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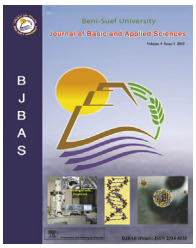

Full Length Article

Synthesis and antimicrobial evaluation of certain purine, benzothiazole and thiazole systems substituted with dialkylaminoalkyl-o-cresols

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ABSTRACT

Novel series of dialkylaminoalkyl-o-cresols incorporated with purine nucleus **2a–b**, benzothiazole nucleus **5a–b**, **8a–b** and thiazole nucleus **11a–d**, **13a–d** were synthesized through Mannich reaction. Structures of the newly synthesized compounds have been deduced on the basis of elemental analysis and spectral data. Antimicrobial activity evaluation was carried out for all synthesized compounds; most of them exerted comparable activity to ciprofloxacin and flucanazole. The thiazole derivatives **11a**, **13c**, **13d** are the most potent compounds.

1. Introduction

The resistance to antimicrobial drugs is widespread; there is an increasing need for identification of novel structure leads that may be of use in designing new, potent and less toxic antimicrobial agents. The previous literature survey revealed that certain phenolic Mannich bases (Magarian and Sorenson, 1976; Pernak et al., 1999), purine (Bakkestuen et al., 2000; Gundersen et al., 2002), benzothiazole (Abdel-Rahman and Morsy, 2007; Hilal et al., 2006; Vicini et al., 2008; Yamazaki et al., 2005) and thiazole (Bozdag-Dundar et al., 2007; Karegoudar et al., 2008; Ozdemir et al., 2007; Vicini et al., 2006) derivatives exhibited significant antibacterial and antifungal activities. At the same time, presence of azo group (Bondock et al., 2007) or azomethine group (Chohan et al., 2003) enhances the antimicrobial activity of the above mentioned nuclei. Also, the introduction of an amidic linkage into the heteroaromatic ring systems increases its biological activity by increasing the lipophilicity of these compounds which facilitates their penetration into the bacterial and fungal cells (Turan-Zitouni et al., 2004, 2005; Zimenkovskii et al., 2006). In view of the aforementioned findings, it was

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interesting to make a link between phenolic Mannich bases and some heteroaromatic ring systems *via* one of the above pharmacophoric groups. Accordingly and in continuation of our previous work (Abdellatif et al., 2014), we now report combination between purine nucleus with dialkylamino-*o*-cresol moieties *via* an amidic linkage (**2a–b**), or an incorporation of a benzothiazole ring system with phenolic Mannich bases through the pharmacophoric azomethine group (**5a–b**) or *via* an amidic linkage (**8a–b**). Moreover, a number of substituted thiazoles incorporated with dialkylamino-*o*-cresol moieties *via* azo group (**11a–d**, **13a–d**) with the aim that the target compounds may show good antimicrobial activity.

2. Experimental

2.1. Chemistry

2.1.1. General

Melting points were determined on a Griffin apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Shimadzu 435 Spectrometer, using KBr discs and values were represented in cm-1. ^{1}H NMR spectra were measured on a Varian Gemini 300 MHz spectrometer in $CDCl_3$ or DMSO-d_6 with TMS as the internal standard. Mass spectra were run on Hewlett Packard 5988 spectrometer. Microanalyses were performed for C, H, N (Micro Analytical Centre, Cairo University, Egypt) and were within± 0.4% of theoretical values. Progress of the reactions was monitored by TLC using TLC sheets precoated with UV fluorescent silica gel MERCK 60 F 254 that was visualized by UV lamp. 2-(1,3-Dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-7-yl)-*N*-(4-hydroxyphenyl)acetamide (**1**), 4-benzothiazol-2-yl-phenylamine (**3**) (Shi et al., 1996), 4-[(4-benzothiazol-2-yl-phenylimino)-methyl]-phenol (**4**) (Nagarajan et al., 2010), *N*-(4-benzothiazol-2-yl-phenyl)-2-chloroacetamide (**6**) (Chua et al., 1999; Pan et al., 2013), 4-phenylthiazol-2-ylamine (**9a**) (Dodson and King, 1945), 4-(4-methylphenyl)thiazol-2-ylamine (**9b**) (King and Hlavacek, 1950), 2-amino-4-methylthiazole-5-carboxylic acid ethyl ester (**9c**) (Dodson and King, 1945), 4-(4-phenylthiazol-2-ylazo) phenol (**10a**), and 2-(4-hydroxy-phenylazo)-4-methylthiazole-5-carboxylic acid ethyl ester (**10b**) were prepared according to reported procedures.

*2.1.1.1. N-(3-Diethylaminomethyl-4-hydroxyphenyl)-2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-purin-7-yl)acetamide (**2a**).* To a solution of the purine derivative **1** (1.64 gm, 5 mmol) in dimethylformamide (15 mL), a mixture of diethylamine (6 mmol) and formaldehyde solution (37–40%) (0.62 mL, 7.5 mmol) in glacial acetic acid (5 mL) was added drop wise. After complete addition, the reaction mixture was stirred at room temperature for 12 h then poured onto ice-cooled water. The formed precipitate was filtered, dried and crystallized from dimethylformamide/water to afford 1.39 g of **2a** (67% yield); mp 249–250°; IR (cm^{-1}) 3306, 3257 (OH & NH), 3162, 3086 (CH aromatic), 2984, 2878 (CH aliphatic), 1703, 1651 (amidic C=O), 1555 (C=N), 1518 (C=C aromatic); ^{1}H NMR (DMSO-d_6) δ 0.95 (t, J = 7.5 *Hz*, 6H, $\underline{H}_3C-H_2C-N-CH_2-C\underline{H}_3$), 2.74 (q, J = 7.5 *Hz*, 4H, $H_3C-\underline{H}_2C-N-C\underline{H}_2-CH_3$), 3.17 (s, 3H, purinyl N3–$C\underline{H}_3$), 3.42 (s, 3H, purinyl N1–$C\underline{H}_3$), 3.55 (s, 2H, $C\underline{H}_2N$); 5.13 (s, 2H, $C\underline{H}_2CO$), 6.68–6.72 (m, 2H, phenyl H-2, H-6), 7.35 (d, J = 8.1 *Hz*, 1H, phenyl H-5), 8.04 (s, 1H, purinyl H-8), 9.33 (s, 1H, N$\underline{H}$, D_2O exchangeable), 10.17 (s, 1H, O$\underline{H}$, D_2O exchangeable); EIMS 414 (M$^+$). Anal. Calcd for $C_{20}H_{26}N_6O_4$: C, 57.96; H, 6.32; N, 20.28. Found: C, 58.22; H, 6.29; N, 20.40.

*2.1.1.2. 2-(1,3-Dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-7-yl)-N-[4-hydroxy-3-(4-methylpiperidin-1-ylmethyl)-phenyl]-acetamide (**2b**).* The title compound **2b** was synthesized, using a similar procedure to that described for the preparation of **2a**, by using 4-methylpiperidine in place of diethylamine, in 80% yield as a pale brown powder; mp 188–190 °C; IR (cm^{-1}) 3300, 3200 (OH & NH), 3160, 3058 (CH aromatic), 2948, 2809 (CH aliphatic), 1705, 1659 (amidic C=O), 1548 (C=N), 1495 (C=C aromatic); ^{1}H NMR (DMSO-d_6) δ 0.92 (d, J = 7.2 *Hz*, 3H, piperidine $C\underline{H}_3$), 1.27–1.43 (m, 5H, piperidinyl H-3, H-4, H-5), 2.13 (t, J = 5.4 *Hz*, 4H, piperidinyl H-2, H-6), 3.41 (s, 3H, purinyl N3–$C\underline{H}_3$), 3.58 (s, 3H, purinyl N1–$C\underline{H}_3$), 3.66 (s, 2H, $C\underline{H}_2N$), 4.99 (s, 2H, $C\underline{H}_2CO$), 6.70–6.73 (m, 2H, phenyl H-2, H-6), 7.33 (d, J = 8.1 *Hz*, 1H, phenyl H-5), 7.77 (s, 1H, purinyl H-8), 9.31 (s, 1H, N$\underline{H}$, D_2O exchangeable), 10.41 (s, 1H, O$\underline{H}$, D_2O exchangeable); EIMS 441 (M + 1). Anal. Calcd for $C_{22}H_{28}N_6O_4$: C, 59.99; H, 6.41; N, 19.08. Found: C, 60.14; H, 6.19; N, 18.84.

*2.1.1.3. 4-[(4-benzothiazol-2-yl-phenylimino)methyl]-2-dimethylaminomethylphenol (**5a**).* A mixture of dimethylamine (0.005 mol) and formaldehyde solution (37–40%) (0.4 mL, 0.005 mol) in absolute ethanol (5 mL) was added to a solution of 4-[(4-benzothiazol-2-yl-phenylimino)-methyl]-phenol (**4**) (1.65 gm, 0.005 mol) in absolute ethanol (10 mL). The reaction mixture was heated under reflux for 5 h, allowed to cool to room temperature and poured onto ice cooled water. The formed precipitate was filtered off, dried and crystallized from aqueous ethanol to give 1.94 g of **5a** (65% yield); mp 161–163 °C; IR (cm^{-1}) 3420 (OH), 3104, 2955 (CH aromatic), 2924, 2851 (CH aliphatic), 1594 (C=N), 1500 (C=C aromatic); ^{1}H NMR (DMSO-d_6) δ 2.27 (s, 6H, $\underline{H}_3C-N-C\underline{H}_3$), 3.58 (s, 2H, $C\underline{H}_2N$), 7.34–8.12 (m, 12H, phenol H-3, H-5, H-6, phenyl H-2, H-3, H-5, H-6, benzothiazolyl H-4, H-5, H-6, H-7, N=C$\underline{H}$), 10.27 (s, 1H, O$\underline{H}$, D_2O exchangeable); EIMS 387 (M$^+$). Anal. Calcd for $C_{23}H_{21}N_3OS$: C, 71.29; H, 5.46; N, 10.84. Found: C, 70.98; H, 5.29; N, 10.58.

*2.1.1.4. 4-[(4-benzothiazol-2-yl-phenylimino)methyl]-2-(piperidin-1-ylmethyl)phenol (**5b**).* The title compound **5b** was synthesized, using a similar procedure to that described for the preparation of **5a**, by using piperidine in place of dimethylamine, in 62% yield as a pale brown powder; mp 110–112 °C; IR (cm^{-1}) 3420 (OH), 3096, 3027 (CH aromatic), 2932, 2801 (CH aliphatic), 1596 (C=N), 1516 (C=C aromatic); ^{1}H NMR (DMSO-d_6) δ 1.00–1.12 (m, 6H, piperidine H-3, H-4, H-5), 2.22 (t, 4H, J = 5.1 *Hz*, piperidine H-2, H-6), 3.96 (s, 2H, $C\underline{H}_2N$), 7.34–8.12 (m, 12H, phenol H-3, H-5, H-6, phenyl H-2, H-3, H-5, H-6, benzothiazolyl H-4, H-5, H-6, H-7, N=C$\underline{H}$), 10.27 (s, 1H, O$\underline{H}$, D_2O exchangeable); EIMS 427 (M$^+$). Anal. Calcd for $C_{26}H_{25}N_3OS$: C, 73.04; H, 5.89; N, 9.83. Found: C, 73.23; H, 5.73; N, 10.13.

*2.1.1.5. N-(4-Benzothiazol-2-ylphenyl)-2-(4-hydroxyphenylamino)acetamide (**7**).* A mixture of *N*-(4-benzothiazol-2-ylphenyl)-2-chloroacetamide (**6**) (1.5 g, 0.005 mol), 4-aminophenol (0.5 g, 0.005 mol) and few crystals

of potassium iodide in absolute ethanol (80 mL) was heated under reflux for 11 h. The formed precipitate was filtered while hot, washed with hot ethanol (5 mL). After drying, the precipitate was suspended in water and neutralized with sodium carbonate solution (20%) then filtered, washed with water, dried and crystallized from n-butanol to afford **7**. m.p. 235–237°, yield: 1.0 g, 53.7%; IR (cm^{-1}) 3424, 3291 (OH & NH), 3102, 3031 (CH aromatic), 2905 (CH aliphatic), 1676 (amidic C=O), 1596 (C=N), 1528 (C=C aromatic); ^1H NMR (DMSO-d_6) δ 4.31 (s, 2H, $C\underline{H}_2C{=}O$), 5.44 (s, 1H, $CH_2N\underline{H}$, D_2O exchangeable), 6.50–8.09 (m, 12H, phenol H-2, H-3, H-5, H-6, phenyl H-2, H-3, H-5, H-6, benzothiazolyl H-4, H-5, H-6, H-7), 8.58 (s, 1H, $N\underline{H}CO$, D_2O exchangeable), 10.26 (s, 1H, $O\underline{H}$, D_2O exchangeable); EIMS 375 (M^+). Anal. Calcd for $C_{21}H_{17}N_3O_2S$: C, 67.18; H, 4.56; N, 11.19. Found: C, 66.91; H, 4.59; N, 11.39.

*2.1.1.6. N-(4-Benzothiazol-2-ylphenyl)-2-(3-dimethylaminomethyl-4-hydroxyphenylamino)aceta-mide (**8a**).* A mixture of dimethylamine (0.005 mol), formaldehyde solution (37–40%) (0.4 mL, 0.005 mol) and absolute ethanol (5 mL) was added portion wise to a suspension of compound **7** (1.87 g, 0.005 mol) in absolute ethanol (10 mL). The reaction mixture was heated under reflux for 5 h then allowed to cool to room temperature. The obtained precipitate was filtered, dried and crystallized from dimethylformamide/water to give **8a** 1.36 g (63%); mp 209–211 °C; IR (cm^{-1}) 3417, 3275 (OH & NH), 3104, 3054 (CH aromatic), 2919, 2836 (CH aliphatic), 1681 (amidic C=O), 1601 (C=N), 1518 (C=C aromatic); ^1H NMR (DMSO-d_6) δ 2.28 (s, 6H, $\underline{H}_3C{-}N{-}C\underline{H}_3$), 3.99 (s, 2H, $C\underline{H}_2N$), 4.49 (s, 2H, $C\underline{H}_2CO$), 5.41 (s, 1H, $CH_2N\underline{H}$, D_2O exchangeable), 6.50–8.14 (m, 11H, hydroxyphenyl H-2, H-5, H-6, phenyl H-2, H-3, H-5, H-6, benzothiazolyl H-4, H-5, H-6, H-7), 8.51 (s, 1H, $N\underline{H}CO$, D_2O exchangeable), 10.05 (s, 1H, $O\underline{H}$, D_2O exchangeable); EIMS 432 (M^+). Anal. Calcd for $C_{24}H_{24}N_4O_2S$: C, 66.64; H, 5.59; N, 12.95. Found: C, 66.40; H, 5.29; N, 12.77.

*2.1.1.7. N-(4-Benzothiazol-2-yl-phenyl)-2-[4-hydroxy-3-(4-methylpiperidin-1-ylmethyl)phenyl-amino]acetamide (**8b**).* The title compound **8b** was synthesized, using a similar procedure to that described for the preparation of **8a**, by using 4-methylpiperidine in place of dimethylamine, in 69% yield as a pale brown powder; mp 170–172 °C; IR (cm^{-1}) 3420, 3250 (OH & NH), 3103, 3054 (CH aromatic), 2949, 2869 (CH aliphatic), 1694 (amidic C=O), 1601 (C=N), 1519 (C=C aromatic); ^1H NMR (DMSO-d_6) δ 0.92 (d, J = 7.2 *Hz*, 3H, piperidine $C\underline{H}_3$), 1.27–1.43 (m, 5H, piperidine H-3, H-4, H-5), 2.13 (t, 4H, J = 5.4 *Hz*, piperidine H-2, H-6), 3.66 (s, 2H, $C\underline{H}_2N$), 4.49 (s, 2H, $C\underline{H}_2CO$), 5.41 (s, 1H, $CH_2N\underline{H}$, D_2O exchangeable), 6.50–8.14 (m, 11H, hydroxyphenyl H-2, H-5, H-6, phenyl H-2, H-3, H-5, H-6, benzothiazolyl H-4, H-5, H-6, H-7), 8.51 (s, 1H, $N\underline{H}CO$, D_2O exchangeable), 10.05 (s, 1H, $O\underline{H}$, D_2O exchangeable); EIMS 486 (M^+). Anal. Calcd for $C_{28}H_{30}N_4O_2S$: C, 69.11; H, 6.21; N, 11.51. Found: C, 69.09; H, 5.81; N, 11.67.

*2.1.2. General procedure for the preparation of 4-(thiazol-2-ylazo)-2-substituted-methyl-phenol derivatives (**11a–d**)*

To a solution of the appropriate azo dye **10a** or **10b** (1.4 g, 0.005 mol) in absolute ethanol (10 mL), a mixture of the respective secondary amine (0.005 mol) and formaldehyde solution (37–40%) (0.4 mL, 0.005 mol) in absolute ethanol (5 mL) was added. The reaction mixture was heated under reflux for 5 h then set-aside overnight. The solvent was distilled off under reduced pressure. The residue left was dissolved in the least amount of ethanol, precipitated with water, filtered, dried and crystallized from aqueous ethanol to afford the Mannich bases **11a–d**. Physical and spectral data for **11a–d** are listed below.

*2.1.2.1. 2-Diethylaminomethyl-4-(4-phenylthiazol-2-ylazo)-phenol (**11a**).* Yield, 60%; mp 99–101 °C; IR (cm^{-1}) 3422 (OH), 3105, 2957 (CH aromatic), 2924, 2822 (CH aliphatic), 1596 (C=N), 1543 (N=N), 1501 (C=C aromatic); ^1H NMR (DMSO-d_6) δ 0.96 (t, J = 7.2 *Hz*, 6H, $\underline{H}_3C{-}H_2C{-}N{-}CH_2{-}C\underline{H}_3$), 2.74 (q, J = 7.2 *Hz*, 4H, $H_3C{-}\underline{H}_2C{-}N{-}C\underline{H}_2{-}CH_3$), 4.04 (s, 2H, $C\underline{H}_2N$), 6.76–8.21 (m, 9H, phenol H-2, H-5, H-6, 5 phenyl protons, thiazolyl H-5), 9.87 (s, 1H, $O\underline{H}$, D_2O exchangeable); EIMS 366 (M^+). Anal. Calcd for $C_{20}H_{22}N_4OS$: C, 65.55; H, 6.05; N, 15.29. Found: C, 65.26; H, 5.79; N, 15.55.

*2.1.2.2. 2-(4-methylpiperazin-1-ylmethyl)-4-(4-phenylthiazol-2-ylazo) phenol (**11b**).* Yield, 51%; mp 179–182 °C; IR (cm^{-1}) 3422 (OH), 3102, 2941 (CH aromatic), 2881, 2824 (CH aliphatic), 1582 (C=N), 1542 (N=N), 1487 (C=C aromatic); ^1H NMR (DMSO-d_6) δ 2.29 (s, 3H, piperazine $C\underline{H}_3$), 2.45–2.49 (m, 8H, piperazine H-2, H-3, H-5, H-6), 3.97 (s, 2H, $C\underline{H}_2N$), 6.79–8.24 (m, 9H, phenol H-2, H-5, H-6, 5 phenyl protons, thiazolyl H-5), 9.86 (s, 1H, $O\underline{H}$, D_2O exchangeable); EIMS 393 (M^+). Anal. Calcd for $C_{21}H_{23}N_5OS$: C, 64.10; H, 5.89; N, 17.80. Found: C, 64.39; H, 5.71; N, 17.74.

*2.1.2.3. 2-(4-Hydroxy-3-piperidin-1-ylmethylphenylazo)-4-methylthiazole-5-carboxylic acid ethyl ester (**11c**).* Yield, 66%; mp 87–90 °C; IR (cm^{-1}) 3424 (OH), 3040, 2932 (CH aromatic), 2856, 2808 (CH aliphatic), 1712 (C=O ester), 1602 (C=N), 1583 (N=N), 1522 (C=C aromatic); ^1H NMR (DMSO-d_6) δ 1.0–1.1 (m, 6H, piperidine H-3, H-4, H-5), 1.39 (t, J = 7.2 *Hz*, 3H, $OCH_2C\underline{H}_3$), 2.30 (t, J = 5.4 *Hz*, 4H, piperidine H-2, H-6), 2.81 (s, 3H, $C\underline{H}_3$), 3.75 (s, 2H, $C\underline{H}_2N$), 4.36 (q, J = 7.2 *Hz*, 2H, $OC\underline{H}_2CH_3$), 6.94 (d, J = 8.1 *Hz*, 1H, phenyl H-5), 7.73 (s, 1H, phenyl H-2); 7.91 (d, J = 8.1 *Hz*, 1H, phenyl H-6), 10.25 (s, 1H, $O\underline{H}$, D_2O exchangeable); EIMS 388 (M^+). Anal. Calcd for $C_{19}H_{24}N_4O_3S$: C, 58.74; H, 6.23; N, 14.42. Found: C, 58.54; H, 6.62; N, 14.78.

*2.1.2.4. 2-(4-hydroxy-3-morpholin-4-ylmethylphenylazo)-4-methylthiazole-5-carboxylic acid ethyl ester (**11d**).* Yield, 42%; mp 100–102 °C; IR (cm^{-1}) 3397 (OH), 3040, 2930 (CH aromatic), 2847, 2822 (CH aliphatic), 1712 (C=O ester), 1581 (C=N), 1544 (N=N), 1518 (C=C aromatic); ^1H NMR (DMSO-d_6) δ 1.38 (t, J = 7.2 *Hz*, 3H, $OCH_2C\underline{H}_3$), 2.12 (t, J = 5.7 *Hz*, 4H, $\underline{H}_2C{-}N{-}C\underline{H}_2$ of morpholine moiety), 2.54 (s, 3H, $C\underline{H}_3$), 3.98 (s, 2H, $C\underline{H}_2N$), 4.37 (q, J = 7.2 *Hz*, 2H, $OC\underline{H}_2CH_3$), 4.68 (t, J = 5.7 *Hz*, 4H, $\underline{H}_2C{-}O{-}C\underline{H}_2$ of morpholine moiety), 6.95 (d, J = 8.1 *Hz*, 1H, phenyl H-5), 7.74 (s, 1H, phenyl H-2), 7.89 (d, J = 8.1 *Hz*, 1H, phenyl H-6), 10.23 (s, 1H, $O\underline{H}$, D_2O exchangeable); EIMS 390 (M^+). Anal. Calcd for $C_{18}H_{22}N_4O_4S$: C, 55.37; H, 5.68; N, 14.35. Found: C, 55.57; H, 5.87; N, 14.25.

*2.1.3. General procedure for the preparation of 4-(2-amino-4-substituted-thiazol-5-ylazo) phenols (**12a–b**)*

4-aminophenol (1.1 g, 0.01 mol) was dissolved in a mixture of concentrated sulphuric acid (0.7 mL), water (5 mL) and

crushed ice (5 gm) and cooled in ice to 5 °C. A solution of sodium nitrite (0.76 g, 0.011 mol) in water (2 mL) was added drop wise while stirring to the above solution, stirring was continued for further 20 min. The diazonium salt solution was filtered onto a well cooled solution of 4-substituted-thiazol-2-ylamine **9a** or **9b** (0.01 mol) and sodium acetate (2.5 gm, 0.03 mol) in aqueous ethanol (30 mL) and stirred at 5 °C for 2 h. The formed precipitate was filtered, washed well with water, dried and crystallized from the appropriate solvent to yield **12a–b** as orange crystals. Physical and spectral data for **12a–b** are listed below.

2.1.3.1. 4-(2-Amino-4-phenylthiazol-5-ylazo)phenol (12a). Yield, 91%; mp 198–200 °C (crystallized from methanol); IR (cm^{-1}): 3486–3333 (OH & NH_2), 3133, 3020 (CH aromatic), 1601 (C=N), 1580 (N=N), 1498 (C=C aromatic); ^1H NMR (DMSO-d_6) δ 5.80 (s, 2H, NH_2, D_2O exchangeable), 6.87–8.18 (m, 9H, 5 phenyl protons and 4 phenol protons), 8.50 (s, 1H, OH, D_2O exchangeable); EIMS 297 (M + 1). Anal. Calcd for $C_{15}H_{12}N_4OS$: C, 60.79; H, 4.08; N, 18.91. Found: C, 60.89; H, 4.05; N, 18.53.

2.1.3.2. 4-(2-Amino-4-p-tolyl–thiazol-5-ylazo)phenol (12b). Yield, 80%; mp 247–248 °C (crystallized from chloroform/diethyl ether); IR (cm^{-1}): 3475, 3345 (OH & NH_2), 3100, 3028 (CH aromatic), 2922, 2853 (CH aliphatic), 1624 (C=N), 1582 (N=N), 1528 (C=C aromatic); ^1H NMR (DMSO-d_6) δ 2.36 (s, 3H, CH_3); 6.85–8.12 (m, 10H, 4 tolyl protons and 4 phenol protons & NH_2 (D_2O exchangeable)), 10.0 (s, 1H, OH, D_2O exchangeable); EIMS 311 (M + 1). Anal. Calcd for $C_{16}H_{14}N_4OS$: C, 61.92; H, 4.55; N, 18.05. Found: C, 62.08; H, 4.52; N, 17.98.

2.1.4. General procedure for the preparation of 4-(2-amino-4-substituted-thiazol-5-ylazo)-2-substituted-methylphenols (13a–d)

To a solution of the thiazole derivative **12a** or **12b** (0.01 mol) in absolute ethanol (10 mL), a mixture of the respective secondary amine (0.01 mol) and formaldehyde solution (37–40%) (0.8 mL, 0.01 mol) in absolute ethanol (5 mL) was added. The reaction mixture was heated under reflux for 5 h then cooled and poured onto ice cooled water. The formed precipitate was filtered, dried and crystallized from aqueous ethanol to afford **13a–d**. Physical and spectral data for **13a–d** are listed below.

2.1.4.1. 4-(2-Amino-4-phenylthiazol-5-ylazo)-2-Diethylaminomethylphenol (13a). Yield, 75%; mp 155–158 °C; IR (cm^{-1}) 3374–3284 (OH & NH_2), 3060, 2971 (CH aromatic), 2929, 2862 (CH aliphatic), 1584 (C=N), 1528 (N=N), 1478 (C=C aromatic); ^1H NMR (DMSO-d_6) δ 1.32 (t, J = 7.2 *Hz*, 6H, CH_2CH_3), 3.84 (s, 2H, CH_2N); 4.28 (q, J = 7.2 *Hz*, 4H, CH_2CH_3), 6.13 (s, 2H, NH_2, D_2O exchangeable), 6.81–8.13 (m, 8H, 5 phenyl protons and 3 phenol protons), 9.22 (s, 1H, OH, D_2O exchangeable); EIMS 381 (M^+). Anal. Calcd for $C_{20}H_{23}N_5OS$: C, 62.97; H, 6.08; N, 18.36. Found: C, 63.11; H, 6.32; N, 18.26.

2.1.4.2. 4-(2-Amino-4-phenylthiazol-5-ylazo)-2-piperidin-1-ylmethylphenol (13b). Yield, 62%; mp 145–148 °C; IR (cm^{-1}): 3336–3278 (OH & NH_2), 3060, 2960 (CH aromatic), 2929, 2872 (CH aliphatic), 1595 (C=N), 1516 (N=N), 1477 (C=C aromatic); ^1H NMR (DMSO-d_6) δ 1.01–1.11 (m, 6H, piperidine H-3, H-4, H-5), 2.30 (t, 4H, J = 5.7 *Hz*, piperidine H-2, H-6), 4.10 (s, 2H, CH_2N); 6.23 (s, 2H, NH_2, D_2O exchangeable); 6.84–8.16 (m, 8H, 5 phenyl protons and 3 phenol protons), 9.18 (s, 1H, OH, D_2O exchangeable); EIMS 393 (M^+). Anal. Calcd for $C_{21}H_{23}N_5OS$: C, 64.10; H, 5.89; N, 17.80. Found: C, 64.40; H, 5.91; N, 17.49.

2.1.4.3. 4-(2-Amino-4-p-tolylthiazol-5-ylazo)-2-dimethylaminomethylphenol (13c). Yield, 45%; mp 100–102 °C; IR (cm^{-1}): 3312, 3199 (OH & NH2), 3028, 2972 (CH aromatic), 2925, 2894 (CH aliphatic), 1599 (C=N), 1536 (N=N), 1489 (C=C aromatic); ^1H NMR (DMSO-d_6) δ 2.28 (s, 6H, $H_3C–N–CH_3$), 2.33 (s, 3H, CH_3), 3.85 (s, 2H, CH_2N), 6.83–8.06 (m, 9H, 4 tolyl protons and 3 phenol protons & NH_2 (D_2O exchangeable)), 10.49 (s, 1H, OH, D_2O exchangeable); EIMS 367 (M^+). Anal. Calcd for $C_{19}H_{21}N_5OS$: C, 62.10; H, 5.76; N, 19.06. Found: C, 62.43; H, 5.99; N, 19.00.

2.1.4.4. 4-(2-Amino-4-p-tolyl-thiazol-5-ylazo)-2-morpholin-4-ylmethylphenol (13d). Yield, 58%; mp 119–121 °C; IR (cm^{-1}): 3362, 3292 (OH & NH2), 3066, 2984 (CH aromatic), 2917, 2857 (CH aliphatic), 1589 (C=N), 1535 (N=N), 1488 (C=C aromatic); ^1H NMR (DMSO-d_6) δ 2.13 (t, 4H, J = 5.7 *Hz*, $H_2C–N–CH_2$ of morpholine moiety), 2.32 (s, 3H, CH_3), 3.88 (s, 2H, CH_2N), 4.69 (t, 4H, J = 5.7 *Hz*, $H_2C–O–CH_2$ of morpholine moiety), 6.86–8.08 (m, 9H, 4 tolyl protons and 3 phenol protons & NH_2 (D_2O exchangeable)), 10.45 (s, 1H, OH, D_2O exchangeable); EIMS 409 (M^+). Anal. Calcd for $C_{21}H_{23}N_5O_2S$: C, 61.59; H, 5.66; N, 17.10. Found: C, 61.65; H, 5.37; N, 17.18.

2.2. *Antimicrobial evaluation*

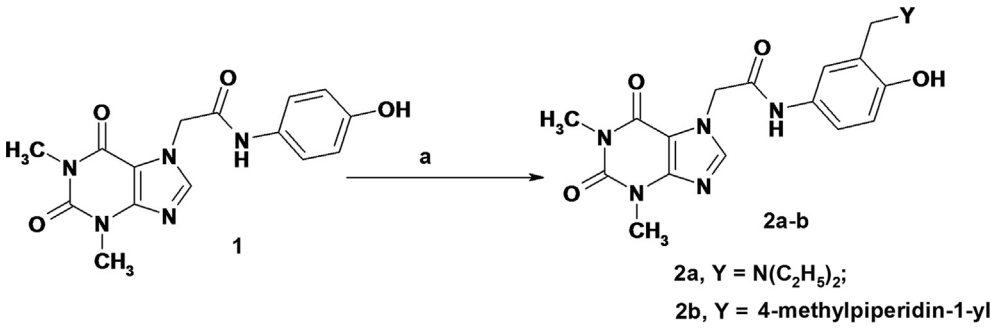
The antimicrobial activity was determined using the agar dilution technique using ciprofloxacin and fluconazole as positive control and the solvent, dimethylsulfoxide (DMSO) as a negative control according to a previously reported procedure (Lorian, 1980).

3. Results and discussion

3.1. *Chemistry*

Reacting solution of 2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropurin-7-yl)-*N*-(4-hydroxyphenyl)acetamide (**1**) in dimethylformamide with a mixture of the appropriate secondary amine (diethylamine or 4-methylpiperidine) and formaldehyde in glacial acetic acid yielded **2a** and **2b** in high yields (67% and 80%) respectively (Scheme 1). The structures of **2a** and **2b** were confirmed on the basis of their elemental and spectral data. The ^1H NMR spectrum of **2a** showed the appearance of a triplet signal at δ 0.95 and a quartet one at δ 2.74 attributed to ($H_5C_2–N–C_2H_5$) in addition to a singlet at δ 3.55 indicating CH_2N. Also, the ^1H NMR spectrum of **2b** displayed the presence of a doublet peak at δ 0.92 indicating the methyl protons attached to the piperidine moiety, a multiplet peak at δ 1.27–1.43 with 5H integration attributed to piperidine H-3, H-4, H-5, a triplet peak at δ 2.13 with 4H integration attributed to piperidine H-2, H-6, and a singlet signal at δ 3.66 corresponding to CH_2N.

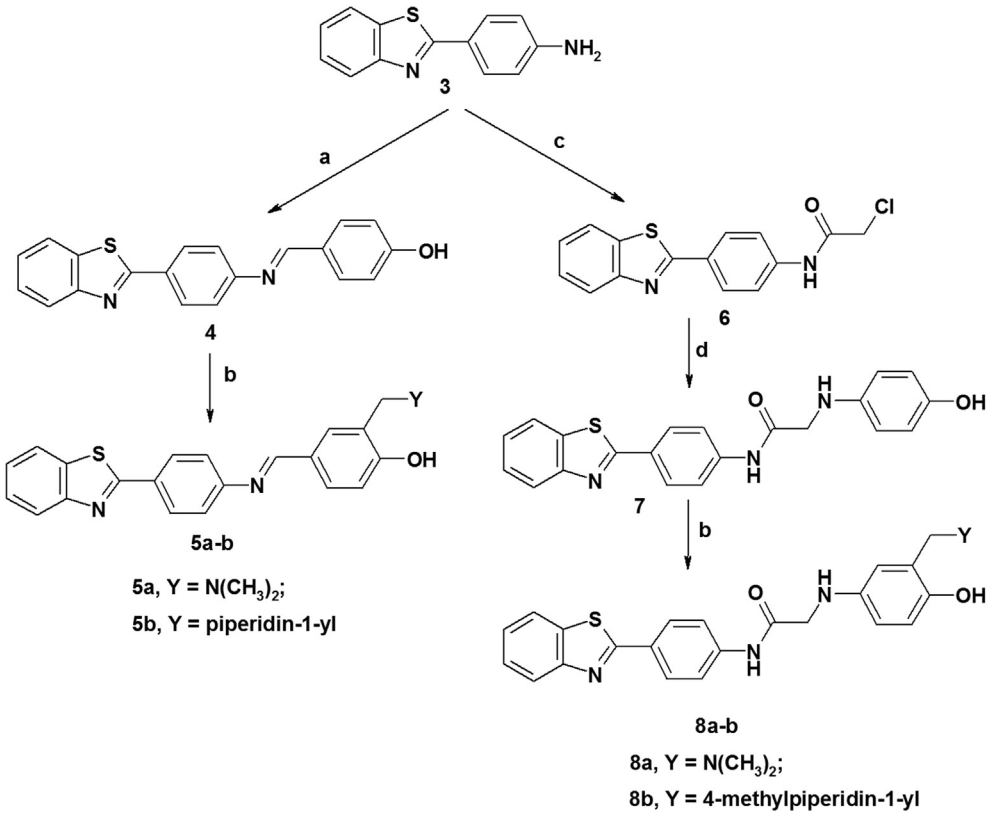
Furthermore, heating equimolar amounts of 4-benzothiazol-2-yl-phenylamine (**3**) with 4-



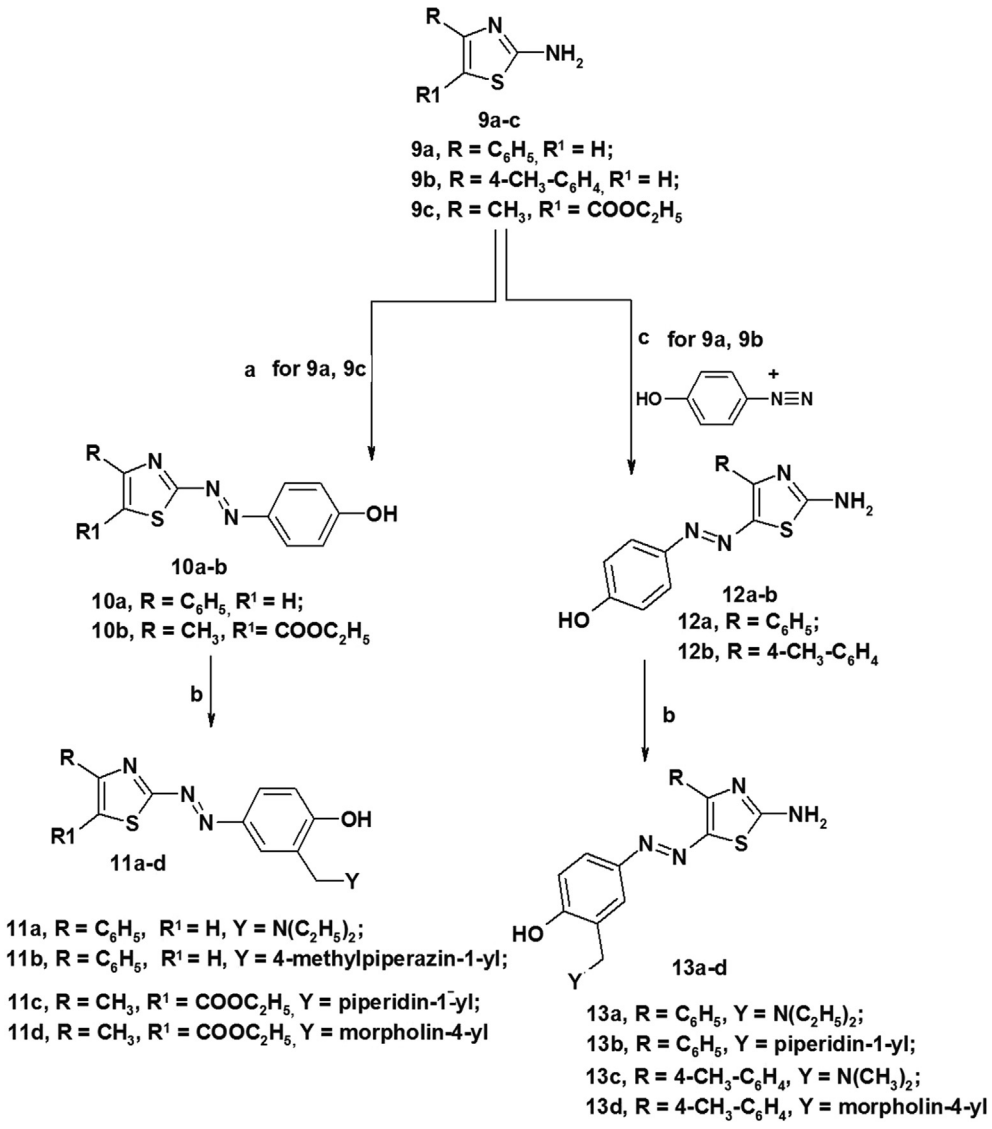
Scheme 1 – Synthetic protocol for compounds 2a–b. Reagents and conditions: (a) diethylamine or 4-methylpiperidine, formaldehyde, ethanol, reflux, 5 h.

hydroxybenzaldehyde in ethanol containing catalytic amount of glacial acetic acid yielded 4-[(4-benzothiazol-2-yl-phenyl-imino)methyl]phenol (**4**). Subjecting **4** to Mannich reaction using formaldehyde and secondary amine (dimethylamine or piperidine) in ethanol afforded **5a** and **5b** as revealed in their ^{1}H NMR spectra which showed singlet at 3.96 or 3.98 ppm assigned to the methyline protons of the CH_2N in addition to signals assigned to the alkylamino groups. Moreover, reacting **3** with chloroacetyl chloride yielded *N*-(4-benzothiazol-2-yl-phenyl)-2-chloroacetamide (**6**) which upon reaction with 4-aminophenol led to the corresponding acetamide **7**. Treating the latter with formaldehyde and secondary amines (dimethylamine or 4-methylpiperidine) gave the target Mannich bases **8a** and **8b** (Scheme 2). The ^{1}H NMR spectrum of **8a** and **8b** showed the characteristic singlet at 3.99 or 3.66 ppm assigned to the methyline protons of the CH_2N in addition to signals assigned to the dimethylamino and 4-methylpiperidino moieties.

On the other hand, the 4-(4-arylthiazol-2-ylazo)phenol (**10a, 10b**) could be obtained by diazotizing the corresponding starting amines in acetic acid with nitrosyl sulphuric acid. Reacting the phenolic azo dye **10a, 10b** with formaldehyde



Scheme 2 – Synthetic protocol for compounds 5a–b and 8a–b. Reagents and conditions. (a) 4-hydroxybenzaldhyde, ethanol, acetic acid, 20 h; (b) dimethylamine or piperidine for 4 and dimethylamine or 4-methylpiperidine for 7, formaldhyde, ethanol, reflux, 5 h; (c) chloroacetyl chloride, benzene, reflux, 30 min; (d) 4-aminophenol, potassium iodide, ethanol, reflux 11 h.



Scheme 3 – Synthetic protocol for compounds 11a–d and 13a–d. Reagents and conditions: (a) i- $NaNO_2$, H_2SO_4, acetic acid, 5 °C, 2 h, ii-phenol, NaOH, 5 °C, 2 h; (b) diethylamine or 4-methylpiperazine for 10a, piperidine or morpholine for 10b, diethylamine or piperidine for 12a and dimethylamine, or morpholine for 12b, formaldehyde, ethanol, reflux, 5 h; (c) sodium acetate, ethanol, 5 °C, 2 h.

and secondary amine (diethylamine, 4-methylpiperazine, piperidine or morpholine) in ethanol afforded the target compounds **11a–d**. The ^{1}H NMR spectrum of **11a–d** showed the characteristic singlet at 3.75–4.04 ppm assigned to the methyline protons of the CH_2N in addition to signals assigned to the diethylamino, 4-methylpiperazino, piperidino and morpholino moieties. Moreover, reacting 4-substituted-thiazole-2-ylamine **9a, 9b** with *p*-aminophenol diazonium salt solution in presence of sodium acetate yielded the corresponding azo phenols **12a, 12b** in high yields (80% and 91% respectively). The reaction of **12a, 12b** according to Mannich reaction conditions with the appropriate secondary amine (diethylamine, piperidine, dimethylamine or morpholine) yielded the target compounds **13a–d** (Scheme 3). The ^{1}H-NMR spectrum of **13a–d** showed the characteristic singlet at 3.84–4.10 ppm assigned to the methyline protons of the CH_2N in addition to signals assigned to the diethylamino, piperidino, dimethylamino or morpholino groups.

3.2. *Pharmacological screening*

The antimicrobial activity of the synthesized compounds **2a–b, 5a–b, 8a–b, 11a–d**, and **13a–d** was studied using the agar dilution technique using ciprofloxacin and fluconazole as positive control and the solvent, dimethylsulfoxide (DMSO) as a negative control. The synthesized compounds were screened for their *in vitro* antimicrobial testing using six selected standard isolates as a representative examples of different types of microorganisms as follows: gram-positive both non sporulated bacteria as *Staphylococus aureus* and

Table 1 – MIC (μg/mL) of heterocyclic systems substituted with dialkylaminoalkyl-o-cresols.

Comp no.	*Ps. aeruginosa*	*E. coli*	*B. subtilis*	*S. aureus*	MRSA	*C. albicans*
2a	>800	>800	>800	>800	>800	>800
2b	>800	>800	>800	>800	>800	>800
5a	>800	800	50	50	50	50
5b	>800	>800	>800	>800	>800	>800
8a	>800	800	200	50	50	200
8b	>800	>800	800	100	100	200
11a	>800	800	12.5	12.5	12.5	800
11b	>800	800	25	25	25	25
11c	>800	800	400	200	200	12.5
11d	>800	>800	>800	>800	>800	>800
13a	>800	400	25	25	25	400
13b	>800	400	50	200	200	>800
13c	>800	200	12.5	12.5	12.5	12.5
13d	>800	400	12.5	12.5	12.5	>800
DMSO	>800	>800	>800	>800	>800	>800
Ciprofloxacin	<6.25	50	<6.25	<6.25	12.5	400
Fluconazole	>800	>800	>800	>800	>800	6.25

micithillin resistant *Staphylococus aureus* (MRSA), sporulated as *Bacillus subtilis*, gram-negative bacteria both sensitive as *Escherichia coli*, resistant as *Pseudomonas aeruginosa* and a fungus as *Candida albicans*. The results are recorded in Table 1 and it was clear that there is variability in the susceptibilities of the different organisms to the different tested compounds. Gram-negative bacteria were the most resistant organism while gram-positive bacteria were the most sensitive. Some compounds showed both antibacterial and antifungal activity, while others showed antibacterial activity with no antifungal activity and vice versa. Compound **13c** showed the highest activity against gram-positive and gram-negative bacteria and fungi, also compound **5a** showed a potent activity against both gram-positive bacteria and fungi. Compounds **11a**, **13a** and **13d** have a significant activity against gram-positive bacteria and no activity against fungi. A moderate activity against gram-positive bacteria and fungi was shown by compound **8a** and **11b**.

4. Conclusion

We can conclude that the combination of phenolic Mannich bases with a thiazole ring system *via* azo group (**11a**, **13a**, **13b**, **13c**) showed a potent antimicrobial activity while the incorporation of benzothiazole nucleus with alkylamino-o-cresols through an amidic linkage (**8a**) exhibited a moderate antimicrobial activity. On the other hand, the incorporation of Mannich bases with purine nucleus (**2a**–**b**) resulted in products with no antimicrobial activity.

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