IJPSR (2011), Vol. 2, Issue 1 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 02 August, 2010; received in revised form 14 November, 2010; accepted 26 December, 2010

SYNTHESIS, CHARACTERIZATION, BIOLOGICAL EVALUATION AND ADME STUDIES USING IN SILICO TECHNIQUES OF NOVEL DERIVATIVES OF BENZOTHIAZOLYL-AMIDES AS NON-ACIDIC ANTI-INFLAMMATORY AGENTS

V. S. Velingkar*, D. C. Ahire, N. S. Kolhe, M.M. Shidore and G. Pokharna

Department of Pharmaceutical Chemistry, Principal K. M. Kundnani College of Pharmacy, Jote joy Bldg. Plot no. 23, Rambhau Salgaonkar Marg, Cuffe Parade, Colaba, Mumbai, Maharashtra, India

Keywords:

Benzothiazole,
Non-Acidic NSAIDs,
QSAR,
QikProp,
ADME

Correspondence to Author:

V. S. Velingkar

Department of Pharmaceutical Chemistry, Principal K. M. Kundnani College of Pharmacy, Jote joy Bldg. Plot no. 23, Rambhau Salgaonkar Marg, Cuffe Parade, Colaba, Mumbai, Maharashtra, India

ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs) have been used to treat various ailments for over 100 years. As a class, these drugs possess anti-inflammatory, anti-allergy, analgesic and antipyretic activity. A series of benzothiazolyl-amides has been synthesized and tested for anti-inflammatory activity. Basic NSAIDs are gaining interest because of their improved biological properties with the aim of improving the therapeutic index through prevention of gastrointestinal complications. The title compounds were identified by performing Thin Layer Chromatography and recording their physical constants. They were further characterized by spectral analysis (IR, ^{1H}NMR, Mass spectroscopy). The effect of the synthesized compounds on inflammation, using the carageenan induced mouse paw edema model was studied. In general, the studied compounds were found to be potent anti-inflammatory agents. Anti-inflammatory activity was influenced by some structural characteristics of the synthesized compounds. Some physicochemical parameters and ADME Properties were computed by using software programs.

INTRODUCTION: Non-steroidal anti-inflammatory drugs (NSAIDs) have been used to treat various ailments for over 100 years. As a class, these drugs possess anti-inflammatory, anti-allergy, analgesic and antipyretic activity and are widely used to treat chronic inflammatory states such as arthritis, psoriasis and asthma. These agents exhibit an inhibitory action on the cyclooxygenase (COX) that catalyses the biosynthesis of prostaglandins and thromboxane from arachidonic acid.

In recent years two COX enzymes, COX-1 and COX-2 have been discovered and it is agreed, that inhibition of COX-2 is more selective for anti-inflammatory effect [1-3]. All NSAIDs are approximately equivalent in terms of anti-inflammatory efficacy but also cause untoward side effects (e.g. in gastrointestinal), in a significant fraction of treated patients and this frequently limits therapy.

Most NSAIDs are weak acids with pK_a values ranging from 3 to 5 [4] and their side effects locally connected to their acidic character. Thus, there is an increased interest in the development of effective non-acidic anti-inflammatory agents. Basic NSAIDs are gaining interest because of their improved biological properties.

Benzothiazolyl ring derivatives are known to possess anti-inflammatory as well as antipyretic activities [5, 6]. Number of benzothiazolyl-aminoketones was found to be strong anti-inflammatory agents [7, 8, 9] prompted us to investigate other analogs. NSAIDs have a broad spectrum of effects and it has been suggested that the variations in both efficacy and their tolerability are partly due to differences in their physicochemical properties which determine their distribution in the body and their ability to pass through and to enter the interior of membranes [10, 11]. Thus, partition coefficients (log *P* values), % Human Oral Absorption in GI, QP log BB for brain/blood were predicted.

MATERIALS AND METHODS:

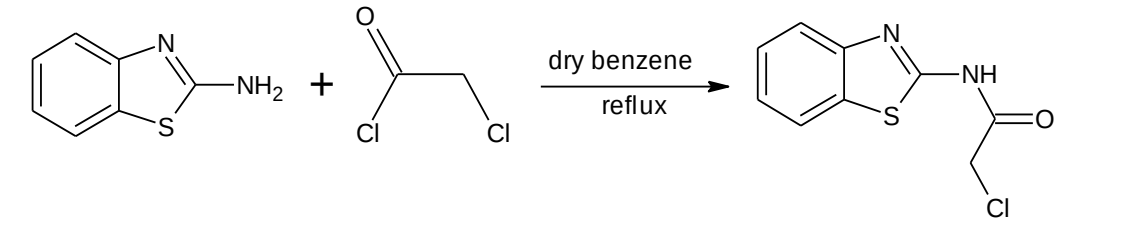
Materials: All other chemicals used were of analytical grade procured from institutional store and solvents were of Analytical grade utilized after distillation. Melting points were determined by open capillary method and reported without correction, which were further confirmed by visual melting point apparatus (Lab India). Thin layer chromatography (TLC) was carried out on precoated silica gel plates (Merck, 60GF254) using UV light as detecting agent. UV spectra were recorded on a UV-Visible Spectrophotometer (Jasco V-30). IR spectra were recorded with a FTIR-4200, Schimadzu spectrometer. 1H NMR spectra were recorded in $CDCl_3$ using 300 MHz ^{1}HNMR Spectrometer (JEOL-FT-NMR-300 mHz, Japan). Mass spectra were recorded on GCMS-QP-2010-Schimadzu, at Indian Institute of Technology, Powai, Mumbai. Prior permission has been taken for the conduction of *In vivo* studies in animals.

Synthesis:

Synthesis of precursors [1]: 2-Aminothiazole was synthesized as described previously using a general synthetic procedure for the synthesis of the 2-chloroacetamido benzothiazole[1]. In a 100 ml round bottom flask fitted with water condenser, were placed, 2-aminobenzothiazole (1.5 gm, 0.02 mole) and 4 ml of dry benzene. To this, chloroacetyl chloride (1.8 ml, 0.02 mole) was added drop wise. Then reaction mixture was refluxed on water bath at 80°C for 3 hr. after completion of reaction, excess of chloroacetylchloride was removed under reduced pressure. The resulting solid residue was washed with aqueous solution of 5% sodium bicarbonate (50 ml) followed by ice cold water. The resulting crude product of 2-chloroacetamido benzothiazole thus obtained was recrystallized from ethanol (**Fig. 1**).

General Procedure for the syntheses of Substituted 2-acetamido-*N*-1, 3-benzothiazole (Title compounds): In a 100 ml round bottom flask equipped with water condenser, were placed *N*- 1, 3- benzothiazol- 2- yl- 2-

chloroacetamide, substituted amine and 0.5 gm of anhydrous sodium carbonate. To this reaction mixture 15ml absolute ethanol added and refluxed for 3-6 hr. After completion of reaction, resulting solution was concentrated under reduced pressure. The residue was washed several times with ice cold water and dried. Purification of the title compounds thus obtain was carried out by using column chromatography using various solvent systems.



1,3-benzothiazol-2-amine chloroacetyl chloride *N*-1,3-benzothiazol-2-yl-2-chloroacetamide

FIG. 1: SYNTHESIS OF PRECURSORS

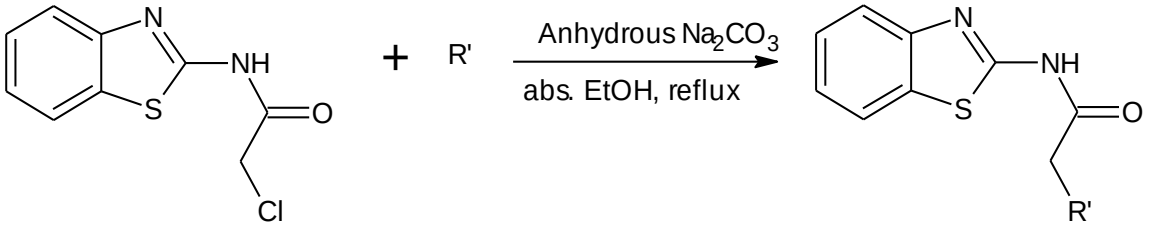


FIG. 2: GENERAL PROCEDURE FOR THE SYNTHESES OF SUBSTITUTED 2- ACETAMIDO- *N*- 1, 3- BENZOTHIAZOLE (TITLE COMPOUNDS)

R': Substituted Primary / Secondary Amine

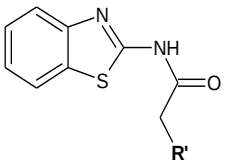


TABLE 1: REACTION DETAILS FOR THE SYNTHESES OF TITLE COMPOUNDS

R'	Yield (%)	M. P. (°C)	R_f	Mass	Molecular structure
o-toludine	52	118-120	0.75	297.37	$C_{16}H_{15}N_3OS$
m-toludine	45	110-112	0.72	297.37	$C_{16}H_{15}N_3OS$
p-anisidine	52	108-110	0.70	313.37	$C_{16}H_{15}N_3O_2S$
o-anisidine	48	112-114	0.65	313.37	$C_{16}H_{15}N_3O_2S$
Aniline	68	130-13	0.58	283.34	$C_{15}H_{13}N_3OS$

- ***N*- 1, 3- benzothiazol- 2-yl- 2-[(2-methylphenyl) amino] acetamide**:

IR: Frequency (Cm^{-1}): 1731 (-CONH stretch), 3323 (N-H stretch), 1633 (Aromatic stretch), 2856-2985 (Aliphatic C-H stretch).

1H-NMR: (DMSO) δ: 8.319 (S, 1H,–NH of amide), 2.490 (S, 3H, CH_3 of Benzene), 6.97-7.60 (M, 8H, phenyl), 3.806 (S, 2H, Methylene linkage), 4.103 (S, 1H, Aromatic C-NH).

MS: M^+ 298 (94%)

- ***N*-1, 3-benzothiazol-2-yl-2-[(3-methylphenyl) amino] acetamide:**

IR: Frequency (Cm^{-1}): 2950-2991(Aliphatic C-H stretch), 3328 (N-H stretch (amide), 1731(-CONH stretch), 1600-1668(Aromatic stretch).

1H-NMR: (DMSO) δ: 6.41-7.80 (M, 9H, Aromatic Region and NH of amide), 2.490 (S, 3H, CH_3 of Benzene), 3.899 (S, 2H, Methylene linkage), 4.10 (S, 1H, Aromatic C-NH)

MS: M^+298(41%).

- ***N*- 1, 3- benzothiazol- 2- yl- 2- [(2-methoxyphenyl) amino] acetamide:**

IR: Frequency (Cm^{-1}): 1600-1630 (Aromatic stretch), 2850-2950 (Aliphatic C-H stretch), 3329 (N-H stretch), 1695 (-CONH stretch), 1570-1620 (Aromatic stretch)
1H-NMR: (DMSO) δ: 6.80-7.63 (M, 8H, Aromatic Region), 8.86 (S, 1H, –NH of amide), 3.763 (S, 3H, CH_3 of Benzene), 3.899 (S, 2H, Methylene linkage), 5.027 (S, 1H, Aromatic C-NH)
MS: M^+298(41%).

- ***N*- 1, 3- benzothiazol- 2-yl-2-[(4-methoxyphenyl) amino] acetamide:**

IR: Frequency (cm^{-1}): 2960 (Aliphatic C-H stretch), 3400 (N-H stretch, amide), 1695 (-CONH stretch), 1628-1650 (Aromatic stretch).
1H-NMR: (DMSO) δ: 6.97-7.73 (M, 8H, Aromatic Region), 6.726 (S, 1H, –NH of amide), 3.461 (S, 3H, CH_3 of Benzene), 3.691 (S, 2H, Methylene linkage), 4.177 (S, 1H, Aromatic C-NH).
MS: M^+312 (52%).

- **2-anilino-*N*-1, 3-benzothiazol-2-yl-acetamide:**

IR: Frequency (Cm^{-1}): 2926 (Aliphatic C-H stretch), 3420 (N-H stretch, amide), 1675 (-CONH stretch), 1632-1700 (Aromatic stretch).
1H-NMR: (DMSO) δ: 7.08-7.96 (M, 8H, Aromatic Region), 6.064 (S, 1H, –NH of amide), 4.077 (S, 1H, Aromatic –NH), 3.667 (S, 2H, Methylene linkage).
MS: M^+284 (33%).

Physicochemical studies: Thin Layer Chromatography (TLC) was performed on silica gel plates. Mobile phase: methanol/water mixture (70:30, v/v) containing 2% aqueous ammonia (27%). The plates were developed in closed chromatography tanks saturated with the mobile phase at 24°C. Spots were detected under UV light. Theoretical calculations of lipophilicity as clog *P* and Suzuki-Kudo's method was performed. The program CLOG *P* has been designed to calculate the lipophilicity of a molecule using software programmer *Quiprop.*

Inhibition of Carrageenan induced paw oedema: Oedema was induced in the right hind paw of Albino wistar rats weighing between 100-150 gms were divided into 5 groups of 6 rats each. The rats were injected subcutaneously 0.1ml of 1% (w/v) of carageenan into the planter region of each hind-paw.

Title compounds were evaluated at single dose level; Diclofenac was used as standard anti-inflammatory drug for comparison. The control animals were treated with vehicle (sodium CMC solution) instead of drugs. The paw edema volumes were measured using plethysmometer at various time intervals like 0, 1, 2, 3, 4, 6 hrs after carageenan injection. The hind paw edema inhibition at doses of test drug and standard was calculated by comparing with vehicle treated control rats. Figure 00, Table 00. The anti-inflammatory activity of test drug was studied at single dose level. The % inhibition of paw edema volume by the test compound or standard anti-inflammatory drug (Diclofenac) was calculated by the formula:

$$\%\text{inhibition of paw edema} = \frac{(Vt\text{-}Vo)_{control} - (Vt\text{-}Vo)_{treated}}{(Vt\text{-}Vo)_{control}} \times 100$$

Where,

Vt is the rat paw volume at time't'; Vo is the initial rat paw volume (before egg albumin injection); $(Vt\text{-}Vo)_{control}$ is edema produced in control group and $(Vt\text{-}Vo)_{treated}$ is edema produced in treatment groups.

The results obtained for anti-inflammatory activity are shown in **Table 2**.

RESULTS AND DISCUSSION:

Chemistry: The general method employed is shown in Fig. 1. The structure of the derivatives and their physicochemical properties are given in Table 1. The compounds of the title were prepared in two steps. Initial amino/substituted amino benzothiazoles were treated with chloracetyl chloride in benzene to give the corresponding chloroacetamides, which in the

second step undergo condensation with different primary, secondary amines in ethanol to give the title compounds. Overall the reactions proceed smoothly in good yields. The amides were identified both by elemental analyses as well as by their spectroscopic analyses. The structure of all compounds was identified both by elemental analyses as well as by spectroscopic analysis (IR, ^{1}H-NMR, MS). The IR spectra were in agreement with the proposed structures: In IR spectra showed a sharp band in the region 1695-1731 cm−1 (NHC=O), as well as in region 1600-1650 cm−1 (aromatic). Coordination of aromatic and side chain protons was observed in the^{1}H-NMR spectra and the exact number of protons was given by integration. In the MS spectra almost all substances gave stable molecular ions. The lipophilicity of the synthesized compounds expressed as R_M values ranging from −0.204 - 0.618, as well as calculated theoretically log *P* values are given in Table 2. Poor correlation was obtained between R_M and clog *P* values.

Biological studies: Basic anti-inflammatory agents are gaining interest because they possess better pharmacokinetic properties and cause less gastric irritation compared to acidic agents. The in vivo anti-inflammatory effects of the synthesized amides were assessed by using the functional model of carageenan induced mouse paw edema the most frequently used model for anti-inflammatory activity (**Table 3**), as percentage of weight increase at the right hind paw in comparison to the uninjected left hind paw. We have previously reported that Benzothiazolyl-amides demonstrated well anti inflammatory activity. Thus, with modifications performed with the synthesis of more compounds presented herein we further elucidated the relative significance of structural features for activity.

TABLE 2: LIPOPHILICITY VALUES

R'	Clog *P* for octanol/water	QP log P for water/gas	QP log P for octanol/gas	QP log BB for brain/blood	CPE %
o-toludine	3.337	11.004	17.108	-0.254	68
m-toludine	3.286	11.012	17.131	-0.372	67
p-anisidine	3.135	11.546	17.434	-0.391	54
o-anisidine	3.087	11.522	17.407	-0.424	62
Aniline	2.990	11.315	16.677	-0.350	68

Lipophilicity prediction using software programmer:

i) Quiprop version 3.0

ii) Hyperchem version 6.0

a) Clog *P* for octanol/water (-2.0 / 6.5)

b) QP log P for water/gas (4.0 / 45.0)

c) QP log P for octanol/gas (8.0 / 35.0)

d) QP log BB for brain/blood (-3.0 / 1.2)

e) In vivo carageenan rat paw oedema % inhibition (CPE %)

TABLE 3: THE RESULTS OBTAINED FOR ANTI-INFLAMMATORY ACTIVITY

Treatment and Dose (mg/kg)	Edema volume (ml) Time Interval (hr)					
	0 hr.	1 hr.	2 hr.	3 hr.	4 hr.	6 hr.
Control	0.84 ± 0.021	1.14± 0.0293	1.24± 0.053	1.47± 0.032	1.30±0.032	1.24±0.0761
Test 1 (10 mg/kg)	0.89± 0.027	0.708±0.05748(45)**	0.57± 0.052(57)	0.52±0.0395(68)**	0.47+0.026(54)**	0.6±0.081(47)**
Test 2 (10 mg/kg)	0.77±0.032	0.698±0.040(39)**	0.43±0.078(65)	0.483±0.060(67)**	0.493+0.062(62)**	0.58±0.057(53)**
Test 3 (10 mg/kg)	0.89±0.04279	0.72±0.06108 (45)**	0.55±0.07079(57)	0.48±0.08449(68)**	0.51±0.07055(54)**	0.65±0.05845(47)**
Test 4 (10 mg/kg)	0.75±0.06318	0.57±0.075089(58)**	0.75±0.063 (41)**	0.50±0.079 (67)**	0.52±0.072 (62)**	0.65±0.059 (48)**
Test 5 (10 mg/kg)	0.87±0.015	0.723±0.043 (45)**	0.53± 0.047(57)	0.49±0.077(68)**	0.54+0.062(54)**	0.62±0.051(47)**
Standard (10 mg/kg)	0.861±0.048	0.67±0.011(50)**	0.67±0.052(61)	0..52±0.080(72)**	0.44±0.085(56)**	0.64±0.0964(49)**

The main chemical features of the synthesized amides can be divided into four basic parts:

1. A substituted aromatic heterocyclic group (benzothiazolyl)
2. An amide substitution
3. A central ligand consisting of a NHCO amide group
4. A methylenic linkage (CH_2) n

The nature of the moieties (1) and (4) seems to have a major effect on the activity. We tried to delineate the contribution of the different primary, secondary amines in the biological activity. Compounds showed higher lipophilicity values. Title compounds were compared with the previous (piperazine derivatives) compounds it seems that the new compounds inhibit more the carageenan induced mice paw oedema. The tested compounds did not present any side effects from the gastrointestinal route because of its non-acidic in nature.

ACKNOWLEDGEMENTS: The authors are thankful to Prin. K. M. Kundnani College of Pharmacy Colaba, Mumbai, for providing splendid library and laboratory facilities to carry out research work.

REFERENCES:

1. Kasture A V, Synthesis and biological activities of some substituted benzimidazole and benzothiazole derivatives. Phd. Thesis, Nagpur University, 1980. 50.
2. Smith C J, Zhang Y, Koboldt C M, Muhammad J, Zweifel B S, Shaffer A, Talley J J, Masferre J L, Seibert K, Iskson P C, Pharma-cological analysis of cycloxygenase-1 in inflammation, Proc. Natl. Acad. Sci. USA 95 (1998) 1313–13318.
3. Winter C, Risley E, Nuss G, Carrageenan-induced oedema in hind paw of the rat as an assay for anti-inflammatory drugs, Proc. Soc. Exp. Biol. Med. 111 (1962) 544–547.
4. Hawkey C J, COX-2 inhibitors, Lancet 353 (1999) 307–331.
5. Brooks P M, Day R O, Non steroidal anti- inflammatory drugs- differences and similarities, N. Engl. J. Med. 324 (1988) 1716–1725.
6. Shanbag V R, Crider M A, Gohkale R, Harpalani A, Dick R M, Ester amide and prodrugs of ibuprofen and naproxen. Synthesis, anti-inflammatory activity and gastrointestinal toxicity, J. Pharm. Sci. 81 (1992) 149–154.
7. Pignatello R, Mazzone S, Panico A M, Mazzone G, Penissi G, Castano R, Matera M, Blandino G, Synthesis and biological evaluation of thiazolo-triazole derivatives, Eur. J. Med. Chem. 26 (1991), 929–938.
8. Hadjipavlou-Litina D, Geronikaki A, Sotiropoulou E, Anti inflammatory activity of aminoketones derivatives of 2,4-disubstituted thiazoles, Res. Commun. Chem. Pathol. Pharmacol. 79 (1993) 355–362.
9. Geronikaki A, Hadjipavlou -Litina D, Lipophilicity and biological studies of some 2-(amino-acetylamino) thiazole derivatives with anti-inflammatory activity, Pharmazie 48 (1993) 948–949.
10. Hadjipavlou-Litina D, Geronikaki A, Mgonzo R, Doytchinova I, Thiazolyl-*N*-substituted amides: Agroup of effective anti-inflammatory agents with potential for local anesthetic properties. Synthesis, biological evaluation, and QSAR approach, Drug Dev. Res. 48 (1999) 53–60.
11. Day R O, Graham G G, Williams K M, Brooks P M, Variability in response to NSAIDs. Fact or Fiction? Drugs 36 (1988) 643–651.
12. Ellis G A, Blake D R, Why are non-steroidal anti inflammatory drugs so variable in their efficacy? A description of ion trapping, Ann. Rheum. Dis. 52 (1993) 241–243.
13. A. Geronikaki, Theophilidis G, Synthesis of 2-(aminoacetylamino) thiazole derivatives and comparison of their local anaesthetic activity by the method of action potential, Eur. J. Med. Chem. 27 (1992) 1–9.
14. Suzuki T, Kudo Y, Automatic log p estimation based on combined additive modelling methods, J. Comput. Aided Mol. Des. 4 (1990), 155–198.
15. Clog P. Biobyte Corp. Claremont, CA 91711, USA.

****