July - Sep © 2017 Transactions; 4(15): 568-575

International Journal of Research and Applications

ISSN (online): 2349-0020 ISSN (print): 2394-4544 http://www.ijraonline.com/

Research Article

Biological screening, evaluation and Docking studies of substituted thiazolylhydrazinomethylidene-pyrazoles derivatives

Jagadeesh Kumar Ega[1] and Kavitha Siddoju [1*]

Corresponding Author:
jkjagadeeshkumare@gmail.com

DOI:
http://dx.doi.org/
10.17812/IJRA.4.15(95)2017

Manuscript:
Received: 4th Aug, 2017
Accepted: 17th Sep, 2017
Published: 20th Sep, 2017

Publisher:
Global Science Publishing Group, USA
http://www.globalsciencepg.org/

ABSTRACT

Pyrazoles with various functional groups at position-4 such as cyano or oxime, aldehyde or carboxylate etc., have been known to show good antimicrobial properties. In this paper we are discussing about the biological activity of a series of thiazolylhydrazinomethylidene-pyrazoles (4a-o) are screened In vitro antibacterial, In vivo anti-inflammatory activity and also tested for docking studies. They are extensively used as useful synthons in organic synthesis. Diversely substituted pyrazolines and their derivatives are important biological agents and possess a wide variety of medicinal as well as agrochemical applications.

Keywords: Pyrazoles, antibacterial, anti-inflammatory, docking study.

[1*] Department of Chemistry, Chaitanya (AUTONAMOUS) Post graduate College, Warangal, T.S-506001.
[1] Department of Chemistry, Kakatiya University, Warangal, Telangana State-506009.

IJRA - Year of 2017 Transactions:
Month: July - September
Volume – 4, Issue – 15, Page No's: 568-575
Subject Stream: Chemistry

Paper Communication: Author Direct

Paper Reference Id: IJRA-2017: 4(15)568-575

DOI: 10.17812/IJRA.4.15(95)2017

International Journal of Research and Applications
July - Sep © 2017 Transactions 4(15): 568-575

eISSN : 2349 – 0020
pISSN : 2394 – 4544
www.ijraonline.com

CHEMISTRY RESEARCH ARTICLE

Biological screening, evaluation and Docking studies of substituted thiazolylhydrazinomethylidene-pyrazoles derivatives

Jagadeesh Kumar Ega [1] and Kavitha Siddoju [1*]

[1*] Department of Chemistry, Chaitanya (AUTONAMOUS) Post Graduate College, Hanamkonda, Warangal, Telangana State 506001.

[1] Department of Chemistry, Kakatiya University, Warangal, Telangana State 506009.

ABSTRACT

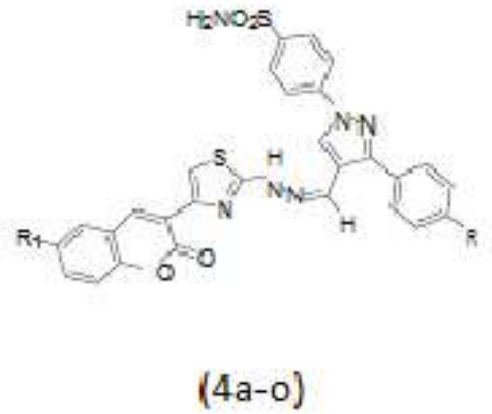
Pyrazoles with various functional groups at position-4 such as cyano or oxime, aldehyde or carboxylate etc., have been known to show good antimicrobial properties. In this paper we are discussing about the biological activity of a series of thiazolylhydrazinomethylidene-pyrazoles (4a-o) are screened In vitro antibacterial, In vivo anti-inflammatory activity and also tested for docking studies. They are extensively used as useful synthons in organic synthesis. Diversely substituted pyrazolines and their derivatives are important biological agents and possess a wide variety of medicinal as well as agrochemical applications.

Keywords: Pyrazoles, antibacterial, anti-inflammatory, docking study.

1. INTRODUCTION

Many alkaloids, vitamins, antibiotics as well as many synthetic medicines and dyestuffs are heterocyclic, and so are many other substances such as nucleic acids which are fundamental to any life process on planet earth. Simple fact that the heterocycles are able to get involved in an extraordinarily wide range of reaction types which are, in general, not feasible with carbocycles explains the reason as to why nature utilizes heterocycles at such a scale. Pyrazolines are well known and important nitrogen containing 5-membered heterocycles having two adjacent nitrogen atoms and three carbons within the ring, bearing only one endocyclic double (C=N) bond and are basic in nature.

Incorporation of heterocyclic rings in general and pyrazoles, pyrazolines, thiazoles or coumarins in particular into prospective pharmaceutical candidates is an established strategy to improve activity and safety of active molecules. Amongst five membered nitrogen heterocycles, pyrazoles have attained a special status in the eyes of chemists and biologists owing to their easy methods of syntheses and enormous pharmacological applications. Nitrogen containing heterocycles are perhaps by far the most explored heterocyclic compounds because of their occurrence in a myriad of natural products and biologically active compounds. For this reason, synthetic chemists continue to be interested in the construction and functionalization of these heterocycles.



(4a-o)

Fig-1: structure of thiazolylhydrazinomethylidene-pyrazoles derivatives

4	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o
R	H	H	H	CH_3	CH_3	CH_3	OCH_3	OCH_3	OCH_3	F	F	F	Cl	Cl	Cl
R_1	H	Cl	Br	H	Cl	Br	H	Cl	Br	H	Cl	Br	H	Cl	Br

Table-1: substitutes of thiazolylhydrazinomethylidene-pyrazoles derivatives

2. MATERIALS AND METHOD

Antimicrobial studies

All the newly synthesized pyrazole derivatives thiazolylhydrazinomethylidenepyrazoles (**4a-4o**) were evaluated for their *in vitro* antibacterial activity against two Gram-positive and two Gram-negative bacteria. In addition to this, these compounds were also evaluated for their *in vitro* antifungal activity against two fungi. All the microbial cultures used in the present study were procured from the Microbial Type Culture Collection (MTCC).

Four bacterial and two fungal strains for testing activity of each compound were selected on the basis of their clinical importance in causing diseases in humans. *Staphylococcus aureus* (*S. aureus*) (MTCC 96), *Bacillus subtilis* (*B. subtilis*) (MTCC121) representing Gram-positive bacteria and *Escherichia coli* (*E. coli*) (MTCC 1652), *Pseudomonas aeruginosa* (*P. aeruginosa*) (MTCC 741) representing Gram-negative bacteria were used for evaluating antibacterial activity of the compounds. For evaluation of antifungal activity, either *Aspergillus niger* (*A. niger*) (MTCC 282) and *Aspergillus flavus* (*A. flavus*) (MTCC 871) or *Candida albicans* (*C. albicans*) (MTCC227) and *Saccharomyces cerevisiae* (*S. cervisiae*) (MTCC 170) representing pathogenic yeasts were used.

***In vitro* antibacterial assay**

The *in vitro* antibacterial activity of all the target compounds and antifungal activity (against yeasts, *C. albicans* and *S. cerevisiae*) of thiazolylhydrazinomethylidenepyrazoles **(4a-o)** were evaluated by agar well diffusion method. All the microbial cultures were adjusted to 0.5 McFarland standard, which is visually comparable to a microbial suspension of approximately 1.5×10^8 cfu/mL. 20 mL of Mueller Hinton agar media was poured into each petri plate and the agar plates were swabbed with 100 µL inocula of the test microorganisms and kept for 15 min for adsorption. Using sterile cork borer of 8 mm diameter, wells were bored into the seeded agar plates and these were loaded with a 100 µL volume with concentration of 4.0 mg/mL of each compound reconstituted in dimethylsulphoxide (DMSO). All the plates were incubated at 37 °C for 14 h. Antibacterial activity against bacteria, and antifungal activity against yeasts, indicated by an inhibition zone surrounding the well containing the compounds, was recorded if the zone of inhibition was greater than 8 mm by using zone reader (HI Antibiotic zone scale). The experiments were performed in triplicate. DMSO was used as a negative control whereas ciprofloxacin was used as a positive control in case of bacteria, and amphotericin-B for fungal yeasts.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation. MIC of various compounds against bacterial strains was tested either through a macro dilution tube method.

In macrodilution tube method, various test concentrations of compounds were prepared from 128 to 0.25 µg/mL in sterile tubes No. 1 to 10. 100 µL sterile Mueller Hinton Broth (MHB) was poured in each sterile tube followed by addition of 200 µL solution of test compound in two fold serial dilutions were carried out from the tube 1 to the tube 10 and excess broth (100 µL) was discarded from the last tube No. 10. To each tube, 100 µL of standard inoculum (1.5×10^8 cfu/mL) was added. Turbidity was observed after incubating the inoculated tubes at 37 °C for 24 h.

In modified agar well diffusion method, a twofold serial dilution of each tested compound was prepared by first reconstituting the compound in DMSO followed by dilution in sterile distilled water to achieve a decreasing concentration range of 256 to

0.5µg/mL. A 100 µL volume of each dilution was introduced into wells (in triplicate) in the agar plates already seeded with 100 µL of standardized inoculum (10^6 cfu/mL) of the test microbial strain. All test plates were incubated aerobically at 37 °C for 24 h and observed for the inhibition zones. MIC, shown by a clear zone of inhibition, words, ciprofloxacin was used as positive control against bacteria and amphotericin-B against fungi as well as yeasts while DMSO as negative control.

3. RESULTS AND DISCUSSION

In vivo* anti-inflammatory activity of *(4a-4o)

Increase in the volume of paw and the percentage of edema inhibition for each rat and each group is summarized in Table 2.

Table 2 *In vivo* anti-inflammatory activity of thiazolylhydrazinomethylidene-pyrazoles **4**

Compounds	Volume of edema (mL)[b] and %AI[c]			
	1h	2h	3h	4h
Control	0.99±0.12	1.98±0.23	1.73±0.22	1.77±0.33
Indomethacin	0.04±0.02*	0.19±0.03*	0.26±0.02*	0.17±0.03*
	(95.95)	(90.40)	(84.97)	(90.39)
4a	0.23±0.08*	1.44±0.12	1.210±0.19	1.90±0.18
4b	(76.76)	(27.27)	(30.05)	(-7.34)
	0.12±0.02*	1.37±0.24	1.378±0.16	1.29±0.03
4c	(87.87)	(30.80)	(20.35)	(27.11)
	0.74±0.2*	0.67±0.14*	0.79±0.16*	0.45±0.18*
4d	(25.25)	(66.16)	(54.33)	(74.57)
	0.28±0.07*	0.24±0.09*	0.20±0.15*	0.16±0.08*
4e	(71.71)	(87.87)	(88.43)	(90.96)
	0.16±0.04*	1.04±0.1*	1.09±0.32	0.67±0.10*
4f	(83.83)	(47.47)	(36.99)	(62.14)
	0.31±0.03*	0.39±0.05*	0.29±0.07*	0.15±0.04*
4g	(68.68)	(80.30)	(83.23)	(91.52)
	0.28±0.03*	0.41±0.05*	0.37±0.04*	0.28±0.02*
4h	(71.71)	(79.29)	(78.61)	(84.18)
	0.21±0.07*	0.27±0.06*	0.32±0.07*	0.12±0.04*
4i	(78.78)	(86.36)	(81.50)	(93.22)

	0.26±0.12*	1.03±0.18*	0.4±0.04*	1.4±0.25
4j	(73.73)	(47.97)	(72.25)	(20.90)
	0.30±0.04*	0.32±0.03*	0.28±0.08*	0.19±0.06*
4k	(69.69)	(84.84)	(83.81)	(89.26)
	0.32±0.07*	0.28±0.04*	1.56±0.18	1.70±0.27
4l	(67.67)	(85.85)	(9.82)	(3.95)
	0.36±0.08*	1.55±0.16	1.44±0.14	1.46±0.13
4m	(63.63)	(43)	(16.71)	(17.51)
	0.24±0.08*	1.03±0.23*	1.24±0.23	1.26±0.02
4n	(75.75)	(47.97)	(28.32)	(28.81)
	0.21±0.02*	0.22±0.05*	0.22±0.06*	0.11±0.16*
4o	(78.78)	(88.88)	(87.18)	(95.95)
	0.98±0.1	1.38±0.25	1.414±0.10	1.19±0.22
	(1.01)	(30.30)	(18.16)	(32.76)

*Significantly different compared to respective control values, $P < 0.01$.
[a]Dose levels: test compounds (50 mg/kg body wt.), Indomethacin (10 mg/kg body wt.).
[b]Values are expressed as mean ± SEM (no. of animals = 6) and analyzed by ANOVA.
[c]Values in parentheses (percentage anti-inflammatory activity, AI%).

***In vitro* antimicrobial activity**

All the newly synthesized thiazolylhydrazinomethylidenepyrazoles (**4a**-**4o**) were assayed for their *in vitro* antimicrobial activity against *S. aureus* and *B. subtilis* (Gram-positive bacteria), *E. coli* and *P. aeruginosa* (Gram-negative bacteria), and *S. cerevisiae* and *C. albicans* (fungal yeasts) using agar well diffusion method (Table 3) and MIC was tested through macro dilution tube method (Table 4).

Table 3 *In vitro* antimicrobial activity of thiazolylhydrazinomethylidenepyrazoles **4**

Compounds		Diameter of growth of inhibition zone (mm)[b]				
	S.	*B.*	*E. coli*	*P. aeruginosa*	*S.*	*C.*
	aureus	*subtilis*			*cerevisiae*	*albicans*
4a	15.6	17.3	–	–	10.3	–
4b	16.3	18.3	–	–	11.4	–
4c	14.6	16.3	–	–	10.3	–

4d	16.0	18.3	–	–	11.3	–
4e	17.3	18.0	–	–	10.6	–
4f	17.6	20.3	–	–	13.3	–
4g	18.6	20.6	–	–	13.6	–
4h	14.3	16.3	–	–	10.0	–
4i	15.6	17.6	–	–	12.6	–
4j	14.3	17.6	–	–	10.3	–
4k	13.6	16.3	–	–	11.0	–
4l	14.0	15.6	–	–	10.2	–
4m	15.2	16.0	–	–	11.6	–
4n	15.6	17.3	–	–	11.3	–
4o	17.3	18.6	–	–	13.0	–
Ciprofloxacin	26.6	24.0	25.0	22.0	Nt	Nt
amphotericin-B	Nt	Nt	Nt	Nt	19.3	16.6

[a]Concentration 4.0 mg/mL.
[b]Values, including diameter of the well (8 mm), are means of three replicates.
Nt: Not tested.–No activity

Table 4 Minimum inhibitory concentration (MIC) (in μg/mL) of compounds **4**

Compounds	*S. aureus*	*B. subtilis*	*S. cerevisiae*
4a	128	128	>256
4b	128	64	256
4c	256	128	>256
4d	128	64	256
4e	128	64	>256
4f	128	32	128
4g	64	32	128
4h	256	128	>256
4i	128	128	128

4j	256	128	>256
4k	256	128	256
4l	256	128	>256
4m	128	128	256
4n	128	128	256
4o	128	64	128
Ciprofloxacin	5	5	Nt
amphotericin-B	Nt	Nt	20

[a] Concentration 4.0 mg/mL. Nt: Not tested.

Results revealed that in general, all the tested compounds possessed moderate antibacterial activity against both the Gram-positive bacteria (*S. aureus*, *B. subtilis*) and moderate antifungal activity against one of the yeasts, *S. cerevisiae*. However, none of the compounds was found to be effective against any Gram-negative bacteria (*E. coli*, *P. aeruginosa*) and the other tested yeast, *C. albicans*. On the basis of zone of inhibition against the test bacterium, compound **4g** was found to be the most effective against *S. aureus* and compounds **4b**, **4d**, **4e**, **4f**, **4g** and **4o** against *B. subtilis* producing ≥18.0 mm zone of inhibition (Table 3) when compared with standard drug ciprofloxacin which showed the zone of inhibition 26.6 mm against *S. aureus* and 24.0 mm against *B. subtilis*.

Rest of the compounds didn't show significant antibacterial activity against any of the Gram-positive bacteria. However, in terms of MIC, none of the compounds was found to possess appreciable antibacterial activity. Amongst all the compounds, the MIC ranged between 32 and 256 µg/mL against Gram-positive bacteria as compared to standard drug ciprofloxacin having MIC of 5 µg/mL (Table 4).

In case of fungal yeasts, compounds **4f**, **4g** and **4o** were found to be the most effective against *S. cervisiae* showing zone of inhibition ≥13.0 mm (Table 3) when compared with standard drug amphotericin-B producing zone of inhibition of 19.3 mm. However, in terms of MIC, most of the compounds failed to possess appreciable antifungal activity. Amongst all the compounds, the MIC ranged between 128 and ≥256 µg/mL against *S. cerevisiae* as compared to standard drug amphotericin-B having MIC of 20 µg/mL (Table 4).

No correlation between the structural variations and the activity could be drawn within the series in terms of antibacterial or antifungal activities.
Amongst fifteen compounds (**4a**-**4o**) tested, four compounds (**4d**, **4f**, **4h** and **4n**) possessed AI activity surpassing that of the standard drug indomethacin 4h after carrageen an injection while two other compounds **4g** and **4j** possessed excellent AI activity comparable to the standard drug. Compound **4c** showed moderate AI activity with 54% inhibition after 3h but good activity with 74% inhibition after 4h. It suggests that these seven compounds do not get easily metabolized in the system, maintaining AI activity for a long period of time. Amongst rest of the compounds, many of them showed fair activity after 1h and 2h but failed to maintain its AI activity after 3h.

Some novel thiazolylhydrazinomethylidenepyrazoles (**4a**-**4o**) consisting a total of 15 compounds bearing benzene sulfonamide and coumarin moieties were synthesized and were investigated for dual anti-inflammatory—antimicrobial activities. Amongst the tested compounds, six compounds (**4d**, **4f**, **4g**, **4h**, **4j** and **4n**) showed pronounced anti-inflammatory activity comparable to that of standard drug indomethacin 3h as well as 4h after carrageen an injection, out of which four compounds (**4d**, **4f**, **4h** and **4n**) were found to be the most potent anti-inflammatory agents in the present study possessing AI activity greater than that of indomethacin after

4h. Docking analysis of the most active compound **4n** and the least active compound **4a** with both COX-1 and COX-2 active sites also supported the observed AI results. Most of the compounds exhibited moderate antibacterial activity against Gram-positive bacteria as well as moderate antifungal activity against fungal yeast, *S. cerevisiae*.

Docking analysis of (4a-4o)

Although, only crystallographic data can fully clarify the binding mode of chemical compounds, the docking studies can be utilized to gain an insight about the structure–activity relationships and to elucidate the essential structural requirements for molecules acting on the same receptor/enzyme. Molecular docking studies were performed using the automated GOLD docking program on the most active (**4n)** and the least active (**4a**) compound of the series in the anti-inflammatory studies along with the control drug indomethacin to gain an insight about the probable nature of their binding at the COX-1 and COX-2 active site using crystal structure data obtained from the RCSB Protein Data Bank (PDB).

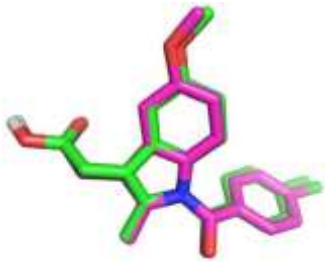


Figure 2.1. Comparison of conformation for indomethacin in the co-crystallized (pink colored carbon) and docked (CY and colored car bon) binding pose of COX-2.

The binding site analysis of indomethacin showed that it penetrates deep into the cycloxygenase active site (Figure 2.2A). The benzoyloxy gen interacts with Ser530 amino acid res idue which is the acetylating site of aspirin in both COX-1 and COX-2.[123] The carboxylate group of indomethacin is positioned towards the mouth of the active site near Arg120 and Tyr355 and the methoxy group of indole points towards the secondary pocket of the COX-2 active site. Additional close cont acts with Ser353, Ala527 and Val523 were also seen (Figure 2.2 A). The bin ding site analysis of the most active com pound **4n** showed that the benzene sulfonamide group is positioned in the vicinity of secondary pocket of CO X-2 isozyme, surrounded by His90, Tyr355, Arg5 13, Ser353, Gln192 and Leu352 (Figure 2.2 A and 2.2B).

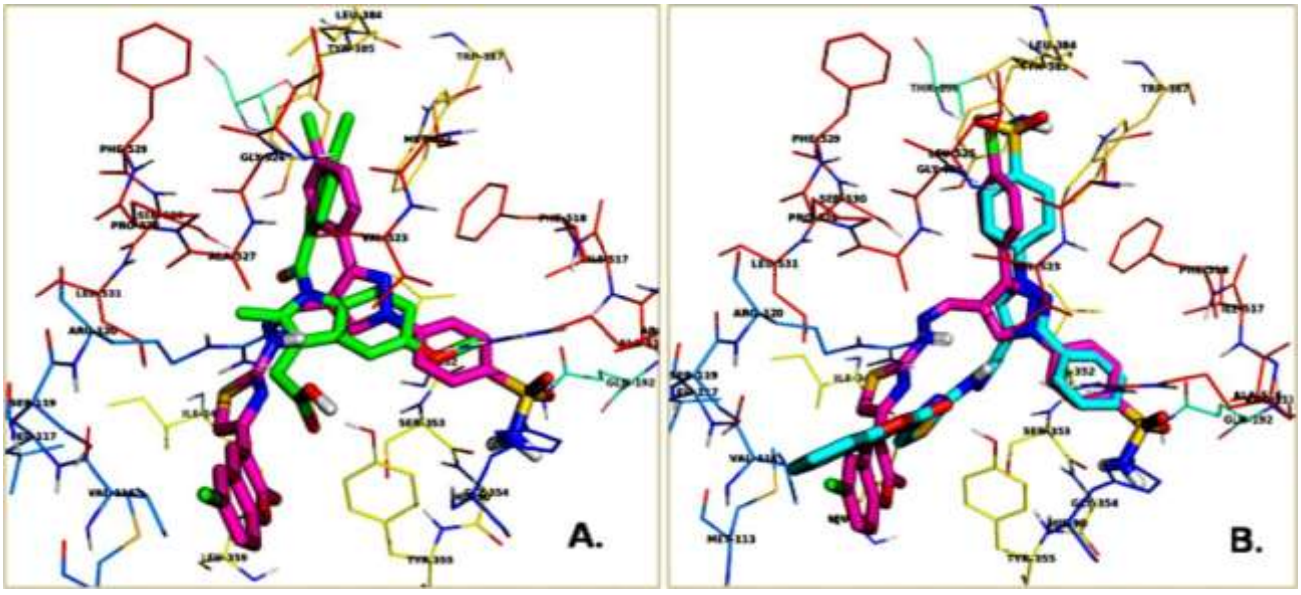


Figure 2.2. Comparative 3D binding pose view of **A.** standard drug indomethacin (green colored carbon) along with the most active compound of series **4n** (pink colored carbon); **B.** most active compo due to the flipping of the benzene sulfonamide group but showed interaction of the thiazole nitrogen and carbonyl oxygen of coumarin with the Arg120 an d Tyr355 at the entrance of CO X-2 active site (Figure 2.2B).

A comparison of the position of indomethacin and compound **4 n** within the COX-2 active site as illustrated in Figure 2.2A showed that methoxy substituent of the indole moiety in indomethacin and benzene sulfonamide moiety in com pound **4 n** assume s a position near the secondary po cket of CO X-2 active site, but the extra bulk of benzene sulfonamide is we ll accommodated by the secondary pocket and shows electrostatic interaction with COX-2 active site residues, plausibly explaining better activity profile of **4n** in com parison to indomethacin. Likewise, reduced activity of **4a** in comparison to **4n** can be explain ed by the incapability of **4a** to interact wit h or accommodate within the secondary pocket of CO X-2 (Fig. 2.2B). T heir binding affinities are further illustrate d in Figures 2.3, 2.4 and 2.5 which show 2D binding and **4n** (pink colored carbon) along with least active compound **4a** (blue colored carbon) of the series at the active site of COX-2.

4. CONCLUSION

The objective of the present work was to synthesize and investigate the anti-inflammatory and/or antimicrobial activities of newly synthesized pyrazole derivatives in the search of potential new structural probes possessing better/enhanced respective activities. The comparison of AI results with starting materials displayed an increase in activity by construction of thiazole ring in compounds containing methyl, methoxy or fluoro substituent on pyrazole (**4d**, **4g**, **4j**) while decrease in activity in those containing no or chloro substituent on pyrazole (**4a**, **4m**). In case of the least active com pound **4a**, the docking results showed that this compound failed to show it's inter action with the secondary pocket residues pose views of indomethacin compound **4n** (most active) and **4a** (least active), respectively at COX-2 active site.

REFERENCES

1) Kumar, P.; Chandak, N.; Kaushik, P.; Sharma, C.; Kaushik, D.; Aneja, K. R.; Sharma, P. K. *Med. Chem. Res.* **2012**, *21*, 3396-3405.
2) Sharma, P. K.; Sawhney, S. N. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2427-2430.
3) Sawhney, S. N.; Gupta, A.; Singh, G. B.; Bani, S. *Indian J. Chem.* **1998**, *37B*, 376-381.
4) Discovery Studio, version 2.1; Accelrys Software Inc.: San Diego, CA, **2008**.
5) Khare, P.; Gupta, A. K.; Gajula, P. K.; Sunkari, K. Y.; Jaiswal, A. K.; Das, S.; Bajpai, P.; Chakraborty, T. K.; Dube, A.; Saxena, A. K. *J. Chem. Inf. Model.* **2012**, *52*, 777-791.
6) Kumar, P.; Chandak, N.; Sharma, P. K. *Med. Chem. Res.* **2012**, *21*, 3396-3405.
7) Hantsch, A.; Weber, H. *Chem. Ber.* **1887**, *20*, 3118-3132.
8) Ali, T. E.-S. *Eur. J. Med. Chem.* **2009**, *44*, 4385-4392.
9) Zhao, P. L.; Wang, Yang, G. F. *J. Agric. Food Chem.* **2008**, *56*, 10767-10773.