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# Synthesis of 2-Mercaptobenzimidazole Derivatives as Potential Anti-microbial and Cytotoxic Agents

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A series of novel 2-(1*H*-benzimidazol-2-ylsulfanyl)-*N*-(4-oxo-2-phenyl-thiazolidin-3yl)-acetamide **5a–j** have been synthesized from various aldehydes and 2-(5-phenyl-[1,3,4]-oxadiazol-2-ylmethylsulfanyl)-1*H*-benzimidazole **6a–j** from various benzoic acids. These compounds were screened for their *in-vitro* anti-bacterial activity against *Staphylococcus aureus* and *Enterococcus faecalis* as Gram positive, *Klebsiella pneumoniae* and *Escherichia coli* as Gram negative bacterial strains and for *in-vitro* anti-fungal activity against *Asperigillus fumigatus* and *Candida albicans*. The *in vitro* cytotoxic properties were studied using brine shrimp bioassay. Results revealed that, compounds **5b**, **5d**, **5g**, **5i**, **6b**, **6e**, **6f**, and **6i** showed excellent activity against a panel of microorganisms. The cytotoxic activities of **5b**, **5g**, **5i**, **6b**, **6f**, **6h**, and **6i** were found to be good. All the newly synthesized compounds were characterized by elemental analysis, IR, $^{1}$H-NMR, $^{13}$C-NMR and MS.

**Keywords:** Antibacterial / Antifungal / Cytotoxicity / 1,3,4-Oxadiazole / 4-Thiazolidinone

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## Introduction

Research and development of potent and effective anti-microbial agents represents one of the most important advances in therapeutics, not only in the control of serious infections, but also in the prevention and treatment of some infectious complications of other therapeutic modalities such as cancer chemotherapy and surgery. However, in recent years, much attention has been focused on addressing the problem of multi-drug resistant (MDR) bacteria and fungi resulting from the widespread use and misuse of classical anti-microbial agents [1]. Recent observations suggested that substituted benzimidazoles, benzoxazoles, and related heterocycles, which are the structural isosters of nucleotides owing to the fused heterocyclic nuclei in their structures, interact easily with biopolymers and possess potential activity with lower toxicities in the chemotherapeutic approach in man [2, 3].

The aim of this research article is to explore the importance of heterocyclic compounds, trends in novel synthesis of most potential 4-thiazolidinone and 1,3,4-oxadiazole derivatives, their biological impact, and also the future potential of this protocol [4, 5]. Interest in the biological and industrial potentialities of 4-thiazolidinone [6] and 1,3,4-oxadiazole [7] derivatives has resulted in the development of various synthetic procedures for the introduction of such heterocyclic moieties into 2-mercapto benzimidazole. Because of significant potential therapeutic properties and industrial applications, a prominent place and much interest is generated in the synthesis of new class of heterocyclic systems such as 4-thiazolidinone [8] and 1,3,4-oxadiazole [9] derivatives, thereby to explore their biological properties and industrial applications.

It is well-known that a number of heterocyclic compounds containing nitrogen and sulphur exhibit a wide variety of biological activities. 2-Mercapto benzimidazole derivatives are known to possess broad spectrum of activities and clinical applications [10]. Benzimidazoles are a component of Vitamin $B_{12}$ and are related to the DNA base purine and the stimulant caffeine. Several five membered aromatic systems having three heteroatoms at symmetrical positions have been studied because of their interesting physiological properties. Hence our study involves the synthesis of 4-thiazolidinones and 1,3,4-oxadiazoles.

4-Thiazolidinones are the derivatives of thiazolidine with a carbonyl group at 4-position. Introduction of sulphur was

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expected to bring in a bulky polarizable atom, thus altering geometry, polarizability, stability, lipophilicity, steric and electronic characteristics of the molecule. It was anticipated that the thiazolidine ring improves the activity of the lead molecules. 4-Thiazolidinones have many interesting activity profiles namely anti-bacterial, anti-fungal [11], anti-inflammatory [12], anti-convulsant [13], COX-1 inhibitors [14], inhibitors of the bacterial enzyme MurB [15], non-nucleoside inhibitors of HIV-RT [16], and anti-histamine agents [17]. Recently, they have also been utilized as hypolipidemics and hypocholesteremics [18]. Besides this they are used as Maillard reaction inhibitors and for treatment of diabetes complications [19].

1,3,4-Oxadiazoles are thermally stable and neutral hetero-aromatic molecules which exhibit one of the active class of compounds possessing various pharmacological properties such as anti-inflammatory [20, 21], anti-viral [22], anti-bacterial [23, 24] activities. The substituted oxadiazoles are heterocyclic compounds, which serve both as biomimetic and reactive pharmacophores and many are key elements with potential biological activities. Additionally substituted 1,3,4-oxadiazole derivatives are becoming an important member in the heterocyclic family because of their wide usage as dyes, photosensitive electrical materials, liquid crystals and organic light emitting diodes. The incorporation of 4-thiazolidinone and 1,3,4-oxadiazole moiety into 2-mercapto benzimidazole scaffold enhances its activity.

The present work has been designed considering the extensive applications of 4-thiazolidinone and 1,3,4-oxadiazole derivatives as industrial and biological products and benzimidazole moiety as an important pharmacophore and privileged structure in medicinal chemistry [25, 26]. Here, an attempt has been made to synthesize 4-thiazolidinone and 1,3,4-oxadiazole derivatives containing 2-mercapto benzimidazole in good yield without microwave irradiation [27, 28].

## Results and discussion

### Chemistry

We synthesized the target title compounds according to Scheme 1. 2-Mercapto benzimidazole (**1**) was prepared according to the reported method [16]. Condensation of **1** with ethylchloroacetate in presence of anhydrous $K_2CO_3$ yielded ethyl-2-(benzimidazolyl thio)-acetate **2**. The appearance of the signal at $\delta$ 1.32 ppm due to $-CH_3$ and $\delta$ 4.33 ppm due to $-CH_2$ in $-COOCH_2CH_3$ in $^1$H-NMR confirmed the formation of ester. This was also confirmed by IR due to a band at 1720 $cm^{-1}$ which appeared because of C = O of ester. Using ethanol as the reaction media, **2** on ammonolysis with hydrazine hydrate afforded [2-(1*H*-benzimidazolylsulfanyl)-acetyl]-hydrazine (**3**). $^1$H-NMR confirms this compound with a signal at $\delta$ 8.02 ppm and $\delta$ 4.13 ppm due to −CONH and $-NH_2$, respectively. In IR, the >C=O band for amide appears at 1641 $cm^{-1}$ and $-NHNH_2$ around 3207 and 3346 $cm^{-1}$ also confirms the formation of **3**. **3** on treatment with aromatic aldehydes in ethanol gave {1*H*-benzimidazol-2-ylsulfanyl}-(*N*-2-substituted phenyl) hydrazide **4a–j**. The hydrazone was confirmed by a signal at $\delta$ 4.02 ppm due to the −N=CH− in $^1$H-NMR and the signal at 65.8 ppm due to >CH-N< in $^{13}$C-NMR and 1640 $cm^{-1}$ due to −N=CH− in IR also confirms this. In the process of synthesis of compounds **4a–j**, it was found that the substituted aromatic aldehyde having an electron withdrawing group Ex: −Cl ends up the reaction quickly with high yield in the absence of catalyst. When there is an electron releasing group Ex: $-OCH_3$ in the benzene ring, the reaction could be finished just in the presence of glacial acetic acid as catalyst.

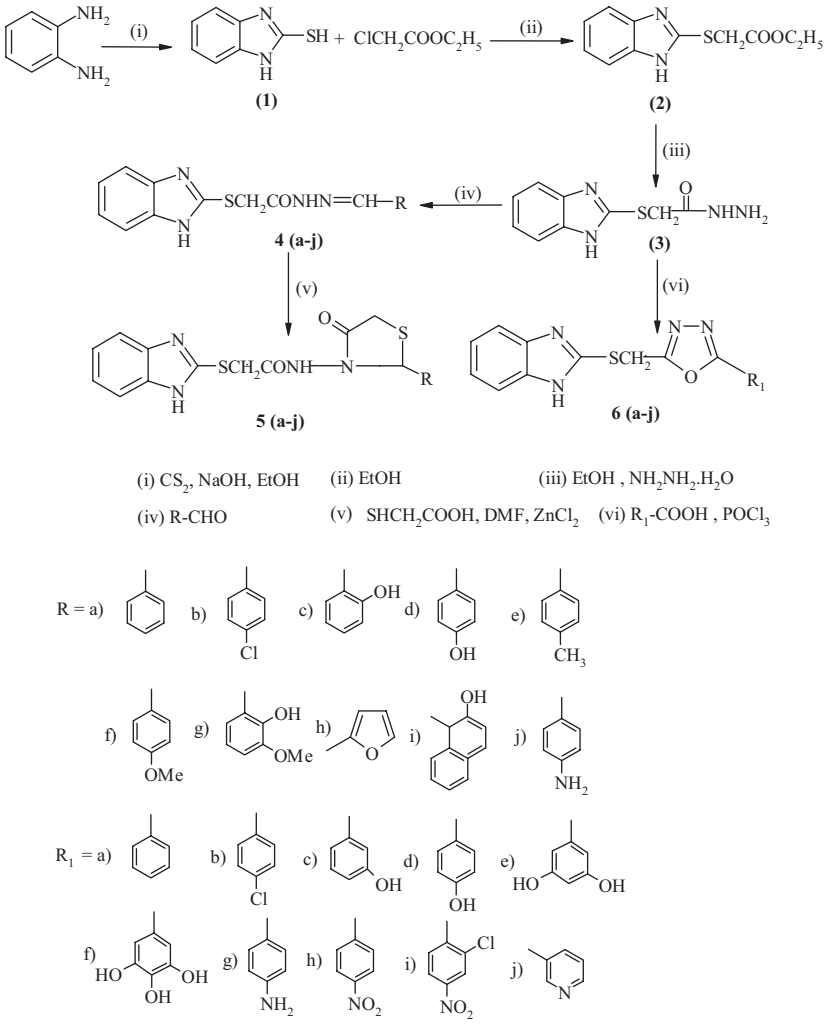
Compounds **4a–j** when treated with thioglycolic acid in presence of anhydrous $ZnCl_2$ afforded 2-(1*H*-bezimidazol-2-ylsulfanyl)-*N*-(4-oxo-2-phenyl-thiazolidin-3-yl)-acetamide **5a–j**. A signal at $\delta$ 3.70 ppm and 3.65 ppm due to >CH-Ar- and $-S-CH_2-$ confirms the formation of thiazolidinone ring. In the $^1$H-NMR spectra of **4a–j,** the proton of −N=CH appears at about $\delta$ 4.02–4.10 ppm. But, in the spectrum of **5a–j** this proton signal is shifted slightly downfield in contrast with compound **4a–j**. The reason was due to the deshielding affected by heterocycles which shifted the resonances to about $\delta$ 3.70–3.95 ppm. In $^{13}$C-NMR, signals of ring $S-CH_2$ and ring >C=O appeared at about $\delta$ 31.63 ppm and 172.35 ppm, respectively.

Treatment of a suspension of 2-sulfanyl acid hydrazide (**3**) with equimolar amount of various acids in the presence of phosphorous oxytrichloride at room temperature and then refluxing for 18–20 h gave 1,3,4-oxadiazoles **6a–j** in yields ranging from 70–75%. The appearance of (C–O–C) signal at 1267.7 $cm^{-1}$ confirms the formation of the oxadiazole moiety.

### Biological Evaluation

All the compounds prepared herein were screened for their potential biological activities such as anti-bacterial, anti-fungal and cytotoxic activities. Bacterial strains *Staphylococcus aureus* [ATCC-25923] and *Enterococcus faecalis* [ATCC-29212] as Gram positive, *Klebsiella pneumoniae* [ATCC-13883] and *Escherichia coli* [ATCC-25922] as Gram negative and *Candida albicans* [ATCC-10145], *Asperigillus fumigatus* [36607] as fungal strains are used for the *in-vitro* study. The *in-vitro* anti-microbial activity of the compounds was tested by tube dilution technique. Each of the test compounds and standards ciprofloxacin and fluconazole were dissolved in DMSO at an initial concentration of 250 μg/mL and then were serially diluted in culture medium as follows: 125, 62.5, 31.250, 16, 8, 4, 2, and 1 μg/mL concentrations. The minimum inhibitory concentrations (MIC) were defined as the




**Scheme 1.** Synthesis of 4-thiazolidinone and 1,3,4-oxadiazole derivatives containing 2-mercapto benzimidazole moiety.

lowest concentrations of the compounds that prevented visible growth. It was determined that the solvent had no anti-microbial activity against any of the test microorganisms.

## Anti-bacterial activity assay

MIC values for the *in vitro* anti-bacterial studies of the compounds **5a–j**, **6a–j** and the standard are represented in Table 1. The anti-bacterial activity of all the compounds against *S. aureus* and *E. faecalis* as Gram positive, *K. pneumoniae* and *E. coli* as Gram negative bacteria showed good potencies compared to control drug ciprofloxacin. Among the synthesized compounds **5b**, **5d**, **5i**, **6b**, **6e**, **6f**, and **6i** showed very good to moderate activity with MIC values of 16–2 μg/mL against all the bacterial strains. Electron withdrawing nature of the substituents $-NO_2$, −Cl, −OH on the aromatic ring highly influenced the activity. It was observed that as the number of −OH group increases, the activity also enhanced which could be seen for compounds **6e** by two-fold and **6f** by four-fold compared to others.

## Anti-fungal activity assay

MIC values for the *in vitro* anti-fungal studies of the compounds **5a–j**, **6a–j** and the standard are represented in Table 2. Among test compounds **5b**, **5c**, **5d**, **5g**, **5i**, **6b**, and **6i** induced markedly anti-fungal activity compared to standard fluconazole against *C. albicans* and *A. fumigatus* compared to control fluconazole with MIC values of 8–2 μg/mL. The compounds **5e**, **5f**, **5h**, **6a**, **6g**, **6j** showed at least an activity of 62.5–125 μg/mL against both the fungi. The presence of an −OH group as per the structural demand of fluconazole was behind the enhancement in activities of these compounds. Also the presence of halogen −Cl and the electron withdrawing group $-NO_2$ equally influenced the activities.

**Table 1.** *In vitro* anti-bacterial activity of synthesized compounds **5a–j** and **6a–j** in MIC (μg/mL).

| Comp. | *S. aureus* (25923)[a] | *E. faecalis* (29212) | *K. pneumonia* (13883) | *E. coli* (25922) |
|---|---|---|---|---|
| **5a** | 16.125 | 31.250 | 62 | 31.250 |
| **5b** | 4 | 8 | 8 | 8 |
| **5c** | 16.125 | 62.5 | 4 | 16.125 |
| **5d** | 08 | 08 | 16.125 | 16.125 |
| **5e** | 62.5 | 62.5 | 31.250 | 31.250 |
| **5f** | 31.250 | 31.250 | 62.5 | 31.250 |
| **5g** | 62.5 | 16 | 2 | 16.125 |
| **5h** | 31.250 | 31.250 | 31.250 | 31.250 |
| **5i** | 16.125 | 16.125 | 16.125 | 16.125 |
| **5j** | 62.5 | 62.5 | 16.25 | 31.250 |
| **6a** | 125 | 125 | 62.5 | 62.5 |
| **6b** | 4 | 4 | 02 | 08 |
| **6c** | 8 | 8 | 16.125 | 08 |
| **6d** | 8 | 8 | 16.125 | 08 |
| **6e** | 4 | 4 | 2 | 4 |
| **6f** | 1 | 1 | 1 | 2 |
| **6g** | 62.5 | 62.5 | 31.25 | 62.5 |
| **6h** | 16.125 | 16.125 | 16.125 | 8 |
| **6i** | 8 | 2 | 4 | 4 |
| **6j** | 31.250 | 62.5 | 125 | 62.5 |
| Ciprofloxacin | 0.78 | 0.70 | 0.19 | 0.19 |

[a] ATCC number.
The active compounds are marked in bold letters.

## Cytotoxic activity assay

All synthesized compounds **5a–j**, **6a–j** were screened for their cytotoxicity (brine shrimp bioassay) using the protocol of Meyer et al. [29]. From the data recorded in Table 3, it is evident that the compounds **5b**, **5g**, **5i**, **6b**, **6f**, **6h** and **6i** displayed potent cytotoxic activity against *Artemia salina*, while the other compounds have shown moderate activity in this assay. Compound **6f** showed a maximum activity ($LD_{50} = 6.330 \times 10^{-4}$ M) in the present series of compounds. Thus, the structure activity relationship studies of these

**Table 2.** *In vitro* anti-fungal activity of synthesized compounds **5a–j** and **6a–j** in MIC (μg/mL).

| Comp. | *C. albicans* (10145)[a] | *A. fumigatus* (36607) |
|---|---|---|
| **5a** | 62.5 | 62.5 |
| **5b** | 16.125 | 8 |
| **5c** | 16.125 | 4 |
| **5d** | 8 | 8 |
| **5e** | 62.5 | 125 |
| **5f** | 125 | 62.5 |
| **5g** | 16.125 | 16.125 |
| **5h** | 31.250 | 31.25 |
| **5i** | 16.125 | 16.125 |
| **5j** | 16.125 | 31.250 |
| **6a** | 31.250 | 62.5 |
| **6b** | 4 | 8 |
| **6c** | 8 | 8 |
| **6d** | 4 | 8 |
| **6e** | 4 | 2 |
| **6f** | 2 | 2 |
| **6g** | 62.5 | 62.5 |
| **6h** | 4 | 8 |
| **6i** | 4 | 4 |
| **6j** | 31.250 | 62.5 |
| Fluconazole | 2.0 | 2.0 |

[a] ATCC number.
The active compounds are marked in bold letters.

**Table 3.** Brine shrimp bioassay data for compounds **5a–j** and **6a–j**.

| Comp. | $LD_{50}$ (mM) |
|---|---|
| **5a** | 3.425 |
| **5b** | 0.5825 |
| **5c** | 4.28 |
| **5d** | 3.725 |
| **5e** | 3.21 |
| **5f** | 2.458 |
| **5g** | 0.5616 |
| **5h** | 2.023 |
| **5i** | 0.5994 |
| **5j** | 3.720 |
| **6a** | 4.789 |
| **6b** | 0.6221 |
| **6c** | 4.390 |
| **6d** | 4.781 |
| **6e** | 3.693 |
| **6f** | 0.6330 |
| **6g** | 5.23 |
| **6h** | 0.5628 |
| **6i** | 0.5956 |
| **6j** | 5.616 |

The active compounds are marked in bold letters.

compounds revealed that R and $R_1$ with functional groups −Cl, −OH at 2, 3, or 4 positions, -$NO_2$ at 4 position showed good cytotoxicity.

## Conclusion

The main aim of the present work is to synthesize thiazolidinone and oxadiazole containing benzimidazole derivatives and investigate for various bioassays with the hope of discovering new structure leads serving as potential broad spectrum pharmacological agents. Among the synthesized compounds **5b**, **6b**, **6e**, and **6f** were found to be remarkably anti-microbial active showing excellent MIC values compared to reference drugs such as ciprofloxacin and fluconazole. SAR studies revealed the critical role of −OH function in the target compounds that showed very promising *in-vitro* activities. Compounds with −Cl, -$NO_2$ and −OH functional groups exhibited attractive cytotoxicity properties.

## Experimental

### Materials and methods

All reagents and solvents were used as obtained from the supplier or recrystallized/redistilled as necessary. Melting points of the synthesized compounds 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 Bruker Avanace-300 (300 MHz) model spectrophotometer in $CDCl_3$ and DMSO as solvent and TMSi as internal standard with $^1$H 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. TLC was performed on alumina silica gel 60 $F_{254}$ (Merck). The mobile phase was ethyl acetate and *n*-hexane (1:1) and detection was made using UV light and iodine vapors. All the compounds gave C, H, and N analysis within ±0.5% of the theoretical values.

### Chemistry

*General procedure for the preparation of compounds*

*Synthesis of 2-mercapto benzimidazole* ***1*** Compound **1** was prepared according to the reported procedure [16].

*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 recrystallized from chloroform to furnish compound **2** (1.055 g, 70%). White solid; m. p.: 60–62°C; IR (KBr) $\nu$: 3042 (aromatic ring), 638 (C-S), 1719 (>C=O of ester), 1684 (-C=N-), 1320 and 1234 (C−O−C), 830 (C-S-C) and 2955, 2889, 1443, 714, (−$CH_2$ and −$CH_3$) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ 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, 55.89; H, 5.01; N, 11.86.

*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 from ethanol to obtain compound **3** (1.77 g, 75%). Pinkish white solid; m. p.: 195°C; IR (KBr) $\nu$: 3311, 3369 (−$NHNH_2$), 1680 (>C=O of amide) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ 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, 48.59; H, 4.79; N, 25.18.

*Synthesis of (1H-Benzimidazol-2-ylsulfanyl)-acetic acid hydrazide* ***4***

A mixture of compound **3** (2.22 g, 0.01 mol) and benzaldehyde (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 was recrystallized from chloroform/methanol mixture to yield the required compound. The compounds **4b–j** were prepared similarly by treating with respective aldehydes.

*(1H-Benzimidazol-2-ylsulfanyl)-acetic acid benzylidene hydrazide* ***4a***

Pale yellow crystals; m. p.: 218–220°C; IR (KBr) $\nu$: 3320,1342 (−NH−), 1682 (>C=O), 1640 (−N=CH−) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 4.02 (s, 1H, −N=CH−), 6.87–7.78 (m, 4H, Ar-H), 7.95 (s, 1H, −CONH−), 10.5 (s, 1H, −NH−benzimidazole). Anal. calcd. for $C_{16}H_{14}N_4OS$: C, 61.92; H, 4.55; N, 18.05. Found: C, 61.88; H, 4.57; N, 18.12.

*(1H-Benzoimidazol-2-ylsulfanyl)-acetic acid (4-chloro-benzylidene)-hydrazide* ***4b***

Colorless crystals; m. p.: 255–257°C; IR (KBr) $\nu$: 3315, 1330 (−NH−), 1660 (>C=O), 1635 (−N=CH−) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 4.07 (s, 1H, −N=CH−), 6.54–7.69 (m, 4H, Ar-H), 7.85 (s, 1H, −CONH−), 10.0 (s, 1H, −NH−benzimidazole). Anal. calcd. for $C_{16}H_{13}ClN_4OS$: C, 55.73; H, 3.80; N, 16.25. Found: C, 55.69; H, 3.79; N, 16.17.

*(1H-Benzimidazol-2-ylsulfanyl)-acetic acid (2-hydroxy-benzylidene)-hydrazide* ***4c***

Colorless crystals; m. p.: 202–204°C; IR (KBr) $\nu$: 3325, 1340 (−NH−), 1671 (>C=O), 1639 (−N=CH−) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 4.10 (s, 1H, −N=CH−), 6.59–7.70 (m, 4H, Ar-H), 7.82 (s, 1H, −CONH−), 10.01 (s, 1H, −NH−benzimidazole). Anal. calcd. for $C_{16}H_{14}N_4O_2S$: C, 58.88; H, 4.32; N, 17.17. Found: C, 58.80; H, 4.29; N, 17.20.

*(1H-Benzimidazol-2-ylsulfanyl)-acetic acid (4-hydroxy-benzylidene)-hydrazide* ***4d***

Colorless crystals; m. p.: 238–240°C; IR (KBr) $\nu$: 3321, 1339 (−NH−), 1669 (>C=O), 1641 (−N=CH−) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$)

$\delta$ ppm: 4.08 (s, 1H, −N=CH−), 6.65–7.75 (m, 4H, Ar-H), 7.79 (s, 1H, −CONH−), 9.75 (s, 1H, −NH−benzimidazole). Anal. calcd. for $C_{16}H_{14}N_4O_2S$: C, 58.88; H, 4.32; N, 17.17. Found: C, 58.81; H, 4.28; N, 17.19.

### *(1H-Benzimidazol-2-ylsulfanyl)-acetic acid (4-methyl-benzylidene)-hydrazide* ***4e***

Pale yellow crystals; m. p.: 206–208°C; IR (KBr) $\nu$: 3330, 1325 (−NH−), 1672 (>C=O), 1645 (−N=CH−) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 4.05 (s, 1H, −N=CH−), 6.62–7.70 (m, 4H, Ar-H), 7.81 (s, 1H, −CONH−), 9.91 (s, 1H, −NH−benzimidazole). Anal. calcd. for $C_{17}H_{16}N_4OS$: C, 62.94; H, 4.97; N, 17.27. Found: C, 62.90; H, 4.90; N, 17.22.

### *(1H-Benzimidazol-2-ylsulfanyl)-acetic acid (4-methoxy-benzylidene)-hydrazide* ***4f***

Light brown crystals; m. p.: 212–214°C; IR (KBr) $\nu$: 3329, 1331 (−NH−), 1675 (>C=O), 1642 (−N=CH−) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 4.09 (s, 1H, −N=CH−), 6.59–7.81 (m, 4H, Ar-H), 7.79 (s, 1H, −CONH−), 10.01 (s, 1H, −NH−benzimidazole). Anal. calcd. for $C_{17}H_{16}N_4O_2S$: C, 59.98; H, 4.74; N, 16.46. Found: C, 59.92; H, 4.72; N, 16.40.

### *(1H-Benzimidazol-2-ylsulfanyl)-acetic acid (2-hydroxy-3-methoxy-benzylidene)-hydrazide* ***4g***

Light brown crystals; m. p.: 180–182°C; IR (KBr) $\nu$: 3333, 1345 (−NH−), 1670 (>C=O), 1650 (−N=CH−) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 4.05 (s, 1H, −N=CH−), 6.55–7.80 (m, 4H, Ar-H), 7.81 (s, 1H, −CONH−), 10.05 (s, 1H, −NH−benzimidazole). Anal. calcd. for $C_{17}H_{16}N_4O_3S$: C, 57.29; H, 4.52; N, 15.72. Found: C, 57.15; H, 4.54; N, 15.78.

### *(1H-Benzimidazol-2-ylsulfanyl)-acetic acid furan-2-ylmethylene-hydrazide* ***4h***

Light brown crystals; m. p.: 222–224°C; IR (KBr) $\nu$: 3330, 1341 (−NH−), 1672 (>C=O), 1655 (−N=CH−) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 4.02 (s, 1H, −N=CH−), 6.52–7.77 (m, 4H, Ar-H), 7.79 (s, 1H, −CONH−), 10.00 (s, 1H, −NH−benzimidazole). Anal. calcd. for $C_{17}H_{16}N_4O_3S$: C, 57.29; H, 4.52; N, 15.72. Found: C, 57.20; H, 4.54; N, 15.78.

### *(1H-Benzimidazol-2-ylsulfanyl)-acetic acid (2-hydroxy-naphthalen-1-ylmethylene)-hydrazide* ***4i***

Pale yellow crystals; m. p.: 218–220°C; IR (KBr) $\nu$: 3329, 1335 (−NH−), 1667 (>C=O), 1649 (−N=CH−) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 4.07 (s, 1H, −N=CH−), 6.64–7.72 (m, 4H, Ar-H), 7.82 (s, 1H, −CONH−), 10.04 (s, 1H, −NH−benzimidazole). Anal. calcd. for $C_{20}H_{16}N_4O_2S$: C, 63.81; H, 4.28; N, 14.88. Found: C, 63.75; H, 4.24; N, 14.87.

### *(1H-Benzimidazol-2-ylsulfanyl)-acetic acid (4-amino-benzylidene)-hydrazide* ***4j***

Pale yellow crystals; m. p.: 198–200°C; IR (KBr) $\nu$: 3342, 1328 (−NH−), 1659 (>C=O), 1642 (−N=CH−) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 4.10 (s, 1H, −N=CH−), 4.65 (s, 2H, $-NH_2$), 6.26–7.29 (m, 4H, Ar-H), 7.65 (s, 1H, −CONH−), 10.21 (s, 1H, −NH−benzimidazole). Anal. calcd. for $C_{16}H_{15}N_5OS$: C, 59.06; H, 4.65; N, 21.52. Found: C, 59.02; H, 4.66; N, 21.49.

### *Synthesis of (1H-benzimidazol-2-ylsulfanyl)-thiazolidin-4-one* ***5***

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 into 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.

### *3-(1H-Benzimidazol-2-ylsulfanylmethyl)-2-phenyl-thiazolidin-4-one* ***5a***

Pale yellow crystals; m. p.: 230–232°C; IR (KBr) $\nu$: 3212.4, 1378 (−NH−), 1522.9 (-C=N), 1593.7 (−CONH), 1640.0 (ring >C=O) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 3.60 (s, 2H, $-CH_2$, ring), 3.65 (s, 2H, S−$CH_2$), 3.70 (d, 1H, CH-Ar), 7.0–7.94 (m, 9H, Ar-H), 8.08 (s, 1H, −CONH−), 11.9 (br, 1H, −NH−benzimidazole, $D_2O$ exchangeable); $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$ 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); MS *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, 56.30; H, 4.20; N, 14.50.

### *3-(1H-Benzimidazol-2-ylsulfanylmethyl)-2-(4-chloro-phenyl)-thiazolidin-4-one* ***5b***

Yellow crystals; m. p.: 235–237°C; IR (KBr) $\nu$: 3206.3, 1377.0 (−NH−), 1531.3 (C=N), 1595.8 (−CONH), 1639.1 (ring >C=O), Ar−Cl (825.6) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 3.50 (s, 2H, S−$CH_2$), 3.58 (s, 2H, $-CH_2$, ring), 3.72 (d, 1H, CH-Ar), 6.98–7.90 (m, 9H, Ar-H), 8.12 (s, 1H, −CONH−), 11.78 (br, 1H, −NH−benzimidazole, $D_2O$ exchangeable); $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$ 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 (amide >C=O), 173.87 (ring >C=O); MS *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, 51.58; H, 3.61; N, 13.30. Found: C, 51.56; H, 3.65; N, 13.28.

### *3-(1H-Benzimidazol-2-ylsulfanylmethyl)-2-(2-hydroxy-phenyl)-thiazolidin-4-one* ***5c***

Brown crystals; m. p.: 135–137°C; IR (KBr) $\nu$: 3235, 1355 (−NH−), 1586.4 (C=N), 1620.5 (−CONH), 1657.5 (ring >C=O) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 3.58 (s, 2H, $-CH_2$, ring), 3.62 (s, 2H, S−$CH_2$), 3.75 (d, 1H, CH-Ar), 7.0–7.85 (m, 9H, Ar-H), 8.25 (s, 1H, −CONH−), 11.54 (br, 1H, −NH−benzimidazole, $D_2O$ exchangeable); $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$ 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 (amide >C=O), 172.98 (ring >C=O); MS *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, 13.91.

### *3-(1H-Benzimidazol-2-ylsulfanylmethyl)-2-(4-hydroxy-phenyl)-thiazolidin-4-one* ***5d***

Yellow powder; m. p.: 140–142°C; IR (KBr) $\nu$: 3207.6, 1380.9 (−NH−), 1515.0 (C=N), 1604.3 (−CONH), 1641.7 (ring >C=O),

3448.8 (−OH) $cm^{-1}$; $^1$H-NMR ($CDCl_3$) $\delta$ ppm: 3.62 (s, 2H, ring $-CH_2$), 3.69 (s, 2H, S$-CH_2$), 3.72 (d, 1H, CH-Ar), 7.0–7.99 (m, 9H, Ar-H), 8.06 (s, 1H, −CONH−), 12.06 (br, 1H, −NH−benzimidazole, $D_2O$ exchangeable); $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$ 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 (amide >C=O), 172.58 (ring >C=O); MS *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, 53.92; H, 4.00; N, 13.94.

### *3-(1H-Benzimidazol-2-ylsulfanylmethyl)-2-p-tolyl-thiazolidin-4-one* ***5e***

Pale white crystals; m. p.: 238–240°C; IR (KBr) $\nu$: 3224, 1346.3 (−NH−), 1590.6 (C=N), 1639.9 (−CONH), 1680.5 (ring >C=O) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 3.60 (s, 2H, S$-CH_2$), 3.62 (s, 2H, $-CH_2$, ring), 3.81 (d, 1H, CH-Ar), 7.08–8.01 (m, 9H, Ar-H), 8.28 (s, 1H, −CONH−), 11.55 (br, 1H, −NH−benzimidazole, $D_2O$ exchangeable); $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$ ppm: 21.89 ($-CH_3$), 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 (amide >C=O), 173.52 (ring >C=O); MS *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, 4.45; N, 14.10.

### *3-(1H-Benzimidazol-2-ylsulfanylmethyl)-2-(4-methoxy-phenyl)-thiazolidin-4-one* ***5f***

White crystals; m. p.: 219–220°C; IR (KBr) $\nu$: 3208.0, 1378.9 (−NH−), 1513.8 (C=N), 1606.0 (−CONH), 1637.6 (ring >C=O), 2831.8 (Ar-$OCH_3$) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 3.65 (s, 2H, S$-CH_2$), 3.66 (s, 2H, $-CH_2$, ring), 3.94 (d, 1H, >CH-Ar), 7.54–7.91 (m, 9H, Ar-H), 8.28 (s, 1H, −CONH−), 11.52 (br, 1H, −NH−benzimidazole, $D_2O$ exchangeable); $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$ 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 (amide >C=O), 174.31 (ring >C=O); MS *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, 13.50. Found: C, 55.10; H, 4.30; N, 13.45.

### *3-(1H-Benzimidazol-2-ylsulfanylmethyl)-2-(2-hydroxy-3-methoxy-phenyl)-thiazolidin-4-one* ***5g***

Yellow crystals; m. p.: 220–222°C; IR (KBr) $\nu$: 3280.9, 1382.7 (−NH−), 1585.0 (C=N), 1637.1 (−CONH), 1670.1 (ring >C=O), 3410.9 (−OH), 2812 (Ar-$OCH_3$) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 3.60 (s, 2H, S$-CH_2$), 3.65 (s, 2H, $-CH_2$, ring), 3.82 (d, 1H, CH-Ar), 7.55–8.01 (m, 9H, Ar-H), 8.08 (s, 1H, −CONH−), 11.9 (br, 1H, −NH−benzimidazole, $D_2O$ exchangeable); $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$ 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 (amide >C=O), 174.29 (ring >C=O); MS *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, 53.08; H, 4.15; N, 12.99.

### *3-(1H-Benzimidazol-2-ylsulfanylmethyl)-2-furan-2-yl-thiazolidin-4-one* ***5h***

Brown crystals; m. p.: 155–157°C; IR (KBr) $\nu$: 3225.2, 1384.3 (−NH−), 1590.5 (C=N), 1685.0 (−CONH), 1697.3 (ring >C=O), 1256.2 (C−O−C) $cm^{-1}$; $^1$H-NMR ($CDCl_3$) $\delta$ (ppm): 3.62 (s, 2H, $-CH_2$, ring), 3.65 (s, 2H, S$-CH_2$), 3.70 (d, 1H, CH-Ar), 7.0–7.94 (m, 9H, Ar-H), 8.21 (s, 1H, −CONH−), 11.54 (br, 1H, −NH−benzimidazole, $D_2O$ exchangeable); $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$ 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 (amide >C=O), 174.2 (ring >C=O); MS *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, 3.71; N, 14.95.

### *3-(1H-Benzimidazol-2-ylsulfanylmethyl)-2-(3-hydroxy-naphthalen-2-yl)-thiazolidin-4-one* ***5i***

Pale yellow crystals; m. p.: 240–242°C; IR (KBr) $\nu$: 3202.6, 1383.0 (−NH−), 1585.0 (C=N), 1624.6 (−CONH), 1678.8 (ring >C=O), 3450.5 (−OH) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 3.55 (s, 2H, S$-CH_2$), 3.62 (s, 2H, $-CH_2$, ring), 3.72 (d, 1H, >CH-Ar), 7.52–7.99 (m, 9H, Ar-H), 8.20 (s, 1H, −CONH−), 11.62 (br, 1H, −NH−benzimidazole, $D_2O$ exchangeable); $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$ 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 (amide >C=O), 172.90 (ring >C=O); MS *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, 12.40.

### *3-(1H-Benzimidazol-2-ylsulfanylmethyl)-2-(4-amine-phenyl)-thiazolidin-4-one* ***5j***

Yellow crystals, (ethanol). Yield: 70.25%; m. p.: 218–220°C; IR (KBr, $cm^{-1}$) $\nu$: 3346.6, 1376.6 (−NH−), 1575.0 (C=N), 1620.6 (−CONH), 1675.2 (ring >C=O), 3450.5 (−OH); $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 3.52 (s, 2H, S$-CH_2$), 3.64 (s, 2H, $-CH_2$, ring), 3.75 (d, 1H, >CH-Ar), 7.50–7.85 (m, 9H, Ar-H), 8.20 (s, 1H, −CONH−), 11.60 (br, 1H, −NH− benzimidazole, $D_2O$ exchangeable); $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$ 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 (amide >C=O), 167.90 (ring >C=O); MS *m/z*: 399 [$M^+$], 400 [M + 1], 401 [M + 2], 262, 259, 245, 230, 216, 164, 115, 105, 58, 28. Anal. calcd. for $C_{22}H_{18}N_4O_3S_2$ (450.54): C, 54.12; H, 4.29; N, 17.53. Found: C, 54.10; H, 4.26; N, 17.57.

### *Synthesis of 2-(5-phenyl-[1,3,4]-oxadiazole-2-ylmethylsulfanyl)-1H-benzimidazole* ***6a–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 into crushed ice with stirring and the precipitated product was neutralized with ammonia, filtered, washed with water and recrystallized from chloroform to get the respective oxadiazole.

### *2-(5-Phenyl-[1,3,4]-oxadiazole-2-ylmethylsulfanyl)-1H-benzimidazole* ***6a***

Dark brown powder; m. p.: 198–200°C; IR (KBr) $\nu$: 3237.6, 1338.7 (−NH−), 1267.1 (C−O−C), 1654.7 (C=N) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$) $\delta$ ppm: 3.39 (s, 2H, S$-CH_2$), 7.17–8.53 (m, 9H, Ar-H), 11.45 (br, 1H, −NH−benzimidazole, $D_2O$ exchangeable); $^{13}$C-NMR (75 MHz, $CDCl_3$) $\delta$ 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); MS *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, 62.30; H, 3.88; N, 18.10.

### *2-(5-Chloro-phenyl-[1,3,4]-oxadiazole-2-ylmethylsulfanyl)-1H-benzimidazole* ***6b***

Dark brown powder; m. p.: 202–204°C; IR (KBr) *ν*: 3322.4, 1340.1 (–NH–), 1275.8 (C–O–C), 1618.4 (C=N), 3412.3 (–OH) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$) *δ* ppm: 3.86 (s, 2H, S–$CH_2$), 7.57–8.13 (m, 8H, Ar-H), 11.02 (br, 1H, –NH–benzimidazole, $D_2O$ exchangeable); $^{13}$C-NMR (75 MHz, $CDCl_3$) *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); MS *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, 56.09; H, 3.28; N, 16.31.

### *3-[5-(1H-Benzimidazol-2-ylsulfanylmethyl)-[1,3,4]-oxadiazole-2-yl]-naphthalen-2-ol* ***6c***

Pale yellow crystals; m. p.: 205–207°C; IR (KBr) *ν*: 3386.5, 1388.5 (–NH–), 1241.1 (C–O–C), 1636.4 (C=N) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$) *δ* ppm: 3.42 (s, 2H, S–$CH_2$), 7.18–8.11 (m, 10H, Ar-H), 11.09 (br, 1H, –NH– benzimidazole, $D_2O$ exchangeable), 12.41 (s, 1H, –OH, $D_2O$ exchangeable); $^{13}$C-NMR (75 MHz, $CDCl_3$) *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); MS *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, 64.12; H, 3.72; N, 14.95.

### *4-[5-(1H-Benzimidazol-2-ylsulfanylmethyl)-[1,3,4]-oxadiazole-2-yl]-phenol* ***6d***

Yellow powder; m. p.: 215–217°C; IR (KBr) *ν*: 3320.9, 1342.5 (–NH), 1280.4 (C–O–C), 1621.4 (C=N) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$) *δ* ppm: 3.38 (s, 2H, S–$CH_2$), 7.07–7.95 (m, 8H, Ar-H), 11.15 (br, 1H, –NH–benzimidazole, $D_2O$ exchangeable), 12.38 (s, 1H, –OH, $D_2O$ exchangeable); $^{13}$C-NMR (75 MHz, $CDCl_3$) *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); MS *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, 3.73; N, 17.29.

### *6-[5-(1H-Benzimidazol-2-ylsulfanylmethyl)-[1,3,4]-oxadiazole-2-yl]-naphthalene-1,7-diol* ***6e***

Brown crystals; m. p.: 187–189°C; IR (KBr) *ν*: 3322.4, 1340.1 (–NH–), 1275.8 (C–O–C), 1618.4 (C=N), 3412.3 (–OH) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$) *δ* ppm: 3.60 (s, 2H, S–$CH_2$), 6.92–8.52 (m, 9H, Ar-H), 11.42 (br, 1H, –NH–benzimidazole, $D_2O$ exchangeable), 12.20 (s, 1H, –OH, $D_2O$ exchangeable); $^{13}$C-NMR (75 MHz, $CDCl_3$) *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); MS *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, 61.56; H, 3.65; N, 14.32.

### *5-[5-(1H-Benzimidazol-2-ylsulfanylmethyl)-[1,3,4]-oxadiazole-2-yl]-benzene-1,2,3-triol* ***6f***

Brown crystals; m. p.: 196–198°C; IR (KBr) *ν*: 3166.1, 1387.8 (–NH–), 1269.5 (C–O–C), 1642.7 (C=N), 3499.0 (–OH) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$) *δ* ppm: 3.29 (s, 2H, S–$CH_2$), 7.18–8.53 (m, 6H, Ar-H), 10.42 (br, 1H, –NH–benzimidazole, $D_2O$ exchangeable), 12.05 (s, 1H, –OH, $D_2O$ exchangeable); $^{13}$C-NMR (75 MHz, $CDCl_3$) *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); MS *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, 3.45; N, 15.69.

### *4-[5-(1H-Benzimidazol-2-ylsulfanylmethyl)-[1,3,4]-oxadiazole-2-yl]-phenylamine* ***6g***

Light brown crystals; m. p.: 228–230°C; IR (KBr) *ν*: 3346.6, 1376.6 (–NH–), 1269.5 (C–O–C), 1603.8 (C=N) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$) *δ* ppm: 3.29 (s, 2H, S–$CH_2$), 7.17–8.55 (m, 8H, Ar-H), 10.92 (br, 1H, –NH–benzimidazole, $D_2O$ exchangeable); $^{13}$C-NMR (75 MHz, $CDCl_3$) *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); MS *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, 59.48; H, 4.10; N, 21.69.

### *2-[5-(4-Nitro-phenyl)-[1,3,4]-oxadiazole-2-ylmethylsulfanyl]-1H-benzimidazole* ***6h***

Brown crystals; m. p.: 164–166°C; IR (KBr) *ν*: 3348.1, 1380.1 (–NH–), 1269.2 (C–O–C), 1618.7 (C=N) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$) *δ* ppm: 3.11 (s, 2H, S–$CH_2$), 7.28–8.21 (m, 8H, Ar-H), 10.85 (br, 1H, –NH–benzimidazole, $D_2O$ exchangeable); $^{13}$C-NMR (75 MHz, $CDCl_3$) *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); MS *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 54.32; H, 3.10; N, 19.85.

### *2-[5-(2-Chloro-4-nitro-phenyl)-[1,3,4]-oxadiazole-2-ylmethylsulfanyl]-1H-benzimidazole* ***6i***

Light brown crystals; m. p.: 208–210°C; IR (KBr) *ν*: 3409.8, 1390.6 (–NH–), 1251.1 (C–O–C), 1657.4 (C=N), 890.8 (C–Cl) $cm^{-1}$; $^1$H-NMR (300 MHz, $CDCl_3$) *δ* ppm: 3.12 (s, 2H, S–$CH_2$), 7.65–8.11 (m, 7H, Ar-H), 11.25 (br, 1H, –NH–benzimidazole, $D_2O$ exchangeable); $^{13}$C-NMR (75 MHz, $CDCl_3$) *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); MS *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, 2.55; N, 18.02.

### *2-(5-Pyridin-3-yl-[1,3,4]-oxadiazole-2-ylmethylsulfanyl]-1H-benzimidazole* ***6j***

Light brown crystals; m. p.: 201–202°C; IR (KBr) *ν*: 3348.5, 1354.0 (–NH–), 1275.1 (C–O–C), 1610 (C=N) $cm^{-1}$; $^1$H-NMR (300 MHz,$CDCl_3$) *δ* ppm: 3.15 (s, 2H, S–$CH_2$), 7.46–8.28 (m, 8H, Ar-H), 11.45 (br, 1H, –NH–benzimidazole, $D_2O$ exchangeable); $^{13}$C-NMR (75 MHz, $CDCl_3$) *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); MS *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, 58.20; H, 3.55; N, 22.59.

## Biological evaluation

### *Anti-bacterial activity assay*

The *in vitro* anti-microbial activity of the compounds was tested by the tube dilution technique [30]. The cultures were obtained

in Mueller-Hinton Broth (Difco) for all the bacteria after 18–24 h of incubation at 37 ± 1°C. Testing was carried out in Mueller-Hinton Broth at pH = 7.4 and twofold dilution technique was applied. A set of tubes containing only inoculated broth was kept as controls. After incubation for 18–24 h at 37 ± 1°C, the last tube with no growth of microorganism was recorded to represent MIC expressed in μg/mL. Ciprofloxacin was used as standard drug.

### *Anti-fungal activity assay*

The yeasts were maintained in Sabouraud Dextrose Broth (Difco) after incubation for 48 h at 25 ± 1°C. Testing was performed in Sabouraud Dextrose Broth at pH = 7.4 and the twofold dilution technique was applied. A set of tubes containing only inoculated broth was kept as controls. After incubation for 48 h at 25 ± 1°C, the last tube with no growth of yeast was recorded to represent MIC expressed in μg/mL. Fluconazole was used as standard drug.

### *Cytotoxic activity assay*

Brine shrimp (*Artemia salina* leach) eggs were hatched in a shallow rectangular plastic dish (22 × 32 cm), filled with artificial seawater, which was prepared with commercial salt mixture and double distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. Approximately 50 mg of eggs were sprinkled into the large compartment, which was darkened while the other compartment was opened to ordinary light. After two days nauplii were collected by a pipette from the lighted side. A sample of the test compound was prepared by dissolving 20 mg of each compound in 2 mL of DMF. From this stock solutions 500, 50, and 5 μg/mL were transferred to nine vials (three for each dilutions were used for each test sample and $LD_{50}$ is the mean of three values) and one vial was kept as control having 2 mL of DMF only. The solvent was allowed to evaporate overnight. After two days, when shrimp larvae were ready, 1 mL of sea water and ten shrimps were added to each vial (30 shrimps/dilution) and the volume was adjusted with sea water to 5 mL per vial. After 24 h the number of survivors was counted [29]. Data was analyzed by a Finney computer program to determine the $LD_{50}$ values [31].

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## References

[1] E. Akbas, I. Berber, *Eur. J. Med. Chem.* **2005**, *40*, 401–405.

[2] C. D. Moldovan, A. Costescu, G. Katona, M. V. Diudea, *Match* **2008**, *60* (*3*), 977–984.

[3] C. Kus, F. Sozudonmez, N. Altanlar, *Arch. Pharm.* **2009**, *342* (*1*), 54–60.

[4] F. C. Brown, *Chem. Rev.* **1961**, *61*, 463–521.

[5] E. P. Nesynov, A. P. Grekov, *Russ. Chem. Rev.* **1964**, *33* (*10*), 508–514.

[6] S. P. Singh, S. S. Parmar, K. Raman, V. I. Stenberg, *Chem. Rev.* **1981**, *81*, 175–203.

[7] J. Tao, L. H. Cao, C. F. Wang, D. Z. Wang, *J. Chin. Chem. Soc.* **2006**, *53*, 1193–1197.

[8] A. Verma, S. K. Saraf, *Eur. J. Med. Chem.* **2008**, *43*, 897–905.

[9] K. Mogilaiah, K. Srinivas, G. R. Sudhakar, *Ind. J. Chem.* **2004**, *43B*, 2014–2017.

[10] M. Alamgir, D. C. Black, N. Kumar, *Topics in Heterocyclic Chemistry*, Springer-Verlag, Berlin Heidelberg **2007**, 87–118

[11] K. G. Desai, K. R. Desai, *J. Saudi. Chem. Soc.* **2005**, *9* (*3*), 631–640.

[12] A. Kumar, S. C. Rajput, S. K. Bhat, *Bioorg. Med. Chem.* **2007**, *15*, 3089–3096.

[13] H. D. Troutman, L. M. Long, *J. Am. Chem. Soc.* **1948**, *70*, 3436–3439.

[14] G. C. Look, J. R. Schullek, C. P. Homes, J. P. Chinn, E. M. Gordon, M. A. Gallop, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 707–712.

[15] C. J. Andres, J. J. Bronson, S. V. D'Andrea, M. S. Deshpande, P. J. Falk, K. A. Grant-Young, W. E. Harte, H. Ho, P. F. Misco, J. G. Robertson, D. Stock, Y. Sun, A. W. Walsh, *Bioorg. Med. Chem. Lett.* **2000**, *10*, 715–717.

[16] M. L. Barreca, A. Chimirri, L. D. Luca, A. M. Monforte, P. Monforte, A. Rao, M. Zappala, J. Balzarini, E. D. Clereq, C. Pannecouque, M. Witvrouw, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1793–1796.

[17] M. V. Diurno, O. Mazzoni, E. Piscopo, A. E. Calignano, F. Giordano, A. Bolognese, *J. Med. Chem.* **1992**, *35*, 2910–2912.

[18] G. K. Nampurnath, S. P. Mathew, V. Khanna, R. T. Zachariah, S. Kanji, M. R. Chamallamudi, *Chem. Biol. Interact.* **2008**, *171* (*3*), 363–368.

[19] S. P. Hiremath, H. K. S. Swamy, B. H. M. Mruthyunjayaswamy, S. M. Patil, K. M. K. Swamy, S. M. Shantakumar, *J. Indian Chem. Soc.* **1995**, *72*, 391–395.

[20] D. H. Boschelli, D. T. Connor, D. A. Barnemeier, R. D. Dyer, J. A. Kennedy, P. J. Kuipers, G. C. Okonkwo, D. J. Schrier, C. D. Wright, *J. Med. Chem.* **1993**, *36*, 1802–1810.

[21] P. C. Unangst, G. P. Shrum, D. T. Connor, R. D. Dyer, D. J. Schrier, *J. Med. Chem.* **1992**, *35*, 3691–3698.

[22] N. Srivastava, S. Bahadur, H. N. Verma, M. M. Khan, *Curr. Sci.* **1984**, *53* (*5*), 235–239.

[23] H. Saad, *Ind. J. Chem.* **1996**, *35B*, 980–984.

[24] X. P. Hui, C. H. Chu, Z. Y. Zang, Q. Wang, *Ind. J. Chem.* **2002**, *41B*, 2176–2179.

[25] P. N. Preston, *The Chemistry of Heterocyclic Compounds of Benzimidazoles and Congeneric Tricyclic Compounds*, 1st Ed., Vol. *40*, John Wiley & Sons, New York **1981**, Chapter 10.

[26] D. A. Horton, G. T. Bourne, M. L. Smythe, *Chem. Rev.* **2003**, *103*, 893–930.

[27] F. Ginah, Multicyclic compounds for use as melanin concentrating hormone antagonists in the treatment of obesity and diabetes, Patent PCT **2003** WO 2003097047, A1 200311127.

[28] A. K. Szikszai, T. Patonay, J. Jekob, *Arkivoc* **2001**, *3*, 40–50.

[29] B. N. Meyer, N. R. Ferrigni, J. E. Putnam, L. B. Jacobsen, D. E. Nichols, J. L. McLaughlin, *Planta Medica* **1982**, *45*, 31–34.

[30] D. F. Sahm, J. A. Washington, in *Manual of Clinical Microbiology* (Eds: A. Barlowes, W. J. Hausler, K. L. Hermann, H. D. Shadomy), 5th ed., American Society for Microbiology, Washington **1991**, 1105–1116.

[31] D. J. Finney, *Probit Analysis*, 3rd ed., Cambridge University Press, Cambridge **1971**.

 