Research Article

In-silico Design, Synthesis and *In vivo* Anti-inflammatory Evaluation of Novel 1, 2, 4 - triazole Derivatives

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ABSTRACT

The present study was aimed to design, synthesize and evaluate the *in vivo* anti-inflammatory activity of some new 1,2,4–triazole derivatives. With this view, totally 55 proposed derivatives were subjected to *in-silico* molecular evaluation by using various software like ACD Lab Chemsketch, Molinspiration, Prediction of activity spectra for substances (PASS) and Schrodinger Glide XP (Grid based ligand docking with energetics). The designed molecules having required physico-chemical properties, drug likeness and obeying Lipinski's rule of five were selected for the synthesis. The synthesized compounds were characterized by TLC, melting point determination, FTIR, ^{1}H NMR, ^{13}C NMR and Mass spectroscopic studies. Anti-inflammatory activity of selected compounds was evaluated by carrageenan-induced paw edema method using Diclofenac sodium as standard. Regarding with the results, two derivatives (MB-17 and MB-18) were selected for the synthesis with the help of *in-silico* modeling. The selected derivatives were synthesized by conventional method. They showed characteristic peaks in FTIR and ^{1}H NMR ^{13}C NMR and Mass spectroscopic evaluation. In the *in vivo* evaluation both MB-17 and MB-18 showed anti-inflammatory activity. But the compound MB-18 showed significant anti-inflammatory activity, compared with standard drug, Diclofenac sodium. It was clear that these results are useful for further investigation.

Keywords: 1, 2, 4-triazole derivatives, conventional synthesis, spectral study, *in vivo* anti-inflammatory activity.

INTRODUCTION

Recently, the design and development of high nitrogen containing heterocyclic compounds receive major attention in pharmaceutical and agrochemical sectors, particularly; triazole derivatives gain significance due to their diverse biological activities[1-4]. A wide variety of activities such as antibacterial[5-8], antifungal[5-11], antitumor[12], antiviral[13], anti-inflammatory, anticonvulsant, anti-tubercular[8], analgesic, enzyme inhibitor, herbicides and plant growth regulators[10] were found in different 1, 2, 4-triazole derivatives[3-4].

Nowadays, *in-silico* molecular modification study is one of the important preliminary steps in novel drug designing. In our previous efforts, various 1, 2, 4-triazole derivatives were designed with *in-silico* methods and eligible candidates were synthesized and their biological activities such as anticancer, anti-tubercular activities were evaluated successfully. Now our ongoing investigations have been directed toward the design and synthesis of some new 1, 2, 4-triazole derivatives and the evaluation of anti-inflammatory activity of them, an attempt to provide a direction for further research.

MATERIALS AND METHODS

In-silico molecular modification

Various software was used for the screening of the physiochemical properties of proposed derivatives. In these, ACD Lab Chemsketch was used for 3-D drawing, optimizing and calculating various physicochemical descriptors of the proposed molecules. Calculation of logP values, Lipinski's rule of five and drug likeness evaluation were done with the help of Molinspiration software. PASS (Prediction of activity spectra for substances) software was employed for the prediction of general biological activities of proposed molecules. Docking studies were done by using Schrodinger Glide XP (Grid based ligand docking with energetics) software. The docking results were analyzed by using Open Babel and Discovery studio docking analysis tool. Based on the results of *in-silico* molecular modification, two compounds, MB-17 and MB-18 were selected for synthesis and further study.

Synthesis of selected 1, 2, 4-triazole derivatives

The selected compounds were synthesized by conventional method in four steps as per the procedure of[3-4]. For the synthesis of MB-17, Para dimethyl amino benzaldehyde and piperidine were used in step-3 and step-4 respectively, and for the MB-18, Para dimethyl amino benzaldehyde and morpholine were used in step-3 and step-4 respectively. The Purity of the synthesized compounds was ascertained by TLC [solvent system: n-hexane: ethyl acetate (1:1)] and melting point determination by open capillary method.

Characterization of synthesized compounds by spectral study

IR Spectrum and ^{1}H NMR Spectral studies were done as per the procedure of previous study[4].

^{13}C NMR spectral study of the synthesized compounds was performed by using Bruker Avance 100 MHz NMR instrument. About 30-40 mg of ligand was dissolved in

$CDCl_3$: DMSO-d_6, 90:10 in an NMR tube. All the spectra obtained were corrected with equivalent to the solvent signal.

Mass Spectra of the compounds were recorded with TOF MS ES Mass Spectroscopy using electron impact process.

Elemental analyses of the selected compounds were performed on a Perkin Elmer 2400 Series II Elemental CHNS analyzer.

Evaluation of *in vivo* anti-inflammatory activity

Anti-inflammatory activities of selected compounds were evaluated by carrageenan-induced paw edema method. Diclofenac sodium was used as standard. Healthy young adult male Albino Wistar rats weighing 180 – 200g were utilized for the experiments. They were obtained from the Central animal house, K.M College of Pharmacy, Madurai, Tamil Nadu, India. The selected animals were housed in ambient temperature (25 ± 2°C), relative humidity (55 ± 5%) and 12h light / dark cycle and fed with commercial diet and water *ad libitum*. All animal experiments were carried out in accordance with the guidelines of a committee for the purpose of control and supervision on experiments on animals (OECD 423). The approval from the institutional animal ethics committee has obtained for conducting animal experiments (IAEC/KMCP/142/2013-14).

The study was conducted in reference to the procedure of [14]. Experimental animals were divided into 4 groups of 6 each. Group 1 served as normal control receives saline 5ml.kg^{-1} body weight. Group II served as standard control which was treated with injection Diclofenac sodium at the dose of 10 mg.kg^{-1} body weight. Group III served as treatment control received test compound MB-17 dissolved in 0.5ml of DMSO at the dose of 10 mg.kg^{-1} body weight. Group IV served as treatment control received test compound MB-18 dissolved in 0.5ml of DMSO at the dose of 10 mg.kg^{-1} body weight. Half an hour before the administration of Diclofenac sodium / test compounds, an injection of 0.1ml of 1 % w/v carrageenan in saline was given to the sub plantar region in the right hind paw of the animals in the entire group. The standard and the test compounds were given to the animals in the entire group except group 1 by intra-peritoneal injection.

The paw volume of the injected animal was measured plethysmometrically at 1/2, 1, 3, and 6 h after the carrageenan injection. The percentage of inhibition of inflammation was calculated using the formula

$$\% \text{ Inhibition of inflammation} = \frac{Vc - Vt}{Vc} \text{x } 100$$

Where, Vt - mean increase in paw volume in rats treated with test compounds; Vc - mean increase in paw volume in a control group of rats.

The results of study were expressed as mean ± Standard Error Mean (SEM) of 6 rats in each group. Statistical significance was calculated by ANOVA followed by Newmann Keul's multiple range tests. P-values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Totally 55 proposed derivatives were subjected to *in-silico* molecular evaluation. On docking with 3CTQ, among the 9 selected compounds, two compounds, MB-17 and MB-18 showed a profound binding interaction and highest glide score, implying the prospective efficiency as agents for inflammatory diseases. Docking score for anti-inflammatory activity is presented in Table 1 and the image is shown in Figure 1.

Table 1: Schrodinger Glide XP scores of selected 1, 2, 4-triazole derivatives for anti- inflammatory activity

Target – PDB ID: 3CTQ

Compound	Glide score
MB-2	-5.83
MB-8	-5.42
MB-9	-6.13
MB-10	-7.12
MB-12	-4.28
MB-13	-6.11
MB-14	-5.99
MB-17	**-8.39**
MB-18	**-8.43**

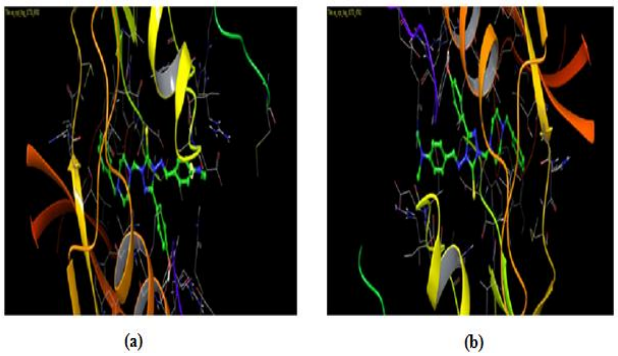


Figure 1: Docking images of Compound MB-17 (a) & 18 (b) to 3CTQ

Docking results of these compounds were analyzed by Open Babel and Discovery Studio docking analysis tool and compared with standard anti-inflammatory drug, Diclofenac sodium which are shown in Figure 2.

Standard Drug – Diclofenac sodium

- 3CTQ– prepared and minimized
- Diclofenac–prepared and minimized
- 3CTQ-Diclofenac– docking
- 10 poses
- Top pose: -vecdocker energy-3.22616kcal/mol
- Interacting residues: Leu167, Ille84, His107, Ala51, Leu167, Tyr35, Phe169, Val138, Lys53, Leu104, Met109.

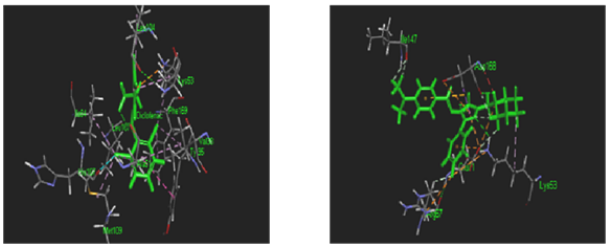
Compound No.17

- T17- prepared and minimized
- 3CTQ-T17– docking
- 10 poses
- Top pose: -vecdocker energy-1.122887kcal/mol
- Interacting residues: Illu147, Asp168, Glu71, Arg67, Lys53.

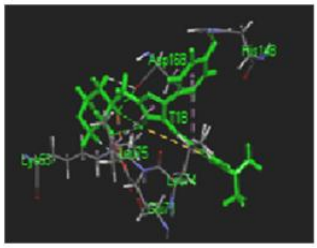
Compound No.18

- T18- prepared and minimized
- 3CTQ-T18– docking
- 10 poses
- Top pose: -vecdocker energy-9.80715kcal/mol

Interacting residues: Lys53, Leu75, Glu7, Leu74, Asp168, His148.



(a) (b)

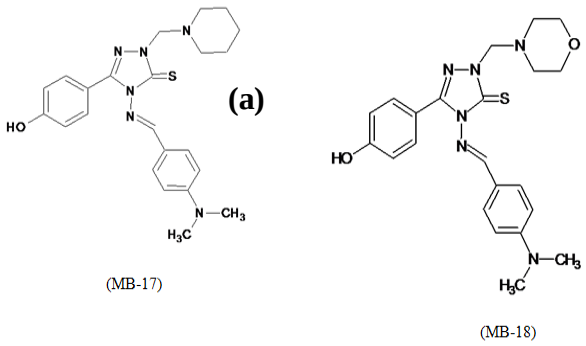


(c)

Figure 2: Docking images of standard drug Diclofenac (a), Compounds MB-17 (b) & 18 (c) to 3CTQ by Discovery studio.

Based on these results, the compounds MB-17 and MB-18 were selected for the synthesis and biological evaluation. They are chemically 2- (piperidin-1-yl methyl) -5- (4-hydroxy phenyl) -4- {[(4-dimethyl amino) phenyl methylidene] amino}-1, 2, 4-triazolin-3-thione (MB-17)

And 2- (morpholin-4-yl methyl) -5- (4-hydroxy phenyl) -4-{[(4-dimethyl amino) phenyl methylidene] amino}-1, 2, 4-triazolin-3-thione (MB-18) their chemical structures are shown in Figure 3.



(a) (MB-17) (MB-18)

Figure 3: Structure of selected compounds for synthesis and biological evaluation

The selected compounds were synthesized by conventional method. Results of evaluation of purity by TLC and melting point determination by an open capillary method are shown in Table 2.

Table 2: Characterization data of synthesized 1, 2, 4-triazole derivatives

Compound	Yield (%)	Melting point (°C)	R_f value
MB-17	54	258	0.82
MB-18	52	267 – 268	0.9

Results of characterization of synthesized compounds by FTIR, ^{1}H NMR, ^{13}C NMR and mass spectroscopic methods are shown in Table 3.

The in-*vivo* anti-inflammatory activity of synthesized compounds, MB-17 and MB-18 was evaluated by carrageenan induced paw edema method. The results showed that both the compounds, MB-17 and MB-18 inhibited the inflammation induced by carrageenan. The results of the study are shown in Table 5 and 6.

Table 3: Spectral data of synthesized compounds

Method	MB-17	MB-18
FTIR	O-H str (3634), C-H str of Mannich base (2922, 2827), C=N str of triazole (1678), C=S (1174), C-H str DMAB (3063).	O-H str (3765), C-H str of Mannich base (2920, 2850), C=N str of triazole (1666), C-O-C str (1087), C=S str(1219), C-H str DMAB (3068)
^{1}H NMR	1.28-2.20 (m, 10H, 5xCH_2 of piperidine), 3.53 (s, 6H, 2xCH_3 of DMA), 4.76 (s, 2H, N-CH_2-N), 6.77-7.31 (m, 8H, ArH), 8.08 (s, 1H, carbimino H), 9.76 (s, 1H, ArOH, D_2O exchangeable)	2.51-2.78 (q, 8H, morpholine H), 3.28 (s, 6H, 2xCH_3 of DMA), 5.11 (s, 2H, N-CH_2-N), 6.76 – 7.41 (m, 8H, ArH), 8.11 (s, 1H, carbimino H), 9.35 (s, 1H, ArOH, D_2O exchangeable)
^{13}CNMR	22.2, 40.7, 55.3, 78.4, 111.8, 115.6, 121. 9, 129.7, 130.7, 142.1, 152.1, 160.1, 189.1.	40.5, 79.4, 115.9, 116.3, 124.7, 130.2, 149.5, 150.0, 160.1, 186.0.
Mass	437 $[M + H]^+$	439 $[M + H]^+$

Results of elemental analysis are shown in Table 4.

Table 4: Elemental analysis of the synthesized compounds

S. No	Compound	Molecular formula	Molecular weight	% Calculated				
				C	H	N	O	S
1	MB-17	$C_{23}H_{28}N_6OS$	436	63.28	6.46	19.25	3.66	7.34
2	MB-18	$C_{22}H_{26}N_6O_2S$	438	60.25	5.98	19.16	7.30	7.31

Table 5: The anti-inflammatory effect of synthesized compounds by carrageenan-induced paw edema in rat

Paw volume in ml					
Group	Treatment	30min	1h	3h	6h
I	Control (Saline 5 ml.kg^{-1})	0.52±0.09	0.55±0.05	0.63±0.08	0.70±0.03
II	Diclofenac sodium (10 mg.kg^{-1})	0.32±0.04**	0.39±0.07*	0.43±0.03*	0.39±0.04**
III	MB-17 (10 mg.kg^{-1})	0.41±0.02*	0.45±0.04*	0.47±0.06*	0.48±0.02*
IV	MB- 18 (10 mg.kg^{-1})	0.33±0.04*	0.38±0.05**	0.42±0.02*	0.40±0.01*

All values are expressed as mean ± SEM for 6 animals in each group; *P<0.05, **P<0.01 compared.

Table 6: Percentage inhibition of the anti-inflammatory effect of MB-17 and MB-18 by carrageenan-induced paw edema in rats

Treatment	% inhibition			
	30min	1h	3h	6h
Diclofenac sodium (10 mg.kg^{-1})	38.46	29.09	31.74	44.28
MB-17 (10 mg.kg^{-1})	21.15	18.18	25.39	31.42
MB-18 (10 mg.kg^{-1})	36.53	30.90	33.33	42.85

The induction of paw edema by carrageenan is a biphasic event. Inflammation involves three distinct phases of mediator release. The inhibitory effect produced by compounds MB-17 and MB-18 was observed at the 1st hour and the effect reached maximum and lasted till the 6th hour. The first phase observed during the first hour is attributed to the release of histamine and serotonin. The second phase is due to the release of prostaglandins, proteases and lysosome and the third one by granuloma formation. The synthesized compounds MB-17 and MB-18 have significant reduction in carrageenan induced paw edema when compared to control. But the compound MB-18 at a dose of 10 mg.kg^{-1}bw produced percentage inhibition of inflammation near to the effect of standard drug Diclofenac sodium. The percentage inhibition was significant (p<0.01) at a dose level of 10mg when compared to standard.

CONCLUSION

In summary, triazole derivatives have gained remarkable importance in recent years due to their wide variety of biological activities. Various 1, 2, 4-triazole derivatives were designed, synthesized and evaluated for their anticancer and anti-tubercular activities in our previous efforts. In continuation of the present study, it was aimed to design, synthesize and evaluate the anti-inflammatory activity of some new 1, 2, 4-triazole derivatives. Based on the particular objective, 55 derivatives were proposed

and subjected to *in-silico* molecular evaluation by using suitable software. Among them, two compounds, named MB-17 and MB-18 were found as eligible candidate for the synthesis and *in-vivo* evaluation of anti-inflammatory activity. These two were synthesized by conventional methods and characterized by FTIR, ^{1}H NMR, ^{13}C NMR and Mass spectral evaluation. The *in-vivo* anti-inflammatory evaluation of both MB-17 and MB-18 showed anti-inflammatory activity. But the compound MB-18 exhibited significant anti-inflammatory activity indicating its potential. Therefore, MB-18 should be extensively studied further to develop a new lead compound for the treatment of inflammation.

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