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Utility of 4-arylidene-3-methyl-1*H*-pyrazole on Michael Addition reactions for biological evaluation

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ABSTRACT

*As an effort to synthesize new heterocyclic compounds, which would expected to have pharmacological and biological activities. We report here the reactivity of 1H-indazole-5-carboxamide **2**, as Michael acceptors under different conditions, towards different Michael donors namely, hydrazine hydrate, hydroxylamine hydrochloride and O-phenylene diamine forming adducts **4** – **8**. Pyrazolopyrimidine derivative **9** used as precursor for preparation some fused heterocyclic compounds **10** – **12**. The structures of the products obtained were confirmed by elemental analysis, IR, ^{1}HNMR, ^{13}C NMR and mass spectra. The biological activities for some synthesized products are screened.*

Key words: Pyrazolopyrimidine, Indazole, Pyrazolopyrazole, Antimicrobial activity.

INTRODUCTION

Pyrazoles, benzodiazepines, pyrazolopyrimidines and thiazolopyrimidines are of current interest by versatile biological activities. Pyrazoles moieties possess anticancer activity against various human cancer cells [1], active against human cytomegalo virus [2] and hydroxylase inhibitor [3]. Benzodiazepines (Zalephon) drug have been shown to improve sleep variable, sedative-hyponotic agent [4] and tolerant to wheat powdery mildew disease [5]. Pyrazolopyrimidines derivatives have been found to possess antitumor and antileukemia activity [6-9] and are used as GSK-3 inhibitors [10], cyclotoxic toward parasticprotozoa [11], incorporated into DNA [12]. Thiazolopyrimidines possess kinase inhibitors [13], in addition to microbial activities [14]. In view of the above and in continuation of our search program [15-21]. This information encouraged us to synthesis some new pyrazolopyrimidine, benzodiazepine and pyrazolothiazolopyrimidine to obtain new functions in an attempt to improve their biological activities.

MATERIALS AND METHODS

Experimental

Chemistry

All melting points are uncorrected and measured using Electro-thermal IA 9100 apparatus, (Shimadzu, Tokyo, Japan). IR spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 spectrophotometer (Perkin-Elmer, Norwalk, CT, USA). [^{13}C and ^{1}H] NMR were determined on a Jeol-Ex-270 NMR spectrometer (JEOL, Tokyo, Japan) and chemical shifts were expressed as part per million; ppm (δ values) against TMS as internal reference. Mass spectra were recorded on VG 2AM-3F mass spectrometer (Thermo electron corporation, USA) Microanalyses were operated using Mario El Mentar apparatus, Organic Microanalysis Unit, and the results were within the accepted range (± 0.2%) of the calculated values. Follow up of the reactions and checking the purity of the compounds was made by TLC on silica gel-coated aluminum sheets (Type 60 F254, Merck, Darmstadt, Germany).

4-(Aryl)-3-methyl-6-oxo-*N*-phenyl-3a,4,5,6-tetrahydro-1*H*-indazole-5-carbox-amide (2a,b)

A mixture of compound **1a,b** (0.01 mole), acetoacetanilide (1.77 g, 0.01 mole) was refluxed in 50 mL ethanol containing sodium hydroxide (0.89 g, 0.02 mole) for 8 h. The solvent was evaporated under vacuum and the solid residue was separated by column as follows:
2a (20% chloroform & 80% pet. ether 60 – 80)
2b (10% ethyl acetate & 90% pet. ether 60 – 80)

4-(Aryl)-5-methyl-3a,4,4a,7-tetrahydro-2*H*-benzo[1,2-*C*; 5, 4-c`]dipyrazolo-3-one (4a,b)

A mixture of compound **2a,b** (0.01 mole) and hydrazine hydrate (0.64 g, 0.02 mole) was refluxed in 30 mL glacial acetic acid for 4 h. The reaction mixture was cooled and separated solid was filtered off and recrystallized from acetic acid to give compounds **4a,b** respectively.

4-(Aryl)-4, 4a-dihydro-5-methyl-3a*H*-isoxazolo[4,3-*f*]indazol-3(7*H*)-one (5a,b)

A mixture of compound **2a,b** (0.01 mole) hydroxylamine hydrochloride (0.7 g, 0.01 mole) was refluxed in 30 mL glacial acetic acid contain sodium acetate (0.86 g, 0.01 mole) for 3 h. The reaction mixture was cooled, poured into cold water 100 mL. The formed solid was filtered off and recrystallized from the proper solvent to give compounds **5a,b** respectively.

4-(Aryl)-6-hydrozono-3-methyl-*N*-phenyl-3a,4,5,6-tetra-hydro-1*H*-indazole-5-carboxamide (6a,b)

A mixture of compound **2a,b** (0.01 mole) and hydrazine hydrate (0.64 g, 0.02 mole) was refluxed in 50 mL absolute ethanol for 2 h. The solvent was concentrated under reduced pressure. The formed solid was formed was recrystallized from the proper solvent to give compounds **6a,b** respectively.

4-(Aryl)-6-hydroxyimino-3-methyl-*N*-phenyl-3a,4,5,6-tetra-hydro-1*H*-indazole-5-carboxyamide (7a,b)

A mixture of compound **2a,b** (0.01 mole), hydroxylamine hydrochloride (0.7 g, 0.01 mole) and sodium acetate (0.86 g, 0.01 mole) was refluxed in ethanol 50 mL for 3 h. The reaction mixture was cooled, poured into cold water and recrystallized from methanol to give compounds **7a,b** respectively.

4-(Aryl)-3-methyl-3a,4,5,6-tetrahydro-indazolo[3,4-d]benzo[2,3-a]diazepin-5-one (8a,b)

A mixture of compound **2a,b** (0.01 mole) and *O*-phenylene diamine (1.08 g, 0.01 mole) was refluxed in glacial acetic acid 30 mL for 2 h. The resulting solid was filtered and recrystallized from dioxane to give compounds **8a,b** respectively.

4-(Aryl)-4,5-dihydro-3-methyl-pyrazolo[3,4-d]pyrimidine-6-thione (9a,b)

A mixture of (3-methyl-4-arylidene)pyrazol-5-one **1a,b** (0.01 mole) and thiourea (1.14 g, 0.015 mole) was refluxed in 30 mL absolute ethanol contaning sodium hydroxide (0.04 g, 0.01 mole) for 4 h. The reaction mixture was cooled and poured into water. The formed solid was filtered off and recrystallized from dioxane to give compounds **9a,b** respectively.

4-(Aryl)-1,4-dihydro-3-methyl-pyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidine-7-one (10a,b)

A mixture of compound **9a,b** (0.01 mole), chloroacetic acid (0.96 g, 0.01 mole) and anhydrous sodium acetate (3 g) was refluxed in glacial acetic acid (30 mL)/ acetic anhydride (10 mL) for 3 h. The reaction mixture was cooled and poured gradually with stirring into cooled water 100 mL. The formed solid was filtered off and recrystallized from the proper solvent to give compounds **10a,b** respectively.

4-(Aryl)-3,6-dimethyl-1,4-dihydro-pyrazolo[3,4-*d*]thiazolo[3,2-*a*]pyrimidine-7-one (11a,b)

A mixture of compound **9a,b** (0.01 mole), α-bromopropionic acid (1.54 g, 0.01 mole) and anhydrous sodium acetate (3 g) was refluxed in glacial acetic acid (30 mL)/ acetic anhydride (10 mL) for 3 h. The reaction mixture was cooled and poured gradually with stirring into cooled water 100 mL. The formed solid was filtered off and recrystallized from ethanol to give compounds **11a,b** respectively.

4-(Aryl)-6-benzylidene-1,4-dihydro-3-methyl-pyrazolo[3,4-d] thiazolo[3,2-a] pyrimidine-7-one (12a,b)

A mixture of compound **10a,b** (0.01 mole), benzaldehyde (0.94 g, 0.01 mole) and anhydrous sodium acetate (3 g) was refluxed in glacial acetic acid (30 mL)/ acetic anhydride (10 mL) for 3 h. The reaction mixture was cooled and poured gradually with stirring into cooled water 100 mL. The formed solid was filtered off and recrystallized from dioxane to give compounds **12a,b** respectively.

Antimicrobial activity

In vitro antimicrobial screening

The newly synthesized compounds were screened for their antibacterial activity against one gram negative bacteria, *Escherichia coli* NRRL B-210 and one gram positive bacteria *Bacillus subtilis* NRRL B-543, and yeast *Candida albican*s NRRL Y-477. These microorganisms were obtained from Northern Utilisation Research and Development Division, U.S. Departement of Agricultural Peoria, Illinois, USA. Chloramphenicol and Fluconazole were purchased (pure form) from Egyptian market and used in a concentration of 2 mg/ml as references for antibacterial and antifungal activities. These compounds were assayed by the agar diffusion method [23]. The assay medium flasks containing 50 mL of nutrient agar medium for bacteria and Czapek's-Dox agar media for yeast. The holes each of 9 mm diameter were made by scooping out medium with a sterilized cork borer in a Petri dish which was seeded with the organisms. The solutions of each test compound (0.10 mL) were added separately in the holes and Petri dishes were subsequently incubated. The incubation was carried out at 37 °C for 24h. Simultaneously, controls were maintained by employing 0.10 mL of dimethylsulfoxide (DMSO) which did not reveal any inhibition and zones of inhibition produced by each compound was measured in mm. The results of antimicrobial studies are given in Table 3.

Minimal inhibitory concentration (MIC) measurement

Minimum inhibitory concentration (MIC) of the test compounds was determined by agar streak dilution method. Stock solutions of the synthesized compounds were made using

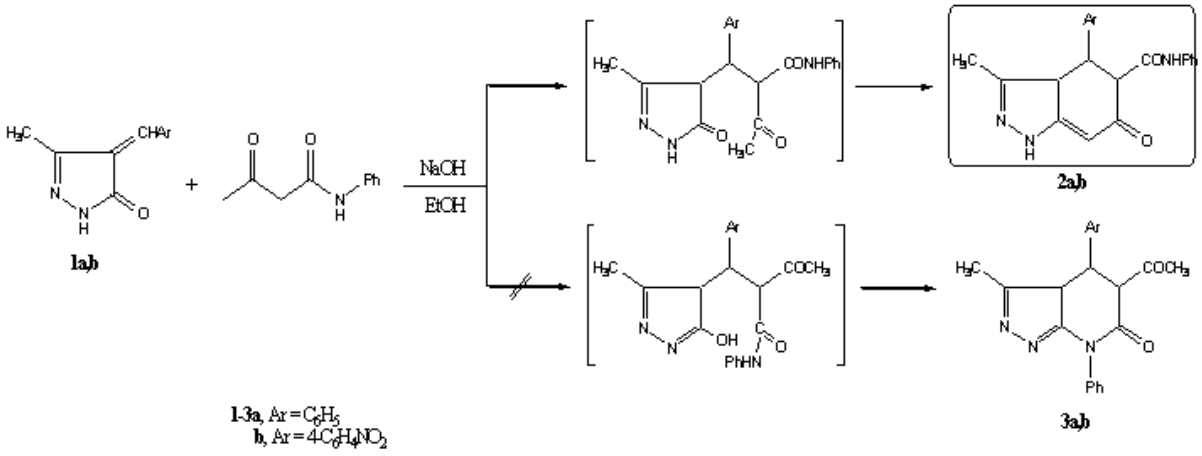
DMSO as a solvent (68 $mgmL^{-1}$). From this stock solution, the sense of concentrations prepared was (0.17, 0.34, 0.68, 0.85 and 1.7 $mgmL^{-1}$) of the tested compounds solutions were mixed with known quantities of molten sterile agar medium aseptically. About 20 mL of the medium containing the tested compound was dispensed into sterile Petri dish. Then the medium was allowed to get solidified. Microorganisms were then streaked one by one on the agar plates aseptically. After streaking all the plates were incubated at 37 °C for 24-48 h for antibacterial and antifungal activity, respectively. The lowest concentration of the synthesized compounds above which inhibite the growth of the given bacteria/fungus was considered as minimum inhibitory concentration (MIC) of the test compounds. The MIC values are tabulated in Table 4.

RESULTS AND DISCUSSION

Chemistry

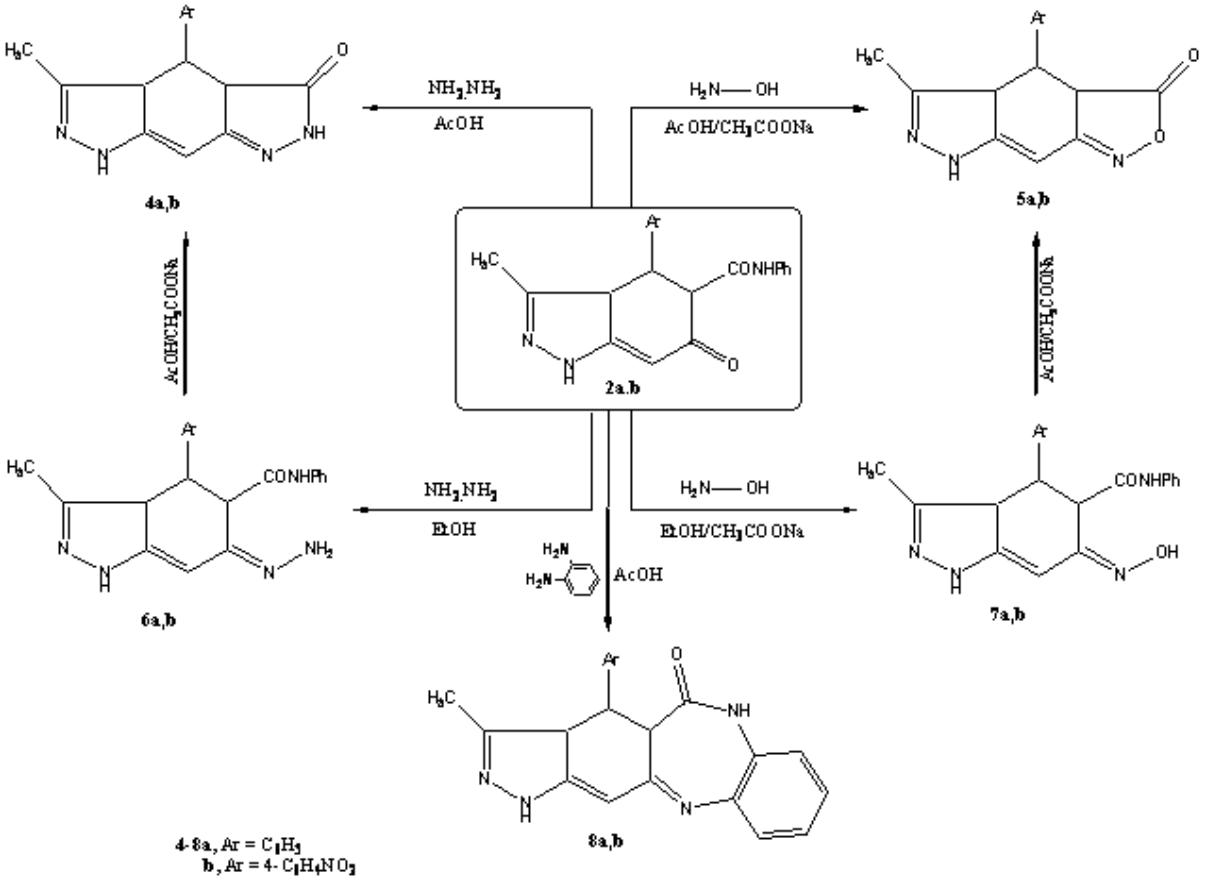
4-Arylidene-3-methyl-1*H*-pyrazol-5-one derivatives (**1**) [22] were reacted with acetoacetanilide via Michael addition to give 1*H*-indazole-5-carboxamide **2** and not pyrazolopyridine derivative **3** (Scheme 1). The structures of all products were based on elemental analysis, IR, [^{1}H, ^{13}C NMR] and Mass spectral data. The IR spectrum of compound **2a** showed the absorption bands at 3153, 3168 for 2NH and 1690, 1703 for 2C=O; ^{1}H NMR showed signals at δ 2.5, 3.2, 3.7 and 4.8 ppm for cyclohexenone ring protons and at δ 4.28, 9.5 ppm for 2NH (exchangeable with D_2O); ^{13}C NMR spectrum revealed that signals at δ 21.5, 172.12 and 194.80 ppm for CH_3 and 2C=O groups; the mass spectrum showed its molecular ion peaks at m/z (%) 345 (56), 226 (100) due to expulsion of anilide. When compounds **2a,b** reacted with hydrazine hydrate or hydroxylamine hydrochloride in acetic acid was afforded pyrazolone and oxazolone derivatives **4**, **5** respectively (Scheme 2). The mass spectrum of compound **4b** showed the molecular ion peak at m/z (%) 311 (89).

Scheme 1.



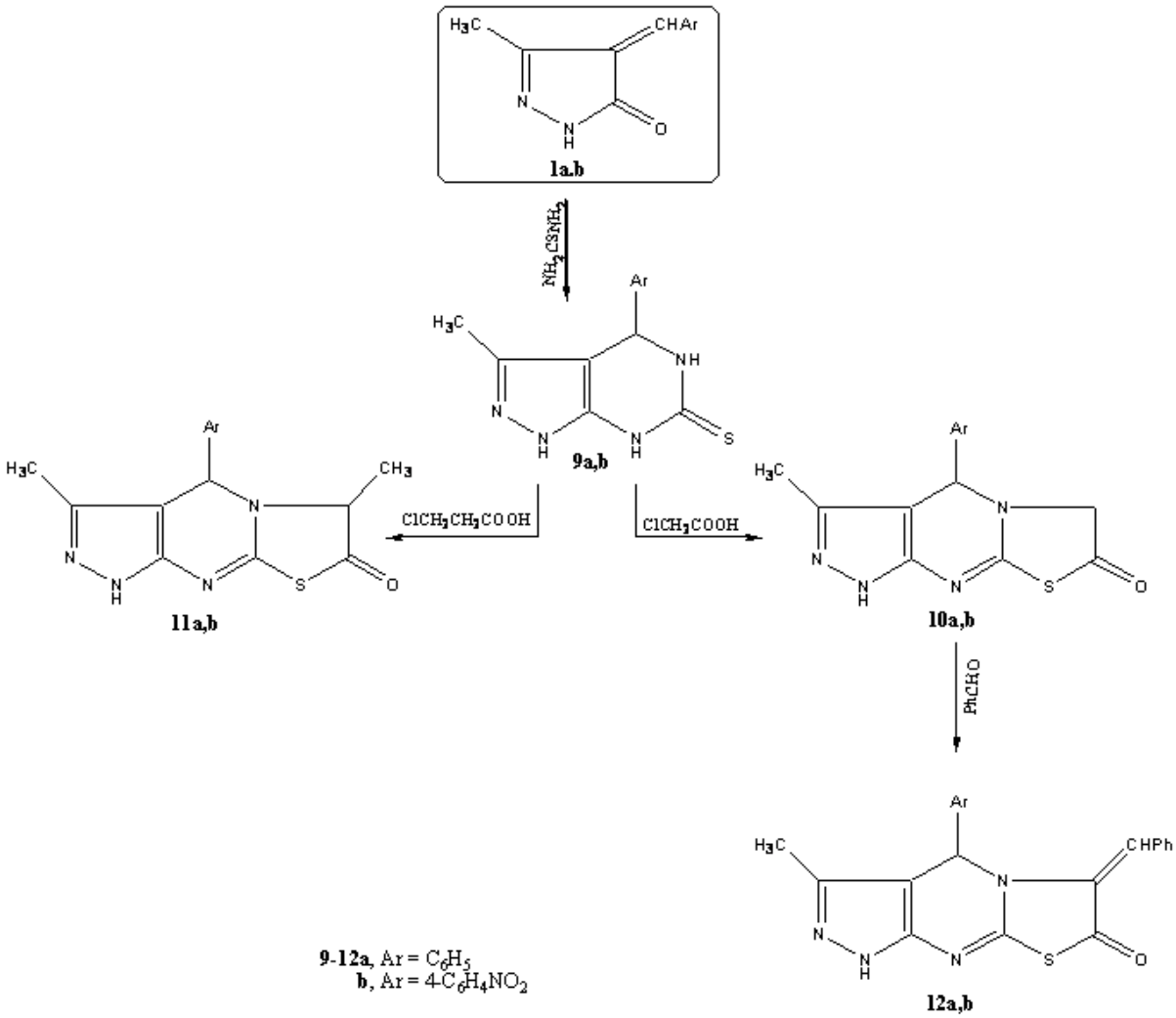
The latter compound may be involved formation of hydrozone or oxime firstly then cyclization to pyrazolone or oxazolone under the effect of acetic acid and sodium acetate. In order to identify such the mechanism, compound **2** was treated with hydrazine hydrate or hydroxylamine hydrochloride in ethanol (see exp. Part), it afforded hydrazone and oximes **6**, **7** respectively. As example the mass spectrum of **6a** showed molecular ion peak at m/z (%) 359 (75). ^{1}H NMR of **6b** showed signals at δ 1.03 ppm for CH_3 and at δ 2.46, 2.69, 3.24 and 3.97 ppm for cyclohexenone ring protons and at δ 4.2, 4.58 and 9.87 ppm for 2NH and NH_2 (exchangeable with D_2O). When compounds **6**, **7** were boiled in acetic acid containing

anhydrous sodium acetate they afforded **4**, **5** respectively which were established by melting point and T.L.C. Also, when compound **2** reacted with *O*-phenylenediamine in boiling acetic acid gave benzodiazepinone derivative **8a,b**. The mass spectrum of **8a** as example, showed the molecular ion peak at m/z (%) 342 (60); ^{1}H NMR showed signals at δ 0.95 ppm for CH_3 and at δ 2.43, 3.19, 3.41 and 3.87 ppm for cyclohexenone ring protons and at δ 9.6 and 10.3 ppm for 2NH (exchangeable with D_2O).



Scheme 2.

On the other hand, compound **1** was reacted with thiourea via Michael condensation to give pyrazolopyrimidine derivative **9 (**Scheme 3.). The IR spectrum of compound **9a** showed characteristic absorption bands for NH, CS and the absence of CO. Also, ^{1}H NMR spectra showed signals at δ 9.6, 10.5, 11.2 ppm due to 3NH (exchangeable with D_2O); ^{13}C NMR spectrum showed signals at δ 16.35 and 178.95 ppm for CH_3 and C=S; the mass spectrum gave the molecular ion peak at m/z (%) 244 (100) supporting its molecular formula. When compound **9** reacted with chloroacetic acid and α-bromopropionic acid afforded pyrazolothiazolopyrimidinone derivatives **10**, **11** respectively. ^{1}H NMR for compound **10a** as example showed signals at δ 3.42 ppm characteristic for thiazolone protons and its mass spectrum gave the molecular ion peak at m/z (%) 284 (73). While the IR of compound **11a** showed absorption bands at 3155, 1696 for NH and C=O respectively. On the other hand, compound **10a** was confirmed chemically via condensation with benzaldehyde to afford 6-benzylidene-pyrazolo[3,4]thiazolo[3, 2-a]pyrimidine derivative (**12a,b**). The mass spectrum of **12a** gave the molecular ion peak at m/z (%) 372 (63). The structures of all prepared compounds were confirmed via elemental analysis and spectral data (**Tables 1, 2**).



Scheme 3.

Table 1. Physical and analytical properties of the new compounds

Compd. No.	Formula (M. wt.)	M.p. (°C) (Cryst. solvent)	Yield (%)	Analysis (%) (calcd./found) C	H	N	S
2a	$C_{21}H_{19}N_3O_2$	171-172	48	73.03	5.54	12.17	—
	(345.39)			72.96	5.50	12.09	
2b	$C_{21}H_{18}N_4O_4$	203-204	50	64.61	4.65	14.35	—
	(390.39)			64.65	4.59	14.40	
4a	$C_{15}H_{14}N_4O$	256-257	63	67.65	5.30	21.04	—
	(266.30)	Acetic acid		67.59	5.24	20.99	
4b	$C_{15}H_{13}N_5O_3$	289-290	73	57.87	4.21	22.50	—
	(311.30)	Acetic acid		57.90	4.25	22.42	
5a	$C_{15}H_{13}N_3O_2$	233-234	65	67.40	4.90	15.72	—
	(267.28)	Benzene		67.48	4.85	15.80	
68	$C_{15}H_{12}N_4O_5$	296-298	60	57.68	3.87	17.94	—
	(312.34)	Dioxane		57.74	3.81	17.99	
6a	$C_{21}H_{21}N_5O$	189-190	70	70.17	5.89	19.48	—
	(359.42)	Methanol		70.09	5.92	19.40	
6b	$C_{21}H_{20}N_6O_3$	258-259	61	62.37	4.98	20.78	—
	(404.42)	Ethanol		62.44	5.02	20.80	
7a	$C_{21}H_{19}N_4O_2$	181-182	60	69.98	5.59	15.55	—
	(360.41)	Methanol		70.02	5.62	15.49	
7b	$C_{21}H_{19}N_5O_4$	234-235	52	62.22	4.72	17.27	—
	(405.41)	Methanol		62.15	4.68	17.36	
8a	$C_{21}H_{18}N_4O$	262-263	51	73.67	5.30	16.36	—

	(342.39)	Dioxane		73.60	5.39	16.45	
8b	$C_{21}H_{17}N_5O_3$	303-304	60	65.11	4.42	18.08	—
	(387.39)	Dioxane		65.02	4.37	17.99	
9a	$C_{12}H_{12}N_4S$	223-224	86	58.99	4.95	22.93	13.12
	(244.32)	Dioxane		59.02	4.97	23.00	13.20
9b	$C_{12}H_{11}N_5O_2S$	288-289	53	49.82	3.83	24.21	11.08
	(289.31)	Dioxane		49.78	3.91	24.27	11.01
10a	$C_{14}H_{12}N_4OS$	192-193	67	59.14	4.25	19.70	11.28
	(284.34)	Ethanol		59.20	4.30	19.62	11.24
10b	$C_{14}H_{11}N_5O_3S$	278-280	78	51.06	3.37	21.27	9.74
	(329.34)	Dioxane		51.10	3.42	21.24	9.80
11a	$C_{15}H_{14}N_4OS$	180-181	72	60.38	4.73	18.78	10.75
	(298.36)	Ethanol		60.30	4.80	18.84	10.69
11b	$C_{15}H_{13}N_5O_3S$	242-243	67	52.47	3.82	20.40	9.34
	(343.36)	Ethanol		52.52	3.73	20.48	9.30
12a	$C_{21}H_{16}N_4OS$	276-277	68	67.72	4.33	15.04	8.61
	(372.44)	Dioxane		67.65	4.39	14.98	8.65
12b	$C_{21}H_{15}N_5O_3S$	292-293	67	60.42	3.62	16.78	7.68
	(417.44)	Dioxane		60.40	3.69	16.80	7.71

Table 2. Spectral characterization of the new compounds

Compd. No.	IR (KBr, cm-1), ^{1}H, ^{13}C NMR (CDCl3, δ ppm) & MS [m/z (%)]	
2a	IR	3153, 3168 (2NH), 1690, 1703 (2C=O);
	^{1}H.NMR	1.1 (s, 3H, CH_3), 2.51 (d, 1H, *J*= 2.4 *Hz*, cyclohexene-H), 3.20 (t, 1H, *J*= 2.3 *Hz*, cyclohexene-H), 3.73 (d, 1H, *J*= 2.6 *Hz*, cyclohexene-H), 4.28 (s, 1H, NH exchangeable with D_2O), 4.80 (s, 1H, cyclohexene-H), 7.0-7.74 (m, 10H, Ar-H), 9.5 (s, 1H, NH exchangeable with D_2O)
	^{13}C.NMR	21.5 (CH_3), 22.3, 45.10, 63.53 (3CH), 100.15-148.52 (12Ar-C + 2 ethylenic-C), 155.56 (C=N), 172.12, 194.80 (2C=O)
	MS	345 [M^+, 56], 226 (100)
2b	IR	3160, 3175 (2NH), 1687, 1709 (2C=O)
	^{1}H.NMR	1.0 (s, 3H, CH_3), 2.45 (d, 1H, *J*= 2.5 *Hz*, cyclohexene-H), 3.1 (t, 1H, *J*= 2.3 *Hz*, cyclohexene-H), 3.89 (d, 1H, *J*= 2.3 *Hz*, cyclohexene-H), 4.31 (s, 1H, NH exchangeable with D_2O), 4.72 (s, 1H, cyclohexene-H), 7.1-7.57 (m, 9H, Ar-H), 9.3 (s, 1H, NH exchangeable with D_2O)
	MS	390 [M^+, 75], 345 (81), 226 (56)
4a	IR	3150, 3217 (2NH), 1700 (C=O)
	^{1}H.NMR	1.0 (s, 3H, CH_3), 2.6 (d, 1H, *J*= 2.4 *Hz*, cyclohexene-H), 2.8 (d, 1H, *J*= 2.7 *Hz*, cyclohexene-H), 3.2 (t, 1H, *J*= 2.4 *Hz*, cyclohexene-H), 3.7 (s, 1H, cyclohexene-H), 7.02-7.20 (m, 5H, Ar-H), 9.6, 9.9 (2s, 2H, 2NH exchangeable with D_2O)
	MS	266 [M^+, 100]
4b	IR	3159, 3197 (2NH), 1714 (C=O)
	^{1}H.NMR	1.12 (s, 3H, CH_3), 2.49 (d, 1H, *J*= 2.3 *Hz*, cyclohexene-H), 2.85 (d, 1H, *J*= 2.8 *Hz*, cyclohexene-H), 3.31 (t, 1H, *J*= 2.4 *Hz*, cyclohexene-H), 3.84 (s, 1H, cyclohexene-H), 7.25 (d, 2H, *J*= 8.5 *Hz*, Ar-H), 7.46 (d, 2H, *J*= 8.5 *Hz*, Ar-H), 9.5, 10.0 (2s, 2H, 2NH exchangeable with D_2O)
	^{13}C.NMR	19.81 (CH_3), 23.5, 46.75, 49.17 (3CH), 105.6-153.9 (6Ar-C + 2 ethylenic-C), 155.31, 155.45 (2C=N), 176.10 (C=O)
	MS	311[M^+, 89]
5a	IR	3157 (NH), 1705 (C=O)
	^{1}H.NMR	0.9 (s, 3H, CH_3), 2.44 (d, 1H, *J*= 2.4 *Hz*, cyclohexene-H), 2.78 (d, 1H, *J*= 2.6 *Hz*,cyclohexene-H), 3.42 (t, 1H, *J*= 2.5 *Hz*, cyclohexene-H), 3.70 (s, 1H, cyclohexene-H), 7.03-7.15 (m, 5H,

		Ar-H), 9.8 (s, 1H, NH exchangeable with D_2O)
	MS	267 [M^+, 95]
5b	IR	3181 (NH), 1698 (C=O)
	^{1}H.NMR	1.1 (s, 3H, CH_3), 2.52 (d, 1H, *J*= 2.4 *Hz*, cyclohexene-H), 2.85 (d, 1H, *J*= 2.4 *Hz*, cyclohexene-H), 3.36 (t, 1H, *J*= 2.5 *Hz*, cyclohexene-H), 3.8 (s, 1H, cyclohexene-H), 7.19 (d, 2H, *J*= 8.8 *Hz*, Ar-H), 7.35 (d, 2H, *J*= 8.8 *Hz*, Ar-H), 9.61 (s, 1H, NH exchangeable with D_2O)
	^{13}C.NMR	20.18 (CH_3), 22.20, 47.25, 50.13 (3CH), 107.1-158.32 (6Ar-C + 2 ethylenic-C), 156.21, 159.03 (2C=N), 177.16 (C=O)
	MS	312 [M^+, 55]
6a	IR	3150, 3217, 3360 (NH, NH_2), 1688 (C=O)
	^{1}H.NMR	0.93 (s, 3H, CH_3), 2.51 (d, 1H, *J*= 2.4 *Hz*, cyclohexene-H), 2.86 (d, 1H, *J*= 2.5 *Hz*, cyclohexene-H), 3.1 (t, 1H, *J*= 2.4 *Hz*, cyclohexene-H), 3.82 (s, 1H, cyclohexene-H), 4.10 (s, 1H, NH exchangeable with D_2O), 4.52 (s, 2H, NH_2 exchangeable with D_2O), 7.1-7.8 (m, 10H, Ar-H), 9.64 (s, 1H, NH exchangeable with D_2O)
	^{13}C.NMR	18.99 (CH_3), 21.48, 40.31, 46.01 (3CH), 105.9-152.9 (12Ar-C + 2 ethylenic-C), 155.40, 160.81 (2C=N), 172.86 (C=O)
	MS	359 [M^+, 75]
6b	IR	3142, 3198, 3331 (NH, NH_2), 1701 (C=O)
	^{1}H.NMR	1.03 (s, 3H, CH_3), 2.46 (d, 1H, *J*= 2.5 *Hz*, cyclohexene-H), 2.69 (d, 1H, *J*= 2.4 *Hz*, cyclohexene-H), 3.24 (t, 1H, *J*= 2.5 *Hz*, cyclohexene-H), 3.97 (s, 1H, cyclohexene-H), 4.2 (s, 1H, NH exchangeable with D_2O), 4.58 (s, 2H, NH_2 exchangeable with D_2O), 7.23-7.65 (m, 9H, Ar-H), 9.87 (s, 1H, NH exchangeable with D_2O)
	MS	404 [M^+, 59]
7a	IR	3160, 3205, 3450 (NH, OH)
	^{1}H.NMR	1.0 (s, 3H, CH_3), 2.56 (d, 1H, *J*= 2.6 *Hz*, cyclohexene-H), 2.80 (d, 1H, *J*= 2.6 *Hz*, cyclohexene-H), 3.22 (t, 1H, *J*= 2.5 *Hz*, cyclohexene-H), 3.84 (s, 1H, cyclohexene-H), 4.16 (s, 1H, NH exchangeable with D_2O), 7.08-7.68 (m, 10H, Ar-H), 9.5, 9.8 (2s, 2H, NH, OH exchangeable with D_2O)
	^{13}C.NMR	21.5 (CH_3), 22.54, 43.19, 46.74 (3CH), 105.1-157.59 (12Ar-C + 2 ethylenic-C), 154.90, 161.97 (2C=N), 172.21 (C=O)
	MS	360 [M^+, 85]
7b	IR	3175, 3195, 3469 (NH, OH)
	^{1}H.NMR	1.16 (s, 3H, CH_3), 2.49 (d, 1H, *J*= 2.5 *Hz*, cyclohexene-H), 2.86 (d, 1H, *J*= 2.5 *Hz*, cyclohexene-H), 3.32 (t, 1H, *J*= 2.5 *Hz*, cyclohexene-H), 3.81 (s, 1H, cyclohexene-H), 4.27 (s, 1H, NH exchangeable with D_2O), 7.17-7.74 (m, 9H, Ar-H), 9.39, 9.85 (2s, 2H, NH, OH exchangeable with D_2O)
	MS	405 [M^+, 73]
8a	IR	3175, 3156 (2NH), 1688 (C=O)
	^{1}H.NMR	0.95 (s, 3H, CH_3), 2.43 (d, 1H, *J*= 2.7 *Hz*, cyclohexene-H), 3.19 (t, 1H, *J*= 2.8 *Hz*, cyclohexene-H), 3.41 (d, 1H, *J*= 2.7 *Hz*, cyclohexene-H), 3.87 (s, 1H, cyclohexene-H), 6.54-7.51 (m, 9H, Ar-H), 9.6, 10.3 (2s, 2H, 2NH exchangeable with D_2O)
	MS	342 [M^+, 60]
8b	IR	3161, 3179 (2NH), 1692 (C=O)
	^{1}H.NMR	1.05 (s, 3H, CH_3), 2.59 (d, 1H, *J*= 2.8 *Hz*, cyclohexene-H), 3.02 (t, 1H, *J*= 2.8 *Hz*, cyclohexene-H), 3.35 (d, 1H, *J*= 2.8 *Hz*, cyclohexene-H), 3.74 (s, 1H, cyclohexene-H), 6.94-7.46 (m, 8H, Ar-H), 9.7, 10.0 (2s, 2H, 2NH exchangeable with D_2O)
	MS	387 [M^+, 80]
9a	IR	3157, 3212, 3250 (3NH), 1250 (C=S)
	^{1}H.NMR	2.8 (s, 3H, CH_3), 5.25 (s, 1H, pyrimidine-H), 7.0-7.2 (m, 5H, Ar-H), 9.6, 10.5, 11.2 (3s, 3H, 3NH exchangeable with D_2O)

	^{13}C.NMR	16.35 (CH_3), 45.37 (CH), 107.18-148.94 (8Ar-C), 149.96 (C=N), 178.95 (C=S)
	MS	244 [M^+, 100]
9b	IR	3168, 3198, 3241 (3NH), 1259 (C=S)
	^{1}H.NMR	2.71 (s, 3H, CH_3), 5.09 (s, 1H, pyrimidine-H), 7.39 (d, 2H, *J*= 8.8 *Hz*, Ar-H), 7.69 (d, 2H, *J*= 8.8 *Hz*, Ar-H), 9.38, 10.3, 11.05 (3s, 3H, 3NH exchangeable with D_2O)
	MS	289 [M^+, 60]
10a	IR	3160 (NH), 1690 (C=O)
	^{1}H.NMR	2.78 (s, 3H, CH_3), 3.42 (s, 2H, thiazole-H), 5.18 (s, 1H, pyrimidine-H), 7.05-7.15 (m, 5H, Ar-H), 9.7 (s, 1H, NH exchangeable with D_2O)
	MS	284 [M^+, 73]
10b	IR	3169 (NH), 1685 (C=O)
	^{1}H.NMR	2.59 (s, 3H, CH_3), 3.45 (s, 2H, thiazole-H), 5.29 (s, 1H, pyrimidine-H), 7.19 (d, 2H, *J*= 8.9 *Hz*, Ar-H), 7.49 (d, 2H, *J*= 8.9 *Hz*, Ar-H), 10.0 (s, 1H, NH exchangeable with D_2O)
	MS	329 [M^+, 62]
11a	IR	3155 (NH), 1696 (C=O)
	^{1}H.NMR	1.30 (s, 3H, CH_3), 2.79 (s, 3H, CH_3), 3.63 (s, 1H, thiazole-H), 5.19 (s, 1H, pyrimidine-H), 7.06-7.20 (m, 5H, Ar-H), 9.6 (s, 1H, NH exchangeable with D_2O)
	^{13}C.NMR	17.03, 19.36 ($2CH_3$), 48.86, 65.15 (2CH), 117.91-146.19 (6Ar-C + 2 ethylenic-C), 139.25, 159.52 (2C=N), 194.49 (C=O)
	MS	298 [M^+, 70]
11b	IR	3142 (NH), 1702 (C=O)
	^{1}H.NMR	0.98 (s, 3H, CH_3), 2.82 (s, 3H, CH_3), 3.68 (s, 1H, thiazole-H), 5.06 (s, 1H, pyrimidine-H), 7.16 (d, 2H, *J*= 9.0 *Hz*, Ar-H), 7.29 (d, 2H, *J*= 8.9 *Hz*, Ar-H), 10.2 (s, 1H, NH exchangeable with D_2O).
	MS	343 [M^+, 81]
12a	IR	3158 (NH), 1717 (C=O)
	^{1}H.NMR	2.74 (s, 3H, CH_3), 5.20 (s, 1H, pyrimidine-H), 7.0-7.32 (m, 11H, Ar-H, methylene proton), 9.5 (s, 1H, NH exchangeable with D_2O)
	MS	372 [M^+, 63]
12b	IR	3135 (NH), 1711 (C=O)
	^{1}H.NMR	2.62 (s, 3H, CH_3), 5.17 (s, 1H, pyrimidine-H), 7.24-7.39 (m, 10H, Ar-H, methylene proton), 9.59 (s, 1H, NH exchangeable with D_2O)
	MS	417 [M^+, 67]

Antimicrobial Activity

The *in vitro* antimicrobial activity of the tested compounds was evaluated by measuring the zone diameters and the results were compared with those of well known drugs (standard) (**Table 3, 4**). Among the tested compounds, for gram-positive and gram-negative bacteria, it was noticed that the indazole derivative **6a**, **7b** (IZ = 20–21 mm and MIC = 0.34 μg/ml) demonstrated inhibitory activities more than pyrazolone **4a,b** and oxazolone **5a,b** (IZ = 10–18 mm). However, the benzodiazepinone derivatives **8a,b** revealed the most significant antibacterial activities (IZ = 22–23 mm and MIC = 0.34–0.68 μg/ml). Pyrazolopyrimidine derivatives **9-12** show significant antibacterial activities (IZ = 15–19 mm and MIC = 0.68–0.85 μg/ml). On the other hand, the benzodiazepinone derivative **8a,b** revealed most effective antifungal activity than the other tested compounds showing (IZ = 18–19 mm and MIC = 1.7–0.85 μg/ml). In general, the benzodiazepinone derivative **8a,b** showed the highest antibacterial and antifungal potency among the tested compounds.

Table 3. The inhibition zones diameter (IZ) in mm.

Compound No.	*Escherichia coli*	*Bacillus subtilis*	*Candida albicans*
2a	–	–	12
4a	16	14	15
4b	18	16	11
5a	11	10	14
5b	10	12	10
6a	20	20	–
7b	21	20	–
8a	23	23	19
8b	23	22	18
9a	18	16	11
10a	17	19	14
10b	15	18	14
11a	–	–	10
11b	–	–	13
12a	16	16	–
12b	17	15	–
Chloramphenicol	24	24	–
Fluconazole	–	–	26

Highly active (inhibition zone > 20 mm); Moderately active; (inhibition zone16–19 mm); Slightly active (inhibition zone 11–15 mm);(–) no inhibition zone

Table 4. MIC in µg/ml of the most active compounds

Compound No.	*Escherichia coli*	*Bacillus subtilis*	*Candida albicans*
6a	0.34	0.34	1.7
7b	0.34	0.34	0.85
8a	0.68	0.34	1.7
8b	0.68	0.34	0.85
10a	0.85	0.85	0.85
10b	0.68	0.34	1.7
12a	0.68	0.68	1.7
12b	0.85	0.68	0.68
Chloramphenicol	0.12	0.15	–
fluconazole	–	–	0.03

CONCLUSION

The overall results indicated that, the tested compounds showed promising antimicrobial activity against bacteria and Fungi. Among the tested compounds, for gram-negative and gram-positive bacteria, it was noticed the indazole derivative **6a**, **7b** demonstrated inhibitory activities more than pyrazolone **4a,b** and oxazolone **5a,b**. However, the benzodiazepinone derivative **8a,b** and pyrazolopyrimidine derivatives **9-12** revealed significant antibacterial activities. On the other hand, the benzodiazepinone derivative **8a,b** revealed the most effective activity against yeast than the other tested compounds.

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