PREPARATION AND CHARACTERIZATION OF NEW 1,3,5-TRISUBSTITUTED-2-PYRAZOLINES DERIVATIVE FOR THEIR ANTI-INFLAMMATORY ACTIVITY

ABSTRACT

The objective of the paper was to design, synthesis and characterization of new 1,3,5-trisubstituted-2-pyrazolines derivative and evaluate for anti-inflammatory potential. The 1,3,5-tri-substituted-2-pyrazolines derivatives has been synthesized by the reaction of chalcone derivatives with 4-hydrazinylbenzene sulfonamide hydrochloride and phenyl hydrazine hydrochloride. Total Sixteen compounds has been synthesized and characterized by the IR, 1HNMR and mass spectral analysis. Proposed compounds have been evaluated for anti-inflammatory activity. Anti-inflammatory activity of the compounds carried out by two animal model i.e. Carrageenan induced, paw edema in rats and Inhibition of formalin induced paw edema in rats. Anti-inflammatory activity of the compounds C7, C8 and C2 were shown 98.26, 92.77 and 96.24 percentages of inhibition and compounds D7, D8 and D2 were shown 81.50, 83.81 and 78.32 percentages of inhibition as compared to the standard drug Diclofenac at 10 mg/kg was inhibit the inflammation 99.42 % after 6h. These result is a evident that synthesized compounds show relevant degree of anti-inflammatory activity as compared to the standard drug. It is also concluded that the presence of SO_2NH_2 group, Cl, CH_3, OCH_3 and $N(CH_3)_2$ group may provide the active compounds when attached to the pyrazoline group. But the addition of OH, Br and no substitution in phenyl ring may diminish the activity.

Keywords: Anti-inflammatory, Paw edema, Diclofenac, Pyrazolines,

INTRODUCTION

Pyrazole has a chemical behaviour that is unusual not just among heterocyclic compounds, but also among related azoles. [1] This is because pyrazole has the normal aromatic system features, which are really rather prominent in these derivatives, as well as the high ring liability under specific conditions. Despite the fact that pyrazole derivatives have been known for over 80 years, research into their chemistry has progressed slowly. Earlier research focused mostly on the creation of synthetic approaches. [2] The study of chemical properties, particularly the peculiarities of the behaviour of pyrazole derivatives and the clarification of their physicochemical features, has recently gotten a lot of interest. This allowed for the collection of fresh data that was crucial. Pyrazole derivatives have a long history of application in agrochemicals as herbicides and insecticides and in pharmaceutical industry as antipyretic and anti-inflammatory. One of the first synthetic drugs was antipyrine. [3,4] Celecoxib [5],Phenzone (phenazon or antipyrine),Lonazolac[6],Rimonabant, Fipronil, Tepoxalin, Fomepizole or 4-methylpyrazole are all pyrazole-based antidotes for methanol or ethylene glycol poisoning. The sulfonamide group in sulfaphenazole is utilised as an antibacterial agent. Chalcone is a 1,3-diphenyl-2-propene-1-one compound with two aromatic rings connected by a three-carbon, -unsaturated carbonyl system. These are found in large quantities in edible plants and are thought to be precursors of flavonoids and isoflavonoids. Chalcones, which feature conjugated double bonds and a totally delocalized -electron system on both benzene rings, have a low melting point due to their low intermolecular force. Molecules with this structure have comparatively low redox potentials

and are more likely to conduct electron transfer reactions [7]. Various chalcone derivatives with suitable substitutions may be synthesised, which then undergo a cyclo-addition process with substituted hydrazine to provide desirable 1,3,5-tri substituted pyrazole derivatives. As a result, several chalcone derivatives were synthesised and employed as intermediates in the synthesis of 1,3,5 tri substituted pyrazole in the current work.

The objective of the paper was to design, synthesis and characterization of new 1,3,5-trisubstituted-2-pyrazolines derivative and evaluate for anti-inflammatory potential.

MATERIAL AND METHOD

Hi-media in New Delhi provided the chemical 3'amino-4'chloro-acetophenone. CDH (Chemical Drug House), New Delhi, India, provided benzaldehyde, 4-chlorobenzaldehyde, 4-bromobenzaldehyde, 4-nitrobenzaldehyde, 4-methylbenzaldehyde, 4-methoxybenzaldehyde, 4-ethylbenzaldehyde, and 4-(dimethyl amino) benzaldehyde. Sigma Aldrich, New Delhi, provided 4-hydrazinylbenzenesulfonamide hydrochloride and Phenyl hydrazine hydrochloride. Chemicals of synthetic grade were utilised in the experiments. In open glass capillaries, the melting points of the produced compounds were determined. ALPHA (Bruker) FTIR Spectrometer was used to record IR spectra. Elemental analysis was carried out, and the results were determined to be within 0.4 percent of the theoretical values. On a Bruker Avance 400 spectrophotometer with a 400 MHz, 5mm multi-nuclear inverse probe head, low and high-temperature facility, and HRMAS accessory, 13C NMR spectra were acquired. ESI used mass spectrometers Jeol SX-102 (FAB) to record the spectra.

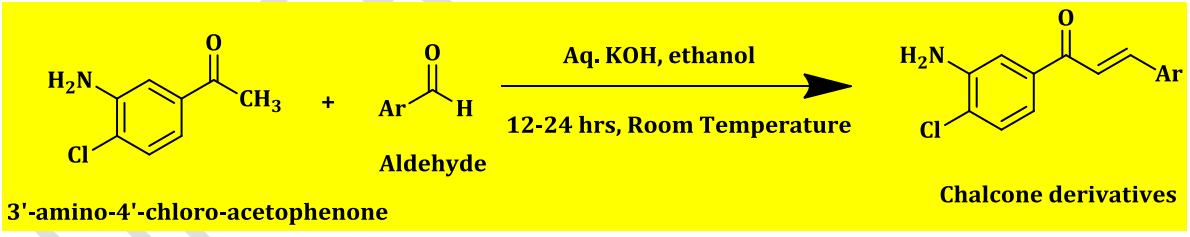
Chemistry

Present synthesis comprises

A. Synthesis scheme-I: Synthesis of chalcone of 3'-amino-4'-chloroacetophenone by Claisen Schmidt condensation

B. Synthesis scheme-II: Synthesis of 1,3,5- tri-substituted-2-pyrazolines derivatives.

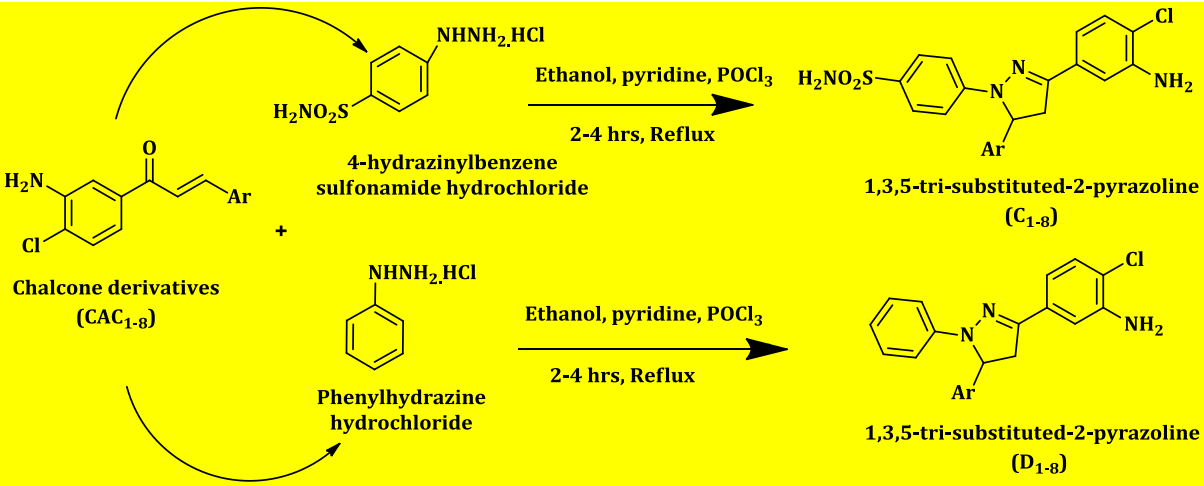
Synthesis scheme-I: Synthesis of the chalcone of 3'-amino-4'-chloroacetophenone



In the aforesaid procedure, an equimolar amount (0.01 M) of 3-amino-4-chloro-acetophenone (0.84g) was combined with an equimolar amount (0.01M) of benzaldehyde and replaced benzaldehyde. In ethanol, the mixture was dissolved. The mixture was stirred for 5 minutes before adding a 50 percent aqueous potassium hydroxide solution, which was steadily added at room temperature for 24 hours [8]. The TLC was used to monitor the reaction's completion. The synthesis was then finished by pouring the liquid onto crushed ice and obtaining a solid result; however, if a solid product was not produced, it was acidified with dilute hydrochloric acid. The resulting solid was filtered, dried, and purified using a solvent system in column chromatography (hexane: ethyl acetate). In synthesis scheme-I, the reaction is shown. Infrared spectroscopy and proton NMR spectroscopy were used to analyse the produced compounds, and they were confirmed to be trustworthy with a likely structure.

The obtained compounds were analysed using IR and 1HNMR and found to have a structure that was compatible with what was predicted. 1500-1520 (C=C Quadrant of Ar), 1430-1470 (CH=CH), 1105 (C-F), 848 (C-Cl), 1015 (C-Br), and 1160 (O-H str) (OCH3). These compound further confirmed by proton NMR revealed the characteristic ethylenic protons of the chalcone system in between δ 6.94 and 8.19 confirm the compound. The reaction was monitored by the TLC using Hexane: ethyl acetate as mobile phase.

Synthesis scheme-II: Synthesis of 1,3,5- tri-substituted-2-pyrazolines



The synthesised chalcone derivatives were combined in absolute alcohol with equimolar amounts of 4-hydrazinylbenzene sulfonamide hydrochloride and phenyl hydrazine hydrochloride (0.005M) and a little amount of pyridine and POCl3 (0.01M) (5-7 ml). The reaction mixture was refluxed for 2-6 hours at 65°C. TLC was used to monitor the reaction, which used ethyl acetate:hexane as the mobile phase. The solvent was entirely evaporated before being placed into ice cold water and constantly stirred to covert the liquid form into a solid product, which yielded the synthesised product. [9] Scheme-II depicted the synthesis. This substance was filtered and dried under vacuum. Purified by column chromatography, the synthesised chemical was produced as a pale yellow solid colour powder. For phenyl hydrazine hydrochloride, the similar technique was followed, in which chalcone derivatives interacted with the phenyl hydrazine hydrochloride.

Pharmacological screening

Anti-inflammatory Activity

The synthesized 1,3,5-pyrazole derivatives were subjected to pharmacological screening as anti-inflammatory activity. Screening was done by using various models to identify the mechanism of action [10-12].

Determination of LD50 value and acute toxicity

In this study, healthy and mature male albino Swiss rats weighing 120-150 g were employed. The animals were fasted for 24 hours before being separated into six groups. The test chemicals were given intraperitoneally at dosages of 10 mg, 100 mg, 1000 mg, and 2000 mg per kg body weight, suspended in sodium carboxymethyl cellulose solution (1%) Only the vehicle was given to the animals in the control group (1 percent sodium CMC). To record the mortality, the animals were watched for 48 hours after the test chemicals were administered. Even at high doses, all of the synthesised

compounds used in the pharmacological screening were determined to be devoid of toxicity and hazardous effects and hence they were considered safe.

Rats in groups of 18 were given varied doses orally using the staircase approach, starting at 10 mg/kg and increasing dosage by a factor 1.5 if no death occurred [14], and lowering succeeding dose by a factor 0.7 if mortality occurred. [15] The hit and try approach was used to identify the least tolerated (100 percent mortality) and most tolerated (0% mortality). The following formulae were used to correct for 0% and 100% mortality: For 0% mortality, use 100 (0.25/n), whereas for 100% mortality, use 100 (n0.025/n). LD50 S.E. = Log Dose with Highest Mortality- Log Dose with Lowest Mortality/ n.

Between these two, doses were chosen, and any mortality was tracked for 24 hours, with the number of fatalities recorded. To calculate the dosage for probit value 5, which was assumed to represent LD_{50}, a log dose versus probit value curve was drawn. For this investigation, a dose range significantly below LD50 was used. Even at a high dosage of 1000 mg/kg body weight, all of the 2-pyrazolines used in the pharmacological screening were determined to be devoid of toxicity and toxic symptoms, and hence were deemed safe.

Animal grouping and dose planning

The dosages employed were comparable to or less than $1/10^{th}$ of the doses corresponding to LD_{50} values, with no major behavioural or CVS abnormalities. When opposed to the standard, which exhibited some alterations at greater dosages, there were no CNS or CVS effects even at larger levels. [16,17]

Anti-inflammatory properties

The anti-inflammatory activity of the 16 synthesised compounds in the current investigation was determined using the carrageenan-induced rat paw edoema technique.

Experimental Procedure

Albino rats, either sexed, weighing 120-150 gm, were placed into eighteen groups, each with six animals. All of these groups were subjected to an overnight fast with just ad libitum access to water. To induce inflammation in all of the groups, 0.05 ml of 1% carrageenan suspension was progressively injected subcutaneously into the subplantar area of the left hind paw. Synthetic chemical derivatives (10 mg/kg) were given to groups 3 to 19. Group 1 was given simply 1% sodium CMC suspension (1ml/kg) as a carrageenan-treated control, whereas group 2 was given diclofenac (10 mg/kg). All of these dosages were given orally, and the amount of produced paw edoema in each group was quantified to see how effective they were at reducing inflammation.

Screening for anti-inflammatory

Inhibition of Carrageenan induced, paw edema in rats [18]

Rats were divided into groups of 06 each, total 18 group is created (120-150 gm).

1. Sodium CMC suspension (1%) as control
2. Untreated diseased animals
3. Reference group treated with diclofenac before carrageenan
4. Experimental Groups

Group in which no changes are made One hour before the carrageenan infusion, I was given Sodium CMC suspension (1%) to drink. The experimental groups were given varied dosages of different fractions in 0.5ml of ordinary saline, infused intraperitoneally one hour before to infusion of 0.1 ml of 1% carrageenan arrangement in the right rear paw under the plantar aponeurosis (s.c) for edoema treatment. Plethysmometer was used to limit the amount of paw edoema, and a measurement reaction relationship was created for both oral and i.p. doses, as well as a link between i.p. and oral dosages, which had the greatest mitigating effect. Diclofenac 10mg/kg was administered to the reference group 1 hour before the carrageenan infusion. Rate restraint of edema in respect to a control gathering was ascertained as depicted by Winter *et al.* 2012. [19]

Inhibition of formalin induced paw edema in rats

The animals were divided into six groups of six. One hour after i.p., a subaponeurotic infusion of 0.1 ml of 2 percent formalin triggered acute inflammation.

Synthetic compound organisation (10 mg/kg), Diclofenac (10 mg/kg), or simply. In individual gatherings, use a vehicle (2.5 percent DMSO and 2.5 percent tween 20 solution). One, two, four, and six hours after the injection, the volume of the paw was resolved. By using a plethysmometer, formalin is measured. Think about the aforementioned animals for endless aggravation.were then given the components, Diclofenac or vehicle, once a day for 9 days.On the third day, a formalin infusion was administered for the second time. The day to day changes in the volume of paw were measured plethysmographically.

RESULT AND DISCUSSION

Spectral analysis

The synthesized compounds was characterized by the Infra-red spectroscopy and proton NMR spectroscopy and was found reliable with probable structure. Obtained compounds were characterized by IR, 1HNMR and were found consistent with an expected structure. 3400-3410 (O-H str.); 1590-1600 (C=N); 1490-1510 (C=C Quadrant of Ar), 1428-1440 (CH=CH) and 1110 (C-F), 845 (C-Cl), 1020 (C-Br) and 1168 (OCH3). These compound further confirmed by proton NMR revealed the characteristic protons of the system in between δ 6.76 and 7.67 confirm the compound . The reaction was monitored by the TLC using Hexane: ethyl acetate as mobile phase.

The infrared spectra of the synthesized compounds showed characteristic absorption band between 3402(O-H str.); 1592 (C=N); 1489 [C=C (Qurdrant of Ar)]; 1174 (-C_6H_5); 1430 (CH=CH str.); 3418 (SO_2-NH str.); 1330, 1168 (S=O); 853 (C-Cl); 2938 (C-H aromatic), 853 (C-Cl), 1024 (C-Br); 1120 (C-F) and 1072 (OCH_3).In 1H-NMR spectra of the synthesized compounds represents the 6.21 (m, 2H, C_6H_5-NH_2), 6.84–7.34 (m, 3H, Ar–H); 8.52 (s, 1H, pyrazole–CH), 7.45-7.72 (d, 3H, Ar–H, J=8.6 Hz), 3.60 (1H, dd); 5.36 (1H, dd), 6.82-7.62 (m, 2H, Ar–H); 2.55 (m, 3H, C_6H_5 $N(CH_3)_2$). 3.81 (m, 3H, C_6H_5-OCH_3), 2.15 (m, 3H, C_6H_5-CH_3), 7.15 (bs, 2H, SO_2NH_2), 3.81 (m, 3H, C_6H_5-$0CH_3$) respectively. Methyl carbons were observed at 2.15 ppm. Elemental analysis of all synthesized compound were within the ±0.4% of the theoretical values. Generation of dense sooty flame and formation of oily layer after nitration of the compounds confirmed the presence of aromatic ring in all the synthesized compounds. In the FAB mass spectra two prominent peaks were observed. Thin layer chromatography (TLC) has been executed for the monitored of reaction and purity of the synthesized

compounds by using silica gel G in various solvent systems like Hexane: Ethyl acetate and iodine chamber has been used for the visualization and in some cases UV chamber used. All these characterization parameter showed that the structure of the synthesized compounds were near to expected.

Compound C1: 4-(3-(3-amino-4-chlorophenyl)-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl) benzenesulfonamide

Molecularformula: $C_{22}H_{21}N_3O_4S$; Molecular weight: 423.48; TLC(Rf value): 0.44; Elemental Analysis (Found/ Calc.): Nitrogen (%) 13.10/13.12, Sulphur (%) 7.51/7.51, Oxygen (%) 7.49/ 7.50; IR (cm^{-1}): 3418 (O-H str.); 1675 (C=N); 1483(C=C); 1170 (-C_6H_5); 1420 (CH=CH str.); 3420 (SO_2NH str.); 1326 (S=O), 852 (C-Cl), 2935 (C-H aromatic); IHNMR (ppm): 7.15 (bs, 2H, SO2NH2), 6.84–7.34 (m, 3H, Ar–H); 8.52 (s, 1H, pyrazole–CH), 7.86-7.90 (d, 2H, Ar–H, J=8.6 Hz), 3.60 (1H, dd); 5.36 (1H, dd), 7.10-7.51 (m, 3H, Ar–H); 6.21 (m, 2H, C6H5-NH2). FAB Mass (m/z): 426.09 (Quassi-molecular ion peak (M+H)+)

Compound C2:4-(3-(3-amino-4-chlorophenyl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide

Molecular formula: $C_{21}H_{18}Cl_2N_4O_2S$; Molecular weight: 461.36; TLC (Rf value):0.54; Elemental Analysis (Found/Calc.): Nitrogen (%)12.12/12.14, Sulphur (%)6.92/6.95, Oxygen (%)6.94/6.94; IR (cm^{-1}):3417 (O-H str.), 1662 (C=N),1487 (C=C), 1174 (C_6H_5), 1458 (CH=CH str.); 3415 (SO_2NH str.); 1322 (S=O), 838 (C-Cl), 2935 (C-H); 1HNMR (ppm):7.15 (bs, 2H, SO2NH2), 6.84–7.34 (m, 3H, Ar–H); 8.52 (s, 1H, pyrazole–CH), 7.86-7.90 (d, 2H, Ar–H, J=8.6 Hz), 3.60 (1H, dd); 5.36 (1H, dd) , 7.55-7.98 (m, 2H, Ar–H); 6.21 (m, 2H, C_6H_5-NH_2).FAB Mass (m/z): 460.05 (Quassi-molecular ion peak (M+H)+).1NMR spectra was shown in Figure 1.

Compound C3: 4-(3-(3-amino-4-chlorophenyl)-5-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl) benzenesulfonamide

Molecularformula: $C_{21}H_{18}BrClN_4O_2S$; Molecular weight: 505.82; TLC (Rf value): 0.55; Elemental Analysis (Found/Calc.): Nitrogen (%)11.05/11.08, Sulphur (%)6.28/6.34, Oxygen (%)6.32/6.33; IR (cm^{-1}): 3409 (O-H str.); 1672C=N; 1492C=C; 1172 (C_6H_5); 1432 (CH=CH str.); 3420 (SO_2NH str.); 1332 (S=O); 856 (C-Cl); 1024 (C-Br) 2936 (C-H);IHNMR (ppm):7.15 (bs, 2H, SO_2NH_2), 6.84–7.34 (m, 3H, Ar–H); 8.52 (s, 1H, pyrazole–CH), 7.86-7.90 (d, 2H, Ar–H, J=8.6 Hz), 3.60 (1H, dd); 5.36 (1H, dd) , 7.66-7.78 (m, 2H, Ar–H); 6.21 (m, 2H, C_6H_5-NH_2).FAB Mass (m/z): 506.0 (Quassi-molecular ion peak (M+H)+).

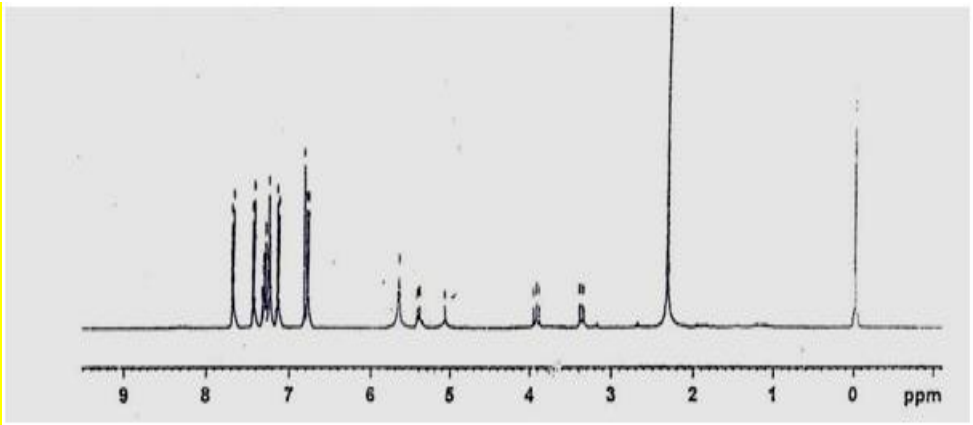


Fig. 1: 1NMR spectra of compound C2.

Compound C4: 4-(3-(3-amino-4-chlorophenyl)-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide

Molecular formula: $C_{21}H_{18}ClFN_4O_2S$; Molecular weight: 444.81; TLC (Rf value): 0.64; Elemental analysis (Found/Calc.)%: Nitrogen(12.56/12.59); Sulphur (7.21/7.21), Oxygen (7.18/7.19); IR (cm^{-1}): 3408O-H str.; 1670C=N; 1491 C=C; 1170C_6H_5; 1424 CH=CH; 3415SO_2NH str.; 1325S=O; 850C-Cl; 1120C-F; 2932C-H; IHNMR (ppm):7.15 (bs, 2H, SO_2NH_2), 6.84–7.34 (m, 3H, Ar–H); 8.52 (s, 1H, pyrazole–CH), 7.86-7.90 (d, 2H, Ar–H, J=8.6 Hz), 3.60 (1H, dd); 5.36 (1H, dd) , 7.66-7.78 (m, 2H, Ar–H); 6.21 (m, 2H, C_6H_5-NH_2).FAB Mass (m/z): 444.08 (Quassi-molecular ion peak (M+H)+)

Compound C5:4-(3-(3-amino-4-chlorophenyl)-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol -1-yl) benzene sulfonamide

Molecularformula: $C_{21}H_{18}ClN_5O_4S$; Molecular weight: 471.92; TLC (Rf value): 0.28; Element (Found/Calculated) %: Nitrogen (14.82/14.84); Sulphur (6.75/6.79), Oxygen (13.52/13.56); IR (cm^{-1}): 3415 (O-H str.), 1662 (C=N); 1498 (C=C); 1172 (C_6H_5); 1416 (CH=CH str.); 3415 (SO_2NH str.), 1322 (S=O), 850 (C-Cl), 1575 (N=0 str.); 1364 (N-O str.), 2935 (C-H); 1HNMR (ppm):7.15 (bs, 2H, SO_2NH_2), 6.84–7.34 (m, 3H, Ar–H); 8.52 (s, 1H, pyrazole–CH), 7.86-7.90 (d, 2H, Ar–H, J=8.6 Hz), 3.60 (1H, dd); 5.36 (1H, dd) , 7.68-7.82 (m, 2H, Ar–H); 6.21 (m, 2H, C_6H_5-NH_2).FAB Mass (m/z): 471.08 (Quassi-molecular ion peak (M+H)+)

Compound C6: 4-(3-(3-amino-4-chlorophenyl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl) benzenesulfonamide

Molecularformula: $C_{22}H_{21}ClN_4O_2S$; Molecular weight: 440.95; TLC (Rf value): 0.39; Element (Found/Calc.) %: Nitrogen (12.70/12.71); Sulphur (7.27/7.27); Oxygen (7.25/7.26); IR (cm^{-1}): 3415 (O-H str.); 1668 (C=N); 1480 (C=C); 1172 (C_6H_5); 1424(CH=CH str.); 3414 (SO_2NH str.); 1324 (S=O); 850 (C-Cl); 2934 (C-H);1HNMR: 7.15 (bs, 2H, SO_2NH_2), 6.84–7.34 (m, 3H, Ar–H); 8.52 (s, 1H, pyrazole–CH), 7.86-7.90 (d, 2H, Ar–H, J=8.6 Hz), 3.60 (1H, dd); 5.36 (1H, dd), 7.29-7.67 (m, 2H, Ar–H); 6.21 (m, 2H, C6H5-NH2), 2.15 (m, 3H, C_6H_5-CH_2).FAB Mass (m/z): 440.11 (Quassi-molecular ion peak (M+H)+).

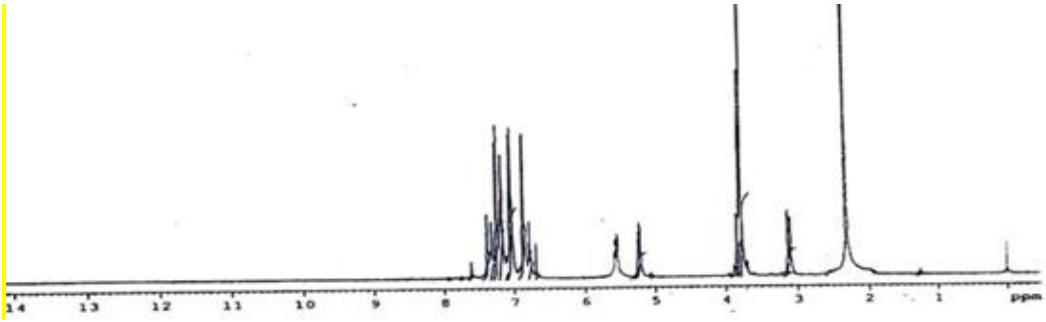


Fig. 2: 1NMR spectra of compound D7

Compound C7: 4-(3-(3-amino-4-chlorophenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide

Molecularformula: $C_{22}H_{21}ClN_4O_3S$; Molecular weight: 456.95; TLC (Rf value): 0.34; Element (Found/Calc.) %: Nitrogen (12.20/12.26); Sulphur (6.98/7.02); Oxygen (10.48/10.50); IR (cm^{-1}):3414(O-H str.); 1634(C=N); 1485(C=C); 1170(C_6H_5); 1428(CH=CH str.); 3414(SO_2NH str.); 1324(S=O); 850(C-Cl); 1070(OCH_3); 2935(C-H); 1HNMR (ppm):7.15 (bs, 2H, SO_2NH_2), 6.84–7.34 (m, 3H, Ar–H); 8.52 (s, 1H, pyrazole–CH), 7.86-7.90 (d, 2H, Ar–H, J=8.6 Hz), 3.60 (1H, dd); 5.36 (1H, dd) , 7.29-7.67 (m, 2H, Ar–H); 6.21 (m, 2H, C_6H_5-NH_2), 3.81 (m, 3H, C_6H_5-OCH_3).FAB Mass (m/z): 456.10 (Quassi-molecular ion peak (M+H)+)

Compound C8: 4-(3-(3-amino-4-chlorophenyl)-5-(4-(dimethylamino)phenyl)-4,5-dihydro-1H-pyrazol-1-yl) benzenesulfonamide

Molecularformula: $C_{23}H_{24}ClN_5O_2S$; Molecular weight: 469.99; TLC (Rf value):0.34; Element (Found/Calc.)%: Nitrogen (14.89/14.90); Sulphur (6.78/6.82); Oxygen (6.80/6.81); IR (cm^{-1}): 3412 (O-H str.); 1664 (C=N); 1482 (C=C); 1170 (C_6H_5);1428(CH=CH str.); 3420 (SO_2NH str.); 1324 (S=O); 850 (C-Cl); 2936 (C-H); 1HNMR (ppm):7.15 (bs, 2H, SO_2NH_2), 6.84–7.34 (m, 3H, Ar–H); 8.52 (s, 1H, pyrazole–CH), 7.86-7.90 (d, 2H, Ar–H, J=8.6 Hz), 3.60 (1H, dd); 5.36 (1H, dd), 6.82-7.61 (m, 2H, Ar–H); 6.21 (m, 2H, C_6H_5-NH_2), 2.55 (m, 3H, C_6H_5-$N(CH_3)_2$); FAB Mass (m/z): 469.13 (Quassi-molecular ion peak (M+H)+)

Compound D1: 2-chloro-5-(1,5-diphenyl-4,5-dihydro-1H-pyrazol-3-yl)aniline

Molecular formula: $C_{21}H_{18}ClN_3$; Molecular weight: 347.84; TLC (Rf value): 0.40; Element (Found/Calc.)%:Nitrogen (12.05/12.08); Chlorine (10.16/10.19); IR (cm^{-1}):3412(O-H str.); 1595(C=N); 1491(C=C); 1172(C_6H_5); 1428(CH=CH str.); 850(C-Cl);2935(C-H); 1HNMR:6.21 (m, 2H, C_6H_5-NH_2), 6.84–7.34 (m, 3H, Ar–H); 8.52 (s, 1H, pyrazole–CH), 7.45-7.72 (d, 3H, Ar–H, J=8.6 Hz), 3.60 (1H, dd); 5.36 (1H, dd), 7.10-7.41 (m, 3H, Ar–H).FAB Mass (m/z): 347.12 (Quassi-molecular ion peak (M+H)+)

Compound D2: 2-chloro-5-(5-(4-chlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl) aniline

Molecularformula:$C_{21}H_{17}Cl_2N_3$; Molecular weight: 382.29; TLC (Rf value): 0.48; Element (Found/Calc.)%: Nitrogen (10.90/10.99); Chlorine (18.52/18.55); IR (cm^{-1}): 3409 (O-H str.); 1595 (C=N); 1491 (C=C); 1170(C_6H_5); 1424 (CH=CH str.); 850 (C-Cl); 2935 (C-H);1HNMR:6.21 (m, 2H, C_6H_5-NH_2), 6.84–7.34 (m, 3H, Ar–H); 8.52 (s, 1H, pyrazole–CH), 3.60 (1H, dd); 5.36 (1H, dd), 7.45-7.72 (d, 3H, Ar–H, J=8.6 Hz), 7.55.7.98 (m, 2H, Ar–H).FAB Mass (m/z): 381.08 (Quassi-molecular ion peak (M+H)+)

Compound D3: 5-(5-(4-bromophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-2-chloro aniline

Molecular formula: $C_{21}H_{17}BrClN_3$; Molecular weight: 426.74; TLC (Rf value): 0.33; Element (Found/Calc.)%: Nitrogen (9.80/9.85); Chlorine (8.30/8.31); IR (cm^{-1}): 3410(O-H str.); 1596 (C=N); 1489 (C=C); 1172 (C_6H_5); 1428 (CH=CH str.); 850 (C-Cl); 1022(C-Br); 2930 (C-H); 1HNMR: 6.21 (m, 2H, C_6H_5-NH_2), 6.84–7.34 (m, 3H, Ar–H); 8.52 (s, 1H, pyrazole–CH), 7.45-7.72 (d, 3H, Ar–H, J=8.6 Hz), 3.60 (1H, dd); 5.36 (1H, dd)7.18-7.92 (m, 2H, Ar–H).FAB Mass (m/z): 427.03 (Quassi-molecular ion peak (M+H)+)

Compound D4: 2-chloro-5-(5-(4-fluorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl) aniline

Molecular formula: $C_{21}H_{17}BrClN_3$; Molecular weight: 365.83; TLC (Rf value): 0.58; Element (Found/Calc.) %: Nitrogen(11.45/11.49); Chlorine (9.60/9.69); IR (cm^{-1}): 3408 (O-H str.); 1590 (C=N); 1484 (C=C); 1172 (C_6H_5); 1428 (CH=CH str.); 850 (C-Cl); 1115 (C-F); 2934 (C-H); 1HNMR:6.21 (m, 2H, C_6H_5-NH_2), 6.84–7.34 (m, 3H, Ar–H); 8.52 (s, 1H, pyrazole–CH), 3.60 (1H, dd); 5.36 (1H, dd), 7.45-7.72 (d, 3H, Ar–H, J=8.6 Hz), 7.30-8.15 (m, 2H, Ar–H).FAB Mass (m/z): 365.11 (Quassi-molecular ion peak (M+H)+).

Compound D5: 2-chloro-5-(5-(4-nitrophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)aniline

Molecular formula: $C_{21}H_{17}ClN_4O_2$; Molecular weight: 392.84; TLC (Rf value): 0.52; Element (Found/Calc.)%: Nitrogen (14.20/14.26); Oxygen (8.13/8.15); Chlorine (9.00/9.02); IR (cm^{-1}): 3410 (O-H str.); 1592 (C=N); 1482 (C=C); 1174 (C_6H_5); 1428(CH=CH str.); 853 (C-Cl); 1514 (N=0 str.); 1325 (N-O str.); 2934 (C-H); 1HNMR: 6.21 (m, 2H, C_6H_5-NH_2), 6.84–7.34 (m, 3H, Ar–H); 8.52 (s, 1H, pyrazole–CH), 3.60 (1H, dd); 5.36 (1H, dd), 7.45-7.72 (d, 3H, Ar–H, J=8.6 Hz), 8.00-8.32 (m, 2H, Ar–H). FAB Mass (m/z): 392.10 (Quassi-molecular ion peak (M+H)+)

Compound D6: 2-chloro-5-(1-phenyl-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-3-yl)aniline

Moleculartformula: C22H20ClN3; Molecular weight: 361.87; TLC (Rf value)0.56; Element (Found/Calc.): Nitrogen (11.60/11.61); Chlorine (9.78/9.80); IR (cm^{-1}): 3402 (O-H str.); 1590 (C=N); 1489 (C=C); 1168 (C_6H_5); 1420 (CH=CH str.); 850 (C-Cl); 2935 (C-H); 1HNMR: 6.21 (m, 2H, C_6H_5-NH_2), 6.84–7.34 (m, 3H, Ar–H); 8.52 (s, 1H, pyrazole–CH), 7.45-7.72 (d, 3H, Ar–H, J=8.6 Hz), 3.60 (1H, dd); 5.36 (1H, dd), 7.29-7.67 (m, 2H, Ar–H), 2.15 (m, 3H, C_6H_5-CH_3); FAB Mass (m/z): 361.13 (Quassi-molecular ion peak (M+H)+)

Compound D7 : 2-chloro-5-(5-(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl) aniline

Molecular formula:$C_{22}H_{20}ClN_3O$; Molecular weight: 377.87; TLC (Rf value): 0.66; Element (Found/Calc.)%: Nitrogen (11.10/11.12); Oxygen (4.22/4.23); Chlorine (9.36/9.38): IR (cm^{-1}): 3402 (O-H str.); 1590 (C=N); 1489 (C=C); 1170 (C_6H_5); 1428 (CH=CH str.); 3420 (SO_2NH str.);1322(S=O); 852 (C-Cl); 1064 (OCH_3); 2935(C-H); IHNMR: 6.21 (m, 2H, C_6H_5-NH_2), 6.84–7.34 (m, 3H, Ar–H); 8.52 (s, 1H, pyrazole–CH), 7.45-7.72 (d, 3H, Ar–H, J=8.6 Hz), 3.60 (1H, dd); 5.36 (1H, dd), 7.05-7.55 (m, 2H,Ar–H), OCH_3 3.81 (m, 3H, C_6H_5-OCH_3); FAB Mass (m/z): 377.13 (Quassi-molecular ion peak (M+H)+).1NMR spectra of compound D7 was shown in Figure 2

Compound D8: 4-(3-(3-amino-4-chlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)-N,N-dimethylaniline

Molecular formula: $C_{23}H_{23}ClN_4$; Molecular weight: 390.91; TLC (Rf value): 0.62; Element (Found/Calc.)%: Nitrogen (14.32/14.33); Chlorine (9.07/9.07); IR (cm^{-1}): 3405 (O-H str.); 1590 (C=N);

1491 (C=C); 1170 (C_6H_5); 1428 (CH=CH str.); 850 (C-Cl); 2935 (C-H); IHNMR: 6.21 (m, 2H, C6H5-NH2), 6.84–7.34 (m, 3H, Ar–H); 8.52 (s, 1H, pyrazole–CH), 7.45-7.72 (d, 3H, Ar–H, J=8.6 Hz), 3.60 (1H, dd); 5.36 (1H, dd), 6.82-7.62 (m, 2H, Ar–H); 2.55 (m, 3H, C_6H_5-$N(CH_3)_2$); FAB Mass (m/z): 390.16 (Quassi-molecular ion peak (M+H)+).

RESULT AND DISCUSSION

Pharmacological assessment

Acute toxicity test

Administration of 2000 mg/kg, p.o. of synthesized compounds did not produce any behavioral abnormalities and mortality. So the dose selected for further study was used is10 mg/kg, p.o. for each synthesized compounds.

Anti-inflammatory activity

Anti-inflammatory activity of 1,3,5-pyrazole derivatives (C1-C8 and D1-D8) was evaluated by the following two animal model i.e. carrageenan induced paw edema in rat model and formalin induced paw edema in rats.

Effect of oral administration of synthesized compounds on carrageenan induced paw edema in rats

There was a gradual increase in paw volume of rats in the carrageenan control group. In the test groups, C1-C8 series C7 (98.26%), C8 (92.77%) and C2 (96.24%) were shown of percent inhibition and D1-D8 series D7 (81.50%), D8 (83.81%) and D2 (78.32%) were shown of percent inhibition. Diclofenac (10 mg/kg) caused significant ($p<0.001$) inhibition of increase in paw volume and inhibitory effect of the diclofenac at 10 mg/kg was at 99.42 after 6h **(Table 1)**.

Table 1: Effect of oral administration of synthesized pyrazole derivatives on carrageenan induced paw edema in rats.

Group No.	Treatment	Change in paw volume (ml)				
		1 h	2h	3h	4 h	6 h
1.	Control	1.43±0.24	2.56 ± 0.10	2.78 ± 0.07	3.10 ± 0.02	3.46 ±0.05
2.	Diclofenac (10 mg/kg)	1.10±0.11 (23.07)	1.37 ± 0.04 (46.48)	1.27 ± 0.04 (54.31)	0.35 ± 0.03 (79.47)	0.02±0.01 (99.42)
3.	C1 (10 mg/kg)	1.37±0.17 (04.19)	1.08 ± 0.05 (24.47)	1.52 ± 0.11** (31.74)	1.50 ± 0.14 (46.24)	1.10 ±0.02 (68.20)
4.	C2 (10 mg/kg)	1.12±0.13 ** (21.67)	1.65 ± 0.11 (35.54)	1.22 ± 0.05** (56.11)	0.50 ± 0.15 (75.14)	0.13±0.06 (96.24)
5.	C3 (10 mg/kg)	1.15±0.16** (19.58)	1.53 ± 0.12 * (40.23)	1.78 ± 0.18**(30.46)	0.90 ± 0.17** (63.58)#	0.60±0.03 (82.65)
6.	C4 (10 mg/kg)	1.26±0.14** (11.88)	1.68 ± 0.11* (39.56)	1.38 ± 0.11** (42.54)	1.20± 0.10** (54.91)	0.75±0.02 (78.32)
7.	C5 (10 mg/kg)	1.28±0.16 ** (10.48)	1.78 ± 0.18* (30.46)	1.35 ± 0.11** (43.54)	1.30 ± 0.13** (52.02)	0.85±0.06 **(75.43)
8.	C6 (10 mg/kg)	1.13±0.17 (20.97)	1.08 ± 0.05 (24.47)	1.25 ± 0.07** (55.03)	0.60 ± 0.15 (72.25)	0.15±0.07 (95.66)

9.	C7 (10 mg/kg)	1.10 ± 0.14 (23.07)	1.68 ± 0.10 (39.56)	1.22 ± 0.05** (56.11)	0.55± 0.16 (73.69)	0.25±0.04 (92.77)
10.	C8 (10 mg/kg)	1.09 ± 0.14 (23.77)	1.68 ± 0.13 (39.56)	1.25 ± 0.07 ** (55.03)	0.40 ± 0.11** (78.03)	0.06 ±0.04 (98.26)
11.	D1 (10 mg/kg)	1.4 ± 0.15 (02.09)	1.53 ± 0.12 (40.23)	1.78 ± 0.18** (30.46)	1.72 ± 0.12** (39.88)	1.35 ±0.03 (60.98)
12.	D2 (10 mg/kg)	1.23 ± 0.16* (13.98)	1.78 ± 0.18* (30.46)	1.65 ± 0.11* (35.54)	1.22 ± 0.16** (54.33)	0.75 ±0.07 (78.32)
13.	D3 (10 mg/kg)	1.37 ± 0.12 (04.19)	1.65 ± 0.11 (35.54)	1.09 ± 0.15** (23.77)	1.62 ± 0.16* (42.77)	1.21±0.03 (65.02)
14.	D4 (10 mg/kg)	1.38 ± 0.15 (03.49)	1.53 ± 0.14 (40.23)	1.13 ± 0.14** (20.97)	1.75±0.13** (39.01)	1.25 ±0.02 (63.87)
15.	D5 (10 mg/kg)	1.36 ± 0.16 (04.89)	1.78 ± 0.18 (30.46)	1.65 ± 0.12 (35.54)	1.44±0.10** (47.97)	0.99 ±0.06 (71.38)
16.	D6 (10 mg/kg)	1.32 ± 0.17 (07.69)	1.08 ± 0.05 (24.47)	1.13 ± 0.17 (20.97)	1.28 ± 0.17* (52.60)	0.84 ±0.04 (75.72)
17.	D7 (10 mg/kg)	1.24 ± 0.14 (13.28)	1.65 ± 0.11 (35.54)	1.09 ± 0.13 (23.77)	1.04 ± 0.15** (59.53)	0.64±0.05 (81.50)
18.	D8 (10 mg/kg)	1.27 ± 0.14 (11.18)	1.53 ± 0.12** (40.23)	1.25 ± 0.12 (55.03) ***	0.89 ± 0.13** (63.87)	0.56±0.06** (83.81)

Values are expressed as mean ± S.E.M.; n=6 rats per group. Two way ANOVA followed by Bonferroni post hoc test when compared with carrageenan control *p<0.05, **p<0.01, ***p<0.001. The Figs in parenthesis indicate the percent inhibition.()#- data in bracket denotes percent of inhibition

Inhibition of formalin induced paw edema in rats: The synthesized compounds were screened for their capacity to inhibit formalin induced paw edema in rats and doses given orally. The observed percent inhibitions was tabulated in Table 2.

Table 2: Inhibition of formalin induced paw edema in rat model by synthesized compounds *at* 10 mg/kg dose

G. No.	Isolated fraction	Dose mg/kg i.p.	Formalin			
			MEV and PI (Percent Inhibition)			
			1hr	2h	4h	6h
1.	Normal	Saline (1ml)	0.58±0.06	0.68±0.03	0.75±0.04	0.82±0.07
2.	Diclofenac	10 mg	0.28±0.02 (51.72 %)	0.30±0.05 (55.88 %)	0.35±0.02 (60.00%)	0.09 ±0.05 (89.02)
3.	C1	10 mg	0.35±0.04 (39.65 %)	0.38±0.07** (44.11 %)	0.40±0.04** (42.66 %)	0.30±0.05 (63.41)

4.	C2	10 mg	0.43±0.06 (25.86%)	0.45±0.05 (33.82 %)	0.48±0.05 (36.00 %)	0.11±0.03 (86.58)
5.	C3	10 mg	0.28±0.04 (51.72 %)	0.30±0.04 (55.88 %)	0.35±0.04 (60.00%)	0.21±0.06 (74.39)
6.	C4	10 mg	0.29±0.07 (50.00 %)	0.31±0.03** (54.41%)	0.33±0.03** (56.00 %)	0.36±0.08 (75.60)
7.	C5	10 mg	0.29±0.04 (50.00 %)	0.31±0.07** (54.41%)	0.33±0.06** (56.00 %)	0.32±0.05 (71.95)
8.	C6	10 mg	0.27±0.07 (53.44 %)	0.29±0.03** (57.35 %)	0.31±0.03** (58.66 %)	0.25±0.04 (70.73)
9.	C7	10 mg	0.45±0.05 (22.41%)	0.48±0.07 (29.41 %)	0.52±0.07 (30.66 %)	0.27±0.07 (67.07)
10.	C8	10 mg	0.35±0.04 (39.65 %)	0.38±0.04** (44.11 %)	0.40±0.04** (42.66 %)	0.09±0.08 (89.02)
11.	D1	10 mg	0.29±0.08 (50.00 %)	0.31±0.03** (54.41%)	0.33±0.04** (56.00 %)	0.38±0.06*** (53.65)
12.	D2	10 mg	0.43±0.04*** (25.86%)	0.45±0.08*** (33.82 %)	0.48±0.04*** (36.00 %)	0.24±0.05*** (70.73)
13.	D3	10 mg	0.31±0.04 (46.55%)	0.32±0.04** (52.94 %)	0.34±0.03** (54.66 %)	0.34±0.07 (58.53)
14.	D4	10 mg	0.45±0.04 (22.41%)	0.48±0.03 (29.41 %)	0.52±0.06 (30.66 %)	0.35±0.05 (57.31)
15.	D5	10 mg	0.28±0.08 (51.72 %)	0.30±0.06 (55.88 %)	0.35±0.07 (60.00%)	0.29±0.08 (64.63)
16.	D6	10 mg	0.37±0.08 (36.20%)	0.41±0.04 (39.70 %)	0.43±0.07 (40.00 %)	0.26±0.03 (68.29)
17.	D7	10 mg	0.29±0.05*** (50.00 %)	0.31±0.07*** (54.41%)	0.33±0.03*** (56.00 %)	0.22±0.03*** (73.17)
18.	D8	10 mg	0.41±0.05*** (29.31 %)	0.43±0.04*** (36.76 %)	0.47±0.05*** (37.33 %)	0.20±0.06*** (75.60%)

***p<0.05; **p<0.01; *** p <0.001, as compared with arthritic control**

After 6 hours, the anti-inflammatory activity of formalin-induced paw edoema in rats revealed that C2, C4, and C8 showed 86.58, 75.60, and 89.02 percentages of inhibition, respectively, and D1-D8 series only compound D8 showed 75.60 percentages of inhibition above the 75 percent inhibition, as compared to the standard drug Diclofenac (89.02). These findings reveal that some substances have a significant amount of anti-inflammatory effect when compared to the conventional medication.

RESULT AND DISCUSSION

The anti-inflammatory and analgesic activities of the produced compounds were investigated. The analgesic and anti-inflammatory properties of the thirty-two substances were assessed. Two animal

models were used to test anti-inflammatory activity: (a) carrageenan-induced hind-paw edoema and (b) formalin-induced paw edoema.

Anti-inflammatory properties

Carrageenan-induced rat paw edoema rat model and Formalin-induced rat paw edoema rat model were used to assess the anti-inflammatory activities of certain newly synthesised chemical series C1-C8 and D1-D8. The evaluation's findings were analysed using Declofenac as the standard medication.

Carrageenan induced paw edema in rat model

The anti-inflammatory activity of carrageenan induced paw edoema in rats revealed that in the C1-C8 series, C7, C8, and C2 showed 98.26, 92.77, and 96.24 percent inhibition, respectively, and in the D1-D8 series, D7, D8, and D2 showed 81.50, 83.81, and 78.32 percent inhibition, respectively, when compared to the standard drug Diclofenac at 10 These findings reveal that some substances have a significant amount of anti-inflammatory effect when compared to the conventional medication. When linked to the pyrazoline group, the presence of SO2NH2 group, Cl, CH3, OCH3 and N(CH3)2 group may give active compounds, according to the findings. But the addition of OH, Br and no substitution in phenyl ring may diminish the activity. Percent inhibition by best synthesized compounds on carrageenan induced paw edema in rat model was represented in Figure 3.

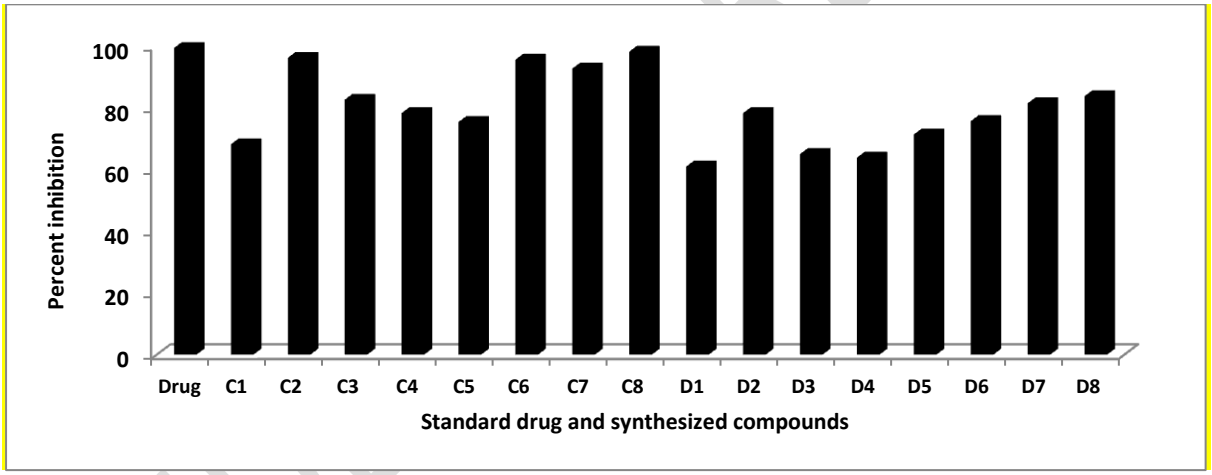


Fig. 3: Percent inhibition by best synthesized compounds on carrageenan induced paw edema in rat model.

Formalin induced paw edema in rat model

The results of anti-inflammatory activity by the formalin induced paw edema in rat model revealed that In C1-C8 series C2, C4 and C8 were shown 86.58, 75.60 and 89.02 percentages of inhibition and In D1-D8 series only compound D8 was shown 75.60 percentages of inhibition above the 75% inhibition as compared to the standard drug Diclofenac (89.02) after 6h. These results is a evident that some compounds show relevant degree of anti-inflammatory activity as compared to the standard drug.**Percent inhibition by best synthesized compounds on Formalin induced paw edema in rat model was shown in Figure 4.**

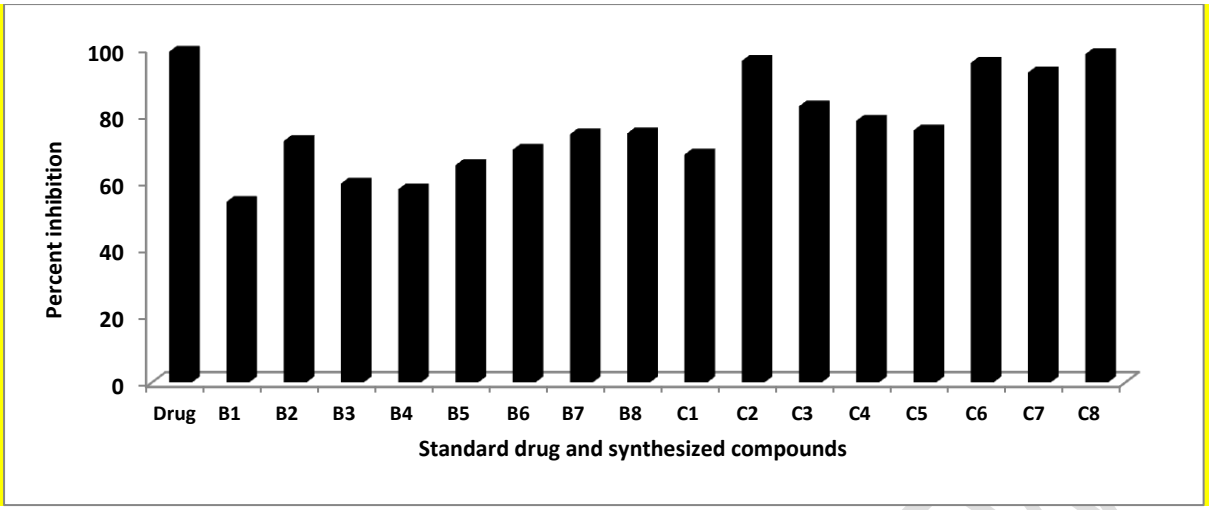


Fig. 4: Percent inhibition by best synthesized compounds on Formalin induced paw edema in rat model

CONCLUSION

The anti-inflammatory potential of 16 produced compounds was investigated using IR, 1HNMR, mass, and elemental analyses. The anti-inflammatory and analgesic activities of the produced compounds were investigated. Two animal models were used to test anti-inflammatory activity: (a) carrageenan-induced hind-paw edoema and (b) formalin-induced paw edoema. In a rat model of carrageenan-induced paw edoema, compound C7, C8, and C2 showed 98.26, 92.77, and 96.24 percent inhibition, respectively, whereas compound D7, D8, and D2 showed 81.50, 83.81, and 78.32 percent inhibition, respectively (Diclofenac). These findings suggest that these chemicals have a significant amount of anti-inflammatory effect. When linked to the pyrazoline group, the presence of SO2NH2 group, Cl, CH3, OCH3 and N(CH3)2 group may give active compounds, according to the findings. The addition of OH, Br, and no alteration in the phenyl ring, on the other hand, may reduce the activity. The most active molecule among the produced compounds belongs to the series C. The presence of SO2NH2 is required for anti-inflammatory action, while the presence of methyl, chloro, methoxy, and N(CH3)2 groups linked to the phenyl ring enhances anti-inflammatory activity.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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