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Research Article

Investigation of Anti-inflammatory and Antioxidant Activities of Promising 1,4,5-Trisubstituted Pyrazoles Derivatives of Chalcone Ditosylates

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ABSTRACT

Patients suffering from chronic pain and inflammatory disorders need novel COX-2 inhibitors to be developed with minimum toxicity to the kidneys, heart, and gastrointestinal tract and with excellent anti-inflammatory activity. The current study centers on an array of 1,4,5-trisubstituted pyrazoles produced *via* the reaction of ditosylates of chalcones with hydrochloride salt of phenylhydrazine. Chalcone was reacted with HTIB to produce a variety of derivatives of α, β chalcone ditosylates. Phenylhydrazine hydrochloride treatment of these chalcone ditosylates produced distinct 1,4,5-trisubstituted pyrazoles. The conversion process is mediated by 1,2-aryl migrations. IR, ^{1}H-NMR, and elemental analysis were used to characterize the compounds after they had been purified by recrystallization. Using ascorbic acid as a reference, the DPPH (1,1-diphenyl-2-picrylhydrazyl) technique was used to assess the compounds' *in-vitro* antioxidant activity. The paw edema technique caused by carrageenan was utilized to assess the compounds' *in-vivo* anti-inflammatory properties. The standard medication used was diclofenac sodium. A plethysmograph was used to measure the volume of the rats' paws. When compared to the standard, the compounds V5D5PH5 and V7D7PH7 showed modest antioxidant activity. When the synthetic pyrazoles were examined for their *in-vivo* anti-inflammatory properties, substances V4D4PH4 and V7D7PH7 outperformed the reference. Here, we attempted to create new, safe, and effective drugs for the treatment of inflammatory disorders and pain by utilizing synthetic pyrazole moiety derivatives. Simple experimentation is used in the proposed study to improve pharmacological activity and yields. In the near future, chalcone ditosylate derivatives will be a powerful tool for selective modification.

Introduction

Nonsteroidal anti-inflammatory medicines (NSAIDs) are commonly used to relieve pain and inflammation. Due to their inseparable gastrointestinal and renal adverse effects from their pharmacological actions, the majority of NSAIDs now in use have limited therapeutic applications. By inhibiting the cyclooxygenase enzyme, these substances stop the production of prostaglandins. The primary enzymes in the production of prostaglandin H2, a precursor to the manufacture of prostaglandins, thromboxanes, and prostacyclins, are cyclooxygenases (COXs). It was shown that this enzyme has two isomers: COX-1, which is constitutive, and COX-2, which is inducible in the gastrointestinal tract (GIT).[1-3] The constitutively produced enzyme COX-1 protects cells, while the inducible COX-2 promotes pain, inflammation, and oncogenesis and traditional NSAIDs inhibit both enzymes.[4-6]

In comparison to COX-2, the majority of them exhibit higher selectivity for COX-1. As a result, long-term usage of nonselective NSAIDs may result in gastrointestinal issues such as bleeding and GI ulcers in addition to stomach distress.[7,8]The majority of clinical NSAIDs have an acidic

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carboxyl (COOH) group, which can irritate the gastrointestinal tract when it comes into direct contact with the GIT. As part of the new NSAID generation, selective COX-2 inhibitors with improved safety profiles are also being sold. However, coxibs containing thiazole rings have shown unanticipated side effects in the cardiovascular system.[9, 10] The use of NSAIDs as a safer medication for the treatment of pain and inflammation is restricted due to these severe adverse effects. Thus, it's critical to discover novel anti-inflammatory medications that are safe and have the potential for therapeutic application. Among the most significant heterocycles are pyrazoles because of their unique structures and wide range of biological activity. As a synthon, chalcone is a crucial component in the synthesis of many bioactive molecules. Due to their diverse biological and pharmacological characteristics, pyrazoles produced from chalcones have garnered a lot of interest. Because of their many uses over the past 30 years, pyrazoles have attracted a lot of attention.[11] As a class of chemicals with a wide range of biological actions, including anti-inflammatory,[12–17] antifungal,[18] anticancer[19–22] and antiviral[23] properties, pyrazoles have garnered significant attention in the field of novel drug development. A3 adenosine receptor antagonists,[25] neuropeptide YY5 receptor antagonists,[26] kinase inhibitors for the treatment of type 2 diabetes, hyperlipidemia, obesity,[27] and thrombopiotinimetics[28] were reported to work as anti-angiogenic drugs in addition to pyrazole derivatives. It has recently been shown that pyrazole urea compounds are strong p38 kinase inhibitors.[29] Traditional pyrazole dyes,[30] herbicide couplings,[31] luminescent and fluorescent substances,[32,33] antiarrhythmic,[34] antipyretic, analgesic, anti-inflammatory,[35-37] and activities that inhibit cholesterol synthesis[38] are a few of these uses. Recently, pyrazoles have drawn interest due to their prospective uses as intermediates in the synthesis of fused pyrazoles, ligand moieties to enhance regio- and stereoselectivity, and chiral catalysts.[39,40] Because of the significance of pyrazoles in biology, medicine, and industry, organic chemists have devised several synthetic methods for their synthesis. [41–42]

In light of the above, the current study aimed to ditosylate α-β chalcones in order to produce 1,4,5-trisubstituted pyrazole derivatives. α-β chalcone ditosylates (3) phenylhydrazine hydrochloride to produce 1,2 aryl shift, which offers a novel method for the production of 1,4,5-trisubstituted pyrazoles. Following the method developed by Rebrovic and Koser, chalcones were reacted with Koser's reagent (HTIB) to yield several chalcone ditosylates. The versatile Koser's reagent [Hydroxy (tosyloxy)iodo] benzene (HTIB) can produce a variety of advantageous conversions. [43] Here, we made use of synthetic pyrazole moiety derivatives in an attempt to develop novel, secure, and potent medications for the management of pain and inflammatory diseases.

Materials And Methods

Synthesis

The melting points (uncorrected) of compounds were ascertained and thin layer chromatography was used to track the reaction and verify purity. Using a KBr pallet, the Shimadzu FTIR 8400 spectrophotometer was used to record the IR spectra. ^{1}H-NMR spectra were collected in CDCl3. An internal standard of tetramethylsilane (TMS) was taken. In Hertz (Hz), coupling constants (J) are expressed. Merck and Sigma Aldrich were the suppliers of all the chemicals and reagents utilized.

General Process for the Synthesis of Compounds (V1D1-V7D7)

Hydroxyl (tosyloxy)iodobenzene (HTIB) (3.96 g, 0.01 mol) was added to a solution of corresponding Chalcone (0.005 mol) in dichloromethane (40 mL). The reaction mixture was agitated for 3 hours at room temperature. To get rid of the p-toluenesulphonic acid that developed as a byproduct, the solution was gently rinsed with water in a separating funnel. Following separation, the organic layer evaporated in a vacuum. To get rid of the iodobenzene, the gummy substance was triturated using petroleum ether. To obtain pure matching α-β Chalcone ditosylates, the resulting solid was further recrystallized using acetonitrile. (2a-2f)

3-Phenyl-1-naphthyl-2,3-ditosyloxypropanone (V1D1)

Yield 57%, m.p. 124–126°C. IR (νmax/cm^{-1}): 1675 (C=O); ^{1}H-NMR (DMSO-d_6): δ 2.23 (s, 3H, --CH_3); 2.26 (s, 3H, -CH_3); 5.12 (d, 1H, -C-H); 6.96 (d, 1H, C-H); 7.16–7.25 (m, 4H, -C_6H_5); 7.23-7.55 (m, 2H, -C_6H_5); 7.39–7.58 (m, 4H, -C_6H_5); 7.58-7.89 (m, 3H, -C_6H_5); 6.55–6.96 (m,7H,ArH); Anal. Calcd. for $C_{40}H_{46}O_2S_2$; C 77.12, H 7.44 C 77.13, H 7.45 Found C 77.13, H 7.45

3-(4-Chlorophenyl)-1-naphthyl-2,3-ditosyloxypropanone (V2D2)

Yield 82%, m.p. 110-–112°C. IR (νmax/cm^{-1}): 1690 cm^{-1} (C=O stretch); ^{1}H-NMR (DMSO-d_6): 2.42 (s, 3H, -CH_3); 2.36 (s, 3H, -CH_3); 4.99 (d, 1H, C-H); 7.01 (d, 1H, C-H); 7.31 (d, 2H, -C_6H_5); 7.16–7.29 (m, 4H, -C_6H_5); 7.53 (d, 2H, -C_6H_5); 7.62–7.78 (m, 4H, -C_6H_5); 6.54–6.90 (m,7H, -C_6H_5); Anal. Calcd. for $C_{40}H_{45}O_2S_2Cl$; C 73.08, H 6.90 Found C 73.09, H 6.91

3-(4-Nitrophenyl)-1-naphthyl-2,3-ditosyloxypropanone (V3D3)

Yield 64%, m.p. 109–111°C. IR (νmax/cm^{-1}): 1684 cm^{-1} (C=O stretch) ; ^{1}H NMR (DMSO-d_6): 2.32 (s, 3H, CH_3); 2.35 (s, 3H, -CH_3); 4.29 (d, 1H, -C-H); 7.02 (d, 1H, -CH); 7.29 (d, 2H, -C_6H_5); 7.12–7.19 (m, 4H, -C_6H_5); 7.48 (d, 2H, -C_6H_5); 7.58–7.69 (m, 4H, -C_6H_5); 6.59–6.85 (m,7H, -C_6H_5); Anal. Calcd. for $C_{40}H_{45}O_4S_2N$; C 71.93, H 6.79 Found C 71.93, H 6.80

3-(4-Methoxyphenyl)-1-naphthyl-2, 3-ditosyloxypropanone (V4D4)

Yield 59%, m.p. 196–198°C. IR (νmax/cm^{-1}): 1692 cm^{-1} (C=O stretch); ^{1}H NMR (DMSO-d_6): 2.41 (s, 3H, -CH_3); 2.43 (s, 3H, -CH_3); 3.79 (s, 3H, -O-CH_3); 5.29 (d, 1H, -C-H); 7.04 (d, 1H, -C-H); 7.12–7.22 (m, 4H, -C_6H_5); 7.41–7.54 (m, 4H, -C_6H_5);7.59–7.68 (m, 4H, -C_6H_5) 6.62–6.87 (m,7H, -C_6H_5); Anal. Calcd. for $C_{41}H_{48}O_3S_2$; C 75.42, H 7.41 Found C 75.42, H 7.42

3-(4-Florophenyl)-1-naphthyl-2,3-ditosyloxypropanone (V5D5)

Yield 62%, m.p. 125–127°C. IR (νmax/cm^{-1}): 1684 cm^{-1} (C=O stretch); ^{1}H NMR (DMSO-d_6): 2.42 (s, 3H, -CH_3); 2.48 (s, 3H, -CH_3); 5.31 (d, 1H, -CH); 7.01 (d, 1H, -CH); 7.31 (d, 2H, -C_6H_5); 7.17–7.27 (m, 4H, -C_6H_5); 7.63 (d, 2H, -C_6H_5); 7.67–7.78 (m, 4H, -C_6H_5); 6.70–6.89 (m,7H, -C_6H_5); Anal. Calcd. for $C_{40}H_{45}O_2S_2F$; C 74.96, H 7.08 Found C 74.97, H 7.08

3-(4-Methylphenyl)-1-naphthyl-2,3-ditosyloxypropanone (V6D6)

Yield 62%, m.p. 92-94°C. IR (νmax/cm^{-1}): 1691 cm^{-1} (C=O stretch)
^{1}H NMR (DMSO-d_6): 2.32 (s, 3H, -CH_3); 2.39 (s, 3H, -CH_3); 2.42 (s, 3H, -CH_3); 5.30 (d, 1H, C-H); 6.99 (d, 1H, C-H); 7.07–7.18 (m, 4H, -C_6H_5); 7.38–7.55 (m, 4H, -C_6H_5); 7.59–7.68 (m, 4H, -C_6H_5); 6.59-6.89 (m,7H, -C_6H_5); Anal. Calcd. for $C_{41}H_{48}O_2S_2$; C 77.31, H 7.60 Found C 77.32, H 7.60

3-(4-Hydroxyphenyl)-1-naphthyl-2,3-ditosyloxypropanone (V7D7)

Yield 72%, m.p. 102–104°C. IR (νmax/cm^{-1}): 1690 cm^{-1} (C=O stretch); ^{1}H NMR (DMSO-d_6): 2.41 (s, 3H, -CH_3); 2.39 (s, 3H, -CH_3); 5.29 (d, 1H, -C-H); 6.99 (d, 1H, C-H); 7.28 (d, 2H,-C-H); 7.09–7.17 (m, 4H, -C_6H_5); 7.43 (d, 2H, -C_6H_5); 7.57–7.68 (m, 4H, -C_6H_5); 6.59–6.89 (m,7H, -C_6H_5); Anal. Calcd. for $C_{40}H_{46}O_3S_2$; C 75.19, H 7.26 Found C 75.20, H 7.26

General process for the synthesis of compounds (V1D1PH1-V7D7PH7)

A three-hour reflux was performed on a chalcone ditosylate (0.566 g) and phenylhydrazine (0.162 g) mixture in ethanol. On top of ice-cold water, the mixture was added. The mixture that was obtained was then divided into three sections using dichloromethane (3×50 mL). A layer of anhydrous sodium sulphate was used to dry and filter the organic extract. Column chromatography on silica gel (100–200 mesh) was used to purify the crude product obtained from vacuum-evaporated dichloromethane to produce pure pyrazoles (V1D1PH1–V7D7PH7).

5-(2-methoxynaphthalen-6-yl)-1,4-diphenyl-1H-pyrazole (V1D1PH1)

Yield 59%, m.p. 124–126°C. IR (νmax/cm^{-1}): Absence of peak in C=O region; ^{1}H-NMR (DMSO-d_6): δ 3.83 (s, 3H, OCH_3), 7.15–7.24 (m, 4H, -C_6H_5); 8.54 (s, 1H, C_3-pyrazole); 7.13–7.23 (m, 10H, -C_6H_5); 6.70–6.88 (m, 7H, -C_6H_5); Anal. Calcd. for $C_{26}H_{20}N_2O$; C 81.90, H 5.30 Found C 81.60, H 5.20

4-(4-chlorophenyl)-5-(2-methoxynaphthalen-6-yl)-1-phenyl-1H-pyrazole (V2D2PH2)

Yield 67%, m.p. 109–110°C. IR (νmax/cm^{-1}): Absence of peak in C=O region; ^{1}H-NMR (DMSO-d_6): δ 3.72 (s, 3H, OCH_3), 7.02 (d, 2H, -C_6H_5); 7.22 (d, 2H, -C_6H_5); 7.29–7.25 (m, 5H, -C_6H_5); 8.45 (s, 1H, C_3- pyrazole); 6.68–6.88 (m,7H, -C_6H_5); Anal. Calcd. for $C_{26}H_{19}ClN_2O$; C 76.20, H 4.6 Found C 76.30, H 4.58

5-(2-methoxynaphthalen-6-yl)-4-(4-nitrophenyl)-1-phenyl-1H-pyrazole (V3D3PH3)

Yield 75%, m.p. 129–131°C. IR (νmax/cm^{-1}): Absence of peak in C=O region; ^{1}H-NMR (DMSO-d_6): δ 3.71 (s, 3H, OCH_3),7.09 (d, 2H, -C_6H_5); 7.11 (d, 2H, -C_6H_5); 7.11–7.15 (m, 5H, -C_6H_5); 8.68 (s, 1H, C_3- pyrazole); 6.8–6.91 (m,7H, -C_6H_5); Anal. Calcd. for $C_{26}H_{19}N_3O_3$; C74.10, H 4.54 Found C 74.20, H 4.30

5-(2-methoxynaphthalen-6-yl)-4-(4-methoxyphenyl)-1-phenyl-1H-pyrazole (V4D4PH4)

Yield 87%, m.p. 147–149°C IR (νmax/cm^{-1}): Absence of peak in C=O region; ^{1}H-NMR (DMSO-d_6): δ 3.69 (s, 3H, OCH_3),6.90 (d, 2H, ArH); 7.16 (d, 2H, ArH); 7.22–7.30 (m, 5H, -C_6H_5); 8.66 (s, 1H, C_3- pyrazole); 6.61–6.58 (m,7H, -C_6H_5); 3.80 (s, 3H, OCH_3); Anal. Calcd. for $C_{27}H_{22}N_2O_2$; C 79.78, H 5.46 Found C 79.65, H 4.99

4-(4-fluorophenyl)-5-(2-methoxynaphthalen-6-yl)-1-phenyl-1H-pyrazole (V5D5PH5)

Yield 67%, m.p. 105–107°C. IR (νmax/cm^{-1}): Absence of peak in C=O region; ^{1}H-NMR (DMSO-d_6): δ 3.68 (s, 3H, OCH_3) 7.01 (d, 2H, -C_6H_5); 7.20 (d, 2H, -C_6H_5); 7.18–7.26 (m, 5H, -C_6H_5); 8.54 (s, 1H, C_3- pyrazole); 6.57–6.88 (m,7H, -C_6H_5); Anal. Calcd. for $C_{26}H_{19}FN_2O$; C 79.17, H 4.86 Found C 79.15, H 4.83

4,5-dihydro-5-(2-methoxynaphthalen-6-yl)-1-phenyl-4-p-tolyl-1H-pyrazole (V6D6PH6)

Yield 42%, m.p. 177–179°C. IR (νmax/cm^{-1}): Absence of peak in C=O region; ^{1}H-NMR (DMSO-d_6): δ 3.68 (s, 3H, OCH_3) ; 7.15 (d, 2H, ArH); 7.16 (d, 2H, -C_6H_5); 7.47–7.55 (m, 5H, -C_6H_5); 8.69 (s, 1H, C_3- pyrazole); 6.69–6.87 (m,7H, -C_6H_5); 2.43 (s, 3H, CH_3); Anal. Calcd. for $C_{27}H_{24}N_2O$; C 82.62, H 6.16 Found C 82.61, H .10

4-(4,5-dihydro-5-(2-methoxynaphthalen-6-yl)-1-phenyl-1H-pyrazol-4-yl)phenol (V7D7PH7)

Yield 61%, m.p. 143-145°C. IR (νmax/cm^{-1}): Absence of peak in C=O region; ^{1}H-NMR (DMSO-d_6): δ 3.78 (s, 3H, -OCH_3) ; 7.06 (d, 2H, -C_6H_5); 7.23 (d, 2H, -C_6H_5); 7.49–7.55 (m, 5H, -C_6H_5); 8.85 (s, 1H, C_3- pyrazole); 6.44–6.67 (m,7H, -C_6H_5); Anal. Calcd. for $C_{26}H_{22}N_2O2$; C 79.14, H 5.61 Found C 79.12, H 5.59

Results And Discussion

Chemistry

Using the open capillary tube method and the Digital Melting Point Apparatus, the melting point of the named analogues was determined and found to be incorrect. The Perkin Elmer RX1 spectrophotometer was used to perform infrared spectroscopy. On a Brucker advanced 300 or 400 MHz spectrometer, nuclear magnetic resonance (NMR) spectra were acquired in a CDCl3 solution using tetramethylsilane (TMS) as an internal standard. Thin layer chromatography (TLC) was used to monitor the reactions' progress. Using a Buchi Rota Evaporator, the solvents were extracted, recovered, or distilled under reduced pressure before being dried over anhydrous sodium sulphate.

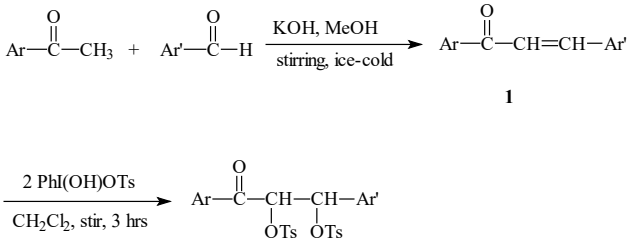
Using the Koser method, Chalcone 1 and HTIB were reacted to produce a variety of derivatives of α-β chalcone ditosylates 3 (Scheme 1).

Phenylhydrazine hydrochloride was used to treat these chalcone ditosylates, resulting in various 1,4,5-trisubstituted pyrazoles (Scheme 2). 1,2-aryl migrations are the method through which ditosylates are converted to 1,4,5-trisubstituted pyrazoles. Only a monochrome product and an excellent yield in the range of 65 to 72% were the results of the reaction. The compounds underwent vacuum drying and recrystallization from ethanol to achieve purification.

Antioxidant Activity

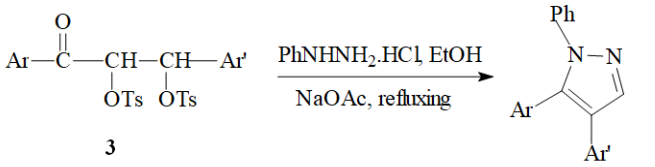
Using the 1,1-diphenyl-2-picrylhydrazyl technique, the compounds' antioxidant activity was assessed.[44] To create a reserve solution with a concentration of 100 µg/mL, the provided chemical was combined with 95% methanol. Various solutions with concentrations of 10, 20, 40, 60 and 100 µg/mL were made from this solution. Various quantities of ascorbic acid were generated in relation to the test substance, with ascorbic acid serving as the standard. After a 15 minutes incubation time at 37°C, 2.5 mL solution of varying concentrations was added to the final reaction mixture. The combination was then allowed to react at ambient temperature. About 517 nm was used to compute absorbance.

Using ascorbic acid as a benchmark, the synthetic compounds' antioxidant properties were assessed based on their capacity to quench DPPH. Table 1 displays all of the findings. Every synthetic molecule exhibited lower potency compared to the reference. When compared to the standard, the compounds V5D5PH5 and V7D7PH7 showed modest antioxidant activity.



Product No.	*Ar*	*Ar'*
V1D1	$C_{11}H_9O$	C_6H_5
V2D2	$C_{11}H_9O$	4-ClC_6H_4
V3D3	$C_{11}H_9O$	4-$NO_2C_6H_4$
V4D4	$C_{11}H_9O$	4-$MeOC_6H_4$
V5D5	$C_{11}H_9O$	4-FC_6H_4
V6D6	$C_{11}H_9O$	4-MeC_6H_4
V7D7	$C_{11}H_9O$	4-OHC_6H_4

Scheme 1: Synthetic scheme of Compounds (V1D1-V7D7)



Product No.	*Ar*	*Ar'*
V1D1PH1	$C_{11}H_9O$	C_6H_5
V2D2PH2	$C_{11}H_9O$	4-ClC_6H_4
V3D3PH3	$C_{11}H_9O$	4-$NO_2C_6H_4$
V4D4PH4	$C_{11}H_9O$	4-$MeOC_6H_4$
V5D5PH5	$C_{11}H_9O$	4-FC_6H_4
V6D6PH6	$C_{11}H_9O$	4-MeC_6H_4
V7D7PH7	$C_{11}H_9O$	4-OHC_6H_4

Scheme 2: Synthesis of 1,4,5-trisubstituted pyrazoles (V1D1PH1-V7D7PH7)

Animals and IAEC

The study involved the collection of adult Wistar rats (150–180 g) of both sexes. Under normal lighting and temperature settings, the animals had unrestricted access to food and drink. Adhering closely to the standards set forth by the Maharishi Markendashwar College of Pharmacy's Institutional Animal Ethics Committee, M.M.U (Deemed to be University) Mullana, standard experimental protocols were used. Protocol was duly authorized by the Institutional Animal Ethics Committee (Reg number. 1355/PO/Re/S/10/CPCSEA with Protocol Ref number. MMCP-IAEC-190), and all interventions and animal care procedures were carried out in compliance with ethical norms. Water displacement was used to assess the increase in foot volume with a Plethysmograph, and a sub-plantar injection of carrageenan caused rats' left paw edema.

Anti-inflammatory Activity

The acute carrageenan-induced paw edema standard technique in rats was used to assess the anti-inflammatory

Table 1: Findings of the 1,4,5 trisubstituted pyrazole's antioxidant properties

S. No.	*Product*	*Percent inhibition*					*IC_{50} value*
		Concentration (µg/mL)					
		10	*20*	*40*	*60*	*100*	
1	V1D1PH1	3.48	7.89	14.88	19.34	23.78	209.61
2	V2D2PH2	1.27	8.97	13.55	18.98	22.39	216.50
3	V3D3PH3	2.76	3.09	12.80	19.65	22.09	212.57
4	V4D4PH4	24.8	32.98	38.63	43.66	52.76	108.42
5	V5D5PH5	27.7	39.32	45.77	53.98	65.34	55.30
6	V6D6PH6	9.73	13.12	23.57	32.09	37.98	128.25
7	V7D7PH7	21.65	32.73	45.89	52.92	61.23	63.06
8	Ascorbic acid	42.01	59.94	68.76	79.32	91.56	9.541

Table 2: Findings of the 1,4,5-trisubstituted pyrazole derivatives' anti-inflammatory activity

Compound	*Paw volume (mm) and after time (hours)*					*%Inhibition*
	0 hour	*1 hour*	*2 hours*	*3 hours*	*4 hours*	
V1D1PH1	0.90 ± 0.049	1.14 ± 0.080	1.19 ± 0.067	1.25 ± 0.046	1.27 ± 0.024	8.63
V2D2PH2	0.95 ± 0.095	1.17 ± 0.071	1.24 ± 0.051	1.28 ± 0.035	1.35 ± 0.078	2.87
V3D3PH3	0.99 ± 0.038	1.18 ± 0.077	1.26 ± 0.037	1.29 ± 0.015	1.36 ± 0.039	2.15
V4D4PH4	0.86 ± 0.045	0.97 ± 0.034	0.94 ± 0.031	0.83 ± 0.021	0.86 ± 0.045	38.12
V5D5PH5	0.92 ± 0.084	1.11 ± 0.065	1.21 ± 0.052	1.26 ± 0.039	1.33 ± 0.047	4.31
V6D6PH6	0.87 ± 0.022	1.04 ± 0.047	1.10 ± 0.050	1.20 ± 0.056	1.24 ± 0.030	10.79
V7D7PH7	0.90 ± 0.067	1.06 ± 0.036	1.14 ± 0.081	1.15 ± 0.062	1.14 ± 0.083	17.98
Control	0.98 ± 0.001	1.16 ± 0.013	1.26 ± 0.003	1.31 ± 0.076	1.39 ± 0.011	...
Diclofenac	0.88 ± 0.009	0.98 ± 0.007	0.93 ± 0.002	0.84 ± 0.023	0.83 ± 0.008	40.28

Readings expressed as mean ± SEM (standard error mean)
Readings calculated and compared to control using one-way ANOVA followed by Dunnet's test

activity of seven representative substances *in-vivo*.[45] A comparison of the obtained results (Table 1) indicates that several newly produced compounds (V6D6PH6 and V7D7PH7) showed stronger anti-inflammatory activities (10.79 and 17.98% inhibition of edema), comparable to that of diclofenac (40.28% inhibition of edema). Moreover, compound V4D4PH4 demonstrated superior activity (38.12% inhibition of edema), suggesting that it is the most potent prepared anti-inflammatory drug. The carrageenan-induced paw edema technique was used to assess the compounds' anti-inflammatory properties. The anti-inflammatory effect was assessed in adult male rats weighing approximately 250 g. There were nine groups of animals. Every group has six creatures in it. Rats with paw edema caused by carrageenan were used to evaluate the anti-inflammatory properties of test substances. The various doses of pretreatment were administered to the various groups of rats. Each rat's left hind paw's sub-plantar region was given 0.1 mL of a 1% carrageenan suspension after an hour, and the paw volume was measured at 0, 1, 2, 3, and 4 hours using a plethysmometer. The standard medication used was diclofenac sodium. Overnight, rats were fasted. The usual dosage of the medication was 20 mg/kg. The synthesized compounds were delivered orally at a dose of 150 mg/kg. Carrageenan was produced in a 1% saline suspension. To cause edema, 0.05 mL of this suspension was injected into the left hind paw's planter tissue. Equal volumes of saline were injected into the animals as a control. Rats' paw volumes were measured using a Plethysmograph. Results are shown in Table 2. The compound V4D4PH4 shows outstanding activity against inflammation.

Conclusion

Compounds (V1D1-V7D7) were synthesized by treating of a variety of chalcones with hydroxyl (tosyloxy) iodobenzene (HTIB) using dichloromethane as a solvent. The melting point of the compounds was determined and the %yield of the compounds was in the range 59 to 73%. The purity and characterization of structures of all newly prepared titled analogs have been elucidated by employing elemental analysis, ^{1}H-NMR and IR data.

The IR spectrum of each ditosylate showed band in the region 1675 to 1682 $cm^{-1,}$ indicating carbonyl stretching.

The ^{1}H-NMR spectra showed two doublets, one at δ 5.30to 5.36 and other at δ 6.90 to 7.01, each peak integrating to one proton having a coupling constant of 8.1 Hz. These peaks can be ascribed to two-methine protons of α,β-chalcone ditosylate. Besides these doublets, the spectra also showed two singlets at δ 2.42 and 2.44 that may be assigned to two *p*-methyl groups of tosylate substituent. Chalcone ditosylates were reacted with phenylhydrazine hydrochloride to create a series of 1,4,5-trisubstituted pyrazole derivatives (V1D1PH1-V7D7PH7). An acceptable yield in the range of 60 to 75% and a colorless product were the results of the reaction. Recrystallization from ethanol allowed the chemicals to be refined. IR, 1NMR, and elemental analysis were used to characterize the synthesized chemicals. The IR spectrum of the product did not show any peak in the carbonyl region. The ^{1}H-NMR spectrum of the product showed a singlet corresponding to one proton at δ 7.8 that can be attributed to the C_3-H of the pyrazole ring in addition to the multiplets for protons of phenyl moieties, on the basis of ^{1}H-NMR data the possibility of isomeric 1,3,5-trisubstituted pyrazole, which was expected to show the singlet of C_4-pyrazolyl proton at upfield δ 6.9 was excluded. The compounds V5D5PH5 and V7D7PH7 displayed considerable antioxidant properties compared to the standard substance. *In-vivo* tests for anti-inflammatory effects were conducted on newly synthesized pyrazoles, highlighting the favorable activity of V4D4PH4 and V7D7PH7 when contrasted with the standard compound.

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References

1. Cristiano S. Revisiting the Structure and Chemistry of 3(5)-Substituted Pyrazoles. Molecules. 2020; 25:42. Available from: doi: 10.3390/molecules25010042
2. Sharanabasappa Patil B. Medicinal Significance of Pyrazole Analogues: A Review. Journal of Pharmaceutical Sciences and Research. 2020;12(3);402-404. Available from: doi.org/10.53730/ijhs.v6nS2.5177
3. Aziz H, Zahoor AF, Shahzadi I and Irfan A. Recent synthetic methodologies towards the synthesis of pyrazoles. Polycyclic Aromatic Compounds. 2021;41(4):698-720 Available from: DOI:10.1080/10406638.2019.1614638
4. Mullins ST. Five-membered heterocyclic compounds with two nitrogen atoms in the ring. Supplements to the of Rodd's Chemistry of Carbon Compounds, A Modern Comprehensive Treatise, 2nd Edition, 4, 1-93 (1975)
5. Zhang S, Gao Z, Lan D, Jia Q, Liu N, Zhang J, Kou K. Recent Advances in Synthesis and Properties of Nitrated Pyrazoles Based Energetic Compounds. Molecules. 2020; 25(3475): 01-42. Available from: https://doi.org/10.3390/molecules25153475
6. Ahmed M, Sophy E and Reheim MA. Synthesis of Some New 1, 3, 4-Oxadiazole, Pyrazole, and Pyrimidine Bearing Thienopyrazole Moieties. Mini-Reviews in Medicinal Chemistry. 2020; 17(8):661-670. Available from: DOI: 10.2174/1570179417999200730215318
7. Zeinab AM, Alshehrei F, Mohie EMZ, Thoraya AF and Abdallah MA. Synthesis of Novel Bis-pyrazole Derivatives as Antimicrobial Agents. Mini-Reviews in Medicinal Chemistry. 2019; 19(15):1276-1290. Available from: DOI: 10.2174/1389557519666190313095545
8. Hassana AS, Moustafab GO, Morsya NM , Abdoud AM and Hafez TS. Design, Synthesis and Antibacterial Activity of N-Aryl-3-(arylamino)-5-(((5-substituted furan-2-yl)methylene)amino)-1Hpyrazole-4- carboxamide as Nitrofurantoin Analogues. Egypt. J. Chem. 2020;63(11):4469- 4481. Available from: DOI: 10.21608/EJCHEM.2020.26158.2525
9. Awasthi SK, Mishra N, Kumar B, Sharma M, Bhattacharya A, Mishra LC, Bhasin VK. Potent antimalarial activity of newly synthesized substituted chalcone analogs *in-vitro*. Med. Chem. Res. 2019;18:407-420. Available from: DOI:10.1007/s00044-008-9137-9
10. Szabo G, Fischer J, Kis-Varga A, Gyires K. New Celecoxib derivatives as anti-inflammatory agents. J Med Chem. 2008;51:142–147. Available from: DOI: 10.1021/jm070821f
11. Wolfe MM, Lichtenstein DR, Singh G. Gastrointestinal toxicity of nonsteroidal antiinflammtory drugs. N Engl J Med. 1999;340:1888–1899. Available from: DOI: 10.1056/NEJM199906173402407
12. Nakamura T, Sato M, Kakinuma H, et al. Pyrazole and isoxazole derivatives as new, potent, and selective 20-hydroxy-5,8,11,14-eicosatetraenoic acid synthase inhibitors. J Med Chem. 2003;46:5416–27. Available from: DOI: 10.1021/jm020557k
13. Cheng H, DeMello KML, Li J, et al. Synthesis and SAR of heteroaryl-phenyl-substituted pyrazole derivatives as highly selective and potent canine COX-2 inhibitors. Bioorg Med Chem Lett. 2006;16:2076–80. Available from: DOI: 10.1016/j.bmcl.2006.01.059
14. Bekhit AA, Ashour HMA, Ghany YSA, et al. Synthesis and biological evaluation of some thiazolyl and thiadiazolyl derivatives of 1H-pyrazole as anti-inflammatory antimicrobial agents. Eur J Med Chem. 2008;43:456–63. Available from: DOI: 10.1016/j.ejmech.2007.03.030
15. El-Sayed MA-A, Abdel-Aziz NI, Abdel-Aziz AAM, et al. Design, synthesis, and biological evaluation of substituted hydrazone and pyrazole derivatives as selective COX-2 inhibitors: molecular docking study. Bioorg Med Chem. 2011;19:3416–24. Available from: DOI: 10.1016/j.bmc.2011.04.027
16. Nagarapu L, Materi J, Gaikwad HK, et al. Synthesis and anti-inflammatory activity of some novel 3-phenyl-N-[3-(4-phenylpiperazin-1yl)propyl]-1H-pyrazole-5-carboxamide derivatives. Bioorg Med Chem Lett 2011;21:4138–40. Available from: DOI: 10.1016/j.bmcl.2011.05.105
17. Ahlstrom MM, Ridderstrom M, Zamora I, Luthman K. CYP2C9 Structure-metabolism relationships: optimizing the metabolic stability of COX-2 inhibitors. J Med Chem. 2007;50:4444–52. Available from: https://doi.org/10.1021/jm0705096
18. Prakash O, Kumar R, Parkash V. Synthesis and antifungal activity of some new 3-hydroxy-2-(1-phenyl-3-aryl-4-pyrazolyl) chromones. Eur J Med Chem. 2008;43:435–40. Available from: DOI: 10.1016/j.ejmech.2007.04.004
19. Brough PA, Aherne W, Barril X, et al. 4,5-Diarylisoxazole Hsp90 chaperone inhibitors: potential therapeutic agents for the treatment of cancer. J Med Chem. 2008;51:196–218. Available from: DOI: 10.1016/j.ejmech.2007.04.004
20. Vera-Di Vaio MAF, Freitas ACC, Castro HCA, et al. Synthesis, antichagasic *in-vitro* evaluation, cytotoxicity assays, molecular modeling and SAR/QSAR studies of a 2-phenyl-3-(1-phenyl-1H-pyrazol-4-yl)-acrylic acid benzylidene-carbohydrazide series. Bioorg Med Chem. 2009;17:295–302. Available from: DOI:10.1016/j.bmc.2008.10.085
21. Singh P, Paul K, Holzer W. Synthesis of pyrazole-based hybrid molecules: search for potent multidrug resistance modulators. Bioorg Med Chem. 2006;14:5061–71. Available from: DOI: 10.1016/j.bmc.2006.02.046
22. Hsu TC, Robins RK, Cheng CC. Studies on 4APP: antineoplastic action *in-vitro*. Science. 1956;13:848–68. Available from: DOI: 10.3109/14756366.2013.873037

23. Storer R, Ashton CJ, Baxter AD, The synthesis and antiviral activity of 4-fluoro-1-β-d-ribofuranosyl-1h-pyrazole-3-carboxamide. Nucleosides, Nucleotides, Nucleic Acids. 1999;18:203–16. Available from: DOI: 10.1080/15257779908043068
24. Genin MJ, Biles C, Keiser BJ, et al. Novel 1,5-diphenylpyrazole nonnucleoside hiv-1 reverse transcriptase inhibitors with enhanced activity versus the delavirdine-resistant P236L mutant: lead identification and SAR of 3- and 4-substituted derivatives. J Med Chem. 2000;43:1034–40. Available from: DOI: 10.1021/jm990383f
25. Qiao JX, Pinto DJ, Orwat MJ, et al. Preparation of 1,1-disubstituted cycloalkyl derivatives as factor Xa inhibitors for treating a thromboembolic disorder. PCT Int Appl WO 03 99, 276 (Chem Abstr 2004;140:16722g)
26. Baraldi PG, Bovero A, Fruttarolo F, et al. New strategies for the synthesis of A3 adenosine receptor antagonists. Bioorg Med Chem. 2003;11:4161–9. Available from: DOI: 10.1016/s0968-0896(03)00484-x
27. Stamford AW, Wu Y. Preparation of N-(phenyl)pyrazolyl-N′-piperidinylureas as neuropeptide Y Y5 receptor antagonists. PCT Int Appl WO 2004, 5262 (Chem. Abstr. 2004;140:11141. Available from: DOI: 10.3109/14756366.2013.873037
28. Brown ML, Cheung M, Dickerson SH, et al. Preparation of pyrazolopyrimidines as kinase inhibitors for the treatment of type 2 diabetes. PCT Int Appl WO 2004, 9596 (Chem. Abstr. 2004;140:128436y. Available from: DOI: 10.1021/acsinfecdis.0c00803
29. Clive DM, Stoff JS. Renal syndromes associated with nonsteroidal anti-inflammatory drugs. N Eng J Med. 1984;310:563–72. Available from: DOI: 10.1056/NEJM198403013100905
30. Duma J, Hatoum-Mokdad H, Sibley R, et al. 1-Phenyl-5-pyrazolyl ureas: potent and selective p38 kinase inhibitors. Bioorg Med Chem Lett. 2000;10:2051–4. Available from: DOI: 10.1016/s0960-894x(00)00272-9
31. Khanna IK, Yu Y, Huff RM, et al. Selective Cyclooxygenase-2 inhibitors: heteroaryl modified 1,2-diarylimidazoles are potent, orally active anti-inflammatory agents. J Med Chem. 2000;43: 3168–85. Available from: DOI: 10.1021/jm0000719
32. Abdel-Rahman H.M, Hussien M.A. Synthesis of β-hydroxypropanoic acid derivatives as potential anti-inflammatory, analgesic and antimicrobial agents. Arch Pharm Chem Life Sci. 2006;339:378–387. Available from: DOI: 10.1002/ardp.200600016
33. Mohamed Ahmed Elian Sophy and Mohamed Ahmed Mahmoud Abdel Reheim, Synthesis of Some New 1, 3, 4-Oxadiazole, Pyrazole, and Pyrimidine Bearing Thienopyrazole Moieties. 2020; 17(8):661-670. Available from: DOI: 10.2174/1570179417999200730215318
34. Zeinab AM, Fatimah Alshehrei, Mohie EMZ, Thoraya A. Farghaly and Magda A. Abdallah. Synthesis of Novel Bis-pyrazole Derivatives as Antimicrobial Agents, Mini-Reviews in Medicinal Chemistry. 2019; 19(15):1276-1290. Available from: DOI: 10.2174/1389557519666190313095545
35. Alina Secrieru, Paul Michael O'Neill, Maria Lurdes, Santos Cristiano, Revisiting the Structure and Chemistry of 3(5)-Substituted Pyrazoles. Molecules. 2020; 25:42-44. Available from: doi:10.3390/molecules25010042
36. Wenquan Z, Honglei X, Rujing Y, Jiaheng Z, Kangcai W, Qinghua Z. Synthesis and Properties of 3,6-Dinitropyrazolo[4,3-c]- pyrazole (DNPP) Derivatives. Propellents. Explosives Pyrotechnics. 2020;45(4):546- 553. Available from: https://doi.org/10.1002/prep.201900205
37. Madhusudana P, Anuradha C. M. and Chitta S. K. Design, synthesis, and evaluation of pyrazolo-pyrazole derivatives on Methylisocitratelyase of Pseudomonas aeruginosa: in silico and *in-vitro* study. Journal of Biomolecular Structure and Dynamics. 2017;35(11):2509-2529. Available from: DOI: 10.1080/07391102.2016.1223754
38. Sobhi M, Hassan G. M, Abdel-aziz A, El-Reedy A.M. Facile Synthesis of Pyrazolo[3,4-c]pyrazoles Bearing Coumarin Ring as Anticancer Agents. Journal of Heterocyclic Chemistry. 2018;55(8):1960-1965. Available from: https://doi.org/10.1002/jhet.3235
39. Silva VLM and Silva AMS. Recent Advances in the synthesis, functionalization and applications of Pyrazole-Type Compounds. Molecules. 2021;26(16):4989. Available from: https://doi.org/10.3390/molecules26164989
40. Mallikarjunna RR, Musthak AM and Sreeramulu J. Synthesis and antimicrobial activity of linked heterocycles containing pyrazolyle-indole derivatives. Journal of Pharmacy Research. 2012;5:1518-1521. Available from: DOI:10.1016/j.ejmech.2014.06.001
41. Steinbach G, Lynch PM, Robin KSP, Wallace MH, Hawk E, Gordon GB, Wakabayashi N, Saunders B. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N. Engl. J. Med. 2000;342:1946–1952. Available from: DOI: 10.1056/NEJM200006293422603
42. Mandawad GG, Dawane BS, Beedkar SD, Khobragade CN, Yemul OS. Trisubstituted thiophene analogues of 1-thiazolyl-2-pyrazoline, superoxidase inhibitors and free radical scavenger. Bioorg. Med. Chem. 2013;2:365-72. Available from: DOI: 10.1016/j.bmc.2012.09.060
43. Prakash O, Sharma D, Kamal R, Kumar R, Nair RR. The chemistry of α,β-ditosyloxyketones: new and convenient route for the synthesis of 1,4,5- trisubstituted pyrazoles from a,b-chalcone ditosylates. Tetrahedron. 2009;65:10175–10181. Available from: DOI:10.1016/j.tet.2009.10.001
44. Pisoschi AM, Cheregi MC and Danet, AF. Total antioxidant capacity of some commercial fruit juices: electrochemical and spectrophotometrical approaches. Molecules. 2009;14:480-493. Available from: https://doi.org/10.3390/molecules14010480
45. Winter CA, Fisley EA, Nuss GW. Carrageenin-induced edema in hind paws of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med. 1964;111:544–547. Available from: DOI: 10.3181/00379727-111-27849

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