Research Article **ISSN: 2349 – 4492**

Asian Journal of Research in Biological and Pharmaceutical Sciences

Journal home page: www.ajrbps.com

SYNTHESIS, DOCKING AND ANTI-INFLAMMATORY ACTIVITY OF NOVEL SERIES OF AZETIDINONE DERIVATIVES

Alex Martin*[1] and Abhay Kumar Verma[1]

*[1]Department of Pharmaceutical Chemistry, Spectrum Institute of Pharmaceutical Sciences and Research, Uttar Pradesh Technical University, Greater Noida, India.

ABSTRACT

The present work is undertaken to explore more possibilities of finding β-lactam derivatives with enhanced anti-inflammatory activity. The scheme involves two steps. In the first step different Schiff bases are synthesized from 4-Amino antipyrine and six differently substituted aromatic aldehydes. In the second step, 2-azetidinone was synthesized by ketene-imine cyclization (Schrodinger) by adding chloroacetyl chloride. The lead compounds are docked against COX enzyme and it was calculated that the derivative AZ5 shows the best docking score which was compared with that of the *in-vivo* anti-inflammatory activity. It was found that the result of the anti-inflammatory activity correlated with the docking score of the concerned derivative.

KEYWORDS

2-azetidinone, 4-aminoantipyrine, Docking and Anti-inflammatory activity.

Author of correspondence:
Alex Martin,
Department of Pharmaceutical Chemistry,
Spectrum Institute of Pharmaceutical Sciences and Research,
Uttar Pradesh Technical University, Greater Noida, India.

Email: aalexmartin@rediffmail.com.

INTRODUCTION[1-4]

A β-lactam ring, is a four membered lactam. It is named as such, because the nitrogen atom is attached to the β-carbon atom relative to the carbonyl group. The simplest β-lactam possible is 2-azetidinone.

2-Azetidinones (Figure No.1) shows various biological activities such as antifungal, antibacterial, antitubercular, anticonvulsant, analgesic, anti-inflammatory and antiviral activity. It is also known for its potent mechanism based inhibitor of several enzymes like human tryptase, chymase, thrombin, leukocyte elastase, human cytomegalovirus proteus and serine protease enzyme. The four membered hetero cyclic ring is well known for its anti-

inflammatory property. The present work is undertaken to explore more possibilities of finding β-lactam derivatives with enhanced anti-inflammatory activity. In the article, docking scores have also been reported and its results have been compared to the result obtained from *in-vivo* anti-inflammatory tests of the lead compounds, thus indicating the authenticity of the docking software.

MATERIALS AND METHODS

Synthetic Scheme

The general scheme of the work includes two steps. The Schiff bases (Figure No.2) are synthesized from 4-Amino antipyrine with different aldehydes and then cyclized to give the azetidin-2-one derivatives (Figure No.3).

Experimental Work[5-8]

Preparation of Schiff Base

Conventional method

0.01mol (2.03g) of 4- Amino antipyrine was dissolved in 30ml ethanol containing few drops of glacial acetic acid. Different substituted benzaldehydes (0.01mol) was added to the reaction mixture and refluxed for 3-5 hours. It was then cooled and poured into crushed ice. The solid obtained was filtered, dried and recrystallized with ethanol.

Preparation of Azetidinone

Conventional method

To a well stirred solution of the synthesized Schiff base, 0.01mol and triethylamine (1ml) in 1,4-dioxan (50ml), chloroacetyl chloride (0.01 mol, 0.8ml) was added dropwise for 20min at room temperature. The reaction mixture was stirred for 3 hours and left at room temperature for 48 hours. The resultant mixture was concentrated, cooled and poured into ice-cold water. The azetidinones formed were dried and recrystallized from 1, 4-dioxan and ethanol mixture (3:2).

Docking Studies[9-12]

Molecular docking using Schrodinger

Computational studies of the newer derivatives were performed using Schrodinger software. The target site for the study of anti-inflammatory activity of the azetidinone derivatives was selected from the PDB. The PDB Id is 3LN1. It is a COX enzyme with Celecoxib as the ligand. The resolutions and other criteria's were checked and confirmed.

The sample structures were uploaded manually into the Maestro Project table. The protein was downloaded from the PDB into the work space. The preparation for the ligands and the protein were done using 'LigPrep' and 'Protein Preparation Wizard' respectively. The chain A of the protein was maintained along with interactive water molecules. The different conformations of the ligands were designed and the best pose of the molecules were selected.

Then the receptor grid was generated using the option 'Receptor Grid Generation'. Now that the target site was identified the natural ligand was replaced with the input structures (sample ligands) automatically by the software for getting the docking scores. This was performed by 'GLIDE' using the option 'Ligand Docking' from the toolbar.

The job was monitored periodically and the results were obtained in the project table.

Anti- Inflammatory Activity[13-17]

Carrageenan induced rat paw edema method

The synthesized compound, AZ_5 was tested for their anti-inflammatory activity by using carrageenan induced rat paw edema method in albino rat (200-225g). The 1% w/v solution of carrageenan for injection was prepared in normal saline (0.9%) and 0.1ml was injected underneath plantar region. The standard drug and synthesized compound were administered in animals by oral route feeding tube through tuberculin syringe. The stock suspensions of the standard and synthesized compound were prepared in concentration of 10 mg/ml of 2% w/v CMC in distilled water. The test compound was given in two doses 50mg/kg and 100mg/kg. The percentage edema inhibition shown by the test and standard compounds at time interval of 0.5, 1.0 and 2.0 hours was measured.

RESULTS AND DISCUSSION

Physical Characterization of the Synthesized Compounds

The molecular formulas, molecular weights, melting

point, R_f value and color of the synthesized compounds are tabulated in Table No.1.

Molecular Docking Studies

Molecular docking using Schrodinger

Computational studies of the newer derivatives were performed using Schrodinger software. The target site for the study of anti-inflammatory activity of the azetidinone derivatives was selected from the PDB. The PDB Id is 3LN1. It is a COX enzyme with Celecoxib as the ligand. The resolutions and other criteria's were checked and confirmed.

The binding affinities of the synthesized derivatives for their anti-inflammatory activity with COX-2 enzyme were determined using Schrodinger software. The docking scores and other interactive parameters are tabulated in Table No.2. The ligand interactions and the best mode of the molecule for binding with the protein are given in the Figures No.4.1 to 4.6.

Anti- Inflammatory Activity

The synthesized azetidinone derivative (AZ_5) and the standard drug (Ibuprofen) were tested for their anti-inflammatory activity by using Carrageenan induced rat paw edema method in Albino rats (200-225g). The results are given in Table No.3 and are depicted in Figure No.5.

The percentage edema was calculated using the formula,

$$V_c - V_t / V_c \times 100$$

Where

V_c and V_t are the mean edema volume of the control and the test group respectively.

Table No.1: Physical Property Data of Schiff Bases and Azetidinone Derivatives

S.No	Compound	Mol. Formula	Mol. Weight	Melting point (˚C)	R_f value	Color
1	SB_1	$C_{18}H_{18}N_3OCl$	343.85	158-160	0.60	Light Yellow
2	SB_2	$C1_8H_{18}N_4O_3$	354.40	170-172	0.59	Orange
3	SB_3	$C_{22}H_{29}N_3O_4$	399.48	160-162	0.61	Yellow
4	SB_4	$C_{20}H_{25}N_3O_3$	355.43	170-172	0.70	Brown
5	SB_5	$C_{19}H_{23}N_3O_2$	325.40	164-166	0.65	Light Brown
6	AZ_1	$C_{20}H_{19}N_3O_2Cl_2$	404.30	176-178	0.62	Pale Yellow
7	AZ_2	$C_{20}H_{19}N_4O_4Cl$	414.85	202-204	0.57	Cream
8	AZ_3	$C_{18}H_{18}N_3O_5Cl$	459.93	180-182	0.72	White
9	AZ_4	$C_{18}H_{18}N_3O_4Cl$	415.88	190-192	0.51	White
10	AZ_5	$C_{18}H_{18}N_3O_3Cl$	385.85	198-200	0.54	White

Table No.2: Docking Score of the Compounds against Cox -1 Enzyme

S.No	Ligand	Dock score
1	**AZ_1**	-5.24
2	**AZ_2**	-6.03
3	**AZ_3**	-6.59
4	**AZ_4**	-6.67
5	**AZ_5**	-6.79

Table No.3: Anti-Inflammatory Activity of Compound Az_5

S.No	Compound	Body Weight	Mean Swelling Volume (ml)	% inhibition of edema
1	Control	210	0.80	--
2	Ibuprofen	220	0.30	62.50
3	AZ_5 (50µg)	210	0.35	56.25
4	AZ_5 (100µg)	220	0.32	60.00

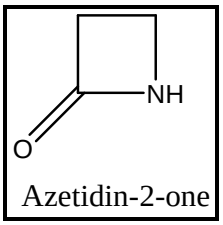

Figure No.1: Structure of 2-azetidinone

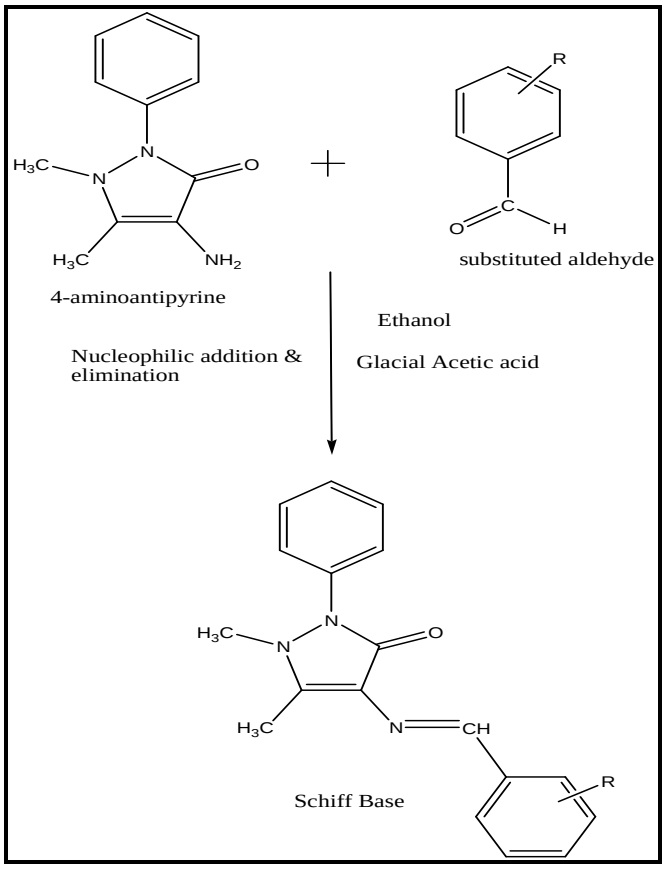

Figure No.2: Synthesis of Schiff bases

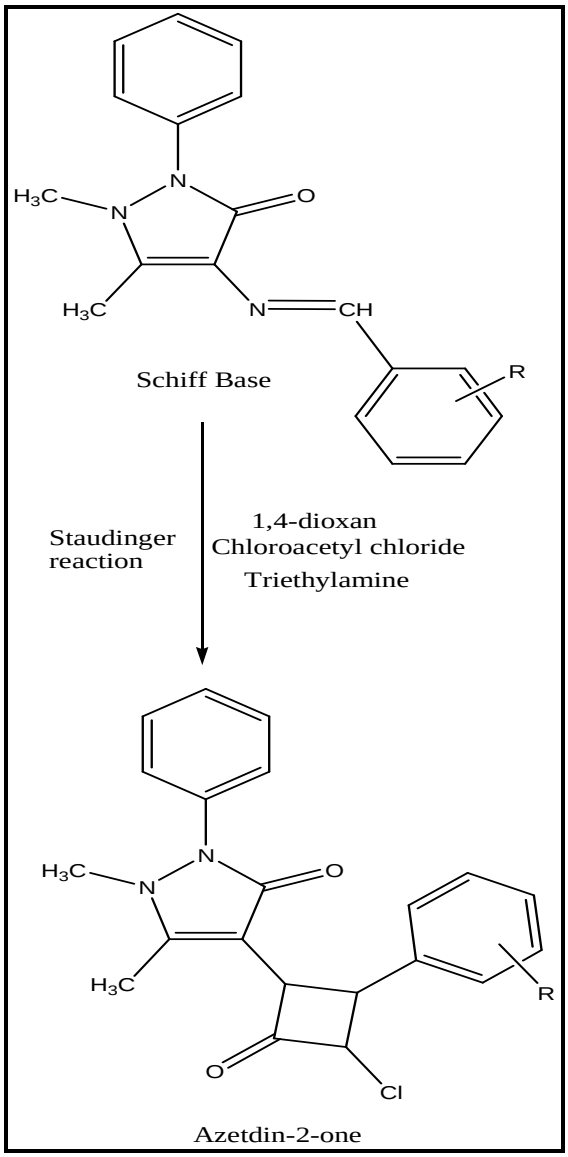

Figure No.3: Synthesis of Azetidinones

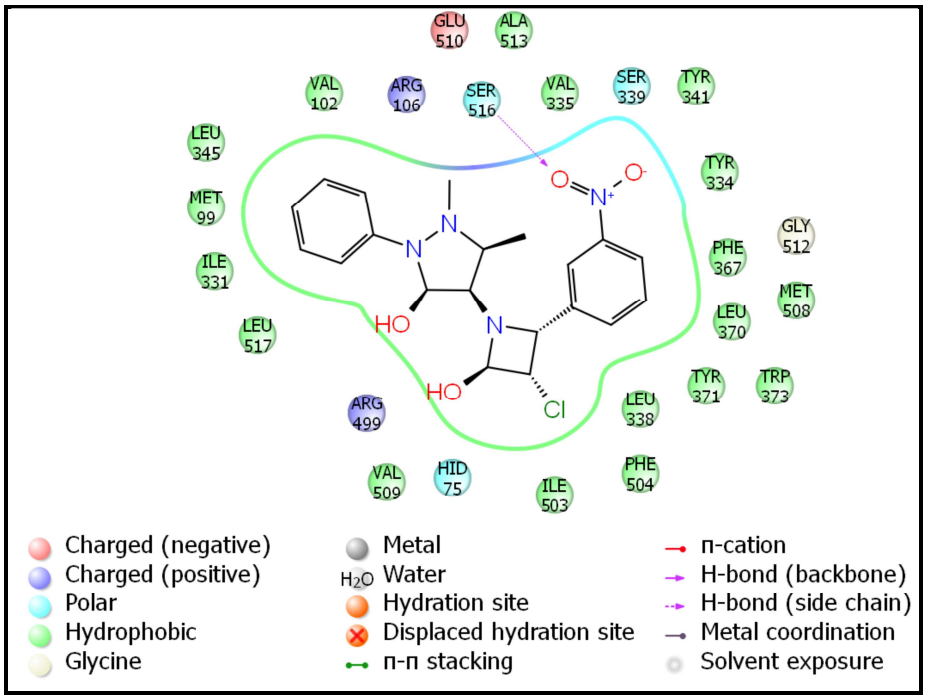

Figure No.4.1: Interactions of AZ1

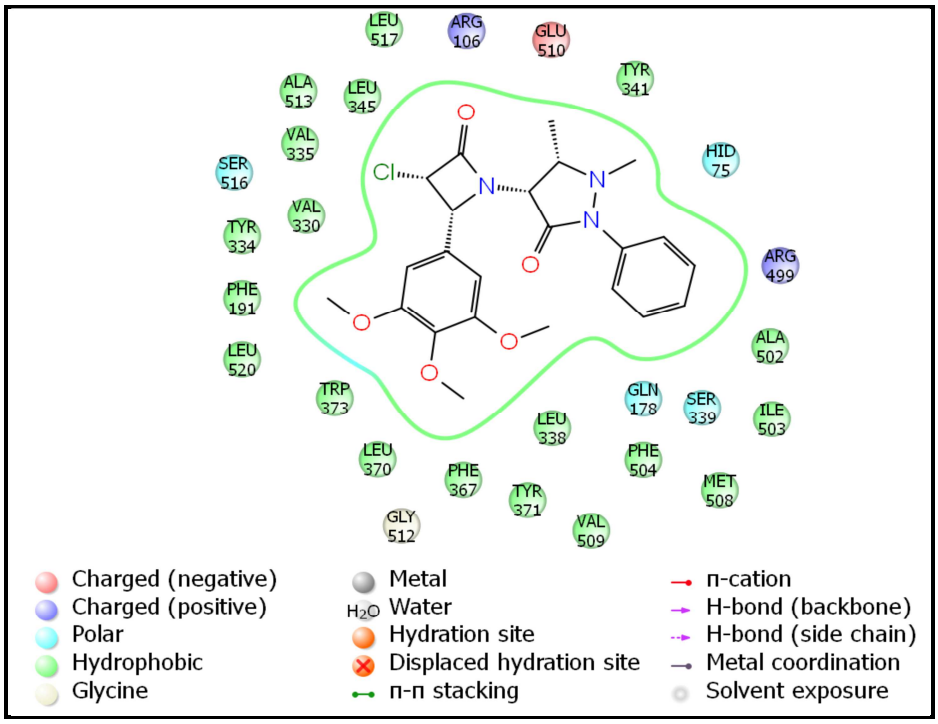

Figure No.4.2: Interactions of AZ2

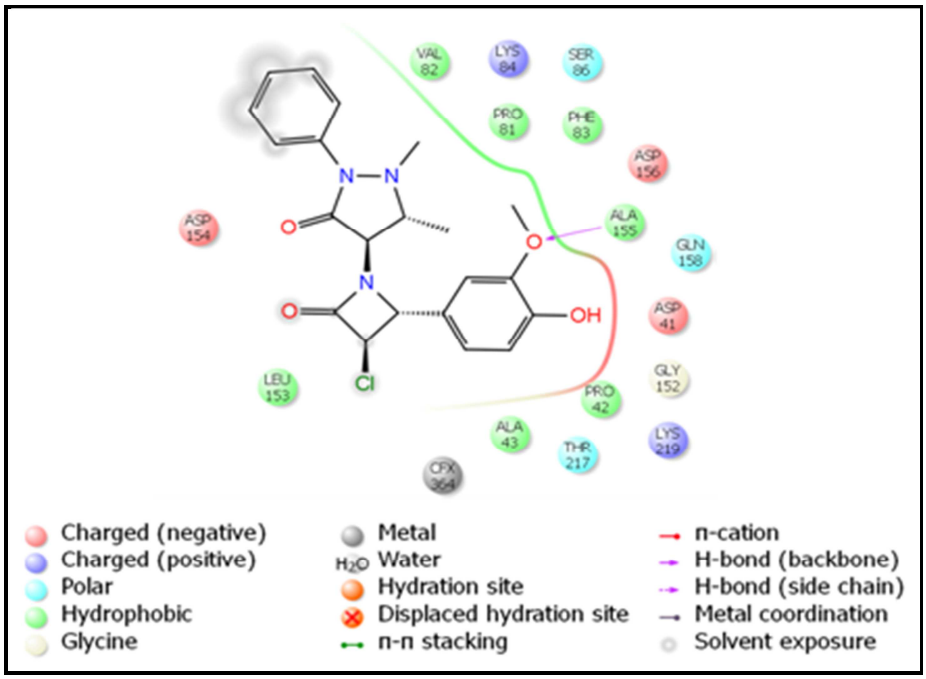

Figure No.4.3: Interactions of AZ3

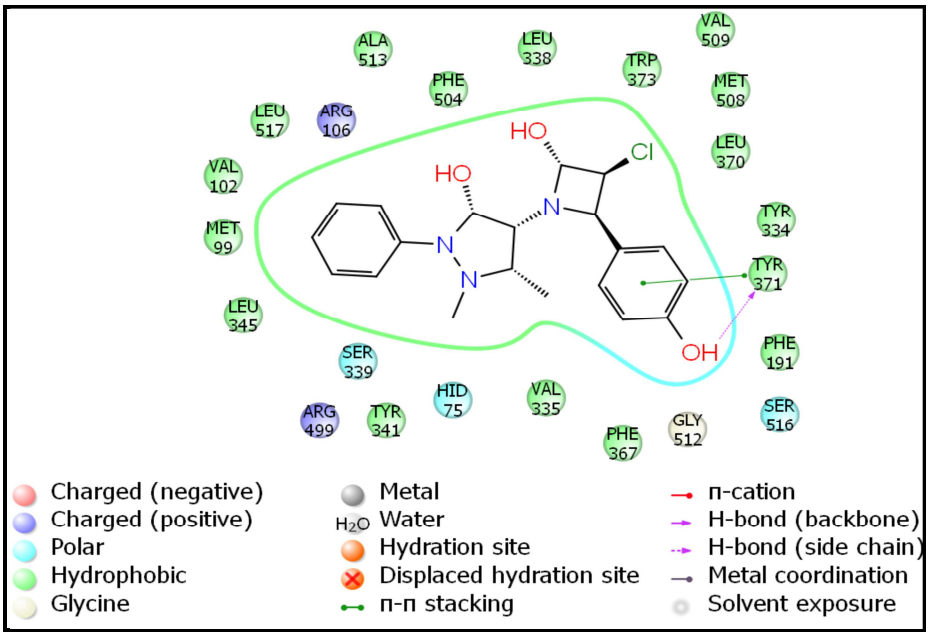

Figure No.4.4: Interactions of AZ4

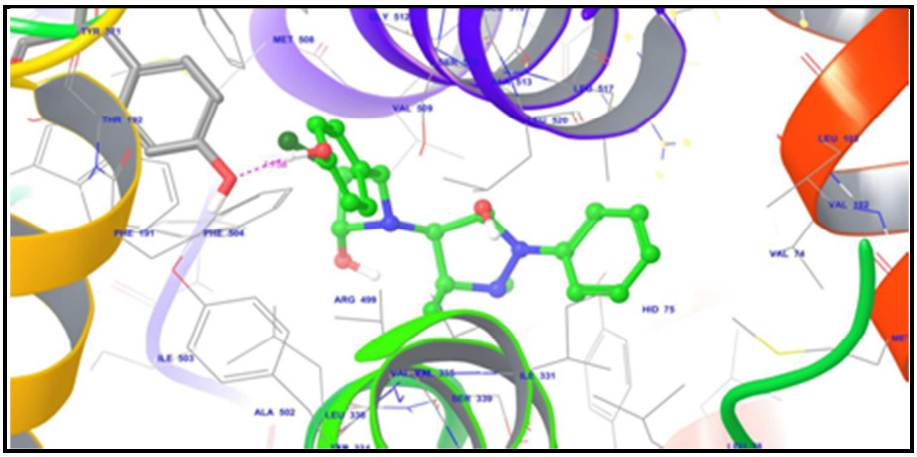

Figure No.4.5: Mode of Docking

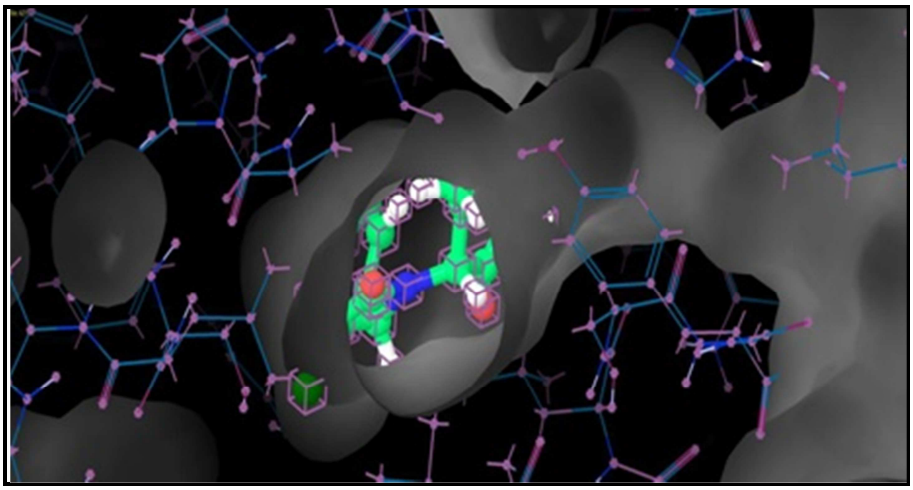

Figure No.4.6: Mode of Docking

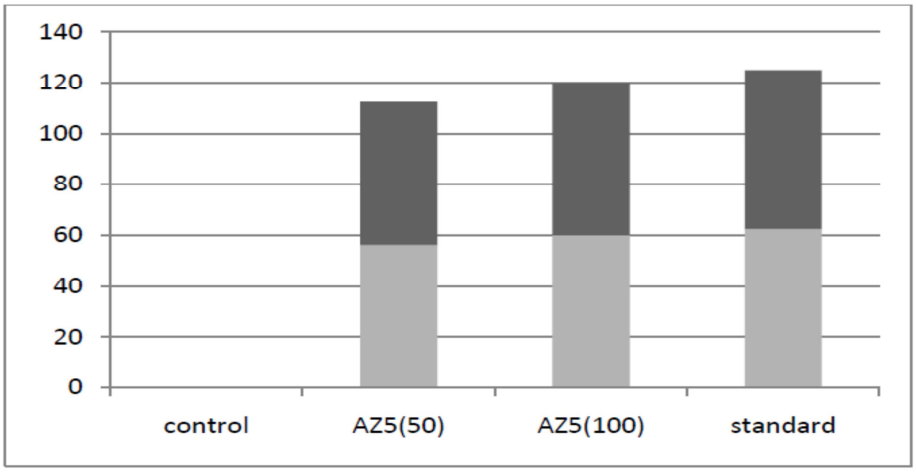

Figure No.5: Chart of anti-inflammatory activity

CONCLUSION

In the first step different Schiff bases are synthesized from 4-Amino antipyrine and six differently substituted aromatic aldehydes. In the second step, 2-azetidinone was synthesized by ketene-imine cyclization (Staudinger) by adding chloroacetyl chloride. The percentage yields of all the synthesized compounds were 50-70%. The Rf values and melting points confirm the purity of the compounds synthesized. The docking study for the anti-inflammatory activity against COX-2 was done using Schrodinger software. The possible ligand interactions and the binding ability of the derivatives were predicted. The software provided the best possible mode of binding and the possible interactions. The reports shows that the derivative AZ_5 has the best binding capability and also that all the derivatives have almost equal range of activity. The compound AZ_5 at a dose of 50µg/ml shows 56.25% anti-inflammatory activity and at a dose of 100µg/ml shows 60.00 % anti-inflammatory activity. On the other hand, Ibuprofen at the standard dose shows 62.50 % anti-inflammatory activity. This shows that the compound AZ_5 shows anti-inflammatory activity comparable to that of the standard. The reports of the docking studies are well correlated with screened anti-inflammatory activity. Docking score calculated for AZ_5 with COX-1 enzyme was -6.79 and as reported by the docking scores, the compound AZ_5 shows good anti-inflammatory activity.

ACKNOWLEDGEMENT

The authors are thankful to Dr. Ramanpreet Walia, Principal, Dept. of Pharmaceutical Chemistry, Spectrum Institute of Pharmaceutical sciences and Research, Greater Noida, for providing Laboratory facilities, guidance and financial support.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Ameya A. Chavan and Nandini R. Pai. Synthesis and biological Activity of N-substituted-3-chloro-2-azetidinones, *Molecules*, 12, 2007, 2467-2477.
2. Freddy H. Havaldar and Sushil Kumar J. Mishra. Synthesis of some azetidi-2-ones and thiazolidin-4-ones as potential antimicrobial Agents, *Indian J. Heterocyclic Chemistry*, 13, 2004, 197-200.
3. Patel K H and Mehta A G. Synthesis and antifungal activity of azetidinones and thiazolidinones derivative of 2-amino-6- (2-naphthalenyl) thiazolo [3, 2-d] thiadiazole, *European Journal of Chemistry*, 3(13), 2006, 267-273.
4. Jubie S, Gowramma B, Nitin K. Muthal, Kalirajan R, Gomathi S and Elango K. Synthesis and Antimicrobial Evaluation of some 2-Azetidinone derivatives, *International Journal of Chem Tech Research*, 1(2), 2014, 153-157.
5. Rajasekaran A, Periasamy M and Venkatesan S. Synthesis, characterization and biological activity of some novel azetidinones, *Journal of Developmental Biology and Tissue Engineering*, 2(1), 2010, 5-13.
6. Senthil Kumaran J, Priya S, Jayachandramani N and Mahalakshmi S. Synthesis, Spectroscopic Characterization and Biological Activities of Transition Metal Complexes Derived from a Tridentate Schiff Base, *Journal of Chemistry*, 3(1), 2013, 1-10.
7. Vijay Kumar M M J, Nagaraja T S, Shameer H, Jayachandran E, Sreenivasa G M. N-Substituted-3-chloro-2-azetidinones: Synthesis and characterization of new novel anti-inflammatory agents, *Journal of Pharmaceutical Sciences and Research*, 1(2), 2009, 83 -92.
8. Ravi Sankar Reddy D and Risy Namratha. Synthesis and biological evaluation of 6-fluoro benzothiazole substituted pyrazolo azetidinones, *Der Pharma Chemica*, 5(1), 2013, 235-240.
9. Rohan V. Bamane, Trupti S. Chitre, Vijay K. Rakholiya. Molecular docking studies of quinoline-3-carbohydrazide as novel PTP1B inhibitors as potential antihyperglycemic agents, *Scholars Research Library*, 3(4), 2011, 227-237.
10. Kamalika Banerjee, Utkarsh Gupta, Sanjay Gupta, Gulshan Wadhwa, Reema Gabrani,

Sanjeev Kumar Sharma and Chakresh Kumar Jain. Molecular docking of glucosamine-6-phosphate synthase in *Rhizopus oryzae*, *Bioinformation*, 7(6), 2011, 285-290.

11. Pappu srinivasan, Arumugam sudha, Ramar manikandan and Chinnasamy arulvasu. Molecular docking studies of 1, 2 disubstituted idopyranose from *vitex negundo* with anti-diabetic activity of type 2 diabetes, *International journal of pharma and bio sciences*, 2(1), 2011, 68-83.
12. Natchimuthu Santhi and Sekar Aishwarya. Insights from the molecular docking of withanolide derivatives to the target protein PknG from Mycobacterium tuberculosis, *Bioinformation*, 7(1), 2011, 1-4.
13. InmaculadaPosadas, MariarosariaBucci, Fiorenti naRoviezzo, AntoniettaRossi, LucaParente, Lidia Sautebin and Giuseppe Cirino. Carrageenan-induced mouse paw oedema is biphasic, age-weight dependent and displays differential nitric oxide cyclooxygenase-2 expression, *British Journal of Pharmacology*, 142(2), 2014, 331-338.
14. Sarika Amdekar, Purabi Roy, Vinod Singh, Avnish Kumar, Rambir Singh and Poonam Sharma. Anti-Inflammatory Activity of Lactobacillus on Carrageenan-Induced Paw Edema in Male Wistar Rats, *International Journal of Inflammation*, 6(2), 2012, 1-6.
15. Srinivasa K, Chandrasekhar B V S, Srinivasa J. Anti-inflammatory activity of theophylline on carrageenan-induced paw edema in male wistar rats, *International Journal of Basic Clinical Pharmacology*, 2(3), 2013, 298-301.
16. Armita Banani, Nasrin Maleki-Dizaji, Alireza Garjani, Hamid Soraya, Sara Mostafalou and Mojtaba Ziaee. Clavulanic acid exhibits anti-inflammatory effects on carrageenan-induced paw edema model of inflammation in rats, *Scholars Research Library*, 3(7), 2012, 3312-3320.
17. Daniela Salvemini, Zhi-Qiang Wang, Pamela S. Wyatt, David M. Bourdon, Margaret H. Marino, Pamela T. Manning and Mark G. Currie. Nitric oxide: Key mediators in the early and late phase of carrageenan-induced rat paw inflammation, *British Journal of Pharmacology*, 7(2), 1996, 829-838.

Please cite this article in press as: Alex Martin and Abhay Kumar Verma. Synthesis, Docking and Anti-Inflammatory Activity of Novel Series of Azetidinone Derivatives, *Asian Journal of Research in Biological and Pharmaceutical Sciences*, 2(3), 2014, 112- 120.