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Bioorganic & Medicinal Chemistry Letters 17 (2007) 3335–3338
Bioorganic & Medicinal Chemistry Letters

Benzimidazo[1,2-*c*][1,2,3]thiadiazole-7-sulfonamides as inhibitors of carbonic anhydrase

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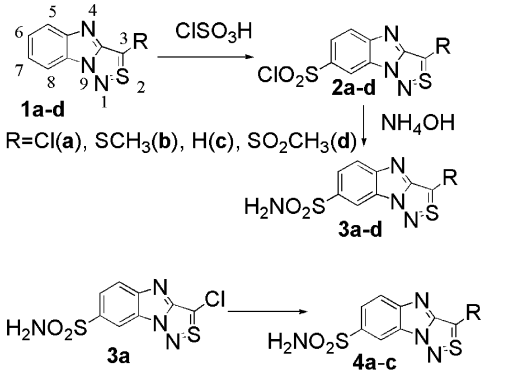
Received 26 February 2007; revised 28 March 2007; accepted 30 March 2007
Available online 5 April 2007

Abstract—A series of benzimidazo[1,2-*c*][1,2,3]thiadiazole-7-sulfonamides were synthesized and their binding to two carbonic anhydrase isozymes measured by isothermal titration calorimetry (ITC). Human carbonic anhydrase I (hCAI) and bovine carbonic anhydrase II (bCAII) bound the inhibitors with observed association constants in the range from 1.1×10^6 to 2.6×10^7 M^{-1}.

Carbonic anhydrases are zinc-containing metalloproteins widespread in all life kingdoms.[1] There are 16 carbonic anhydrase isoforms identified in mammals (humans have 15 isoforms) that differ in their subcellular localization and catalytic activity.[2,3] These enzymes are efficient catalysts for reversible hydration of carbon dioxide to bicarbonate ($CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$), and are thus involved in essential physiological processes such as respiration, pH and CO_2 homeostasis, electrolyte secretion, bone resorption, calcification, biosynthetic reactions (e.g., lipogenesis, gluconeogenesis, and ureagenesis), tumorigenicity and many other physiological or pathological processes.[2,4] Many of these isozymes are important targets for design of inhibitors with clinical applications. The most prominent class of CA inhibitors is aromatic/heterocyclic sulfonamides which have been studied for the development of antiglaucoma, antitumor, antiobesity or anticonvulsant drugs.[5,6] However, many sulfonamides possess high affinity for all major isozymes. Although some progress was done on the development of the isozyme specific inhibitors[7,8], a constant need exists for the design of novel CA inhibitors belonging to different classes of compounds, since such derivatives may lead to potential pharmacological applications. The goal of the present study was the design and synthesis of novel CA inhibitors with bulky and rigid structure that are useful for the design of enzyme-specific inhibitors.

The chemistry employed for the design of the new compounds reported here is shown in Schemes 1 and 2. A series of benzimidazo[1,2-*c*][1,2,3]thiadiazole-7-sulfonamides (**3a–d**, **4a–c**) was synthesized. Incorporation of the sulfonamide group in benzimidazo[1,2-*c*][1,2,3]thiadiazole ring system was accomplished according to Scheme 1. 1,2,3-Thiadiazole derivatives **1a–d** bearing different substituents (chloro-, methylthio-, hydrogen-, methylsulfonyl-) at ring position three were reacted with chlorosulfonic acid, leading to the sulfonyl chlorides **2a–d** which were converted to sulfonamides **3a–d**.[9] The attempt to synthesize sulfonamide **3d** directly from **3b** by oxidation with hydrogen peroxide in acetic acid or with 3-chloroperbenzoic acid in acetic acid failed. The formation of two compounds was observed, and they



Scheme 1. Synthesis of compounds **2a–d**, **3a–d**, **4a–c**.

Keywords: Aromatic sulfonamides; Binding enthalpy; Observed binding constant; Carbonic anhydrase; Inhibition.
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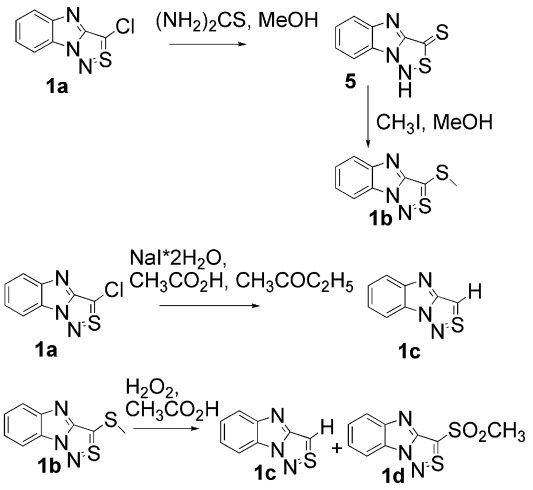
doi:10.1016/j.bmcl.2007.03.100



Scheme 2. Synthesis of compounds **1a**–**d**.

decomposed readily during separation by column chromatography. Sulfonamide derivatives **4a**–**c** were prepared by nucleophilic substitution reactions of compound **3a** with morpholine, *N*-methylpiperazine, and thiophenol.[10]

The starting materials **1b**–**d** were synthesized from 3-chlorobenzimidazo[1,2-*c*][1,2,3]thiadiazole (**1a**)[11] which was prepared as previously reported.[12] The methylthio intermediate **1b** was obtained from **1a** by the two-step reaction. First, **1a** was treated with thiourea leading to thione **5** whose different synthesis method was described previously.[12] Then the reaction of thione **5** with methyl iodide yielded methylthio derivative **1b**. Treatment of **1a** with sodium iodide dihydrate in the mixture of acetic acid and **2**-butanone yielded starting material **1c**. Methylsulfonyl derivative **1d** was prepared by oxidation of **1b**. It should be mentioned that oxidation of compound **1b** with hydrogen peroxide in acetic acid afforded a mixture of compounds **1c** and **1d**. The main product which was separated by flash chromatography was methylsulfonyl derivative **1d**.

The binding affinity of benzimidazo[1,2-*c*][1,2,3]thiadiazole-7-sulfonamides to human carbonic anhydrase I and bovine carbonic anhydrase II was determined by isothermal titration calorimetry, a method widely used to measure inhibitor binding to CA[13] (Fig. 1). The observed dissociation constants and binding enthalpies of compounds **3a**–**d** and **4a**–**c** are listed in Tables 1 and 2, respectively.

Isothermal titration calorimetry (ITC)[16–18] measurements were performed on the Nano-ITC III calorimeter (Calorimetry Sciences Corp., USA). Human carbonic anhydrase I (hCAI) and bovine carbonic anhydrase II (bCAII) were purchased from, 'Sigma'. Binding measurements were performed at pH 7.0 in 50 mM tris–chloride buffer containing 50 mM NaCl. Titrations were carried out at 25 °C. A typical titration consisted of 25 injections of a tested compound (10 μl per injection) at 3 min intervals into the sample cell containing protein sample.

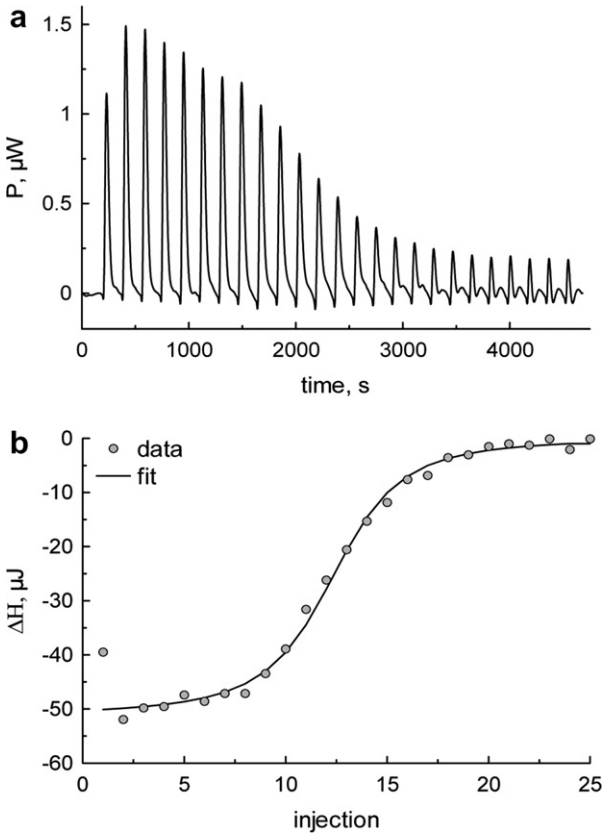


Figure 1. Typical isothermal titration calorimetry curves of carbonic anhydrase binding to compounds (**4a** binding to bCAII). a—raw data (power (P) dependence on time), b—integrated data (enthalpy (ΔH) evolved per injection).

Table 1. Dissociation constants of inhibitor **3a**–**d** and **4a**–**c** binding to human carbonic anhydrase I and bovine carbonic anhydrase II

Compound	Binding to hCAI K_d[a] (μM)	Binding to bCAII K_d[a] (μM)
3a	0.435	0.141
3b	0.217	0.322
3c	0.588	0.286
3d	0.141	0.500
4a	0.048	0.038
4b	0.197	0.391
4c	0.909	0.285
AZM[b]	1.163[c]	0.045[c], 0.025[d]

[a] Values are means of at least two experiments, determined by ITC.
[b] AZM—acetazolamide, commonly used as CA inhibitor.
[c] Data from Ref.14.
[d] Data obtained by surface plasmon resonance.[15]

The strongest binder to both isozymes of carbonic anhydrase was compound **4a** with the observed K_d of about 0.04 μM. The most specific binder of hCAI was compound **3d** that bound about fourfold stronger to hCAI than to bCAII. The **3a** compound bound threefold tighter to bCAII than to hCAI. The differences in the enthalpies of binding among the compounds were much greater than the binding constants. The enthalpies of binding to bCAII were more exothermic than hCAI. The compound **4b**, containing *N*-methylpiperazine

Table 2. The observed enthalpies of inhibitor **3a-d** and **4a-c** binding to human carbonic anhydrase I and bovine carbonic anhydrase II

Compound	Binding to hCAI ΔH[a] (kJ/mol)	Binding to bCAII ΔH[a] (kJ/mol)
3a	−26.5	−44.5
3b	−37.4	−49.0
3c	−25.0	−46.8
3d	−31.3	−39.0
4a	−23.9	−34.6
4b	−66.6	−72.8
4c	−17.5	−16.0
AZM	−44.4[b]	−48.1[b]

[a] Values are means of at least two experiments, determined by ITC.
[b] Data from Ref. 14.

moiety, bound with exceptionally large exothermic enthalpy change, while the **4c** had exceptionally small enthalpy change. Based on enthalpy and Gibbs free energy changes upon binding it may be possible to design more specific inhibitors of carbonic anhydrases with the therapeutic application.

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9. Preparation of 3-chlorobenzimidazo[1,2-*c*][1,2,3]thiadiazole-7-sulfonyl chloride (**2a**) as a representative of substituted benzimidazo[1,2-*c*][1,2,3]thiadiazole-7-sulfonyl chlorides **2b**, **2c**. Sulfonyl chloride group goes to the seventh position because electrophilic substitution at mild conditions was shown to occur at the seventh position. This was demonstrated by studying bromination and nitration reactions. The structure of 6-nitro substituted **1a** obtained by an alternative route was determined by X-ray crystallography. The 3-chlorobenzimidazo[1,2-*c*][1,2,3]thiadiazole (**1a**) (0.3 g, 1.43 mmol) was added slowly with stirring to chlorosulfonic acid (3 mL) at −5 °C. The reaction mixture was allowed to warm to room temperature and remained under stirring for 24 h. The excess of acid was then hydrolyzed with ice. The product was extracted with chloroform. The combined chloroform layers were washed with water, dried over anhydrous sodium sulfate, yielding chlorosulfonyl compound after evaporation. The crude material was used for next step without further purification. Yield: 0.38 g (86%), mp 184–185 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.1 (1H, d, J = 9 Hz, CH(5)), 8.23 (1H, dd, J = 2 and 9 Hz, CH(6)), 8.94 (1H, d, J = 2 Hz, CH(8)). 3-Methylthiobenzimidazo[1,2-*c*][1,2,3]thiadiazole-7-sulfonyl chloride (**2b**). Yield: 86%, mp 190 °C (dec). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 2.99 (3H, s, SCH_3), 8.05 (1H, dd, J = 0.6 and 9 Hz, CH(5)), 8.17 (1H, dd, J = 2 and 9 Hz, CH(6)), 8.90 (1H, dd, J = 0.6 and 2 Hz, CH(8)). Benzimidazo[1,2-*c*][1,2,3]thiadiazole-7-sulfonyl chloride (**2c**). Yield: 64%, mp 188–189 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 8.1 (1H, dd, J = 0.6 and 9 Hz, CH(5)), 8.24 (1H, dd, J = 2 and 9 Hz, CH(6)), 8.74 (1H, s, CH(3)), 9.00 (1H, dd, J = 0.6 and 2 Hz, CH(8)). 3-Methylsulfonylbenzimidazo[1,2-*c*][1,2,3]thiadiazole-7-sulfonyl chloride (**2d**). The 3-methylsulfonylbenzimidazo[1,2-*c*][1,2,3]thiadiazole (**1d**) (0.05 g, 0.2 mmol) was added slowly with stirring to chlorosulfonic acid (0.6 mL) at −5 °C. The reaction mixture was allowed to warm to room temperature and remained under stirring for 24 h. The excess of acid was then hydrolyzed with ice. The solid that precipitated was filtered. The crude material was used for next step without further purification. Yield: 0.05 g (72%), mp 210 °C (dec). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.73 (3H, s, SO_2CH_3), 8.1 (1H, d, J = 9 Hz, CH(5)), 8 (1H, dd, J = 2 and 9 Hz, CH(6)), 8.42 (1H, s, CH(8)). Preparation of 3-chlorobenzimidazo[1,2-*c*][1,2,3]thiadiazole-7-sulfonamide (**3a**) as representative of substituted benzimidazo[1,2-*c*][1,2,3]thiadiazole-7-sulfonamides **3b**, **3c**. To a solution of 3-chlorobenzimidazo[1,2-*c*][1,2,3] thiadiazole-7-sulfonyl chloride (**2c**) (0.04 g, 0.13 mmol) in tetrahydrofurane (2 mL) was added dropwise with stirring an aqueous ammonia solution (0.1 mL, 25%). After addition the reaction mixture was stirred at ambient temperature for 15 min. The solid that precipitated was filtered, washed ($NaHCO_3$/H_2O, H_2O). Recrystallization was accomplished from acetic acid. Yield: 0.03 g (80%), mp 242–243 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.49 (2H, s, NH_2), 8.01 (2H, s, CH(5), CH(6)), 8.62 (1H, s, CH(8)). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 112.00, 121.59, 125.77, 127.34, 127.37, 136.18, 154.13, 154.83. MS (+ESI) (*m*/*z*, %): $(M+H)^+$ 289, 100%, $(M+H)^+$ 291, 50%. Analysis ($C_8H_5ClN_4O_2S_2$): calcd C 33.28%, H 1.75%, N 19.40%; found: C 33.39%, H 1.8%, N 19.63%. 3-Methylthiobenzimidazo[1,2-*c*][1,2,3]thiadiazole-7-sulfonamide (**3b**). Recrystallization was accomplished from acetic acid. Yield: 53%, mp 260–261 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.99 (3H, s, SCH_3), 7.47 (2H, s, NH_2), 8.00 (2H, s, CH(5), CH(6)), 8.58 (1H, s, CH(8)). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 18.58, 112.00, 121.47, 125.04, 126.69, 135.95, 138.72, 154.00, 154.98. Analysis ($C_9H_8N_4O_2S_3$): calcd C 35.99%, H 2.68%, N 18.65%; found: C 36.16%, H 2.62%, N 18.80%. Benzimidazo[1,2-*c*][1,2,3]thiadiazole-7-sulfonamide (**3c**). Recrystallization was accomplished from acetic acid. Yield: 70%, mp 245–246 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.46 (2H, s, NH_2), 7.98 (2H, s, CH(5), CH(6)), 8.63 (1H, s, CH(8)), 9.3 (1H, s, CH(3)). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 112.08, 121.22, 122.69, 125.18, 125.94, 135.37, 155.3, 157.75. Analysis ($C_8H_6N_4O_2S_2$): calcd C 37.79%, H 2.38%, N 22.03%; found: C 37.86%, H 2.41%, N 22.18%. 3-Methylsulfonylbenzimidazo[1,2-*c*][1,2,3]thiadiazole-7-sulfonamide (**3d**). To a solution of 3-methylsulfonylbenzimidazo[1,2-*c*][1,2,3]thiadiazole-7-sulfonyl chloride (**2d**) (0.032 g, 0.09 mmol) in dioxane (20 mL) was added dropwise with stirring an aqueous ammonia solution (0.1 mL, 25%). After addition the reaction mixture was stirred at ambient temperature for 0.5 h. The reaction solvent was evaporated and residue was washed ($NaHCO_3$/H_2O, H_2O). Recrystallization was accomplished from acetic acid. Yield: 0.018 g (60%), mp 249–250 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 3.69 (3H, s, SO_2CH_3), 7.56 (2H, s, NH_2), 8.1 (2H, s, CH(5), CH(6)), 8.7 (1H, s, CH(8)).

^{13}C NMR (75 MHz, DMSO-d_6): 44.96, 112.13, 121.7, 126.44, 126.95, 132.96, 137.24, 152.79, 155.73. Analysis ($C_9H_8N_4O_4S_3$): calcd C 32.52%, H 2.43%, N 16.86%; found: C 32.61%, H 2.44%, N 16.75%.

10. Preparation of 3-morpholinbenzimidazo[1,2-*c*][1,2,3]thiadiazole-7-sulfonamide (**4a**) as a representative of substituted benzimidazo[1,2-*c*][1,2,3]thiadiazole-7-sulfonamides **4b**, **4c**. The 3-chloro position of **1a** is amenable to substitution as discussed previously.[12] The mixture of 3-chlorobenzimidazo[1,2-*c*][1,2,3]thiadiazole-7-sulfonamide (**3a**) (0.02 g, 0.069 mmol), morpholine (0.012 g, 0.14 mmol), and ethanol (20 mL) was refluxed for 1.5 h. The reaction mixture was cooled to room temperature and the precipitate was filtered, washed (cold H_2O). Recrystallization was accomplished from acetic acid. Yield: 0.02 g (85%), mp 253–254 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.9 (8H, s, $(CH_2)_4$), 7.41 (2H, s, NH_2), 7.88 (2H, s, CH(5), CH(6)), 8.44 (1H, s, CH(8)). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 50.38, 65.79, 111.6, 121.27, 124.00, 126.4, 135.15, 146.94, 151.7, 154.23. Analysis ($C_{12}H_{14}N_5O_3S_2$): calcd C 42.47%, H 3.86%, N 20.63%; found: C 42.56%, H 3.94%, N 20.47%. 3-(*N*-methylpiperazin)benzimidazo[1,2-*c*][1,2,3]thiadiazole-7-sulfonamide (**4b**). Recrystallization was accomplished from ethanol. Yield: 61%, mp 260–262 °C (dec). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.29 (3H, s, CH_3), 2.6 (4H, s, $(CH_2)_2$), 3.9 (4H, s, $(CH_2)_2$), 7.42 (2H, s, NH_2), 7.88 (2H, s, CH(5), CH(6)), 8.43 (1H, s, CH(8)). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 46.34, 50.4, 53.96, 111.62, 121.3, 123.95, 126.47, 135.11, 146.88, 151.67, 154.09. Analysis ($C_{13}H_{16}N_6O_2S_2$): calcd C 44.30%, H 4.58%, N 23.85%; found: C 44.18%, H 4.45%, N 23.72%. 3-Phenylthiobenzimidazo[1,2-*c*][1,2,3]thiadiazol-7-sulfonamide (**4c**). Recrystallization was accomplished from acetic acid. Yield: 85%, mp 225–226 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 7.3–7.6 (5H, m, SC_6H_5), 7.65 (2H, s, NH_2), 7.96 (2H, s, CH(5), CH(6)), 8.59 (1H, s, CH(8)). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 112.00, 121.54, 125.43, 127.13, 129.9, 130.9, 131.39, 131.97, 132.51, 136.15, 154.76, 155.7. Analysis ($C_{14}H_{10}N_4O_2S_3$): calcd C 46.39%, H 2.78%, N 15.46%; found: C 46.26%, H 2.87%, N 15.37%.

11. 3-Methylthiobenzimidazo[1,2-*c*][1,2,3]thiadiazole (**1b**). The mixture of benzimidazo[1,2-*c*][1,2,3]thiadiazolo-3-thione (**5**) (0.2 g, 0.96 mmol), methyl iodide (0.2 g, 1.4 mmol), and methanol (40 mL) was refluxed for 0.5 h. The reaction solvent was evaporated and residue was washed ($NaHCO_3/H_2O$, H_2O). Recrystallization was accomplished from mixture of H_2O/ethanol (2:1).Yield: 0.18 g (86%), mp 120–121 °C. ^{1}H NMR (300 MHz, DMSO-d_6/CCl_4) δ ppm 2.97 (3H, s, SCH_3), 7.26 (1H, t, J = 8 Hz, CH(7)), 7.51 (1H, t, J = 8 Hz, CH(6)), 7.77 (1H, d, J = 8 Hz, CH(5)), 8.09 (1H, d, J = 8 Hz, CH(8)). ^{13}C NMR (75 MHz, DMSO-d_6/CCl_4) δ ppm 18.49, 113.25, 120.33, 121.18, 127.66, 128.21, 134.79, 153.04, 153.63. Analysis ($C_9H_7N_3S_2$) calcd C 48.85%, H 3.19%, N 18.99%; found: C 48.93%, H 2.96%, N 18.89%. Benzimidazo[1,2-*c*][1,2,3]thiadiazole (**1c**). The mixture of 3-chlorobenzimidazo[1,2-*c*][1,2,3]thiadiazole (**1a**) (0.2 g, 0.95 mmol), NaI*2H_2O (0.9 g, 4.7 mmol), acetic acid (10 mL), and 2-butanone (50 mL) was refluxed for 5 h. The reaction solvent was evaporated and residue was poured into $Na_2S_2O_3/H_2O$ solution and was mixed up carefully. The precipitate was filtered and recrystallized from mixture of H_2O/ethanol (10:1). Yield: 0.06 g (38%), mp 158–160 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 7.34 (1H, t, J = 8 Hz, CH(7)), 7.6 (1H, t, J = 8 Hz, CH(6)), 7.94 (1H, d, J = 8 Hz, CH(5)), 8.2 (1H, d, J = 8 Hz, CH(8)), 8.47 (1H, s, CH(3)). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 113.29, 116.76, 120.47, 121.15, 127.71, 128.28, 154.98, 155.38. MS (EI) (*m*/*z*,%): M^+ 175, 93%. Analysis ($C_8H_5N_3S$): calcd C 54.84%, H 2.88%, N 23.98%; found: C 54.65%, H 2.37%, N 23.60%. 3-Methylsulfonylbenzimidazo[1,2-*c*][1,2,3]thiadiazole (**1d**). H_2O_2 (0.65 g, 35%) was poured to a solution of 3-methylthiobenzimidazo[1,2-*c*][1,2,3]thiadiazole (**1b**) (0.3 g, 1.36 mmol) in acetic acid (7 mL). The reaction mixture was kept at room temperature for five days. The reaction solvent was evaporated and residue was poured into $NaHCO_3/H_2O$ solution and was mixed up carefully. The precipitate contained a mixture of compounds **1c** and **1d**. The compounds were separated by flash chromatography (ethylacetate). Compound **1c**. Yield: 0.03 g (13%), mp 158–160 °C. R_f = 0.13 (ethylacetate). Compound **1d**. Yield: 0.19 g (56%), mp 186-187°C. R_f = 0,73 (ethylacetate). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 3.64 (3H, s, SO_2CH_3), 7.47 (1H, t, J = 8 Hz, CH(7)), 7.71 (1H, t, J = 8 Hz, CH(6)), 8.03 (1H, d, J = 8 Hz, CH(5)), 8.24 (1H, d, J = 8 Hz, CH(8)). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 44.82, 113.39, 121.68, 122.2, 128.35, 129.86, 141.86, 149.59, 155.6. Analysis ($C_9H_7N_3O_2S_2$), calcd C 42.67%, H 2.79%, N 16.59%; found: C 42.86%, H 2.75%, N 16.48%. Benzimidazo[1,2-*c*][1,2,3]thiadiazolo-3-thione (**5**). The mixture of 3-chlorobenzimidazo[1,2-*c*][1,2,3]thiadiazole (**1a**) (0.5 g 2.38 mmol), thiourea (0.4 g, 5.26 mmol), and methanol (20 mL) was refluxed for 0.5 h. The reaction mixture was cooled to room temperature and the precipitate was filtered, washed (cold methanol). The precipitate was dissolved in sodium hydroxide solution (0.2 M). This solution was filtered, acidified with acetic acid (pH $\sim$ 5). Recrystallization was accomplished from dioxane. Yield: 0.34 g (69%), mp 236–237 °C.

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