Synthesis of Some Novel Thiocyanotopurine Derivatives and Investigation of Their Antimicrobial Activity and DNA Interactions

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A series of 6-thiocyanatopurine derivatives introduced with different alkyl groups in position 9 was synthesized. The structures of the synthesized compounds were evaluated *via* spectroscopic methods and elemental methods of analyses. All the synthesized compounds were screened for their antibacterial activities against Gram-positive and Gram-negative bacteria and for their antifungal activities against yeast strains. All the synthesized compounds showed better antibacterial activities against Gram-positive bacteria compared to Gram-negative bacteria. DNA interactions with pBR322 DNA were determined. Most of the compounds caused conformational changes in DNA.

Key words purine derivative; antimetabolite; antimicrobial activity; DNA interaction

Purines are one of the most important classes of heterocyclic compounds in nature, fulfilling functional roles as nucleic acids, coenzymes, and constituents in metabolic processes, cell signaling, and energy storage.[1)] Cells obtain purine nucleotides through two separate metabolic pathways, *de novo* purine synthesis and salvage of extracellular purine bases.[2)] Therefore, *de novo* purine syntheses are important targets for antimetabolites.[3)]

Purine antimetabolites are of great importance to chemists as well as to biologists as they possess antitubercular, antiulcer, antimicrobial, antifungal, antineoplastic, antitumor, antiviral and cardiotonic properties.[3–10)] The purine nucleoside analogs are also used in the treatment of autoimmune diseases.[11)] Current evidence indicates that purine analogs alone or in combination with other chemotherapeutic agents are very effective against almost any fast-growing cells.[4,5)] Therefore, the potent activities of modified purines have accelarated the continuation of the studies on this subject.

The substitution of oxygen atom in purines with other heteroatoms has been the subject of numerous synthetic studies.[12)] Replacement of the oxygen atom at position 6 in guanine and in hypoxanthine with sulfur gave clinically widely used anticancer drugs, thioguanine (NSC752) and 6-mercaptopurine (NSC755).[13)] Structurally related nucleoside sulfinosine [2-amino-9-(β-D-ribofuranosyl)purine-6-sulfinamide] also exhibited unique antitumor properties.[14)] 6-Methylmercapto-9-(β-D-ribofuranosyl)purine is one of the most potent inhibitors of *de novo* purine synthesis.[15)] 6-Thioinosine, 6-thiocyanatoguanine, 6-benzylthiopurine, 6-methylthioinosine, 6-methylthiopurine, 6-thiocyanatoguanosine, 6-phenacylthioinosine, 6-nitrobenzlythioinosine, 6-benzylthioguanine, 6-(*p*-chlorobenzyl)thioinosine, 6-(*p*-nitrobenzyl)thioguanosine, 6-benzylthioinosine, 6-ethylthioinosine, and 6-thiocyanatoinosine are the other biologically active sulfur-containing purine antimetabolites.[16)]

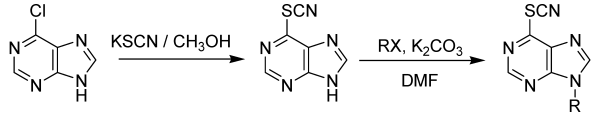
9-Alkyl-6-substituted purines are one of the most important classes of biologically active compounds. They were reported to have some antagonistic activities on adenosine receptors and an influence on numerous enzymatic systems.[17–20)] They also displayed inhibitory activities on metabolic pathway of cytokinins and cyclin dependent kinases.[21)]

6-Thiocyanatopurine was reported to show some effects on Adenocarcinoma 755 and several fungi,[22–24)] but the subject has not been studied systematically. Looking at the importance of 9-substituted purines,[25–27)] it was thought that it would be worthwhile to design and synthesize some thiocyanatopurine derivatives to obtain more active substances. Therefore, we synthesized 6-thiocyanatopurine derivatives by introducing different alkyl substituents in position 9 in order to observe the change in antimicrobial activities and DNA interactions.

Results and Discussion

Synthesis 6-Thiocyanato-9*H*-purine (**1**) was prepared from 6-chloropurine by nucleophilic substitution of the chlorine atom with KSCN.[28)] The structure of 6-thiocyanato-9*H*-purine (**1**) was confirmed by comparing its physical and spectral data with the reported ones.[23,24,28)]

Treatment of 6-thiocyanato-9*H*-purine **1** with some appropriate alkyl halide in the presence of potassium carbonate generated 9-substituted 6-thiocyanatopurines (**2**–**10**) (Chart 1). The products were separated and purified with column



1 **2-10**

R= (**2**: -CH_3; **3**: -C_2H_5; **4**: -C_3H_7; **5**: -$CH(CH_3)_2$; **6**: -C_6H_{13}; **7**: -$CH_2C_6H_5$; **8**: $CH_2COOC_2H_5$; **9**: -$CH_2C_4H_7$; **10**: -$CH_2C_6H_{11}$)

Chart 1. Synthetic Route for Compounds **1**–**10**

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Table 1. Antibacterial Activities of Compounds **1**–**10**

Compounds	Inhibition zone diameter (mm)									
	S. aureus	*B. cereus*	*B. subtilis*	*E. faecalis*	*P. aeruginosa*	*E. coli* 35218	*E. coli* 25922	*P. vulgaris*	*Candida albicans*	*Candida tropicalis*
1	34.7±0.5	19.0±1.0	21.0±1.0	—	10.5±0.7	24.5±0.7	24.3±0.5	22.7±1.1	—	—
2	40.0±0.0	26.3±1.1	25.5±0.7	18.0±2.0	10.3±0.5	21.3±0.5	20.0±0.0	—	23.0±1.4	11.3±0.5
3	35.3±0.5	20.3±0.5	25.5±0.7	—	—	—	14.0±0.0	—	15.0±0.0	9.0±0.0
4	44.3±0.5	25.7±0.5	32.5±0.7	19.3±0.5	—	17.3±0.5	12.0±0.0	—	25.0±0.0	14.0±0.0
5	31.5±0.7	—	12.7±0.5	—	—	—	—	—	24.0±0.0	—
6	40.0±1.0	23.0±1.0	25.3±0.6	—	—	—	—	—	19.3±0.6	15.3±0.6
7	39.0±1.7	20.0±0.0	23.7±0.5	—	—	—	—	—	17.0±0.0	—
8	36.3±0.5	22.7±0.5	25.7±0.5	—	—	—	—	—	—	—
9	40.0±1.0	—	23.7±0.5	14.0±1.0	23.3±0,5	—	—	—	25.0±0.0	15.0±0.0
10	28.0±1.0	—	14.7±0.5	—	—	—	—	—	—	—
Amp	25.0±0.0	11.0±0.0	16.5±1.5	35.0±0.0	28.0±0.0	—	30.0±0.0	11.0±0.0	NS	NS
C	25.0±0.0	24.0±0.0	26.0±1.0	31.3±0.6	21.9±0.9	10.3±0.6	25.0±0.6	—	NS	NS
Keto	NS	NS	NS	NS	NS	NS	NS	NS	30.0±0.0	29.5±0.5
Control (DMSO)	—	—	—		—	—	—	—	—	—

Antibiotics; Amp: ampicilin and C: chloramphenicol (antibacterial), antifungal; Keto: ketoconazole, NS: not studied.

Table 2. Minimum Inhibitory Concentrations (MIC, μM) of Compounds **1**–**10**

Compounds	MIC values (μM)									
	S. aureus	*B. cereus*	*B. subtilis*	*E. fecalis*	*P. aeruginosa*	*E. coli* 35218	*E. coli* 25922	*P. vulgaris*	*Candida albicans*	*Candida tropicalis*
1	1250	2500	2500	—	2500	2500	2500	2500	—	—
2	19,50	312,50	9,75	9,75	2500	9,75	9,75	—	2500	2500
3	625	1250	312,50	—	—	—	1250	—	2500	2500
4	625	19,50	625	1250	—	312,50	1250	—	5000	5000
5	1250	—	625	—	—	—	—	—	2500	—
6	625	39	1250	—	—	—	—	—	2500	2500
7	2500	2500	2500	—	—	—	—	—	2500	—
8	1250	2500	1250	—	—	—	—	—	—	—
9	2500	—	2500	2500	2500	—	—	—	1250	2500
10	2500	—	2500	—	—	—	—	—	—	—

chromatography to provide the compounds **2**–**10** in moderate to good yields. The structure of compounds **2**–**10** were confirmed by FT-IR, ^{1}H-NMR, ^{13}C-NMR and MS spectra or elemental analyses. The IR spectra of the synthesized compounds exhibited a band in the region of 2164–2181 cm^{-1} which can be assigned to the characteristic band of stretching vibrations of CN group. The disappearance of band at 3443 cm^{-1} (–N–H stretching frequency) is good evidence for the structure of the compounds **2**–**10**. ^{13}C-NMR spectra showed a characteristic peak at δ 106 ppm for CN group.

The MS spectra or elemental analyses also complied with the expected values. All the target compounds (**2**–**10**) were reported for the first time. The purity of the synthesized compounds was monitored by TLC.

Antimicrobial Activity Results of tests with bacteria and fungi are often used as a preliminary indicator of biological activity in the choice of chemotherapeutic agents.[22] Therefore, all the synthesized compounds were tested for their antimicrobial activities.

The *in vitro* antibacterial activities of compounds **1**–**10** were undertaken against Gram-positive bacteria including *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* NRLL B-3008, *Bacillus subtilis* ATCC 29213 and *Enterobacter faecalis* ATCC 292112 and Gram-negative bacteria including *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 35218, *Escherichia coli* ATCC 25922 and *Proteus vulgaris* ATCC 8427 by disk diffusion technique.[29,30] In addition, the *in vitro* antifungal activities of compounds **1**–**10** were undertaken against *Candida albicans* ATCC 10231 and *Candida tropicalis* ATCC 13803. The lowest concentration (highest dilution) required to completely inhibit growth of a microorganism was regarded as minimum inhibitory concentration (MIC, μM). Chloramphenicol and ampicillin were used as the reference antibacterial agents and ketoconazole was used as a reference antifungal agent under similar conditions for comparison. Dimethyl sulfoxide (DMSO) was used as a control. The results of antimicrobial activity data of the compounds against microorganisms are listed in Table 1. The results showed that all of the synthesized compounds displayed significant activity against *S. aureus* when compared to standard drugs chloramphenicol and ampicillin. All of the compounds were also very influential on *B. subtilis*. Most of the compounds showed good to mild activity towards *B. cereus*. However, less activity was noted against *Escherichia coli*, *P. vulgaris*, *E. faecalis* and *P.*

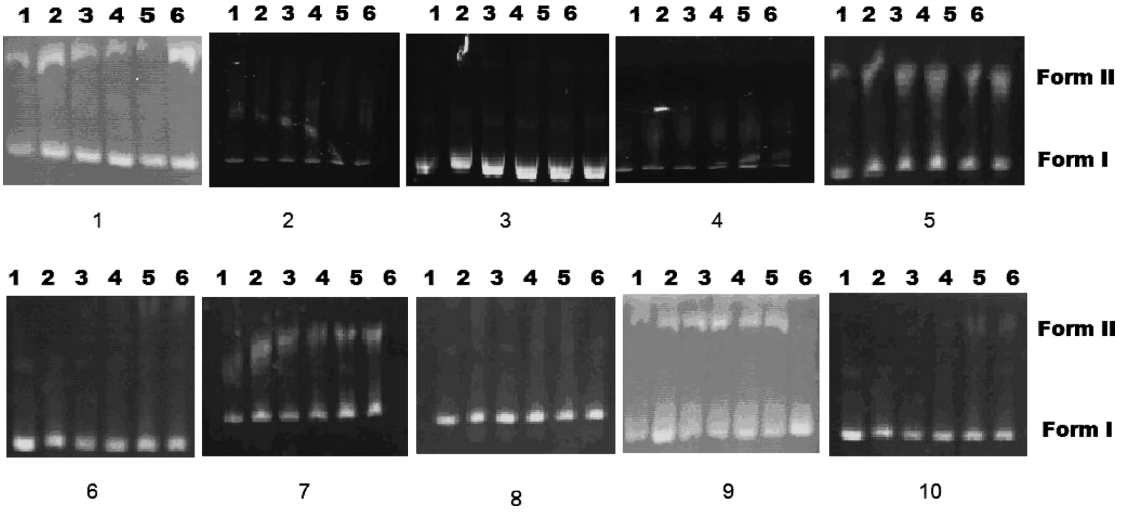

Fig. 1. Gel Electrophoretic Mobility of pBR322 Plasmid DNA When Incubated with Various Concentrations of Compounds **1**–**10**

Concentrations (in μM) are as follows: lane 1, untreated pBR322 plasmid DNA; lane 2, 5000; lane 3, 2500; lane 4, 1250; lane 5, 625; lane 6, 312. The top and the bottom bands correspond to form II (singly-nicked) and form I (covalently closed circular) plasmids, respectively.

aeruginosa. The screening results revealed that all the synthesized compounds showed better antibacterial activity against Gram-positive bacteria compared to Gram-negative bacteria. Among them, compounds **2**, **4** and **6** were found to be the most active antimicrobial members in this investigation. Compound **10** was the least active compound tested. Compounds **2**–**7** and **9** were found to possess antifungal activity against *C. albicans*, but this activity was lower than the action of ketoconazole. Compounds **2**–**4**, **6** and **9** were also influential on *C. tropicalis*.

Table 2 summarizes the minimum inhibitory concentrations of the compounds **1**–**10**. MIC values were ranged from 9.75 to 5000 μM. As shown in Table 2, compound **2** showed a very strong influence on the strains tested. On the other hand, only compound **1** showed potent activity against *P. vulgaris*.

It is believed that the strong lipophilic character of the molecule plays an essential role in producing antimicrobial effect. These properties are seen as an important parameter related to membrane permeation in biological system. By increasing the hydrophobicity (in other words, the length of the alkyl chain), compounds become able to interact with the cytoplasmic membrane. Thus, it was expected that compounds having the longest alkyl chains would show the highest antibacterial activity. In fact, homolongation of the chain with one carbon atom (compound **2**) demonstrated an increase in the inhibitory activity against Gram-positive bacteria and fungi. However, an increase in chain length with two carbon atoms (compound **3**) resulted a small decrease in antimicrobial activity. On the other hand, when the chain length was increased with three carbon atoms (compound **4**), antimicrobial activity increased again. It was also observed that a reduction in the antibacterial activity was obtained with the introduction of rigidity in position 9 (compounds **5**, **10**). On the other hand, introduction of cyclobutylmethyl group in position 9 (compound **9**) improved the antibacterial activity against *S. aureus*.

DNA and Compound Interaction Small molecules that bind to DNA are useful biochemical tools for the visualization of DNA *in vitro*.[31] Many antitumor, antineoplastic, antimalarial, antibiotic, and antifungal agents are the small compounds that interact with DNA.[32] The interaction of DNA with compounds can convert covalently supercoiled form I of DNA into singly nicked relaxed form II, and then into doubly nicked form III DNA. Therefore, interaction of the synthesized compounds **1**–**10** with supercoiled pBR322 DNA was studied by agarose gel electrophoresis.[33,34] pBR322 DNA was incubated with decreasing concentrations of compounds ranging from 5000 to 312 μM at 37°C for 24 h. Figure 1 gives the electrophoretograms applying to the interaction of pBR322 DNA with decreasing concentrations of compounds **1**–**10** ranging from 5000 to 312 μM. In the electrophoretograms, the untreated pBR plasmid DNA was used as a control. pBR plasmid DNA was initially found to be a mixture of mainly supercoiled form I and a small amount of singly-nicked form II.

As pBR plasmid DNA was allowed to interact with compound **1**, the mobility of form I band increased whereas that of form II band decreased. Compound **1** was effective on both form I and form II bands. When pBR plasmid DNA was allowed to interact with compound **2**, the mobility of form II band increased whereas that of form I band remained unchanged so that the two bands came closer together. In addition to the alteration in the mobility of nicked DNA, relaxed form was induced. Compounds **3**, **4** and **8** had no effect on form I and form II bands. When pBR plasmid DNA was allowed to interact with compound **5**, the mobility of form I band decreased slightly whereas that of form II band increased. For compounds **6** and **10**, the mobility of form II band decreased at high concentrations. The decrease in the mobility of form II plasmid DNA was observed at 5000 μM and 2500 μM. The intensity of form II band also decreased at high concentrations. The decrease in intensity at higher concentrations indicates the occurrence of DNA damage caused by the binding of these compounds. As pBR plasmid DNA was allowed to interact with compound **7**, the mobility form II band decreased. For compound **9**, the migration of DNA was slower than the control DNA at all concentrations tested.

Conclusion

In conclusion, several 9-substituted 6-thiocyanatopurine derivatives were synthesized. The pharmacological study was undertaken to evaluate the effects of substituents on the

antibacterial and antifungal activities. All the synthesized compounds showed good antimicrobial activity against Gram-positive bacteria and fungi. However, it can not be deduced from the results that a linear correlation exists between the size of alkyl chain and the inhibition zone diameter. Gel electrophoresis data indicated that the compounds **2**, **4** and **7** effectively converted closely circular plasmid DNA into a singly nicked DNA and linear form III band of plasmid DNA.

Experimental

Chemistry General All chemicals and solvents used were reagent grade (Merck or Aldrich or Sigma) and were used without additional purification. Reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F254 plates from Merck and plates were visualized by UV light. Column chromatography was performed using Merck Silica Gel 60 F_{254} (particle size: 0.63–0.200 mm; 70–230 mesh ASTM). Melting points were determined with an Electrothermal 9100 melting point apparatus and were uncorrected. Elemental analyses for C, H, N, S were performed by TUBITAK Laboratory (Ankara, Turkey) on a CHNS analyzer, model Leco-932, and were within ±0.4% of theoretical values. IR spectra (4000–400 cm^{-1}) were recorded using KBr disk on a Mattson 1000 FT-IR spectrometer and were reported in cm^{-1} units. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker spectrometer (300 MHz for ^{1}H- and 75 MHz for ^{13}C-NMR). Chemical shifts were reported in δ ppm units with respect to tetramethylsilane (TMS) as internal standard in DMSO-d_6 solution and coupling constants (J) were reported in Hz units. Mass spectra measurements were recorded on a Waters ACQUITY ultra performance liquid chromatography combined with Micromass LCT Premier^{TM} XE TOF-MS.

Synthesis of 6-Thiocyanato-9*H*-purine (1) 6-Chloropurine (2.00 g, 0.013 mol) was refluxed with potassium thiocyanate (1.26 g, 0.013 mol) in 30 mL of methanol for 4 h. The reaction mixture was cooled and filtered. The crude precipitate was washed with methanol and recrystallized from water.[28] Yield 89%, mp >235°C (decomp.). IR (KBr) cm^{-1}: 2181 (–CN), 3080 (Ar-H), 3443 (–N–H). ^{1}H-NMR (DMSO-d_6) δ: 8.75 (s, 1H, Ar-H), 8.91 (s, 1H, Ar-H), 14.05 (s, 1H, –N–H). ^{13}C-NMR (DMSO-d_6) δ: 106.99, 130.74, 146.39, 147.53, 151.93, 152.46. *Anal.* Calcd for $C_6H_3N_5S$: C, 40.67; H, 1.71; N, 39.53; S, 18.10. Found: C, 40.75; H, 1.76; N, 39.19; S, 18.72.

General Procedure for the Preparation of 9-Alkyl-6-thiocyanatopurines (2–10) K_2CO_3 (0.39 g, 0.0028 mol) was suspended in a stirred solution of 6-thiocyanato-9*H*-purine (0.50 g, 0.0028 mol) in *N*,*N*-dimethylformamide (DMF) (30 mL). The appropriate alkyl halide (0.0028 mol) was added to the mixture and the reaction mixture was stirred for 24 h at room temperature. 20 mL water was added and the crude product was collected by filtration. The product was purified by column chromatography on silica gel using 1 : 1 ethyl acetate–hexane and recrystallized from ethyl acetate.

Synthesis of 9-Methyl-6-thiocyanato-9*H*-purine (2) The compound was prepared from methyl iodide as described above. Yield 30%, mp 179–180°C. IR (KBr) cm^{-1}: 2169 (–CN), 2934 (–C–H), 3063 (Ar-H). ^{1}H-NMR (DMSO-d_6) δ: 3.90 (s, 3H, –N–CH_3), 8.75 (s, 1H, Ar-H), 8.90 (s, 1H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 30.57, 106.95, 131.40, 148.10, 148.55, 150.96, 152.43. HR-MS for $C_7H_6N_5S^+$ ($[M-H]^+$) Calcd: 192.0344; Found: 192.0353.

Synthesis of 9-Ethyl-6-thiocyanato-9*H*-purine (3) The compound was prepared from ethyl iodide as described above. Yield 51%, mp 156–157°C. IR (KBr) cm^{-1}: 2171 (–CN), 2980 (–C–H), 3076 (Ar-H). ^{1}H-NMR (DMSO-d_6) δ: 1.47 (t, 3H, J=7,3 Hz, –CH_3), 4.34 (q, 2H, J=7,3 Hz, –N–CH_2), 8.76 (s, 1H, Ar-H) 8.95 (s, 1H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 15.25, 39.70, 106.82, 131.54, 147.57, 148.18, 150.38, 152.27. *Anal.* Calcd for $C_8H_7N_5S$: C, 46.82; H, 3.44; N, 34.12; S, 15.62. Found: C, 46.89; H, 3.42; N, 34.04; S, 15.44.

Synthesis of 9-Propyl-6-thiocyanato-9*H*-purine (4) The compound was prepared from 1-bromopropane as described above. Yield 51%, mp 134–136°C. IR (KBr) cm^{-1}: 2166 (–CN), 2966 (–C–H), 3099 (Ar-H). ^{1}H-NMR (DMSO-d_6) δ: 0.86 (t, 3H, J=7,4 Hz, –CH_3), 1.88 (m, 2H, –CH_2), 4.28 (t, 2H, J=7,1 Hz, –N–CH_2), 8.76 (s, 1H, Ar-H), 8.94 (s, 1H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 11.56, 23.15, 45.57, 106.87, 131.51, 147.96, 148.30, 150.65, 152.36. *Anal.* Calcd for $C_9H_9N_5S$: C, 49.30; H, 4.14; N, 31.94; S, 14.62. Found: C, 49.33; H, 4.09; N, 31.66; S, 14.30.

Synthesis of 9-Isopropyl-6-thiocyanato-9*H*-purine (5) The compound was prepared from 2-bromopropane as described above. Yield 68%, mp 118–119°C. IR (KBr) cm^{-1}: 2167 (–CN), 2985 (–C–H), 3057 (Ar-H). ^{1}H-NMR (DMSO-d_6) δ: 1.59 (d, 6H, J=6,8 Hz, –CH_3), 4.91 (m, 1H, –C–H), 8.84 (s, 1H, Ar-H), 8.94 (s, 1H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 21.52, 48.38, 106.92, 131.88, 146.22, 148.31, 150.12, 152.15. *Anal.* Calcd for $C_9H_9N_5S$: C, 49.30; H, 4.14; N, 31.94; S, 14.62. Found: C, 49.24; H, 4.16; N, 31.91; S, 14.69.

Synthesis of 9-Hexyl-6-thiocyanato-9*H*-purine (6) The compound was prepared from 1-bromohexane as described above. Yield 79%, mp 80–82°C. IR (KBr) cm^{-1}: 2170 (–CN), 2957 (–C–H), 3097 (Ar-H). ^{1}H-NMR (DMSO-d_6) δ: 0.81 (m, 3H, –CH_3), 1.24 (m, 6H, –CH_2), 1.85 (m, 2H, –CH_2), 4.28 (t, 2H, J=7,1 Hz, –N–CH_2), 8.74 (s, 1H, Ar-H), 8.92 (s, 1H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 14.22, 22.23, 26.02, 29.40, 31.02, 44.23, 106.82, 131.50, 147.96, 148.31, 150.61, 152.35. HR-MS for $C_{12}H_{16}N_5S^+$ ($[M-H]^+$) Calcd: 262.1126; Found: 262.1123.

Synthesis of 9-Benzyl-6-thiocyanato-9*H*-purine (7) The compound was prepared from benzyl bromide as described above. Yield 43%, mp 131–133°C. IR (KBr) cm^{-1}: 2164 (–CN), 2923 (–C–H), 3062 (Ar-H). ^{1}H-NMR (DMSO-d_6) δ: 5.61 (s, 2H, –N–CH_2), 7.42 (m, 5H, Ar-H), 8.94 (s, 1H, Ar-H), 9.01 (s, 1H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 47.47, 106.88, 128.18, 128.53, 129.24, 131.54, 136.38, 147.87, 148.66, 150.51, 152.67. *Anal.* Calcd for $C_{13}H_9N_5S$: C, 58.41; H, 3.39; N, 26.20; S, 12.00. Found: C, 58.35; H, 3.44; N, 26.14; S, 11.31.

Synthesis of Ethyl 2-(6-Thiocyanato-9*H*-purin-9-yl)acetate (8) The compound was prepared from ethyl bromoacetate as described above. Yield 66%, mp 115–116°C. IR (KBr) cm^{-1}: 1730 (–C=O ester), 2167 (–CN), 2945 (–C–H), 3059 (Ar-H). ^{1}H-NMR (DMSO-d_6) δ: 1.22 (t, 3H, J=7,1 Hz, –CH_3), 4.19 (q, 2H, J=7,1 Hz, –O–CH_2), 5.30 (s, 2H, –N–CH_2), 8.71 (s, 1H, Ar-H), 8.96 (s, 1H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 14.43, 45.09, 62.18, 106.85, 131.16, 148.34, 148.80, 150.76, 152.82, 167.76. *Anal.* Calcd for $C_{10}H_9N_5O_2S$: C, 45.62; H, 3.45; N, 26.60; S, 12.18. Found: C, 45.56; H, 3.35; N, 26.61; S, 12.23.

Synthesis of 9-(Cyclobutylmethyl)-6-thiocyanato-9*H*-purine (9) The compound was prepared from (bromomethyl)-cyclobutane as described above. Yield 76%, mp 128–130°C. IR (KBr) cm^{-1}: 2167 (–CN), 2969 (–C–H), 3070 (Ar-H).

^{1}H-NMR (DMSO-d_6) δ: 1.83 (m, 2H, –CH_2), 1.96 (m, 4H, –CH_2), 2.84 (m, 1H, –C–H), 4.32 (d, 2H, J=7,5 Hz, –N–CH_2), 8.73 (s, 1H, Ar-H), 8.92 (s, 1H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 17.90, 25.47, 35.07, 48.94, 106.88, 131.40, 148.23, 148.38, 150.69, 152.43. HR-MS for $C_{11}H_{12}N_5S^+$ ([M−H]$^+$) Calcd: 246.0813; Found: 246.0813.

Synthesis of 9-(Cyclohexylmethyl)-6-thiocyanato-9*H*-purine (10) The compound was prepared from (bromomethyl)cyclohexane as described above. Yield 20%, mp 152–153°C. IR (KBr) cm^{-1}: 2167 (–CN), 2923 (–C–H), 3093 (Ar-H). ^{1}H-NMR (DMSO-d_6) δ: 1.10 (m, 2H, –CH_2), 1.50 (m, 4H, –CH_2), 1.63 (m, 4H, –CH_2), 1.90 (m, 1H, –C–H), 4.15 (d, 2H, J=7,2 Hz, –N–CH_2), 8.71 (s, 1H, Ar-H), 8.92 (s, 1H, Ar-H). ^{13}C-NMR (DMSO-d_6) δ: 25.45, 26.14, 30.31, 37.97, 49.93, 106.92, 131.45, 148.24, 148.39, 150.86, 152.45. HR-MS for $C_{13}H_{16}N_5S^+$ ([M−H]$^+$) Calcd: 274.1126; Found: 274.1120.

Antibacterial and Antifungal Testing Method The *in vitro* antibacterial activity of 9-substituted 6-thiocyanatopurine derivatives (compounds **1**–**10**) was performed against American Type Culture Collection (ATCC) reference bacterial strain and fungi by disk diffusion technique.[29,30] Gram-negative and Gram-positive bacteria were grown in nutrient agar medium and incubated at 37°C for 24 h. The yeast strains were grown in Sabouraud dextrose agar medium and incubated at 27°C for 72 h. Chloramphenicol and ampicillin were used as the reference antibacterial agents and ketoconazole was used as a reference antifungal agent under similar conditions for comparison. All the testings were repeated for three times, and the mean values were calculated. The minimum inhibitory concentrations (MIC) of the synthesized compounds were determined by using microdilution method.[29,30]

DNA and Compound Interaction The interaction of the compounds **1**–**10** with pBR322 plasmid DNA was studied by agarose gel electrophoresis.[33,34] pBR322 DNA were incubated with decreasing concentrations of compounds ranging from 5000 to 312 μM in a shaking water bath at 37°C for 24 h and electrophoresed in 1% agarose gel. Electrophoresis was carried under 1× TAE (Tris acetic acid EDTA) buffer for approximately 3 h at 60 V. At the end of the electrophoresis, the gel was stained with ethidium bromide. DNA was observed by UV-transillumination.

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References

1) Strouse J. J., Jeŝelnik M., Arterburn J. B., *Acta Chim. Slov.*, **52**, 187–199 (2005).
2) Lech-Maranda E., Korycka A., Robak T., *Mini Rev. Med. Chem.*, **6**, 575–581 (2006).
3) Hu Y. L., Ge Q., Lu M., Lu H. F., *Bull. Chem. Soc. Ethiop.*, **24**, 425–432 (2010).
4) Lino Carmo A. M., Braga F. G., De Paula M. L., Ferreira A. P., Teixeira H. C., Da Silva A. D., Coimbra E. S., *Lett. Drug Des. Discov.*, **5**, 122–126 (2008).
5) Rida S. M., Ashour F. A., El-Hawash S. A. M., El-Semary M. M., Badr M. H., *Arch. Pharm. Chem. Life Sci.*, **340**, 185–194 (2007).
6) Steurer M., Pall G., Richards S., Schwarzer G. Bohlius J., Greil R. on behalf of the Cochrane Haematologic Malignancies Group, *Cancer Treat. Rev.*, **32**, 377–389 (2006).
7) Zaza G., Yang W., Kager L., Cheok M., Downing J., Pui C. H., Cheng C., Relling M. V., Evans W. E., *Blood*, **104**, 1435–1441 (2004).
8) Dolan M. E., Pegg A. E., *Clin. Cancer Res.*, **3**, 837–847 (1997).
9) Mitra S., Kaina B., *Prog. Nucleic Acid Res. Mol. Biol.*, **44**, 109–142 (1993).
10) Morimoto K., Dolan M. E., Scicchitano D., Pegg A. E., *Carcinogenesis*, **6**, 1027–1031 (1985).
11) Robak T., Lech-Maranda E., Korycka A., Robak E., *Curr. Med. Chem.*, **13**, 3165–3189 (2006).
12) Jayakanthan K., Johnston B. D., Pinto B. M., *Carbohydr. Res.*, **343**, 1790–1800 (2008).
13) Hocek M., Holý A., Votruba I., Dvoráková H., *J. Med. Chem.*, **43**, 1817–1825 (2000).
14) Pesić M., Podolski A., Rakić L., Ruzdijić S., *Invest. New Drugs*, **28**, 482–492 (2010).
15) Nair V., Young D. A., *Synthesis*, **6**, 450–453 (1986).
16) Akiyama S. I., Kuwano M., Komiyama S., Saneyoshi M., *Cancer Lett.*, **9**, 305–311 (1980).
17) Harada H., Asano O., Hoshino Y., Yoshikawa S., Matsukura M., Kabasawa Y., Niijima J., Kotake Y., Watanabe N., Kawata T., Inoue T., Horizoe T., Yasuda N., Minami H., Nagata K., Murakami M., Nagaoka J., Kobayashi S., Tanaka I., Abe S., *J. Med. Chem.*, **44**, 170–179 (2001).
18) Camaioni E., Costanzi S., Vittori S., Volpini R., Klotz K. N., Cristalli G., *Bioorg. Med. Chem.*, **6**, 523–533 (1998).
19) Česnek M., Holý A., Masojídková M., Zídek Z., *Bioorg. Med. Chem.*, **13**, 2917–2926 (2005).
20) Thompson R. D., Secunda S., Daly J. W., Olsson R. A., *J. Med. Chem.*, **34**, 2877–2882 (1991).
21) Havlícek L., Hanus J., Veselý J., Leclerc S., Meijer L., Shaw G., Strnad M., *J. Med. Chem.*, **40**, 408–412 (1997).
22) Bradner W. T., Clarke D. A., *Cancer Res.*, **18**, 299–304 (1958).
23) Saneyoshi M., Tokuzen R., Maeda M., Fukuoka F., *Chem. Pharm. Bull.*, **16**, 505–508 (1968).
24) Saneyoshi M., Chihara G., *Chem. Pharm. Bull.*, **15**, 909–914 (1967).
25) Platzer N., Galons H., Bensaid Y., Miocque M., Bram G., *Tetrahedron*, **43**, 2101–2108 (1987).
26) Shugar D., *Pure Appl. Chem.*, **57**, 423–440 (1985).
27) Martin J. C., Smee D. F., Verheyden J. P. H., *J. Org. Chem.*, **50**, 755–759 (1985).
28) Elion G. B., Goodman I., Lange W., Hitchings G. H., *J. Am. Chem. Soc.*, **81**, 1898–1902 (1959).
29) "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard (M7-A6)," 6th ed., The National Committee for Clinical Laboratory Standards (NCCLS), Wayne, 2003.
30) "Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard (M27-A3)," 3rd ed., Clinical and Laboratory Standards Institute, Wayne, 2008.
31) Palchaudhuri R., Hergenrother P. J., *ACS Chem. Biol.*, **6**, 21–33 (2011).
32) Palchaudhuri R., Hergenrother P. J., *Cur. Opin. Biotech.*, **18**, 497–503 (2007).
33) Asmafiliz N., Kiliç Z., Oztürk A., Hökelek T., Koç L. Y., Açik L., Kısa O., Albay A., Ustündağ Z., Solak A. O., *Inorg. Chem.*, **48**, 10102–10116 (2009).
34) Huq F., Tayyem H., Yu J. Q., Beale P., Fisher K., *Med. Chem.*, **5**, 372–381 (2009).