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Synthesis and antimicrobial evaluation of some new substituted purine derivatives

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ABSTRACT

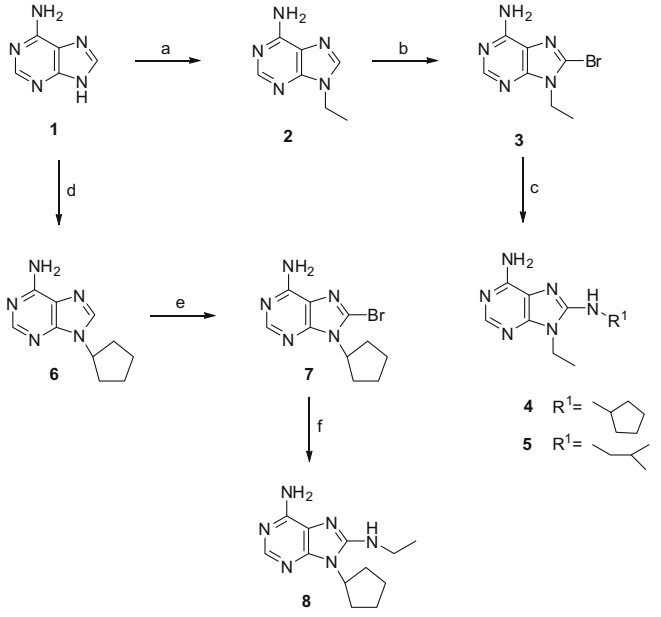
A series of 8,9-disubstituted adenines (**4**, **5**, **8**), 6-substituted aminopurines (**10–13**) and 9-(*p*-fluorobenzyl/cyclopentyl)-6-substituted aminopurines (**16**, **17**, **19–30**) have been prepared and the antimicrobial activities of these compounds against *Staphylococcus aureus,* methicillin-resistant *S. aureus* (MRSA, standard and clinical isolate), *Bacillus subtilis*, *Escherichia coli* and *Candida albicans* were evaluated. 6-[(*N*-phenylaminoethyl)amino]-9*H*-purine (**12**) which has no substitution at N-9 position and 9-cyclopentyl-6-[(4-fluorobenzyl)amino]-9*H*-purine (**24**) exhibited excellent activity against *C. albicans* with MIC 3.12 μg/mL. These compounds displayed better antifungal activity than that of standard oxiconazole. Furthermore, compound **22** carrying 4-chlorobenzylamino group at the 6-position of the purine moiety exhibited comparable antibacterial activity with that of the standard ciprofloxacin against both of the drug-resistant bacteria (MRSA, standard and clinical isolate).

1. Introduction

Nosocomial bloodstream infections are important causes of morbidity and mortality. *Staphylococcus aureus* remains a significant reason of nosocomial bloodstream infection.[1] Methicillin-resistant *S. aureus* (MRSA) isolates came into existence soon after the introduction of methicillin and MRSA is now a major pathogen worldwide. MRSA isolates have been associated with nosocomial infections and rapidly developed resistance to multiple drug classes.[2]

Candida infections have increased dramatically over the past three decades. Candida spp. are the fourth most common cause of nosocomial bloodstream infections in many hospitals and represent 10% of all bloodstream infections.[3,4]

Purine, purines nucleosides and their analogs have been extensively investigated due to their potential activity as enzyme inhibitors,[5–8] cytotoxic,[9–12] antiviral,[13–16] antihyperglycemic,[17] immunostimulator,[18] antifungal and antibacterial agents.[19–25] On the basis of these observations, in this study, we designed and synthesized novel substituted purine derivatives (**4–6**, **8**, **10–13**, **16**, **17**, **19–30**) and evaluated their antibacterial, antifungal activities.



Scheme 1. (a) Iodoethane, K_2CO_3, DMF; (b) NBS, DMF; (c) the appropriate amine; (d) cyclopentyl bromide, K_2CO_3, DMF, (e) Br_2, H_2O; (e) ethylamine (70% in H_2O).

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2. Result and discussions

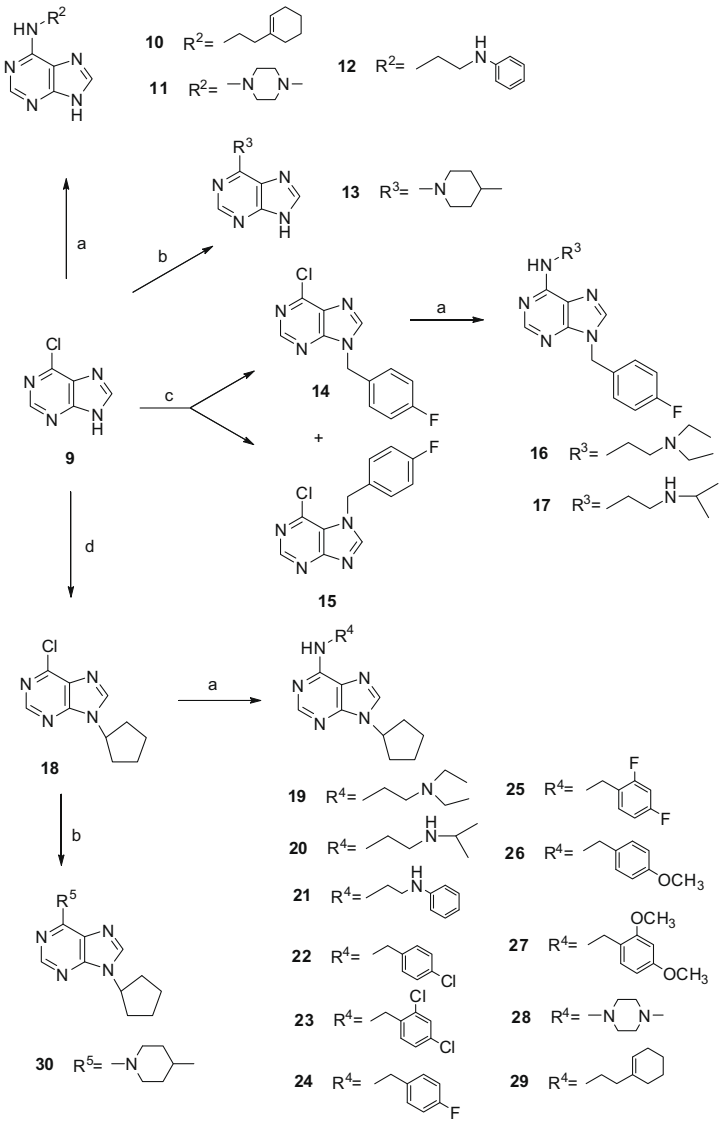
2.1. Chemistry

The synthesis of the 8,9-disubstituted adenine derivatives **4**, **5**, **8** was carried out starting from commercially available adenine (**1**) (Scheme 1). For preparation of 9-ethyl-8-(alkylamino)adenines (**4**, **5**), **1** was alkylated with iodoethane to give intermediate 9-ethyladenine (**2**[26]). The reaction of **2** *N*-bromosuccinimide gave the 8-bromo-9-ethyladenine (**3**[26]). The final products (**4**, **5**) were obtained by nucleophilic substitution of compound **3** with cyclopentylamine or isobutylamine. The 9-cyclopentyl analoge **6** was previously prepared from 5-amino-1-cyclopentylimidazole-4-carboxamidine,[27] or from 6-chloro-9-cyclopentyl purine,[28] or from *t*-butoxycarbonyl protected adenine derivative and cyclopentanol with Mitsunobu reaction.[29] Here, 9-cyclopentyl adenine (**6**) was obtained from **1** with potassium carbonate and cyclopentylbromide. Reaction of **6** with bromine[30] followed by ethylamine alkylation furnished 9-cyclopentyl-8-ethyladenine (**8**) in good yield.

6-Substituted aminopurines (**10–13**) and 9-(*p*-fluorobenzyl/cyclopentyl)-6-substituted aminopurines (**16**, **17**, **19–30**) were synthesized as shown in Scheme 2. Compounds **10–13** were synthesized by nucleophilic substitution of chlorine of commercially available 6-chloropurine (**9**) with the appropriate amines. Compound **9** was alkylated with *p*-fluorobenzyl chloride to give mixture of 7- and 9-alkylated purines.[31] Isomers were separated by column chromatography to obtain the 9-isomers (**14**) in 55% and 7-isomers (**15**) in 21% yield. Treatment of 9-*p*-fluorobenzyl-6-chloropurine (**14**) with substituted ethylenediamines afforded the 6-(substituted ethylenediamino)-9-(*p*-fluorobenzyl)purines (**16**, **17**). Finally, compounds **19–30** were synthesized via N-9 alkylation of 6-chloropurine **9** with cyclopentylbromide,[32] and by amination of 6-chloro-9-cyclopentylpurine **18** with substituted amines. The alkylation reaction occurred only at the N-9 atom. X-ray analysis also confirmed the structure of compound **18** (Fig. 1).

2.2. Microbiology

The newly obtained purine derivatives (**4–6, 8, 10–13, 16, 17, 19–30**) were evaluated for their in vitro antibacterial activity against Gram positive *S. aureus,* methicillin-resistant *S. aureus* (MRSA, standart and clinical isolate), *Bacillus subtilis*, Gram negative *Escherichia coli* and antifungal activity against *Candida albicans* by tube dilution technique.[33,34] Sultamicillin, ampicillin and ciprofloxacin were used



Scheme 2. (a) The appropriate amine, EtOH; (b) 4-methylpiperidine; (c) 4-fluorobenzyl chloride, NaH, DMF; (d) cyclopentyl bromide, K_2CO_3, DMF.

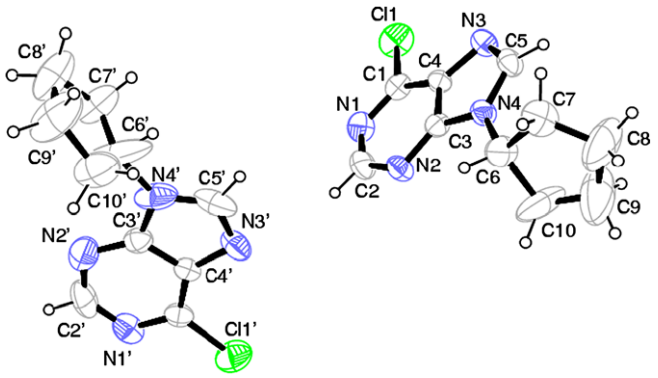


Figure 1. Two molecules in the asymmetric unit of **18**, showing the atom numbering scheme; displacement ellipsoids are drawn at the 40% probability level.

as antibacterial standard drugs, while miconazole and oxiconazole were used as antifungal standard drugs whose minimum inhibitory concentration (MIC) values are provided (Table 1).

As it indicated in Table 1 most of the compounds showed good activity against *C. albicans*. 6-[(*N*-phenylaminoethyl)amino]-9*H*-purine (**12**) which have no substitution at N-9 position and 9-cyclopentyl-6-[(4-fluorobenzyl)amino]-9*H*-purine (**24**) were the most active compounds, they were two times as active as oxiconazole against *C. albicans* with MIC 3.12 μg/mL. When compared with miconazole, compounds **12** and **24** showed similar activity.

All the 6-(substitutedbenzylamino)-9-cyclopentylpurines (**22–27**) possess good antifungal activity, with MIC values against *C. albicans* in the range of 3.12–25 μg/mL. From these derivatives compound **22**, with 4-chlorobenzylamino substituted at C-6, showed also comparable activity against MRSA standard with the standard ciprofloxacin. Again, this compound possessed a better activity against MRSA clinical isolate than the standard ciprofloxacin with MIC 6.25 μg/mL. 6-Substitutedbenzylamino compounds **22**, **24** exhibited a better activity against both of the drug-resistant bacteria than the standard ampicillin with MIC 6.25–25 μg/mL.

From this screening, the results reported in Table 1 show that most of the compounds were effective against *B. Subtilis*; **4, 6, 10, 11, 13, 16, 19–24, 27** and **28** showed better activity than the standard ampicillin with MIC 25 μg/mL.

The synthesized compounds were also tested against Gram-negative bacteria *E. coli* and **10–12, 27, 29** displayed similar activity with the standard sultamicillin with MIC 25 μg/mL. These derivatives possessed also a better activity against *E. Coli* than the standard ampicillin.

3. Conclusion

In this work, we have synthesized novel 8,9-disubstituted adenine derivatives (**4**, **5, 8**), 6-substituted aminopurines (**10–13**) and 9-(*p*-fluorobenzyl/cyclopentyl)-6-substituted aminopurine analogs (**16**, **17**, **19–30**) and screened for their antimicrobial activities. All the new compounds except **19,** demonstrated potent antifungal activity against *C. albicans*. Particularly, 6-substituted aminopurine analogs **12** having no substituents on the 9-position of the purine structure and **24** which is among 9-cyclopentyl-6-substituted aminopurine derivatives displayed better antifungal efficacy than that of the standard drug oxiconazole. Also these compounds showed same activity as miconazole against *C. albicans*. Against both of the drug-resistant bacteria, compound **22** carrying 4-chlorobenzylamino group at the 6-position of the purine exhibited comparable antibacterial activity with that of the clinically used drug ciprofloxacin. Therefore, w**e** need to do studies on in vivo and mode of action mechanisms of the new purines to better determine the potential of their antifungal and antibacterial activity.

4. Experimental

4.1. Chemistry

Melting points were recorded with a capillary melting point apparatus (Electrothermal 9100) and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on VARIAN Mercury 400 FT-NMR spectrometer (operated at 400) and are referenced to internal tetramethylsilane (TMS) at 0.0 ppm. The spin multiplicities are indicated by the symbols s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet), and br (broad). Mass spectra were taken on Waters Micromass ZQ by using (ESI+) method. Elemental analyses (C, H, N) were determined on a Leco CHNS 932 instrument and gave values within ±0.4% of the theoretical values. All instrumental analysis was performed at Ankara University, Faculty of Pharmacy. Column chromatography was accomplished on silica gel 60 (40–63 mm particle size). The chemical reagents used in synthesis were purchased from E. Merck, Fluka, Sigma, Acros and Aldrich.

4.1.1. 6-Amino-9-ethyl-8-(cyclopentylamino)-9*H*-purine (9-ethyl-8-(cyclopentylamino)-9*H*-adenine, 4)

A solution of **3** (0.18 g, 0.74 mmol) in excess cyclopentylamine (2 mL) was heated at 120 °C for 14 h. The reaction mixture was concentrated by evaporation and the residue was purified by column chromatography eluting with $CHCl_3$–MeOH (10:1) to give **4** (0.12 g, 65%) as a cream-colored solid: mp 221 °C. ^{1}H NMR (DMSO-d_6) δ 1.18 (t, 3H), 1.53–1.71 (m, 6H), 1.95–1.99 (m, 2H), 4.00 (q, 2H), 4.20–4.25 (m, 1H), 6.36 (s, 2H), 6.51 (d, 1H), 7.90 (s, 1H). MS (ESI+) *m/z*: 247 (100%) (M+H). Anal. Calcd for $C_{12}H_{18}N_6$: C, 58.51; H, 7.37; N, 34.12. Found: C, 58.22; H, 7.15; N, 33.85.

4.1.2. 6-Amino-9-ethyl-8-(isobutylamino)-9*H*-purine (9-ethyl-8-(isobutylamino)-9*H*-adenine, 5)

A solution of **3** (0.08 g, 0.33 mmol) in excess isobutylamine (1.5 mL) was refluxed with stirring for 48 h. The reaction mixture was concentrated by evaporation and the residue was purified by column chromatography eluting with $CHCl_3$–isopropanol (10:2) to give **5** (0.04 g, 52%) as a cream-colored solid: mp 188–190 °C. ^{1}H NMR (DMSO-d_6) δ *0.90–0.94* (3, 9H), 1.76–1.88 (m, 1H), 3.22 (t, 2H), 4.10 (q, 2H), 7.60–7.84 (br s, 3H), 8.21 (s, 1H). MS (ESI+) *m/z*: 235 (100%) (M+H). Anal. Calcd for $C_{11}H_{18}N_6$: C, 56.39; H, 7.74; N, 35.87. Found: C, 56.54; H, 7.80; N, 35.73.

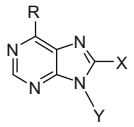
4.1.3. 6-Amino-9-cyclopentyl-9*H*-purine (9-cyclopentyl-9*H*-adenine, 6)

To a suspension of **1** (0.25 g, 1.85 mmol) in 4 mL of DMF was added K_2CO_3 (0.29 g, 2.12 mmol). The reaction mixture was stirred at 60 °C for 30 min. A solution of cyclopentyl bromide (0.3 mL, 2.77 mmol) in 3 mL DMF was added to the reaction mixture and the mixture was stirred at 65 °C for 72 h. The reaction mixture was concentrated by evaporation and the residue was purified by column chromatography eluting with EtOAc–MeOH (10:1) to give **6** (0.18 g, 48%) as a white solid**:** mp 154 °C. ^{1}H NMR (DMSO-d_6) δ *1.67–2.16* (m, 8H), 4.81–4.87 (m, 1H), 7.23 (s, 2H), 8.15 (s, 1H), 8.22 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 23.8, 32.8, 56.0, 119.9, 138.5, 150.1, 152.7, 155.8. MS (ESI+) *m/z*: 204 (100%) (M+H). Anal. Calcd for $C_{10}H_{13}N_5$: C, 59.10; H, 6.45; N, 34.46. Found: C, 58.72; H, 6.51; N, 34.53.

4.1.4. 6-Amino-8-bromo-9-cyclopentyl-9*H*-purine (8-bromo-9-cyclopentyl-9*H*-adenine, 7)

A solution of bromine (0.2 mL, 3.9 mmol) in water (15 mL) was added to **6** (0.1 g, 0.49 mmol). The reaction mixture was stirred at room temperature for 17 h. The reaction mixture was concentrated

Table 1
Antibacterial and antifungal activity of compounds **4–6**, **8**, **10–13**, **15**, **16**, **18–29** (MIC minimum inhibitory concentration μg/mL)



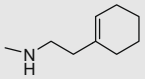
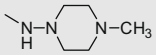
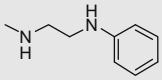
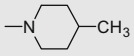
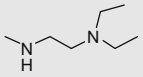
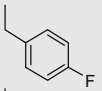
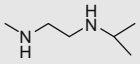
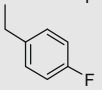
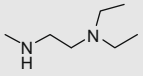
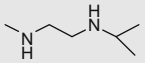
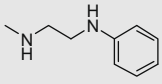
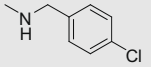
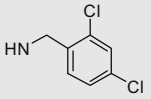
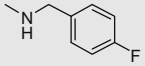
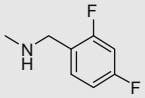
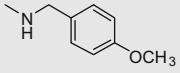
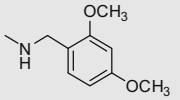
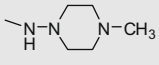
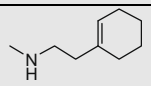
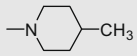
Compound	R	X	Y	*S. aureus* (25923)[a]	MRSA[b] (431300)	MRSA[c]	*E. coli* (25922)	*B. subtilis* (6633)	*C. albicans* (10145)
4	$-NH_2$		$-C_2H_5$	50	50	50	50	25	12.5
5	$-NH_2$		$-C_2H_5$	50	50	50	50	50	25
6	$-NH_2$	–H		50	50	50	50	25	12.5
8	$-NH_2$			25	50	50	50	50	6.25
10		–H	–H	50	50	50	25	25	6.25
11		–H	–H	50	50	50	25	25	12.5
12		–H	–H	50	50	50	25	50	3.12
13		–H	–H	25	50	50	50	25	25
16		–H		50	50	50	50	25	25
17		–H		50	50	50	50	50	25
19		–H		50	50	50	50	25	50
20		–H		25	50	50	50	25	25
21		–H		50	50	50	50	25	12.5
22		–H		12.5	6.25	6.25	50	25	12.5
23		–H		12.5	25	50	50	25	6.25
24		–H		25	25	25	50	25	3.12
25		–H		50	50	50	50	50	25
26		–H		25	50	25	50	50	12.5
27		–H		50	50	50	25	25	12.5
28		–H		50	50	50	50	25	25

Table 1 (*continued*)

Compound	R	X	Y	*S. aureus* (25923)[a]	MRSA[b] (431300)	MRSA[c]	*E. coli* (25922)	*B. subtilis* (6633)	*C. albicans* (10145)
29		–H		12.5	25	25	25	50	12.5
30		–H		50	50	50	50	50	12.5
Sultamicillin				1.56	25	25	25	0.78	
Ampicillin				0.78	50	50	50	50	
Ciprofloxacin				0.78	6.25	12.5	0.39	0.19	
Miconazole									3.12
Oxiconazole									6.25

[a] ATCC number.
[b] MRSA: methicillin-resistant *S. aureus* (standart).
[c] MRSA: methicillin-resistant *S. aureus* (clinical isolate).

by evaporation and the residue was purified by column chromatography eluting with hexane–EtOAc (1:1) to give **7** (0.11 g, 79%) as a cream-colored solid: mp 193 °C. ^{1}H NMR (DMSO-d_6) δ *1.66–2.34* (m, 8H), 4.84–4.92 (m, 1H), 7.36 (s, 2H), 8.11 (s, 1H). MS (ESI+) *m/z*: 282 (98%) (M+H), 284 (100%) (M+H+2). Anal. Calcd for $C_{10}H_{12}BrN_5$: C, 42.57; H, 4.29; N, 24.82. Found: C, 42.95; H, 4.56; N, 24.79.

4.1.5. 6-Amino-8-(ethylamino)-9-cyclopentyl-9*H*-purine (8-(ethylamino)-9-cyclopentyl-9*H*-adenine, 8)

A solution of **7** (0.07 g, 0.25 mmol) in excess ethylamine (70% in water, 2 mL) was stirred at 30 °C for 48 h. The reaction mixture was concentrated by evaporation and the residue was purified by column chromatography eluting with $CHCl_3$–MeOH (10:1) to give **8** (0.046 g, 75%) as a cream-colored solid: mp 188 °C. ^{1}H NMR (DMSO-d_6) δ 1.17 (t, 3H), 1.56–2.26 (m, 8H), 3.35 (m, 2H), 4.56 (m, 1H), 6.29 (s, 2H), 6.54 (t, 1H), 7.84 (s, 1H). MS (ESI+) *m/z*: 247 (100%) (M+H). Anal. Calcd for $C_{12}H_{18}N_6 \cdot 0.1CHCl_3 \cdot 0.1MeOH$: C, 56.04; H, 7.13; N, 32.14. Found: C, 56.40; H, 6.79; N, 32.02.

4.1.6. 6-(2-Cyclohexenylethyl)amino-9*H*-purine (10)

To a solution of **9** (0.15 g, 0.97 mmol) in absolute EtOH (5 mL) was added 2-(1-cyclohexenyl)ethylamine (0.27 mL, 1.94 mmol) and this mixture was refluxed for 40 h. The reaction mixture was concentrated by evaporation and the residue was purified by column chromatography eluting with EtOAc–MeOH (10:1) to give **10** (0.16 g, 68%) as a white solid: mp 199 °C. ^{1}H NMR (DMSO-d_6) δ 1.51 (dd, 4H), 1.94 (d, 4H), 2.22 (t, 2H), 3.55 (br s, 2H), 5.41 (s, 1H), 7.52 (br s, 1H), 8.07 (s, 1H), 8.17 (br s, 1H), 12.88 (br s, 1H). MS (ESI+) *m/z*: 244 (100%) (M+H). Anal. Calcd for $C_{13}H_{17}N_5$: C, 64.17; H, 7.04; N, 28.78. Found: C, 63.85; H, 6.65; N, 28.39.

4.1.7. 6-(4-Methylpiperazin-1-yl)amino-9*H*-purine (11)

To a solution of **9** (0.13 g, 0.84 mmol) in absolute EtOH (5 mL) was added 1-amino-4-methylpiperazine (0.2 mL, 1.68 mmol) and this mixture was refluxed for 4 h. The reaction mixture was concentrated by evaporation. The product was washed with $CHCl_3$ and crystallized from MeOH–EtOAc to give **11** (0.14 g, 71%) as a cream-colored solid: mp 299 °C. ^{1}H NMR (DMSO-d_6) δ 2.51 (s, 3H), 2.77 (s, 4H), 3.15 (br s, 4H), 8.23 (s, 1H), 8.31 (br s, 1H), 9.04 (br s, 1H), 12.20 (br s, 1H). MS (ESI+) *m/z*: 234 (100%) (M+H). Anal. Calcd for $C_{10}H_{15}N_7$: C, 51.49; H, 6.48; N, 42.03. Found: C, 51.57; H, 6.36; N, 41.92.

4.1.8. 6-[2-(Phenylamino)ethyl]amino-9*H*-purine (12)

To a solution of **9** (0.15 g, 0.97 mmol) in absolute EtOH (5 mL) was added *N*-phenylethylenediamine (0.25 mL, 1.94 mmol) and this mixture was refluxed for 15 h. The reaction mixture was concentrated by evaporation and the product was crystallized from DMF–MeOH to give **13** (0.19 g, 77%) as a white solid: mp 253 °C. ^{1}H NMR (DMSO-d_6) δ 3.31 (q, 2H), 3.71 (br s, 2H), 5.84 (t, 1H), 6.57 (t, 1H, J = 7.2 Hz), 6.68 (d, 2H, J = 8.4 Hz), 7.13 (t, 2H, J = 7.6 Hz), 7.79 (br s, 1H), 8.16 (s, 1H), 8.28 (br s, 1H), 13.00 (br s, 1H). MS (ESI+) *m/z*: 255 (100%) (M+H). Anal. Calcd for $C_{13}H_{14}N_6$: C, 61.40; H, 5.55; N, 33.05. Found: C, 61.04; H, 5.46; N, 32.95.

4.1.9. 6-(4-Methylpiperidin-1-yl)-9*H*-purine (13)

To a solution of **9** (0.15 g, 0.97 mmol) in absolute EtOH (5 mL) was added 4-methylpiperidine (0.23 mL, 1.94 mmol) and this mixture was refluxed for 16 h. The reaction mixture was concentrated by evaporation and the product was crystallized from MeOH–EtOAc to give **12** (0.15 g, 71%) as a white solid: mp 265 °C. ^{1}H NMR (DMSO-d_6) δ 0.92 (d, 3H), 1.04–1.16 (m, 1H), 1.72 (m, 4H), 3.03 (m, 4H), 8.09 (s, 1H), 8.17 (s, 1H), 13.00 (br s, 1H). ^{13}C NMR (DMSO-d_6) δ 22.5, 31.3, 34.6, 45.5, 119.4, 138.4, 152.0, 152.5, 153.8. MS (ESI+) *m/z*: 218 (100%) (M+H). Anal. Calcd for $C_{11}H_{15}N_5$: C, 60.81; H, 6.96; N, 32.23. Found: C, 60.47; H, 6.58; N, 32.30.

4.1.10. 6-Chloro-9-(4-fluorobenzyl)-9*H*-purine (14) and 6-chloro-7-(4-fluorobenzyl)-9*H*-purine (15)

A suspension of **9** (0.25 g, 1.62 mmol) in DMF (8 mL) was treated with NaH (0.07 g, 3.23 mmol) and stirred at room temperature for 20 min. 4-Fluorobenzyl chloride (0.38 mL, 3.23 mmol) was added and the mixture stirred for 45 h at room temperature. H_2O was added and then extracted with EtOAc. The extract was dried over Na_2SO_4, the solvent was evaporated in vacuo, and the residue was purified by column chromatography eluting with EtOAc–hexane (7:3) to give first **14** (0.23 g, 55%) and afterwards **15** (0.09 g, 21%). Compound **14** mp 132 °C. ^{1}H NMR ($CDCl_3$) δ 5.44 (s, 2H), 7.07 (t, 2H, J = 8.4 Hz), 7.31–7.35 (m, 2H), 8.11 (s, 1H), 8.79 (s, 1H). MS (ESI+) *m/z*: 263 (100%) (M+H), 265 (32%) (M+H+2). Anal. Calcd for $C_{12}H_8ClFN_4$: C, 54.87; H, 3.07; N, 21.33. Found: C, 54.52; H, 2.69; N, 21.26. Compound **15** mp 168 °C. ^{1}H NMR ($CDCl_3$) δ 5.67 (s, 2H), 7.09 (t, 2H, J = 8.8 Hz), 7.17–7.21 (m, 2H), 8.24 (s, 1H), 8.91 (s, 1H). MS (ESI+) *m/z*: 263 (100%) (M+H), 265 (32%) (M+H+2). Anal. Calcd for $C_{12}H_8ClFN_4$: C, 54.87; H, 3.07; N, 21.33. Found: C, 54.49; H, 2.72; N, 21.30.

4.1.11. 6-[2-(*N*,*N*-Diethylamino)ethyl]amino-9-(4-fluorobenzyl)-9*H*-purine (16)

To a solution of **14** (0.10 g, 0.38 mmol) in absolute EtOH (4 mL) was added *N*,*N*-diethylethylenediamine (0.21 mL, 1.52 mmol) and this mixture was refluxed for 5 h. The reaction mixture was concentrated by evaporation and the residue was purified by column

chromatography eluting with EtOAc–MeOH–NH_3 (10:2:0.2) to give **16** (0.09 g, 69%) as a cream-colored solid: mp 94 °C. ^{1}H NMR ($CDCl_3$) δ 1.11 (t, 6H), 2.70 (q, 4H), 2.84 (t, 2H), 3.78 (br s, 2H), 5.35 (s, 2H), 6.75 (br s, 1H), 7.02 (t, 2H), 7.27–7.32 (m, 2H), 7.84 (s, 1H), 8.41 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 11.3, 38.0, 46.6, 47.0, 51.6, 116.0, 116.2, 129.8 (2), 131.9 (2), 139.9, 153.6, 155.1, 161.5, 164.0. MS (ESI+) *m/z*: 343 (100%) (M+H). Anal. Calcd for $C_{18}H_{23}FN_6{\cdot}0.6H_2O{\cdot}0.2CH_3OH$: C, 60.78; H, 7.00; N, 23.37. Found: C, 60.44; H, 6.65; N, 23.08.

4.1.12. 9-(4-Fluorobenzyl)-6-[2-(*N*-isopropylamino)ethyl]amino-9*H*-purine (17)

This compound was prepared from **14** (0.09 g, 0.34 mmol) and N-isopropylethylenediamine (0.1 mL, 1.36 mmol) according to the procedure described in for **16** as a cream-colored solid **17** (0.07 g, 62%): mp 90 °C. ^{1}H NMR (DMSO-d_6) δ 0.81 (d, 6H), 2.54–2.60 (m, 3H), 3.40 (br s, 2H), 5.21 (s, 2H), 7.02 (t, 2H), 7.23–7.26 (m, 2H), 7.54 (br s, 1H), 8.08 (br s, 1H), 8.12 (s, 1H). MS (ESI+) *m/z*: 329 (100%) (M+H). Anal. Calcd for $C_{17}H_{21}FN_6{\cdot}0.05H_2O{\cdot}0.1CH_3COOC_2H_5$: C, 61.81; H, 6.53; N, 24.86. Found: C, 61.94; H, 6.17; N, 24.55.

4.1.13. 6-Chloro-9-cyclopentyl-9*H*-purine (18)

To a suspension of **9** (0.4 g, 2.58 mmol) in 5 mL of DMSO was added K_2CO_3 (0.53 g, 3.88 mmol). The reaction mixture was stirred at 40 °C for 20 min. **A** solution of cyclopentyl bromide (0.4 mL, 3.88 mmol) in 4 mL DMF was added to the reaction mixture and the mixture was stirred at 40 °C for 48 h. The reaction mixture was treated with H_2O and extracted with EtOAc. The extract was dried over Na_2SO_4, the solvent was evaporated in vacuo, and the residue was purified by column chromatography eluting with hexanes–EtOAc (10:1) to give **18** (0.32 g, 55%) as a cream-colored crystal: mp 97 °C. ^{1}H NMR ($CDCl_3$) δ 1.76–2.14 (m, 6H), 2.26–2.44 (m, 2H), 4.95–5.06 (m, 1H), 8.17 (s, 1H), 8.75 (s, 1H). MS (ESI+) *m/z*: 223 (100%) (M+H), 225 (34%) (M+H+2). Anal. Calcd for $C_{10}H_{11}ClN_4$: C, 53.94; H, 4.98; N, 25.16. Found: C, 53.78; H, 4.70; N, 25.03.

4.1.14. General procedure for the synthesis of compounds 19–30

To a suspension of 6-chloro-9-cyclopentyl-9*H*-purine (**18**) (0.5 mmol) in absolute EtOH (5 mL) was added the appropriate amine (excess) and the mixture was refluxed for 8–13 h. The reaction mixture was concentrated in vacuo and the residue was purified by column chromatography.

4.1.14.1. 6-[2-(*N*,*N*-Diethylamino)ethyl]amino-9-cyclopentyl-9*H*-purine (19).

The compound was prepared from **18** and *N*,*N*-diethylethylenediamine according to general procedure and was purified by column chromatography (EtOAc/MeOH/NH_3 8:2:0.2) to give **19** (0.13 g, 95%) as a cream-colored solid: mp 155 °C. ^{1}H NMR (DMSO-d_6) δ 1.26 (t, 6H), 1.68–2.15 (m, 8H), 3.18 (q, 4H), 3.19 (t, 2H), 3.87 (br s, 2H), 4.82–4.90 (m, 1H), 7.96 (br s, 1H), 8.28 (s, 1H), 10.84 (br s, 1H). MS (ESI+) *m/z*: 303 (100%) (M+H). Anal. Calcd for $C_{16}H_{26}N_6$: C, 63.54; H, 8.67; N, 27.79. Found: C, 63.67; H, 8.55; N, 27.68.

4.1.14.2. 6-[2-(*N*-isopropylamino)ethyl]amino-9-cyclopentyl-9*H*-purine HCl (20).

The compound was prepared from **18** and *N*-isopropylethylenediamine according to general procedure. The residue was purified by column chromatography (EtOAc/MeOH/NH_3 5:2:0.4) and HCl salt was prepared with conc. HCl to give **20** (0.15 g, 93%) as a white solid: mp 233 °C. ^{1}H NMR (DMSO-d_6) δ 1.13 (d, 6H), 1.50–2.12 (m, 8H), 3.09 (m, 1H), 3.86 (t, 2H), 4.21–4.82 (m, 3H), 8.35–8.67 (m, 2H), 9.21–9.43 (m, 2H). MS (ESI+) *m/z*: 289 (100%) (M+H). Anal. Calcd for $C_{15}H_{24}N_6{\cdot}3HCl$: C, 45.29; H, 6.84; N, 21.13. Found: C, 45.05; H, 6.71; N, 21.01.

4.1.14.3. 6-[2-(Phenylamino)ethyl]amino-9-cyclopentyl-9*H*-purine (21).

The compound was prepared from **18** and *N*-phenylethylenediamine according to general procedure and was purified by column chromatography (EtOAc/MeOH 10:1) to give **21** (0.08 g, 50%) as a cream-colored solid: mp 123 °C. ^{1}H NMR (DMSO-d_6) δ *1.66–2.18* (m, 8H), 3.24 (q, 2H), 3.65 (br s, 2H), 4.80–4.88 (m, 1H), 5.77 (t, 1H), 6.51 (t, 1H, J = 7.2 Hz), 6.62 (d, 2H, J = 8.0 Hz), 7.07 (t, 2H, J = 7.6 Hz), 7.81 (br s, 1H), 8.22 (s, 1H), 8.25 (br s, 1H). ^{13}C NMR (DMSO-d_6) δ 24.1, 33.0, 40.5, 44.5, 56.2, 113.0, 117.7, 129.5, 138.3, 148.2, 152.9, 155.4. MS (ESI+) *m/z*: 323 (100%) (M+H). Anal. Calcd for $C_{18}H_{22}N_6.0.1CH_3COOC_2H_5$: C, 66.72; H, 6.94; N, 25.37. Found: C, 66.83; H, 6.55; N, 25.08.

4.1.14.4. 6-(4-Chlorobenzyl)amino-9-cyclopentyl-9*H*-purine (22).

The compound was prepared from **18** and 4-chlorobenzylamine according to general procedure and was purified by column chromatography (EtOAc/hexane 2:1) to give **22** (0.13 g, 71%) as a cream-colored solid: mp 153.5 °C. ^{1}H NMR (DMSO-d_6) δ *1.65–2.09* (m, 8H), 4.64 (br s, 2H), 4.74–4.87 (m, 1H), 7.32 (s, 4H), 8.16 (s, 1H), 8.20 (s, 1H), 8.32 (br s, 1H). MS (ESI+) *m/z*: 328 (100%) (M+H). Anal. Calcd for $C_{17}H_{18}ClN_5{\cdot}0.1CH_3COOC_2H_5$: C, 62.08; H, 5.62; N, 20.80. Found: C, 62.33; H, 5.25; N, 20.58.

4.1.14.5. 6-(2,4-Dichlorobenzyl)amino-9-cyclopentyl-9*H*-purine (23).

The compound was prepared from **18** and 2,4-dichlorobenzylamine according to general procedure and was purified by column chromatography (EtOAc/hexane 2:1) to give **23** (0.12 g, 74%) as a cream-colored solid: mp 135 °C. ^{1}H NMR (DMSO-d_6) δ *1.66–2.10* (m, 8H), 4.68 (br s, 2H), 4.78–4.86 (m, 1H), 7.24–7.33 (m, 2H), 7.57 (s, 1H), 8.15 (s, 1H), 8.24 (s, 1H), 8.33 (br s, 1H). MS (ESI+) *m/z*: 362 (100%) (M+H). Anal. Calcd for $C_{17}H_{17}Cl_2N_5$: C, 56.36; H, 4.73; N, 19.33. Found: C, 56.67; H, 4.80; N, 18.95.

4.1.14.6. 6-(4-Fluorobenzyl)amino-9-cyclopentyl-9*H*-purine (24).

The compound was prepared from **18** and 4-fluorobenzylamine according to general procedure and was purified by column chromatography (EtOAc/hexane 2:1) to give **24** (0.10 g, 71%) as a cream-colored solid: mp 143 °C. ^{1}H NMR (DMSO-d_6) δ *1.64–2.13* (m, 8H), 4.64 (br s, 2H), 4.77–4.85 (m, 1H), 7.08 (t, 2H), 7.35 (t, 2H), 8.17 (s, 1H), 8.20 (s, 1H), 8.30 (br s, 1H). ^{13}C NMR (DMSO-d_6) δ 24.2, 32.6, 42.9, 56.1, 115.5, 115.7, 120.2, 129.8 (2), 137.1, 140.0, 149.5, 152.8, 154.9, 160.6, 162.9. MS (ESI+) *m/z*: 312 (100%) (M+H). Anal. Calcd for $C_{17}H_{18}FN_5.0.15H_2O$: C, 65.01; H, 5.87; N, 22.30. Found: C, 65.34; H, 5.98; N, 21.94.

4.1.14.7. 6-(2,4-Difluorobenzyl)amino-9-cyclopentyl-9*H*-purine (25).

The compound was prepared from **18** and 2,4-difluorobenzylamine according to general procedure and was purified by column chromatography (EtOAc/hexane 3:1) to give **25** (0.11 g, 74%) as a cream-colored solid: mp 127 °C. ^{1}H NMR (DMSO-d_6) δ *1.65–2.12* (m, 8H), 4.67 (br s, 2H), 4.77–4.88 (m, 1H), 6.97 (t, 1H), 7.17 (t, 1H), 7.35 (q, 1H), 8.17 (s, 1H), 8.22 (s, 1H), 8.27 (br s, 1H). MS (ESI+) *m/z*: 330 (100%) (M+H). Anal. Calcd for $C_{17}H_{17}F_2N_5{\cdot}0.1H_2O$: C, 61.66; H, 5.23; N, 21.15. Found: C, 62.03; H, 5.13; N, 20.78.

4.1.14.8. 6-(4-Methoxybenzyl)amino-9-cyclopentyl-9*H*-purine (26).

The compound was prepared from **18** and 4-methoxybenzylamine according to general procedure and was purified by column chromatography (EtOAc/hexane 3:1) to give **26** (0.12 g, 82%) as a cream-colored solid: mp 138 °C. ^{1}H NMR ($CDCl_3$) δ *1.77–1.99* (m, 6H), 2.24–2.31 (m, 2H), 3.79 (s, 3H), 4.81 (br s, 2H), 4.87–4.93 (m, 1H), 6.44 (br s, 1H), 6.85 (d, 2H, J_o = 8.4 Hz), 7.31 (d, 2H, J_o = 8.0 Hz), 7.66 (br s, 1H), 8.41 (br s, 1H). ^{13}C NMR ($CDCl_3$) δ

23.8, 32.7, 44.0, 52.3, 55.8, 114.0, 120.0, 129.0, 130.8, 137.9, 149.4, 152.9, 154.8, 159.0. MS (ESI+) *m/z*: 324 (100%) (M+H). Anal. Calcd for $C_{18}H_{21}N_5O$: C, 66.85; H, 6.55; N, 21.66. Found: C, 66.59; H, 6.78; N, 21.67.

4.1.14.9. 6-(2,4-Dimethoxybenzyl)amino-9-cyclopentyl-9*H*-purine (27). The compound was prepared from **18** and 2,4-dimethoxybenzylamine according to general procedure and was purified by column chromatography (EtOAc/hexane 3:1) to give **27** (0.07 g, 44%) as a white solid: mp 142 °C. ^{1}H NMR ($CDCl_3$) δ *1.77–2.04* (m, 6H), 2.22–2.30 (m, 2H), 3.78 (s, 3H), 3.84 (s, 3H), 4.76 (br s, 2H), 4.87–4.92 (m, 1H), 6.19 (br s, 1H), 6.41 (d, 1H, J_o = 8 Hz), 6.46 (s, 1H), 7.30 (d, 1H, J_o = 7.6 Hz), 7.75 (br s, 1H), 8.41 (br s, 1H). MS (ESI+) *m/z*: 354 (100%) (M+H). Anal. Calcd for $C_{19}H_{23}N_5O_2$: C, 64.57; H, 6.56; N, 19.82. Found: C, 64.25; H, 6.31; N, 19.55.

4.1.14.10. 6-(4-Methylpiperazin-1-yl)amino-9-cyclopentyl-9*H*-purine (28). The compound was prepared from **18** and 1-amino-4-methylpiperazine according to general procedure and was purified by column chromatography ($CHCl_3$/MeOH 10:2) to give **28** (0.07 g, 52%) as a cream-colored solid: mp 91 °C. ^{1}H NMR (DMSO-d_6) δ *1.65–2.00* (m, 6H), 2.06–2.16 (m, 5H), 2.39 (t, 4H), 2.84 (t, 4H), 4.77–4.85 (m, 1H), 8.17 (s, 1H), 8.20 (s, 1H), 8.66 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 24.0, 32.9, 45.9, 51.0, 54.4 (2), 56.2 (2), 119.2, 136.9, 138.6, 149.9, 153.5 (2). MS (ESI+) *m/z*: 302 (100%) (M+H). Anal. Calcd for $C_{15}H_{23}N_7$: C, 59.78; H, 7.69; N, 32.53. Found: C, 59.55; H, 7.32; N, 32.47.

4.1.14.11. 6-(2-Cyclohexenylethyl)amino-9-cyclopentyl-9*H*-purine (29). The compound was prepared from **18** and 2-(1-cyclohexenyl)ethylamine according to general procedure and was purified by column chromatography (EtOAc/hexane 1:1) to give **29** (0.10 g, 71%) as a cream-colored solid: mp 88 °C. ^{1}H NMR (DMSO-d_6) δ *1.43–2.20* (m, 18H), 3.51 (br s, 2H), 4.76–4.84 (m, 1H), 5.38 (s, 1H), 7.56 (br s, 1H), 8.16 (s, 2H). ^{13}C NMR (DMSO-d_6) δ 22.6, 23.1, 24.2, 25.4, 28.5, 32.6, 38.1, 39.0, 56.0, 120.2, 122.4, 135.9, 139.6, 149.2, 152.8, 155.1. MS (ESI+) *m/z*: 312 (100%) (M+H). Anal. Calcd for $C_{18}H_{25}N_5 \cdot 0.1CH_3COOC_2H_5$: C, 68.62; H, 8.14; N, 21.75. Found: C, 68.78; H, 7.81; N, 21.40.

4.1.14.12. 6-(4-Methylpiperidin-1-yl)-9-cyclopentyl-9*H*-purine (30). The compound was prepared from **18** and 4-methylpiperidine according to general procedure and was purified by column chromatography ($CHCl_3$/MeOH 10:1) to give **30** (0.09 g, 70%) as a cream-colored solid: mp 79 °C. ^{1}H NMR (DMSO-d_6) δ 0.87 (d, 3H), 0.98–1.12 (m, 1H), 1.60–2.16 (m, 12H), 2.99 (m, 4H), 4.78–4.86 (m, 1H), 8.18 (d, 2H). ^{13}C NMR (DMSO-d_6) δ 22.4, 24.2, 31.3, 32.5, 34.5, 45.6, 55.9, 120.1, 138.7, 151.1, 152.2, 153.8. MS (ESI+) *m/z*: 286 (100%) (M+H). Anal. Calcd for $C_{16}H_{23}N_5$: C, 67.34; H, 8.12; N, 24.54. Found: C, 67.18; H, 7.76; N, 24.47.

4.2. Microbiology

Determination of the minimal inhibitory concentration (MIC) of the compounds by dilution method.

4.2.1. Sample preparation

Each of the test compounds and standards (sultamicillin, ampicillin, ciprofloxacin, miconazole, oxiconazole) were dissolved in 12.5% DMSO, at concentrations of 200 μg/mL. Further dilutions of the compounds and standards in the test medium were prepared at required quantities of 100, 50, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 and 0.19 μg/mL.

4.2.2. Culture of microorganisms

Bacteria and fungal species used were obtained from Microbiology Department of Ankara University Faculty of Pharmacy and Refik Saydam Health Institution of Health Ministry, Ankara, Turkey, namely *S. aureus* (ATCC 25923), MRSA (standart, ATCC 431300), MRSA (clinical isolate), *E. coli* (ATCC 25922), *B. Subtilis* (ATCC 6633), *C. albicans* (ATCC 10145). The bacterial strains were maintained on MHA (Mueller–Hinton Agar) medium for 24 h at 37 °C and fungi were maintained on SDA (Sabouraud Dextrose Agar) for 2–5 days at 25 ± 1 °C. The bacteria and fungi inocula were prepared by suspension in 9 mL of sterile water for colonies from culture on MHA and SDA medium.

4.2.3. Tube dilution technique

The in vitro antibacterial and antifungal activity of compounds were tested by the tube dilution technique.[33,34] The tube dilution technique was followed to determine the minimum inhibitory concentration (MIC) of all the synthesized compounds. MHB (Mueller–Hinton Broth) was used for bacteria, SDB (Sabouraud Dextrose Broth) was used for *C. albicans*. The cell density of each inoculum was adjusted in sterile water of 0.5 Mc Farland standard. A final concentration of approximately 10^5 CFU/mL and 10^4 CFU/mL for the bacteria and fungi, respectively. Microbial inocula were added to the two-fold diluted samples. After incubation for bacteria 18–24 h at 37 ± 1 °C and for fungi 2–5 days 25 ± 1 °C, the last tube with no growth of microorganism was recorded to represent MIC value expressed in μg/mL.

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