

Synthesis, antimicrobial activity and docking studies of new *N*-ethyl-3-indolyl heterocycles

Weal A. El-Sayed¹ · Hebat-Allah S. Abbas^{1,2} · Randa E. Abdel Mageid¹ · Tomasz Magdziarz³

Received: 7 March 2015 / Accepted: 15 December 2015 / Published online: 23 December 2015
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Abstract We report here, the synthesis of 1-(1-ethyl-1*H*-indol-3-yl)-3-pyridin-4-yl-prop-2-en-1-one (**2**) which was used as a base to the synthesis of new 3-(pyrimidin-4-yl)-1*H*-indole derivatives; their thioglycoside and *N*-glycoside derivatives **3–10a, b**; pyrane derivatives and pyranopyrimidine derivative **11–13**; and tricyclic pyranotriazololo[1,5-*a*]pyrimidine **14**. Moreover, reaction of *N*-ethyl-3-acetylindole **2** with phenylhydrazine and hydroxylamine hydrochloride gave pyrazolyl-indole and oxazolyl-indole derivatives **15** and **16**, respectively. The structures of the products obtained were confirmed by elemental analysis, IR, ¹H NMR and ¹³C NMR. The newly synthesized compounds were investigated for their antimicrobial activity, and some of them showed high growth inhibition activities. Anti-bacterial activity of the synthesized compounds was further analyzed by the molecular docking approach, a method of simulation of fitting ligands into binding site(s) of macromolecular targets. AutoDock Vina results showed that compounds **5a** and **6a, b** were located in a pocket in the active site.

Keywords Indole · Pyrimidine · Pyrane · Glycosides · Antimicrobial · Docking

Tomasz Magdziarz on leave from Institute of Chemistry, The University of Silesia, Katowice, Poland.

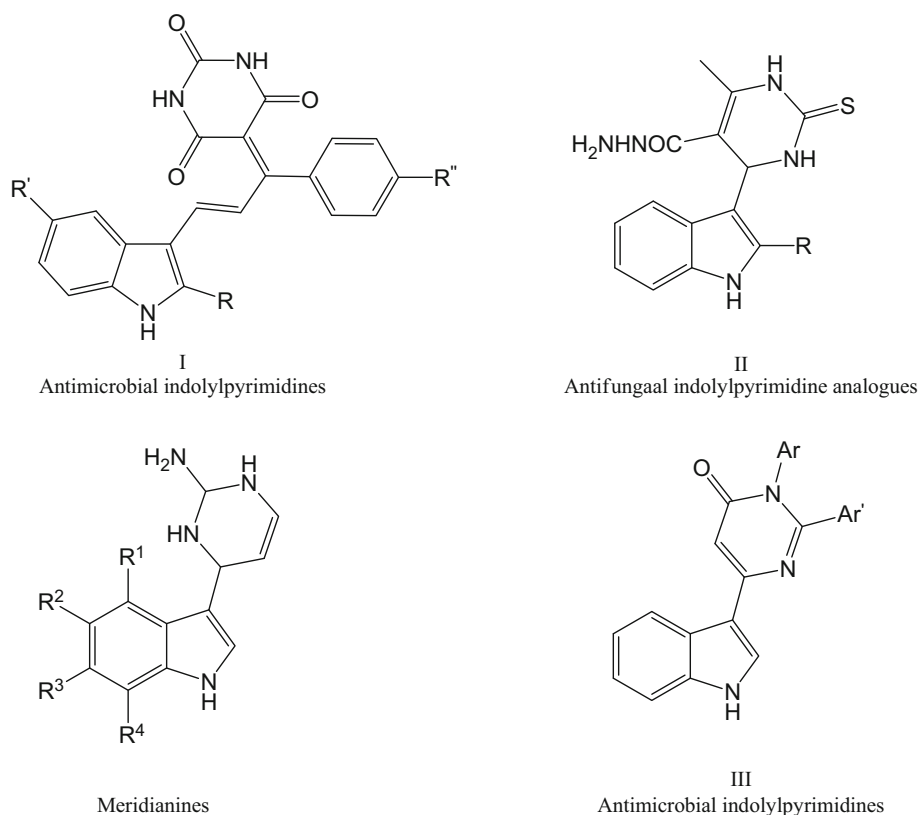
✉ Hebat-Allah S. Abbas
hebanrc@yahoo.com

- ¹ Photochemistry Department, National Research Centre, 12622 Dokki, Giza, Egypt
- ² Chemistry Department, College of Science, King Khalid University, Abha, Saudi Arabia
- ³ Molecular Networks GmbH - Computerchemie, Henke-str. 91, 91052 Erlangen, Germany

Introduction

The chemistry of indole compounds has received a great interest because of their interesting biological activities (El-Sayed *et al.*, 2011; Chang *et al.*, 2005). Indole derivatives have been found to possess anti-inflammatory (Ozdemir *et al.*, 2015), anti-bacterial (Baharfar *et al.*, 2015), anti-viral (Bal *et al.*, 2005), analgesic (Dilli *et al.*, 2012) and anti-tumor activities (El-Sayed *et al.*, 2011). Many indole compounds were effective in causing a marked increase in growth inhibition activity against various types of bacteria, fungi and viruses (Velezheva *et al.*, 2004). On the other hand, substituted pyrimidines are of great interest due to their diverse biological activities such as HIV-1 inhibition (Gadhachanda *et al.*, 2007), anti-bacterial (Hegab *et al.*, 2007), anticancer (Donger *et al.*, 2014) and anti-inflammatory (Ahmed *et al.*, 2014) activities. The thiopyrimidine bases and their *S*-, *N*-disubstituted analogues have shown therapeutic properties as anti-viral, anti-thyroid and anti-tumor activities (Tanaka *et al.*, 1995; Nugent *et al.*, 1998) due to their incorporation into polynucleic acids and inhibition of protein and polynucleic acid syntheses. As the literature survey revealed that indole and pyrimidine rings possess potential antimicrobial activity, it was thought that is worthwhile to link indole-pyrimidine-conjugated system to sugar molecule so as to get enhanced bioactivity. Substituted indolyl-pyrimidine analogues **I** (Fig. 1) have been reported of high antimicrobial potency (Jaiprakash and Sasidhar, 2012). Recently, new indolyl-pyrimidine analogues **II** (Fig. 1) were found to be equally potent as to the anti-fungal drugs Griseofulvin and Nystatin (Gaffar *et al.*, 2012). Also, triazolopyrimidine constitutes a well-established scaffolding in crop protection chemistry (Lamberth, 2007). Such compounds revealed anti-fungal (Serey *et al.*, 2007) and anti-mycobacterial

Fig. 1 Interesting biologically active indolyl-pyrimidines



(Abdel-Rahman *et al.*, 2009) activities. On the other hand, it is well known that DNA gyrase A protein from *Escherichia coli* is a macromolecular target for several antimicrobial drugs (Morris *et al.*, 1998; Ostrov *et al.*, 2007). Interestingly, such target possesses several potential sites at which ligands can be bound, probably those located in the proximity of residues taking key roles in catalytic functions.

Led by the previous facts and coupled with our ongoing project aimed at investigating new bioactive molecules derived from heterocycles attached to carbohydrate moieties (Abdel-Aal *et al.*, 2006, 2008; El-Sayed *et al.*, 2009a, b, c), the present work involves the synthesis and antimicrobial activity of new pyrimidine glycosides and thio-glycoside derivatives.

Experimental

Chemistry

General procedures

All melting points are uncorrected and measured using an Electro-thermal IA 9100 apparatus (Shimadzu, Japan). Yields listed are of the isolated compounds. The IR spectra

were recorded as potassium bromide pellets on a PerkinElmer 1650 Spectrophotometer, National Research Centre, Cairo, Egypt. ^1H NMR and ^{13}C NMR spectra were determined on a Jeol-Ex-300 NMR spectrometer, and chemical shifts were expressed as parts per million, ppm (δ values) against TMS as an internal reference (Faculty of Science, Cairo University, Cairo, Egypt). Microanalyses were operated using Mario Elmentar apparatus, Organic Microanalysis Unit, National Research Centre, Cairo, Egypt. The progress of the reactions was monitored by TLC using aluminum silica gel plates 60 F245. Column chromatography was performed on (Merck) Silica gel 60 (particle size 0.06–0.20 mm). Antimicrobial activities were measured at National Research Centre, Cairo, Egypt. The chemical names given for the prepared compounds are according to the IUPAC system. The starting compound 1-(1-ethyl-1H-indol-3-yl)ethanone **1** was prepared according to a previously reported procedure (Ottoni, *et al.*, 1998).

1-(1-Ethyl-1H-indol-3-yl)-3-pyridin-4-yl-prop-2-en-1-one (2)

To a solution of 1-(1-ethyl-1H-indol-3-yl)ethanone **1** (1.87 g, 0.01 mol) in ethanol (50 mL), potassium hydroxide (0.5 g) in water (5 mL) was added over a period of

30 min at 0 °C. Then 4-pyridine carboxaldehyde (1.07 g, 0.01 mol) was added slowly to the reaction mixture with stirring at room temperature for 12 h. Then the reaction mixture was poured into water. The solid that formed was filtered, dried and recrystallized from dioxane to afford compound **2** as a pale yellow powder, 2.62 g, (90 %); m.p. 172–174 °C. IR (KBr) cm^{-1} : ν (C=O) 1695, ν (C=N) 1608; ^1H NMR (DMSO- d_6 , 300 MHz): δ 1.48 (t, 3H, $J = 6.2$ Hz, CH_3), 4.12 (q, 2H, $J = 6.2$ Hz, CH_2), 6.25 (d, 1H, $J = 7.2$ Hz, CH), 6.88 (d, 1H, $J = 7.2$ Hz, CH), 7.38 (m, 3H, Ar-H), 7.52–7.82 (m, 3H, Ar-H), 8.55 (s, 1H, indole H-2), 8.72 (d, 2H, $J = 7.6$ Hz, Ar-H); ^{13}C NMR (DMSO- d_6 , 75 MHz): δ 16.7 (CH_3), 44.6 (CH_2), 109.1, 110.7, 120.1, 121.9, 123.7, 124.4, 124.9, 135.1, 136.5, 144.5, 149.3, 151.2, 152.5 (Ar-13C, 2 CH), 180.8 (C=O). Anal. Calcd. for $\text{C}_{18}\text{H}_{16}\text{N}_2\text{O}$ (276.33): C, 78.24; H, 5.84; N, 10.14. Found: C, 78.08; H, 5.98; N, 10.01.

4-(1-Ethyl-1H-indol-3-yl)-6-(pyridin-4-yl)-5,6-dihydropyrimidine-2(1H)-thione (3)

A mixture of compound **2** (2.76 g, 0.01 mol), thiourea (0.1 g, 0.014 mol) and NaOH (1.0 g, 0.025 mol) in ethanol (30 mL) was refluxed for 6 h. The reaction mixture was concentrated, cooled and filtered. The formed precipitate was recrystallized from ethanol to give **3** as a pale yellow powder, 2.74 g, (82 %); m.p. 182–184 °C. IR (KBr) cm^{-1} : ν (NH) 3312, ν (C=N) 1610, ν (C=S) 1180; ^1H NMR (DMSO- d_6 , 300 MHz): δ 1.47 (t, 3H, $J = 6.2$ Hz, CH_3), 3.07 (dd, 1H, $J = 12.8$ Hz, $J = 4.8$ Hz, pyrimidyl H-5), 3.67 (dd, 1H, $J = 8.8$ Hz, $J = 12.8$ Hz, pyrimidyl H-5'), 3.84–4.02 (m, 3H, CH_2 and CH_2CH), 7.38–7.50 (m, 3H, Ar-H), 7.62 (d, 2H, $J = 7.4$ Hz, Ar-H), 8.27–8.32 (m, 3H, Ar-H), 8.62 (s, 1H, indole H-2), 9.18 (s, 1H, NH exchangeable with D_2O); ^{13}C NMR (DMSO- d_6 , 75 MHz): δ 16.7 (CH_3), 44.6 (CH_2), 49.8 (pyrimidyl C-5), 58.2 (pyrimidyl C-6), 109.2, 111.3, 120.4, 121.8, 123.1, 124.4, 125.1, 135.1, 136.1, 144.3, 149.2 (Ar-13C), 161.1 (pyrimidyl C-4), 182.8 (C=S). Anal. Calcd. for $\text{C}_{19}\text{H}_{18}\text{N}_4\text{S}$ (334.44): C, 68.23; H, 5.42; N, 16.75. Found: C, 68.19; H, 5.38; N, 16.64.

General procedure for synthesis of compounds 5a, b

To a solution of the compound **3** (3.34 g, 0.01 mol) in aqueous potassium hydroxide [(0.01 mol in distilled water (16 mL)], a solution of 2, 3, 4, 6-tetra-*O*-acetyl- α -D-galactopyranosyl bromide or 2, 3, 4-tri-*O*-acetyl- α -D-xylopyranosyl bromide (0.011 mol) in acetone (20 mL) was added. The reaction mixture was stirred at room temperature for 12 h. The solvent was evaporated under reduced pressure at 40 °C, and the residue was washed with distilled water to remove potassium bromide formed. The

product was dried and crystallized from ethanol to give compounds **5a**, **b**, respectively.

*1-Ethyl-3-{2-[(2, 3, 4, 6-tetra-*O*-acetyl- β -D-galactopyranosyl)thio]-6-(pyridin-4-yl)-5,6-dihydropyrimidin-4-yl}-1H-indole (5a)*

4.59 g, (69 %), pale yellow powder, m.p. 139–141 °C. IR (KBr) cm^{-1} : ν (C=O) 1742, ν (C=N) 1612; ^1H NMR (DMSO- d_6 , 300 MHz): δ 1.47 (t, 3H, $J = 6.2$ Hz, CH_3), 1.95, 2.05, 2.11, 2.14 (4s, 12H, 4 CH_3CO), 3.11 (dd, 1H, $J = 5.2$ Hz, $J = 12.8$ Hz, pyrimidyl H-5), 3.72 (dd, 1H, $J = 8.6$ Hz, $J = 12.8$ Hz, pyrimidyl H-5'), 3.95–4.07 (m, 2H, CH_2CH and H-6), 4.11–4.15 (m, 3H, CH_2 and H-6'), 4.17 (dd, 1H, $J = 2.8$ Hz, $J = 11.4$ Hz, H-5), 4.96 (t, 1H, $J_{3,4} = 9.3$ Hz, H-4), 5.24 (dd, 1H, $J_{2,3} = 9.6$ Hz, $J_{3,4} = 9.3$ Hz, H-3), 5.38 (t, 1H, $J_{2,3} = 9.6$ Hz, H-2), 5.72 (d, 1H, $J_{1,2} = 10.2$ Hz, H-1), 7.40–7.47 (m, 3H, Ar-H), 7.55 (d, 1H, $J = 7.4$ Hz, Ar-H), 8.18 (d, 2H, $J = 7.6$ Hz, Ar-H), 8.59 (s, 1H, indole H-2), 8.72 (s, 2H, $J = 7.6$ Hz, Ar-H); ^{13}C NMR (DMSO- d_6 , 75 MHz): δ 16.7 (CH_3), 19.3, 19.5, 20.1, 20.2 (4 CH_3CO), 44.6, (CH_2), 50.8 (pyrimidyl C-5), 56.2 (pyrimidyl C-6), 62.7 (C-6), 64.2 (C-4), 68.7 (C-3), 71.2 (C-2), 71.9 (C-5), 88.9 (C-1), 109.3, 111.1, 121.4, 122.2, 123.5, 125.1, 126.1, 134.6, 136.0, 143.7, 149.6, 150.9, 152.2 (Ar-13C), 159.2 (pyrimidyl C-4), 165.1 (pyrimidyl C-2), 169.4, 170.5, 171.2, 171.7 (4C=O). Anal. Calcd. for $\text{C}_{33}\text{H}_{36}\text{N}_4\text{O}_9\text{S}$ (664.73): C, 59.63; H, 5.46; N, 8.43. Found: C, 59.48; H, 5.38; N, 8.52.

*1-Ethyl-3-{2-[(2, 3, 4-tri-*O*-acetyl- β -D-xylopyranosyl)thio]-6-(pyridin-4-yl)-5,6-dihydro-pyrimidin-4-yl}-1H-indole (5b)*

2.91 g, (49 %), pale yellow powder, m.p. 141–143 °C. IR (KBr) cm^{-1} : ν (C=O) 1739, ν (C=N) 1619; ^1H NMR (DMSO- d_6 , 300 MHz): δ 1.48 (t, 3H, $J = 6.2$ Hz, CH_3), 1.95, 2.05, 2.12 (3 s, 9H, 3 CH_3CO), 3.12 (dd, 1H, $J = 5.2$ Hz, $J = 12.8$ Hz, pyrimidyl H-5), 3.72 (dd, 1H, $J = 8.8$ Hz, $J = 12.8$ Hz, pyrimidyl H-5'), 3.82–4.09 (m, 2H, CH_2CH and H-5), 4.12–4.17 (m, 3H, CH_2 and H-5'), 4.20 (dd, 1H, $J = 2.8$ Hz, $J = 11.4$ Hz, H-4), 5.26 (dd, 1H, $J_{2,3} = 9.6$ Hz, $J_{3,4} = 9.3$ Hz, H-3), 5.38 (t, 1H, $J_{2,3} = 9.6$ Hz, H-2), 5.75 (d, 1H, $J_{1,2} = 9.8$ Hz, H-1), 7.44–7.51 (m, 3H, Ar-H), 7.59–7.65 (m, 3H, Ar-H), 8.42 (s, 1H, indole H-2), 8.73 (d, 2H, $J = 7.8$ Hz, Ar-H) ppm; ^{13}C NMR (DMSO- d_6 , 75 MHz): δ 16.7 (CH_3), 19.3, 19.5, 20.2 (3 CH_3CO), 44.6 (CH_2), 49.8 (pyrimidyl C-5), 55.2 (pyrimidyl C-6), 62.7 (C-5), 64.2 (C-4), 68.8 (C-3), 71.2 (C-2), 89.1 (C-1), 110.8, 111.9, 120.2, 122.9, 123.7, 124.0, 126.0, 134.1, 136.4, 141.6, 143.1, 144.3, 150.9 (Ar-13C), 159.2 (pyrimidyl C-4), 165.1 (pyrimidyl C-2), 169.4, 170.5, 171.2 (3C=O) ppm. Anal. Calcd. for $\text{C}_{30}\text{H}_{32}\text{N}_4\text{O}_7\text{S}$

(592.66): C, 60.80; H, 5.44; N, 9.45. Found: C, 60.67; H, 5.36; N, 9.31.

General procedure for the synthesis of compounds **6a**, **b**

The protected glycoside **5a** or **5b** (0.01 mol) was dissolved in methanolic ammonia (20 mL) at 0 °C and stirred for 0.5 h at the same temperature. The reaction mixture was further stirred at room temperature for about 5 h. The solvent was evaporated under reduced pressure at 40 °C to give a solid residue, which was crystallized from ethanol to give compounds **6a**, **6b**, respectively.

1-Ethyl-3-[2-[(β-D-galactopyranosyl)thio]-6-(pyridin-4-yl)-5, 6-dihydropyrimidin-4-yl]-1H-indole (6a)

2.13 g, (43 %), pale yellow powder, m.p. 189–191 °C. IR (KBr) cm^{-1} : ν (OH) 3471–3435, ν (C=N) 1622; ^1H NMR (DMSO- d_6 , 300 MHz): δ 1.48 (t, 3H, $J = 6.2$ Hz, CH_3), 3.18–3.34 (m, 3H, H-6, H-6' and pyrimidyl H-5), 3.51–3.55 (m, 1H, H-5), 3.70–3.79 (m, 3H, H-3, H-4 and pyrimidyl H-5'), 3.95–4.12 (m, 3H, CH_2 and CH_2CH), 4.21 (t, 1H, $J_{2,3} = 9.2$ Hz, H-2), 4.28 (t, 1H, $J = 6.4$ Hz, OH exchangeable with D_2O), 4.36 (m, 1H, OH exchangeable with D_2O), 4.42 (t, 1H, $J = 6.4$ Hz, OH exchangeable with D_2O), 4.84 (m, 1H, OH exchangeable with D_2O), 5.76 (d, 1H, $J_{1,2} = 9.8$ Hz, H-1), 7.39–7.45 (m, 2H, Ar-H), 7.54 (d, 2H, $J = 7.4$ Hz, Ar-H), 8.18 (s, 1H, indole H-2), 8.59 (d, 2H, $J = 7.6$ Hz, Ar-H), 8.72 (d, 2H, $J = 7.8$ Hz, Ar-H); ^{13}C NMR (DMSO- d_6 , 75 MHz): δ 16.3 (CH_3), 43.9 (CH_2), 48.2 (pyrimidyl C-5), 55.3 (pyrimidyl C-6), 63.9 (C-6), 64.1 (C-5), 65.3 (C-4), 69.5 (C-3), 70.7 (C-2), 89.6 (C-1), 109.0, 111.4, 121.8, 122.6, 123.5, 125.3, 126.0, 134.6, 136.3, 143.7, 148.6, 151.9, 153.8 (Ar-13C), 159.6 (pyrimidyl C-4), 165.8 (pyrimidyl C-2). Anal. Calcd. for $\text{C}_{25}\text{H}_{28}\text{N}_4\text{O}_5\text{S}$ (496.58): C, 60.47; H, 5.68; N, 11.28. Found: C, 60.14; H, 5.48; N, 11.54.

1-Ethyl-3-[2-[(β-D-xylopyranosyl)thio]-6-(pyridin-4-yl)-5, 6-dihydropyrimidin-4-yl]-1H-indole (6b)

1.91 g, (41 %), pale yellow powder, m.p. 192–194 °C. IR (KBr) cm^{-1} : ν (OH) 3465–3440, ν (C=N) 1614; ^1H NMR (DMSO- d_6 , 300 MHz): δ 1.43 (t, 3H, $J = 6.2$ Hz, CH_3), 3.18–3.39 (m, 3H, H-5, H-5' and pyrimidyl H-5), 3.71–3.77 (m, 3H, H-3, H-4 and pyrimidyl H-5'), 3.92–4.12 (m, 3H, CH_2 and CH_2CH), 4.24 (t, 1H, $J_{2,3} = 9.2$ Hz, H-2), 4.31 (t, 1H, $J = 6.4$ Hz, OH exchangeable with D_2O), 4.42 (m, 1H, OH exchangeable with D_2O), 4.84 (m, 1H, OH exchangeable with D_2O), 5.77 (d, 1H, $J_{1,2} = 9.8$ Hz, H-1), 7.42–7.50 (m, 3H, Ar-H), 7.54–8.64 (m, 3H, Ar-H), 8.18 (s, 1H, indole H-2), 8.71 (d, 2H, $J = 7.6$ Hz, Ar-H); ^{13}C NMR (DMSO- d_6 , 75 MHz): δ 16.7 (CH_3), 44.6 (CH_2),

48.2 (pyrimidyl C-5), 55.2 (pyrimidyl C-6), 63.8 (C-5), 65.2 (C-4), 69.1 (C-3), 70.8 (C-2), 89.5 (C-1), 108.9, 110.7, 121.4, 122.5, 123.4, 126.1, 126.8, 134.6, 136.6, 143.7, 149.6, 150.2, 153.0 (Ar-13C), 159.4 (pyrimidyl C-4), 165.2 (pyrimidyl C-2). Anal. Calcd. for $\text{C}_{24}\text{H}_{26}\text{N}_4\text{O}_4\text{S}$ (466.55): C, 61.78; H, 5.62; N, 12.01. Found: C, 61.54; H, 5.52; N, 11.92.

4-(1-Ethyl-1H-indol-3-yl)-6-(pyridin-4-yl)pyrimidin-2-amine (7)

To a solution of guanidine hydrochloride (0.95 g, 0.01 mol) in ethanol (50 mL), sodium ethoxide (0.011 mol) was added. The reaction mixture was refluxed for 2 h, then compound **2** (2.76 g, 0.01 mol) was added, and the reflux was continued for 8 h. The reaction mixture was cooled and poured into water. The solid that formed was filtered, dried and recrystallized from dioxane to afford compound **7** as a yellow powder, 1.86 g, (59 %), m.p. 202–204 °C. IR (KBr) cm^{-1} : ν (NH_2) 3412, 3388, ν (C=N) 1612; ^1H NMR (DMSO- d_6 , 300 MHz): δ 1.43 (t, 3H, $J = 6.2$ Hz, CH_3), 4.16 (q, 2H, $J = 6.2$ Hz, CH_2), 6.25 (s, 2H, NH_2 exchangeable with D_2O), 7.37–7.49 (m, 3H, Ar-H), 7.55–7.69 (m, 4H, Ar-H), 8.25 (s, 1H, indole H-2), 8.70 (d, 2H, $J = 7.8$ Hz, Ar-H); ^{13}C NMR (DMSO- d_6 , 75 MHz): δ 16.7 (CH_3), 44.6 (CH_2), 109.3, 111.1, 121.4, 122.2, 124.5, 125.4, 126.0, 134.4, 136.8, 141.3, 143.7, 149.6, 150.9, 152.3 (Ar-13C and pyrimidyl C-5), 160.1, 164.2, 165.1 (pyrimidyl C-2, C-4, C-6). Anal. Calcd. for $\text{C}_{19}\text{H}_{17}\text{N}_5$ (315.37): C, 72.36; H, 5.43; N, 22.21. Found: C, 72.18; H, 5.21; N, 22.02.

General procedure for the synthesis of compounds **9a**, **b**

A mixture of compound **7** (3.15 g, 0.01 mol), D-galactose and/or D-xylose (0.011 mol) in ethanol (30 mL), and a catalytic amount of acetic acid (3 drops) were heated at reflux temperature for 2 h. The formed precipitate was filtered on hot, washed with water several times, dried and recrystallized from ethanol to give to give compounds **9a**, **b**, respectively.

4-(1-Ethyl-1H-indol-3-yl)-N-(β-D-galactopyranosyl)-6-(pyridin-4-yl)pyrimidin-2-amine (9a)

2.91 g, (61 %), pale yellow powder, m.p. 196–197 °C. IR (KBr) cm^{-1} : ν (OH) 3515–3440, ν (NH) 3287, ν (C=N) 1615; ^1H NMR (DMSO- d_6 , 300 MHz): δ 1.42 (t, 3H, $J = 6.2$ Hz, CH_3), 3.40 (m, 2H, H-6, H-6'), 3.51 (m, 1H, H-5), 3.73–3.78 (m, 2H, H-3, H-4), 4.11 (q, 2H, $J = 6.2$ Hz, CH_2), 4.24 (t, 1H, $J_{2,3} = 9.2$ Hz, H-2), 4.31 (t, 1H, $J = 6.4$ Hz, OH exchangeable with D_2O), 4.37 (m, 1H, OH exchangeable with D_2O), 4.45 (t, 1H, $J = 6.4$ Hz,

OH exchangeable with D₂O), 4.88 (*m*, 1H, OH exchangeable with D₂O), 5.87 (*d*, 1H, $J_{1,2} = 9.8$ Hz, H-1), 7.38–7.52 (*m*, 3H, Ar-H), 7.70–7.94 (*m*, 4H, Ar-H), 8.36 (*s*, 1H, indole H-2), 8.53 (*d*, 2H, $J = 7.8$ Hz, Ar-H), 10.12 (*s*, 1H, NH exchangeable with D₂O); ¹³C NMR (DMSO-d₆, 75 MHz): δ 16.7 (CH₃), 44.6 (CH₂), 62.8 (C-6), 65.2 (C-4), 69.1 (C-3), 70.8 (C-2), 72.6 (C-5), 92.2 (C-1), 109.2, 110.4, 120.8, 121.4, 124.5, 125.5, 126.5, 134.6, 136.4, 143.7, 149.1, 150.4, 151.0 (Ar-13C and pyrimidyl C-5), 161.0, 164.2, 165.1 (pyrimidyl C-2, C-4, C-6). Anal. Calcd. for C₂₅H₂₇N₅O₅ (477.51): C, 62.88; H, 5.70; N, 14.67. Found: C, 62.69; H, 5.53; N, 14.49.

4-(1-Ethyl-1H-indol-3-yl)-N-(β-D-xylopyranosyl)-6-(pyridin-4-yl)pyrimidin-2-amine (9b)

2.59 g, (58 %), pale white powder, m.p. 197–199 °C. IR (KBr) cm⁻¹: ν (OH) 3510–3448, ν (NH) 3293, ν (C=N) 1612; ¹H NMR (DMSO-d₆, 300 MHz): δ 1.45 (*t*, 3H, $J = 6.2$ Hz, CH₃), 3.42 (*m*, 2H, H-5, H-5'), 3.75 (*m*, 2H, H-3, H-4), 4.14 (*q*, 2H, $J = 6.2$ Hz, CH₂), 4.25 (*t*, 1H, $J_{2,3} = 9.2$ Hz, H-2), 4.32 (*m*, 1H, OH exchangeable with D₂O), 4.38 (*m*, 1H, OH exchangeable with D₂O), 4.88 (*m*, 1H, OH exchangeable with D₂O), 5.89 (*d*, 1H, $J_{1,2} = 9.6$ Hz, H-1), 7.38–7.51 (*m*, 3H, Ar-H), 7.54–7.69 (*m*, 4H, Ar-H), 8.58 (*s*, 1H, indole H-2), 8.62 (*d*, 2H, $J = 7.8$ Hz, Ar-H), 10.08 (*s*, 1H, NH exchangeable with D₂O); ¹³C NMR (DMSO-d₆, 75 MHz): δ 16.7 (CH₃), 44.6 (CH₂), 62.8 (C-5), 65.2 (C-4), 69.1 (C-3), 70.9 (C-2), 92.8 (C-1), 110.1, 111.1, 121.4, 122.6, 123.1, 123.9, 125.9, 134.6, 136.2, 143.7, 149.6, 150.9, 151.3, 151.9 (Ar-13C and pyrimidyl C-5), 160.9, 164.2, 165.2 (pyrimidyl C-2, C-4, C-6). Anal. Calcd. for C₂₄H₂₅N₅O₄ (447.49): C, 64.42; H, 5.63; N, 15.65. Found: C, 64.11; H, 5.45; N, 15.41.

General procedure for the preparation of compounds 10a, b

To a well-stirred solution of the glycoside **9a, b** (0.01 mol) in pyridine (7 mL), acetic anhydride (0.03 mol) was added. The mixture was stirred at room temperature for 15–20 h (TLC). The resulting solution was poured onto crushed ice, and the product that separated out was filtered, washed with sodium hydrogen carbonate (30 %, 50 mL) solution, followed by water (50 mL) and then dried. The products were recrystallized from ethanol–water (2:1) to give compounds **10a, b**, respectively.

4-(1-Ethyl-1H-indol-3-yl)-N-(2, 3, 4, 6-tetra-O-acetyl-β-D-galactopyranosyl)-6-(pyridin-4-yl)pyrimidin-2-amine (10a)

2.71 g, (42 %), pale yellow powder, m.p. 137–138 °C. IR (KBr) cm⁻¹: ν (NH) 3288, ν (C=O) 1744, ν (C=N) 1615; ¹H NMR (CDCl₃, 300 MHz): δ 1.46 (*t*, 3H, $J = 6.2$ Hz,

CH₃), 1.94, 2.04, 2.11, 2.15 (4*s*, 12H, 4 CH₃CO), 4.05 (*m*, 1H, H-6), 4.12–4.15 (*m*, 3H, CH₂ and H-6'), 4.15 (dd, 1H, $J = 2.8$ Hz, $J = 11.4$ Hz, H-5), 4.98 (*t*, 1H, $J_{3,4} = 9.3$ Hz, H-4), 5.25 (dd, 1H, $J_{2,3} = 9.6$ Hz, $J_{3,4} = 9.3$ Hz, H-3), 5.39 (*t*, 1H, $J_{2,3} = 9.6$ Hz, H-2), 5.71 (*d*, 1H, $J_{1,2} = 9.8$ Hz, H-1), 7.42–7.55 (*m*, 3H, Ar-H), 7.59–7.72 (*m*, 4H, Ar-H), 8.42 (*s*, 1H, indole H-2), 8.61 (*d*, 2H, $J = 7.8$ Hz, Ar-H), 10.08 (*s*, 1H, NH exchangeable with D₂O); ¹³C NMR (CDCl₃, 75 MHz): δ 16.7 (CH₃), 19.3, 19.5, 20.2, 20.2 (4CH₃CO), 44.6 (CH₂), 62.7 (C-6), 64.2 (C-4), 68.8 (C-3), 71.2 (C-2), 72.0 (C-5), 87.1 (C-1), 110.1, 111.1, 121.3, 122.1, 123.4, 124.7, 126.1, 134.1, 136.0, 141.8, 143.7, 149.6, 150.9, 151.2 (Ar-13C and pyrimidyl C-5), 161.1, 164.2, 165.2 (pyrimidyl C-2, C-4, C-6), 169.4, 170.5, 171.3, 171.7 (4C=O). Anal. Calcd. for C₃₃H₃₅N₅O₉ (645.66): C, 61.39; H, 5.46; N, 10.85. Found: C, 61.14; H, 5.31; N, 10.73.

4-(1-Ethyl-1H-indol-3-yl)-N-(2, 3, 4-tri-O-acetyl-β-D-xylopyranosyl)-6-(pyridin-4-yl) pyrimidin-2-amine (10b)

3.21 g, (56 %), pale white powder, m.p. 143–145 °C. IR (KBr) cm⁻¹: ν (NH) 3294, ν (C=O) 1742, ν (C=N) 1618; ¹H NMR (DMSO-d₆, 300 MHz): δ 1.47 (*t*, 3H, $J = 6.2$ Hz, CH₃), 1.94, 2.03, 2.14 (3 *s*, 9H, 3CH₃CO), 4.04 (*m*, 1H, H-5), 4.12–4.15 (*m*, 3H, CH₂ and H-5'), 4.17 (dd, 1H, $J = 2.6$ Hz, $J = 11.4$ Hz, H-4), 5.26 (dd, 1H, $J_{2,3} = 9.6$ Hz, $J_{3,4} = 9.3$ Hz, H-3), 5.38 (*t*, 1H, $J_{2,3} = 9.6$ Hz, H-2), 5.73 (*d*, 1H, $J_{1,2} = 9.8$ Hz, H-1), 7.44–7.57 (*m*, 3H, Ar-H), 7.59–7.73 (*m*, 4H, Ar-H), 8.45 (*s*, 1H, indole H-2), 8.71 (*d*, 2H, $J = 7.8$ Hz, Ar-H), 10.14 (*s*, 1H, NH exchangeable with D₂O); ¹³C NMR (DMSO-d₆, 75 MHz): δ 16.5 (CH₃), 19.4, 19.4, 20.2 (3CH₃CO), 44.7 (CH₂), 64.4 (C-4), 68.6 (C-3), 71.2 (C-2), 72.9 (C-5), 87.1 (C-1), 109.3, 111.1, 120.3, 121.4, 122.2, 123.5, 125.1, 126.1, 134.6, 136.0, 143.7, 149.6, 150.9, 151.6 (Ar-13C and pyrimidyl C-5), 161.4, 164.9, 165.2 (pyrimidyl C-2, C-4, C-6), 169.9, 170.1, 171.3 (3C=O). Anal. Calcd. for C₃₀H₃₁N₅O₇ (573.60): C, 62.82; H, 5.45; N, 12.21. Found: C, 62.54; H, 5.35; N, 11.93.

2-Amino-6-(1-ethyl-1H-indol-3-yl)-4-(pyridin-4-yl)-2H-pyran-3-carbonitrile (11)

A mixture of the compound **2** (2.76 g, 0.01 mol) and malononitrile (0.66 g, 0.01 mmol) in pyridine (20 mL) was refluxed for 10 h. The solution was cooled and poured onto ice/HCl, and the solid that formed was filtered, washed several times with water, dried and recrystallized from ethanol to give **11** as a yellow powder, 1.68 g, (49 %), m.p. 221–223 °C. IR (KBr) cm⁻¹: ν (NH₂) 3415, 3390, ν (CN) 2212, ν (C=N) 1610; ¹H NMR (DMSO-d₆, 300 MHz): δ

1.45 (*t*, 3H, $J = 6.2$ Hz, CH₃), 4.17 (*q*, 2H, $J = 6.2$ Hz, CH₂), 5.25 (bs, 2H, NH₂ exchangeable with D₂O), 5.35 (*t*, 1H, $J = 5.2$ Hz, pyran H-2), 5.64 (*s*, 1H, pyran H-5), 7.35–7.48 (*m*, 3H, Ar-H), 7.52–7.66 (*m*, 3H, Ar-H), 8.56 (*s*, 1H, Indole H-2), 8.70 (*d*, 2H, $J = 7.8$ Hz, Ar-H); ¹³C NMR (DMSO-d₆, 75 MHz): δ 16.1 (CH₃), 44.6 (CH₂), 85.1 (pyran C-2), 104.5 (pyran C-5), 109.0, 109.9, 111.1, 119.4, 123.2, 123.8, 125.6, 126.4, 132.6, 134.3, 135.7, 140.9, 143.7, 149.6, 152.2, 154.7 (Ar-13C, CN and pyran C-3, C-4, C-6). Anal. Calcd. for C₂₁H₁₈N₄O (342.39): C, 73.67; H, 5.30; N, 16.36. Found: C, 73.49; H, 5.20; N, 16.09.

Ethyl N-(3-cyano-6-(1-ethyl-1H-indol-3-yl)-4-(pyridin-4-yl)-2H-pyran-2-yl)formimidate (12)

A mixture of compound **11** (3.42 g, 0.01 mol) and triethylorthoformate (3 mL) was heated at 100 °C for 5 h. The excess triethylorthoformate was evaporated under reduced pressure. Ether (10 mL) was added, and the solid product obtained was filtered and crystallized from ethanol to give **12** as a yellow powder, 2.27 g, (57 %), m.p. 186–188 °C. IR (KBr) cm⁻¹: ν (CN) 2210, ν (C=N) 1611; ¹H NMR (CDCl₃, 300 MHz): δ 1.35 (*t*, 3H, $J = 6.6$ Hz, CH₃), 1.46 (*t*, 3H, $J = 6.2$ Hz, CH₃), 3.98 (*q*, 2H, $J = 6.6$ Hz, CH₂), 4.21 (*q*, 2H, $J = 6.2$ Hz, CH₂), 5.37 (*t*, 1H, $J = 5.2$ Hz, pyran H-2), 5.65 (*s*, 1H, pyran H-5), 7.35–7.48 (*m*, 3H, Ar-H), 7.53–7.67 (*m*, 4H, Ar-H and CH=N), 8.54 (*s*, 1H, Indole H-2), 8.72 (*d*, 2H, $J = 7.8$ Hz, Ar-H); ¹³C NMR (CDCl₃, 75 MHz): δ 15.2, 16.1 (2CH₃), 43.1, 44.6 (2CH₂), 81.2 (pyran C-2), 103.5 (pyran C-5), 109.3, 110.1, 119.1, 121.4, 122.5, 123.3, 125.7, 126.0, 134.3, 135.4, 143.3, 148.1, 150.4, 151.3, 152.4, 153.4, 155.1 (Ar-13C, CN, CH=N, and pyran C-3, C-4, C-6). Anal. Calcd. for C₂₄H₂₂N₄O₂ (398.46): C, 72.34; H, 5.57; N, 14.06. Found: C, 72.10; H, 5.31; N, 13.84.

7-(1-Ethyl-1H-indol-3-yl)-4-imino-5-(pyridin-4-yl)-4, 8a-dihydro-3H-pyranof[2, 3-d] pyrimidin-3-amine (13)

A mixture of compound **12** (3.98 g, 0.01 mol) and hydrazine hydrate (1 mL, 0.017 mol) in ethanol (30 mL) was heated under reflux for 10 h. The solvent was reduced under reduced pressure and left overnight at 5 °C. The solid product obtained was filtered, washed with cold ethanol and crystallized from ethanol as pale white powder, 1.80 g, (47 %), m.p. 234–236 °C. IR (KBr) cm⁻¹: ν (NH₂, NH) 3418, 3385, 3328, ν (C=N) 1615; ¹H NMR (DMSO-d₆, 300 MHz): δ 1.49 (*t*, 3H, $J = 6.2$ Hz, CH₃), 4.19 (*q*, 2H, $J = 6.2$ Hz, CH₂), 5.37 (*t*, 1H, $J = 5.2$ Hz, pyran H-2), 5.69 (*s*, 1H, pyran H-5), 6.74 (bs, 2H, NH₂ exchangeable with D₂O), 7.48–7.57 (*m*, 3H, Ar-H), 7.61–7.77 (*m*, 4H, CH = N and Ar-H), 8.55 (*s*, 1H, indole

H-2), 8.72 (*d*, 2H, $J = 7.8$ Hz, Ar-H), 11.26 (bs, 1H, NH exchangeable with D₂O); ¹³C NMR (DMSO-d₆, 75 MHz): δ 16.1 (CH₃), 44.6 (CH₂), 84.1 (pyran C-2), 105.5 (pyran C-5), 109.2, 110.1, 121.4, 122.0, 123.2, 125.0, 126.1, 133.5, 136.6, 143.1, 149.9, 150.9, 152.2, 155.9 (Ar-13C, CH = N and pyran C-3, C-4, C-6), 158.1 (C=NH). Anal. Calcd. for C₂₂H₂₀N₆O (384.43): C, 68.73; H, 5.24; N, 21.86. Found: C, 68.59; H, 5.18; N, 21.61.

8-(1-Ethyl-1H-indol-3-yl)-10-(pyridin-4-yl)-3, 6a-dihydro-2H-pyrano[3, 2-e][1, 2, 4] triazolo[1, 5-c]pyrimidine-2-thione (14)

To a stirred suspension of compound **13** (3.84 g, 0.01 mol) in ethanol (15 mL), ethanolic potassium hydroxide (20 mL, 0.01 mol) and CS₂ (2 mL) were added dropwisely. The reaction mixture was then heated under reflux for 8 h. After cooling and evaporation of the solvent, the potassium salt obtained was dissolved in water and acidified with 2 N aqueous HCl. The solid product formed was collected and recrystallized from ethanol to give **14** as a yellow powder, 2.47 g, (58 %), m.p. 179–180 °C. IR (KBr) cm⁻¹: ν (NH) 3319, ν (C=N) 1618, ν (C=S) 1189; ¹H NMR (DMSO-d₆, 300 MHz): δ 1.48 (*t*, 3H, $J = 6.2$ Hz, CH₃), 4.16 (*q*, 2H, $J = 6.2$ Hz, CH₂), 5.35 (*t*, 1H, $J = 5.2$ Hz, pyran H-2), 5.69 (*s*, 1H, pyran H-5), 7.47–7.59 (*m*, 3H, Ar-H), 7.63–7.80 (*m*, 4H, CH = N and Ar-H), 8.59 (*s*, 1H, indole H-2), 8.72 (*d*, 2H, $J = 7.8$ Hz, Ar-H), 12.98 (bs, 1H, NH exchangeable with D₂O); ¹³C NMR (DMSO-d₆, 75 MHz): δ 16.2 (CH₃), 44.6 (CH₂), 84.2 (pyran C-2), 106.7 (pyran C-5), 109.4, 110.9, 120.1, 121.7, 124.1, 125.7, 126.6, 134.2, 136.4, 143.4, 149.8, 150.6, 152.4, 155.4 (Ar-13C, CH = N and pyran C-3, C-4, C-6), 159.1 (C=NH), 179.1 (C=S). Anal. Calcd. for C₂₃H₁₈N₆OS (426.49): C, 64.77; H, 4.25; N, 19.70. Found: C, 64.55; H, 4.31; N, 19.58.

1-Ethyl-3-(1-phenyl-5-pyridin-4-yl-1H-pyrazol-3-yl) 1H-indol (15)

A mixture of the compound **2** (2.76 g, 0.01 mol) and phenylhydrazine (1.08 g, 0.01 mol) in ethanol (20 mL) was heated under reflux for 10 h. Then the reaction mixture was cooled, filtered, dried and recrystallized from ethanol to give the compound **15** as a pale yellow powder, 2.20 g, (60 %), m.p. 182–184 °C. IR (KBr) cm⁻¹: ν (C=N) 1605; ¹H NMR (DMSO-d₆, 300 MHz): δ 1.44 (*t*, 3H, $J = 6.2$ Hz, CH₃), 3.22 (dd, 1H, $J = 4.8$ Hz, $J = 14.6$ Hz, pyrazoline H-4), 3.82 (dd, 1H, $J = 8.8$ Hz, $J = 14.6$ Hz, pyrazoline H-4'), 4.16 (*q*, 2H, $J = 6.2$ Hz, CH₂), 5.32 (dd, 1H, $J = 4.8$ Hz, $J = 8.8$ Hz, pyrazoline H-5), 7.14–7.26 (*m*, 3H, Ar-H), 7.47–7.59 (*m*, 4H, Ar-H), 7.69–7.82 (*m*, 4H, Ar-H), 8.57 (*s*, 1H, indole H-2), 8.65 (*d*, 2H,

$J = 7.6$ Hz, Ar–H); ^{13}C NMR (DMSO- d_6 , 75 MHz): δ 16.2 (CH₃), 44.1, 44.5 (2CH₂), 66.0 (CH), 110.1, 110.9, 111.3, 112.5, 120.8, 122.7, 123.5, 125.6, 126.4, 134.9, 136.2, 140.3, 143.8, 149.6, 150.9, 152.0, 152.9 (Ar-19C), 152.7 (pyrazole C-3). Anal. Calcd. for C₂₄H₂₂N₄366.46): C, 78.66; H, 6.05; N, 15.29. Found: C, 78.48; H, 5.89; N, 15.02.

1-Ethyl-3-(5-pyridin-4-yl-isoxazol-3-yl) 1H-indol (16)

A solution of compound **2** (2.76 g, 0.01 mol) and hydroxylamine hydrochloride (0.33 g, 0.01 mol) in ethanol (15 mL) containing (0.4 g) sodium hydroxide was refluxed for 8 h. The reaction mixture was cooled and poured onto crushed ice. The precipitate was filtrated, washed with water and recrystallized from ethanol to give the compound **16** as a yellow powder, 2.03 g, (70 %), m.p. 178–179 °C. IR (KBr) cm⁻¹: ν (C=N) 1608; ^1H NMR (DMSO- d_6 , 300 MHz): δ 1.49 (*t*, 3H, $J = 6.2$ Hz, CH₃), 3.18 (dd, 1H, $J = 4.6$ Hz, $J = 14.8$ Hz, isoxazoline H-4), 3.82 (dd, 1H, $J = 9.2$ Hz, $J = 14.8$ Hz, isoxazoline H-4'), 4.21 (*q*, 2H, $J = 6.2$ Hz, CH₂), 5.28 (dd, 1H, $J = 4.6$ Hz, $J = 9.2$ Hz, isoxazoline H-5), 7.48–7.60 (*m*, 3H, Ar–H), 7.70–7.83 (*m*, 3H, Ar–H), 8.61 (*s*, 1H, indole H-2), 8.75 (*d*, 2H, $J = 7.8$ Hz, Ar–H); ^{13}C NMR (DMSO- d_6 , 75 MHz): δ 16.1 (CH₃), 44.1, 44.5 (2CH₂), 66.1 (CH), 110.14, 121.4, 122.9, 123.5, 125.1, 126.4, 134.6, 136.0, 143.1, 149.6, 150.0, 150.4 (Ar-13C), 153.1 (oxazole C-3). Anal. Calcd. for C₁₈H₁₇N₃O (291.35): C, 74.20; H, 5.88; N, 14.42. Found: C, 74.02; H, 5.65; N, 14.20.

Molecular docking

All docking simulations were performed with AutoDock Vina v1.1.2 software (Trott and Olson, 2010). Initial 3D conformations of ligands were generated with CORINA 3D structure generator v3.47. Preparation of protein and further processing of ligand data were done in AutoDock Tools suite v1.5.6. Result analysis and visualizations were performed with PyMOL v1.5.0.3.

The DNA gyrase A protein was represented by 1AB4 PDB entry. The biological assembly dimeric form was directly downloaded from RCSB Internet site [RCSB <http://www.rcsb.org> accessed 28/04/2013]. The protein structure was preprocessed using the AutoDock Tools suite. Partial charges were automatically added, and AutoDock-specific atom types were assigned. In order to increase accuracy of docking simulation side chains of following residuals in both chains were annotated as flexible: R32, H78, S83, D87, Y122. Initial confirmations of all 17 compounds used in docking simulations were found using the CORINA 3D structure generator. Further pre-

processing was performed with AutoDock Tools suite. Partial charges were assigned, and rotatable bonds were selected.

Docking simulations were performed with AutoDock Vina. The size of the search space was set to 40 × 40 × 40 Å, and it was centered at expected binding site residues, i.e., at R32, H78, S83, D87 and Y122 residues (Cartesian coordinates of the center were 60, 60, 40 Å)—see Fig. 6. Two docking simulations were performed. In the first simulation, all 17 compounds were docked. Twenty different docking poses were obtained for each compound. The maximal allowed energy range between the best and the worst binding mode was set to 3 kcal/mol. The global search exhaustiveness parameter controlling the search algorithm accuracy was set to 12 (default value is 8; 12 means high accuracy). The second simulation was done for **3**, **5a**, **5b**, **6a** and **6b** compounds only. The energy range parameter was set to 4.5 kcal/mol and the exhaustiveness to 20. Inspection and visualization of the docking results were done in PyMOL.

Antimicrobial activity

The anti-bacterial activity of the synthesized compounds was tested against *Bacillus subtilis*, *Streptococcus lactis* (Gram-positive bacteria), *E. coli*, *Pseudomonas* sp. (Gram-negative bacteria) using the nutrient agar medium. The anti-fungal activity of the compounds was tested against the yeast-like pathogenic fungi (*Candida albicans*, *Candida glabrata*) using Sabouraud dextrose agar medium.

Agar diffusion medium

All compounds were in vitro screened for their antimicrobial activity by agar diffusion method (Cruickshank *et al.*, 1975). A suspension of the organisms was added to sterile nutrient agar media at 45 °C, and the mixture was transferred to sterile Petri dishes and allowed to solidify. Holes of 10 mm in diameter were made using a cork borer. An amount of 0.1 mL of each of the synthesized compounds was poured inside the holes. A hole filled with DMSO was also used as a control. The plates were left for 1 h at room temperature as a period of preincubation diffusion to minimize the effects of variation in time between the applications of the different solutions. The plates were then incubated at 37 °C for 24 h and observed for anti-bacterial activity. The diameters of zone of inhibition were measured and compared with that of the standard drugs, and the values are tabulated in Table 1. Nalidixic acid[®] and Nystatin[®] were used as standard drugs for anti-bacterial and anti-fungal activity, respectively.

Table 1 Inhibition zone in mm as a criterion of anti-bacterial and anti-fungal activities

Compound	Gram-positive bacteria		Gram-negative bacteria		Fungi	
	<i>Bacillus subtilis</i>	<i>Streptococcus lactis</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeuroginosa</i>	<i>Candida albicans</i>	<i>Candida gabrata</i>
1	10	9	7	6	6	1
2	14	12	13	15	6	2
3	8	20	8	10	13	19
5a	19	16	18	16	15	16
5b	18	9	13	12	6	8
6a	23	23	18	16	17	21
6b	24	18	21	18	17	16
7	12	25	9	19	12	3
9a	22	15	13	12	16	19
9b	20	15	10	17	9	4
10a	12	12	16	12	8	13
10b	14	14	15	12	5	5
11	18	18	21	16	7	7
12	16	21	12	15	5	19
13	19	15	17	13	5	4
14	25	14	17	18	11	8
15	11	16	12	17	7	6
16	17	20	17	26	9	6
Nalidixic acid	23	22	13	14	–	–
Nystatin	–	–	–	–	15	14

Inhibition zones: 3–12 mm; low activity, 13–21 mm; moderate activity, >22 mm; high activity

Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) of the test compounds was determined by the agar streak dilution method. A stock solution of 68 mg/mL of the synthesized compounds was made using DMSO as the solvent. From this stock solution, the following concentrations (0.17; 0.34; 0.68; 0.85; and 1.7 mg/mL) of the tested compound solutions were mixed with the known quantities of molten sterile agar media aseptically. About 20 mL of the media containing the tested compound was dispensed into each sterile Petri dish. Then the media were allowed to get solidified. Microorganisms were then streaked one by one on the agar plates aseptically. After streaking, all the plates were incubated at 37 °C for 24 h/48 h for bacterial and fungus activities, respectively. Then the plates were observed for the growth of microorganisms. The lowest concentrations of the synthesized compounds inhibiting the growth of the given bacteria/fungi were considered as the minimum inhibitory concentration (MIC) of the tested compounds against that bacteria or fungi on the plate. The MIC values of each compound against various bacteria and fungus are tabulated in Table 2. MIC values of the compounds against the tested bacteria/fungi (Table 2) were

calculated as the concentration which resulted in >99 % inhibition of the microorganism on the plate.

Results and discussion

Chemistry

In this investigation, the *N*-alkylated indole derivative **1** was reacted (El-Sayed *et al.*, 2009a, b, c) with 4-pyridine carboxaldehyde in the presence of potassium hydroxide to afford the substituted indolyl enone derivative **2**. The later enone derivative was used as a key precursor for the preparation of 4, 6-disubstituted pyrimidine and thiopyrimidine derivatives (Scheme 1). Thus, the reaction of **2** with thiourea in the presence of sodium hydroxide led to the formation of 4, 6-disubstituted thiopyrimidine **3** in 82 % yield (El-Sayed *et al.*, 2009a, b, c; Rathod and Kulkarni, 2011; Patel *et al.*, 2011), while in reaction with guanidine hydrochloride the amino-4, 6-disubstituted pyrimidine derivative **7** was obtained (Scheme 1). Formation of compound **3** may be explained according to a reported mechanism starting with a 1, 2-addition product which is then dehydrated to form an intermediate

Table 2 MIC in µg/mL of the newly synthesized compounds

Compound	Gram-positive bacteria		Gram-negative bacteria		Fungi	
	<i>Bacillus subtilis</i>	<i>Streptococcus lactis</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeuroginosa</i>	<i>Candida albicans</i>	<i>Candida gabrata</i>
3	–	30	–	–	–	40
5a	40	40	40	50	50	50
5b	40	50	50	50	50	50
6a	30	30	30	40	40	30
6b	20	30	20	30	50	40
7	–	20	–	30	–	–
9a	30	50	40	40	40	40
9b	30	50	50	20	50	50
10a	50	50	30	50	50	40
10b	50	50	30	50	50	50
11	30	30	20	30	50	50
12	50	30	50	30	–	40
13	30	40	30	40	50	50
14	20	40	30	30	50	50
Nalidixic acid	20	20	30	30	–	–
Nystatin	–	–	–	–	30	30

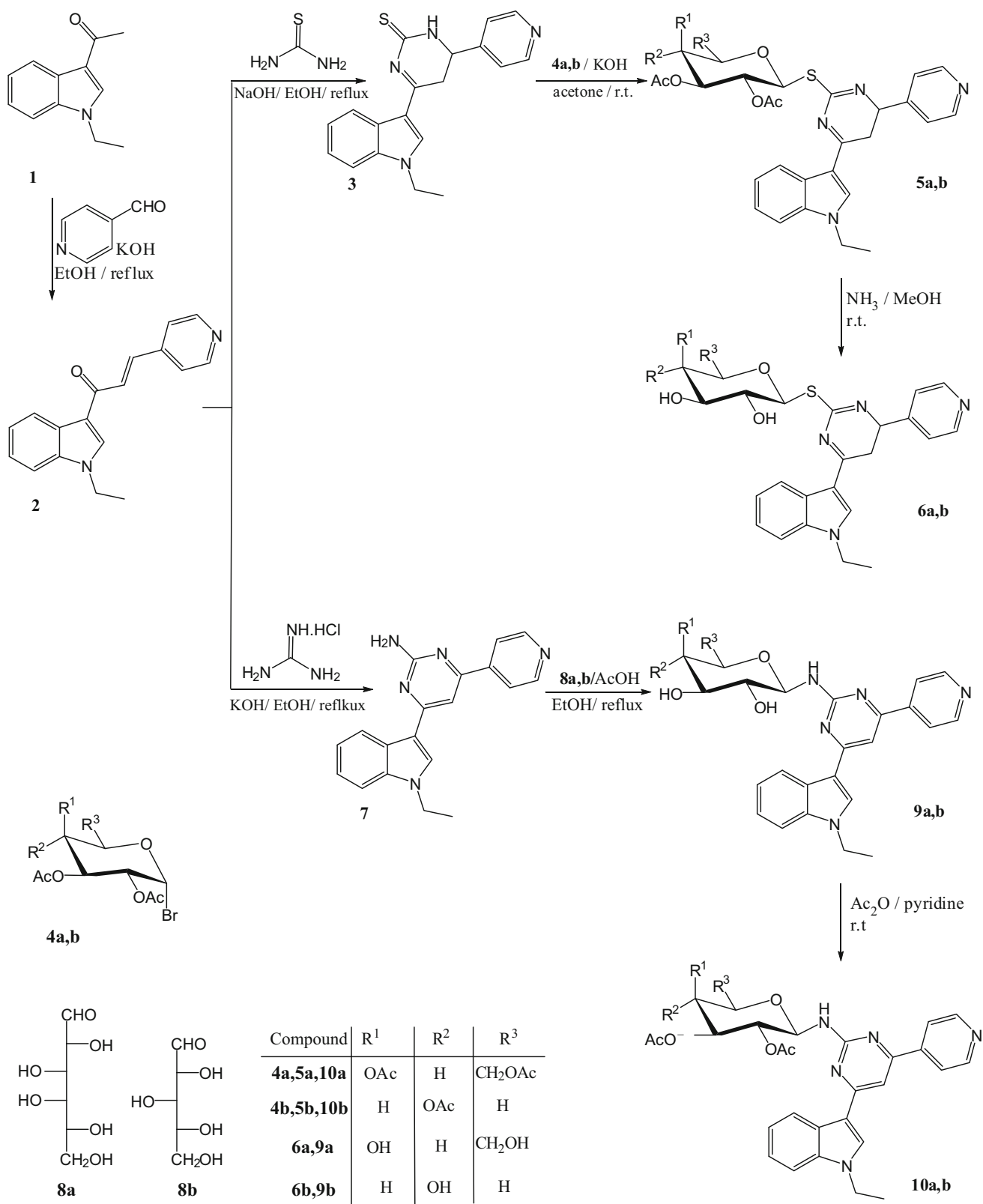
possessing a sufficiently activated olefinic double bond to undergo an internal nucleophilic attack and thus forming the 5, 6-dihydro derivative (Ebraheem, 2013; Brown *et al.*, 1985). Another mechanistic route starts with an initial 1, 4-addition to the activated olefinic double bond², and the resulting Micheal addition product then cyclizes to tetrahydropyrimidine derivative. The later derivative readily loses a water molecule to afford the 5, 6-dihydro derivative. As the reaction was not carried out under nitrogen, no hydrogen transfer to the chalcone occurred, and thus, fully aromatic compound was not formed (Brown *et al.*, 1985).

Glycosylation of the thiopyrimidine derivative **3** was carried out by using glycosyl halides **4a, b** in the presence of potassium hydroxide to produce the corresponding thioglycosides **5a, b** (Scheme 1). The regioselectivity of forming thioglycosides was confirmed on the basis of their spectral data. The ¹H NMR spectra of the produced thioglycosides showed the anomeric protons of the sugar moieties in the range δ 5.72 and 5.75 ppm as doublets, with coupling constants equal to 10.2 and 9.8 Hz indicating the β-orientation of the thioglycosidic bond. The anomeric proton of β-*N*-glycosides having an adjacent C=S was reported to appear at higher chemical shift (δ 6.9–7.2 ppm) due to the anisotropic deshielding effect of the C=S (Ibrahim, 1996; Mansour *et al.*, 1999). The ¹³C NMR spectra of **5a, b** showed a signal at δ 89.1 and 88.9 ppm corresponding to the anomeric C-1, which also confirmed the β-configuration. The absence of a peak corresponding

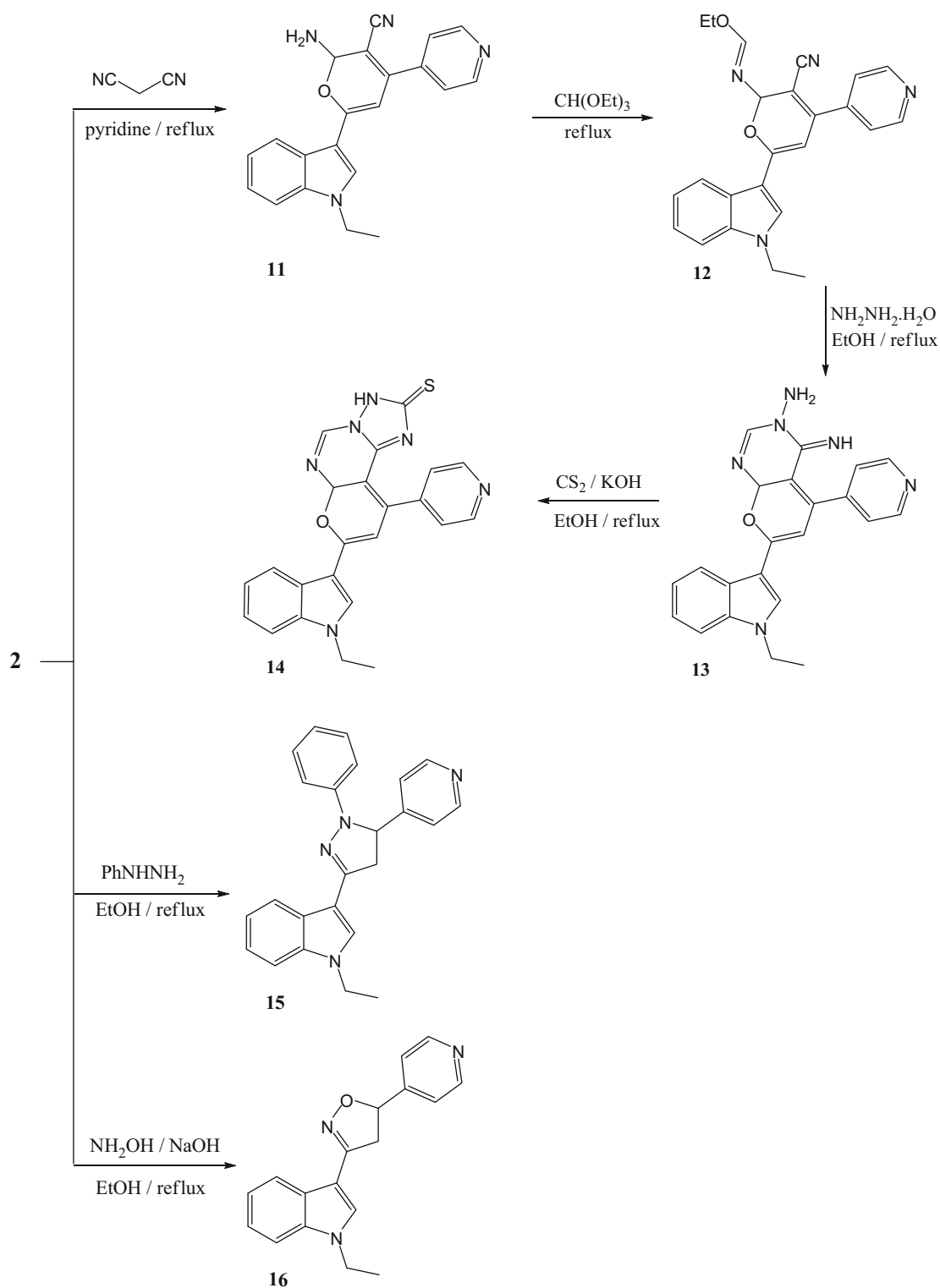
to the C=S group indicates that the attachment of the sugar has taken place at the sulfur atom and not on the nitrogen atom. Treatment of the acetylated thioglycosides **5a, b** with methanolic ammonia afforded the free hydroxyl glycosides **6a, b**, respectively. The spectral data of the obtained derivatives were agreements with the assigned structures.

Reaction of aminopyrimidine derivative **7** with D-galactose and D-xylose in the presence of acetic acid gave stereoselectively the corresponding β-*N*-glycosides **9a, b**, respectively (Scheme 1). The characterization and assignment of the acyclic–cyclic nature of the produced amino sugars were investigated by their NMR spectra. Both sugars afforded products whose NMR spectral data indicated the cyclic structure of the sugar moiety. The observed lower chemical shift values assigned for the H-1 proton at δ 5.87 and 5.89 ppm with coupling constants 9.8 and 9.6 in addition to the absence of the doublet signal in the downfield region at 7.50–7.70 (for the acyclic form) indicated the cyclic form with β-configuration. Moreover, the appearance of the C-1 signal in the ¹³C NMR spectra at δ 92.2 and 92.8 ppm in addition to the absence of the signal in the downfield region at 153–158 (for the acyclic form) evidenced the cyclic structure. The reaction of the β-*N*-glycosides **9a, b** with acetic anhydride at room temperature led to the acetylation of the sugar hydroxyls and formation of compounds **10a, b**, respectively (Scheme 1).

On the other hand, the *N*-ethylindolyl enone **2** was used as a starting compound for the preparation of fused tricyclic and heterocycles. Reaction of compound **2** with



Scheme 1 General methods for the preparation of compounds **2–10a, b**



Scheme 2 General methods for the preparation of compounds 11–16

malononitrile afforded the aminocyano-functionalized pyran derivative **11** (Scheme 2) (Mohamed *et al.*, 2008). Reaction of the obtained pyrane **11** with triethylorthoformate led to the formation of the formamdate derivative **12**

which when allowed to react with hydrazine hydrate afforded the pyranopyrimidine derivative **13** (Scheme 2). Its ¹H NMR spectrum showed triplet and quartet signals at δ 1.49, 4.19 ppm assigned for the ethyl protons and broad

signals at δ 6.74, 11.26 ppm corresponding to NH_2 and NH , respectively. When compound **13** was reacted with carbon disulfide, the tricyclic derivative **14** was obtained in 58 % yield (Scheme 2). The ^1H NMR spectrum of **14** showed the signals corresponding to ethyl group as triplet and quartet, pyran H-5 in addition to the aromatic protons and the NH signal. Its ^{13}C NMR spectrum showed the signal at 179.1 ppm for the $\text{C}=\text{S}$ group.

Reaction of the *N*-ethylindolyl enone **2** with phenylhydrazine and hydroxylamine hydrochloride resulted in the formation of pyrazolyl-indole and oxazolyl-indole derivatives **15** and **16**, respectively (Scheme 2). Their ^1H NMR spectra revealed the presence of the signals corresponding to the methylene and methane protons in the newly formed rings in their structures.

Antimicrobial activity

The synthesized compounds were tested for their in vitro antimicrobial activity against a panel of standard strains of the Gram-positive bacteria (*B. subtilis* and *S. lactis*), the Gram-negative bacteria (*E. coli* and *Pseudomonas aeruginosa*) and the yeast-like pathogenic fungi (*C. albicans*, *C. gabrata*). The primary screening was carried out using the agar disk diffusion method using Muller-Hinton agar medium. The results of the preliminary anti-bacterial and the anti-fungal activities are shown in Table 1. The results revealed that the compounds showed varying degrees of inhibition against the tested microorganisms. In general, the best anti-bacterial activity was displayed by compounds **5a, b**; **6a, b**; **9a, b**; **11**; **13**; **14**; and **16**. Compounds **6a, b**; **9a, b**; and **14** showed strong activity against Gram-positive bacteria *B. subtilis* (inhibition zone 22–25 mm), while compounds **5a, b**; **11**; and **13** were moderate activity (inhibition zone 18–21 mm). Compounds **6a** and **7** showed good activity against *S. lactis*, while compounds **3**, **12** and **16** were moderately active. Compounds **2**; **5a, b**; **6a, b**; **9a**; **10a, b**; **11**; **13**; **14**; and **16** produced high activity against the Gram-negative bacteria *E. coli*. The compounds **2**; **5a**; **6a**; **7**; **9b**; **11–13**; and **15** showed good activity against *Pseudomonas sp.*, while compounds **6b**, **14** and **16** produce the highest activity. The anti-fungal activities depicted in Table 1 revealed that compounds **3**, **5a**; **6a, b**; **9a**; **10a**; and **12** exhibited good anti-fungal activities.

The minimum inhibitory concentration (MIC) of the most active compounds compared to the antibiotic Nalidixic acid[®] and the anti-fungal drug Nystatin[®] is explained in Table 2. The results showed that the MICs ranged between 20 and 50 $\mu\text{g}/\text{disk}$. The MICs for the synthesized compounds were in accordance with the results obtained in the primary screening.

The antimicrobial activity results and structure–activity relationship indicated that the attachment of glycosyl

moieties to the pyrimidine ring in the indolyl-pyrimidine ring system through *N*- or *S*-glycosidic linkage resulted in a marked increase in growth inhibition activity of different microbial strains. Additionally, the anti-bacterial activity observed for the *N*-substituted pyrimidine glycosides **6a, b** and **9a, b** indicated the importance of the free hydroxyl groups of *S*-glycopyranosyl and *N*-glycopyranosyl moieties, and the activity was reduced when these groups were replaced with the corresponding *O*-acetylated analogues. Moreover, the anti-bacterial activity observed for the xylopyranosyl derivative was relatively higher than that of the corresponding galactopyranosyl. In addition, the inhibition activity of the thioglycoside **6b** against Gram-positive bacteria was found to be relatively higher than that of the similar *N*-glycoside **9b**. The anti-bacterial activity results also indicated that the attachment of pyrano[3, 2-*e*][1, 2, 4]triazolo[1, 5-*c*]pyrimidine thione moiety to the *N*-ethyl indole ring as compound **14** resulted in a marked increase in activity when compared with the amino derivative **13**.

For the anti-fungal properties, the results revealed that the growth inhibition activity of the thioglycoside **6b** against fungi under test was found to be relatively higher than that of the similar *N*-glycoside **9b**. Furthermore, the indolyl-pyrimidine thione **3** and the formamidate derivative **12** showed higher activity against *C. gabrata*. In addition to the glycosides and tricyclic pyridotriazolopyrimidine, the 2-amino-substituted pyrimidine, the *N*-phenylpyrazole and oxazole attached to the indolyl ring systems showed increased inhibition activities against Gram-negative bacteria *E. coli* and *P. aeruginosa*.

Molecular docking

The anti-bacterial activity of the synthesized compounds was further analyzed by the molecular docking approach, a method of simulation of fitting ligands into binding site(s) of macromolecular targets. The docking algorithm manipulates ligands and macromolecules in the way that leads to the optimal fit. Analysis of the docking results can lead to better understanding of the molecular basis of the biological effects in question. The obvious prerequisite of successful docking is that the macromolecule and ligands selected for studies can interact in the living organisms and that this interaction plays an important role in production of the observed biological effect (Trott and Olson, 2010). One should keep in mind that the direct analysis of docking results assumes a priori that the interaction between a drug and a docking target is the main mechanism of action. This assumption can often be false. The actual mechanism is usually much more complicated and involves multiple steps or multitarget interactions, metabolic transformations and other events.

As a docking macromolecular target for compounds described in this report, DNA gyrase A protein from *E. coli* was used (Morris *et al.*, 1998). DNA gyrase is a well-

Table 3 Estimated binding affinities compared for convenience with growth inhibition of *E. coli*

Compound	Inhibition <i>-E. coli</i>	Score			
		Min	Max	Mean	Std.
2	13	-5.6	-4.9	-5.3	0.16
3	8	-7.0	-5.7	-6.2	0.33
5a	18	-7.8	-6.5	-6.9	0.37
5b	13	-6.8	-5.8	-6.4	0.27
6a	18	-8.0	-6.0	-6.8	0.61
6b	21	-7.8	-6.3	-7.0	0.40
7	9	-8.0	-6.2	-6.7	0.61
9a	13	-7.7	-7.0	-7.4	0.23
9b	10	-7.7	-6.9	-7.3	0.25
10a	16	-6.7	-6.1	-6.4	0.17
10b	15	-7.7	-6.7	-7.3	0.27
11	21	-7.2	-6.2	-6.7	0.30
12	12	-7.3	-5.9	-6.4	0.35
13	17	-7.8	-6.5	-6.9	0.32
14	17	-7.8	-6.8	-7.2	0.26
15	12	-7.2	-6.1	-6.5	0.29
16	17	-7.4	-6.3	-6.9	0.28

known target for several antimicrobial drugs which have some structural features common with our compounds (Ostrov *et al.*, 2007). It has several potential sites where ligands can be bound. From the perspective of inhibitors, the most potent sites are probably those located in the proximity of residues taking key roles in catalytic functions. According to the literature, the most important residues constituting the active site are R32, H78 and Y122 (Morris *et al.*, 1998; Ostrov *et al.*, 2007). Moreover, it was reported that the residues S83 and D87 are often substituted in fluoroquinolone-resistant forms (Ostrov *et al.*, 2007). All these residues are in the relative mutual proximity and are located around the dimer interface of the protein. We have used this part of the protein in our simulations, and side chains of the above-mentioned residues were kept flexible (10 residues in total for both chains of the dimer).

Performed simulations resulted in 20 different binding poses for each of 17 compounds. Table 3 summarizes the estimated binding affinities compared for convenience with growth inhibition of *E. coli* (Table 1). Figure 2 shows the plot of minimal, maximal and mean values (together with standard deviation) of scoring function plotted against the inhibition zone.

The lack of correlation between the inhibition and scoring function values is evident. Quantitative reliability of scoring functions used in docking simulations has been often questionable. However, we have found in our

Fig. 2 Minimal, maximal and mean values of scoring function plotted against the inhibition zones

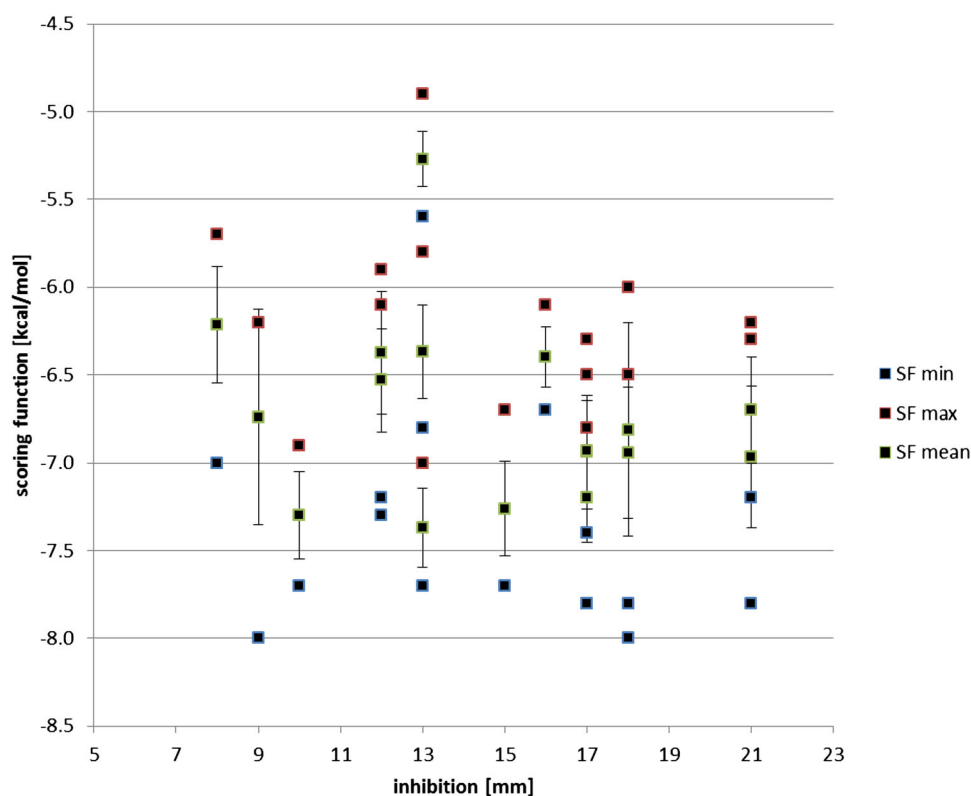


Fig. 3 Compound **5a** located in the pocket located between R32, P43, H78 and Y122 residues

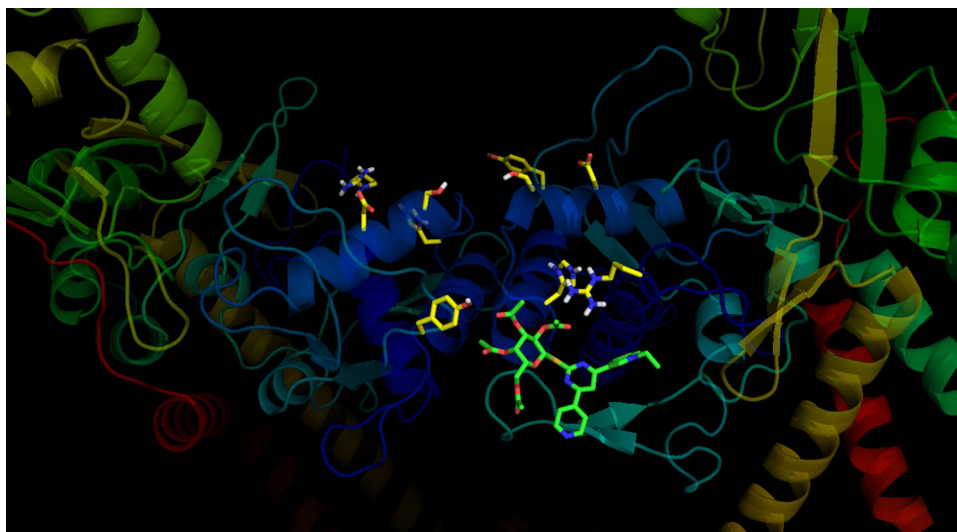


Fig. 4 Compound **6a** located in the pocket located between R32, P43, H78 and Y122 residues



previous studies that docking results can be successfully discussed in a qualitative manner (Magdziarz *et al.*, 2009). Careful analysis of docking results can reveal some interesting differences between the active and the inactive compounds.

Thiopyrimidine derivatives

Compounds **3**, **5a**, **5b**, **6a** and **6b** form a group of thiopyrimidine derivatives (Scheme 1). Compounds **3** and **5b** are not very active against *E. coli*, whereas **5a**, **6a** and **6b** are quite active ones. Compound **3** is much smaller than the other compounds lacking the sugar moieties, and this could be the reason of its low activity.

Inspection of docking poses reveals one interesting difference between compounds **3**, **5a**, **b** and **6a**, **b**. The formers do not bind in the pocket located between R32, P43, H78 and Y122 residues. This pocket seems to be important because it is constituted by the residues playing key roles in the catalytic function of the protein, and Y122 residue in this pocket is located at the opposite chain. Inhibitors located in this pocket, probably, interfere with these important residues and influence with the dimer interface of the protein.

Figures 3, 4 and 5 show compounds **5a** and **6a**, **b** located in this pocket as found by AutoDock Vina (Trott and Olson, 2010). Compound **5b** is not placed in this pocket what seems to correspond with its slightly lower inhibition activity.

Fig. 5 Compound **6b** located in the pocket located between R32, P43, H78 and Y122 residues

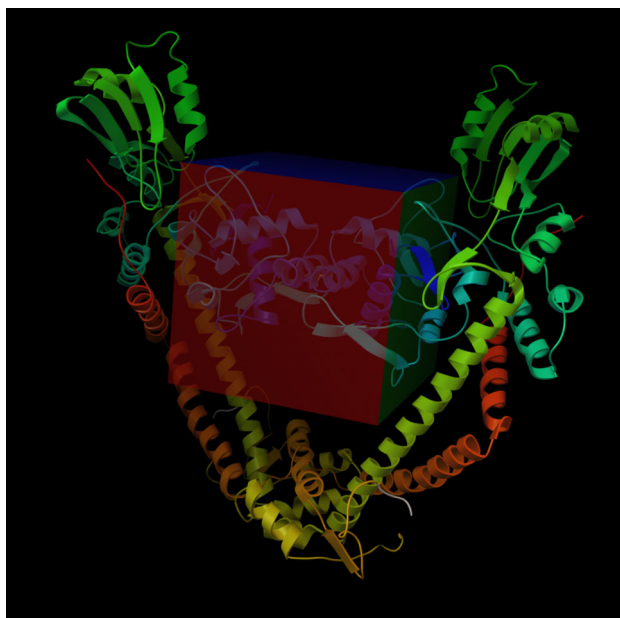
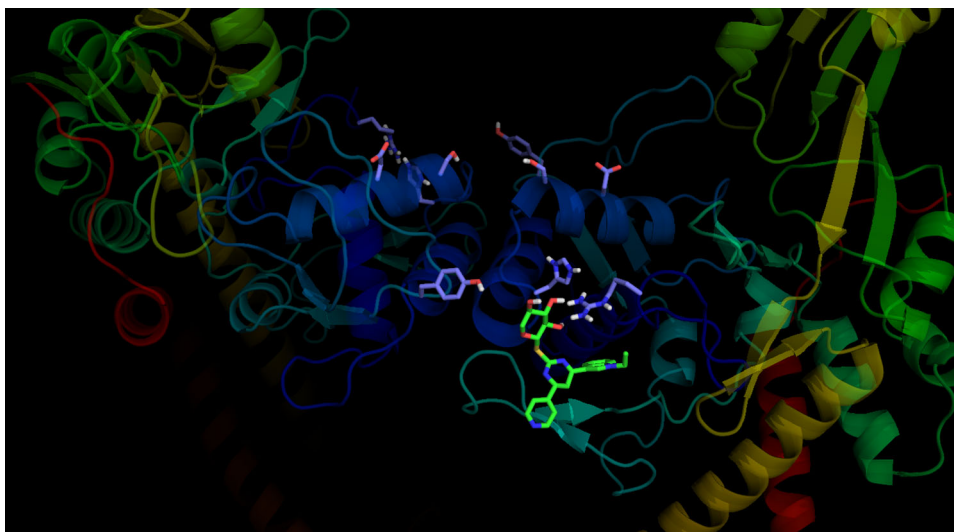


Fig. 6 Size of the search space was set to $40 \times 40 \times 40 \text{ \AA}$, and it was centered at expected binding site residues, i.e., at R32, H78, S83, D87 and Y122 residues, Cartesian coordinates of the center were 60, 60, 40 \AA

Pyrimidine derivatives

This is another group formed by 5 compounds **7**, **9a**, **b** and **10a**, **b**. In this case, there is no clear dependency between the inhibition rate and the propensity to the R32, P43, H78 and Y122 binding sites. This seems to contradict the hypothesis of the importance of this binding pocket. On the other hand, the lack of specificity in binding can be explained by slightly less overall activity of this group as compared to thiopyrimidine derivatives. It is also very likely that the lack of sulfur atom in these compounds changes completely their interaction mode with the protein. No specific docking modes were observed for the compounds **2** and **11–16** (Fig. 6).

Thiopyrimidine derivatives: repeated simulations

In order to verify results observed for the thiopyrimidine derivatives, docking simulations were repeated using slightly different settings. The parameters controlling allowed energy range and the accuracy of search algorithm were increased—see experimental section for details. Table 4 compares the results of the first and repeated simulations.

Table 4 Comparison of the results of the first and repeated simulations

Compound	Inhibition <i>-E. coli</i>	First simulation		Repeated simulation	
		Min score	Poses in pocket	Min score	Poses in pocket
3	8	-7.0	None	-6.9	None
5a	18	-7.8	1, 2, 4, 5, 9	-7.1	1, 2, 5, 7, 12, 19
5b	13	-6.8	None	-7.3	1, 2
6a	18	-8.0	1, 4, 19	-8.1	1, 8
6b	21	-7.8	4	-7.9	3, 10, 13, 16

It is interesting to notice that for compounds **5a**, **b** and **6b**, the modified settings allowed to find more poses located in the binding pocket. In particular, compound **5b** was located in the pocket 2 times top two poses. Compound **6a** was placed in the pocket only 2 times, but this is somehow compensated by its lowered minimal score. In spite of significantly increased search accuracy, compound **3** was not placed in the pocket what is in good agreement with its low activity.

Comparison of results between **5a** and **5b** shows apparent dependency between inhibition potency of these compounds and their propensity to bind to this pocket. One has to keep in mind that docking simulations performed with AutoDock Vina have stochastic nature (Trott and Olson, 2010). Therefore, further repetition of docking simulations should be performed in order to fully support observations described in this report. Moreover, additional studies should be done to compare thiopyrimidine derivatives binding modes with compounds **11**, **13**, **14** and **16** (all displaying high activity against *E. coli*).

Conclusion

In the present work, new 1*H*-indole derivatives were synthesized and characterized using spectral and elemental analyses. The synthesized compounds were investigated for their antimicrobial activity, and some of them showed promising anti-bacterial and anti-fungal activities. Anti-bacterial activity against *E. coli* of the synthesized compounds was further analyzed by the molecular docking approach. Obtained results showed qualitative dependency between activity and binding mode in the thiopyrimidine derivatives. Lack of specific binding of pyrimidine derivatives may indicate that this group exhibits different mechanism of *E. coli* inhibition

Acknowledgments The authors would like to thank Dr. El-Sayed E. Mostafa, Department of Microbial Chemistry, National Research Center, Cairo, Egypt, for performing the antimicrobial activity of the synthesized compounds.

Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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