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Synthesis and antimicrobial evaluation of novel 3-(thiophen-2-yl) -Pyrazoline-5-yl derivatives

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Abstract

Ten derivatives of N1 substituted/unsubstituted 5-(4-chlorophenyl)-3-(2-thienyl) pyrazoline were synthesised from chalcone-like intermediate and substituted phenyl hydrazines, hydrazine hydrate, and semi/thiosemicarbazide. The chemical structure of compounds was confirmed by means of IR, ^{1}HNMR, mass spectroscopy, and elemental analysis. The antimicrobial activities (antibacterial and antifungal activities) of the synthesized compounds were evaluated against *Staphylococcus aureus, Staphylococcus faecalis, Escherichia coli, Salmonella typhi, and Candida albicans* by agar diffusion cup plate method. Compound IId (5-[(4-chlorophenyl)-1-(4-methoxyphenyl)]-3-thiophen-2-yl)-4,5-dihydro-2-pyrazoline), IIg (5-(4-chlorophenyl)-1-(2-methylphenyl)-3-(thiophen-2-yl)-4,5-dihydro-2-pyrazoline) showed very good activity. IIi (5-(4-chlorophenyl)-1-thiocarbamoyl-3-(thiophen-2-yl))-2-pyrazoline), IIj (5-(4-chlorophenyl)-1-carbamoyl-3-(thiophen-2-yl))-2-pyrazoline) exhibit mild to moderate inhibitory response against all the tested microbial strains. Compound IId shows excellent significant zone of inhibition (mm) against all the species of microbes, thereby producing promising antimicrobial respectively.

Keywords: Chalcone, Pyrazoline, antimicrobial activity.

1. Introduction

Antimicrobials are the most important weapons in combating bacterial infections and have greatly benefitted the health related quality of human life since their introduction [1]. Combat against bacterial infections has resulted in the development of a wide variety of antibiotics. However, continuous use of antibiotics particularly leads to resistance against the microbes, which is presently the major area of research, thereby, contribution towards finding a new lead molecule. So there is recommendation of use of new antibacterial agents with enhanced broad spectrum of activity and lesser side effects [2, 3].

Compounds with pyrazoline ring have received widespread attention in recent years. They have been reported as possessing a wide range of biological activities such as anti-inflammatory [4], antidepressant [5], anticonvulsant [6], Antitubercular [7], Anti-diabetic [8], antiandrogenic [9], anti-thrombotic [10], properties, In addition to these effects, in the last decade pyrazolines and substituted pyrazolines have emerged as promising antimicrobial agents [11], The present study was aimed to develop some novel pyrazoline derivatives and evaluate their possible antimicrobial activity.

2. Materials and Methods

2.1. Reagents and Apparatus.

All reagents and solvents used in the study were of analytical grade purity and procured from Sigma–Aldrich Ltd (Mumbai, India). The progress of the reaction was monitored by thin layer chromatography over a commercial available adsorbent (silica gel 60 F_{254} aluminium sheets) procured from Merck Ltd., Germany. The mobile phase used for developing the chromatogram was hexane and ethyl acetate (3:2).

The products obtained from the reaction process were purified by re-crystallisation. Melting points were determined in open capillaries using Stuart SMP10 (Barloworld Scientific Ltd., UK), electrothermal melting point apparatus and were not corrected. IR spectra were recorded on a Shimadzu 8400S FTIR (Shimadzu Corporation, Japan) spectrophotometer using KBr pellets and ῡ were recorded in cm^{-1}. ^{1}HNMR (300 MHz) spectra were acquired on a JEOL AL300 FT-NMR (Jeol Ltd., Japan) in CDCl3 using TMS as the internal standard and the chemical shifts were reported in δ. The mass spectrum was obtained on a Hewlett Packard model GCD-1800A (Hewlett Packard, USA) electron impact mass spectrometer at 70 eV ionising beam and using a direct insertion probe. Elemental analyses for C, H, and N were performed on Exeter CE-440 (Hewlett–Packard, USA) elemental analyser.

2.2. Synthesis:

2.2.1. Synthesis of (E)-3-(4-chlorophenyl)-1-(thiophen-2-yl) prop-2-en-1-one (I) : Intermediate

Equimolar concentrations (0.04 mol) of 1-thiophen-2-yl-ethanone and 4-chlorobenzaldehyde were added to the 10 % aqueous solution of NaOH and ethanol (30mL) in order to carry out Claisen–Schmidt condensation. The reaction mixture was then stirred at room temperature for 3 h and the product thus obtained was filtered, dried, and re-crystallised from ethanol [12]**.**

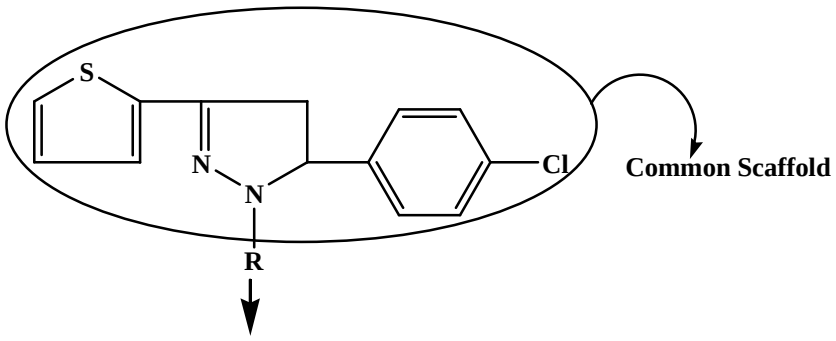
2.2.2. General procedure for the synthesis of pyrazoline derivatives: [13]

2.2.2.1. Synthesis of compounds IIa–IIh:

Equimolar concentrations (0.1 mol) of **I** and 2,4-substituted phenylhydrazine and hydrazine hydrate in ethanol were refluxed for 4h. The reaction mixture was then poured into ice-cold water and the precipitate so obtained was washed with water, dried, and recrystallised from ethanol to afford the target compounds IIa–IIh.

2.2.2.2. Synthesis of compounds IIi–IIj:

A mixture of compound I (1.0 mol) and semi/thiosemicarbazide (1.0 mol) in ethanolic NaOH (0.02 mol, 50 mL) was refluxed for about 2 h. The reaction mixture was then poured into ice-cold water and the precipitate so obtained was separated by filtration, washed with water, dried, and re-crystallised from ethanol to afford the target compounds IIi and IIj.



"Fig. 1". Scaffold of the designed pyrazoline derivatives

3. Pharmacological Activity:

The biological study was approved by the GIPS animal ethical committee (GIPS/IAEC/9). All the chemicals and solvents used for the pharmacological activity were purchased from Sigma-Aldrich. The newly synthesized compounds (IIa-IIj) were tested for their antimicrobial activity.

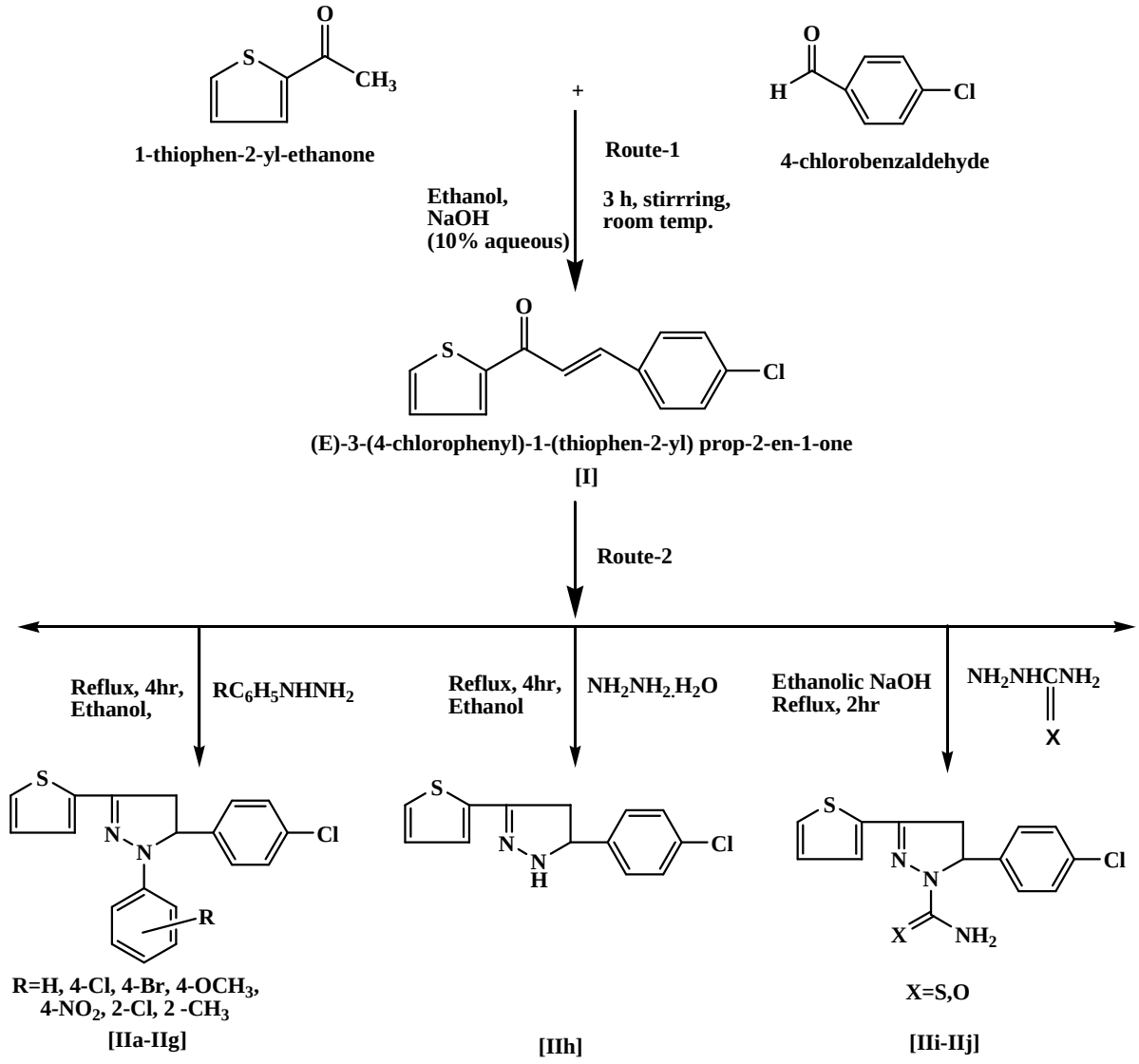
3.1. Anti microbial activity:

The microbial evaluations (antibacterial and antifungal activities) of the compounds were evaluated by agar diffusion cup plate method [14, 15]. All the compounds were screened for antimicrobial activity at 100 µg/mL concentration against the following bacterial strains: *Staphylococcus aureus, Staphylococcus feacalis, Escherichia coli,* and *Salmonella typhi*. Antifungal activity was tested on Sabouraud dextrose agar (Himedia Pvt., Ltd) plates (260C, 48-72 h) by cup plate method against *Candida albicans* at the concentration level of 100 µg/mL. Ciprofloxacin and Clotrimazole were used as a reference standard for comparison of antibacterial and antifungal activity under the similar conditions. DMF was used as a solvent control for both antibacterial and anti fungal activities.

4. Results and Discussion

4.1. Spectral characterization:

The proposed derivatives were synthesised as illustrated in **Fig. 2**. Physical properties and elemental analysis (**Table 1**) as well as all the spectral data (**Table 2**) are in accordance with the structures of the synthesized compounds. The spectra of I displayed the characteristic —C=O stretching at 1708 cm^{-1} and a sharp peak at 1658 cm^{-1} due to *α, β* —CH=CH. The derivatives showed diagnostic infrared absorptions at 1578–1598 cm^{-1} for —C=N stretching of the pyrazoline nucleus. In addition, all the compounds displayed C_4—H deformation (1354–1442 cm^{-1}) and C_5—N_1 stretching (1068–1142 cm^{-1}). Compounds Iii and IIj showed additional thiocarbamoyl NH stretching vibration (3481 cm^{-1}), carbamoyl NH stretching (3464–3481 cm^{-1}), —C=S stretching at a lower frequency of 1345 cm^{-1}, and —C=O stretching at 1684 cm^{-1}. ^{1}HNMR spectra of IIa–IIj exhibited three spectral ranges in which each appears as a double doublet due to the presence of non-magnetically equivalent pyrazoline Ha, Hb, and Hx protons. The geminal pyrazoline proton in position 4 represented by the Ha and Hb methylene protons displayed signals at δ 3.04–3.13 (upfield) and δ 3.13–3.93 (downfield), respectively. The methine proton Hx appeared further downfield at δ 5.16–5.98 and the aromatic and thienyl protons at chemical shift in the range of δ 6.44–7.82. All the other protons belonging to the methyl and methoxy groups were seen according to the expected chemical shift. The mass spectra of the compounds were studied and the molecular ion peaks ((M)+), which were found consistent for all the compounds. The elemental analysis results were within ± 0.4 % of the theoretical values.



"Fig. 2": Synthesis of compounds IIa-IIj

Table 1: Physico-Chemical Characterization

Comp Code	Mol. Formula	Mol.wt	Wi (Calc.)/% Wi (Found)/%			Yield (%)	MP(0°C)
			C	H	N		
I	$C_{13}H_9ClOS$	248.81	62.78	3.65	----	82	104-106
			63.01	3.62	----		
IIa	$C_{19}H_{15}ClN_2S$	338.12	67.35	4.46	8.27	71	135-137
			67.53	4.61	8.25		
IIb	$C_{19}H_{14}Cl_2N_2S$	372.67	61.13	3.78	7.50	69	130-132
			60.92	3.65	7.48		
IIc	$C_{19}H_{14}BrClN_2S$	417.38	54.63	3.38	6.71	70	121-123
			54.81	3.37	6.69		
IId	$C_{20}H_{17}ClN_2OS$	368.74	65.12	4.65	7.59	67	140-142
			64.89	4.63	7.56		
IIe	$C_{19}H_{14}ClN_3O_2S$	383.09	59.45	3.68	10.95	63	137-139
			59.53	3.69	10.91		
IIf	$C_{19}H_{14}Cl_2N_2S$	372.12	61.13	3.78	7.50	66	125-127
			61.24	3.79	7.48		
IIg	$C_{20}H_{17}ClN_2S$	352.21	68.07	4.86	7.94	68	134-136
			67.42	4.84	7.96		
IIh	$C_{13}H_{11}ClN_2S$	362.13	59.42	4.22	10.66	70	133-135
			59.27	4.29	10.68		
IIi	$C_{14}H_{12}ClN_3S_2$	321.04	52.25	3.76	13.06	75	180-182
			52.06	3.75	13.01		
IIj	$C_{14}H_{12}ClN_3OS$	305.06	54.99	3.96	13.74	87	195-198
			54.85	3.97	13.77		

Table-2:Spectral Characterization of Newly Prepared Compounds

Compound	Spectral data
I	IR, υ˜/cm^{-1}: 855 (C-Cl stretching), 1658 (α, β CH=CH), 1708 (C=O). ^{1}H-NMR ($CDCl_3$), δ: 6.65 (d, 1H,-CO-CH=), 7.20-7.68 (m, 7H, Ar-H and thiophene), 7.81 (d, 1H, =CH-Ar). MS, m/z: 248.81 (M^+)
IIa	IR, υ˜/cm^{-1}: 1069 (C5-N1 stretching), 1373 (C4-H deformation), 1591 (C=N), 3010 (CH thienyl). ^{1}H-NMR ($CDCl_3$),δ: 3.05 (1H, dd, Ha), 3.89 (1H, dd, Hb), 5.26 (1H, dd, Hx), 6.93–7.71 (12H, m, thiophene and Ar-H).MS, m/z: 338.12 (M^+)
IIb	IR, υ˜/cm^{-1}: 1076 (C5-N1 stretching), 1089 (C-S-C stretching), 1383 (C4-H deformation), 1597 (C=N), 3012 (CH thienyl). ^{1}H-NMR ($CDCl_3$), δ: 3.07 (1H, dd, Ha), 3.83 (1H, dd, Hb), 5.21 (1H, dd, Hx), 6.94–7.68 (11H, m, thiophene and Ar-H).MS, m/z: 372.67 (M^+)
IIc	IR, υ˜/cm^{-1}: 1081 (C5-N1 stretching), 1085 (C-S-C stretching), 1367 (C4-H deformation), 1593 (C=N), 3050 (CH thienyl).^{1}H-NMR ($CDCl_3$),δ:3.07 (1H, dd, Ha), 3.86 (1H, dd, Hb), 5.24 (1H, dd, Hx), 6.85–7.64 (11H, m, thiophene and Ar-H).MS, m/z: 417.38 (M^+)
IId	IR, υ˜/cm^{-1}: 1068 (C5-N1 stretching), 1082 (C-S-C stretching), 1132 (C-O stretching), 1358 (C4-H deformation), 1595 (C=N), 3041 (CH thienyl)1H-NMR ($CDCl_3$),δ:2.78(3H,s,OCH), 3.04 (1H, dd, Ha), 3.82 (1H, dd, Hb), 5.16 (1H; dd, Hx), 6.73–7.73 (11H,33m, thiophene and Ar-H). MS, m/z: 368.74 (M^+)
IIe	IR, υ˜/cm^{-1}: 1072 (C5-N1 stretching), 1088 (C-S-C stretching), 1350 (N-O stretching), 1359 (C4-H deformation), 1586 (C=N), 3017 (CH thienyl).^{1}H-NMR ($CDCl_3$),δ:3.08 (1H, dd, Ha), 3.75 (1H, dd, Hb), 5.53 (1H, dd, Hx), 7.05–7.82 (11H, m, thiophene and Ar-H). MS, m/z: 383.09 (M^+)
IIf	IR, υ˜/cm^{-1}: 1087 (C-S-C stretching), 1138 (C5-N1 stretching), 1442 (C4-H deformation), 1595 (C=N), 3045(CH thienyl).^{1}H-NMR ($CDCl_3$),δ: 3.13 (1H, dd, Ha), 3.71 (1H, dd, Hb), 5.27 (1H, dd, Hx), 6.79–7.60 (11H, m, thiophene and 3Ar-H).MS, m/z: 372.12 (M^+)
IIg	IR, υ˜/cm^{-1}: 1079 (C-S-C stretching), 1142 (C5-N1 stretching), 1378 (C4-H deformation), 1578 (C=N), 3032(CH thienyl).^{1}H-NMR ($CDCl_3$),δ:2.12(1H,s,CH), 3.10 (1H, dd, Ha), 3.78 (1H, d, Hb), 5.22 (1H, d, Hx), 6.89–7.65 (11H, m,133thiophene and Ar-H).MS, m/z: 352.21 (M^+)
IIh	IR, υ˜/cm^{-1}: 1075 (C5-N1 stretching), 1091 (C-S-C stretching), 1354 (C4-H deformation), 1597 (C=N), 3012 (CH thienyl), 3290 (NH streching). ^{1}H-NMR ($CDCl_3$),δ: 3.12 (1H, dd, Ha), 3.93 (1H, dd, Hb), 5.29 (1H, dd, Hx), 6.44–7.49 (7H, m, thiophene and Ar-H), 7.51 (1H, s, NH_2).MS, m/z: 362.13 (M^+)
IIi	IR, υ ˜/cm^{-1}: 1071 (C-S-C stretching), 1085 (C5-N1 stretching), 1345 (C=S stretching), 1425 (C4-H deformation), 1592 (C=N stretching), 3024 (CH thienyl), 3481 (NH stretching). ^{1}H-NMR ($CDCl_3$), δ: 3.12 (1H, dd, Ha), 3.81 (1H, dd, Hb), 5.98 (1H; dd, Hx), 7.07–7.63 (7H, m, thiophene and Ar-H), 7.75 (1H, s, NH_2). MS, m/z: 321.04 (M^+)
IIj	IR, υ ˜/cm^{-1}: 1072 (C-S-C stretching), 1095 (C5-N1 stretching), 1433 (C4-H deformation), 1595 (C=N stretching), 1684 (C=O stretching), 3021 (CH thienyl), 3464 (NH stretching).^{1}H-NMR ($CDCl_3$),δ: 3.08 (1H, dd, Ha), 3.84 (1H, dd, Hb), 5.29 (1H, dd, Hx), 7.04–7.52 (7H, m, thiophene and Ar-H), 7.56 (2H, s, NH_2). MS, m/z: 305.06 (M+)

a) Ha, Hb protons denote the methylene protons and Hx protons denote the methine proton of pyrazoline nucleus

4.2. Antimicrobial activity:

The microbial evaluations (antibacterial and antifungal activities) of the compounds were evaluated by agar diffusion cup plate method. The zone of inhibition was ascertained by disk diffusion method. Pharmacological data of the compounds are shown in Table 3. Compound **IId and IIg** exerted pronounced inhibitory response against all the antimicrobial species. This may be due to the electron releasing substituent present in phenyl hydrazine ring attached to position 1 of pyrazoline ring. Compound **IIi, IIj** exerted mild to moderate inhibitory response against all the antimicrobial species. Compound IIa, IIb, IIc, IIe, IIf, IIh does not show good activity. This may be due to the presence of electro withdrawing group present in the phenyl hydrazine moiety which is attached to the position of pyrazoline ring.

Table 3: Antimicrobial activity of synthesized compounds

Compound	Substituent	Conc. (µg/ml)	Zone of inhibition (mm)				
IIa	H	100	***Staphylococcus aureus***	***Staphylococcus faecalis***	***Escherichia coli***	***Salmonella typhi***	***Candida albicans***
IIb	4-Cl	100	14	13	17	13	15
IIc	4-Br	100	14	17	18	19	17
IId	4-OCH_3	100	**28**	**29**	**21**	**25**	**23**
IIe	4-NO_2	100	15	12	16	18	17
IIf	2-Cl	100	15	14	15	14	16
IIg	2-CH_3	100	**26**	**27**	**25**	**24**	**25**
IIh	H	100	16	13	15	12	18
IIi	S	100	**21**	**23**	**20**	**21**	**20**
IIj	O	100	**20**	**17**	**19**	**18**	**21**
Ciprofloxacin	----	10	30	33	29	28	---
Clotrimazole	----	20	------	---	----	-----	30

Conclusion

A new series of pyrazoline derivatives with a common skeleton was synthesised and evaluated for their antimicrobial properties. A few of them are considered to be promising compounds due to their respective activities. Two compounds (**IId, IIg**) exhibited a good activity profile against *Staphylococcus aureus, Staphylococcus faecalis, Escherichia coli, Salmonella typhi, and Candida albicans*. Therefore, the study deserves further investigation, particularly with respect to that of the in vitro antimicrobial activity and simultaneous derivatisation of the building block 5-(4-chlorophenyl)-3-(2-thienyl) pyrazoline derivatives.

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References

1. Sarkar A, Kumar KA, Dutta NK, Chakraborty P, Dastidar SG (2003) Evaluation of in vitro and in vivo antibacterial activity of dobutamine hydrochloride. Indian J. Med. Microbiol 21(3):172-178.
2. Mohamed AM, Magdy MG, Magda NN, Waleed AHB (2004) Arch. Pharm. Pharm Med Chem 337:427-433.
3. Ozdemir A, Zitouni GT, Zafar AK, Gilbert R, Kiymet G (2007) Synthesis and antimicrobial activity of 1-(4-aryl-2-thiazolyl)3-(2-thienyl)-5-aryl-2-pyrazoline derivatives. Eur J Med Chem 42: 403-409.
4. Fioravanti R, Bolasco A, Manna F, Rossi F, Orallo F, Ortuso F, Alcaro S, Cirilli R (2010) Synthesis and biological evaluation of N-substituted-3,5-diphenyl-2-pyrazoline derivatives as cyclooxygenase (COX-2) inhibitors. Eur J Med Chem 45(12): 6135–6138.
5. Rajendra Prasad Y , Lakshmana Rao A , Prasoona L , Murali K , Ravi Kumar (2005) Synthesis and antidepressant activity of some 1,3,5-triphenyl-2-pyrazolines and 3-(2"-hydroxy naphthalene-1"-yl)-1,5-diphenyl-2-pyrazolines. Bioorg Med Chem Lett 15(22):5030-5034.
6. Das N, Dash B, Dhanawat M, Shrivastava SK (2012) Design, synthesis, preliminary pharmacological evaluation, and docking studies of pyrazoline derivatives. Chemical Paper 66(1): 67–74.
7. Ali MA, Yar MS, Kumar M, Pandian, GS (2007) Synthesis and antitubercualr activity of substituted novel pyrazoline derivatives. Natural Product Research; 21(7):575-579.

8. Bertrand C, Patrick T, Christophe M, Aline P, Chenault J (2002) Synthesis and hypoglycaemic evaluation of substituted pyrazole-4-carboxylic acids. Bioorg Med Chem Lett 12: 2105-2108.
9. Amr Ael-G, Abdel-Latif NA, Abdalla MM (2006) Synthesis and antiandrogenic activity of some new 3- substituted androstano[17,16-c]-5'-aryl-pyrazoline and their derivatives. Bioorg Med Chem Lett 14(2): 373–384.
10. Casimiro-Garcia A, Dudley DA, Heemstra RJ, Filipski KJ, Bigge CF, Edmunds JJ (2006) Progress in the discovery of factor Xa inhibitors. Exp Opi Ther Pat 16: 119–145.
11. Azarifar D, Sharbanzadeh M. (2002) Synthesis and characterization of new 3, 5-dinaphthyl substituted 2-pyrazolines and study of their antimicrobial activity. Molecules 7(12):885-895.
12. Manna F, Chimenti F, Bolasco A, Secci D, Bizzarri B, Befani O, Turini, P., Mondovi B, Alcaro S, Tafi A (2002) Inhibition of amine oxidases activity by1-acetyl-3,5-diphenyl-4,5-dihydro-(1H)-pyrazole derivatives. Bioorg Med Chem Lett 12: 3629–3633.
13. Ozdemir, Z., Kandilci, H. B., Gumusel, B., Calis, U., Bilgin, A A (2007) Synthesis and studies on antidepressant and anticonvulsant activities of some 3-(2-furyl)-pyrazoline derivatives. Euro J Med Chem 42: 373-379.
14. Sahu SK, Banerjee M, Samantray A, Behera C, Azam MA (2008) Synthesis, Analgesic, Anti-inflammatory and Antimicrobial Activities of Some Novel Pyrazoline Derivatives. Trop J Pharm Res 7(2): 961-968.
15. Nada MA, Hamdi MH, Nadia GK, Omar AM (2008) Synthesis and Antimicrobial Activity of Some New Pyrazole, Fused Pyrazolo[3,4-d]-pyrimidine and Pyrazolo[4,3-e][1,2,4]- triazolo [1,5-c] pyrimidine Derivatives. Molecules 13(7):1501-1517.

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