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Synthesis, molecular docking and evaluation of anti-inflammatory activity of some 5-alkyl/aryl 2-amino-1,3,4 thiadiazole derivatives

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ABSTRACT

The nonsteroidal anti-inflammatory drugs like Aspirin, Ibuprofen, Mefenamic acid inhibit Cyclooxygenase(COX) enzyme and control release of inflammatory mediators responsible for inflammation. COX exist as isoenzymes COX-1 and COX-2.Treatment with NSAID has some side effects in different patients such as gastric irritation, ulceration due to inhibition of protective COX-1 enzyme. Larger active site of COX-2 than COX-1 is basis for selectivity in design of selective COX-2 inhibitors. 5-alkyl/ aryl 2-amino 1,3,4-thiadiazole derivatives were designed and which can interact with binding site of the COX-2 enzyme more selectively. These synthesized compounds were confirmed by TLC, MP and spectral analysis. The docking studies were performed using Vlife MDS4.3 software using COX-2 receptor. It has been observed that, phenyl substitution on 1,3,4 Thiadiazole ring shows higher dock score than aliphatic substitution. Further substitution on ortho, meta & para position of phenyl ring with hydroxy group shows remarkable binding affinity for receptor but substitution on ortho, meta & para position of phenyl ring with chloro or nitro group shows reduction in dock score indicating less binding affinity for receptor. The in-vitro anti-inflammatory activity binding was performed using carrageenan induced paw edema method in rats for the compounds which has shown significant dock score .Among the synthesized compounds, 5(2,5 dihydroxy phenyl) 2 amino 1,3,4 thiadiazole has shown significant anti-inflammatory activity.

Keywords: 1,3,4-thiadiazoles, Anti-inflammatory activity, cyclooxygenase enzyme.

INTRODUCTION

Non steroidal Anti-inflammatory Drugs:-Non steroidal anti-inflammatory drugs are widely used for treatment of minor aches, inflammation and pain. Inflammation is normal and essential response to any noxious stimulus. The nonsteroidal anti-inflammatory drugs like Aspirin, Ibuprofen, Mefenamic acid inhibit Cyclooxygenase(COX) enzyme and control release of inflammatory mediators responsible for inflammation. COX exist as isoenzymes COX-1 and COX-2.Treatment with NSAID has some side effects in different patients such as gastric irritation, ulceration due to inhibition of protective COX-1 enzyme.[1]

Active site of Cylooxygenase enzyme :- Studies have demonstrated that residues that form the substrate binding channel ,the catalytic site and residues immediately adjacent of COX-1 and COX-2 are identical except for two variations Isoleucine in COX-1 is exchanged for valine in COX-2 at position 434 and 523. Valine is less bulky than isoleucine hence it increases volume of active site .Larger active site of COX-2 than COX-1 is basis for selectivity in design of selective COX-2 inhibitors. The large active site of COX-2 is due to polar hydrophilic side pocket that forms due to substitution of Isoleucine 434, Histidine 513, Isoleucine 523 of COX-1 by valine 434,Arginine 513, valine 523..Substitution of isoleucine 434 for valine also allows the side chain of Phenyl alanine 518 to move back

and make some extra space this results in interaction of substrate with Arginine 513 which is replacement for Histidine 573 of COX-1 enzyme. Arginine 513 is thought to be key residue for binding of coxibs (celecoxib, rofecoxib, valdecoxib, etoricoxib)which are selective COX-2 inhibitors. [2,3,4,5,6]

From the exhaustive literature survey it was observed that large number of thiadiazoles have been reported to have different pharmacological activities. The marketed drugs containing 1,3,4-thiadiazole ring system are acetazolamide, methazolamide(diuretic) and megazol (trypanocidal agent). The compounds bearing thiadiazole ring posses anti-inflammatory and analgesic activity with reduced ulcerogenic index and side effects.[7] Therefore it was thought to design and synthesize derivatives of 5-alkyl/ aryl 2-amino 1,3,4-thiadiazole derivatives which can interact with binding site of the COX-2 enzyme more selectively.

The molecular docking approach is rational and authentic approach in drug design and discovery for study of molecular interactions of selected target and ligand. Binding affinities of synthesized compounds are compared to reference.

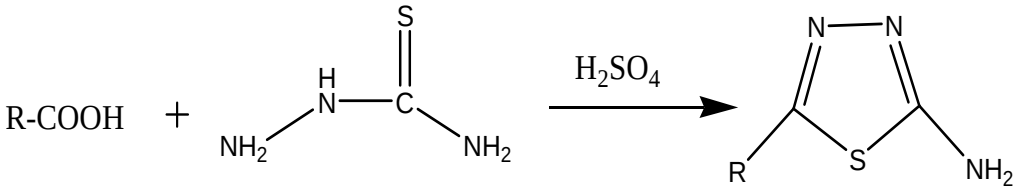
MATERIALS AND METHODS

All materials of AR grade were purchased from Modern research laboratory, Nasik. Solvents were dried and distilled before use. Thin layer chromatography were carried out using silica gel G LR on glass plates and were visualized by exposure to iodine vapours and in CAMAG UV cabinet with dual wavelength UV lamp at 254nm and 366nm wavelength. Melting points were determined on digital scientific melting point apparatus KUMAR model were uncorrected .Infra red spectra were recorded on KBr pellets on 8400S SHIMADZU Spectrometer. ^{1}H NMR spectra were recorded at 300MHz using DMSO solvent in department of chemistry in Savitribai Phule Pune university, Pune with TMS as a internal standard. Chemical shifts (δ) were expressed in parts per million (δ PPM).GC-MS spectra and chromatograms were recorded on GCMS-QP 2010 SHIMADZU instrument. Molecular modeling of compound is done with help of V Life MDS 4.3 software.

2-Amino,5-substituted 1,3,4 thiadiazole derivatives were synthesized by heating corresponding benzoic acid derivatives with thiosemicarbazide using conc.sulphuric acid as a dehydrating agent. [8,9]

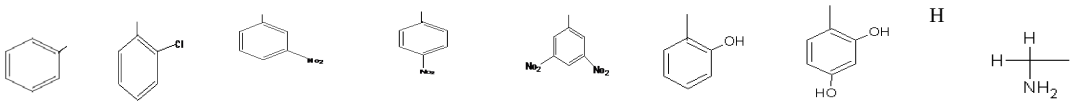
The method followed for synthesis of Thiadiazole derivatives is described here.

Scheme for synthesis of 2-amino 5-substituted 1,3,4 thiadizole



Benzoic acid Thiosemicarbazide 2-Amino 5-subt.1,3,4 Thiadiazole

Table1-Where R=



5-phenyl 1,3,4 thiadiazole 2-amine (PAT):-

Benzoic acid 8gm (0.1mole)and thiosemicarbazide 7.8gm (0.1mole) were placed in RBF . To this conc. Sulphuric acid (30ml) was added and mixed thoroughly, with cooling and rapid stirring. It was then kept overnight to that solution 90 ml water added and refluxed for 4hrs. The completion of reaction was monitored with TLC. The mixture was allowed to cool and filtered; the filtrate was neutralized with ammonia solution. The precipitate was filtered, dried and recrystalised from ethanol.

Mol. Formula- $C_8H_7N_3S$, Mol wt:- 177.04, M.P:-219-222^{0}c,Yield-57%; IR (K Br) (C=C str) 1635 ,(C=N str) 1575 ,(N-H str) 3285.85 ,(AR CH str) 3109.35 ,(C-S-C str) 760.94

5- (2-chloro phenyl)-1,3,4 thiadiazole 2-amine (CLT)
It was synthesized using similar procedure as above using o-cholro benzoic acid. Mol. Formula:-$C_8H_6ClN_3S$, Mol wt:- 211, M.P:-187-189^{0}c,Yield-52%; IR (K Br) (C=N str) 1640 , (N-H str) 3294.53 ,(AR CH str) 2323.64 , (C-S-C str) 661, (C-CL str) 759.01

5- (3-nitro phenyl)-1,3,4 thiadiazole 2-amine (3-NT):-
It was synthesized using similar procedure as above using 3-Nitro benzoic acid. Mol. Formula:-$C_8H_6N_4O_2S$, Mol wt:- 222, M.P:- 208 – 210 ^{O}C, Yield-70%; IR (K Br) (C=N str) 1629.45 , (N-H str) 3122.86 ,(AR CH str) 2386.62 , (C-S-C str) 661.61, (nitro str) 1539.25, (AR.ring) 720

5- (4-nitro phenyl)-1,3,4 thiadiazole 2-amine (4-NT):-
It was synthesized using similar procedure as above using 4-Nitro benzoic acid. Mol. Formula:-$C_8H_6N_4O_2S$, Mol wt:- 222, M.P:-232-234^{0}c**,** Yield-41%; IR (K Br) (C=N str) 1653 , (N-H str) 3240.53, (AR CH str) 2361.91 , (C-S-C str) 617.24, (nitro str) 1516.44, (AR.ring) 751

5- (3,5-dinitro phenyl)-1,3,4 thiadiazole 2-amine (DNT) :-
It was synthesized using similar procedure as above using 3,5di-Nitro benzoic acid. Mol. Formula:-$C_8H_5N_5O_4S$, Mol wt:- 267.22, M.P:-224-228^{0}c**,** Yield-41%; IR (K Br) (C=N str) 1623.39 , (N-H str) 3313, (AR CH str) 2362.88 , (C-S-C str) 611.25, (nitro str) 1535.39, (AR.ring) 796.63

5-(2-hydroxy phenyl)-1,3,4 thiadiazole 2-amine (TAP):-
It was synthesized using similar procedure as above using salicylic acid. Mol. Formula:- $C_8H_7N_3OS$, Mol wt:- 193, M.P:-260-262^{0}c, Yield-65%; IR (K Br) (C=N str) 1629.90 , (N-H str) 3263.66, (AR CH str) 2350 , (C-S-C str) 619.17, (AR.ring) 785.05 ,(OH str) 3099.71

5- (2,4-dihydroxy phenyl)-1,3,4 thiadiazole 2-amine (BAT):-
It was synthesized using similar procedure as above using 4-hydoxy benzoic acid. Mol. Formula:-$C_8H_7N_3O_2S$, Mol Wt:- 209, M.P:-208-210^{0}c,Yield-56%; IR (K Br) (C=N str) 1629.90 , (N-H str) 3300, (AR CH str) 2303.08 , (C-S-C str) 619.17, (AR.ring) 760

1,3,4 thiadiazole 2-amine (AT):-
It was synthesized using similar procedure as above using formic acid. Mol. Formula:-$C_2H_3N_3S$, Mol wt:- 101, M.P:-204-206^{0}C**,**Yield45%; IR (K Br) (C=N str) 1633.76 , (N-H str) 3449.80, (AR CH str) 2362.88 , (C-S-C str) 622.06, (AR.ring) 764

5- (Methyl)-1,3,4 thiadiazole 2-amine (MAT):-
It was synthesized using similar procedure as above using acetic acid. Mol. Formula:-$C_3H_5N_3S$, Mol wt:- 115, M.P:-230-232^{0}c, Yield-70%; IR (K Br) (C=N str) 1602.90 , (N-H str) 3531.78, (AR CH str) 2358.06 , (C-S-C str) 612.42, (AR.ring) 760

5- (Amino Methyl)-1,3,4 thiadiazole 2-amine (AMAT):-
It was synthesized using similar procedure as above using 2-amino acetic acid (glycine). Mol. Formula:- $C_3H_6N_4S$, Mol wt:- 130, M.P:-212-214^{0}c, Yield-78%; IR (K Br) (C=N str) 1629.90 , (N-H str) 3350, (AR CH str) 2360.95 , (C-S-C str) 619.17, (AR.ring) 686.68 ,(C-CH3 str) 2933.83

DOCKING TOOL AND ALGORITHM:
Molecular docking was completed using Vlife MDS version 4.3. In Grip based docking structures of synthesized compounds were drawn in 2D and converted to 3D and were optimized for docking study. The docking algorithm biopredicta B based on genetic algorithm ,offers a successful docked conformer's space.

The genetic algorithm method was performed to study and predict binding mode of newly synthesized compound with target enzyme 1CX2 of receptor Cyclooxygenase available in PDB format for anti-inflammatory activity.

IN VIVO ANTI-INFLAMMATORY ACTIVITY:
All the experimental procedures and protocol used in this study approved by the institutional animal ethics committee (IAEC) of MGV'S Pharmacy College, Panchavati, Nashik constituted under the provisions of committee for purpose of control and supervision of experiments on animal (CPCSCA), Ministry of Environment and forestry, Government of India. Ethical guideline was strictly followed during all the experiment.

Carrageenan-induced paw edema method:-
Animal**:-** Albino rat of Wister strain of either sex (197-207 gm), were obtained from serum institute, Pune. Animal were housed in groups of 5 at ambient temperature of 25 ± 1^0C. Animals had free access to water and food (Hindustan lever, India). They were deprived of food but not water on day before the experiments. The experiments were carried out between 11am to 5pm.

The experiment was performed on albino rats of Wister strain of either sex, weighing (197-207 gm) . The animals were randomly divided into groups of five. Group I was kept as control, and received only 0.5 % carboxyl methyl cellulose (CMC) solution. Group II and III were kept as standard and test group and received ibuprofen 70mg/kg, and test drug (BAT):100mg/kg respectively orally suspended in 0.5% CMC. Carrageenan solution in a volume of 0.1 ml was injected subcutaneously into the sub-planter region of the right hind paw of each rat, half hr after the administration of the test compounds and standard drugs. The right hind paw volume was measured before and after carrageenan treatment for 3 hours at the interval of one hour by means of a plethysmometer. The percent anti-inflammatory activity was calculated according to the following formula.

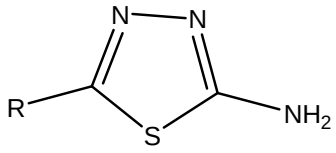
Percent anti-inflammatory activity = (Vc-Vt/Vc) ×100

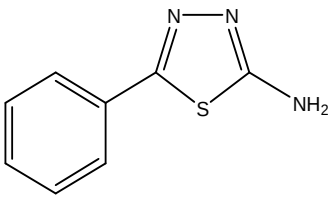
Where, Vt represents the mean increase in paw volume in rats treated with test compound, and
Vc represents the mean increase in paw volume in control group of rats.

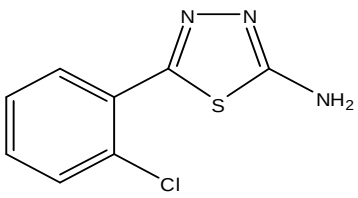
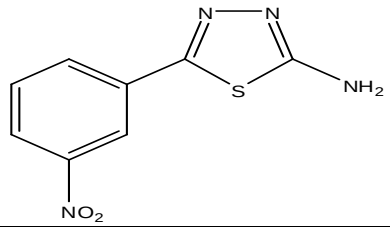
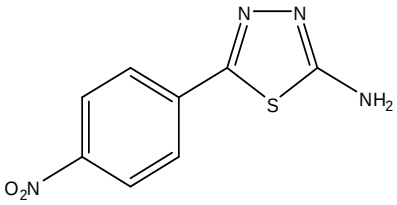
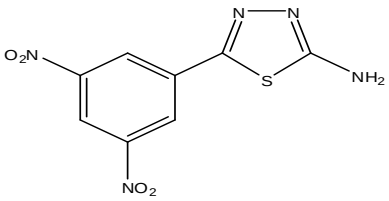
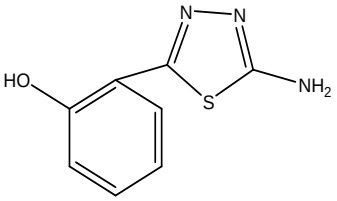
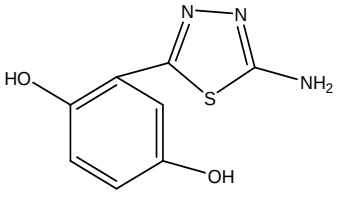
RESULTS

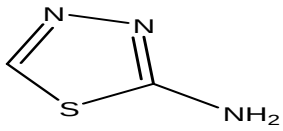
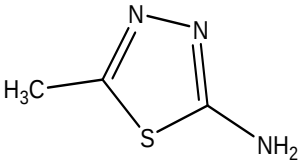
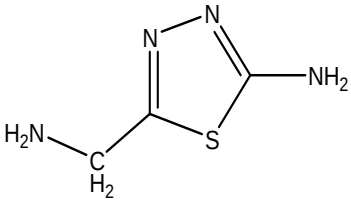
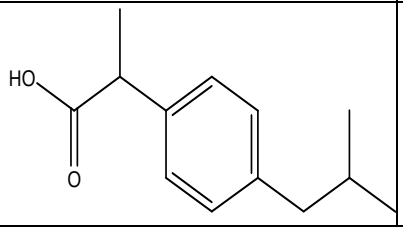
General structure

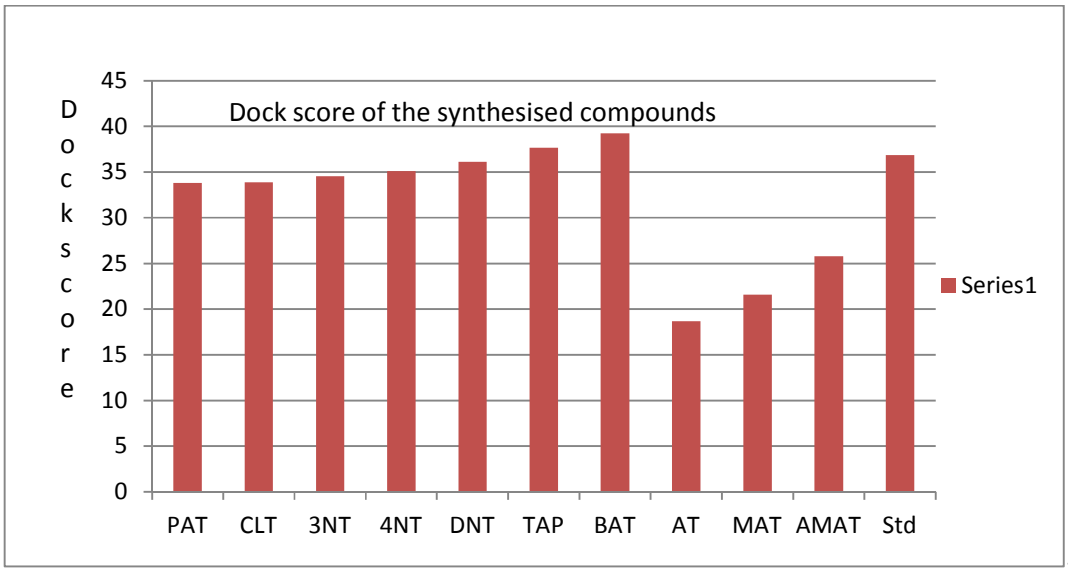
Table2- Dock Score of the synthesized compounds



Sr.No	Structure	Abbrevation	Dock score
1		PAT	-33.81

2		CLT	-33.99
3		3NT	-34.55
4		4NT	-35.08
5		DNT	-36.10
6		TAP	-37.68
7		BAT	-39.25

8		AT	-18.67
9		MAT	-21.58
10		AMAT	-25.77
11		(Standard) Ibuprofen	-36.87



Graph1-Dock score of the synthesized compounds with COX-2 receptor

Compound BAT binding with the COX-2 receptor

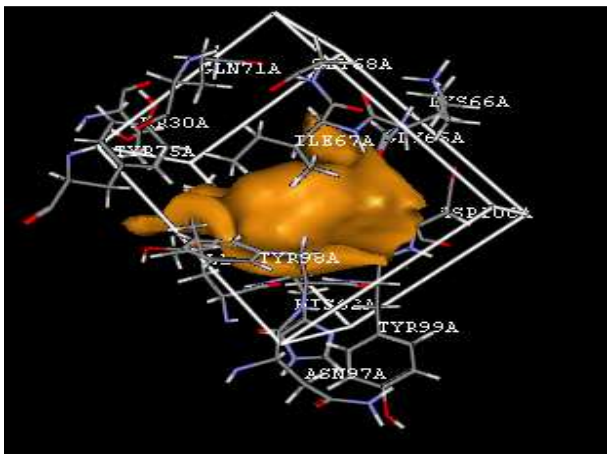


Figure-1. Cavity binding of BAT with the COX-2 receptor

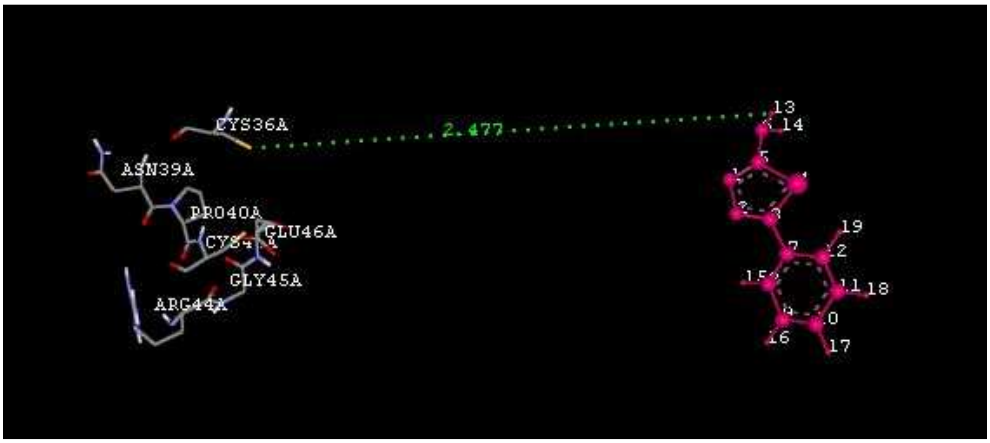


Figure 2.-Hydrogen bonding of amino group of BAT with amino acid cystein 36A of COX-2

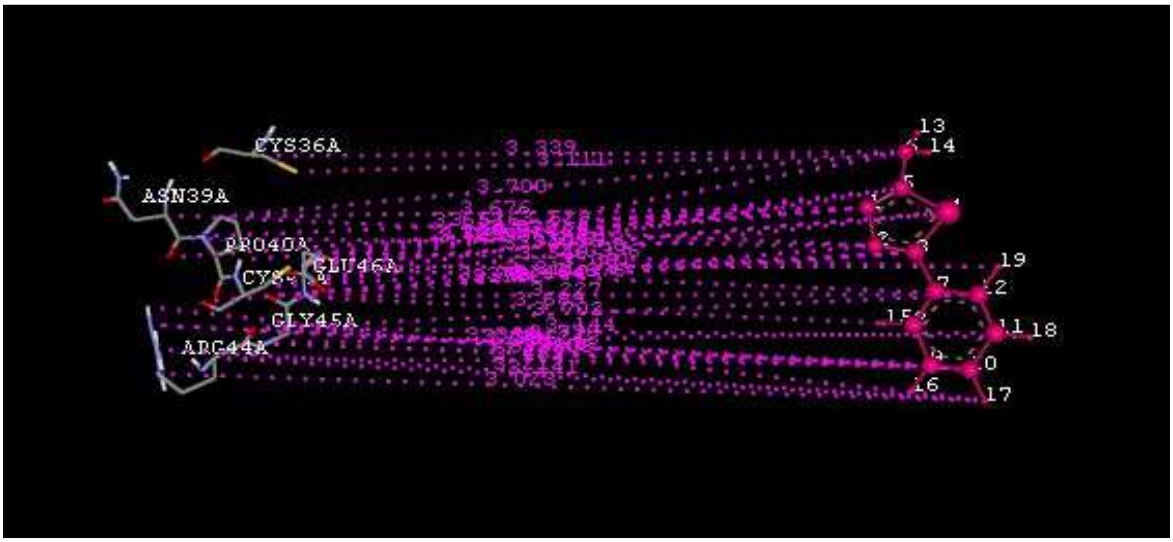


Figure 3- Vanderwall binding of BAT with amino acids cystein 36A, Aspergine 39A of COX-2

Table3-Anti-inflammatory activity of synthesized compound(BAT)

Sr.NO.	Compound	Time	Caragenan induced rat paw edema	
			Mean increase in paw volume+/- S.D. mL	**%** inhibition
1	Control	1 Hr	1.86	-
2	Test		0.74	60.21
3	Standard		0.78	58.06
4	Control	2Hr	2.66	-
5	Test		0.13	95.11
6	Standard		0.60	77.00
7	Control	3 Hr	2.55	-
8	Test		0.28	85.33
9	Standard		0.11	95.68

CONCLUSION

The thiadiazole derivatives were synthesized in pure form. The identity of products was confirmed by melting points and FTIR, NMR and GCMS studies. Dock score of synthesized compounds was calculated with cox-2 receptor. From docking studies, it is concluded that 5- phenyl substitution on 2-amino1,3,4- thiadiazole nucleus shows good binding affinity for COX-2 receptor, further substitution on ortho, meta and para position of 5- phenyl ring with –OH group shows remarkable binding affinity for COX-2 receptor than substitution on ortho, meta and para position of 5- phenyl ring with Cl or NO_2 groups.

Synthesized compound BAT was found to have highest binding affinity with receptor. The in vivo anti-inflammatory activity of synthesized compound BAT was determined using carrageenan induced paw edema method in rats and compared with ibuprofen as reference standard .The compound BAT shown significant in vivo anti-inflammatory activity after 2 Hrs and 3 Hrs.

Acknowledgement
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