Full Paper

Synthesis and *In-vivo* Evaluation of Carbonyl-amide Linkage Based New Benzimidazole Derivatives

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In search of pharmacologically active potent compounds, a series of carbonyl-amide linkage based new benzimidazole derivatives were synthesized from acid, aldehydes and isocyanide at ambient temperature *via* Passerini reaction. All the compounds synthesized were screened for their potential anti-inflammatory, antidiabetic and anticonvulsant properties. The results revealed that compounds **2i** and **2j** were found to be the most potent anti-inflammatory agents, while compounds **2a**, **2c**, **2e**, **2f**, **2i** and **2j** showed increased antidiabetic activity than the reference drugs and **2a**, **2g**, **2h**, **2i** and **2j** were found to be the main structural requirement for maintaining anticonvulsant activity.

Keywords: Anticonvulsant property / Antidiabetic property / Anti-inflammatory property / Benzimidazole derivatives / One-pot synthesis

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Introduction

Inflammation is a local reaction of the vascular and supporting elements of a tissue to injury resulting in the formation of protein-rich exudates; it is a protective response of the nonspecific immune system that serves to localize, neutralize, or to destroy an injurious agent in preparation for the process of healing. The cardinal signs of inflammation are rubor (redness), calor (heat), dolor (pain), tumor (swelling) and functio laesa (loss of function). Cause of inflammation includes physical agents, chemical agents, immunological reactions and infection by pathogenic organism [1]. Non-steroidal anti-inflammatory drugs (NSAIDs) are useful tools in the treatment of acute and chronic inflammation [2], pain [3] and fever [4]. However, long-term clinical usage of NSAIDs is associated with significant side effects of gastrointestinal lesions, bleeding and nephrotoxicity [5, 6]. Therefore, the discovery of new and safer anti-inflammatory drugs represents a challenging goal for such a research area [7]. As resistance to anti-inflammatory drugs is widespread, there is an increasing need for identification of novel structures that may be of use in designing new, potent and less toxic anti-inflammatory agents.

Diabetes mellitus is a common endocrine disorder characterized by chronic hyperglycemia, alterations in carbohydrate and lipid metabolism. The hyperglycemia causes extensive tissue damage appearing in eyes, kidneys, nerves, heart and blood vessels [8]. The prevalence of diabetes keeps increasing with age, reaching a plateau at 10–20% in people over 70 years old. The number of diabetic people is expected to rise from present estimate of 150 million to 230 million in 2025 [9, 10]. Nowadays, benzimidazoles play a vital role for the treatment of diabetes in spite of other therapeutic options.

Epilepsy is one of the most common diseases of the brain affecting at least 50 million persons worldwide. There is currently a need of improved agents for the treatment of seizures, since currently available drugs are effective only in 60–80% of epileptic patients [11]. Polytherapy with antiepileptic drugs (AEDs) is necessary in clinical practice because of the limited efficacy of monotherapy [12]. During the past decade several new drugs were approved (Rufinamide, Retigabine, Pregabaline, Remacemide). However, none of the available antiepileptic drugs is an ideal one as these can be associated with chronic and toxic side effects [13]. Thus, the search for the new anti-convulsant drugs continues to be an active area of investigation in medicinal chemistry.

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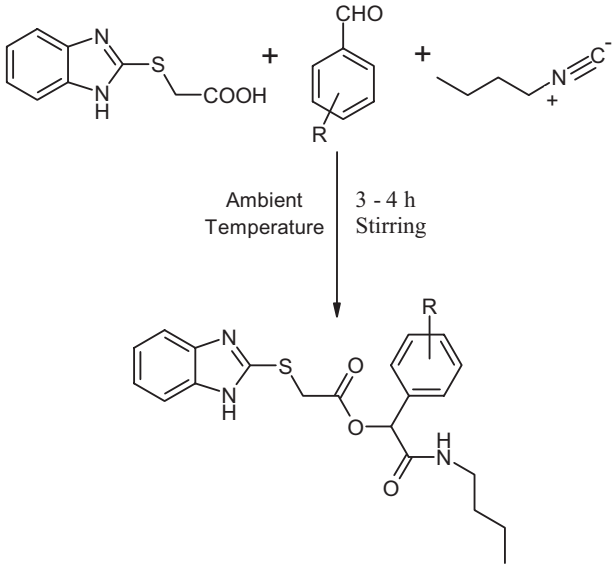
Benzimidazole moiety plays an important heterocyclic pharmacophore in the pharmaceutical chemistry and drug discovery. These compounds carrying different substituents in benzimidazole biomolecules are associated with a wide range of biological and pharmacological activities including anticancer [14], antiviral [15], antimicrobial [16], antihelmentic [17], anti-inflammatory [18], antihistaminic [19], antioxidant [20], antihypertensive [21] and anticoagulant [22] properties. These derivatives also exhibit significant activities against several viruses such as HIV [23].

Some of the important benzimidazole derivatives have been reported as proton pump inhibitor [24], anti-dopaminergic Domperidone [25], anti-psychotic Pimozide [26], thyroid receptor agonists [27], gonadotropin releasing hormone receptor antagonists [28] and interestingly alkynylbenzimidazoles as modulators of metabotropic glutamate receptors [29]. Recently, we have also published some series of biologically active benzimidazole derivatives [30, 31]. There is a lack of literature on the synthesis and biological evaluation of carbonyl-amide linkage based benzimidazole derivatives. Owing to the immense biological importance of benzimidazole libraries, we wish to report synthesis of a new class of benzimidazole derivatives and their biological activity screening studies.



Where R= 2a) H, 2b) 2-CH_3, 2c)4-CH_3, 2d)2-OCH_3, 2e)4-OCH_3, 2f) 4-C_2H_5, 2g) 2-NO_2, 2h) 4-NO_2, 2i) 2-Cl, 2j) 4-Cl, 2k)2,4-NO_2, 2l) 2,4-Cl

Scheme 1. Schematic representation of compounds **2a–2l**.

Results and discussion

Chemistry

In the present protocol, we are reporting one-pot synthesis of new benzimidazole derivatives using 1*H*-benzimidazol-2-ylsulfanyl acetic acid, substituted aldehydes and butyl-isocyanide (1:1:1) with stirring at ambient temperature in the presence of water to give desired products as shown in Table 1 (see also Scheme 1). Aromatic aldehydes substituted with both electron withdrawing and electron donating groups could be used successfully. The yield of the products was excellent and varied according to the substitution pattern of the aromatic aldehydes. Structure of the synthesized compounds was confirmed on the basis of IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data. The IR spectra of all compounds (**2a–l**) have aromatic –NH stretching bands in the range of 3488–3420 cm^{-1}, whereas aliphatic-NH stretching bands appear in between 3157–3138 cm^{-1}. While C=O stretching bands of carboxylic acid appeared from 1688–1632 cm^{-1} and similarly C=O stretching bands of CONH was observed from 1524-1578 cm^{-1}. In the ^{1}H-NMR spectra of the target substituted benzimidazoles, the –NH proton of benzimidazole ring resonated as a broad singlet in the region δ 3.95–4.17 ppm and NH proton of CONH was observed as singlet in the region δ 7.77–7.96 ppm and was exchangeable on D_2O addition confirming the formation of carbonyl-amide linkage. A singlet in the range of δ 6.43–6.93 can be assigned to O–CH. The other protons appeared at the expected chemical shifts. ^{13}C-NMR spectral data for the title compounds most characteristic peak around δ 169.6–171.9 ppm (CONH) indicated the formation of carbonyl-amide linkage. Mass spectra showed accurate molecular ion peak with respect to the targeted compounds.

Table 1. Physical Data of Synthesized Compounds (**2a–2l**).

Compound	R	Time (hrs)	Yield[a] (%)	Melting Point (°C)
2a	H	3.25	90	225–228
2b	2-CH_3	3.45	91	231–232
2c	4-CH_3	3.55	83	185–187
2d	2-OCH_3	3.15	87	218–220
2e	4-OCH_3	3.20	79	201–202
2f	4-C_2H_5	3.35	85	175–176
2g	2-NO_2	3.30	78	242–244
2h	4-NO_2	3.10	90	159–161
2i	2-Cl	3.40	82	195–197
2j	4-Cl	3.50	86	212–214
2k	2,4-NO_2	3.25	88	168–169
2l	2,4-Cl	3.40	84	238–240

[a] Isolated yields

Pharmacology

All the compounds prepared herein were screened for their potential *in-vivo* biological activities such as anti-inflammatory, antidiabetic and anticonvulsant activities. Anti-inflammatory activity was evaluated using carrageenan induced hind paw edema method [32]. Evaluation of antidiabetic

Table 2. Anti-inflammatory activity of synthesized compounds (**2a–2l**) by carrageenan-induced hind paws edema method.

Groups	Dose	Paw volume (mean ± SEM)					
	mg/kg p.o.	0 hr	1 hr	2 hr	3 hr	4 hr	6 hr
Control	Tween 80	1.356 ± 0.001	1.303±0.001	1.332 ± 0.001	1.334 ± 0.001	1.448 ± 0.002	1.493 ± 0.001
Std. drug Diclofenac sodium	200 mg/kg	0.862 ± 0.002	0.664 ± 0.002	0.647 ± 0.001	0.588 ± 0.001	0.636 ± 0.002	0.768 ± 0.001
2a	400 mg/kg	1.153 ± 0.002	1.285 ± 0.005	1.226 ± 0.003	1.189 ± 0.002	1.343 ± 0.003	1.288 ± 0.005
2b	400 mg/kg	0.855 ± 0.003***	0.778 ± 0.004**	0.623 ± 0.002***	0.887 ± 0.001**	0.768 ± 0.005**	0.987 ± 0.003**
2c	400 mg/kg	0.752 ± 0.004**	0.893 ± 0.005**	0.778 ± 0.003**	0.682 ± 0.001**	0.830 ± 0.005**	0.785 ± 0.006**
2d	400 mg/kg	1.150 ± 0.002	1.196 ± 0.003	1.328 ± 0.003	1.067 ± 0.005	1.112 ± 0.004	1.406 ± 0.003
2e	400 mg/kg	1.252 ± 0.001	1.293 ± 0.001	1.328 ± 0.001	1.387 ± 0.001	1.442 ± 0.002	1.485 ± 0.001
2f	400 mg/kg	0.656 ± 0.001	0.593 ± 0.001	0.328 ± 0.001	0.458 ± 0.001	0.642 ± 0.002	0.544 ± 0.001
2g	400 mg/kg	1.226 ± 0.002	1.218 ± 0.003	1.208 ± 0.001	1.209 ± 0.001	1.211 ± 0.004	1.216 ± 0.004
2h	400 mg/kg	0.551 ± 0.002	0.599 ± 0.003	0.457 ± 0.001	0.684 ± 0.004	0.542 ± 0.004	0.583 ± 0.002
2i	400 mg/kg	0.848 ± 0.004***	0.793 ± 0.005**	0.828 ± 0.003**	0.687 ± 0.002**	0.442 ± 0.001**	0.785 ± 0.004***
2j	400 mg/kg	0.658 ± 0.004**	0.892 ± 0.005**	0.721 ± 0.002**	0.725 ± 0.002**	0.842 ± 0.001**	0.679 ± 0.003***
2k	400 mg/kg	0.851 ± 0.003***	0.793 ± 0.002**	0.628 ± 0.004***	0.687 ± 0.001**	0.642 ± 0.005**	0.685 ± 0.002**
2l	400 mg/kg	1.151 ± 0.002	1.123 ± 0.001	1.148 ± 0.003	1.237 ± 0.001	1.242 ± 0.004	1.185 ± 0.001

Data was analyzed by unpaired one-way ANOVA test.
Each value represents the mean ± SEM (n = 6).
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with control Student's 't' test.

activity was done by blood glucose test and oral glucose tolerance test (OGTT) [33, 34]. Male Wister albino rats (150–200 g) of either sex were selected. The anticonvulsant activities of the synthesized compounds were tested through an *in-vivo* rodent model of convulsions (maxima electro shock, MES test) [35, 36].

Anti-inflammatory evaluation

In the acute oral toxicity study, no mortality was noticed within 48 h by any of the compounds up to 1500 mg/kg p.o. dose level in the present study. However, some behavioral changes were noticed depending at the higher dose (400 mg/kg). The effect of the tested compounds as well as reference standard were measured before and 1, 2, 3, 4, 5 and 6 h after carrageenan injection. Most of the tested compounds showed a reasonable inhibition of edema size in comparison with standard drug Diclofenac sodium. From the obtained results (Table 2), it has been observed that newly prepared compounds (**2a–2l**) reveal better anti-inflammatory properties comparable to that of Diclofenac sodium which was used as a reference standard. Structure–activity relationships based on the observed results indicated that the type of group substitution attached to the phenyl ring plays a controlling role for developing the exhibited pharmacological properties. It has been noticed that substitution of an electron-withdrawing group, a chlorine atom at the *o*-position (**2i**) and *p*-position (**2j**) on the phenyl ring, seems more favorable for constructing an anti-inflammatory active agent than the case of substitution with an electron-donating group, a methyl residue (**2b**, **2c**). On the other hand, comparing the activity of the compounds **2d–2h**, it was observed that replacing the hydrogen atom by a methoxy, ethyl and nitro group reduces the anti-inflammatory activity.

Antidiabetic evaluation

In the acute toxicity study, all the synthesized compounds showed better toxic effects when observed for the parameters during 2^{nd} h, then frequently for 4^{th} h and finally for 9^{th} day. No mortality was observed, the substance is found to be safe at the tested dose level of 350 mg/kg body weight. The results of blood glucose of control and all the synthesized compounds are shown in Table 3. Oral anti-diabetic efficiencies of twelve compounds (**2a–2l**) were assessed using an oral glucose tolerance test (OGTT) in normal rat model. The marketed drug Glibenclamide was used as standard drug. The sugar lowering effect of these compounds is presented in Table 3. Compounds **2c**, **2d**, **2h**, **2j** and **2l** showed better reduction in the blood glucose levels on 9^{th} day compared with diabetic control rats. Oral administrations of the same compounds processed good reduction in the glucose levels in diabetic rats. Compounds **2a**, **2c**, **2e**, **2f**, **2i** and **2k** showed increased activity compared to the reference drug ($P < 0.001$) Glibenclamide at 90 min interval (Table 3). The acute toxicity study of all the compounds showed no death of these rats even under high levels indicating the margin of safety. These results indicate compounds mentioned above showed better anti-diabetic activity, maybe due to its protective role against pancreatic injury in diabetic rats.

Table 3. Anti-diabetic activity of synthesized compounds (**2a–2l**) by oral glucose tolerance test.

Treatment & Groups	Blood glucose level (mg/dL)		Oral glucose tolerance test (OGTT) in non-diabetic rats		
	Basal	9th day	Fasting	30 min	90 min
Control	73.80 ± 1.36	80.29 ± 0.40	82.12 ± 1.57	180.32 ± 3.68	136.66 ± 2.85
Diabetic control (Alloxan)	330.52 ± 2.64	336.68 ± 1.79	–	–	–
Glibenclamide (SD)	332.20 ± 4.31	140.18 ± 2.64	82.33 ± 3.29	178.40 ± 2.95	104.82 ± 2.23**
2a	328.20 ± 4.24	384.68 ± 12.55	78.32 ± 1.89	170.62 ± 6.34	104.43 ± 3.28**
2b	342.20 ± 12.52	373.18 ± 25.62	72.39 ± 3.20	173.40 ± 5.98	128.04 ± 4.54
2c	353.23 ± 6.58	182.20 ± 16.21***	81.28 ± 2.59	163.80 ± 6.42	107.25 ± 2.80**
2d	326.33 ± 5.13	166.26 ± 15.20***	80.83 ± 3.55	166.62 ± 3.03	127.24 ± 2.89
2e	321.20 ± 7.28	334.62 ± 12.54	79.30 ± 2.81	160.66 ± 4.38	106.42 ± 3.27**
2f	340.34 ± 12.53	371.14 ± 25.68	74.36 ± 3.33	173.42 ± 5.82	116.06 ± 4.52**
2g	342.20 ± 6.54	372.27 ± 16.23	83.28 ± 2.45	162.80 ± 6.44	128.18 ± 2.71
2h	334.33 ± 5.20	175.26 ± 15.26***	82.73 ± 3.52	168.66 ± 3.33	118.22 ± 2.82
2i	337.17 ± 4.29	379.63 ± 12.57	78.30 ± 1.73	171.62 ± 6.37	116.43 ± 3.29**
2j	323.33 ± 5.10	165.25 ± 15.20***	81.86 ± 3.55	163.62 ± 3.09	125.21 ± 2.86
2k	369.22 ± 6.55	364.27 ± 16.29	88.28 ± 2.57	164.80 ± 6.40	108.24 ± 2.82**
2l	324.33 ± 5.10	163.25 ± 15.20***	82.80 ± 3.54	165.62 ± 3.02	125.27 ± 2.86

Data was analyzed by unpaired one-way ANOVA followed by Dunnet's test.
Each value represents the mean ± SEM.
$P > 0.05$ is considered as non-significant (ns), $P < 0.05$ is considered as significant.
** $P < 0.001$ as compared to normal control group.
*** $P < 0.001$ as compared to normal control group.
Control = Normal saline.
Diabetic control = Alloxan.
SD = Standard drug Glibenclamide.

Anticonvulsant evaluation

Results of anticonvulsant activity with standard drug Phenytoin are tabulated in Table 4. The compounds presented in Table 4 showed good protection and most were devoid of toxicity at the dose tested. From the structure activity relationship, it is evident that the presence of a chloro- and nitro-function at *ortho*- and *para*-position of the phenyl ring as seen with compounds **2g**, **2h**, **2i** and **2j** was

Table 4. Anti-convulsant activity of synthesized compounds (**2a–2l**) by maximal electroshock model.

Treatment	Dose (mg/kg)	Time (s) in various phases of convulsion (mean ± SEM)				
		Flexon	Extensor	Clonus	Stupor	Recovery/Death
Control Gum acacia	1 % w/w	3.16 ± 0.32	11.07 ± 0.58	16.78 ± 2.54	219.34 ± 5.79	–
Phenytoin (SD)	20	2.87 ± 0.35^{ns}	0.0 ± 0.00**	4.85 ± 4.29^{ns}	160.4 ± 20.54^{ns}	Recovery
2a	80	3.30 ± 0.36^{ns}	4.21 ± 0.39**	16.30 ± 2.24**	213.8 ± 5.68*	Death
2b	84	4.23 ± 1.08^{ns}	5.33 ± 0.3*	1.08 ± 1.27*	208.6 ± 4.81^{ns}	Death
2c	65	4.57 ± 1.04^{ns}	12.2 ± 0.58*	1.35 ± 1.35*	214.5 ± 20.54^{ns}	Death
2d	70	3.96 ± 1.16^{ns}	3.73 ± 0.42**	1.02 ± 1.21*	202.1 ± 20.55^{ns}	Death
2e	84	3.22 ± 0.27^{ns}	9.24 ± 0.71*	1.29 ± 1.22*	193.2 ± 13.26^{ns}	Death
2f	62	4.55 ± 1.04^{ns}	12.0 ± 0.59*	1.35 ± 1.36*	214.5 ± 20.54^{ns}	Death
2g	75	3.14 ± 0.30^{ns}	4.38 ± 0.44**	15.64 ± 2.12*	208.7 ± 4.96*	Death
2h	70	3.14 ± 0.31^{ns}	4.38 ± 0.46**	15.67 ± 2.13*	208.6 ± 4.95*	Death
2i	85	3.08 ± 0.27*	3.99 ± 0.45**	1.83 ± 1.29*	209.5 ± 13.42*	Death
2j	76	3.16 ± 0.25*	5.67 ± 0.21**	15.40 ± 2.15*	210.7 ± 4.83*	Death
2k	72	3.17 ± 0.38^{ns}	11.0 ± 0.56*	14.62 ± 2.17^{ns}	207.3 ± 4.97^{ns}	Death
2l	60	3.02 ± 0.25^{ns}	9.50 ± 0.49*	12.65 ± 2.28^{ns}	208.4 ± 5.66^{ns}	Death

Data was analyzed by unpaired one-way ANOVA test.
Each value represents the mean ± SEM (n = 6).
$P > 0.05$ is considered as non-significant (ns), $P < 0.05$ is considered as significant.
** $P < 0.001$ as compared to normal control group.
$^{***}P < 0.001$ as compared to standard drug.

found to be the main structural requirement for maintaining anti-convulsant activity. The chloro-substitution led to compounds (**2i**, **2j**) with slightly different behavior, but all characterized by a very low toxicity: derivatives **2i** and **2j**, having a chlorine on the phenyl ring in the 2 and 4 position, demonstrated good activity in rats at 85 mg/kg and 76 mg/kg, while the introduction of methyl and methoxy groups on the phenyl ring (**2b**, **2c**, **2d** and **2e**) was detrimental for the protection against electrical induced seizures in rats. The nitro-substitution afforded good protection, especially for the derivatives **2g** and **2h**. Finally, the introduction of the ethyl substituent led to a decrease of potency (**2f**). All the compounds showed protection against seizures in extensor phase in both phenytoin and tested compounds with control group. The extensor phase time was remarkably reduced for these compounds. Compound **2a** showed remarkable change in the clonus stage, while compounds **2b–j** remains moderate and compounds **2k–l** were non-significant. These compounds exhibited their ability to diminish the magnitude of tonic-clonus seizures. In flexon and stupor no significant change was observed. Only five compounds namely **2a**, **2g**, **2h**, **2i** and **2j** showed rapid and prolonged action in stupor stage. These compounds showed excellent ($P < 0.001$) anticonvulsant activity when compared with standard Phenytoin drug as tabulated in Table 4. Finally, these compounds represent new structure that could be further optimized for future development of more potent and selective anticonvulsant agents.

Conclusion

In present study, we have demonstrated one-pot synthesis of new benzimidazole derivatives in water at ambient temperature *via* Passerini reaction. All the synthesized compounds were screened for *in-vivo* anti-inflammatory, antidiabetic and anticonvulsant activities. From the results, it can be concluded that compounds **2i** and **2j** was found to be the most potent anti-inflammatory compound, whereas compounds **2b**, **2c** and **2k** showed the moderate inhibitory effect. 5 compounds (**2c**, **2d**, **2h**, **2j** and **2l**) at the dose of 20 mg/kg exhibited considerably potent blood glucose lowering activity. The anti-diabetic evaluation against oral glucose tolerance test (OGTT) indicated that compounds **2a**, **2c**, **2e**, **2f**, **2i**, and **2k** showed increased activity than their reference drug Glibenclamide. All compounds exhibited protection in the seizure models, *viz*., MES and **2a**, **2g**, **2h**, **2i** and **2j** have emerged as the most active compounds in these models with no neurotoxicity. SAR studies revealed the critical role of chloro-function in the target compounds that showed very promising *in-vivo* activities. So we conclude that, further structural modifications of these molecules might lead to the discovery of more potent *in-vivo* agents with still lower neurotoxicity.

Experimental

Instrumentation

All compounds were routinely checked by thin-layer chromatography (TLC) on aluminum-backed silica gel plates. Melting points were determined with open capillary method and are uncorrected. IR spectra were recorded on a Nicolet 5700 FT-IR instrument (Nicolet, Madison, WI, USA) as KBr discs. The ^{1}H-NMR and ^{13}C-NMR spectra were measured with a Bruker-300 (Bruker Bioscience, USA), 300 MHz instrument using DMSO as solvent and TMS as internal standard. All chemical shifts were reported as δ values (ppm). Mass spectrometer with ionization energy maintained at 70 eV using on Shimadzu Mass Spectrometer (LCMS) and the elemental analysis was carried out by using Heraus CHN rapid analyzer. The biological activities were carried out at Luqman College of Pharmacy, Gulbarga, Rajiv Gandhi University of Health Science, India.

General procedure for the synthesis of compounds

A mixture of (1*H*-benzimidazol-2-yl-sulfanyl)-acetic acid (0.01 mol), butyl-isocyanide (0.01 mol) and substituted aldehyde (0.01 mol) was given into a round bottom flask. The reaction was stirred at ambient temperature. After 40–50 min, precipitation starts forming on the sides of the flask and the stirring should be continued for further 3–4 h The progress of the reaction is monitored by TLC using *n*-hexane/ethyl acetate (80:20) as eluents. The reaction mixture was filtered to get a desired product and recrystallized from methanol.

*(1H-Benzimidazol-2-yl-sulfanyl)-acetic acid butylcarbamoylphenyl-methyl ester (**2a**)*

Pale yellow solid, yield 1.8774 g; IR (KBr) ν_{max} cm^{-1}: 3422 (br, arom-NH), 3140 (aliph-NH), 1679 (CO of acid), 1576 (CO of CONH); ^{1}H-NMR (300 MHz, DMSO) δ ppm: 0.94 (t, $J = 3.8$, 6.6 Hz, 3H, CH_3), 1.47 (q, $J = 5.0$ Hz, 2H, CH_2), 1.62 (d, $J = 6.6$ Hz, CH_2), 3.47 (s, 2H, CH_2), 3.71 (s, 2H, CH_2), 4.15 (br, 1H, NH of imidazole), 6.43 (s, 1H, O–CH), 7.21–7.26 (m, 5H, arom-H), 7.64–7.73 (q, 4H, arom-H), 7.96 (s, 1H, aliph-NH); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm: 12.2, 21.6, 114.9, 122.5, 143.3, 170.6, 172.4; MS *m/z*: 398.02 (M^+); anal. calcd. for $C_{21}H_{23}N_3O_3S$: C, 63.45; H, 5.83; N 10.57%, Found: C, 63.43; H, 5.85; N, 10.60%.

*(1H-Benzimidazol-2-yl-sulfanyl)-acetic acid butylcarbamoyl-otolyl-methyl ester (**2b**)*

White solid, yield 1.9058 g; IR (KBr) ν_{max} cm^{-1}: 3435 (br, arom-NH), 3142 (aliph-NH), 1632 (CO of acid), 1524 (CO of CONH); ^{1}H-NMR (300 MHz, DMSO) δ ppm: 0.90 (t, $J = 3.3$, 5.7 Hz, 3H, CH_3), 1.30 (d, $J = 10$ Hz, 2H, CH_2), 1.62 (d, $J = 6.6$ Hz, 2H, CH_2), 2.34 (s, 3H, CH_3), 3.16 (s, 2H, CH_2), 3.87 (s, 2H, CH_2), 3.95 (br, 1H, NH of imidazole), 6.80 (s, 1H, O–CH), 7.15–7.21 (m, 4H, arom-H), 7.65–7.68 (q, $J = 6.6$ Hz, 4H, arom-H), 7.90 (s, 1H, aliph-NH); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm: 12.9, 15.6, 21.5, 115.8, 123.3, 142.1, 170.8, 172.6; MS *m/z*: 410.41 (M); anal. calcd. for $C_{22}H_{25}N_3O_3S$: C, 61.21; H, 6.12; N, 10.21%, Found: C, 61.23; H, 6.10; N, 10.19%.

*(1H-Benzimidazol-2-yl-sulfanyl)-acetic acid butylcarbamoyl-ptolyl-methyl ester (**2c**)*

Light brown solid, yield 1.7463 g; IR (KBr) ν_{max} cm^{-1}: 3433 (br, arom-NH), 3146 (alp-NH), 1679 (CO of acid), 1576 (CO of CONH); ^{1}H-NMR (300 MHz, DMSO) δ ppm: 0.92 (d, $J = 10$ Hz, 3H, CH_3),

1.29 (s, 2H, CH_2), 1.65 (d, $J = 6.6$ Hz, 2H, CH_2), 2.32 (s, 3H, CH_3), 3.37 (s, 2H, CH_2), 3.87 (s, 2H, CH_2), 3.99 (br, 1H, NH of imidazole), 6.87 (s, 1H, O–CH), 7.22–7.27 (m, 4H, arom-H), 7.65–7.71 (m, 4H, arom-H), 7.80 (s, 1H, aliph-NH); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm: 13.1, 18.9, 21.6, 116.1, 122.8, 142.9, 170.0, 171.9; MS *m/z*: 412.06 (M^+); anal. calcd. for $C_{22}H_{25}N_3O_3S$: C, 61.21; H, 6.12; N, 10.21%, Found: C, 61.19; H, 6.15; N, 10.23%.

*(1H-Benzimidazol-2-yl-sulfanyl)-acetic acid butylcarbamoyl (2-methoxy-phenyl) methyl ester (**2d**)*

White solid, yield 1.821 g; IR (KBr) ν_{max} cm^{-1}: 3476 (br, arom-NH), 3145 (aliph-NH), 1679 (CO of acid), 1574 (CO of CONH); ^{1}H-NMR (300 MHz, DMSO) δ ppm: 0.87 (d, $J = 10.3$ Hz, 3H, CH_3), 1.24 (s, 2H, CH_2), 1.63 (s, 2H, CH_2), 3.19 (s, 2H, CH_2), 3.83 (s, 3H, OCH_3), 3.87 (s, 2H, CH_2), 4.15 (br, 1H, NH of imidazole), 6.93 (s, 1H, O–CH), 7.04–7.14 (m, 4H, arom-H), 7.46 (q, $J = 4.9$ Hz, 4H, arom-H), 7.89 (s, 1H, aliph-NH); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm: 13.9, 22.1, 56.9, 116.1, 123.1, 144.8, 170.6, 172.8; MS *m/z*: 426.40 (M); anal. calcd. for $C_{22}H_{25}N_3O_4S$: C, 61.81; H, 5.89; N, 9.83%, Found: C, 61.79; H, 5.91; N, 9.80%.

*(1H-Benzimidazol-2-yl-sulfanyl)-acetic acid butylcarbamoyl (4-methoxy-phenyl) methyl ester (**2e**)*

White solid, yield 1.6575 g; IR (KBr) ν_{max} cm^{-1}: 3454 (br, arom-NH), 3148 (aliph-NH), 1634 (CO of acid), 1574 (C=O of CONH); ^{1}H-NMR (300 MHz, DMSO) δ ppm: 0.93 (t, $J = 4.1$, 6.8 Hz, 3H, CH_3), 1.24 (d, $J = 6.6$ Hz, 2H, CH_2), 1.70 (s, 2H, CH_2), 3.17 (s, 2H, CH_2), 3.82 (s, 3H, OCH_3), 3.87 (s, 2H, CH_2), 4.13 (br, 1H, NH of imidazole), 6.91 (s, 1H, O–CH), 7.03–7.13 (m, 4H, arom-H), 7.42–7.45 (m, 4H, arom-H), 7.91 (s, 1H, aliph-NH); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm: 14.2, 20.9, 58.3, 115.8, 123.5, 142.4, 171.0, 171.8; MS *m/z*: 426.38 (M); anal. calcd. for $C_{22}H_{25}N_3O_4S$: C, 61.81; H, 5.89; N 9.8%, Found: C, 61.83; H, 5.87; N, 9.84%.

*(1H-Benzimidazol-2-yl-sulfanyl)-acetic acid butylcarbamoyl-(4-ethyl-phenyl) ethyl ester (**2f**)*

White solid, yield 1.7767 g; IR (KBr) ν_{max} cm^{-1}: 3441 (br, arom-NH), 3151 (aliph-NH), 1634 (CO of acid), 1575 (CO of CONH); ^{1}H-NMR (300 MHz, DMSO) δ ppm: 0.97 (t, $J = 3.3$, 6.6 Hz, 3H, CH_3), 1.30 (d, $J = 6.6$ Hz, 2H, CH_2), 1.46 (s, 3H, ar-CH_3), 1.62 (s, 2H, CH_2), 2.40 (s, 2H, ar-CH_2), 3.22 (s, 2H, CH_2), 3.81 (s, 2H, CH_2), 4.16 (br, 1H, NH of imidazole), 6.64 (s, 1H, O–CH), 7.19–7.27 (m, 4H, arom-H), 7.66 (d, $J = 7.1$ Hz, 4H, arom-H), 7.90 (s, 1H, aliph-NH); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm: 14.3, 17.6, 23.3, 35.3, 115.2, 122.6, 144.1, 171.1, 172.6; MS *m/z*: 426.02 (M^+); anal. calcd. for $C_{23}H_{27}N_3O_3S$: C, 64.92; H, 6.40; N, 9.87%, Found: C, 62.95; H, 6.41; N, 9.85%.

*(1H-Benzimidazol-2-yl-sulfanyl)-acetic acid butylcarbamoyl-(2-nitro-phenyl) methyl ester (**2g**)*

White solid, yield 1.6255 g; IR (KBr) ν_{max} cm^{-1}: 3420 (br, arom-NH), 3151 (aliph-NH), 1664 (CO of acid), 1578 (CO of CONH); ^{1}H-NMR (300 MHz, DMSO) δ ppm: 0.98 (d, $J = 6.6$ Hz, 3H, CH_3), 1.33 (d, $J = 10$ Hz, 2H, CH_2), 1.63 (m, 2H, CH_2), 3.18 (s, 2H, CH_2), 3.87 (s, 2H, CH_2), 4.15 (br, 1H, NH of imidazole), 6.52 (s, 1H, O–CH), 7.23–7.33 (m, 4H, arom-H), 7.44–7.75 (m, 4H, arom-H), 7.96 (s, 1H, aliph-NH); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm: 12.6, 22.5, 114.3, 123.8, 142.6, 170.1, 172.5; MS *m/z*: 440.36 (M); anal. calcd. for $C_{21}H_{22}N_4O_5S$: C, 57.00; H, 5.01; N, 12.66%, Found: C, 56.97; H, 5.03; N, 12.70%.

*(1H-Benzimidazol-2-yl-sulfanyl)-acetic acid butylcarbamoyl-(4-nitro-phenyl) methyl ester (**2h**)*

White solid, yield 1.8741 g; IR (KBr) ν_{max} cm^{-1}: 3424 (br, arom-NH), 3252 (aliph-NH), 1683 (CO of acid), 1578 (CO of CONH); ^{1}H-NMR (300 MHz, DMSO) δ ppm: 0.89 (t, $J = 3.3$, 5.6 Hz, 3H, CH_3), 1.29 (d, $J = 6.6$ Hz, 2H, CH_2), 1.62 (s, 2H, CH_2), 3.29 (s, 2H, CH_2), 3.72 (s, 2H, CH_2), 3.96 (br, 1H, NH of imidazole), 6.60 (s, 1H, O–CH), 7.20–7.28 (m, 4H, arom-H), 7.56–7.77 (m, 4H, arom-H), 7.79 (s, 1H, aliph-NH); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm: 14.1, 20.3, 112.2, 125.5, 143.0, 171.3, 172.8; MS *m/z*: 440.44 (M); anal. calcd. for $C_{21}H_{22}N_4O_5S$: C, 57.00; H, 5.01; N, 12.66%, Found: C, 57.02; H, 5.03; N, 12.62%.

*(1H-Benzimidazol-2-yl-sulfanyl)-acetic acid butylcarbamoyl (2-chloro-phenyl) methyl ester (**2i**)*

Yellow solid, yield 1.7146 g, IR (KBr) ν_{max} cm^{-1}: 3488 (br, arom-NH), 3151 (aliph-NH), 1680 (CO of acid), 1571 (CO of CONH); ^{1}H-NMR (300 MHz, DMSO) δ ppm: 0.94 (s, 3H, CH_3), 1.39 (s, 2H, CH_2), 1.63 (d, $J = 4.9$, 2H, CH_2), 3.20 (s, 2H, CH_2), 3.75 (s, 2H, CH_2), 4.17 (br, 1H, NH of imidazole), 6.66 (s, 1H, O–CH), 7.10–7.27 (s, 4H, arom-H), 7.34–7.67 (s, 4H, arom-H), 7.77 (s, 1H, aliph-NH); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm: 13.3, 21.8, 112.7, 124.1, 145.5, 169.6, 172.3; MS *m/z*: 430.52 (M); anal. calcd. for $C_{21}H_{22}ClN_3O_3S$: C, 58.39; H, 5.13, N, 9.73%, Found: C, 58.42; H, 5.10; N, 9.71%.

*(1H-Benzimidazol-2-yl-sulfanyl)-acetic acid butylcarbamoyl (4-chloro-phenyl) methyl ester (**2j**)*

White solid, yield 1.8014 g; IR (KBr) ν_{max} cm^{-1}: 3482 (br, arom-NH), 3138 (aliph-NH), 1684 (CO of acid), 1575 (CO of CONH); ^{1}H-NMR (300 MHz, DMSO) δ ppm: 0.99 (t, $J = 5.3$ Hz, 3H, CH_3), 1.23 (d, $J = 6.8$, 2H, CH_2), 1.58 (s, 2H, CH_2), 3.23 (s, 2H, CH_2), 3.76 (s, 2H, CH_2), 4.14 (br, 1H, NH of imidazole), 6.91 (s, 1H, O–CH), 7.02–7.14 (m, 4H, arom-H), 7.43–7.65 (m, 4H, arom-H), 7.89 (s, 1H, aliph-NH); ^{13}C-NMR (DMSO, 75 MHz): δ (ppm) 12.8, 20.9, 113.4, 122.6, 145.8, 168.7, 172.2; MS *m/z*: 432.02 (M^+); anal. calcd. for $C_{21}H_{22}ClN_3O_3S$: C, 58.39; H, 5.13; N, 9.73%, Found: C, 58.42; H, 5.11; N, 9.74%.

*(1H-Benzimidazol-2-yl-sulfanyl)-acetic acid butylcarbamoyl-(2,4-dinitro-phenyl) methyl ester (**2k**)*

White solid, yield 1.8387 g; IR (KBr) ν_{max} cm^{-1}: 3466 (br, arom-NH), 3149 (aliph-NH), 1688 (CO of acid), 1574 (CO of CONH); ^{1}H-NMR (300 MHz, DMSO) δ ppm: 0.98 (s, 3H, CH_3), 1.27 (s, 2H, CH_2), 1.66 (s, 2H, CH_2), 3.18 (s, 2H, CH_2), 3.87 (s, 2H, CH_2), 4.14 (br, 1H, NH of imidazole), 6.93 (s, 1H, O–CH), 7.11–7.14 (m, 4H, arom-H), 7.43–7.46 (s, 4H, arom-H), 7.92 (1H, s, aliph-NH); ^{13}C-NMR (DMSO, 75 MHz): δ (ppm) 14.5, 20.9, 116.2, 123.8, 144.3, 170.8, 172.3; MS *m/z*: 488.12 (M^+): anal. calcd. for $C_{21}H_{21}N_5O_7S$: C, 51.74; H, 4.34; N, 14.37%, Found: C, 51.76; H, 4.37; N, 14.35%.

*(1H-Benzimidazol-2-yl-sulfanyl)-acetic acid butylcarbamoyl (2,4-dichloro-phenyl) methyl ester (**2l**)*

White solid, yield 1.7531 g; IR (KBr) ν_{max} cm^{-1}: 3488 (br, arom-NH), 3157 (aliph-NH), 1674 (CO of acid), 1575 (CO of CONH); ^{1}H-NMR (300 MHz, DMSO) δ ppm: 0.91 (t, $J = 3.8$ Hz, 3H, CH_3), 1.24 (s, 2H, CH_2), 1.72 (s, 2H, CH_2), 3.17 (s, 2H, CH_2), 3.82 (s, 2H, CH_2), 4.13 (br, 1H, NH of imidazole), 6.88 (s, 1H, O–CH), 7.00–7.13 (m, 4H, arom-H), 7.42–7.45 (s, 4H, arom-H), 7.91 (1H, s, aliph-NH); ^{13}C-NMR (DMSO, 75 MHz): δ (ppm) 14.7, 22.1, 115.2, 123.2, 143.6,

170.3, 172.2; MS *m/z*: 464.29 (M); anal. calcd. for $C_{21}H_{21}Cl_2N_3O_3S$: C, 54.08; H, 4.54; N, 9.01%, Found: C, 54.11; H, 4.52, N, 8.98%.

Pharmacological assay

Animals used

Albino-Wistar rats (150–200 g) were used for *in-vivo* activities. Animals were maintained under standard laboratory conditions (24 ± 2°C; relative humidity 60–70%). Study protocol was approved by the institutional Animal Ethics Committee (IAEC, Reg.No. 346/CPCSEA: dated 03-01-2001, Department of Pharmacy, Luqman College of Pharmacy and Research, Gulbarga) before experiment. The animals were kept in polypropylene cages and maintained on balanced ration with free access to clean drinking water. All experimental procedures were conducted in accordance with the guide for care and use of laboratory animals and in accordance with the local animal care and use committee.

Acute toxicity study

For testing the acute toxicity potential of the test compounds, Albino-Wistar rats of either sex, weighing (20–25 g) were used. The dosage was varied from 100 up to 3000 mg/kg body weight. The animals were continuously observed for 8 h for any signs of acute toxicity such as increased-decreased motor activity, ataxia, tremors, convulsions, sedation, lacrimation, etc. After 24 h the animals were sacrificed, stomach, intestine, and liver were inspected under the magnifying lenses for any ulcer-hemorrhagic spots.

Anti-inflammatory activity screening

Anti-inflammatory activity was evaluated by using carrageenan-induced hind paw edema method [32, 37]. Albino-Wistar rat strains of either sex between 150–200 g were selected for the studies. The animals were kept on diet and allowed food and water *ad libitum*. They were housed in polypropylene cages maintained under standard condition (12 h light/12 h dark cycles at 25 ± 3°C temperature, 35–60% humidity). The rats were divided into fourteen groups of six rats each as described in Table 2. The control group received Tween-80 (1%) 10 mL/kg p.o. The test groups received 400 mg/kg p.o. of synthetic compounds. Diclofenac sodium 200 mg/kg p.o. served as standard. All the suspensions were administrated 30 min before the injection of carrageenan (0.1 mL of 1%). The paw edema volume was measured with the help of plethysmograph by mercury displacement method at 0, 1, 2, 3, 4 and 6 h.

Antidiabetic activity screening

Effect of blood glucose level

Fasted rats were divided into three groups of six each. Group I, the control group, received normal saline. Group II received compounds **2a–2l**, respectively, at a dose of 250 mg/kg body weight by oral route and group III for Glibenclamide standard. The rats of all groups were given glucose (2 g/kg body weight, p.o.) 30 min after administration of the drug. Blood samples were collected from the tail vein just prior to glucose administration at 2nd h, 4th h and 9th day after the glucose loading. Serum was separated and blood glucose levels were measured immediately by glucose–oxidase method [38].

OGTT Screening

Oral glucose tolerance test (OGTT) method was used to evaluate anti-diabetic activity. Albino-Wistar rats of either sex (150–200 g) were induced diabetic by a single i.p. injection of 150 mg/kg body weight of alloxan monohydrate in sterile normal saline. The rats were maintained on 5% glucose solution for next 24 h to prevent hypoglycemia [39]. Nine days later blood samples were collected by retro orbital puncture of each rat under mild anesthesia in 1-mL Eppendorff tubes containing 50 mL of anticoagulant heparin. The diabetic rats were divided into three groups, each containing six animals. Controls rats (group I) were given normal saline orally, while compounds **2a–2l** were given to group II, respectively, at a dose of 250 mg/kg, orally. Group III received Glibenclamide at a dose of 10 mg/kg. Blood samples were collected from the tail vein just prior to 30 min and 90 min after drug administration. The blood glucose level was determined by glucose–oxidase method.

Anti-convulsant activity screening

Evaluation of anti-convulsant activity was done by maxima electroshock model [35, 36] and Albino-Wistar rats (150–200 g) were used for the above method. In MES convulsions electroshock is applied through the corneal electrode; from this optic stimulation cortical excitation is produced. The MES convulsions are divided into five phases such as a) tonic flexion, b) tonic extensor, c) clonic convulsions, d) stupor and e) recovery or death. The time (s) spent by the animal in each phase of the convulsions is noted down. A substance is known to possess anti-convulsant property if it reduces or abolishes the extensor phase of MES convulsions.

Statistical analysis

The experimental results for anti-inflammatory, anti-diabetic and anti-convulsant activities were expressed as the mean ± standard error of mean (SEM), evaluated by compared one-way ANOVA test. The statistical significance was evaluated by using the Student's '*t*' and Dunnet's test. Values of $P < 0.01$ were considered as statistically significant [37, 40].

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