SYNTHESIS AND ANTIMICROBIAL EVALUATION OF NEW ISOXAZOLE AND PYRAZOLE DERIVATIVES

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تم تشييد عدد من المركبات التى تحتوى على 3- ميثايل -4- مشتقات (فينايل آزو) ايزوكسازولين -5- اون(5a,b) والبيرازولين -5- اون (6a-b) وكذلك مشتقات البيرازوليدين -3 و 5- ثنائى آون (10a-c) وقد تم اختبار عدد من هذه المركبات لمعرفة مدى فاعليتها كمضادات للميكروبات و تشتمل هذه المقاله على طرق تشييد هذه المركبات و ماتم عمله من دراسات طيفيه و حيويه عليها.

A series of 3-methyl-4-substituted (phenylazo) isoxazolin-5-ones (5a,b), pyrazolin-5-ones (6a-d) and pyrazolidin-3,5-diones (10a-c) have been synthesized and evaluated for their antimicrobial activities. The detailed synthesis, spectroscopic and biological data are reported.

Key words *:* Isoxazolin-5-ones, pyrazolin-5-ones, pyrazolidin-3,5-diones, antimicrobial activity.

Introduction

Many natural and synthetic products containing heterocyclic rings as pyrazoles(1-4), pyrazolones and isoxazolidines(5-10) and pyrazolidinediones (11,12) were reported to possess varied pharmacological activities. Many of these biological activities were attributed to the presence of N-bridge heterocyclic nuclei of some pyrazoles(13,14) and isoxazoles(15) which are described to have antiviral and antimicrobial activity (16-18). Moreover, pyrazoles attached to a sulphanilamido moiety through an azo linkage have been reported to exhibit antibacterial activity(19). This has initiated us to prepare some pyrazoles, isoxazoles and pyrazolidinediones to be examined as possible antibacterial agents. It has been considered worthwhile to incorporate a nitro, chloro and fluoro group into some of these derivatives to potentiate their expected antibacterial activity (20,21).

Experimental

Chemistry synthesis

Melting points (uncorrected) were determined using Fischer – Johns apparatus. The IR spectra were recorded on Hewlett Packard Laser Jet 6L. Spectrometer (υ,cm^{-1}). ^{1}H-NMR spectra Varian EM 390 (90 MH_Z) spectrometer using TMS as an internal standard (chemical shift, δppm). Microanalytical data (C, H, N) agreed with the proposed structures within ± 0.4% of the theoretical values.

Test organism; Staphylococcus aureus *ATCC 06538,* Escherichia coli *ATCC 10536. Fungi:* Candida albicans *ATCC 1023 (American Type Culture Collection; Manassas, VA 20108 USA)*

General method for preparation of 1-(4-aminophenyl)-1- aryliminoethanes3a,b

A mixture of the appropriate amine 2a,b (0.01 mol) and 4- aminoacetophenone (1.35g, 0.01 mol) in DMF (20 ml) was heated under reflux for 4h. After cooling to room temperature, the precipitated solid was collected by filtration, washed with water, dried and crystallized Compounds 3a,b IR (KBr, cm^{-1}) Showed 3400 (NH_2), 1640 (C=N), 3a: ^{1}HNMR (DMSO-d_6) δ: 2.70-(s. 3H, CH_3), 6.90 (s, 2H, NH_2 , D_2O-exchangeable), 7.0 – 7.6 (m, 6H, ArH). 3b: ^{1}HNMR (DMSO-d_6) δ: 2.72 (s. 3H, CH_3), 6.88 (s, 2H, NH_2 , D_2O-exchangeable), 7.22 – 7.82 (m, 8H, ArH).

General method for preparation of ethyl 2-(4-substituted arylazo) -3- oxobutyrates 4a,b.

Sodium nitrite (0.69 g, 0.01 mol) in water (10 ml) was added to an ice cooled mixture of **3a,b** (0.0 1 mol) in conc. HCl (10 ml) and water (10 ml),

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with cooling in an ice-bath. The diazotized compound was filtered off and dropped while cooling with stirring over a mixture of ethyl acetoacetate(1.39 g,0.01 mol) and sodium acetate (2 g in 10 ml water) in ethanol (20 ml). The reaction mixture was stirred for 6h at room temperature, the precipitated solid was collected by filtration, washed with water, dried and crystallized.Compounds **4a,b** IR (KBr, cm^{-1}) showed 1760 (C=O), 1630 (C=N), 1560 (N=N).

4a : ^{1}HNMR (DMSOd_6) δ: 1.22 (t, 3H, $OCH_2\underline{CH_3}$), 2.60 (s, 3H, CH_3), 2.90 (s, 3H, CH_3), 3.12 (s, 1H, CH), 3.90-4.20 (q, 2H, $O\underline{CH_2}CH_3$), 6.80-7.70 (m,6H, ArH). **4b** : ^{1}HNMR (DMSOd_6) δ: 1.21 (t, 3H, $OCH_2\underline{CH_3}$), 2.60 (s, 3H, CH_3), 2.88 (s, 3H, CH_3), 3.0 (s, 1H, CH), 4.20-4.22 (q, 2H, $O\underline{CH_2}CH_3$), 6.70-7.60 (m,8H, ArH).

General method for preparation of 3- methyl 4- [(4-substituted phenyl) azo] isoxazol -5- ones 5a,b.

A suspension of **4a,b** (0.01 mol) in ethanol (10 ml) and hydroxylamine hydrochloride (0,69 g, 0.01 mol) in presence of ammonium acetate (2g) was heated under reflux for 5h. After cooling to room temperature the precipitated solid was collected by filtration, washed with water, dried and crystallized from the appropriate solvent. Compound **5a,b** IR (KBr, cm^{-1}) showed 1750 (C=O). 1650 (C=N) 1560 (N=N) , **5a** : ^{1}HNMR (DMSO-d_6) δ: 2.70-2.80 (2s, 6H,$2CH_3$), 5.80 (s, 1H, isoxazolin-5- one), 7.0-7.72 (m, 6H, ArH), **5b**: ^{1}HNMR (DMSO-d_6) δ: 2.84-2.70 (2s, 6H, $2CH_3$), 5.68 (s, 1H, isoxazolin-5-one), 6.82-7.22 (m, 8H, ArH).

General method for preparation of 3- methyl 4-[(4-substituted phenyl) azo] pyrazolin-5-ones 6a-d

Hydrazine hydrate or phenyl hydrazine (0.0 1mol) was added to a suspension of compounds **5a,b** (0.01 mol) in ethanol (20 ml. the reaction mixture was heated under reflux for 10-14h. The excess solvent was then removed under vacuo and the residue was purified by crystallization. Compounds **6a-d** IR (KBr, cm^{-1}) showed 1725 (C=O), 1630 (C=N), 1540 (N=N).

6a: ^{1}HNMR (DMSO-d_6) δ 2.64-2.82 (2s, 6H, $2CH_3$), 5.80 (s, 1H, pyrazolin-5-one),7.20-7.90(m, 6H, ArH), 8.20-9.2 (s, 1H, NH, D_2O exchangeable) **6b**: ^{1}HNMR (DMSO-d_6) δ 2.60-2.80 (2s,6H,$2CH_3$), 5.75 (s, 1H, pyrazolin-5-one), 6.80-7.40 (m, 8H, ArH), 8.40 (s, 1H, NH, D_2O-exchangeable). **6c**: ^{1}HNMR (DMSO-d_6) δ: 2.68- 2.80 (2s, 6H, $2CH_3$), 5.75 (s, 1H, pyrazolin-5- one), 6.80- 8.22 (m, 11H, ArH,). **6d**: ^{1}HNMR (DMSO-d_6) δ: 2.60- 2.80 (2s, 6H, $2CH_3$), 5.75 (s, 1H, pyrazolin-5- one), 6.80- 7.40(m, 13H, ArH,).

General method for preparation of α,β. unsaturated ketones 8a-c

A mixture of 4-aminoacetophenone **1** (1.35g, 0.01 mol) in 2N HCl (30 ml) and the appropriate aldehydes (**7a-c**) (0.01 mol) was refluxed for 6-10 h. The reaction mixture was cooled and then poured onto ice-water (100 ml). The precipitated solid was filtered off, dried and crystallized. **8a,c** IR (KBr, cm^{-1}) showed 3400 (NH_2), 1675 (C=O) 1560 , **8a** : ^{1}HNMR (DMSO-d_6) 6.60 (s, 2H, NH_2 , D_2O-exchangeable), **7**.0-7.80 (m, 9H, ArH). **8b** : ^{1}HNMR (DMSO-d_6) 6.80 (s, 2H, NH_2 , D_2O-exchangeable), **7**.70-7.80 (m, 9H, ArH). **8c** : ^{1}HNMR (DMSO-d_6) 3.88(s, 6H, $2OCH_3$), 6.70 (s, 2H, NH_2 , D_2O-exchangeable), **7**.37-7.90 (m, 9H, ArH).

General method for preparation of diethy1-4-substituted arylazo-malonate 9a-c

Sodium nitrite (0.69 gm, 0.01 mol) in water (10 ml) was added to an ice cooled mixture of **8a,b** (0.0 1 mol) in conc. HCl (10 ml) and water (10 ml), with cooling in an ice-bath. The diazotized compound was filtered off and dropped while cooling with stirring over a mixture of diethyl malonate(1.60 g,0.01 mol) and sodium acetate (2 g in 10 ml water) in ethanol (20 ml). The reaction mixture was stirred for 6h at room temperature, the precipitated solid was collected by filtration, washed with water, dried and crystallized. **9a-c** IR (KBr, cm^{-1}) showed 1560(N=N), 1730 (C=O) , **9a** : ^{1}HNMR (DMSO-d_6) 1.24-1.30 (t, 6H, $2OCH_2$-CH_3), 3.0 (s, 1H, CH), 3.8-4.20(q,4H, $2OCH_2$-CH_3), 7.20-8.0(m,9H, ArH). **9b** : ^{1}HNMR (DMSO-d_6) 1.22-1.25 (t, 6H, $2OCH_2$-CH_3), 3.2 (s, 1H, CH), 4.0-4.2 (q, 4H, $2OCH_2$-CH_3), 7.0-7.8(m,9H, ArH). **9c** : ^{1}HNMR (DMSO-d_6) 1.24-1.28 (t, 6H, $2OCH_2$-CH_3), 3.2 (s, 1H, CH),3.84(s, 6H,OCH_3) 3.79-4.21(q,4H, $2OCH_2$-CH_3), 6.90-7.72 (m,9H, ArH).

General method for preparation of 4-[4-[5-(substituted aryl) pyrazol -3- yl] phenylazo] pyrazolidin-3,5-diones10a-c

A solution of **9a-c** (0.01 mol) and hydrazine hydrate (1.5g, 0.03 mol) in absolute ethanol (25 ml) was refluxed for 5h. Excess solvent was removed in vacuo and the residue was purified by crystallization

Table 1. Characterization of the new compounds

Com. No.	Formulae (M.W.)	Solvent of Crystn.	M. P. °C	yield %	Analysis % Calc./Found		
					C	H	N
3a	$C_{11}H_{11}N_3S$ (217.30)	EtOH	201-204	55	60.80 60.6	5.10 5.4	19.34 19.5
3b	$C_{13}H_{13}N_3S$ (211.27)	EtOH	97 -100	52	73.91 74.0	6.20 6.5	19.89 19.7
4a	$C_{17}H_{18}O_3N_4S$ (358.43)	EtOH	165-167	60	56-97 56.7	5.06 5.3	15.63 15.4
4b	$C_{19}H_{20}N_4O_3S$ (352.40)	EtOH	120-122	50	64.76 64.3	5.72 6.0	15.90 16.1
5a	$C_{15}H_{13}N_5O_2$ (327.38)	Pet – ether	210-212	58	63.54 63.4	4 .7 4.9	21.79 21.5
5b	$C_{17}H_{15}N_5O_2$ (321.35)	EtOH – H_2O	225-227	54	55.03 55.4	4.0 4.3	21.39 21.6
6a	$C_{15}H_{14}N_6OS$ (326.39)	Isopropanol	240-242	66	55.20 55.0	4.32 4.6	25.75 25.5
6b	$C_{17}H_{16}N_6O$ (320.36)	Isopropanol	185-187	58	63.74 63.4	5.03 5.3	26.24 26.4
6c	$C_{21}H_{18}N_6OS$ (402.49)	Isopropanol	215-217	54	62.67 62.3	4.51 4.6	20.86 20.6
6d	$C_{23}H_{20}N_6O$ (396.46)	Ethyl acetate	207-209	50	69.68 69.9	5.08 5.2	21.20 21.0
8a	$C_{15}H_{11}ClFNO$ (275.72)	EtOH-H_2O	110-112	58	65.34 65.6	4.02 4.3	5.08 5.2
8b	$C_{15}H_{11}ClN_2O_3$ (302.73)	EtOH-H_2O	148-150	52	59.51 59.2	3.66 4.0	9.25 9.4
8c	$C_{17}H_{17}NO_3$ (283.33)	EtOH-H_2O	180-183	55	72.7 72.3	6.05 6.3	4.94 4.6
9a	$C_{22}H_{20}ClFN_2O_5$ (446.88)	EtOH	233-235	60	59.13 59.4	4.51 4.8	6.27 6.5
9b	$C_{22}H_{20}ClN_3O_7$ (473.88)	EtOH	170-172	62	55.76 55.4	4.25 4.5	8.87 9.0
9c	$C_{24}H_{26}N_2O_7$ (454.49)	EtOH-H_2O	190-192	64	63.43 63.7	5.77 5.9	6.16 6.3
10a	$C_{18}H_{14}ClFN_6O_2$ (400.82)	AcOH	233-235	50	53.9 53.6	3.52 3.7	20.97 21.0
10b	$C_{18}H_{14}ClN_7O_4$ (427.82)	AcOH	220-222	53	5.53 50.7	3.29 3.4	22.92 22.7
10c	$C_{20}H_{20}N_6O_4$ (408.43)	AcOH – H_2O	198-202	55	58.82 59.1	4.94 5.0	20.58 20.3

.Compounds **10a-c** : IR (KBr, cm^{-1}) showed 3200 (NH) 1730-1710(C=O), 1640 (C=N) , 1540 (N=N) **10a** : ^{1}HNMR (DMSO-d_6) 3.30-3.42 (dd, 1H, J=5.80 Hz,5- pyrazoline C_4-H), 3.90-4.10 (dd, 1H,J=11.5 Hz pyrazoline C_4-H), 5.40-5.42 (dd, 1H,J=5.80Hz pyrazolin C_5-H), 5.89 (s, 1H, pyrazolidin- 3,5diones C_4-H), 6.80-7.80 (m, 7H, ArH), 9.50-9.52 (brs, 3H, 3NH, D_2O-exchangeable) . **10b** : ^{1}HNMR (DMSO-d_6) 3.32-3.44 (dd, 1H, J=5.80 Hz, 5- pyrazoline C_4-H), 3.92-4.20 (dd, 1H,J=11.5 Hz pyrazoline C_4-H), 5.42-5.44 (dd, 1H,J=5.80Hz pyrazolin C_5-H), 5.90 (s, 1H, pyrazolidin- 3,5diones C_4-H), 6.90-7.92 (m, 7H, ArH), 9.52-9.70 (brs, 3H, 3NH, D_2O-exchangeable) **10c**: ^{1}HNMR (DMSO-d_6)δ: 3.30-3.45 (dd, 1H, J=5.80 Hz pyrazolin-C_4-H), 3.90-4.10 (dd, 1H, J=11.6 Hz pyrazolin C_4-H), 5.50-5.54 (dd, 1H,J=5.80 pyrazolin-C_5 - H), 5.80 (s, 1H, pyrazolidin-3,5-diones C_4-H), 6.90-7.95 (m, 7H, ArH), 9.40-9.60 (brs, 3H, 3NH, D_2O-exchangeable) .

Biological evaluation

Antibacterial and antifungal screening was carried out using the agar diffusion technique (22). Twelve of the synthesized new compounds were selected for comparison in preliminary antibacterial screening (Table II). The compounds were tested for their activities against the gram-positive bacteria, *Staphylococcus aureus*, Gram-negative bacteria, *Escherichia coli* in addition to pathogenic fungi, *candida albicans*.

Antibacterial testing:

Evaluation of antimicrobial activity

The tested compounds were first dissolved in dimethyl sulfoxide and then diluted with water at the required quantities (1mg / ml). In order to ensure that the solvent had no effect on bacteria growth, an inoculated control test was performed with only dimethyl sulfoxide at the same dilution used in our experiment and found inactive in culture media. Cultures were incubated for 24 hr. at 37 ^oC for bacteria and 48 hr. at 25 ^oC for fungi. Ampicillin, streptomycin and nystatin were used as reference compounds. Results are recorded as average diameter of inhibition zone in mm. The lowest concentration compounds that completely inhibit the growth was considered to be the minimum inhibitory concentration (MIC) expressed in μg/ml. MIC was the mean of three measurements. A minimum in hibitory concentration (MIC) experiment was performed for the active compound using the broth dilution technique (23). The result are shown in (Table III).

Result and Discussion

Chemistry:

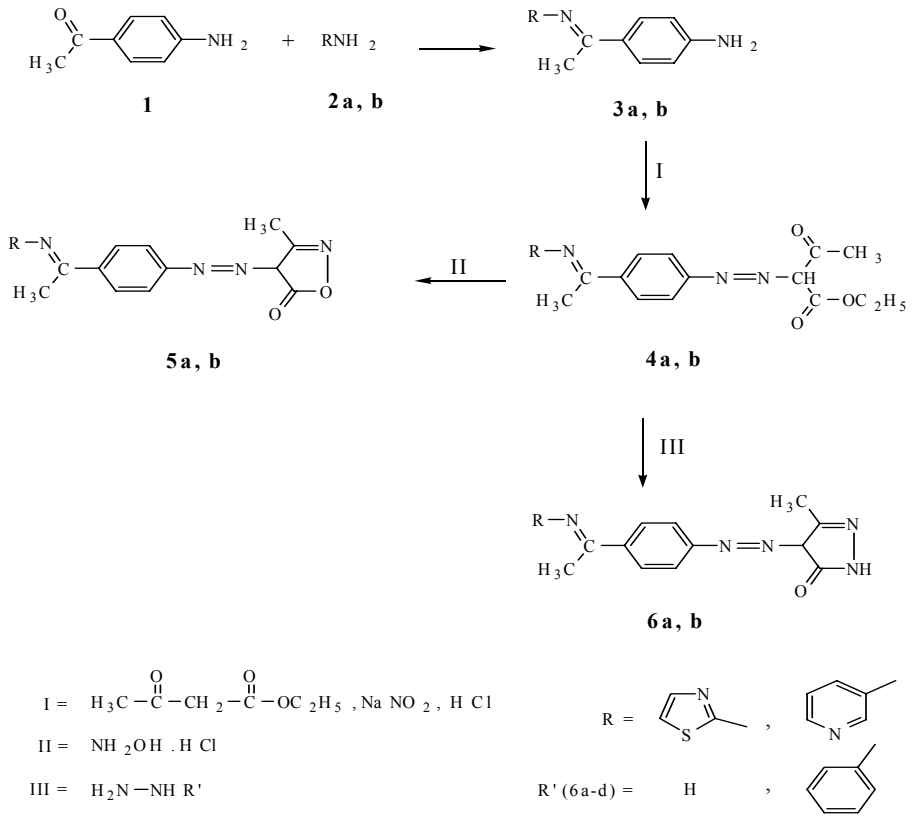
The syntheses of compounds **5a,b** and **6a,b** are outlined (Scheme 1,Table 1). Treatment of 4-aminoacetophenone **1** with different appropriate **2a,b** afforded a quantitative amount of the intermediates **3a,b.** Diazotization of compounds **3a,b** followed by coupling with ethyl acetoacetate in buffer solution furnshing oxobutyrates **4a,b**. Subsequent cyclization of compounds **4a,b** with hydroxylamine in ethanol in the presence of ammonium acetate, yielded the corresponding isoxazolin-5-ones **5a,b.** Similarly, reaction of compounds **4a,b** with hydrazine hydrate or phenyl hydrazine afforded the corresponding pyrazolin-5-ones **6a-d.** Preparation of 4-arylazopyrazolidin-3,5-diones **10a-c** was illustrated (Scheme 2,Table 1). Condensation of compound **1** with the appropriate aldehydes **7a-c** in acidic medium afforded compounds **8a-c**, diazotization of the free amino group of the intermediates **8a-c** followed by coupling with diethyl malonate yielded the corresponding malonate esters **9a-c**. Condensation of the latter malonate ester compounds with hydrazine hydrate furnished the target compounds **10a-c** .

Table 2. Antimicrobial screening results of the tested compounds.

Comp. No.	S. aureus	E. cali	C. albicans
5a	13	*NA*	*NA*
5b	18	13	NA
6a	NA	NA	9
6b	NA	NA	8
6c	14	NA	9
6d	14	NA	NA
10a	18	NA	9
10b	20	NA	NA
10c	18	18	NA

NA, no activity, inhibition zone <7 mm, weak activity (7-10 mm), moderate activity (11-15 mm), high activity (>15 mm).

Scheme 1.



Scheme 2.

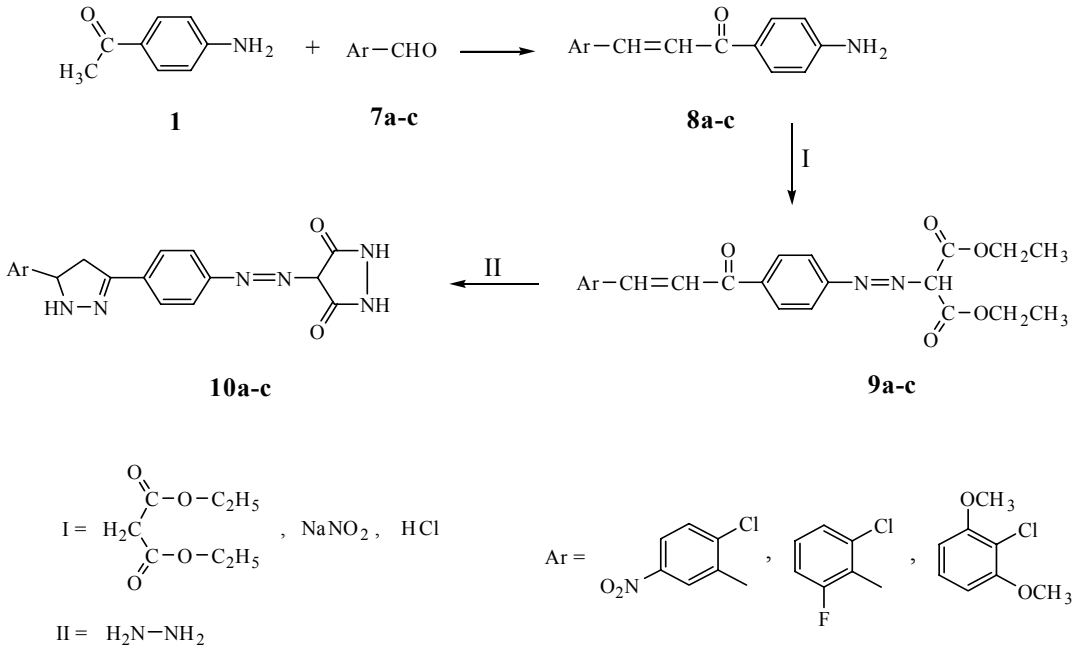


Table 3. Minimum inhibitory concentration (μg/ml) of the tested compounds.

Comp. No.	S. aureus	E. cali	C. albicans
5a	6.25	-	-
5b	1.8	6.25	-
6a	-	-	-
6b	-	-	12.5
6c	6.25	-	12.5
6d	6.25	-	-
10a	6.25	-	-
10b	1.8	-	-
10c	12.5	1.8	-
Ampicillin	1	_	-
Streptomycin	4	3	-
Nystatin	-	-	2

Conclusion

As shown in (Table 11), the obtained data revealed that compound **5b** in which the ring closure to isoxazole showed high activity against *S. aureus* and moderate activity against *E. coli.* Conversion of the ring clodure im **5a,b** into pyrazolone ring **6a-d** reduced the potency against *S. aureus*. Ring closure of ethyl malonates into pyrazolidin-3,5-diones **10a-c** showed the best results against *S.aureus*. compound **10b** bearing Cl and F groups exhibit high activity in addition compound 10c showed high activity against *E.Coli*. Compounds **6a,b** and **10b** showed weak activity *C. albicans* . These results revealed that the substituted Azopyrazolidin-3,5-diones showed the best results among the corresponding comparable structures.

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