ABSTRACT

Objective: Chalcones are one of the major classes of the natural products, which exhibit a wide range of pharmacological properties. Also, chalcones are well-known intermediates for synthesizing various heterocyclic compounds like pyrazoline and pyrimidine derivatives. The present work is designed to synthesize new 3-indolylheterocycles starting from N-benzyl and N-benzoyl-1H-indole-3-carboxaldehydes and evaluating their in vitro antimicrobial activity. In addition, the probability of the most promising antimicrobial compounds to inhibit ATPase, enoyl reductase and dihydrofolate reductase were studied theoretically via molecular docking.

Methods: A new series of 3-indolylchalcones 2a,b were prepared and allowed to react with hydrazine hydrate, phenyl hydrazine, hydroxylamine, urea, thiourea and thiocyanic acid to afford the corresponding pyrazoles 3a,b,6a-b and pyrimidines 7a,b,9a.b. On the other hand, the reaction of 2a,b with malononitrile afforded 10a,b, which upon condensation with formic acid, formamide, urea or thiourea yielded the fused pyridine [2,3-dipyriridine 11a,b-14a,b. Moreover, cyclo-condensation of 2a,b with thiourea afforded thiazole derivatives 15a,b. Under cyclization with phenacyl bromide afforded thiazole derivatives 16a and 16b. While the reaction of 2a,b with cyano thiocarbamide afforded 2-mercaptonicotinonitriles 17a,b. The reaction of 17a,b with some halo-compounds gave S-alkyl derivatives 18a-d and 19a-d, respectively. All the newly prepared compounds were evaluated for their in vitro antimicrobial activity. In addition, molecular docking study of the most promising antimicrobial compounds against ATPase, enoyl reductase and dihydrofolate reductase theoretically is discussed.

Results: Compounds 17a and 17b were found to be the most potent compounds with MIC of 0.98, 0.49 and 0.98μg/ml against S. pneumoniae (RCMB 010010), E. coli (RCMB 010052) and A. fumigatus (RCMB 02568), respectively compare to the reference drugs. Also, compounds 17a and 17b exhibited good docking scores and could act as inhibitors of enzymes understudied.

Conclusion: Further work is recommended to confirm the activity of compounds 17a and 17b to inhibit ATPase, enoyl reductase and dihydrofolate reductase in a specific bioassay.

Keywords: Indole-3-carboxaldehyde, Heterocycle, α,β-unsaturated ketone, Antimicrobial, Molecular docking

INTRODUCTION

Chalcones [1, 3-diaryl-2-propen-1-ones], one of the major classes of the natural products with widespread occurrence in fruits, vegetables, spices, tea and soy-based food stuff [1]. Literature review reveals that natural and synthetic chalcones display a wide range of pharmacological properties, including cytotoxicity towards different cancer cell lines [2], antibacterial [3], antimalarial [4], anti-inflammatory [5] and antiviral activities [6]. Also, chalcones are well-known intermediates for synthesizing various heterocyclic compounds like pyrazoline and pyrimidine derivatives [7,8]. In addition, indole derivatives which form potent pharmacodynamic nuclei have been reported to possess a wide variety of biological properties, viz, anti-inflammatory, anti-cancer and antimicrobial [9-14]. Encouraged by the above observations, and in continuation of our work on the preparation of new bioactive indole derivatives [7,14-16], the present work is designed to synthesize new 3-indolylheterocycles starting from N-benzyl and N-benzoyl-1H-indole-3-carboxaldehydes and evaluating their antimicrobial activity. In addition, molecular docking study of the most biologically active compounds against three different enzymes namely, ATPase, enoyl reductase and dihydrofolate reductase theoretically is discussed.

MATERIALS AND METHODS

General

Melting points were determined on digital melting point apparatus (Electrothermal 9100, Electrothermal Engineering Ltd, serial No. 8694, Rochford, United Kingdom) and are uncorrected. The micromethodal data were achieved on a Perkin-Elmer 2400 analyzer (Perkin-Elmer, 940 Winter Street, Waltham, Massachusetts 02451, USA) and were found within±0.4 % of the theoretical values. IR spectra were recorded on a Perkin-Elmer 1600 Fourier Transform Infrared Spectrophotometer (Perkin-Elmer). The 1H NMR spectra were recorded on a Bruker Avance digital spectrometer (BRUKER Germany) in DMSO-d6, and chemical shifts (δ) are reported in ppm units relative to the standard tetramethylsilane (TMS). Mass spectra (EI) were recorded at 70eV with JEOl-JMS-AX500 mass spectrometer (JEOL Ltd. 1-2, Musashino 3-chome Akishima, Tokyo 196-8558, Japan). The chemical and solvents were of commercial grade and used without further purification. 1-benzyl and 1-benzoyl-1H-indole-3-carboxaldehyde, cyano thiocarbamide were prepared as reported [17-19].

Synthesis

General procedure for the preparation of α,β-unsaturated ketones 2a, 2b

To solution of 2-acetyl naphthalene (0.01 mol) and compound 1a or 1b (0.01 mol) in absolute ethanol (10 ml), aqueous potassium hydrosulphide solution (5 ml, 25%) was added. The reaction mixture was stirred for 2h at room temperature and then left overnight in the refrigerator. The reaction mixture was neutralized with diluted hydrochloric acid (1:1) and the solid that formed was filtered off, washed with water, air-dried and crystallized from absolute ethanol.

1-(1-Benzyl-1H-indol-3-yl)-3-(naphthalen-2-yl) prop-2-ene-1-one (2a)

Yield: 95 %; MP: 164-166°C; IR (KBr): ν 1675 (C=O), 1621 cm⁻¹ (C=C); 1H NMR (270 MHz, CDCl₃); δ 8.85 (1H, s, H-2 indole), 7.26-8.19 (16H, m, Ar-H), 8.02, 7.19 (2H, 2d, CH=CH), 5.37 ppm (2H, s, N-
1-Benzyl-3-[(3-naphthalen-1-yl)-1-phenyl-4, 5-dihydro-1H-pyrazol-5-yl]-1H-indole (5b)

Yield: 99 %; MP: 169-171°C; IR (KBrs): v 1665 (C=O), 1595 (C=N), 1570 cm⁻¹ (C=C); MS (m/z): 491 [M⁺]; Anal. C₃₂H₂₃N₂O (491.58)
Calcd: C, 83.07; H, 5.13; N, 8.55; Found: C, 83.11; H, 5.23; N, 8.41.

Synthesis of compounds 6a, 6b

A mixture of compound 2a or 2b (0.01 mol), hydroxylamine hydrochloride (0.01 mol) and anhydrous sodium acetate (0.01 mol) in absolute ethanol (10 ml) was heated under reflux for 6-8h. After cooling the reaction mixture was poured onto ice-water (50 ml) and the solid formed was filtered off, air-dried and crystallized from absolute ethanol.

4, 5-Dihydro-3-(1-benzyl-1H-indol-3-yl)-5-(naphthalen-2-ylene) isoxazole (6a)

Yield: 81 %; MP: 140-142°C; IR (KBrs): v 1620 (C=O), 1539 cm⁻¹ (C=O); H NMR (270 MHz, DMSO-d₆): δ 7.17-8.12 (17H, m, Ar-H), 5.54 (1H, dd, CH-pyrazoline), 4.64 (2H, s, N-CH₂); 2.75 (1H, dd, CH₂-pyrazoline equatorial); 1.76 ppm (1H, dd, CH₂-pyrazoline axial); Anal. C₃₂H₂₃N₂O (424.49)
Calcd: C, 83.36; H, 5.51; N, 6.96; Found: C, 83.50; H, 5.42; N, 6.99.

4,5-Dihydro-3-(1-benzyl-1H-indol-3-yl)-5-(naphthalen-2-ylene) isoxazole (6b)

Yield: 90 %; MP: 240-242°C; IR (KBrs): v 1670 (C=O), 1619 (C=O), 1538 cm⁻¹ (C=O); MS (m/z): 491 [M⁺]; Anal. C₃₂H₂₃N₂O₂ (461.47)
Calcd: C, 80.75; H, 4.84; N, 6.73; Found: C, 80.74; H, 4.82; N, 6.70.

Synthesis of compounds 7a, 7b

A mixture of compound 2a or 2b (0.01 mol) and urea (0.0076, 0.01 mol) in dry ethanol (10 ml) containing glacial acetic acid (0.5 ml) was refluxed for 6-8h. After cooling the reaction mixture was poured onto ice-water (50 ml) and the solid formed was filtered off, air-dried and crystallized from absolute ethanol.

4-(1-Benzyl-1H-indol-3-yl)-6-(1-naphthalen-2-yl) pyrimidin-2(1H)-one (7a)

Yield: 71 %; MP: 100-103°C; IR (KBrs): v 3420 (NH), 1667 (C=O), 1620 (C=O), 1553 cm⁻¹ (C=O); H NMR (270 MHz, DMSO-d₆): δ 8.91 (1H, s, NH), 8.53 (1H, s, H-5 pyrimidinyl), 7.00-8.21 (17H, m, Ar-H), 5.53 (2H, s, CH₂-N); Anal. C₃₃H₂₁N₅O (575.50)
Calcd: C, 81.48; H, 4.95; N, 9.53; Found: C, 81.35; H, 4.89; N, 9.68.

4-(1-Benzyl-1H-indol-3-yl)-6-(1-naphthalen-2-yl) pyrimidin-2(1H)-one (7b)

Yield: 80%: MP: 128-130°C; IR (KBrs): v 3133 (NH), 1702 (C=O), 1623 (C=O), 1590 cm⁻¹ (C=O); H NMR (270 MHz, DMSO-d₆): δ 8.91 (1H, s, NH), 8.19 (1H, s, H-5 pyrimidinyl), 8.63 (1H, s, H-2 indolyl), 7.15-8.36 ppm (16H, m, Ar-H); Anal. C₃₃H₂₁N₅O (544.48)
Calcd: C, 80.79; H, 4.34; N, 9.52; Found: C, 80.19; H, 4.23; N, 9.48.

Synthesis of compounds 8a, 8b

A mixture of compound 2a or 2b (0.01 mol) and thiourea (0.0076, 0.01 mol) in dry ethanol (10 ml) containing glacial acetic acid (0.5 ml) was heated under reflux for 6-8h. After cooling the reaction mixture was poured onto ice-water (50 ml) and the solid formed was filtered off, air-dried and crystallized from absolute ethanol.

4-(1-Benzyl-1H-indol-3-yl)-6-(1-naphthalen-2-yl) pyrimidin-2(1H)-thione (8a)

Yield: 70%; MP: 140-142°C; IR (KBrs): v 3431 (NH), 1599 (C=O), 1553 (C=O), 1240 cm⁻¹ (C=S); H NMR (270 MHz, DMSO-d₆): δ 11.65 (1H, s, H-5 pyrimidinyl), 7.84 (1H, s, H-2 indolyl), 7.11-7.53 (16H, m, Ar-H), 5.65 (2H, s, N-CH₂); Anal. C₂₅H₁₇N₃S (443.56)
Calcd: C, 78.53; H, 4.77; N, 9.47; Found: C, 78.48; H, 4.65; N, 9.51.

4-(1-Benzyl-1H-indol-3-yl)-6-(1-naphthalen-2-yl) pyrimidin-2(1H)-thione (8b)

Yield: 79%; MP: 184-186°C; IR (KBrs): v 3432 (NH), 1665 (C=O), 1603 (C=O), 1558 cm⁻¹ (C=S); H NMR (270 MHz, DMSO-d₆):
heated under reflux for 8h. The reaction mixture was then cooled, filtered, and washed with water, air-dried and crystallized from absolute ethanol.

4-(1-Benzyl-1H-indol-3-yl)-6-naphthalen-2-yl) pyrimidine-2-amine (9a)

Yield: 72%; MP: 70-72°C; IR (KBr): v 3416 and 3165 (NH), 1634 (C=N); 1571 cm⁻¹ (C-C); δ H NMR (270 MHz, DMSO-d₆): δ 8.91 (2H, s, N-H), 8.53 (2H, s, NH₂), 5.63 ppm (2H, s, CH₂-N); Anal. C₉H₁₀N₄ (246.51): Calc.: C, 81.28; H, 5.65; N, 13.22.

4-(1-Benzyl-1H-indol-3-yl)-6-naphthalen-2-yl) pyrimidine-2-amine (9b)

Yield: 83%; MP: 171-73°C; IR (KBr): ν 3416 (NH), 1668 (C=O); 1621 (C-C); δ H NMR (270 MHz, DMSO-d₆): δ 11.41 (1H, s, NH), 8.77 (1H, s, H-5 pyrimidinyl), 7.23-8.26 ppm (19H, m, Ar-H); Anal. C₂₉H₂₉N₄ (440.50): Calc.: C, 81.28; H, 5.65; N, 13.22.

Synthesis of compounds 10a, 10b

A mixture of compound 2a or 2b (0.001 mol) malononitrile (0.01 mol) pyridine-3-carbonitrile (10a) 4-(1-Benzyl-1H-indol-3-yl)-6-naphthalen-2-yl) pyrimidine-2-amine (9a) 4-(1-Benzyl-1H-indol-3-yl)-6-naphthalen-2-yl) pyrimidine-2-amine (9b) 4-Amino-5-(1-benzoyl-1H-indol-3-yl)-7-(naphthalen-2-yl) pyridro[2,3-d]pyrimidine-2(1H)-one (13a)

Yield: 75%; MP: 200-202°C; IR (KBr): v 3350 (NH₂), 3195 (NH), 1694 (C=O), 1604 (C=C); δ H NMR (270 MHz, DMSO-d₆): δ 8.99 (1H, s, NH), 7.37-8.32 (18H, m, Ar-H), 5.03 (2H, s, N-H₂), 2.47 ppm (2H, s, N-CH₂); Anal. C₂₉H₂₉N₄O (493.19): Calc.: C, 77.87; H, 4.70; N, 14.19; Found: C, 77.64; H, 4.7; N, 14.01.

Amino-5-(1-benzyl-1H-indol-3-yl)-7-(naphthalen-2-yl)pyridro[2,3-d]pyrimidine-2(1H)-one (13b)

Yield: 83%; MP: 170-172°C; IR (KBr): ν 3424 (NH₂), 3316 (NH), 1694 (C=O); 1605 (C=C); δ H NMR (270 MHz, DMSO-d₆): δ 8.94 (1H, s, NH), 7.35-8.31 (18H, m, Ar-H), 5.01 ppm (2H, s, N-CH₂), 2.47 ppm (2H, s, N-CH₂); Anal. C₂₉H₂₉N₄O (491.54): Calc.: C, 78.19; H, 4.31; N, 14.25; Found: C, 78.22; H, 4.21; N, 14.01.

Synthesis of compounds 14a, 14b

A mixture of compound 10a or 10b (0.005 mol) and thiourea (0.005 mol) in formamide (20 ml) containing few drops of triethylamine was heated under reflux for 6-7h. After cooling, the formed solid was filtered off, air-dried and crystallized from absolute ethanol.

Amino-5-(1-benzyl-1H-indol-3-yl)-7-(naphthalen-2-yl)pyridro[2,3-d]pyrimidine-2(1H)-thione (14a)

Yield: 79%; MP: 260-262°C; IR (KBr): ν 3410 (NH₂), 3214 (NH), 1730 (C=O), 1643 (C=C); δ H NMR (270 MHz, DMSO-d₆): δ 11.89 (3H, s, NH and NH₂), 7.13-8.27 (18H, m, Ar-H); Anal. C₁₅H₁₅N₅O (507.17): Calc.: C, 75.73; H, 4.17; N, 13.80; Found: C, 75.64; H, 4.1; N, 13.67.

Amino-5-(1-benzyl-1H-indol-3-yl)-7-(naphthalen-2-yl)pyridro[2,3-d]pyrimidine-2(1H)-thione (14b)

Yield: 83%; MP: 261-263°C; IR (KBr): ν 3410 (NH₂), 3214 (NH), 1730 (C=O), 1643 (C=C); δ H NMR (270 MHz, DMSO-d₆): δ 11.89 (3H, s, NH and NH₂), 7.13-8.27 (18H, m, Ar-H); Anal. C₁₅H₁₅N₅O (507.17): Calc.: C, 75.73; H, 4.17; N, 13.80; Found: C, 75.64; H, 4.1; N, 13.67.

Synthesis of compounds 14a, 14b

A mixture of compound 10a or 10b (0.005 mol) and thiourea (0.005 mol) in formamide (20 ml) containing few drops of thiourea was heated under reflux for 6-7h. After cooling, the formed solid was filtered off, air-dried and crystallized from absolute ethanol.

5-(1-Benzyl-1H-indol-3-yl)-7-(naphthalen-2-yl) pyrido[2,3-d]pyrimidin-4-amine (12a)

Yield: 85%; MP: 220-2°C; IR (KBr): ν 3264, 3103 (NH₂), 1639 (C=O), 1604 cm⁻¹ (C=C); δ H NMR (270 MHz, DMSO-d₆): δ 8.73-8.78 (1H, m, Ar-H), 5.24 (2H, s, N-H₂), 2.46 ppm (2H, s, N-H₂); Anal. C₂₉H₂₉N₅ (457.55): Calc.: C, 76.13; H, 4.15; N, 14.12; Found: C, 76.22; H, 4.23; N, 9.01.

5-(1-Benzyl-1H-indol-3-yl)-7-(naphthalen-2-yl) pyrido[2,3-d]pyrimidin-4-amine (12b)

Yield: 85%; MP: 201-2°C; IR (KBr): ν 3100, 3128 (NH₂), 1723 (C=O), 1639 (C=C); δ H NMR (270 MHz, DMSO-d₆): δ 8.10 (2H, s, N-H₂), 7.15-8.41 ppm (1H, m, Ar-H); Anal. C₂₉H₂₉N₅O (491.54): Calc.: C, 78.19; H, 4.31; N, 14.25; Found: C, 78.22; H, 4.21; N, 14.01.
Calcd: C, 79.63; H, 4.53; N, 8.99; Found: C, 79.60; H, 4.44; N, 9.01.

Synthesis of compounds 16a and 16b

To a solution of sodium (0.23 g, 0.01 mol) in absolute methanol (20 ml) was added compound 1a or 1b (0.01 mol) and phenacyl bromide (1.99 g, 0.01 mol). The reaction mixture was heated under reflux for 10-12 h. After cooling, the solid that formed was filtered off, air-dried and crystallized from absolute ethanol.

6-(1-Benzyl-1H-indol-3-yl)-2-mercaptop-4-(naphthalen-2-yl) pyridine-3-carbonitrile (18a)

Yield: 95%; MP: 242-244 °C; IR (KBr): ν 3471 (NH), 2207 (CN), 1640, 1592 (C=O), 1530 (C=N), 1513 (C=C); Anal. C_{27}H_{23}NS (585.19): Calcd: C, 77.51; H, 4.30; N, 9.70.

Synthesis of compounds 17a and 17b

A mixture of compound 2a or 2b (0.01 mol) and cyano thioacetamide (1.0 g, 0.01 mol) was heated under reflux in absolute ethanol (20 ml) for 7 h. After cooling, the solid formed was filtered off, air-dried and crystallized from absolute ethanol.

6-(1-Benzyl-1H-indol-3-yl)-2-mercaptop-4-(naphthalen-2-yl) pyridine-3-carbonitrile (20a)

Yield: 85%; MP: 225-227 °C; IR (KBr): ν 3160, 3107 NH2, 2215 (CN), 1764, 1640 (C=O), 1592 (C=N), 1513 (C=C); Calcd: C, 74.05; H, 4.44; N, 7.40; Found: C, 74.00; H, 4.45; N, 7.35.

Synthesis of compounds 18a-d, 19a-d

A solution of compound 17a or 17b (0.001 mol) and potassium hydroxide (0.6 g, 0.01 mol) in N,N-dimethylformamide (20 ml) was stirred for 2 h at room temperature, then halo-compound namely, chloro-acetonitrile, ethyl chloroacetate, phenacyl bromide or 2-chloro-N-[thiazol-2-yl]acetamide (0.001 mol) was added and the reaction mixture kept stirring for another 2 h. The resulting solid was filtered off, washed with water, air-dried and crystallized from dimethylformamide and absolute ethanol.

6-(1-Benzyl-1H-indol-3-yl)-2-cyanomethylsulfonyl-4-(naphthalen-2-yl) pyridine-3-carbonitrile (18a)

Yield: 85%; MP: 120-122 °C; IR (KBr): ν 3426, 3383 (NH), 1670 (C=O), 1600 (C=N), 1280 cm⁻¹ (C=S); Anal. C_{27}H_{23}NS (544.63): Calcd: C, 78.32; H, 4.33; N, 10.99; Found: C, 78.32; H, 4.32; N, 10.99.

6-(1-Benzyl-1H-indol-3-yl)-3-cyano-4-(naphthalene-2-yl) pyridine-2-ylsulfanyl]acetic acid ethyl ester (18b)

Yield: 85%; MP: 130-2 °C; IR (KBr): ν 2204 (CN), 1634 (C=O), 1600 (C=N), 1569 cm⁻¹ (C=O); Anal. C_{27}H_{23}NS (544.63): Calcd: C, 78.32; H, 4.33; Found: C, 78.32; H, 4.32; N, 10.99.

6-(1-Benzyl-1H-indol-3-yl)-3-cyano-4-(naphthalene-2-yl) pyridine-2-ylsulfanyl]acetic acid ethyl ester (18c)

Yield: 82%; MP: 140-2 °C; IR (KBr): ν 2204 (CN), 1634 (C=O), 1600 (C=N), 1584 cm⁻¹ (C=O); Anal. C_{27}H_{23}NS (585.19): Calcd: C, 79.62; H, 4.65; N, 7.40; Found: C, 79.65; H, 4.51; N, 7.22.

Phenyl thiocacetic acid S-[6-(1-benzyl-1H-indol-3-yl)-3-cyano-4-(naphthalene-2-yl) pyridine-2-yl] acid ethyl ester (18d)

Yield: 81%; MP: 120-2 °C; IR (KBr): ν 3398 (NH), 2208 (CN), 1635 (C=O), 1570 (C=N), 1527 cm⁻¹ (C=O); Anal. C_{27}H_{23}NS (585.19): Calcd: C, 79.62; H, 4.65; N, 7.40; Found: C, 79.65; H, 4.51; N, 7.22.

6-(1-Benzyl-1H-indol-3-yl)-2-cyanomethylsulfonyl-4-(naphthalene-2-yl) pyridine-3-carbonitrile (19a)

Yield: 50%; MP: 222.7-224 °C; IR (KBr): ν 3430 (NH), 2210 (CN), 1769 (C=O), 1513 (C=N), 1508 (C=C); Anal. C_{27}H_{23}NS (520.60): Calcd: C, 76.22; H, 3.98; N, 10.70; Found: C, 76.28; H, 4.00; N, 10.72.

6-(1-Benzyl-1H-indol-3-yl)-3-cyano-4-(naphthalene-2-yl) pyridine-2-ylsulfanyl]acetic acid ethyl ester (19b)

Yield: 95%; MP: 242-244 °C; IR (KBr): ν 3471 (NH), 2210 (CN), 1764, 1671 (C=O), 1561 (C=N), 1513 cm⁻¹ (C=O); Anal. C_{27}H_{23}NS (585.19): Calcd: C, 77.60; H, 4.33; N, 10.99; Found: C, 77.63; H, 4.30; N, 10.97.

6-(1-Benzyl-1H-indol-3-yl)-3-cyano-4-(naphthalene-2-yl) pyridine-3-carbonitrile (20b)

Yield: 90%; MP: 242-244 °C; IR (KBr): ν 3430 (NH), 2210 (CN), 1673 (C=O), 1634 (C=N), 1568 cm⁻¹ (C=O); Anal. C_{27}H_{23}NS (544.63): Calcd: C, 78.32; H, 4.33; N, 10.99; Found: C, 78.32; H, 4.32; N, 10.97.

Phenyl thiocacetic acid S-[6-(1-benzyl-1H-indol-3-yl)-3-cyano-4-(naphthalene-2-yl) pyridine-2-yl] acid ethyl ester (20c)

Yield: 95%; MP: 242-244 °C; IR (KBr): ν 3471 (NH), 2210 (CN), 1764, 1671 (C=O), 1561 (C=N), 1513 cm⁻¹ (C=O); Anal. C_{27}H_{23}NS (585.19): Calcd: C, 77.60; H, 4.33; N, 10.99; Found: C, 77.63; H, 4.30; N, 10.97.

6-(1-Benzyl-1H-indol-3-yl)-2-cyanomethylsulfonyl-4-(naphthalene-2-yl) pyridine-3-carbonitrile (20d)

6-(1-Benzyl-1H-indol-3-yl)-3-cyano-4-(naphthalene-2-yl) pyridine-2-ylsulfanyl]acetic acid ethyl ester (20b)

Yield: 85%; MP: 100-2 °C; IR (KBr): ν 3127 (NH), 1732 (C=O), 1640 (C=N), 1518 cm⁻¹ (C=O); Anal. C_{27}H_{23}NS (544.63): Calcd: C, 78.25; H, 4.38; N, 11.06; Found: C, 78.02; H, 4.21; N, 10.99.
Antimicrobial evaluation
dihydrothino[2,3-b]pyridine-3-amine (20c)

Yield: 85%; MP: 186-8°C; IR (KBr): ν 3100 (CH), 1648 (C=O), 1614 (C=N), 1573 (C=C); MS (m/z): 541 [M-NH$_3$ and CO]; Anal. Calcd: C, 76.13; H, 3.87; N, 10.76; Found: C, 76.00; H, 3.91; N, 10.67.

Yield: 75%; MP: 270-2°C; IR (KBr): ν 3377, 3216 (NH, NH$_2$), 1664 (C=O), 1544 (C=N), 1517 cm$^{-1}$ (C=C); MS (m/z): 585 (M), 541 (M-44); Anal. Calcd: C, 75.92; H, 4.92; N, 7.59; Found: C, 75.81; H, 5.00; N, 7.60.

Yield: 91%; MP: 140-2°C; IR (KBr): ν 3100 (NH, NH$_2$), 1647 (C=O), 1614 cm$^{-1}$ (C=N); MS (m/z): 541 [M-NH$_3$ and CO]; Anal. Calcd: C, 75.92; H, 4.92; N, 7.59; Found: C, 75.81; H, 5.00; N, 7.60.

Yield: 75%; MP: 100-2°C; IR (KBr): ν 3391, 3171 (NH, NH$_2$), 1700 (C=O), 1653 cm$^{-1}$ (C=N); MS (m/z): 541 [M-NH$_3$ and CO]; Anal. Calcd: C, 75.92; H, 4.92; N, 7.59; Found: C, 75.81; H, 5.00; N, 7.60.

3-Amino-1-(benzyl-1H-indol-3-yl)-4-(naphthalen-2-yl)-2,3-dihydrothino[2,3-b]pyridine-2-carboxylic acid thiazol-2-yl-amide (20d)

Yield: 95%; MP: 100-2°C; IR (KBr): ν 3391, 3171 (NH, NH$_2$), 1700 (C=O), 1653 cm$^{-1}$ (C=N); MS (m/z): 541 [M-NH$_3$ and CO]; Anal. Calcd: C, 75.92; H, 4.92; N, 7.59; Found: C, 75.81; H, 5.00; N, 7.60.

3-Amino-1-(benzyl-1H-indol-3-yl)-4-(naphthalen-2-yl)-2,3-dihydrothino[2,3-b]pyridine-2-carboxylic acid ethyl ester (21b)

Yield: 95%; MP: 170-2°C; IR (KBr): ν 3216, 3171 (NH, NH$_2$), 1703, 1681 cm$^{-1}$ (C=O), 1648 (C=N), 1617 cm$^{-1}$ (C=N); MS (m/z): 541 [M-NH$_3$ and CO]; Anal. Calcd: C, 75.92; H, 4.92; N, 7.59; Found: C, 75.81; H, 5.00; N, 7.60.

3-Amino-1-(benzyl-1H-indol-3-yl)-4-(naphthalen-2-yl)-2,3-dihydrothino[2,3-b]pyridine-3-amine (21c)

Yield: 91%; MP: 140-2°C; IR (KBr): ν 3200, 3158 (NH, NH$_2$), 1705 (C=O), 1651 cm$^{-1}$ (C=N); MS (m/z): 541 [M-NH$_3$ and CO]; Anal. Calcd: C, 75.92; H, 4.92; N, 7.59; Found: C, 75.81; H, 5.00; N, 7.60.

3-Amino-1-(benzyl-1H-indol-3-yl)-4-(naphthalen-2-yl)-2,3-dihydrothino[2,3-b]pyridine-2-carboxylic acid thiazol-2-yl-amide (21d)

Yield: 91%; MP: 140-2°C; IR (KBr): ν 3200, 3158 (NH, NH$_2$), 1705 (C=O), 1651 cm$^{-1}$ (C=N); MS (m/z): 541 [M-NH$_3$ and CO]; Anal. Calcd: C, 75.92; H, 4.92; N, 7.59; Found: C, 75.81; H, 5.00; N, 7.60.

Biological assays

Antimicrobial evaluation

The antimicrobial activity of the synthesized compounds was evaluated in vitro using agar well diffusion method [20] against a variety of pathogenic microorganisms: Streptococcus pneumoniae (PDB ID: 1AI9), Staphylococcus aureus (RCMB 05036) and Eschericha coli (RCMB 00043), Aspergillus fumigatus (585.72), Candida albicans (RCMB 05036) and Aspergillus fumigatus (RCMB 05036).

Dimethylformamide (DMF) was used as a solvent for impregnation. Ampicillin and ciprofloxacin were used as reference drugs for bacteria, whereas, amphotericin B was used as reference drug for fungi.

Method of testing

The sterilized media was poured onto the sterilized Petri dishes (20 ml, each Petri dish) and allowed to solidify. Wells of 6 mm diameter were made in the solidified media (Nutrient and MacConkey agar media for bacteria and on Sabouraud dextrose agar for fungus) with the help of sterile borer. A sterile swab was used to distribute microbial suspension evenly over the surface of solidified media and solutions of the tested samples (5 mg/ml) were added to each well with the help of micropipette. The inhibition zones (IZ) of the test compounds were measured after 24-48 h incubation at 37 °C for bacteria and after 5 d incubation at 28 °C for fungi. The experiment was performed in triplicate, and the average zone of inhibition was calculated. All strains were provided from the culture collection of the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt.

Minimum inhibitory concentration (MIC)

The MIC was determined by the broth microdilution method using 96-well microplates [21]. The inoculate of the microbial strains was prepared from 24 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. Each sample (1.0 mg) was dissolved in DMOS (1 ml) to obtain 1000 μg/ml stock solution. A number of wells were reserved in each plate for positive and negative controls. Sterile broth (100 μl) was added to the well from row B to H. The stock solutions of samples (100 μl) were added to the wells in rows A and B. Then, the mixture of samples and sterile broth (100 μl) in row B was transferred to each well in order to obtain a two-fold serial dilution of the stock samples [concentration of 500, 250, 125, 62.5, 31.3, 15.6 and 7.813, 3.91, 0.98 and 0.49 μg/ml]. The inoculums (100 μl) were added to each well and a final volume 200 μl was obtained in each well. Plates were incubated at 37 °C for 24 h in case of antibacterial activity and 48 h at 25 °C for antifungal activity. Microbial growth was indicated by the presence of turbidity of the well. The lowest concentration showing no growth was taken as the minimum inhibitory concentration (MIC).

Molecular docking study

Molecular docking study of the most active compounds 8b, 17a, 17b, 19d were performed by Molecular Operating Environment (MOE) 2008.10 (http://www.chemcomp.com). The target compounds were docked against three different biological targets including the crystal structures of ATPase enzyme in complex with (3E)-(3-3-pyridin-3-ylmethylidene)-1,3-dihydro-2H-indol-2-one (EVO) (PDB ID: 5CPH) as promising gram-positive bacteria target [22]; enoyl reductase enzyme in complex with triclosan (TCL) (PDB ID: 1C14) as promising gram-negative bacteria target [23], and dihydrofolate reductase enzyme in complex with dihydro nicotinamide adenine dinucleotide phosphate (NDP) (PDB ID: 1A9F) as promising fungi target [24]. The protein crystal structures were downloaded from protein data bank (http://www.rcsb.org/pdb) and prepared for docking process.

The co-crystalline ligands were re-docked in the active pockets to validate the docking protocol.

The structure of the target compounds was drawn in ChemDraw Ultra 10.0 (ChemOffice package) and the energy was minimized using the MMFF94x force field until an RMSD (root-mean-square deviation) of atomic position gradient of (0.01) Kcal mol$^{-1}$. MMFF94x was reported as the efficient force field for minimizing ligand-protein complexes [25].

The docking Algorithm was done by MOE-DOCK default which uses flexible, a rigid technique for post the molecule inside the cavity. All rotatable bonds of ligands are allowed to undergo free rotation to explore the conformational space inside the rigid receptor binding site. The docking scores were expressed in negative energy terms; the lower the binding free energy, the better the binding affinity [26] and the ligand interactions (hydrogen bonding, hydrophobic and Van der Waals interaction) with active sites were determined.

RESULTS AND DISCUSSION

Chemistry

The synthetic routes of the target compounds are outlined in Schemes 1, 2 and 3. Condensation of N-benzyl (1a) and N-benzoyl-1H-indole-3-carboxaldehydes (1b) with 2-acetyl napththalene in ethanol and in the presence of potassium hydroxide (50%) [Caisen-Schmidt reaction] led to the formation of the corresponding Ω-unaturated ketones 2a,b (Scheme 1). Cyclocondensation of 2a and 2b via reaction with hydrazine hydrate under reflux in absolute ethanol and in the presence of few drops of glacial acetic acid led to the formation of the corresponding pyrazoles 3a and 3b (Scheme 1).

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Whereas reaction of 2a and 2b with hydrazine hydrate under reflux in a mixture of acetic anhydride and glacial acetic acid (2:1) afforded the corresponding N-acetylpiprazoles 4a and 4b, respectively (Scheme 1). Also, condensation of 2a and 2b with phenyl hydrazine under reflux in absolute ethanol and in the presence of few drops of glacial acetic acid led to the formation of the corresponding pyrazoles 5a and 5b. On the other hand, condensation of 2a and 2b with hydroxylamine hydrochloride in the presence of anhydrous sodium acetate yielded isoxazoles derivative 6a and 6b (Scheme 1). Whereas reaction of compound 2a or 2b with guanidine hydrochloride in the presence of anhydrous sodium acetate afforded the corresponding pyrimidin-2(1H)-ones 9a and 9b (Scheme 1).

\[ \text{\textsuperscript{1}H NMR spectra of pyrazole compounds 3a,b, 4a,b and 5a,b besides} \]

\[ \text{CH-} \]

\[ \text{pyrazoline axial (H} \]

\[ \text{equatorial), 1.76 ppm (1H, dd, 1H, dd, CH- pyrazoline equatorial (H} \]

\[ \text{and CH- pyrazoline axial (H} \]

\[ \text{respectively.} \]

The reaction of compound 2a or 2b with urea or thiourea in absolute ethanol in the presence of few drops of glacial acetic acid afforded the corresponding pyrimidin-2(1H)-ones 7a,b and pyrimidin-2(1H)-thiones 8a,b, respectively. Whereas, the reaction of compound 2a or 2b with guanidine hydrochloride in the presence of anhydrous sodium acetate yielded pyridine-2-amines 9a and 9b (Scheme 1).

It was reported that one-pot three-component α,β-unsaturated ketone, malononitrile, ammonium acetate in absolute ethanol led to the formation of 2-aminoisonicotinonitrile derivative [27]. In the present work and under the previous reaction, reaction of α,β-unsaturated ketone compounds 2a and 2b with malononitrile afforded the corresponding 2-amino-4-{[1-benzyl-1H-indol-3-yl]-6-(naphthalen-2-yl) nicotine nitrile (10a) and 2-amino-4-{[1-benzoyl-1H-indol-3-yl]-6-(naphthalen-2-yl)nicotine nitrile (10b) (Scheme 2). IR (KBr) spectra of this compounds show the absence of C=O characteristic absorption bands and show new bands at 2211 and 2225 cm⁻¹, respectively characteristic for the CN group besides new absorption bands at ~ 3279-3389 cm⁻¹ characteristic for NH₂ group which confirm the formed of 2-aminoisonicotinonitrile derivatives 10a and 10b.

Compounds 10a and 10b were used as starting material for building up of fused heterocyclic systems through the reactions of α,β-bifunctional amino, cyano groups. Cyclocondensation of compounds 10a and 10b either with formic acid solution 85% or formamide under reflux condition led to the formation of the fused pyridine[2,3-d]pyrimidine derivatives 11a,b and 12a,b, respectively (Scheme 2).

On the other hand, the reaction of 10a and 10b with urea or thiourea in absolute ethanol and in the presence of triethylamine as a catalyst under reflux condition led to the formation of the fused pyrido[2,3-d]pyrimidine derivatives 13a,b and 14a,b, respectively (Scheme 2).
Antimicrobial activity

The \(^1\)H NMR spectrum of 18b as an example revealed signals at 8.89 (1H, s, H-3 pyridine), 7.25-7.93 (17H, m, Ar-H), 5.98 (2H, s, S-CH\(_2\)) respectively (Scheme 3). IR (KBr) spectra of this compound showed the absence of CN absorption bands and revealed new absorption bands at ~ 2934 cm\(^{-1}\) for \(\text{CH}_2\) and ~ 3107-3391 cm\(^{-1}\) characteristic for NH\(_2\). Their \(^1\)H NMR spectra lacked the presence of singlet signals of S-CH\(_2\) and revealed a new singlet signals at 5.75 (2H, s, N-CH\(_2\)).

Compounds 18a-d or 19a-d on heating under reflux in absolute ethanol in the presence of piperidine as a catalyst led to the formation of fused moiety, namely triazolopyridine derivatives 20a-d and 21a-d, in the presence of piperidine as a catalyst led to the formation of fused 3107-3391 cm\(^{-1}\). compounds with the inhibition zone ranging from of 19.6 to 22.4 mm compared to the reference drug ampicillin of 27.4 mm compared to the reference drug ampicillin.

Antifungal activity

Compounds 20a-d and 21a-d showed moderate antifungal activity against Candida albicans (RCMB 05036) with the inhibition zone of 23.3 mm compared to the reference drug amphotericin B of 25.4 mm (Table 1). Whereas, compounds 17a and 17b were nearly to be the most potent activity with the inhibition zone of 23.3 mm against Aspergillus fumigatus (RCMB 02568).

Minimum inhibitory concentration

Compounds 8b, 17a, 17b and 19d which showed high antimicrobial activity were used to calculate MICs, which were in the lowest concentration required to inhibit the growth of tested microorganisms. Ampicillin, ciprofloxacin, and amphotericin B were used as reference drugs (Table 2). From the data obtained it was found that, compounds 17a and 17b showed the same equipotent inhibition activity (MIC of 0.98 μg/ml) as the reference drug ampicillin against S. pneumoniae (RCMB 010010). In the case of gram-negative bacteria, compounds 17a and 17b were found to be more potent with MIC of 0.49 μg/ml against E. coli (RCMB 010052) higher than ciprofloxacin (MIC 0.98 μg/ml).

Moreover, in the case of fungi compounds 17a, 17b and 19d were found to be exhibited equipotent activity (MIC 0.98 μg/ml) as amphotericin B against A. fumigatus (RCMB 02568).

From the data obtained it is clear that compound 17a and 17b were the most active compounds, and their activity may be due to the presence of the 2-mercaptopyridine-3-carbonitrile at position 3 of indole moiety.

Molecular docking study

Three different enzymes namely, ATPase, enoyl reductase and dihydrofolate reductase belong to focused microorganisms were chosen as major antimicrobial drug targets and essential for the biological process in the microorganism cell life. The inhibition of such enzymes leads to overall cell death. In the present work, the molecular docking studies of the most active antimicrobials compound 8b, 17a, 17b and 19d were carried out to understand the interaction binding modes of these compounds with the active site of the three enzymes.

Theoretically, all the synthesized compounds showed minimum binding energies better than the binding energies of co-crystallized ligands (Table 3) and exhibited good fitting inside the active pocket via a hydrogen bond, electrostatic forces or Van der Waals interaction.

Docking study against G+ve bacteria

From the data obtained it was found that (Table 3, fig. 1a,b-2a,b), compounds 17a and 17b exhibited good fitting inside the binding site of the protein molecular surface and having minimum binding energy of -19.97 and -21.00 kJ mol\(^{-1}\) with the formation of arene-cation interaction between Arg(A84) and naphthalene ring, respectively. In comparison to co-crystallized ligand EVO which exhibited binding energy of -14.41 kJ mol\(^{-1}\) (Rmsd 1.52) and formed a hydrogen bond between NH of isatin ring and C=O of Asp481 (2.80 Å).

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Also, compounds 17a and 17b exhibited good fitting inside the active
minimum binding energy of -20.71 and -16.81 kJ mol
3a,b-4a,b), compound 17a and 17b exhibited docking score with a
The result of molecular docking studies showed that (table 3, fig.

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a: each value is the mean of three values, NA: no activity, RCMB: The Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt

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RCMB: The Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt; - no activity

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Docking study against G-ve bacteria

The result of molecular docking studies showed that (table 3, fig. 3a-4a,b), compound 17a and 17b exhibited docking score with a minimum binding energy of -20.71 and -16.81 kJ mol⁻¹ respectively. Also, compounds 17a and 17b exhibited good fitting inside the active site via formation of one hydrogen bond between CN group of pyridine moiety and OH of Ser9 (2.96 Å) or between C-O of benzoil moiety and NH of Lys163 (2.60 Å) respectively, in comparison to co-crystallized ligand tricosan which has binding energy of -18.53 kJ mol⁻¹ (Rmsd 1.70) and formed hydrogen bond between OH group and C=O of Tyr156 (2.41 Å).
Docking studies against fungi

The data obtained showed that (table 3, fig. 5a,b-6a,b), compound 17a and 17b exhibited excellent docking score of 17.77 and -21.06 kJ mol\(^{-1}\) with arene-arene and arene-cation interaction between naphthalene ring and LysA57 and PheA36, respectively compared to co-crystalline ligand (NDP) of -12.45 kJ mol\(^{-1}\) and Rmsd 1.45 (table 3).
Fig. 4a and 4b: The 3D depiction of the docked conformation of 17a and 17b into active side of enoyl reductase enzyme (PDB ID: 1C14)

Fig. 3b and 4b: The 2D depiction of the docked conformation of 17a and 17b with co-crystalline ligand (TCL). Both co-crystalline ligand (red color) and the active compounds (green color) are aligned in the binding pocket and compared in each fig.

Fig. 5a and 5b: The 3D depiction of the docked conformation of 17a and 17b into active side of dihydrofolate reductase enzyme in complex (PDB ID: 1A19)

Fig. 6a and 6b: The 3D depiction of the docked conformation of 17a and 17b into active side of dihydrofolate reductase enzyme in complex (PDB ID: 1A19)

Fig. 4a and 4b: The 2D depiction of the docked conformation of 8b and 19d respectively with the co-crystalline ligand (NDP). Both co-crystalline ligand (red color) and the active compounds (green) are aligned in the binding pocket and compared in each fig.

CONCLUSION
A series of pyrazoles, isoxazoles, pyrimidines and pyridines derivatives incorporated to N-alkyl indole at their 3-positions were prepared. Compounds 17a and 17b were found to be the most potent compounds with MIC of 0.98, 0.49 and 0.98μg/ml against S. pneumoniae (RCMB 010010), E. coli (RCMB 010052) and A. fumigates (RCMB 02568), respectively compare to the reference.
drugs ampicillin [MIC of 0.98 μg/ml], ciprofloxacin [MIC of 0.98 μg/ml] and amphotericin B [MIC of 0.98 μg/ml]. The active compounds were employed for docking study towards three different enzymes namely, ATPase, enoyl reductase and dihydrofolate reductase belongs to focused microorganisms. The result obtained revealed that all compounds exhibited good fitting inside the binding site of the proteins molecular surface with minimum binding energy compares to co-crystalline ligands. Compounds 17a and 17b could act as inhibitors of enzymes understudied and led to overall microorganism’s cells death.

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CONFLICT OF INTERESTS
Declare none

REFERENCES

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