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Regioselective synthesis of isoxazole–mercaptobenzimidazole hybrids and their in vivo analgesic and anti-inflammatory activity studies

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ABSTRACT

Regioselective synthesis of isoxazole–mercaptobenzimidazole hybrids and their efficiency in in vivo analgesic and anti-inflammatory activity was described. A comparison of structure–activity relationship for there compounds was also emphasized.

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most widely prescribed drug categories against various inflammation mediated diseases, such as arthritis, rheumatism as well as to relieve the aches and pain of daily life.[1–8] Inflammation is the non-specific protective mechanistic action of the immune system, local biological response of vascular and supporting elements in the tissues at injury to harmful stimuli, resulting in the formation of protein-rich exudates. These exudates mainly include inflammatory mediators such as prostaglandin and histamine, which are responsible for the clinical sign and manifests it as swelling (*tumor*). In this context, the NSAIDs mainly act by lowering the prostaglandin production through inhibition of the enzyme cyclooxygenase-2.[9,10,4]

Generally, in designing new bio-active agents for various therapeutic areas, besides the development of completely new agents, there is another approach involving the synthesis of hybrid molecules.[3,11–18] In the present study, we aim at designing and developing of new NSAIDs of hybrid molecules through the combination of different azole pharmacophores in one structure. Indeed this concept allows the fine tuning of electronic effects in the hybrid structure, provides synergistic effect, to deduce structure–activity relationship (SAR) to improve the bio-potency. Azole heterocycle (imidazole, triazole, pyrazole, oxazole, isoxazole etc.) is an important structural motif in several naturally occurring bio-molecules[19–25] and have been used as privileged scaffolds to synthesize selective drugs of interest in numerous therapeutic areas including HIV-RT inhibitor,[26] anti-cancer,[27] anti-ulcer,[28,29] anti-microbial,[30,31] antihistamine,[32] anthelmintic,[33,34] antioxidant,[35,36] antihypertensive,[37] anti-viral[38] and anticoagulant properties.[39]

This manuscript describes a facile route for the regioselective synthesis of *N*-isoxazole-bound 2-mercaptobenzimidazole hybrids by readily obtainable materials via catalytic nitrile oxide-alkyne 1,3-dipolar cycloaddition and the in vivo screening of the resultant cycloadducts by analgesic and anti-inflammatory activities.

We have employed some newly synthesized 2-mercaptobenzimidazole derived terminal alkynes as partners to nitrile oxide in cycloaddition to obtain 3,5-disubstiuted isoxazole bound 2-mercaptobenzimidazole hybrid molecules. The new terminal alkynes that is *N*-propargyl 2-mercaptobenzimidazoles were obtained in a three step synthesis (Scheme 1). Firstly, the 2-mercaptobenzimidazoles

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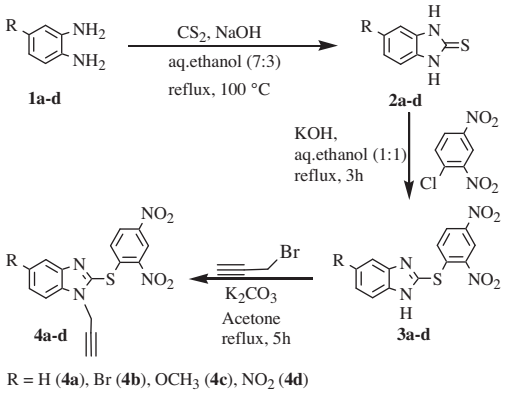


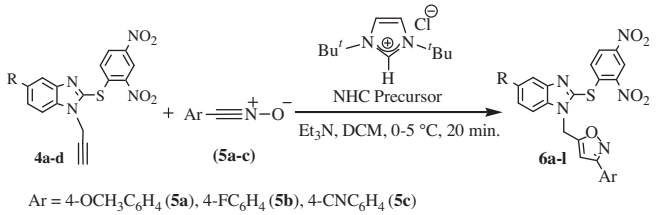
Scheme 1. Synthesis of *N*-propargyl 2-mercaptobenzimidazoles (**4a–d**).

(**2a–d**) were obtained by the condensation of *o*-phenylenediamines (OPDA) (**1a–d**) with carbon disulphide in presence of NaOH as a base. The as-synthesized **2a–d** were then treated with 2,4-dinitrochlorobenzene in the presence of a base to obtain 2-mercaptobenzimidazoles **3a–d**. N-propargylation of **3a–d** has given the terminal alkynes (**4a–d**).

Now the terminal alkynes were subjected to cycloaddition reaction with nitrile oxides (**5a–c**) in the presence of an organo-*N*-heterocyclic carbene (NHC) catalyst to obtain *N*-isoxazole-bound 2-mercaptobenzimidazoles (**6a–l**) (Scheme 2). The in situ generation and stabilization of aryl nitrile oxides was reported by us recently.[40]

In a more detailed explanatory way, the results of the reactions shown in Scheme 2 are summarized in Table 1. Firstly, the conditions for cycloaddition were optimized by using nitrile oxide (**5a**) and terminal alkyne (**4a**) as model substrates. The cycloaddition between **5a** and **4a** studied without a catalyst was so sluggish (~16 h) and produced a mixture of two regioisomers of disubstituted (3,4- and 3,5-) isoxazole (entry 1, 68% of combined mixture in ~3:7 ratio). In this situation, the cycloaddition between **5a** and **4a** performed in the presences of nucleophilic organo-NHC catalyst (*N,N′*-ditertiarybutylimidazolium chloride) has enhanced the reactivity of terminal alkyne and thereby 3,5-disubstituted isoxazole product (**6a**) regioselectivity (Table 1, entry 2). After the synthesis of **6a**, we have also studied the catalytic cycloaddition of terminal alkynes (**4b–d**) with nitrile oxides (**5a–c**) and synthesized only 3,5-disubstituted isoxazoles (**6b-l**) as a sole product (see Table 1) in a shorter reaction period of ~20 min.

The synthesized 2-mercaptobenzimidazole containing 3,5-disubstituted isoxazole compounds (**6a–l**) were characterized by



Scheme 2. Organo-NHC catalyzed 1,3-dipolar cycloaddition synthesis of isoxazoles (**6a–l**).

Table 1
Results of organo-NHC-catalyzed cycloadditon of terminal alkyne (**4a–d**) with aryl nitrile oxides (**5a–c**)[a]

Entry	R	Ar	Product	Yield[b] (%)
1	H (**4a**)	4-OMeC$_6$H$_4$ (**5a**)	**6a**	68 (3:7)[c]
2	**4a**	**5a**	**6a**	92
3	**4a**	4-FC$_6$H$_4$ (**5b**)	**6b**	90
4	**4a**	4-CNC$_6$H$_4$ (**5c**)	**6c**	94
5	Br (**4b**)	**5a**	**6d**	92
6	**4b**	**5b**	**6e**	90
7	**4b**	**5c**	**6f**	95
8	OCH$_3$ (**4c**)	**5a**	**6g**	92
9	**4c**	**5b**	**6h**	95
10	**4c**	**5c**	**6i**	94
11	NO$_2$ (**4d**)	**5a**	**6j**	92
12	**4d**	**5b**	**6k**	95
13	**4d**	**5c**	**6l**	94

[a] All products were characterized by NMR and mass spectral analysis.
[b] Isolated yields after column chromatography.
[c] Without NHC catalyst.

IR, ^{1}H/^{13}C NMR, mass and elemental analysis (Experimental Section). The absence of ^{1}H NMR signals of terminal alkyne at δ = ~2.10, and emerging of a new signal at δ = ~6.30 corresponds to 4th C–H proton of isoxazole provides a good support for the cycloaddition to form 3,5-disubstituted isoxazoles. The same features are reflected in ^{13}C NMR spectra, where the signal belongs to terminal carbon of alkyne was disappeared and a new signal belongs to 4th C–H ring carbon, was appeared at δ = ~99 after cycloaddition.

All the compounds prepared herein (**6a–l**) were screened for their in vivo analgesic and anti-inflammatory activities. These activities were carried out by measuring the physiological responses of animals to the thermal and chemical stimuli. For analgesic activity, hot plate method in mice was used[41] at a dose of 100 mg/kg b.w (body weight) was performed. These activity results were compared with standard drug pentazocine (50 mg/kg b.w). For anti-inflammatory activity, carrageenan induced inflammation on rat hind paw oedema method of Winter et al.,[42] in mice at a dose of 100 mg/kg b.w. was carried out. The percentage inhibition was determined for synthesized compounds as well as standard drug diclofenac (50 mg/kg b.w). The animal study was conducted according to the protocol approved by animal ethics committee, Kakatiya University, India.

Table 2
Analgesic activity of tested compounds (100 mg/kg b.w) and pentazocine (50 mg/kg b.w) The Bold values specify the compounds with superior activity.

Entry	Compound	Reaction time in seconds ($X \pm SD$)[a]
1	Control	6.07 ± 0.057
2	**6a**	13.28 ± 0.106***
3	**6b**	13.58 ± 0.134***
4	**6c**	13.50 ± 0.147***
5	**6d**	13.80 ± 0.085***
6	**6e**	**13.92 ± 0.093*****
7	**6f**	**13.87 ± 0.093*****
8	**6g**	13.60 ± 0.126***
9	**6h**	13.65 ± 0.057***
10	**6i**	13.65 ± 0.102***
11	**6j**	13.68 ± 0.118***
12	**6k**	**13.80 ± 0.061*****
13	**6l**	**13.77 ± 0.061*****
14	Pentazocine	14.10 ± 0.05***

***p <0.05. The active compounds are marked in bold letters.
[a] Data represent mean values ± SD (standard deviation) of six mice per group, shown at the final value for each group (saline, pentazocine and tested compounds) after 120 min. Data were analyzed using one-way ANOVA followed by Newman–Keuls multiple comparison test.

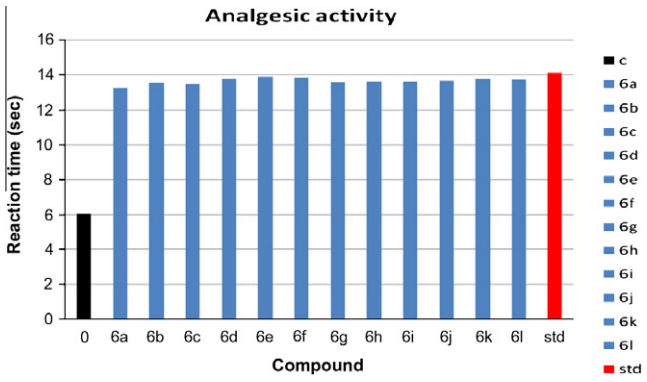


Figure 1. Analgesic activity of *N*-isoxazole-bound 2-mercaptobenzimidazoles (**6a–l**) by hot plate method.

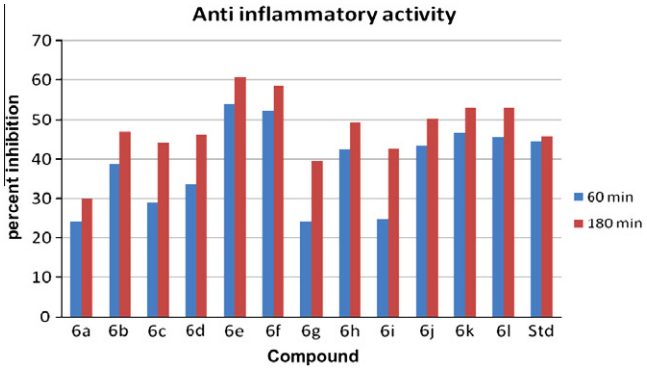


Figure 2. Anti-inflammatory activity of *N*-isoxazole-bound 2-mercaptobenzimidazoles (**6a–l**).

The analgesic activity of the synthesized compounds (**6a–l**) was assessed by hot plate method. According to the structure–activity relationship (SAR) studies, almost all the compounds have shown very potent analgesic activity when compared with standard drug pentazocine. Amongst all the compounds, **6e** and **6f** with potent analgesic activity, the compounds **6k** and **6l** has shown moderate activity, and was found to be more potent than the standard pentazocine. The remaining compounds **6a–d** and **6g–j** had shown poor activity (see Table 2, Fig. 1).

The SAR study was evaluated by changing substituent on 5-position of benzimidazole and 3-position of isoxazoles as shown in Table 1 to see the influence electronic effects. Among the 12 compounds investigated for in vivo analgesic activity, when the 5th position of benzimidazole and 3rd position of substituted aryls of isoxazoles containing electron withdrawing groups of bromo, fluoro, nitro and cyano as in **6e**, **6f**, **6k** and **6l** showed superior activity over the compounds **6a–d** and **6g–j** that contain atleast one electron donating group or without any subtituent on benzimidazole ring.

The activity of the tested compounds **6a–l** as well as reference standard were measured after the administration of carrageenan inflammation induced in rats. The percent oedema inhibition was calculated as a regard to saline control group, as depicted in Figure 2, Table 3. Most of the tested compounds have shown good results in comparison with standard drug diclofenac. Among all the compounds investigated, **6e** (60.76%) and **6f** (58.46%) have shown potent anti-inflammatory activity, the compounds **6j**, **6k** and **6l** have shown moderate activity (50.38, 53.07 & 53.07%). However, all the results were found to be more potent than the standard diclofenac. On the other hand, the compounds **6a**, **6g** have shown poor activity.

Further, the efficacious anti-inflammatory activity of these compounds was analyzed based on the structure–activity relationships considering the basic two structural derivatives: nature of the group attached to 3-aryl substituted isoxazole ring and nature of the substituents (functional group) at 5th position of 2-mercaptobenzimidazole as described in in vivo analgesic activity.

As observed in in vivo analgesic activity, compounds **6e**, **6f**, **6k** and **6l** that containing electron withdrawing groups on 5th position of benzimidazole and 3rd position of substituted aryls of isoxazoles exhibited greater activity over the compounds that contain atleast one electron donating group or without any subtituent on benzimidazole ring.

In conclusion, a facile catalytic method for the regioselective synthesis of 3,5-disubstituted isoxazole bound benzimidazole hybrid molecules and their effective analgesic and anti-inflammatory activities and structure–activity relationship (SAR) was demonstrated. These hybrid compounds possessing an electron-withdrawing group displayed higher activity than for compounds containing electron-donating groups and were found to be more potent than the standard pentazocine for analgesic and diclofenac for anti-inflammatory activity.

Table 3
Anti-inflammatory activity of the tested compounds (100 mg/kg b.w) and diclofenac (50 mg/kg b.w). The Bold values specify the compounds with superior activity.

Entry	Compound	Paw oedema thickness (mm)			
		60 min ($X \pm SD$)	% Oedema inhibition	180 min ($X \pm SD$)[a]	% Oedema inhibition[b]
1	Control	2.65 ± 0.10	–	2.60 ± 0.06	–
2	**6a**	2.01 ± 0.05**	24.15	1.82 ± 0.01**	29.99
3	**6b**	1.62 ± 0.02**	38.86	1.38 ± 0.02**	46.92
4	**6c**	1.88 ± 0.02*	29.05	1.45 ± 0.05*	44.23
5	**6d**	1.76 ± 0.06*	33.58	1.40 ± 0.04*	46.15
6	**6e**	**1.22 ± 0.03****	53.95	**1.02 ± 0.02****	**60.76**
7	**6f**	**1.26 ± 0.01****	52.44	**1.08 ± 0.04****	**58.46**
8	**6g**	2.01 ± 0.13*	24.15	1.57 ± 0.02*	39.61
9	**6h**	1.52 ± 0.02*	42.63	1.32 ± 0.04*	49.23
10	**6i**	1.99 ± 0.02*	24.90	1.49 ± 0.02*	42.70
11	**6j**	**1.50 ± 0.04****	43.39	**1.29 ± 0.02****	**5.38**
12	**6k**	**1.41 ± 0.01****	46.78	**1.22 ± 0.02****	**53.07**
13	**6l**	**1.44 ± 0.03****	45.65	**1.22 ± 0.03****	**53.07**
14	Diclofenac	1.47 ± 0.01***	44.52	1.41 ± 0.04***	45.76

[a] Data represent mean values ± SD (standard deviation) of six mice per group and the percent changes versus 60 and 180 min post-carrageenan injection. Data were analyzed using one-way ANOVA followed by Newman–Keuls multiple comparison test. Significant difference from the control value at $p < 0.05$.
[b] Percent oedema inhibition was calculated as regards saline control group and the active compounds are marked in bold letters.

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Supplementary data

Supplementary data (experimental procedures for **3a–d**, **4a–d**, **6a–l** and spectral data of compounds **3a–d** and **6a–l**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.12.101.

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