Synthesis, Characterization, Antitumor, Antibacterial and Urease Inhibitory Activity of a Small Series of *N*-tosyl benzimidazoles

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Summary: Benzimidazole derivatives exhibited a broad range of biological activities, *e.g.*, antimicrobial, antiviral, anthelmintic, anti-inflammatory, anticancer and as an anti-ulcer/proton pump inhibitor. Keeping in view the large number of reported drugs containing benzimidazole moiety on one hand and sulfonamide on the other hand, a small series of *N*-tosyl benzimidazoles **(4a-e)** have been synthesized. The present work describes the synthesis, characterization and bio-evaluation of five new *N*-tosyl benzimidazoles with the objective to develop new compounds with improved anticancer, antibacterial and urease inhibitory activities. The substituted 1,2-phenylenediamines in the first step were condensed with aliphatic carboxylic acids to synthesize the substituted benzimidazoles. In the second step the tosyl chloride was reacted with substituted benzimidazoles in basic conditions to afford the title *N*-tosyl benzimidazoles **(4a-e)**. The screening for their antitumor activities was performed against *Agrobacterium tumefaciens* by following the potato disc tumor assay. The compound (**4e**) exhibited excellent antitumor activity with IC_{50} values 474.45µgml^{-1} compared to other synthesized compounds. Antibacterial activity results revealed that compounds **4d** and **4e** having methyl and ethyl substitution respectively at the imidazole ring showed excellent zone inhibition against both gram positive and gram negative strains. The urease inhibitory activity results showed that derivative **4e** exhibited highest potential to inhibit the urease enzyme compared to all other derivatives. Based upon our investigation it is proposed that compound **(4e)** may serve as lead structure to design more potent biological active compounds having multitargets inhibition activities.

Keywords: Benzimidazole, Sulfonamides, *N*-tosyl benzimidazoles, Anticancer activity, Antitumor activity, Urease inhibitory activity, Antibacterial activity, X-ray crystallography

Introduction

Benzimidazole is a significant heterocyclic moiety for the discovery of new drugs. A wide range of 1,2-disubstituted benzimidazoles have been synthesized with a variety of substitutions at different positions on the phenyl ring of the benzimidazoles [1]. Benzimidazole and its derivatives have been researched in pharmaceutical industry as an NSAID (non-steroidal anti-inflammatory drug) drug [2]. Benzimidazole derivatives have also provided a broad range of biological activities, e.g., antimicrobial [3], antiviral [4], anthelmintic, anti-inflammatory [5,6], anticancer [7,8] and as an anti-ulcer/proton pump inhibitor [9]. It has also been reported that benzimidazole derivatives bearing sulfonamide moiety possess carbonic anhydrase inhibitory activity [10-12]. Benzimidazole derivatives are also found to be active in the treatment of several diseases such as epilepsy, diabetes, and tuberculosis. Recently benzimidazole containing compounds such as regioisomers of 5(6)-bromo-1-[(phenyl)sulfonyl]-2-[(4-nitrophenoxy)methyl]-1H-benzimidazole have been reported as an antitubercular agents [13]. The potent anticancer, antibacterial, antifungal and antiprotozoal activities were also reported for halogenated 2-trifluoromethyl- and 2-pentafluoroethyl-benzimidazoles [14-16].

The tosyl substituted benzimidazoles have also been reported to possess significant biological activity Scheme-I. Omeparazole **1** (prilosec, losec) and esomeprazole (Nexium) drugs are used to control the gastric acid secretions and are also used as an antimicrobial drug. The benzimidazole derivative **2** having piperazine ring at 2 position is reported as 5-HT3 receptor antagonists is potent against viruses [17]. Sulfonamide containing benzimidazole derivative **3**, a non-peptidic LHRH (luteinizinghormone-releasing hormone) antagonist is expected to be e□ective for the oral treatment of

dysfunctional sex steroid production [18]. A number of clinically used drugs possess benzimidazole nucleus and potential of sulfonyl moiety in drugs is also well-established. Keeping in view the importance of these two moieties a series of benzimidazoles containing sulfonyl functionality i.e. tosyl benzimidazoles were synthesized and characterized. Furthermore the synthesized tosyl benzimidazoles were also screened for their antitumor and antibacterial activities against selected strains.

Experimental

Reagents and chemicals

Instrumentation

FTIR spectra (4000-400cm^{-1}) were recorded using KBr pellets in Nicolet iS10 (Thrmo Scientific) spectrophotomer. ^{1}HNMR spectra were recorded using AVANCE AV-III 300 Hz NMR spectrophotometer. EIMS were carried out by using JEOL JMS 600-H. X-ray crystallography was carried out at Keene State College (Keene, NH) whereby individual crystals of compounds **4a** and **4b** were mounted on a CryoLoop (Hampton Research) and placed in a -100^{o}C compressed air stream on an Agilent Gemini-EOS Single Crystal Autodiffractometer. Crystallographic data were collected using graphite monochromated 0.71073 Å Mo-Kα radiation and integrated and corrected for absorption using the *CrysAlisRed* [19] software package. The structures were solved using the OLEX^{2} software package [20] using Superflip [21] for structure solution and Shelx 2013 [22] for structure refinement. All other pertinent crystallographic details such as θ ranges, and R-factors can be found in Table 1. Melting points were measured on a Gallen Kamp, cat. NO. MPD350.BM 3.5, Sayno, U.K instrument.

General procedure for the preparation of compounds **(3a-e)**

The equimolar amounts of 1,2-phenylenediamine and monobasic acids (formic acid, acetic acid and propionic acid separately) were refluxed for 4 hours in 4N HCl. After the completion of reaction the mixture was cooled at room temperature, diluted with water (250 ml) and brought to pH 6.5 with Na_2CO_3. The products were precipitated out, filtered them and recrystallized in (ethanol/water 1:3) to afford the desired substituted benzimidazoles. This general procedure was followed for the synthesis of title benzimidazoles **(3a-e)**.

1H-benzimidazole (**3a**)Yield: 69 %; m.p: 167 ^{o}C; IR (KBR); 1652 (C=N), 1003 (C-N), and 3300 (N-H) cm^{-1}; ^{1}HNMR (DMSO) δ: 7.55 (dd, 2H, *J*=5.7;3.3Hz) , 7.17 (dd, 2H, *J*=6.9;3.9Hz); ^{13}CNMR (DMSO) δ: 143 (C-2), 112 (C-4,7), 102 (C-5, 6), 128 (C-8, 9) ppm. EIMS: m/z 118.0 (M+, 100%).

2-Methyl-1H-benzimidazole (**3b**) Yield: 40 %; m.p:170 ^{o}C; IR (KBR); 1621 (C=N), 1027 (C-N), and 3273 (N-H) cm^{-1} ; ^{1}HNMR (DMSO) δ: 2.46 (s, 3H), 7.41 (dd, 2H, *J*=6.9;3Hz), 7.08 (dd, 2H, *J*=6.9;3.9 Hz); ^{13}CNMR (DMSO) δ: 145 (C-2), 113 (C-4, 7), 102 (C-5, 6), 130 (C-8, 9), 31 (C-1') ppm; EIMS: m/z 132 (M+, 100%).

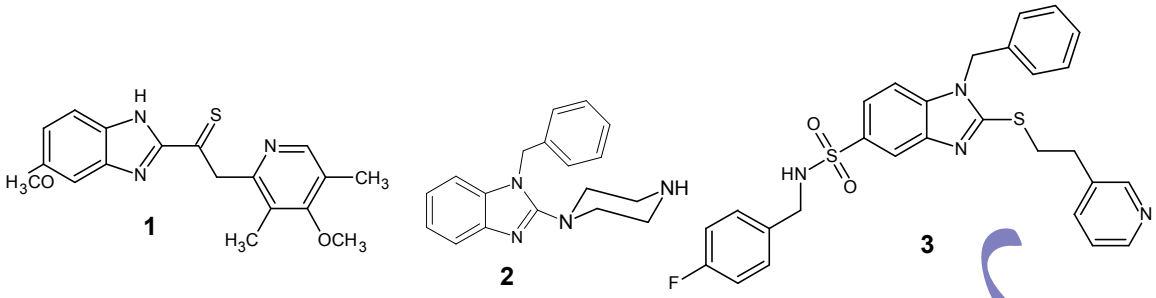
6-Nitro-1H-benzimidazole (**3c**) Yield: 78%; m.p: 195 ^{o}C; IR (KBR); 1338-1517 (NO_2), 1629 (C=N), 1065 (C-N), and 3452 (N-H) cm^{-1} ; ^{1}HNMR (DMSO) δ: 8.08 (s, 1H) , 8.63 (d, 1H, *J*=2.1Hz),8.19 (d, 1H, *J*=9 Hz), 7.96 (d, 1H, *J*=9 Hz); ^{13}CNMR (DMSO) δ: 143 (C-2), 113 (C-4), 155 (C-5) , 111 (C-6), 102 (C-7) , 129 (C-8, 9) ppm; EIMS: m/z 164 (M+, 100%).

2-Methyl-6-nitro-1H-benzimidazole (**3d**) Yield: 47 %; m.p:220 ^{o}C; IR (KBr); 1338-1517 (NO_2), 1629 (C=N), 1065 (C-N), and 3452 (N-H) cm^{-1} ; ^{1}HNMR (DMSO) δ: 2.55 (s, 3H) , 8.35 (d, 1H, *J*=2.1 Hz),8.05 (dd, 1H, *J*=9; 2.4 Hz), 7.62 (d, 1H, *J*=9); ^{13}CNMR (DMSO) δ: 143 (C-2), 117 (C-4), 152 (C-5),115 (C-6), 110 (C-7), 129 (C-8, 9), 40 (C-1') ppm; EIMS: m/z 176.9 (M+, 100%).

2-Ethyl-6-nitro-1H-benzimidazol (**3e**) Yield: 46 %; m.p: 155 ^{o}C; IR (KBR); 1341-1516 (NO_2), 1625 (C=N), 1062 (C-N), and 3435 (N-H) cm^{-1} ; ^{1}HNMR (DMSO) δ: 2.90 (q, 2H, *J*=7.8 Hz), 1.33 (t, 3H, *J*=7.8 Hz), 8.36 (d, 1H, *J*=2.1 Hz),8.05 (dd, 1H, *J*=9; 2.4 Hz), 7.62 (d, 1H, *J*=9 Hz); ^{13}CNMR (DMSO) δ: 146 (C-2), 142 (C-5), 129 (C-8, 9), 118 (C-4), 116 (C-6), 110 (C-7), 60 (C-2'), 30 (C-1') ppm; EIMS: m/z 190.9 (M+, 100%).

General procedure for the preparation of compounds **(4a-e)**

The substituted benzimidazoles **(3a-e)** (10mmol) were dissolved in 10% NaOH (25 ml). A concentrated solution of 4-methylbenezenesulfonyl chloride (19mmol) in acetone (5ml) was added slowly with continuous stirring to the benzimidazoles solution. The reaction mixture was stirred until white precipitates were appeared in the reaction flask. The crude product was filtered off, washed with water and recrystallized from ethanol to afford the *N*-tosyl benzimidazoles. This general procedure was adopted for the synthesis of the *N*-tosyl benzimidazoles **(4a-e)**.



Scheme-I: Medicinally important benzimidazole derivatives.

1-(Toluene-4-sulfonyl)-1H-benzimidazole (**4a**) Yield: 54%; m.p: 118°C; Rf: 0.24 (n-Hexane / Ethyl acetate 8:2); IR (KBr);1594 (C=N), 1256 (C-N), and 1161-1373 (S=O) cm^{-1} ; ^{1}HNMR (DMSO) δ: 8.82 (s, 1H), 7.40 (m), 8.03 (d, 2H, *J*=8.1 Hz), 7.74 (d, 2H, *J*=7.5 Hz), 2.33 (s,3H); ^{13}CNMR (DMSO) δ: 146, 143, 133, 129, 128, 119, 117,110, 30 ppm; EIMS: m/z 272 (M+, 81.8%).

2-Methyl-1-(toluene-4-sulfonyl)-1H-benzimidazole (**4b**) Yield:72 %; m.p: 120 °C; Rf: 0.29 (n-Hexane / Ethyl acetate 8:2); IR (KBr);1597 (C=N), 1274 (C-N), and 1173-1371 (S=O) cm^{-1} ; ^{1}HNMR (DMSO) δ: 2.78 (s, 3H), 7.37 (m, 4H), 7.96 (d, 2H, *J*=6 Hz), 7.44 (d, 2H, *J*=6 Hz), 2.33 (s,3H); ^{13}CNMR (DMSO) δ: 146 (C-2), 130 (C-4, 7),128 (C-5, 6), 143 (C-8, 9), 60 (C-1') 133 (C-a), 119 (C-b, f),110 (C-c, e), 30 (C-a') ppm; EIMS: m/z 285.9 (M+, 100%).

5/6-nitro-1-(toluene-4-sulfonyl)-1H-benzimidazole (**4c**) Yield: 60 %; m.p: 150 °C; Rf: a=0.23, b=0.63 (n-Hexane / Ethyl acetate 8:2); IR (KBr); 1338-1517 (NO_2), 1587 (C=N), 1300 (C-N), and 1173-1371 (S=O) cm^{-1} ; ^{1}HNMR (DMSO) δ: 8.32 (d, 1H, *J*=1.8 Hz), 8.62 (d, 1H, *J*= not clear), 8.02 (d, 1H, *J*=9 Hz), 8.10 (d, 2H, *J*=9.3 Hz), 7.50 (d, 2H, *J*=8.1Hz), 2.36 (s,3H); ^{13}CNMR (DMSO) δ: 146 (C-2), 130 (C-4, 7),128 (C-5, 6), 143 (C-8, 9), 133 (C-a), 119 (C-b, f),110 (C-c, e), 30 (C-a') ppm; EIMS: m/z 316 (M+, 39.4%).

2-Methyl-1-[(4-methylphenyl) sulfonyl]-5/6-nitro-1H-benzimidazole (**4d**) Yield: 61 %; m.p: 153 °C; Rf: a=0.25, b=0.12 (n-Hexane / Ethyl acetate 8:2); IR (KBr); 1348-1519 (NO_2), 1596 (C=N), 1270 (C-N), and 1173-1371 (S=O) cm^{-1} ; ^{1}HNMR (DMSO) δ: 2.83 (s, 3H), 8.25 (d, 1H, *J*=1.5 Hz), 8.45 (d, 1H, *J*= not clear), 8.15 (d, 1H, *J*=9 Hz), 8.02 (d, 2H, *J*=8.1 Hz), 7.48 (d, 2H, *J*=7.8 Hz), 2.36 (s,3H); ^{13}CNMR (DMSO) δ: 149 (C-2), 121 (C-4),155 (C-5), 120 (C-6), 116 (C-7), 148 (C-8, 9), 24 (C-1'), 131 (C-a), 128 (C-b, f),127 (C-d), 123 (C-c, e), 33 (C-a') ppm; EIMS: m/z 330.9 (M+, 100%).

2-Ethyl-5/6-nitro-1-(toluene-4-sulfonyl)-1H-benzimidazole (**4e**) Yield: 61 %; m.p: 136°C; Rf: a= 0.37, b=0.22 (n-Hexane / Ethyl acetate 8:2); IR (KBr); 1347-1525 (NO_2), 1595 (C=N), 1276 (C-N), and 1164-1376 (S=O) cm^{-1} ; ^{1}HNMR (DMSO) δ: 2.34 (q, 2H), 3.18(t, 3H), 8.25 (d, 1H, *J*=2.1 Hz), 8.75 (d, 1H, *J*= not clear), 7.90 (d, 1H, *J*=9 Hz), 8.00 (d, 2H, *J*=8.1 Hz), 7.46 (d, 2H, *J*=8.1 Hz), 2.36 (s,3H); ^{13}CNMR (DMSO) δ: 149 (C-2), 121 (C-4),155 (C-5), 120 (C-6), 116 (C-7), 148 (C-8, 9), 24 (C-1'), 16 (C-2'), 131 (C-a), 128 (C-b, f),127 (C-d), 123 (C-c, e), 33 (C-a') ppm; EIMS: m/z 345.0 (M+, 40.6%).

Potato Disc Antitumor Assay

The *N*-tosyl benzimidazoles **(4a-e)** were tested for their antitumor activities by using potato disc antitumor method [23]. In this assay a 48h old culture of an AT-10 strain of *Agrobacterium tumefaciens* was used. Inoculum with three concentrations (1000, 100, 10µgml^{-1}) of each test sample containing bacterial culture was prepared. Red skinned potatoes were surface sterilized in 0.1% mercuric chloride ($HgCl_2$) solution in distilled water, for 7-10 minutes and then washed with autoclaved distilled water inside LFH. Potato cylinders were made with the help of a sterilized cork borer (8 mm). These cylinders were cut into (5 mm x 8 mm) thick discs and were placed on solidified agar plates (10 discs per plate). Then 50 µl of inoculums were applied to the top of each disk and each petriplate was wrapped with parafilm strips to avoid contamination and loss of moisture during the incubation period. These petri-plates were placed in a 28°C incubator for 21 days. The number of tumors was counted after staining with the Lugol's solution (10% KI and 5% I_2) with the help of a dissecting microscope and the percentage inhibition was determined as follows:

$$\text{Percentage inhibition} = 100 - \left[\frac{\text{Average number of tumors of sample}}{\text{Average number of tumors of negative control}} \times 100 \right]$$

Antibacterial Activities

In vitro evaluation of the antibacterial activity of the compounds was carried out by agar well diffusion assay against six different Gram positive and Gram negative bacteria [24]. Antibacterial activity was determined by using a Mueller Hinton Agar (MHA). The fresh inoculums of these bacteria were prepared and diluted by sterilized normal saline. The turbidity of these cultures was adjusted by using 0.5Mc-Farland. A homogeneous bacterial lawn was developed by sterile cotton swabs. The inoculated plates were bored by a 6 mm sized borer to make the wells. Sample dilutions were prepared by dissolving each sample (1.0mg) in 1.0 mL of DMSO used as negative control in this bioassay. The equimolar concentration of Levofloxacin (1.0mg/ml), a broad spectrum antibiotic (positive control) was prepared. These plates were incubated at 37 °C for 24 hours. Antibacterial activity of the compounds was determined by measuring the diameter of zone of inhibition (mm, ± standard deviation).

Urease Inhibitory Activity

The urease inhibitory activity of the synthesized *N*-tosyl benzimidazoles was determined by measuring the amount of ammonia produced by the indophenols method described by Weatherburn [25]. The reaction mixtures, comprising 20 µL of enzyme (Jack bean urease, 5 U/mL) and 20µL of test compounds in 50µL buffer (100mM urea, 0.01 M K_2HPO_4, 1mM EDTA and 0.01 M $LiCl_2$, pH 8.2), were incubated for 10 min at 37 ºC in 96-well plate. Briefly, 40µL each of phenol reagents (1%, w/v phenol and 0.005%, w/v sodium nitroprusside) and 70µL of alkali reagent (0.5%, w/v NaOH and 0.1% active chloride NaOCl) were added to each well. The absorbance at 625nm was measured after 30min, using a microplate reader ($OPTI_{Max}$, Tunable). All reactions were performed in triplicate. The urease inhibition activities were calculated according to the following formula:

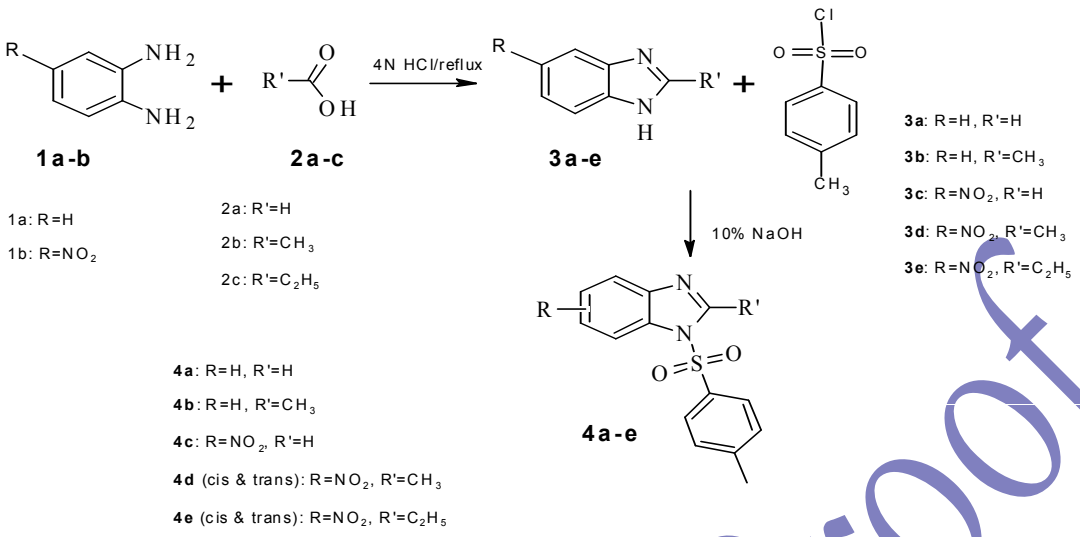
$$\text{Urease inhibition activity (\%)} = (OD_{control} - OD_{sample} \times 100) / OD_{control}$$

where $OD_{control}$ and OD_{sample} represent the optical densities in the absence and presence of sample respectively. Thiourea was used as the standard inhibitor for urease.

Results and discussion

Chemistry

Substituted benzimidazoles (**3a-e**) were synthesized by following the already reported method [26] with slight modifications. 1,2-phenylene diamine (**1a-b**) were reacted with monobasic acids (**2a-c**) in presence of 4N HCl to obtain substituted benzimidazoles (**3a-e**) Scheme II. *N*-tosyl benzimidazoles (**4a-e**) were synthesized by reacting 2-alkyl substituted-benzimidazoles with tosyl chloride in 10% sodium hydroxide and acetone solution according to the methods reported earlier [27-28]. The molecular structure of **4b** was determined by single X-ray diffraction technique.^{1}HNMR of 4c, 4d and 4e revealed a characteristic set of signals for aromatic protons indicating the presence of regioisomers. The ^{1}HMNR spectra of 4a and 4b were also consistent with the proposed structures. Ar-H (4, 5, 6, and 7) displayed multiplet signals in range of 7.23-7.40 ppm. Moreover, Ar-H (2', 3') for N-tosyl substitution displayed the doublet at 8.10 ppm (*J*=9.3 Hz) for 4c, 8.02 ppm (*J*=8.1Hz) for 4d, 8.00 ppm (*J*=8.1Hz) for 4e, 8.03ppm (*J*=8.1Hz) for 4a and at 7.96 ppm (*J*=6 Hz) represented ortho coupling. FTIR spectra of (3a-e) showed a strong peak for N-H in the range of 3500-3300cm^{-1}, whereas it was not observed in (**4a-e**) which confirmed the formation of desired *N*-tosyl benzimidazoles (**4a-e**). X-ray crystallography exhibited a monoclinic crystal system for 4b with a moiety formula $C_{15}H_{14}N_2O_2S$, Mr=286.34.



Scheme-II: Synthesis of N-tosyl benzimidazoles **(4a-e).**

Table-1: Crystal data for $C_{14}H_{12}N_2O_2S$ **(4a)** and $C_{15}H_{14}N_2O_2S$ **(4b).**

Compounds	4a	4b
Empirical formula	$C_{14}H_{12}N_2O_2S$	$C_{15}H_{14}N_2O_2S$
Formula weight	272.32	286.34
Temperature (K)	173 (2)	173 (2)
Crystal system	triclinic	monoclinic
Space group	P -1	C c
Unit cell dimensions (Å, °)		
a	8.6305(11)	10.9624(4)
b	8.9318 (11)	16.2917 (5)
c	17.3824 (13)	8.0762 (3)
α	81.070 (8)°	90
β	81.690 (9)°	106.089 (4)
γ	80.757 (11)°	90
Volume (Å^3)	1296.7 (2)	1385.88 (9)
Z	4	4
Absorption coefficient μ (mm^{-1})	0.248	0.236
F(000)	568	600
θ range for data collection (°)	3.106-32.83	3.0567-32.7824
Calculated density (mg m^{-3})	1.395	1.372
Final R indexes [I>=2□(I)]	R_1=-.0504, wR_2=0.1181	R_1=-.0391, wR_2=0.0973
Final R indexes [all data]	R_1=0.1016,wR_2=0.1302	R_1=0.0459,wR_2=0.1026
Goodness-of-fit on F^2	0.869	1.059
Number of data collected	11589	7882
Largest diff. peak/hole / e Å^{-3}	0.45/-0.39	0.32/-0.21

Table 2 Selected bond lengths (Å) and angles (°) for $C_{14}H_{12}N_2O_2S$ **(4a)** and $C_{15}H_{14}N_2O_2S$ **(4b)**

$C_{14}H_{12}N_2O_2S$ (4a)			
S1A-O1A	1.4281(17))	N1A-C1A	1.396(3)
S1A-O2A	1.4140(19)	N1A-C7A	1.406(3)
S1A-N1A	1.6719(19)	N2A-C1A	1.291(3)
S1A-C8A	1.745(2)	N2A-C2A	1.387(3)
S1B-O1B	1.4241(16)	N1B-C1B	1.388(3)
S1B-O2B	1.4188(18)	N1B-C7B	1.403(3)
S1B-N1B	1.674(2)	N2B-C1B	1.289(3)
S1B-C8B	1.751(2)	N2B-C2B	1.403(3)
O1A-S1A-O2A	121.11(11)	O1A-S1A-N1A	105.11(10)
O1A-S1A-C8A	109.58(10)	O2A-S1A-N1A	105.74(10)
O2A-S1A-C8A	110.36(11)	N1A-S1A-C8A	103.25(9)
O1B-S1B-O2B	120.84(10)	O1B-S1B-N1B	105.15(10)
O1B-S1B-C8B	109.39(10)	O2B-S1B-N1B	106.03(10)
O2B-S1B-C8B	110.68(11)	N1B-S1B-C8B	103.06(9)
$C_{15}H_{14}N_2O_2S$ (4b)			
S1-O1	1.423 (2)	N1-C1	1.416 (3)
S1-O2	1.4273 (19)	N1-C7	1.403 (3)
S1-N1	1.687 (2)	N2-C1	1.296 (3)
S1-C8	1.743 (2)	N2-C2	1.397 (3)
01-S1-O2	120.96 (12)	O1-S1-N1	106.12 (11)
O1-S1-C8	109.75 (12)	O2-S1-N1	105.14 (11)
O2-S1-C8	109.38 (11)	N1-S1-C8	104.02 (10)

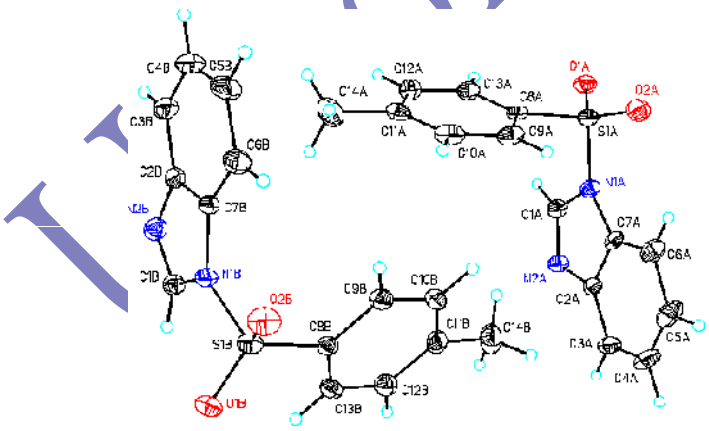
Table-3: Weak intermolecular interactions for $C_{14}H_{12}N_2O_2S$ **(4a)** and $C_{15}H_{14}N_2O_2S$ **(4b)** [Å and °].

D-H...A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/°
		$C_{14}H_{12}N_2O_2S$ (4a)		
C1A---H1A…O1A[1]	0.95	2.42	3.351(3)	166.2
	0.95	2.61	3.465(3)	150.3
	0.95	2.42	3.353(3)	166.0
	0.95	2.62	3.391(3)	138.0
		$C_{15}H_{14}N_2O_2S$ (4b)		
C6---H6…N2[5]	0.95	2.56	3.356(3)	142.0

[1]–x, 1-y, -z; [2]+x, -1+y, +z; [3]1-x, 2-y, 1-z; [4]1+x, +y, +z; [5]-1/2+x, ½-y, -1/2+z

Description of the X-ray single crystal structures

The solid state structure of the compounds **(4a)** and **(4b)** were investigated by single crystal x-ray diffraction technique. The structure of **4a** shows two independent molecules (A and B) in asymmetric unit as given in Fig. 1. The selected crystallographic data are summarized in Table 1 while selected geometrical parameters are listed in Table 2. The table 3 identifies the weak intermolecular interactions in the crystal packing. The crystal structure refines as a twin (BASF = 0.46861). As a whole, molecule is essentially non-planar although the benzimidazole ring and toluene-4-sulphonyl groups are individually planar. Dihedral angle between the mean planes of these two groups is 89.4(2)° (A), 89.0(6)° (B) while the N1A/S1A/C8A/C9A and N1B/S1B/C8B/C9B torsion angles are 79.4(2)° and 82.1(2)°, respectively. The S1A--N1A and S1B--N1B bond lengths are in good agreement with data observed in the literature [27]. Crystal structure of the molecule is further stabilized by some non-classical weak intermolecular C---H…O, C-H···N interactions which link the molecules into chains along [1 0 0] and [0 0 1] forming an extended 2-dimensional supramolecular network. The compound crystallizes in the *triclinic* crystal system with space group P-1, a = 8.6305(11) Å, b = 8.9318 (11) Å, c = 17.3824 (13) Å and α = 81.070 (8)°, β = 81.690 (9)°, γ = 80.757 (11)°.



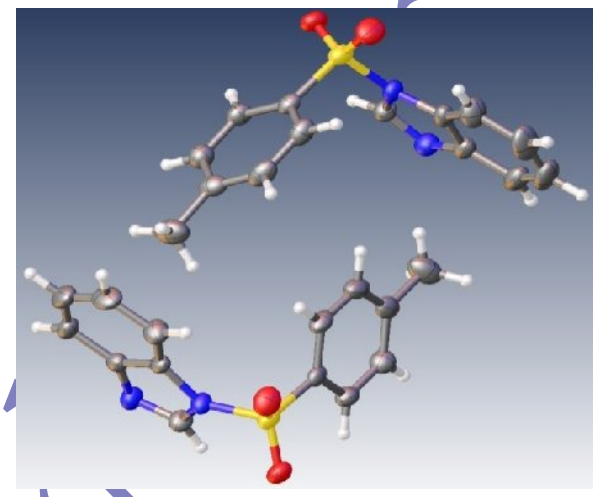


Fig. 1: Molecular structure and screenshot of $C_{14}H_{12}N_2O_2S$ (4a) showing the atom labeling scheme and 30% probability displacement ellipsoids.

The crystal structure of the compound **(4b)** is shown in Fig. 2. The asymmetric unit reveals a single molecule of **(4b)**. The molecule **(4b)** crystallizes in *monoclinic* crystal system with space group *C c*, a = 10.9624(4) Å, b = 16.2917 (5) Å, c = 8.0762 (3) Å and β = 106.089 (4)°. As a whole, molecule is essentially non-planar although the benzimidazole ring and toluene-4-sulphonyl groups are individually planar. Dihedral angle between the mean planes of these two groups is 88.1(5)° and the N1/S1/C8/C9 torsion angle is 95.1(2)°. The S1-N1 bond length is in good agreement with data observed in the literature. Crystal structure of the molecule is further stabilized by some non-classical weak inter-molecular C-H···N forces which link molecules into chains along [1 0 0]. Both molecules in asymmetric unit have similar bond lengths and angles. Both molecules are essentially non-planar but individual benzimidazole and toluene-4-suphlonyl groups are planar. The dihedral angles between these groups is 79.4(2)° in molecule A (N1A/S1A/C8A/C9A) and 81.2° in molecule B(N1B/S1B/C8B/C9B). Similar to 4b, the crystal structure of compound 4a is stabilized by non-classic intermolecular hydrogen bonding C-H···O and C-H···N.

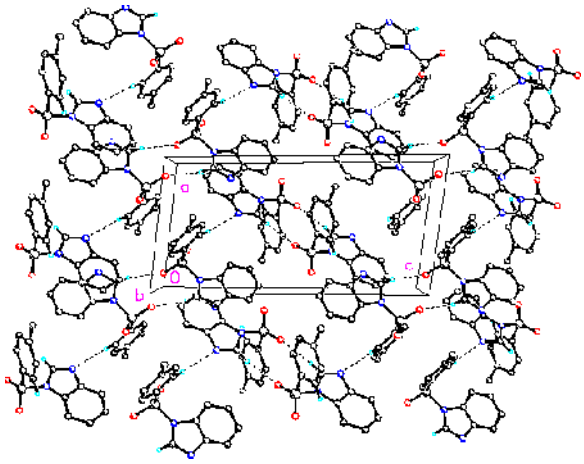


Fig. 2: Packing diagram of $C_{14}H_{12}N_2O_2S$ (4a) viewed along the b axis. Dashed lines indicate weak C---H…N and C---H…O intermolecular interactions which link the molecules into chains along [1 0 0] and [0 0 1] forming an extended 2-dimensional supramolecular network. H atoms not involved in intermolecular interactions have been removed for clarity.

Biological activities

All of the synthesized *N*-tosyl benzimidazoles **(4a-e)** were examined for antitumor activity by the potato disc method. Table 4 presented the antitumor activity results of the synthesized *N*-tosyl benzimidazoles **(4a-e)**. The compound **(4e)** which possesses nitro functionality at benzimidazole ring exhibited excellent antitumor activity compared to other derivatives. The presence of electron withdrawing nitro group at benzimidazole ring play significant role in antitumor activity. The compound **(4c)** showed good antitumor activity with IC_{50} value 1000µgml^{-1} while IC_{50} value of most potent derivative **(4e)** is 474.45µgml^{-1}.

Table-4: Antitumor activity of synthesized N-tosyl benzimidazoles **(4a-e).**

Sr. No.	Compound Code	% Inhibition at different concentrations			IC_{50} Value (µg/ml)
		1000 µg/ml	100 µg/ml	10 µg/ml	
1	4a	-	-	-	-
2	4b	32	21	3	>1000
3	4c	50	28	6	1000
4	4d	32	14	4	>1000
5	4e	67	21	8	474.45

Antibacterial activity of synthesized *N*-tosyl benzimidazoles **(4a-e)** was determined and results were presented in table 5. Antibacterial activity results revealed that compound **(4d)** and **(4e)** which possesses methyl and ethyl substitution, respectively, at the imidazole ring exhibited superb zone inhibition against gram positive and gram negative strains. The compound **(4e)** exhibited 80% growth inhibition against *Bacillus subtilis* and 78% inhibition against *Escherichia coli* compared to the standard drug. *Staphylococcus aureus, Pseudomona putida* and *Pseudomona aeruginosa* are the most resistant bacteria among the tested microorganisms. Among all the *N*-tosyl benzimidazole derivatives, the most active was **(4e)**. Antibacterial activity results revealed that the presence of a benzimidazole nucleus as well as the presence of polar or non-polar substituents greatly affected the result.

Table 5 Antibacterial activity of synthesized *N*-tosyl benzimidazoles (4a-e)

Bacterial Strains	Compounds					
	4a	4b	4c	4d	4e	standard
Proteus mirabilis	7	10	16	20	23	30
Bacillus subtilis	6	9	13	17	20	25
Escherichia coli	10	15	15	19	25	32
Staphylococcus aureus	-	-	-	20	26	34
Pseudomonas putida	-	-	12	16	22	36
Pseudomonas aeruginosa	11	-	-	14	20	28

Antibacterial activity of the compounds was determined by measuring the diameter of zone of inhibition in millimeter (mm), (-) No Activity.

The urease inhibitory potential of the synthesized *N*-tosyl benzimidazoles derivatives **(4a-e)** was also determined and results were presented in Table 6. The derivative **4e** exhibited highest potential to inhibit the urease enzyme compared to all other derivatives. The compound **4e** displayed IC_{50} value 31.1 ± 0.85µM while IC_{50} value for the standard thiourea is 20.9 ± 0.92 µM. The presence of electron withdrawing substituent at benzimidazole phenyl ring and alkyl substitution at heterocyclic ring play vital role in the urease inhibitory activity.

Table-6: Urease Inhibitory activity of synthesized *N*-tosyl benzimidazoles **(4a-e).**

Compounds	IC_{50} µM
4a	83.3 ± 1.32
4b	83.5 ± 1.07
4c	35.5 ± 0.93
4d	158.6 ± 1.81
4e	31.1 ± 0.85
Thiourea	20.9 ± 0.92

Conclusion

The *N*-tosyl benzimidazoles **(4a-e)** were efficiently synthesized starting from substituted *o*-phenylene diamine. The spectral data of compounds **(4a-e)** and X-ray crystallographic data in case of compounds **(4a, 4b)** assured the structures of the

desired products. The benzimidazole derivative **(4e)** exhibited excellent antitumor activity compared to other derivatives with IC_{50} value 474.45µgml^{-1}. The presence of electron withdrawing nitro group at benzimidazole ring play significant role in antitumor activity. In case of antibacterial activity compounds **(4d)** and **(4e)** which possesses methyl and ethyl substitution respectively at the imidazole ring exhibited significant zone inhibition against gram positive and gram negative bacteria. The compound **(4e)** exhibited 80% growth inhibition against *Bacillus subtilis* and 78% inhibition against *Escherichia coli* compared to the standard drug. Antibacterial activity results revealed that the presence of polar and non-polar substituents in the synthesized derivatives had significant influenced on their activities. The urease inhibitory activity results showed that derivative **4e** exhibited highest potential to inhibit the urease enzyme compared to all other derivatives. Based upon our investigation it is proposed that compound **(4e)** may serve as lead structure to design more potent biological active compounds having multi-targets inhibition activities.

Conflict of Interest

The authors declared no conflict of interests.

References

1. B. Zou, Q. Yuan, D. Ma, Synthesis of 1,2-Disubstituted Benzimidazoles by a Cu-Catalyzed Cascade Aryl Amination/Condensation Process, *Angewandte Chemie*, *International Edition*, **46**, 2598 (2007).
2. R. M. Garavito, The Cyclooxygenase-2 Structure: New Drugs for an Old Target? *Nature Struct Biol*, **3**, 897 (1996).
3. Y. F. Li, G. F. Wang, P. L. He, W. G. Huang, F. H. Zhu, H. Y. Gao, W. Tang, Y. Luo, C. L. Feng, L. P. Shi, Y. D. Ren, W. Lu and J. P. Zuo, Synthesis and Anti-hepatitis B Virus Activity of Novel Benzimidazole Derivatives, *J. Med. Chem.*, **49**, 4790 (2006).
4. Y. Tong, J. J. Bouska, P. A. Ellis, E. F. Johnson, J. Leverson, X. Liu, P. A. Marcotte, A. M. Olson, D. J. Osterling, M. Przytulinska, L. E. Rodriguez, Y. Shi, N. Soni, J. Stavropoulos, S. Thomas, C. K. Donawho, D. J. Frost, Y. Luo, V. L. Giranda, T. D. Penning, Synthesis and Evaluation of a New Generation of Orally Efficacious Benzimidazole-Based Poly(ADP-ribose) Polymerase-1 (PARP-1) Inhibitors as Anticancer Agents. *J. Med. Chem*., **52**, 6803 (2009).
5. Y. Bansal, O. Silakari, The Therapeutic Journey of Benzimidazoles: A Review, *Bioorg. Med. Chem.*, **20**, 6208 (2012).
6. Y. F. Li, G. F. Wang, Y. Luo, W. G. Huang, W. Tang, C. L. Feng, L. P. Shi, Y. D. Ren, J. P. Zuo, W. Lu, Identification of 1-isopropylsulfonyl-2-amine Benzimidazoles as a New Class of Inhibitors of Hepatitis B Virus. *Euro. J. Med. Chem.,* **42**, 1358 (2007).
7. K. C. S. Achar, K.M. Hosamani, H. R. Seetharamareddy, *In-vivo* Analgesic and Anti-Inflammatory Activities of Newly Synthesized Benzimidazole Derivatives. *Euro. J. Med. Chem*, **45**, 2048 (2010).
8. H. M. Refaat, Synthesis and Anticancer Activity of Some Novel 2-Substituted Benzimidazole Derivatives. *Euro. J. Med. Chem*., **45**, 2949 (2010).
9. L. Olbe, E. Carlsson, P. Lindberg, A Proton-Pump Inhibitor Expedition: the Case Histories of Omeperazole and Esomeperazole. *Nature Reviews Drug Discovery*, **2**, 132 (2003).
10. M. Abdoli, A. Angeli, M. Bozdag, F. Carta, A. Kakanejadifard, H. Saeidian, C. T. Supuran, Synthesis and Carbonic Anhydrase I, II, VII, and IX Inhibition Studies with a Series of Benzo[d]Thiazole-5- and 6-Sulfonamides, *Journal of Enzyme Inhibition and Medicinal Chemistry,* **32 (1),** 1071 (2017).
11. C. T. Supuran**,** Carbonic Anhydrases: Novel Therapeutic Applications for Inhibitors and Activators**,** *Nature reviews. Drug discovery*, **7** (2), 168 (2008).
12. A. Scozzafava, T. Owa, A. Mastrolorenzo, C. T. Supuran, Anticancer and Antiviral Sulfonamides*, Current Medicinal Chemistry*, **10 (11)**, 925 (2003).
13. P. K. Ranjith, P. Rajeesh, K. R. Haridas, N. K. Susanta, T. N. G. Row, R. Rishikesan, N. S. Kumari, Design and Synthesis of Positional Isomers of 5 and 6-Bromo-1-[(phenyl)sulfonyl]-2-[(4-nitrophenoxy)methyl]-1*H*-benzimidazoles as Possible Antimicrobial and Antitubercular Agents. *Bioorg. Med. Chem. Lett*., **23**, 5228 (2013).
14. G. N. Vázquez, R. Cedillo, A. H. Campos, L. Yépez, F. H. Luis, J. Valdez, R. Morales, R. Cortés, M. Hernández, R. Castillo. Synthesis and Antiparasitic Activity of 2-(trifluoromethyl) Benzimidazole Derivatives, *Bioorg. Med. Chem. Lett.*, **11**, 187, (2001).
15. M. Andrzejewska, L. Yépez-Mulia, R. Cedillo-Rivera, A. Tapia, L. Vilpo, J. Vilpo, Z. Kazimierczuk. Synthesis, Antiprotozoal and Anticancer Activity of Substituted 2-Trifluoromethyl- and 2-

Pentafluoroethylbenzimidazoles, *Eur. J. Med. Chem.*, **37**, 973-978, (2002).
16. Z. Kazimierczuk, M. Andrzejewska, J. Kaustova and V. Klimešova. Synthesis, Antiprotozoal and Anticancer Activity of Substituted 2-Trifluoromethyl- and 2-Pentafluoroethylbenzimidazoles, *Eur. J. Med. Chem.*, **40**, 203, (2005).
17. A. Orjales, R. Mosquera, L. Labeaga, R. Rodes, New 2-Piperazinylbenzimidazole Derivatives as 5-HT3 Antagonists; Synthesis and Pharmacological Evaluation. *J. Med. Chem.,* **40**, 586 (1997).
18. T. Imada, N. Cho, T. Imaeda, Yoji. Hayase, S. Sasaki, S. Kasai, M. Harada, H. Matsumoto, S. Endo, N. Suzuki, S. Furuya, Benzimidazole Derivatives as Novel Non-Peptide Luteinizing Hormone-Releasing Hormone (LHRH) Antagonists. Part 1: Benzimidazole-5-sulfonamides. *Bioorg. Med. Chem. Lett*., **15**, 799 (2005).
19. Agilent. *CrysAlis PRO* and *CrysAlis RED*, Agilent Technologies, Yanton, England, (2012).
20. O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, R, J. A. Howard, H. Puschmann,. OLEX2: a Complete Structure Solution, Refinement and Analysis Program. *Journal of Applied Crystallography*, ***42*(2)**, 339 (2009).
21. L. Palatinus, S. J. Prathapa, S. V. Smaalen,. EDMA: A Computer Program for Topological Analysis of Discrete Electron Densities. *Journal of Applied Crystallography*, ***45*(3)**, 575 (2012).
22. G. M. Sheldrick,. A Short History of SHELX. *Acta Crystallographica Section A: Foundations of Crystallography*, ***64*(1)**, 112 (2008).
23. G. Bibi, N. Ullah, A. Mannan, B. Mirza,. Antitumor, Cytotoxic and Antioxidant Potential of Aster Thomsonii Extracts. *African Journal of Pharmacy and Pharmacology*, **5(2)**, 252 (2011).
24. Z. Ashraf, A. Saeed, H. Nadeem, Design, Synthesis and Docking Studies of Some Novel Isocoumarin Analogues as Antimicrobial Agents. *RSC Adv*. **4**, 53842 (2014).
25. M.W. Weatherburn. Phenol-Hypochlorite Reaction for Determination of Ammonia. *Anal. Chem*. **39**, 971-974, (1967).
26. M. A. Phillips The Formation of 2-substituted Benziminazoles, *J Chem Soc*., 2393, (1928).
27. M. Hasan, N. Rashid, M. C. G. Alvarez, F. Malik, Preparation, Physical Properties, Mass and Nuclear Magnetic Resonance Spectra of Some 2-Alkyl Benzimidazoles, *J Chem Soc Pak,* **9**, 65 (1985).
28. M. Hassan, N. Rashid, F. Malik, K. Akhtar, O. Osman, H. Huddeck, Preparation, Separation, and NMR Spectral Peak Assignment of Region-Isomeric Unsymmetrical Benzimiadozles. *J Chem Soc Pak*., **14**, 54 (1992).
29. N. Rashid, M. Hasan, M. N. Yusof, M. B. Yamin, 1-(4-Methylphenylsulfonyl)-5-nitro-2-[(E)-prop-1-enyl]-1*H*-benzimidazole, *Acta Cryst E*., **62**, 5455 (2006).