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COMPUTER AIDED DRUG DESIGNING AND DEVELOPMENT OF NEW NON-STEROIDAL ANTI-INFLAMMATORY DRUGS CONSIDERING MEFENAMIC ACID/ DICLOFENAC AS A LEAD COMPOUND FOLLOWED BY THEIR SYNTHESIS AND EVALUATION

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ABSTRACT

Discovery of new and new non-steroidal anti-inflammatory drug (NSAID) always has attracted greater attention over the years. In the present work series of derivatives of Mefenamic acid and Diclofenac (known non-steroidal anti-inflammatory drugs) were designed and different physicochemical properties were calculated such as Log P, H-Donor, H-Acceptor, Molecular weight and P ka .and these properties are compared with Diclofenac/ Mefenamic Acid. All the new non-steroidal anti-inflammatory drugs (NSAID) selected for designing have to follow Lipinski's Rule of Five. Out of them seven compounds having similar physicochemical properties were selected for the synthesis. They were found to have potential anti-inflammatory activity.

KEYWORDS: Computer aided drug designing, Anti-inflammatory activity, physicochemical properties

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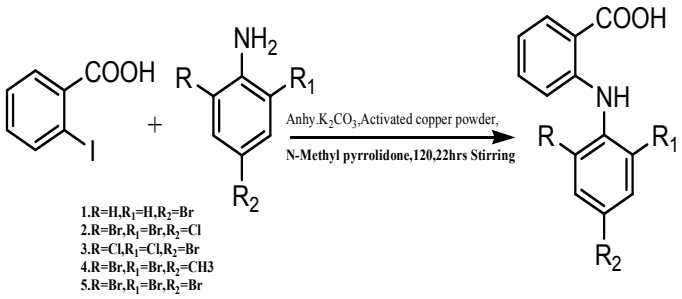
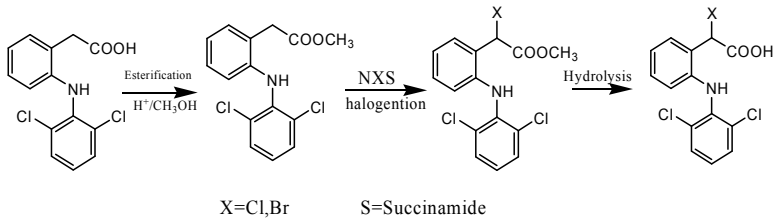
INTRODUCTION

Use of computational techniques in drug discovery and development process is rapidly gaining popularity, implementation and appreciation. Term Computer-Aided Drug Discovery and Development (CADDD) [1] will be employed to cover the entire process. Both computational and experimental techniques have important roles in drug discovery and development and CADDD is being utilized to identify hits (active drug candidates), select leads (most likely candidates for further evaluation), and optimize leads i.e. transform biologically active compounds into suitable drugs by improving their physicochemical, pharmaceutical, ADMET/PK (pharmacokinetic) properties. Virtual screening is used to discover new drug candidates from different chemical scaffolds by searching commercial, public, or private 3- dimensional chemical structure databases. It is intended to reduce the size of chemical space and thereby allow focus on more promising candidates for lead discovery and optimization. The goal is to enrich set of molecules with desirable properties (active, drug-like, lead-like) and eliminate compounds with undesirable properties (inactive, reactive, toxic, poor ADMET/PK). In another words, *in silico* modeling is used to significantly to minimize time and resource requirements of chemical synthesis and biological testing [2]. Role of computational models is to increase prediction based on existing knowledge [3]. Computational methods are playing increasingly larger and more important role in drug discovery and development .In early 1960s, Corwin.Hansch [4] extended the concept of Linear –free energy relationship (LFER) to describe the effectiveness of biologically-active molecule. Generating useful Hansch equation can be very challenging and even a good Hansch equation will not give perfect predication of activity. For this reason new methods have somewhat replaced the traditional Hansch analysis. In the late 1980s and early 1990s combinatorial chemistry emergent diminished the importance of QSAR. Since the middle 1990s, a technique called comparative molecular field Analysis (COMFA) [5] has emerged. This method uses highly complicated statistical analysis with large number of variable to correlate practical molecular properties to activity. However, Drug designing is still a big challenge. We wish to report here a simple hypothesis, in which series of derivatives of Mefenamic acid and Diclofenac (known non-steroidal anti-inflammatory drugs) were designed and different physicochemical properties were calculated such as Log P, H-Donor, H-Acceptor, Molecular weight and P ka .and these properties are compared with Diclofenac/ Mefenamic Acid. All the new non-steroidal anti-inflammatory drugs (NSAID) selected for designing have to follow Lipinski's Rule of Five [6], in general, an orally active drug has no more than one violation of the following criteria: Not more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms)Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms) A molecular mass less than 500 daltons An octanol-water partition coefficient Log P not greater than 5. Note that all numbers are multiples of five, which is the origin of the rule's name. Out of them seven compounds having similar physicochemical properties were selected for the synthesis. They were found to have potential anti-inflammatory activity.



Scheme1



Scheme II

MATERIALS AND METHODS

In general, reagents, solvents, and other chemicals were used as purchased without further purification. Melting points were taken in open capillaries and are uncorrected. . IR spectra were recorded on Perkin–Elmer FT-IR-1710 instrument, 1H NMR spectra were recorded on Varian 300MHz spectrometer in $CDCl_3$ as a solvent and TMS as an internal standard. Peak values are shown in *d*(ppm). Mass spectra were recorded on a GCMS-QP2010 Shimadzu.

2. General procedure for synthesis of derivatives of mefenamic acid.

A mixture of compound O-iodobenzoic acid (1.54g.5.925 mmol)) with of P-bromoaniline (5.3028g.29.625 mmol), anhydrous potassium carbonate (2.8g.20.28 mmol) and activated copper powder [7,11] (0.1875g.2.95 mmol) in (7mL) of N-methyl pyrrolidione was refluxed for 22h at $120C^0$with stirring. The completion of reaction was monitored by TLC. And the hot reaction mixture filtered with celite Filtrate cooled at room temperature and the N-methyl pyrrolidione layer was removed and the aqueous layer treated by (25mL) of 2N HCl and (50mL) of ether. The mixture was vigorously shaken and layers were separated and the solvent was evaporated under reduced pressure and the resulting product was purified using silica gel column chromatography eluting with chloroform.

2{-(4-bromo phenyl) amino} benzoic acid (3)

Yellow crystalline m.p 218^0C; Yield (45%), IR (KBr, cm^{-1}) 3522, 3200-2800 broad peak,1680,1585,1516,1342,1196,903,794and709;1HNMR7.10(d,Ar2H),7.3(s,Ar4H),8.4 (dd,Ar2H)8.9(s,NH)11.5(sCOOH). Molecular formula for $C_{13}H_{10}BrNO_2$: MS 293.

2-(2, 6, dibromo 4- chloro phenyl amino) benzoic acid (**5**)

Yellow crystalline m.p148^0 C; Yield(40%),IR(KBr,cm^{-1})3500,32002800 broad peak,*-1660,1584,1458,1377,1153,722;1HNMR),6.98. 2(m,Ar6H),9.1(s,NH),10.5(s,COOH)

Molecular formula for $C_{13}H_8ClBr_2NO_3$: MS 407.

2-(2,6-dichlorop-bromophenylamino)benzoicacid. (**6**)
Yellow crystalline.m.p142^0 C; Yield(32%);IR(KBr,cm^{-1})3332,3065to2800 broad peak, 1663,1584,1452,1250,1072,750;1HNMR,),6.8-8.2(m,6ArH,9.1(s,NH),10.5(s,COOH) Molecular formula for .$C_{13}H_8Cl_2BrNO_2$: MS 360.

2-(2,6 di bromo p-methyl phenyl amino) benzoic acid (**7**)
Yellowcrystalline.m.p143^{0}C; Yield(35%),IR(KBr,cm^{-1})3400,3200-2800 broad peak, 1660,1611,1460,1377,1156,722;1HNMR2.4(s,1H),6.4,8.4(m,6ArH),9.1(s,NH)10.4(s,COOH).Molecular formula for $C_{14}H_{11}Br_2NO_2$: MS 385.

3. General Procedure for the preparation of methyl ester of Diclofenac
Methyl esters of diclofenac were synthesized using direct esterification method. Diclofenac (0.004mols) and methyl alcohol (0.25mols) were taken in a round bottom flask fitted with reflex condenser. 1ml of conc. Sulphuric acid was added gradually to this reaction mixture. The reaction mixture was refluxed with continuous stirring at 80^{o}C until the esterification was completed. The progress of the reaction was monitored by TLC. After the reaction completion excess alcohol was removed by distillation under reduced pressure. Crude product was dissolved in 30ml dichloromethane and organic layer was washed by 30ml of 1% of NaOH solution, this was followed by water washing (30X3) ml water and dried over anhydrous sodium sulphate followed by filtration and solvent was removed under vacuum to furnish the crude product which was purified by column chromatography using silica gel as stationary phase and 5% Ethyl acetate in pet ether as an eluent. Haloester of diclofenac was then hydrolyzed by 2N NaOH, were taken in a round bottom flask fitted with a condenser. The reaction mixture refluxed at 80^{0}C until hydrolysis was completed. The progress of reaction was monitored by thin layer chromatography. The reaction mixture was extracted with dichloromethane (30ml) taken aqueous layer was acidified by concentrated HCl then extracted by DCM. Then organic layer was washed with 1% NaOH (30ml).This was washed by water (30mlx3) .The organic layer was dried over anhydrous sodium sulphate. DCM was removed by distillation and vacuum evaporation and the product was collected.

2-(2,4,6, tribromophenyl amino) benzoic acid (**32**)
Yellow crystalline m.p127^{0}C;Yield(34%);IR(KBr,cm^{-1})34000,3000-2800 broad peak, 1660,1458,1377,1155,,722cm1HNMR6.8,8.2(m, 6ArH),9.4(s,NH).10.4(m,COOH),Molecular formula for $C_{13}H_8Br_3NO_2$.MS 450.

2-(2-(2,6-dichlorophenylamino)phenyl)-2-bromoacetic acid (**35**)
Dark brown viscous liquid, Yield: (34%). IR(KBr,cm^{-1}): 3451,3208-2916 (broad), 1732, 1612, 1565, 1489, 1360, 1317,1240, 1194, 1169, 1092,1020, 950, 750. 1HNMR(δppm): 3.7(s,NH) 3.8 (s,COOH),5.4 (s,1H)6.3-7.6 (m, Ar 7H). Molecular formula for $C_{14}H_{10}BrCl_2NO_2$; MS 373

2-(2-(2,6-dichlorophenylamino)phenyl)-2-chloroacetic acid (**37**)
Yellowish brown viscous liquid, Yield: (30%). IR(KBr,cm^{-1}):3439, 3377-2953 (broad), 1732 1615, 1575, 1489, 1441, 1354,1315, 1240, 1198, 1169, 1107, 1020, 908,735cm-1
1HNMR(δppm):3.54 (s,COOH)3.56 (s,NH), 5.15(s,1H), 6.4-7.35 (m,Ar 7H)
Molecular formula for $C_{14}H_{10}C_{l3}NO_2$; MS 329.

RESULTS AND DISCUSSIONS

Out of several derivative of mefenamic acid designed (Figure1), and calculated values of various physical properties (Figure2) five novel compounds were selected for their synthesis [7] by condensing o-iodobenzoic acid and corresponding substituted aniline as shown in (Scheme I) and similarly methyl ester of diclofenac(Figure3,4) respectively were synthesized [8] and alpha position was

halogenated [9] as shown in (Scheme II) and their structures were confirmed using infrared spectroscopy, 1HNMR, and Mass Spectrometry. Preliminary pharmacological evaluation has been done for the designed compounds and it has been found that these compounds exhibit anti-inflammatory activity compared to that with Mefenamic acid. At time (1h) there is a maximum increase in paw edema i.e. maximum edema occurs in all compounds. There is no difference between reference drug (Mefenamic acid) and compound 6 in the reduction of paw edema. At time (2h) compound 5 show comparable affect to that of Mefenamic acid and compound 7 has more anti-inflammatory affect compared with Mefenamic acid.

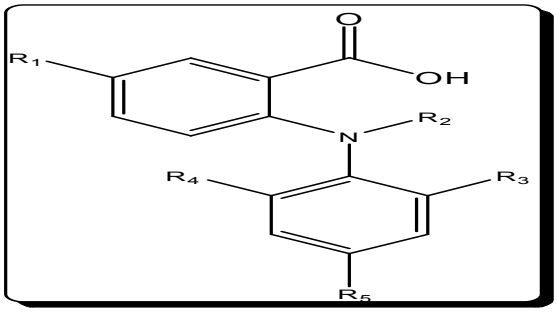


Table 1
Substituted Mefenamic acid

Compound	R_1	R_2	R_3	R_4	R_5	Mol. Formula
1	H	H	H	H	CH_3	$C_{14}H_{13}NO_2$
2	H	H	Cl	Cl	Cl	$C_{13}H_8Cl_3NO_2$
3	H	H	Br	Br	Br	$C_{13}H_8Br_3NO_2$
4	H	H	Cl	Cl	CH_3	$C_{14}H_{11}Cl_2NO_2$
5	H	H	H	H	Br	$C_{13}H_{10}BrNO_2$
6	H	H	Br	Br	CH_3	$C_{14}H_{11}Br_2NO_2$
7	H	H	Cl	Cl	Br	$C_{13}H_8Cl_2BrNO_2$
8	CH_3O	CH_2CH_3	CH_3	CH_3	Br	$C_{18}H_{20}BrNO_3$
9	CH_3O	CH_2CH_3	H	H	H	$C_{16}H_{17}NO_3$
10	CH_3O	CH_3	H	H	Br	$C_{15}H_{14}BrNO_3$
11	CH_3O	CH_3	CH_3	CH_3	Cl	$C_{17}H_{18}ClNO_3$
12	CH_3O	CH_2CH_3	H	H	NCH_3CH_3	$C_{18}H_{22}N_2O_3$
13	CH_3O	CH_3	H	H	I	$C_{15}H_{14}INO_3$
14	CH_3O	CH_2CH_3	CH_3	CH_3	Br	$C_{18}H_{20}BrNO_3$
15	CH_3O	CH_2CH_3	Br	Br	Br	$C_{16}H1_4Br_3NO_3$
16	CH_3O	CH_2CH_3	CH_3	CH_3	CH_3	$C_{19}H_{23}NO_3$
17	CH_3O	CH_2CH_3	H	H	Br	$C_{16}H_{i6}BrNO_3$
18	CH_3O	CH_2CH_3	Cl	Cl	H	$C_{16}H_{15}Cl_2NO_3$
19	OH	H	Cl	Cl	Br	$C_{15}H_{14}BrNO_3$
20	OH	H	H	H	H	$C_{13}H_{11}NO_3$
21	OH	H	H	H	Br	$C_{13}H_{10}BrNO_3$
22	OH	H	Br	Br	Cl	$C_{15}H_{14}ClNO_3$
23	OH	H	H	H	NCH_3CH_3	$C_{15}H_{16}N_2O_3$
24	OH	H	H	H	I	$C_{13}H_{10}INO_3$
25	CH_3O	H	Cl	Cl	Br	$C_{18}H_{20}BrNO_3$
26	CH_3O	H	Cl	Br	H	$C_{14}H_{11}Cl_2NO_3$
27	CH_3O	H	H	H	Br	$C_{14}H_{12}BrNO_3$
28	CH_3O	H	CH_3	CH_3	Cl	$C_{16}H_{16}ClNO_3$
29	CH_3O	H	H	H	NCH_3CH_3	$C_{14}H_{18}N_2O_3$
30	CH_3O	H	H	H	I	$C_{14}H_{12}INO_3$
31	CH_3O	H	CH_3	CH_3	H	$C_{16}H_{17}NO_3$
32	CH_3O	H	Br	Br	Cl	$C_{13}H_8ClBr_2NO_3$
33	CH_3O	H	CH_3	CH_3	CH_3	$C_{17}H_{19}NO_3$

Table 2
Calculated values of various physical properties such as log P, P Ka etc..

Compound	Mol. Formula	M.W	H.Donor	H.acceptor	Log P	P ka
Mefenamic acid	$C_{15}H_{15}NO_2$	241.29	2	4	4.0	3.89
Diclofenac	$C_{14}H_{11}Cl_2NO_2$	295.02	2	6	4.4	4.30
3	$C_{13}H_8Br_3NO_2$	449.92	2	7	5.5	3.81
5	$C_{13}H_{10}BrNO_2$	292.13	2	5	3.8	3.88
6	$C_{14}H_{11}Br_2NO_2$	385.05	2	6	5.0	3.81
7	$C_{13}H_8Cl_2BrNO_2$	361.02	2	7	5.0	3.79
32	$C_{13}H_8ClBr_2NO_3$	405.47	2	7	5.2	3.81

Table 3
Calculated values of various physical properties such as Log P, p Ka etc..

Compound	Mol. Formula	M.W	H.Donor	H.acceptor	Log P	P ka
Diclofenac	$C_{14}H_{11}Cl_2NO_2$	295.02	2	6	4.4	4.30
1	$C_{16}H_{15}Cl_2NO_2$	324.20	1	6	4.69	4.18
2	$C_{14}H_{10}Cl_3NO_2$	330.59	2	7	5.26	3.61
3	$C_{21}H_{17}Cl_2NO_3$	402.27	2	7	5.72	3.91
4	$C_{14}H_{10}BrCl_2NO_2$	375.04	2	7	4.81	3.15
5	$C_{15}H_{13}Cl_2NO_3$	326.17	2	7	3.99	3.77
6	$C_{14}H_{11}Cl_2NO_3$	312.14	3	7	3.73	3.82
7	$C_{14}H_{11}Cl_2NO_4$	328.14	4	8	3.34	3.61
8	$C_{14}H_{11}Cl_2NO_3$	312.14	3	7	3.73	3.76
9	$C_{15}H_{13}Cl_2NO_4$	342.17	3	8	3.60	3.59
10	$C_{14}H_{11}ICINO_2$	387.60	2	6	4.92	3.54
11	$C_{15}H_{13}ClFNO_2$	293.72	2	6	4.20	4.11
12	$C_{15}H_{13}ClFNO_3$	309.72	2	7	3.59	3.90
13	$C_{14}H_{11}ClFNO_2$	279.69	2	6	3.72	3.99
14	$C_{14}H_{11}Cl_2NO_3$	312.14	3	7	3.73	3.81
15	$C_{15}H_{11}Cl_2NO_4$	340.15	3	9	3.67	3.63
16	$C_{16}H_{15}Cl_2NO_4$	356.20	2	8	3.86	3.70
17	$C_{15}H_{15}NO_2$	241.28	2	4	3.49	4.69
18	$C_{14}H_{12}FNO_2$	245.24	2	5	3.16	4.28
19	$C_{15}H_{12}Cl_2O_2$	295.16	1	5	4.74	4.04
20	$C_{14}H_{11}ClO_3$	262.68	1	4	3.63	3.74
21	$C_{14}H_{11}FO_3$	246.23	1	4	3.23	3.75
22	$C_{14}H_{12}O_3$	228.24	1	3	3.07	3.99
23	$C_{14}H_{10}ClBr_2NO_2$	463.94	2	7	5.22	3.04

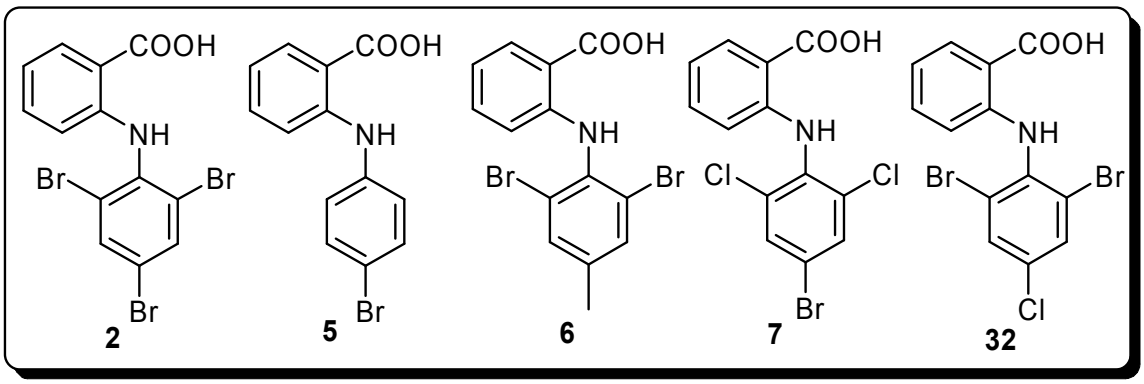


Figure I
Schematic representation of Mefenamic acid derivatives

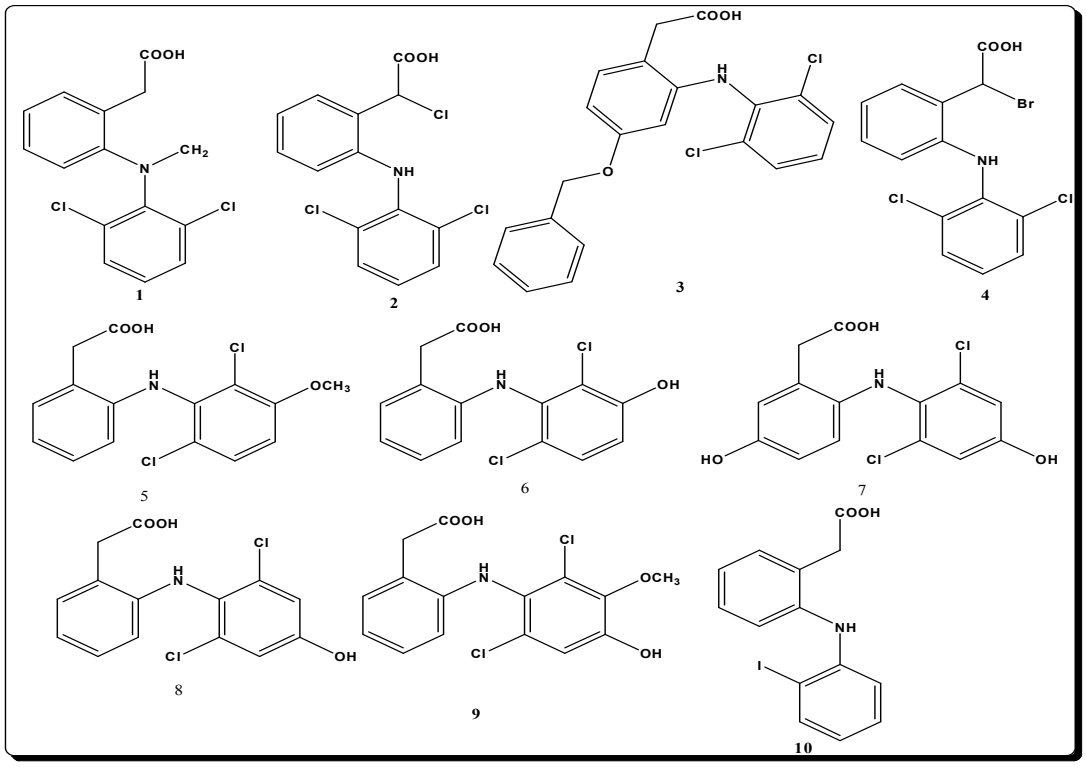


Figure 2
Schematic representations of Diclofenac derivatives

Table 4
Calculated values of various physical properties such as log P, pKa etc

Compound	Mol. Formula	M.W	H.Donor	H.acceptor	Log P	P ka
Mefenamic acid	$C_{15}H_{15}NO_2$	241.29	2	4	4.0	3.89
Diclofenac	$C_{14}H_{11}Cl_2NO_2$	295.02	2	6	4.4	4.30
35	$C_{14}H_{10}Cl_3NO_2$	330.59	2	7	5.2	3.61
37	$C_{14}H_{10}BrCl_2NO_2$	375.04	2	7	4.8	3.15

PHARMACOLOGICAL STUDIES

Anti- inflammatory screening - Carrageenan induced hind paw edema bioassay

The synthesized compounds were tested for anti-inflammatory activity by selected analogs (5compounds) from Mefenamic acid and (2 compounds) from Diclofenac derivatives namely; 3, 5, 6,7 ,32and 35,37. They were evaluated by screening protocol; namely, the formalin-induced paw edema 33 and turpentine oil-induced granuloma pouch 34 bioassays, using Mefenamic acid (10 mg/kg) as a reference standard anti-inflammatory agent. The paw edema was employed as a model for acute and sub-acute inflammation condition. The data obtained were presented in (Table5) and expressed as means ± SE. Statistical differences of control and test groups was carried out using the Analysis of Variance (ANOVA). The difference in results was considered significant when $P > 0.05$.

Table 5
Bioassay results for the newly synthesized compounds

Compound[a]	Volume of edema(mL)[b]			
	0hr	0.5hr	1hr	2hr
3	1.125±0.04787*	1.650±0.09574*	1.425±0.02500*	1.250±0.05000*
5	1.075±0.04787*	1.310±0.04787*	1.400±0.04082*	1.115±0.04787*
6	1.025±0.07500*	1.575±0.04787*	1.275±0.04787*	1.125±0.04787*
7	1.425±0.06292*	1.675±0.04787*	1.325±0.04787*	1.175±0.07500*
32	1.200±0.04082*	1.700±0.04082*	1.300±0.04082*	1.150±0.06455*
Mefenamic acid	1.075±0.09500*	1.219±0.04087*	1.475±0.04787*	1.105±0.04787*
35	1.118± 0.561*	1.691 ±0.427*	1.390 ±0.322*	1.121± 0.332*
37	1.391± 0.436*	1.879 ±0.332*	1.231 ±0.627*	1.141±0.319*

* ***Significantly different compared to respective control values, P > 0.05.***
[a] ***Dose levels, po: test compound Mefenamic acid (10 mg/kg b.wt.).***
[b] ***Values are expressed as mean ± SE (number of animals N = 20 rats)***

CONCLUSION

In conclusions, Computer-Aided Drug Discovery and Development (CADDD) is used for virtual screening of various compounds to discover a new compound as a possible drug. In this way seven novels anti-inflammatory compounds are selected by virtual screening and these are only synthesized. Further, these novel NSAIDs are evaluated and found to have comparable anti inflammatory activity to Mefenamic acid and Diclofenac.

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REFERENCES

1. Kapetanovic I M, Chem Biol Interact*.,* 1711, 65–176. (2008).
2. Van de Waterbeemd H, Gifford E, Nat Rev Drug Discov., 2,192–204. (2003).
3. Kumar N, Hendriks B S , Janes KA, de Graaf D,Lauffenburger DA. Drug Discovery Today.,11, 806–811.(2006).
4. Hansch C, Muir R M, Fujita P.Maloney T, Geiger F, and M.Streich. J. Am. Chem Soc., 85, 2817-2824. (1963).
5. Cramer R D, Patterson D E, Bunce J D, J. Am. Chem. Soc.,110 ,5959–5967. (1988).
6. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ: Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Del Rev, 46**:**3-26. (2001).
7. Rhea Mohan, Ramaa C S, Indian Journal of Chemistry B. l46, 1164-1168.(2007).
8. Sallmann A, and Pfister R, German Patent, 1,815. 1969.
9. Juby P F, Hudyma T W, Brown M, J.Med.Chem, 11,111-116. (1968).
10. Peter Moser, Alferd Sallmann, and Irmgard Wiesenberg, J.Med. Chem,33(9), 2358-2368. (1990).
11. Somasundaram S, Rafi S, Hayllar J, Sigthorsson G, Jacob M, Price AB, Macpherson A, Mahmod T, Scott D, Wrigglesworth JM, et al. Mitochondrial damage: a possible mechanism of the "topical" phase of NSAID induced injury to the rat intestine. Gut. Sep, 41(3):344–353. (1997).