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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of some novel amino and thiotetrazole purine derivatives and investigation of their antimicrobial activity and DNA interactions

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Abstract A series of amino and thiotetrazole purine derivatives introduced with different alkyl groups in position 9 was synthesized. The structures of the synthesized compounds were characterized using spectroscopic methods. 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. The effect of the compounds on pBR322 plasmid DNA was studied by gel electrophoretic mobility measurements. The results of antimicrobial activity show that attachment of tetrazole group to purine bases results in disappearance of antimicrobial activity The results of the plasmid DNA interaction and the restriction studies suggest that while aminotetrazole purine derivatives cause DNA damages, thiotetrazole purine derivatives are believed to form a range of interstrand GG adducts with duplex DNA that induce global changes in the DNA conformation.

Keywords Tetrazole · Purine derivative · Antimetabolite · Antimicrobial activity · DNA interaction

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Introduction

Bioisosteric replacement of a functional group is a widely used technique in medicinal chemistry for the generation of more selective and more potent analog of a lead compound (Matta *et al.*, 2010). Tetrazoles are of particular interest to the medicinal chemists since they constitute the most commonly used bioisostere of the carboxylate moiety (Herr, 2002). Like their carboxylic acid counterparts, tetrazoles are ionized at physiological pH and they are more lipophilic than the corresponding carboxylates while having similar acidity (Herr, 2002). The interchange of carboxylic acid for tetrazole often results in useful drugs.

Tetrazoles are screened for various biological activities such as antiviral, antibacterial, antifungal, antiallergic, antiulcer, anticonvulsant, anti-inflammatory, and antitubercular activities (Shemyakina *et al.*, 2011; Kumar *et al.*, 2011; Chermahini *et al.*, 2008; Mohite and Bhaskar, 2011). 1,5-substituted tetrazoles are known for their pharmaceutical activities as stimulants or depressants on the central nervous system (Shemyakina *et al.*, 2011; Mohite and Bhaskar, 2010). Tetrazoles are currently used in cancer and AIDS treatment (Tamura *et al.*, 1998; Abell and Foulds, 1997).

Purine analogs are of great importance to chemists as well as to biologists as they possess antitubercular, antiulcer, antimicrobial, antifungal, antineoplastic, antitumor, antiviral, and cardiotonic properties (Hu *et al.*, 2010; Carmo *et al.*, 2008; Rida *et al.*, 2007; .Steurer *et al.*, 2006; Dolan and Pegg, 1997; Mitra and Kaina, 1993; Morimoto *et al.*, 1985). The purine antimetabolites are also used in the treatment of autoimmune diseases (Robak *et al.*, 2006). Current evidence indicates that purine analogs alone or in combination with other chemotherapeutic agents are very effective against almost any fast-growing cells (Rida *et al.*,

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2007). Therefore, attention has been drawn to the structural modification of purine bases as well as introduction of different functional groups into these bases. Looking at the intense research activity in the tetrazole field, it was thought the combined effects of purine and tetrazole pharmacophores could result in interesting biological activity. Therefore, this study describes the synthesis of some novel amino and thiotetrazole purine derivatives, and evaluation of their antimicrobial activities and DNA interactions.

Result and discussion

Synthesis

Treatment of 6-chloropurine with some appropriate alkyl halides in the presence of potassium carbonate generated 6-chloro-9-alkyl-9*H*-purines. The structures of 6-chloro-9-alkyl-9*H*-purines were confirmed by comparing their physical and spectral data with the reported ones (Okamura *et al.*, 2007; Lambertucci *et al.*, 2009; Hanna *et al.*, 1994; Montgomery and Temple, 1961).

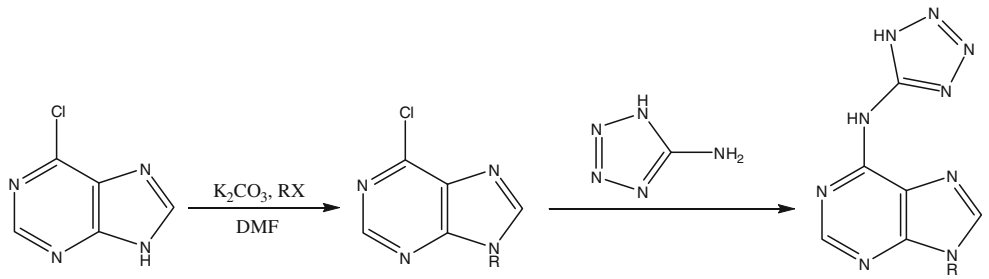
9-Alkyl-*N*-(1*H*-tetrazol-5-yl)-9*H*-purin-6-amines **(1–9)** were prepared by refluxing 6-chloro-9-alkyl-9*H*-purines with 5-aminotetrazole in ethanol (Scheme 1). The products were separated and purified using column chromatography to provide compounds **1–9**. The structures of compounds **1–9** were confirmed by FTIR, ^{1}H-NMR, ^{13}C-NMR, and MS spectra. All spectroscopic data were in accordance with the assigned structures. IR spectra of the synthesized compounds exhibited a band in the region of 3,300–3,400 cm^{-1} which could be assigned to the characteristic NH absorption band of tetrazole ring. NH absorption band appeared as a broad band due to tautomeric structure of the tetrazole. ^{1}H-NMR spectra showed two singlets at 11.90 and 15.80 ppm due to NH protons of aminotetrazole. The MS spectra also complied with the expected values.

9-Alkyl-6-(1-methyl-1*H*-tetrazol-5-ylthio)-9*H*-purines (**10–19**) were synthesized by refluxing 6-chloro-9-alkyl-9*H*-purines with 1-methyl-5-mercaptotetrazole in dichloromethane (Scheme 2). The structures of compounds **10–19** were confirmed by FTIR, ^{1}H-NMR, ^{13}C-NMR, and MS spectra and all spectroscopic data were in accordance with the assigned structures. ^{1}H-NMR spectra showed a singlet at 4.10 ppm due to methyl protons of thiotetrazole. All the target compounds (**1–19**) 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 (Bradner and Clarke, 1958). Therefore, all the synthesized compounds were tested for their antimicrobial activities.

The in vitro antibacterial activities of compounds **1–19** 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, *E. coli* ATCC 25922, and *Proteus vulgaris* ATCC 8427 by disk diffusion technique (Clinical and Laboratory Standards Institute, 2006, 2007). In addition, the in vitro antifungal activities of compounds **1–19** were undertaken against *Candida albicans* ATCC 10231 and *Candida tropicalis* ATCC 13803. Chloramfenicol and ampicillin were used as the reference antibacterial agents, and ketoconozole was used as a reference antifungal agent under similar conditions for comparison.

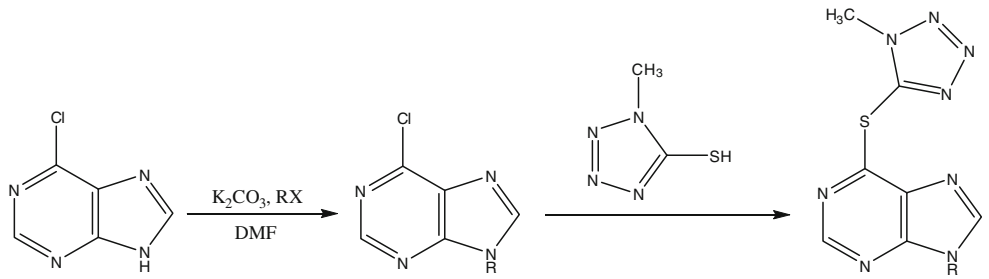


1-9

R= (**1**: -H; **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**: -$CH_2C_4H_7$).

Scheme 1 Synthesis of aminotetrazole purine derivatives **1–9. R** = (**1** –H, **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** –$CH_2C_4H_7$)



10-19

R= (**10**: -H; **11**: -CH_3; **12**: -C_2H_5; **13**: -C_3H_7; **14**: -$CH(CH_3)_2$ **15**: -C_6H_{13}; **16**: -$CH_2C_6H_5$;

17: - $CH_2COOC_2H_5$; **18**: -$CH_2C_4H_7$; **19**: -$CH_2C_6H_{11}$).

Scheme 2 Synthesis of thiotetrazole purine derivatives **10–19**. **R** = (**10** –H, **11** –CH_3, **12** –C_2H_5, **13** –C_3H_7, **14** –$CH(CH_3)_2$, **15** –C_6H_{13}, **16** –$CH_2C_6H_5$, **17** –$CH_2COOC_2H_5$, **18** –$CH_2C_4H_7$, **19** –$CH_2C_6H_{11}$)

Dimethyl sulfoxide (DMSO) was used as the control. The results show that none of the synthesized compounds displayed antimicrobial activity against tested bacteria and fungi.

Since a carboxylic acid group can bioisosterically be replaced by a tetrazole, compounds **1–9** could be interpreted as a potential bioisostere of the corresponding purine-6-carbamic acid derivatives and compounds **10–19** could be interpreted as a potential bioisostere of the corresponding *O*-alkyl-*S*-purin-6-yl ester derivatives, yet the compounds **1–19** failed to exhibit antimicrobial activity against tested bacteria and fungi.

DNA and compound interactions

Small molecules that bind to DNA are useful biochemical tools for the visualization of DNA in vitro (Palchaudhuri and Hergenrother, 2011). Many antitumor, antineoplastic, antimalarial, antibiotic, and antifungal agents are the small compounds that interact with DNA (Palchaudhuri and Hergenrother, 2007). The interaction of DNA with compounds could convert covalently supercoiled form I DNA into singly nicked circular form II, and then into linear form III DNA (Asmafiliz *et al.*, 2009; Huq *et al.*, 2009). This cleavage reaction could be monitored by gel electrophoresis. Therefore, interactions of compounds **1–19** with supercoiled pBR322 plasmid DNA have been studied. The compounds were incubated over a range of concentrations with supercoiled pBR322 plasmid DNA in the dark at 37 °C for 24 h. The DNA cleavage by these compounds has been examined by observing the conversion of supercoiled DNA to the open circular form II and linear form III DNA. Figure 1 shows the electrophoretograms for the interaction of pBR322 plasmid DNA with compounds **1–19** at concentrations of compounds ranging from 5,000 to 312 μM. Lane P applies to the untreated pBR322 plasmid DNA (control DNA), showing the major supercoiled (form I) and minor nicked (form II) forms. Lanes 1–5 apply to pBR322 plasmid DNA incubated with the compounds with concentrations ranging from 5,000 to 312 μM.

As pBR322 plasmid DNA was allowed to interact with decreasing concentrations of compounds **1**, **2**, **3**, **5**, and **6**, the mobility of both form I and form II bands remained essentially unchanged. On the other hand, the intensity of both form I and form II bands changed at high concentrations. It was found that although the unreacted DNA was not very intense, there was a pronounced increase in the intensity of form I band at all concentrations of the compounds. The increase in intensity of the band may be due to the covalent binding of the compounds. Moreover, there were small DNA fragments on the gel. These small DNA pieces could be the result of DNA cleavage.

The initial sharp increase in the intensity of the form II band due to the presence of a small amount of compounds points to DNA damage in which form I DNA is changed to form II DNA. The decrease in intensity at higher concentrations of compounds is due to a partial damage of the DNA caused by the covalent binding of the compounds.

When the pBR plasmid DNA interacted with decreasing concentrations of compound **4**, the mobility of form I decreased slightly in all concentrations tested and small DNA bands appeared. In the case of compounds **7**, **8**, and **9**, the mobility of form I DNA decreased with increasing concentrations of the compounds, and there were small DNA fragments on the gel of compounds **8** and **9**. As pBR322 plasmid DNA was allowed to interact with compound **10**, the mobility of form I gradually decreased with increasing concentration of the compound. In the case of compounds **11** and **12**, opposite effect was seen. When the pBR322 plasmid DNA interacted with compounds **11** and **12**, mobility of form I increased with increasing concentrations of the compounds. At high concentrations of compound **12**, form II

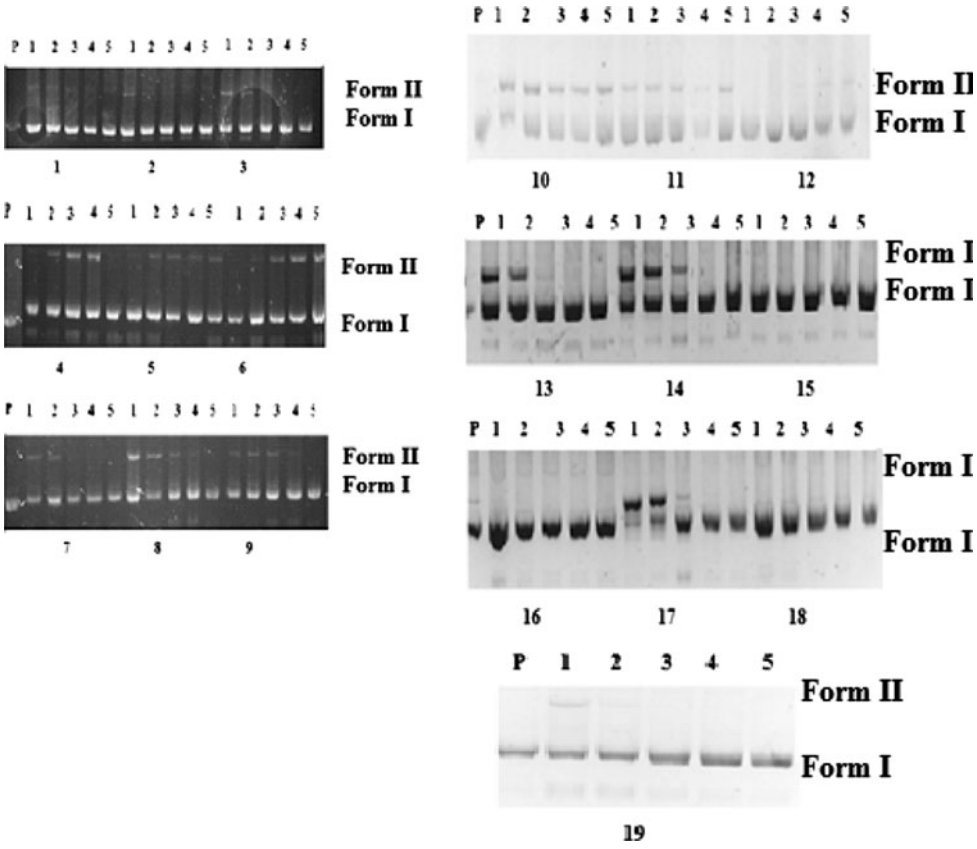


Fig. 1 Gel electrophoretic mobility of pBR322 plasmid DNA when incubated with various concentrations of compounds **1**–**19**. Concentrations (in μM) as follows: *lane P*, untreated pBR322 plasmid DNA; *lane 1*, 5000; *lane 2*, 2500; *lane 3*, 1250; *lane 4*, 625; *lane 5*, 312. The *top* and the *bottom* bands correspond to form II (singly-nicked) and form I (covalently closed circular) plasmids, respectively

band was disappeared. The disappearance of form II band is due to the damage of the DNA caused by the covalent binding of the compound. In the case of compounds **13** and **14**, the mobility of form I changed slightly and small DNA fragments were observed. Furthermore, the intensity of form II bands decreased with decreasing concentration of the compounds. This comfirms cleavage of the two strands of the DNA. When the pBR322 plasmid DNA interacted with decreasing concentrations of compound **15**, the mobility of form I decreased and form II band was disappeared. In the case of compounds **16**, **17**, and **18**, it was observed that the intensity of form II bands changed with the concentrations of the compounds. Moreover, there were small DNA fragments on the gel. The plasmid DNA interaction with these compounds lead to DNA cleavage. Compound **19** also changes the intensities of both form I and form II. While the intensity of form I increased, the intensity of form II decreased at low concentrations. The concentrations required to partial damage of the DNA caused by covalent binding of the compound were 312, 625, and 1250 μM. There were also small DNA fragments on the gel.

In order to find out whether the synthesized compounds bind or cleave DNA, restriction analyses were performed (Huq *et al.*, 2004; Gümüş *et al.*, 2009). The compound–DNA adducts were digested with *Bam*HI and *Hin*dIII enzymes. *Bam*HI is known to recognize the sequence 5′-G/GATCC-3′ and hydrolyze the phosphodiester bond between adjacent guanine sites (Huq *et al.*, 2004). pBR322 contains a single restriction site for *Bam*HI, which converts pBR322 plasmid DNA from supercoiled form I and singly nicked circular form II to linear form III DNA. *Hin*dIII enzymes bind at the recognition sequence 5′-A/AGCTT-3′ and cleave the sequence just after 5′-adenine site and, as a result, convert form I and form II DNA to linear form III DNA (Gümüş *et al.*, 2009). In order to assess whether the synthesized compounds show affinity toward guanine–guanine (GG) and/or adenine–adenine (AA) regions, we carried out restriction endonuclease analysis of the compound–pBR322 plasmid DNA adducts digested by *Bam*HI and *Hin*dIII enzymes. Only one concentration for each compound was used for the restriction analysis (1,250 μM). Figure 2 shows the electrophoretograms applying to incubated mixtures of pBR322 plasmid DNA and compounds **1**–**19** followed by their restriction with *Bam*HI (Fig. 2a, c) and *Hin*dIII (Fig. 2b, d). Lane P applies to the untreated and undigested pBR322 plasmid DNA, Lane P + B and P + H apply to pBR322 plasmid DNA restricted with enzymes *Bam*HI and *Hin*dIII, respectively. Lanes 1–19 apply to pBR322 plasmid DNA interacted with the compounds **1**–**19** followed by their digestion with *Bam*HI (Fig. 2a, c) and *Hin*dIII (Fig. 2b, d).

When untreated, pBR322 plasmid DNA was digested with *Bam*HI and *Hin*dIII, only one band corresponding to

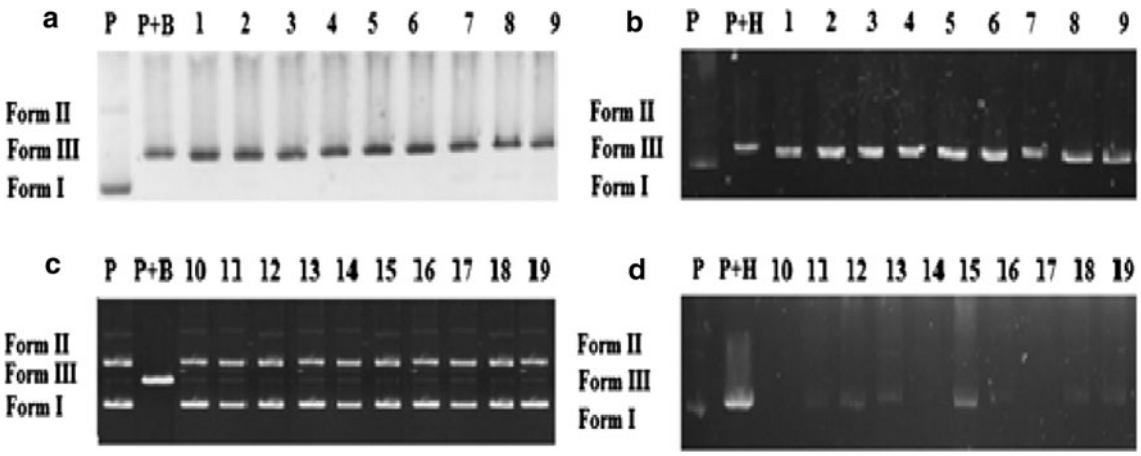


Fig. 2 Electrophoretograms applying to incubated mixtures of pBR322 plasmid DNA and compounds **1–19** followed by their restriction with *Bam*HI (**a**, **c**) and *Hin*dIII (**b**, **d**). *Lane P*, untreated and undigested pBR322 plasmid DNA; *lane* $P + B$ and $P + H$ pBR322 plasmid DNA restricted with enzymes *Bam*HI and *Hin*dIII, respectively; *lanes 1–19,* pBR322 plasmid DNA interacted with the compounds **1–19** followed by their digestion with *Bam*HI (**a**, **c**) and *Hin*dIII (**b**, **d**)

form III band was observed. In the untreated and undigested pBR322 plasmid DNA, generally two bands corresponding to form I and form II were observed (form I band has the highest velocity, form II has the lowest velocity, form III band has the intermediate velocity).

In the case of incubated mixtures of pBR322 plasmid DNA and compounds **10–19** followed by their restriction with *Bam*HI (Fig. 2c), two bands corresponding to form I and form II were observed. This result shows that compounds **10–19** prevent digestion with *Bam*HI enzyme. This may be due to the conformational change in the DNA brought about by the covalent binding of the compounds with the plasmid DNA. On the other hand, *Bam*HI digestion at the specific GG site is not prevented in the presence of compounds **1–9** (Fig. 2a). All the synthesized compounds also inhibit *Hin*dIII enzyme activity (Fig. 2b, d).

Conclusion

Several amino and thiotetrazole purine derivatives were synthesized and antimicrobial activities of the synthesized compounds were investigated. The results of antimicrobial activity show that attachment of tetrazole group to purine bases results in disappearance of antimicrobial activity. Level of binding with DNA and nature of interaction with pBR322 plasmid DNA have also been determined. The prevention of *Bam*HI digestion of form I and form II pBR322 plasmid DNA with thiotetrazole purine derivatives is believed to be due to interstrand binding that brings about global changes in DNA conformation. On the other hand, *Hin*dIII enzyme cuts all of the compounds interacted with DNA.

Experimental

Chemistry

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. IR spectra (4,000–400 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-NMR and 75 MHz for ^{13}C-NMR). Chemical shifts were reported in δ ppm units with respect to tetramethylsilane (TMS) as an internal reference in DMSO-d_6 or $CDCl_3$ 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.

General procedure for the preparation of 6-chloro-9-alkyl-9H-purines

K_2CO_3 (1.79 g, 0.013 mol) was suspended in a stirred solution of 6-chloropurine (2.0 g, 0.013 mol) in DMF (30 ml). The appropriate alkyl halide (0.013 mol) was added to the reaction mixture and the resultant was stirred at room temperature for 24 h. The solvent was evaporated

under reduced pressure. The mixture was poured into water (50 ml), and extracted with chloroform (4 × 25 ml). The combined organic layer was dried over $MgSO_4$ and filtered. The solvent was evaporated in vacuo, and the residue was purified by column chromatography on silica gel using 1:1 ethyl acetate–hexane as eluent.

Synthesis of 6-chloro-9-methyl-9H-purine The compound was prepared from methyl iodide (1.84 g, 0.013 mol) as described above and recrystallized from diethyl ether. Yield 45 %, mp 137–138 °C. IR (KBr) cm^{-1}: 2922 (–C–H), 3078 (Ar–H). ^{1}H-NMR (DMSO-d_6) δ: 3.83 (s, 3H, –N–CH_3), 8.64 (s, 1H, Ar–H), 8.75 (s, 1H, Ar–H). ^{13}C-NMR (DMSO-d_6) δ: 30.57, 131.08, 148.49, 149.21, 151.82, 152.65. HR-MS for $C_6H_6ClN_4^+$ ($[M-H]^+$) Calcd: 169.0281; Found: 169.0285.

Synthesis of 6-chloro-9-ethyl-9H-purine The compound was prepared from ethyl iodide (2.02 g, 0.013 mol) as described above and recrystallized from ethyl acetate–hexane. Yield 48 %, mp 78–80 °C. IR (KBr) cm^{-1}: 2938 (–C–H), 3079 (Ar–H). ^{1}H-NMR (DMSO-d_6) δ: 1.46 (t, 3H, $J = 7.3$ Hz, –CH_3), 4.32 (q, 2H, $J = 7.3$ Hz, –N–CH_2), 8.73 (s, 1H, Ar–H) 8.76 (s, 1H, Ar–H). ^{13}C-NMR (DMSO-d_6) δ: 15.20, 39.47, 131.21, 147.40, 149.27, 151.59, 152.02. HR-MS for $C_7H_8ClN_4^+$ ($[M-H]^+$) Calcd: 183.0437; Found: 183.0437.

Synthesis of 6-chloro-9-propyl-9H-purine The compound was prepared from 1-bromopropane (1.60 g, 0.013 mol) as described above. Yield 63 %. IR (KBr) cm^{-1}: 2935 (–C–H), 3073 (Ar–H). ^{1}H-NMR (DMSO-d_6) δ: 0.81 (t, 3H, $J = 7.4$ Hz, –CH_3), 1.84 (m, 2H, –CH_2), 4.22 (t, 2H, $J = 7.1$ Hz, –N–CH_2), 8.68 (s, 1H, Ar–H), 8.69 (s, 1H, Ar–H). ^{13}C-NMR (DMSO-d_6) δ: 11.26, 22.90, 45.79, 131.20, 147.86, 149.37; 151.73, 152.32. HR-MS for $C_8H_{10}ClN_4^+$ ($[M-H]^+$) Calcd: 197.0594; Found: 197.0589.

Synthesis of 6-chloro-9-isopropyl-9H-purine The compound was prepared from 2-bromopropane (1.60 g, 0.013 mol) as described above and recrystallized from ethyl acetate. Yield 32 %, mp 108–109 °C. IR (KBr) cm^{-1}: 2933 (–C–H), 3069 (Ar–H).). ^{1}H-NMR (DMSO-d_6) δ: 1.58 (d, 6H, $J = 6.8$ Hz, –CH_3), 4.89 (m, 1H, –C–H), 8.70 (s, 1H, Ar–H), 8.80 (s, 1H, Ar–H). ^{13}C-NMR (DMSO-d_6) δ: 22.28, 48.31, 131.63, 146.19, 149.42, 151.63, 151.88. HR-MS for $C_8H_{10}ClN_4^+$ ($[M-H]^+$) Calcd: 197.0594; Found: 197.0591.

Synthesis of 6-chloro-9-hexyl-9H-purine The compound was prepared from 1-bromohexane (2.13 g, 0.013 mol) as described above and recrystallized from ethyl acetate. Yield 50 %, mp 60–62 °C. IR (KBr) cm^{-1}: 2929 (–C–H), 3099 (Ar–H).). ^{1}H-NMR (DMSO-d_6) δ: 0.78 (m, 3H, –CH_3), 1.23 (m, 6H, –CH_2), 1.83 (m, 2H, –CH_2), 4.27 (t, 2H, $J = 7.1$ Hz, –N–CH_2), 8.71 (s, 1H, Ar–H), 8.74 (s, 1H, Ar–H). ^{13}C-NMR (DMSO-d_6) δ: 14.20, 22.32, 26.02, 29.40, 31.02, 44.23, 131.24, 147.92, 149.40, 151.82, 152.36. HR-MS for $C_{11}H_{16}ClN_4^+$ ($[M-H]^+$) Calcd: 239.1063; Found: 239.1057.

Synthesis of 6-chloro-9-benzyl-9H-purine The compound was prepared from benzyl bromide (2.21 g, 0.013 mol) as described above and recrystallized from hexane. Yield 51 %, mp 80–82 °C. IR (KBr) cm^{-1}: 2939 (–C–H), 3062 (Ar–H). ^{1}H-NMR (DMSO-d_6) δ: 5.33 (s, 2H, –N–CH_2), 7.33 (m, 5H, Ar–H), 8.79 (s, 1H, Ar–H), 8.85 (s, 1H, Ar–H). ^{13}C-NMR (DMSO-d_6) δ: 47.49, 128.13, 128.47, 129.21, 131.29, 136.47, 147.90, 149.63, 152.16, 152.26. HR-MS for $C_{12}H_{10}ClN_4^+$ ($[M-H]^+$) Calcd: 245.0594; Found: 245.0590.

Synthesis of ethyl 2-(6-chloro-9H-purin-9-yl)acetate The compound was prepared from ethyl bromoacetate (2.16 g, 0.013 mol) as described above and recrystallized from heptane. Yield 49 %, mp 89–90 °C. IR (KBr) cm^{-1}: 1737 (–C=O ester), 2930 (–C–H), 3094 (Ar–H). ^{1}H-NMR ($CDCl_3$) δ: 1.33 (t, 3H, $J = 7.1$ Hz, –CH_3), 4.30 (q, 2H, $J = 7.1$ Hz, –O–CH_2), 5.07 (s, 2H, –N–CH_2), 8.21 (s, 1H, Ar–H), 8.77 (s, 1H, Ar–H). ^{13}C-NMR ($CDCl_3$) δ: 14.07, 44.56, 62.70, 131.22, 145.45, 151.29, 151.92, 152.26, 166.44. HR-MS for $C_9H_{10}ClN_4O_2^+$ ($[M-H]^+$) Calcd: 241.0492; Found: 241.0485.

Synthesis of 6-chloro-9-(cyclobutylmethyl)-9H-purine The compound was prepared from (bromomethyl)cyclobutane (1.92 g, 0.013 mol) as described above. Yield 46 %, mp 52–55 °C. IR (KBr) cm^{-1}: 2974 (–C–H), 3092 (Ar–H). ^{1}H-NMR (DMSO-d_6) δ: 1.80 (m, 2H, –CH_2), 1.95 (m, 4H, –CH_2), 2.83 (m, 1H, –C–H), 4.49 (d, 2H, $J = 7.4$ Hz, –N–CH_2), 8.75 (s, 1H, Ar–H), 8.85 (s, 1H, Ar–H). ^{13}C-NMR (DMSO-d_6) δ: 17.89, 25.26, 36.21, 51.41, 122.38, 142.51, 151.18, 151.89, 161.95. HR-MS for $C_{10}H_{12}ClN_4^+$ ($[M-H]^+$) Calcd: 223.0750; Found: 223.0749.

Synthesis of 6-chloro-9-(cyclohexylmethyl)-9H-purine The compound was prepared from (bromomethyl)cyclohexane (2.29 g, 0.013 mol) as described above. Yield 49 %, mp 70–72 °C. IR (KBr) cm^{-1}: 2932 (–C–H), 3047 (Ar–H). ^{1}H-NMR ($CDCl_3$) δ: 1.15 (m, 2H, –CH_2), 1.21 (m, 4H, –CH_2), 1.74 (m, 4H, –CH_2), 1.90 (m, 1H, –C–H), 4.31 (d, 2H, $J = 7.2$ Hz, –N–CH_2), 8.22 (s, 1H, Ar–H), 8.88 (s, 1H, Ar–H). ^{13}C-NMR ($CDCl_3$) δ: 25.39, 25.95, 30.02, 39.50, 53.43, 122.44, 143.02, 149.48, 152.21, 161.95. HR-MS for $C_{12}H_{16}ClN_4^+$ ($[M-H]^+$) Calcd: 251.1063; Found: 251.1072.

*Synthesis of N-(1H-tetrazol-5-yl)-9H-purin-6-amine (**1**)* 6-Chloropurine (0.31 g, 2.0 mmol) was refluxed with 5-aminotetrazole (0.17 g, 2.0 mmol) in ethanol (25 ml) for 5 days. The solvent was evaporated in vacuo. The product was purified by column chromatography on silica gel using 2:1 ethyl acetate–methanol. Yield 51 %, mp >219 °C (decomp.). IR (KBr) cm^{-1}: 3053 (Ar–H), 3400–3300 (–N–H). ^{1}H-NMR (DMSO-d_6) δ: 8.40 (s, 1H, Ar–H), 8.60 (s, 1H, Ar–H), 11.90 (s, 1H, –N–H), 12.15 (s, 1H, –N–H), 13.50 (s, 1H, –N–H). ^{13}C-NMR (DMSO-d_6) δ: 152.31, 159.36, 158.47, 163.20, 166.96. HR-MS for $C_6H_6N_9^+$ ($[M-H]^+$) Calcd: 204.0746; Found: 204.0686.

*General procedure for the preparation of 9-alkyl-N-(1H-tetrazol-5-yl)-9H-purin-6-amine compounds (**2**–**9**)*

The appropriate 6-chloro-9-alkyl-9*H*-purine (2.0 mmol) was refluxed with 5-aminotetrazole (0.17 g, 2.0 mmol) in ethanol (25 ml) for 5 days. The solvent was evaporated in vacuo. The residue was purified by column chromatography on silica gel to give the corresponding aminotetrazole purine derivatives (**2**–**9**).

*Synthesis of 9-methyl-N-(1H-tetrazol-5-yl)-9H-purin-6-amine (**2**)* The compound was prepared from 6-chloro-9-methyl-9*H*-purine (0.34 g, 2.0 mmol) as described above and purified by column chromatography on silica gel using 2:1 ethyl acetate–hexane as eluent. Yield 35 %, mp >305 °C (decomp.). IR (KBr) cm^{-1}: 2979 (–C–H), 3073 (Ar–H), 3400–3300 (–N–H). ^{1}H-NMR (DMSO-d_6) δ: 3.83 (s, 3H, –N–CH_3), 8.43 (s, 1H, Ar–H), 8.58 (s, 1H, Ar–H), 11.97 (s, 1H, –N–H), 15.78 (s, 1H, –N–H). ^{13}C-NMR (DMSO-d_6) δ: 30.19, 120.18, 144.58, 149.47. HR-MS for $C_7H_8N_9^+$ ($[M-H]^+$) Calcd: 218.0903; Found: 218.0904.

*Synthesis of 9-ethyl-N-(1H-tetrazol-5-yl)-9H-purin-6-amine (**3**)* The compound was prepared from 6-chloro-9-ethyl-9*H*-purine (0.36 g, 2.0 mmol) as described above and purified by column chromatography on silica gel using 2:1 ethyl acetate–hexane as eluent. Yield 34 %, mp 277–278 °C. IR (KBr) cm^{-1}: 2977 (–C–H), 3093 (Ar–H), 3400–3300 (–N–H). ^{1}H-NMR (DMSO-d_6) δ: 1.45 (t, 3H, $J = 7.2$ Hz, –CH_3), 4.30 (q, 2H, $J = 7.2$ Hz, –N–CH_2), 8.49 (s, 1H, Ar–H), 8.56 (s, 1H, Ar–H), 12.10 (s, 1H, –N–H), 15.80 (s, 1H, –N–H). ^{13}C-NMR (DMSO-d_6) δ: 15.59, 38.98, 120.42, 143.67, 149.55, 151.16, 151.60. HR-MS for $C_8H_{10}N_9^+$ ($[M-H]^+$) Calcd: 232.1059; Found: 232.1062.

*Synthesis of 9-propyl-N-(1H-tetrazol-5-yl)-9H-purin-6-amine (**4**)* The compound was prepared from 6-chloro-9-propyl-9*H*-purine (0.39 g, 2.0 mmol) as described above and purified by column chromatography on silica gel using 4:1 ethyl acetate–hexane as eluent. Yield 81 %, mp 262–263 °C. IR (KBr) cm^{-1}: 2932 (–C–H), 3074 (Ar–H), 3400-3300 (–N–H). ^{1}H-NMR (DMSO-d_6) δ: 0.86 (t, 3H, $J = 7.4$ Hz, –CH_3), 1.87 (m, 2H, –CH_2), 4.22 (t, 2H, $J = 7.1$ Hz, –N–CH_2), 8.55 (s, 1H, Ar–H), 8.48 (s, 1H, Ar–H), 11.91 (s, 1H, –N–H), 15.80 (s, 1H, –N–H). ^{13}C-NMR (DMSO-d_6) δ: 11.38, 23.14, 45.36, 120.36, 144.06, 149.65, 151.42. HR-MS for $C_9H_{12}N_9^+$ ($[M-H]^+$) Calcd: 246.1216; Found: 246.1014.

*Synthesis of 9-isopropyl-N-(1H-tetrazol-5-yl)-9H-purin-6-amine (**5**)* The compound was prepared from 6-chloro-9-isopropyl-9*H*-purine (0.39 g, 2.0 mmol) as described above and purified by column chromatography on silica gel using ethyl acetate as eluent. Yield 32 %, mp >276 °C (decomp.). IR (KBr) cm^{-1}: 2926 (–C–H), 3076 (Ar–H), 3400–3300 (–N–H). ^{1}H-NMR (DMSO-d_6) δ: 1.57 (d, 6H, $J = 6.8$ Hz, –CH_3), 4.85 (m, 1H, –C–H), 8.54 (s, 1H, Ar–H), 8.57 (s, 1H, Ar–H), 11.90 (s, 1H, –N–H), 15.80 (s, 1H, –N–H). ^{13}C-NMR (DMSO-d_6) δ: 22.51, 47.53, 120.72, 142.02, 149.79, 150.81, 151.37, 151.99. HR-MS for $C_9H_{12}N_9^+$ ($[M-H]^+$) Calcd: 246.1216; Found: 246.1213.

*Synthesis of 9-hexyl-N-(1H-tetrazol-5-yl)-9H-purin-6-amine (**6**)* The compound was prepared from 6-chloro-9-hexyl-9*H*-purine (0.48 g, 2.0 mmol) as described above and purified by column chromatography on silica gel using methanol as eluent. Yield 30 %, mp 222–223 °C. IR (KBr) cm^{-1}: 2924 (–C–H), 3078 (Ar–H), 3400–3300 (–N–H). ^{1}H-NMR (DMSO-d_6) δ: 0.82 (m, 3H, –CH_3), 1.25 (m, 6H, –CH_2), 1.85 (m, 2H, –CH_2), 4.24 (t, 2H, $J = 7.1$ Hz, –N–CH_2), 8.47 (s, 1H, Ar–H), 8.56 (s, 1H, Ar–H), 12.00 (s, 1H, –N–H), 15.70 (s, 1H, –N–H). ^{13}C-NMR (DMSO-d_6) δ: 14.27, 22.37, 26.06, 29.64, 31.05, 43.74, 120.32, 144.04, 149.57, 151.34. HR-MS for $C_{12}H_{18}N_9^+$ ($[M-H]^+$) Calcd: 288.1685; Found: 288.1682.

*Synthesis of 9-benzyl-N-(1H-tetrazol-5-yl)-9H-purin-6-amine (**7**)*

The compound was prepared from 6-chloro-9-benzyl-9*H*-purine (0.49 g, 2.0 mmol) as described above and purified by column chromatography on silica gel using 1:1 chloroform–methanol as eluent. Yield 55 %, mp 271–273 °C. IR (KBr) cm^{-1}: 2975 (–C–H), 3010 (Ar–H), 3050 (Ar–H), 3400–3300 (–N–H). ^{1}H-NMR (DMSO-d_6) δ: 5.50 (s, 2H, –N–CH_2), 7.40 (m, 5H, Ar–H), 8.57 (s, 1H, Ar–H), 8.59 (s, 1H, Ar–H), 12.00 (s, 1H, –N–H), 15.80 (s, 1H, –N–H). ^{13}C-NMR (DMSO-d_6) δ: 47.02, 120.33, 128.03, 128.37, 129.22, 137.09, 142.02, 149.73, 151,27. HR-MS for $C_{13}H_{12}N_9^+$ ($[M-H]^+$) Calcd: 294.1216; Found: 294.1224.

*Synthesis of ethyl 2-(6-(1H-tetrazol-5-ylamino)-9H-purin-9-yl)acetate (**8**)*

The compound was prepared from ethyl 2-(6-chloro-9*H*-purin-9-yl)acetate (0.48 g, 2.0 mmol) as described above and purified by column chromatography on silica gel using ethyl acetate as eluent. Yield 30 %, mp 241–242 °C. IR (KBr) cm^{-1}: 1726 (–C=O ester), 2995 (–C–H), 3078 (Ar–H), 3400–3300 (–N–H). ^{1}H-NMR (DMSO-d_6) δ: 1.22 (t, 3H, $J = 7.1$ Hz, $–CH_3$), 4.18 (q, 2H, $J = 7.1$ Hz, $–O–CH_2$), 5.20 (s, 2H, $–N–CH_2$), 8.45 (s, 1H, Ar–H), 8.55 (s, 1H, Ar–H), 12.09 (s, 1H, –N–H), 15.65 (s, 1H, –N–H). ^{13}C-NMR (DMSO-d_6) δ: 14.45, 44.75, 61.99, 119.93, 138.97, 144.44, 149.69, 151.97, 168.13. HR-MS for $C_{10}H_{12}N_9O_2^+$ ($[M-H]^+$) Calcd: 290.1114; Found: 290.1103.

*Synthesis of 9-(cyclobutylmethyl)-N-(1H-tetrazol-5-yl)-9H-purin-6-amine (**9**)* The compound was prepared from 6-chloro-9-(cyclobutylmethyl)-9*H*-purine (0.45 g, 2.0 mmol) as described above and purified by column chromatography on silica gel using 2:1 ethyl acetate–hexane as eluent. Yield 30 %, mp >255 °C (decomp.). IR (KBr) cm^{-1}: 2928 (–C–H), 3099 (Ar–H), 3400–3300 (–N–H). ^{1}H-NMR (DMSO-d_6) δ: 1.82 (m, 2H, $–CH_2$), 1.96 (m, 4H, $–CH_2$), 2.85 (m, 1H, –C–H), 4.28 (d, 2H, $J = 7.4$ Hz, $–N–CH_2$), 8.47 (s, 1H, Ar–H), 8.56 (s, 1H, Ar–H), 12.00 (s, 1H, –N–H), 15.80 (s, 1H, –N–H). ^{13}C-NMR (DMSO-d_6) δ: 17.43, 25.11, 33.25, 48.03, 119.72, 143.46, 149.10, 150,90. HR-MS for $C_{11}H_{14}N_9^+$ ($[M-H]^+$) Calcd: 272.1372; Found: 272.1312.

*Synthesis of 6-(1-methyl-1H-tetrazol-5-ylthio)-9H-purine (**10**)* 6-chloropurine (0.31 g, 2.00 mmol) was refluxed with 1-methyl-5-mercaptotetrazole (0.23 g, 2.00 mmol) in dichloromethane (25 ml) for 4 days. The solvent was evaporated in vacuo. The product was purified by column chromatography on silica gel using 2:1 ethyl acetate–hexane. Yield 25 %, mp 210–215 °C. IR (KBr) cm^{-1}: 2959 (–C–H), 3098 (Ar–H), 3428 (–N–H). ^{1}H-NMR (DMSO-d_6) δ: 4.05 (s, 3H, $–N–CH_3$), 8.58 (s, 1H, Ar–H), 8.61 (s, 1H, Ar–H), 13.80 (s, 1H, –N–H). ^{13}C-NMR (DMSO-d_6) δ: 35.12, 145.55, 146.97, 151.79, 152.15, 152.99. HR-MS for $C_7H_7N_8S^+$ ($[M-H]^+$) Calcd: 235.0514; Found: 235.0470.

*General procedure for the preparation of 9-alkyl-6-(1-methyl-1H-tetrazol-5-ylthio)-9H-purine compounds (**11**–**19**)*

The appropriate 6-chloro-9-alkyl-9*H*-purine (2 mmol) was refluxed with 1-methyl-5-mercaptotetrazole (0.23 g, 2.00 mmol) in dichloromethane (25 ml) for 4 days. The solvent was evaporated in vacuo.

*Synthesis of 9-methyl-6-(1-methyl-1H-tetrazol-5-ylthio)-9H-purine (**11**)* The compound was prepared from 6-chloro-9-methyl-9*H*-purine (0.34 g, 2.00 mmol) as described above, washed with diethyl ether, and recrystallized from ethyl acetate. Yield 58 %, mp 230–235 °C. IR (KBr) cm^{-1}: 2951 (–C–H), 3074 (Ar–H). ^{1}H-NMR (DMSO-d_6) δ: 3.83 (s, 3H, $–N–CH_3$), 4.05 (s, 3H, $–N–CH_3$), 8.56 (s, 1H, Ar–H), 8.65 (s, 1H, Ar–H). ^{13}C-NMR (DMSO-d_6) δ: 30.37, 35.11, 130.75, 146.90, 147.70, 150.78, 152.06, 153.54. HR-MS for $C_8H_9N_8S^+$ ($[M-H]^+$) Calcd: 249.0671; Found: 249.0667.

*Synthesis of 9-ethyl-6-(1-methyl-1H-tetrazol-5-ylthio)-9H-purine (**12**)* The compound was prepared from 6-chloro-9-ethyl-9*H*-purine (0.36 g, 2.00 mmol) as described above, washed with diethyl ether, and recrystallized from ethyl acetate. Yield 75 %, mp 134–135 °C. IR (KBr) cm^{-1}: 2974 (–C–H), 3091 (Ar–H). ^{1}H-NMR (DMSO-d_6) δ: 1.44 (t, 3H, $J = 7.3$ Hz, $–CH_3$), 4.05 (s, 3H, $–N–CH_3$), 4.29 (q, 2H, $J = 7.3$ Hz, $–N–CH_2$), 8.61 (s, 2H, Ar–H). ^{13}C-NMR (DMSO-d_6) δ: 15.34, 35.12, 39.30, 130.96, 146.80, 146.88, 150.27, 151.98, 153.69. HR-MS for $C_9H_{11}N_8S^+$ ($[M-H]^+$) Calcd: 263.0827; Found: 263.0750.

*Synthesis of 9-propyl-6-(1-methyl-1H-tetrazol-5-ylthio)-9H-purine (**13**)* The compound was prepared from 6-chloro-9-propyl-9*H*-purine (0.39 g, 2.00 mmol) as described above, washed with diethyl ether, and recrystallized from ethyl acetate. Yield 68 %, mp 168–172 °C. IR (KBr) cm^{-1}: 2937 (–C–H), 3074 (Ar–H). ^{1}H-NMR (DMSO-d_6) δ: 0.90 (t, 3H, $J = 7.3$ Hz, $–CH_3$), 1.90 (m, 2H, $–CH_2$), 4.05 (s, 3H, $–N–CH_3$), 4.48 (t, 2H, $J = 7.2$ Hz, $–N–CH_2$), 8.60 (s, 1H, Ar–H), 8.80 (s, 1H, Ar–H). ^{13}C-NMR (DMSO-d_6) δ: 11.11, 24.79, 35.14, 49.07, 123.23, 146.72, 146.80, 150.75, 152.08, 160.26. HR-MS for $C_{10}H_{13}N_8S^+$ ($[M-H]^+$) Calcd: 277.0984; Found: 277.0914.

*Synthesis of 9-isopropyl-6-(1-methyl-1H-tetrazol-5-ylthio)-9H-purine (**14**)* The compound was prepared from 6-chloro-9-isopropyl-9*H*-purine (0.39 g, 2.00 mmol) as described above, purified by column chromatography on silica gel using 2:1 ethyl acetate–hexane as eluent, and re-crystallized from ethyl acetate. Yield 46 %, mp 94–98 °C. IR (KBr) cm^{-1}: 2934 (–C–H), 3058 (Ar–H). ^{1}H-NMR (DMSO-d_6) δ: 1.56 (d, 6H, $J = 6.8$ Hz, $–CH_3$), 4.08 (s, 3H, $–N–CH_3$), 4.86 (m, 1H, –C–H), 8.64 (s, 1H, Ar–H), 8.71 (s, 1H, Ar–H). ^{13}C-NMR (DMSO-d_6) δ: 22.56, 35.13, 48.10, 131.24, 145.31, 146.88, 149.95, 151.77, 153.77. HR-MS for $C_{10}H_{13}N_8S^+$ ($[M-H]^+$) Calcd: 277.0984; Found: 277.0951.

*Synthesis of 9-hexyl-6-(1-methyl-1H-tetrazol-5-ylthio)-9H-purine (**15**)* The compound was prepared from 6-chloro-9-hexyl-9*H*-purine (0.48 g, 2.00 mmol) as described above,

washed with diethyl ether, and recrystallized from ethyl acetate. Yield 67 %, mp 119–122 °C. IR (KBr) cm^{-1}: 2928 (–C–H), 3057 (Ar–H). ^{1}H-NMR (DMSO-d_6) δ: 0.85 (m, 3H, $–CH_3$), 1.33 (m, 6H, $–CH_2$), 1.95 (m, 2H, $–CH_2$), 4.08 (s, 3H, $–N–CH_3$), 4.51 (t, 2H, $J = 7.3$ Hz, $–N–CH_2$), 8.60 (s, 1H, Ar–H), 8.80 (s, 1H, Ar–H). ^{13}C-NMR (DMSO-d_6) δ: 14.27, 22.41, 25.97, 31.08, 31.65, 35.14, 47,64, 123.24, 146.67, 146.82, 150.70, 152.07, 160.28. HR-MS for $C_{13}H_{19}N_8S^+$ ($[M-H]^+$) Calcd: 319.1453; Found: 319.1359.

*Synthesis of 9-benzyl-6-(1-methyl-1H-tetrazol-5-ylthio)-9H-purine (**16**)* The compound was prepared from 6-chloro-9-benzyl-9*H*-purine (0.49 g, 2.00 mmol) as described above and purified by column chromatography on silica gel using 2:1 ethyl acetate–hexane as eluent. Yield 87 %, mp 115–118 °C. IR (KBr) cm^{-1}: 2954 (–C–H), 3020 (Ar–H), 3066 (Ar–H). ^{1}H-NMR (DMSO-d_6) δ: 4.10 (s, 3H, $–N–CH_3$), 5.50 (s, 2H, $–N–CH_2$), 7.34 (m, 5H, Ar–H), 8.65 (s, 1H, Ar–H), 8.80 (s, 1H, Ar–H). ^{13}C-NMR (DMSO-d_6) δ: 35.14, 47.31, 128.20, 128.33, 128.49, 130.85, 136.55, 146.81, 147.01, 150.32, 152.31, 154.04. HR-MS for $C_{14}H_{13}N_8S^+$ ($[M-H]^+$) Calcd: 325.0984; Found: 325.0934.

*Synthesis of ethyl 2-(6-(1-methyl-1H-tetrazol-5-ylthio)-9H-purin-9-yl)acetate (**17**)* The compound was prepared from ethyl 2-(6-chloro-9*H*-purin-9-yl)acetate (0.48 g, 2.00 mmol) as described above, washed with diethyl ether, and recrystallized from ethyl acetate. Yield 79 %, mp 88–92 °C. IR (KBr) cm^{-1}: 1736 (–C=O ester), 2945 (–C–H), 3072 (Ar–H). ^{1}H-NMR (DMSO-d_6) δ: 1.20 (t, 3H, $J = 7.1$ Hz, $–CH_3$), 4.10 (s, 3H, $–N–CH_3$), 4.18 (q, 2H, $J = 7.1$ Hz, $–O–CH_2$), 5.25 (s, 2H, $–N–CH_2$), 8.60 (s, 1H, Ar–H), 8.70 (s, 1H, Ar–H). ^{13}C-NMR (DMSO-d_6) δ: 14.43, 35.14, 44.92, 62.12, 130.47, 146.79, 147.47, 150.57, 152.42, 154.14, 167.85. HR-MS for $C_{11}H_{13}N_8O_2S^+$ ($[M-H]^+$) Calcd: 321.0882; Found: 321.0867.

*Synthesis of 9-(cyclobutylmethyl)-6-(1-methyl-1H-tetrazol-5-ylthio)-9H-purine (**18**)* The compound was prepared from 6-chloro-9-(cyclobutylmethyl)-9*H*-purine (0.45 g, 2.00 mmol) as described above, purified by column chromatography on silica gel using 2:1 ethyl acetate–hexane as eluent, and recrystallized from ethyl acetate. Yield 70 %, mp 90–94 °C. IR (KBr) cm^{-1}: 2937 (–C–H), 3062 (Ar–H). ^{1}H-NMR (DMSO-d_6) δ: 1.80 (m, 2H, $–CH_2$), 1.95 (m, 4H, $–CH_2$), 2.85 (m, 1H, –C–H), 4.10 (s, 3H, $–N–CH_3$), 4.29 (d, 2H, $J = 7.4$ Hz, $–N–CH_2$), 8.61 (s, 1H, Ar–H), 8.63 (s, 1H, Ar–H). ^{13}C-NMR (DMSO-d_6) δ: 17.89, 25.66, 34.14, 35.13, 48.77, 130.72, 146.83, 146.90, 150.47, 152,01, 153.77. HR-MS for $C_{12}H_{15}N_8S^+$ ($[M-H]^+$) Calcd: 303.1140; Found: 303.1039.

*Synthesis of 9-(cyclohexylmethyl)-6-(1-methyl-1H-tetrazol-5-ylthio)-9H-purine (**19**)* The compound was prepared from 6-chloro-9-(cyclohexylmethyl)-9*H*-purine (0.50 g, 2.00 mmol) as described above, washed with diethyl ether, and recrystallized from ethyl acetate. Yield 80 %, mp 108–110 °C. IR (KBr) cm^{-1}: 2923 (–C–H), 3067 (Ar–H). ^{1}H-NMR (DMSO-d_6) δ: 1.15 (m, 2H, $–CH_2$), 1.20 (m, 4H, $–CH_2$), 1.60 (m, 4H, $–CH_2$), 1.85 (m, 1H, –C–H), 4.07 (s, 3H, $–N–CH_3$), 4.12 (d, 2H, $J = 7.2$ Hz, $–N–CH_2$), 8.59 (s, 1H, Ar–H), 8.64 (s, 1H, Ar–H).^{13}C-NMR (DMSO-d_6) δ: 25.45, 26.15, 31.41, 35.14, 37.89, 49.75, 130.75, 146.87, 147.40, 150.66, 152.08, 153.80. HR-MS for $C_{14}H_{19}N_8S^+$ ($[M-H]^+$) Calcd: 331.1453; Found: 331.1417.

Antibacterial and antifungal testing methods

The in vitro antibacterial activity of synthesized amino and thiotetrazole purine derivatives (compounds **1**–**19**) was performed against American Type Culture Collection (ATCC) reference bacterial strain and fungi by disk diffusion technique (Clinical and Laboratory Standards Institute 2006, 2007). 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 three times.

DNA and compound interaction

The interaction of compounds **1**–**19** with pBR322 plasmid DNA was studied by agarose gel electrophoresis (Asmafiliz *et al.*, 2009; Huq *et al.*, 2009). The compounds were dissolved in DMSO and the plasmid DNA were added to the aliquots of decreasing concentrations of compounds ranging from 5,000 to 312 μM. The mixtures were incubated in the dark at 37 °C for 24 h and electrophoresed in 1 % agarose gel. Electrophoresis was carried under TAE buffer for approximately 3 h at 60 V. At the end of the electrophoresis, the gel was stained with ethidium bromide. The gel was visualized under UV light using BioDoc Analyzer, Biometra. The illuminated gel was photographed with a video-camera and saved as a TIFF file. The experiments were repeated three times.

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