyl substituent at N9 (Supplemental material Fig. S5). Using the latter reaction conditions with commercially available 2-arylethene and arylsulfonyl chlorides, the respective *N*-9-sulfonylated products **3**-**7** were obtained in very good (50-77%) yields (Table 1, entries 2-6).

All 6-chloro-9-sulfonyl derivatives **2**-**7** were obtained as white crystals, and they were stable for more than a month at room temperature. However, we have soon noticed that **2**-**7** were not stable in DMSO solution. After 6-18 hours standing at room temperature, the decomposition products were observed. The NMR spectra were consistent with the cleavage of the N-SO2 bond and the formation of free 6-chloropurine **1** and the corresponding aryl sulfonic acid, caused by water commonly present as a trace

impurity in the deuterated DMSO (Supplemental material Fig. S6). Furthermore, in the reaction of 6-chloropurine **1** with mesyl chloride, the product was detected by thin layer chromatography. However, it was not possible to isolate the 6-chloro-9-mesylated purine from the reaction mixture due to the instability of the compound (Table 1, entry 7).

Our previous work[35] showed that the *N*-9-sulfonyladenine derivatives were relatively stable in solutions and therefore we decided to replace chlorine with amino group and morpholine, whose ring is an integral part of many biologically active compounds.[52]

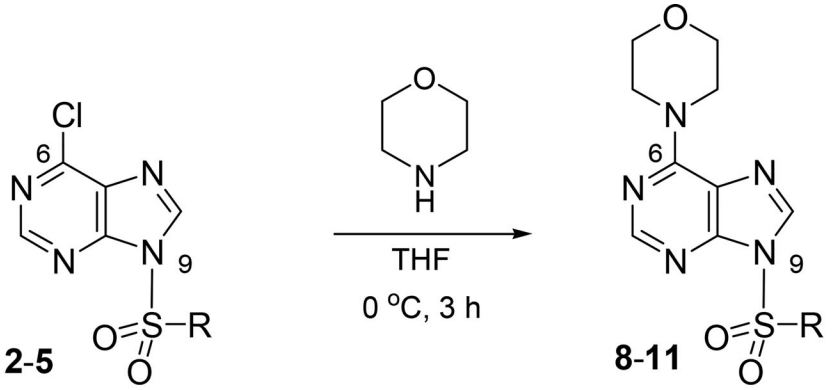
Huang *et al.*[53] published the nucleophilic displacement reaction of 6-chloropurine with various nucleophiles under focused microwave irradiation. For example, 6-morpholino purine was synthesized in excellent 94% yield, by heating 6-chloropurine **1** at 80 °C with 10 equivalents of morpholine. When the tosyl derivative **2** was heated in a microwave reactor with 10 molar excess of morpholine for one minute at 80 °C, 6-morpholinopurine precipitated from the reaction mixture, indicating the instability of the N-SO_2 bond at elevated temperature and the presence of the base. We investigated the effect of temperature, solvent, reaction time and amount of morpholine on the relative yields of 6-morpholino-9-tosylpurine **8**. The best yield was obtained when 6-chloro-9-tosylpurine **2** was reacted with a four-fold molar excess of morpholine in tetrahydrofuran at 0 °C. In this way, chlorine derivatives **2**-**5** have been successfully converted to 6-morpholine derivatives **8**-**11** in 61-93% yields (Table 2).

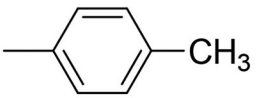
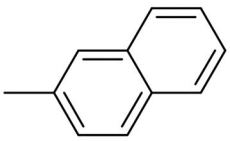
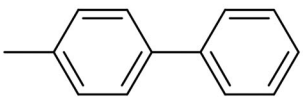
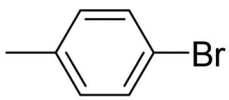
However, as we expected, with 6-chloro derivatives **6** and **7**, the situation was more complicated. In addition to the chlorine substitution at the C6 position of **6**, chlorine on the aromatic ring of the sulfonyl substituent (activated by the *ortho*-nitro group) was also substituted (Scheme 1). The main product, disubstituted morpholino derivative **12** was isolated in 79% yield. Small amounts of unstable monosubstituted **13** and desired product **14** were also isolated (1-2%).

In the reaction of **7** with morpholine, chlorine substitution on purine ring and morpholine addition to the double bond of a styrenesulfonyl substituent were simultaneously obtained (Scheme 2). Derivative **15** was isolated in the yield of 73% as the only product.

Since sulfonylation of 6-chloropurines **6** and **7** failed to yield the desired 6-morpholino products, we have selected a reversed synthetic approach. First, 6-morpholinopurine **16** was synthesized according to a known procedure.[53] Then, applying the conditions for the synthesis of compounds **2**-**7**, the morpholine derivatives **14** and **17** were prepared in good yields (Table 3, entry 1 and 2). This synthetic route also provided mesyl derivative **18** (Table 3, entry 3).

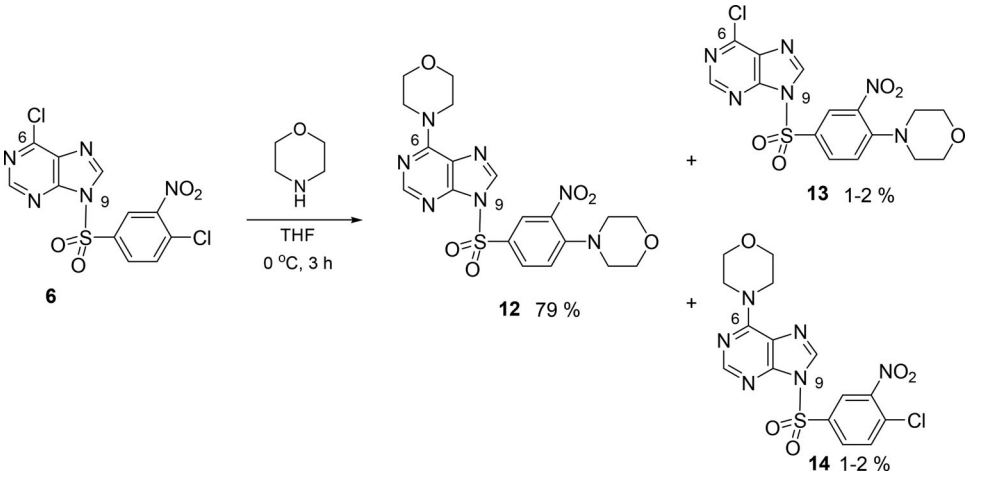
Table 2. Substitution of C6-chlorine in **2**-**7** derivatives with morpholine and the synthesis of 6-morpholino-*N*-9-sulfonylpurines **8**-**11**.



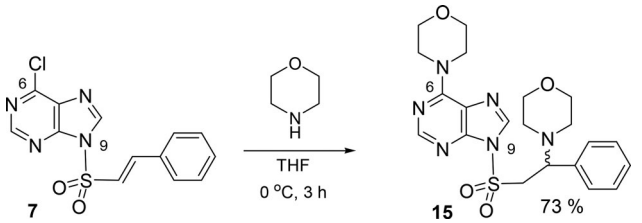
Entry	R	Product	Yield / %[a]
1		**8**	61
2		**9**	61
3		**10**	63
4		**11**	93

[a]Yields of analytically pure products.

In the ^{1}H NMR spectra of 6-morpholino-*N*-9-sulfonylpurines (**8**-**11**, **14**, **17** and **18**) as well as dimorpholine derivatives (**12** and **15**) the signals of purine protons H-8 and H-2 are slightly shifted upfield ($\Delta\delta_{\text{H-8}} = 0.5$ and $\Delta\delta_{\text{H-2}} = 0.6$ ppm), compared to the corresponding 6-chloro derivatives. The broad singlet at $\delta \sim 4.2$ ppm and the broad triplet at $\delta \sim 3.7$ ppm correspond to C6 morpholine functionality in monosubstituted derivatives, while disubstituted derivatives **12** (3.68 and 3.23 ppm) and **15** (3.24 – 1.88 ppm) show additional signals for another morpholine unit. The most striking difference refers to the signals of purine carbon atoms C-8 and C-2 in ^{13}C NMR spectra of all morpholine derivatives. The signals of purine C-8 are



Scheme 1. The reaction of **6** with morpholine.



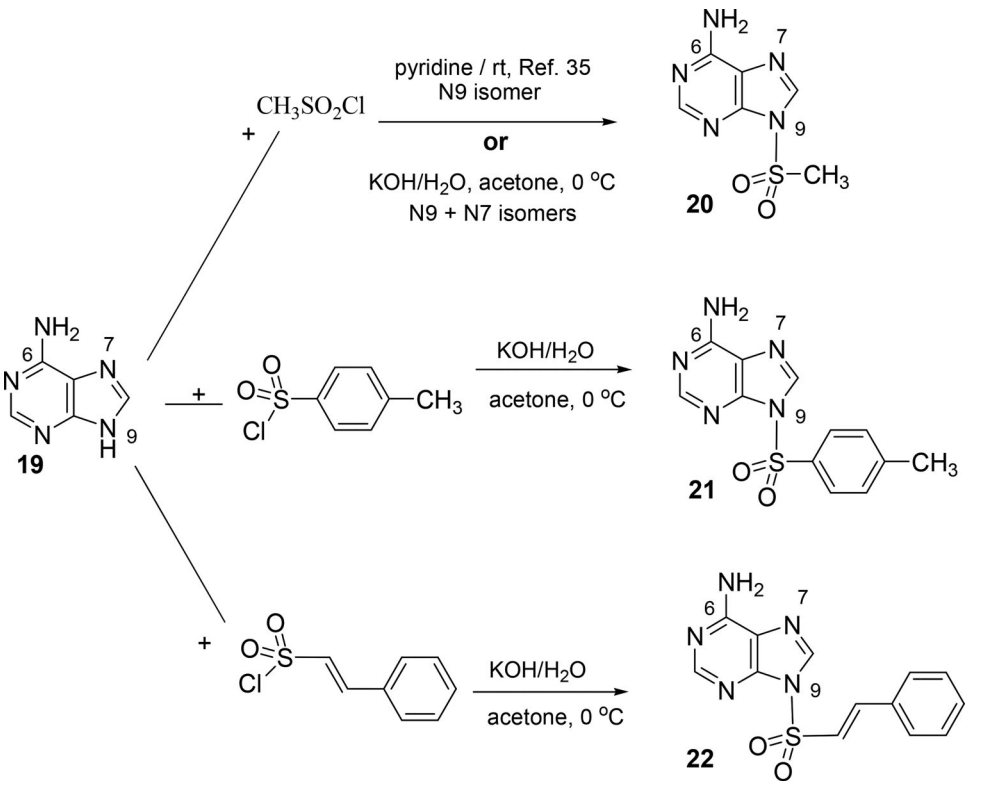
Scheme 2. The reaction of **7** with morpholine.

shifted upfield for ~7.1 ppm, and C-5 are more shielded for ~12.6 ppm, compared to the corresponding signals of 6-chloro derivatives.

The stability of 6-morpholino-*N*-9-sulfonylpurines (**8**-**11**, **14**, **17** and **18**), dimorpholine derivatives (**12** and **15**), and *N*-9-sulfonyladenine derivatives **20**-**22** were investigated by the time-dependent ^{1}H NMR experiments. All compounds except **15** were stable for at least 4 days.

The time-dependent ^{1}H NMR spectra of compound **15**, after 6 hours in DMSO solution at room temperature, showed the cleavage of N-SO_2 bond and the formation of 6-morpholino purine base **16** and the corresponding aryl sulfonic acid **B** (Scheme 4; Supplemental material Figs. S29 and S30). Interestingly, we have also observed that at elevated temperature (40 °C) in DMSO/H_2O or methanolic solution, the faster reaction was the elimination of morpholine present in the aryl-N-SO_2 moiety, giving solely the product **17**.

Therefore, we decided to include compound **15** in *in vitro* experiments, assuming that, under the conditions of *in vitro* experiment, **15** will



Scheme 3. The condensation reactions of adenine **19** with sulfonyl chlorides.

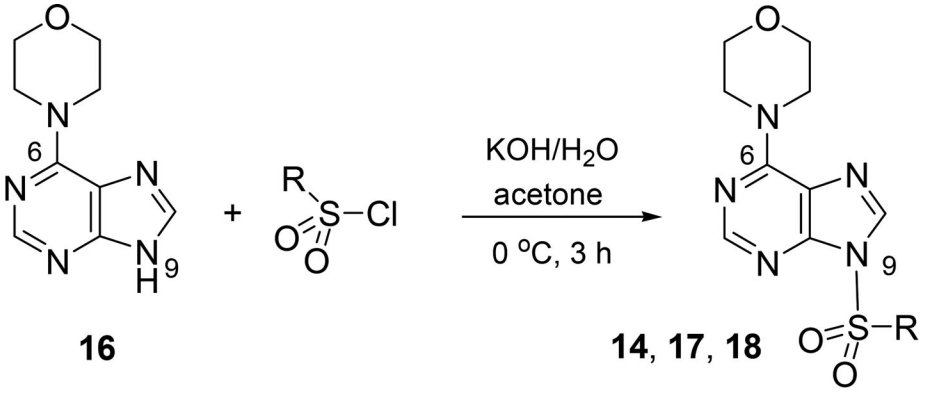
transform rapidly enough into compound **17** and give the corresponding effects. It was found that 6-morpholinopurine **16** exhibited weak antiproliferative activity. Since a similar activity was obtained for **15** and **17** obtained by a different route, the plausible explanation is that **15** transforms to **17** in the course of *in vitro* experiment (Supplemental material, Table S1).

The results of stability experiments show that the purine 6-morpholino/amino substitution results in the formation of stable *N*-9-sulfonylpurines. This fact opens the way toward the preparation of a new series of 6-amino substituted *N*-9-sulfonylpurines with increased stability, which is favorable for *in vitro* and *in vivo* screening experiments.

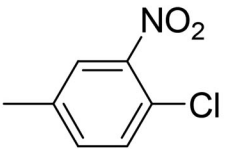
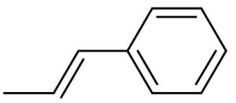
2.2. Computational analysis

Computational DFT analysis was initiated by inspecting the tautomeric properties of variously C6-substituted purines focusing on the relative Gibbs free energy differences among the matching N9 and N7 tautomers. A range of substituents was considered and the calculated ΔG_{taut} are

Table 3. Condensation of 6-morpholinopurine **16** with sulfonyl chlorides and synhesis of 6-morpholino-*N*-9-sulfonylpurines **14**, **17** and **18**.



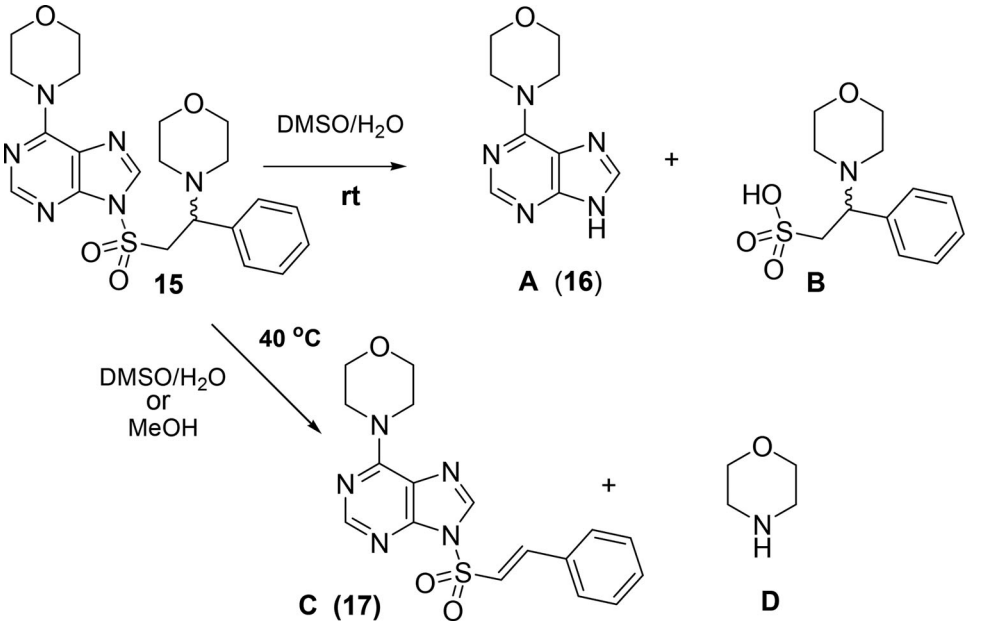
16 **14**, **17**, **18**

Entry	R	Product	Yield / %[a]
1		**14**	68
2		**17**	58
3	—CH_3	**18**	42

[a]Yields of analytically pure products.

presented in Table 4 with an emphasis that negative ΔG_{taut}values signify a larger stability of the N7 tautomer, while positive ΔG_{taut} values indicate preference of the matching N9 tautomer, the latter being desired for the further synthetic protocols described here.

The obtained results point to a predominance of the N9 tautomers in a majority of considered systems, which justifies their successful conversion into the matching 9-sulfonyl derivatives. In purine (X = H), the difference among tautomers is only marginal (0.3 kcal mol^{-1}) and both analogs are present in solution, which would likely aggravate a direct conversion to the N9-substituted products. Introduction of the C6-chlorine atom, as in **1**, stabilizes the N9 tautomer, making it 1.4 kcal mol^{-1} more stable than the N7 analogue, thus indicating around 90% prevalence of the former, which is



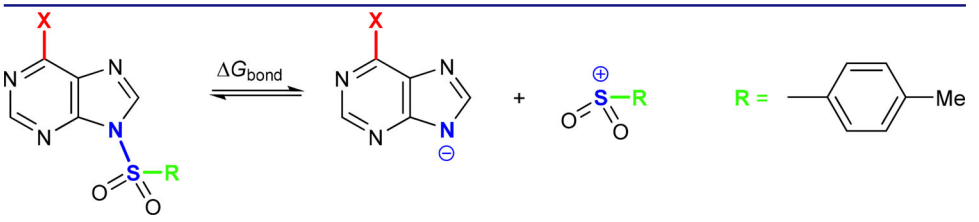
Scheme 4. Transformation of dimorpholine derivative **15** in DMSO/H_2O solution at room temperature (mixture **A** (**16**)+**B**) and DMSO/H_2O or MeOH solutions at 40 °C (mixture **C** (**17**)+**D**), as determined by time-dependent ^{1}H NMR spectra.

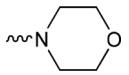
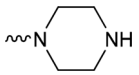
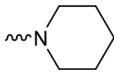
beneficial for the synthesis. The replacement of 6-Cl with other halogen atoms would not considerably change this picture, with a notion that bromine slightly improves the stability of the N9 tautomer, while fluorine reduces it. However, a much stronger electron-withdrawing $-NO_2$ group reverses the stability in favor of the N7 tautomer, making it 1.3 kcal mol^{-1} more stable than the N9 analogue, which would disfavor the subsequent N9 derivatization and is not recommended for the present purposes.

On the other hand, the introduction of electron-donating 6-amino substituents significantly improves the stability of the N9 tautomers, which is desired. Simple $-NH_2$ moiety, as in adenine, already results in 1.9 kcal mol^{-1} larger stability of the N9 tautomer, in accordance with earlier reports.[54] Interestingly, further enhancement of the electron-donating ability, either by methylation to $-NMe_2$ or by introducing triphenylphosphazeno moiety, works toward lowering the difference among tautomers, or even reversing it in favor of the N7 derivative for the latter by 1.2 kcal mol^{-1}.

Nevertheless, cyclic amines provide a very favorable option for the following N9 derivatization, as all three investigated substituents promote the stability of N9 tautomer by over 3 kcal mol^{-1}. These results are strongly in line with experiments reported here and help explaining higher yields of the prepared 6-morpholino-*N*-9-sulfonylpurines relative to, for example, 6-

Table 4. Relative stabilities of N9 and N7 tautomers of C6 substituted purines (all values in kcal mol^{-1}).

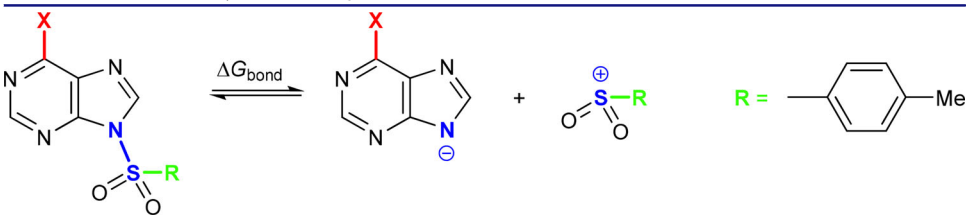


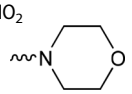
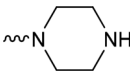
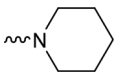
X	ΔG_{taut}	X	ΔG_{taut}
–H	0.3		
–F	1.0	$-NH_2$	1.9
–Cl	1.4	$-N(Me)_2$	1.3
–Br	1.8	$-N=P(Ph)_3$	–1.2
$-NO_2$	–1.3	–OH	0.9
	3.0	–OMe	2.2
	3.8	–SH	0.7
	3.2	–SMe	–0.6

chloro analogs. It is worth pointing that potentially replacing the employed 6-morpholino derivative with the analogous 6-piperazine could likely improve the synthetic outcomes. We note in passing that oxygen and sulfur substituents position themselves somewhere between halogen- and nitrogen-containing groups and their utilization is not recommended. In concluding this section, it should be emphasized that a significant predominance of the undesired N7 tautomer is observed only when the C6 substituent is either very nucleophilic and contains an excess negative charge, as with the $-NO_2$ group, or when it involves a very basic moiety, as with the imino nitrogen in the phosphazeno fragment.[55,56] In both cases, the stability of the N7 tautomer is favored due to the vicinity of the N7–H moiety on the C6 substituent, which form favorable hydrogen bonding interactions that contribute to the stability.

Secondly, we turned our attention to evaluate the stability of the $N–SO_2$ bond in the prepared *N*-9-sulfonylpurines in order to rationalize the observed stability trends. For that purpose we calculated the Gibbs free energy required for the cleavage of the mentioned bond in the *N*-9-tosyl derivatives again varying substituents on the C6 position (Table 5). In doing so, we have considered all three possible $N–SO_2$ bond cleavage

Table 5. Calculated heterolytic bond energies (ΔG_{bond}, in kcal mol^{-1}) for the considered C6 substituted N-9-sulfonylpurines together with related Hammett's substituent constants.



X	ΔG_{bond}	σ_m	σ_p	X	ΔG_{bond}	σ_m	σ_p
–H	52.1	0.00	0.00				
–F	48.9	0.34	0.06	$-NH_2$	55.7	–0.16	–0.66
–Cl	47.8	0.37	0.23	$-N(Me)_2$	57.2	–0.16	–0.83
–Br	49.8	0.39	0.23	$-N=P(Ph)_3$	59.1	–0.33	–0.77
$-NO_2$	44.7	0.71	0.78	–OH	52.1	0.12	–0.37
	55.0	–	–	–OMe	49.8	0.25	0.15
	54.8	–	–	–SH	51.4	0.12	–0.27
	56.0	–	–	–SMe	51.3	0.15	0.00

routes, namely homolytic cleavage that produces two radical species, and both heterolytic pathways varying positive and negative charges on the cleaved fragments. In all cases, the heterolytic route was energetically much more favored and will be considered here (Table 5). Specifically, upon cleavage, the *N*-9-tosyl derivatives turn into a negatively charged purine fragment and the sulfonyl part accommodating an excess positive charge, being in line with the electronegativity of the bonding nitrogen and sulfur atoms in the cleaved N–SO_2 bond.

In purine (X = H), the considered N–SO_2 bond is moderately stable with 52.1 kcal mol^{-1} required for its cleavage. In systems containing C6-halogen atom, the corresponding bond energies are even lower by 2–4 kcal mol^{-1}, thus indicating a reduced stability. For the C6-chloro derivative, particularly relevant for the present study, this assumes ΔG_{bond} = 47.8 kcal mol^{-1}, thus revealing around 3 orders of magnitude lower stability relative to purine. The lowest stability is calculated for the C6-nitro derivative (ΔG_{bond} = 44.7 kcal mol^{-1}) and this substitution pattern is not recommended if the stability of the prepared sulfonylpurines is at stake.

On the other hand, introduced C6-amino substituents significantly improve the stability. Specifically, a very relevant comparison between chloro- and morpholino-analogs indicates 7.2 kcal mol^{-1}, or 5 orders of magnitude, higher stability of the latter system. This notion strongly agrees

with experimental results reported here and with a demonstrated prolonged stability of all C6-morpholino prepared derivatives. Additionally, this goes in line with the fact that C6-amino derivatives should be somewhat even more stable than analogous morpholino systems, also observed here. Lastly, as a useful guidance, the obtained results point to a conclusion that replacing C6-amino group with its dimethylamino analogue would provide an extra 1.5 kcal mol^{-1} of the stability to such system, and will be considered in our future synthetic strategies.

The obtained results reveal a trend where, relative to the unsubstituted purine (X = H), electron-withdrawing substituents at the position C6 lower the stability of the N–SO_2 bond, whereas their electron-donating analogs increase it, which is beneficial for our purpose. Such a notion would be in line with a proposed heterolytic nature of the cleavage process, where the purine fragment accommodates an excess negative charge. In this context, it is not surprising that electron-donating groups disfavor such a cleavage, which results in prolonged stability. To further confirm this conclusion, we have correlated the calculated ΔG_{bond} values with the matching Hammett's substituent constants,[57] σ_m and σ_p, corresponding to the introduced C6-moieties (Supplemental material Fig. S31). The results reveal a high correlativity ($R^2 > 0.9$) among both sets of data, which (i) confirm resonance connection between C6 substituents and the N9 position, (ii) validate the proposed heterolytic cleavage pathway, and (iii) suggest that C6-electron-donating substituents should be retained in future attempts to prepare *N*-9-sulfonylpurines derivatives which will likely assure their stability.

2.3. Biological activity

2.3.1. Antiproliferative activity

Antiproliferative effect of 6-morpholino and 6-amino-9-sulfonylpurine derivatives (6-morpholino **8**-**11**, **14**, **17** and **18**; dimorpholine derivatives **12** and **15**; adenine derivatives **20**-**22**; in parallel with purine bases 6-morpholinopurine **16**, adenine **19**; and 5-fluorouracil (5-FU), as a standard antitumor drug, were tested on normal (BJ) cells and human tumor cells with different histological origin such as carcinoma (HeLa, CaCo-2 and NCI-H358), leukemia (K562, MOLT4) and lymphoma (Raji) cell lines. Obtained results are given in Table 6 and the Supplemental material (Table S1). Significant reduced cellular growth driven by 6-morpholino and 6-amino-9-sulfonylpurine derivatives is apparent against leukemia and lymphoma cells, with decreased influence on carcinoma cells. N9 unsubstituted purine bases 6-morpholino purine **16** and adenine **19** did not show a significant antitumor effect. Within the group of 6-substituted-9-sulfonylpurine

Table 6. Antiproliferative activities of selected 6-substituted-9-sulfonyl-purine derivatives **17**, **22** and **5-FU** on human tumor and normal cells.

Compounds:		17		22		5-FU	
	Cells	IC_{50} (μM)	SI	IC_{50} (μM)	SI	IC_{50} (μM)	SI
Normal	**BJ**	14.3 ± 1.6		15.0 ± 2.6		74.0 ± 3.1	–
Leukemia Lymphoma	**K562**	1.3 ± 0.1	**11**	2.4 ± 0.4	**6.3**	9.8 ± 0.5	**7.6**
	MOLT4	1.7 ± 0.9	**8.4**	1.5 ± 0.8	**10**	76.3 ± 11.4	-
	Raji	2.9 ± 0.4	**4.9**	2.4 ± 1.0	**6.3**	>100	-
Carcinoma	**HeLa**	22.3 ± 2.1	–	34.6 ± 8.8	–	8.2 ± 1.9	**9.0**
	CaCo-2	10.6 ± 1.8	1.4	6.6 ± 3.2	2.8	5.9 ± 0.7	**12.5**
	NCI-H358	3.4 ± 0.2	4.2	3.8 ± 0.7	4	8.0 ± 1.1	**9.3**

The data represents the mean IC_{50} (μmol dm^{-3}) values ± standard deviation (SD) of three independent experiments; IC_{50} represents the concentration of tested compound that inhibited cell proliferation by 50%. Assessment of cell proliferation was analyzed by MTT after incubation of 72 h. SI = IC_{50} for normal cell line, BJ/IC_{50} for cancer cell line. **5-FU**: 5-Flurouracil.

derivatives, the most efficient derivatives were **15**, **17**, and **22** (Supplemental material, Table S1).

However, as described by separate NMR experiments (Scheme 4), it was shown that **15** tend to transform into **17** under the conditions of *in vitro* experiments (Supplemental material, Table S1). Hence, the most effective compound with scaffold based on 6-morpholinopurine was derivative **17** with an IC_{50} 3.4 ± 0.2 μmol dm^{-3} on NCI-H358 cells and IC_{50} below 24 μmol dm^{-3} on HeLa and CaCo-2 cells. The prominent inhibitory concentration of derivative **17** was achieved on leukemia and lymphoma cell lines (K562, MOLT 4 and Raji) at a range of applied concentrations between 1.0 and 1.7 μmol dm^{-3}. As shown in Table 6, derivatives **17** and **22** possessed highly selective activity against leukemia and lymphoma cells with a selectivity index (SI) between 4.9 and 11. Similar molecules with morpholino-purine scaffold showed noticeable antiproliferative effects on tumor cells *in vitro* as well.[58,59] Within *N*-9-sulfonyladenine derivatives, the most effective was derivative **22** with IC_{50} below 2.4 μmol dm^{-3} against leukemia and lymphoma cells and with remarkable selectivity index 10, on acute lymphoblastic leukemia (MOLT4) cells. A weaker antiproliferative effect was observed against carcinoma (CaCo-2 and NCI-H358) cells.

The styryl group bounded to the purine scaffold *via* the sulfonyl group is a structural link between the most potent derivatives **17** and **22**. Since the significant antitumor potential of compounds with styryl sulfonyl group has been already demonstrated by heteroaryl styryl sulfone molecules which induced tumor cell cycle arrest with minimal or no consequences on normal cells[60] and while derivatives **17** and **22** showed a remarkable effect on leukemia and lymphoma cells, these derivatives were selected for further testing on biological effects.

When the cell membrane is compromised lactate dehydrogenase (LDH) is released into extracellular space and the presence of this enzyme in the culture medium can be used as a cell death marker. In order to detect the

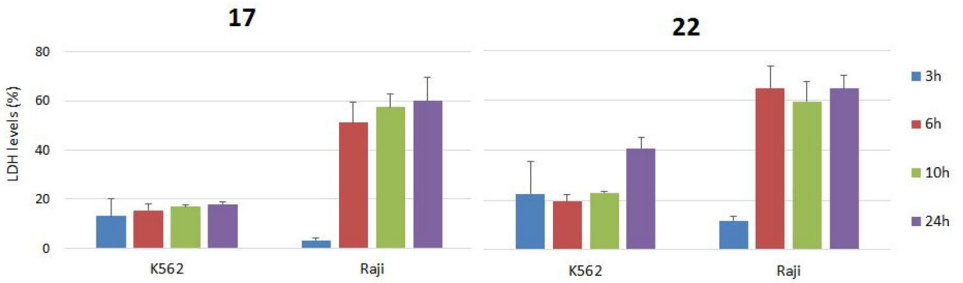


Figure 1. Cytotoxicity of derivatives **17** and **22** on K562 and Raji cells.
Levels of released LDH in the medium were determined after 3, 6, 10, and 24 hours of treatment with 5 μmol dm^{-3} of selected derivatives. The results represent the mean percentage of value of three independent experiments ± SD.

ability of derivatives **17** and **22** to induce disruption of cell membrane integrity of treated K562 and Raji cells, LDH assay was performed after 3, 6, 10 and 24 h of treatment. The results are shown in Fig. 1. Compared to non-treated cells, a notable increased LDH leak of LDH (40.7 ± 4.2%), as a sign of the loss of membrane integrity, was observed in K562 cells only after 24 h of treatment with 5 μM of derivative **22**. In comparison to K562 cells, Raji cells were significantly vulnerable to derivatives **17** and **22** and showed loss of membrane integrity already after 6 h of treatment with 51.2 ± 8.1% and 64.6 ± 8.8%, respectively.

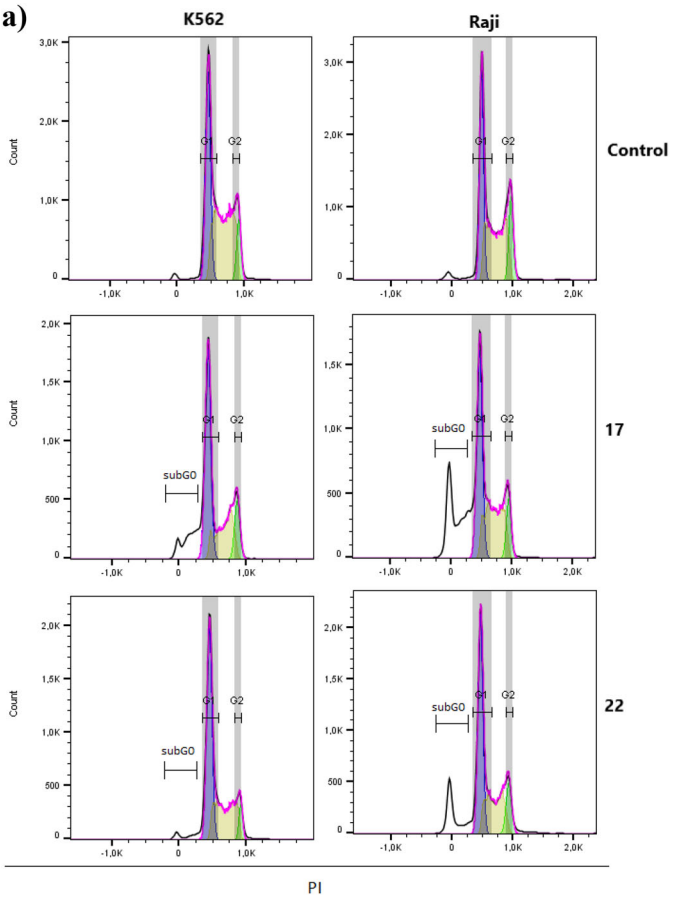
2.3.2. Cell cycle analysis

To clarify the mechanism of treated cells' growth inhibition we tested changes in cell cycle progression of K562 and Raji cells after 24 h of treatment with derivatives **17** and **22**. As is shown in Table 7 both tested derivatives induced re-distribution in the cell cycle of the treated cells compared to control untreated cells. Aggregation of cells in the subG0 phase with noticeable cell reduction in S and G2/M cell cycle phases was observed. The derivative **17** induced significant reduction of K562 cells (26.6%, $p < 0.001$) in S phase and accumulation of cells in G0/G1 (41.6 ± 3.4%) and G2/M (11.9 ± 0.1%, $p < 0.037$) phases of the cell cycle. Almost 19% of the K562 cells were accumulated in the subG0 phase. After treatment with derivative **17** Raji cells showed reduced percentage of cells in S (30.3 ± 2.3%, $p < 0.051$) and G2/M (7.7 ± 0.4%, $p < 0.025$) phases of the cell cycle, while more than 28% was accumulated in the subG0 phase.

Derivative **22** induced statistically significant accumulation cells in the G0/G1 phase of 48.4% ($p < 0.026$) for K562 and 40.0% ($p < 0.009$) for Raji cells. Decreased percentage of Raji and K562 cells in S (> 10%) and G2/M phases of the cell cycle were observed as well. Similar to the K562 cells treated by derivative 17, almost 19% of the Raji cells were found to be accumulated in the subG0 phase. Cells that are dominantly accumulated in

Table 7. Cell cycle analysis: a) distribution histograms b) respective values for each phase.

a)



(b)

		subG0[#]	G0/G1	S	G2/M
K562	control	6.3 ± 0.7	32.1 ± 4.4	52.0 ± 2.2	7.8 ± 1.6
	17	18.9 ± 0.6	41.6 ± 3.4	26.6 ± 0.6*	11.9 ± 0.1*
	22	5.8 ± 0.4	48.4 ± 1.3*	36.0 ± 0.0*	6.2 ± 0.4
Raji	control	6.0 ± 1.1	32.1 ± 1.3	43.0 ± 2.7	16.6 ± 2.3
	17	28.7 ± 0.9	32.0 ± 1.3	30.3 ± 2.3	7.7 ± 0.4*
	22	18.6 ± 0.7	40.0 ± 0.2*	31.4 ± 4.5	9.3 ± 1.9*

Results are shown as: **a)** distribution histograms **b)** respective values for each phase. K562 and Raji cells were treated during 24 h with 1 μM of tested derivatives. Cell cycle data were analyzed using Watson (Pragmatic) model in FloJo software. [#]subG0 - calculated by manually gating. Statistical significant p value is defined as $p < 0.05$ (Dunnett - two sided analysis).

the subG0 phase of the cell cycle have fragmented DNA and represent cells that have entered the apoptosis.[61] Recently published studies on pyrimidine and purine derivatives have shown that they can regulate the cell cycle by inhibition of cyclin-dependent kinases (CDKs).[62,63] Mojzych *et al.*[62] characterized 8-azopurine and pyrazole[4,3-*d*]pyrimidines as CDK inhibitors. Furthermore, it has been shown that the purine derivative CYC202 inhibits CDK with the promotion of apoptosis in lung, prostate, breast, and

colon carcinoma as well as in multiple myeloma.[63] Since derivatives **17** and **22** induced significant changes in the cell cycle of the treated cells, further research is aimed at the elucidation of their mechanism of action.

3. Conclusions

This paper describes efficient synthetic methods for the preparation of new 6-morpholino/amino-9-sulfonylpurine derivatives with enhanced anticancer activity. The synthesis of the 6-substituted-9-sulfonylpurine derivative was carried out in two different ways. The first involves the condensation of 6-chloropurine with different sulfonyl chlorides in the presence of aqueous KOH in acetone at 0 °C, resulting in 6-chloro-9-sulfonylpurines **2**-**7** in good 50-77% yields. The reactions were regioselective, and the products were sufficiently stable to be transformed into 6-morpholino-9-sulfonylpurines **8**-**11** in good yields 61-93%. The second method was used in the case of nonselective sulfonylation due to the character of the sulfonyl substituent, or due to the instability of the 6-chloro-9-sulfonyl derivatives. In those cases 6-morpholinopurine **16** and commercially available adenine **19** bases were reacted with alkyl, 2-arylethene and arylsulfonyl chlorides in the presence of aqueous KOH in acetone at 0 °C, giving the 6-morpholino **14**, **17**, **18**, and 6-amino **20**-**22**, 9-sulfonylated products.

Computational analysis convincingly demonstrated that C6 substituted systems containing electron-donating groups provide much better starting materials for the synthesis as, unlike electron-withdrawing group, these substituents promote the predominance of the N9 tautomers and help assuring the stability of the final products, which should be kept in mind in future synthetic strategies.

The obtained results of the antiproliferative effect show that the influence of newly synthesized 6-morpholino and 6-amino-9-sulfonylpurine derivatives depends on the cell type, the chemical structure, as well as the concentration applied. Derivatives **17** and **22**, which have a styryl group bounded to the purine scaffold showed a better cytotoxic potential and selectivity between normal and tumor cells compared to other investigated newly synthesized compounds. Both derivatives influenced the cell cycle of treated tumor cells so the accumulation in the S phase is reduced with noticeable aggregation in the subG0 phase. Observed points to apoptosis as a mechanism of treated tumor cell death. Further studies are necessary to determine their mechanism of antitumor action in more details.

4. Experimental

General Information: Solvents were distilled from appropriate drying agents shortly before use. TLC was carried out on DC-plastikfolien

Kieselgel 60 F254, and flash column chromatography was performed on silica gel Merck 0.040–0.063 mm, and preparative thick layer (2 mm) chromatography was done on Merck 60 F254 plates (Merck KGaA, Darmstadt, Germany). Melting points were determined on a Kofler hot-stage apparatus and were uncorrected. UV spectra were taken on a Philips PU8700 UV/VIS spectrophotometer (Philips Analytical, Cambridge, Great Britain). IR spectra were obtained in KBr pellets on a Perkin-Elmer 297 spectrophotometer (Perkin-Elmer, Waltham, MA). NMR spectra were recorded on AV600 and AV300 MHz spectrometers (Bruker BioSpin GmbH, Rheinstetten, Germany), operating at 150.92 or 75.47 MHz for ^{13}C and 600.13 or 300.13 MHz for ^{1}H nuclei using DMSO-d_6 as the internal standard. High-resolution mass spectra (HRMS) in the positive mode were obtained with a Micromass QTof2 hybrid quadrupole time-of-flight mass spectrometer (Micromass, Cary, NC, USA).

General procedure 1. *Synthesis of 6-chloro-N-9-sulfonylpurine derivatives (**2-7**), 6-morpholino-N-9-sulfonylpurine derivatives (**14, 17, 18**), and N-9-sulfonyladenine derivatives (**20-22**)* Aqueous potassium hydroxide ($c = 0.4$ mol dm^{-3}, 5 mL) was added to a suspension of 6-substituted purine (1 mmol; 6-chloropurine **1**, 6-morpholinopurine **16**, or adenine **19**) in acetone (10 mL). The mixture was stirred at room temperature for 20 min and then cooled to 0 °C. Appropriate sulfonyl chloride (1 mmol) was added, and the reaction mixture was stirred at 0 °C for 3 h. The mixture was neutralized with hydrochloric acid ($c = 1$ mol dm^{-3}). The raw product was filtered off, washed with water, and purified by recrystallization from the mixture of hot acetone and methanol.

General procedure 2. *Synthesis of 6-morpholino-N-9-sulfony-lpurine derivatives (**8-11**) and dimorpfolino derivatives (**12** and **15**).* A solution of appropriate 6-chloro-*N*-9-sulfonylpurine (1 mmol) in tetrahydrofuran (30 mL) was cooled to 0 °C, and morpholine (4 mmol) was added. The reaction mixture was stirred at 0 °C for 3 h. Addition of methanol caused the precipitation of the morpholino derivative. The product was filtered off, washed with methanol and then purified by recrystallization from the mixture of hot acetone and methanol.

6-Chloro-9-(4-methylphenylsulfonyl)-9H-purine (2). 6-Chloropurine **1** (250 mg, 1.6 mmol) and tosyl chloride (305 mg, 1.6 mmol) were used according to general procedure 1. The product **2** was isolated as a white crystalline solid (317 mg, 64%). M.p. = 174–175 °C; R_f = 0.8 (CH_2Cl_2:EtOAc/9:1); UV (MeOH) λ_{max}/nm: 237; log ε/dm^3 mol^{-1} cm^{-1}: 4.52; IR (KBr) ν_{max}/cm^{-1}: 3113 (w), 1587 (m), 1553 (m), 1433 (m), 1418 (w), 1387 (s), 1364 (m), 1333 (w), 1190 (s), 1178 (s), 1167 (s), 1140 (m), 1090 (s), 1070 (s); (m) ^{1}H NMR (DMSO-d_6) δ/ppm: 9.12 (s, 1H, H8), 8.90 (s, 1H, H2), 8.13 (d, $J = 8.5$ Hz, 2H, Ar), 7.51 (d, $J = 8.1$ Hz, 2H, Ar), 2.38

(s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ/ppm: 153.3 (C2), 150.4 (C4 or C6), 150.1 (C4 or C6), 147.3 (C_q), 144.3 (CH, C8), 132.8 (C_q), 131.6 (C5), 130.4 (CH-Ar), 128.3 (CH-Ar), 21.1 (CH_3); (Supplemental material Figs. S2–S5). HRMS: *m/z*: 360.1118 $[M+H]^+$; calculated $C_{16}H_{17}N_5O_3S$ H^+: 360.1125.

6-Chloro-9-(naphtalen-2-ylsulfonyl)-9H-purine (3). 6-Chloropurine **1** (250 mg, 1.6 mmol) and 2-naphtalenesulfonyl chloride (366 mg, 1.6 mmol, 99%) were used according to general procedure 1. The product **3** was isolated as a white crystalline solid (378 mg, 68%). M.p. = 217–219 °C; R_f = 0.8 (CH_2Cl_2:EtOAc/9:1); UV (MeOH) λ_{max}/nm: 236, 265, 280 and 332; log ε/dm^3 mol^{-1} cm^{-1}: 4.70, 3.97, 3.76 and 3.19; IR (KBr) ν_{max}/cm^{-1}: 3111 (m), 3059 (w), 2922 (w), 2854 (w), 1626 (w), 1589 (m), 1560 (s), 1504 (w), 1475 (w), 1435 (m), 1389 (m), 1354 (m), 1236 (w), 1178 (s), 1163 (m), 1132 (m), 1074 (s); ^1H NMR (DMSO-d_6) δ/ppm: 9.22 (s, 1H, H8), 9.02 (d, J = 0.9 Hz, 1H, Ar), 8.89 (s, 1H, H2), 8.29 (d, J = 8.2 Hz, 1H, Ar), 8.22 (d, J = 8.8 Hz, 1H, Ar), 8.16 (dd, J = 8.8 Hz, J = 1.8 Hz, 1H, Ar), 8.07 (d, J = 8.2 Hz, 1H, Ar), 7.80 (t, J = 7.4 Hz, 1H, Ar), 7.74 (t, J = 7.5 Hz, 1H, Ar); ^{13}C NMR (DMSO-d_6) δ/ppm: 153.3 (C2), 150.4 (C4 or C6), 150.2 (C4 or C6), 144.4 (C8), 135.5 (C_q), 132.6 (C_q), 131.7 (C5), 131.3 (C_q), 131.0 (CH-Ar), 130.6 (CH-Ar), 130.2 (CH-Ar), 129.9 (CH-Ar), 128.3 (CH-Ar), 128.0 (CH-Ar), 121.9 (CH-Ar); (Supplemental material Figs. S7–S8). HRMS: *m/z*: 309.0206 $[M+H]^+$; calculated $C_{12}H_9ClN_4O_2S$ H^+: 309.0207.

9-[(1,1′-Biphenyl)-6-chloro-4-sulfonyl]-9H-purine (4). 6-Chloropurine **1** (250 mg, 1.6 mmol) and biphenyl-4-sulfonyl chloride (405 mg, 1.6 mmol, 95%) were used according to general procedure 1. The product **4** was isolated as a white crystalline solid (457 mg, 77%). M.p. = 196–197 °C; R_f = 0.9 (CH_2Cl_2:EtOAc/9:1); UV (MeOH) λ_{max}/nm: 270; log ε/dm^3 mol^{-1} cm^{-1}: 4.48; IR (KBr) ν_{max}/cm^{-1}: 3117 (w), 1589 (s), 1556 (s), 1481 (w), 1474 (w), 1439 (s), 1391 (s), 1366 (m), 1339 (m), 1232 (w), 1211 (w), 1175 (s), 1144 (m), 1088 (s), 1070 (s), 1005 (w); ^1H NMR (DMSO-d_6) δ/ppm: 9.19 (s, 1H, H8), 8.94 (s, 1H, H2), 8.39–8.27 (m, 2H, Ar), 8.09–7.93 (m, 2H, Ar), 7.74 (dd, J = 8.1 and 1,5 Hz, 2H, Ar), 7.58–7.41 (m, 3H, Ar); ^{13}C NMR (DMSO-d_6) δ/ppm: 153.5 (C2), 150.5 (C4 or C6), 150.2 (C4 or C6), 147.4 (C_q), 144.5 (C8), 137.7 (C_q), 134.3 (C_q), 131.8 (C5), 129.3 (br, 2xCH-Ar), 129.1 (CH-Ar), 128.2 (CH-Ar), 127.4 (CH-Ar); (Supplemental material Fig. S9). HRMS: *m/z*: 371.0359 $[M+H]^+$; calculated $C_{17}H_{11}ClN_4O_2S$ H^+: 371.0364.

9-(4-Bromophenylsulfonyl)-6-chloro-9H-purine (5). 6-Chloropurine **1** (250 mg, 1.6 mmol) and 4-bromobenzenesulfonyl chloride (411 mg, 1.6 mmol, 98%) were used according to general procedure 1. The product **5** was isolated as a pale yellow crystalline solid (457 mg, 77%). M.p. = 193–195 °C; R_f = 0.9 (CH_2Cl_2: EtOAc/9:1); UV (MeOH) λ_{max}/nm: 242 and 315, log ε/dm^3 mol^{-1} cm^{-1}: 4.23 and 3.56; IR (KBr) ν_{max}/cm^{-1}: 3125 (w), 3074 (w), 3009 (w), 1589 (m), 1572 (s), 1556 (s), 1477 (w), 1435 (m), 1394

(s), 1356 (m), 1192 (m), 1178 (m), 1163 (s), 1144 (m), 1088 (s), 1070 (s), 1007 (w); ^{1}H NMR (DMSO-d_6) δ/ppm: 9.14 (s, 1H, H8), 8.91 (s, 1H, H2), 8.17 (d, $J = 8.8$ Hz, 2H, Ar), 7.95 (d, $J = 8.9$ Hz, 2H, Ar); ^{13}C NMR (DMSO-d_6) δ/ppm: 153.5 (C2), 150.5 (C4 or C6), 150.3 (C4 or C6), 144.4 (C8), 134.9 (C_q), 133.2 (CH-Ar), 131.8 (C5), 130.7 (C_q), 130.4 (CH-Ar); (Supplemental material Fig. S10). HRMS: *m/z*: 372.9158 $[M+H]^+$; calculated $C_{13}H_9ClN_4O_2S$ H^+: 372.9156.

6-Chloro-9-(4-chloro-3-nitrophenylsulfonyl)-9H-purine (6). 6-Chloropurine **1** (250 mg, 1.6 mmol) and 4-chloro-3-nitrobenzenesulfonyl chloride (422 mg, 1.6 mmol, 98%) were used according to general procedure 1. The product **6** was isolated as a white crystalline solid (399 mg, 66%). M.p. = 204–206 °C; R_f = 0.8 (CH_2Cl_2:EtOAc/9:1); UV (MeOH) λ_{max}/nm: 225, log ε/dm^3 mol^{-1} cm^{-1}: 4.13; IR (KBr) ν_{max}/cm^{-1}: 3132 (m), 3078 (w), 2920 (w), 1589 (s), 1560 (s), 1549 (s), 1479 (w), 1443 (m), 1400 (s), 1362 (s), 1339 (w), 1188 (s), 1161 (m), 1136 (m), 1097 (m), 1074 (s), 1049 (m); ^{1}H NMR (DMSO-d_6) δ/ppm: 9.13 (s, 1H, H8), 8.93 (s, 1H, H2), 8.89 (d, $J = 2.2$ Hz, 1H, Ar), 8.51 (dd, $J = 8.7$ and 2.2 Hz, 1H, Ar), 8.14 (d, $J = 8.7$ Hz, 1H, Ar); ^{13}C NMR (DMSO-d_6) δ/ppm: 153.5 (C2), 150.5 (C4 or C6), 150.3 (C4 or C6), 147.6 (C_q), 144.4 (C8), 135.4 (C_q), 133.7 (CH-Ar), 133.2 (CH-Ar), 133.2 (C_q), 131.9 (C5), 126.3 (CH-Ar); (Supplemental material Figs. S11–S12). HRMS: *m/z*: 373.9530 $[M+H]^+$; calculated $C_{13}H_9ClN_4O_2S$ H^+: 373.9512.

(E)-6-Chloro-9-(styrylsulfonyl)-9H-purine (7). 6-Chloropurine **1** (249 mg, 1.6 mmol) and *trans*-β-stirenesulfonyl chloride (338 mg, 1.6 mmol, 97%) were used according to general procedure 1. The product **7** was isolated as a white crystalline solid (373 mg, 73%). M.p. = 173–174 °C; R_f = 0.8 (CH_2Cl_2:EtOAc/9:1); UV (MeOH): λ_{max}/nm: 272; log ε/dm^3 mol^{-1} cm^{-1}: 4.93; IR (KBr) ν_{max}/cm^{-1}: 3119 (vw), 1610 (m), 1591 (m), 1562 (s), 1472 (w), 1445 (m), 1389 (s), 1339 (m), 1236 (w), 1204 (w), 1171 (s), 1157 (s), 1140 (s), 1072 (s); ^{1}H NMR (DMSO-d_6) δ/ppm: 9.02 (s, 1H, H8), 8.92 (s, 1H, H2), 8.13 (d, $J = 15.3$ Hz, 1H, CH), 7.81 (d, $J = 15.3$ Hz, 1H, CH, overlap with Ar), 7.84–7.73 (m, 2H, Ar, overlap with CH), 7.57–7.42 (m, 3H, Ar); ^{13}C NMR (DMSO-d_6) δ/ppm: 153.3 (C2), 150.4 (C4 or C6), 150.2 (C4 or C6), 148.3 (CH), 144.6 (C8), 132.4 (CH-Ar or CH), 131.7 (C5), 131.4 (C_q), 129.7 (CH-Ar), 129.1 (CH-Ar), 122.7 (CH-Ar or CH); (Supplemental material Fig. S13). HRMS: *m/z*: 321.0215 $[M+H]^+$; calculated $C_{13}H_9ClN_4O_2S$ H^+: 321.0208.

6-Morpholino-9-(4-methylphenylsulfonyl)-9H-purine (8). 6-Chloro-9-(4-methylphenylsulfonyl)-9*H*-purine (**2**) (100 mg, 0.3 mmol) and morpholine (105 µL, 1.2 mmol, 99%) were used according to general procedure 2. The product **8** was obtained as a white solid (70 mg, 61%). M.p. = 178–180 °C; R_f = 0.3 (CH_2Cl_2:EtOAc/9:1); UV (MeOH): λ_{max}/nm 235 and 272 log

ε/dm^3 mol^{-1} cm^{-1} = 4.80 and 4.88; IR (KBr) ν_{max}/cm^{-1}: 3106 (w), 2959 (w), 2860 (w), 1587 (s), 1560 (m), 1474 (m), 1450 (m), 1383 (m), 1331 (w), 1292 (m), 1184 (m), 1171 (s), 1150 (s), 1115 (s), 1092 (m); ^{1}H NMR (DMSO-d_6) δ/ppm: 8.66 (s, 1H, H8), 8.32 (s, 1H, H2), 8.08 (d, J = 8.4 Hz, 2H, Ar) 7.49 (d, J = 8.1 Hz, 2H, Ar), 4.14 (brs, 4H, CH_2), 3.68 (t, J = 4.8 Hz, 4H, CH_2), 2.38 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ/ppm: 153.5 (C2), 153.2 (C4 or C6), 149.4 (C4 or C6), 146.7 (C_q), 137.2 (C8), 133.3 (C_q), 130.3 (CH-Ar), 128.1 (CH-Ar), 119.1 (C5), 66.0 (CH_2), 45.3 (CH_2), 21.1 (CH_3); (Supplemental material Figs. S14–S15). HRMS: *m/z*: 360.1118 $[M+H]^+$; calculated $C_{16}H_{17}N_5O_3S$ H^+: 360.1125.

6-Morpholino-9-(naphtalen-2-ylsulfonyl)-9H-purine (9). 6-Chloro-9-(naphtalen-2-ylsulfonyl)-9*H*-purine (**3**) (48 mg, 0.1 mmol) and morpholine (35 µL, 0.4 mmol, 99%) were used according to general procedure 2. The product **9** was obtained as a white solid (34 mg, 61%). M.p. = 198–200 °C; R_f = 0.4 (CH_2Cl_2:EtOAc/9:1); UV (MeOH): λ_{max}/nm 238 and 276, log ε/dm^3 mol^{-1} cm^{-1} 4.71 and 4.37; IR (KBr) ν_{max}/cm^{-1}: 3109 (w), 1596 (s), 1560 (w), 1471 (w), 1447 (w), 1384 (m), 1333 (w), 1286 (w), 1252 (m), 1205 (w), 1190 (w), 1151 (s), 1115 (m), 1072 (w); ^{1}H NMR (DMSO-d_6) δ/ppm: 8.97 (s, 1H, H8), 8.74 (s, 1H, H2), 8.33–8.15 (m, 3H, Ar), 8.16–8.01 (m, 2H, Ar), 7.85–7.68 (m, 2H,Ar), 4.13 (brs, 4H, CH_2), 3.67 (t, J = 4.7 Hz, 4H, CH_2); ^{13}C NMR (DMSO-d_6) δ/ppm: 153.5 (C2), 153.2 (C4 or C6), 149.5 (C4 or C6), 137.2 (C8), 135.3 (C_q), 133.1 (C_q), 131.3 (C_q), 130.6 (CH-Ar), 130.4 (CH-Ar), 130.1 (CH-Ar), 129.8 (CH-Ar), 128.2 (CH-Ar), 128.0 (CH-Ar), 121.9 (CH-Ar), 119.1 (C5), 65.9 (CH_2); (Supplemental material Fig. S16). HRMS: *m/z*: 396.1115 $[M+H]^+$; calculated $C_{19}H_{17}N_5O_3SH^+$: 396.1125.

9-[(1,1′-Biphenyl)-6-morpholino-4-sulfonyl]-9H-purine (10). 9-[(1,1′-Biphenyl)-4-sulfonyl]-6-chloro-9*H*-purine (**4**) (32 mg, 0.1 mmol) and morpholine (32 µL, 0.4 mmol, 99%) were used according to general procedure 2. The product **10** was obtained as a white solid (24 mg, 63%). M.p. = 188–190 °C; R_f = 0.4 (CH_2Cl_2:EtOAc/9:1); UV (MeOH): λ_{max}/nm: 274, log ε/dm^3 mol^{-1} cm^{-1} 4.64; IR (KBr) ν_{max}/cm^{-1}: 3101 (w), 1589 (s), 1570 (m), 1479 (w), 1393 (w), 1286 (w), 1250 (w), 1169 (m), 11519 (s), 1113 (m); ^{1}H NMR (DMSO-d_6) δ/ppm: 8.71 (s, 1H, H8), 8.35 (s, 1H, H2), 8.28 (d, J = 8.7 Hz, 2H, Ar), 7.99 (d, J = 8.7 Hz, 2H, Ar), 7.77–7.70 (m, 2H, Ar), 7.55–7.42 (m, 3H, Ar), 4.15 (brs, 4H, CH_2), 3.69 (t, J = 4.8 Hz, 4H, CH_2); ^{13}C NMR (DMSO-d_6) δ/ppm: 153.6 (C2), 153.2 (C4 or C6), 149.4 (C4 or C6), 146.9 (C_q), 137.7 (C_q), 137.2 (C8), 134.8 (C_q), 129.1 (CH-Ar), 129.1 (CH-Ar), 128.8 (CH-Ar 127.9 (CH-Ar), 127.2 (CH-Ar), 119.1 (C5), 65.9 (CH_2); (Supplemental material Fig. S17). HRMS: *m/z*: 422.1281 $[M+H]^+$; calculated $C_{21}H_{19}N_5O_3SH^+$: 422.1281.

9-(4-Bromphenylsulfonyl)-6-Morpholino-9H-purine (11). 9-(4-Bromphenylsulfonyl)-6-chloro-9*H*-purine (**5**) (100 mg, 0.3 mmol) and

morpholine (105 µL, 1.2 mmol, 99%) were used according to general procedure 2. The product **11** was obtained as a white solid (105 mg, 93%). M.p. = 180–183 °C; R_f = 0.5 (CH_2Cl_2:EtOAc/9:1); UV (MeOH): λ_{max}/nm: 246 and 272, log ε/dm^3 mol^{-1} cm^{-1}: 4.42 and 4.43; IR (KBr) ν_{max}/cm^{-1}: 3105 (w), 3001 (w), 2854 (w), 1589 (s), 1508 (w), 1477 (s), 1450 (s), 1394 (s), 1371 (m), 1327 (m), 1308 (w), 1285 (m), 1248 (s), 1192 (s), 1177 (s), 1161 (s), 1148 (s), 1117 (s), 1088 (m), 1069 (s), 1053 (m), 1009 (m); ^{1}H NMR (DMSO-d_6) δ/ppm: 8.67 (s, 1H, H8), 8.32 (s, 1H, H2), 8.13 (d, J = 8.7 Hz, 2H, Ar), 7.93 (d, J = 8.7 Hz, 2H, Ar), 4.14 (bs, 4H, CH_2), 3.69 (t, J = 8.7 Hz, 4H, CH_2); ^{13}C NMR (DMSO-d_6) δ/ppm: 153.6 (C2), 153.2 (C4 or C6), 149.4 (C4 or C6), 137.1 (C8), 135.4 (C_q), 133.0 (CH-Ar), 130.1 (CH-Ar), 119.0 (C5), 65.9 (CH_2); (Supplemental material Fig. S18). HRMS: *m/z*: 424.0072 $[M+H]^+$; calculated $C_{15}H_{14}BrN_5O_3SH^+$: 424.0073.

6-Morpholino-9-(4-morpholino-3-nitrophenylsulfonyl)-9H-purine (12). 6-Chloro-9-(4-chloro-3-nitrophenylsulfonyl)-9*H*-purine (**6**) (100 mg, 0.3 mmol), morpholine (105 µL, 1.2 mmol, 99%) were used according to general procedure 2. The raw mixture was filtered off and purified by preparative chromatography (CH_2Cl_2/CH_3OH 9:1) to give the product **12** as a yellow solid (100 mg, 79%), and **13** and **14** were isolated as traces (1-2%). Product **12**: m.p. = 185–190 °C; R_f = 0.2 (CH_2Cl_2:EtOAc/9:1); UV (MeOH): λ_{max}/nm: 274 and 304, log ε/dm^3 mol^{-1} cm^{-1}: 4.32 and 4.22; IR (KBr) ν_{max}/cm^{-1}: 3105 (w), 3001 (w), 2854 (w), 1593 (s), 1518 (m), 1477 (w), 1450 (w), 1389 (s), 1348 (w), 1281 (w), 1250 (m), 1180 (s), 1155 (s), 1115 (s), 1043 (w); ^{1}H NMR (DMSO-d_6) δ/ppm: 8.63 (s, 1H, H8), 8.58 (d, J = 2.4 Hz, 1H, Ar), 8.34 (s, 1H, H2), 8.20 (dd, J = 2.4 and 9.2 Hz, 1H, Ar), 7.47 (d, J = 9.2 Hz, 1H, Ar), 4.15 (bs, 4H, CH_2), 3.68 (m, 8H, CH_2), 3.23 (m, 4H, CH_2); ^{13}C NMR (DMSO-d_6) δ/ppm: 153.5 (C2), 153.2 (C4 or C6), 149.3 (C4 or C6), 148.8 (C_q), 137.1 (C8), 137.1 (C_q), 132.5 (CH-Ar), 128.5 (CH-Ar), 123.5 (C_q), 120.6 (CH-Ar), 119.1 (C5), 66.0 (CH_2), 65.5 (CH_2), 50.0 (CH_2); (Supplemental material Fig. S19). HRMS: *m/z*: 476.1349 $[M+H]^+$; calculated $C_{19}H_{21}N_7O_6SH^+$: 476.1347.

6-Chloro-9-(4-morpholino-3-nitrophenylsulfonyl)-9H-purine (13). Compound **13** was isolated as a by-product (1-2%) in the reaction of morpholine and 6-chloro-9-(4-chloro-3-nitrophenylsulfonyl)-9*H*-purine (**6**) according to general procedure 2. Yellow solid, m.p. = 145–147 °C; R_f = 0.4 (CH_2Cl_2: EtOAc/9:1); UV (MeOH): λ_{max}/nm: 257, log ε/dm^{3}mol^{-1}cm^{-1}: 4.19; IR (KBr) λ_{max}/cm^{-1}: 3443 (s), 1605 (m), 1385 (m), 1350 (w), 1263 (w), 1236 (w), 1169 (m), 1115 (s); ^{1}H NMR (DMSO-d_6) δ/ppm: 9.09 (s, 1H, H8), 8.92 (s, 1H, H2), 8.59 (d, J = 2.3 Hz, 1H, Ar), 8.22 (dd, J = 9.2 and 2.3 Hz, 1H, Ar), 7.49 (d, J = 9.2 Hz, 1H, Ar), 3.74–3.57 (m, 4H, CH_2), 3.25–3.15 (m, 4H, CH_2); (Supplemental material Fig. S20). ^{13}C NMR spectra are not measured because of the instability of the compound in DMSO solution.

9-(4-Chloro-3-nitrophenylsulfonyl)-6-morpholino-9H-purine (14). Compound **14** was isolated as a by-product (1-2%) in the reaction of morpholine and 6-chloro-9-(4-chloro-3-nitrophenylsulfonyl)-9*H*-purine (**6**) according to general procedure 2. However in the condensation reaction of 6-morpholinopurine **16** (80 mg, 0.4 mmol) with 4-chloro-3-nitrobenzenesulfonyl chloride (102 mg, 0.4 mmol, 98%) according to general procedure 1, compound **14** was obtained as a white solid (96 mg, 68%). M.p. = 145–150 °C; R_f = 0.7 (CH_2Cl_2:EtOAc/9:1); UV (MeOH): λ_{max}/nm: 221 and 272, log ε/dm^3 mol^{-1} cm^{-1}: 4.39 and 4.30; IR (KBr) λ_{max}/cm^{-1}: 3048 (w), 1595 (s), 1541 (w), 1477 (w), 1389 (m), 1356 (w), 1296 (w), 1248 (w), 1191 (s), 1163 (s), 1113 (m), 1049 (w); ^{1}H NMR (DMSO-d_6) δ/ppm: 8.89 (d, J = 2.1 Hz, 1H, Ar), 8.68 (s, 1H, H8), 8.50 (dd, J = 2.2 and 8.6 Hz, 1H, Ar), 8.34 (s, 1H, H2), 8.13 (d, J = 2.1 Hz, 1H, Ar), 4.15 (bs, 4H, CH_2), 3.73–3.65 (m, 4H, CH_2); ^{13}C NMR (DMSO-d_6) δ/ppm: 153.6 (C2), 153.2 (C4 or C6), 149.4 (C4 or C6), 147.5 (C_q), 137.1 (C8), 135.8 (C_q), 133.6 (CH-Ar), 133.0 (CH-Ar), 132.8 (C_q), 126.0 (CH-Ar), 119.0 (C5), 66.0 (CH_2); (Supplemental material Fig. S21). HRMS: *m/z*: 425.0424 $[M+H]^+$; calculated $C_{15}H_{13}ClN_6O_5SH^+$: 425.0429.

6-Morpholino-9-(2-morpholino-2-phenylethylsulfonyl)-9H-purine (15). (*E*)-6-Chloro-9-(styrylsulfonyl)-9*H*-purine (**7**) (26 mg, 0.1 mmol) and morpholine (35 µL, 0.4 mmol, 99%) were used according to general procedure 2. Dimorpholine derivative **15** was obtained as a white solid (27 mg, 73%). M.p. = 169–171 °C; R_f = 0.1 (CH_2Cl_2:EtOAc/9:1); UV (MeOH): λ_{max}/nm 273, log ε/dm^3 mol^{-1} cm^{-1} 4.17; IR (KBr) ν_{max}/cm^{-1}: 3126 (w), 2867 (w), 1590 (s), 1566 (m), 1480 (w), 1450 (w), 1376 (m), 1329 (w), 1286 (w), 1250 (w), 1240 (w), 1158 (s), 1130 (w), 1113 (s), 1048 (w); ^{1}H NMR (DMSO-d_6) δ/ppm: 8.43 (s, 1H, H8), 8.39 (s, 1H, H2), 7.32–7.22 (m, 3H, Ar), 7.20–7.10 (m, 2H, Ar), 4.78 (dd, J = 15.0, 8.9 Hz, CH), 4.41–4.06 (m, 6H, CH_2), 3.81–3.61 (m, 4H, CH_2), 3.24–3.11 (m, 2H, CH_2), 3.05–2.89 (m, 2H, CH_2), 2.22–2.06 (m, 2H, CH_2), 2.06–1.88 (m, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ/ppm: 153.3 (C2), 153.2 (C4 or C6), 150.0 (C4 or C6), 137.3 (C8), 133.0 (C_q), 128.7 (CH-Ar), 127.9 (CH-Ar), 127.8 (CH-Ar), 119.2 (C5), 66.1 (CH_2), 65.6 (CH_2), 63.6 (CH), 54.3 (CH_2), 48.6 (CH_2); (Supplemental material Fig. S22). HRMS: *m/z*: 459.1814 $[M+H]^+$; calculated $C_{21}H_{26}N_6O_4SH^+$: 459.1809.

6-Morpholino-9H-purine (16). 6-Morpholino-9*H*-purine **16** was prepared according to known procedure (183 mg, 69%).[53] ^{1}H NMR (DMSO-d_6) δ/ppm: 13.06 (s, 1H, NH), 8.23 (s, 1H, H8 or H2), 8.13 (s, 1H, H8 or H2), 4.20 (s, 4H, CH_2), 3.82–3.59 (m, 4H, CH_2); ^{13}C NMR (DMSO-d_6) δ/ppm: 153.19 (s, C4 or C6), 151.76 (d, C2), 151.51 (s, C4 or C6), 138.35 (d, C8), 118.81 (s, C5), 66.19 (t, CH_2), 45.16 (t, CH_2).

(E)-6-Morpholino-9-(styrylsulfonyl)-9H-purine (17). 6-Morpholinopurine **16** (50 mg, 0.2 mmol) and *trans*-β-styrenesulfonyl

chloride (51 mg, 0.2 mmol, 97%) were used according to general procedure 1. The product **17** was obtained as a colorless crystalline solid (52 mg, 58%). M.p. = 156–157 °C; R_f = 0.3 (CH_2Cl_2:EtOAc/9:1); UV (MeOH): λ_{max}/nm: 279; log ε/dm^3 mol^{-1} cm^{-1}: 4.64; IR (KBr) ν_{max}/cm^{-1}: 3109 (m), 3032 (w), 3015 (w), 1593 (s), 1558 (m), 1474 (m), 1450 (m), 1377 (s), 1337 (w), 1304 (w), 1286 (w), 1275 (w), 1254 (m), 1203 (m), 1188 (m), 1176 (w), 1161 (s), 1151 (m), 1117 (m), 1053 (w), 989 (w); ^{1}H NMR (DMSO-d_6) δ/ppm: 8.56 (s, 1H, H8), 8.34 (s, 1H, H2), 8.06 (d, J = 15.3 Hz, 1H, CH), 7.82–7.76 (m, 3H, CH, Ar,), 7.52 (t, J = 7.3 Hz, 1H, Ar), 7.46 (t, J = 7.4 Hz, 2H, Ar), 4.17 (bs, 4H, CH_2), 3.78–3.60 (m, 4H, CH_2); ^{13}C NMR (DMSO-d_6) δ/ppm: 153.5 (C2), 153.2 (C4 or C6), 149.6 (C4 or C6), 147.8 (CH), 137.2 (C8), 132.3 (CH-Ar or CH), 131.5 (C_q), 129.6 (CH-Ar), 129.2 (CH-Ar), 123.0 (CH-Ar or CH), 119.2 (C5), 66.1 (CH_2); (Supplemental material Fig. S23). HRMS: *m/z*: 372.1137 $[M+H]^+$; calculated $C_{17}H_{17}N_5O_3SH^+$: 372.1130.

9-(Methylsulfonyl)-6-morpholino-9H-purine (18). 6-Morpholino-purine **16** (51 mg, 0.2 mmol) and mesyl chloride (38 µL, 0.2 mmol), were used according to general procedure 1. Purification by thin layer chromatography (CH_2Cl_2:MeOH/9:1) and recrystallization from the hot acetone and methanol yielded **18** as a colorless crystalline solid (30 mg, 42%). M.p. = 186–187 °C; R_f = 0.8 (CH_2Cl_2:MeOH/9:1); UV (MeOH): λ_{max}/nm: 272; log ε/dm^3 mol^{-1} cm^{-1}: 4.18; IR (KBr) λ_{max}/cm^{-1}: 3132 (w), 3032 (w), 3001 (w), 2922 (w), 2870 (w), 1591 (s), 1560 (m), 1506 (w), 1475 (m), 1454 (m), 1366 (s), 1327 (m), 1303 (w), 1286 (w), 1275 (m), 1246 (m), 1209 (w), 1188 (s), 1165 (s), 1149 (s), 1115 (s), 1067 (w), 1055 (w); ^{1}H NMR (DMSO-d_6) δ/ppm: 8.47 (s, 1H, H8), 8.41 (s, 1H, H2), 4.21 (bs, 4H, CH_2), 3.79 (s, 3H, CH_3), 3.76–3.70 (m, 4H, CH_2); ^{13}C NMR (DMSO-d_6) δ/ppm: 153.46 (d, C2), 153.26 (s, C4 or C6), 149.68 (s, C4 or C6), 136.84 (d, C8), 119.17 (s, C5), 66.08 (t, CH_2), 41.71 (q, CH_3); (Supplemental material Fig. S24). HRMS: *m/z*: 284.0822 $[M+H]^+$; calculated $C_{10}H_{13}N_5O_3SH^+$: 284.0817.

6-Amino-9-(methylsulfonyl)-9H-purine (20). **A)** 9-Mesyladenine **20** was prepared following the described procedure[35] in 87% yield as a white solid: R_f = 0.4 (CH_2Cl_2:MeOH/9:1); m.p. 228 °C (Ref[35] m.p. = 228–230 °C); ^{1}H NMR (DMSO-d_6) δ/ppm: 8.40 (s, 1H, H8), 8.29 (s, 1H, H2), 7.67 (brs, 2H, NH_2), 3.77 (s, 3H, CH_3); (Supplemental material Fig. S25).

B) Adenine **19** (1 g, 7.4 mmol) and mesyl chloride (0.574 mL, 7.4 mmol) were used according to general procedure 1. The raw mixture of N9 and N7 isomers was filtered off (see NMR spectrum Supplemental material Fig. S26) and purified by preparative chromatography (CH_2Cl_2/CH_3OH 9:1) to give the product **20** (482 mg, 31%) as a white crystalline solid.

6-Amino-9-(4-methylphenylsulfonyl)-9H-purine (21). Adenine **19** (1 g, 7.4 mmol) and tosyl chloride (1.4 g, 7.4 mmol) were used according to

general procedure 1. The product **21** was isolated as a white crystalline solid (1.4 g, 66%): R_f = 0.6 (CH_2Cl_2:MeOH/9:1); m.p. 206–207 °C (Ref.[35] m.p. = 206–207 °C); ^{1}H NMR (DMSO-d_6) δ/ppm: 8.60 (s, 1H, H8), 8.20 (s, 1H, H2), 8.08 (d, 2H, J = 8.2 Hz, Ar), 7.65 (s, 2H, NH_2), 7.49 (d, 2H, J = 8.2 Hz, Ar), 2.37 (s, 3H, CH_3); (Supplemental material Fig. S27).

(E)-6-Amino-9-(styrylsulfonyl)-9H-purine (22). Adenine **19** (0.654 g, 4.8 mmol) and *trans*-β-styrenesulfonyl chloride (1 g, 4.8 mmol, 97%) were used according to general procedure 1. The product **22** was isolated as a white crystalline solid (1.01 g 70%). M.p. = 186–190 °C; R_f = 0.7 (CH_2Cl_2/MeOH, 9:1); UV (MeOH): λ_{max}/nm: 266; log ε/dm^3 mol^{-1} cm^{-1}: 4.72; IR (KBr) λ_{max}/cm^{-1}: 3290 (w), 3176 (w), 3053 (w), 1680 (m), 1607 (m), 1574 (m), 1498 (w), 1417 (w), 1374 (m), 1273 (m), 1178 (m), 1159 (s), 1141 (s), 1031 (w); ^{1}H NMR (DMSO-d_6) δ/ppm: 8.49 (s, 1H, H8), 8.22 (s, 1H, H2), 8.04 (d, J = 15.4 Hz, 1H, CH), 7.82–7.76 (m, 3H, CH and Ar), 7.63 (s, 2H, NH_2), 7.54-7.74 (m, 3H, Ar); ^{13}C NMR (DMSO-d_6) δ/ppm: 156.3 (C6), 154.3 (C2), 148.5 (C4), 147.3 (CH), 138.0 (C8), 132.2 (CH-Ar or CH), 131.6 (C_q), 129.6 (CH-Ar), 129.2 (CH-Ar), 123.3 (CH-Ar or CH), 118.8 (C-5); (Supplemental material Fig S28). HRMS: *m/z*: 302.0712 $[M+H]^+$; calculated $C_{13}H_{11}N_5O_2SH^+$: 302.0724.

4.1. Computational details

All geometrical parameters were optimized employing the density functional theory (DFT) M06–2X functional together with the 6–31+G(d) basis set, followed by the vibrational analysis using the Gaussian 16 software.[64] Thermal corrections were extracted from the matching uncorrected vibrational frequencies, also used to confirm the obtained structures as true minima by the lack of imaginary frequencies. Solvent effects were considered through the implicit SMD polarizable continuum model with all parameters for pure DMSO (ε = 46.7), giving the (SMD)/M06–2X/6–31+G(d) model employed here. As such, all computational values correspond to differences in Gibbs free energies obtained at a room temperature of 298 K and a normal pressure of 1 atm. The choice of this computational setup was prompted by its recent success in reproducing kinetic and thermodynamic parameters of various organic,[65,66] organometalic,[67,68] and enzymatic reactions.[69,70]

4.2. Biological studies

4.2.1. Cell culture

Biological effects of 6-morpholino and 6-amino-9-sulfonylpurine derivatives (6-morpholino **8**-**11**, **14**, **17** and **18**; dimorpholine derivatives **12** and **15**; adenine derivatives **20**-**22**; 6-morpholinopurine **16**, adenine **19**, and 5-FU,

were investigated on a panel of human tumor cells: cervix adenocarcinoma (HeLa), colon adenocarcinoma (CaCo-2), bronchioalveolar carcinoma (NCI-H358), chronic myeloid leukemia in blasted crisis (K562), acute lymphoblastic leukemia (MOLT-4), and Burkitt lymphoma (Raji). As normal cells human fibroblast (BJ) cell line was used.

Adherent cells were cultured in Dulbecco's modified Eagle medium – DMEM (Gibco, EU) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco, EU), 2 mM glutamine, and 100 U/0.1 mg penicillin/streptomycin. Cells on suspension and NCI-H358 were cultured in RPMI 1640 (Gibco, EU) medium supplemented with 10% FBS (Gibco, EU), 2 mM glutamine, 1 mM sodium pyruvate, 10 mM HEPES. Cells were grown in humidified atmosphere under the conditions of 37 °C/5% of CO_2 gas in the CO_2 incubator (IGO 150 CELLlife^{TM}, JOUAN, Thermo Fisher Scientific, Waltham, MA, USA).

4.3. Antiproliferative effect evaluation by MTT assay[71]

4.3.1. Adherent cells

Adherent cells (HeLa, CaCo-2, NCI-H358, and BJ) were plated in 96-well flat bottom plates (Greiner, Frickenhausen, Austria) at a concentration of 2×10^4 cells/mL. The trypan blue dye exclusion test was used to determine number of viable cells before each experiment. Cells were incubated at 37 °C for 24 h, and on day 1 treated with 6-substituted-9-sulfonylpurine derivatives at a concentration rang of 100 to 0.1 μmol dm^{-3}. Upon completion of the incubation period growth medium was discarded and 5 mg/mL of MTT was added. After 4 h incubation at 37 °C water insoluble MTT-formazan crystals were dissolved in DMSO.

4.3.2. Suspension cells

Suspension cells (K562, MOLT-4, and Raji) were plated in 96-well flat bottom plates (Sarstead, Newton, USA) at a concentration of 1×10^5 cells/mL with addition of concentration range (100 to 0.1 μmol dm^{-3}) of 6-substituted-9-sulfonylpurine derivatives. The trypan blue dye exclusion test was used to determine number of viable cells before each experiment. After expired time of incubation, 5 mg/mL of MTT was added in each well and incubated for 4 h in CO_2 incubator. Formed MTT-formazan crystals were dissolved in 10% SDS with 0.01 mol/L HCl overnight. Elisa micro plate reader (iMark, BIO RAD, Hercules, CA, USA) was used for measurement of absorbance at 595 nm. Experiment was performed in triplicate.

Statistical analyses were carried out with *Statistica for Windows v. 13.1* software. Independent samples were analyzed by nonparametric Kolmogorov-Smirnov test. Data is presented as a mean values ± SD of three

independent experiments. Values were considered significantly different at the level of $p < 0.05$.

Percent of life cells was calculated as follows:

$$\% = \text{OD (sample)} - \text{OD (background)}/\text{OD (control)} - \text{OD (background)} \times 100$$

Optical density (OD) of background for adherent cells is the OD of MTT solution and DMSO; OD (background) for suspension cells is OD of the culture medium with MTT and 10% SDS with 0.01 mol/L; OD (control) is the OD of the cells growth without tested compounds.

Selectivity index (SI) was calculated as follows: $SI = IC_{50}$ for normal cell line/IC_{50} for respective tumor cell line.[72]

4.3.3. Assessment of cell membrane integrity by LDH assay

K562 and Raji cells were plated in 96-well plates at a concentration of 1×10^5 cells/mL. Cultured medium was RPMI (1% FBS, 2 mM glutamine, 1 mM Na-piruvate, and 10 mM HEPES) Derivatives **17** and **22** were applied on cells at concentration of 5 µmol dm^{-3} and supernatants were collected after 3, 6, 10 and 24 h of incubation. Supernatant was analyzed according to the kit protocol (LDH Cytotoxicity Detection Kit, TAKARA BIO INC.). Microplate reader (Viktor 3 V 1420, Perking Elmer) was used for measured absorbance on 490 nm.

Percent of cell membrane integrity was calculated as follows:

$$\% = \text{OD (sample)} - \text{OD (low control)}/\text{OD (high control)} - \text{OD (low control)}\ \text{x100}$$

Low control represents supernatant from cells which were cultivated with fresh medium and high control is supernatant from cells treated with 1% Triton X.

4.3.4. Flow cytometry analysis of cell cycle

K562 and Raji cells were plated at a concentration of 5×10^5 cells and same day treated with selected derivatives **17** and **22** (1 µmol dm^{-3}). After 24 h of incubation cells were collected, fixated with cold 70% ethanol, and kept at 4 °C. Before analysis collected cells were washed in PBS and stained with 15 µg/mL of propidium iodide at room temperature. Before staining on cells were added 0.2 µg/µL of RNA-ase A for 5 minutes at room temperature. Cells were analyzed by flow cytometry (FacsCanto II, BD Biosciences, USA) using FlowJo software. One way statistical analysis of variances (ANOVA) was performed in XSTAT with Dunnett (two sided) analysis ($p < 0.05$).

Conflicts of interest

The authors declare no conflict of interest.

Funding

The authors gratefully acknowledge financial support from the Ministry of Science, Education and Sport of Croatia (Grant No 098-0982914-2935); financial support from Croatian Science Foundation (Grant HRZZ-1477) and Faculty of Medicine, University of Osijek supporting grant to Lj. Glavas-Obrovac (VIF2018-MEFOS-6).

ORCID

Josipa Matić http://orcid.org/0000-0003-1774-0446
Marijana Jukić http://orcid.org/0000-0002-3326-4868
Dijana Saftić http://orcid.org/0000-0002-0657-1635
Željka Ban http://orcid.org/0000-0002-6723-5811
Robert Vianello http://orcid.org/0000-0003-1779-4524
Teuta Opačak-Bernardi http://orcid.org/0000-0001-8563-8684
Ljubica Glavaš-Obrovac http://orcid.org/0000-0001-7497-296X
Biserka Žinić http://orcid.org/0000-0002-1536-7142

References

[1] Kolaczek, A.; Fusiarz, I.; Lawecka, J.; Branowska, D. Biological Activity and Synthesis of Sulfonamide Derivatives: A Brief Review. *Chemik* **2014**, *68*, 620–628.
[2] VanMeter, K. C.; Hubert, R. J. *Microbiology for the Healthcare Professional*; Elsevier: St. Louis, MO, **2016**.
[3] Scozzafava, A.; Briganti, F.; Mincione, G.; Menabuoni, L.; Mincione, F.; Supuran, C. T. Carbonic Anhydrase Inhibitors: Synthesis of water-soluble, aminoacyl/dipeptidyl sulfonamides possessing long-lasting intraocular pressure-lowering properties via the topical route. *J. Med. Chem.* **1999**, *42*, 3690–3700. DOI: 10.1021/jm9901879.
[4] Jaiswal, M.; Khadikar, P. V.; Supuran, C. T. Topological Modeling of Lipophilicity, Diuretic Activity, and Carbonic Inhibition Activity of benzene sulfonamides: a molecular connectivity approach. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5661–5666. DOI: 10.1016/j.bmcl.2004.08.051.
[5] Nishimori, I.; Vullo, D.; Innocenti, A.; Scozzafava, A.; Mastrolorenz, A.; Supuran, C. T. Carbonic Anhydrase Inhibitors: Inhibition of the Transmembrane Isozyme XIV with Sulfonamides. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3828–3833. DOI: 10.1016/j.bmcl.2005.06.055.
[6] Ogden, R. C.; Flexner, C. W. *Protease Inhibitors in AIDS Therapy*; Marcel Dekker: New York, Basel, **2001**.
[7] Casini, A.; Scozzafava, A.; Mastrolorenzo, A.; Supuran, C. T. Sulfonamides and Sulfonylated Derivatives as Anticancer Agents. *Ccdt.* **2002**, *2*, 55–75. DOI: 10.2174/1568009023334060.
[8] Ismail, M. M.; Ghorab, M. M.; Noaman, E.; Ammar, Y. A.; Heiba, H. I.; Sayed, M. Y. Novel Synthesis of Pyrrolo[2,3-*d*]Pyrimidines Bearing Sulfonamide Moieties as Potential Antitumor and Radioprotective Agents. *Arzneim. Forsch* **2006**, *56*, 301–308.

[9] Rostom, S. A. Synthesis and *in-Vitro* Antitumor Evaluation of Some Indeno [1,2-*c*]Pyrazol(in)es Substituted with Sulfonamide, Sulfonyl-Urea(-Thiourea) Pharmacophores, and Some Derived Thiazole Ring Systems. *Bioorg. Med. Chem* **2006**, *14*, 6475–6485. DOI: 10.1016/j.bmc.2006.06.020.

[10] Supuran, C. T.; Scozzafava, A.; Mastrolorenzo, A. Bacterial Proteases: current Theraputic Use and Future Prospects for the Development of New Antibiotics. *Exp. Opin. Therap. Pat* **2001**, *1*, 221–259.

[11] Scozzafava, A.; Supuran, C. T. Carbonic Anhydrase and Matrix Metalloproteinase Inhibitors: Sulfonylated Amino Acid Hydroxamates with MMP Inhibitory Properties Act as Efficient Inhibitors of CA Isozymes I, II, and IV, and N-hydroxysulfonamides inhibit both these zinc enzymes . *J. Med. Chem*. **2000**, *43*, 3677–3687. DOI: 10.1021/jm000027t.

[12] Žinić, B.; Krizmanić, I.; Žinić, M. Synthesis of the sulfonylpyrimidine derivatives with anticancer activity. E.P. Patent 0 877 022 B1, April 16, **2003**.

[13] Saftić, D.; Vianello, R.; Žinić, B. 5-Triazolyluracils and Their N^1-Sulfonyl Derivatives: Intriguing Reactivity Differences in the Sulfonation of Triazole $N^{1'}$-Substituted and $N^{1'}$-Unsubstituted Uracil Molecules. *Eur. J. Org. Chem* **2015**, *35*, 7695–7704.

[14] Matić, J.; Nekola, I.; Višnjevac, A.; Kobetić, R.; Martin-Kleiner, I.; Kralj, M.; Žinić, B. C5-Morpholinomethylation of *N*1-Sulfonylcytosines by a One-Pot Microwave Assisted Mannich Reaction. *Org. Biomol. Chem*. **2018**, *16*, 2678–2687. DOI: 10.1039/c8ob00253c.

[15] Glavaš-Obrovac, L.; Karner, I.; Žinić, B.; Pavelić, K. Antineoplastic Activity of Novel *N*-1-Sulfonypyrimidine Derivatives. *Anticancer Res* **2001**, *21*, 1979–1986.

[16] Supek, F.; Kralj, M.; Marjanović, M.; Šuman, L.; Šmuc, T.; Krizmanić, I.; Žinić, B. Atypical Cytostatic Mechanism of *N*-1-Sulfonylcytosine Derivatives Determined by *in Vitro* Screening and Computational Analysis. *Invest New Drugs*. **2008**, *26*, 97–110. DOI: 10.1007/s10637-007-9084-1.

[17] Glavaš-Obrovac, L.; Jukić, M.; Mišković, K.; Marković, I.; Saftić, D.; Ban, Ž.; Matić, J.; Žinić, B. Antiproliferative and Proapoptotic Activity of Molecular Copper(II) Complex of *N*-1-Tosylcytosine. *J Trace Elem Med Biol*. **2019**, *55*, 216–222. DOI: 10.1016/j.jtemb.2017.10.009.

[18] Kašnar-Šamprec, J.; Glavaš-Obrovac, L.; Pavlak, M.; Mihaljević, I.; Štambuk, N.; Konjevoda, P.; Žinić, B. Synthesis, Spectroscopic Characterization and Biological Activity of *N*-1-Sulfonylcytosine Derivatives. *Croat. Chem. Acta* **2005**, *78*, 261–267.

[19] Pavlak, M.; Stojković, R.; Radačić-Aumiler, M.; Kašnar-Šamprec, J.; Jerčić, J.; Vlahović, K.; Žinić, B.; Radačić, M. Antitumor Activity of Novel *N*-Sulfonylpyrimidine Derivatives on the Growth of Anaplastic Mammary Carcinoma *in Vivo*. *J Cancer Res Clin Oncol*. **2005**, *131*, 829–836. DOI: 10.1007/s00432-005-0026-z.

[20] Kašnar-Šamprec, J.; Ratkaj, I.; Mišković, K.; Pavlak, M.; Baus-Lončar, M.; Kraljević Pavelić, S.; Glavaš-Obrovac, L.; Žinić, B. *In Vivo* Toxicity Study of *N*-1-Sulfonylcytosine Derivatives and Their Mechanisms of Action in Cervical Carcinoma Cell Line. *Invest New Drugs*. **2012**, *30*, 981–990. DOI: 10.1007/s10637-011-9657-x.

[21] Glavaš-Obrovac, L.; Karner, I.; Štefanić, M.; Kašnar-Šamprec, J.; Žinić, B. Metabolic Effects of Novel *N*-1-Sulfonylpyrimidine Derivatives on Human Colon Carcinoma Cells. *Farmaco* **2005**, *60*, 479–483. DOI: 10.1016/j.farmac.2005.04.006.

[22] Rossi, A. The Clinical Uses of Nucleoside Analogues in Malignant Disease. In *Nucleoside Analogues: Chemistry, Biology, and Medical Applications*; Walker, R.T., De Clercq, E., Eckstein, F. Eds.; Plenum Press: New York; **1979**; pp. 409–436

[23] Nielsen, O. H.; Vainer, B.; Rask-Madsen, J. Review article: the treatment of inflammatory bowel disease with 6-mercaptopurine or azathioprine . *Aliment. Pharmacol. Ther*. **2001**, *15*, 1699–1708. DOI: 10.1046/j.1365-2036.2001.01102.x.

[24] Lukenbill, J.; Kalaycio, M. Fludarabine: A Review of the Clear Benefits and Potential Harms. *Leuk. Res*. **2013**, *37*, 986–994. DOI: 10.1016/j.leukres.2013.05.004.

[25] Leclerc, M.; Suarez, F.; Noël, M. P.; Vekhoff, A.; Troussard, X.; Claisse, J. F.; Thieblemont, C.; Maloisel, F.; Beguin, Y.; Tamburini, J.; et al. Rituximab Therapy for Hairy Cell Leukemia: A Retrospective Study of 41 Cases. *Ann. Hematol*. **2015**, *94*, 89–95. DOI: 10.1007/s00277-014-2175-0.

[26] Liliemark, J. The Clinical Pharmacokinetics of Cladribine. *Clin. Pharmacokinet*. **1997**, *32*, 120–131. DOI: 10.2165/00003088-199732020-00003.

[27] De Clercq, E. Antiviral Drugs in Current Clinical Use. *J. Clin. Virol*. **2004**, *30*, 115–133. DOI: 10.1016/j.jcv.2004.02.009.

[28] De Clercq, E.; Field, H. J. Antiviral Prodrugs - the Development of Successful Prodrug Strategies for Antiviral Chemotherapy. *Br. J. Pharmacol*. **2006**, *147*, 1–11. DOI: 10.1038/sj.bjp.0706446.

[29] De Clercq, E. Milestones in the Discovery of Antiviral Agents: nucleosides and Nucleotides. *Acta Pharm. Sin. B* **2012**, *2*, 535–548. DOI: 10.1016/j.apsb.2012.10.001.

[30] Laufer, S. A.; Domeyer, D. M.; Scior, T. R. F.; Albrecht, W.; Hauser, D. R. J. Synthesis and Biological Testing of Purine Derivatives as Potential ATP-Competitive Kinase Inhibitors. *J. Med. Chem*. **2005**, *48*, 710–722. DOI: 10.1021/jm0408767.

[31] Endo, K.; Deguchi, K.; Matsunaga, H.; Tomaya, K.; Yamada, K. 8-Substituted 2-alkynyl-N(9)-propargyladenines as A2A adenosine receptor antagonists . *Bioorg. Med. Chem*. **2014**, *22*, 3072–3082. DOI: 10.1016/j.bmc.2014.04.041.

[32] Hocek, M.; Naus, P.; Pohl, R.; Votruba, I.; Furman, P. A.; Tharnish, P. M.; Otto, M. J. Cytostatic 6-Arylpurine Nucleosides. 6. SAR in anti-HCV and Cytostatic Activity of Extended Series of 6-Hetarylpurine Ribonucleosides. *J. Med. Chem*. **2005**, *48*, 5869–5873. DOI: 10.1021/jm050335x.

[33] Martirosyan, Z. A.; Gunar, V. I.; Zav′yalov, S. I. *Izv. Akad. Nauk SSSR, Ser. Khim*. **1970**, 1841–1844.

[34] Megati, S.; Phadtare, S.; Zemlicka, J. Unsaturated Phosphonates as Acyclic Nucleotide Analogs. Anomalous Michaelis-Arbuzov and Michaelis-Becker Reactions with Multiple Bond Systems. *J. Org. Chem*. **1992**, *57*, 2320–2327. DOI: 10.1021/jo00034a025.

[35] Žinić, B.; Krizmanić, I.; Vikić-Topić, D.; Srzić, D.; Žinić, M. Synthesis, NMR and MS Study of Novel *N*-Sulfonylated Purine Derivatives. *Croat. Chem. Acta* **2001**, *74*, 399–414.

[36] Scozzafava, A.; Mastrolorenzo, A.; Supuran, C. T. Antimycobacterial Activity of 9-Sulfonylated/Sulfenylated-6-Mercaptopurine Derivatives. *Bioorg. Med. Chem. Lett*. **2001**, *11*, 1675–1678. DOI: 10.1016/s0960-894x(01)00266-9.

[37] Bakkestuen, A. K.; Gundersen, L.-L.; Utenova, B. T. Synthesis, Biological Activity, and SAR of Antimycobacterial 9-Aryl-, 9-Arylsulfonyl-, and 9-Benzyl-6-(2-Furyl)Purines. *J. Med. Chem*. **2005**, *48*, 2710–2723. DOI: 10.1021/jm0408924.

[38] Kucukdumlu, A.; Tuncbilek, M.; Guven, E. B.; Atalay, R. C. Synthesis of Some Substituted 6-Phenyl Purine Analogues and Their Biological Evaluation as Cytotoxic Agents. *Acta Chim Slov* **2017**, *64*, 621–632. DOI: 10.17344/acsi.2017.3419.

[39] Demir, Z.; Guven, E. B.; Ozbey, S.; Kazak, C.; Atalay, R. C.; Tuncbilek, M. Synthesis of Novel Substituted Purine Derivatives and Identification of the Cell Death Mechanism. *Eur. J. Med. Chem.* **2015**, *89*, 701–720. DOI: 10.1016/j.ejmech.2014.10.080.

[40] (a) Díaz-Gavilán, M.; Gómez-Vidal, J. A.; Rodríguez-Serrano, F.; Marchal, J. A.; Caba, O.; Aránega, A.; Gallo, M. A.; Espinosa, A.; Campos, J. M. Anticancer activity of (1,2,3,5-tetrahydro-4,1-benzoxazepine-3-yl)-pyrimidines and -purines against the MCF-7 cell line: Preliminary cDNA microarray studies. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1457–1460. (b) Kode, N.; Chen, L.; Murthy, D.; Adewumi, D.; Phadtare, S. New bis-*N*9-(methylphenylmethyl)purine derivatives: Synthesis and antitumor activity. *Eur. J. Med. Chem.* **2007**, *42*, 327–333.

[41] (a) Ikejiri, M.; Ohshima, T.; Fukushima, A.; Shimotohno K.; Maruyama, T. Synthesis and evaluation of 5′-modified 2′-deoxyadenosine analogues as anti-hepatitis C virus agents. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4638–4641. (b) Ikejiri, M.; Saijo, M.; Morikawa, S.; Fukushi, S.; Mizutani, T.; Kurane I.; Maruyama, T. Synthesis and biological evaluation of nucleoside analogues having 6-chloropurine as anti-SARS-CoV agents. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2470–2473. (c) Maruyama, T.; Sato, Y.; Oto, Y.; Takahashi, Y.; Snoeck, R.; Andrei, G.; Witvrouw, M.; De Clercq, E. Synthesis and antiviral activity of 6-chloropurine arabinoside and its 2'-deoxy-2'-fluoro derivative. *Chem. Pharm. Bull.* **1996**, *44*, 2331–2334.

[42] Aleksandrova, E. V.; Kochergin, P. M. The Use of Protecting Groups in the Synthesis of Purine Derivatives (Review). *Chem. Heterocycl. Comp.* **2009**, *45*, 1–27. DOI: 10.1007/s10593-009-0220-z.

[43] Jain, Z. J.; Gide, P. S.; Kankate, R. S. Biphenyls and Their Derivatives as Synthetically and Pharmacologically Important Aromatic Structural Moieties. *Arab. J. Chem* **2017**, *10*, S2051–S2066. DOI: 10.1016/j.arabjc.2013.07.035.

[44] Tarade, D.; Ma, D.; Pignanelli, C.; Mansour, F.; Simard, D.; van den Berg, S.; Gauld, J.; McNulty, J.; Pandey, S. Structurally Simplified Biphenyl Combretastatin A4 Derivatives Retain *in Vitro* anti-cancer activity dependent on mitotic arrest. *PLoS One.* **2017**, *12*, e0171806. DOI: 10.1371/journal.pone.0171806.

[45] Benfodda, Z.; Fritz, V.; Henriquet, C.; Fattorusso, C.; Cebrián-Torrejón, G.; Persico, M.; Di Dato, A.; Menna, M.; Blancou, H.; Fajas, L. Synthesis, Anticancer Activity and Computational SAR Analysis of Acylsulfonylpiperazines Derivatives. *Med. Chem. (Los Angeles)* **2017**, *7*, 257–267.

[46] Fernández-Tornero, C.; Lozano, R. M.; Redondo-Horcajo, M.; Gómez, A. M.; López, J. C.; Quesada, E.; Uriel, C.; Valverde, S.; Cuevas, P.; Romero, A.; Giménez-Gallego, G. Leads for Development of New Naphthalenesulfonate Derivatives with Enhanced Antiangiogenic Activity: cristal Structure of Acidic Fibroblast Growth Factor in Complex with 5-Amino-2-Naphathalenesulfonate. *J. Biol. Chem* **2003**, *278*, 21774–21781. DOI: 10.1074/jbc.M212833200.

[47] Mishra, R. C.; Gundala, S. R.; Karna, P.; Lopus, M.; Gupta, K. K.; Nagaraju, M.; Hamelberg, D.; Tandon, V.; Panda, D.; Reid, M. D.; Aneja, R. Design, Synthesis and Biological Evaluation of Disubstituted Noscapine Analogs as Potent and Microtubule-Targeted Anticancer Agents. *Bioorg. Med. Chem. Lett* **2015**, *25*, 2133–2140. DOI: 10.1016/j.bmcl.2015.03.076.

[48] Ferenc, G.; Pádár, P.; Szolomájer, J.; Kovács, L. *N*-Alkylated Guanine Derivatives. *Coc.* **2009**, *13*, 1085–1135. DOI: 10.2174/138527209788680718.

[49] Zhong, M.; Robins, M. J. Regiospecific N9 Alkylation of 6-(heteroaryl)purines: shielding of N7 by a proximal heteroaryl C-H1. *J. Org. Chem*. **2006**, *71*, 8901–8906. DOI: 10.1021/jo061759h.

[50] Meshram, G. A.; Patil, V. D. A Simple and Efficient Method for Sulfonylation of Amines, Alcohols and Phenols with Cupric Oxide under Mild Conditions. *Tetrahedron Lett* **2009**, *50*, 1117–1121. DOI: 10.1016/j.tetlet.2008.12.085.

[51] Brik, A.; Wu, C.-Y.; Best, M. D.; Wong, C.-H. Tetrabutylammonium Fluoride-Assisted Rapid N9-Alkylation on Purine Ring: application to Combinatorial Reactions in Microtiter Plates for the Discovery of Potent Sulfotransferase Inhibitors in Situ. *Bioorg. Med. Chem*. **2005**, *13*, 4622–4626. DOI: 10.1016/j.bmc.2005.02.066.

[52] Al-Ghorbani, M.; Begum, B. A.; Zabiulla, S.; Mamatha, S. V.; Khanum, S. A. Piperazine and Morpholine: Synthetic Preview and Pharmaceutical Applications. *J. Chem. Pharm. Res* **2015**, *7*, 281–301.

[53] Huang, L.-K.; Cherng, Y.-C.; Cheng, Y.-R.; Jang, J.-P.; Chao, Y.-L.; Cherng, Y.-J. An Efficient Synthesis of Substituted Cytosines and Purines under Focused Microwave Irradiation. *Tetrahedron* **2007**, *63*, 5323–5327. DOI: 10.1016/j.tet.2007.02.124.

[54] Hanus, M.; Kabeláč, M.; Rejnek, J.; RyjáčEk, F.;.; Hobza, P. ; Correlated *ab Initio* Study of Nucleic Acid Bases and Their Tautomers in the Gas Phase, in a Microhydrated Environment, and in Aqueous Solution. Part 3. Adenine. *J. Phys. Chem. B*. **2004**, *108*, 2087–2097. DOI: 10.1021/jp036090m.

[55] Tandarić, T.; Vianello, R. Design of Exceptionally Strong Organic Superbases Based on Aromatic Pnictogen Oxides: Computational DFT Analysis of the Oxygen Basicity in the Gas Phase and Acetonitrile Solution. *J. Phys. Chem. A*. **2018**, *122*, 1464–1471. DOI: 10.1021/acs.jpca.7b11945.

[56] Despotović, I.; Vianello, R. Engineering Exceptionally Strong Oxygen Superbases with 1,8-Diazanaphthalene di-*N*-Oxides. *Chem Commun (Camb) .)* **2014**, *50*, 10941–10944. DOI: 10.1039/c4cc05125d.

[57] Hansch, C.; Leo, A.; Taft, R. W. A Survey of Hammett Substituent Constants and Resonance and Field Parameters. *Chem. Rev*. **1991**, *91*, 165–195. DOI: 10.1021/cr00002a004.

[58] Zask, A.; Verheijen, J. C.; Richard, D. J. Recent Advances in the Discovery of Small-Molecule ATP Competitive mTOR Inhibitors: A Patent Review. *Expert Opin Ther Pat* **2011**, *21*, 1109–1127. DOI: 10.1517/13543776.2011.584871.

[59] Chen, Y.; Wang, X.; Xiang, W.; He, L.; Tang, M.; Wang, F.; Wang, T.; Yang, Z.; Yi, Y.; Wang, H.; et al. Development of Purine-Based Hydroxamic Acid Derivatives: Potent Histone Deacetylase Inhibitors with Marked *in Vitro* and *in Vivo* Antitumor Activities. *J. Med. Chem*. **2016**, *59*, 5488–5504. DOI: 10.1021/acs.jmedchem.6b00579.

[60] Long, Y.; Yu, M.; Li, P.; Islam, S.; Goh, A. W.; Kumarasiri, M.; Wang, S. Synthesis and Biological Evaluation of Heteroaryl Styryl Sulfone Derivatives as Anticancer Agents. *Bioorg. Med. Chem. Lett*. **2016**, *26*, 5674–5678. DOI: 10.1016/j.bmcl.2016.10.062.

[61] Haneef, J.; Parvathy, M.; M. P.; Thankayyan R, S. K.; Sithul, H.; Sreeharshan, S. Bax translocation mediated mitochondrial apoptosis and caspase dependent photosensitizing effect of *Ficus religiosa* on cancer cells. *PloS ONE* **2012**, *7*, e40055. DOI: 10.1371/annotation/ab341223-61ed-4b19-95c7-21455c321e06.

[62] Mojzych, M.; Šubertová, V.; Bielawska, A.; Bielawski, K.; Bazgier, V.; Berka, K.; Gucký, T.; Fornal, E.; Kryštof, V. Synthesis and Kinase Inhibitory Activity of New Sulfonamide Derivatives of Pyrazolo[4,3-e][1,2,4]Triazines. *Eur. J. Med. Chem* **2014**, *78*, 217–224. DOI: 10.1016/j.ejmech.2014.03.054.

[63] Wang, Q.; Su, L.; Liu, N.; Zhang, L.; Xu, W.; Fang, H. Cyclin Dependent Kinase 1 Inhibitors: A Review of Recent Progress. *Curr. Med. Chem*. **2011**, *18*, 2025–2043. DOI: 10.2174/092986711795590110.

[64] , Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Petersson, G. A.; Nakatsuji, H, Gaussian 16, Revision C.01Gaussian, Inc., Wallingford CT, **2016**.

[65] Cindrić, M.; Sović, I.; Mioč, M.; Hok, L.; Boček, I.; Roškarić, P.; Butković, K.; Martin-Kleiner, I.; Starčević, K.; Vianello, R.; et al. Experimental and Computational Study of the Antioxidative Potential of Novel Nitro and Amino Substituted Benzimidazole/Benzothiazole-2-Carboxamides with Antiproliferative Activity. *Antioxidants* **2019**, *8*, 477. DOI: 10.3390/antiox8100477.

[66] Tandarić, T.; Hok, L.; Vianello, R. From Hydrogen Peroxide-Responsive Boronated Nucleosides towards Antisense Therapeutics – a Computational Mechanistic Study. *Croat. chem. Acta. (Online)* **2019**, *92*, 287–295. DOI: 10.5562/cca3592.

[67] Pantalon Juraj, N.; Muratović, S.; Perić, B.; Šijaković Vujičić, N.; Vianello, R.; Žilić, D.; Jagličič, Z.; Kirin, S. I. Structural Variety of Isopropyl-Bis(2-Picolyl)Amine Complexes with Zinc(II) and Copper(II). *Cryst. Growth Des* **2020**, *20*, 2440–2453. DOI: 10.1021/acs.cgd.9b01625.

[68] Pantalon Juraj, N.; Miletić, G. I.; Perić, B.; Popović, Z.; Smrečki, N.; Vianello, R.; Kirin, S. I. Stereochemistry of Hexacoordinated Zn(II), Cu(II), Ni(II), and Co(II) Complexes with Iminodiacetamide Ligands. *Inorg. Chem*. **2019**, *58*, 16445–16457. DOI: 10.1021/acs.inorgchem.9b02200.

[69] Tandarić, T.; Vianello, R. Computational Insight into the Mechanism of the Irreversible Inhibition of Monoamine Oxidase Enzymes by the Antiparkinsonian Propargylamine Inhibitors Rasagiline and Selegiline. *ACS Chem Neurosci* **2019**, *10*, 3532–3542. DOI: 10.1021/acschemneuro.9b00147.

[70] Maršavelski, A.; Vianello, R. What a Difference a Methyl Group Makes: The Selectivity of Monoamine Oxidase B Towards Histamine and N-Methylhistamine. *Chemistry* **2017**, *23*, 2915–2925. DOI: 10.1002/chem.201605430.

[71] Mickisch, G.; Fajta, S.; Bier, H.; Tschada, R.; Alken, P. Cross-Resistance Patterns Related to Glutathione Metabolism in Primary Human Renal Cell Carcinoma. *Urol. Res.* **1991**, *19*, 99–103. DOI: 10.1007/BF00368184.

[72] Badisa, R. B.; Darling-Reed, S. F.; Joseph, P.; Cooperwood, J. S.; Latinwo, L. M.; Goodman, C. B. Selective Cytotoxic Activities of Two Novel Synthetic Drugs on Human Breast Carcinoma MCF-7 Cells. *Anticancer Res.* **2009**, *29*, 2993–2996.