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SYNTHESIS AND ANTIMICROBIAL EVALUATION OF NOVEL PYRAZOLE, IMIDAZOLE AND PYRIMIDINE DERIVATIVES POSSESSING IMIDAZO[4,5-B]INDOL MOIETY

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Abstract. In this study, new pyrazole, imidazole, pyrimidine derivatives having imidazo[4,5-b]indol moiety were successfully synthesized, elucidated by spectroscopic techniques, and evaluated as potential antimicrobial agents. The structure-activity relationship was investigated to obtain a better understanding of the relationship between the chemical structure of the synthesized compounds and their corresponding biological activity. Compounds **2b** and **3b** exhibited potent antibacterial activities against *Bacillus subtilis* bacteria comparable to that of Ampicillin standard. Structure-activity relationship studies revealed that the presence of withdrawing carbonyl group on 5-position of pyrazole moiety **2b**, phenylpyrazole moiety **3b** led to an enhancement in the antibacterial activity of pyrazole derivatives. Furthermore, the presence of carbonyl group on 2-position of the pyrimidine ring of compounds **4a**, **5a** and **6a** has a significant effect on their antibacterial activity against *Bacillus subtilis*. The antifungal studies indicated that compounds **3b**, **4b**, **7** and **9** have comparable antifungal activity to that of standard Amphotericin B against *Candida albicans* and *Aspergillus flavus* fungi.

Keywords: pyrazole, imidazole, pyrimidine, indol, antimicrobial activity.

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Introduction

Pyrazole derivatives are well considered in the literature as significant biologically active heterocyclic compounds [1-4]. Medicinal chemistry studies have shown that pyrazole systems, as biomolecules, display a broad spectrum of pharmacological activities including antimicrobial [5,6], anti-inflammatory [7-9], antitumor [10], anticonvulsant [11] and neuroprotective activity. Synthetic drugs have endured an emergence in recent decades because of the increasing population of multidrug-resistant (MDR) bacteria, particularly the "ESKAPE" pathogens, such as *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* [12-15].

Further studies showed that imidazole, a five-member heterocyclic aromatic compound, occupied an exclusive position in heterocyclic chemistry and its derivatives have attracted considerable interest in recent years for their ability to cure several diseases [16]. Imidazole derivatives show anti-inflammatory, anticancer [17,18], antibacterial [19], analgesic [20], and anti-tubercular [21-22] activity.

The existence of pyrimidine base in uracil, cytosine, and thymine - the main building blocks of nucleic acids DNA and RNA, is among the reasons for their extensive pharmacological applications [23]. The literature surveys depict that compounds encompassing pyrimidine moiety constitute an important class of natural and non-natural products, many of which display a wide range of biological activity [24,25]. Various pyrimidine derivatives have been found to exhibit remarkable antibacterial [26], antifungal [27], antihypertensive [28], antipyretic [29] and anticancer activity [30].

The recent drug design tendency is to reassemble two or three heterocyclic molecules possessing distinct sites of action to serve as new core structures of molecules towards the obtaining of new biologically active agents. In view of our global interest in the design of new potent antimicrobial agents, the main purpose of our study is to synthesize a new set of antimicrobial

agents based on pyrazole, imidazole, pyrimidine derivatives bearing the imidazo[4,5-b]indol moiety.

In this study, the identification of the designed compounds was carried out by IR spectroscopy, NMR and mass spectrometry. The biological activity of these compounds was realized to evaluate their antibacterial and antifungal properties against various strains.

Experimental

Generalities

Unless otherwise mentioned, reagents were provided from Sigma Aldrich (Bayouni Trading Co. Ltd., Al-Khobar, Saudi Arabia) and used without further purification. Reaction progress was monitored using thin-layer chromatography (TLC) on silica gel pre-coated F254Merck plates (Darmstadt, Germany) and spots were visualized by ultraviolet irradiation.

The *melting point* values were determined by Gallenkamp electrothermal melting point device (Weiss-Gallenkamp, Loughborough, UK) and are uncorrected.

IR spectra were recorded on ϕ Bruker-Vector 22 Fourier transform infrared spectrophotometer (Billerica, MA) using potassium bromide disks.

The ^{1}H and ^{13}C *NMR spectra* were measured on a Varian MercuryVXR-300 NMR spectrometer (Palo Alto, CA) at 400 and 125 MHz for using DMSO-d_6 as solvents.

Mass spectra were recorded on a Hewlett Packard MS-5988 spectrometer (Palo Alto, CA) at 70 eV.

Elemental analysis was carried out at the Micro-analytical Center of Cairo University, Giza, Egypt.

General synthesis of compounds (1a-c)

A mixture of isatins (1 mmol), urea (1 mmol), and different active methylene reagents (malononitrile, ethylcyanoacetate, ethylacetoacetate) (1 mmol) dissolved in ethanol (10 mL) was stirred under reflux for 2 h until the reaction was completed. Thin-layer chromatography (TLC) on silica gel plates was carried out for reaction monitoring using ethanol : ethyl acetate (1:2). The reaction mixture was left to cool at room temperature and filtered off. The solution was concentrated under vacuum to afford the product **1a-c**, which was purified by recrystallization in ethanol to afford the corresponding **1a-c** in good yield.

2-(imidazo[4,5-b]indol-2(4H)-ylidene)malononitrile ***1a***, red crystals in 80% yield, m.p. 250-252°C. IR (KBr, cm^{-1}): 3382 and 3166 (NH), 2183 (CN), cm^{-1}. ^{1}H NMR: δ 6.92 -7.22 (m, 4H, Ar-H), 10.31 (s, H, NH) ppm. ^{13}C NMR: δ 72.35, 113.08, 114.57, 117.08, 119.88, 131.06, 132.26, 156.72, 165.82, 166.53, 169.08 ppm. MS (ESI): m/z 219.2 (M^+). Anal. calcd. for $C_{12}H_5N_5$: C, 65.54; H, 2.26; N, 31.89 %. Found: C, 65.75; H, 2.30; N, 31.95.

ethyl 2-cyano-2-(imidazo[4,5-b]indol-2(4H)-ylidene)acetate ***1b***, reddish brown crystals in 77% yield, m.p. 285-287°C. IR (KBr, cm^{-1}): 3379 and 3154 (NH), 2163 (CN), 1716 (C=O) cm^{-1}. ^{1}H NMR: δ 1.29 (s, 3H, CH_3), 4.32 (q, 2H, J= 7.57 Hz, CH_2), 6.89 -7.21 (m, 4H, Ar-H), 10.25 (s, H, NH) ppm. ^{13}C NMR: δ 15.21, 61.32, 93.24, 113.12, 116.32, 117.05, 118.42, 131.14, 132.31, 156.31, 167.12, 169.25, 175.03 ppm. MS (ESI): m/z 226.25 (M^+). Anal. calcd. for $C_{14}H_{10}N_4O_2$: C, 63.34; H, 3.75; N, 21.11 %. Found: C, 63.15; H, 3.79; N, 21.04.

diethyl 2-(imidazo[4,5-b]indol-2(4H)-ylidene)malonate ***1c***, orange solid in 70% yield, m.p. 230-232°C. IR (KBr, cm^{-1}): 3386 and 3167 (NH), 1724 (C=O) cm^{-1}. ^{1}H NMR: δ 1.32 (2s, 6H, $2CH_3$), 4.21 (q, 4H, J= 7.53 Hz, $2CH_2$), 6.93-7.36 (m, 4H, Ar-H), 10.39 (s, H, NH) ppm. ^{13}C NMR: δ 15.31, 62.33, 115.23, 116.21, 119.22, 131.34, 126.52, 156.13, 166, 167, 169.62 ppm. MS (ESI): m/z 313.31 (M^+). Anal. calcd. for $C_{16}H_{15}N_3O_4$: C, 61.30; H, 4.81; N, 13.29 %. Found: C, 61.34; H, 4.83; N, 13.41.

General procedure for the synthesis of pyrazole derivatives (2a-c)

A mixture of compounds **1a-c** (mmol) and hydrazine hydrate (15 mL) in ethanol was refluxed until the reaction was completed. The reaction was checked by TLC using (ethyl acetate : hexane, 3:1) and the reaction mixture was cooled at room temperature to obtain a solid colorless product **2a-c**. Subsequently, the precipitate was filtered and recrystallized with ethanol and dried.

4-(imidazo[4,5-b]indol-2-yl)-1H-pyrazole-3,5-diamine ***2a***, pale yellow crystals, yield 72%, m.p. 160-162°C. IR (KBr, cm^{-1}): 3491-3229 (NH_2), 3236 (NH), 1620 (C=N) cm^{-1}. ^{1}H NMR: δ 4.93 (s, 4H, 2 NH_2), 7.20-7.41 (m, 4H, C_6H_4), 9.85 (s, H, NH) ppm. ^{13}C NMR: δ 124.81, 128.26, 131.44, 133.23, 150.19, 151.14, 152.17, 164.51, 165.55, 170.22 ppm. MS (ESI): m/z 251.25 (M^+). Anal. calcd. for: ($C_{12}H_9N_7$: C, 57.37; H, 3.61; N, 39.02. Found: C, 57.42; H, 3.64; N, 39.13.

3-amino-1,2-dihydro-4-(imidazo[4,5-b]indol-2-yl)pyrazol-5-one ***2b***, reddish crystals, yield 74%, m.p. 177-179°C. IR (KBr, cm^{-1}): 3924-3134 (NH_2), 3231 (NH), 1679 (C=O), 1619 (C=N) cm^{-1}.

^{1}H NMR: δ 7.24-7.48 (m, 4H, C_6H_4), 9.34 (s, H, NH), 9.86 (s, H, NH) ppm. ^{13}C NMR: δ 123.24, 125.16, 128.41, 131.22, 133.35, 150.36, 164.55, 166.33, 172. 78 ppm. MS (ESI): m/z 252.23 (M^+). Anal. calcd. for: $C_{12}H_8N_6O$: C, 57.14; H, 3.20; N, 33.32. Found: C, 57.19; H, 3.25; N, 33.39.

1,2-dihydro-4-(imidazo[4,5-b]indol-2-yl)-5-methylpyrazol-3-one ***2c***, green crystals, yield 68%, m.p. 140-142°C. IR (KBr, cm^{-1}): 3230 (NH), 1676 (C=O), 1616 (C=N) cm^{-1}. ^{1}H NMR: δ 2.35 (s, 3H, CH_3), 7.21-7.43 (m, 4H, C_6H_4), 9.87 (s, H, NH), 10.02 (s, H, NH) ppm. ^{13}C NMR: δ 18.21, 97.51, 123.22, 125.37, 128.42, 131.19, 133.31, 144.32, 150.41, 166.25, 167.22 ppm. MS (ESI): m/z 251.24 (M^+). Anal. calcd. for: ($C_{13}H_9N_5O$: C, 62.15; H, 3.61; N, 27.87. Found: C, 62.19; H, 3.67; N, 27.93.

General procedure for the synthesis of compounds (3a-c)

A mixture of compounds **1a-c** (1 mmol) and phenylhydrazine (15 mL) in ethanol/triethylamine was refluxed and the progress of the reaction was monitored by TLC using ethyl acetate: hexane (3:1). After completion of the reaction, the contents were cooled at room temperature, and the solid thus obtained was filtered and washed with ethanol and dried.

3,2-(5-methyl-1-phenyl-1H-pyrazol-4-yl)imidazo[4,5-b]indole ***3a***, brown crystals, yield 65%, m.p. 135-137°C; IR (KBr, cm^{-1}): 3431-3156 (NH_2), 1679 (C=O), 1624 (C=N) cm^{-1}. ^{1}H NMR: δ 6.86 (s, 4H, $2NH_2$), 7.20-7.36 (m, 9H, C_6H_4, C_6H_5) ppm. ^{13}C NMR: δ 123.31, 125.31, 127.25, 128.21, 128.33, 130.41, 131.17, 133.23, 140.21, 140.44, 150.32, 159.16, 170.26 ppm. MS (ESI): m/z 327.34 (M^+). Anal. calcd. $C_{18}H_{13}N_7$: C, 66.04; H, 4.00; N, 29.95. Found: C, 66.09; H, 4.07; N, 30.01.

3-amino-1,2-dihydro-4-(imidazo[4,5-b]indol-2-yl)-2-phenylpyrazol-5-one ***3b***, orange crystals, yield 70%, m.p. 150-152°C, IR (KBr, cm^{-1}): 3431-3156 (NH_2), 3213 (NH), 1679 (C=O), 1624 (C=N) cm^{-1}. ^{1}H NMR: δ 6.86 (s, 2H, NH_2), 7.20-7.36 (m, 9H, C_6H_4, C_6H_5), 9.23 (s, 2H, NH_2) ppm. ^{13}C NMR: δ 75.28, 113.24, 120.11, 120.55, 123.22, 125.36, 128.23, 129.42, 130.15, 131.33, 133.12, 136.41, 150.44, 164.27, 166.37 ppm. MS (ESI): m/z 278.27(M^+). Anal. calcd. ($C_{18}H_{12}N_6O$; 328.33): C, 65.85; H, 3.68; N, 25.60. Found C, 65.60; H, 3.71; N, 25.64.

2-(5-methyl-1-phenyl-1H-pyrazol-4-yl)imidazo[4,5-b]indole ***3c***, reddish brown crystals, yield 60%, m.p. 125-127°C; IR (KBr, cm^{-1}): 3420-3256 (NH_2), 3203 (NH), 1673 (C=O), 1618 (C=N) cm^{-1}. ^{1}H NMR: δ 4.86 (s, 4H, $2NH_2$), 7.20-7.36 (m, 9H, C_6H_4, C_6H_5), 10.35 (s, 2H, NH_2) ppm. ^{13}C NMR: δ 77.26, 113.33, 114.25, 117.46, 119.29, 126.46, 130.44, 131.36, 131.42, 144.13, 146.52, 155.32, 156.28, 164.17 ppm. MS (ESI): m/z 311.34 (M^+). Anal. calcd. $C_{19}H_{13}N_5$: C, 73.30; H, 4.21; N, 22.49. Found: C, 73.36; H, 4.26; N, 22.53.

General procedure for the synthesis of 4a, 4b, 5a, 5b, 6a and 6b

A mixture of compounds **1a-c** (0.1 mmol), trimethylamine (few drops), and urea or thiourea (0.1 mmol) in ethanol (15 mL) was refluxed for 8 h. After the reaction was completed, the mixture was concentrated and the obtained solid was collected by filtration and recrystallized from ethanol.

4,6-diamino-5-(imidazo[4,5-b]indol-2-yl)pyrimidin-2(1H)-one ***4a***, yellow crystals, yield 60%, m.p. 215-217°C; IR (KBr, cm^{-1}): 3420-3256 (NH_2), 3203 (NH), 1673 (C=O), 1618 (C=N) cm^{-1}. ^{1}H NMR: δ 4.86 (s, 4H, $2NH_2$), 7.23-7.52 (m, 4H, C_6H_4), 10.35 (s, 2H, NH_2) ppm. ^{13}C NMR: δ 123.22, 128.33, 131.32, 133.34, 150.24, 162.23, 164.47, 167.31, 167.51 ppm. MS (ESI): m/z 278.27(M^+). Anal. calcd. for $C_{13}H_9N_7O$: C, 55.97; H, 3.28; N, 35.15. Found: C, 55.91; H, 3.25; N, 35.11.

4,6-diamino-5-(imidazo[4,5-b]indol-2-yl)pyrimidine-2-thiol ***4b***, white crystals, yield 64%, m.p. 225-227°C; IR (KBr, cm^{-1}): 3432-3234 (NH_2), 3221 (NH), 1678 (C=O), 1623 (C=N) cm^{-1}. ^{1}H NMR: δ 5.32 (s, 4H, $2NH_2$), 7.03-7.58 (m, 4H, C_6H_4), 10.35 (s, H, NH) ppm. ^{13}C NMR: δ 123.44, 125.27, 128.41, 131.33, 133.19, 150.31, 167.32, 175.26, 182.41 ppm. MS (ESI): m/z 295.32 (M^+). Anal. calcd. for $C_{13}H_9N_7S$: C, 52.87; H, 3.07; N, 33.20; S, 10.86. Found: C, 52.89; H, 3.08; N, 33.24; S, 10.90.

4-amino-5-(imidazo[4,5-b]indol-2-yl)pyrimidin-2(1H)-one ***5a***, pale yellow crystals, yield 66%, m.p. 234-236°C; IR (KBr, cm^{-1}): 3370-3172 (NH_2), 3150 (NH), 1676 (C=O), 1620 (C=N). ^{1}H NMR: δ 5.36 (s, 2H, NH_2), 7.15-7.32 (m, 4H, C_6H_4), 9.78 (s, H, NH) ppm. ^{13}C NMR: δ 99.33, 123.23, 125.22, 127.32, 133.31, 131.51, 133.35, 150.21, 157.44, 164.41, 167.23, 167.32 ppm. MS (ESI): m/z 264.24 (M^+). Anal. calcd. for $C_{13}H_8N_6O$: C, 59.09; H, 3.05; N, 31.80. Found: C, 59.21; H, 3.07; N, 31.84.

4-amino-5-(imidazo[4,5-b]indol-2-yl)pyrimidine-2(1H)-thione ***5b***, greenish crystals, yield 69%, m.p. 242-244°C; IR (KBr, cm^{-1}): 3437-3233 (NH_2), 3130 (NH), 1682 (C=O), 1619 (C=N), 1334 (C=S) cm^{-1}. ^{1}H NMR: δ 5.02 (s, 2H, NH_2), 6.98 -7.21 (m, 4H, C_6H_4), 10.09 (s, H, NH) ppm. ^{13}C NMR: δ 123.21, 125.22, 128.14, 133.33,

150.42, 146.11, 165.36, 168.51, 181.24 ppm. MS (ESI): m/z 280.31 (M+). Anal. calcd. for $C_{13}H_8N_6S$: C, 55.70; H, 2.88; N, 29.98; S, 11.44. Found: C, 55.75; H, 2.92; N, 30.02; S, 11.47.

5-(imidazo[4,5-b]indol-2-yl)-6-methylpyrimidine-2,4(1H,3H)-dione ***6a***, greenish yellow crystals, yield 59%, m.p. 190-192°C; IR (KBr, cm^{-1}): 3239 (NH), 1673 (C=O), 1617 (C=N) cm^{-1}. ^1H NMR: δ 7.03-7.58 (m, 4H, C_6H_4), 9.68 (s, H, NH_2), 10.06 (s, H, NH) ppm. ^{13}C NMR: δ 128.33, 131.42, 132.17, 134.14, 150.22, 152.15, 153.12, 163.33, 165.17 ppm. MS (ESI): m/z279.25 (M^+). Anal. calcd. for $C_{14}H_9N_5O_2$: C, 60.21; H, 3.25; N, 25.08. Found: C, 60.26; H, 3.31; N, 25.19.

2,3-dihydro-5-(imidazo[4,5-b]indol-2-yl)-6-methyl-2-thioxopyrimidin-4(1H)-one ***6b***, reddish brown crystals, yield 63%, m.p. 170-172°C. IR (KBr, cm^{-1}): 3231 (NH), 1665 (C=O), 1612 (C=N), 1336 (C=S) cm^{-1}. ^1H NMR: δ 7.03-7.58 (m, 4H, C_6H_4), 9.32 (s, H, NH), 10.01 (s, H, NH) ppm ^{13}C NMR: δ 15.28, 123.41, 128.34, 131.16, 133.22, 150.29, 165.33, 167.36, 172.55, 175.46 ppm. MS (ESI): m/z 295.32 (M^+). Anal. calcd. for $C_{14}H_9N_5OS$: C, 56.94; H, 3.07; N, 23.71; S, 10.86. Found: C, 57.04; H, 3.19; N, 23.79; S, 10.92.

General procedure for the synthesis of compounds 7, 8 and 9

A mixture of compounds **1a-c** (10 mmol), guanidine hydrochloride (12.0 mmol), anhydrous K_2CO_3 (15.0 mmol), and absolute ethanol (20 mL) was heated and refluxed for 7 h. After cooling, the mixture was poured into ice water and neutralized with acetic acid. A solid product formed was filtered off and recrystallized from EtOH to afford the corresponding compounds **7**, **8** and **9** with good yields.

5-(imidazo[4,5-b]indol-2-yl)pyrimidine-2,4,6-triamine ***7***, orange crystals, yield 73%, m.p. 286-288°C; IR (KBr, cm^{-1}): 3450-3250 (NH_2), 3200 (NH), 1626 (C=N) cm^{-1}. ^1H NMR: δ 4.75 (s, 4H, $2NH_2$), 5.35 (s, 2H, NH_2), 7.01 -7.33 (m, 4H, C_6H_4) ppm. ^{13}C NMR: δ 107.27, 113.25, 117.55, 119.48, 131.22, 132.34, 156.33, 167.38ppm. MS (ESI): m/z 278.27(M^+). Anal. calcd. for $C_{13}H_{10}N_8$: C, 56.11; H, 3.62; N, 40.27. Found C, 56.21; H, 3.66; N, 40.32.

5-(imidazo[4,5-b]indol-2-yl)pyrimidine-2,4-diamine ***8***, deep brown, yield 67%, m.p. 246-247°C; IR (KBr, cm^{-1}): 3362-3252 (NH_2), 3150 (NH), 1680 (C=O) cm^{-1}. ^1H NMR: δ 4.32 (s, 2H, NH_2), 5.62 (s, 2H, NH_2), 7.02 -7.32 (m, 4H, C_6H_4), 9.32 (s, 1H, NH) ppm. ^{13}C NMR: δ 99.33, 125.33, 125.66, 128.27, 128.32, 131.44, 133.12, 150.17, 157.47, 165.55, 170. 11 ppm. MS (ESI): m/z 263.26 (M^+). Anal. calcd. for $C_{13}H_9N_7$: C, 59.31; H, 3.45; N, 37.24. Found: C, 59.37; H, 3.82; N, 37.29.

5-(imidazo[4,5-b]indol-2-yl)-4-methylpyrimidin-2-amine ***9***, red crystals, yield 77%, m.p. 254-256°C; IR (KBr, cm^{-1}): 3392-3235 (NH_2), 1612 (C=N) cm^{-1}. ^1H NMR: δ 4.83 (s, H, NH_2), 6.96-7.02 (m, 4H, C_6H_4) ppm. ^{13}C NMR: δ 113.35, 123.14, 125.44, 128.26, 131.22, 133.11, 150.15, 157.55, 166.45 ppm. MS (ESI): m/z 262.27 (M^+). Anal. calcd. for $C_{14}H_{10}N_6$: C, 64.11; H, 3.84; N, 32.04. Found: C, 64.17; H, 3.89; N, 32.15.

Antimicrobial activity assays

Antimicrobial activity (antibacterial and antifungal) of the tested samples was checked out according to a modified Kirby–Bauer disk diffusion method [31]. Briefly, 100 µL of each test bacteria/fungi were grown in 10 mL of fresh media until reaching a count of nearly 108 cells/mL for bacteria and 105 cells/mL for fungi. Subsequently, 100 µL of the microbial suspension was spread out onto Mueller–Hinton agar plates corresponding to the broth in which they were maintained. Plates impregnated with filamentous fungi like *Aspergillus flavus* at 25°C for 48 h; gram-positive bacteria as *Staphylococcus aureus*, *Bacillus subtilis*; gram-negative bacteria as *Escherichia coli*, *Pseudomonas aeuroginosa* were incubated at 35-37°C for 24-48 h and yeast as *Candida albicans* was incubated at 30°C for a period varying between 24 and 48 h. Subsequently, the diameters of the inhibition zone were measured in millimeters. The standard disk of ampicillin (antibacterial agent), amphotericin B (an antifungal agent), served as a positive control for antimicrobial activity and filter disks impregnated with 10 µL of solvent (distilled water, chloroform, and DMSO) were used as a negative control. All experiments were repeated and carried out in triplicate in the case of a significant difference in the results and mean values were reported. The mean inhibition zone diameters were measured in mm/mg sample.

Results and discussion

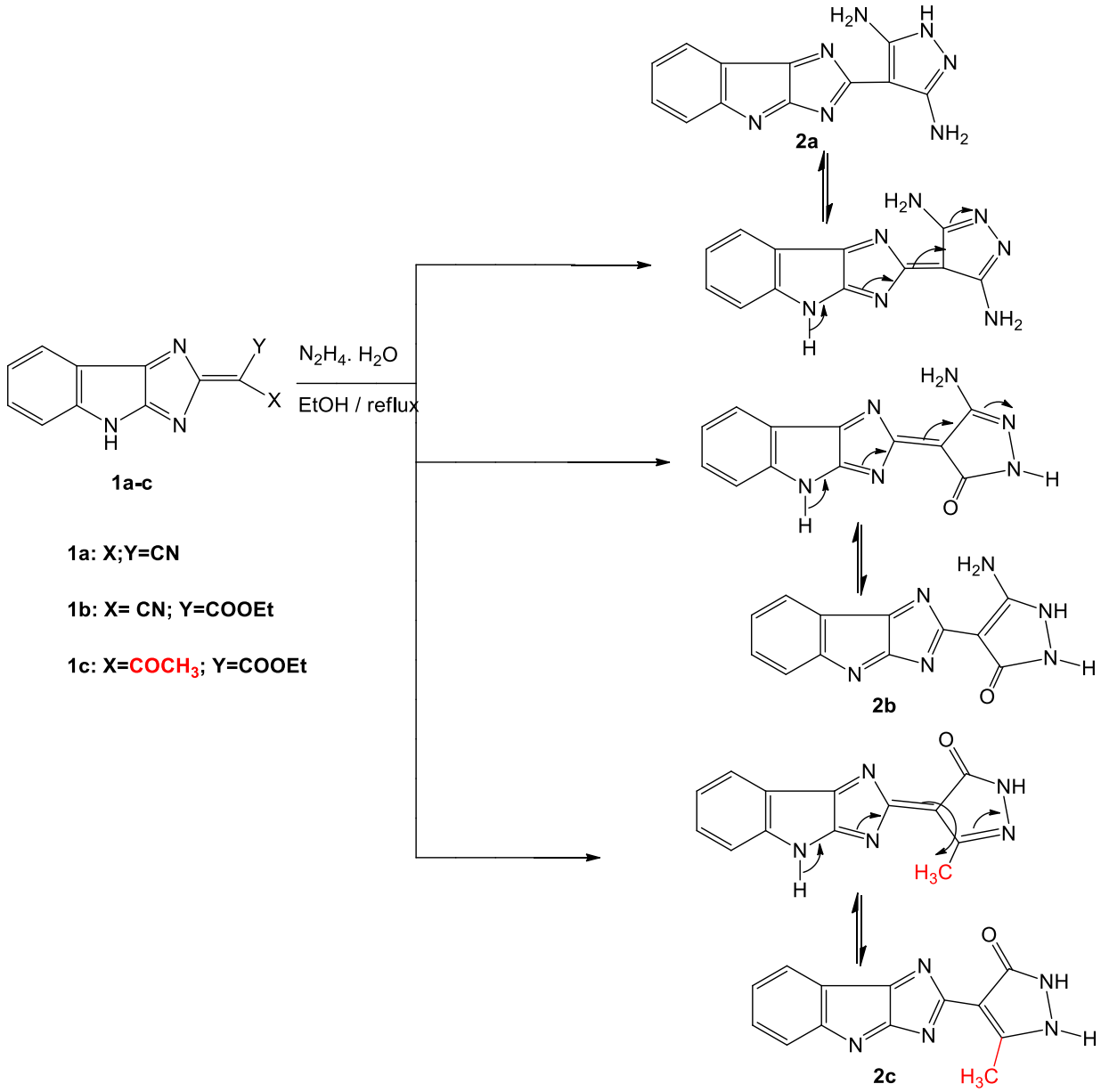
Structure determination

The novel pyrazole, imidazole, and pyrimidine derivatives were synthesized according to the regioselective attack that occurred on the cyano group present in 2-(imidazo[4,5-b]indol-2(4*H*)-ylidene) derivatives **1a**,**b**. Refluxing of **1a-c** derivatives with hydrazine hydrate or phenyl hydrazine in the presence of ethanol gave pyrazole compounds derivatives **2a-c** and **3a-c** (Schemes 1 and 2).

These reactions involve 1,2-dinucleophile cyclization on CN moiety in compounds **1a-c** and concomitant aromatization in the presence of hydrazine and ethanol under reflux.

All reactions were prepared with satisfactory yields varying from 68% to 74% and the obtained products **2a-c** were confirmed by spectral data including IR, ^{1}H and ^{13}C NMR. The IR spectra of 4-(imidazo[4,5-b]indol-2-yl)-1*H*-pyrazole-3,5-diamine **2a** showed the characteristic NH_2 amine absorption bands in the 3421-3257 cm^{-1} range and the absence of absorption bands corresponding to the cyano group. ^{1}H NMR spectrum of compound **2a** displayed two different broad signals concentrated at 10.29, 13.25 ppm and attributed to NH_2 and NH groups. Furthermore, the ^{13}C NMR spectrum of this compound showed signals at 146.25 ppm related to CH=N and further signals appeared in the regular regions were mentioned in the experimental data.

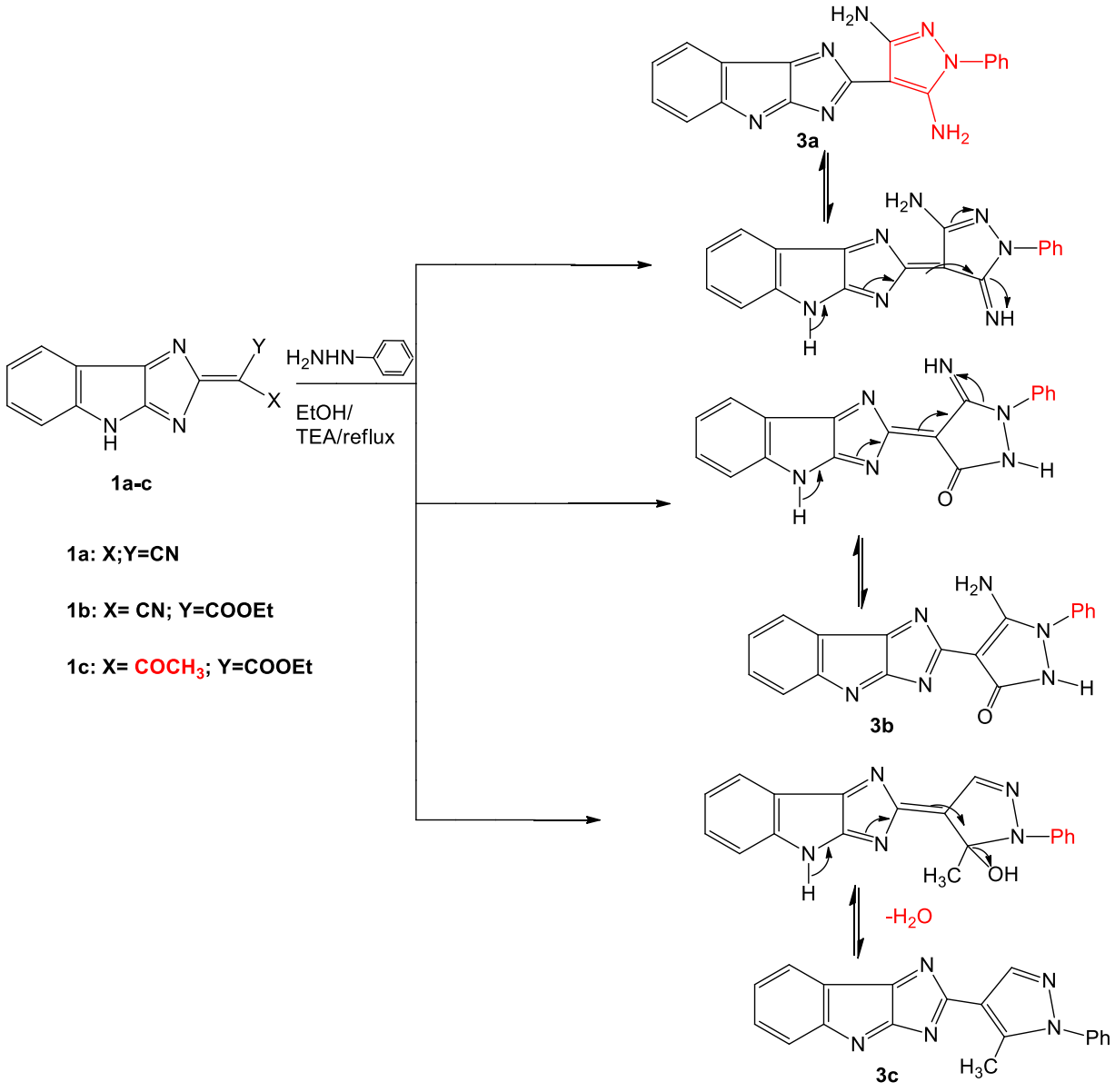
The reaction between ethyl 2-cyano-2-(imidazo[4,5-b]indol-2(4*H*)-ylidene)acetate **1b** and hydrazine hydrate in ethanol was carried out under reflux and gave compound 3-amino-1,2-dihydro-4-(imidazo[4,5-b]indol-2-yl)pyrazol-5-one **2b**. The data obtained from the IR spectrum of compound **2b** confirmed the presence of an absorption band at 1687 cm^{-1} ascribed to C=O. Its ^{1}H NMR spectrum showed a signal at 9.45 ppm corresponding to NH and a singlet signal peak at 2.35 ppm for (CH_3).



Scheme 1. Synthetic routes for the compounds 2a-c.

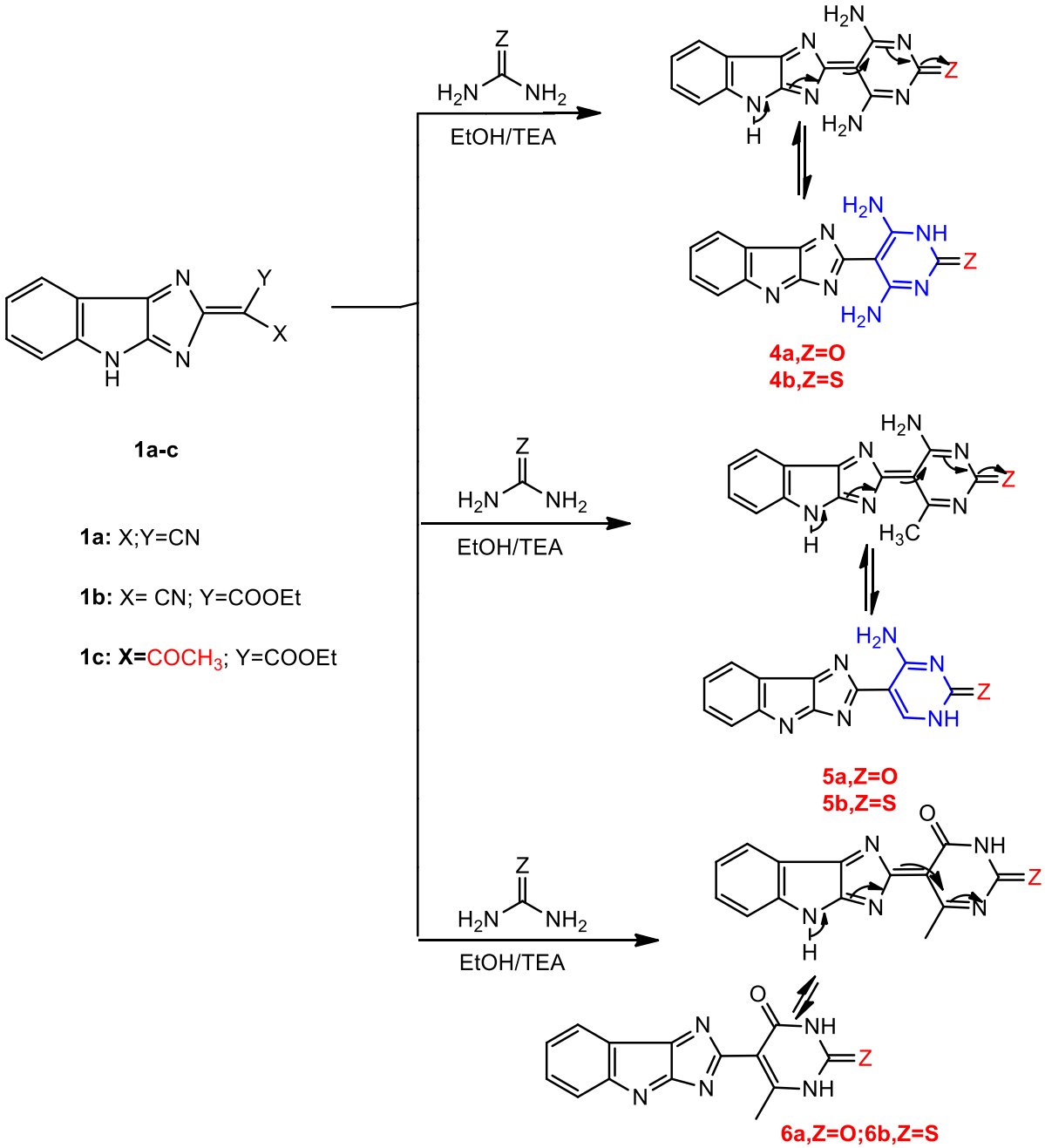
In this study, 1,2-dihydro-4-(imidazo[4,5-b]indol-2-yl)-5-methylpyrazol-3-one **2c** was prepared by reaction of diethyl 2-(imidazo[4,5-b]indol-2(4H)-ylidene) malonate **1c** with hydrazine hydrate in ethanol under reflux. Its IR spectrum showed the characteristic absorption band of (C=O) at 1673 cm^{-1} and another absorption band at 3421, 3232 cm^{-1} correspondings to NH_2 group. The ^1H NMR spectrum of compound **2c** exhibited two singlets; the first appeared at 8.25 ppm and the second at 11.25 ppm. Another singlet peak was observed at 4.27 ppm, and assigned to NH_2 group. The data further ascertain the chemical structures of the synthesized compounds.

As an extension of our work that aims to obtain novel 2-(imidazole)[4-b] indole derivatives possessing different substituents with significant bioactive properties, additional experiments were conducted by reacting compounds **1a-c** with adequate amount of phenylhydrazine in ethanol under reflux to yield compounds **3a-c** as presented in (Scheme 2). IR spectrum of compound 3,2-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)imidazo[4,5-b]indole **3a** showed the characteristic absorption bands due to the NH_2 stretching. The corresponding ^1H NMR spectrum showed a multiplet at δ 7.20-7.36 ppm due to aromatic protons of phenyl ring (C_6H_5). The IR spectrum of compound 3-amino-1,2-dihydro-4-(imidazo[4,5-b]indol-2-yl)-2-phenylpyrazol-5-one **3b** showed absorption at 3431-3156 cm^{-1}, which is due to the NH stretching vibrations. The observed bands at 1679 and 1624 cm^{-1} were attributed to C=O and C=N respectively. The ^1H NMR spectrum of compound **3b** showed a multiplet at δ 7.20-7.36 due to the phenyl ring and singlets at 6.86 and 9.23 ppm related to NH_2.



Scheme 2. Synthetic routes for the compounds 3a-c.

The appearance of bands between 3420-3256 (NH_2), 3203 (NH), 1673 (C=O) and 1618 (C=N) cm^{-1} in the IR spectrum; a multiplet at δ value 7.20-7.36 ppm for phenyl ring support the formation of 2-(5-methyl-1-phenyl-1*H*-pyrazol-4-yl)imidazo[4,5-b]indole **3c**. Compounds **1a-c** were subjected to condensation reactions with urea and thiourea under reflux to give pyrimidine derivatives **4a**,**b**, **5a**,**b** and **6a**,**b** as shown in (Scheme 3). The chemical structures of these compounds were confirmed by spectral data (see experimental section). The IR spectrum of all compounds displayed the characteristic absorption bands of C=C and C=N stretching frequencies at 1475 and 1620 cm^{-1} corresponding to pyrimidine moiety. The absorption of C=O at 1673 cm^{-1} was observed in the IR spectrum of compound **4a**. The ^{1}H NMR spectrum of compounds **4a**,**b** displayed a multiplet at δ value 7.20-7.36 ppm for C_6H_4, two singlets at 10.35 and 4.86 ppm for NH_2 groups. Moreover, the ^{1}H NMR spectrum of compounds **5a**,**b** exhibited two singlets for each NH of pyrimidine ring in the range of 11.25 and 9.26 ppm. The IR spectrum of compounds **8a**,**b** revealed the presence of absorption bands at 1673 cm^{-1} due to (C=O). The absence of the CN group in compounds **6a,b** (IR spectrum) and the singlet at 7.23 ppm related to NH_2 (^{1}H NMR) ascertain their formation.



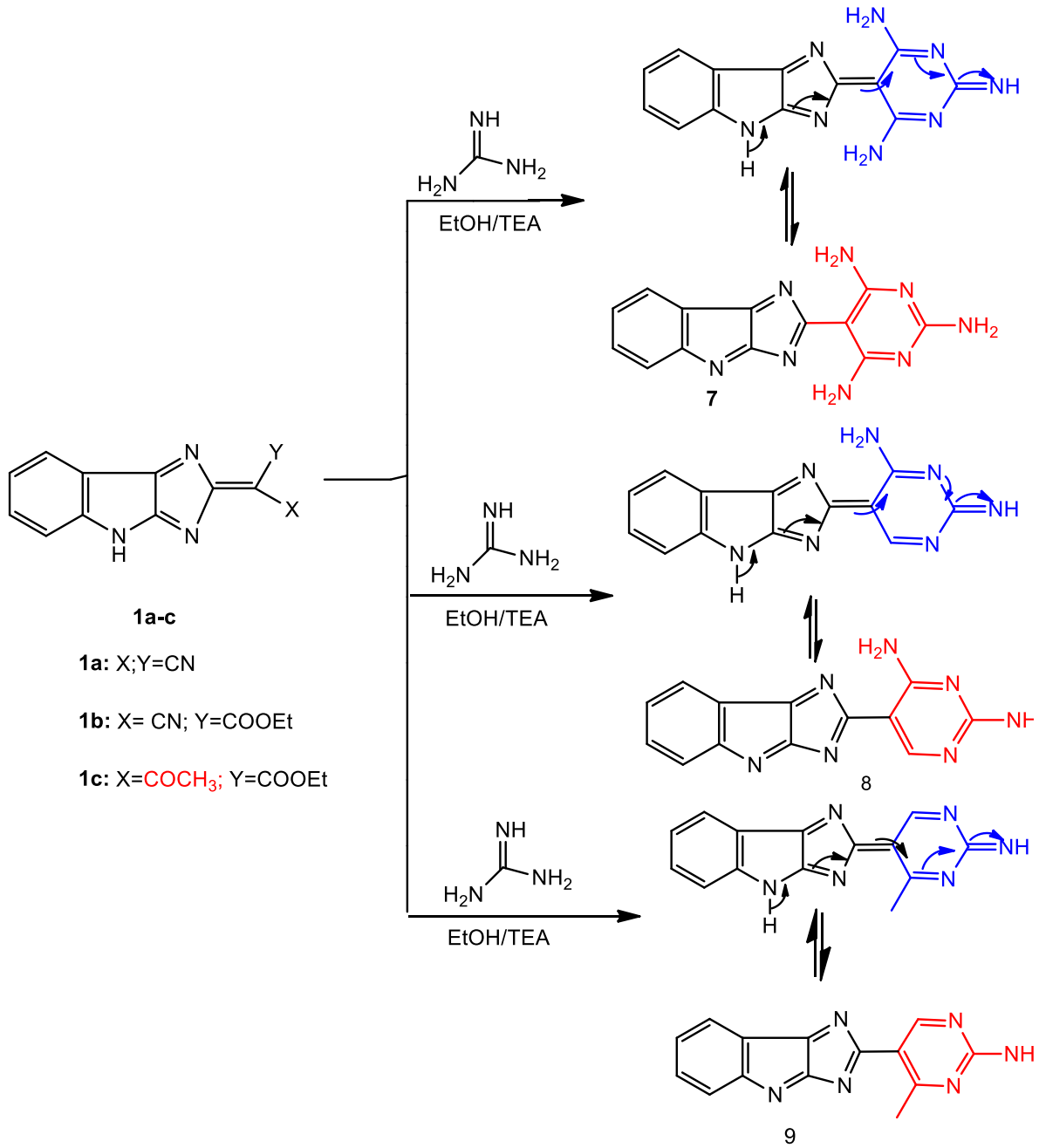
Scheme 3. Synthetic routes for the compounds 4a,b, 5a,b and 6a,b.

In this work, another procedure was adopted to design novel substituted pyrimidines having imidazo[4,5-b]indol moiety (Scheme 4). For this purpose, compounds **1a-c** were treated with guanidine hydrochloride anhydrous K_2CO_3 in ethanol (20 mL) under reflux to give novel pyrimidine derivatives **7-9**. All spectral data confirmed the formation of these compounds. ^{1}H NMR spectrum of compounds **7-9** displayed three singlets in different positions ranging from 4.83 to 5.62 for NH_2 groups. Compound **9** showed a singlet peak at 2.36 ppm for CH_3 group and a singlet peak at 4.25 ppm corresponding to NH_2.

Antimicrobial activity assays

Antibacterial activity

After successful synthesis and elucidation of the chemical structures of the newly synthesized compounds, the *in vitro* screening for their antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* bacteria was performed. The obtained results are presented in Table 1. Structure-activity relationship (SAR) studies allowed a better understanding of the relationship between the chemical structure of the synthesized compounds and their corresponding biological activity against the tested bacteria.



Scheme 4. Synthetic routes for the compounds 7-9.

Most of the synthesized compounds displayed moderate to good activities and the SARs of these compounds were discussed. Among these compounds, compounds **2b** and **3b** showed comparable efficacy as ampicillin against *Bacillus subtilis* strains (d= 25 mm/mg sample). Firstly, the introduction of carbonyl group on the 5-position of pyrazole moiety of compound **2b** and phenylpyrazole moiety of compound **3b** afforded more potent antibacterial activities against the tested strains than compounds **2a,c** and **3a,c** containing amino and methyl groups on the 5-position (Table 1, Figure 1). Meanwhile, the relationship between various substituents on the 5-position of pyrazole ring were investigated, the presence of electro-donating groups such as amino **2a** and methyl **2c** showed lower activities against the bacterial strains compared to compound **2b** having a carbonyl withdrawing group in 5-position of the pyrazole ring.

Secondly, compounds **4a** and **5a** with C=O group on the 2-position of pyrimidine moiety exhibited higher activity against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa bacteria* strains compared to compounds **4b** and **5b** containing C=S group on the 2-position. The substitution of amino groups on 4-position in compounds **4a,b**, and **5a,b** with a carbonyl group in **6a,b** improved the antibacterial activity against *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa bacteria.* It is also noteworthy to indicate that compound **6a** bearing a carbonyl group on 2-position of pyrimidine ring exhibited higher antibacterial properties against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* strains than compound **6b** possessing a thiol (C=S) group on the same position.

These finding ascertain that the presence of more withdrawing groups on the 5-position of pyrazole moiety **2b, 3b** and on 2-position of pyrimidine ring **4a, 5a** and **6a** of the synthesized compounds enhanced their antibacterial activities against the tested bacterial strains. Compound **9** exhibited better antibacterial activity against *Escherichia coli,* this finding was assigned to the absence of electro-donating amino group on 6-position and to the presence of methyl group in 6-position of the pyrimidine ring. Furthermore, compound **7** exhibited higher antibacterial activity against *Bacillus subtilis* compared to **8** and **9**.

Table 1

Antibacterial activity of the synthesized compounds.

Sample	*Bacterial strains*			
	Gram-positive		*Gram-negative*	
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
	Mean inhibition zone diameter (mm/mg sample) (n=3)			
1a	15	14	14	12
1b	13	12	14	11
1c	12	13	13	11
2a	18	**1**5	15	13
2b	25	19	18	15
2c	19	14	15	15
3a	18	17	14	16
3b	25	18	14	15
3c	19	15	13	12
4a	17	15	12	11
4b	16	14	13	15
5a	15	13	13	14
5b	14	13	11	13
6a	18	15	13	12
6b	12	11	10	12
7	19	14	13	16
8	17	14	12	14
9	16	14	15	13
Control (DMSO)	0	0	0	0
Ampicillin	27	23	25	26

Antifungal activity

Antifungal properties of the synthesized compounds were tested against *Aspergillus flavus* and *Candida albicans* fungi using the standard Amphotericin B antifungal agent. The obtained results are summarized in Table 2. Antifungal data of targeted compounds have obviously shown that distinct electronic varieties are responsible for wide spectrum activity.

Table 2

Antifungal activity of the synthesized compounds.

Sample	*Fungi*	
	Aspergillus flavus	*Candida albicans*
	Mean inhibition zone diameter (mm/mg sample) (n=3)	
Control (DMSO)	0	0
Amphotericin B	15	19
1a	12	15
1b	11	15
1c	10	12
2a	14	13
2b	14	15
2c	12	10
3a	13	17
3b	16	18
3c	12	17
4a	13	16
4b	14	17
5a	13	14
5b	11	12
6a	13	15
6b	12	11
7	15	18
8	13	14
9	14	18

Compounds **3b, 4b, 7** and **9** displayed similar antifungal activities as that of standard Amphotericin B against *Candida albicans* and *Aspergillus flavus* fungi. Compound **3b** afforded more important antifungal activity against the tested fungi compared to compounds **3a** and **3c**, this finding was attributed to the presence of the carbonyl withdrawing group on the 5-position of the phenylpyrazole moiety of compound **3b**. On the other hand, the antifungal activity of compound **4b** against *Candida albicans* was associated with the presence of the C=S group on the 2-position of the pyrimidine ring. SARs studies also indicated that the introduction of the electron releasing groups such as amino (2,4,6-position) in compound **7** and methyl (4-position) in compound **9** enhanced their antifungal activity against *Candida albicans* fungi.

Conclusions

In this study, novel pyrazole, imidazole, pyrimidine derivatives bearing imidazo[4,5-b]indol moiety were successfully synthesized and their chemical structures were identified and confirmed by different spectral techniques. All the synthesized compounds were assessed for their antibacterial activities against four bacterial strains (*Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa*) and antifungal activities against two fungi (*Aspergillus flavus and Candida albicans*).

Structure-activity relationship studies revealed that the introduction of the electron-withdrawing group contributes substantially to the antibacterial activity of the synthesized compounds. It was interesting to note that the compounds bearing a carbonyl group on 5-position of pyrazole moiety **2b**, phenylpyrazole moiety **3b** displayed outstanding antibacterial activity against *Bacillus subtilis* bacteria and are almost similar to the Ampicillin standard. Furthermore, the presence of carbonyl group in 2-position of the pyrimidine ring of compounds **4a**, **5a** and **6a** has significantly improved their antibacterial activity against *Bacillus subtilis* bacteria and the other tested bacterial strains.

The antifungal studies indicated that compounds **3b**, **4b**, **7** and **9** displayed comparable antifungal activity to that of standard Amphotericin B against *Candida albicans* and *Aspergillus flavus* fungi. Structure-activity relationship studies indicated that the presence of electrodonating groups such as amino (2,4,6-position) in compound **7** and methyl (4-position) in compound **9** enhanced their antifungal activity against *Candida albicans* fungi. These synthesized compounds could find fruitful applications as antibacterial and antifungal agents in pharmaceutical chemistry and additional studies are currently conducted by our group to evaluate their anti-inflammatory and anti-cancer activity.

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