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SYNTHESIS AND FLUORESCENCE PROPERTIES OF 4,5-, 4,6- AND 5,6-DISUBSTITUTED BENZOFURAZAN (2,1,3-BENZOXADIAZOLE) COMPOUNDS

Natsuko Okiyama, Seiichi Uchiyama, Maki Onoda, Kazuhiro Imai, and Tomofumi Santa*

Laboratory of Bio-Analytical Chemistry, Graduate School of Pharmaceutical Sciences,
The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
E-mail: santa@mol.f.u-tokyo.ac.jp

Abstract – New 4,5-, 4,6- and 5,6-disubstituted benzofurazan (2,1,3-benzoxadiazole) compounds having various substituent groups were synthesized. Their fluorescence properties were obtained and the discussions were made to develop new sensitive and selective fluorescent reagents.

INTRODUCTION

Until now, over forty 4,7-disubstituted benzofurazan (2,1,3-benzoxadiazole) compounds (the benzofurazan skeleton was shown in Figure 1) have been reported as fluorogenic and fluorescent labeling

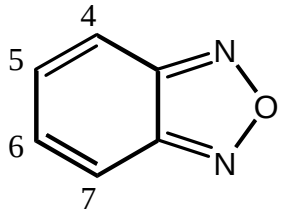

Figure 1: Benzofurazan (2,1,3-benzoxadiazole) skeleton

reagents.[1] For example, 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F),[2] ammonium 7-fluoro-2,1,3-benzoxadiazole-4-sulfonate (SBD-F),[3] 4-mercapto-7-methylthio-2,1,3-benzoxadiazole (MTBD-SH),[4] 7-phenylsulfonyl-4-(2,1,3-benzoxadiazolyl) isocyanate (PSBD-NCO),[5] and 7-methylthio-4-(2,1,3-benzoxadiazolyl) isothiocyanate (MTBD-NCS)[6] are now used as highly-sensitive fluorogenic reagents for amines, thiols, carboxylic acids, alcohols, and peptides, respectively. Thus, sensitive and selective determinations of biologically important compounds have been achieved with these benzofurazan reagents.[1]

While the synthesis and fluorescence properties of various 4,7-disubstituted benzofurazan compounds including the reagents mentioned above have been reported,[7] there are few reports on the synthesis and the fluorescence properties of 4,5-, 4,6- and 5,6-disubstituted benzofurazan compounds.[8] In this paper, we

report a synthesis of new 4,5-, 4,6- and 5,6-disubstituted benzofurazan compounds having -NHAc, -NH_2, -Cl, -NAc_2, -NMe_2, -F, -OMe, -SMe and -NO_2 groups and their fluorescence properties to develop new useful fluorescent reagents.

RESULTS AND DISCUSSION

Synthesis

The new benzofurazan compounds synthesized in this study are summarized in Table 1. In this paper, the disubstituted benzofurazan compounds are denoted using substituent groups and position numbers, *e.g.* 7-fluoro-4-nitro-2,1,3-benzoxadiazole (NBD-F) was denoted as **4-NO_2/7-F**.

Table 1: Synthesis of 4,5-, 4,6-, and 5,6- benzofurazan compounds

Product	From	Yield (%)	Product	From	Yield (%)
Cyclization to benzofurazan structure			*Reduction of nitro group*		
4-Cl/6-Cl	2,4-dichloro-6-nitroaniline	37	**4-NH_2/5-NMe_2**	**4-NO_2/5-NMe_2**	24
4-Cl/6-NO_2	6-chloro-2,4-dinitroaniline	68	**4-NH_2/5-Cl**	**4-NO_2/5-Cl**	67
5-F/6-NO_2	2,4-dinitro-5-fluoroaniline	41	**4-NH_2/5-OMe**	**4-NO_2/5-OMe**	45
			4-NH_2/5-SMe	**4-NO_2/5-SMe**	13
Replacement of halogen group with nucleophile			**4-SMe/5-NH_2**	**4-SMe/5-NO_2**	85
4-Cl/6-NMe_2	**4-Cl/6-Cl**	9.6	**5-NH_2/6-F**	**5-NO_2/6-F**	4.7
4-NO_2/5-NMe_2	**4-NO_2/5-Cl**	76			
4-NO_2/5-SMe	**4-NO_2/5-Cl**	35	*Acetylation of amino group*		
4-SMe/5-NO_2	**4-Cl/5-NO_2**	93	**4-NHAc/5-NMe_2**	**4-NH_2/5-NMe_2**	83
5-SMe/6-NH_2	**5-NH_2/6-F**	57	**4-NAc_2/5-Cl**	**4-NH_2/5-Cl**	45
4-NO_2/5-OMe	**4-NO_2/5-Cl**	63	**4-NAc_2/5-SMe**	**4-NH_2/5-SMe**	12

Construction of a benzofurazan ring. In general, the benzofurazan ring is synthesized from *o*-nitroaniline by four steps: (1) diazotization, (2) reaction of diazonium salt with sodium azide, (3) cyclization for the construction of a benzofuroxan (2,1,3-benzoxadiazole *N*-oxide) ring, and (4) reduction to a benzofurazan ring with triphenylphosphine (TPP).[9] **4-Cl/6-Cl, 4-Cl/6-NO_2,** and **5-F/6-NO_2** were obtained by these procedures. On the other hand, benzofurazan compounds having a nitro group at the 4-position could not be synthesized in a similar way. The reaction of benzofuroxan compounds having a nitro group at the 4-position with TPP did not produce the corresponding benzofurazan compounds because of the strong electron attractive nitro group.

Nucleophilic substitution. A halogeno group of benzofurazan compounds can be replaced by various nucleophilic groups such as –NR_1R_2, -SR, and -OR.[1] The fluoro group is more reactive to nucleophiles than the chloro group and the 5-(or 6-)position of the benzofurazan skeleton is also more reactive than the 4-(7-) position.[10] Therefore, **4-Cl/6-NMe_2** and **4-NO_2/5-NMe_2** were synthesized from the corresponding halogenobenzofurazans using dimethylamine solution. **4-NO_2/5-SMe**, **4-SMe/5-NO_2**, and **5-SMe/6-NH_2**

were obtained with methyl mercaptan sodium salt, while **4-NO_2/5-OMe** was with methanol. The results indicated that the 4,5-, 4,6-, and 5,6-disubstituted benzofurazan compounds with the halogeno group could be used as derivatization reagents for amines, thiols, and alcohols as same as 4,7-disubstituted benzofurazan reagents such as NBD-F and SBD-F.

Reduction of a nitro group to an amino group. An amino group can be inserted into the benzofurazan compounds with changing a nitro group using iron and hydrochloric acid.[11] Thus, **4-NH_2/5-NMe_2**, **4-NH_2/5-Cl**, **4-NH_2/5-OMe**, **4-NH_2/5-SMe**, **4-SMe/5-NH_2**, and **5-NH_2/6-F** were obtained. The amino group can be easily converted to reactive isocyano and isothiocyano groups for the useful derivatization reagents.

Acetylation of an amino group. 4-NHAc/5-NMe_2, **4-NAc_2/5-Cl**, and **4-NAc_2/5-SMe** were synthesized from the corresponding amines with an excess of acetic anhydride. **4-NHAc/5-Cl** and **4-NHAc/5-SMe** were not obtained from **4-NH_2/5-Cl** under this condition, while the reason was unknown.

Table 2: Absorption and fluorescence properties of the disubstituted benzofurazan compounds

	in cyclohexane				in acetonitrile			
Compounds	$\lambda_{ab.}$ /nm	ε /10^3 $M^{-1}cm^{-1}$	$\lambda_{em.}$ /nm*	Φ*	$\lambda_{ab.}$ /nm	ε /10^3 $M^{-1}cm^{-1}$	$\lambda_{em.}$ /nm*	Φ*
4-SMe/5-NH_2	378	2.14	490	0.31	394	3.26	528	0.048
4-Cl/6-NMe_2	401	4.28	503	0.26	438	4.88	561	0.036
5-SMe/6-NH_2	323	3.24	465	0.16	332	7.76	509	0.077
5-NH_2/6-F	339	2.26	413	0.11	354	4.08	454	0.077
4-NH_2/5-SMe	395	2.12	516	0.092	403	2.42	582	0.0026
4-NHAc/5-NMe_2	394	2.58	515	0.071	398	4.98	549	0.0014
4-NH_2/5-Cl	379	5.52	488	0.048	392	2.92	537	0.017
4-NAc_2/5-SMe	353	2.56	426	0.044	362	4.60	468	0.028
4-NH_2/5-OMe	401	3.22	527	0.043	448	2.96	-	0
4-NH_2/5-NMe_2	398	3.40	542	0.014	451	3.06	-	0
5-F/6-NO_2	230	9.96	340	0.0040	244	8.26	-	0
4-NO_2/5-NMe_2	409	5.90	-	0	420	9.18	-	0
4-NH_2/5-NO_2	399	4.96	-	0	408	5.02	-	0
4-NO_2/5-SMe	391	3.42	-	0	400	6.38	-	0
4-NO_2/5-OMe	334	4.44	-	0	347	5.30	-	0
4-Cl/6-NO_2	325	3.78	-	0	250	14.5	-	0
4-Cl/5-NO_2	321	4.34	-	0	321	2.92	-	0
4-Cl/6-Cl	316	4.50	-	0	321	3.42	-	0
4-NAc_2/5-Cl	294	3.04	-	0	307	4.12	-	0
4-NO_2/5-Cl	285	8.56	-	0	291	7.38	-	0

* excited at maximum absorption wavelength ($\lambda_{ab.}$)

Fluorescence properties. The absorption and fluorescence properties of the twenty 4,5-, 4,6-, 5,6-disubstituted benzofurazan compounds including **4-Cl/5-NO_2**,[12] **4-NH_2/5-NO_2**,[12] and **4-NO_2/5-Cl**[13]

synthesized previously were summarized in Table 2. The absorption and especially fluorescence properties of these compounds were quite different from each other, and the difference was derived from the different substituent groups and their positions. The results shown in Table 2 suggested that the nucleophilic group such as -NH_2, -SMe, and -OMe was necessary for the benzofurazan compound to be fluorescent. Compared the absorption and fluorescence properties of fluorescent 4,5-, 4,6-, and 5,6-disubstituted benzofurazan compounds in Table 2 with those of 4,7-disubstituted benzofurazan compounds,[14] Φ values of the 4,5-, 4,6-, and 5,6-disubstituted benzofurazan compounds are greatly influenced by the solvent polarity more than those of 4,7-disubstituted benzofurazan compounds. These results indicated that the 4,5-, 4,6-, and 5,6-disubstituted benzofurazan structures such as **4-SMe/5-NH_2** and **4-Cl/6-NMe_2** would be polarity sensitive fluorophores. Moreover, the benzofurazan skeletons studied in this paper are also suitable to fluorogenic reagents. For instance, non-fluorescent **4-Cl/6-Cl** is converted to fluorescent **4-Cl/6-NMe_2** with dimethylamine, meaning that **4-Cl/6-Cl** is the fluorogenic reagent for amines.

CONCLUSION

Twenty 4,5-, 4,6-, and 5,6-disubstituted benzofurazan compounds were synthesized and their fluorescence properties were obtained. The fluorescence properties of these compounds were quite different from each other by the substituent groups and positions, suggesting us the new coming fluorogenic 4,5-, 4,6-, or 5,6-disubstitued benzofurazan reagents. If the relationship between the chemical structures and the fluorescence properties of these compounds is elucidated, new highly-sensitive fluorogenic reagents would be developed as previously reported of 4,7-disubstituted benzofurazan compounds.[7]

EXPERIMENTAL

Materials. Triphenyl phosphine (TPP), 2,4-dichloro-6-nitroaniline, and 2,4-dinitro-5-fluoroaniline were purchased from Aldrich (Milwaukee, WI, USA). Hydrochloric acid and cyclohexane were obtained from Wako Pure Chemicals (Osaka, Japan). Methyl mercaptan sodium salt solution (15 % in water) and 6-chloro-2,4-dinitroaniline were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Iron powder, dimethylamine solution (50 % in water), acetic anhydride, acetic acid, dichloromethane, diethylene glycol, *n*-hexane, ethyl acetate, chlorobenzene, toluene, acetonitrile, methanol, triethylamine, *o*-dichlorobenzene, sulfuric acid, sodium nitrite, anhydrous sodium sulfate, and sodium hydrogen carbonate were obtained from Kanto Chemicals (Tokyo, Japan). Sodium azide was purchased from Nacalai tesque (Kyoto, Japan). Silica gel 60 was supplied by Merck (Darmstadt, Germany). Water was purified using a Milli-Q system (Millipore, Bedford, MA, USA). All reagents were of analytical, HPLC or guaranteed grade and used

without further purification.

Apparatus. Melting points were measured on a Yanagimoto Micro Point Apparatus (Tokyo, Japan) and are uncorrected. ^{1}H-NMR spectra were obtained using a JEOL LA-500 spectrometer (Tokyo, Japan) with tetramethylsilane as the internal standard in $CDCl_3$. J values are given in Hz. MS spectra were measured using a Hitachi M-1200H mass spectrometer (atmospheric pressure chemical ionization (APCI) system) (Tokyo, Japan). UV-VIS absorption spectra (5 μM) were measured using a JASCO (Japan Spectroscopic Co., Ltd.) Ubest-50 spectrophotometer (Tokyo, Japan). Fluorescence spectra (5 μM) were measured using a Hitachi F-4010 fluorescence spectrophotometer (Tokyo, Japan). The fluorescence quantum yields (Φ) were determined using quinine sulfate in 0.1 M sulfuric acid (Φ = 0.55; $\lambda_{ex.}$, 355 nm) as the standard.

Synthesis. 4-Chloro-5-nitro-2,1,3-benzoxadiazole (**4-Cl/5-NO$_2$**),[12] 4-amino-5-nitro-2,1,3-benzoxadiazole (**4-NH$_2$/5-NO$_2$**),[12] and 5-chloro-4-nitro-2,1,3-benzoxadiazole (**4-NO$_2$/5-Cl**)[13] was synthesized and purified as described previously.

4,6-Dichloro-2,1,3-benzoxadiazole (4-Cl/6-Cl). 2,4-Dichloro-6-nitroaniline (550 mg, 2.7 mmol) was dissolved in a mixture of acetic acid (6 mL) and conc. sulfuric acid (2 mL). After the addition of sodium nitrite (200 mg, 2.9 mmol) solution (5 mL) at 0 °C, sodium azide (190 mg, 2.9 mmol) solution (5 mL) was added and the mixture was stirred at rt for 30 min. The mixture was concentrated *in vacuo* and the residue was chromatographed on silica gel with CH_2Cl_2–*n*-hexane (2:5) to afford 2,4-dichloro-6-nitrophenyl azide. 2,4-Dichloro-6-nitrophenyl azide was dissolved in diethylene glycol (5 mL) and stirred for 1 h at 150 °C. The mixture was chromatographed on silica gel with CH_2Cl_2–*n*-hexane (2:5) to afford 4,6-dichlorobenzofuroxan (300 mg, 55%) as yellow crystals. 4,6-Dichlorobenzofuroxan (300 mg, 1.4 mmol) was dissolved in chlorobenzene (5 mL). TPP (416 mg, 1.6 mmol) was added and refluxed for 1 h. The reaction mixture was chromatographed on silica gel with *n*-hexane to afford **4-Cl/6-Cl** (185 mg, 68%) as white crystals: mp 55 – 56 °C. δ_H 7.78 (1H, s), 7.40 (1H, s). Anal. Calcd for $C_6H_2N_2OCl_2$: C, 38.13; H, 1.07; N, 14.82. Found: C, 38.13; H, 1.32; N, 14.81.

4-Chloro-6-nitro-2,1,3-benzoxadiazole (4-Cl/6-NO$_2$). 6-Chloro-2,4-dinitroaniline (129 mg, 0.59 mmol) was dissolved in a mixture of acetic acid (8 mL) and conc. sulfuric acid (4 mL). After the addition of sodium nitrite (50 mg, 0.72 mmol) solution (5 mL) at 0 °C, sodium azide (48 mg, 0.74 mmol) solution (5 mL) was added and the mixture was stirred at rt for 1 h. The mixture was concentrated *in vacuo* and the residue was chromatographed on silica gel with *n*-hexane–ethyl acetate (3:1) to afford 2-chloro-4,6-dinitrophenyl azide as yellow oil. 2-Chloro-4,6-dinitrophenyl azide was dissolved in *o*-dichlorobenzene (5 mL) and refluxed for 1. The mixture was chromatographed on silica gel with *n*-hexane–ethyl acetate (3:1) to afford 4-chloro-6-nitrobenzofuroxan (100 mg, 78%) as yellow crystals. 4-Chloro-6-nitrobenzofuroxan (28 mg, 0.13 mmol) was dissolved in toluene (3 mL). After the addition of TPP (38 mg, 0.15 mmol), the mixture was refluxed for 1 h and concentrated *in vacuo*. The residue was

chromatographed on silica gel with CH_2Cl_2–*n*-hexane (1:2) to afford **4-Cl/6-NO_2** (23 mg, 88%) as white needles: mp 72 °C. δ_H 8.81 (1H, s), 8.24 (1H, s). Anal. Calcd for $C_6H_2N_3O_3Cl$: C, 36.11; H, 1.01; N, 21.06. Found: C, 36.33 ; H, 1.12 ; N, 21.30.

5-Fluoro-6-nitro-2,1,3-benzoxadiazole (5-F/6-NO_2). 2,4-Dinitro-5-fluoroaniline (1.1 g, 5.2 mmol) was dissolved in a mixture of acetic acid (40 mL) and conc. sulfuric acid (20 mL). After the addition of sodium nitrite (400 mg, 5.8 mmol) solution (10 mL) at 0 °C, sodium azide (480 mg, 7.4 mmol) solution (5 mL) was added and the mixture was stirred at rt for 1 h. After the extraction with ethyl acetate (3×50 mL), the organic layer was refluxed for 2.5 h. The reaction mixture was concentrated *in vacuo* and the residue was chromatographed on silica gel with *n*-hexane–ethyl acetate (4:1) to afford 5-fluoro-6-nitrobenzofuroxan (810 mg, 78%) as yellow crystals. 5-Fluoro-6-nitrobenzofuroxane (810 mg, 4.1 mmol) was dissolved in ethyl acetate (10 mL). After the addition of TPP (1.3 g), the mixture was refluxed for 1 h. The mixture was chromatographed on silica gel with CH_2Cl_2–*n*-hexane (1:2) to afford 5-fluoro-6-nitro-2,1,3-benzoxadiazole (390 mg, 52%) as yellow needles: mp 33 °C. δ_H 8.62 (1H, d, J = 6.7), 7.73 (1H, m). Anal. Calcd for $C_6H_2N_3O_3F$: C, 39.54; H, 1.10; N, 22.95. Found: C, 39.34; H, 1.35; N, 23.07.

4-Chloro-6-*N*,*N*-dimethylamino-2,1,3-benzoxadiazole (4-Cl/6-NMe_2). 4-Cl/6-Cl (20 mg, 0.11 mmol) was dissolved in acetonitrile (2 mL). After the addition of 50% dimethylamine solution (1.5 mL, 12 mmol), the mixture was refluxed for 4 h. The reaction mixture was chromatographed on silica gel with CH_2Cl_2–*n*-hexane (1:2) to afford **4-Cl/6-NMe_2** (2.0 mg, 9.6%) as yellow crystals: mp 60 – 61 °C. δ_H 7.02 (1H, s), 5.95 (1H, s), 3.34 (6H, s). Anal. Calcd for $C_8H_8N_3OCl$: C, 48.62; H, 4.08; N, 21.26. Found: C, 48.87; H, 4.28; N, 21.41. APCI-MS: *m/z* 198 $[M + H]^+$.

5-*N*,*N*-Dimethylamino-4-nitro-2,1,3-benzoxadiazole (4-NO_2/5-NMe_2). 4-NO_2/5-Cl (29 mg, 0.15 mmol) was dissolved in acetonitrile (2 mL). After the addition of 50% dimethylamine solution (200 μL, 1.6 mmol), the mixture was stirred at rt for 2 h. The reaction mixture was chromatographed on silica gel with ethyl acetate–*n*-hexane (2:1) to afford **4-NO_2/5-NMe_2** (23 mg, 76%) as yellow crystals: mp 166 °C. δ_H 7.83 (1H, d, J = 10.7), 7.42 (1H, d, J =10.7), 3.24 (6H, s). Anal. Calcd for $C_8H_8N_4O_3$: C, 46.16; H, 3.87; N, 26.91. Found: C, 46.17 ; H, 3.82 ; N, 26.96. APCI-MS: *m/z* 209 $[M + H]^+$.

5-Methylthio-4-nitro-2,1,3-benzoxadiazole (4-NO_2/5-SMe). 4-NO_2/5-Cl (200 mg, 1.0 mmol) was dissolved in the mixture of acetonitrile (10 mL) and 50 mM borate buffer (pH 9.3, 4 mL). After the addition of 15 % methyl mercaptan sodium salt solution (800 μL, 1.9 mmol), the mixture was stirred at rt for 1.5 h. The reaction mixture was concentrated *in vacuo* and the residue was chromatographed on silica gel with CH_2Cl_2–*n*-hexane (2:1) to afford **4-NO_2/5-SMe** (75 mg, 35%) as orange crystals: mp 137 °C. δ_H 8.08 (1H, d, J = 9.8), 7.64 (1H, d, J = 9.8), 2.70 (3H, s). Anal. Calcd for $C_7H_5N_4O_3S$: C, 39.81; H, 2.39; N, 19.90. Found: C, 39.89; H, 2.66; N, 19.68.

5-Methylthio-6-amino-2,1,3-benzoxadiazole (5-SMe/6-NH_2). 5-Amino-6-fluoro-2,1,3-benzoxadiazole

(**5-NH$_2$/6-F**, 15 mg, 0.098 mmol) was dissolved in acetonitrile (12 mL). After the addition of 15 % methyl mercaptan sodium salt solution (1.6 mL, 3.7 mmol), the mixture was stirred at rt for 10 h. The reaction mixture was concentrated *in vacuo* and the residue was chromatographed on silica gel with dichloromethane to afford **5-SMe/6-NH$_2$** (10 mg, 57%) as yellow crystals: mp 183 – 184 °C. δ_H 7.42 (1H, s), 6.69 (1H, s), 4.49 (2H, br), 2.58 (3H, s). Anal. Calcd for $C_7H_7N_3OS$: C, 46.39; H, 3.89; N, 23.19. Found: C, 46.50; H, 4.04; N, 22.94. APCI-MS: *m/z* 182 $[M + H]^+$.

5-Methoxy-4-nitro-2,1,3-benzoxadiazole (4-NO$_2$/5-OMe). 4-NO$_2$/5-Cl (300 mg, 1.5 mmol) was dissolved in methanol (15 mL). After the addition of triethylamine (0.5 mL, 3.6 mmol), the mixture was stirred at rt for 4 h. The reaction mixture was concentrated *in vacuo* and the residue was chromatographed on silica gel with CH_2Cl_2–*n*-hexane (1 : 1) to afford **4-NO$_2$/5-OMe** (185 mg, 63%) as yellow crystals: mp 120 °C. δ_H 8.15 (1H, d, *J* = 9.7), 7.54 (1H, d, *J* = 9.7), 4.21 (3H, s). Anal. Calcd for $C_7H_5N_3O_4$: C, 43.09; H, 2.58; N, 21.53. Found: C, 43.06; H, 2.78; N, 21.48. APCI-MS: *m/z* 196 $[M + H]^+$.

4-Amino-5-*N*,*N*-dimethylamino-2,1,3-benzoxadiazole (4-NH$_2$/5-NMe$_2$). 4-NO$_2$/5-NMe$_2$ (207 mg, 1.0 mmol) was dissolved in the mixture of dichloromethane (8 mL), conc. hydrochloric acid (4 mL) and methanol (10 mL). After the addition of iron powder (800 mg, 14 mmol), the mixture was stirred at rt for 1 h. The reaction mixture was poured into water and extracted using dichloromethane. The organic layer was dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was chromatographed on silica gel with CH_2Cl_2–*n*-hexane (2 : 1) to afford **4-NH$_2$/5-NMe$_2$** (42 mg, 24%) as red crystals: mp 75 °C. δ_H 7.31 (1H, d, *J* = 9.4), 7.12 (1H, d, *J* = 9.4), 4.69 (2H, br), 2.67 (6H, s). Anal. Calcd for $C_8H_{10}N_4O$: C, 53.92; H, 5.66; N, 31.44. Found: C, 54.13; H, 5.46; N, 31.48. APCI-MS: *m/z* 179 $[M + H]^+$.

4-Amino-5-chloro-2,1,3-benzoxadiazole (4-NH$_2$/5-Cl). 4-NO$_2$/5-Cl (200 mg, 1.0 mmol) was dissolved in the mixture of dichloromethane (4 mL), conc. hydrochloric acid (3 mL) and methanol (10 mL). After the addition of iron powder (500 mg, 9.0 mmol), the mixture was stirred at rt for 1 h. The reaction mixture was poured into water and extracted using dichloromethane. The organic layer was dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was chromatographed on silica gel with CH_2Cl_2–*n*-hexane (1 : 1) to afford **4-NH$_2$/5-Cl** (113 mg, 67%) as yellow crystals: mp 130 °C. δ_H 7.24 (1H, d, *J* = 9.4), 7.11 (1H, d, *J* = 9.4), 4.91 (2H, br). Anal. Calcd for $C_6H_4N_3OCl$: C, 42.50; H, 2.38; N, 24.78. Found: C, 42.44; H, 2.51; N, 24.62. APCI-MS: *m/z* 168 $[M - H]^-$.

4-Amino-5-methoxy-2,1,3-benzoxadiazole (4-NH$_2$/5-OMe). 4-NO$_2$/5-OMe (50 mg, 0.26 mmol) was dissolved in the mixture of dichloromethane (4 mL), conc. hydrochloric acid (2 mL) and methanol (6 mL). After the addition of iron powder (200 mg, 3.6 mmol), the mixture was stirred at rt for 1 h. The reaction mixture was poured into water and extracted using dichloromethane. The organic layer was dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was chromatographed on silica gel with dichloromethane to afford **4-NH$_2$/5-OMe** (19 mg, 45%) as red crystals: mp 88 °C. δ_H 7.28 (1H, d, *J* =

9.4), 7.17 (1H, d, *J* = 9.4), 4.40 (2H, br), 3.92 (3H, s). Anal. Calcd for $C_7H_7N_3O_2$: C, 50.91; H, 4.27; N, 25.44. Found: C, 50.77; H, 4.40; N, 25.17. APCI-MS: *m/z* 166 $[M + H]^+$.

4-Amino-5-methylthio-2,1,3-benzoxadiazole (4-NH_2/5-SMe). 4-NO_2/5-SMe (200 mg, 0.95 mmol) was dissolved in the mixture of dichloromethane (15 mL), conc. hydrochloric acid (9 mL) and methanol (35 mL). After the addition of iron powder (1.1 g, 20 mmol), the mixture was stirred at rt for 30 min. The reaction mixture was poured into water and extracted using dichloromethane. The organic layer was dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was chromatographed on silica gel with CH_2Cl_2–*n*-hexane (2 : 1) to afford **4-NH_2/5-SMe** (23 mg, 13%) as orange crystals: mp 66 °C. δ_H 7.36 (1H, d, *J* = 9.2), 7.07 (1H, d, *J* = 9.2), 5.27 (2H, br), 2.35 (3H, s). Anal. Calcd for $C_7H_7N_3OS$: C, 46.39; H, 3.89; N, 23.19. Found: C, 46.37; H, 3.90; N, 23.13.

5-Amino-4-methylthio-2,1,3-benzoxadiazole (4-SMe/5-NH_2). 4-Cl/5-NO_2 (162 mg, 0.81 mmol) was dissolved in the mixture of acetonitrile (12 mL) and saturated sodium hydrogen carbonate solution (3 mL). After the addition of 15 % methyl mercaptan sodium salt solution (100 μL, 0.23 mmol), the mixture was stirred at rt for 20 min. The reaction mixture was concentrated *in vacuo* and the residue was chromatographed on silica gel with CH_2Cl_2–*n*-hexane (1:1) to afford 4-methylthio-5-nitro-2,1,3-benzoxadiazole (**4-SMe/5-NO_2**, 93%) as yellow crystals. δ_H 8.06 (1H, d, *J* = 5.8), 7.60 (1H, d, *J* = 5.8), 3.04 (3H, s). **4-SMe/5-NO_2** (160 mg, 0.76 mmol) was dissolved in the mixture of dichloromethane (20 mL), conc. hydrochloric acid (1.1 mL) and methanol (8 mL). After the addition of iron powder (517 mg, 9.3 mmol), the mixture was stirred at rt for 20 min. The reaction mixture was poured into water and extracted using dichloromethane. The organic layer was dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was chromatographed on silica gel with CH_2Cl_2–*n*-hexane (5 : 2) to afford **4-SMe/5-NH_2** (117 mg, 85%) as yellow needles: mp 102 °C. δ_H 7.60 (1H, d, *J* = 9.4), 6.96 (1H, d, *J* = 9.4), 5.03 (2H, br), 2.41 (3H, s). Anal. Calcd for $C_7H_7N_3OS$: C, 46.39; H, 3.89; N, 23.19. Found: C, 46.15; H, 3.83; N, 23.10. APCI-MS: *m/z* 182 $[M + H]^+$.

5-Amino-6-fluoro-2,1,3-benzoxadiazole (5-NH_2/6-F). 5-NO_2/6-F (391 mg, 2.1 mmol) was dissolved in the mixture of dichloromethane (50 mL), conc. hydrochloric acid (3 mL) and methanol (20 mL). After the addition of iron powder (1.1 g, 20 mmol), the mixture was stirred at rt for 15 min. The reaction mixture was poured into water and extracted using dichloromethane. The organic layer was dried over anhydrous sodium sulfate and concentrated *in vacuo*. The residue was chromatographed on silica gel with dichloromethane to afford **5-NH_2/6-F** (15 mg, 4.7%) as white crystals: mp 186 °C. δ_H 7.34 (1H, d, *J* = 10.3), 6.74 (1H, d, *J* = 7.9), 4.47 (2H, br). Anal. Calcd for $C_6H_4N_3OF$: C, 47.07; H, 2.63; N, 27.44. Found: C, 46.94; H, 2.84; N, 27.28. APCI-MS: *m/z* 154 $[M + H]^+$.

4-Acetylamino-5-*N*,*N*-dimethylamino-2,1,3-benzoxadiazole (4-NHAc/5-NMe_2). 4-NH_2/5-NMe_2 (5.0 mg, 0.028 mmol) was dissolved in acetic anhydride (2 mL, 22 mmol). The mixture was stirred at rt for 8

h and chromatographed on silica gel with ethyl acetate to afford **4-NHAc/5-NMe$_2$** (5.1 mg, 83%) as yellow crystals: mp 210 – 211 °C. δ_H 7.57 (1H, d, *J* = 9.7), 7.29 (1H, d, *J* = 9.7), 2.99 (6H, s), 2.24 (3H, s). Anal. Calcd for $C_{10}H_{12}N_4O_2$: C, 54.54; H, 5.49; N, 25.44. Found: C, 54.64; H, 5.36; N, 25.31. APCI-MS: *m/z* 221 $[M + H]^+$.

5-Chloro-4-diacetylamino-2,1,3-benzoxadiazole (4-NAc$_2$/5-Cl). 4-NH$_2$/5-Cl (30 mg, 0.18 mmol) was dissolved in acetic anhydride (2 mL, 22 mmol). The mixture was stirred at 130 °C for 2 h and chromatographed on silica gel with ethyl acetate–*n*-hexane (1 : 2) to afford **4-NAc$_2$/5-Cl** (20 mg, 45%) as white crystals: mp 110 °C. δ_H 7.90 (1H, d, *J* = 9.4), 7.50 (1H, d, *J* = 9.7), 2.33 (6H, s). Anal. Calcd for $C_{10}H_8N_3O_3Cl$: C, 47.35; H, 3.18; N, 16.57. Found: C, 47.47; H, 3.43; N, 16.38. APCI-MS: *m/z* 253 $[M]^-$.

4-Diacetylamino-5-methylthio-2,1,3-benzoxadiazole (4-NAc$_2$/5-SMe). 4-NH$_2$/5-SMe (20 mg, 0.11 mmol) was dissolved in acetic anhydride (2 mL, 22 mmol). The mixture was heated at 100 °C for 2 h and chromatographed on silica gel with ethyl acetate–*n*-hexane (2 : 1) to afford **4-NAc$_2$/5-SMe** (3.5 mg, 12%) as yellow crystals: mp 142 – 143 °C. δ_H 7.84 (1H, d, *J* = 9.5), 7.41 (1H, d, *J* = 9.5), 2.53 (3H, s), 2.28 (6H, s). Anal. Calcd for $C_{11}H_{11}N_3O_3S$: C, 49.80; H, 4.18; N, 15.84. Found: C, 49.92; H, 4.44; N, 15.57. APCI-MS: *m/z* 266 $[M + H]^+$.

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