Synthesis and Biological Evaluation of new acetylated pyrazoline analogues

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Summary

One series of novel substituted 3-(2-indoloyl)-3-phenylprop-2-en-1-one were synthesized by a base catalyzed Claisen-Schmidt condensation reaction of 3-Acetyl indole with different halogen substituted benzaldehydes. Synthesized chalcones were converted into pyrazoline derivative by reacting with hydrazine hydrate. All the compounds were purified by recrystallization and characterized by ^{1}HNMR, ^{13}CNMR and MS spectroscopy. Synthesized compounds were screened for its anti-inflammatory activity by membrane stabilization method. These compounds showed effective stabilization of RBC membrane and can become a promising leads for the development of new anti-inflammatory agents in future.

Keywords: COX-2, Inflammation, Chalcone, Pyrazoline, Membrane Stabilization.

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Introduction

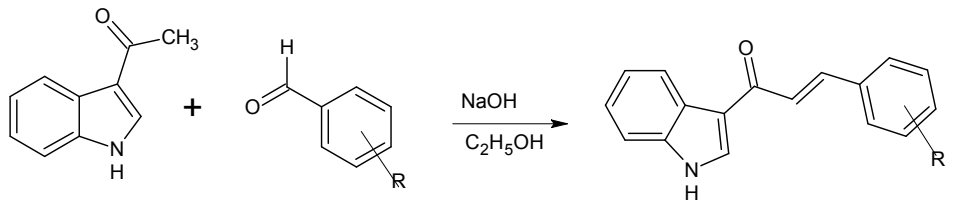
Pyrazolines are compounds with noteworthy applications. Pyrazolines are well known and important nitrogen-containing five member heterocyclic compounds [1]. Numerous pyrazoline derivatives have been found to possess considerable biological activities, which stimulated the research activity in this field. 2-pyrazoline moiety [2]. It has been demonstrated to have an important therapeutic potential, mainly as anti-inflammatory, antidepressant, antipyretic, antibacterial, antifungal, and antitumor agents have been cited in literature [3]. Pyrazoline derivatives such as antipyrine, aminopyrine, and dipyrone are known as antipyretic and analgesic substances and their pharmacological action and has been widely surveyed and have found their clinical application as NSAIDs [4]. After the pioneering work of Fischer and Knovenagel in the late nineteenth century, various methods have been worked

out for the synthesis of pyrazoline derivatives, the reaction of α, β-unsaturated aldehydes and ketones with hydrazines became one of the most popular methods for the preparation of 2-pyrazolines [5]. Cyclooxygenase-2 (COX-2) is the target for choice for design of some new and safer anti-inflammatory agents. Cyclooxygenase are key enzymes in the synthesis of prostaglandin H_2 which is a precursor for the biosynthesis of prostaglandins [7]. COX enzymes exist in two isoforms, COX-1 and COX-2. The COX-1 enzyme is constitutively expressed and is critical for protection of gastric mucosa, platelet aggregation, and renal blood flows whereas the COX-2 enzyme is inducible an expressed during inflammation, pain and oncogenesis [9]. Since COX-2 is involved in inflammation and pain, molecules that inhibit enzymatic activity would be of therapeutic value. Well-known medicinal pyrazolone, dipyrone, has shown to inhibit the activities in a dose-dependent manner of cyclooxygenase enzyme COX-1 and its variant COX-3, which catalyze the rate-limiting step of prostaglandin synthesis.

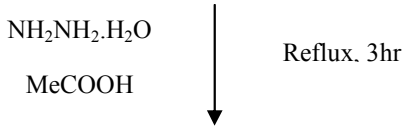
Methodology

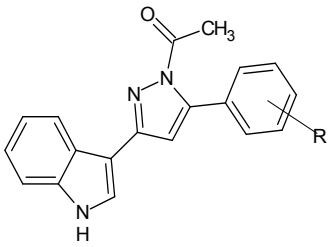
A solution of 5mL 10% NaOH and 3mL rectified spirit is taken in a 100ml round bottom flask and immersed in a crushed ice-bath. 0.01mol freshly distilled 3-Acetyl Indole is poured into the flask and stirred mechanically. To this mixture, 0.01mol of halogen substituted benzaldehyde is added and stirred vigorously until mixture becomes so thick that stirring is not effective (2-3 hours) and keep it overnight in refrigerator. The product was filtered and washed with cold water until washings are neutral and then with ice cold rectified spirit. Crude chalcone was collected after drying in air and purified by recrystalization [1].

All the chalcones were treated with hydrazine hydrate in hot glacial acetic acid to afford 1- acetyl-3, 5-diaryl-2-pyrazolines in good yields. It is done by refluxing the desired chalcones (5 mmol) with hydrazine hydrate (25 mmol) in hot acetic acid (30 ml) for three hours and the completion of reaction was confirmed by TLC using the mobile phase Ethyl acetate and Hexane in various proportions (1:9, 2:8, 3:7…). The reaction mixture was poured over crushed ice (250 ml) and the precipitate was collected by filtration. The product was washed with water, dried and recrystallized from methanol [3].



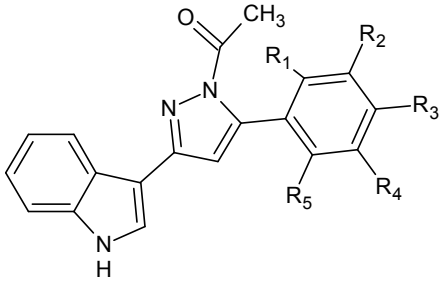
3-Acetylindole(1) Substituted benzaldehyde(2a-d) Chalcones (3a-d)





Pyrazolines (4a-d)

Fig- 1 Synthetic Scheme of Acetylated pyrazolines



4(a-d)

Table 1: Pyrazolines Representation

COMPOUND CODE	R_1	R_2	R_3	R_4	R_5
(4a)-3-[1-acetyl-5-(4-fluorophenyl)-1*H*-pyrazol-3-yl]-1*H*-indole	H	H	F	H	H
(4b)- 3-[1-acetyl-5-(4-chlorophenyl)-1*H*-pyrazol-3-yl]-1*H*-indole	H	H	Cl	H	H
(4c)- 3-[1-acetyl-5-(2-bromophenyl)-1*H*-pyrazol-3-yl]-1*H*-indole	Br	H	H	H	H
(4d)- 3-[1-acetyl-5-(2-fluorophenyl)-1*H*-pyrazol-3-yl]-1*H*-indole	F	H	H	H	H

***In vitro* membrane stabilization studies.**

Anti-inflammatory activity of the synthesized pyrazoline compounds were screened by Human RBC membrane stabilization method. The compounds were tested at different concentration respectively. Ketoprofen was served as a standard drug for comparision of the results.Results are presented in Table-3

Results And Discussions

Synthesis of Chalcones

Chalcone intermediates (3a– 3d) was prepared by Claisen-Schmidt condensation of various halogen substituted benzaldehyde and 3-Acetyl Indole in ethanol and in presence of 10% sodium hydroxide solution. Completion of the reaction was monitored by thin layer chromatography. The chalcones were fully characterized by melting point, 400MHz ^{1}H NMR, and Mass spectrometry. Melting point

was determined in open capillary tubes method. Number and nature of proton present were analyzed by ^{1}H NMR spectra and molecular mass was confirmed from mass spectra for all the synthesized compounds.

Spectral Data

3-(4-fluorophenyl)-1-(1*H*-indol-3-yl) prop-2-en-1-one- Dark yellow solid (94.67%) mp - 54-56ºC, ^{1}H NMR 400 MHz ($CDCl_3$) δ 8.55-7.10 (m,9H,CH,Ar), 2.5 (d,2H,CH), 8.85 (s,1H,NH), ^{13}C NMR 500MHz δ 193.68 (C=O),140.18-122.40 (14C,Ar), 40-27.6 (2C,Aliphatic), MS m/z (rel intensity) 265.28 (M^-,100).

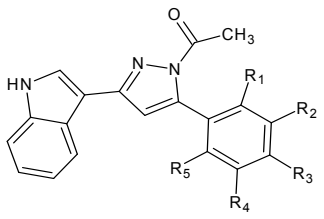
3-(4-chlorophenyl)-1-(1*H*-indol-3-yl)prop-2-en-1-one – Off white solid, (95.74%) mp – 60-62ºC,^{1}H NMR 400 MHz ($CDCl_3$) δ 8.40-7.25(m,9H,CH,Ar),2.55 (d,2H,CH), 8.82(s,1H,NH), ^{13}CNMR 500 MHz δ 193.66(C=O),136.36-111.3(14C,Ar),41.1-27.6(2C,Aliphatic), MS m/z (rel intensity) 281.73 (100).

3-(2-bromophenyl)-1-(1*H*-indol-3-yl)prop-2-en-1-one – pale yellow solid, (58.73%) mp -72-74ºC, ^{1}H NMR 400MHz ($CDCl_3$) δ 8.82-7.25(m,9H,CH,Ar), 2.55 (d,2H,CH), 9.74(s,1H,NH),^{13}C NMR 500 MHz δ 190.37(C=O),136.36-111.01(14C,Ar),40.05-27.63(2C,Aliphatic), MS m/z (rel intensity) 325.18 (M^-,100).

3-(2-fluorophenyl)-1-(1*H*-indol-3-yl)prop-2-en-1-one – Brownish yellow solid, (57.42%), mp-75-78ºC, ^{1}H NMR 400MHz ($CDCl_3$) δ 7.91-7.31 (m,9H,CH,Ar), 8.5-8.4 (d,2H,CH), 9.09 (s,1H,NH),^{13}C NMR 500 MHz δ 193.7(C=O),136.4-111.3(14C,Ar),41.5-27.6 (2C,Aliphatic), MS m/z (rel intensity) 265.28 (100)

Synthesis of Pyrazolines

Pyrazolines (4a- 4d) were prepared by refluxing desired chalcones with hydrazine hydrate in the presence of hot glacial acetic acid for three to four hours. The pyrazolines were fully characterized by melting point ^{1}H NMR, and Mass spectrometry. Melting point was determined by open capillary tubes. Number and nature of proton present were analyzed by ^{1}H NMR spectra and molecular mass was confirmed from mass spectra for all the synthesized compounds.



Pyrazolines 4(a-d)

Table 2. Physical Properties of Pyrazolines

Compound code	Mol. formula	M.w	m.p	Color	Yield %
4a	$C_{19}H_{14}FN_3O$	319.33	128-130°C	Pale yellow	56.87
4b	$C_{19}H_{14}ClN_3O$	335.78	120-121°C	Dark yellow	48.74
4c	$C_{19}H_{14}BrN_3O$	380.21	122-124°C	Pale yellow	65.21
4d	$C_{19}H_{14}FN_3O$	319.33	132-134°C	Brownish yellow	58.42

Spectral Data

3-[1-acetyl-5-(4-fluorophenyl)-1*H*-pyrazol-3-yl]-1*H*-indole – Pale yellow solid, (56.87%), mp 128-130ºC, ^{1}H NMR 400 MHz ($CDCl_3$) δ 8.53-6.8 (m,10H,CH,Ar), 2.61(s,3H,CH_3), 8.69 (s,1H,NH),^{13}C NMR 500 MHz δ168.53(C=O,Acetyl), 137.98-115.60(17C,Ar),58.02 (1C,Aliphatic),MS m/z (rel intensity) 319.33 (100).

3-[1-acetyl-5-(4-chlorophenyl)-1*H*-pyrazol-3-yl]-1*H*-indole –Dark yellow solid, (48.74%), mp-120-121ºC, ^{1}H NMR 400 MHz ($CDCl_3$) δ 8.73-6.9 (m,10H,CH,Ar), 2.6(s,3H,CH_3), 8.89 (s,1H,NH),^{13}C NMR 500 MHz δ168.43(C=O, Acetyl), 139.68-114.60(17C,Ar),57.12 (1C,Aliphatic), MS m/z (rel intensity) 335.78 (100)s

3-[1-acetyl-5-(2-bromophenyl)-1*H*-pyrazol-3-yl]-1*H*-indole – Pale yellow solid, (65.21%), mp-122-124ºC, ^{1}H NMR 400 MHz ($CDCl_3$) δ 8.4-7.07 (m,10H,CH,Ar), 2.58 (s,3H,CH_3), 8.61 (s,1H.NH), ^{13}C NMR 500 MHz δ168.50(C=O, Acetyl), 151.00-108.03(17C,Ar),58.57 (1C,Aliphatic), MS m/z (rel intensity) 379.03 (M^-,100).

3-[1-acetyl-5-(2-fluorophenyl)-1*H*-pyrazol-3-yl]-1*H*-indole – Brownish yellow solid, (58.4%), mp-132-134ºC, ^{1}H NMR 400MHz ($CDCl_3$) δ 8.4-7.0 (m,10H,CH,Ar), 2.61 (s,3H,CH_3), 8.5 (s,1H,NH), ^{13}C NMR 500 MHz δ168.54(C=O, Acetyl), 151-110.06(17C,Ar),53.25(1C,Aliphatic), MS m/z (rel intensity) 319.33 (100).

Membrane Stabilization Studies

The anti-inflammatory capacity of the synthesized compounds were performed by membrane stabilization method and the data were compared with a standard drug ketoprofen, and the results were tabulated in table no.3

Table 3. Membrane Stabilization activity of pyrazoline series

Conc.(µg/ml)	% Membrane Stabilization				
	4a	**4b**	**4c**	**4d**	**Ketoprofen**
25	52.4	51.6	42.3	40.2	49
50	53.4	54.2	44.7	49.7	51.2
100	66.4	57.2	50.8	64.4	55.2

The compound 4a (3-[1-acetyl-5-(4-fluorophenyl)-1*H*-pyrazol-3-yl]-1*H*-indole) is exhibiting best Membrane stabilization activity among the synthesized pyrazolines. The compound 4d (3-[1-acetyl-5-(2-fluorophenyl)-1*H*-pyrazol-3-yl]-1*H*-indole**)** and 4b (3-[1-acetyl-5-(4-chlorophenyl)-1*H*-pyrazol-3-yl]-1*H*-indole also exhibits almost good membrane stabilization activity when compared to the standard drug Ketoprofen.

Conclusion

Structure of all the Pyrazolines were confirmed by ^{1}H NMR, ^{13}C NMR and MASS spectroscopic techniques. Pyrazolines were screened for *Invitro* anti-inflammatory activity by RBC membrane stabilization studies. All compounds were found to possess considerable membrane stabilizing activity. The compound 4a (3-[1-acetyl-5-(4-fluorophenyl)-1*H*-pyrazol-3-yl]-1*H*-indole) is exhibiting best Membrane stabilization activity among all the synthesized compounds. The best orders of Membrane stabilization effect of synthesized compounds are as follows.

4a> 4b> 4d> Ketoprofen> 4c

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