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Synthesis of A New Series of *N*-Substituted-3-Indolyl-Heterocycles for Antimicrobial Evaluation

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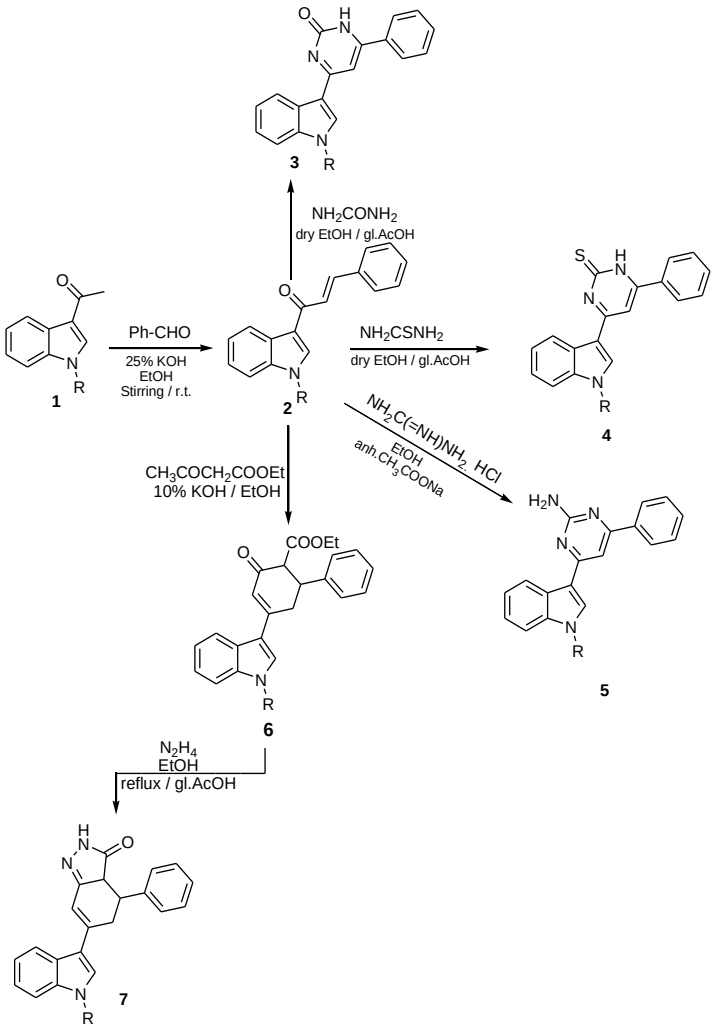
CLAISEN-SCHMIDT reaction of *N*-ethyl (1a), *N*-benzyl (1b), *N*-benzoyl (1c), *N*-methylsulphonyl (1d) and *N*-benzenesulphonyl-3-acetylindoles (1e) with benzaldehyde gave 1-(*N*-substituted-1*H*-indol-3-yl)-3-phenyl-prop-2-ene-1-ones(2a-e). Cyclocondensation of 2a-e with urea, thiourea or guanidine led to the formation of pyrimidine derivatives 3a-e -5a-e, respectively. Base catalyzed reaction of 2a-e with ethyl acetoacetate gave cyclohexanone derivatives 6a-e, which were reacted with hydrazine hydrate to afford the indazole derivatives 7a-e. On the other hand, reaction of 2a-c with some hydrazine derivatives namely, hydrazine hydrate, acetyl hydrazine, phenyl hydrazine and benzyl hydrazine hydrochloride yielded the pyrazole derivatives 8a-c–11a-c, respectively. Moreover, reaction of 2a-c with hydroxyl amine hydrochloride gave the isoxazole derivatives 12a-c. The newly synthesized compounds were tested for their antimicrobial activity and the results revealed that, 4-(*N*-benzyl-1*H*-indol-3-yl)-6-phenyl-pyrimidin-2-amine (5b) showed potent growth inhibition activity at a concentration of 20 and 10 µg against *Candida albicans* (ATCC 10231) compared to reference drug cycloheximide.

Keywords: Claisen-Schmidt reaction, 3-Acetylindoles, Pyrimidine, Pyrazole, Isoxazole and antimicrobial activity.

Chalcones, one of the major classes of natural products with widespread occurrence in fruits, vegetables, spices and soy-based foodstuff, have been reported to possess several biological activities[1,2]. An important feature of chalcones is their ability to act as an intermediate for the synthesis of biologically active heterocyclic compounds, *e.g.*, pyrimidine, cyclohexanone, pyrazole and isoxazole derivatives[3-6]. Additionally, indole which is the potent basic pharmacodynamic nucleus has been reported to possess a wide variety of biological properties such as, anti-inflammatory, anti-cancer and antimicrobial activities[7-10]. Based on these interesting biological activities and in continuation of our search[7,9,10], herein, we reported the synthesis of some new *N*-substituted-3-indolyl-heterocycles starting from *N*-substituted-3-indolylchalcones and evaluating their antimicrobial activity against some Gram-positive bacteria, Gram-negative bacteria and fungi.

Results and Discussion

Claisen-Schmidt reaction of *N*-ethyl (1a), *N*-benzyl (1b), *N*-benzoyl (1c), *N*-methylsulphonyl (1d) and *N*-benzenesulphonyl-3-acetylindoles (1e) with benzaldehyde in ethanol and in the presence of aqueous potassium hydroxide (25%) led to the formation of 1-(*N*-substituted-1*H*-indol-3-yl)-3-phenylprop-2-ene-1-ones (2a-e) (Scheme 1). Compounds 2d and 2e are previously reported[11] and the newly compounds 2a-c were confirmed by their correct elemental analyses and spectral data.



Scheme 1. Compounds 1-7; R, a= -CH_2CH_3; b=- CH_2Ph; c=-COPh; d=-SO2 CH_3; e=-SO2ph.

Cyclocondensation of 2a-e with urea in dry ethanol and in the presence of glacial acetic acid gave 4-(*N*-substituted-1*H*-indol-3-yl)-6-phenyl-pyrimidin-2(1*H*)-ones (3a-e) (Scheme 1). Similarly, reaction of chalcones 2a-e with thiourea gave pyrimidin-2(1*H*)-thiones (4a-e) (Scheme 1).

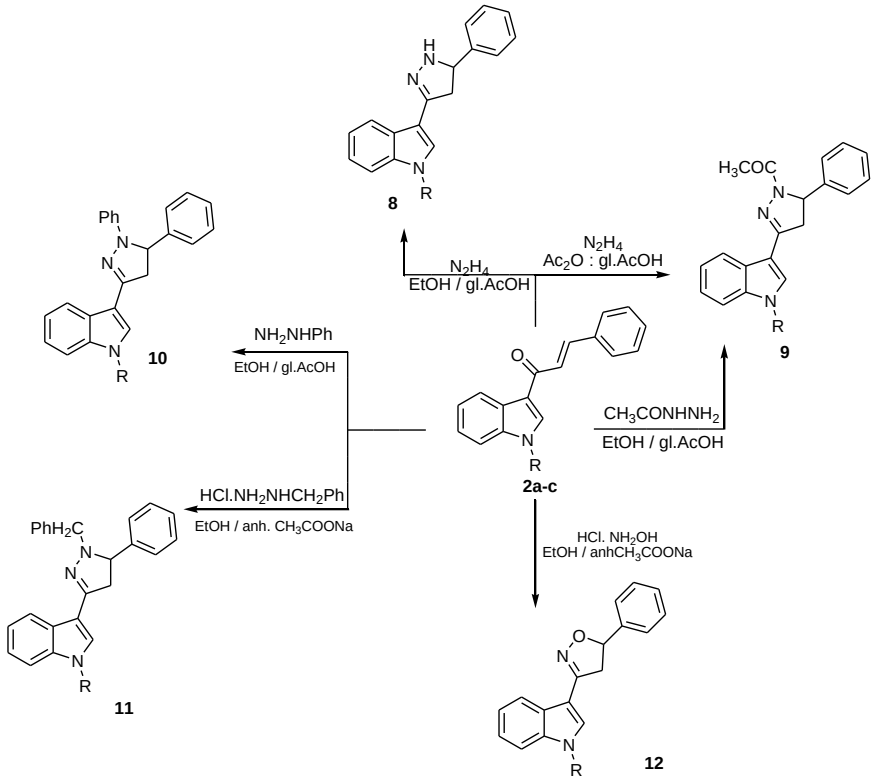
Moreover, reaction of 2a-e with guanidine hydrochloride in dry ethanol in the presence of anhydrous sodium acetate yielded 4-(*N*-substituted-1*H*-indol-3-yl)-6-phenyl-pyrimidin-2-amines (5a-e) (Scheme 1).

It has been reported that, reaction of α, B- unsaturated ketone with ethyl acetoacetate in the presence of aqueous potassium hydroxide 10 % gives rise to the corresponding cyclohexanone derivative [6]. In the present work and under the above mentioned conditions, compounds 2a-e reacted with ethyl acetoacetate (1:1) to give ethyl-4-(*N*-substituted-1*H*-indol-3-yl)-6-phenyl-2-oxo-cyclohexa-3-ene- carboxylates (6a-e) (Scheme 1).

The reaction of compound 6a with hydrazine hydrate under reflux in ethanol in the presence of glacial acetic acid gave compound with molecular ion peak at *m/z* = 355 (1%). Its IR (KBr) spectrum showed characteristic absorption band at 3350 (NH), 1645 (C=O), 1620 (C=N) & 1579 cm^{-1} (C=C) and its ^{1}H NMR (DMSO) revealed signals at 8.71 (s, NH), 8.23 (s, H-2 indole), 7.66-7.01 (m, Ar-H), 6.66 (s, CH=C), 4.44 (q, CH_2-N), 2.91 & 3.34 (2d, CH-CH) 2.19& 2.40 (dd, CH-$\underline{CH_2}$) and 1.61 ppm (t, CH_3). Based on these data, the assigned structure of the product was proposed as 4,5-dihydro-4-phenyl-6-(*N*-ethyl-1*H*-indol-3-yl)-2*H*-indazol-3(*H*)one (7a) (Scheme 1). So, similar to the preparation of indazole 7a, compounds 7b-e were prepared through the reaction of 6b-e with hydrazine hydrate (Scheme 1).

On the other hand, condensation of 2a-c with hydrazine hydrate in absolute ethanol in the presence of few drops of glacial acetic acid, afforded 4,5-dihydro-3-(*N*-substituted-1*H*-indol-3-yl)-5-phenyl-pyrazoles (8a-c) (Scheme 2). Whereas, reaction of 2a-c with hydrazine hydrate under reflux in a mixture of acetic anhydride and glacial acetic acid (2:1), afforded the corresponding *N*-acetylpyrazole derivatives 9a-c (Scheme 2). Incidentally, compounds 9a-c were obtained *via* the reaction of 2a-c with acetic acid hydrazide in absolute ethanol in the presence of few drops of glacial acetic acid which showed no depression in admixed m.p.s with that previously obtained (Scheme 2).

Additionally, reaction of 2a-c with phenylhydrazine gave *N*-phenylpyrazole derivatives 10a-c. While, reaction of 2a-c with benzylhydrazine hydrochloride in the presence of anhydrous sodium acetate gave *N*-benzylpyrazole derivatives 11a-c (Scheme 2). Furthermore, reaction of 2a-c with hydroxyl amine hydrochloride in the presence of anhydrous sodium acetate led to the formation of 4,5-dihydro-3-(*N*-substituted-1*H*-indol-3-yl)-5-phenyl-isoxazoles (12a-c) (Scheme 2).



Scheme 2. Compounds 2-12; R, a = -CH_2CH_3; b=-CH_2ph; e=-COph.

The newly synthesized compounds were confirmed by their correct elemental analyses (Table 1) as well as spectral data, IR, NMR and MS spectra (Table 2).

The antimicrobial activity of the newly synthesized compounds were evaluated against a variety of pathogenic microorganisms: *Salmonella typhimurium* (ATCC 14028), *Pseudomonas fluorescens* (S 97) (Gram-positive bacteria), *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6635) (Gram-negative bacteria) and two strain of fungi *Candida albicans* (ATCC 10231) and *Aspergillus fumigatus* (isolated from clinical samples and identified to the species level according to different API 20E system (Analytab Products) (bioMerieux, Australia) using the disk diffusion method [12] at a concentrations of 10 and 20 µg per disc. From the data obtained (Table 3) it is clear that, only compound 4-(*N*-benzyl-1*H*-indol-3-yl)-6-phenyl-pyrimidin-2-amine(5b), showed potent growth inhibition activity at a concentration of 20 and 10 µg against *Candida albicans* (ATCC 10231) compared to reference drug cycloheximide. While, the rest of synthesized compound showed no inhibition against all the tested microorganisms

TABLE 1. Physical and analytical properties of the newly synthesized compounds.

Compd. No.	Formula (M_r)	M.p. (°C)	Yield (%)	Analysis (%) (calculated / found)		
				C	H	N
2a	$C_{19}H_{17}NO$ (275.34)	78-80	71	82.88/83.05	6.22/6.01	5.09/5.21
2b	$C_{24}H_{19}NO$ (337.41)	105-7	77	85.43/85.25	5.68/5.46	4.15/4.32
2c	$C_{24}H_{17}NO_2$ (351.4)	168-70	80	82.03/82.21	4.88/5.00	3.99/3.78
3a	$C_{20}H_{17}N_3O$ (315.38)	97-9	73	76.17/76.00	5.43/5.61	13.32/13.21
3b	$C_{25}H_{19}N_3O$ (377.45)	79-81	40	79.55/79.32	5.07/4.84	11.13/11.32
3c	$C_{25}H_{17}N_3O_2$ (391.42)	195-7	52	76.71/76.53	4.38/4.26	10.74/10.91
3d	$C_{19}H_{15}N_3O_3S$ (365.41)	91 dec.	41	62.45/62.24	4.14/4.32	11.50/11.31
3e	$C_{24}H_{17}N_3O_3S$ (427.48)	181-3	47	67.43/67.22	4.01/4.20	9.83/9.61
4a	$C_{20}H_{17}N_3S$ (331.45)	107-9	95	72.48/72.29	5.17/4.95	12.68/12.46
4b	$C_{25}H_{19}N_3S$ (393.52)	94-6	60	76.31/76.20	4.87/4.65	10.68/10.48
4c	$C_{25}H_{17}N_3OS$ (407.49)	201-3	58	73.69/73.47	4.21/4.43	10.31/10.49
4d	$C_{19}H_{15}N_3O_2S_2$ (381.47)	161 dec.	40	59.82/59.65	3.96/3.80	11.02/10.90
4e	$C_{24}H_{17}N_3O_2S_2$ (443.54)	174 dec.	50	64.99/64.75	3.86/4.00	9.47/9.63
5a	$C_{20}H_{18}N_4$(314.15)	90-2	66	76.41/76.22	5.77/5.44	17.82/18.00
5b	$C_{25}H_{20}N_4$(376.45)	128-130	70	79.76/79.65	5.35/5.21	14.88/14.65
5c	$C_{25}H_{18}N_4O$(390.44)	117 dec.	67	76.91/77.00	4.65/4.54	14.35/14.20
5d	$C_{19}H_{16}N_4O_2S$ (364.42)	98 dec.	82	62.62/62.44	4.43/4.30	15.37/15.11
5e	$C_{24}H_{18}N_4O_2S$ (426.49)	210-2	80	67.59/67.44	4.25/4.01	13.14/13.00
6a	$C_{25}H_{25}NO_3$(387.47)	202-4	55	77.49/77.33	6.50/6.33	3.61/3.45
6b	$C_{30}H_{27}NO_3$(449.54)	79-81	45	80.15/79.99	6.05/6.22	3.12/3.40
6c	$C_{30}H_{25}NO_4$(463.52)	116-8	60	77.74/77.54	5.44/5.21	3.02/3.24
6d	$C_{24}H_{23}NO_5S$(437.51)	290-2	62	65.89/65.77	5.30/5.11	3.20/3.44
6e	$C_{29}H_{25}NO_5S$ (499.58)	162-4	57	69.72/69.55	5.04/5.22	2.80/3.01
7a	$C_{23}H_{21}N_3O$ (355.45)	70-2	66	77.72/77.99	5.96/6.01	11.82/11.66
7b	$C_{28}H_{23}N_3O$ (417.51)	166-8	75	80.55/80.42	5.55/5.32	10.06/10.22
7c	$C_{28}H_{21}N_3O_2$(431.49)	250-2	70	77.94/78.00	4.91/4.77	9.74/9.65
7d	$C_{22}H_{19}N_3O_3S$ (405.47)	112-3	60	65.17/65.40	4.72/4.55	10.36/10.22
7e	$C_{27}H_{21}N_3O_3S$ (467.54)	85-7	65	69.36/69.11	4.53/4.40	8.99/9.02
8a	$C_{19}H_{19}N_3$ (289.37)	90-2	42	78.86/78.69	6.62/6.46	14.52/14.35
8b	$C_{24}H_{21}N_3$ (351.44)	126-8	86	82.02/81.94	6.02/6.19	11.96/11.78
8c	$C_{24}H_{19}N_3O$ (365.43)	120-2	55	78.88/79.00	5.24/5.41	11.50/11.67
9a	$C_{21}H_{21}N_3O$ (331.41)	98-100	67	76.11/76.00	6.39/6.23	12.68/12.50
9b	$C_{26}H_{23}N_3O$ (393.48)	112-4	95	79.36/79.17	5.89/6.00	10.68/10.79
9c	$C_{26}H_{21}N_3O_2$ (407.46)	174-6	88	76.64/76.81	5.19/5.04	10.31/10.15
10a	$C_{25}H_{23}N_3$(365.47)	150-2	55	82.16/82.33	6.34/6.20	11.50/11.42
10b	$C_{30}H_{25}N_3$(427.54)	117-9	75	84.28/84.11	5.89/6.01	9.83/9.74
10c	$C_{30}H_{23}N_3O$(441.52)	207-9	46	81.61/81.54	5.25/5.11	9.52/9.45
11a	$C_{26}H_{25}N_3$(379.50)	84-6	60	82.29/82.11	6.64/6.42	11.07/11.21
11b	$C_{31}H_{27}N_3$(441.57)	182-4	66	84.32/84.55	6.16/6.32	9.52/9.33
11c	$C_{31}H_{25}N_3O$(455.55)	260 dec.	70	81.73/81.56	5.53/5.44	9.22/9.00
12a	$C_{19}H_{18}N_2O$ (290.36)	186-8	30	78.59/78.40	6.25/6.06	9.65/9.50
12b	$C_{24}H_{20}N_2O$ (352.43)	75-7	38	81.79/81.61	5.72/5.56	7.95/8.10
12c	$C_{24}H_{18}N_2O_2$ (366.41)	190-2	95	78.67/78.49	4.95/4.80	7.65/7.79

TABLE 2. Spectral characterization of the newly synthesized compounds.

Compd. No.	IR (ν_{max} cm^{-1})	NMR (δ, ppm)	Mass (*m/z*, %)
2a	1721 (C=O), 1640 (C=C)		
2b	1738 (C=O), 1506 (C=C)	^{1}H NMR: 8.53 (1H, s, H-2 ind.), 8.37 (1H, d, H-7 ind.), 8.21 (1H, m, H-6 ind.), 7.81 (1H, d, H-4 ind.), 7.64 (1H, d, CH=C), 7.58 (1H, m, H-5 ind.), 7.50-7.19 (10H, m, Ar-H), 7.00 (1H, d, CH=C), 5.50 (2H, s, CH_2)	
2c	1704 & 1654 (C=O), 1576 (C=C)		
3a	3410 (NH), 1644 (C=O), 1601 (C=N), 1577 (C=C)	^{1}H NMR: 8.82 (1H, s, NH), 8.36 (1H, s, H-2 ind.), 7.86-7.23 (10H, m, Ar-H), 4.35 (2H, q, CH_2), 1.51 (3H, t, CH_3) ^{13}C NMR: 193.39 (C=O), 139.43-112.14 (Ar-C), 51.02 (CH_2), 28.40 (CH_3)	315 (M^+, 1), 275 (100), 247 (41), 170 (64), 144 (22)
3b	3340 (NH), 1665 (C=O), 1601 (C=N), 1573 (C=C)		
3c	3320 (NH), 1669 (C=O), 1620 (C=N), 1557 (C=C)		391 (M^+, 1), 144 (100), 116 (22), 77 (11)
3d	3323 (NH), 1640 (C=O), 1609 (C=N), 1571 (C=C), 1379 & 1151 (SO_2)		
3e	3318 (NH), 1640 (C=O), 1618 (C=N), 1517 (C=C), 1375 & 1148 (SO_2)	^{1}H NMR: 12.08 (1H, s, NH), 8.79 (1H, s, H-2 ind.), 8.73 (1H, s, CH-pyrimidine), 8.36 (1H, d, H-7 ind.),7.86-7.21 (13H, m, Ar-H)	
4a	3382 (NH), 1640 (C=N), 1569 (C=C), 1220 (C=S)		
4b	3371 (NH), 1642 (C=N), 1579 (C=C), 1236 (C=S)	^{1}H NMR: 8.99 (1H, s, NH), 8.56 (1H, s, H-2 ind.), 8.37 (1H, d, H-7 ind.), 8.21 (1H, d, H-4 ind.), 7.86-7.20 (13H, m, Ar-H), 5.55 (2H, s, CH_2) ^{13}C NMR: 184.14 (C=S), 160.63 (C=N), 139.43-112.14 (Ar-C), 51.02 (CH_2)	
4c	3418 (NH), 1736 (C=O), 1640(C=N), 1567 (C=C), 1238 (C=S)		407 (M^+, 1), 247 (100), 144 (85), 117 (34), 89 (61)
4d	3420 (NH), 1640 (C=N), 1565 (C=C), 1375 & 1154 (SO_2), 1240 (C=S)		
4e	3418 (NH), 1640 (C=N), 1569 (C=C), 1375 & 1151 (SO_2), 1239 (C=S)	^{1}H NMR: 12.09 (1H, s, NH), 8.70 (1H, s, H-2 ind.), 8.32 (1H, d, H-7 ind.), 7.84-7.22 (14H, m, Ar-H)	443 (M^+, 1), 282 (10), 247 (100), 218 (51), 116 (58), 77 (20)
5a	3450 (NH_2), 1620 (C=N), 1577 (C=C)	^{1}H NMR: 10.05 (2H, s, NH_2), 8.21 (1H, s, H-2 ind.), 7.88 - 7.20 (15H, m, Ar-H)	
5b	3318 (NH_2), 1618 (C=N), 1587 (C=C)		
5c	3300 (NH_2), 1666 (C=O), 1620 (C=N), 1570 (C=C)		
5d	3350 & 3228 (NH_2), 1621 (C=N), 1587 (C=C), 1375 & 1163 (SO_2)		364 (M^+, 10), 332 (20), 144 (100), 117 (50), 77 (18)

TABLE 2 .Cont.

Compd. No.	IR (ν_{max} cm^{-1})	NMR (δ, ppm)	Mass (*m/z*, %)
5e	3348 (NH_2), 1619 (C=N), 1544 (C=C), 1375 & 1170 (SO_2)		
6a	1703 & 1688 (C=O), 1598 (C=C)	^{1}H NMR: 8.21 (1H, s, H-2 ind.), 7.66-7.20 (9H, m, Ar-H), 6.66 (1H, s, CH=C), 4.44 (2H, q, CH2-CO), 4.21 (2H, q, CH2-N), 3.59-2.65 (4H, m, -CH-CH-CH2 cyclohexanone), 1.99 (3H, t, CH3CH2CO), 1.61 (3H, t, CH3CH2N) ^{13}C NMR: 182.17 & 168.32 (2 C=O), 110.11-152.11 (Ar-C), 61.32 (CH2CO), 52.65 (CH2N), 42.62 (CH), 15.17 (2CH3)	
6b	1701 & 1687 (C=O), 1601 (C=C)	^{1}H NMR: 8.22 (1H, s, H-2 ind.), 7.66-7.20 (14H, m, Ar-H), 6.66 (1H, s, CH=C), 5.55 (2H, s, CH2-N), 4.42 (2H, q, CH2-CO), 3.59-2.66 (4H, m, -CH-CH-CH2 cyclohexanone), 1.91 (3H, t, CH3CH2CO)	
6c	1700 & 1699 (C=O), 1577 (C=C)		
6d	1730 & 1687 (C=O), 1577 (C=C), 1370 & 1163 (SO2)		
6e	1703 & 1667 (C=O), 1577 (C=C), 1363 & 1151 (SO2)		
7a	3350 (NH), 1645 (C=O), 1620 (C=N), 1579 (C=C)	^{1}H NMR: 8.71 (1H, s, NH), 8.24 (1H, s, H-2 ind.), 7.66-7.01 (9H, m, Ar-H), 6.66 (s, 1H, CH=C), 4.42 (2H, q, CH2-N), 2.91 & 3.30 (2H, 2d, CH-CH) 2.12 & 2.41 (2H, dd, CH-CH2), 1.61 (3H, t, CH3CH2N)	355 (M+, 1), 325 (10), 145 (100), 117 (70), 77 (50)
7b	3100 (NH), 1688 (C=O), 1620 (C=N), 1577 (C=C)		417 (M+, 1), 350 (10), 144 (100), 117 (70), 77 (50)
7c	3118 (NH), 1686 (C=O), 1618 (C=N), 1577 (C=C)		
7d	3210 (NH), 1656 (C=O), 1619 (C=N), 1365 & 1163 (SO2)		
7e	3245 (NH), 1645 (C=O), 1620 (C=N), 1556 (C=C), 1375 & 1153 (SO2)		
8a	3397 (NH), 1585 (C=N), 1527 (C=C)		289 (M+, 18), 275 (32), 172 (100), 145 (29)
8b	3400 (NH), 1590 (C=N), 1526 (C=C)	^{1}H NMR: 8.51 (1H, s, H-2 ind.), 8.01 -7.67 (4H, m, Ar-H of ind.), 7.01 (10H, s, Ph-H), 5.55 (2H, s, CH2), 4.46(1H, dd, CH-pyrazoline), 4.21 (1H, dd, CH2-pyrazoline equatorial), 3.21 (1H, dd, CH2-pyrazoline axial), 2.61 (1H, s, NH)	

TABLE 2 Cont.

Compd No.	IR (ν_{max} cm^{-1})	NMR (δ, ppm)	Mass (*m/z*, %)
8c	3216 (NH), 1736 (C=O), 1619 (C=N), 1521 (C=C)		365 (M+, 1), 259 (10), 144 (100), 116 (33), 177 (10), 77 (14)
9a	1752 (C=O), 1644 (C=N), 1578 (C=C)		331 (M+, 1), 275 (100), 172 (33), 144 (13), 130 (11), 116 (17)
9b	1729 (C=O), 1641 (C=N), 1575 (C=C)	^{1}H NMR: 8.51 (1H, s, H-2 ind.),8.33 (1H, d, H-7 ind.), 8.16-7.25 (13H, m, Ar-H), 5.52 (2H, s, CH2), 5.48 (1H, dd, CH-pyrazoline), 3.94 (1H, dd, CH2-pyrazoline equatorial), 2.45 (1H, dd, CH2-pyrazoline axial), 2.49 (3H, s, CH3)	
9c	1719 (C=O), 1642 (C=N), 1580(C=C)-N)	^{1}H NMR: 8.36 (1H, s, H-2 ind.), 8.00-7.18 (14H, m, Ar-H), 2.84 (1H, dd, CH-pyrazoline), 2.78 (1H, dd, CH2-pyrazoline equatorial), 2.58 (1H, dd, CH2-pyrazoline axial), 2.46 (3H, s, CH3) 13C NMR: 192.28 & 183.88 (C=O), 139.74-111.03 (Ar-C), 49.90 (CH and CH2), 27.30 (CH3)	407 (M+ , 1), 246 (32), 144 (100), 117 (12), 89 (16)
10a	1619 (C=N), 1599 (C=C)		365(M+, 10), 350 (20), 144 (45), 177 (16), 77 (100)
10b	1633 (C=N), 1577 (C=C)	^{1}H NMR: 8.21 (1H, s, H-2 ind.), 8.01 -7.43 (19H, m, Ar-H), 5.55 (2H, s, CH2), 4.46 (1H, dd, CH-pyrazoline), 3.91 (1H, dd, CH2-pyrazoline equatorial), 2.99 (1H, dd, CH2-pyrazoline axial)	
10c	1686 (C=O), 1620 (C=N), 1576 (C=C)		
11a	1620 (C=N), 1567 (C=C)		
11b	1619 (C=N), 1567 (C=C)		
11c	1645 (C=O), 1620 (C=N), 1578 (C=C)	^{1}H NMR: 8.21 (1H, s, H-2 ind.), 8.01 -7.00 (19H, m, Ar-H), 5.55 (2H, s, CH2-N), 4.22 (1H, dd, CH-pyrazoline), 3.79 (1H, dd, CH2-pyrazoline equatorial), 2.58 (1H, dd, CH2-pyrazoline axial)	
12a	1616 (C=N), 1626 (C=C)		290 (M+, 100), 275 (32), 145 (53), 130 (31), 77 (30)
12b	1636 (C=N), 1611C=C)		352 (M+, 1), 377 (1), 350 (1), 116 (1), 91 (100)
12c	1738 (C=O), 1638 (C=N), 1569 (C=C)	^{1}H NMR: 8.21 (1H, s, H-2 ind.), 8.01 -7.21 (14H, m, Ar-H), 4.99 (1H, dd, CH-isoxazole), 4.12 (1H, dd, CH2-isoxazole equatorial), 3.01 (1H, dd, CH2-isoxazole axial)	366 (M+, 1), 314 (64), 299 (60), 185 (80), 107 (100)

TABLE 3. Antimicrobial activity* of the newly synthesized compounds.

Compd. No.	Inhibition zone (mm)											
	Gram-positive bacteria				Gram-negative bacteria				Fungi			
	S. typhimurium (ATCC 14028)		*P. fluorescens* (S 97)		*S. aureus* (ATCC 25923)		*B. subtilis* (ATCC 6635)		*C. albicans* (ATCC 10231)		*A. fumigatus***	
	Concentration µg											
	20	10	20	10	20	10	20	10	20	10	20	10
3a	-	-	-	-	-	-	-	-	-	-	-	-
3b	-	-	-	-	-	-	-	-	-	-	-	-
3c	-	-	-	-	-	-	-	-	-	-	-	-
3d	-	-	-	-	-	-	-	-	-	-	-	-
3e	-	-	-	-	-	-	-	-	-	-	-	-
4a	-	-	-	-	-	-	-	-	-	-	-	-
4b	-	-	-	-	-	-	-	-	-	-	-	-
4c	-	-	-	-	-	-	-	-	-	-	-	-
4d	-	-	-	-	-	-	-	-	-	-	-	-
4e	-	-	-	-	-	-	-	-	-	-	-	-
5a	-	-	-	-	-	-	-	-	-	-	-	-
5b	-	-	17	-	13	-	13	-	27	22	-	-
5c	-	-	-	-	-	-	-	-	-	-	-	-
5d	-	-	-	-	-	-	-	-	8	-	-	-
5e	-	-	-	-	-	-	-	-	-	-	-	-
6a	-	-	-	-	-	-	-	-	-	-	-	-
6b	-	-	-	-	-	-	-	-	-	-	-	-
6c	-	-	-	-	-	-	-	-	-	-	-	-
5d	-	-	-	-	-	-	-	-	-	-	-	-
6e	-	-	-	-	-	-	-	-	-	-	-	-
7a	-	-	-	-	-	-	-	-	-	-	-	-
7b	-	-	-	-	-	-	-	-	-	-	-	-
7c	-	-	-	-	-	-	-	-	-	-	-	-
7d	-	-	-	-	-	-	-	-	-	-	-	-
7e	-	-	-	-	-	-	-	-	-	-	-	-
8a	-	-	-	-	-	-	-	-	-	-	-	-
8b	-	-	-	-	-	-	-	-	-	-	-	-
8c	-	-	-	-	-	-	-	-	-	-	-	-
9a	-	-	-	-	-	-	-	-	-	-	-	-
9b	-	-	-	-	-	-	-	-	-	-	-	-
9c	-	-	-	-	-	-	-	-	-	-	-	-
10a	-	-	-	-	-	-	-	-	-	-	-	-
10b	-	-	-	-	-	-	-	-	-	-	-	-
10c	-	-	-	-	-	-	-	-	-	-	-	-
11a	-	-	-	-	8	-	-	-	-	-	-	-
11b	-	-	-	-	-	-	-	-	-	-	-	-
11c	-	-	14	8	8	-	8	-	8	-	-	-
12a	-	-	-	-	-	-	-	-	-	-	-	-
12b	-	-	-	-	-	-	-	-	-	-	-	-
12c	-	-	-	-	-	-	-	-	-	-	-	-
Control a	42	28	37	31	-	-	-	-	-	-	-	-
Control b	-	-	-	-	38	29	38	30	-	-	-	-
Control c	-	-	-	-	-	-	-	-	39	28	40	31

***:** Disk diffusion method

****:** isolated from clinical samples and identified to the species level according to different API 20E system (Analytab Products) (bioMerieux, Australia)**.**

Control a: chloramphenecol; reference drug for Gram- positive bacteria; control b :cephalothin; reference drug for Gram- negative bacteria; control c: cycloheximide; reference drug for fungi

Inhibition values: (-mm) non sensitive; (8-12 mm) low activity; (13-21 mm) intermediate activity; (22-30 mm) high activity.

Expermintal

Melting points were determined in open capillary tubes on an Electrothermal 9100 digital melting point apparatus (Büchi, Switzerland) and were uncorrected. Elemental analyses were performed on a Perkin-Elmer 2400 analyzer (USA) and were found within ± 0.4 % of the theoretical values. IR spectra were recorded on a Perkin-Elmer 1600 FTIR (USA) in KBr pellets. The NMR spectra were measured with a Bruker Avance digital spectrophtometer (300 and 125 MHz) in DMSO-d_6 and chemical shifts were recorded in δ ppm relative to TMS as internal stander solvent. Mass spectra (EI) were run at SECTOR-FILD MS and GC-MS (single-phase) 200V (50/60 Hz) 30 A (Germany). *N*-ethyl (1a), *N*-benzyl (1b), *N*-benzoyl (1c), *N*-methylsulphonyl (1d) and *N*-benzene-sulphonyl-3-acetylindoles (1e) have been prepared as reported [13].

1-(N-substituted-1H-indol-3-yl)-3-phenylprop-2-ene-1-ones (2a-e)

To a solution of *N*-substituted-3-acetylindoles 1a-e (0.001 mol) in ethanol (10 ml) containing potassium hydroxide solution (5 ml, 25 %) benzaldehyde (0.1 ml, 0.001 mol) was added. The reaction mixture was stirred for 2hr at room temperature, and then left overnight at 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 recrystallized from absolute ethanol.

4-(N-substituted-1H-indol-3-yl)-6-phenylpyrimidin-2(1H)-ones (3a,b)

A mixture of 2a-e (0.01 mol) and urea (0.6 gm. 0.01 mol) in dry ethanol (10 ml) containing glacial acetic acid (0.5 ml) was refluxed for 6-8 hr. After cooling, the reaction mixture was poured onto ice-water (50 ml) and the solid that formed was filtered off, air dried and recrystallized from absolute ethanol.

4-(N-substituted-1H-indol-3-yl)-6-phenylpyrimidin-2(1H)-thiones (4a- e)

A mixture of 2a-e (0.01 mol) and thiourea (0.76 gm, 0.01 mol) in dry ethanol (10 ml) containing glacial acetic acid (0.5 ml) was refluxed for 6-8hr. After cooling, the reaction mixture was poured onto ice-water (50 ml) and the solid that formed was filtered off, air dried and recrystallized from absolute ethanol.

4-(N-substituted-1H-indol-3-yl)-6-phenylpyrimidin-2-amines (5a-e)

A mixture of 2a-e (0.01 mol), guanidine hydrochloride (0.96 gm, 0.01 mol) and anhydrous sodium acetate (0.82 gm, 0.01mol) in dry ethanol (15 ml) was refluxed for 2-3 hr. After cooling, the solid that formed was filtered off, air dried and re crystallized from absolute ethanol.

Ethyl-4- (N-substituted-1H- indol-3-yl)-6- phenyl-2-oxo- cyclohexa-3-ene-carboxylates (6a-e)

A mixture of 2a-e (0.01 mol) and ethyl acetoacetate (1.30 ml, 0.01mol) in absolute ethanol containing potassium hydroxide solution (1 ml, 10%). The reaction mixture was refluxed for 2hr then, left overnight at room temperature. The solid that formed was filtered off, air dried and recrystallized from ethanol.

4,5-Dihydro-4-phenyl-6-(N-substituted-1H-indol-3-yl)-2H-indazol-3(H) ones (7a-e)

A mixture of compound 6a-e (0.1 mol) and hydrazine hydrate 99% (5 ml, 0.1 mol) in absolute ethanol (15 ml) containing glacial acetic acid (0.5 ml) was refluxed for 2hr. After cooling, the solid that formed was filtered off, air dried and recrystallized from chloroform.

4,5-Dihydro-3-(N-substituted-1H-indol-3-yl)-5-phenyl-pyrazoles (8a-c)

To a solution of compound 2a-c **(**0.01 mol) in absolute ethanol (10 ml) containing glacial acetic acid (0.5 ml), hydrazine hydrate 99% (1 ml, 0.02 mol) was added. The reaction mixture was refluxed for 4hr. After cooling, the reaction mixture was poured onto ice-water (50 ml) and the solid that formed was filtered off, air dried and recrystallized from ethanol.

4,5-Dihydro-3-(N-substituted-1H-indol-3-yl)-5-phenyl-N-acetyl-pyrazoles (9a-c)

Method A

To a solution of compound 2a-c (0.01 mol) in a mixture of (10 ml) acetic anhydride and glacial acetic acid (2:1) was added hydrazine hydrate 99% (1 ml, 0.02 mol). The reaction mixture was refluxed for 6-8 hr. After cooling, the reaction mixture was poured onto ice-water (50 ml) and the solid that formed was filtered off, air dried and recrystallized from aqueous ethanol.

Method B

A mixture of compound 2a-c (0.01 mol) and acetic acid hydrazide (0.74 gm, 0.01 mol) in absolute ethanol (10 ml) containing glacial acetic acid (0.5 ml) was refluxed for 2hr. After cooling, the solid that formed was filtered off, air dried and recrystallized from aqueous ethanol.

4,5-Dihydro-3- (N-substituted-1H- indol-3-yl)-5- phenyl-N- phenyl- pyrazoles (10-ac)

A mixture of compound 2a-c (0.01 mol) and phenylhydrazine (1.08 ml, 0.01 mol) in absolute ethanol (10 ml) containing glacial acetic acid (0.5 ml) was refluxed for 2hr. After cooling, the solid that formed was filtered off, air dried and recrystallized from absolute ethanol.

4,5-Dihydro-3-(N-substituted-1H-indol-3-yl)-5-phenyl-N-benzyl-pyrazoles(11a-c)

A mixture of compound 2a-c (0.01 mol), benzylhydrazine hydrochloride (1.95 gm, 0.01 mol) and anhydrous sodium acetate (0.67 gm, 0.01 mol) in dry ethanol (15 ml) was refluxed for 2-3 hr. After cooling, the solid that formed was filtered off, air dried and re crystallized from absolute ethanol.

4,5-Dihydro-3-(N-substituted-1H-indol-3-yl)-5-phenyl-isoxazoles (12a-c)

A mixture of compound 2a-c (0.01 mol), hydroxylamine hydrochloride (0.69 gm, 0.01 mol) and anhydrous sodium acetate (0.67 gm, 0.01 mol) in absolute ethanol (10 ml) was refluxed for 6-8 hr. After cooling, the reaction mixture was poured onto ice-water (50 ml). The solid that formed was filtered off, air dried and recrystallized from absolute ethanol.

Biological assay

Antimicrobial evaluation

The antimicrobial activity of the synthesized compounds was determined *in vitro* by using the disc diffusion method([12]) against a variety of pathogenic microorganisms: *Salmonella typhimurium* (ATCC 14028), *Pseudomonas fluorescens* (S 97) (Gram-positive bacteria), *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6635) (Gram-negative bacteria) and two strain of fungi *Candida albicans* (ATCC 10231) and *Aspergillus fumigates* (isolated from clinical samples and identified to the species level according to different API 20E system (Analytab Products) (bioMerieux, Australia). The antimicrobial activity of the tested compounds were estimated by placing presterilized filter paper discs (6 mm in diameter) impregnated with 10 and 20 μg per disc in Nutrient and MacConky agar media for bacteria and on Sabouraud dextrose agar for fungus. Dimethyl formamide (DMF) which showed no inhibition zone was used as a solvent for impregnation. The inhibition zones (IZ) of the tested compounds were measured after 24-48 hr incubation at 37°C for bacteria and after 5 days incubation at 28 °C for fungi. Chloramphenicol and cephalothin were used as reference drugs for bacteria. Whereas, cycloheximide was used as reference drug for fungi.

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سلسلة جديدة من مشتقات ن-مستبدل-3-حلقات غير متجانسة اندول كمضادات للميكروبات

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تفاعل كلايزين شميدت ل ن-إيثيل (1أ) ن-بنزيل (1ب) ن-بنزويل (1ج) ن-ميثيل سالفونيل (1د) ون- بنزين سالفونيل-3-أسيتيل إندول (1ه) مع البنزالدهيد أعطى 1-(ن-مستبدل-إندول-3-يل)-3-فينيل-بروب-2-إين-1-أون (2 أ-ه). و بحولقة المركبات (2 أ-ه) من خلال تفاعلها مع اليوريا، الثايويوريا أو الجوانيديين أعطت مشتقات البريميدين المقابلة من (3 أ-ه) إلى (5 أ-ه) على التوالى. بينما التفاعل القاعدى للمركبات (2 أ-ه) مع الايثيل أسيتو أسيتات أعطى حلقات السيكلوهيكسانون المقابلة (6 أ-ه) والتى بدورها تم تفاعلها مع الهيدرازيين هيدرات وأعطت مشتقات الاندازول (7 أ-ه).

من جهة أخرى ، تم تفاعل المركبات (2 أ-ج) مع مشتقات الهيدرازيين مثل الهيدرازيين هيدرات، الاسيتيل هيدرازيين، الفينيل هيدرازيين أو البنزيل هيدرازيين هيدروكلوريد لتعطى مشتقات البيرازول من (8 أ-ج) حتى (11 أ-ج). وأخيرا تم تفاعل الشالكونات (2 أ-ج) مع الهيدروكسيل أمين هيدروكلورايد لتعطى مشتقات الايزوكسازول (12 أ-ج).

المركبات التى تم تحضيرها تم دراسة نشاطها ضد الميكروبات وأظهرت النتائج أن، المركب 4-(ن-بنزيل-إندول-3-يل)-6-فينيل-بيريميديين-2-أمين (5ب)، أظهر فاعلية ضد الكانديدا البيكانس (ATCC 10231) عند كلا من التركيزيين 20 و 10 ميكروجرام مقارنة بالدواء المرجعى السيكلوهيكساميد .