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Synthesis and Biological Evaluation of Quinazolinone Fused Chalcone Derivatives as Anti-inflammatory Agents

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ABSTRACT

In the present study we have designed a new pharmacophore by pharmacophore hybridization approach of drug design. Quinazolinone nucleus has potent anti-inflammatory activities with nearly negligible ulcer index unlike other non steroidal anti-inflammatory drugs. Chalcone also have anti-inflammatory activity. Therefore, here we had tried to synthesize some of the novel quinazolinone fused chalcone derivatives and tested them for their anti-inflammatory activity. The structures of the compound have been confirmed by spectral analysis. Newly synthesized compounds were tested for anti-inflammatory activity using Rat hind paw method. Among the synthesized compounds 3-[1-(3, 4-dimethoxyphenyl)-3-(3-nitrophenyl) allylideneamino]-2-phenylquinazolin-4(3H)-one (4) were found to be most active.

Keywords: Quinazolinone, Schiff base, chalcone, anti-inflammatory activity.

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1. INTRODUCTION

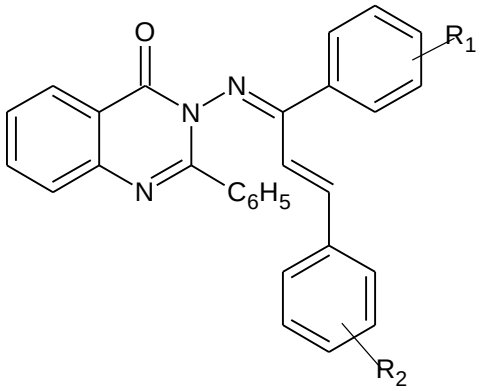
Non-steroidal anti-inflammatory drugs (NSAIDs) are used to treat a wide variety of illnesses and inflammatory diseases, cancer, diabetes, Alzheimer's and Parkinson's[1,2,3]. The anti-inflammatory action is based on the mechanism by inhibiting cyclooxygenase (COX) enzyme, and catalyze the bis-dioxygenation and subsequent reduction of arachidonic acid (AA) to prostaglandins (PGs) [4]. Cyclo-oxygenase enzyme exists in two isoforms COX-1 and COX-2, which are regulated differently [5]. COX-1 is a constitutive isoform and it is found mainly in normal cells and tissues and called the housekeeping enzyme[6,7], it is stimulated by growth factor and hormones and it plays essential roles in the generation of PGs, gastric protection and control of renal blood flow and it regulates the process of platelet aggregation[8]. The major side effects of NSAID's are their gastrointestinal ulcerogenic activity and bronchospasm because of decreased levels of PGs [9]. COX-2 is induced by proinflammatory stimuli such as endotoxin, bacterial lipopolysaccharide, growth factors, cytokines, mitogens, and tumor-promoting agents [10,11] It has been revealed that the undesirable side effects of NSAID's are may be due to COX-1 inhibition while the beneficial effects, such as reduction of swelling and analgesia, are related to COX-2 inhibition [12]. Selective COX-2 inhibitors having cardiovascular toxicity is due to the inhibition of the synthesis of prostacyclin (PGI2), which has anti-thrombotic properties, while sparing the synthesis of thromboxane A2 (TXA2), a pro-thrombotic substance, PGE2 is the PG primarily associated with inflammation. Therefore, selective COX-2 inhibitors could be a rational approach for reducing inflammation without producing the cardiovascular and GI toxicity associated with NSAIDs. COX-2 inhibitors block the production of prostaglandin in inflammatory cells without affecting homeostasis and gastro-protective actions mediated by COX-1[13].

The search continues to develop new drugs that have potent anti-inflammatory activity with minimum side effects. Although many NSAID's are in the market, the present therapeutic approach and chemical design of NSAID's are now targeted towards the development of selective COX-2 inhibitors.

There are large numbers of synthetic compounds with substituted quinazolinone nucleus used for anti- inflammation [14], anti – HIV [15], anticonvulsants, sedation anxiolytic activities [16], antihyper-glycemic activity [17], antimicrobial activity [18], anxiolytic and antidopaminergic activity [19], antitumour activity [20].

From the literature it have also been concluded that the quinazolinone and chalcone, both the moieties have good anti-inflammatory and analgesic activities. In the present study we have used pharmacophore hybridization technique of drug design and designed a pharmacophore model 'chalcone quinazolinone'.

Table 1: The list of synthesized compounds



(1-4)

Synthesized Compounds	R_1	R_2
1	H	H
2	4-C_2H_5	4-Cl
3	3,4-OCH_3	4-F
4	3,4-OCH_3	3-NO_2

2. MATERIALS AND METHODS

The starting materials were commercially available and purchased from acros organics. Melting points were measured on a Veego Amp-1 melting point apparatus. Thin layer chromatography (TLC, silica gel-G) was used to monitor reactions and check product homogeneity. The structure of synthesized compounds was determined by spectral analysis. The λmax of synthesized compounds was determined by using Shimadzu UV-1700 spectrophotometer. IR spectra were recorded in Bruker spectrometer using ABS technique. 1H-NMR spectra were recorded on a JEOL GSX 270 MHz spectrometer using $CDCl_3$ or DMSO as solvent and TMS as internal reference (chemical shifts in ∂ ppm) Splitting patterns are described as singlet (s), doublet (d), and multiplet (m).

2.1 Chemistry

Synthesis of compounds was completed in three steps as described in scheme 1. First step involves the treatment of Anthranilic acid with benzoyl chloride in pyridine resulted in the formation of 3-amino-2-phenylquinazolin-4(3H)-one.The second step involves the treatment of aromatic aldehydes and substituted acetophenones in presence of aqueous potassium hydroxide solution for the formation of chalcones. Both the reaction summarised in step 1 & 2 respectively. The final step involves the admixing of 3-amino-2-phenylquinazolin-4(3H)-one and chalcones in the presence of HCl to yield the final product, quinazolinone derivatives (1-4). The final step reaction is summarised in step 3. The list of the synthesized compound is reported in table 1.

Step-1: Synthesis of 3-amino-2-phenylquinazolin-4(3H)-one

Anthranilic acid (20mmol/2.74g) was dissolved in 30ml of pyridine (dry). To this solution benzoyl chloride (20mmol/2.54ml) was added drop wise with constant stirring at low temperature. Reaction mixture was treated with 10% sodium bicarbonate and filtered and washed repeatedly with water to remove inorganic material. The crude product (2-phenyl 3, 1-benzoxazinone) was obtained and recrystallized from ethanol. Then 2-phenyl 3, 1-benzoxazinone was reacted with hydrazine hydrate and refluxed in n-butanol for 4hrs. The corresponding 3-amino-2-phenylquinazolin-4(3H)-one was formed in good yield, which was filtered, dried and recrystallized from ethanol.

Step-2: Synthesis of chalcones

Equimolar quantities (10mmol, 1equiv) of the aromatic aldehydes and substituted acetophenones were dissolved in approximately 25 ml of ethanol. The mixture was allowed to stir for several minutes at 5-10°C. In the mixture 10 ml of a 40% aqueous potassium hydroxide solution was added drop wise to the reaction flask. The reaction solution was allowed to stir at room temperature for approximately 4 hrs. Most commonly, a precipitate formed was then collected by suction filtration.

Step-3: Synthesis of substituted quinazolinone derivatives

3-amino-2-phenylquinazolin-4(3H)-one in 25ml of ethanol added on equimolar quantity of appropriate chalcone derivative previously dissolved in ethanol, then few drops of conc. HCl was added and continuously stirred for 4-5 hrs. The obtained product was filtered, dried and recrystallised from hot ethanol.

Synthesis of 3-(1, 3-diphenylallylideneamino)-2-phenylquinazolin-4(3H)-one (1)

Yield 73.2%; m. p. 79-80°C; λmax 310 nm; IR (ABS): 749 cm^{-1} (C-H bending), 1209 cm^{-1} (C-C), 1333 cm^{-1} (C-N), 1602 cm^{-1} (C=N), 1660 cm^{-1} (C=C),1720 cm^{-1} (C=O), 2928 cm^{-1} (C-H streching), 3054 cm^{-1} (=C-H); 1H NMR ($CDCl_3$): ∂ 7.235-8.012 (m, 19H, Ar-H), 1.637 (s, 1H, CH).

Synthesis of 3-[3-(4-chlorophenyl)-1-(4-ethylphenyl) allylideneamino]-2-phenylquinazolin-4(3H)-one (2)

Yield 80%; m. p. 109-111°C; λmax 306 nm; IR (ABS): 758 cm^{-1} (C-H bending), 818 cm^{-1} (C-Cl), 1323 cm^{-1} (C-N), 1599 cm^{-1} (C=N), 1657 cm^{-1} (C=C), 1715 cm^{-1} (C=O), 2964 cm^{-1} (C-H streching), 3031cm^{-1} (=C-H); ^{1}H NMR ($CDCl_3$): ∂ 7.078-7.965(m, 17H, Ar-H), 1.279 (s, 1H, CH), 2.705-2.761 (m, 3H, Ar-CH_3).

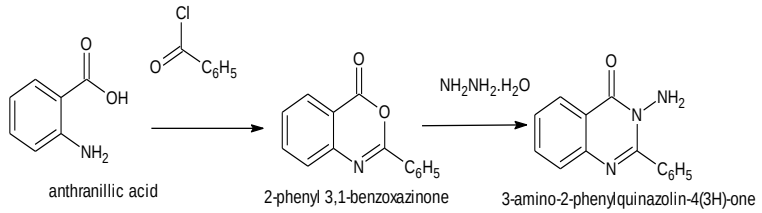
Synthesis of 3-[1-(3, 4-dimethoxyphenyl)-3-(4-fluorophenyl) allylideneamino]-2-phenyl quinazolin-4(3 H)-one (3)

Yield 72.5%; m.p. 129-130°C; λmax 318 nm; IR (ABS): 799 cm^{-1} (C-H bending), 1023 cm^{-1} (C-F), 1201 cm^{-1} (C-O-C), 1344 cm^{-1} (C-N), 1594 cm^{-1} (C=N), 1657 cm^{-1} (C=C), 1708 cm^{-1} (C=O), 2942 cm^{-1} (C-H streching), 3076 cm^{-1} (=C-H) ; ^{1}H NMR ($CDCl_3$): ∂ 3.438-4.580(m, 3H, O-CH3), 7.234-8.014 (m, 16H, Ar-H).

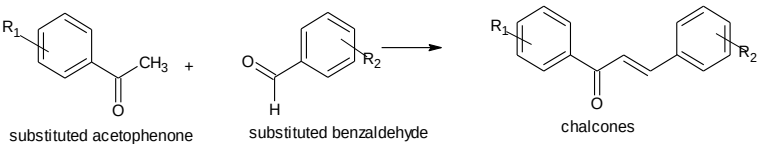
Synthesis of 3-[1-(3, 4-dimethoxyphenyl)-3-(3-nitrophenyl) allylideneamino]-2-phenyl quinazoin-4(3H)-one (4)

Yield 70.5%; m.p. 88-90°C; λmax 315 nm; IR (ABS): 803 cm^{-1} (C-H bending), 1019 cm^{-1} (C-F stretching), 1156 cm^{-1} (C-O-C stretching), 1321 cm^{-1} (C-N stretching), 1506 cm^{-1} (N-O stretching), 1590 cm^{-1} (C=N stretching), 1653 cm^{-1} (C=C stretching), 1720 cm^{-1} (C=O stretching) 2928 cm^{-1} (C-H stretching), 3018 cm^{-1} (=C-H) ; ^{1}H NMR ($CDCl_3$): ∂ 1.633 (s, 1H, CH), 7.397-8.120 (m, 16H, Ar-H), 3.544-3.585 (m, 3H, O-CH_3).

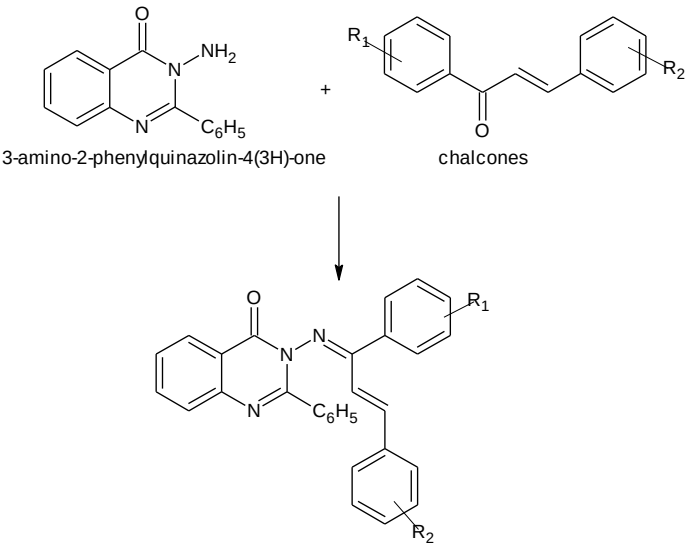
Step 1: Synthesis of 3-amino-2-phenylquinazolin-4(3H)-one



Step 2: Synthesis of chalcones



Step 3: Synthesis of substituted quinazolinone derivatives



Scheme: Synthesis Quinazolinone fused chalcone derivatives.

2.2 Anti-inflammatory Activity

Carrageenan induced paw edema assay: Male and female albino rats (150- 200g) were used. The animals were fed with commercial feed pellets and were given water ad libitum.

Carrageenan was obtained from s d fine-chem. Ltd. Paw edema was measured by UGO BASILE 7140 Plethysmometer. The protocol adopted for the experimentation of animals was approved by the Institutional Animal Ethics Committee (approval No: 778/PO/a/03/CPCSEA)

The rats were divided into 6 groups consisting of six animals each. First group served as control, Second group animals received standard drug indomethacin (10mg/kg) [21] and rest of the groups of animals received the test compounds (50mg/kg) orally in 0.5% sodium carboxy methyl cellulose. After one hour, 0.05ml of 1% suspension of carrageenan was injected into the sub plantar region of hind paw of all the rats.

For the inflammation, the volume of the injected paw was measured by water displacement in a digital plethysmograph immediately after carrageenan injection. The paw volume was again measured after 3 hrs. A mark was made at the lateral maleolous of the right paw and the foot was dipped to the same distance of the mark into the arm of plethysmograph. Average edema volumes for test compounds treated and positive control rats were compared statistically with those of the vehicle control animals and expressed as percent edema inhibition which is calculated using the formula [22]. The results are shown in table 2.

Percentage edema inhibition= 100 (1-Vt/Vc)

Where, Vt =volume of edema in treated group, Vc = volume of the edema in the control group.

3. RESULT AND DISCUSSION

All the newly synthesized compounds were examined for physiochemical characterization and anti-inflammatory activity. Indomethacin is used as standard against all synthesized compounds. The melting point of all compounds was observed, different from ingredients melting point which was confirmed the synthesis of product. The purity of synthesized compounds was checked by observing single spot on TLC plate.

It indicates the purity of sample. The UV λmax of synthesized compounds was observed at range between 300-318nm. This range of λmax was showed the presence of β-unsaturated carbonyl moiety. The IR spectra of synthesized compounds showed characteristic absorption peak for the functional group present in compounds. The stretching vibrations for Ar-H bending (749 to 803), C=O Stretching (1700-1715), C-F stretching (1019 to 1023), C-Cl stretching (818), C-N stretching (1321 to 1344), C=N stretching (1590 to 1657), C-O-C stretching (1156 to 1201), N-O stretching (1506), C-H stretching (2928 to 2964), =C-H stretching (3018 to 3076) were showed for the aryl, Carboxy, carbonyl, alkene, nitro, ether and halogen functionality respectively present in the synthesized compounds.

The ^{1}H NMR spectrum of synthesized compound (1) explained the presence of Aromatic ring Ar-H 7.7078 to 8.120, Ar-CH_3 2.705-2.761, O-CH_3 3.438 to 4.580. The order of compounds with respect to their anti-inflammatory activity is: 4 > 3 > 2 > 1. The Compound (1) does not have any substitution at R1 and R2 position it is less active than all synthesized compounds. Compound (2) is monosubstituted with ethyl group and chloro group at R1 and R2 respectively. It have moderate activity against the standard compound. Among all compounds, compound (4) and (3) demonstrated maximum anti-inflammatory activity and this may be due to the presence of electro-negative nitro group and fluoro group respectively in the nucleus. The percentage inhibition of compound (4) was found 45.4% which is to be maximum of the entire synthesized compound. Compound 3, 2, 1 also shows 36.3%, 31.8%, 29.5% percentage inhibition induced carrageenan respectively. This concludes that the presence of electro-negative group in the nucleus enhances the biological activity of the compound.

4. CONCLUSION

Quinazolinone fused chalcone derivatives were synthesized and characterized for their structure elucidation. Synthesized compounds effectively prevented the increase in inflammation induced by carrageenan. Combinations of these subunits showed better anti-inflammatory drugs and can create new entities with superior therapeutic activity and better safety profiles.

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Table 2: Anti-inflammatory activity of synthesized compounds by carrageenan induced rat hind paw edema method

Groups	Dose (mg/kg)	Mean paw volume (mm)±SEM				% Inhibition $(1-V_t/V_c)*100$
		0 h	1 h	2 h	3 h	
I (Control)	----	0.45±0.02	0.46±0.02	0.46±0.01	0.44±.002	----
II (Std.)	10	0.43±0.02	0.36±0.03*	0.27±0.05*	0.16±0.01*	63.6%
III (1)	50	0.46±0.03	0.40±0.01*	0.37±0.05*	0.31±0.05*	29.5%
IV (2)	50	0.43±0.05	0.39±0.04*	0.35±0.01*	0.30±0.01*	31.8%
V (3)	50	0.44±0.05	0.38±0.05*	0.31±0.02*	0.28±0.03*	36.3%
VI (4)	50	0.43±0.01	0.37±0.05*	0.30±0.03*	0.24±0.02*	45.4%

Values are mean ± SEM; n= 6 albino rats per groups; *P<0.05 as compared with control group

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