European Journal of Medicinal Chemistry 45 (2010) 1753–1759

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Derivatives of benzimidazole pharmacophore: Synthesis, anticonvulsant, antidiabetic and DNA cleavage studies

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ARTICLE INFO

Article history:
Received 30 September 2009
Received in revised form
4 January 2010
Accepted 6 January 2010
Available online 14 January 2010

Keywords:
4-Thiazolidinones
1,3,4-Oxadiazoles
2-Mercapto benzimidazole
Anticonvulsant
Antidiabetic
DNA cleavage

ABSTRACT

In seeking broad spectrum pharmacological activities of benzimidazole derivatives, a group of 4-thiazolidinones **5**(**a**–**j**) and 1,3,4-oxadiazoles **6**(**a**–**j**) containing 2-mercapto benzimidazole moiety were synthesized and screened for *in vivo* anticonvulsant activity by Maximal Electroshock (MES) model and antidiabetic activity using Oral Glucose Tolerance Test (OGTT). Compounds (**5c**), (**5d**), (**5g**) and (**5i**) exhibited potent anticonvulsant results and (**6c**), (**6d**), (**6h**) and (**6i**) showed excellent antidiabetic activities and also pharmacophore derived from active molecules suggested that presence of –OH group was a common feature in all active compounds. In DNA cleavage studies, compound (**5d**) cleaved DNA completely as no trace of DNA was found. On the other hand, a sharp streak was found for compounds (**5c**), (**6a**) and (**6d**).

1. Introduction

Epilepsy, being one of the most common and serious neurological disorders is characterized by recurrent seizures which results from a temporary electrical disturbance of the brain due to an imbalance between excitory and inhibitory neurotransmitters. The mechanisms of action of the antiepileptic drugs (AEDs) consist in the blockade of voltage-dependent Na^{+} channels or T-type Ca^{++} channels, inhibition of glutamatergic transmission and facilitation of γ-aminobutyric acid (GABA) inhibitory neurotransmission. About one third of patients do not respond well to current multiple drugs therapy [1,2]. Phenytoin, Carbamazepine and Sulfamate topiramate (TPM) are recent antiepileptic drugs which have been clinically effective against different types of seizures [3].

Diabetes mellitus (DM) is a non-communicable disease and the most daunting challenges posed by chronic, complex and a multifarious group of disorders that disturbs the metabolism of carbohydrates, fats and proteins resulting from insulin deficiency or insulin resistance. Insulin resistance is associated with a deficit in protein tyrosine phosphorylation in insulin signal transduction cascade. Diabetic nephropathy, a serious chronic diabetic microvascular complication has become most important cause of end-stage renal disease [4], followed by neuropathy, cataracts and retinopathy, which practically are not controlled by insulin. Sulfonylureas are the most widely used antidiabetic agents. These agents are acting on pancreatic β-cells stimulating insulin secretion. Next to these are thiazolidinediones which enhance insulin action. Glibenclamide, Ciglitazone and Troglitazone belong to this group which is effective towards enhancing insulin in a large number of Type-2 patients. Metformin which belongs to the third class of oral-lowering agents i.e., Biguanides, enhances insulin action at the postreceptor level in peripheral tissues such as muscle [5].

DNA is a helical polyanion built by the union of two linear polymeric strands that are composed of sugars (deoxyribose) linked by phosphates. It is an important target of antitumoral drugs because it plays a central role in replication, transcription, and regulation of genes. Substantial progress has been made during the past few decades to develop metal-based small molecules as DNA foot-printing as well as therapeutic agents that are capable of binding and cleaving DNA under physiological conditions [6–8]. DNA cleavage can occur by a nucleophilic attack to a phosphate in nucleic acid backbone [9,10]. The design of DNA and RNA-specific agents capable of controlled chemical cleavage are of paramount importance due to their potential use as drugs, regulators of gene expression and tools for molecular biology. The ability to selectively target and cleave DNA with high affinity and to report on the binding event by changes in luminescence is of great current interest [11,12].

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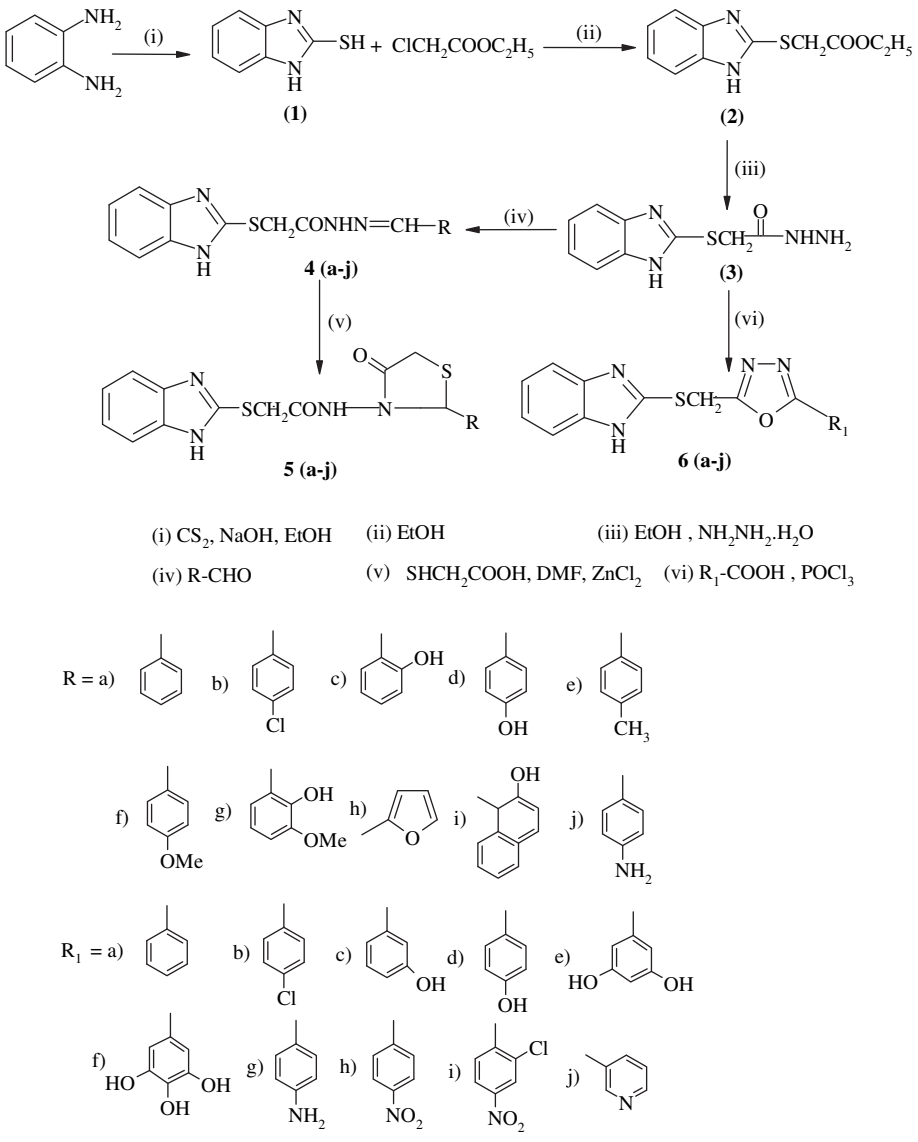
doi:10.1016/j.ejmech.2010.01.007

The target thiazolidinone and oxadiazole pharmacophores are being introduced to 2-mercapto benzimidazole in search of potent drugs. The introduction of sulphur was expected to bring in a bulky polarisable atom for potent pharmacological activities, thus altering its geometry, polarisability, stability, lipophilicity, steric and electronic characteristics of the molecule. It was also anticipated that, thiazolidinone ring along with sulphur atom improves the activity of lead molecules. Wherein, 4-thiazolidinones have many interesting activity profiles [13,14]. Similarly, 1,3,4-oxadiazoles [15] are thermally stable and neutral heteroaromatic molecules which exhibit one of the active class of compounds possessing various pharmacological properties such as antimicrobial [16], antiinflammatory [17], antimalarial [18], antimycobacterial [19] and anti HIV [20] activities.

Considering extensive applications of benzimidazole moiety in medicinal chemistry and in continuation of our ongoing project biologically active heterocycles [21–25], an attempt has been made to synthesize 4-thiazolidinone and 1,3,4-oxadiazole derivatives containing 2-mercapto benzimidazole and evaluate for their *in vivo* bioassays.

2. Chemistry

2-Mercapto benzimidazole (**1**) was prepared according to reported method [26]. The synthetic routes to compounds **5**(**a**–**j**) and **6**(**a**–**j**) are illustrated in Scheme 1. The appearance of signals at δ 1.32 ppm due to $-CH_3$ and δ 4.33 ppm due to $-CH_2$ in $-COOCH_2CH_3$ in ^{1}H NMR confirms the formation of ester (**2**). This was also confirmed by IR spectra which shows a band at 1720 cm^{-1} because of C=O of ester. Compound (**3**) was confirmed by signals at δ 8.02 ppm and δ 4.13 ppm due to –CONH and $-NH_2$ respectively. The >C=O band for amide in IR appears at 1641 cm^{-1} and $-NHNH_2$ around 3207 and 3346 cm^{-1} confirms the formation of (**3**). A signal at δ 3.70 ppm and 3.65 ppm due to >CH–Ar– and $-S-CH_2-$ confirms the formation of thiazolidinone ring. In the ^{1}H NMR spectra of **4**(**a**–**j**), the proton of –N=CH appears at about δ 4.02–4.10 ppm. But, in the spectra of **5**(**a**–**j**), this proton signal is shifted slightly downfield in contrast with compounds **4**(**a**–**j**). The reason being the deshielding affected by heterocycles, which shifted the resonances to about δ 3.70–3.95 ppm. The aromatic protons appeared as a multiplet in the region δ 6.98–8.01 ppm. In ^{13}C NMR, signals of



Scheme 1. Synthetic pathway to generate benzimidazole derivatives.

ring S–CH_2 and ring >C=O appeared at about δ 31.63 and 172.35 ppm respectively. The appearance of (C–O–C) signal at 1267.7 cm^{-1} confirms the formation of the oxadiazole moiety.

3. Pharmacology

All the compounds prepared herein were screened for their potential *in vivo* biological activities such as anticonvulsant, antidiabetic and DNA cleavage studies. The anticonvulsant activities of the synthesized compounds were tested through an *in vivo* rodent model of convulsions (Maxima ElectroShock, MES test) [27]. Evaluation of antidiabetic activity was done by blood–glucose test and oral glucose tolerance test (OGTT) [28,29]. Male Wister albino rats (150–200 g) of either sex were selected. DNA cleavage of selected compounds is also studied by electrophoresis method.

4. Results and discussion

4.1. Pharmacology

4.1.1. Anticonvulsant activity

Results of anticonvulsant activity with standard drug phenytoin are discussed in Table 1. The existence of a hydrophobic unit in benzimidazole ring, an electron donor group and hydrogen-bonding domain was essential for anticonvulsant activity as depicted by the models and also evidenced by active drug phenytoin and carbamazepine. A study of structure–activity relationship revealed that compounds (**5c**), (**5d**), (**5g**) and (**5i**) exhibited their ability to diminish tonic-extensor seizures. The presence of a hydroxyl –OH function at 2 and 4 position of the phenyl ring as seen with compounds (**5c**), (**5d**), (**5g**) and (**5i**) was found to be the main structural requirement for maintaining anticonvulsant activity. The extensor phase time was remarkably reduced for these compounds. This requirement was further evidenced by compounds (**5b**) and (**5e**) where –OH function was replaced by a –Cl, $-CH_3$ function, which resulted in complete loss of activity due to the disappearance of this function and can be explained in terms of interaction at the binding site by the pharmacophoric models. From the present study, four compounds have emerged as lead moieties. Further structural modifications might lead to the discovery of more potent anticonvulsant agents.

4.1.2. Antidiabetic study

In vivo efficacy of compounds **6**(**a**–**j**) was evaluated for antidiabetic activity and the results are discussed in Table 2. Four compounds showed better reduction in blood–glucose levels on 9th day compared with glibenclamide. They were considered as significant compared to diabetic control group. Therefore, the best potent activity was found to be associated with (**6c**), (**6d**), (**6h**) and (**6i**). Several other compounds (**6a**), (**6b**), (**6c**), (**6f**), (**6h**) and (**6i**) were significant compared to normal control group during Oral Glucose Tolerance Test at 90th min.

4.1.3. DNA cleavage study

The results of DNA cleavage for compounds **5**(**a**–**f**) and **6**(**a**–**f**) studied by agarose gel electrophoresis method. The gel after electrophoresis clearly revealed that, compound (**5d**) did cleave the DNA completely, as no traces of DNA were found. (**5c**) was also found to be significant than other compounds. In case of **6**(**a**–**f**), a sharp streak was found for compounds (**6a**) and (**6d**). Other compounds were seen to be nearly completely inactive to cleave DNA.

5. Conclusion

By choosing proper experimental conditions we have been able to synthesize thiazolidinone and oxadiazole containing 2-mercapto benzimidazole derivatives and investigate for various bioassays with the hope of discovering new structure leads serving as potential broad spectrum pharmacological agents. SAR studies revealed the critical role of –OH function in the target compounds that showed very promising activities. Compounds (**5c**), (**5d**), (**5g**) and (**5i**) were considered significant compared to control group for anticonvulsant activity. Meanwhile compounds (**6c**), (**6d**), (**6h**) and (**6i**) showed excellent activity against Glibenclamide and were considered significant compared to diabetic control group. Compound (**5d**) cleaved DNA completely as no traces of DNA were found. Other compounds were seen to be nearly completely inactive to cleave DNA.

6. Experimental section

6.1. Chemistry

All reagents and solvents were used as obtained from the supplier or recrystallized/redistilled unless otherwise noted. Melting points were determined in open capillaries and are uncorrected. Infrared spectra were recorded using KBr pellets on Nicolet 5700 FT-IR instrument. The ^{1}H NMR and ^{13}C NMR spectra were recorded on Brucker Avanace-300 (300 MHz) model

Table 1
Anticonvulsant activity of 4-thiazolidinones **5**(**a**–**j**) using Maximal Electroshock (MES) Model.

S. No treatment (dose)		Time (s) in various phases of convulsion (mean ± SEM)				
		Flexion	Extensor	Clonus	Stupor	Recovery/Death
Control Gumacacia	1% w/w	3.12 ± 0.31	11 ± 0.57	16.80 ± 2.50	218.33 ± 5.80	–
Phenytoin (Std. drug)	20 mg/kg	2.87 ± 0.31^{ns}	0 ± 0.00**	4.90 ± 4.20^{ns}	158.6 ± 20.51^{ns}	Recovery
5a	80 mg/kg	3.20 ± 0.25^{ns}	9.20 ± 0.70*	1.20 ± 1.20*	192.5 ± 13.20^{ns}	Death
5b	75 mg/kg	4.56 ± 1.02^{ns}	12.1 ± 0.57*	1.36 ± 1.32*	215.5 ± 20.54^{ns}	Death
5c	85 mg/kg	**3.14 ± 0.30^{ns}**	**4.33 ± 0.42****	**15.62 ± 2.10***	**209.3 ± 4.96***	Death
5d	70 mg/kg	**3.96 ± 1.16^{ns}**	**3.73 ± 0.42****	**1.02 ± 1.20***	**202.1 ± 20.54^{ns}**	Death
5e	70 mg/kg	3.12 ± 0.30^{ns}	11.0 ± 0.57^{ns}	14.62 ± 2.10^{ns}	207.3 ± 4.96^{ns}	Death
5f	75 mg/kg	3.02 ± 0.27^{ns}	9.50 ± 0.57*	12.62 ± 2.10 ^{ns}	208.0 ± 5.66^{ns}	Death
5g	82 mg/kg	**3.30 ± 0.36^{ns}**	**4.21 ± 0.39****	**16.20 ± 2.14****	**213.80 ± 5.68***	Death
5h	65 mg/kg	4.20 ± 1.08^{ns}	5.30 ± 0.31	1.00 ± 1.20*	208.6 ± 4.80^{ns}	Death
5i	60 mg/kg	**3.08 ± 0.28***	**3.98 ± 0.44****	**1.80 ± 1.20***	**172.5 ± 13.40^{ns}**	Death
5j	60 mg/kg	3.15 ± 0.25*	5.66 ± 0.21**	15.40 ± 2.15*	210.5 ± 4.85*	Death

The active compounds are marked in bold letters.
$P > 0.05$ 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 diabetic control group.

Table 2
Antidiabetic activity of 1,3,4-oxadiazoles **6**(**a**–**j**) using Oral Glucose Tolerance Test (OGTT) test.

Treatment & Groups	Blood glucose level in mg/dl		Oral Glucose Tolerance Test (OGTT) in non-diabetic rats		
	Basal	9th day	Fasting	30 min	90 min
Control Normal saline	74.67 ± 1.38	79.17 ± 0.48	83.33 ± 1.54	183.2 ± 3.68	135.7 ± 2.9
Diabetic control (Alloxan)	336.6 ± 2.651^{+++}	339.8 ± 1.815^{+++}	–	–	–
Glibenclamide (Std drug)	332.3 ± 4.349	139.2 ± 2.52***	79.17 ± 3.15	176.8 ± 3.04	105.7 ± 2.20**
6a	320.2 ± 7.58	337.2 ± 13.08	76.33 ± 2.98	180.2 ± 6.40	**107.7 ± 3.30****
6b	352.0 ± 12.53	386.7 ± 25.68	74.83 ± 1.62	173.2 ± 5.50	**120.0 ± 4.50****
6c	353.0 ± 6.74	**181.2 ± 17.49*****	80.33 ± 2.55	162.8 ± 6.60	**107.3 ± 2.81****
6d	327.3 ± 5.00	**166.2 ± 15.20*****	80.83 ± 3.33	167.5 ± 3.05	125.0 ± 2.70
6e	320.0 ± 7.58	337.2 ± 13.08	81.50 ± 2.14	155.7 ± 2.20	106.5 ± 2.10
6f	352.0 ± 12.53	386.4 ± 25.60	73.33 ± 3.72	174.2 ± 3.38	**110.0 ± 3.70****
6g	320.0 ± 7.58	335.5 ± 12.42	80.85 ± 2.55	155.5 ± 2.20	106.5 ± 2.15
6h	322.2 ± 1.80	**158.7 ± 4.76*****	78.33 ± 2.30	154.5 ± 5.77	**110.0 ± 4.11****
6i	327.4 ± 5.00	**187.7 ± 29.4*****	77.17 ± 2.57	155.3 ± 10.38	**121.7 ± 10.6****
6j	325.5 ± 7.58	343.3 ± 14.40	81.34 ± 2.92	181.8 ± 3.09	122.8 ± 2.85

The active compounds are marked in bold letters.
One way ANOVA followed by Dunnets'*t*' test. Values are expressed as ± SEM.
$P > 0.05$ is considered as non-significant.
$P < 0.05$ is considered as significant.
$^{+++}P < 0.001$ as compared to normal control group.
$^{**}P < 0.001$ as compared to normal control group.
$^{***}P < 0.001$ as compared to diabetic control group.

spectrophotometer in $CDCl_3$ and DMSO as a solvent and TMSi as internal standard with ^1H resonant frequency of 300 MHz and ^{13}C resonant frequency of 75 MHz D_2O exchange was applied to confirm the assignment of the signals of NH protons. The chemical shifts were measured in ppm downfield from internal TMSi at $\delta = 0$. The mass spectra were recorded on Schimadzu GCMS-QP2010S at 70 eV. All reactions were monitored by thin layer chromatography (TLC) using E. Merck $60F_{254}$ procoated silica gel plates detected by UV light (254 nm) or iodine vapours. The elemental analysis was carried out by using Heraus CHN rapid analyzer. All the compounds gave C, H and N analysis within ±0.5% of the theoretical values. The biological activities were carried out at Luqman Pharmacy College, Gulbraga, Rajiv Gandhi University, India.

*6.1.1. General method for the synthesis of 2-mercapto benzimidazole (**1**)*

Compound (**1**) was prepared according to the reported procedure [26].

*6.1.2. Synthesis of ethyl 2-(benzimidazolyl-thio) acetate (**2**)*

An equimolar solution of 2-mercapto benzimidazole (**1**) (1.50 g, 0.01 mol) and ethylchloroacetate (1.22 mL, 0.01 mol) in dry acetone (4 mL) in presence of anhydrous K_2CO_3 (1 g) was refluxed on a water bath for 6 h. The solvent was removed by vacuum distillation and the residue was dried and recrystallized to furnish compound (**2**). White solid, yield: 70%; m.p. 60–62 °C; IR (KBr): υ_{max} in cm^{-1}: 3042 (aromatic ring), 638 (C–S), 1719 (>C═O of ester), 1684 (–C═N–), 1320 and 1234 (C–O–C), 830 (C–S–C), 2955, 2889, 1443, 714, (–CH_2 and –CH_3); ^1H NMR ($CDCl_3$, 300MHz) δ ppm: 1.40 (t, 3H, J = 7 Hz, –$COOCH_2CH_3$), 3.85 (q, 2H, J = 6.5 Hz, –$COOCH_2CH_3$), 4.20 (s, 2H, S–CH_2–), 6.90–7.85 (m, 4H, Ar–H), 10.9 (s, 1H, –NH–benzimidazole); Anal. Calcd for $C_{11}H_{12}N_2O_2S$: C, 55.93; H, 5.10; N, 11.86%. Found: C, 55.90; H, 5.12; N, 11.85%.

*6.1.3. Synthesis of [(2-benzimidazolylthio)-acetyl]-hydrazine (**3**)*

Compound (**2**) (2.36 g, 0.01 mol) and hydrazine hydrate (0.9 mL, 0.02 mol) in ethanol (20 mL) were refluxed for about 5 h on a steam bath. After cooling the resulting solid was filtered, dried and recrystallized to obtain compound (**3**). Pinkish white solid, yield: 75%; m.p. 195 °C; IR (KBr) υ_{max} in cm^{-1}: 3311, 3369 (–$NHNH_2$), 1680 (>C═O of amide); ^1H NMR ($CDCl_3$, 300MHz) δ ppm: 3.90 (s, 2H, –NH_2), 4.35 (s, 2H, S–CH_2), 6.95–7.90 (m, 4H, Ar–H), 7.55 (s, 1H, –CONH–), 10.25 (s, 1H, –NH–benzimidazole). Anal. Calcd for $C_9H_{10}N_4OS$: C, 48.64; H, 4.80; N, 25.22%. Found: C, 48.67; H, 4.83; N, 25.26%.

*6.1.4. Synthesis of (1H-benzimidazol-2-ylsulfanyl)-acetic acid hydrazide (**4**)*

A mixture of compound (**3**) (2.22 g, 0.01 mol), respective aldehyde (1.06 mL, 0.01 mol) and 2–3 drops of glacial acetic acid in ethanol (20 mL) was refluxed on a water bath for about 6 h. The solvent was removed and the residue recrystallized from chloroform–methanol mixture to yield the required compound. The other compounds **4**(**a**–**j**) were prepared similarly by treating with corresponding aldehydes.

*6.1.5. Synthesis of (1H-benzimidazol-2-ylsulfanyl)-thiazolidin-4-one **5**(**a**–**j**)*

To a solution of (**4**) (0.01 mol) in DMF (30 mL) was added mercapto acetic acid (0.9 mL, 0.01 mol) and $ZnCl_2$ (1 g) and the reaction mixture was refluxed for 8 h, cooled and poured onto crushed ice, the separated solid was filtered and washed with 10% $NaHCO_3$. The crude product was dried and recrystallized from DMF to obtain the desired compound.

*6.1.5.1. 3-(1H-Benzimidazol-2-ylsulfanylmethyl)-2-phenyl-thiazolidin-4-one (**5a**).* Pale yellow crystals, yield: 75.66%; m.p. 230–232 °C; IR (KBr) υ_{max} in cm^{-1}: 3212.4, 1378 (–NH–), 1522.9 (–C═N), 1593.7 (–CONH), 1640.0 (ring >C═O); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 3.60 (s, 2H, –CH_2, ring), 3.65 (s, 2H, S–CH_2), 3.70 (d, 1H, J = 15.8 Hz, CH–Ar), 7.0–7.94 (m, 9H, Ar–H), 8.08 (s, 1H, –CONH–), 11.9 (b, 1H, –NH–benzimidazole, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 31.63 (ring S–CH_2), 36.63 (S–CH_2), 54.98 (–CH), 121.50, 122.4, 127.53, 129.21, 129.46, 130.05, 135.52, 143.15 (heteroaromatics), 163.18 (Amide >C═O), 172.35 (ring >C═O); EIMS m/z: 384 [M^+], 385 (M + 1), 386 (M + 2), 197, 195, 191, 180, 174, 164, 163, 150, 149, 117, 105, 28; Anal. Calcd for $C_{18}H_{16}N_4O_2S_2$: C, 56.23; H, 4.19; N, 14.57%. Found: C, 56.27; H, 4.22; N, 14.56%.

*6.1.5.2. 3-(1H-Benzimidazol-2-ylsulfanylmethyl)-2-(4-chloro-phenyl)-thiazolidin-4-one (**5b**).* Yellow crystals, yield: 78.24%; m.p. 235–237 °C; IR (KBr) υ_{max} in cm^{-1}: 3206.3, 1377.0 (–NH–), 1531.3 (C═N), 1595.8 (–CONH), 1639.1 (ring >C═O), Ar–Cl (825.6); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 3.50 (s, 2H, S–CH_2), 3.58 (s, 2H, –CH_2,

ring), 3.72 (d, 1H, $J = 15.6$ Hz, CH–Ar), 6.98–7.90 (m, 9H, Ar–H), 8.12 (s, 1H, –CONH–), 11.78 (b, 1H, –NH–benzimidazole, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 31.11 (ring S–CH_2), 36.79 (S–CH_2), 55.15 (–CH), 112.82, 120.0, 122.42, 129.23, 129.45, 130.28, 132.9, 134.28, 135.3, 145.85 (heteroaromatics), 164.72 (>C=O), 173.87 (ring >C=O); EIMS *m/z*: 418 [M^+], 419 (M + 1), 420 (M + 2), 231, 215, 202, 200, 191, 194, 187, 170, 149, 117, 28; Anal. Calcd for $C_{18}H_{15}N_4O_2S_2Cl$: C, 51.61; H, 3.61; N, 13.37%. Found: C, 51.59; H, 3.65; N, 13.34%.

*6.1.5.3. 3-(1H-Benzimidazol-2-ylsulfanylmethyl)-2-(2-hydroxy-phenyl)-thiazolidin-4-one (**5c**).* Brown crystals, yield: 69.84%; m.p. 135–137 °C; IR (KBr) υ_{max} in cm^{-1}: 3235, 1355 (–NH–), 1586.4 (C=N), 1620.5 (–CONH), 1657.5 (ring >C=O); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 3.58 (s, 2H, –CH_2, ring), 3.62 (s, 2H, S–CH_2), 3.75 (d, 1H, $J = 14.6$ Hz, CH–Ar), 7.0–7.85 (m, 9H, Ar–H), 8.25 (s, 1H, –CONH–), 11.54 (b, 1H, –NH–benzimidazole, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 32.5 (ring S–CH_2), 35.4 (S–CH_2), 58.10 (–CH), 112.0, 121.0, 127.9, 128.85, 129.21, 129.58, 130.54, 136.9, 146.85, 156.5 (heteroaromatics), 164.53 (>C=O), 172.98 (ring >C=O); EIMS *m/z*: 400 [M^+], 401 (M + 1), 402 (M + 2), 213, 194, 190, 169, 152, 106, 89, 44, 15; Anal. Calcd for $C_{18}H_{16}N_4O_3S_2$: C, 53.98; H, 4.03; N, 13.99%. Found: C, 53.95; H, 4.00; N, 14.03%.

*6.1.5.4. 3-(1H-Benzimidazol-2-ylsulfanylmethyl)-2-(4-hydroxy-phenyl)-thiazolidin-4-one (**5d**).* Yellow powder, yield: 74.29%; m.p. 140–142 °C; IR (KBr) υ_{max} in cm^{-1}: 3207.6, 1380.9 (–NH–), 1515.0 (C=N), 1604.3 (–CONH), 1641.7 (ring >C=O), 3448.8 (–OH); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 3.62 (s, 2H, ring –CH_2), 3.69 (s, 2H, S–CH_2), 3.72 (d, 1H, $J = 14.6$ Hz, CH–Ar), 7.0–7.99 (m, 9H, Ar–H), 8.06 (s, 1H, –CONH–), 12.06 (b, 1H, –NH–benzimidazole, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 31.94 (ring S–CH_2), 36.26 (S–CH_2), 55.42 (–CH), 120.55, 128.21, 129.11, 129.81, 129.99, 139.88, 142.55, 154.9 (heteroaromatics), 164.53 (>C=O), 172.58 (ring >C=O); EIMS *m/z*: 400 [M^+], 401 (M + 1), 402 (M + 2), 213, 209, 194, 169, 152, 119, 115, 106, 94, 92, 28; Anal. Calcd for $C_{18}H_{16}N_4O_3S_2$: C, 53.98; H, 4.03; N, 13.99%. Found: C, 54.02; H, 4.00; N, 13.97%.

*6.1.5.5. 3-(1H-Benzimidazol-2-ylsulfanylmethyl)-2-p-tolyl-thiazolidin-4-one (**5e**).* Pale white crystals, yield: 78.54%; m.p. 238–240 °C; IR (KBr) υ_{max} in cm^{-1}: 3224, 1346.3 (–NH–), 1590.6 (C=N), 1639.9 (–CONH), 1680.5 (ring >C=O); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 3.60 (s, 2H, S–CH_2), 3.62 (s, 2H, –CH_2, ring), 3.81 (d, 1H, $J = 15.3$ Hz, CH–Ar), 7.08–8.01. (m, 9H, Ar–H), 8.28 (s, 1H, –CONH–), 11.55 (b, 1H, –NH–benzimidazole, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 21.89 (–CH3), 32.54 (ring S–CH_2), 37.83 (S–CH_2), 55.42 (–CH), 121.5, 128.5, 129.58, 129.82, 129.88, 138.25, 145.28 (heteroaromatics), 168.8 (>C=O), 173.52 (ring >C=O); EIMS *m/z*: 398 [M^+], 399 (M + 1), 400 (M + 2), 210, 195, 181, 165, 149, 105, 91, 58, 44, 15; Anal. Calcd for $C_{19}H_{18}N_4O_2S_2$: C, 57.26; H, 4.55; N, 14.06%. Found: C, 57.25; H, 4.57; N, 14.10%.

*6.1.5.6. 3-(1H-Benzimidazol-2-ylsulfanylmethyl)-2-(4-methoxy-phenyl)-thiazolidin-4-one (**5f**).* White crystals, yield: 75.54%; m.p. 219–220 °C; IR (KBr) υ_{max} in cm^{-1}: 3208.0, 1378.9 (–NH–)1513.8 (C=N),1606.0 (–CONH), 1637.6 (ring >C=O), 2831.8 (Ar–OCH_3); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 3.65 (s, 2H, S–CH_2), 3.66 (s, 2H, –CH_2, ring), 3.94 (d, 1H, $J = 15.5$ Hz, CH–Ar), 7.54–7.91 (m, 9H, Ar–H), 8.28 (s, 1H, –CONH–), 11.52 (b, 1H, –NH– benzimidazole, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 31.12 (ring S–CH_2), 36.55 (S–CH_2), 55.98 (–CH), 112.12, 121.77, 129.0, 129.51, 129.82, 130.55, 135.81, 142.42 (heteroaromatics), 163.14 (>C=O), 174.31 (ring >C=O); EIMS *m/z*: 414 [M^+], 415 (M + 1), 416 (M + 2), 226, 211, 195, 180, 167, 119, 108, 92; Anal. Calcd for $C_{19}H_{18}N_4O_3S_2$: C, 55.05; H, 4.38; N, 13.52%. Found C, 55.08; H, 4.34; N, 13.49%.

*6.1.5.7. 3-(1H-Benzimidazol-2-ylsulfanylmethyl)-2-(2-hydroxy-3-methoxy-phenyl)-thiazolidin-4-one (**5g**).* Yellow crystals, yield: 776.35%; m.p. 220–222 °C; IR (KBr) υ_{max} in cm^{-1}: 3280.9, 1382.7 (–NH–), 1585.0 (C=N), 1637.1 (–CONH), 1670.1 (ring >C=O), 3410.9 (–OH), 2812 (Ar–OCH3); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 3.60 (s, 2H, S–CH_2), 3.65 (s, 2H, –CH_2, ring), 3.82 (d, 1H, $J = 15.8$ Hz, CH–Ar), 7.55–8.01 (m, 9H, Ar–H), 8.08 (s, 1H, –CONH–), 11.9 (b, 1H, –NH–benzimidazole, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 31.55 (ring S–CH_2), 36.85 (S–CH_2), 55.26 (–CH), 115.4, 121.0, 129.31, 129.80, 129.84, 130.45, 135.32, 142.45 (heteroaromatics), 163.17 (>C=O), 174.29 (ring >C=O); EIMS *m/z*: 430 [M^+], 431 (M + 1), 432 (M + 1), 240, 228, 224, 215, 191, 163, 150, 118, 105, 58, 14; Anal. Calcd for $C_{19}H_{18}N_4O_4S_2$: C, 53.01; H, 4.21; N, 13.01%. Found: C, 53.05; H, 4.24; N, 12.99%.

*6.1.5.8. 3-(1H-Benzimidazol-2-ylsulfanylmethyl)-2-furan-2-yl-thiazolidin-4-one (**5h**).* Brown crystals, yield: 76.5%; m.p. 155–157 °C; IR (KBr) υ_{max} in cm^{-1}: 3225.2, 1384.3 (–NH–), 1590.5 (C=N), 1685.0 (–CONH), 1697.3 (ring >C=O), 1256.2 (C–O–C); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 3.62 (s, 2H, –CH_2, ring), 3.65 (s, 2H, S–CH_2), 3.70 (d, 1H, $J = 15.80$ Hz, CH–Ar), 7.0–7.94 (m, 9H, Ar–H), 8.21 (s, 1H, –CONH–), 11.54 (b, 1H, –NH–benzimidazole, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 32.55 (ring S–CH_2), 36.85 (S–CH_2), 54.99 (–CH), 102.8, 114.8, 122.99, 140.22, 141.2, 150.5 (heteroaromatics), 165.81 (>C=O), 174.2 (ring >C=O); EIMS *m/z*: 374 [M^+], 375 (M + 1), 377 (M + 2), 194, 183, 167, 155, 149, 119, 107, 92, 68, 39, 29; Anal. Calcd for $C_{16}H_{14}N_4O_3S_2$: C, 51.32; H, 3.77; N, 14.96%. Found: C, 51.28; H, 3.80; N, 14.99%.

*6.1.5.9. 3-(1H-Benzimidazol-2-ylsulfanylmethyl)-2-(3-hydroxy-naphthalen-2-yl)-thiazolidin-4-one (**5i**).* Pale yellow crystals, yield: 68.65%; m.p. 240–242 °C; IR (KBr) υ_{max} in cm^{-1}: 3202.6, 1383.0 (–NH–), 1585.0 (C=N), 1624.6 (–CONH), 1678.8 (ring >C=O), 3450.5 (–OH); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 3.55 (s, 2H, S–CH_2), 3.62 (s, 2H, –CH_2, ring), 3.72 (d, 1H, $J = 15.5$ Hz, CH–Ar), 7.52–7.99 (m, 9H, Ar–H), 8.20 (s, 1H, –CONH–), 11.62 (b, 1H, –NH–benzimidazole, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 32.5 (ring S–CH_2), 37.82 (S–CH_2), 56.28 (–CH), 121.18, 122.4, 125.29, 127.92, 129.19, 129.87, 129.95, 130.11, 135.98, 143.45 (heteroaromatics), 164.05 (>C=O), 172.90 (ring >C=O). EIMS *m/z*: 450 [M^+], 451 (M + 1), 453 (M + 2), 262, 259, 245, 230, 216, 164, 144, 115, 105, 58, 32, 28, 14; Anal. Calcd for $C_{22}H_{18}N_4O_3S_2$: C, 58.65; H, 4.03; N, 12.44%. Found: C, 58.66; H, 4.02; N, 12.41%.

*6.1.5.10. 3-(1H-Benzimidazol-2-ylsulfanylmethyl)-2-(4-amine-phenyl)-thiazolidin-4-one (**5j**).* Yellow crystals, yield: 70.25%; m.p. 218–220 °C; IR (KBr) υ_{max} in cm^{-1}: 3346.6, 1376.6 (–NH–), 1575.0 (C=N), 1620.6 (–CONH), 1675.2 (ring >C=O), 3450.5 (–OH); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 3.52 (s, 2H, S–CH_2), 3.64 (s, 2H, –CH_2, ring), 3.75 (d, 1H, $J = 15.6$ Hz, CH–Ar), 7.50–7.85 (m, 9H, Ar–H), 8.20 (s, 1H, –CONH–), 11.60 (b, 1H, –NH–benzimidazole, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 32.9 (ring S–CH_2), 37.75 (S–CH_2), 56.15 (–CH), 121.18, 122.9, 125.29, 127.92, 129.19, 129.8, 129.95, 130.11, 135.98, 141.9, 145.9 (heteroaromatics), 171.05 (>C=O), 167.90 (ring >C=O); EIMS *m/z*: 399 [M^+], 400 (M + 1), 401 (M + 2), 262, 259, 245, 230, 216, 164, 115, 105, 58, 28; Anal. Calcd for $C_{22}H_{18}N_4O_3S_2$: C, 54.12; H, 4.29; N, 17.53%. Found: C, 54.10; H, 4.26; N, 17.57%.

*6.1.6. Synthesis of 2-(5-phenyl-[1, 3, 4]-oxadiazole-2-ylmethylsulfanyl)-1H-benzimidazole **6**(**a**–**j**)*

A solution of hydrazide (**3**) (2.22 g, 0.01 mol) and corresponding acid (0.01 mol) in $POCl_3$ (30 mL) was refluxed for 18–20 h. Excess solvent was removed by vacuum distillation and the solution was poured onto crushed ice with stirring and the precipitated product

was neutralized with ammonia, filtered, washed with water and recrystallized from chloroform to get respective oxadiazole.

*6.1.6.1. 2-(5-Phenyl-[1,3,4]-oxadiazole-2-ylmethylsulfanyl)-1H-benzimidazole (**6a**).* Dark brown powder, yield: 65.42%; m.p. 198–200 °C; IR (KBr) υ_{max} in cm^{-1}: 3237.6, 13387 (–NH–), 1267.1 (C–O–C), 1654.7 (C═N); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 3.39 (s, 2H, S–CH_2), 7.17–8.53 (m, 9H, Ar–H), 11.45 (b, 1H, –NH–benzimidazole, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 37.23 (S–CH_2), 117.2, 120.9, 129.5, 135.0, 136.8, 140.0 (heteroaromatics), 155.8 (C_2, oxa), 172.6 (C_5, oxa); EIMS *m*/*z*: 308 [M^+], 309 (M + 1), 310 (M + 2), 163, 150, 145, 119, 105, 91, 78, 70, 44; Anal. Calcd for $C_{16}H_{12}N_4OS$: C, 62.32; H, 3.92; N, 18.17%. Found: C, 61.30; H, 3.89; N, 18.20%.

*6.1.6.2. 2-(5-Chloro-phenyl-[1,3,4]-oxadiazole-2-ylmethylsulfanyl)-1H-benzimidazole (**6b**).* Dark brown powder, yield: 68.42%; m.p. 202–204 °C; IR (KBr) υ_{max} in cm^{-1}: 3322.4, 1340.1 (–NH–), 1275.8 (C–O–C), 1618.4 (C═N), 3412.3 (–OH); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 3.86 (s, 2H, S–CH_2), 7.57–8.13 (m, 8H, Ar–H), 11.02 (b, 1H, –NH–benzimidazole, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 36.65 (S–CH_2), 122.7, 129.6, 136.25, 145.25, (heteroaromatics), 155.54 (C_2, oxa), 172.81 (C_5, oxa); EIMS *m*/*z*: 342 [M^+], 343 (M + 1), 344 (M + 2), 163, 108, 91, 70, 111, 51, 35; Anal. Calcd for $C_{16}H_{11}N_4O_3Cl$: C, 56.06; H, 3.23; N, 16.34%. Found: C, 56.10; H, 3.28; N, 16.30%.

*6.1.6.3. 3-[5-(1H-Benzimidazol-2-ylsulfanylmethyl)-[1,3,4]-oxadiazole-2-yl]-naphthalene-2-ol (**6c**).* Pale yellow crystals, yield: 69.54%; m.p. 205–207 °C; IR (KBr) υ_{max} in cm^{-1}: 3386.5, 1388.5 (–NH–), 1241.1 (C–O–C), 1636.4 (C═N); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 3.42 (s, 2H, S–CH_2), 7.18–8.11 (m, 10H, Ar–H), 11.09 (b, 1H, –NH–benzimidazole, D_2O exchangeable), 12.41 (s, 1H, –OH, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 37.26 (S–CH_2), 122.85, 129.89, 136.85, 145.28 (heteroaromatics), 155.2 (C_2, oxa), 172.65 (C_5, oxa); EIMS *m*/*z*: 374 [M^+], 375 (M + 1), 376 (M + 2), 149, 91, 117, 143, 32, 28. Anal. Calcd for $C_{20}H_{14}N_4O_2S$: C, 64.16; H, 3.77; N, 14.96%. Found: C, 64.14; H, 3.80; N, 14.95%.

*6.1.6.4. 4-[5-(1H-Benzimidazol-2-ylsulfanylmethyl)-[1,3,4]-oxadiazole-2-yl]-phenol (**6d**).* Yellow powder, yield: 69.55%; m.p. 215–217 °C; IR (KBr) υ_{max} in cm^{-1}: 3320.9, 1342.5 (–NH), 1280.4 (C–O–C), 1621.4 (C═N); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 3.38 (s, 2H, S–CH_2), 7.07–7.95 (m, 8H, Ar–H), 11.15 (b, 1H, –NH–benzimidazole, D_2O exchangeable), 12.38 (s, 1H, –OH, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 35.82 (S–CH_2), 122.10, 129.43, 135.87, 145.69 (heteroaromatics), 155.62 (C_2, oxa), 172.87 (C_5, oxa). EIMS *m*/*z*: 324 [M^+], 325 (M + 1), 326 (M + 2), 165, 149, 106, 94, 70, 15; Anal. Calcd for $C_{16}H_{12}N_4O_2S$: C, 59.25; H, 3.73; N, 17.27%. Found: C, 59.28; H, 3.71; N, 17.29%.

*6.1.6.5. 6-[5-(1H-Benzimidazol-2-ylsulfanylmethyl)-[1,3,4]-oxadiazole-2-yl]-naphthalene −1,7-diol (**6e**).* Brown crystals, yield: 64.22%; m.p. 187–189 °C; IR (KBr) υ_{max} in cm^{-1}: 3322.4, 1340.1 (–NH–), 1275.8 (C–O–C), 1618.4 (C═N), 3412.3 (–OH); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 3.60 (s, 2H, S–CH_2), 6.92–8.52 (m, 9H, Ar–H), 11.42 (b, 1H, –NH–benzimidazole, D_2O exchangeable), (s, 1H, –OH, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 35.93 (S–CH_2), 123.45, 128.65, 136.85, 146.73, 148.0 (heteroaromatics), 154.73 (C_2, oxa), 171.96 (C_5, oxa); EIMS *m*/*z*: 390 [M^+], 391 (M + 1), 392 (M + 2), 163, 150, 105, 160, 71, 28; Anal. Calcd for $C_{20}H_{14}N_4O_3S$: C, 61.53; H, 3.61; N, 14.35%. Found: C, 61.57; H, 3.65; N, 14.32%.

*6.1.6.6. 5-[5-(1H-Benzimidazol-2-ylsulfanylmethyl)-[1,3,4]-oxadiazole-2-yl]-benzene-1,2,3-triol (**6f**).* Brown crystals, yield: 69.54%; m.p. 196–198 °C; IR (KBr) υ_{max} in cm^{-1}: 3166.1, 1387.8 (–NH–), 1269.5 (C–O–C), 1642.7 (C═N), 3499.0 (–OH); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 3.29 (s, 2H, S–CH_2), 7.18–8.53 (m, 6H, Ar–H), 10.42 (b, 1H, –NH–benzimidazole, D_2O exchangeable), 12.05 (s, 1H, –OH, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 35.44 (S–CH_2), 122.90, 133.83, 137.25, 140.56, 145.2, 145.8 (heteroaromatics), 155.0 (C_2, oxa), 172.11 (C_5, oxa); EIMS *m*/*z*: 356 [M^+], 357 (M + 1), 357 (M + 2), 163, 149, 128, 105, 70, 28; Anal. Calcd for $C_{16}H_{12}N_4O_4S$: C, 53.93; H, 3.39; N, 15.72%. Found: C, 53.95; H, 3.43; N, 15.70%.

*6.1.6.7. 4-[5-(1H-Benzimidazol-2-ylsulfanylmethyl)-[1,3,4]-oxadiazole-2-yl]-phenylamine (**6g**).* Light brown crystals, yield: 68.65%; m.p. 228–230 °C; IR (KBr) υ_{max} in cm^{-1}: 3346.6, 1376.6 (–NH–), 1269.5 (C–O–C), 1603.8 (C═N); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 3.29 (s, 2H, S–CH_2), 7.17–8.55 (m, 8H, Ar–H), 10.92 (b, 1H, –NH–benzimidazole, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 36.82 (S–CH_2), 155.21 (C_2, oxa), 171.73 (C_5, oxa), 145.42, 141.8, 137.2, 127.54, 122.3 (heteroaromatics); EIMS *m*/*z*: 326 [M^+], 327 (M + 1), 328 (M + 2), 149, 70, 93, 77, 16; Anal. Calcd for $C_{16}H_{13}N_5OS$: C, 59.43; H, 4.05; N, 21.66%. Found: C, 59.47; H, 4.08; N, 21.69%.

*6.1.6.8. 2-[5-(4-Nitro-phenyl)-[1, 3, 4]-oxadiazole-2-ylmethylsulfanyl]-1H-benzimidazole (**6h**).* Brown crystals, yield: 68.75%; m.p. 164–166 °C; IR (KBr) υ_{max} in cm^{-1}: 3348.1, 1380.1 (–NH–), 1269.2 (C–O–C), 1618.7 (C═N); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 3.11 (s, 2H, S–CH_2), 7.28–8.21 (m, 8H, Ar–H), 10.85 (b, 1H, –NH–benzimidazole, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 36.14 (S–CH_2), 122.82, 124.34, 133.82, 149.79 (heteroaromatics), 156.73 (C_2, oxa), 172.6 (C_5, oxa); EIMS *m*/*z*: 356 [M^+], 357 (M + 1), 358 (M + 2), 105, 117, 122, 46, 15; Anal. Calcd for $C_{16}H_{11}N_5O_3S$: C, 54.38; H, 3.14; N, 19.82%. Found: C 54.42; H, 3.12; N, 19.80%.

*6.1.6.9. 2-[5-(2-Chloro-4-nitro-phenyl)-[1,3,4]-oxadiazole-2-ylmethylsulfanyl]-1H-benzimidazole (**6i**).* Light brown crystals, yield: 64.25%; m.p. 208–210 °C; IR (KBr) υ_{max} in cm^{-1}: 3409.8, 1390.6 (–NH–), 1251.1 (C–O–C), 1657.4 (C═N), 890.8 (C–Cl); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 3.12 (s, 2H, S–CH_2), 7.65–8.11 (m, 7H, Ar–H), 11.25 (b, 1H, –NH–benzimidazole, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 33.52 (S–CH_2), 121.42, 122.58, 133.85, 138.9, 140.0, 145.57 (heteroaromatics), 154.99 (C_2, oxa), 171.42 (C_5, oxa); EIMS *m*/*z*: 387 [M^+], 388 (M + 1), 391 (M + 2), 163, 157, 149, 112, 105, 32; Anal. Calcd for $C_{16}H_{10}ClN_5O_3S$: C, 49.55; H, 2.60; N, 18.06%. Found: C, 49.58; H, 2.58; N, 18.02%.

*6.1.6.10. 2-(5-Pyridin-3-yl-[1,3,4] oxadiazole-2-ylmethylsulfanyl)-1H-benzimidazole (**6j**).* Light brown crystals, yield: 69.86%; m.p. 201–202 °C; IR (KBr) υ_{max} in cm^{-1}: 3348.5, 1354 (–NH–), 1275.1 (C–O–C), 1610 (C═N); ^1H NMR ($CDCl_3$, 300 MHz) δ ppm: 3.15 (s, 2H, S–CH_2), 7.46–8.28 (m, 8H, Ar–H), 11.45 (b, 1H, –NH–benzimidazole, D_2O exchangeable); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm: 36.42 (S–CH_2), 122.0, 134.2, 138.2, 142.9, 148.15, 149.82, (heteroaromatics), 156.2 (C_2, oxa), 172.64 (C_5, oxa). EIMS *m*/*z*: 309 [M^+], 310 (M + 1), 311 (M + 2), 161, 149, 70, 52, 27; Anal. Calcd for $C_{15}H_{11}N_5OS$: C, 58.24; H, 3.58; N, 22.64%. Found: C, 58.27; H, 3.59; N, 22.63%.

6.2. Pharmacology

6.2.1. Anticonvulsant activity

The anticonvulsant activities of the synthesized compounds were tested through an *in vivo* rodent model of convulsions (the Maxima ElectroShock, MES test) [27]. In MES-convulsions electroshock is applied through the corneal electrode. Through 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 anticonvulsant property if it reduces or abolishes the extensor phase of MES-convulsions.

6.2.2. Antidiabetic activity

Evaluation of antidiabetic activity was done by blood–glucose test and oral glucose tolerance test (OGTT) [28,29]. Male wistar albino rats (150–200 g) of either sex were selected. Diabetes was induced by single intraperitonial injection of freshly prepared aqueous solution of alloxan monohydrate (150 mg/kg) to overnight fasted rats. After 48hrs of alloxan injection, the animals which did not develop hyperglycemia i.e. glucose level >200 mg/dL were rejected/replaced with new animals. Treatment was continued for 9 days. Before the treatment (0 day) and at the end of 9th day, blood samples were collected by retro orbital puncture of each rat under mild anesthesia in 1 mL Ependorff's tubes containing 50 mL of anticoagulant heparin. And serum separated by centrifugation of blood at 4000 rpm for 10 min was subjected for estimation of glucose by Glucose oxidase method [30].

6.2.3. DNA cleavage study

6.2.3.1. Preparation of culture media. DNA cleavage experiments were done according to the literature [31]. Nutrient broth [peptone, 10; Yeast extract, 5; NaCl, 10; in (g/l)] was used for culturing of *Escherichia coli*. 50 mL media was prepared, autoclaved for 15 min at 121 °C under 15 lb pressure. The autoclaved media were inoculated for 24 h at 37 °C.

6.2.3.2. Isolation of DNA. The fresh bacterial culture (1.5 mL) is centrifuged to obtain the pellet which is then dissolved in 0.5 mL of lysis buffer (100 mM tris pH 8.0, 50 mM EDTA, 10% SDS). To this 0.5 mL of saturated phenol was added and incubated at 55 °C for 10 min, then centrifuged at 10,000 rpm for 10 min and to the supernatant, equal volume of chloroform: isoamyl alcohol (24:1) and 1/20th volume of 3 M sodium acetate (pH 4.8) was added. Again centrifuging at 10,000 rpm for 10 min and to the supernatant, 3 volumes of chilled absolute alcohol were added. The precipitated DNA was separated by centrifugation and the pellet was dried and dissolved in TAE buffer (10 mM tris pH 8.0, 1 mM EDTA) and stored in cold condition.

6.2.3.3. Agarose gel electrophoresis. Cleavage products were analyzed by agarose gel electrophoresis method. Test samples (1 mg/mL) were prepared in DMF. The samples (25 mg) were added to the isolated DNA of *E. coli*. The samples were incubated for 2 h at 37 °C and then 20 mL of DNA sample (mixed with bromophenol blue dye at 1:1 ratio) was loaded carefully into the electrophoresis chamber wells along with standard DNA marker containing TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA/1 L) and finally loaded on agarose gel and passed the constant 50 V of electricity for around 30 min. Removing the gel and being stained with 10.0 mg/mL ethidium bromide for 10–15 min, the bands were observed under Vilberlourmate Gel documentation system and then photographed to determine the extent of DNA cleavage. Henceforth the results were compared with standard DNA marker.

Acknowledgments

This research work is financially supported by the University Grants Commission, (UGC), New Delhi 110 012. (Ref: No. 33-296/2007 (SR) dated 28-02-2008).

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