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Anti-inflammatory activity of novel 1,2-Dihydroquinoline derivatives

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Abstract:

Earlier we reported the synthesis, characterisation and antimicrobial activity of twelve novel 1,2-Dihydroquinoline derivatives. In extension of this work, anti-inflammatory activity of these 12 novel compounds using carrageenan-induce paw oedema model in rodent was carried out. From the analysis, it was observed that most of the compounds exhibited varied degree of anti-inflammatory activity. Among tested compounds 402 and 407 exhibited significant reduction in paw volume and percentage inhibition of paw edema compared to other derivatives. The rest of the compounds exhibited moderate anti-inflammatory activity. The observed beneficial effect of active compounds may be due to inhibition of cyclooxygenase and/or lipoxygenase enzymes involved in inflammation.

Keywords: 1,2-Dihydroquinoline derivatives, inflammation, Carrageenan

INTRODUCTION

Inflammation is generally considered as an essentially protective response to tissue injury caused by noxious physical, chemical or microbiological stimulus. It is a complex process involving various mediators, such as prostaglandins, leukotrienes and platelet activating factors, Inflammation is caused by release of chemicals from tissues and migrating cells. Most strongly implicated are the prostaglandins (PGs), leukotrienes (LTs), histamine, bradykinin, and, more recently, platelet- activating factor (PAF) and interleukin [1].

Inflammation is a normal, essential, protective response to any noxious stimulus that may threaten the whole organism. Rheumatic diseases are inflammatory conditions causing major

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disability. Moreover, inflammation is simply a physiologic response process generated by the body in response to injury, infection, or irritation. In acute stages, the inflammatory process is vital to the healing process.

The chemistry of heterocyclic compounds has been an interesting field of study for a long time. The synthesis of novel quinoline analogs and investigation of their chemical and pharmacological behaviour have gained more importance in recent decades for medicinal reasons.

The chemistry of quinoline derivatives has been of increasing interest since many of these compounds have been found useful as chemotherapeutic agents against malaria[2] parasite and microbes[3].Literature surveys indicate that quinoline derivatives possess diverse pharmacological activities, including antimicrobial [4], antimalarial [2], antiviral [5], antitumor [6], immunomodulatory [7], caspase-3 inhibition[8], local anaesthetic [9], antiarrhythmic [9] and anti-inflammatory activities [10].

The dihydroquinoline structure exists in a large number of natural products and biologically active molecules.[11] Particularly, many of these naturally occurring 1,2-dihydroquinolines and their synthetic analogs are important precursors for the synthesis of natural products and pharmaceuticals.[12] Therefore, the development of new and efficient synthetic routes for the preparation of dihydroquinoline analogs is of importance to both organic synthetic and medicinal chemistry.[13-16] These facts provoked us to carry out the anti-inflammatory activity for the synthesized compounds.

MATERIALS AND METHODS

Synthesis and characterization of 12 novel 1,2- Dihydroquinoline derivatives were achieved and their antimicrobial activity was earlier reported by us [17]. Briefly, total 12 derivatives were synthesised from three diverged schemes (Scheme 1, 2 & 3). The characterization of these derivatives was carried out by Melting points, IR, ^{1}H NMR, ^{13}C NMR and LC-MS data.

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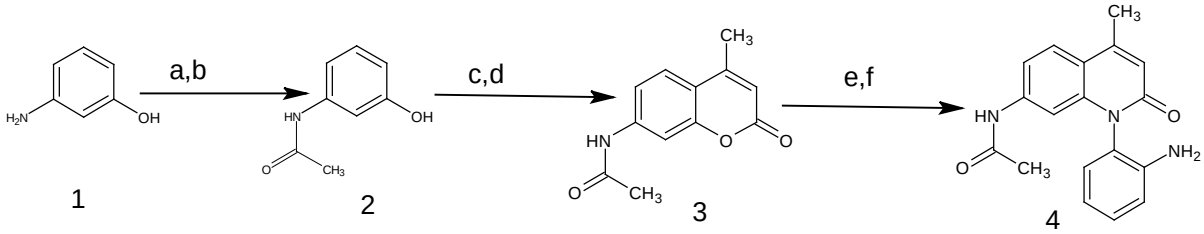
FT-IR Spectra was recorded using Agilent Carry 630 FTIR with ATR instrument. ^{1}H NMR and ^{13}C NMR were recorded in Bruker model avance II (399.65 MHz, 1H NMR) and Bruker model avance II (100.50 MHz, 13C NMR) instruments respectively and analysis were carried out either DMSO-d_6 or CD_3OD depending on solubility of the compound. All the chemical shifts were reported in parts per million (ppm). LC-MS was recorded using Waters Alliance 2795 separations module and Waters Micromass LCT mass detector. Elemental analysis (C, H and N) was performed on an Elementar vario MICRO cube. The purity of the compound was confirmed using TLC on pre-coated silica gel plate and further purification was done using column chromatography.

Synthetic route for preparation of Metacetamol derivatives was shown in scheme 1



Procedure for the preparation of N-(3-hydroxyphenyl) acetamide (metacetamol) (2): Compound (1) (0.11 mol, 25g) was dissolved in acetic anhydride (a; 80 mL) and the reaction mixture was stirred at 60 °C for 8 h at room temperature under nitrogen atmosphere. The excess acetic anhydride was removed under reduced pressure; the residue was dissolved in methylene dichloride (b; MDC), washed with water. The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated to obtain compound (2).

Procedure for the preparation of *N*-(4-methyl-2-oxo-2*H*-chromen-7-yl) acetamide (3) :

A mixture of 3- hydroxy acetanilide (metacetamol) (0.1 mol, 15.1g) and ethylacetoacetate (c; 0.1 mol) with 70% sulphuric acid (d, 50 mL) was heated carefully for 5 h. The resulting solution was cooled and poured over crushed ice (250 g). The crude product was filtered off

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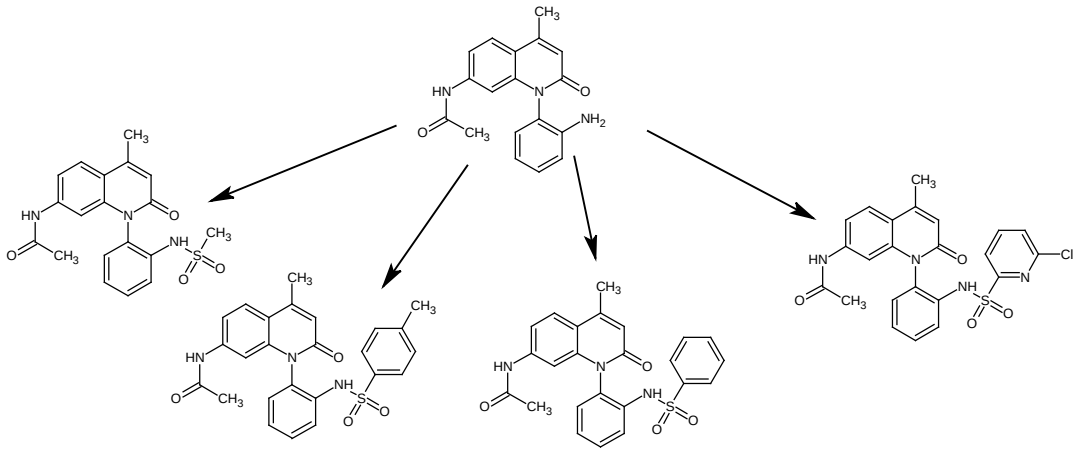
and washed repeatedly with water, dried and recrystallized from hot water to result in title compound (3).

Procedure for the preparation of N-[1-(2-aminophenyl)-4-methyl-2-oxo-1,2-dihydroquinolin-7- yl]acetamide (4): A mixture of N-(4-methyl-2-oxo-2H-chromen-7-yl) acetamide (0.01 mol, 2.17g), o-phenylenediamine (e, 0.01 mol, 1.08g) and sodium acetate (f, 5 g) in glacial acetic acid (15 mL) was refluxed for 8 h and cooled [18,19]. The separated solid was filtered and recrystallized from methanol: water (1:2) to give title compound (4).

General Procedure for the preparation of sulphonamides containing dihydroquinoline nucleus (0401 to 0404):

Equimolar quantities of compound (4) (0.001 mol, 0.5 g), and different substituted sulphonyl chlorides (0.001 mol) such as methyl-, p-tolyl-, phenyl- and 2-chloro pyridyl-sulfonyl chloride, and tetra ethyl amine (TEA, 0.003 moles, 0.57 g) were stirred in dry MDC (10 mL) under nitrogen condition at room temperature for 12 h. The reaction was monitored by TLC; mixture was washed with water and brine. The organic phase was dried over Na_2SO_4 and evaporated on vacuum. Residue was purified by column chromatography using petroleum ether:ethyl acetate as eluent (7:3) to get sulphonamides dihydroquinoline nucleus (0401 to 0404) in good yield **(Scheme 2)**.



04 01 **04 02** **0403** **0404**

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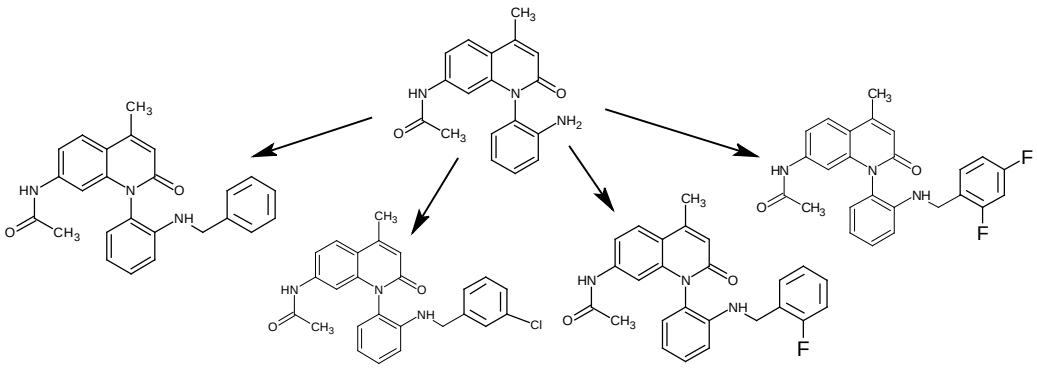
Scheme 2

General Procedure for the preparation of benzylated dihydroquinoline nucleus (0405 to 0408): Equimolar quantities of compound (4) (0.5 g, 0.001 mol) and different substituted benzyl bromides (0.001 mol) such as benzyl-, 2-chloro benzyl-, 2-fluoro benzyl- and 2,4-difluoro benzyl bromides, K_2CO_3 (0.003 moles, 0.57 g), were stirred in dry ACN (10 mL) under nitrogen at room temperature for 10 h. The reaction was monitored by TLC and reaction mixture was filtered. The organic phase was dried over Na_2SO_4 and evaporated on vacuum. Residue was purified by column chromatography using petroleum ether: ethyl acetate as eluent (8:2) to get benzylated dihydroquinoline nucleus (0405 to 0408) in good yield **(Scheme 3)**.



0405 **0406** **0407** **0408**

Scheme 3

General Procedure for the preparation of amides containing dihydroquinoline nucleus (0409 to 0412): Equimolar quantities of compound (4) (0.001 mol, 0.5 g) and different substituted acid chlorides (0.001 mol) such as 3-fluoro benzoyl-, 3-trifluoromethoxy benzoyl-3,5-dichloro benzoyl- and 3-chloro benzoylchlorides, TEA (0.003 moles, 0.57 g), were stirred in dry MDC (10 mL) under nitrogen at room temperature for 10 h[19,20]. The reaction was monitored by TLC, reaction mixture was washed with water and brine. The organic phase was dried over Na_2SO_4 and evaporated on vacuum. Residue was purified by column chromatography using petroleum ether:ethyl acetate as eluent (7:3) to get amides containing

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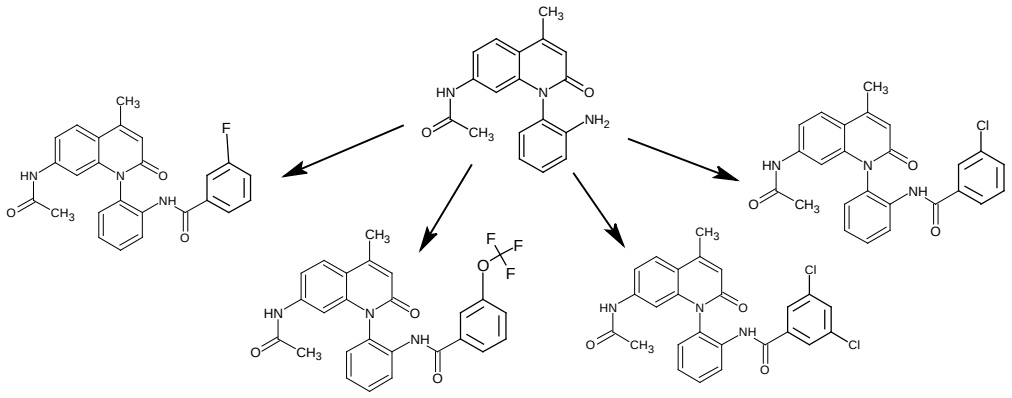
dihydroquinoline nucleus (0409 to 0412) in good yield. These derivatives are represented in **Scheme 4**.



0409 **0410** **0411** **0412**

Scheme 4

Animal experimentation

Animals: Adult Wistar rats and mice of both sex weighing between 200-250 g and 25-30 grespectively were used for experiment. They were housed in standard environmental condition like, ambient temperature (25 ± 1° C), relative humidity (55±5%) and 12/12h light dark cycle. Animals had free access to standard pellet diet and water. All animal experiments were carried out in accordance with the guidelines of CPCSEA. The institute animal ethical committee gave the approval for conducting animal experiments

Anti-inflammatory activity

Carrageenan induced rat paw edema method

Anti-inflammatory activity was assessed by the method described by Winter et al.[21] Rats were divided in to seven groups of five each. Group-I received 0.9% normal saline as normal control (2ml/kg), Group- II received 0.1ml of 1% carrageenan suspension in normal saline and was injected into the subplanter region of left hind paw to induce edema, Group-III to Group VI received10mg/kg of diclofenac, compound 404, 407, 410 and

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411respectively.After 1 h of respective treatment, 0.1ml of 1% suspension of carrageenan in normal saline was injected into the sub-planter region of left hind paw to induce edema to all group except normal control. The paw volume was measured at 0 min, 30min, 60min and 180min after carrageenan injection using digital Plethysmometer (520-R, IITC Life Science - USA). The difference between the initial and subsequent values gave the actual edema volume which was compared with disease control.

The inhibition of inflammation was calculated using the formula,

$$\% \text{ Inhibition} = 100\left(\frac{Vc-Vt}{Vc}\right)$$

Where 'Vc' represents edema volume in disease control and 'Vt' edema volume in group treated with test drugs.

Carrageenan induced mice paw edema method

Micewere divided in to eleven groups of five each. Group-I received 0.9% normal saline as normal control (2ml/kg), Group- II received 0.1ml of 1% carrageenan suspension in normal saline and was injected into the subplanter region of left hind paw to induce edema, Group-III to Group IX received10mg/kg of diclofenac, compound 401, 402, 403, 405, 406, 408, 409 and 412respectively.After 1 h of respective treatment, 0.1ml of 1% suspension of carrageenan in normal saline was injected into the sub-planter region of left hind paw to induce edema to all group except normal control and measurement of edema was carried out as explained above and represented.

Statistical analysis: Data analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison Test $p<0.05$ was considered statistically significant.

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RESULTS AND DISCUSSION

The characterization of 12 synthesised derivatives was carried out by Melting points, IR, ^{1}H NMR, ^{13}C NMR and LC-MS data and the details are presented in our earlier report [17].

At the inflammatory site, the release of chemical mediators, which cause edema as a result of extravasations of fluid and proteins from the local microvasculature and accumulation of polymorphonuclear leukocytes resulted in acute inflammation. Carrageenan model is conventional, sensitive, and accepted for screening of newer anti-inflammatory agents as it induces inflammation resulting from a complex of diverse mediators.**[**21,22**]** Further, this model reliably predicts the anti-inflammatory efficacy based on inhibition of prostaglandin amplification [23**]**.

Anti-inflammatory activity of compounds in Carrageenan induced rat paw edema method

Sub-planter injection of carrageenan to left hind paw of rats resulted in significant ($p<0.01$; $p<0.001$) increase in paw volume at different tested time intervals compared to normal control animals. Administration of 1,2-dihydroquinoline analogues (10 mg/kg, p.o) to rats with paw edeam exhibited significant reduction in paw edema at different time points. Administration of compound 407 exhibited significant percentage inhibition of edema at 30 min (20.42%) 60 min (23.03%) and 180 min (29.41%) post carrageenan injection compared to inducer control. Furthermore, compound 407 was found to be most active out of four derivatives (404, 407, 410 and 411) tested (Table 1 & 2).

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Table 1. Percentage inhibition of edema by 1,2-Dihydroquinoline derivatives in Carrageenan induced paw edema in rats.

Sl No	Treatment	Dose (mg/kg)	% of Inhibition of Edema		
			30 min	60 min	180 min
1	Diclofenac	10 mg/kg	23.94±5.15	29.60±2.46	36.88±2.05
2	404 (A1)	10 mg/kg	9.15±3.42	15.13±4.83	28.10±3.91
3	407 (A2)	10 mg/kg	20.42±4.51	23.03±2.69	29.41±0.40
4	411 (A3)	10 mg/kg	13.38±5.86	16.44±6.35	21.56±6.50
5	410 (A4)	10 mg/kg	4.26±4.03	11.84±3.33	18.95±6.23

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Table 2. Anti-inflammatory activity of 1,2-Dihydroquinoline derivatives in Carrageenan induced paw edema in rats.

Sl No	Treatment	Dose(mg/kg)	Mean paw volume ± SEM		
			30 min	60 min	180 min
1	Normal control	1ml/kg	0.90±0.055	0.904±0.061	0.892±0.054
2	Carrageenan	0.1ml	1.42±0.012$^{###}$	1.52±0.033$^{###}$	1.53±0.025$^{###}$
3	Diclofinac	10mg/kg	1.08±0.0186**	1.07±0.027*	0.966±0.043***
4	404(A1)	100mg/kg	1.29±0.053	1.29±0.056*	1.10±0.049***
5	407(A2)	100mg/kg	1.13±0.057**	1.17±0.030***	1.08±0.018***
6	411(A3)	100mg/kg	1.23±0.082	1.27±0.074*	1.20±0.085**
7	410(A4)	100mg/kg	1.36±0.050	1.34±0.023	1.24±0.059**

Values are given as Mean ± S.E.M (n = 5). The inter group variation was measured by one-way ANOVA followed by tukey's test. $^{###}p<0.001$ compared to normal group; **p<0.001, **p<0.01 & *p<0.05 compared with Carrageenan treated group.

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Anti-inflammatory activity of compounds in Carrageenan induced mice paw edema method

Administration of carrageenan to left hind paw of mice exhibited significant ($p<0.05$; $p<0.01$) increase in paw volume at 30 min, 60 min & 180 min compared to normal control animals. Oral administration of 10 mg/kg dose of 1,2-dihydroquinoline analogues (401, 402, 403, 405, 406, 408, 409 and 412) to carrageenan induced mice exhibited significant reduction in paw edema at different time points. Administration of compound 402 exhibited significant percentage inhibition of edema at 30 min (19.58%), 60 min (21.48%) and 180 min (28.76%) post carrageenan injection compared to inducer control. Compound 402 was found to be most active out of eight tested derivatives (Table 3 & 4). Furthermore, compound 401, 403, 405 and 406 also exhibited moderate activity (Table 3 & 4).

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Table 3. Percentage inhibition of edema by 1,2-Dihydroquinoline derivatives in Carrageenan induced paw edema in mice.

Sl No	Treatment	Dose(mg/kg)	% of Inhibition of Edema		
			30 min	60 min	180 min
1	Diclofenac	10mg/kg	20.99±4.97	28.19±7.73	31.37±6.94
2	401	10 mg/kg	18.88±6.19	21.48±4.81	27.45±5.11
3	402	10 mg/kg	19.58±8.10	21.48±7.98	28.76±6.94
4	403	10 mg/kg	18.88±7.48	17.45±9.15	28.76±5.64
5	405	10 mg/kg	16.78±4.07	22.82±2.40	28.10±4.58
6	406	10 mg/kg	15.38±4.08	18.804.05	25.49±5.57
7	408	10 mg/kg	11.89±4.65	18.80±4.05	19.61±2.26
8	409	10 mg/kg	11.89±4.65	20.13±5.02	26.14±6.10
9	412	10 mg/kg	15.38±3.63	23.33±2.79	22.22±5.02

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Table 4. Anti-inflammatory activity of 1,2-Dihydroquinoline derivatives in Carrageenan induced paw edema in mice.

Sl No	Treatment	Dose(mg/kg)	Mean paw volume ± SEM		
			30 min	60 min	180 min
1	Normal saline	2 ml/kg	0.220±0.010	0.210±0.012	0.206±0.006
2	Carrageenan Control	0.1 ml	0.286±0.010$^{\#}$	0.298±0.011$^{\#\#}$	0.306±0.007$^{\#\#}$
3	Diclofenac	10 mg/kg	0.226±0.017	0.214±0.017*	0.210±0.018**
4	401	10 mg/kg	0.232±0.015	0.234±0.021	0.222±0.016*
5	402	10 mg/kg	0.230±0.019	0.234±0.023	0.218±0.021*
6	403	10 mg/kg	0.232±0.021	0.246±0.023	0.218±0.018*
7	405	10 mg/kg	0.238±0.005	0.230±0.007	0.220±0.008*
8	406	10 mg/kg	0.242±0.007	0.242±0.007	0.228±0.019*
9	408	10 mg/kg	0.252±0.010	0.242±0.007	0.246±0.011
10	409	10 mg/kg	0.252±0.0102	0.238±0.015	0.226±0.016*
11	412	10 mg/kg	0.242±0.004	0.234±0.010	0.238±0.017

Values are given as Mean ± S.E.M (n=5). The inter group variation was measured by one-way ANOVA followed by tukey's test. $^{\#}p<0.05$ &$^{\#\#}p<0.01$ compared to normal group;**$p<0.01$ & *$p<0.05$ compared with Carrageenan treated group.

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CONCLUSION

Taken together, the present study reveals that compound 402 and 407 exhibited better anti-inflammatory activity than other tested compounds. A structural Activity Resealtionship (SAR) study reveals that 2-fluoro benzyl and 4-methyl phenyl sulphonamide moieties are responsible for the observed anti-inflammatory activity and considered to be the pharamcophoric groups. Further studies are needed to explore these active derivatives in chronic inflammation models.

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