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Synthesis of 4-benzyl-1,3-thiazole derivatives as potential anti-inflammatory agents: An analogue-based drug design approach

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Abstract

A series of novel 4-Benzyl-1,3-thiazole derivatives was synthesized by applying analogue-based drug design approach and they were screened for anti-inflammatory activity. Darbufelone (CI 1004) a dual COX/LOX inhibitor, served as a lead molecule for designing a molecular scaffold. The derivatives with the 1,3 thiazole molecular scaffold bearing a side chain at position-2 resembling that of Romazarit (Ro-31-3948) were synthesized. The substitution at the second position of thiazole scaffold consisted of either carbalkoxy amino or aryl amino side chain. The introduction of an NH linker at the second position was the bioisoteric approach to impart the metabolic stability to the carbalkoxy side chains in designed molecules so as to avoid the likelihood of generating toxic moieties, like in Romazarit, which was withdrawn due to its toxicity profile. An important outcome of this study is the optimization of the substitution at the second position of the thiazole scaffold in eliciting better biological activity. The biological activity exhibited by the two designed series were in the order of carbalkoxy amino series > phenyl amino series. Molecule RS31 had emerged to be best compound in the whole series, having the side chain -NH-(C=O)O-R which resemble to Romazerit with 1,3 thiazole scaffold and substituted phenyl carbonyl group at fifth position derived from the retro-analysis of Darbufelone. This novel three-point pharmacophore, which is necessarily evolved from a lead-based drug design strategy, has opened up new avenues in designing of molecules acting on more than one rate-limiting step along the inflammatory cascade.

Keywords: *Romazarit, anti-inflammatory, 4-benzyl-1,3-thiazole, Darbufelone, analogue design, bioisosterism*

Introduction

Treatment of inflammatory diseases today is largely based on interrupting the synthesis or action of various mediators that drive the host's response to injury. The blockade of prostaglandin synthesis by inhibition of cyclo-oxygenase enzyme is the common basis by which the several NSAIDs act to get therapeutic effect. However, several structurally and functionally unrelated small-molecule inhibitors used in the symptomatic treatment of inflammation exhibit considerable side effects. Moreover discovery of COX-2 isoform and subsequently their inhibitors, which were thought to have lesser gastrointestinal irritation or hemorrhage then traditional NSAIDs, have also been reported to possess increased risk of myocardial infarction and cardiovascular thrombotic events. Likewise two major isoforms 5- and 15-LOX are known to induce undesirable physiological effects in humans and are associated with inflammation, hypersensitivity, asthma and atherosclerosis [1].

Considering the multi-origin nature of inflammatory diseases with several pathways and involving a

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number of rate limiting steps, it seemed appropriate to look for candidates acting on more than one pathway [2]. In comparison with simple COX inhibitors, compounds with dual inhibitory activities against COX and 5-LOX, such as KME-4, E-5110, S-2474, Tenidap, CI-987 and 3,5-di-tert-butyl-4-hydroxyphenyl derivatives are reported to be superior and have been studied as potential anti-inflammatory agents with an improved safety profile [3–5].

Darbufelone (CI-1004), ((*Z*)-5-[[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]methyllene]-2-imino-4-thiazolidinone, methanesulfonate salt is a novel anti-inflammatory agent. It is di-*tert*-butyl phenol with thiazole molecular scaffold and has been identified as a dual inhibitor of cellular PGF_2-alpha (COX pathway) and LTB_4 (LOX pathway) production [4,6]. Darbufelone is orally active and has been shown to be non-ulcerogenic in animal model of inflammation and arthritis [4,6].

The interesting pharmacological profile of Darbufelone was sufficient to consider its structure appropriate for probable pharmacophore. Studies on derivatives of thiazole, a pi-excessive and pi-deficient ring as molecular scaffold in designing better anti-inflammatory agents conducted by our group established that the benzyl substitution at fourth position serves as a pharmacophore [7]. We anticipated that some novel potential dual acting anti-inflammatory agents could be designed through careful modification of thiazole molecular scaffold obtained as a result of the retro-analysis of Darbufelone (Scheme 1), that contains phenyl carbonyl side chain at fifth position, and carbalkoxy amino or phenyl amino side chain at second position [8,9].

The side chains substituted at second position in the designed molecule as well as overall structure was so selected to resemble the structure of Romazarit. Romazarit, the Roche candidate (Ro-31-3948), (2-[{2-(4-chlorophenyl)-4-methyl-5-oxazolyl}methoxy]-2-methyl propionic acid sodium salt, which is known to show a desirable activity profile in various biological test systems and animal models indicating its efficacy in arresting more than one destructive pathway in the inflammatory cascade. It was withdrawn from the phase II clinical trials due to the adverse effects (bladder tumor) that emerged during chronic toxicity studies [10]. The peroxy derivative and alpha-methyl acrylic acid is generated from the metabolic hydroxylation of the CH_2 group at fifth position of Romazarit (Scheme 2), either of these could be responsible for the occurrence of bladder tumor [11].

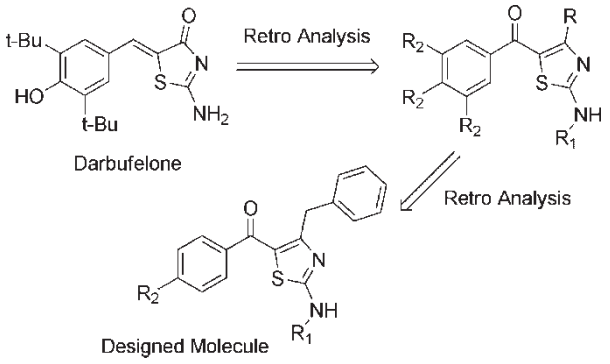
Thus we attempted to design thiazole analogues (Scheme 3) taking Darbufelone as the lead and by bioisosteric replacement of CH_2 at fifth position of Romazarit side chain with NH such that they are unlikely to produce either peroxy derivatives or alpha-methyl acrylic acid (Scheme 4).

The present research deals with the design, synthesis and pharmacological evaluation of some 4-benzyle-1,3-thiazole derivatives with phenyl carbonyl and carbalkoxy amine or phenyl amine as the side chains, exemplifying the importance of analog-based drug design leading to development of potential dual acting anti-inflammatory agents.

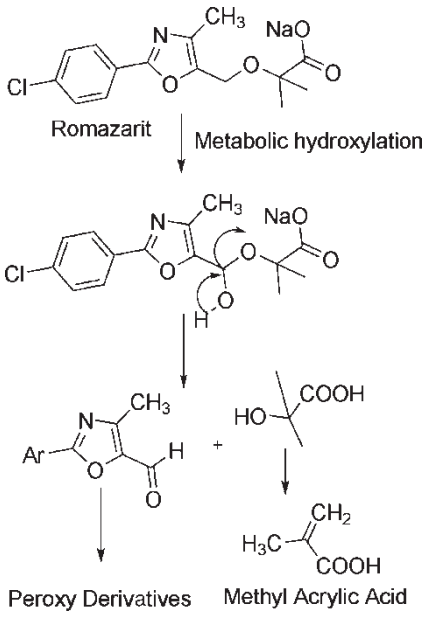
Materials and methods

Chemistry

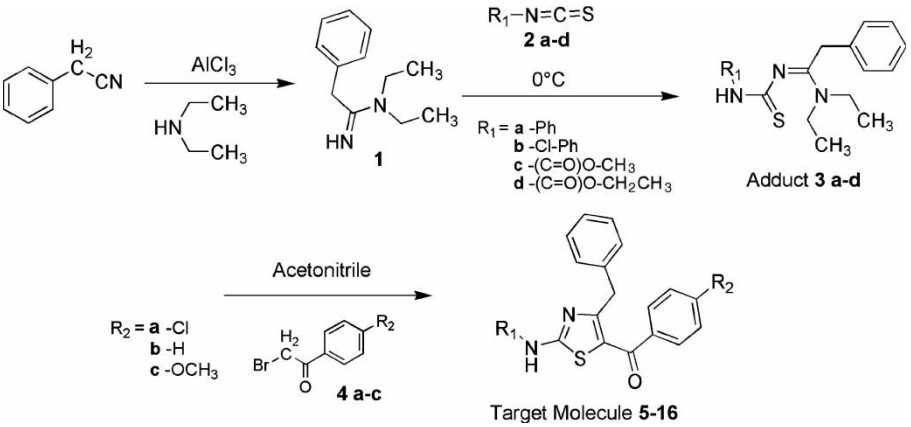
Carrageenan was purchased from spectrochem. Ibuprofen was a gift sample from AVIK Pharmaceuticals, Mumbai. Rofecoxib was procured from Cadila Pharmaceuticals, Ahmedabad. Caffeic acid, and nordihydroguaiaretic acid (NDGA) were purchased from Cayman Chemicals Inc., Ann Arbor, MI, USA. Bromine was



Scheme 1. Retroanalysis of Darbufelone (CI1004), a dual COX/LOX inhibitor. Pharmacophore generated from structure analysis of Darbufelone.

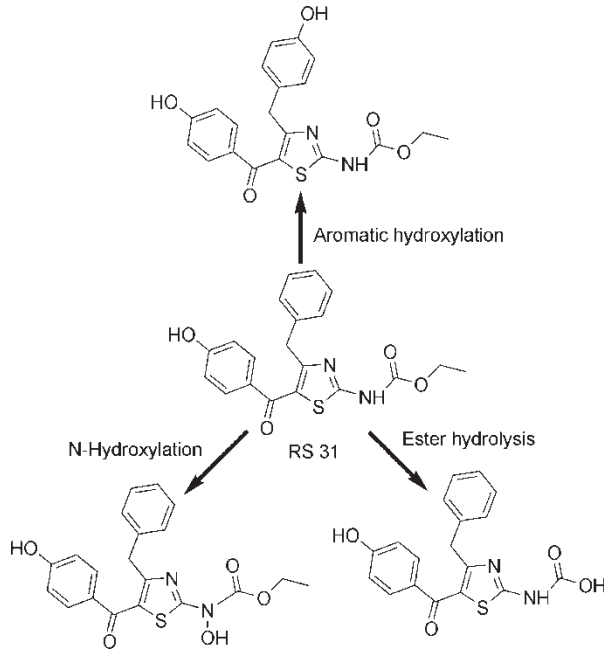


Scheme 2. Probable mechanism of metabolic toxicity exhibited by Romazarit. Peroxy type radicals or methyl acrylic acid moiety might be responsible for the bladder tumor formation.



Scheme 3. Synthetic route for the different 4-benzyl-1,3-thiazole derivatives **5-16**.

purchased from S.D. fine chemicals. The melting point of the compounds was taken in open capillaries and is uncorrected. The infrared spectra were recorded using KBr as the medium, utilizing Buck Scientific M-500 Infrared spectrophotometer. The mass of all the compounds was recorded on a Perkin Elmer Sciex atmospheric pressure ionization liquid chromatography mass instrument (LC-MS) at PERD centre, Ahmedabad. ^{1}H NMR was recorded on 60-300 MHz instruments from different sources. Elemental analysis was taken on Heraeus Carlo Erba 1108 elemental analyzer at CDRI, Lucknow. All the reactions were monitored using TLC plates precoated with Silica gel G or GF_{254} and were detected under UV at 254 nm.



Scheme 4. Probable metabolic pathways of most potent compound **RS31**. The bioisosteric replacement of CH_2 group at second position by NH obstructs the pathway that may generate peroxy type radicals or methyl acrylic acid moiety.

The designed compounds were synthesized by the previously reported protocol [12] as depicted in Scheme 3. The starting material N,N-diethyl benzylamidine was synthesized using modified benzamidine synthesis [13]. Aromatic isothiocyanates were synthesized using the modified Kaluza method and carbonyl isothiocyanate by Esmail method [14,15].

*Synthesis of 4-benzyl-1,3-thiazole derivatives (**RS11- 43**)*. All the target compounds (RS 11-43) were synthesized by employing same procedure using different adducts (**3a-d**) and alpha-heloketones (**4a-c**) [12]. For example adduct **3c** (0.50 gm, 1.56 mmol) and 2-bromo-1(4-chlorophenyl)ethanone **4a** (0.36 gm, 1.56 mmol) were mixed with acetonitrile and stirred for 4-6 h. Reaction was monitored by TLC using toluene: ethyl acetate (7:3) as mobile phase. The solid obtained was filtered and washed by hexane. The crude product thus obtained was recrystallized using methanol to get pure crystals of target compound (**RS33**). All the synthesized compounds were subjected to physical and spectral characterizations.

*(2-Anilino-4-benzyl-1,3-thiazol-5-yl) (4-methoxyphenyl)methanone (**RS11**)*. Mp (°C) 136-137, R_f 0.67, Anal. Calcd /found for C_{24} H_{20} N_2 O_2 S: C, 71.29/ 71.89; H, 4.94/ 4.90; N, 7.23/6.99%. IR KBr (cm^{-1}) 3173 (NH), 2930 (CH_2), 1623 (C=O). LC-MS (M + 1)m/z 401.3. ^{1}H-NMR ($CDCl_3$ δ ppm): 10.12 (1H, s, NH); 7.81-7.79 (2H, d, C=O-Ar-$H_{2,6}$); 7.64-7.62 (2H, d, NH-Ar $H_{2,6}$); 7.33-6.81 (10H, m, Ar-H); 3.83 (3H, s, O-CH_3); 3.80 (2H, s, CH_2).

*(2-Anilino-4-benzyl-1,3-thiazol-5-yl) (phenyl)methanone (**RS12**)*. Mp (°C) 176-178, R_f 0.66, Anal. Calcd / found for C_{23} H_{18} N_2 O S: C, 73.92/73.78; H, 4.79/ 4.67; N, 7.84/7.79%. IR KBr (cm^{-1}) 3168 (NH), 2925 (CH_2), 1598 (C=O). LC-MS (M + 1)m/z 371.3. ^{1}H-NMR ($CDCl_3$ δ ppm): 10.10 (1H, s, NH); 7.89-7.88 (2H, d, C=O-Ar-$H_{2,6}$); 7.72-6.80 (11H, m, Ar-H); 3.81(2H, s, CH_2).

*(2-Anilino-4-benzyl-1,3-thiazol-5-yl) (4-chlorophenyl)methanone (**RS13**)*. Mp (°C) 140-141, R_f 0.73, Anal. Calcd /found for C_{23} $H_{17}Cl$ N_2 O S: C, 68.22/ 68.68; H, 4.23/ 4.15; N, 6.92/6.81%. IR KBr (cm^{-1}) 3171 (NH), 2930 (CH_2), 1628 (C=O). LC-MS (M + 1)m/z 405.5. ^{1}H-NMR ($CDCl_3$ δ ppm): 9.92 (1H, s, NH); 7.96-7.89 (2H, d, C=O-Ar-$H_{2,6}$); 7.69-7.67 (2H, d, Cl-Ar-$H_{2,6}$); 7.64-7.61 (2H, d, NH-Ar-$H_{2,6}$); 7.34-6.81 (8H, m, Ar-H); 3.80 (2H, s, CH_2).

*{4-Benzyl-2-[(4-chlorophenyl) amino]-1,3-thiazol-5-yl}(4-methoxyphenyl) methanone (**RS21**)*. Mp (°C) 161-163, R_f 0.69, Anal. Calcd /found for C_{24} $H_{19}Cl$ N_2 O_2 S: C, 66.28/ 65.34; H, 4.40/ 4.54; N, 6.44/5.98%. IR KBr (cm^{-1}) 3217 (NH), 2926 (CH_2), 1623 (C=O). LC-MS (M + 1)m/z 435.2. ^{1}H-NMR ($CDCl_3$ δ ppm): 10.11(1H, s, NH); 7.80-7.79 (2H, d, C=O-Ar $H_{2,6}$); 7.67-7.66 (2H, d, NH-Ar-$H_{2,6}$); 7.33-7.13 (9H, m, Ar-H); 3.84 (3H, s, OCH_3); 3.81(2H, s, CH_2).

*{4-Benzyl-2-[(4-chlorophenyl) amino]-1,3-thiazol-5-yl}(phenyl)methanone (**RS22**)*. Mp (°C) 151-153, R_f 0.70, Anal. Calcd /found for C_{23} $H_{17}Cl$ N_2 O S: C, 67.43/ 67.21; H, 4.12/ 3.95; N, 7.15/6.92%. IR KBr (cm^{-1}) 3213 (NH), 2928 (CH_2), 1626 (C=O). LC-MS (M + 1)m/z 405.9. ^{1}H-NMR ($CDCl_3$ δ ppm): 10.21 (1H, s, NH); 7.90-7.89 (2H, d, C=O-Ar-$H_{2,6}$); 7.72-7.22 (12H, m, Ar-H); 3.82 (2H, s, CH_2).

*{4-Benzyl-2-[(4-chlorophenyl) amino]-1,3-thiazol-5-yl}(4-chlorophenyl) methanone (**RS23**)*. Mp (°C) 151-153, R_f 0.70, Anal. Calcd /found for C_{23} $H_{16}Cl_2N_2$ O S: C, 62.88/ 61.32; H, 3.67/ 3.23; N, 6.38/6.12%. IR KBr (cm^{-1}) 3168 (NH), 2930 (CH_2), 1620 (C=O). LC-MS (M + 1)m/z 439.3. ^{1}H-NMR ($CDCl_3$ δ ppm): 9.98 (1H, s, NH); 7.97-7.95 (2H, d, C=O-Ar-$H_{2,6}$); 7.68-7.22 (11H, m, Ar-H); 3.82 (2H, s, CH_2).

*Ethyl 4-benzyl-5-(4-methoxybenzoyl)-1,3-thiazol-2-ylcarbamate (**RS31**)*. Mp (°C) 142-143, R_f 0.62, Anal. Calcd /found for C_{21} $H_{20}N_2$ O_4 S: C, 63.62/ 63.34; H, 5.08/ 4.87; N, 7.07/6.89%. IR KBr (cm^{-1}) 3168 (NH), 2983 (CH_2), 1727 (Ester), 1639 (C=O). LC-MS (M + 1)m/z 397.3. ^{1}H-NMR ($CDCl_3$ δ ppm): 9.81 (1H, s, NH); 7.91-7.72 (2H, d, C=O-Ar-$H_{2,6}$); 7.32-7.11 (10H, m, Ar-H); 4.4 (2H, q, CH_2); 3.83 (3H, s, OCH_3); 3.80 (2H, s, CH_2); 1.41 (3H, t, CH_3).

*Ethyl 5-benzoyl-4-benzyl-1,3-thiazol-2-ylcarbamate (**RS32**)*. Mp (°C) 139-140, R_f 0.70, Anal. Calcd /found for C_{20} $H_{18}N_2$ O_3 S: C, 65.55/ 64.77; H, 4.95/ 4.32; N, 7.64/7.99%. IR KBr (cm^{-1}) 3148 (NH), 2910 (CH_2), 1737 (Ester), 1637 (C=O). LC-MS (M + 1)m/z 367.3. ^{1}H-NMR ($CDCl_3$ δ ppm): 9.83(1H, s, NH); 7.89-7.81 (2H, d, C=O-Ar-$H_{2,6}$); 7.73-7.21 (8H, m, Ar-H); 4.13 (2H, q, CH_2); 3.81(2H, s, CH_2); 1.38 (3H, t, CH_3).

*Ethyl 4-benzyl-5-(4-chlorobenzoyl)-1,3-thiazol-2-ylcarbamate (**RS33**)*. Mp (°C) 145-146, R_f 0.68, Anal. Calcd /found for C_{20} $H_{17}ClN_2$ O_3 S: C, 59.92/ 59.37; H, 4.27/ 4.33; N, 6.99/6.80%. IR KBr (cm^{-1}) 3173 (NH), 2969 (CH_2), 1740 (Ester), 1642 (C=O). LC-MS (M + 1)m/z 401.6. ^{1}H-NMR ($CDCl_3$ δ ppm): 9.81(1H, s, NH); 7.91-7.72 (2H, d, C=O-Ar-$H_{2,6}$); 7.32-7.11 (7H, m, Ar-H); 4.42 (2H, q, CH2); 3.82 (2H, s, CH_2); 1.40 (3H, t, CH_3).

*Methyl 4-benzyl-5-(4-methoxybenzoyl)-1,3-thiazol-2-ylcarbamate (**RS41**)*. Mp (°C) 153-154, R_f 0.73, Anal. Calcd /found for C_{20} $H_{18}N_2$ O_4 S: C, 62.81/ 63.09; H, 4.74/ 4.36; N, 7.33/7.07%. IR KBr (cm^{-1}) 3173 (NH), 2959 (CH_2), 1732 (Ester), 1640 (C=O). LC-MS (M + 1)m/z 383.4. ^{1}H-NMR ($CDCl_3$ δ ppm): 10.81(1H, s, NH); 7.81-7.78 (2H, d, C=O-Ar-$H_{2,6}$); 7.41-7.13 (7H, m, Ar-H); 3.83 (3H, s, OCH_3); 3.81 (2H, s, CH_2); 3.68 (3H, s, CH_3).

*Methyl 5-benzoyl-4-benzyl-1,3-thiazol-2-ylcarbamate (**RS42**)*. Mp (°C) 156-157, R_f 0.82, Anal. Calcd /found for C_{19} $H_{16}N_2$ O_3 S: C, 64.76/ 63.99; H, 4.58/ 4.11; N, 7.95/7.17%. IR KBr (cm^{-1}) 3174 (NH), 2956 (CH_2), 1730 (Ester), 1641 (C=O). LC-MS (M + 1)m/z 353.4. ^{1}H-NMR ($CDCl_3$ δ ppm): 10.80 (1H, s, NH); 7.80-7.78 (2H, d, C=O-Ar-$H_{2,6}$); 7.74-7.23 (8H, m, Ar-H); 3.81 (2H, s, CH_2); 3.69 (2H, s, CH_3).

*Methyl 4-benzyl-5-(4-chlorobenzoyl)-1,3-thiazol-2-ylcarbamate (**RS43**)*. Mp (°C) 152-153, R_f 0.88, Anal. Calcd /found for C_{19} $H_{15}ClN_2$ O_3 S: C, 58.99/ 58.78; H, 3.91/ 3.06; N, 7.24/7.21%. IR KBr (cm^{-1}) 3263 (NH), 2951 (CH_2), 1700 (Ester), 1612 (C=O). LC-MS (M + 1)m/z 387.3. ^{1}H-NMR ($CDCl_3$ δ ppm): 11.17 (1H, s, NH); 7.89-7.78 (2H, d, C=O-Ar-$H_{2,6}$); 7.68-7.66 (2H, d, Cl-Ar-$H_{2,6}$); 7.34-7.23 (5H, m, Ar-H); 3.81 (2H, s, CH_2); 3.67 (3H, s, CH_3).

Biological evaluation

Anti inflammatory activity. Sprague–Dawley (male/female) rats weighing 150-250 g were used for the carrageenin induced rat paw edema test (an acute *in vivo* model) [16]. Animals were divided into 17 groups comprising six rats per group. Rats were fasted for 18 h prior to the experiment. The two different compounds were randomly chosen for dose response activity studies (Figure 1). Further, the test drugs (50 mg/kg body weight) were given orally as a suspension, in 0.1% sodium CMC as vehicle. The dose of standard drugs, Rofecoxib, Caffeic acid, nordihydroguaiaretic acid (NDGA) was 50 mg/kg while of Ibuprofen was 100 mg/kg. One hour later, 0.1 mL of 1% carrageenin in saline solution was injected in the sub plantar region of the right hind paw of each rat. After 3 h of the carrageenin injection, the reduction in paw volume compared to control was measured using plethysmography. The percentage

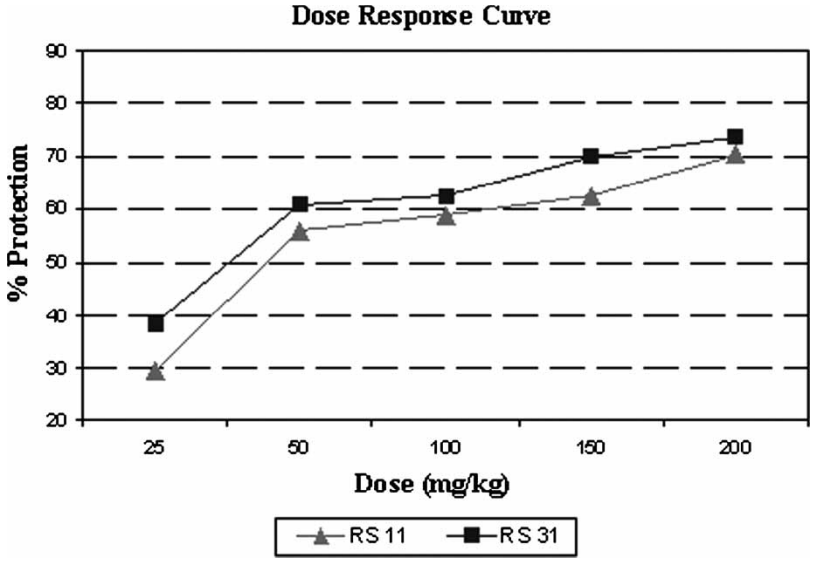


Figure 1. Dose response curves of two randomly chosen compounds **RS11** and **RS31**.

protection given to the inflamed paw was calculated using the formula-

$$\%\text{Protection} = [(\text{Control} - \text{Test})/\text{Control}] \times 100$$

The experimental protocol was duly approved by the Institutional Ethics Committee, constituted under ruling of Ministry of Social Justice and Empowerment, Government of India.

Acute toxicity (LD_{50}) [17]. The median lethal doses (LD_{50}) of the all the compounds were determined in mice. The test compounds in graded doses were injected intra-peritoneal to each of ten male adult albino mice in every group. The percentage of mortality in each group of animals was determined 24 h after injection. Graphical method was used to calculate the LD_{50} of all compounds.

Molecular modeling

The molecular modeling studies were performed on Chem3D Ultra version 8.0.3 software using windows as operating system. The geometry of most active molecule (RS31), Darbufelone and Romazarit were optimized using the molecular mechanics (MM_2) force field.

Superimposition analysis. The superimposition analysis of active molecule (RS31) with Darbufelone and Romazarit was performed using overlay method in Chem3D Ultra to ensure pharmacophore matching. The minimum RMS gradient of 0.010 was employed for this study.

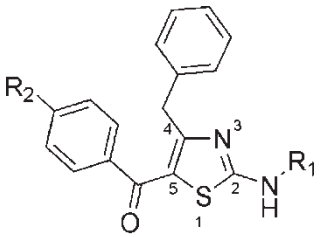
Results and discussion

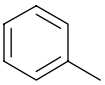
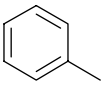
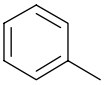
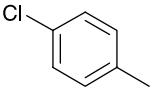
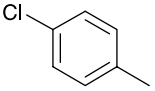
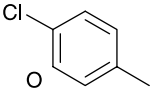
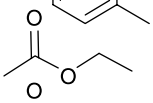
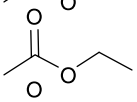
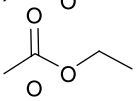
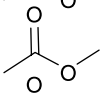
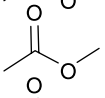
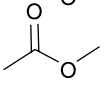
Novel 1,3-thiazole derivatives were designed and synthesized with second and fifth position having varying substitutions and fourth position with benzyl group. Synthesis of target compounds was carried out as per method shown in Scheme 3. Different isothiocyanates (**2a-d**) were reacted with N,N-diethylbenzylamidine (**1**) to give adducts (**3a-d**) that were further subjected to reaction with different alpha haloketones (**4a-c**) to provide target molecules (**RS11-43**). The structures of all the synthesized compounds were elucidated by physical characterization like-Mp., R_f value; elemental analysis and spectral analysis- IR, Mass and NMR spectroscopy.

In the target molecules second position was substituted with aryl amino or alkyl amino group, the amino group served as a linker to the thiazole scaffold. Fifth position was substituted with phenyl carbonyl or substituted phenyl carbonyl group (i.e. p-chloro, p-methoxy), here the carbonyl group served as a linker. Thiazole derivatives thus synthesized can be divided into two series depending on the substitution at position second, i.e. aryl amino function (compounds from RS11 to RS23) and carbalkoxy amino function (compounds from RS31 to RS43).

Both the above series were subjected to anti-inflammatory activity testing by carrageenan induced paw edema model in albino rats. The dose response curves of two randomly chosen compounds RS11 and RS31 (Figure 1) were used to select the oral dose (50 mg/kg). The percentage protections of test compounds were compared with 50 mg/kg of Rofecoxib, Caffeic acid, nordihydroguaiaretic acid (NDGA) and 100 mg/kg of Ibuprofen as reference drugs (Table I).

Table I. *In vivo* anti-inflammatory activity data of the 4-benzyl-1,3-thiazol derivatives.



Compound	Candidate Code	R_1	R_2	Anti-inflammatory activity [% Protection ± S.E.M] After 3 h
5	RS11		OCH_3	55.8 ± 1.21*
6	RS12		H	12.9 ± 3.65**
7	RS13		Cl	44.2 ± 1.89*
8	RS21		OCH_3	35.3 ± 2.53**
9	RS22		H	18.4 ± 3.23**
10	RS23		Cl	42.4 ± 2.21*
11	RS31		OCH_3	60.8 ± 1.45*
12	RS32		H	36.2 ± 2.13**
13	RS33		Cl	44.4 ± 2.43*
14	RS41		OCH_3	52.6 ± 1.56*
15	RS42		H	30.1 ± 2.45**
16	RS43		Cl	45.6 ± 2.31*
Standard 1	Ibuprofen			60.1 ± 1.75*
Standard 2	Rofecoxib			43.6 ± 1.23*
Standard 3	Caffeic acid			11.2 ± 2.03**
Standard 4	NDGA			21.8 ± 1.13*

The test drugs were dosed at 50 mg/kg body weight po. Ibuprofen was dosed at 100 mg/kg body weight po. Rofecoxib, Caffeic acid and NDGA were dosed at 50 mg/kg body weight po. *Significant difference at $P < 0.001$, ** Significant difference at $P < 0.05$.

Biological activity data suggest that in the case of the first series i.e. aryl amino function, activity of compounds with phenyl group (RS11-13) at R1 was higher then compounds with p-chloro phenyl group (RS21-23). The compounds with substituted phenyl carbonyl group at the fifth position were more active than unsubstituted ones. Results show that RS11(p-OCH_3) and RS13 (p-Cl) produced better percent protection of inflammation respectively as

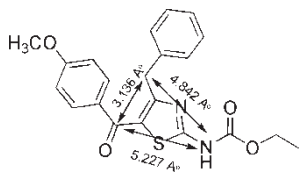


Figure 2. Proposed three point pharmacophore for designing dual acting anti-inflammatory agents.

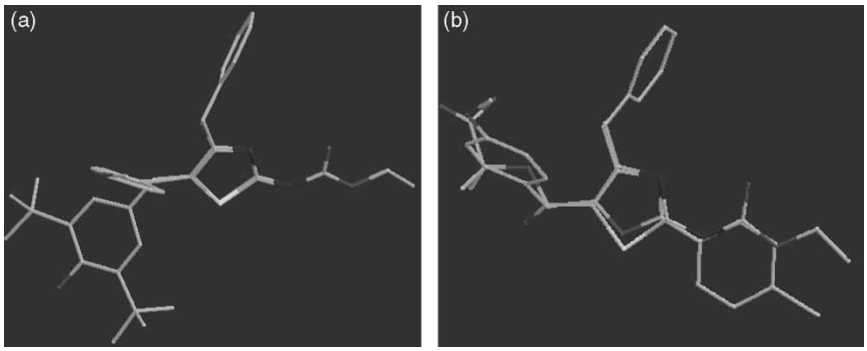


Figure 3. Superimposition analysis of most active molecule RS31 (a) with Darbufelone (b) with Romazarit.

compared to preferential COX-1 inhibitor ibuprofen, selective COX-2 inhibitor rofecoxib, selective 5-LOX inhibitor caffeic acid and selective 15-LOX inhibitor nordihydroguaiaretic acid (NDGA). The compound with unsubstituted phenyl carbonyl group showed less activity probably because unsubstituted phenyl carbonyl group is more prone to metabolism as it is hydroxylated easily and gets conjugated with glucuronic acid for easy elimination as observed in case of Mebendazole [18]. Better efficacy of para substituted phenyl carbonyl group can be attributed to the likelihood of lone pair of electrons on OCH_3 and Cl facilitating the hydrogen bonding with the receptor structure.

As it is evident from Table I, second series i.e. carbalkoxy amino function showed better activity than first series. In carbalkoxy amino series compounds with carbethoxy group at R1 (RS31-33) were more active then carbmethoxy group (RS41-43). Here also the compounds with p-OCH_3 and p-Cl substitution at R2 also showed better activity (RS31 60.8 $\pm$ 1.45% and RS33 44.4 $\pm$ 2.43%) than unsubstituted ones. The substitution on phenyl group reduces the metabolic susceptibility as well as the lone pair of electrons play an important role in the anti inflammatory activity, as discussed above.

Optimization of the substitution at R1 position of the thiazole scaffold was done by attaching phenyl or p-chloro phenyl group in one series and carbethoxy or carbmethoxy group in second series with H, Cl and OCH_3 substitution at R2, for eliciting better anti-inflammatory activity. Amongst all these substituents, highest activity was observed in case of carbethoxy at R1 and OCH_3 at R2 (RS31), while among the derivatives with Cl at R2, carbmethoxy substitution at R1 (RS43) showed highest activity. On the contrary lesser activity was observed in case of phenyl group as well as p-chloro phenyl at R1 as compared to carbalkoxy at R1. Thus from the above discussion, we can infer that carbalkoxy amino group at second position as well as para substituted phenyl carbonyl group at fifth position of the thiazole molecular scaffold, both contribute to anti-inflammatory activity.

Molecule RS31 was found to be the most active compound among all the synthesized compounds having pharmacophore generated from retro-analysis of Darbufelone (CI-1004) and bearing the side chain (-NH-(C=O)O-CH_2CH_3) at second position that resemble to Romazarit (Ro-31-3948). At the same time, it is not likely to metabolize into either methacrylic acid or peroxy derivative responsible for bladder tumor formation because the presence of NH linker prevents metabolic hydroxylation, observed in case of Romazarit. Molecule RS31 is probably metabolized either through aromatic hydroxylation, N-hydroxylation or ester hydrolysis and does not produce the methacrylic acid or peroxy derivative (Scheme 4). The (-NH-(C=O) O-CH_2 CH_3) side chain at the second position as in candidate RS31, the para substituted phenyl carbonyl group at the fifth position, and the benzyl group at the fourth position of the thiazole moiety can be considered as a three-point pharmacophore (Figure 2) for designing better dual acting anti-inflammatory agents.

All the synthesized compounds of both the series were investigated for the LD_{50} values using graphical method. The LD_{50} values of all the compounds were found in range of 725-775 mg/kg while that of most active compounds RS11 and RS31 was 750 mg/kg and 775 mg/kg (i.p.) respectively. The LD_{50} values observed are approximately 15 times higher as compared to effective dose, indicating that the designed molecules are reasonably safe.

The superimposition analysis of most active molecule (RS31) of the deigned series with Darbufelone and also with Romazarit (Figure 3), further corroborates our biological findings. The root mean square deviation (RMSD) value was 0.079 with Darbufelone and 0.098 with Romazarit, which confirms the structural similarity among pharmacophore of RS31 and lead molecules.

Conclusions

The present study discloses a novel three-point pharmacophore for designing better dual acting anti-inflammatory agents that is essentially generated from a lead-based drug design approach. A series of

substituted 1,3 thiazole derivatives was designed based on the pharmacophore produced by the retro analysis of the structure of a dual inhibitor Darbufelone. The optimization at second position of thiazole scaffold was done by incorporating different aryl amino and carbalkoxy amino side chains. The introduction of NH linker at second position of thiazole scaffold was bioisoteric approach to impart the metabolic stability to the carbalkoxy side chains in designed molecule so as to avoid the likelihood of generating toxic moieties, as in Romazarit, which was withdrawn due to its toxicity profile. The rationale for the metabolic stability of the side chain in designed molecule is discussed. The three point superimposition data are presented to further substantiate our hypothesis.

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References

[1] Moreau A, Praveen Rao PN, Knaus EE. Synthesis and biological evaluation of acyclic triaryl (Z)-olefins possessing a 3,5-di-tert-butyl-4-hydroxyphenyl pharmacophore: Dual inhibitors of cyclooxygenases and lipoxygenases. Bioorg Med Chem 2006;15:5340.

[2] Hickenbottorn SL, Grotta J. Neuroprotective therapy. Semin Neurol 1998;18:485.

[3] Masanao I, Tatsuo T, Horokuni J, Takashi O, Katsutoshi Y, Mika K, Yozo H, Akinori A, Kiyoshi Y, Kouji O, Shinji K, Kenzo K, Ryuji S, Miyato K, Shinichi K, Saichi M. Noveln antiarthritic agents with 1,2-isothiazolidine-1,1-dioxide (-sultam) skeleton: Cytokine suppressive dual inhibitors of cyclooxygenase-2 and 5-lipoxygenase. J Med Chem 2000;43:2040.

[4] Unangst PC, Connor DT, Cetenko WA, Sorenson RJ, Kostlan CR, Sircar JC, Wright CD, Schrier DJ, Dyer RD. Synthesis and biological evaluation of 5-[[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]methylene]oxazoles, -thiazoles, and -imidazoles: Novel dual 5-lipoxygenase and cyclooxygenase inhibitors with antiinflammatory activity. J Med Chem 1994;37:322.

[5] Yagami T, Ueda K, Asakura K, Sakaeda T, Kuroda T, Hata S, Kambayashi Y, Fujimoto M. Effects of S-2474, a novel nonsteroidal anti-inflammatory drug, on amyloid beta protein-induced neuronal cell death. Br J Pharmacol 2001;134:673.

[6] Unangst PC, Connor DT, Cetenko WA, Sorenson RJ, Sircar JC, Wright CD, Schrier DJ, Dyer RD. Oxazole, thiazole, and imidazole derivatives of 2,6-di-tert-butylphenol as dual 5-lipoxygenase and cyclooxygenase inhibitors. Bioorg Med Chem Lett 1993;3:1729.

[7] Franklin PX. Design, synthesis and pharmacological evaluation of novel five membered heterocyclic derivatives as potential therapeutic agents. PhD Thesis, Bhavnagar University, India, 2006.

[8] Pillai AD, Rathod PD, Franklin PX, Padh H, Kamala KV, Sudarsanam V. Design, synthesis, and SAR studies of some 5-aliphatic oximino esters of thiophene as potential anti-inflammatory leads: Comparative biological activity profile of aliphatic oximes vs aromatic oximes. Biochem Biophys Res Commun 2004;317:1067.

[9] Pillai AD, Rathod PD, Franklin PX, Padh H, Kamala KV, Sudarsanam V. Design, synthesis, and pharmacological evaluation of some 2-[4-morpholino]-3-aryl-5-substituted thiophenes as novel anti-inflammatory agents: Generation of a novel anti-inflammatory pharmacophore. Bioorg Med Chem 2004;12:4667.

[10] Bradshaw D, Sedgwick AD, Self CR. Romazarit sodium. Drugs Fut 1991;6:868.

[11] Pillai AD, Rathod PD, Franklin PX, Padh H, Sudarsanam V, Kamala KV. Tetra substituted thiophenes as anti-inflammatory agents: Exploitation of analogue-based drug design. Bioorg Med Chem 2005;13:6685.

[12] Thiazole Derivatives, Method of their Production and Use, Indian Patent Application No. 950/MUM/2001.

[13] Hullin RP, Miller J, Short WF. Amidines. part V. preparation of amidines from cyanides and substituted aminomagnesium halides. J Chem Soc 1947;12:394.

[14] Hodgkins JE, Reeves WP. The modified kaluza synthesis. III. The synthesis of some aromatic isothiocyanates. J Org Chem 1964;29:3098.

[15] Esmail R, Kurzer F. The chemistry of ethoxycarbonyl lsothiocyanate and related compounds. Synthesis 1975;5:301.

[16] Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs. Proc Soc Exp Biol Med 1962;111:544.

[17] Safwat MR, Nawal AE, Hoda YH, Tarek A-F. Synthesis of 5-phenyl-1-(3-pyridyl)-1H-1,2,4-triazole-3-carboxylic acid derivatives of potential anti-inflammatory activity. Arch Pharm Chem Life Sci 2006;339:32.

[18] Dawson M, Watson TR. The effect of dose form on the bioavailability of mebendazole in man. Br J Clin Pharmacol 1985;19:87.