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SYNTHESIS, BIOLOGICAL EVALUATION AND MOLECULAR PROPERTIES PREDICTION OF 1, 3, 5-TRISUBSTITUTED 2-PYRAZOLINE DERIVATIVES

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Keywords:

pyrazolines, chalcones, molecular properties prediction, acute toxicity, antifungal, antiinflammatory

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ABSTRACT: A number of trisubstituted 2-pyrazolines which are known for their biological importance like antimicrobial, anti-inflammatory, antitubercular, analgesic, anticancer, antimalarial, anti-viral, antihelmentic activities are prepared by reacting chalcones with phenyl hydrazine in absolute ethanol in presence of pyridine. In the present investigation an attempt has been made for the synthesis of 2-pyrazoline derivatives and subjected to molecular properties prediction by molinspiration, molsoft and osiris softwares. The synthesized compounds have been confirmed by IR, Mass and ^{1}HNMR spectral data. These compounds were also screened for various biological activities like antifungal, anti-inflammatory and acute toxicity activity by standard methods. The synthesized compounds have shown moderate to good antifungal, anti-inflammatory activity and some of the synthesized compounds have shown significant activity as compared with standard. Compound 5d showed good antiinflammatory activity, compounds 5c, 5d, and 5e showed higher antifungal activity and all the synthesized compounds were found to be in conformity with lipinski's rule.

INTRODUCTION: The chemistry and biological study of heterocyclic compounds has been an interesting field in medicinal chemistry for a long time. The title compound Pyrazoline (1) is five-membered heterocyclic having two adjacent nitrogen atoms within the ring as shown in figure-1. It has only one endocyclic double bond and is basic in nature. Among its various derivatives, 2-pyrazolines (2) as in **Figure-1** seem to be the most frequently studied pyrazoline type compounds and can be considered as a cyclic hydrazine moiety as seen in **Figure-1**.

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They display a broad spectrum of potential pharmacological activities and are present in a number of pharmacologically active molecules such as phenazone/ amidopyrene/ methampyrone (analgesic and antipyretic), azolid/tandearil (anti-inflammatory), indoxacarb (insecticide) and anturane (uricosuric). Changes in their structure have offered a high degree of diversity that has proven useful for the development of new therapeutic agents having improved potency and lesser toxicity. In this context, the recently synthesized 2-pyrazoline derivatives possessing important pharmacological activities have been highlighted.

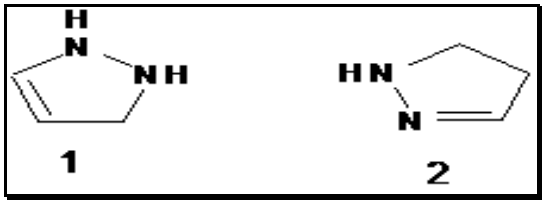


FIGURE: 1

Pyrazoline derivatives were found to have potential anticonvulsant [1], antidepressant [2], anti-inflammatory [3], antimicrobial [4], antibacterial [5], antitubercular [6], antitumour [7], antihepatotoxic [8], analgesic [9], antioxidant [10] and hypotensive [11] activities.

Molecular properties:

A molecular property, drug likeness is a complex balance of various structural features which determines whether a particular molecule is similar to the known drugs. It generally means molecules which contain functional groups and or have molecular properties which are associated with some basic molecular descriptors such as logP (partition coefficient), molecular weight or hydrogen bond acceptor and donor counts in a molecule. Lipinski used molecular properties in formulating his "rule of five". The rule states that most molecules with good membrane permeability have logP ≤5, molecular weight ≤500, number of hydrogen bond acceptors ≤10 and hydrogen bond donors ≤5. Along with the above rules the other molecular descriptors like total polar surface area (TPSA), molecular volume and number of rotatable bonds explain the pharmacodynamic properties[12]. All these properties are calculated by molsoft, molinspiration and osiris softwares in order to filter the drugs for synthesis and biological screening and to reduce enormous wastage of expensive chemicals and precious time.

Molinspiration:

Molinspiration chem. informatics provides calculation of molecular properties relevant to drug design and QSAR, including logP, molecular polar surface area (PSA), nrotb and HBA/HBD counts and the rule of five descriptors[13]. However, this website offers tools to calculate other properties, such as volume and total number of atoms in the molecule. Molinspiration molecular properties and bioactivity calculations of the synthesized compounds (5a-5j) are predicted in the **Table 1A** and **1B** respectively.

TABLE-1A: MOLINSPIRATION CALCULATIONS OF COMPOUNDS 5a-5j:

Cmpd	Clogp	TPSA	MW	noN	noHNH	Nviolation	nrotb	volume
5a	5.739	15.602	346.499	2	0	1	3	326.087
5b	5.454	15.602	350.462	2	0	1	3	314.458
5c	5.968	15.602	366.917	2	0	1	3	323.062
5d	6.574	15.602	401.362	2	0	1	3	336.598
5e	4.922	43.304	422.55	5	0	0	6	386.163
5f	5.181	34.07	376.481	4	0	1	3	333.456
5g	5.164	33.428	412.562	4	0	1	4	348.707
5h	5.626	61.426	391.496	5	0	1	4	349.422
5i	5.855	61.426	411.914	5	0	1	4	346.396
5j	5.249	61.426	377.469	5	0	1	4	332.86
Streptomycin	-5.35	336	582	19	16	3	9	497
Ampicillin	-0.87	113	349	4	24	0	4	299
Fluconazole	-0.12	81.6	306	7	1	0	5	249

MW - Molecular weight; TPSA - Total Polar Surface Area;
noN - no. of Hydrogen acceptors; noHNH - no. of Hydrogen donors;
nrotb - no. of Rotatable bon

TABLE- 1B: MOLINSPIRATION BIOACTIVITY CALCULATIONS OF COMPOUNDS 5a-5j

CMPD	GPCR	ICM	KI	NRLI	PI	EI
5a	-0.41	-0.91	-0.86	-0.09	-0.81	-0.47
5b	-0.38	-0.88	-0.80	-0.04	-0.80	-0.45
5c	-0.39	-0.87	-0.85	-0.08	-0.82	-0.47
5d	-0.38	-0.86	-0.92	-0.07	-0.86	-0.51
5e	-0.38	-0.80	-0.72	-0.15	-0.74	-0.41
5f	-0.37	-0.90	-0.83	-0.13	-0.78	-0.44
5g	-0.45	-0.89	-0.85	-0.33	-0.92	-0.54
5h	-0.53	-0.87	-0.90	-0.17	-0.87	-0.54
5i	-0.50	-0.85	-0.98	-0.13	-0.93	-0.56
5j	-0.49	-0.85	-0.91	-0.14	-0.84	-0.50
Streptomycin	0.09	-0.16	-0.17	-0.18	0.65	0.38
Ampicillin	0.04	-0.47	-0.71	-1.61	0.87	0.25
Fluconazole	-0.04	0.01	-0.09	-0.23	-0.09	0.03

GPCRL - GPCR ligand; ICM - Ion channel modulator KI- Kinase inhibitor;
NRL - Nuclear receptor ligand; NRL - Nuclear receptor ligand; EI- enzyme inhibitor

Molsoft: Molsoft online tool calculates the chemical properties like molecular formula, molecular weight, number of hydrogen bond acceptors (HBA), number of hydrogen bond donors (HBD), molLogP (octanol/water partition coefficient), mollogS (water solubility), polar surface area (molPSA), volume, number of stereo centers, drug likeness model score.

In Molsoft[14] the strategy which leads to success focuses on particular drug classes and development of specific activity scores for each of these classes. The method compares structures of representative ligands active on the particular target with structures of inactive molecules and to identify substructure features (which in turn determine physicochemical properties) typical for active molecules. Molsoft molecular properties calculations of the synthesized compounds (5a-5j) are predicted in the **Table-2**.

TABLE-2: MOLSOFT CALCULATIONS OF COMPOUNDS 5a-5j:

CMPD	MF	MW	NO.HBA	NO.HBD	MLOGP	MLOGS	MPSA	MV	NO.SC	DL
5a	$C_{22}H_{22}N_2S$	346.15	2	0	5.74	-5.52	14.94	349.62	1	-0.21
5b	$C_{21}H_{19}FN_2S$	350.13	2	0	5.60	-5.59	14.94	334.59	1	0.12
5c	$C_{21}H_{19}ClN_2S$	366.10	2	0	6.05	-6.03	14.94	345.87	1	0.29
5d	$C_{21}H_{18}Cl_2N_2S$	400.06	2	0	6.64	-6.51	14.94	362.93	1	0.34
5e	$C_{24}H_{26}N_2O_3S$	422.17	5	0	5.36	-5.00	37.91	423.79	1	-0.01
5f	$C_{22}H_{20}N_2O_2S$	376.12	4	0	5.48	-6.01	32.05	368.51	1	-0.49
5g	$C_{25}H_{24}N_4S$	412.17	3	0	5.35	-5.92	29.65	407.60	1	-0.25
5h	$C_{22}H_{21}N_3O_2S$	391.14	4	0	5.29	-6.01	48.02	374.37	1	-0.63
5i	$C_{21}H_{18}ClN_3O_2S$	411.08	4	0	5.60	-6.40	48.32	370.72	1	-0.20
5j	$C_{21}H_{19}N_3O_2S$	377.12	4	0	5.00	-5.92	48.32	353.67	1	-0.35
Streptomycin	$C_{22}H_{41}N_7O_{12}$	595	15	16	-6.98	-0.86	268.91	515.35	15	0.90
Ampicillin	$C_{16}H_{19}N_3O_4S$	349	6	4	0.31	-2.41	90.99	350.86	4	1.11
Fluconazole	$C_{13}H_{12}F_2N_6O$	304	5	1	-0.09	-2.12	66.19	263.43	0	0.03

MF: Molecular formula; HBA: Hydrogen bond acceptors; HBD: Hydrogen bond donors; MlogP: MolLogP; MlogS, MolLogS; MPSA: Molecular Polar Surface Area; MV: Molecular Volume; SC: no. of stereocentres; DL: Drug Likeness

Osiris: The osiris property explorer is an integral part of actelion's inhouse substance registration system[15]. It lets you draw chemical structures and calculates on-the-fly various drug-relevant properties whenever a structure is valid. Properties with high risks of undesired effects like mutagenicity or a poor intestinal absorption are shown in red whereas green colour indicates drug-conform behavior. Structure based design is now fairly routine but many potential drugs fail to reach the clinic because of ADME toxicity liabilities. One very important class of enzymes, responsible for many ADME problems, is the cytochrome P_{450}. Inhibition of these or production of unwanted metabolites can result in many adverse drug reactions. The results are predicted in the **Table-3**. Most of the synthesized compounds (5a-5j) were found to be in conformity with lipinski's "rule of five" and other parameters, for their onward screening for biological activity as oral active leads/ drugs.

TABLE 3: OSIRIS CALCULATIONS OF COMPOUNDS 5a-5j:

Compd	Toxicity risks				Molecular properties calculation				
	MUT	TUMO	IRRI	REP	CLP	logS	mw	DL	DS
5a					6.1	-5.7	346	2.8	0.38
5b					5.84	-5.67	350	4.45	0.4
5c					6.39	-6.09	366	6.0	0.34
5d					7.01	-6.83	400	4.76	0.28
5e					5.47	-5.41	422	6.51	0.42
5f					5.88	-6.07	376	3.42	0.36
5g					5.33	-5.46	412	3.22	0.42
5h					5.97	-6.16	391	-5.42	0.07
5i					6.26	-6.55	411	-3.17	0.16
5j					5.65	-5.82	377	-6.01	0.20
Streptomycin					-7.83	-0.96	581	0.83	0.43
Ampicillin					-0.04	-1.57	349	10.72	0.91
Fluconazole					-0.21	-2.18	306	-1.13	0.46

MUT: Mutagenic; TUMO: Tumorogenic; IRRI: Irritant; REP: Reproductive Effective; MW: Molecular weight in g/mol; CLP: ClogP; logs: Solubility mol/lit; DL: Drug-Likeness; DS: Drug-Score.

MATERIALS AND METHODS:

Chemicals and Instrumentation

Melting points were determined in one-end open capillary tubes on shital scientific melting point apparatus and are uncorrected. Infrared spectra were recorded on Schimadzu infrared spectrophotometer in KBr pellets. ^{1}HNMR was recorded on a Bruker AVANCE-400 spectrometer. All NMR spectra were measured in $CDCl_3$ and DMSO solution using tetra methyl silane (TMS) as an internal standard. Mass spectra were recorded by using electro spray ionization technique (ESI) on a GV170708H mass spectrometer. Silica gel 60-120 mesh (Merck) was used as an adsorbent for column chromatography. TLC was performed on 5-10 cm aluminium plates coated with silica gel 60F-254 (Merck) in an appropriate solvent.

Acute toxicity:

Healthy and adult male albino swiss mice weighing between 20-25gm were used in this study. Animals were fasted for 24hrs and divided into groups of five animals each. The test compounds suspended in sodium carboxy methyl cellulose solution (1%) were administered intraperitoneally in doses of 100mg to 1000mg per kg body weight. The control group of animals received only the vehicle (1% sodium CMC).

The animals were observed for 48hrs from the time of administration of test compounds to record the mortality. All the pyrazolines employed in screening have found to be free from toxicity[16] as well as toxic symptoms even at high dose of 1000mg/kg body weight and hence they were considered safe.

Anti-inflammatory activity:

Carrageenan required for inducing the inflammation was obtained from hi-media (Mumbai) whereas sodium carboxy methyl cellulose (sodium CMC) was of Merck grade and the required saline (core health care) was purchased from a local supplier. Aceclofenac used as standard was supplied as gift sample by jagsonpal, New Delhi.

Albino rats of either sex weighing between 150-200 gm supplied by M/S Ghosh enterprises, Kolkata were divided into twelve groups of six animals each. All these groups were kept for fasting overnight and only allowed water ad libitum.

0.05 ml of 1% carragenan suspension was slowly injected subcutaneously into the subplantar region of left hind paw to produce inflammation in all the groups. Groups III to XII were treated with trisubstituted pyrazolines 5a-5j (10mg/kg).Group I used as carragenan treated control was given only 1% sodium CMC gel(1ml/kg) whereas group-II received aceclofenac (2mg/kg).All these doses were administered orally and the induced paw oedema in each group was measured to assess the anti-inflammatory activity[17]

Anti fungal activity:

The antifungal activity of test compounds is evaluated by cup plate method[18] taking drug at concentration of 100µg/ml against three fungal organisms *A. niger, C. albicans, R. oryzae*. The zone of inhibition (ZOI) is taken as parameter for antifungal activity. The ZOI of test compound is compared to that of standard drug i.e. flucanazole. Chloroform is taken as control.

Potato dextrose agar medium is dissolved and distributed in 25 ml quantities in 100ml conical flasks and are sterilized in an autoclave 121ºC(15lbs/sq.in) for 20 minutes.

The medium is inoculated at using 48hrs old cultures of test organisms mentioned above aseptically into sterile petridishes and allowed to settle at room temperatures for about 30 minutes.

In a size of 4 inches petridishes, 4 cups of 8mm diameter at equal distance are made in each plate. In each plate, 1 cup is used for standard i.e. flucanazole with 100 µg/ml, other cup for chloroform, other 2 cups with concentrations of test compounds i.e. 50 µg/ml and 100 µg/ml solutions.

The plates thus prepared are left for 90 minutes in a refrigerator for diffusion. After incubation for 72hrs at 27ºc, the plates are examined for inhibition zone (in mm).The zone of inhibition is measured using antibiotic zone reader.

Experimental

Procedure for the synthesis of compound 3

Equimolar quantities (0.005 mol) of 3-acetyl-2, 5-dimethyl thiophene and respective aldehydes were

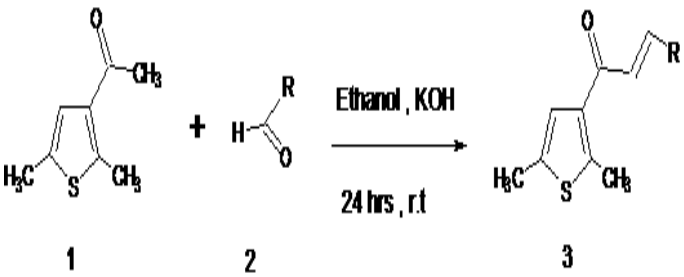
mixed and dissolved in minimum amount of alcohol. To this aqueous potassium hydroxide solution (50%, 7.5ml) was added slowly and mixed occasionally for 24hrs, at room temperature as shown in figure-2. Completion of the reaction was identified by TLC using silica gel-G. After completion of reaction, the mixture was poured onto crushed ice, acidified if necessary with dilute hydrochloric acid, and the solid separated was isolated by filtration, dried and purified by column chromatography on silica gel (100-200 mesh, Merck),with a mixture of ethyl acetate and hexane as the mobile phase as shown in figure-2.

1-(2, 5-dimethylthiophen-3-yl)-3-(4-nitrophenyl) prop-2-en-1-one (3a):

Yield: 80%, m.p: 132ºC, MS m/z: 287, IR (KBr, cm-1):1515(N=O, asymmetric); 1525(-CH=CH-); 1603(C=C of Ar); 1658(C=O); 1348(N=O, symmetric); 739(C-S). ^{1}HNMR (CDCl3): δ2.45[s, 3H, C-S-CH3]; 2.73[s, 3H, C-S-CH3]; 7.10[s, 1H, Ar-H]; 7.40[d, 1H, CO-CH=]; 7.73[d, 1H, =CH-Ar-H]; 7.78 [dd, 2H, Ar-H]; 8.30[dd, 2H, Ar-H]. Anal. Calcd for $C_{15}H_{13}NO_3S$: C, 62.71; H, 4.52; N, 4.87 Found: C, 62.69; H, 4.50; N, 4.86.

Procedure for the synthesis of compound 5

The condensation of chalcones with phenyl hydrazine in absolute ethanol in presence of pyridine, at reflux temperatures for 6hrs resulted in the formation of corresponding 2-pyrazoline derivatives as shown in scheme. Completion of the reaction was established by TLC using silica gel-G. After completion of the reaction, the mixture was poured into crushed ice with constant stirring; the solid that separated was filtered, dried and purified by column chromatography on silica gel, using mixture of ethyl acetate and hexane as the mobile phase. The purified 2-pyrazolines derivatives were obtained as light to bright coloured powders as shown in **Figure-2**.



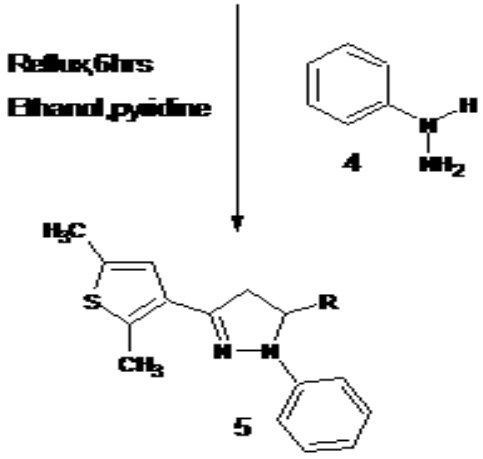


FIGURE- 2

R=5a = 4-nitrophenyl	5f = 3,4methylenedioxyphenyl
5b = 4-methylphenyl	5g = 3,4,5-trimethoxyphenyl
5c = 4-fluorophenyl	5h =3-methyl-1-phenypyrazolyl
5d = 4-chlorophenyl	5i = 3-nitro-4-methylphenyl
5e=2,4-dichlorophenyl	5j = 2-chloro-5-nitrophenyl
R=5a = 4-nitrophenyl	5f = 3,4methylenedioxyphenyl
5b = 4-methylphenyl	5g = 3,4,5-trimethoxyphenyl
5c = 4-fluorophenyl	5h =3-methyl-1-phenypyrazolyl
5d = 4-chlorophenyl	5i = 3-nitro-4-methylphenyl
5e=2,4-dichlorophenyl	5j = 2-chloro-5-nitrophenyl

1-phenyl- 3 - (2, 5-dimethylthiophene-3-yl) - 5-(4-nitrophenyl)-2-pyrazoline (5a):

Yield: 78%, m.p: 135ºC, MS m/z: 377, IR (KBr, cm-1): 1598(C=N); 1504(C=C); 1153(C-N); 1533(N=O, asymmetric); 1398(N=O, symmetric); 691(C-S). 1HNMR (CDCl3): δ2.28[S, 3H, C-S-CH3]; 2.53[S, 3H, C-S-CH3]; 3.13[dd, 1H, HA]; 3.85[dd, 1H, HB]; 5.30[dd, 1H, HX]. 6.95-8.19[M, 10H, Ar-H]. Anal. Calcd for $C_{22}H_{22}N_2S$: C, 66.84; H, 5.03; N, 11.14 Found: C, 66.82; H, 5.01; N, 11.12.

1-phenyl – 3 - (2, 5-dimethylthiophene-3-yl)-5-(4-methylphenyl)-2-pyrazoline (5b):

Yield: 75%, m.p: 124ºC, MS m/z: 346, IR (KBr, cm-1): 1596(C=N); 1502(C=C); 1148(C-N); 688(C-S). 1HNMR (CDCl3): δ2.28[S, 3H, C-S-CH3]; 2.56[S, 3H, C-S-CH3]; 3.10[dd, 1H, HA]; 3.85[dd, 1H, HB]; 5.32[dd, 1H, HX]. 6.68-7.57[M, 10H, Ar-H]. Anal. Calcd for: $C_{21}H_{19}FN_2S$ C, 76.30; H, 6.35; N, 8.09 Found: C, 76.29; H, 6.33; N, 8.07.

1 - phenyl - 3- (2, 5-dimethylthiophene-3-yl)-5-(4-fluorophenyl)-2-pyrazoline (5c):

Yield: 74%, m.p: 135ºC, MS m/z: 350, IR (KBr, cm-1): 1591(C=N); 1502(C=C); 1152(C-N);

858(C-F); 684(C-S). 1HNMR (CDCl3): δ2.40[S, 3H, C-S-CH3]; 2.70[S, 3H, C-S-CH3]; 3.10[dd, 1H, HA]; 3.75[dd, 1H, HB]; 5.10[dd, 1H, HX]. 6.52-7.25[M, 10H, Ar-H]. Anal. Calcd for $C_{21}H_{19}ClN_2S$: C, 72.00; H, 5.42; N, 8.00 Found: C, 72.02; H, 5.40; N, 8.02.

1-phenyl - 3 - (2, 5-dimethylthiophene-3-yl)-5-(4-chlorophenyl)-2-pyrazoline (5d):
Yield: 72%, m.p: 150ºC, MS m/z: 366, IR (KBr, cm-1): 1583(C=N); 1503(C=C); 1153(C-N); 834(C-F); 649(C-S). 1HNMR (CDCl3): δ2.40[S, 3H, C-S-CH3]; 2.70[S, 3H, C-S-CH3]; 3.10[dd, 1H, HA]; 3.76[dd, 1H, H_B]; 5.10[dd, 1H, H_X]. 6.58-7.40[M, 10H, Ar-H]. Anal. Calcd for $C_{21}H_{18}Cl_2N_2S$: C, 68.85; H, 5.19; N, 7.65 Found: C, 68.83; H, 5.17; N, 7.63.

1-phenyl-3-(2, 5-dimethylthiophene-3-yl)-5-(2, 4-dichlorophenyl)-2-pyrazoline (5e):
Yield: 82%, m.p: 128ºC, MS m/z: 400, IR (KBr, cm-1): 1594(C=N); 1504(C=C); 1148(C-N); 836(C-F); 656(C-S). 1HNMR (CDCl3): δ2.28[S, 3H, C-S-CH3]; 2.55[S, 3H, C-S-CH3]; 3.10[dd, 1H, HA]; 3.90[dd, 1H, HB]; 5.58[dd, 1H, HX]. 6.50-7.60[M, 9H, Ar-H]. Anal. Calcd for $C_{24}H_{26}N_2O_3S$: C, 63.00; H, 4.50; N, 7.00 Found: C, 63.02; H, 4.48; N, 7.02.

1-phenyl-3-(2, 5-dimethylthiophene-3-yl)-5-(3, 4-methylenedioxyphenyl)-2-pyrazoline (5f):
Yield: 75%, m.p: 138ºC, MS m/z: 376, IR (KBr, cm-1): 1596(C=N); 1499(C=C); 1251(C-O-C); 1112(C-N); 689(C-S). 1HNMR (CDCl3): δ2.28[S, 3H, C-S-CH3]; 2.55[S, 3H, C-S-CH3]; 3.10[dd, 1H, HA]; 3.75[dd, 1H, HB]; 5.16[dd, 1H, HX]; 5.95(s, 2H, O-CH2-O); 6.70-7.40[M, 9H, Ar-H]. Anal. Calcd for $C_{22}H_{20}N_2O_2S$: C, 70.21; H, 5.31; N, 7.44 Found: C, 70.22; H, 5.29; N, 7.42.

1-phenyl-3-(2, 5-dimethylthiophene-3-yl)-5-(3, 4, 5-trimethoxyphenyl)-2-pyrazoline (5g):
Yield: 79%, m.p: 144ºC, MS m/z: 422, IR (KBr, cm-1): 1598(C=N); 1502(C=C); 1165(O-CH3); 1142(C-N); 684(C-S). 1HNMR (CDCl3): δ2.28[S, 3H, C-S-CH3]; 2.55[S, 3H, C-S-CH3]; 3.10[dd, 1H, HA]; 3.71[dd, 1H, H_B];3.81(S,3H,O-CH_3);3.85(S,6H,2XO-CH_3);5.15[dd, 1H, H_X]; 6.30-7.60[M, 8H, Ar-H]. Anal. Calcd for $C_{25}H_{24}N_4S$: C, 68.24; H, 6.16; N, 6.63 Found: C, 68.22; H, 6.14; N, 6.61.

1-phenyl - 3 - (2, 5-dimethylthiophene-3-yl)-5-(3-methyl – 1 - phenylpyrazole - 4 - yl) – 2 - pyrazoline (5h):
Yield: 81%, m.p: 141ºC, MS m/z: 413, IR (KBr, cm^{-1}): 1578(C=N); 1502(C=C); 1138(C-N); 650(C-S). ^{1}HNMR ($CDCl_3$): δ2.31[S, 3H, Ar-CH_3]; 2.40[S, 3H, Ar-CH_3]; 2.70[S, 3H, Ar-CH_3]; 3.12[dd, 1H, H_A]; 3.75[dd, 1H, H_B]; 5.15[dd, 1H, H_X]. 6.80-7.40[M, 12H, Ar-H]. Anal. Calcd for $C_{22}H_{21}N_3O_2S$: C, 72.63; H, 6.05; N, 13.55 Found: C, 72.61; H, 6.03; N, 13.52.

1-phenyl-3-(2, 5-dimethylthiophene-3-yl)-5-(3-nitro-4-methylphenyl)-2-pyrazoline (5i):
Yield: 78%, m.p: 129ºC, MS m/z: 391, IR (KBr, cm^{-1}): 1598(C=N); 1529(C=C); 1110(C-N); 1063(C-O-C); 658(C-S). ^{1}HNMR ($CDCl_3$): δ2.52[S, 3H, Ar-CH_3]; 2.59[S, 3H, Ar-CH_3]; 2.72[S, 3H, Ar-CH_3]; 3.11[dd, 1H, H_A]; 3.83[dd, 1H, H_B]; 5.20[dd, 1H, H_X]; 6.58-7.40[M, 10H, Ar-H]. Anal. Calcd for $C_{21}H_{18}ClN_3O_2S$: C, 67.51; H, 5.37; N, 10.74 Found: C, 67.49; H, 5.35; N, 10.72.

1-phenyl - 3 -(2, 5 - dimethylthiophene-3-yl) - 5-(2-chloro-5-nitrophenyl)-2-pyrazoline (5j):
Yield: 74%, m.p: 151ºC, MS m/z: 411, IR (KBr, cm^{-1}): 1595(C=N); 1500(C=C); 1172(C-N); 1553(N=O, asymmetric); 1333(N=O, symmetric); 688(C-S). ^{1}HNMR ($CDCl_3$): δ2.30[S, 3H, C-S-CH_3]; 2.50[S, 3H, C-S-CH_3]; 3.12[dd, 1H, H_A]; 3.76[dd, 1H, H_B]; 5.50[dd, 1H, H_X]. 6.55-7.50[M, 9H, Ar-H]. Anal. Calcd for $C_{21}H_{19}N_3O_2S$: C, 61.31; H, 4.37; N, 10.21 Found: C, 61.29; H, 4.35; N, 10.19.

RESULTS AND DISCUSSION:

From the **Tables-1A**, **1B**, 2, 3 it is clearly inferred that most of the synthesized compounds (5a-5j) were found to be in conformity with lipinski's "rule of five" and other parameters, for their onward screening for biological activity as oral active leads/ drugs.

The acute toxicity test conducted on swiss mice had shown that all the synthesized compounds were free of toxic symptoms even at concentration of 1000mg/kg body weight and hence were considered safe compounds.

The anti-inflammatory activity of all pyrazolines had been evaluated using carragenan induced rat

paw oedema method. The results of this activity shown in table-4.The pyrazolines possessed some degree of anti-inflammatory activity when compared to standard drug aceclofenac, but not an identical dose level since the compounds were tested at 10mg/kg whereas drug tested at 2mg/kg body weight dose levels. The compound 5d possessed maximum activity followed by the other compounds like 5c, 5j, 5d, 5a, 5i. However, the activity was not much higher with other derivatives.

A close examination of **Table-5** pertaining to antifungal data of pyrazoline derivatives revealed that all compounds had been found to be effective against all fungi at both 50µg (0.05ml) and 100µg (0.1ml) dose levels, when compared with standard drug flucanazole. Compounds having dihalogen substitution followed mono halogen substitution were found to be more potent. We have developed a novel synthetic approach of pharmacologically active diversely functionalized novel trisubstituted pyrazolines.

TABLE-4: ANTI-INFLAMMATORY ACTIVITY OF TRISUBSTITUTED 2-PYRAZOLINES (5a-5j)

Cmpd	R	% Inhibition ± SEM at various time intervals					
		0.5h	**1.0h**	**2.0h**	**3.0h**	**4.0h**	**6.0h**
5a	4-NO_2	25± 1	40 ±1	60±1	71± 2	80±2	90 ±2
5b	4-CH_3	23 ±2	38 ±2	58 ±1	69 ±2	77 ±1	87 ±2
5c	4-F	28± 1	44± 1	66± 2	78 ±2	90± 2	98± 3
5d	2-Cl	27 ±2	41 ±1*	64 ±1	73± 2	81±2	94± 2
5e	2,4-dichloro	29± 1*	45 ±1	67± 1	80 ±2	95 ±2	98± 2
5f	3,4-methylenedioxy	22± 1	35± 1	56 ±1	65 ±1	74± 2	85± 2
5g	3,4,5-trimethoxy	20 ±2	27± 1	50± 2	60± 2*	65 ±2	80± 2
5h	3-CH_3-1-C_6H4-pyrazolyl	21 ±1	32 ±1	55±1	62± 2*	70 ±1*	82± 2
5i	3-NO_2-4-CH_3	24 ±1	39 ±1	59 ±1	70 ±2	79 ±2*	89 ±2
5j	2-chloro-5-nitrophenyl	26 ±1	42 ±2	62 ±1	74 ±2	81 ±2*	95 ±2
Aceclofenac		35 ±2	48 ±21	70 ±2	84 ±1	98 ±2.52*	99± 2

All values are represented as mean±SEM (n=6).*p<0.01 compared to reference standard Aceclofenac. Student's t-test. Dosage: Aceclofenac – 2mg/kg and test compounds-10mg/kg body weight of rat.

TABLE-5: ANTIFUNGAL ACTIVITY OF TRISUBSTITUTED 2-PYRAZOLINES (5a-5j)

Compound	R	Zone of inhibition (in mm) , Quantity (µg/ml)					
		A.niger		***C.albicans***		***R.oryzae***	
		50	***100***	***50***	***100***	***50***	***100***
5a	4-NO_2	17	19	17	20	17	20
5b	4-CH_3	14	16	14	17	15	17
5c	4-F	21	24	20	24	20	23
5d	2-Cl	20	23	19	23	19	22
5e	2,4-dichloro	22	26	22	26	20	25
5f	3,4-methylenedioxy	13	16	13	16	14	17
5g	3,4,5-trimethoxy	12	15	12	16	13	15
5h	3-CH_3-1-C_6H4-pyrazolyl	15	17	15	18	16	18
5i	3-NO_2-4-CH_3	16	18	16	19	17	19
5j	2-chloro-5-nitrophenyl	19	22	18	22	18	22
Flucanazole		25	28	24	29	22	28

CONCLUSION: A series of trisubstitued 2-pyrazoline derivatives 5a-5j were subjected to molecular properties prediction by different softwares such as molinspiration, molsoft and osiris in order to find suitable molecules for the synthesis and biological screening. All the pyrazolines employed in screening have found to be free from toxicity as well as toxic symptoms and hence were considered safe. The compounds 5d showed better anti-inflammatory activity. The compounds 5e and 5c, 5d showed higher antifungal activity. From the above results it is clear that the trisubstituted 2-pyrazolines play a major role in scientific study for future research.

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ETHICAL MATTER:

As per OECD guidelines institutional animal ethics committee register number is 516/01-A-CPCSEA.

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