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Original Article

SYNTHESIS, ANTIMICROBIAL ACTIVITY AND MOLECULAR DOCKING STUDY OF SOME NEW *N*-BENZYL AND *N*-BENZOYL-3-INDOLYL HETEROCYCLES

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ABSTRACT

Objective: Chalcones are one of the major classes of the natural products, which display 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-1*H*-indole-3-carboxaldehyds and evaluating theirs *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 guanidine to afford the corresponding pyrazoles 3a,b-6a,b and pyrimidines derivatives 7a,b-9a,b. On the other hand, the reaction of 2a, b with malononitrile afforded 10a, b, which upon cyclo-condensation with formic acid, formamide, urea or thiourea yielded the fused pyrido [2,3-*d*]pyrimidine 11a,b-14a,b. Moreover, cyclo-condensation of 2a, b with thiosemicarbazide gave pyrazolin-1-carbothioamides 15a, b, which under cyclization with phenacyl bromide afforded thiazole derivatives 16a and 16b. While the reaction of 2a, b with cyano thioacetamide afforded 2-mercaptonicotinonitriles 17a, b. The reaction of 17a, b with some halo-compounds gave S-alkyl derivatives 18a-d and 19a-d, respectively,which under heating in the presence of piperidine gave the fused thienopyridines 20a-d and 21a-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 ability 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

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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-1*H*-indole-3-carboxaldehyds 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 belonging to focused microorganisms 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 microanalytical 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 ^{1}H NMR spectra were recorded on a Bruker Avance digital spectrometer (BRUKER, Germany) in DMSO-d_6, 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 chemicals and solvents were of commercial grade and used without further purification. 1-benzyl and 1-benzoyl-1*H*-indole-3-carboxaldehyde, cyano thioacetamide 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 hydroxide 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-1*H*-indol-3-yl)-3-(naphthalen-2-yl) prop-2-ene-1-one (2a)

Yield: 95 %; MP: 164-166°C; IR (KBr): υ 1675 (C=O), 1621 cm^{-1} (C=C); ^{1}H NMR (270 MHz, $CDCl_3$): δ 8.57 (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-

CH_2); Anal. $C_{28}H_{21}NO$ (387.16): Calcd: C, 86.79; H, 5.46; N, 3.61; Found: C, 86.58; H, 5.32; N, 3.54.

1-(1-Benzoyl-1*H*-indol-3-yl)-3-(naphthalen-2-yl) prop-2-ene-1-one (2b)

Yield: 90 %; MP: 138-140°C; IR (KBr): υ 1671, 1633 (C=O), 1576 cm^{-1} (C=C); ^1H NMR (270 MHz, $CDCl_3$): δ 8.48 (1H, s, H-2 indole), 7.32-8.35 ppm (18H, m, Ar-H including 2H, 2d, CH=CH); Anal. $C_{28}H_{19}NO_2$ (401.46): Calcd: C, 83.77; H, 4.77; N, 3.49; Found: C, 83.57; H, 4.65; N, 3.51.

Synthesis of compounds 3a, 3b

A mixture of compound 2a or 2b (0.01 mol), hydrazine hydrate (99%, 1 ml, 0.02 mol) in absolute ethanol (10 ml) containing few drops of glacial acetic acid was heated under reflux for 4h. After cooling the reaction mixture was poured onto ice-water (50 ml), and the solid that formed was filtered off, air-dried and crystallized from absolute ethanol.

1-Benzyl-3-(5-naphthalen-2-yl-4, 5-dihydro-1*H*-pyrazol-3-yl)-1*H*-indole (3a)

Yield: 87 %; MP: 120-122°C; IR (KBr): υ 3325 (NH), 1601 (C=N), 1557 cm^{-1} (C=C); ^1H NMR (270 MHz, $CDCl_3$): δ 7.11-8.10 (17H, m, Ar-H), 5.35 (1H, dd, CH-pyrazoline), 5.28 (2H, s, *N*-CH_2), 3.66 (1H, dd, *CH_2*-pyrazoline equatorial), 3.39 (1H, dd, 1H, dd, *CH_2*-pyrazoline axial), 2.96 ppm (1H, s, NH); Anal. $C_{28}H_{23}N_3$ (401.5): Calcd: C, 83.76; H, 5.77; N, 10.47; Found: C, 83.81; H, 5.64; N, 10.55.

1-Benzoyl-3-(5-naphthalen-2-yl-4, 5-dihydro-1*H*-pyrazol-3-yl)-1*H*-indole (3b)

Yield: 87 %; MP: 200-202°C; IR (KBr): υ 3209 (NH), 1665 (C=O), 1616 (C=N), 1542 cm^{-1} (C=C); MS (*m/z*): 451 [M^+]; Anal. $C_{28}H_{21}N_3O$ (415.49): Calcd: C, 80.94; H, 5.09; N, 10.11; Found: C, 80.91; H, 5.11; N, 10.32.

Synthesis of compounds 4a, 4b

To a solution of compound 2a or 2b (0.01 mol) in a mixture of (15 ml) acetic anhydride and glacial acetic acid (2:1), hydrazine hydrate (99%, 1 ml, 0.02 mol) was added. The reaction mixture 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 aqueous ethanol.

1-[3-(1-Benzyl-1*H*-indol-3-yl)-5-naphthalen-2-yl-4, 5-dihydro-pyrazol-1-yl] ethanone (4a)

Yield: 85 %; MP: 74-76°C; IR (KBr): υ 1663 (C=O), 1600 (C=N), 1581 (C=C), 1010 cm^{-1} (C-O-C); ^1H NMR (270 MHz, $CDCl_3$): δ 7.18-8.23 (17H, m, Ar-H), 5.99 (1H, dd, CH-pyrazoline), 5.37(2H, s, CH_2Ph), 4.01 (1H, dd, *CH_2*-pyrazoline equatorial), 3.62 (1H, dd, 1H, dd, *CH_2*-pyrazoline axial), 2.88 ppm (3H, s, CO*CH_3*); Anal. $C_{30}H_{25}N_3O$ (443.54): Calcd: C, 81.24; H, 5.68; N, 9.47; Found: C, 81.44; H, 5.64; N, 9.51.

1-[3-(1-Benzoyl-1*H*-indol-3-yl)-5-naphthalen-2-yl-4, 5-dihydro-pyrazol-1-yl] ethanone (4b)

Yield: 89 %; MP: 153-155°C; IR (KBr): υ 1722 (C=O), 1643 (C=N), 1585 cm^{-1} (C=C); MS (*m/z*): 457 [M^+]; Anal. $C_{30}H_{23}N_3O_2$ (457.52): Calcd: C, 78.75; H, 5.07; N, 9.18; Found: C, 78.70; H, 5.00; N, 9.22.

Synthesis of compounds 5a, 5b

A mixture of compound 2a or 2b (0.01 mol) and phenyl hydrazine (1.08 ml, 0.01 mol) in absolute ethanol (10 ml) containing glacial acetic acid (0.5 ml) was refluxed for 2 h. After cooling, the solid that formed was filtered off, air dried and crystallized from absolute ethanol.

1-Benzyl-3-(3-(naphthalen-1-yl)-1-phenyl-4, 5-dihydro-1*H*-pyrazol-5-yl)-1*H*-indole (5a)

Yield: 95 %; MP: 109-111°C; IR (KBr): υ 1641 and 1593 (C=N), 1557 cm^{-1}(C=C); ^1H NMR (270 MHz, DMSO-d_6): δ 6.72-8.02 (22H, m, Ar-H), 5.85 (1H, dd, CH-pyrazoline), 5.39 (2H, s, N-CH_2), 4.04 (1H, dd, *CH_2*-pyrazoline equatorial), 3.40 ppm (1H, dd, 1H, dd, *CH_2*-pyrazoline axial); Anal. $C_{34}H_{27}N_3$ (477.60): Calcd: C, 85.50; H, 5.70; N, 8.80; Found: C, 85.44; H, 5.75; N, 8.79.

1-Benzoyl-3-(3-(naphthalen-1-yl)-1-phenyl-4, 5-dihydro-1*H*-pyrazol-5-yl)-1*H*-indole (5b)

Yield: 99 %; MP: 169-171°C; IR (KBr): υ 1665 (C=O), 1595 (C=N), 1570 cm^{-1} (C=C); MS (*m/z*): 491 [M^+]; Anal. $C_{34}H_{25}N_3O$ (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 (0.69 g, 0.01 mol) and anhydrous sodium acetate (0.67 g, 0.01 mol) in absolute ethanol (10 ml) was heated under reflux for 6-8h. After cooling the reaction mixture was poured on to 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-(naphthalene-2-yl) isoxazole (6a)

Yield: 81 %; MP: 140-142°C; IR (KBr): υ 1620 (C=N), 1539 cm^{-1}(C=C); ^1H NMR (270 MHz, DMSO-d_6): δ 7.17-8.12 (17H, m, Ar-H), 5.54 (1H, dd, CH-pyrazoline), 4.64 (2H, s, N-CH_2), 2.75 (1H, dd, *CH_2*-pyrazoline equatorial), 1.76 ppm (1H, dd, 1H, dd, *CH_2*-pyrazoline axial); Anal. $C_{28}H_{22}N_2O$ (402.49): Calcd: C, 83.56; 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-(naphthalene-2-yl) isoxazole (6b)

Yield: 90 %; MP: 240-242°C; IR (KBr): υ 1676 (C=O), 1619 (C=N), 1538 cm^{-1} (C=C); MS (*m/z*): 491 [M^+]; Anal. $C_{28}H_{20}N_2O_2$ (416.47): 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 (0.76 g, 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-1*H*-indol-3-yl)-6-(1-naphthalen-2-yl) pyrimidin-2(1*H*)-one (7a)

Yield: 71%; MP: 100-103°C; IR (KBr): υ 3420 (NH), 1667 (C=O), 1620 (C=N), 1553 cm^{-1}(C=C); ^1H NMR (270 MHz, DMSO-d_6): 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, CH_2-N); Anal. $C_{29}H_{21}N_3O$ (427.50): Calcd: C, 81.48; H, 4.95; N, 9.83; Found: C, 81.35; H, 4.89; N, 9.86.

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

Yield: 80%; MP: 128-130°C; IR (KBr): υ 3133 (NH), 1702 (C=O), 1623 (C=N), 1590 cm^{-1}(C=C); ^1H NMR (270 MHz, DMSO-d_6): δ 11.65 (1H, s, NH), 8.91 (1H, s, H-5 pyrimidinyl), 8.63 (1H, s, H-2 indolyl), 7.15-8.36 ppm (16H, m, Ar-H); Anal. $C_{29}H_{19}N_3O_2$ (441.48): Calcd: C, 78.90; H, 4.34; N, 9.52; Found: C, 78.88; H, 4.23; N, 9.48.

Synthesis of compounds 8a, 8b

A mixture of compound 2a or 2b (0.01 mol) and thiourea (0.76g, 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-1*H*-indol-3-yl)-6-(1-naphthalen-2-yl) pyrimidin-2(1*H*)-thione (8a)

Yield: 70%; MP: 140-142°C; IR (KBr): υ 3431 (NH), 1599 (C=N), 1533 (C=C), 1240 cm^{-1} (C=S); ^1H NMR (270 MHz, DMSO-d_6): δ 11.88 (1H, s, NH), 8.34 (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-CH_2); Anal. $C_{29}H_{21}N_3S$ (443.56): Calcd: C, 78.53; H, 4.77; N, 9.47; Found: C, 78.48; H, 4.65; N, 9.51.

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

Yield: 79%; MP: 184-186°C; IR (KBr): υ 3432 (NH), 1665 (C=O), 1603 (C=N), 1558 (C=C), 1241 cm^{-1} (C=S); ^1H NMR (270 MHz, DMSO-

d_6): δ 12.10 (1H, s, NH), 8.77 (1H, s, H-5 pyrimidinyl), 7.23-8.26 (17H, m, Ar-H); Anal. $C_{29}H_{19}N_3OS$ (457.55): Calcd: C, 76.13; H, 4.15; N, 9.18; Found: C, 76.22; H, 4.23; N, 9.01.

Synthesis of compounds 9a, 9b

A mixture of compound 2a or 2b (0.01 mol), guanidine hydrochloride (0.96 g, 0.01 mol) and anhydrous sodium acetate (0.82 g, 0.01 mol) in dry ethanol (15 ml) was heated under reflux for 2-3h. After cooling the solid formed was filtered off, air-dried and crystallized from absolute ethanol.

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

Yield: 72%; MP: 70-72°C; IR (KBr): υ 3416 and 3165 (NH), 1634 (C=N), 1571 cm^{-1} (C=C); ^{1}H NMR (270 MHz, DMSO-d_6): δ 8.91 (2H, s, NH_2), 8.53 7.01-8.37 (18H, m, Ar-H), 5.61 (2H, s, CH_2-N); Anal. $C_{29}H_{22}N_4$ (426.51): Calcd: C, 81.28; H, 5.65; N, 13.07; Found: C, 81.31; H, 5.69; N, 13.22.

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

Yield: 83%; MP: 171-173°C; IR (KBr): υ 3416 (NH), 1668 (C=O), 1621 (C=N), 1573 cm^{-1} (C=C); ^{1}H NMR (270 MHz, DMSO-d_6): δ 11.41 (2H, s, NH_2), 7.09-8.44 (18H, m, Ar-H); Anal. $C_{29}H_{20}N_4O$ (440.50): Calcd: C, 78.71; H, 5.01; N, 12.66; Found: C, 78.66; H, 5.22; N, 12.53.

Synthesis of compounds 10a, 10b

A mixture of compound 2a or 2b (0.01 mol) malononitrile (0.01 mol) and ammonium acetate (0.01 mol) in absolute ethanol (40 ml) was heated under reflux for 8h. The reaction mixture was then cooled, poured into crushed ice and product separated out was filtered off, washed with water, air-dried and crystallized from absolute ethanol.

2-Amino-6-(1-benzyl-1*H*-indol-3-yl)-4-(naphthalen-2-yl) pyridine-3-carbonitrile (10a)

Yield: 90%; MP: 60-62°C; IR (KBr): υ 3373 and 3292 (NH_2), 2250 (CN), 1676 (C=N), 1616 cm^{-1} (C=C); ^{1}H NMR (270 MHz, DMSO-d_6): δ 8.30 (1H, s, H-5 pyridinyl), 8.06 (1H, s, H-2 indolyl), 7.29-7.52 (16H, m, Ar-H), 5.63 (2H, s, NH_2), 5.51 ppm (2H, s, N-CH_2); Anal. $C_{31}H_{22}N_4$ (450.53): Calcd: C, 82.64; H, 4.92; N, 12.44; Found: C, 82.59; H, 4.98; N, 12.31.

2-Amino-6-(1-benzoyl-1*H*-indol-3-yl)-4-(naphthalen-2-yl) pyridine-3-carbonitrile (10b)

Yield: 95%; MP: 88-90°C; IR (KBr): υ 3389, 3279 (NH_2), 2211 (CN), 1662(C=O), 1591 (C=N), 1583 cm^{-1} (C=C); ^{1}H NMR (270 MHz, DMSO-d_6): δ 11.88 (2H, s, NH_2), 7.13-8.26 ppm (18H, m, Ar-H); MS (*m/z*): 464 [M$^+$]; Anal. $C_{31}H_{20}N_4O$ (450.53): Calcd: C, 80.15; H, 4.34; N, 12.06; Found: C, 80.22; H, 4.41; N, 12.00.

Synthesis of compounds 11a, 11b

A solution of compound 10a or 10b (0.005 mol) in formic acid 85% (10 ml) was heated under reflux for 3h. The solid that formed on hot was filtered off, air-dried and crystallized from absolute ethanol.

5-(1-Benzyl-1*H*-indol-3-yl)-7-(naphthalen-2-yl) pyrido [2,3-*d*]pyrimidin-4(3H)-one (11a)

Yield: 97%; MP: 240-2°C; IR (KBr): υ 3277 (NH), 1642 (C=O), 1591 (C=N), 1523 cm^{-1} (C=C); ^{1}H NMR (270 MHz, DMSO-d_6): δ 8.95 (1H, s, NH), 7.22-8.36 (19H, m, Ar-H), 5.54 ppm (2H, s, CH_2-Ph); Anal. $C_{32}H_{22}N_4O$ (478.18): Calcd: C, 80.32; H, 4.63; N, 11.71; Found: C, 80.21; H, 4.60; N, 11.62.

5-(1-Benzoyl-1*H*-indol-3-yl)-7-(naphthalen-2-yl) pyrido[2,3-*d*]pyrimidin-4(3H)-one (11b)

Yield: 97%; MP: <400°C; IR (KBr): υ 3155 (NH), 1695, 1651 (C=O), 1600 (C=N), 1555 cm^{-1} (C=C); ^{1}H NMR (270 MHz, DMSO-d_6): δ 9.93 (1H, s, NH), 7.18-8.27 ppm (19H, m, Ar-H); Anal. $C_{32}H_{20}N_4O_2$ (492.16): Calcd: C, 78.03; H, 4.09; N, 11.38; Found: C, 77.99; H, 4.01; N, 11.21.

Synthesis of compounds 12a, 12b

A solution of compound 10a or 10b (0.005 mol) in formamide (20 ml) was heated under reflux for 2-3hr. The solid that formed on hot was filtered off, air-dried and crystallized from absolute ethanol.

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

Yield: 85%; MP: 220-2 °C; IR (KBr): υ 3264, 3103 (NH_2), 1639 (C=N), 1604 cm^{-1} (C=C); ^{1}H NMR (270 MHz, DMSO-d_6): δ 7.37-8.78 (19H, m, Ar-H), 5.24 (2H, s, N-CH_2), 2.46 ppm (2H, s, NH_2); Anal. $C_{32}H_{23}N_5$ (477.56): Calcd: C, 80.48; H, 4.85; N, 14.66; Found: C, 80.49; H, 4.79; N, 14.45.

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

Yield: 85%; MP: 210-2 °C; IR (KBr): υ 3100, 3128 (NH_2), 1723 (C=O), 1639 (C=N), 1575 cm^{-1} (C=C); ^{1}H NMR (270 MHz, DMSO-d_6): δ 10.15 (2H, s, NH_2).7.15-8.41 ppm (19H, m, Ar-H); Anal. $C_{32}H_{21}N_5O$ (491.54): Calcd: C, 78.19; H, 4.31; N, 14.25; Found: C, 78.22; H, 4.21; N, 14.01.

Synthesis of compounds 13a, 13b

A mixture of compound 10a or 10b (0.005 mol) and urea (g, 0.005 mol) in absolute ethanol (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.

4-Amino-5-(1-benzyl-1*H*-indol-3-yl)-7-(naphthalen-2-yl) pyrido[2,3-*d*]pyrimidin-2(1*H*)-one (13a)

Yield: 75%; MP: 200-2 °C; IR (KBr): υ 3350 (NH2), 3195 (NH), 1694 (C=O), 1604 (C=N), 1550 cm^{-1} (C=C); ^{1}H NMR (270 MHz, DMSO-d_6): δ 8.89 (1H, s, NH), 7.37-8.32 (18H, m, Ar-H), 5.03 (2H, s, N-CH_2), 2.47 (2H, s, NH_2); Anal. $C_{32}H_{23}N_5O$ (493.19): Calcd: C, 77.87; H, 4.70; N, 14.19; Found: C, 77.64; H, 4.76; N, 14.01.

4-Amino-5-(1-benzoyl-1*H*-indol-3-yl)-7-(naphthalen-2-yl) pyrido[2,3-*d*]pyrimidin-2(1*H*)-one (13b)

Yield: 79%; MP: 260-2 °C; IR (KBr): υ 3410 (NH2), 3214 (NH), 1730 (C=O), 1643(C=N), 1579 cm^{-1} (C=C); ^{1}H NMR (270 MHz, DMSO-d_6): δ 11.89 (3H, s, NH and NH_2), 7.13-8.27 (18H, m, Ar-H); Anal. $C_{32}H_{21}N_5O_2$ (507.17): Calcd: C, 75.73; H, 4.17; N, 13.80; Found: C, 75.64; H, 4.01; N, 13.66.

Synthesis of compounds 14a, 14b

A mixture of compound 10a or 10b (0.005 mol) and thiourea (0.38g, 0.005 mol) in absolute ethanol (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.

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

Yield: 76%; MP: 210-2 °C; IR (KBr): υ 3312 (NH), 1621 (C=N), 1547 (C=C), 1234 cm^{-1} (C=S); MS (*m/z*): 477 [M$^+$-S]; Anal. $C_{32}H_{23}N_5S$ (509.62): Calcd: C, 75.42; H, 4.55; N, 13.74; Found: C, 75.22; H, 4.35; N, 13.69.

4-Amino-5-(1-benzoyl-1*H*-indol-3-yl)-7-(naphthalen-2-yl) pyrido[2,3-*d*]pyrimidine-2(1*H*)-thione (14b)

Yield: 78%; MP: 100-2 °C; IR (KBr): υ 3107 (NH), 1720 (C=O), 1644 (C=N), 1584 (C=C), 1202 cm^{-1} (C=S); ^{1}H NMR (270 MHz, DMSO-d_6): δ 11.87 (2H, s, NH_2), 8.67 (1H, s, NH), 7.00-8.29 (18H, m, Ar-H); Anal. $C_{32}H_{21}N_5OS$ (523.15): Calcd: C, 73.40; H, 4.04; N, 13.38; Found: C, 73.45; H, 4.00; N, 13.21.

Synthesis of pyrazolin-1-carbothioamide derivatives 15a, 15b

A solution of compound 2a or 2b (0.001 mol) in dioxane (10 ml) was refluxed with thiosemicarbazide (0.001 mol) in glacial acetic acid (1 ml) for 4h, then the reaction mixture was poured onto crushed ice and was kept overnight at room temperature. The separated solid was filtered off, washed successively with water, air-dried and crystallized from methanol.

3-(1-Benzyl-1*H*-indol-3-yl)-5-(naphthalen-2-yl)-4, 5-dihydro-1*H*-pyrazole-1-carbothio-amide (15a)

Yield: 87%; MP: 121-3 °C; IR (KBr): υ 3102 (NH_2), 1640 (C=O), 1569(C=N), 1239 cm^{-1} (C=S); ^{1}H NMR (270 MHz, DMSO-d_6): δ 10.20 (2H, s, NH_2), 7.13-8.11 (18H, m, Ar-H), 4.48 (2H, 2, N-CH_2); Anal.

$C_{29}H_{22}N_4S$ (458.59): Calcd: C, 75.95; H, 4.84; N, 12.22; Found: C, 75.85; H, 4.70; N, 12.01.

3-(1-Benzoyl-1*H*-indol-3-yl)-5-(naphthalen-2-yl)-4, 5-dihydro-1*H*-pyrazole-1-carbothio-amide (15b)

Yield: 77%; MP: 102-4 °C; IR (KBr): υ 3426, 3383 (NH_2), 1670 (C=O), 1600 (C=N), 1280 cm^{-1} (C=S); ^1H NMR (270 MHz, DMSO-d_6): δ 9.36 (2H, s, NH_2), 6.75-8.20 (18H, m, Ar-H); Anal. $C_{29}H_{20}N_4OS$ (472.58): Calcd: C, 73.71; H, 4.27; N, 11.86; Found: C, 73.54; H, 4.37; N, 11.75.

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 15a or 15b (0.01 mol) and phenacyl bromide (1.99 g, 0.01 mol). The reaction mixture was heated under reflux for 10-12h. After cooling the solid that formed was filtered off, air-dried and crystallized from absolute ethanol.

2-(3-(1-Benzyl-1*H*-indol-3-yl)-5-(naphthalen-2-yl)-4, 5-dihydro-1*H*-pyrazol-1-yl)-5-phenylthiazole (16a)

Yield: 65%; MP: 210-2 °C; IR (KBr): υ 1643 (C=N), 1550 cm^{-1} (C=C); ^1H NMR (270 MHz, DMSO-d_6): δ 8.83 (2H, s, H-3 thiazole and H-5 pyrazole), 8.33 (1H, s, H-2 indole), 7.27-8.22 (21H, m, Ar-H), 5.51 (2H, 2, CH_2); Anal. $C_{37}H_{26}N_4S$ (558.71): Calcd: C, 79.54; H, 4.69; N, 10.03; Found: C, 79.29; H, 4.45; N, 10.00.

2-(3-(1-Benzoyl-1*H*-indol-3-yl)-5-(naphthalen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)-5-phenylthiazole (16b)

Yield: 50%; MP: 225-7 °C; IR (KBr): υ 1638 (C=O), 1572 (C=N), 1515 cm^{-1} (C=C); ^1H NMR (270 MHz, DMSO-d_6): δ 8.91 (1H, s, H-3 thiazole), 7.17-8.41 (23H, m, Ar-H); Anal. $C_{37}H_{24}N_4OS$ (572.69): Calcd: C, 77.60; H, 4.22; N, 9.78; Found: 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 7h. After cooling the solid formed was filtered off air-dried and crystallized from absolute ethanol.

6-(1-Benzyl-1*H*-indol-3-yl)-2-mercapto-4-(naphthalen-2-yl) pyridine-3-carbonitrile (17a)

Yield: 70%; MP: 80-2°C; IR (KBr): υ 3450 (NH), 2197 (CN), 1622 (C=N), 1562 (C=C), 1297 cm^{-1} (C=S); ^1H NMR (270 MHz, DMSO-d_6): δ 8.83 (1H, s, H-3 pyridine), 8.33 (1H, s, H-2 indole), 7.22-8.20 (16H, m, Ar-H),5.57 (1H, s, SH), 5.51 (2H, 2, N-CH_2); Anal. $C_{31}H_{21}N_3S$ (467.15): Calcd: C, 79.63; H, 4.53; N, 8.99; Found: C, 79.60; H, 4.44; N, 9.01.

6-(1-Benzoyl-1*H*-indol-3-yl)-2-mercapto-4-(naphthalen-2-yl) pyridine-3-carbonitrile (17b)

Yield: 75%; MP: 320-2°C; IR (KBr): υ 3395 (NH), 2203 (CN), 1600(C=N), 1567 (C=C), 1273 cm^{-1} (C=S); ^1H NMR (270 MHz, DMSO-d_6): δ 12.06 (1H, s, NH), 8.91 (1H, s, H-3 pyridine), 8.67 (1H, s, H-2 indole), 6.84-8.27 (16H, m, Ar-H); Anal. $C_{31}H_{19}N_3OS$ (481.57): Calcd: C, 77.32; H, 3.98; N, 8.73; Found: C, 77.35; H, 4.01; N, 8.65.

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

A solution of compound 17a or 17b (0.001 mol) and potassium hydroxide (0.6 g, 0.001 mol) in *N,N*-dimethylformamide (20 ml) was stirred for 2h 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 keep stirring for another 2h. The resulting solid was filtered off, washed with water, air-dried and crystallized from dimethyl formamide and absolute ethanol.

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

Yield: 85%; MP: 120-2°C; IR (KBr): υ 2205 (CN), 1643 (C=N), 1568 cm^{-1}(C=C); ^1H NMR (270 MHz, DMSO-d_6): δ 8.74 (1H, s, H-3 pyridine), 8.55 (1H, s, H-2 indole), 7.10-8.05 (16H, m, Ar-H), 6.21 (2H, s, S-CH_2), 6.16 (2H, 2, CH_2-N); Anal. $C_{33}H_{22}N_4S$ (506.16): Calcd: C, 78.23; H, 4.38; N, 11.06; Found: C, 78.02; H, 4.21; 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): υ 2204 (CN), 1634 (C=O), 1600 (C=N), 1569 cm^{-1}(C=C); ^1H NMR (270 MHz, DMSO-d_6): δ 8.89 (1H, s, H-3 pyridine), 7.25-7.93 (17H, m, Ar-H), 5.98 (2H, 2, S-CH_2), 5.75 (2H, 2, N-CH_2), 3.51 (2H, q, CH_2), 1.14 (3H, t, CH_3); Anal. $C_{35}H_{27}N_3O_2S$ (553.18): Calcd: C, 75.92; H, 4.92; N, 7.59; Found: C, 75.95; H, 4.99; N, 7.64.

Phenyl thioacetic acid S-(6-(1-benzyl-1*H*-indol-3-yl)-3-cyano-4-(naphalen-2-yl) pyridine-2-yl) ester (18c)

Yield: 82%; MP: 140-2 °C; IR (KBr): υ 2204 (CN), 1634 (C=O), 1600 (C=N), 1584 cm^{-1}(C=C); MS (*m/z*): 585 [M^+]; Anal. $C_{39}H_{27}N_3OS$ (585.19): Calcd: C, 79.97; H, 4.65; N, 7.17; Found: C, 79.88; H, 4.51; N, 7.22.

2-[6-(1-Benzyl-1*H*-indol-3-yl)-3-cyano-4-(naphthalen-2-yl) pyridine 2yl sulfonyl]-*N*-thiazol-2-yl-acetamide (18d)

Yield: 81%; MP: 120-2 °C; IR (KBr): υ 3398 (NH), 2208 (CN), 1635 (C=O), 1570 (C=N), 1527 cm^{-1}(C=C); ^1H NMR (270 MHz, DMSO-d_6): δ 9.58 (1H, s, NH), 7.23-8.02 (20H, m, Ar-H), 5.66 (2H, 2, S-CH_2), 5.35 (2H, 2, N-CH_2); Anal. $C_{36}H_{25}N_5OS_2$ (607.15): Calcd: C, 71.15; H, 4.15; N, 11.52; Found: C, 71.30; H, 4.00; N, 11.49.

6-(1-Benzoyl-1*H*-indol-3-yl)-2-cyanomethylsulfonyl-4-(naphalen-2-yl) pyridine-3-carbonitrile (19a)

Yield: 82%; MP: >340 °C; IR (KBr): υ 2221 (CN), 1675 (C=O), 1611(C=N), 1570 cm^{-1}(C=C); ^1H NMR (270 MHz, DMSO-d_6): δ 8.81 (1H, s, H-3 pyridine), 8.68 (1H, s, H-2 indole), 7.14-8.38(16H, m, Ar-H), 6.89 (2H, s, S-CH_2); Anal. $C_{33}H_{20}N_4OS$ (520.60): Calcd: C, 76.13; H, 3.87; N, 10.76; Found: C, 76.22; H, 3.78; N, 10.70.

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

Yield: 95%; MP: 242-2 °C; IR (KBr): υ 2209 (CN), 1764, 1671 (C=O), 1561 (C=N), 1513 cm^{-1}(C=C); ^1H NMR (270 MHz, DMSO-d_6): δ 8.80 (1H, s, H-3 pyridine), 7.20-8.32 (17H, m, Ar-H), 4.32 (2H, 2, S-CH_2) 4.13 (2H, q, CH_2), 1.15 (3H, t, CH_3); Anal. $C_{35}H_{25}N_3O_3S$ (567.6): Calcd: C, 74.05; H, 4.44; N, 7.40; Found: C, 74.00; H, 4.51; N, 7.22.

Phenyl thioacetic acid S-(6-(1-benzoyl-1*H*-indol-3-yl)-3-cyano-4-(naphalen-2-yl) pyridine-2-yl) ester (19c)

Yield: 90%; MP: 242-2 °C; IR (KBr): υ 2201 (CN), 1673 (C=O), 1634 (C=N), 1568 cm^{-1}(C=C); ^1H NMR (270 MHz, DMSO-d_6): δ 8.47 (1H, s, H-3 pyridine), 7.19-8.28 (22H, m, Ar-H), 5.14 (2H, 2, S-CH_2); Anal. $C_{39}H_{25}N_3O_2S$ (599.70): Calcd: C, 78.11; H, 4.20; N, 7.01; Found: C, 78.01; H, 4.22; N, 7.30.

2-[6-(1-Benzoyl-1*H*-indol-3-yl)-3-cyano-4-(naphthalen-2-yl) pyridine-2ylsulfonyl]-*N*-thiazol-2-yl-acetamide (19d)

Yield: 82%; MP: >340 °C; IR (KBr): υ 3394 (NH), 2200 (CN), 1683 (C=O), 1600 (C=N), 1572 (C=C) cm^{-1}; MS (*m/z*): 544 [M^+-Ph]; Anal. $C_{36}H_{23}N_5O_2S_2$ (621.73): Calcd: C, 69.55; H, 3.73; N, 11.26; Found: C, 69.45; H, 3.80; N, 11.30.

Synthesis of compounds 20a-d, 21a-d

The solution of compounds 18a-d or 19a-d (0.005 mol) in absolute ethanol (20 ml) containing piperidine (0.3 ml) was heated under reflux for 2h. After cooling, the resulting solid was filtered, air-dried and crystallized from absolute ethanol.

3-Amino-6-(1-benzyl-1*H*-indol-3-yl)-4-(naphthalen-2-yl)-2,3-dihydrothino[2,3-*b*]pyridine-2-carbonitrile (20a)

Yield: 90%; MP: 242-2°C; IR (KBr): υ 3160, 3107 NH2, 2215 (CN), 1576 cm^{-1}(C=C); ^1H NMR (270 MHz, DMSO-d_6): δ 9.69 (2H, s, NH_2), 8.40 (1H, s, H-3 pyridine), 8.04 (1H, s, H-2 indole), 6.55-7.37 (16H, m, Ar-H), 5.17 (2H, 2, N-CH_2); Anal. $C_{33}H_{22}N_4S$ (506.62): Calcd: C, 78.23; H, 4.38; N, 11.06; Found: C, 78.01; H, 4.22; N, 11.00.

3-Amino-6-(1-benzyl-1*H*-indol-3-yl)-4-(naphthalen-2-yl)-2,3-dihydrothino[2,3-*b*]pyridine-2-carboxylic acid ethyl ester (20b)

Yield: 85%; MP: 100-2°C; IR (KBr): υ 3127 (NH_2), 1732 (C=O), 1640 (C=N), 1518 cm^{-1}(C=C); ^1H NMR (270 MHz, DMSO-d_6): δ 9.93 (2H, s,

NH_2), 8.89 (1H, s, A-H), 7.18-8.57 (17H, m, Ar-H), 5.54 (2H, s, N-CH_2), 4.29 (2H, q, CH_2), 1.32 (3H, t, CH_3); Anal. $C_{35}H_{27}N_3O_2S$ (553.67): Calcd: C, 75.92; H, 4.92; N, 7.59; Found: C, 75.81; H, 5.00; N, 7.60.

2-Benzoyl-6-(1-benzoyl-1*H*-indol-3-yl)-4-(naphthalen-2-yl)-2,3-dihydrothino[2,3-*b*] pyridine-3-amine (20c)

Yield: 85%; MP: 186-8°C; IR (KBr): υ 3102 (NH_2), 1640 (C=O), 1570 (C=N), 1517 cm^{-1}(C=C); MS (*m/z*): 541 [M^+-NH_2 and CO]; Anal. $C_{39}H_{27}N_3OS$ (585.72): Calcd: C, 79.97; H, 4.65; N, 7.17; Found: C, 79.80; H, 4.60; N, 7.00.

3-Amino-6-(1-benzyl-1*H*-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: 210-2 °C; IR (KBr): υ 3377, 3216 (NH, NH_2), 1643 (C=O), 1579 (C=N), 1518 cm^{-1}(C=C); ^{1}H NMR (270 MHz, DMSO-d_6): δ 13.25 (1H, s, NH), 10.00 (2H, s, NH_2), 8.73 (1H, s, Ar-H), 7.24-8.35 (19H, m, Ar-H), 5.15 (2H, s, N-CH_2); Anal. $C_{36}H_{25}N_5OS_2$ (607.75): Calcd: C, 71.15; H, 4.15; N, 11.52; Found: C, 71.22; H, 4.22; N, 11.60.

3-Amino-6-(1-benzoyl-1*H*-indol-3-yl)-4-(naphthalen-2-yl)-2,3-dihydrothino[2,3-*b*] pyridine-2-carbonitrile (21a)

Yield: 75%; MP: 100-2°C; IR (KBr): υ 3391, 3171 (NH_2), NH2, 2200 (CN),, 1622(C=N), 1563 cm^{-1}(C=C); ^{1}H NMR (270 MHz, DMSO-d_6): δ 11.86 (2H, s, NH_2), 7.13-8.25 (18H, m, Ar-H); Anal. $C_{33}H_{20}N_4OS$ (520.60): Calcd: C, 76.13; H, 3.87; N, 10.76; Found: C, 76.00; H, 3.91; N, 10.66.

3-Amino-6-(1-benzoyl-1*H*-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): υ 3216, 3171 (NH_2), 1703, 1681 (C=O), 1648 (C=N), 1617 cm^{-1}(C=C); ^{1}H NMR (270 MHz, DMSO-d_6): δ 12.10 (2H, s, NH_2), 8.77 (1H, s, A-H), 7.20-8.77 (17H, m, Ar-H), 4.34 (2H, q, CH_2), 1.07 (3H, t, CH_3); Anal. $C_{35}H_{25}N_3O_3S$ (567.66): Calcd: C, 74.05; H, 4.44; N, 7.40; Found: C, 74.00; H, 4.31; N, 7.39.

2-Benzoyl-6-(1-benzyl-1*H*-indol-3-yl)-4-(naphthalen-2-yl)-2,3-dihydrothino[2,3-*b*] pyridine-3-amine (21c)

Yield: 75%; MP: 270-2°C; IR (KBr): υ 3292, 3171 (NH_2), 1703, 1699 (C=O), 1647 (C=N), 1624 cm^{-1}(C=C); ^{1}H NMR (270 MHz, DMSO-d_6): δ 7.13-8.28 (23H, m, Ar-H), 2.40 (2H, s, NH_2); Anal. $C_{39}H_{25}N_3O_2S$ (599.70): Calcd: C, 78.11; H, 4.20; N, 7.01; Found: C, 78.00; H, 4.32; N, 7.22.

3-Amino-6-(1-benzoyl-1*H*-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): υ 3200, 3158 (NH, NH_2), 1705 (C=O), 1614(C=N), 1571 cm^{-1}(C=C); ^{1}H NMR (270 MHz, DMSO-d_6): δ 12.10 (1H, s, NH), 9.93 (2H, s, NH_2), 8.26, 8.27 (2H, s, Ar-H), 7.18-8.10 (18H, m, Ar-H); Anal. $C_{36}H_{23}N_5O_2S_2$ (621.73): Calcd: C, 69.55; H, 3.73; N, 11.26; Found: C, 69.44; H, 3.71; N, 11.11.

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* (RCMB 010010), *Staphylococcus aureus* (RCMB 010028) (Gram-positive bacteria), *Pseudomonas aeruginosa* (RCMB 010043), *Escherichia coli* (RCMB 010052) (Gram-negative bacteria) and two strains of fungi, *Candida albicans* (RCMB 05036) and *Aspergillus fumigatus* (RCMB 02568). 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 MacConky 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 DMSO (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 twofold serial dilution of the stock samples (concentration of 500, 250, 125, 62.5, 31.3, 15.6 and 7.81,3.9,1.95, 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 (3*E*)-3-(pyridin-3-ylmethylidene)-1,3-dihydro-2*H*-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: 1AI9) 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 $mol^{-1}Å^{-1}$. MMFF94x was reported as the efficient force field for minimizing ligand-protein complexes [25].

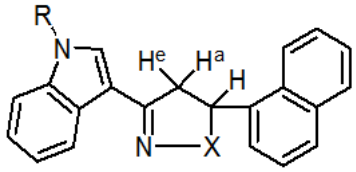
The docking Algorithm was done by MOE-DOCK default which uses flexible, a rigid technique for posting the molecule inside the cavity. All rotatable bonds of ligands are allowed to undergo free rotation to explore the conformational space inside the rigid receptor binding site. The docking scores were 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

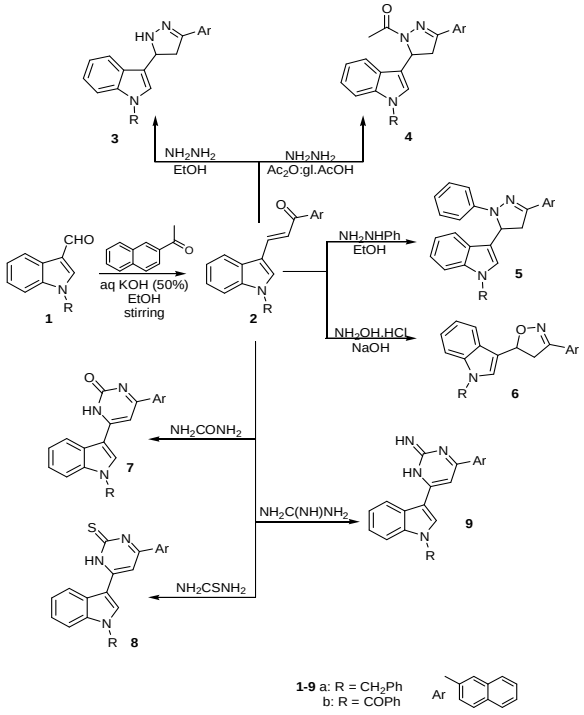
The synthetic routes of the target compounds are outlined in Schemes 1, 2 and 3. Condensation of *N*-benzyl (1a) and *N*-benzoyl-1*H*-indole-3-carboxaldehydes (1b) with 2-acetyl naphthalene in ethanol and in the presence of potassium hydroxide (50%) [Claisen-Schmidt reaction] led to the formation of the corresponding α,β-unsaturated 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).

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*-acetylpyrazoles 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). ^{1}H NMR spectra of pyrazole compounds 3a,b, 4a,b and 5a,b besides isoxazole compounds 6a, black the presence of the double signals of CH=CH of the parent compounds 2a,b and revealed new three doublet of doublet signals due to CH-pyrazoline, CH_2-pyrazoline equatorial (H^e) and CH_2-pyrazoline axial (H^a), respectively



For example, ^{1}H NMR ($CDCl_3$) spectrum of compounds 3a revealed signals at (δ, ppm) 7.11-8.10 (17H, m, Ar-H), 5.35 (1H, dd, CH-pyrazoline), 5.28 (2H, s, CH_2Ph), 3.66 (1H, dd, CH_2-pyrazoline equatorial), 3.39 (1H, dd, 1H, dd, CH_2-pyrazoline axial) and 2.96 (1H, s, NH). Also ^{1}H NMR (DMSO-d_6) spectrum of compounds 6a revealed signals, at (δ, ppm) 7.17-8.12 (17H, m, Ar-H), 5.54 (1H, dd, CH-pyrazoline), 4.64 (2H, s, N-CH_2), 2.75 (1H, dd, CH_2-pyrazoline equatorial), 1.76 ppm (1H, dd, 1H, dd, CH_2-pyrazoline axial).

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(1*H*)ones 7a,b and pyrimidin-2(1*H*)thiones 8a,b, respectively. Whereas, the reaction of compound 2a or 2b with guanidine hydrochloride in the presence of anhydrous sodium acetate yielded pyrimidine-2-amines 9a and 9b (Scheme 1).

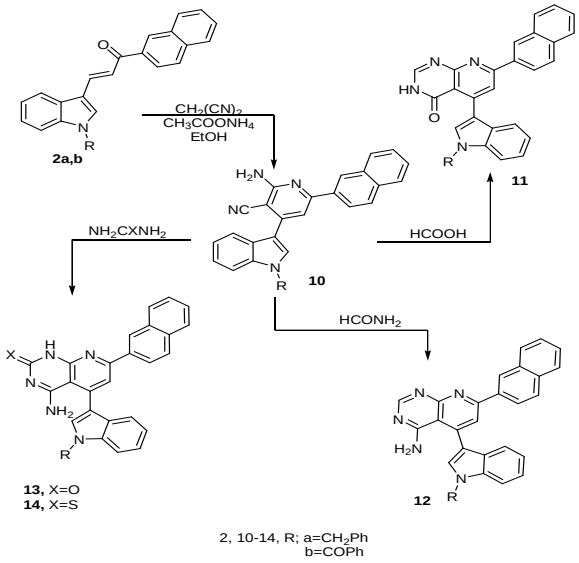


Scheme 1

It was reported that one-pot three-component α,β-unsaturated ketone, malononitrile, ammonium acetate in absolute ethanol led to the formation of 2-aminonicotinonitrile derivative [27]. In the present work and under the previous condition, 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 NH_2 group which confirm the formed of 2-aminonicotinonitrile 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 pyrido[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).



Scheme 2

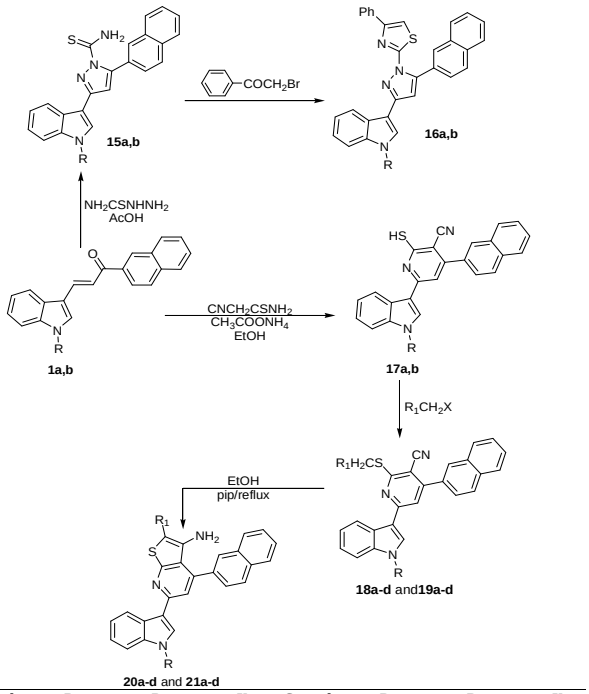
Cyclo-condensation of 2a and 2b with thiosemicarbazide in dioxane containing few drops of acetic acid gave pyrazolin-1-carbothioamide derivatives 15a and 15b (Scheme 3)

Furthermore, cyclo-condensation of enol form of 15a and 15b *via* reaction with phenacyl bromide in absolute ethanol led to the formation of thiazole derivatives 16a and 16b (Scheme 3).

On the other hand, condensation of 2a and 2b with an equal molar ratio of cyano thioacetamide and ammonium acetate in absolute ethanol under reflux condition yielded 2-mercapto-6-(1-benzyl-1H-indol-3-yl)-4-(naphthalen-2-yl) nicotinonitrile (17a) and 2-mercapto-6-(1-benzoyl-1H-indol-3-yl)-4-(naphthalen-2-yl) nicotinonitrile (17b) (Scheme 3). IR (KBr) spectra of this compounds show the absence of C=O characteristic absorption bands and show new bands at 2197 and 2203 cm^{-1}, respectively characteristic for the CN group besides new absorption bands at 1297 and 1273 cm^{-1} characteristic for C=S group which confirm the formed of 2-mercaptonicotinonitrile 17a and 17b.

S-alkylation of enol form of 2-mercaptonicotinonitrile derivatives 17a and 17b *via* reaction with various halo-compounds namely, chloro-acetonitrile, ethyl chloroacetate, phenacyl bromide and/or 2-chloro-*N*-(thiazol-2-yl)acetamide in dimethylformamide and in the presence of sodium hydroxide led to the formation of 2-(substituted thio)-6-(1-benzyl-1*H*-indol-3-yl)-4-(naphthalen-2-yl)nicotinonitriles 18a-d and 2-(substituted thio)-6-(1-benzoyl-1*H*-indol-3-yl)-4-(naphthalen-2-yl) nicotinonitriles 19a-d (Scheme 3). Their ^{1}H NMR spectra revealed new singlet signals at ~ 4.31-6.89 ppm for S-CH_2.

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), 5.75 (2H, s, N-CH_2), 3.51 (2H, q, CH_2), 1.14 (3H, t, CH_3)



Compds	R	R_1	X	Compds	R	R_1	X
2a, 15a,16a, 17a	CH_2Ph	-	-	**2b, 15b, 16b, 17b**	COPh	-	-
18a	CH_2Ph	CN	Cl	**19a**	COPh	CN	Cl
18b	CH_2Ph	$CO_2CH_2CH_3$	Cl	**19b**	COPh	$CO_2CH_2CH_3$	Cl
18c	CH_2Ph	COPh	Br	**19c**	COPh	COPh	Br
18d	CH_2Ph		Cl	**19d**	COPh		Cl
20a	CH_2Ph	CN	-	**21a**	COPh	CN	-
20b	CH_2Ph	$CO_2CH_2CH_3$	-	**21b**	COPh	$CO_2CH_2CH_3$	-
20c	CH_2Ph	COPh	-	**21c**	COPh	COPh	-

Scheme 3

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, respectively (Scheme 3). IR (KBr) spectra of this compound showed the absence of CN absorption bands and revealed new absorption bands at ~ 3107-3391 cm-1characteristic for NH2. Their 1H NMR spectra lacked the presence of singlet signals of S-CH2 and revealed a new singlet signals at 11.86, 9.93, 12.10, 2.40, 8.73 and 9.93, respectively.

Antimicrobial activity

All the newly synthesized compounds were evaluated *in vitro* for their antimicrobial activities against a variety of pathogenic microorganisms: *S. pneumoniae* (RCMB 010010), *S. aureus* (RCMB 010028) (Gram-positive bacteria), *E. coli* (RCMB 010052), *P. aeruginosa* (RCMB 010043) (Gram-negative bacteria) and two strains of fungi, *C. albicans* (RCMB 05036) and *A. fumigatus* (RCMB 02568) using agar well diffusion method at 5 mg/ml (table 1). Ampicillin, ciprofloxacin and amphotericin B were used as reference drugs.

Antibacterial activity

Gram-positive bacteria

The data obtained showed that, compounds 19b, 20a, 20d and 21b showed moderate activity with inhibition zones ranging from of 17.3-19.3 mm against *S. pneumoniae* (RCMB 010010) compare to the reference drug ampicillin of 23.8 mm (table 1). Whereas, compounds 5b, 6b, 8b, 15a, 15b, 16b, 17a, 17b and 19d was found to be the most active compounds with the inhibition zones ranging from of 21.2 to 23.1 mm against *S. pneumoniae* (RCMB 010010) compared to the reference drug ampicillin of 23.8 mm. Only compound 6b showed potent activity against *S. aureus* (RCMB 010028) with an inhibition zone of 23.4 mm compared to the reference drug ampicillin of 27.4 mm, respectively.

Gram-negative bacteria

Compounds 8b, 16b, 17a, 17b and 19d was found to be the high potent compounds with the inhibition zone of 24.3, 23.5, 25.3, 24.2 and 23.7 mm against *E. coli* (RCMB 010052), which was better than the reference drug ciprofloxacin of 23.4 mm. None of the test compounds showed antimicrobial activity against *P. aeruginosa* (RCMB 010043).

Antifungal activity

Compounds 6b, 8b, 15a, 16b, 17a and 17b showed moderate antifungal activity against *Candida albicans* (RCMB 05036) with the inhibition zone ranging from 19.6 to 22.4 mm compare to the reference drug amphotericin B of 25.4 mm (table 1). Whereas, compounds 17a and 17b was nearly be an equipotent activity with the inhibition zone of 23.3 mm against *Aspergillus fumigatus* (RCMB 02568) compare to the reference drug amphotericin B of 23.7 mm.

Minimum inhibitory concentration

Compounds 8b, 17a, 17b and 19d which showed high antimicrobial activity were used to calculate MICs, which corresponds to 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 dar 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 Asp*A*81 (2.80 Å).

Table 1: Antimicrobial activity of most active compounds

Inhibition zone (mm)±Standard deviation[a]						
Compd. No.	***Streptococcus pneumoniae* (RCMB 010010)**	***Staphylococcus aureus* (RCMB 010028)**	***Escherichia coli* (RCMB 010052)**	***Pseudomonas aeruginosa* (RCMB 010043)**	***Candida albicans* (RCMB 05036)**	***Aspergillus fumigatus* (RCMB 02568)**
	Test compounds 5 mg/ml					
2a	12.4±1.5	10.6±0.63	12.7±1.5	NA	10.3±0.58	11.3±1.2
2b	17.1±0.44	16.2±0.63	18.3±1.5	NA	15.2±1.2	17.3±0.58
5b	21.2±1.5	19.3±0.63	21.9±1.2	NA	18.3±0.58	20.6±1.2
6b	21.3±0.19	23.4±0.37	21.3±0.24	NA	20.4±1.5	21.8±0.32
7a	14.2±0.63	12.3±0.58	11.3±1.2	NA	11.6±0.58	12.3±1.2
8a	19.6±0.58	17.9±0.63	21.6±1.2	NA	17.2±1.5	19.3±0.58
8b	22.1±0.55	20.6±0.52	24.3±0.58	NA	19.6±0.35	20.3±0.36
9a	NA	NA	NA	NA	11.2±0.58	9.3±0.58
9b	18.4±0.67	17.3±0.56	19.3±0.72	NA	16.3±0.36	18.1±0.63
15a	22.4±0.44	20.6±0.63	23.2±1.2	NA	20.3±0.58	21.3±0.58
15b	21.3±0.53	19.3±0.44	21.3±0.23	NA	17.4±0.44	19.3±0.53
16a	NA	NA	NA	NA	12.1±0.43	10.3±0.63
16b	21.3±1.2	21.2±0.58	23.5±0.63	NA	20.2±1.2	21.3±1.5
17a	23.1±1.5	21.3±1.2	25.3±0.58	NA	21.4±1.2	23.3±0.58
17b	22.8±0.58	21.3±0.63	24.2±1.5	NA	22.4±0.19	23.3±0.44
18a	11.7±0.52	10.6±0.63	12.4±0.62	NA	10.3±0.53	12.3±0.62
18b	13.6±0.58	12.6±1.2	NA	NA	NA	NA
18d	13.9±1.2	14.4±1.5	15.0±0.72	NA	14.5±1.5	13.7±0.58
19a	11.3±0.44	10.2±0.58	NA	NA	NA	12.3±0.58
19b	17.3±0.63	15.3±1.5	20.6±0.58	NA	13.6±1.2	16.3±0.19
19d	21.3±0.68	19.6±0.36	23.7±0.58	NA	17.9±0.44	20.3±0.25
20a	17.5±0.63	15.7±0.58	20.7±1.2	NA	14.1±1.5	16.9±1.2
20b	13.3±0.19	13.5±0.36	11.4±0.36	NA	14.7±0.58	14.9±0.25
20c	15.4±0.19	16.0±0.58	13.5±0.44	NA	15.2±0.73	15.4±0.44
20d	19.1±1.2	17.2±1.5	21.3±1.2	NA	16.9±0.63	18.4±0.58
21a	16.9±1.5	16.2±1.2	12.5±0.63	NA	14.4±0.58	16.8±0.63
21b	19.3±1.5	18.2±0.58	20.3±0.58	NA	16.3±1.2	17.8±0.58
21d	9.3±0.58	11.4±0.44	NA	NA	NA	NA
Ampicillin	23.8±1.2	27.4±0.72	NA	NA	NA	NA
Ciprofloxacin	NA	NA	23.4±0.63	20.6±1.2	NA	NA
Amphotericin B	NA	NA	NA	NA	25.4±0.58	23.7±1.2

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

Table 2: Antimicrobial activity as MICS (µg/ml) of test samples against test microorganisms

Compd. No.	***Streptococcus pneumonia* (RCMB 010010)**	***Staphylococcus aureus* (RCMB 010028)**	***Escherichia coli* (RCMB 010052)**	***Pseudomonas aeruginosa* (RCMB 010043)**	***Candida albicans* (RCMB 05036)**	***Aspergillus fumigatus* (RCMB 02568)**
8b	1.95	1.95	0.98	-	3.9	3.9
17a	0.98	1.95	0.49	-	1.95	0.98
17b	0.98	1.95	0.49	-	1.95	0.98
19d	1.95	3.9	0.98	-	7.81	0.98
Ampicillin	0.98	0.49	-	-	-	-
Ciprofloxacin	-	-	0.98	1.95	-	-
Amphotericin B	-	-	-	-	0.49	0.98

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

Table 3: Binding energy (kJ mol^{-1}) of the most active compounds which docked with the active pocket of ATPase (5CPH), enoyl reductase (1C14) and dihydrofolate reductase (1AI9)

Compd. No.	**5CPH**	**1C14**	**1AI9**
	Binding Energy (kJ mol^{-1})		
Co-crystalline ligand	-14.41	-18.53	-12.45
8b	-20.70	-20.47	-24.38
17a	-19.97	-20.71	-17.77
17b	-21.00	-16.81	-21.06
19d	-21.83	-26.45	-29.27

Docking study against G-ve bacteria

The result of molecular docking studies showed that (table 3, fig. 3a,b-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 Ser*91* (2.96Å) or between C=O of benzoyl moiety and NH of Lys*163* (2.60 Å) respectively, in comparison to co-crystallized ligand triclosan which has binding energy of–18.53 kJ mol^{-1} (Rmsd 1.70) and formed hydrogen bond between OH group and C=O of Tyr*156* (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 Lys*A57* and Phe*A36*, respectively compare to co-crystalline ligand (NDP) of-12.45 kJ mol^{-1}and Rmsd 1.45 (table 3).

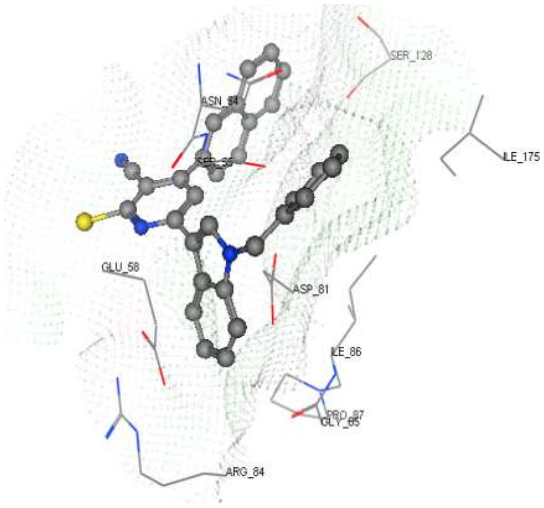


Fig. 1a

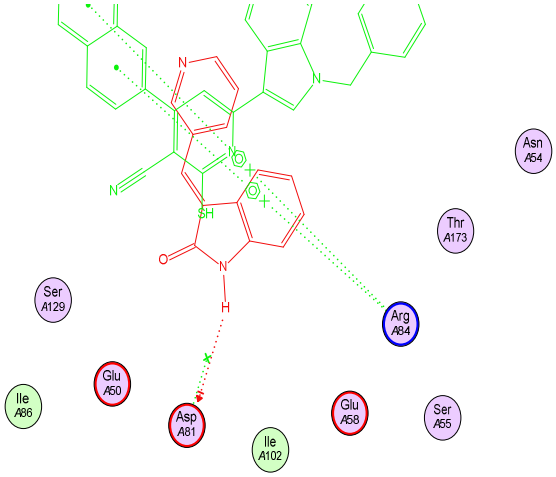


Fig. 1b

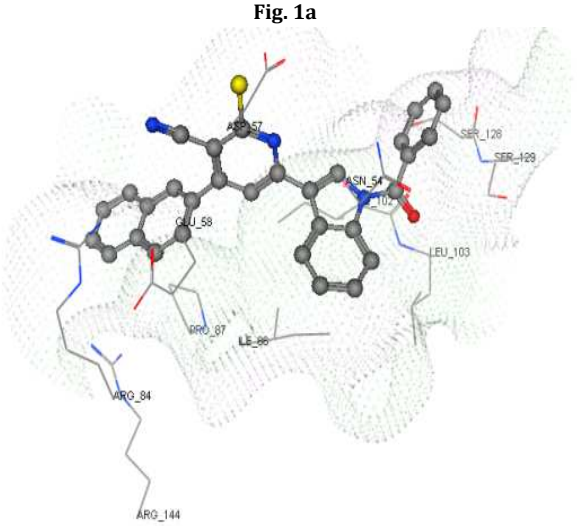


Fig. 2a

Fig. 1a and 1b: The 3D depiction of the docked conformation of 17a and 17b into active side of ATPase enzyme (PDB ID: 5CPH)

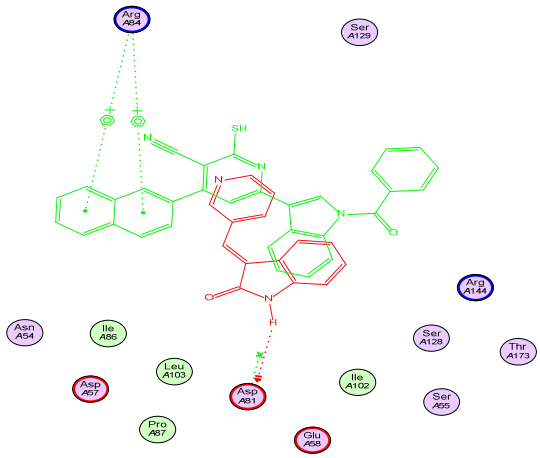


Fig. 2b

Fig. 1b and 2b: The 2D depiction of the docked conformation of 17a and 17b, respectively with the co-crystalline ligand (EVO). Both co-crystalline ligand (red color) and the active compounds (green color) are aligned in the binding pocket and compared in each figure

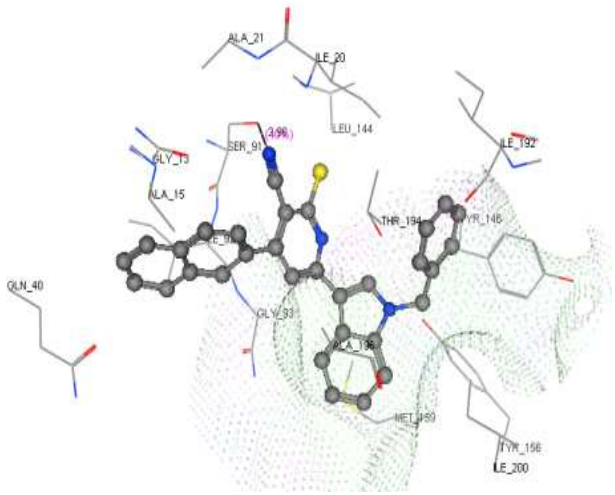


Fig. 3a

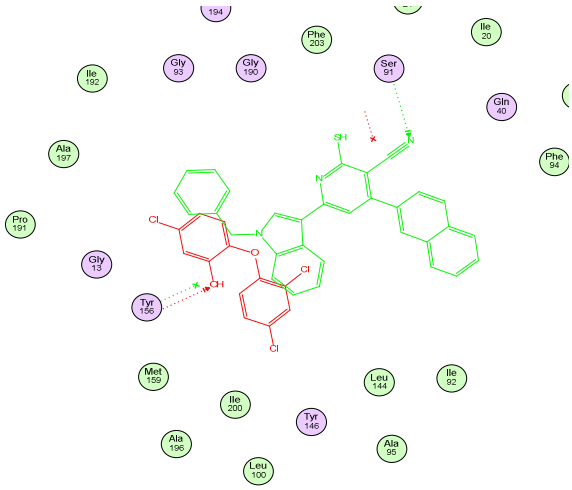


Fig. 3b

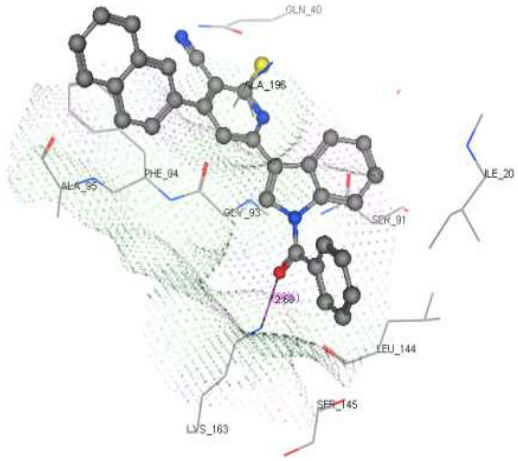


Fig. 4a

Fig. 3a and 4b: The 3D depiction of the docked conformation of 17a and 17b into active side of enoyl reductase enzyme (PDB ID: 1C14)

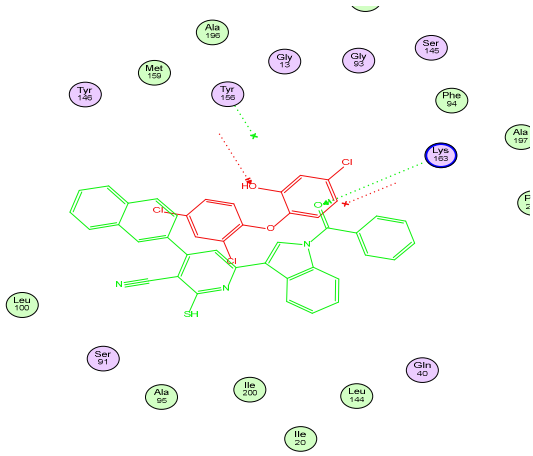


Fig. 4b

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

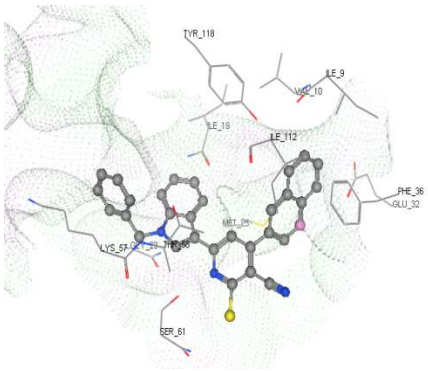


Fig 5a

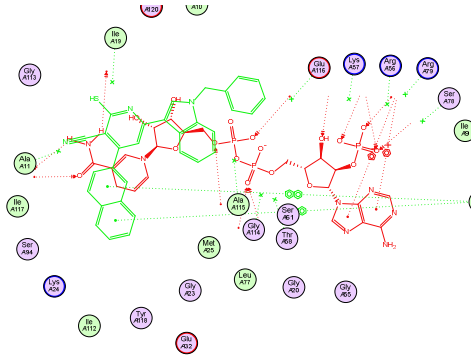


Fig 5b

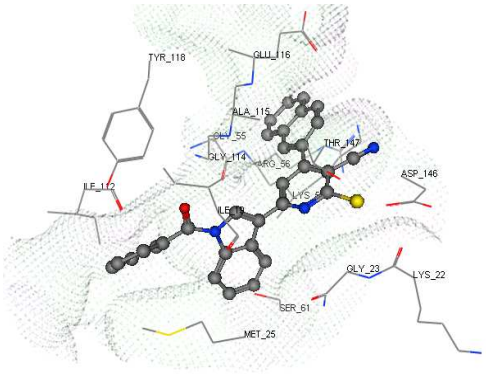


Fig 6a

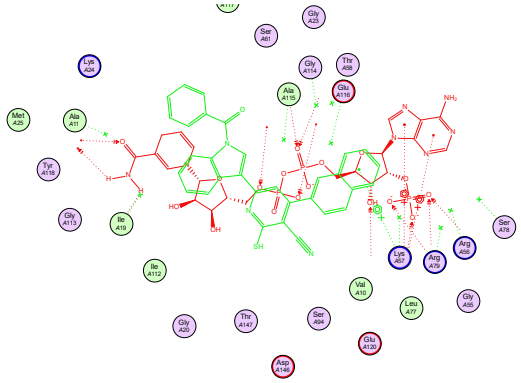


Fig 6b

Fig. 5a and 6b: the 3D depiction of the docked conformation of 17a and 17b into active side of dihydrofolate reductase enzyme in complex (PDB ID: 1AI9)

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

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