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Synthesis of some novel pyrazole derivatives as potent antimicrobial agents

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ABSTRACT

One pot reaction of pyrazolone **(3)** with malononitrile, different aromatic aldehydes in presence of ammonium acetate or piperidine afforded compounds **(6a-c)** and **(7)**. Compound **(3)** reacted with different active methylenes and p-chlorobenzaldehyde to give the corresponding pyrazole derivatives **(8a-c)**, while using p-chlorobenzaldehyde, and acetylacetone gave compound **(9)**. Treatment of compounds 6b and/or 7 with ethanolic potassium hydroxide and excess carbon disulphide produced products **(10a-b)**. On treating compounds **(6b)** and **(7)** with formic and/or acetic acids, the pyrido and pyranopyrimidines **(11a-b)** and **(12a-b)**, were obtained. Compound **(3)** reacted with malononitrile and sulfur in presence of triethylamine produced compound **(13)** that treated with formic and acetic acids to get the thienopyrazoles **(14a-b)**. All derivatives exhibited high antimicrobial activities.

KEYWORDS

Pyrrazole derivatives;
One pot reaction;
Antimicrobial activity.

INTRODUCTION

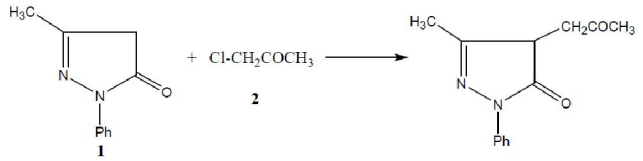
Simple nitrogenated heteroatomic compounds have received much attention over the years. These compounds can be considered as potential building blocks in synthesis and are found in a wide variety of pharmacologically and biologically active compounds[1,2]. Pyrazole derivatives can be considered as good example for these types of compounds. Pyrazole derivatives are well established in the literature as important biologically active heterocyclic compounds. Diversely substituted pyrazolines and their derivatives embedded with variety of functional groups are important biological agents and a significant amount of research activity has been directed towards this class. Pyrazole derivatives have a long history of application in agrochemicals and pharmaceutical industry as herbicides and active pharmaceuticals.

A systematic investigation of this class of heterocyclic revealed that pyrazole containing pharmaco active agents play important role in medicinal chemistry. Pyrazoles are class of heterocyclic compounds that exhibit a broad spectrum of biological activities[3-6]. Series of structurally related 1*H*-pyrazolyl derivatives were synthesized and tested for their in vivo anti-inflammatory and antimicrobial activities[7]. Some 4-pyrazolyl benzenesulphonamide derivatives were also synthesized and examined for their both anti-inflammatory and antimicrobial activities[8]. Research studies for pyrazole derivatives focused on their potential activities as anti-inflammatory[9], antipyretic[10], antimicrobial[11], antiviral[12], antitumor[13], antihistaminic[14], and

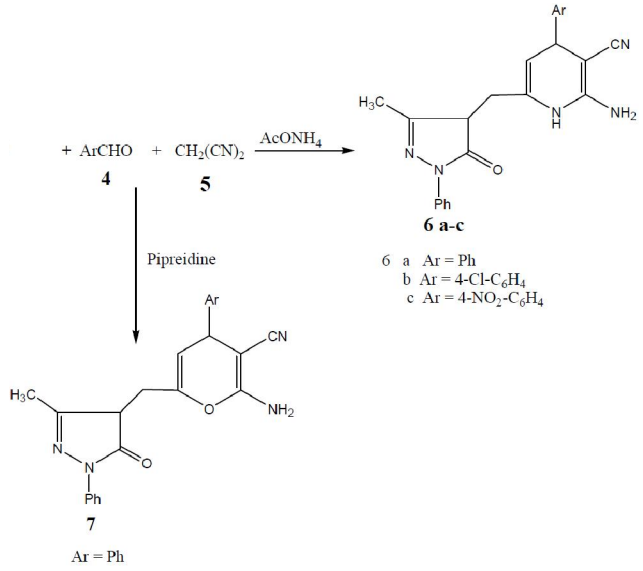
antifungicides[15]. Multicomponent reactions (MCRs) by virtue of their ease of exeuation and generally high yields of products have attracted a considerable attention from the point of view of organic synthesis[16-17]. Therefore, this study aimed to synthesize some new pyrazole derivatives via the one-pot three-component reaction methodology and to evaluate in vitro for their antimicrobial activity.

RESULTS AND DISSCUSSION

Compound **(3)**, 5-Methyl-4-(2-oxo-propyl)-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one was prepared by the reaction of 3-methyl-2,4-dihydro-3H-pyrazol-3-one with chloroacetone, Scheme 1. A mixture of **(3)**, malononitrile, and different aromatic aldehydes was heated under reflux in ethanol containing excess of ammonium acetate in three component reaction, giving the corresponding 2-Amino--1*H*-pyrazol-1,4-dihydropyridine-3-carbonitrile systems **(6a-c)**. On using piperidine instead of ammonium acetate, the pyrano derivative **(7)**, was obtained as shown in Scheme 2.

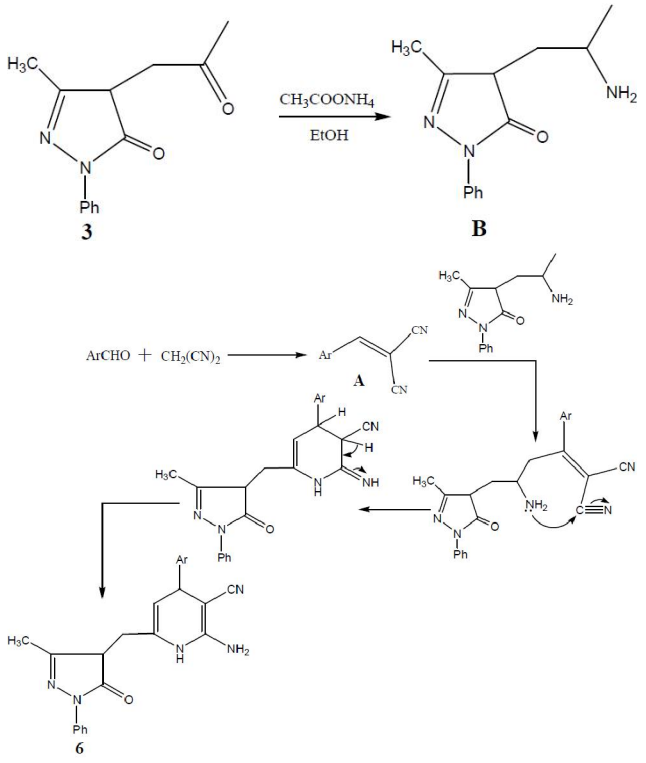


Scheme 1



Scheme 2

It is obvious that the carbonyl group of compound **(3)** is converted to the amino one by the reaction with ammonium acetate to give (B). The formation of **(6a-c)** can be rationalized by initial formation of (arylmethylidene) propanedinitrile (A) by Knoevenagel condensation of aromatic aldehyde and malononitrile with subsequent Micheal-type addition of 1-phenyl-pyrazolo-amine B to the adduct (A) which cyclized *via* loss of water to afford the corresponding products **(6a-c)** Scheme 3.

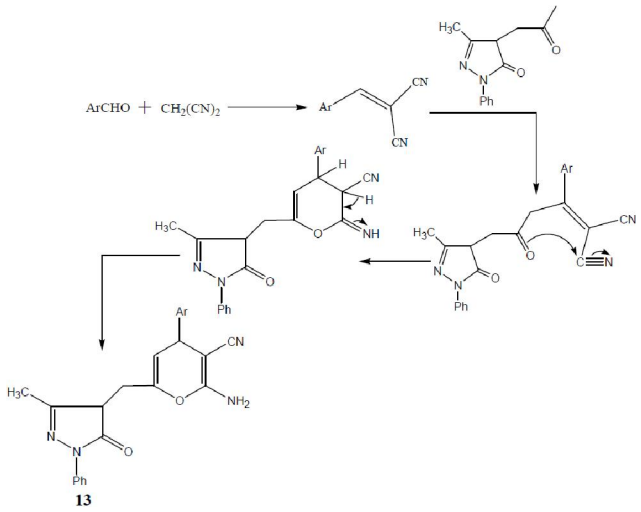


Scheme 3

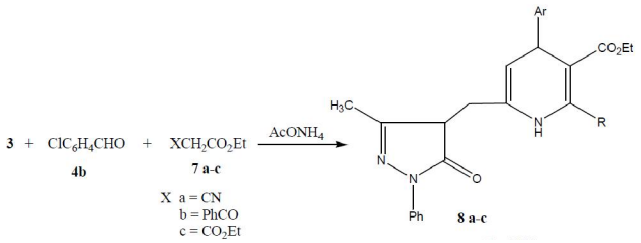
Formation of compound **(7)** can be rationalized by initial formation of (arylmethylidene) propanedinitrile (A) by Knoevenagel condensation of aromatic aldehyde and malononitrile with subsequent Micheal-type addition of compound **(3)** to the adduct (A) which cyclized *via* loss of water to afford the corresponding products **(7)** Scheme 4.

The presence of a hydrogen proton at position 4 in pyridine ring **(6a-c)** at 5.11 ppm in ^{1}HNMR spectrum confirms that the newly synthesized compounds are in accordance with the suggested structures in Scheme 2. Also the disappearance of the characteristic signal of the NH group of piperidine ring confirms the formation of the pyrano derivative **(7)**. Microanalytical and other spectral data are matched well with the expected struc-

tures of Scheme 2. Scheme 5 shows the reaction of the pyrazolo derivative **(3)** with chlorobenzaldehyde and different active methylenated reagents under the same previous reaction conditions gives rise to the formation of the products **(8a-c)**, *c.f*. the experimentl section.

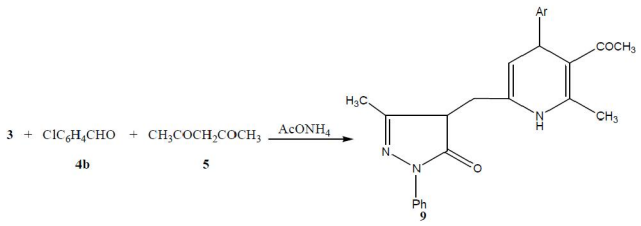


Scheme 4



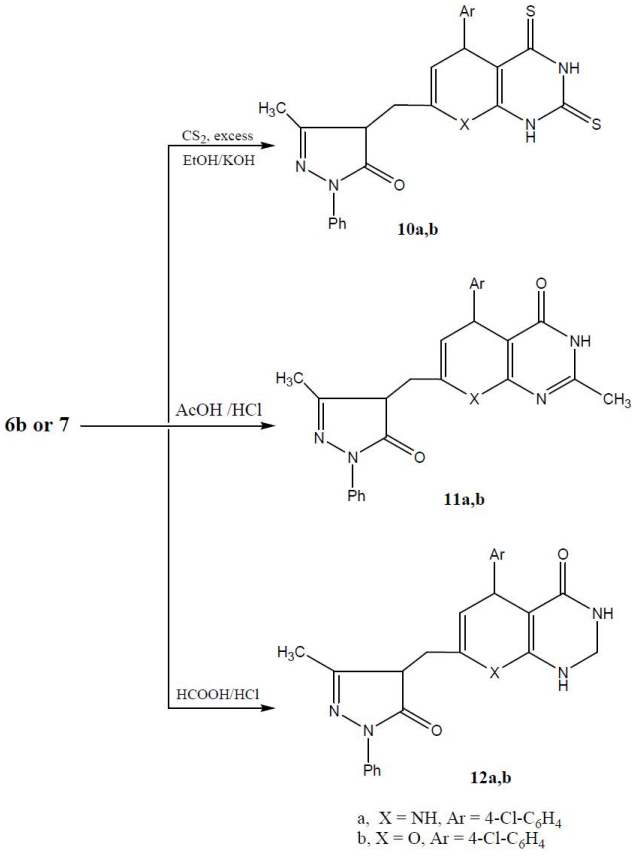
Scheme 5

Similarly, the same pyrazolo derivative derivative reacted with chlorobenzaldehyde and acetyl acetone in presence of excess of ammonium acetate under the same condition to give product **(9)**, Scheme 6. The appearance of C=O group at 1715 ν cm^{-1} in the IR spectrum and the presence of protons at 2.14 and 3.51 ppm corresponding to CH_3 and OCH_3 respectively in the ^{1}HNMR spectrum confirms the formation of product **(9)** structure.



Scheme 6

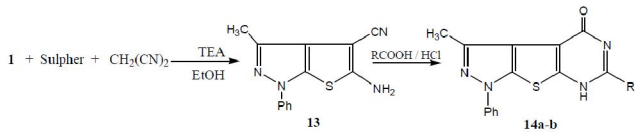
Pyridopyrimidine and pyranopyrimidine derivatives of pyrazole **(10a-b)**, **(11a-b)**, and **(12a-b)**, were obtained on boiling of the pyridine derivative **(6b)** and/or pyran derivative **(7)** with each of CS_2, acetic, or formic acids, Scheme 7. In case of using CS_2, the reaction does not proceed with equimolar ratios of this reagent. Therefore, excess of CS_2 affords products **(10a-b)**. ^{1}HNMR spectra of **(10a-b)** shows the presence of three signals at 8.14, 11.09 and 12.30 ppm for NHs of **(10a)**, where the only NH signal of **(10b)** appears at 11.36 ppm. Compounds **(11a-b)** were confirmed by the existence of only one proton signal that related to NH group in pyrimidine ring at 7.68 and 8.79 ppm for derivative **(11a)** and at 8.35 for **(11b)** respectively, where a signal that related to the terminal CH_3 group appears at 0.99 ppm. On the other hand, it is found that the derivative **(12a)** having three NH protons signals at 7.95, 8.13, and8.96 ppm where the signal at 4.35 ppm is related for CH_2 in the pyrimidine ring. Compound **(12b)** spectrum exhibited two signals for the NH groups at 7.86, 8.27, and 4.30 for the pyrimidine CH_2 respectively.



Scheme 7

Scheme 8 represents the reaction of 5-methyl-2-N-phenyl-3*H*-pyrazol-3-one with sulfur and

malononitrile in presence of triethylamine and absolute ethanol as solvent that heated under reflux to give compound **(13)**. Structure of **(13)** was confirmed using both elemental analysis and spectral data. Treatment of compound **(13)** each of formic and acetic acids giving rise to the formation of products **(14a-b)**. The appearance of only one signal for NH group at 4.10 and 4.12 ppm for derivatives **(14a)**, and **(14b)** respectively explains the formation of these structures.



Scheme 8

Biological activity studies

Antimicrobial activity

The antimicrobial activity of all the synthesized pyrazole derivatives against two strains of Gram positive bacteria, *Staphylococcus aureus* and *Bacillus subtillis*, in addition to two strains of Gram negative bacteria, *Escherichia coli* and *Pseudomonas aeroginosa*, were investigated using disc diffusion (ADDT) and micro dilution (MIC) methods in comparison with the reference antibacterial drug Tetracycline and Fluconazole as antifungal standard drug. The results of the disc diffusion method are shown in TABLE 1 and that of micro dilution method, for estimation of minimal inhibitory concentration (MIC) are given in TABLE 2. All results clearly revealed that, all synthesizedpyrazole derivatives under investigation in the present study exhibited highly significant antibacterial and antifungal activities against the used strains of Gram positive and negative bacteria in addition to *Aspragillus flavus* fungus.

TABLE 1 : The ADDT antimicrobial activity of the synthesized compounds against bacterial and fungal strains isolated from animal origin

Code Sample		G⁻ Bacteria		G⁺ Bacteria	Fungi	
		E-Coli	*Ps. aruginoas*	*S. aureus*	*B. subtillis*	*A. flavus*
Solvent: DMSO		0.0	0.0	0.0	0.0	0.0
Standard	Tetracycline 100 µg/ml	33	28	31	30	-ve
	Fluconazole 20 µg/ml	-ve	-ve	-ve	-ve	12
	3	15	20	12	14	16
	6a	17	15	13	15	14
	6b	13	16	13	12	15
	6c	17	13	12	14	17
	7	24	32	24	18	14
	8a	16	12	16	18	13
	8b	14	18	17	12	10
	8c	15	23	21	15	12
	9	14	21	29	19	11
	10a	16	19	17	18	11
	10b	28	34	36	32	12
	11a	19	18	14	18	13
	11b	37	32	26	34	21
	12a	20	19	18	19	12
	12b	39	37	34	27	22
	13	11	19	17	18	15
	14a	14	16	15	12	18
	14b	17	11	13	16	15

Results in TABLE 1 that represents the disc diffusion method shows that the compound **(12b)**, 5-(4-chlorophenyl)-7-(3-methyl-5-oxo-1-phenyl-4,5-dihydro-1*H*-pyrazol-4-ylmethyl)1,2,3,5-tetrahydro-

1*H*-pyrano[2,3-*d*]pyrimidin-4-one, exhibits the greatest significant activity that reachs 132% and 118% against the two strains of Gram negative bacteria, *Escherichia coli* and *Pseudomonas aeroginosa* when compared with the reference drug. Values of antibacterial activity of this compound against the two Gram positive bacteria, *Staphylococcus aureus* and *Bacillus subtillis* are 109% and 90% respectively in comparison with the tetracycline drug. The same compound, **(12b)**, also showed higher antifungal activity reachs 183% that of the Fluconazole standard agent. The derivative **(10b)** and **(11b)** showed high antibacterial activities against both Gram positive and Gram negative bacteria, in addition to their highly antifungal activity compared with the standard antifungal agent.

On the other hand, most of the pyrazole derivatives showed notable activity with MIC values. Concerning compound **(12b)**, these values ranging from 0.35 to 0.89μg/mL aginst the Gram negative bacteria, *Escherichia coli* and *Pseudomonas aeroginosa* and 054-0.75μg/mL against the Gram positive bacteria, *Staphylococcus aureus* and *Bacillus subtillis*. The MIC values of all compounds exhibited greater antifungal activities compared with the standard antifungal agent.

TABLE 2 : The MIC antimicrobial of the synthesized compounds against bacterial and fungal strains isolated from animal origin

Code Sample		G^- Bacteria		G^+ Bacteria	Fungi	
		E-Coli	*Ps. aruginoas*	*S. aureus*	*B.subtilis*	*A.flavus*
Solvent: DMSO		0.0	0.0	0.0	0.0	0.0
Standard	Tetracycline 100 μg/ml	6.5	50	3.125	3.125	-ve
	Fluconazole 20 μg/ml	-ve	-ve	-ve	-ve	50
3		2.51	2.01	1.32	1.46	5.69
6a		5.73	3.64	5.73	2.82	4.12
6b		2.61	4.82	2.04	3.07	14.35
6c		1.94	12.35	2.29	1.43	11.96
7		0.97	0.48	1.41	0.83	5.03
8a		1.85	1.47	3.28	1.98	2.58
8b		4.99	2.08	4.72	3.25	2.30
8c		1.37	10.21	1.85	5.21	12.9
9		2.38	1.36	1.99	1.69	1.40
10a		1.24	2.05	1.36	1.74	11.75
10b		0.64	0.82	0.78	0.62	1.09
11a		00	1.39	1.28	1.68	3.62
11b		0.48	0.84	1.59	0.97	0.94
12a		1.15	1.19	1.72	1.78	3.84
12b		0.35	.089	0.54	0.75	1.25
13		1.62	4.85	1.86	1.89	2.57
14a		2.37	9.66	2.39	1.52	1.73
14b		6.13	13.24	4.94	2.14	1.99

EXPERIMENTAL

Melting points were measured using an electrothermal apparatus (Buchi 535, Switzerland) in an open capillary tube and are uncorrected. IR spectra expressed in cm^{-1} were recorded in KBr pellets on a PA-9721 IR spectrophotometer. ^1H NMR and ^{13}C NMR spectra were carried out on a varian EM-390 (270 and 500 MHz) spectrometer in DMSO-d_6 as solvent, using TMS as internal reference and chemical shilts (δ) are expressed in ppm. Mass spectra were recorded on Kratos (75ev) Ms Equipment. Elemental analyses were carried out by the Micro analytical Unit at the National

Research Center, Giza, Egypt. All reactions were monitored by thin layer chromatography, carried out on 0.2mm silica gel 60 F-254 (Merck) plates using UV light (254 and 365 nm) for detectio

Synthesis of 5-Methyl-4-(2-oxo-propyl)-2-phenyl-2,4-dihydro-3*H*-pyrazol-3-one (3)

Equimolar ratios of 5-methyl-2-N-phenyl3-H-pyrazol-3-one, chloroacetone (that is obtained from condensation of phenyl hydrazine and ethyl acetoacetate in presence of 1 mL of glacial acetic acid which refluxed for 3 h) and sodium ethoxide were refluxed for 4 h. The reaction mixture was poured on ice water, then HCl drops added till neutralization. The resulted orange precipitate that recrystallized from ethanol has mp. of 142°C.

Orange colour, mp.142-144°C yield (97%). Ms. m/z: 230 [M^+,15.5 %]. IR (KBr, cm^{-1}): υ= 3138 (CH, arom.); 2967 (CH, aliph.); 1671 (C=O); 1605 (C=C, arom.); ^{1}H NMR (270 MHz, DMSO-d_6, TMS, ppm): δ = 1.02 (s, 3H, CH_3); 2.20 (d, 2H,CH_2); 2.82 (s,3H,CH_3); 2.71 (t, 1H, CH); 7.13-7.64 (m, 5H, aromatic protons). ^{13}C-NMR (270 MHz, DMSO-d_6, TMS, ppm), δ = pyrazole: 17.5 (CH_3); 38.5 (C4); 155.6(C3, imine); 173 (C5, amide); 24.1 (CH_3, ester); 37.3 (CH_2, ester); 207.1 (C=O); 120.4, 120.4, 124.1, 128.7, 128.7, 140.8 (6C, phenyl); Calcd. for $C_{13}H_{14}N_2O_2$ (230.262): C, 67.81%; H, 6.13%; N, 12.17%. Found: C, 67 46% ; H, 6.07% ; N, 12.12%.

General procedure of (6a-c) and (7)

Solution of the compound **(3)** (0.01 mole), equimolar ratios of mlononitrile and different aromatic aldehydes, in ethanol, containing excess ammonium acetate / or pipredine were refluxed for 7 h. The reaction mixture was left to cool and then poured ice water that neutralized with HCl. The solution was filtered to get precipitate that collected and recrystallized from the appropriate solvent.

2-Amino-6-[(3-methyl-5-oxo-1-phenyl-4,5-dihydro-1*H*-pyrazol-4-yl)methyl]-4-phenyl-1,4-dihydropyridine-3-carbonitrile (6a)

Pale brown colour, mp. 213-215°C, yield, 72%; Ms. m/z: 383 [M^+, 24.3%]. IR (KBr cm^{-1}): υ= 3425 (NH_2); 3128 (NH); 3142 (CH, arom.); 2985 (CH, aliph.); 2135 (CN); 1669 (C=O); 1610 (C=C, arom.); ^{1}H NMR (270 MHz, DMSO-d_6, TMS, ppm): δ= 1.02 (s, 3H, CH_3); 2.36 (d, 2H, CH_2); 2.68 (t, 1H, CH); 4.13 (s, 1H, pyrid.); 5.11 (s, 1H, CH); 7.13–7.89 (m, 10H, aromatic protons); 7.82 (s, 1H, NH); 8.76 (br, 2H, NH_2). ^{13}C-NMR (270 MHz, DMSO-d_6, TMS): δ = pyrazole: 17.9 (CH_3); 40.5, (C4); 155.6 (C3, imine); 173 (C5, amide); 34.0 (CH_2, bridge); pyridine: 30.3 (C4); 57.6 (C5); 94.5 (C3); 117 (CN); 140.3 (C2); 161 (C6); 120.4, 120.4, 124.1, 124.7, 128.7, 128.7, 140.8 (6C, phenyl); 125.5, 128.4, 128.4, 129.2, 129.2, 137.7 (6C, chlorophenyl); Calcd. for $C_{23}H_{21}N_5O$ (383.44): C, 72.04%; H, 5.52% ; N, 18.26%. Found: C, 72.01%; H, 5.34%; N, 18.09%.

2-Amino-4-(4-chlorophenyl)-6-[(3-methyl-5-oxo-1-phenyl-4,5-dihydro-1*H*-spyrazol-4-yl)methyl]-4-phenyl-1,4-dihydropyridine-3-carbonitrile (6b)

Pale brown colour, mp. 198°C, yield, 81%; Ms. m/z:417 [M^+, 18.6%]. IR (KBr cm^{-1}): υ= 3433 (NH_2); 3125 (NH); 3145 (CH arom.); 2993 (C-H aliph.); 2128 (CN); 1668 (C=O); 1611 (C=C arom.); ^{1}H NMR (270 MHz, DMSO-d_6, TMS, ppm): δ= 1.02 (s, 3H, CH_3); 2.32 (d, 2H, CH_2); 2.73 (t, 1H, CH); 4.12 (s, 1H, pyrid.); 5.11 (s, 1H, CH); 6.34.14–7.59 (m, 9H, aromatic protons); 7.63 (s, 1H, NH); 8.93 (br, 2H, NH_2). Calcd. for $C_{23}H_{20}ClN_5O$ (417): C 66.10%; H, 4.82%; N, 16.76%. Found: C 65.96%; H, 4.28%; N, 16.68%

2-Amino-6-[(3-methyl-5-oxo-1-phenyl-4,5-dihydro-1*H*-pyrazol-4-yl)methyl]-4-(4-nitrophenyl)-1,4-dihydropyridine-3-carbonitrile (6c)

Brown colour, mp. 236-238°C, yield, 81%; Ms. m/z: 428 [M^+, 22.4%]. IR (KBr cm^{-1}): υ= 3432 (NH_2); 3124 (NH); 3140 (CH arom.); 2987 (CH aliph.); 2131 (CN); 1674 (C=O); 1606 (C=C arom.); 1558 (NO_2). ^{1}H NMR (270 MHz, DMSO-d_6, TMS, ppm): δ= 1.02 (s, 3H, CH_3); 2.29 (d, 2H, CH_2); 2.76 (t, 1H, CH); 4.13 (s, 1H, pyrid.); 5.09 (s, 1H, CH); 6.72–7.69 (m, 9H, aromatic protons, s, 1H, NH); 8.81 (br, 2H, NH_2). Calcd. for $C_{23}H_{20}ClN_6O$ (428): C, 64.48%; H, 4.67%; N, 19.62%. Found: C, 64.38%; H, 4.13%; N, 16.27

2-Amino-4-(4-chlorophenyl)-6-[(3-methyl-5-oxo-1-phenyl-4,5-dihydro-1*H*-pyrazol-4-yl)methyl]-4H-pyran-3-carbonitrile (7)

Brown colour, mp. 218-220°C, yield, 92%; Ms. m/z:421 [M^+, 21.9%]. IR (KBr cm^{-1}): υ= 3431 (NH_2); 3138 (CH arom.); 2994 (CH aliph.); 2129 (CN); 1669

(C=O); 1607 (C=C arom.). ^{1}H NMR (270 MHz, DMSO-d_6, TMS, ppm): δ= 1.02(s, 3H, CH_3); 2.13 (d, 2H, CH_2); 2.71 (t, 1H, CH); 4.14 (s, 1H, pyrid.); 5.12 (s, 1H, CH); 6.73–7.66 (m, 9H, aromatic protons); 8.75 (br, 2H, NH_2). Calcd. for $C_{23}H_{18}ClN_4O_2$ (421.5): C, 65.48%; H, 4.27% ; N, 13.28%. Found: C, 65.41%; H, 4.22%; N,13.15%

General procedure of (8a-c)

0.01 mole of ethanolic solution of compound **(3)**, equimolar ratios of different active methelynic reagents and p-chlorobenzaldehyde, in ethanol as a solvent and in presence of excess ammonium acetate were heated under reflux for 9 hrs. The reaction mixture was left to cool and then poured on ice water with HCl addition till neutralization to get solid products on filtration. The collected product was recrystallized from the appropriate solvent.

2-Amino-4-(4-chlorophenyl)-6-(3-metyhl-5-oxo-1-phenyl-4,5-dihydro-1*H*-pyrazol-4-ylmethyl)-1,4-dihydropyridine-3-carboxylic acid ethyl ester (8a)

Deep brown colour, mp. 241-244°C, yield, 81%; Ms. m/z: 464 [M^+, 17.3%]. IR (KB cm^{-1}r): υ= 3427 (NH_2); 3085 (NH); 3138 (CH arom.); 2968 (CH aliph.); 1746 (ester,C=O); 1673 (C=O); 1609 (C=C arom.). ^{1}H NMR (270 MHz, DMSO-d_6, TMS, ppm): δ= 1.02 (s, 3H, CH_3); 1.31 (t, 3H, CH_3), 2.27 (d, 2H, CH_2); 2.52 (t, 1H, CH); 4.15 (q, 2H, CH_2), 4.13 (s, 1H, pyrid.); 5.11 (s, 1H, CH); 6.56–7.58 (m, 9H, aromatic protons); 7.84 (s, 1H, NH); 8.96 (br, 2H, NH_2). ^{13}C-NMR (270 MHz, DMSO-d_6, TMS, ppm): δ = 13.7 (CH_3, ester); 59.9 (CH_2, ester); 165.0 (C, carbonyl); pyrazole: 17.9 (CH_3); 40.0 (C4); 155.6 (C3, imine); 173.0 (C5, amide); 34.0 (CH_2, bridge); pyridine: 30.0 (C4,); 80.0 (C5); 94.5 (C3); 140.0 (C2); 154.3 (C6); 120.4, 120.4, 124.1, 128.7, 128.7, 140.8 (6C, phenyl); 128.8, 128.8, 130.6, 130.6, 130.8, 135.8 (6C, chlorophenyl); Calcd. for $C_{25}H_{25}ClN_4O_3$ (464.94): C, 64.58%; H, 5.37%; N, 12.05%. Found: C, 64.36%; H, 5.14%; N, 11.79%.

4-(4-Chlorophenyl)-6-(3-metyhl-5-oxo-1-phenyl-4,5-dihydro-1*H*-pyrazol-4-ylmethyl)-2-phenyl-1,4-dihydropyridine-3-carboxylic acid ethyl ester (8b)

Brown colour, mp. 227-229°C, yield, 78%; Ms. m/z: 526 [M^+, 21.5%]. IR (KBr cm^{-1}): υ= 3088 (NH); 3137 (CH arom.); 2963 (CH aliph.); 1745 (C=O,ester); 1669 (C=O); 1605 (C=C arom.). ^{1}H NMR (270 MHz, DMSO-d_6, TMS, ppm): δ= 1.02 (s, 3H, CH_3); 1.30 (t, 3H, CH_3), 2.33 (d, 2H, CH_2); 2.72 (t, 1H, CH); 4.18 (q, 2H, CH_2), 4.12 (s, 1H, pyrid.); 5.14 (s, 1H, CH); 6.39–7.25 (m, 9H, aromatic protons); 7.43-7.86 (m, 5H, aromatic protons); 8.09 (s, 1H, NH). Calcd. for $C_{31}H_{28}ClN_3O_3$ (526.02): C,70.72%; H,5.32%; N,7.98%. Found: C, 70.58%; H,5.11%; N, 7.65%

4-(4-Chlorophenyl)-6-(3-metyhl-5-oxo-1-phenyl-4,5-dihydro-1*H*-pyrazol-4-ylmethyl)-2-oxo-1,2,3,4-tetrahydropyridine-3-carboxylic acid ethyl ester (8c)

Brown colour, mp. 227-230°C, yield, 78%; Ms. m/z: 465 [M^+, 14.5%]. IR (KBr cm^{-1}): υ= 3105 (NH); 3138 (CH arom.); 2869 (CH aliph.); 1745 (ester,C=O); 1673 (C=O); 1665 (C=O); 1606 (C=C, arom.). ^{1}H NMR (270 MHz, DMSO-d_6, TMS,ppm): δ= 1.02 (s, 3H, CH_3); 1.31 (t, 3H, CH_3), 2.36 (d, 2H, CH_2); 2.70 (t, 1H, CH); 3.64 (s, 1H, methine CH); 4.12 (q, 2H, CH_2), 4.14 (s, 1H, pyrid.); 5.13 (s, 1H, CH); 6.86–7.64 (m, 9H, aromatic protons); 7.96 (s, 1H, NH). Calcd. for $C_{25}H_{24}ClN_3O_4$ (465.92): C, 64.38%; H,5.15%; N, 9.01%. Found: C, 64.15%; H, 5.11%; N, 8.86%

Synthesis of 4-[5-Acetyl-4-(4-chlorophenyl)-6-methyl-1,4-dihydropyridin-2-yl-methyl]-5-methyl-2-phenyl-2,4-dihydropyrazol-3-one (9)

Equimolar ratios of compound **(3)**, p-chlorobenzaldehyde and acetylacetone in presence of excess of ammonium acetate and absolute ethanol as solvent were refluxed for 5 hrs. The reaction mixture was left to cool, poured on ice water and then reaction mixture neutralized with HCl. The solid products collected on filtration and recrystallized from ethanol

Pale brown colour, mp. 243-246°C, yield, 81.5%, Ms.m/z: 433 [M^+, 17.2%]. IR (KBr cm^{-1}): υ= 3127 (NH); 1715(C=O); 1675 (C=O); 1603 (C=C arom.);. ^{1}H NMR (270 MHz, DMSO-d_6, TMS, ppm): δ = 1.02(s, 3H, CH_3); 2.14 (s, 3H, CH_3); 2.5 (d, 2H, CH_2); 2.70 (t, 1H, CH); 3.51 (s, 3H, OCH_3); 4.11 (s, 1H, CH); 5.19 (s, 1H, CH); 6.51 – 7.88 (m, 9H, aromatic protons, s, 1H, NH). Calcd. for $C_{25}H_{24}ClN_3O_2$ (433.92): C, 69.20%; H,5.57%; N, 9.68%. Found: C, 69.03%; H,5.42%; N, 9.52%.

General procedure of (10a-b)

Compound **(6b)** and/or **(7)** in 15 mL of 10% ethanolic KOH solution and excess carbon disulphide were refluxed for 10 h. The reaction mixture was left to cool and then poured on acidic ice water that filtered to get solid products. The collected product was recrystallized from ethanol.

4-[5-(4-chlorophenyl)-2,4-dithioxo-1,2,3,4,5,8-hexahydropyrido[2,3-*d*]pyrimidin-7-ylmethyl]5-methyl-2-phenyl-2,4-dihydropyrazol-3-one (10a)

Deep yellow colour; mp. 246-248°C; yield 84 %); Ms. m/z: 494[M^+, 13.9%]. IR (KBr cm^{-1}): υ= 3118 (NH); 3146 (CH arom.); 2945 (CH aliph.); 1668 (C=O); 1613 (C=C arom.); 1578 (C=S). ^{1}H NMR (270 MHz, DMSO-d_6,TMS, ppm): δ= 1.01 (s, 3H, CH_3); 2.30 (d, 2H, CH_2) 2.52 (t, 1H, CH); 4.20 (s, 1H, CH); 5.12 (s, 1H, CH); 6.94-7.69 (m, 9H, aromatic protons); 8.14 (s, 1H, NH); 11.09 (s, 1H, NH); 12.30 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6, TMS, ppm): δ = pyrazole: 17.9 (CH_3, pyrazole); 40.5 (C4); 155 (C3, imine); 173 (C5, amide); 34 (CH_2, bridge); pyridopyrimidine: 35.6 (C4); 90.7 (C9); 94.5 (C3); 140.3 (C2); 154.3 (C10); 178 (C7, thioamide); 195 (C5, thioamide). 128.8, 128.8, 130.6, 130.6, 130.8, 135.8 (6C, chlorophenyl); 120.4, 120.4, 124.1, 128.7, 128.7, 140.8 (6C, phenyl); Calcd.for $C_{24}H_{20}ClN_5OS_2$ (494): C,58.35%; H,4.08%; N,14.18%. Found: C, 58.32%; H, 3.86%; N 13.88%.

4-[5-(4-chlorophenyl)-2,4-dithioxo-1,3,4,5-tetrahydro-2*H*-pyrano[2,3-*d*]pyrimidin-7-ylmethyl]5-methyl-2-phenyl-2,4-dihydropyrazol-3-one (10b)

Deep yellow colour; mp. 273-275°C; yield 91 %); Ms. m/z: 495[M^+, 27.3%]. IR (KBr cm^{-1}): υ= 3114 (NH); 3142 (CH arom.); 2967 (CH aliph.); 1679 (C=O); 1605 (C=C arom.); 1569 (C=S). ^{1}H NMR (270 MHz, DMSO-d_6,TMS, ppm): δ= 1.03 (s, 3H, CH_3); 2.26 (d, 2H, CH_2) 2.48 (t, 1H, CH); 4.18 (s, 1H, CH); 4.99 (s, 1H, CH); 6.72-7.83 (m, 9H, aromatic protons); 11.36 (s, 1H, NH). Calcd. for $C_{24}H_{19}ClN_4O_2S_2$; (495): C,58.32%; H,3.87%; N,11.32%. Found: C,58.22%; H,3.47%; N,13.6

General procedure of 11a-b and 12a-b

Equimolar ratios of **(6b)** and/or **(7)** were treated with either acetic or formic acids in presence of few drops of HCl and heated under reflux for 8-10 h. The reaction mixture was left to cool and then treated with alkaline ice water and filtered to get solid products. The collected product was recrystallized from the appropriate solvent.

5-(4-chlorophenyl)-2-methyl-7-(3-methyl-5-oxo-1-phenyl-4,5-dihydro-1*H*-pyrazol-4-ylmethyl)-5,8-dihydro-3*H*-pyrido[2,3-*d*]pyrimidin-4-one (11a)

Brown colour, mp. 239°C, yield, 79.5%, Ms. m/z : 459 [M^+, 28.4 %]. IR (KBr cm^{-1}): υ= 3125 (NH); 3139 (CH arom.); 2962 (CH aliph.); 1672 (C=O); 1658 (C=O, amide); 1601 (C=C arom.). ^{1}H NMR (270 MHz, DMSO-d_6, TMS,ppm): δ = 0.99 (s, 6H, $2CH_3$); 2.30 (d, 2H, CH_2); 2.50 (t, 1H, CH); 4.20 (s, 1H, CH); 4.99 (s, 1H, CH); 6.77 – 7.79 (m, 9H, aromatic protons, s, 1H, NH); 8.79 (s, 1H, NH). Calcd.for $C_{25}H_{24}ClN_3O_2$ (459.92): C,65.29%; H, 4.82%; N,15.23%. Found: C,65.11%; H,4.47%; N, 14.87%.

5-(4-chlorophenyl)-2-methyl-7-(3-methyl-5-oxo-1-phenyl-4,5-dihydro-1*H*-pyrazol-4-ylmethyl)-3,5-dihydropyrano[2,3-*d*]pyrimidin-4-one (11b)

Deep brown colour, mp. 247°C, yield, 89%, Ms. m/z : 460.91 [M^+, 1.72 %]. IR (KBr cm^{-1}): υ= 3137 (NH); 3137 (CH arom.); 2965 (CH aliph.); 1668 (C=O); 1652 (amide C=O); 1610 (C=C arom.). ^{1}H NMR (270 MHz, DMSO-d_6, TMS, ppm): δ = 0.99 (s, 6H, $2CH_3$); 2.32 (d, 2H, CH_2); 2.54 (t, 1H, CH); 4.21(s, 1H, CH); 5.07 (s, 1H, CH); 6.52 – 7.56 (m, 9H, aromatic protons); 8.35 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6, TMS, ppm): δ = pyrazole: 17.9 (CH_3); 39.4 (C4) 155.6 (C3,imine); 173 (C5, amide); 32.8 (CH2, bridge); pyranopyrimidine: 20.3 (CH_3); 29.9 (C4); 94.3 (C3); 100.7 (C9); 155.4 (C2); 161.9 (C10); 164 (C7); 168 (C5); 120.4, 120.4, 124.1, 128.7, 128.7 140.8 (6C, phenyl); 128.8, 128.8 130.6, 130.6 130.8, 135.8 (6C, chlorophenyl). Calcd. for $C_{25}H_{21}ClN_4O_3$ (460.91): C,65.15%; H,4.59%; N,12.16%. Found: C, 64.83%; H,4.36%; N,11.85.

5-(4-chlorophenyl)-7-(3-methyl-5-oxo-1-phenyl-4,5-dihydro-1*H*-pyrazol-4-ylmethyl)2,3,5,8-tetrahydro-1*H*-pyrido[2,3-*d*]pyrimidin-4-one (12a)

Pale brown colour, mp. 273-275°C, yield, 89%,

Ms. m/z : 447 [M^+, 18.14 %]. IR (KBr cm^{-1}): υ= 3116 (NH); 3142 (CH arom.); 2950 (CH aliph.); 1672(C=O); 1658 (amide C=O); 1609 (C=C arom.). ^{1}H NMR (270 MHz, DMSO-d_6, TMS, ppm): δ = 0.99 (s, 3H, CH_3); 2.31 (d, 2H, CH_2); 2.50 (t, 1H, CH); 4.21(s, 1H, CH); 4.35 (s, 2H, CH_2); 5.07 (s, 1H, CH); 6.72 – 7.68 (m, 9H, aromatic protons); 7.95 (s, 1H, NH); 8.13 (s, 1H, NH); 8.96 (s, 1H, NH). Calcd.for $C_{24}H_{22}ClN_5O_2$ (447.91): C,64.35%; H,4.95%; N,15.64%. Found: C, 64.16%; H, 4.91%; N, 15.58.

5-(4-chlorophenyl)-7-(3-methyl-5-oxo-1-phenyl-4,5-dihydro-1*H*-pyrazol-4-ylmethyl)1,2,3,5-tetrahydro-1*H*-pyrano[2,3-*d*]pyrimidin-4-one (12b)

Pale brown colour, mp. 261-264°C, yield, 89%, Ms. m/z : 448 [M^+, 23.6 %]. IR (KBr cm^{-1}): υ= 3137 (NH); 3145 (CH arom.); 2955 (CH aliph.); 1678 (C=O); 1658 (amide C = O); 1609 (C=C arom.). ^{1}H NMR (270 MHz, DMSO-d_6, TMS, ppm): δ = 1.03 (s, 3H, CH_3); 2.30 (d, 2H, CH_2); 2.42 (t, 1H, CH); 3.98 (s, 1H, CH); 4.33 (s, 2H, CH_2); 4.98(s, 1H, CH); 6.62–7.65 (m, 9H, aromatic protons); 7.86 (s, 1H, NH); 8.27 (s, 1H, NH). ^{13}C-NMR (270 MHz, DMSO-d_6, TMS, ppm), δ = pyrazole: 17.9 (CH_3); 39.4 (C4) 155.6 (C3, imine); 173 (C5, amide); 32.8 (CH2, bridge); pyranopyrimidine: 29.7 (C4); 52.6 (C7); 82.7 (C9); 94.2 (C3); 155.4 (C2); 166.8 (C5); 168.2 (C10); 120.4, 120.4, 124.1, 128.7, 128.7 140.8 (6C, phenyl); 128.8, 128.8 130.6, 130.6 130.8, 135.8 (6C, chlorophenyl). Calcd.for $C_{24}H_{21}ClN_4O_3$ (448.90): C,64.21%; H,4.72% ; N,12.48%. Found: C,64.19%; H 4.57%; N,12.25%.

General procedure of (13) and (14a-b)

0.01 mole of compound **(3)** and 0.01 mole of malononitrile, and 0.01 mole of sulfur in presence of few drops of triethylamine and absolute ethanol as solvent were heated under reflux for 5 h. The reaction mixture was left to cool, the solid was collected by filtration and then recrystallized from ethanol to obtain compound **(13)**. The resulted product **(13)** was treated with acetic and/or formic acids in excess, then subjected to reflux for 10 h. The reactin mixture was poured on alkaline ice water, filtered to collect the solid product and then recrystallized from ethanol.

5-amino-3-methyl-1-phenyl-1*H*-thieno[2,3-*c*]pyrazole-4-carbonitrile (13)

Yellow crystals, mp. 119-120°C, yield (66.5%); Ms. m/z (M^+); 254 (M^+, 34%): IR (KBr cm^{-1}): ν= 3425 (NH_2); 3142 (CH, arom.); 2960 (CH,alip.); 2148 (CN). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS, ppm): δ= 2.77 (s, 3H, CH_3); 4.20 (s, 2H, NH_2); 7.23 -7.64 (m, 5H, aromatic protons). Calcd.for $C_{13}H_{10}N_4S$: (254): C,61.40%; H,3.96%; N, 22.03%. Found: C,61.32%; H,3.87%; N, 22.00%.

3-Methyl-1-phenyl-1,7-dihydro-4*H*-pyrazolo[4',3':4,5]thieno[2,3-*d*]pyrimidin-4-one (14a)

Deep Yellow crystals, mp. 239°C, yield (71.5%); Ms. m/z, 282 (M^+, 16.2%): IR (KBr cm^{-1}): ν= 3158 (NH); 3115 (CH, arom.); 2985 (CH,aliph.); 1709 (C=O); ^{1}H-NMR (270 MHz, DMSO-d_6, TMS, ppm) δ= 2.79 (s, 3H, CH_3); 4.10 (s, H, NH); 7.53 (N=CH); 7.12 -7.64 (m, 5H, aromatic protons). Calcd.for $C_{14}H_{10}N_4OS$ (282.32): C,59.56%; H,3.57%; N,19.85%. Found C,59.31%; H,3.26 %; N,19.47%.

3,6-Dimethyl-1-phenyl-1,7-dihydro-8-thia-1,2,5,7-tetraaza-cyclopenta [a]inden-4-one (14b)

Deep Yellow crystals, mp. 274-276°C, yield (71.5%); Ms. m/z, 296.34 (M^+, 16.9%): IR (KBr cm^{-1}): ν= 3245 (NH); 3122 (CH, arom.); 2991 (CH,aliph.); 1709 (C=O). ^{1}H-NMR (270 MHz, DMSO-d_6, TMS, ppm): δ= 0.91 (s, 3H, CH3); 2.68 (s, 3H, CH_3); 4.12 (s, 1H, NH); 7.15 -7.69 (m, 5H, aromatic protons). ^{13}C-NMR (270 MHz, DMSO-d_6, TMS, ppm): δ = 10.9 (CH_3,pyrazole); 19.5 (CH_3, pyrimidine); 107.9 C9); 118.8 (C10); 137 (C12); 142 (C11); 151 (C3); 164 (C6); 186 (C4). Calcd.for $C_{15}H_{12}N_4OS$ (296.34): C,60.79%; H,4.08%;N,18.91%. Found: C,59.88%; H,3.93%; N,18.64%.

BIOLOGICALACTIVITY

Antimicrobial activity

Preparation of microbial suspensions

Antimicrobial activities were carried out against highly pathogenic strains; two Gram positive bacteria

Staphylococcus aureus and *Bacillus subtillis*, two Gram negative bacteria *Escherichia coli* and *Pseudomonas aeroginosa*, and only one mycotic strain (*Aspragillus flavus*) isolated from animal origin. Agar disk diffusion (qualitative method) and minimum inhibitory concentration (MIC) (quantitative method) were used in this study. Wherein a suspension of bacterial and mycotic strains were freshly prepared by inoculating fresh stock culture from each strain into separate broth tubes, each containing 7 ml of Muller Hinton Broth for bacterial strains and Sabaroud Dextrose broth for mycotic strain. The inoculated tubes were incubated at 37°C and 28 °C for 24 hr, respectively. Serial dilutions were carried out for each strain, dilution matching with 0.5 Mc-Farland was selected for screening of antimicrobial activities. Tetracycline 100µg/ml and fluconazole 20µg/ml were used as reference drugs (Oxoid).

Determination of antimicrobial activity by Disk-diffusion method. (Bansod and Rai)

Muller Hinton and Sabaroud Dextrose agar plates were prepared. Bacterial and fungal strains matching with 0.5 Mc-Farland were spread onto the surface of the agar plates using sterile cotton swabs. For evaluation of antibacterial activities, Whatman no1 filter paper disks were saturated with 100 µl of the extract, others were saturated with 100 µL Tetracycline/ (100µg/mL) and others 100 µL DMSO as control negative. The same method was used for evaluation of antimycotic activities using fluconazole (20µg/mL). Disks were placed onto inoculated agar plates and left for 1 hr at 25 °C to allow a period of pre-incubation diffusion in order to minimize the effects of variation in time between the applications of different solutions. The plates were re-incubated at 37°C and 28°C for 24 hrs for bacterial and mycotic strains respectively. After incubation, plates were observed for antimicrobial activities by determining the diameters of the inhibition zone for each of the samples. For an accurate analysis, tests were run in triplicate for each strain to avoid any error[18].

Determination of minimum inhibitory concentration (MIC)

Micro dilution plate quantitative method (Andrews, 2001), i.e. the minimum inhibitory concentration (MIC) was used for evaluation of the antimicrobial activity of the tested compounds against previously tested organisms showing inhibition zone using disc diffusion method. Determination of MIC of compounds against tested isolates was achieved using 96-well sterile micro plates. Initial concentration 100%, then two fold serial dilutions of the compounds and reference drugs (tetracycline or fluconazole) and drugs coated with nanoparticles were inoculated with 100µl of tested isolates (0.5 McFarland, about 1×10^5 cells/ml) and incubated at 37°C-28°C for 24 h for bacterial and fungal isolates respectively. After incubation, plates were examined visually for bacterial or fungal growth precipitation. The experiment was repeated three times. The lowest concentration that showed complete growth inhibition of the microbe was taken as MIC[19].

CONCLUSION

All synthesized pyrazole derivatives showed very high antimicrobial activity in both disc diffusion method (ADDT), and microdilution one (MIC). Many compounds exhibited antibacterial activity, against Gram – ve bacteria higher than that of the standard drug, in particular the pyrano derivatives of pyrazole. Most of the synthesized compounds showed also high antifungal activity when compared with that of the standard antifungal drug. Again, most compounds, in MICs values, indicate high antibacterial and antifungal activities when compared with the corresponding reference drug.

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