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Design, Synthesis, Biological Evaluation and Docking Studies of Some New Diclofenac Analogues

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Authors' contributions

This work was carried out in collaboration between all authors. Author GMN designed the study and wrote the protocol. Author HAO wrote the first draft of the manuscript, conducted the experimental works and performed the statistical analysis. Author GMN managed the literature searches and analyses of data. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: There is still a need for new, selective COX-2 inhibitors with an improved safety profile, therefore, a series of diclofenac analogues were designed and different physicochemical properties were calculated such as log P, hydrogen donor, hydrogen-acceptor, molecular weight and pKa etc and compared with diclofenac and study was aimed to design and calculate different physicochemical properties and attempt to introduce diclofenac derivatives with improved anti-inflammatory profile along with docking focusingon CO X-2.

Materials and Method: Carrageenan-induced paw edema to evaluate the anti-inflammatory activity of the conjugates 4 groups (n = 6) of Wistar rats (150–200 g) were examined. A first group of rats was used as a control without using the drug, group II received Diclofenac 20 mg kg^{-1}, received PEG600-Diclo conjugate and PEG4000-Diclo conjugate (52.60 mg kg^{-1} and 214 mg kg^{-1} respectively), where the dose was molecularly equivalent to the diclofenac.

Results: Result showed a significant ($p<0.05$) dose dependent increase in reaction time in mice in the method at the doses of 150 and 200 mg/kg body weight. Also docking studies specifically on COX-2 exhibited promising anti-inflammatory effect as demonstrated by statistically significant ($p<0.05$) inhibition of paw volume at the dose of

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150 mg/kg body weight and the dose of 500 mg/kg body weight at the three hours of study.
Conclusion: In this study molecular docking results, along with biological assay data, show that all compounds have a potential anti-inflammatory activity comparable to diclofenac.

Keywords: Diclofenac analogues; physicochemical properties; petasis reaction; anti-inflammatory activity; molecular docking.

ABBREVIATIONS

B.E = Binding Energy;
CMC = Carboxymethyl Cellulose;
COX = Cyclooxygenase;
DCM = Dichloromethane;
D.E = Dissociation Energy;
D.M = Dipole Moment;
Elec. E = Electrostatic Energy;
H.A = Hydrogen Acceptor;
H.B = Hydrogen Bond;
H.D = Hydrogen Donor;
H.F = Heat of formation;
M.W = Molecular weight;
NSAID = Non-steroidal anti-inflammatory drug;
PG = Prostaglandin;
VDW = Van Der Waals;

1. INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) play essential role as anti-inflammatory, analgesic, and antipyretic drugs. Diclofenac, 2-(2, 6-dichloranilino) phenylacetic acid is an NSAID that is commonly used for the treatment of arthritis, soft tissue injuries [1]. dysmenorrhoea and menorrhagia [2]. On the other hand, diclofenac treatment may have some adverse effects, such as gastrointestinal damage, platelet dysfunction and convulsion. These effects are likely to be associated with the ability of this phenylacetic acid derivative to compete with arachidonic acid for binding to cyclooxygenase (COX), resulting in decreased prostaglandin formation [3,4]. Traditional synthesis of a series of new compounds utilizing Combinatorial Chemistry and high-throughput screening can be carried out at high cost and also are time consuming whereas on the other hand, docking various ligands to the protein of interest followed by scoring to determine the affinity of binding and to reveal the strength of interactions has become increasingly important in the context of drug discovery.

In the view of this background, the present study was conducted to design, synthesize and preliminarily evaluate some new non-steroidal anti-inflammatory agents with expected selectivity toward COX-2 enzyme. There is evidence to suggest that COX-2 selective inhibitors may inhibit COX-1 and induce GI irritation or ulceration with long term use or at higher doses [5,6] clinical cardiovascular and renal liabilities of at least some COX-2 selective inhibitors have also been reported [7].

Thus, there is still a need for new, selective COX-2 inhibitors with an improved safety profile, therefore we designed and calculated different physicochemical properties such as log P, hydrogen donor, hydrogen-acceptor, molecular weight and pKa etc by using different software such as Winmopac2.0, Dragon and Chemdraw etc and these properties were compared with diclofenac. Out of them five compounds having similar physicochemical properties were selected for the synthesis.

2. MATERIAL AND METHODS

2.1 Materials

All reagents were purchased from Merck and Loba and used without further purification. Melting points were measured in open capillary and are uncorrected. The products were characterized by IR spectra, ^{1}H NMR, and elemental analyses. IR spectra were recorded on Perkin–Elmer FT-IR-1710 instrument. ^{1}H NMR was recorded on BrukerMSL-300 instrument using TMS as an internal standard. Elemental analyses were determined by an elemental analyser (CHNS-O, EA 1108-elemental analyser, Carlo Erba instruments).

2.2 Methods

2.2.1 General method for synthesis of (4a-e) through Petasis reaction

Round-bottom flask containing Diclofenac 1, phenylboronic acid 2 and aldehyde 3 (in a 1:1:1 mmolar ratio) in 15ml DCM. It was then stirred at 30°C temperature for 24 h, the completion of the reaction was monitored by (TLC) and after this time, the solvent and volatile materials were removed under reduced pressure. The residue was crystallized from absolute ethanol.

2.2.2 Molecular modeling (docking) studies

Docking studies were performed using (Auto Dock 4.2.) 2006.02 (CCG Inc.)20 and runs on a cluster of 12 Pentium IV processors. The 2.4 Å resolution coordinates of arachidonic acid bound to the murine COX-2 enzyme were obtained from the RCSB Protein Data Bank, hydrogen atoms were added and the structure was optimized 21 Ligand conformers were constructed using the Builder module and systematic conformer search method and non-redundant conformers up to 7 kcal from global minima conformer were retained (21 conformers).

2.2.3 Carrageenan-induced paw edema

The anti-inflammatory activity was evaluated using carrageenan-induced paw edema on rat method [8]. To evaluate the anti-inflammatory activity of the conjugates 4 groups (n = 6) of Wistar rats (150–200 g) were examined. A first group of rats was used as a control without using the drug, group II received diclofenac 20 mg kg^{-1}, received PEG600-Diclo conjugate and PEG4000-Diclo conjugate (52.60 mg kg^{-1} and 214 mg kg^{-1} respectively), where the dose was molecularly equivalent to the diclofenac. A stock solution of 0.2, 0.52 and 2.14 mg ml was prepared as a homogeneous suspension in aqueous solution of sodium CMC (0.5% w/v) and each animal received 2.0- 2.2 ml of the respective drugs orally. Thirty minutes after administration of drugs, each rat received a sub planter injection of 0.1 ml of 1% carrageenan solution in its left hind paw. The swelling volume of the paw was measured before (time 0) and at 0.5, 1, 2, 3, h after the carrageen an injection. The measurement of

the hind paw volume was carried out using an Ugo Basile. Plethysmometer before any treatment (Vo) and in any interval (Vt) after the administration of the drugs. The percentage increase in the paw volume was calculated from the normal paw volume. The percentage of swelling inhibition was calculated using:

$$\text{Inhibition}(\%) = \frac{(Vt - Vo)_{control} - (Vt - Vo)_{treated}}{(Vt - Vo)_{control}} \times 100$$

(Vt and Vo relates to the average volume in the hind paw of the rats (n = 6) before any treatment and after anti-inflammatory agent treatment, respectively.

2.3 Statistical Analysis

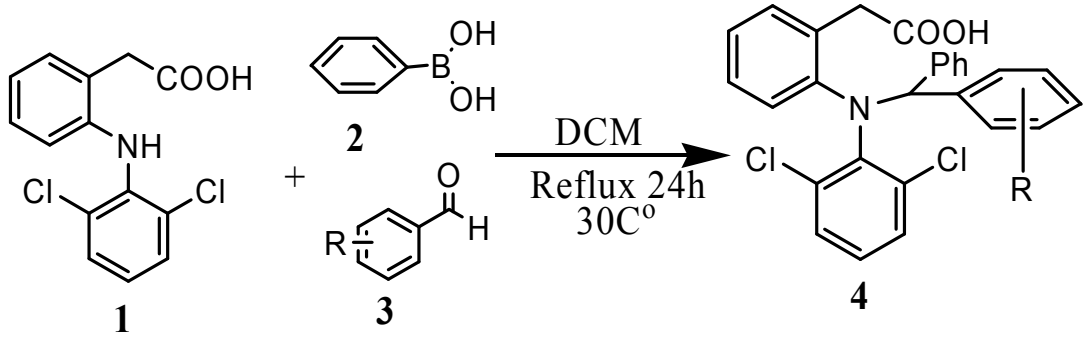
All the results are expressed as mean ± S.E.M. Statistical evaluation was performed using analysis of variance followed by Dunnet's t-test for sub group comparison. A P value <0.05 was considered significant.

3. RESULTS AND DISCUSSION

Thousands of new analogues of diclofenac were designed their physiochemical properties such as log P, HOMO, LUMO and pKa etc. were calculated As a sample various physicochemical properties of 21 analogues of diclofenac along with the diclofenac itself are shown in Table 1 and Out of them five compounds were selected for synthesis. The reaction is depicted in Scheme 1.

In vivo pharmacological evaluation of (4a–e) was carried out to assess their potential anti-inflammatory activity by using the anti-inflammatory rat paw edema assay and this group of five new compounds exhibit anti-inflammatory activity with moderate to good activity Table 2.

Also docking studies specifically on COX-2 exhibited promising anti-inflammatory effect as depicted in Table 3 and Fig. 1.



R= H, -OH ,-OMe,-Cl , -NO_2

Scheme 1. Synthesis of compounds 4a-e

Table 1. Calculated values of various physicochemical properties such as log *P*, pKa etc

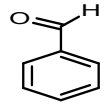
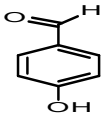
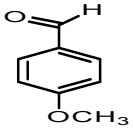
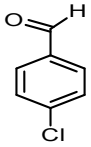
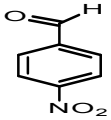
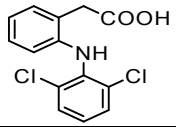
R	M.W	H.F(ev)	D.M(D)	B.E(ev)	H.D	H.A	log P	pka
	552	-6.18	3.34	310.34	1	8	7.42	4.03
	478	-3.29	1.87	266.45	2	6	7.41	4.33
	492.3	-2.98	1.99	278.08	1	6	7.67	4.32
	496.8	-1.67	1.28	261.24	1	6	8.36	4.29
	507.3	-0.78	3.80	269.16	1	7	7.93	4.16
	296.1	-3.27	1.65	144.69	2	5	4.12	4.00

Table 2. Effect of various synthesized compounds on carrageenan –induced paw oedema in Rats in different time intervals

Treatment and dose (100 mg/ml)	Oedema Volume			
	0.5 h	1 h	2 h	3 h
Control	3.49± 0.6	4.12±0.13	4.78±0.19	5.01±0.06
4a	3.75±0.19	3.92±0.15	3.15±0.33	2.98±0.17**
4b	3.84±0.15	3.50±0.13	3.12±0.29	2.45±0.15**
4c	4.37±0.13	3.60±0.19	3.05±0.19	2.53±0.16**
4d	3.56±0.14	3.14±0.15	2.06±0.16	2.21±0.19**
4e	3.69±0.15	3.20±0.18	2.90±0.16	2.40±0.15**
Diclofenac	3.74±0.09	4.03±0.07	3.58±0.70	3.02±0.13**

*Values represent the mean ± S.E. of six rate for each group. Control group animal were given few drop of carboxymethyl cellulose %. *Statistically significant (p< 0.05) from the control normal inflamed group at the corresponding time using one way ANOVA*

Because the score is an approximation of free energy, lower scores represent greater stability, compound 4b exhibited the lowest score even though, all the compounds shown they are selective to COX-2. Thus it can be said that there was a fairly good correlation between the docked results depicted in Table 3 and in vivo study. It was shown that there existed one to two hydrogen bonds were bound to COX-2.Amino acid residues involved in such hydrogen bonds were Arg120, Tyr115, Val89and His90[9]. It is clear in Fig.1for 4b.

Table 3. Docking results on COX-2 enzyme for (4a-e)

Compounds	Docking score	B. E Kcal/ mol	No. of H-B formed	Participating residues with H-B (A°)	Elec. E	VdW + H.B + D.E	IC_{50} (µM)
4b	-11.32	-7.46	2	ARG120*, ARG120	-0.6	-8.62	3.41
4c	-10.21	-7.46	2	Arg120, Arg120	-1.44	-8.11	3.38
4a	-11.19	-7.35	2	TYR115, ARG120	-0.9	-8.54	4.09
4e	-11.29	-7.75	1	Arg120	-2.22	-7.92	2.08
4d	-11.25	-7.52	1	Arg120	-1.13	-8.47	3.08
Diclofenac	-9.42	-7.68	1	ARG120	-0.77	-8.11	2.36

**Compound 4b forms two hydrogen bonds with Arg120*

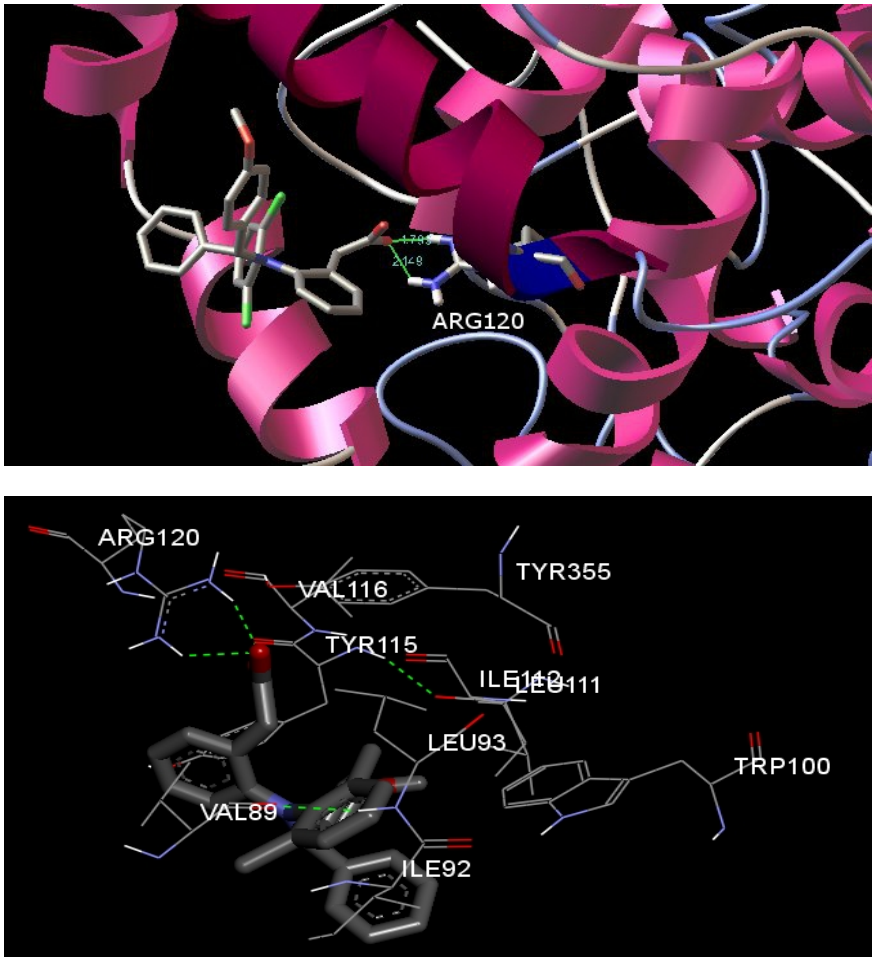


Fig. 1. Diclofenac derivative 4b ligand bound to COX-2. Hydrogen bonds between ligand, protein, water are shown as dashed Green lines. The ligand also makes van der Waals contacts with lobby residues TYR115, VAL89 and ARG120

4. CONCLUSION

In Conclusions discovery of mild and practical route for the synthesis of drug continues to attract the attention of researchers. To accomplish this, we employed multicomponent reactions the newly synthesized analogues of diclofenac exhibited increased biological potency comparable to the parent drug, diclofenac. Further investigations are required such as extra biological activity and comparison incase of docking to complete the study.

CHARACTERIZATION

2-{2-[(2, 6-dichlorophenyl) (diphenylmethyl) amino]phenyl} aceticacid(4a):

as pale yellow solid.Yield:83%.Mp78-81^{o}C.IR(KBr):3000,1474,1300,1610cm^{-1};^{1}HNMR (300MHz,$CDCl_3$)3.8(s,CH_2),5.3(s,CH),6.5-8.3(m,Ar-17H),10.4(br,CO-OH)Anal.Calc.for. $C_{27}H_{21}Cl_2NO_2$ C, 70.14; H, 4.58; Cl, 15.34; N, 3.03. Found: C, 70.18 H, 4.65; Cl, 15.38; N, 3.10.

2-{2-[(2, 6-dichlorophenyl)[(4-hydroxyphenyl)(phenyl)methyl]amino]phenyl}acetic acid (4b):

As pale yellow solid.Yield:78%. Mp 280-282^{o}C. IR (KBr):2990, 1475, 3400, 1650 cm^{-1} ; ^{1}HNMR (300MHz,$CDCl_3$)3.9(s,CH_2),4.8(s,OH),5.3(s,CH),6.5-8.2(m,Ar-16H),9.9(br,CO-OH) Anal.Calc.for $C_{27}H_{21}Cl_2NO_3$ C, 67.79; H, 4.42; Cl, 14.82; N, 2.93. Found: C, 67.84; H, 4.47; Cl, 14.90; N, 2.99.

2-{2-[(2,6-dichlorophenyl)[(4-methoxyphenyl)(phenyl)methyl]amino]phenyl}acetic acid (4c):

As pale yellow solid.Yield:82%. Mp 238-240^{o}C. IR (KBr):3000, 1470, 1290, 1640 cm^{-1} ; ^{1}HNMR (300MHz,$CDCl_3$)3.5(s,OCH3),3.9(s,CH_2),5.3(s,CH),6.5-8.3(m,Ar-16H),10.1(br,CO-OH) Anal.Calc.for $C_{28}H_{23}Cl_2NO_3$ C, 68.30; H, 4.71; Cl, 14.40; N, 2.84. Found: C, 68.33; H, 4.76; Cl, 14.47; N, 2.86.

2-(2-{[(4-chlorophenyl)(phenyl)methyl](2,6-dichlorophenyl)amino}phenyl) aceticacid (4d):

As pale yellow solid.Yield:71%.Mp178-180^{o}C.IR(KBr):3000,1472,1300,1645cm^{-1} ;^{1}HNMR(300MHz,$CDCl_3$)3.8(s,CH_2),5.3(s,CH),6.5-8.2(m,Ar-16H),9.9(br,CO-OHAnal.Calc.for $C_{27}H_{20}Cl_3NO_2$ C, 65.27; H, 4.06; Cl, 21.41; N, 2.82. Found: C, 65.32 H, 4.10, Cl, 21.47; N, 2.86.

2-{2-[(2,6-dichlorophenyl)[(4-nitrophenyl)(phenyl)methyl]amino]phenyl}acetic acid(4e):

As pale yellow solid. Yield:69%.Mp196-198^{o}C. IR(KBr): 3000, 1470, 1330, 1525,1644cm^{-1};^{1}HNMR(300MHz,$CDCl_3$)3.9(s,CH_2),5.3(s,CH),6.4-8.3(m,Ar-16H),10.4 (br,CO-OH) Anal.Calc.for $C_{26}H_{19}Cl_2N_2O_4$ C, 63.92; H, 3.97; Cl, 13.98; N, 5.52. Found: C, 63.97; H, 3.99; Cl, 14.07; N, 5.59.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Todd PA, Sorkin EM. Diclofenac sodium. A reappraisal of its pharmacodynamic and pharmacokinetic properties and therapeutic efficacy, Drugs. 1988;35:244–285.
2. Dawood MY. Non-steroidal anti-inflammatory drugs and reproduction. Am J Obstet Gynecol. 1993;169:1255–1265.
3. Small RE. Diclofenac sodium, Clin. Pharmacol. 1989;8:545–558.
4. Vane JR. Introduction: mechanism of action of NSAIDs, Br. J. Rheumatol. 1996;35:1–3.
5. Francesca C. Leslie. J. C. Cyclooxygenase inhibition and thrombogenicity. Am J Med. 2001;110:28–32.
6. Mukherjee D, Nissen SE, Topol EJ. Risk of cardiovascular events associated with selective COX-2 inhibitors. JAMA ‑ J Am Med Assoc. 2001;286:954–959.
7. Giovanni D, Maria BD, Chiara C. Prevention of thrombosis and vascular inflammation: benefits and limitations of selective or combined COX-1, COX-2 and 5-LOX inhibitors. Trends Pharmacol Sci. 2003,24:245–252.
8. Winter CA, Risley EA, Nuss GW. Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med. 1962;111:544−547.
9. Kurumbail RGA, Stevens M, Gierse JK, McDonald JJ, Stegeman RA, Pak JY, Gildhaus D, Miyashiro JM, Penning TD, Seibert K, Isakson PC, Stallings WC. Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents, Nature. 1996;384:644-648.

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