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Synthesis of novel benzothiozole and benzisoxazole functionalized unsymmetrical alkanes and study of their antimicrobial activity

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Novel unsymmetrical alkanes **4** and **5** have been independently synthesized in single pot from 2-amino 5 / 6-hydroxybenzothiazole, 6-hydroxy-3-methyl-1,2-benzisoxazole and different dihaloalkanes [X-$(CH_2)_n$-X]. The compounds **4** and **5** have been screened for antimicrobial activity and some of them have been found to show promising activity.

Keywords: Unsymmetrical, functionalized alkanes, beznothiazole, benzisoxazole, antibacterial, antifungal

Thiazole[1-4] and isoxazole[5-7] moieties are important constituents in the core structure of many of the biologically active compounds. More specifically, molecules with substituted 1,2-benzisoxazoles often exhibit anti-inflammatory[8], tuberculostatic[9], sedative, analgesic, neuroleptic[10], antibacterial activity[11] and molecules containing 2-amino benzothiazole unit show varied biological activity as anti-inflammatory[12], analgesic[13], antitubercular[14], anti-tumor[15,16], antibacterial[17,18] and muscle relaxant activities[19]. We envisaged that synthesis of new molecules constituted with both of these heterocyles could give entry to novel bioactive compounds. In a preliminary study in this direction, such novel compounds have been synthesized hooking both the heterocyles at the ends of long chain alkane through ether link as shown in **Scheme I**.

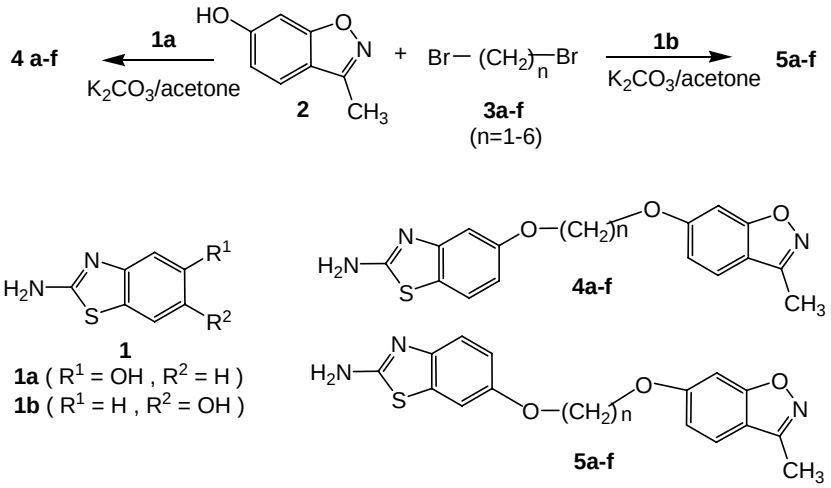
Results and Discussion

Synthesis of 2-amino-5-hydroxybenzothiazole **1a,b** and 6-hydroxy-3-methyl-1,2-benzisoxazole **2** were carried out by literature methods[20,21]. 2-amino-5-hydroxybenzothiazole, **1** and 6-hydroxy-3-methyl-1,2-benzisoxazole, **2** were reacted with dihaloalkane **3** using potassium carbonate as base by refluxing acetone to obtain unsymmetrically end-substituted alkanes **4a-f** and **5a-f** respectively in moderate yields as shown in **Scheme I**. In this reaction, symmetrical substituted products also formed along with **4** and **5** but the present discussion is limited only to unsymmetrical ones.

In this reaction sequence it has been observed selective O-alkylation of 2-amino-5-hydroxybenzothiazole and the products **4** and **5** were isolated by normal column chromatography using ethyl acetate-*n*-hexane (3:7) as eluent. Isolated products were characterized by IR, ^{1}H NMR and mass spectral data.

Antibacterial activity

The newly synthesized compounds were screened for their antibacterial activity against *Escherichia coli* (ATTC-25922), *Staphylococcus aureus* (ATTC-25923), *Pseudomonas aeruginosa* (ATTC-27853), and *Bacillius subtilis* (recultured) bacterial stains by the disk diffusion method[22,23]. Disks measuring 6.25 mm in diameter were punched from Whatman no.1 filter paper. Batches of 100 disks were dispensed to each screw-capped bottles and sterilized by dry heat at 140°C for an hour. The test compounds were prepared with different concentrations using dimethylformamide. Exactly 1 mL containing 100 times the amount of chemical in each disk was added to each bottle, which contained 100 discs. Disks of each concentration were placed in nutrient agar medium inoculated with fresh bacteria strains separately. The incubation was carried out at 37°C for 24 hr. Ciprofloxacin was used as a standard drug at a concentration of 10 µg/mL. Solvent and growth



Scheme I

Table I — Antibacterial activity data of synthesized compounds

Compd	*Ec*	*Sa*	*Pa*	*Bs*
4a	12	14	18	14
4b	13	13	14	16
4c	15	12	17	13
4d	18	19	22	21
4e	15	15	17	15
4f	18	20	22	21
5a	15	14	15	19
5b	12	15	15	20
5c	16	18	18	19
5d	15	14	13	14
5e	17	14	13	14
5f	12	15	12	16
Standard	19	20	23	22

(*Ec*): *Escherichia coli* (ATTC-25922); (*Sa*): *Staphylococcus aureus* (ATTC-25923; (*Pa*): *Pseudomonas aeruginosa* (ATTC-27853); (*Bs*): *Bacillius subtilis* (recultured)
Zone of inhibition in millimeters.

Table II — Antifungal activity data of synthesized compounds

Compd	*Af*	*Ca*	*Af*	*Pm*
4a	14	13	14	14
4b	16	17	15	14
4c	15	15	13	13
4d	16	18	13	16
4e	15	12	14	16
4f	17	18	15	18
5a	13	15	12	14
5b	14	14	14	13
5c	19	19	18	20
5d	16	18	14	12
5e	18	19	17	20
5f	16	13	17	12
Standard	21	20	19	21

(*Af*): *Aspergillus flavus* (NICM No. 524); (*Ca*):*Candida albicans* (NCIM No. 3100); (*Af*): *Aspergillus fumigatus* (NCIM No. 902); (*Pm*): *Penicillium marneffei* (recultured).
Zone of inhibition in millimetres

controls were kept and zones of inhibition were noted. The results of such studies are given in **Table I**.

Antifungal activity

Newly prepared compounds were screened for their antifungal activity against *Aspergillus flavus* (NICM No. 524), *Candida albicans* (NCIM No. 3100), *Aspergillus fumigatus* (NCIM No. 902) *Penicillium marneffei* (recultured) in DMSO by the serial plate dilution method[24,25]. Sabouraud's agar media were prepared by dissolving peptone (1 g), D-glucose (4 g), and agar (2 g) in distilled water (100 mL) and adjusting the *p*H to 5.7. Normal saline was used to make a suspension of the spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of the corresponding species. Agar media (20 mL) was poured into each petri dish. Excess suspension was decanted and the plates were dried by placing in an incubator at 37°C for 1 hr. Wells were made on these seeded agar plates using an agar punch and labelled. A 10 µL solution of the test compounds in DMS was then added into each of these labeled walls. A control was also prepared in the same way using DMSO. The petri dishes was then incubated at 37°C for 3-4 days. The antifungal activity was determined by measuring the diameter of the inhibition zone. Activity of each compound was compared with that of flucanazole as standard (**Table II**).

Experimental Section

Melting points were recorded in open glass capillaries and are uncorrected. Solvents were purified and dried by standard procedures before use. Progress of reaction was monitored by thin layer chromatography (TLC) using GF_{254} silica gel. The spots were detected in an iodine chamber and under UV light. Column chromatography was performed over silica gel (BDH 100-200 mesh) using ethyl acetate: *n*-hexane (3:7) mixture as eluent. IR spectra were recorded on FT-IR Shimadzu Perkin-Elmer 1310 infrared spectrophotometer. ^{1}H NMR spectra were recorded on Varian Gemini 200 MHz spectrometer using tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained using a FAB-MS spectrometer.

Typical Procedure

1-(2-Aminobenzothiazol-5-oxy)-1-(3-methyl-1, 2-benzisoxazol-6-oxy)-methane, 4a

A mixture of 2-amino-5-hydroxybenzothiazole **1a** (1.66 g, 10 mmole), 6-hydroxy-3-methyl-1,2-benzisoxazole (1.49 g, 10 mmole), diiodomethane **3a** (1.73 g, 10 mmole) and anhydrous potassium carbonate (5.5 g, 40 mmole) in dry acetone (20 mL) was refluxed on water-bath for 6 hr. Reaction mixture was cooled to RT and concentrated on rotavapor. The residue was treated with water, extracted with dichloromethane and dried over anhydrous sodium sulphate. The dichloromethane extract was concentrated to give the crude product which was purified by column chromatography using 100-200 mesh silica gel and desired product was eluted with 30% ethyl acetate/ hexane mixture to afford the compound **4a** (1.0g, 30.58%), m.p. 142°C; IR: 3422, 2890, 1635, 1267, 823, 759, 696 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.20 (s, 3H, CH_3), 4.40 (s, 2H, CH_2), 4.81 (s, 2H, NH_2) [this signal disappeared upon D_2O exchange], 6.86 (d,1H, ArH), 7.30-7.42 (m, 3H, ArH), 7.88-7.94 (m, 2H, ArH); MS (FAB): *m/z* 328 (M+1). Anal. Calcd for $C_{16}H_{13}N_3O_3S$: C, 58.71; H, 3.97; N,12.84. Found: C, 58.62; H, 3.93; N, 12.78%.

1-(2-Aminobenzothiazol-5-oxy)-2-(3-methyl-1,2-benzisoxazol-6-oxy)-ethane, 4b

The compound **4b** was prepared following the method described for compound **4a**, employing dibromoethane **3b** (1.87 g, 10 mmole) and crude product was purified by column chromatography to afford the compound **4b** (1.31 g, 38.15%), m.p. 130°C; IR: 3392, 2875, 1623, 1274, 812, 786, 682 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.20 (s, 3H, CH_3), 4.27 (t, 4H, CH_2), 4.83 (s, 2H, NH_2), 6.91 (d,1H, ArH), 7.18-7.29 (m, 3H, ArH), 7.79-7.86 (m, 2H, ArH); MS (FAB): *m/z* 342 (M+1). Anal. Calcd for $C_{17}H_{15}N_3O_3S$: C, 59.82; H, 4.39; N, 12.31. Found: C, 59.73; H, 4.28; N, 12.27%.

1-(2-Aminobenzothiazol-5-oxy)-3-(3-methyl-1,2-benzisoxazol-6-oxy)-propane, 4c

The compound **4c** was prepared following the method described for compound **4a**, employing dibromopropane **3c** (2.01 g, 10 mmole) and crude product was purified by column chromatography to afford the compound **4c** (1.34 g, 37.75%), m.p. 160°C; IR: 3398, 2887, 1629, 1280, 809, 794, 689 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.82-1.89 (m, 2H, CH_2), 2.30 (s, 3H, CH_3), 3.80 (t, 4H, CH_2), 4.92 (s, 2H, NH_2), 6.92 (d,1H, ArH), 7.27-7.39 (m, 3H, ArH), 7.88-7.96 (m, 2H, ArH); MS (FAB): *m/z* 356 (M+1). Anal. Calcd for $C_{18}H_{17}N_3O_3S$: C, 60.84; H, 4.78; N, 11.84. Found: C, 60.80; H, 4.71; N, 11.79%.

1-(2-Aminobenzothiazol-5-oxy)-4-(3-methyl-1,2-benzisoxazol-6-oxy)-butane, 4d

The compound **4d** was prepared following the method described for compound **4a**, employing dibromobutane **3d** (2.15 g, 10 mmole) and crude product was purified by column chromatography to afford the compound **4d** (1.41 g, 37.92%), m.p. 109°C; IR: 3417, 2882, 1611, 1260, 817, 762, 661 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.51-1.62 (m, 4H, CH_2), 2.20(s, 3H, CH_3), 3.90-3.99 (m, 4H, CH_2), 4.91 (s, 2H, NH_2), 6.94 (d, 1H, ArH) 7.28-7.41 (m, 3H, ArH), 7.89-8.12 (m, 2H, ArH); MS (FAB): *m/z* 370 (M+1). Anal. Calcd for $C_{19}H_{19}N_3O_3S$: C, 61.78; H, 5.14; N, 11.38. Found: C, 61.73; H 5.10; N, 11.29%.

1-(2-Aminobenzothiazol-5-oxy)-5-(3-methyl-1,2-benzisoxazol-6-oxy)-pentane, 4e

The compound **4e** was prepared following the method described for compound **4a**, employing dibromopentane **3e** (2.29 g, 10 mmole) and crude product was purified by column chromatography to afforded the compound **4e** (1.52 g, 39.15%), m.p. 114°C; IR: 3390, 2890, 1629, 1284, 816, 795, 696 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.38-1.49 (m, 4H, CH_2), 1.51-1.67(m, 2H, CH_2), 2.38 (s, 3H, CH_3), 4.12 (t, 4H, CH_2), 4.75 (s, 2H, NH_2), 6.87 (d, 1H, ArH),

7.12-7.34 (m, 3H, ArH), 7.81-7.94 (m, 2H, ArH); MS (FAB): *m/z* 384 (M+1). Anal. Calcd for $C_{20}H_{21}N_3O_3S$: C, 62.66; H, 5.48; N, 10.96. Found: C, 62.59; H, 5.39; N, 10.89%.

1-(2-Aminobenzothiazol-5-oxy)-6-(3-methyl-1,2-benzisoxazol-6-oxy)-hexane, 4f

The compound **4f** was prepared following the method described for compound **4a**, employing dibromohexane **3f** (2.43 g, 10 mmole) and crude product was purified by column chromatography to afford the compound **4f** (1.51 g, 37.30%), m.p. 123°C; IR: 3413, 2893, 1639, 1261, 809, 794, 680 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.39-1.49 (m, 4H, CH_2), 1.61-1.69 (m, 4H, CH_2), 2.40 (s, 3H, CH_3), 4.12 (t, 4H, CH_2), 4.81 (s, 2H, NH_2), 6.98 (d, 1H, ArH), 7.29-7.42 (m, 3H, ArH), 7.89-8.1(m, 2H, ArH); MS (FAB): *m/z* 398 (M+1). Anal. Calcd for $C_{21}H_{23}N_3O_3S$: C, 63.47; H, 5.79; N, 10.57. Found: C, 63.41; H, 5.69; N, 10.50%.

1-(2-Aminobenzothiazol-6-oxy)-1-(3-methyl-1,2-benzisoxazol-6-oxy)-methane, 5a

A mixture of 2-amino-6-hydroxybenzothiazole (1.66 g, 10 mmole), 6-hydroxy-3-methyl-1,2-benziso-xazole (1.49 g, 10 mmole), dibromomethane **3a** (1.73 g, 10 mmole) and anhydrous potassium carbonate (5.5 g, 40 mmole) in dry acetone (20 mL) was refluxed on water-bath for 8 hr. Reaction mixture was cooled to RT and concentered on rotavapor. The residue is treated with water, extracted with dichloromethane and dried over sodium sulphate. The dichloromethane extract was concentrated to give the crude product which was purified by column chromatography using 100-200 mesh silica gel and desired product was eluted with 30% ethyl acetate/ hexane mixture to afford the compound **5a** (1.10 g, 33.63%), m.p. 130°C; IR: 3398, 2880, 1635, 1280, 807, 793, 680 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.32 (s, 3H, CH_3), 4.50 (s, 2H, CH_2), 4.93 (s, 2H, NH_2), 6.94 (d, 1H, ArH), 7.22-7.35 (m 3H, ArH), 7.86-7.98 (m, 2H, ArH); MS (FAB): *m/z* 328 (M+1). Anal. Calcd for $C_{16}H_{13}N_3O_3S$: C, 58.71; H, 3.97; N, 12.84. Found: C, 58.64; H, 3.92; N, 12.80%.

1-(2-Aminobenzothiazol-6-oxy)-2-(3-methyl-1,2-benzisoxazol-6-oxy)-ethane, 5b

The compound **5b** was prepared following the method described for compound **5a**, employing dibromoethane **3b** (1.87 g, 10 mmole) and crude product was purified by column chromatography to afford the compound **5b** (1.21 g, 38.15%), m.p.112°C; IR: 3404, 2890, 1611, 1261, 821, 780, 667 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.24 (s, 3H, CH_3), 4.32 (t, 2H, CH_2), 4.76 (s, 2H, NH_2), 6.93 (d, 1H, ArH) 7.23-7.34 (m, 3H, ArH), 7.92-8.10 (m, 2H, ArH); MS (FAB): *m/z* 342 (M+1). Anal. Calcd for $C_{17}H_{15}N_3O_3S$: C, 59.82; H, 4.39; N, 12.31. Found: C, 59.75; H, 4.31; N, 12.29%.

1-(2-Aminobenzothiazol-6-oxy)-3-(3-methyl-1,2-benzisoxazol-6-oxy)-propane, 5c

The compound **5c** was prepared following the method described for compound **5a**, employing dibromopropane **3c** (2.01 g, 10 mmole) and crude product was purified by column chromatography to afford the compound **5b** (1.20 g, 33.8%), m.p.137°C; IR: 3413, 2889, 1639, 1271, 812, 786, 696 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.76-1.82 (m, 2H, CH_2), 2.20 (s, 3H, CH_3), 3.76 (t, 4H, CH_2), 4.8 (s, 2H, NH_2), 6.95 (d, 1H, ArH), 7.29-7.38 (m, 3H, ArH), 7.98-8.10 (m, 2H, ArH); MS (FAB): *m/z* 356 (M+1). Anal. Calcd for $C_{18}H_{17}N_3O_3S$: C, 60.84; H, 4.78; N, 11.84. Found: C, 60.79; H, 4.73; N, 11.80%.

1-(2-Aminobenzothiazol-6-oxy)-4-(3-methyl-1,2-benzisoxazol-6-oxy)-butane, 5d

The compound **5d** was prepared following the method described for compound **5a**, employing dibromobutane **3d** (2.15 g, 10 mmole) and crude product was purified by column chromatography to afford the compound **5d** (1.31 g, 35.23%), m.p. 109°C; IR: 3391, 2855, 1628, 1266, 825, 781, 663 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.71-1.82 (m, 4H, CH_2), 2.30 (s, 3H, CH_3), 4.01-4.12 (m, 4H, CH_2), 4.94 (s, 2H, NH_2), 6.90 (d, 1H, ArH) 7.42-7.55 (m, 3H, ArH), 7.98-8.12 (m, 2H, ArH); MS (FAB): *m/z* 370 (M+1). Anal. Calcd for $C_{19}H_{19}N_3O_3$ S: C, 61.78; H, 5.14; N, 11.38. Found: C, 61.70; H, 5.1; N, 11.30%.

1-(2-Aminobenzothiazol-6-oxy)-5-(3-methyl-1,2-benzisoxazol-6-oxy)-pentane, 5e

The compound **5e** was prepared following the method described for compound **5a**, employing dibromopentane **3e** (2.29 g, 10 mmole) and crude product was purified by column chromatography to afford the compound **5e** (1.50 g, 39.16%), m.p. 151°C; IR: 3391, 2875, 1629, 1286, 808, 782,

678 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.39-1.48 (m, 4H, CH_2), 1.57-1.62 (m, 2H, CH_2), 2.45 (s, 3H, CH_3), 4.27 (t, 2H, CH_2), 4.89 (s, 2H, NH_2), 6.96 (d,1H, ArH) 7.27-7.36 (m, 3H, ArH), 7.89-7.97 (m, 2H, ArH); MS (FAB): *m/z* 348 (M+1). Anal. Calcd for $C_{20}H_{21}N_3O_3S$: C, 62.66; H, 5.48; N, 10.96. Found: C, 62.60; H, 5.42; N, 10.91%.

1-(2-Aminobenzothiazol-5-oxy)-6-(3-methyl-1,2-benzisoxazol-6-oxy) hexane, 5f

The compound **5f** was prepared following the method described for compound **5a**, employing dibromohexane **3f** (2.43 g, 10 mmole) and crude product was purified by column chromatography to afford the compound **5f**. (1.41 g, 35.26%), m.p.140°C; IR: 3411, 2891, 1630, 1278, 823, 794, 652 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.31-1.39, (m, 4H, CH_2), 1.52-1.61 (m, 4H, CH_2), 2.36 (s, 3H, CH_3), 4.07 (t, 4H, CH_2), 4.92 (s, 2H, NH_2), 6.82 (d, 1H, ArH), 7.24-7.38 (m, 3H, ArH), 8.04-8.18 (m, 2H, ArH); MS (FAB): *m/z* 398 (M+1). Anal. Calcd for $C_{21}H_{23}N_3O_3S$: C, 63.47; H, 5.79; N, 10.57. Found: C, 63.39; H, 5.70; N,10.52%.

Conclusion

This article reports a convenient one pot synthesis of some novel unsymmetrical alkanes in good yield. The antibacterial and antifungal screening of **4a-f** and **5a-f** revealed that some of them *i.e.*, **4d**, **4f** and **5c, 5e** showed good inhibition at 10 μg/mL concentration and the remaining compounds showed moderate activity.

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