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1,3-Benzoxazole-4-carbonitrile as a novel antifungal scaffold of β-1,6-glucan synthesis inhibitors

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ABSTRACT

Synthesis and in vitro antifungal evaluations of 1,3-benzoxazole-7-carbonitrile **3**, 1,3-benzoxazole-4-carbonitrile **4**, benzofuran **5**, benzoxazine **7**, and benzimidazole **8** were reported. Among them, 1,3-benzoxazole-4-carbonitrile was found to be a superior scaffold structure with moderate growth inhibition against *Candida* species. 1,3-Benzoxazole-4-carbonitrile **6** showed potent activity against *Candida* species compared to 5-desmethyl compound **4** and triazolopyridine **2**. Compound **6** was efficiently prepared from versatile intermediate **24**, which possessed six different substituents on the benzene ring. Conversion of benzene **24** into various 1,3-benzoxazole derivatives such as 2-aliphatic **34**, 2-amino **35**, and lactone **38** was demonstrated.

1. Introduction

Candida species (spp.), including *Candida albicans*, *Candida glabrata*, and *Candida krusei* are considered to be significant pathogens of systemic fungal infections.[1,2] So far, several kinds of antifungal drugs such as polyenes, azoles, and candins have been launched for the treatment of these opportunistic infections, which show efficacies against *Candida* spp.[2–6] However, the usage of these agents is often limited due to their unsatisfactory antifungal activity, narrow spectrum, and side effects. These unresolved issues of antifungal therapy have caused a high rate of mortality and drug resistance.[6–9] Therefore, new antifungal drugs with a novel mode of action have been constantly required in clinical therapy.

It was reported that pyridobenzimidazole **1** and its derivatives exhibited antifungal activities against *Candida* spp., based on selective inhibition of the biosynthesis of β-1,6-glucan which is conserved in various fungi, but not in mammalian cells (Fig. 1).[10–12] In the course of our program toward the discovery of new antifungal scaffolds, we have found that triazolopyridine **2** inhibited β-1,6-glucan synthesis and showed potent antifungal activities against *Candida* spp. (Fig. 1).[13] Herein, we would like to report the synthesis of various bicyclic scaffolds and the discovery of 1,3-benzoxazole-4-carbonitrile as a novel bicyclic scaffold with potent antifungal activity (Fig. 2). A practical and efficient route for the preparation of 1,3-benzoxazole-4-carbonitrile **6** and its derivatives was established starting from benzene derivative **24**, which has six different functional substituents as a versatile intermediate.

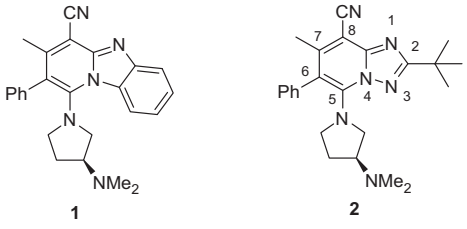
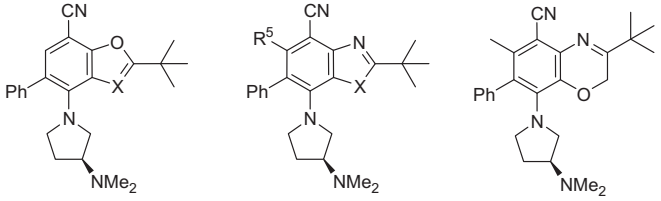


Figure 1. Structure of compounds **1** and **2**.



3 : X = N
5 : X = CH
4 : X = O, R^5 = H
6 : X = O, R^5 = Me
8 : X = NH, R^5 = Me
7

Figure 2. Synthesized bicyclic derivatives **3**–**8**.

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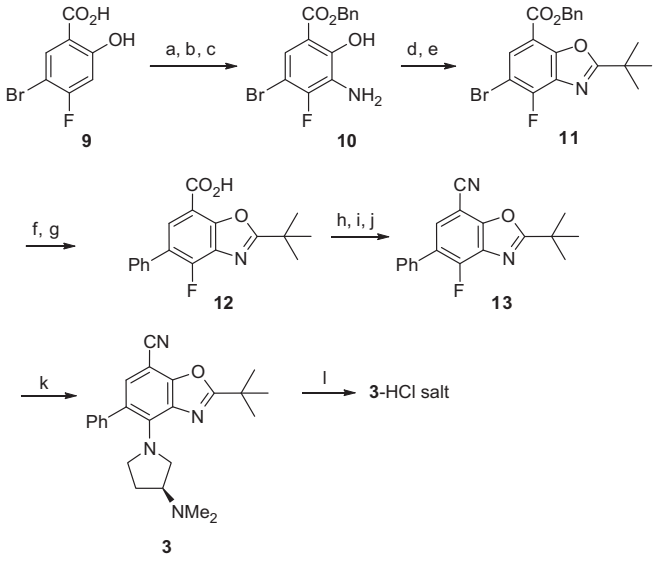
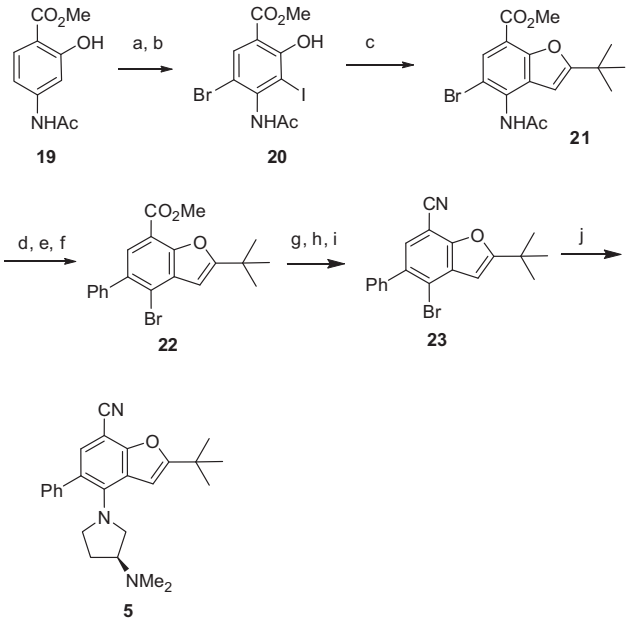


Scheme 1. Synthesis of 1,3-benzoxazole-7-carbonitrile **3**. Reagents and conditions: (a) f.HNO_3, H_2SO_4, 83%; (b) BnBr, K_2CO_3, DMF, 88%; (c) Fe, AcOH, 34%; (d) pivaloyl chloride, pyridine, benzene, 83%; (e) *p*-TsOH·H_2O, benzene, 43%; (f) $PhB(OH)_2$, $Pd(PPh_3)_4$, K_3PO_4, THF, 87%; (g) H_2, 10% Pd–C, EtOAc, 96%; (h) $(COCl)_2$, DMF, CH_2Cl_2; (i) 28% NH_4OH, EtOAc; (j) Tf_2O, 4-DMAP, pyridine, 90% (three steps); (k) **14**, Et_3N, DMSO, 78%; (l) 1 M HCl in EtOH, 91%.



Scheme 3. Synthesis of benzofuran-7-carbonitrile **5**. Reagents and conditions: (a) NBS, DMF, 45%; (b) $BnNMe_3Cl_2I$, $NaHCO_3$, CH_2Cl_2–MeOH, 100%; (c) 3,3-dimethyl-1-butyne, $Cl_2Pd(PPh_3)_2$, CuI, Et_3N, 1,4-dioxane, 54%; (d) $PhB(OH)_2$, $Pd(PPh_3)_4$, 2 M Na_2CO_3 aq, toluene–DME, 91%; (e) 4 M HCl, MeOH, 48%; (f) *tert*-BuONO, $CuBr_2$, MeCN, 99%; (g) KOH, THF–MeOH, 94%; (h) 28% NH_4OH, EDC·HCl, HOBt, DMF, 99%; (i) Tf_2O, Et_3N, CH_2Cl_2, 87%; (j) **14**, $Pd(OAc)_2$, (*R*)-BINAP, NaO*t*Bu, toluene, 33%.

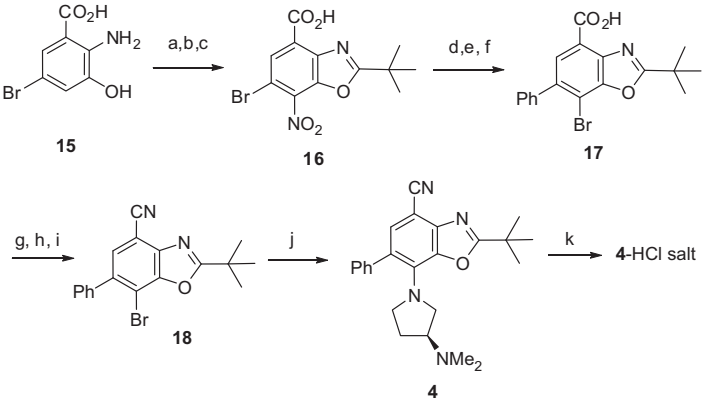
2. Chemistry

As shown in Scheme 1, 1,3-benzoxazole-7-carbonitrile **3** was synthesized. Nitrization of salicylic acid **9**[14] followed by esterification and reduction gave **10**, which was condensed with pivaloyl chloride to afford 1,3-benzoxazole-7-carboxylate **11** as a key intermediate. Compound **12** was obtained from **11** using Suzuki cross-coupling reaction[15] and hydrogenolysis. (3*S*)-3-(Dimethylamino)pyrrolidine (**14**) was introduced to the fluoride **13** to afford **3**, which was subsequently treated with hydrochloric acid to provide HCl salt of **3**.

As shown in Scheme 2, 1,3-benzoxazole-4-carbonitrile **18** was prepared from anthranilic acid **15**[16] following the similar manner described in Scheme 1. Palladium catalyzed cross-coupling reaction[17] of **18** with **14** gave 1,3-benzoxazole-4-carbonitrile **4**, which was converted into HCl salt of **4**.

As outlined in Scheme 3, compound **20**, obtained from methyl 4-acetamido-2-hydroxybenzoate (**19**), was subjected to cross-coupling reaction[18] followed by intramolecular cyclization to give benzofuran **21**. Subsequent Suzuki cross-coupling reaction, deacetylation and Sandmeyer reaction[19] afforded **22**, which was then converted to benzofuran-7-carbonitrile **5** by the same manner as described in Schemes 1 and 2.

As in Scheme 2, our methodology for the synthesis of **4** required multiple steps in quite low yield (1.3% total yield for 11 steps). Moreover, functionalization at the vacant 5-position, which was required for synthesis of **6**, has not been achieved because of the hindered position or highly functionalized benzene structure. Since both potent antifungal analogs **1** and **2** possessed methyl groups, methylation of the 1,3-benzoxazole-4-carbonitrile was particularly interesting. The synthetically and biologically attractive structure of the fully substituted 1,3-benzoxazole-4-carbonitrile **6** motivated us to develop an efficient and versatile synthetic methodology that would allow ready access to various analogs for further biological



Scheme 2. Synthesis of 1,3-benzoxazole-4-carbonitrile **4**. Reagents and conditions: (a) pivaloyl chloride, pyridine, benzene, 98%; (b) f.HNO_3, AcOH, 13%; (c) *p*-TsOH·H_2O, benzene, 98%; (d) $PhB(OH)_2$, $Pd(PPh_3)_4$, 2 M Na_2CO_3 aq, toluene–EtOH; (e) H_2, 10% Pd–C, MeOH, 66% (two steps); (f) *tert*-BuONO, $CuBr_2$, MeCN, 91%; (g) $(COCl)_2$, cat. DMF, CH_2Cl_2; (h) 28% NH_4OH, EtOAc; (i) Tf_2O, 4-DMAP, pyridine, 62% (three steps); (j) **14**, $Pd(OAc)_2$, NaO*t*Bu, *rac*-BINAP, toluene, 34%; (k) 1 M HCl in EtOH, 80%.

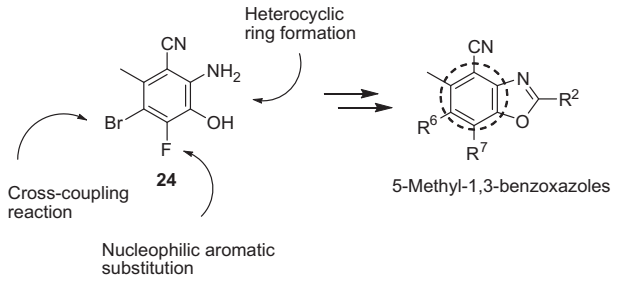


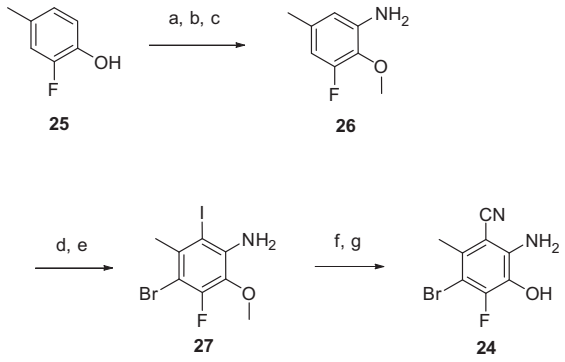
Figure 3. Design of key intermediate **24**.

evaluation. Our strategy was shown in Figure 3. We envisioned that the fully substituted 1,3-benzoxazole-4-carbonitirile derivatives could be formed from hexasubstituted benzene **24** through appropriate functionalization. Bromo group can be replaced to various substituents such as aromatic rings, unsaturated aliphatic groups, and amino moieties by means of cross-coupling reactions. Fluoro group can also be converted into amino substituents by S_NAr reactions. Amino and hydroxyl groups are used for heteroaromatic cyclization to afford 1,3-benzoxazole skeleton.

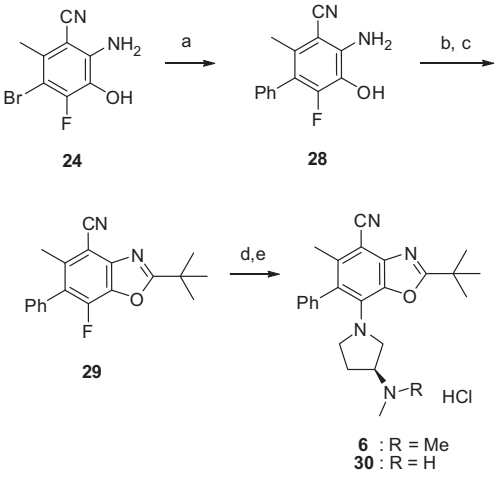
The key intermediate benzene **24** was synthesized as follows: 2-Fluoro-4-methylphenol (**25**) was selectively nitrized at the *ortho*-position of the hydroxyl group, followed by methylation of the hydroxyl group and reduction of the nitro group to afford aniline **26**. Subsequent regioselective halogenations gave fully substituted benzene **27** in good yield. Finally, palladium-catalyzed cyanization and subsequent demethylation gave **24** in seven steps and an overall 33% yield from commercially available **25** (Scheme 4).

As outlined in Scheme 5, methylated derivatives **6** and **30** were prepared from **24** and **14** or (3*S*)-3-(methylamino)pyrrolidine (**31**) in a manner similar to that described in Scheme 1.

Benzenes **24** and **28** were converted into various 1,3-benzoxazoles to demonstrate their utility as versatile intermediates. As shown in Scheme 6, **24** was condensed with various acid chlorides to give **32**. Using this strategy, the 2-position was easily converted. Cross-coupling reaction of **32** followed by the introduction of **14** gave **34**, indicating that this route was applicable to the optimization of the 6-position. Benzene **28**, possessing a phenyl group, was used to synthesize various 2-substituted 1,3-benzoxazoles such as **33**, which was converted into **34** as described above. Benzene **28** was also useful for the preparation of 2-amino analog **35** (Scheme 7). Synthesis of lactone **38** demonstrated that the 4- and 5-positions can be cyclized easily by using a well-known procedure (Scheme 8). To extend the utility of fully substituted benzene, benzoxazine **7** was prepared from **27** (Scheme 9). Benzimidazole **8** was also synthesized from fully substituted benzene **42** (Scheme 10).



Scheme 4. Synthesis of hexa-substituted benzene **24**. Reagents and conditions: (a) f.HNO_3, H_2SO_4, 98%; (b) Me_2SO_4, Na_2CO_3, toluene, 69%; (c) H_2, Pd–C, MeOH, 81%; (d) NBS, DMF, 81%; (e) NIS, AcOH, 98%; (f) $Zn(CN)_2$, $Pd(dba)_2$, DPPF, DMF–H_2O, 80%; (g) BBr_3, CH_2Cl_2, 96%.

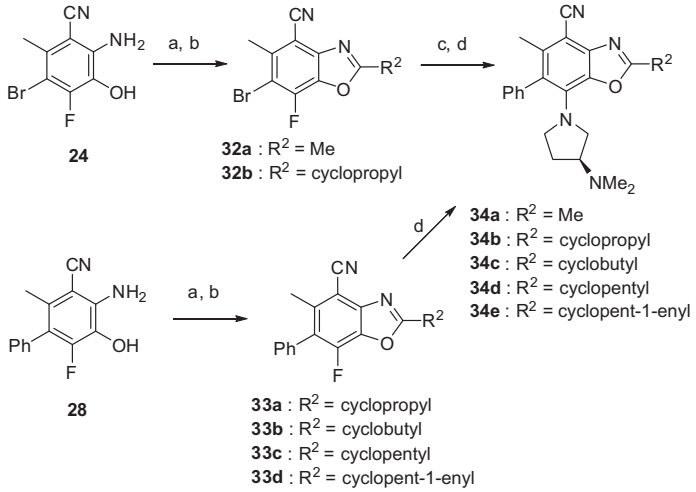


Scheme 5. Synthesis of 5-methyl-1,3-benzoxazole-4-carbonitriles **6** and **30**. Reagents and conditions: (a) $PhB(OH)_2$, $Pd(PPh_3)_4$, K_3PO_4, 1,4-dioxane-H_2O, 91%; (b) pivaloyl chloride, *i*Pr_2NEt, CH_2Cl_2; (c) *p*-TsOH·H_2O, toluene, 93% (two steps); (d) amine **14** or **31**, Et_3N, DMSO; (e) 4 M HCl in 1,4-dioxane.

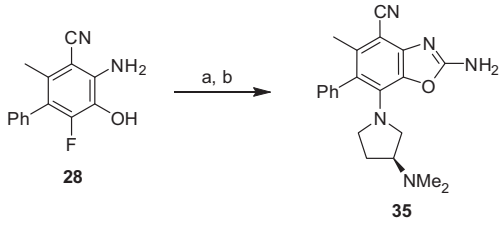
3. Results and discussion

Synthesized bicyclic compounds **3**–**8** and **30** as well as pyridobenzimidazole **1**, triazolopyridine **2**, and fluconazole (FLCZ) as positive control agents were evaluated for their in vitro antifungal activity against *Saccharomyces cerevisiae* YPH500 (*S. cerevisiae*), *Candida albicans* ATCC 24433 (*C. albicans*), *Candida glabrata* ATCC 48435 (*C. glabrata*), *Candida tropicalis* IAM4965 (*C. tropicalis*), and *Candida krusei* ATCC 44507 (*C. krusei*) (Table 1). Minimum inhibitory concentration MIC-2 (the lowest drug concentration showing 50% growth inhibition compared to the control without drug) was determined by using the microdilution method with alamar blue.[10–13,20–22] As shown in Table 1, 1,3-benzoxazole-7-carbonitrile **3** exhibited weak growth inhibition against *S. cerevisiae*. On the other hand, 1,3-benzoxazole-4-carbonitrile **4**, which possessed a different array of nitrogen and oxygen atoms on the benzoxazole scaffold from **3**, showed moderate growth inhibitory activities against *S. cerevisiae* and *C. glabrata* (MIC-2 = 0.25 and 0.25 μg/mL, respectively). Evaluation of benzofuran derivative **5** exhibited weak antifungal activity. These results indicate that the positions of heteroatoms such as nitrogen and oxygen on the bicyclic ring could strongly affect the potencies of their antifungal activities. In addition, the nitrogen atom at the ring juncture, as seen at the 4-position of **2** (Fig. 1), was unessential for antifungal activity. The requirement of the nitrogen atom at the 3-position, as seen in **4** (Fig. 2), also seems to be endorsed by the result obtained in the previous study of fused pyridine derivatives.[13] The 5-methylated analog **6** exhibited potent growth inhibition against various *Candida* spp., which suggests that the introduction of methyl group at the 5-position shows more than fourfold enhancement in antifungal activities compared to 5-desmethyl analog. Compound **30**, possessing monomethylamino pyrrolidine moiety, decreased inhibitory activity. Although **7** had a desired array of heteroatoms on the benzoxazine skeleton and appropriate substituents at each position, it showed little effects. Benzimidazole **8** was less active than benzoxazole **6**. These results implied that slight modification of scaffolds might cause a significant loss of potency.

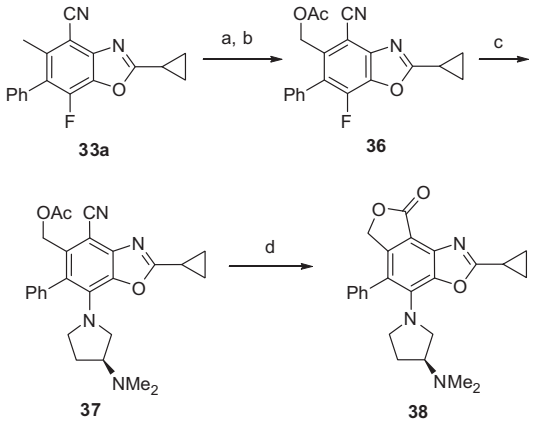
As shown in Table 2, synthesized 1,3-benzoxazole-4-carbonitriles **34**, **35**, and lactone **38** as well as triazolopyridine **2** and FLCZ as positive control agents were evaluated for their in vitro antifungal activity against FLCZ-susceptible *Candida albicans* ATCC 90028 (*C. albicans-s*), FLCZ-resistant *Candida albicans* ATCC MYA-573 (*C. albicans-r*), *Candida glabrata* ATCC 48435 (*C. glabrata*), *Candida tropicalis*



Scheme 6. Synthesis of 2-aliphatic 1,3-benzoxazole-4-carbonitriles **34**. Reagents and conditions: (a) R^2COCl, iPr_2NEt, EtOAc; (b) *p*-TsOH·H_2O, toluene; (c) $PhB(OH)_2$, $Pd(PPh_3)_4$, K_3PO_4, 1,4-dioxane-H_2O; (d) **14**, Et_3N, DMSO.

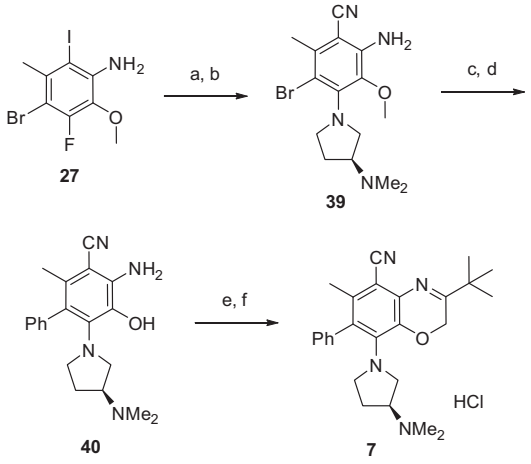


Scheme 7. Synthesis of 2-amino-1,3-benzoxazole-4-carbonitriles **35**. Reagents and conditions: (a) di(1*H*-imidazol-1-yl)methanimine, THF, 87%; (b) **14**, Et_3N, DMSO, 30%.

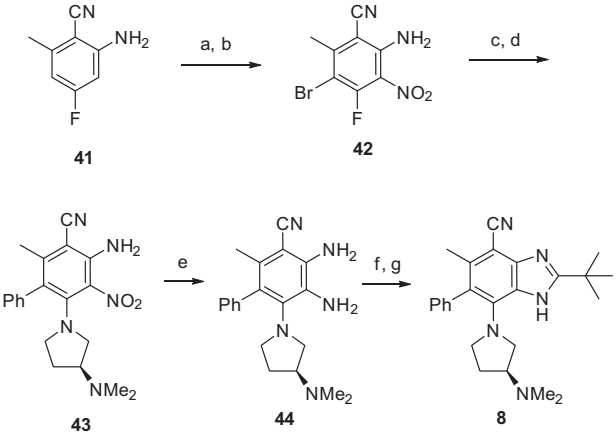


Scheme 8. Synthesis of lactone **38**. Reagents and conditions: (a) NBS, AIBN, CCl_4, 82%; (b) NaOAc, AcOH, 84%; (c) **14**, Et_3N, DMSO, 44%; (d) K_2CO_3, MeOH, 33%.

ATCC 44508 (*C. tropicalis*), and *Candida krusei* ATCC 44507 (*C. krusei*). YPD (1% yeast extract, 2% peptone, and 20% glucose) was used as the medium. MIC-1 (the lowest drug concentration showing 80% growth inhibition compared to the control without drug) was evaluated for each compound. Among various 2-aliphatic 1,3-benzoxazole-4-carbonitriles, *tert*-butyl and cycloalkyl derivatives **6** and **34b–d** showed potent growth inhibition against *Candida* spp. Moreover, these derivatives exhibited antifungal activity against both FLCZ-susceptible and FLCZ-resistant *C. albicans* (MIC-1 = 0.063 and 0.063 μg/mL for **34b**, respectively). Conversion of the 2-substitute to NH_2 decreased growth inhibition. Lactone **38** maintained moderate activity without the 5-methyl and 4-cyano groups.

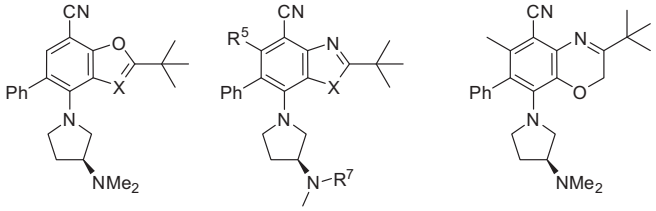


Scheme 9. Synthesis of benzoxazine **7**. Reagents and conditions: (a) $Zn(CN)_2$, $Pd(dba)_2$, DPPF, DMF–H_2O, 80%; (b) **14**, Et_3N, DMSO, 44%; (c) $PhB(OH)_2$, $Pd(PPh_3)_4$, K_3PO_4, 1,4-dioxane-H_2O, 39%; (d) $AlCl_3$, CH_2Cl_2, 62%; (e) 1-bromo-3,3-dimethylbutan-2-one, Bu_4NHSO_4, K_2CO_3, CH_2Cl_2–H_2O; (f) 4 M HCl in 1,4-dioxane, Et_2O, 64% (two steps).



Scheme 10. Synthesis of benzimidazole **8**. Reagents and conditions: (a) NBS, DMF; (b) f.HNO_3, H_2SO_4, 48% (two steps); (c) **14**, Et_3N, DMSO; (d) $PhB(OH)_2$, $Pd(PPh_3)_4$, K_3PO_4, 1,4-dioxane-H_2O, 87% (two steps); (e) $FeCl_3·6H_2O$, Zn, DMF–H_2O, 97%; (f) pivaloyl chloride, $CHCl_3$; (g) AcOH, 15% (two steps).

Table 1
In vitro activity of bicyclic derivatives



Compd	MIC-2 (μg/mL)[a]					Log *D*[b]	Sol.[c]	MS[d]
	S. cerevisiae YPH500	*C. albicans* ATCC 24433	*C. glabrata* ATCC 48435	*C. tropicalis* IAM 4965	*C. krusei* ATCC 44507	pH 7.4	pH 6.8 (μg/mL)	Human (%)
3	4	>4	>4	>4	>16	NT[e]	NT	NT
4	0.25	16	0.25	0.5	16	3.8	16	8
5	2	>4	>4	4	>4	5.4	<2	20
6	⩽0.016	4	⩽0.016	0.063	4	4.6	5	6
30	0.125	16	0.125	0.25	>16	3.4	120	54
7	8	>16	8	16	>16	4.4	56	NT
8	0.5	4	0.5	2	>16	4.7	8	21
1	0.004	>4	0.063	0.25	0.125	3.1[f]	5	29
2	0.063	>4	0.063	0.25	>4	3.1[f]	16	9
FLCZ	4	0.25	2	0.25	16	0.4	>2000	96

[a] MIC-2s (in micrograms per milliliter) were determined by using the microdilution method with alamar blue.
[b] Log *D* values were determined from the partition coefficient for 1-octanol/phosphate buffer saline (BPS) at pH 7.4.
[c] Water solubility was measured at pH 6.8.
[d] Metabolic stability for human liver microsomes.
[e] Not tested.
[f] Log *D* value at pH 6.8.

Physicochemical properties of the obtained compounds were shown in Tables 1 and 2. Although compound **6** exhibited high lipophilicity, poor water solubility, and low metabolic stability, monomethylamino analog **30** demonstrated improved physicochemical properties. 2-Methyl analog **34a** also showed high water solubility. These results suggest that physicochemical properties could be improved by reducing lipophilicity.

To confirm the mode of action of 1,3-benzoxazole-4-carbonitriles such as **30** obtained above, we adopted the biochemical approach based on the methods described by Kitamura et al.[10] Compound **30** was evaluated by incorporation studies with growing cells of *C. albicans* ATCC 90028 using [^{14}C]-glucose. As shown in Figure 4, **30** markedly reduced the radioactivity in the fraction of β-1,6-glucan in a dose-dependent manner. Significant reductions in the fractions of β-1,3-glucan, chitin, and mannan were not observed in each case. The result obtained above suggested that 1,3-benzoxazole-4-carbonitrile such as **30** was also a specific inhibitor of β-1,6-glucan synthesis as well as pyridobenzimidazole **1** and triazolopyridine **2**.

4. Conclusion

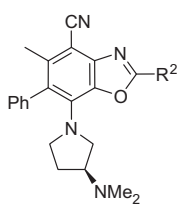
We prepared benzoxazole, benzofuran, benzoxazine, and benzimidazole as scaffolds and evaluated their antifungal activities. Among them, 1,3-benzoxazole-4-carbonitrile was found to be a superior scaffold of β-1,6-glucan synthesis inhibitors with potent antifungal activities. It was revealed that the nitrogen atom at the ring juncture was considered unessential for antifungal activity. On the other hand, the nitrogen atom at the 3-position, as seen in compound **4**, was indispensable for potent growth inhibition. 1,3-Benzoxazole-4-carbonitrile **6** demonstrated that the methyl group at the 5-position enhanced antifungal efficacy significantly compared to 5-desmethyl compound **4**. The structurally and functionally attractive key intermediate benzene **24**, which possessed six different functional groups, was synthesized efficiently. Conversion of benzene **24** into various 1,3-benzoxazoles afforded derivatives such as 2-aliphatic **34**, 2-amino **35**, and lactone **38**, indicating that benzene **24** proved to be a useful intermediate for preparing 1,3-benzoxazoles with various substituents at the 2, 4, 5, 6, and 7-positions.

5. Experimental section

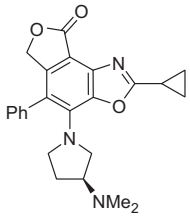
5.1. Chemistry

Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. Melting points were taken on a Yanako MP-500D melting point apparatus and are uncorrected. Optical rotations were measured in a 0.5-dm cell at 25 °C at 589 nm with a HORIBA SEPA-300 polarimeter. ^{1}H NMR spectra were determined on a JEOL JNM-EX400 spectrometer. ^{13}C NMR spectra were determined on a JEOL JNM-ECP500 spectrometer. Chemical shifts are reported in parts per million relative to tetramethylsilane as an internal standard. Significant ^{1}H NMR data are tabulated in the following order: number of protons, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad), and coupling constant(s) in hertz. Infrared (IR) spectra were obtained on a HORIBA FT-720 spectrometer or a JASCO FT/IR-6100 type A. High-resolution mass spectra were obtained on a JEOL JMS-700 mass spectrometer under electron impact ionization

Table 2
In vitro activity of 1,3-benzoxazole derivatives



6, **34**, and **35**



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Compd	R^2	MIC-1 (μg/mL)[a]					Log *D*[b]	Sol.[c]
		C. albicans-s ATCC 90028	*C. albicans-r* ATCC MYA-573	*C. glabrata* ATCC 48435	*C. tropicalis* ATCC 44508	*C. krusei* ATCC 44507	pH 7.4	pH 6.8 (μg/mL)
6	*t*-Bu	0.063	0.125	0.063	0.032	2	4.6	5
30	*t*-Bu	0.25	0.25	0.125	0.063	>4	3.4	120
34a	Me	2	2	0.25	0.25	4	3.3	120
34b	Cyclopropyl	0.063	0.063	0.032	0.032	0.125	3.9	10
34c	Cyclobutyl	0.063	0.125	⩽0.016	⩽0.016	0.5	4.5	7
34d	Cyclopentyl	0.125	0.125	0.032	0.063	2	4.9	<2
34e	Cyclopenten-1-yl	4	4	0.5	1	16	5.2	<2
35	NH_2	1	2	0.125	0.5	2	3.3	18
38	-	1	1	0.25	0.25	2	2.6	310
2	-	0.25	0.5	0.125	0.125	8	3.1[d]	16
FLCZ	-	0.25	>128	16	0.5	32	0.4	>2000

[a] MIC-1s (in micrograms per milliliter) were determined by using the microdilution method with YPD (1% yeast extract, 2% peptone, and 20% glucose) as the medium.
[b] Log *D* values were determined from the partition coefficient for 1-octanol/phosphate buffer saline (BPS) at pH 7.4.
[c] Water solubility was measured at pH 6.8.
[d] Log *D* value at pH 6.8.

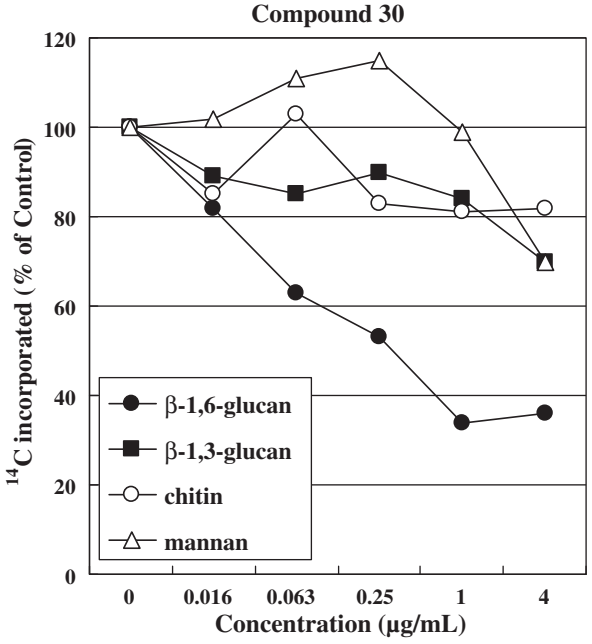

Figure 4. Incorporation studies of **30** with growing cells of *C. albicans* ATCC 90028 using [^{14}C]-glucose.

conditions (EI), electron spray ionization conditions (ESI), or fast atom bombardment ionization conditions (FAB). Column chromatography refers to flash column chromatography conducted on Merck silica gel 60, 230–400 mesh ASTM. Thin-layer chromatography (TLC) was performed with Merck silica gel 60 F_{254} TLC plates, and compound visualization was effected with a 5% solution of molybdophosphoric acid in ethanol, a UV-lamp, iodine, or Wako Ninhydrin Spray.

5.1.1. Benzyl 3-amino-5-bromo-4-fluoro-2-hydroxybenzoate (10)

To a stirred solution of 5-bromo-4-fluoro-2-hydroxybenzoic acid 9[14] (500 mg, 2.13 mmol) in H_2SO_4 (5 mL) was added fuming HNO_3 (97 μL, 2.34 mmol) at 0 °C, and the resultant mixture was stirred at 0 °C for 1.5 h. The reaction mixture was poured into ice water and the mixture was extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to afford 5-bromo-4-fluoro-2-hydroxy-3-nitrobenzoic acid (495 mg, 83%) as a brown solid. HRMS (EI) *m/z*: 278.9169 (Calcd for $C_7H_3{}^{79}BrNO_5$ 278.9170). ^{1}H NMR ($CDCl_3$) δ: 8.21 (1H, d, J = 7.6 Hz). IR (ATR): 3076, 2860, 1668, 1541, 1431, 1228, 1213, 1167, 1072, 685, 654 cm^{-1}.

Benzyl bromide (456 μL, 3.83 mmol) and K_2CO_3 (481 mg, 3.48 mmol) were added to a solution of the obtained compound (195 mg, 0.70 mmol) in DMF (4 mL), and the mixture was stirred at 80 °C for 14 h. The mixture was poured into 1 M HCl aq at 0 °C, and then the mixture was extracted with AcOEt. The obtained organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography eluting with *n*-hexane/AcOEt = 2:1, v/v to afford benzyl 5-bromo-4-fluoro-2-hydroxy-3-nitrobenzoate (226 mg, 88%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ: 5.43 (2H, s), 7.30–7.63 (5H, m), 8.20 (1H, d, J = 7.1 Hz), 11.63 (1H, br s).

A mixture of the obtained compound (100 mg, 2.69 mmol) and iron powder (45 mg, 8.08 mmol) in AcOH (4 mL) was stirred at 120 °C for 3 h. AcOEt was added and the resultant mixture was filtered. The filtrate was concentrated in vacuo and the residue was diluted with $CHCl_3$/MeOH = 10:1, v/v. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography eluting with *n*-hexane/AcOEt = 9:1, v/v to give **10** (31 mg, 34%) as a colorless solid. Mp: 86–88 °C. MS (ESI) *m/z*: 340, 342 $(M+1)^+$. ^{1}H NMR ($CDCl_3$) δ: 3.95 (2H, br s), 5.36 (2H, s), 7.30–7.48 (6H, m), 10.98 (1H, d, J = 2.0 Hz).

5.1.2. Benzyl 5-bromo-2-*tert*-butyl-4-fluoro-1,3-benzoxazole-7-carboxylate (11)

To a solution of **10** (52 mg, 0.15 mmol) in benzene (3 mL) were added pyridine (27 μL, 0.34 mmol) and pivaloyl chloride (42 μL, 0.34 mmol), and then the mixture was stirred at 85 °C for 4 h. The reaction mixture was cooled to room temperature and diluted with AcOEt and 1 M HCl aq. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography eluting with *n*-hexane/AcOEt = 3:1, v/v to give benzyl 5-bromo-3-[(2,2-dimethylpropanoyl)amino]-4-fluoro-2-hydroxybenzoate (85 mg, 83%) as a colorless oil. HRMS (EI) *m/z*: 423.0471 (Calcd for $C_{19}H_{19}{}^{79}BrFNO_4$ 423.0482). ^{1}H NMR ($CDCl_3$) δ: 1.34 (9H, s), 5.38 (2H, s), 7.03 (1H, br s), 7.35–7.43 (5H, m), 7.95 (1H, d, *J* = 7.4 Hz), 11.19 (1H, d, *J* = 1.7 Hz).

To a solution of the obtained compound (82 mg, 0.19 mmol) in benzene (3 mL) was added *p*-toluenesulfonic acid monohydrate (37 mg, 0.19 mmol), and the mixture was stirred at 85 °C for 20 h. The reaction mixture was combined with AcOEt and satd $NaHCO_3$ aq. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography eluting with *n*-hexane/AcOEt = 5:1, v/v to give **11** (38 mg, 43%) as a colorless solid. Mp: 99–101 °C. MS (ESI) *m/z*: 406, 408 $(M+1)^+$. ^{1}H NMR ($CDCl_3$) δ: 1.50 (9H, s), 5.44 (2H, s), 7.35–7.45 (3H, m), 7.50–7.56 (2H, m), 8.17 (1H, d, *J* = 6.4 Hz).

5.1.3. 2-*tert*-Butyl-4-fluoro-5-phenyl-1,3-benzoxazole-7-carboxylic acid (12)

To a stirred solution of **11** (37 mg, 0.09 mmol) in THF (4 mL) were added phenylboronic acid (22 mg, 0.18 mmol), potassium phosphate (39 mg, 0.18 mmol), and tetrakis(triphenylphosphine) palladium(0) (11 mg, 0.09 mmol) at room temperature. The mixture was stirred at 70 °C for 16 h. The reaction mixture was combined with AcOEt and satd NH_4Cl aq. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography eluting with *n*-hexane/AcOEt = 5:1, v/v to give benzyl 2-*tert*-butyl-4-fluoro-5-phenyl-1,3-benzoxazole-7-carboxylate (32 mg, 87%) as a white solid. MS (ESI) *m/z*: 404 $(M+1)^+$. ^{1}H NMR ($CDCl_3$) δ: 1.53 (9H, s), 5.47 (2H, s), 7.33–7.43 (4H, m), 7.45–7.51 (2H, m), 7.52–7.61 (4H, m), 8.10 (1H, d, *J* = 6.9 Hz).

A mixture of the obtained compound (31 mg, 0.08 mmol) and 10% palladium on carbon (6.2 mg) in AcOEt (3 mL) was stirred under a hydrogen atmosphere at room temperature for 1 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to afford **12** (23 mg, 96%) as a white solid. Mp: 219–222 °C. MS (ESI) *m/z*: 314 $(M+1)^+$. ^{1}H NMR (DMSO-d_6) δ: 1.46 (9H, s), 7.44 (1H, t, *J* = 7.3 Hz), 7.51 (2H, t, *J* = 7.3 Hz), 7.57 (2H, t, *J* = 7.3 Hz), 7.89 (1H, d, *J* = 7.3 Hz).

5.1.4. 2-*tert*-Butyl-4-fluoro-5-phenyl-1,3-benzoxazole-7-carbonitrile (13)

To a stirred solution of **12** (22 mg, 0.07 mmol) in CH_2Cl_2 (3 mL) were added DMF (one drop) and oxalyl chloride (18 μL, 0.21 mmol) and the resultant mixture was stirred at room temperature for 13 h. The reaction mixture was concentrated in vacuo and azeotroped twice with toluene, and then the residue was dissolved with AcOEt (2 mL). Then, 28% NH_3 in water (1 mL) was added and the mixture was stirred at room temperature for 3 h. The reaction mixture was combined with $CHCl_3$/MeOH = 10:1, v/v and brine. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was dissolved with pyridine (3 mL). Trifluoromethanesulfonic anhydride (59 μL, 0.35 mmol) and 4-dimethylaminopyridine (1.7 mg, 0.014 mmol) were added, and the resultant mixture was stirred at room temperature for 3 h. The reaction mixture was combined with AcOEt and 1 M HCl aq, and the organic layer was washed with satd $NaHCO_3$ aq and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography eluting with *n*-hexane/AcOEt = 5:1, v/v to give **13** (18.7 mg, 90%) as a white solid. Mp: 119–121 °C. HRMS (EI) *m/z*: 294.1161 (Calcd for $C_{18}H_{15}FN_2O$ 294.1169). ^{1}H NMR ($CDCl_3$) δ: 1.56 (9H, s), 7.41–7.53 (5H, m), 7.69 (1H, d, *J* = 6.4 Hz). ^{13}C NMR ($CDCl_3$) δ: 28.4 (3C), 34.8, 92.1 (d, *J* = 4.8 Hz), 113.9, 126.6 (d, *J* = 12.5 Hz), 128.6, 128.9 (2C), 129.2, 129.2, 130.8 (d, *J* = 3.8 Hz), 131.4 (d, *J* = 18.2 Hz), 133.5, 152.6 (d, *J* = 8.6 Hz), 153.1 (d, *J* = 266.8 Hz), 175.8. IR (ATR): 2231, 1572, 1485, 1402, 1248, 1171, 1117, 1093, 918, 878, 773, 702 cm^{-1}.

5.1.5. 2-*tert*-Butyl-4-[(3*S*)-3-(dimethylamino)pyrrolidin-1-yl]-5-phenyl-1,3-benzoxazole-7-carbonitrile (3)

To a stirred solution of **13** (17 mg, 0.06 mmol) in DMSO (3 mL) were added triethylamine (24 μL, 0.17 mmol) and (3*S*)-3-(dimethylamino)pyrrolidine **14** (15 μL, 0.12 mmol). The mixture was stirred at 90 °C for 18 h, cooled to room temperature, and then combined with AcOEt and satd $NaHCO_3$ aq. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography eluting with $CHCl_3$/MeOH = 9:1, v/v to give **3** (17.5 mg, 78%) as a brown oil. HRMS (EI) *m/z*: 388.2225 (Calcd for $C_{24}H_{28}N_4O$ 388.2263). ^{1}H NMR ($CDCl_3$) δ: 1.47 (9H, s), 1.65 (1H, dq, *J* = 8.8, 10.5 Hz), 1.96–2.03 (1H, m), 2.17 (6H, s), 2.52–2.61 (1H, m), 3.40 (1H, dd, *J* = 8.8, 10.5 Hz), 3.50–3.65 (3H, m), 7.26–7.38 (6H, m). IR (ATR): 2216, 1618, 1471, 1338, 1119, 710 cm^{-1}.

5.1.6. 2-*tert*-Butyl-4-[(3*S*)-3-(dimethylamino)pyrrolidin-1-yl]-5-phenyl-1,3-benzoxazole-7-carbonitrile hydrochloride (3·HCl salt)

To a stirred solution of **3** (17 mg, 0.044 mmol) in Et_2O (3 mL) was added 1 M HCl in EtOH (48 μL, 0.048 mmol), and the mixture was stirred at room temperature for 4 h. The precipitate was collected by filtration and washed with Et_2O to afford the title compound (17 mg, 91%) as a white solid. Mp: 201–202 °C. HRMS (EI) *m/z*: 388.2225 (Calcd for $C_{24}H_{28}N_4O$ 388.2263). ^{1}H NMR (DMSO-d_6) δ: 1.46 (9H, s), 1.96–2.06 (1H, m), 2.10–2.20 (1H, m), 3.18–3.28 (1H, m), 3.31 (6H, s), 3.75–4.00 (3H, m), 7.34–7.48 (6H, m), 10.74 (1H, br). ^{13}C NMR (DMSO-d_6) δ: 26.0, 27.9 (3C), 33.7, 40.2, 40.9, 50.8, 52.4, 63.5, 82.4, 115.5, 125.4, 127.1, 128.3 (2C), 129.2 (2C), 130.8, 132.9, 140.2, 142.3, 150.9, 170.0. IR (ATR): 2295, 2220, 1618, 1477, 1466, 1454, 1354, 1122, 710 cm^{-1}. Anal. Calcd for $C_{24}H_{28}N_4O{\cdot}1.0HCl{\cdot}0.2H_2O$: C, 67.26; H, 6.91; N, 13.07; Cl, 8.27. Found: C, 67.62; H, 6.86; N, 12.67; Cl, 8.12. $[\alpha]_D^{25}$ +13.1 (*c* 1.00, MeOH).

5.1.7. 6-Bromo-2-*tert*-butyl-7-nitro-1,3-benzoxazole-4-carboxylic acid (16)

Following the procedures as described for 11, 5-bromo-2-[(2,2-dimethylpropanoyl)amino]-3-hydroxybenzoic acid was prepared in 98% yield from 15[16] and pivaloyl chloride as a brown solid. HRMS (FAB) *m/z*: 316.0232 (Calcd for $C_{12}H_{15}{}^{79}BrNO_4$ 315.9752). ^{1}H NMR ($CDCl_3$) δ: 1.41 (9H, s), 7.04 (1H, s), 7.45 (1H, d, *J* = 2.2 Hz), 7.80 (1H, d, *J* = 2.2 Hz). IR (ATR): 3360, 1749, 1583, 1466, 1302, 1088, 858, 789, 754 cm^{-1}.

To a stirred solution of the obtained compound (149 mg, 0.47 mmol) in AcOH (3 mL) was added fuming HNO_3 (22 μL, 0.52 mmol) and the resultant mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated in vacuo and the residue was combined with AcOEt and brine. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column

chromatography eluting with *n*-hexane/AcOEt = 3:1, v/v to give 5-bromo-2-[(2,2-dimethylpropanoyl)amino]-3-hydroxy-4-nitrobenzoic acid (22.0 mg, 13%). ^{1}H NMR ($CDCl_3$) δ: 1.43 (9H, s), 7.95 (1H, s).

Following the procedures as described for **11**, **16** was prepared in 98% yield from the obtained compound and *p*-toluenesulfonic acid monohydrate as a brown solid. Mp: 206–209 °C. HRMS (EI) *m/z*: 341.9821 (Calcd for $C_{12}H_{11}{}^{79}BrN_2O_5$ 341.9852), 343.9803 (Calcd for $C_{12}H_{11}{}^{81}BrN_2O_5$ 343.9831). ^{1}H NMR (CD_3OD) δ: 1.52 (9H, s), 8.20 (1H, s). ^{13}C NMR (CD_3OD) δ: 28.6 (3C), 35.7, 109.7, 131.2, 132.5, 136.7, 142.9, 145.3, 168.0, 177.5. IR (ATR): 1705, 1533, 1190, 1136, 808 cm^{-1}.

5.1.8. 7-Bromo-2-*tert*-butyl-6-phenyl-1,3-benzoxazole-4-carboxylic acid (17)

Following the procedures as described for **12**, 2-*tert*-butyl-7-nitro-6-phenyl-1,3-benzoxazole-4-carboxylic acid was prepared from **16** and phenylboronic acid as a brown oil. The obtained compound was used as such without further purification. HRMS (EI) *m/z*: 340.1064 (Calcd for $C_{18}H_{16}N_2O_5$ 340.1059). ^{1}H NMR ($CDCl_3$) δ: 1.41 (9H, s), 7.26–7.32 (2H, m), 7.38–7.45 (3H, m), 7.94 (1H, s). IR (ATR): 2976, 1711, 1533, 1358, 1236, 1115, 812, 698 cm^{-1}.

A mixture of the obtained compound (3.00 g) and 10% palladium on carbon (430 mg) in MeOH (30 mL) was stirred under a hydrogen atmosphere at room temperature for 16 h. The reaction mixture was filtered and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography eluting with $CHCl_3$/MeOH = 49:1, v/v to give 7-amino-2-*tert*-butyl-6-phenyl-1,3-benzoxazole-4-carboxylic acid (1.27 g, 66% in two steps) as a pale brown solid. HRMS (EI) *m/z*: 310.1295 (Calcd for $C_{18}H_{18}N_2O_3$ 310.1318). ^{1}H NMR ($CDCl_3$) δ: 1.52 (9H, s), 4.62 (2H, br), 7.35–7.50 (5H, m) 7.89 (1H, s). IR (ATR): 3323, 3209, 1724, 1635, 1556, 1363, 1329, 1227, 1153, 1109, 775, 702 cm^{-1}.

A mixture of copper(II) bromide (1.98 g, 8.86 mmol) and *tert*-butyl nitrite (1.07 mL, 8.06 mmol) in MeCN (20 mL) was stirred at 60 °C for 5 min. A solution of the obtained compound (1.25 g, 4.03 mmol) in MeCN (110 mL) was added, and the resultant mixture was stirred at the same temperature for 50 min. The mixture was combined with AcOEt and 0.5 M HCl aq. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography eluting with $CHCl_3$/MeOH = 49:1, v/v to give **17** (1.37 g, 91%) as a brown solid. Mp: 211–213 °C. HRMS (EI) *m/z*: 373.0312 (Calcd for $C_{18}H_{16}{}^{79}BrNO_3$ 373.0314), 375.0291 (Calcd for $C_{18}H_{16}{}^{81}BrNO_3$ 375.0295). ^{1}H NMR ($CDCl_3$) δ: 1.55 (9H, s), 7.38–7.52 (5H, m), 8.04 (1H, br s). IR (ATR): 2974, 1689, 1545, 1236, 1117, 931, 742, 698 cm^{-1}.

5.1.9. 7-Bromo-2-*tert*-butyl-6-phenyl-1,3-benzoxazole-4-carbonitrile (18)

Following the procedures as described for **13**, **18** was prepared in 62% yield from **17** as a colorless solid. Mp: 160–163 °C. HRMS (EI) *m/z*: 354.0368 (Calcd for $C_{18}H_{15}{}^{79}BrN_2O$ 354.0368). ^{1}H NMR ($CDCl_3$) δ: 1.56 (9H, s), 7.38–7.51 (5H, m), 7.60 (1H, s). IR (ATR): 2231, 1726, 1556, 1460, 1385, 1250, 1124, 1043, 771, 708 cm^{-1}.

5.1.10. 2-*tert*-Butyl-7-[(3*S*)-3-(dimethylamino)pyrrolidin-1-yl]-6-phenyl-1,3-benzoxazole-4-carbonitrile (4)

A suspension of (±)-2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (13.2 mg, 0.02 mmol) in toluene (2 mL) was heated at 80 °C until the reagent was dissolved completely. Palladium acetate (3.2 mg, 0.014 mmol) was added to the solution and the mixture was stirred at room temperature for 5 min. To the stirred mixture were added **18** (50 mg, 0.14 mmol), **14** (22 μL, 0.17 mmol), and sodium *tert*-butoxide (19 mg, 0.20 mmol) and the resultant mixture was stirred at 80 °C for 14 h. The mixture was cooled, and the precipitate was removed by filtration and washed with AcOEt. The filtrate was diluted with AcOEt and brine. The obtained organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography eluting with $CHCl_3$/MeOH = 19:1, v/v to give the title compound (18.5 mg, 34%) as a brown oil. HRMS (EI) *m/z*: 388.2251 (Calcd for $C_{24}H_{28}N_4O$ 388.2263). ^{1}H NMR ($CDCl_3$) δ: 1.50 (9H, s), 1.63–1.74 (1H, m), 1.98–2.08 (1H, m), 2.17 (6H, s), 2.57–2.66 (1H, m), 3.26 (1H, t, J = 9.5 Hz), 3.40–3.50 (3H, m), 7.30–7.40 (6H, m). IR (ATR): 2218, 1610, 1475, 1410, 1192, 1138, 750, 698 cm^{-1}.

5.1.11. 2-*tert*-Butyl-7-[(3*S*)-3-(dimethylamino)pyrrolidin-1-yl]-6-phenyl-1,3-benzoxazole-4-carbonitrile hydrochloride (4-HCl salt)

Following the procedures as described for **3**-HCl salt, **4-HCl** salt was prepared in 80% yield from **4** as a white solid. Mp: 180–183 °C. MS (ESI) *m/z*: 389 (M+1)$^+$. ^{1}H NMR (CD_3OD) δ: 1.53 (9H, s), 2.05 (1H, dq, J = 12.7, 8.3 Hz), 2.33–2.40 (1H, m), 2.70–2.91 (6H, br m), 3.47–3.61 (3H, m), 3.71 (1H, dd, J = 10.9, 7.4 Hz), 3.87 (1H, dq, J = 7.3, 7.3 Hz), 7.37–7.61 (6H, m). ^{13}C NMR (DMSO-d_6) δ: 26.1, 28.0 (3C), 33.9, 40.0, 41.2, 50.2, 51.7, 63.6, 90.0, 116.9, 126.3, 127.4, 128.3 (2C), 129.2 (2C), 133.1, 136.0, 139.7, 141.1, 142.7, 174.6. IR (ATR): 2216, 1610, 1483, 1406, 1140, 714 cm^{-1}. Anal. Calcd for $C_{24}H_{28}N_4O{\cdot}0.25H_2O{\cdot}1.0HCl$: C, 67.12; H, 6.92; N, 13.05; Cl, 8.25. Found: C, 67.23; H, 6.71; N, 12.82; Cl, 8.17. $[\alpha]_D^{25}$ +79.6 (*c* 0.697, $CHCl_3$/MeOH = 1:1, v/v).

5.1.12. Methyl 4-acetamido-5-bromo-2-hydroxy-3-iodobenzoate (20)

To a solution of **19** (1.1 g, 5.0 mmol) in DMF (8 mL) was added *N*-bromosuccinimide (0.94 g, 5.3 mmol) and the mixture was refluxed for 2 h. AcOEt was added and the mixture was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography eluting with $CHCl_3$/MeOH = 100:1, v/v to give methyl 4-acetamido-5-bromo-2-hydroxybenzoate (640 g, 45%) as a pale red solid. Mp: 171–173 °C. MS (FAB) *m/z*: 288 (M+1 for ^{79}Br)$^+$, 290 (M+1 for ^{81}Br)$^+$. ^{1}H NMR ($CDCl_3$) δ: 2.26 (3H, s), 3.94 (3H, s), 7.73 (1H, br s), 8.00 (1H, s), 8.16 (1H, s), 10.73 (1H, s).

To a solution of the obtained compound (576 mg, 2.0 mmol) in CH_2Cl_2 (20 mL) and MeOH (10 mL) were added $BnNMe_3Cl_2I$ (835 mg, 2.4 mmol) and $NaHCO_3$ (1.1 g, 13.0 mmol). The mixture was stirred at room temperature for 30 h and then filtered. The filtrate was concentrated in vacuo and the residue was diluted with $CHCl_3$ and satd NH_4Cl aq. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography eluting with $CHCl_3$/MeOH = 50:1, v/v to give the title compound (889 mg, 100%) as a pale yellow solid. Mp: 222–224 °C. MS (FAB) *m/z*: 414 (M+1 for ^{79}Br)$^+$, 416 (M+1 for ^{81}Br)$^+$. ^{1}H NMR ($CDCl_3$) δ: 2.26 (3H, s), 4.00 (3H, s), 7.13 (1H, br s), 8.12 (1H, s), 11.73 (1H, s). ^{13}C NMR (DMSO-d_6) δ: 22.7, 53.2, 94.9, 111.7, 112.5, 132.3, 145.6, 159.3, 167.5, 167.6. IR (KBr): 3219, 3178, 1672, 1436, 1232, 794 cm^{-1}. Anal. Calcd for $C_{10}H_9BrINO_4{\cdot}0.2H_2O$: C, 28.76; H, 2.27; N, 3.35; Br, 19.13; I, 30.39. Found: C, 28.86; H, 2.20; N, 3.43; Br, 19.27; I, 30.14.

5.1.13. Methyl 4-acetamido-5-bromo-2-*tert*-butyl-1-benzofuran-7-carboxylate (21)

To a stirred solution of triethylamine (4 mL) in 1,4-dioxane (6 mL) were added **20** (414 mg, 1.0 mmol), bis(triphenylphosphine)palladium(II) dichloride (49 mg, 0.07 mmol), copper(I) iodide (19 mg, 0.1 mmol), and 3,3-dimethyl-1-butyne (0.13 mL, 1.1 mmol) and the mixture was stirred at 30 °C for 2 h and then

refluxed for 2 h. The reaction mixture was concentrated and the residue was purified by silica gel column chromatography eluting with *n*-hexane/AcOEt = 2:1, v/v to give the title compound (165 mg, 54%) as a white solid. Mp: 186–188 °C. MS (FAB) *m/z*: 368 (M+1 for $^{79}Br)^+$, 370 (M+1 for $^{81}Br)^+$. ^1H NMR ($CDCl_3$) δ: 1.40 (9H, s), 2.30 (3H, s), 3.98 (3H, s), 6.38 (1H, s), 7.45 (1H, br s), 8.05 (1H, s). ^{13}C NMR (DMSO-d_6) δ: 24.0, 28.7 (3C), 33.2, 52.3, 99.8, 109.9, 113.1, 128.8, 128.9, 131.4, 153.1, 164.1, 167.9, 168.5. IR (KBr): 2965, 1658, 1539, 1391, 1285, 1269, 1160, 1071, 775 cm^{-1}. Anal. Calcd for $C_{16}H_{18}BrNO_4$: C, 52.19; H, 4.93; N, 3.80; Br, 21.70. Found: C, 52.11; H, 4.86; N, 3.86; Br, 21.98.

5.1.14. Methyl 4-bromo-2-*tert*-butyl-5-phenyl-1-benzofuran-7-carboxylate (22)

Following the procedures as described for **12**, methyl 4-acetamido-2-*tert*-butyl-5-phenyl-1-benzofuran-7-carboxylate was prepared in 91% yield from **21** and phenylboronic acid as a brown solid. MS (FAB) *m/z*: 366 (M+1)$^+$. ^1H NMR ($CDCl_3$) δ: 1.43 (9H, s), 2.11 (3H, br s), 3.98 (3H, s), 6.40 (1H, s), 7.03 (1H, br s), 7.34–7.50 (5H, m), 7.83 (1H, s).

To a solution of the obtained compound (2.28 g, 6.24 mmol) in MeOH (60 mL) was added 4 M HCl in 1,4-dioxane (15.6 mL, 62.4 mmol), and the mixture was stirred at room temperature for 25 h and then stirred at 50 °C for 5 h. The reaction mixture was poured into satd $NaHCO_3$ aq, and the mixture was extracted with AcOEt. The obtained organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography eluting with *n*-hexane/AcOEt = 3:1–1:1, v/v to give methyl 4-amino-2-*tert*-butyl-5-phenyl-1-benzofuran-7-carboxylate (972 mg, 48%) as a pale yellow solid. MS (FAB) *m/z*: 324 (M+1)$^+$. ^1H NMR ($CDCl_3$) δ: 1.43 (9H, s), 3.93 (3H, s), 4.35 (2H, br s), 6.31 (1H, s), 7.31–7.50 (5H, m), 7.74 (1H, s).

Following the procedures as described for **17**, **22** was prepared in 99% yield from the obtained compound (2.50 g, 7.73 mmol), copper(II) bromide, and *tert*-butyl nitrite as a brown oil. HRMS (ESI) Calcd. for $C_{20}H_{19}{}^{79}BrO_3$ + H: 387.0596. Found: 387.0584. ^1H NMR ($CDCl_3$) δ: 1.45 (9H, s), 3.99 (3H, s), 6.52 (1H, s), 7.35–7.47 (5H, m), 7.84 (1H, s). ^{13}C NMR ($CDCl_3$) δ: 28.8 (3C), 33.3, 52.2, 100.3, 113.5, 118.9, 127.6, 127.9, 128.0 (2C), 129.8 (2C), 132.8, 136.8, 140.4, 151.3, 165.0, 169.8. IR (KBr): 2966, 1722, 1594, 1435, 1393, 1241, 1146, 769 cm^{-1}.

5.1.15. 4-Bromo-2-*tert*-butyl-5-phenyl-1-benzofuran-7-carbonitrile (23)

To a solution of **22** (2.90 g, 7.49 mmol) in THF (50 mL) was added a solution of KOH (1.26 g, 22.5 mmol) in MeOH (12 mL) and the mixture was stirred at room temperature for 3.5 h. The mixture was concentrated and then 1 M HCl aq and water was added to the residue. The mixture was extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to afford 4-bromo-2-*tert*-butyl-5-phenyl-1-benzofuran-7-carboxylic acid (2.64 g, 94%) as a yellow solid. MS (FAB) *m/z*: 373 (M+1 for $^{79}Br)^+$, 375 (M+1 for $^{81}Br)^+$. ^1H NMR ($CDCl_3$) δ: 1.46 (9H, s), 6.55 (1H, s), 7.37–7.50 (5H, m), 7.94 (1H, s).

To a solution of the obtained compound (2.62 g, 7.02 mmol) and 1-hydroxybenzotriazole monohydrate (1.14 g, 8.42 mmol) in DMF (80 mL) was added EDC·HCl (1.61 g, 8.42 mmol) and the mixture was stirred at the room temperature for 1 h. To the reaction mixture was added 28% NH_4OH aq (1.42 mL, 21.1 mmol) at 0 °C, and then the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated, diluted with satd $NaHCO_3$ aq and AcOEt, and then extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography eluting with *n*-hexane/AcOEt = 3:2, v/v to give 4-bromo-2-*tert*-butyl-5-phenyl-1-benzofuran-7-carboxamide (2.59 g, 99%) as a yellow solid. MS (FAB) *m/z*: 372 (M+1 for $^{79}Br)^+$, 374 (M+1 for $^{81}Br)^+$. ^1H NMR ($CDCl_3$) δ: 1.45 (9H, s), 6.32 (1H, br s), 6.59 (1H, s), 7.26 (1H, br s), 7.35–7.47 (5H, m), 8.02 (1H, s).

To a solution of the obtained compound (2.56 g, 6.88 mmol) in CH_2Cl_2 (100 mL) were added triethylamine (2.88 mL, 20.7 mmol) and trifluoromethanesulfonic anhydride (1.86 mL, 11.0 mmol). The resultant mixture was stirred at room temperature for 2 h. Water was added and the mixture was extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography eluting with *n*-hexane/AcOEt = 10:1, v/v to give **23** (2.11 g, 87%) as a yellow solid. Mp: 126–128 °C. MS (FAB) *m/z*: 354 (M+1 for $^{79}Br)^+$, 356 (M+1 for $^{81}Br)^+$. ^1H NMR ($CDCl_3$) δ: 1.44 (9H, s), 6.54 (1H, s), 7.35–7.50 (6H, m). ^{13}C NMR ($CDCl_3$) δ: 28.6 (3C), 33.5, 94.8, 101.0, 114.8, 119.6, 128.1, 128.2 (2C), 129.0, 129.6 (2C), 132.5, 137.8, 139.3, 152.2, 170.8. IR (KBr): 3116, 2961, 2236, 1594, 1452, 1439, 1394, 1087, 921 cm^{-1}. Anal. Calcd for $C_{19}H_{16}BrNO$: C, 64.42; H, 4.55; N, 3.95; Br, 22.56. Found: C, 64.50; H, 4.46; N, 3.97; Br, 22.87.

5.1.16. 2-*tert*-Butyl-4-[(3*S*)-3-(dimethylamino)pyrrolidin-1-yl]-5-phenyl-1-benzofuran-7-carbonitrile (5)

Following the procedures as described for **4**, **5** was prepared in 33% yield from **23** as a yellow oil. HRMS (ESI) Calcd. for $C_{25}H_{29}N_3O$ + H: 388.2389. Found: 388.2382. ^1H NMR ($CDCl_3$) δ: 1.39 (9H, s), 1.60–1.74 (1H, m), 1.92–2.04 (1H, m), 2.15 (6H, s), 2.50–2.61 (1H, m), 3.13 (1H, t, *J* = 9.0 Hz), 3.31–3.42 (3H, m), 6.65 (1H, s), 7.24–7.38 (6H, m). ^{13}C NMR ($CDCl_3$) δ: 28.8 (3C), 30.4, 33.0, 44.2 (2C), 51.7, 57.4, 65.5, 84.8, 100.4, 117.0, 118.7, 124.1, 126.7, 128.3 (2C), 129.4 (2C), 132.8, 142.0, 145.1, 155.3, 165.7. IR (ATR): 2969, 2869, 2774, 2219, 1602, 1465 cm^{-1}. $[\alpha]_D^{25}$ −4.16 (*c* 1.07, $CHCl_3$).

5.1.17. 3-Fluoro-2-methoxy-5-methylaniline (26)

Following the procedures as described for **10**, 2-fluoro-4-methyl-6-nitrophenol was prepared in 98% yield from **25** and fuming HNO_3 in H_2SO_4 as a brown solid. Mp: 63–64 °C. HRMS (EI) *m/z*: 171.0326 (Calcd for $C_7H_6FNO_3$ 171.0332). ^1H NMR ($CDCl_3$) δ: 2.35 (3H, s), 7.26 (1H, dd, *J* = 2.2, 10.5 Hz), 7.71 (1H, s), 10.29 (1H, s). ^{13}C NMR ($CDCl_3$) δ: 20.5, 119.6 (d, *J* = 3.8 Hz), 124.3 (d, *J* = 17.3 Hz), 129.1 (d, *J* = 5.8 Hz), 134.4, 142.7 (d, *J* = 15.4 Hz), 152.0 (d, *J* = 250.5 Hz). IR (ATR): 3223, 1638, 1549, 1381, 1237, 1125, 770 cm^{-1}.

To a solution of the obtained compound (8.79 g, 51.4 mmol) in toluene (51 mL) was added Na_2CO_3 (25.6 g, 154 mmol), followed by the dropwise addition of dimethyl sulfate (13.4 mL, 141 mol) at room temperature. After being stirred under reflux for 6 h, the reaction mixture was cooled to room temperature. 10% Na_2CO_3 aq was added, and the resulting mixture was extracted with AcOEt, washed with water and brine, dried over Mg_2SO_4, filtered, and concentrated. Column chromatography on silica gel (elution with *n*-hexane/AcOEt = 9:1, v/v) provided 6.58 g (69%) of 2-fluoro-4-methyl-6-nitroanisole as a pale yellow oil. ^1H NMR ($CDCl_3$) δ: 2.37 (3H, s), 4.02 (3H, d, *J* = 1.5 Hz), 7.13–7.17 (1H, m), 7.37–7.39 (1H, m). ^{13}C NMR ($CDCl_3$) δ: 20.7, 62.6 (d, *J* = 5.8 Hz), 120.3 (d, *J* = 2.9 Hz), 121.7 (d, *J* = 19.2 Hz), 134.1 (d, *J* = 7.7 Hz), 139.8 (d, *J* = 14.4 Hz), 144.3, 155.8 (d, *J* = 250.5). IR (ATR): 2950, 1532, 1498, 1355, 1302, 1245, 1130, 992, 858, 771 cm^{-1}. Anal. Calcd for $C_8H_8FNO_3$: C, 51.90; H, 4.36; N, 7.56; F, 10.26. Found: C, 52.06; H, 4.55; N, 7.47; F, 10.24.

To a solution of the obtained compound (250 mg, 1.35 mmol) in MeOH (5 mL) was added 5% palladium on carbon (100 mg), and the mixture was stirred under a hydrogen atmosphere at room temperature for 30 min. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. Column chromatography on silica gel (elution with *n*-hexane/AcOEt = 9:1,

v/v) provided 170 mg (81%) of **26** as a yellow oil. HRMS (ESI) Calcd for $C_8H_{10}FNO$ + H: 156.0825. Found: 156.0813. ^1H NMR ($CDCl_3$) δ: 2.19 (3H, s), 3.84 (2H, br s), 3.87 (3H, d, J = 1.2 Hz), 6.26–6.32 (2H, m). ^{13}C NMR ($CDCl_3$) δ: 21.0 (d, J = 1.9 Hz), 60.8 (d, J = 4.8 Hz), 106.7 (d, J = 19.2 Hz), 111.5 (d, J = 1.9 Hz), 132.6 (d, J = 13.4 Hz), 134.0 (d, J = 8.6 Hz), 140.7 (d, J = 4.8 Hz), 155.7 (d, J = 242.8). IR (ATR): 3373, 2936, 1629, 1584, 1516, 1352, 1227, 999, 815 cm^{-1}.

5.1.18. 4-Bromo-3-fluoro-6-iodo-2-methoxy-5-methylphenylamine (27)

To a solution of **26** (1.55 g, 10.0 mmol) in DMF (20 mL) was added *N*-bromosuccinimide (1.84 g, 10.2 mmol) in DMF (10 mL) at 0 °C over a period of 25 min. The mixture was heated at room temperature and stirred for 18 h. Brine was added and the resulting mixture was extracted with AcOEt. The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated. Column chromatography on silica gel (elution with *n*-hexane/AcOEt = 4:1, v/v) provided 1.91 g (81%) of 4-bromo-3-fluoro-2-methoxy-5-methylphenylamine as a brown solid. HRMS (ESI) *m/z*: 233.9925, 235.9903 (Calcd for C_8H_9BrFNO + H 233.9930, 235.9909). ^1H NMR ($CDCl_3$) δ: 2.27 (3H, s), 3.80–3.94 (5H, m), 6.43 (1H, s). ^{13}C NMR ($CDCl_3$) δ: 22.2 (d, J = 2.9 Hz), 61.0 (d, J = 4.8 Hz), 99.4 (d, J = 18.2 Hz), 111.8 (d, J = 2.9 Hz), 133.6 (d, J = 15.4 Hz), 133.7, 139.6 (d, J = 4.8 Hz), 152.7 (d, J = 243.8). IR (ATR): 3433, 3312, 2951, 1632, 1575, 1499, 1363, 1248, 989, 976, 816 cm^{-1}. Anal. Calcd for C_8H_9BrFNO: C, 41.05; H, 3.88; N, 5.98; F, 8.12; Br, 34.14. Found: C, 40.84; H, 3.80; N, 6.02; F, 8.25; Br, 34.05.

To a solution of the obtained compound (1.90 g, 8.1 mmol) in AcOH (40 mL) was added *N*-iodosuccinimide (1.91 g, 8.5 mmol), and the mixture was stirred at room temperature for 67 h. Brine was added and the resulting mixture was extracted with AcOEt. The combined organic phases were washed with brine, dried over Na_2SO_4, filtered, and concentrated. Column chromatography on silica gel (elution with *n*-hexane/AcOEt = 19:1, v/v) afforded 2.86 g (98%) of **27** as a pale brown solid. Mp: 89–90 °C. HRMS (ESI) *m/z*: 359.8931, 361.8900 (Calcd for $C_8H_8BrFINO$ + H 359.8896, 361.8876). ^1H NMR ($CDCl_3$) δ: 2.62 (3H, s), 3.91 (3H, d, J = 1.5 Hz), 4.50 (2H, br s). ^{13}C NMR ($CDCl_3$) δ: 29.2 (d, J = 1.9 Hz), 61.1 (d, J = 4.8 Hz), 83.4 (d, J = 2.9 Hz), 98.2 (d, J = 19.2 Hz), 131.9 (d, J = 15.4 Hz), 135.5, 141.2 (d, J = 5.8 Hz), 152.2 (d, J = 242.8). IR (ATR): 3363, 1606, 1464, 1433, 983, 823 cm^{-1}. Anal. Calcd for $C_8H_8BrFINO$: C, 26.69; H, 2.24; N, 3.89; F, 5.28; Br, 22.20; I, 35.25. Found: C, 26.59; H, 2.27; N, 3.90; F, 5.66; Br, 22.38; I, 35.30.

5.1.19. 2-Amino-5-bromo-4-fluoro-3-hydroxy-6-methylbenzonitrile (24)

A mixture of **27** (359 mg, 1.0 mmol), zinc cyanide (59 mg, 0.5 mmol), 1,1′-bis(diphenylphosphino)ferrocene (67 mg, 0.12 mmol), and bis(dibenzylideneacetone)palladium(0) (58 mg, 0.1 mmol) in DMF (10 mL)-water (0.1 mL) was heated at 120–130 °C for 3 h under a nitrogen atmosphere. Brine was added and the resulting mixture was extracted with AcOEt. The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated. Column chromatography on silica gel (elution with *n*-hexane/AcOEt = 9:1, v/v) provided 208 g (80%) of 2-amino-5-bromo-4-fluoro-3-methoxy-6-methylbenzonitrile as a white solid. Mp: 151–152 °C. MS (FAB) *m/z*: 258 (M+1 for $^{79}Br)^+$, 260 (M+1 for $^{81}Br)^+$. ^1H NMR ($CDCl_3$) δ: 2.51 (3H, s), 3.93 (3H, d, J = 1.8 Hz), 4.70 (2H, br s). ^{13}C NMR ($CDCl_3$) δ: 21.2 (d, J = 1.9 Hz), 61.3 (d, J = 5.8 Hz), 93.8 (d, J = 1.9 Hz), 99.9 (d, J = 20.2 Hz), 115.8 (d, J = 1.9 Hz), 132.8 (d, J = 15.4 Hz), 136.9 (d, J = 1.9 Hz), 144.4 (d, J = 7.7 Hz), 153.8 (d, J = 250.5). IR (ATR): 3466, 3345, 2223, 1629, 1486, 1257, 987, 781 cm^{-1}. Anal. Calcd for $C_9H_8BrFN_2O$: C, 41.72; H, 3.11; N, 10.81; F, 7.33; Br, 30.84. Found: C, 42.02; H, 3.07; N, 10.88; F, 7.52; Br, 30.56.

Tribromoborane (100 mL, 100.00 mmol) (1 M in CH_2Cl_2) was added dropwise to a solution of the obtained compound (8.63 g, 33.30 mmol) in CH_2Cl_2 (270 mL) at −20 °C, and the mixture was stirred at room temperature for 15 h. Ice water was added and the resulting mixture was neutralized by satd $NaHCO_3$ aq. AcOEt was added and the organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was recrystallized from *n*-hexane/CH_2Cl_2 = 1:1, v/v to afford 7.86 g (96%) of **24** as a pale brown solid. Mp: 214–215 °C. MS (ESI) *m/z*: 245 $(M+1)^+$. ^1H NMR (DMSO-d_6) δ: 2.36 (3H, s), 5.88 (2H, s). ^{13}C NMR (DMSO-d_6) δ: 20.2 (d, J = 1.9 Hz), 91.6, 96.7 (d, J = 20.2 Hz), 116.3, 129.8 (d, J = 17.3 Hz), 131.0, 143.8 (d, J = 7.7 Hz), 150.3 (d, J = 240.9 Hz). IR (ATR): 3436, 3357, 3198, 2227, 1641, 1588, 1491, 1234, 1088, 888 cm^{-1}. Anal. Calcd for $C_8H_6BrFN_2O$: C, 39.21; H, 2.47; N, 11.43; F, 7.75; Br, 32.61. Found: C, 39.15; H, 2.38; N, 11.47; F, 7.59; Br, 32.33.

5.1.20. 2-Amino-4-fluoro-3-hydroxy-6-methyl-5-phenylbenzonitrile (28)

A solution of **24** (30.0 g, 122 mmol), phenylboronic acid (29.8 g, 245 mmol), K_3PO_4 (52.0 g, 245 mmol) and tetrakis(triphenylphosphine)palladium(0) (13.7 g, 12.2 mmol) in 1,4-dioxane (1000 mL) and water (100 mL) was stirred at 95 °C for 2 days. AcOEt was added to the mixture, and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. Column chromatography on silica gel (elution with $CHCl_3$/AcOEt = 3:1, v/v) provided 27.1 g (91%) of **28** as a colorless solid. Mp: 197–199 °C. ^1H NMR ($CDCl_3$) δ: 2.22 (3H, s), 4.61 (2H, br s), 5.09 (1H, d, J = 4.2 Hz), 7.19–7.46 (5H, m). ^{13}C NMR (DMSO-d_6) δ: 17.8 (d, J = 2.9 Hz), 91.5, 116.9, 118.5 (d, J = 17.3 Hz), 127.4, 128.3 (2C), 129.0 (d, J = 18.2 Hz), 130.0 (d, J = 3.8 Hz), 130.3 (2C), 133.7, 143.3 (d, J = 7.7 Hz), 151.3 (d, J = 240.9 Hz). IR (KBr): 3406, 3277, 2220, 1647, 1493, 1267, 1190 cm^{-1}. Anal. Calcd for $C_{14}H_{11}FN_2O$: C, 69.41; H, 4.58; N, 11.56; F, 7.84. Found: C, 69.50; H, 4.57; N, 11.46; F, 7.61.

5.1.21. 2-*tert*-Butyl-7-fluoro-5-methyl-6-phenyl-1,3-benzoxazole-4-carbonitrile (29)

Following the procedures as described for **11**, **29** was prepared in 93% yield from the obtained compound (9.18 g, 37.46 mmol) as a white solid. Mp: 192–194 °C. MS (ESI) *m/z*: 309 $(M+1)^+$. ^1H NMR ($CDCl_3$) δ: 1.54 (9H, s), 2.41 (3H, s), 7.22–7.28 (2H, m), 7.42–7.55 (3H, m). ^{13}C NMR ($CDCl_3$) δ: 19.2 (d, J = 1.9 Hz), 28.4 (3C), 34.8, 99.9 (d, J = 3.8 Hz), 115.0, 127.2 (d, J = 13.4 Hz), 128.5, 128.8 (2C), 130.0 (2C), 132.7, 136.6 (d, J = 12.5 Hz), 139.3 (d, J = 1.9 Hz), 145.9 (d, J = 3.8 Hz), 146.6 (d, J = 258.2 Hz), 176.6. IR (KBr): 2972, 2229, 1559, 1116, 725 cm^{-1}. Anal. Calcd for $C_{19}H_{17}FN_2O{\cdot}0.2H_2O$: C, 73.15; H, 5.62; N, 8.98; F, 6.09. Found: C, 73.52; H, 5.49; N, 9.07; F, 6.16.

5.1.22. 2-*tert*-Butyl-7-[(3*S*)-3-(dimethylamino)pyrrolidin-1-yl]-5-methyl-6-phenyl-1,3-benzoxazole-4-carbonitrile hydrochloride (6-HCl salt)

Following the procedures as described for **3**-HCl salt, **6**-HCl salt was prepared in 88% yield from **29** as a pale yellow solid. Mp: 165–168 °C. MS (FAB) *m/z*: 403 $(M+1-HCl)^+$. ^1H NMR (DMSO-d_6) δ: 1.47 (9H, s), 1.87–1.97 (1H, m), 2.09–2.17 (1H, m), 2.12 (3H, s), 2.57–2.61 (3H, m), 2.65–2.69 (3H, m), 3.03–3.10 (1H, m), 3.17–3.24 (1H, m), 3.43–3.51 (1H, m), 3.62–3.68 (1H, m), 3.71–3.80 (1H, m), 7.23 (1H, d, J = 6.9 Hz), 7.30 (1H, d, J = 8.6 Hz), 7.41–7.51 (3H, m). ^{13}C NMR (DMSO-d_6) δ: 19.7, 26.0, 28.0 (3C), 33.9, 40.0, 40.9, 49.8, 51.7, 63.3, 91.5, 116.7, 126.8, 127.7, 128.4, 128.6, 130.6, 130.9, 135.9, 138.4, 138.5, 139.7, 142.9, 174.4. IR (KBr): 2969, 2581, 2464, 2208, 1608, 1471, 1367 cm^{-1}. Anal. Calcd for $C_{25}H_{30}N_4O{\cdot}HCl{\cdot}H_2O$: C, 65.70; H, 7.28; N, 12.26; Cl, 7.76. Found: C, 65.85; H, 7.31; N, 12.14; Cl, 7.72. $[\alpha]_D^{25}$ +36.7 (c 1.02, MeOH).

5.1.23. 2-*tert*-Butyl-5-methyl-7-[(3*S*)-3-(methylamino)pyrrolidin-1-yl]-6-phenyl-1,3-benzoxazole-4-carbonitrile hydrochloride (30)

Following the procedures as described for **3**-HCl salt, **30** was prepared in 89% yield from **29** and (3*S*)-3-(methylamino)pyrrolidine (**31**) as a pale yellow solid. Mp: 253–255 °C. MS (FAB) *m/z*: 389 $(M+1)^+$. ^{1}H NMR (DMSO-d_6) δ: 1.47 (9H, s), 1.86–1.94 (1H, m), 2.01–2.09 (1H, m), 2.10 (3H, s), 2.46 (3H, s), 3.18–3.38 (2H, m), 3.38–3.48 (2H, m), 3.55–3.63 (1H, m), 7.23–7.30 (2H, m), 7.41–7.49 (3H, m). ^{13}C NMR (DMSO-d_6) δ: 19.8, 27.1, 28.0 (3C), 30.6, 33.8, 49.1, 52.9, 56.6, 90.7, 116.8, 125.7, 127.6, 128.4, 128.4, 130.8, 131.0, 136.1, 138.6, 138.6, 139.2, 142.8, 174.2. IR (ATR): 3512, 2971, 2218, 1606, 1465, 1365, 1304, 709 cm^{-1}. Anal. Calcd for $C_{24}H_{28}N_4O{\cdot}HCl{\cdot}0.5H_2O$: C, 66.42; H, 6.97; N, 12.91; Cl, 8.17. Found: C, 66.41; H, 6.96; N, 12.57; Cl, 8.13. $[\alpha]_D^{25}$ +30.6 (*c* 1.01, MeOH).

5.1.24. 6-Bromo-7-fluoro-2,5-dimethyl-1,3-benzoxazole-4-carbonitrile (32a)

Acetyl chloride (3.85 mL, 54.2 mmol) was added dropwise to a solution of **24** (8.85 g, 36.1 mmol) and *N,N*-diisopropylethylamine (22.0 mL, 126 mmol) in AcOEt (400 mL) at 0 °C, and then the mixture was stirred at room temperature for 14 h. AcOEt was added and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. A solution of the residue and *p*-toluenesulfonic acid monohydrate (200 mg) in toluene (500 mL) was stirred at reflux for 3 h. The mixture was concentrated and the residue was diluted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was recrystallized from AcOEt to afford 7.07 g (73%) of **32a** as a colorless solid. MS (EI) *m/z*: 269 (M^+). ^{1}H NMR ($CDCl_3$) δ: 2.73 (3H, s), 2.75 (3H, s). IR (ATR): 2229, 1574, 1475, 1406, 1396, 1384, 1333, 1315, 1273, 1198, 1134 cm^{-1}.

5.1.25. 6-Bromo-2-cyclopropyl-7-fluoro-5-methyl-1,3-benzoxazole-4-carbonitrile (32b)

Following the procedures as described for **32a**, **32b** was prepared in 80% yield from **24** as a pale red solid. Mp: 164–168 °C. MS (ESI) *m/z*: 295, 297 $(M+1)^+$. ^{1}H NMR ($CDCl_3$) δ: 1.27–1.34 (2H, m), 1.38–1.42 (2H, m), 2.25–2.32 (1H, m), 2.73 (3H, s). ^{13}C NMR (DMSO-d_6) δ: 8.9, 10.5 (2C), 21.3, 98.3, 106.9 (d, J = 17.3 Hz), 114.2, 135.5 (d, J = 10.6 Hz), 139.3, 145.1 (d, J = 196.7 Hz), 146.3, 172.5. IR (ATR): 3057, 2230, 1625, 1567, 1317, 1131, 1034 cm^{-1}. Anal. Calcd for $C_{12}H_8BrFN_2O$: C, 48.84; H, 2.73; N, 9.49; F, 6.44. Found: C, 49.02; H, 2.75; N, 9.57; F, 6.55.

5.1.26. General procedure for preparation of 7-fluoro-5-methyl-6-phenyl-1,3-benzoxazole-4-carbonitrile (33)

Following the procedures as described for **32**, **33** was prepared from **28**.

5.1.27. 2-Cyclopropyl-7-fluoro-5-methyl-6-phenyl-1,3-benzoxazole-4-carbonitrile (33a)

Yield, 85% from **28** with cyclopropanecarbonyl chloride, as a colorless solid. MS (ESI) *m/z*: 293 $(M+1)^+$. ^{1}H NMR ($CDCl_3$) δ: 1.26–2.01 (2H, m), 2.38–2.42 (2H, m), 2.28–2.34 (1H, m), 2.40 (3H, s), 7.21–7.26 (2H, m), 7.42–7.51 (3H, m). ^{13}C NMR ($CDCl_3$) δ: 9.5, 10.4 (2C), 19.1 (d, J = 1.9 Hz), 99.1 (d, J = 3.8 Hz), 115.0, 126.8 (d, J = 14.4 Hz), 128.5, 128.8 (2C), 130.0 (2C), 132.7, 136.1 (d, J = 13.4 Hz), 139.3, 146.3 (d, J = 3.8 Hz), 146.3 (d, J = 258.2 Hz), 172.0. IR (ATR): 2221, 1562, 1412, 1321, 1122, 1026, 722 cm^{-1}. Anal. Calcd for $C_{18}H_{13}FN_2O$: C, 73.96; H, 4.48; N, 9.58; F, 6.50. Found: C, 73.65; H, 4.47; N, 9.54; F, 6.23.

5.1.28. 2-Cyclobutyl-7-fluoro-5-methyl-6-phenyl-1,3-benzoxazole-4-carbonitrile (33b)

Yield, 77% from **28** with cyclobutanecarbonyl chloride, as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 2.06–2.25 (2H, m), 2.42 (3H, s), 2.45–2.68 (4H, m), 3.83–3.93 (1H, m), 7.23–7.27 (2H, m), 7.44–7.53 (3H, m).

5.1.29. 2-Cyclopentyl-7-fluoro-5-methyl-6-phenyl-1,3-benzoxazole-4-carbonitrile (33c)

Yield, 42% from **28** with cyclopentanecarbonyl chloride, as a colorless amorphous solid. ^{1}H NMR ($CDCl_3$) δ: 1.72–1.89 (4H, m), 1.98–2.22 (4H, m), 2.41 (3H, s), 3.41–3.49 (1H, m), 7.25–7.29 (2H, m), 7.43–7.54 (3H, m).

5.1.30. 2-Cyclopent-1-enyl-7-fluoro-5-methyl-6-phenyl-1,3-benzoxazole-4-carbonitrile (33d)

Yield, 56% from **28** with 1-cyclopentene-1-carbonyl chloride, as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 2.08–2.17 (2H, m), 2.42 (3H, s), 2.66–2.73 (2H, m), 2.92–2.99 (2H, m), 7.07–7.11 (1H, m), 7.25–7.29 (2H, m), 7.44–7.54 (3H, m).

5.1.31. 7-[(3*S*)-3-(Dimethylamino)pyrrolidin-1-yl]-2,5-dimethyl-6-phenyl-1,3-benzoxazole-4-carbonitrile (34a)

Following the procedures as described for **28**, 7-fluoro-2,5-dimethyl-6-phenyl-1,3-benzoxazole-4-carbonitrile was prepared in 45% yield from **32a** as a colorless solid. MS (ESI) *m/z*: 267 $(M+1)^+$. ^{1}H NMR ($CDCl_3$) δ: 2.42 (3H, s), 2.76 (3H, s), 7.23–7.27 (2H, m), 7.40–7.53 (3H, m). To the solid (266 mg, 1.00 mmol) in DMSO (5 mL) were added **14** (190 μL, 1.50 mmol) and triethylamine (300 μL, 2.15 mmol), and then the mixture was heated at 80 °C for 14 h. The reaction mixture was diluted with $CHCl_3$ and the organic layer was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated. Column chromatography on silica gel (elution with $CHCl_3$/MeOH = 19:1, v/v) provided 110 mg (31%) of **34a** as a colorless solid. HRMS (ESI) *m/z*: 361.2000 (Calcd for $C_{22}H_{24}N_4O$ + H 361.2028). ^{1}H NMR ($CDCl_3$) δ: 1.53–1.67 (1H, m), 1.90–1.98 (1H, m), 2.19 (6H, s), 2.28 (3H, s), 2.45–2.58 (1H, m), 2.66 (3H, s), 2.90–2.98 (1H, m), 3.25–3.43 (3H, m), 7.10–7.14 (1H, m), 7.20–7.26 (1H, m), 7.32–7.50 (3H, m). ^{13}C NMR (DMSO-d_6) δ: 14.0, 19.5, 29.0, 43.1 (2C), 50.6, 55.2, 64.7, 90.6, 116.7, 126.2, 127.4, 128.4, 129.6, 130.8, 131.2, 136.8, 138.4, 139.2, 139.9, 143.5, 165.1. IR (ATR): 2210, 1670, 1608, 1585, 1442, 1248, 1153 cm^{-1}. $[\alpha]_D^{25}$ +69.2 (*c* 1.03, $CHCl_3$/MeOH = 1:1, v/v).

5.1.32. 2-Cyclopropyl-7-[(3*S*)-3-(dimethylamino)pyrrolidin-1-yl]-5-methyl-6-phenyl-1,3-benzoxazole-4-carbonitrile (34b)

Following the procedures as described for **34a**, **34b** was prepared in 63% yield from **32b** as a white solid. MS (ESI) *m/z*: 387 $(M+1)^+$. ^{1}H NMR ($CDCl_3$) δ: 1.17–2.30 (4H, m), 1.55–1.67 (1H, m), 1.90–1.98 (1H, m), 2.14 (6H, s), 2.17 (3H, s), 2.24 (1H, ddd, J = 5.1, 8.5, 13.0 Hz), 2.48–2.57 (1H, m), 2.94 (1H, t, J = 9.3 Hz), 3.22–3.39 (3H, m), 7.10 (1H, d, J = 7.6 Hz), 7.23 (1H, d, J = 7.6 Hz), 7.31–7.42 (3H, m). ^{13}C NMR ($CDCl_3$) δ: 9.4 (2C), 9.6, 20.1, 30.3, 44.1 (2C), 50.9, 56.3, 65.4, 91.6, 117.2, 125.9, 127.4, 128.2, 128.6, 131.0, 131.5, 136.5, 139.2, 139.5, 139.6, 144.2, 169.4. IR (ATR): 2206, 1585, 1560, 1466, 1152, 712, 702 cm^{-1}. Anal. Calcd for $C_{24}H_{26}N_4O{\cdot}0.25H_2O$: C, 73.72; H, 6.83; N, 14.33. Found: C, 74.08; H, 6.76; N, 14.35. $[\alpha]_D^{25}$ +97.2 (*c* 0.746, $CHCl_3$).

5.1.33. 2-Cyclobutyl-7-[(3*S*)-3-(dimethylamino)pyrrolidin-1-yl]-5-methyl-6-phenyl-1,3-benzoxazole-4-carbonitrile (34c)

Following the procedures as described for **3**, **34c** was prepared in 47% yield from **33b** as a white amorphous. MS (ESI) *m/z*: 401 $(M+1)^+$. ^{1}H NMR ($CDCl_3$) δ: 1.56–1.68 (1H, m), 2.14 (6H, s), 2.19 (3H, s), 2.06–2.20 (3H, m), 2.41–2.56 (5H, m), 2.99 (1H, t, J = 9.3 Hz), 3.27–3.43 (3H, m), 3.77–3.86 (1H, m), 7.09–7.13 (1H, m), 7.23–7.27 (1H, m), 7.32–7.44 (3H, m). ^{13}C NMR (DMSO-d_6) δ: 18.3, 19.6, 26.5, 26.6, 29.2, 32.8, 43.2 (2C), 50.6, 55.3, 64.7, 90.6, 116.7, 126.1, 127.4, 128.1, 128.3, 130.8, 131.3, 136.9, 138.5, 139.3, 139.6, 143.3, 170.2. .IR (ATR): 2210, 1604, 1583, 1468,

1362, 1153, 701 cm^{-1}. Anal. Calcd for $C_{25}H_{28}N_4O{\cdot}0.25H_2O$: C, 74.14; H, 7.09; N, 13.83. Found: C, 74.50; H, 7.09; N, 13.72. $[\alpha]_D^{25}$ +92.6 (*c* 1.05, $CHCl_3$).

5.1.34. 2-Cyclopentyl-7-[(3*S*)-3-(dimethylamino)pyrrolidin-1-yl]-5-methyl-6-phenyl-1,3-benzoxazole-4-carbonitrile (34d)

Following the procedures as described for **3**, **34d** was prepared in 33% yield from **33c** as a white solid. Mp: 120–122 °C. MS (ESI) *m/z*: 415 $(M+1)^+$. ^1H NMR ($CDCl_3$) δ: 1.53–2.09 (8H, m), 2.13 (6H, s), 2.14–2.18 (2H, m), 2.19 (3H, s), 2.45–2.56 (1H, m), 2.98 (1H, t, *J* = 9.2 Hz), 3.22–3.46 (4H, m), 7.09–7.13 (1H, m), 7.22–7.26 (1H, m), 7.31–7.43 (3H, m). ^{13}C NMR (DMSO-d_6) δ: 19.6, 25.2 (2C), 29.2, 30.9 (2C), 38.2, 43.2 (2C), 50.6, 55.4, 64.7, 90.5, 116.8, 125.9, 127.4, 128.1, 128.3, 130.8, 131.3, 136.9, 138.4, 139.3, 139.6, 143.2, 171.3. IR (ATR): 2952, 2867, 2208, 1604, 1558, 1468, 1363, 1299, 1191, 704 cm^{-1}. Anal. Calcd for $C_{26}H_{30}N_4O{\cdot}0.25H_2O$: C, 74.52; H, 7.34; N, 13.37. Found: C, 74.57; H, 7.31; N, 13.31. $[\alpha]_D^{25}$ +90.4 (*c* 1.04, $CHCl_3$).

5.1.35. 2-Cyclopent-1-enyl-7-[(3*S*)-3-(dimethylamino)pyrrolidin-1-yl]-5-methyl-6-phenyl-1,3-benzoxazole-4-carbonitrile (34e)

Following the procedures as described for **3**, **34e** was prepared in 18% yield from **33d** as a pale yellow solid. Mp: 98–101 °C. MS (ESI) *m/z*: 413 $(M+1)^+$. ^1H NMR ($CDCl_3$) δ: 1.57–1.60 (1H, m), 1.89–1.99 (1H, m), 2.13 (6H, s), 2.05–2.16 (2H, m), 2.20 (3H, s), 2.44–2.55 (1H, m), 2.60–2.68 (2H, m), 2.88–3.03 (3H, m), 3.25–3.42 (3H, m), 6.87 (1H, br s), 7.12 (1H, d, *J* = 7.6 Hz), 7.25–7.27 (1H, m), 7.31–7.44 (3H, m). IR (ATR): 2202, 1603, 1466, 1442, 1362, 1302, 706 cm^{-1}. Anal. Calcd for $C_{26}H_{28}N_4O{\cdot}1.0H_2O$: C, 72.53; H, 7.02; N, 13.01. Found: C, 72.62; H, 6.99; N, 12.70. $[\alpha]_D^{25}$ +97.8 (*c* 0.225, $CHCl_3$).

5.1.36. 2-Amino-7-[(3*S*)-3-(dimethylamino)pyrrolidin-1-yl]-5-methyl-6-phenyl-1,3-benzoxazole-4-carbonitrile (35)

A suspension of **28** (1.66 g, 6.86 mmol) and di(1*H*-imidazol-1-yl)methanimine (2.21 g, 13.7 mmol) in THF (33 mL) was refluxed under nitrogen for 5 days. After the mixture was concentrated, the residue was diluted with $CHCl_3$/MeOH (9:1, v/v) and water, and extracted with $CHCl_3$. The organic layer was separated, dried (Na_2SO_4), and evaporated to give the crude product, which was recrystallized from MeOH to yield 2-amino-7-fluoro-5-methyl-6-phenyl-1,3-benzoxazole-4-carbonitrile (1.60 g, 87%) as a pale yellow solid. A solution of the solid obtained above (200 mg, 0.75 mmol), **14** (190 μL, 1.50 mmol) and triethylamine (209 μL, 1.50 mmol) in DMSO (4 mL) was heated at 150 °C in a sealed tube. After cooling, the mixture was diluted with AcOEt and brine and then extracted with AcOEt. The combined organic layer was washed with brine, dried (Na_2SO_4), and evaporated to give the crude product, which was purified by preparative TLC plates ($CHCl_3$/7 M ammonia in MeOH = 10:1, v/v) to afford **35** (81 mg, 30%) as a brown solid: Mp 249–251 °C. HRMS (EI) *m/z*: 361.1904 (Calcd for $C_{21}H_{23}N_5O$ 361.1903). ^1H NMR ($CDCl_3$) δ: 1.38–1.49 (1H, m), 1.79–1.86 (1H, m), 1.96 (6H, s), 2.01 (3H, s), 2.38–2.47 (1H, m), 2.67 (1H, t, *J* = 8.3 Hz), 3.07–3.27 (3H, m), 7.09–7.12 (1H, m), 7.27–730 (1H, m), 7.32–7.45 (3H, m), 7.73 (2H, s). ^{13}C NMR (DMSO-*d6*) δ: 19.3, 29.4, 43.5 (2C), 50.3, 55.4, 64.7, 87.9, 117.5, 123.7, 127.0, 128.0, 128.3, 130.7, 131.4, 135.1, 135.6, 135.8, 139.5, 147.1, 163.6. IR (ATR): 3228, 2210, 1672, 1560, 1466, 1408, 1286, 739 cm^{-1}. Anal. Calcd for $C_{21}H_{23}N_5O{\cdot}0.25H_2O$: C, 68.92; H, 6.47; N, 19.14. Found: C, 68.68; H, 6.41; N, 18.79. $[\alpha]_D^{25}$ +110 (*c* 0.202, $CHCl_3$).

5.1.37. (4-Cyano-2-cyclopropyl-7-fluoro-6-phenyl-1,3-benzoxazol-5-yl)methyl acetate (36)

A solution of **33a** (1.00 g, 3.42 mmol), *N*-bromosuccinimide (913 mg, 5.13 mmol) and 2,2′-azobis(2-methylpropionitrile) (56 mg, 342 μmol) in CCl_4 (12 mL) was stirred at 80 °C for 13 h. The mixture was concentrated and the resulting residue was purified by silica gel column chromatography eluting with *n*-hexane/AcOEt = 5:1, v/v to give 5-(bromomethyl)-2-cyclopropyl-7-fluoro-6-phenyl-1,3-benzoxazole-4-carbonitrile (1.04 g, 82%) as a white solid. MS (ESI) *m/z*: 371, 373 $(M+1)^+$. ^1H NMR ($CDCl_3$) δ: 1.29–1.35 (2H, m), 1.39–1.45 (2H, m), 2.28–2.36 (1H, m), 4.50 (2H, s), 7.36–7.42 (2H, m), 7.50–7.55 (3H, m). IR (ATR): 2227, 1566, 1469, 1415, 1325, 1265, 1219, 1122, 1036 cm^{-1}.

To a solution of the solid (834 mg, 2.25 mmol) in AcOH (23 mL) was added sodium acetate (922 mg, 11.2 mmol) and the mixture was stirred at 100 °C for 21 h. The reaction mixture was concentrated and the residue was diluted with AcOEt and water. The mixture was extracted with AcOEt and the organic layer was dried (Na_2SO_4) and evaporated to give the crude product, which was purified by silica gel column chromatography eluting with *n*-hexane/AcOEt = 3:1, v/v to afford **36** (664 mg, 84%) as a white solid. MS (ESI) *m/z*: 351 $(M+1)^+$. ^1H NMR ($CDCl_3$) δ: 1.29–1.35 (2H, m), 1.39–1.45 (2H, m), 2.01 (3H, s), 2.28–2.37 (1H, m), 5.08 (2H, s), 7.24–7.29 (2H, m), 7.45–7.50 (3H, m). IR (ATR): 2229, 1732, 1556, 1417, 1365, 1308, 1236, 1227, 1124, 1026 cm^{-1}.

5.1.38. {4-Cyano-2-cyclopropyl-7-[(3*S*)-3-(dimethylamino)pyrrolidin-1-yl]-6-phenyl-1,3-benzoxazol-5-yl}methyl acetate (37)

Following the procedures as described for **3**, **37** was prepared in 44% yield from **36** as a brown oil. MS (ESI) *m/z*: 445 $(M+1)^+$. ^1H NMR ($CDCl_3$) δ: 1.18–1.32 (4H, m), 1.53–1.65 (1H, m), 1.90–1.98 (1H, m), 1.97 (3H, s), 2.12 (6H, s), 2.21–2.29 (1H, m), 2.44–2.54 (1H, m), 2.88–2.96 (1H, m), 3.22–3.41 (3H, m), 4.82 (1H, d, *J* = 12.2 Hz), 4.98 (1H, d, *J* = 12.2 Hz), 7.11–7.17 (1H, m), 7.23–7.28 (1H, m), 7.32–7.40 (3H, m). IR (ATR): 2212, 1739, 1587, 1468, 1367, 1221, 1155 cm^{-1}.

5.1.39. 2-Cyclopropyl-4-[(3*S*)-3-(dimethylamino)pyrrolidin-1-yl]-5-phenylfuro[3,4-*e*][1,3]benzoxazol-8(6*H*)-one (38)

To a solution of **37** (236 mg, 531 μmol) in MeOH (5 mL) was added K_2CO_3 (220 mg, 1.59 mmol) and the mixture was stirred at room temperature for 24 h. The mixture was diluted with $CHCl_3$ and the solution was washed with brine, dried over Na_2SO_4, and evaporated to give the crude product, which was purified by preparative TLC plates (CH_2Cl_2/MeOH = 10:1, v/v) to afford **38** (71 mg, 33%) as a pale brown solid. Mp: 192–194 °C. MS (ESI) *m/z*: 404 $(M+1)^+$. ^1H NMR ($CDCl_3$) δ: 1.17–1.24 (2H, m), 1.29–1.35 (2H, m), 1.58–1.73 (1H, m), 1.96–2.04 (1H, m), 2.15 (6H, s), 2.27–2.35 (1H, m), 2.51–2.62 (1H, m), 3.16 (1H, t, *J* = 9.3 Hz), 3.39–3.54 (3H, m), 4.69 (1H, d, *J* = 15.4 Hz), 4.99 (1H, d, *J* = 15.4 Hz), 7.22–7.29 (2H, m), 7.33–7.43 (3H, m). ^{13}C NMR ($CDCl_3$) δ: 9.4, 9.6 (2C), 30.5, 44.3 (2C), 51.2, 56.7, 65.4, 69.5, 104.0, 117.2, 127.8 (2C), 128.6 (2C), 129.9, 137.2, 137.5, 139.1, 141.8, 147.4, 169.5, 170.3. IR (ATR): 1755, 1603, 1560, 1481, 1367, 1315, 1192, 1153, 1099 cm^{-1}. Anal. Calcd for $C_{24}H_{25}N_3O_3{\cdot}0.25H_2O$: C, 70.66; H, 6.30; N, 10.30. Found: C, 71.01; H, 6.22; N, 10.59. $[\alpha]_D^{25}$ +0.234 (*c* 1.02, $CHCl_3$).

5.1.40. 2-Amino-5-bromo-4-[(3*S*)-3-(dimethylamino)pyrrolidin-1-yl]-3-methoxy-6-methylbenzonitrile (39)

Following the procedures as described for **3**, **39** was prepared in 44% yield from 2-amino-5-bromo-4-fluoro-3-methoxy-6-methylbenzonitrile as a pale brown oil. MS (FAB) *m/z*: 353 (M+1 for $^{79}Br)^+$, 355 (M+1 for $^{81}Br)^+$. ^1H NMR ($CDCl_3$) δ: 1.81–1.94 (1H, m), 2.14–2.25 (1H, m), 2.30 (6H, s), 2.49 (3H, s), 2.77–2.88 (1H, m), 3.35–3.44 (2H, m), 3.47–3.54 (1H, m), 3.58–3.70 (1H, m), 3.65 (3H, s), 4.52 (2H, br s).

5.1.41. 4-Amino-6-[(3*S*)-3-(dimethylamino)pyrrolidin-1-yl]-5-hydroxy-2-methylbiphenyl-3-carbonitrile (40)

Following the procedures as described for **28**, 4-amino-6-[(3*S*)-3-(dimethylamino)pyrrolidin-1-yl]-5-methoxy-2-methylbiphenyl-3-carbonitrile was prepared in 39% yield from **39** as a pale brown

oil. MS (FAB) *m/z*: 351 $(M+1)^+$. ^1H NMR ($CDCl_3$) δ: 1.42–1.56 (1H, m), 1.78–1.90 (1H, m), 2.09 (3H, s), 2.11 (6H, s), 2.30–2.42 (1H, m), 2.80–2.98 (3H, m), 3.10 (1H, dd, J = 6.9, 9.0 Hz), 3.63 (3H, s), 4.51 (2H, br s), 7.04–7.09 (1H, m), 7.11–7.17 (1H, m), 7.26–7.42 (3H, m). To a suspension of $AlCl_3$ (399 mg, 3.00 mmol) in CH_2Cl_2 (2.0 mL) was added the obtained compound (210 mg, 599 μmol) in CH_2Cl_2 (10 mL), and the mixture was refluxed for 15 min. The reaction mixture was cooled to room temperature. Satd $NaHCO_3$ aq and brine were added, and the mixture was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography eluting with $CHCl_3$/MeOH = 100:1, v/v to afford **40** (125 mg, 62%) as a brown solid. MS (FAB) *m/z*: 337 $(M+1)^+$. ^1H NMR ($CDCl_3$) δ: 1.57–1.70 (1H, m), 2.05–2.15 (1H, m), 2.08 (3H, s), 2.34 (6H, s), 2.48–2.54 (1H, m), 2.73 (1H, dt, J = 1.2, 8.7 Hz), 2.89–2.95 (1H, m), 3.01–3.18 (2H, m), 4.51 (2H, br s), 7.05–7.16 (2H, m), 7.24–7.42 (3H, m).

5.1.42. 3-*tert*-Butyl-8-[(3*S*)-3-(dimethylamino)pyrrolidin-1-yl]-6-methyl-7-phenyl-2*H*-1,4-benzoxazine-5-carbonitrile hydrochloride (7)

To a mixture of **40** (113 mg, 336 μmol) and 20% K_2CO_3 aq (1.8 mL) in CH_2Cl_2 (3.6 mL) were added tetrabutylammonium hydrogen sulfate (37.5 mg, 0.111 mmol) and 1-bromo-3,3-dimethylbutan-2-one (54.2 μL, 0.403 mmol), and the reaction mixture was stirred for 15 h at room temperature. Brine was added, and the mixture was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography eluting with $CHCl_3$/MeOH = 100:1, v/v to afford a brown oil. To a solution of the oil in Et_2O (15 mL) was added 4 M HCl in 1,4-dioxane (75.6 μL, 0.302 mmol), and the resultant precipitate was collected and dried to afford **7** (97.2 mg, 64%) as a yellow solid. Mp: 130–132 °C. HRMS (ESI) *m/z*: 417.2617 (Calcd for $C_{26}H_{32}N_4O$ + H 417.2654). ^1H NMR ($CDCl_3$) δ: 1.30 (9H, s), 1.94–2.08 (1H, m), 2.09–2.26 (1H, m), 2.16 (3H, s), 2.30–2.40 (6H, m), 3.10 (2H, t, J = 7.1 Hz), 3.25–3.33 (1H, m), 3.37–3.48 (1H, m), 3.54–3.67 (1H, m), 4.63 (2H, dd, J = 14.7, 19.5 Hz), 7.05–7.15 (2H, m), 7.30–7.45 (3H, m). IR (KBr): 2965, 2563, 2220, 1623, 1458, 1004 cm^{-1}. $[\alpha]_D^{25}$ +104 (*c* 1.02, $CHCl_3$/MeOH = 1:1, v/v).

5.1.43. 2-Amino-5-bromo-4-fluoro-6-methyl-3-nitrobenzonitrile (42)

Following the procedures as described for **20** and **26**, **42** was prepared in 48% yield from **41** as yellow solid. MS (EI) *m/z*: 273, 275 (M^+). ^1H NMR ($CDCl_3$) δ: 2.66 (3H, s), 6.53 (2H, br s). IR (ATR): 3455, 3324, 2227, 1625, 1589, 1560, 1508 cm^{-1}.

5.1.44. 4-Amino-6-[(3*S*)-3-(dimethylamino)pyrrolidin-1-yl]-2-methyl-5-nitrobiphenyl-3-carbonitrile (43)

Following the procedures as described for **39** and **40**, **43** was prepared in 87% yield from **42** as a brown solid. MS (FAB) *m/z*: 366 $(M+1)^+$. ^1H NMR ($CDCl_3$) δ: 1.54 (1H, m), 1.83 (1H, m), 2.06 (6H, s), 2.12 (3H, s), 2.50 (1H, m), 2.65 (1H, m), 2.90–2.99 (3H, m), 5.73 (2H, br s), 7.12–7.16 (2H, m), 7.34–7.50 (3H, m). IR (ATR): 3326, 3210, 2827, 2776, 2208, 1623, 1571 cm^{-1}.

5.1.45. 4,5-Diamino-6-[(3*S*)-3-(dimethylamino)pyrrolidin-1-yl]-2-methylbiphenyl-3-carbonitrile (44)

To a solution of **43** (1.40 g, 3.83 mmol) in DMF (35 mL) and water (14 mL) were added $FeCl_3 \cdot 6H_2O$ (830 mg, 3.06 mmol) and zinc (5.01 g, 76.6 mmol), and the mixture was stirred at room temperature for 1 h. The reaction mixture was filtered, and the filtrate was diluted with AcOEt. The organic layer was washed with satd $NaHCO_3$ aq and brine, dried over Na_2SO_4, filtered, and concentrated to afford **44** (1.25 g, 97%) as a brown solid. MS (FAB) *m/z*: 336 $(M+1)^+$. ^1H NMR ($CDCl_3$) δ: 1.64 (1H, m), 1.80 (1H, m), 2.06 (6H, s), 2.16 (3H, s), 2.35 (1H, m), 2.76 (1H, m), 2.890–2.96 (3H, m), 4.09 (2H, br s), 7.10–7.14 (2H, m), 7.34–7.49 (3H, m). IR (ATR): 3357, 3284, 2944, 2821, 2775, 2200, 1666, 1583, 1459, 1436 cm^{-1}.

5.1.46. 2-*tert*-Butyl-7-[(3*S*)-3-(dimethylamino)pyrrolidin-1-yl]-5-methyl-6-phenyl-1*H*-benzimidazole-4-carbonitrile (8)

To a solution of **44** (1.25 g, 3.73 mmol) in $CHCl_3$ (40 mL) was added pivaloyl chloride (0.46 mL, 3.73 mmol), and the mixture was stirred at room temperature for 3 h. Water was added, and the mixture was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography eluting with $CHCl_3$/MeOH = 19:1, v/v to afford a brown amorphous solid. A solution of the solid in AcOH (5 mL) was stirred at 80 °C for 2.5 h. The mixture was cooled to room temperature, and then poured into aqueous NaOH solution (6.7 M, 15 mL). The resultant solid was collected and purified by silica gel column chromatography eluting with AcOEt/acetone = 1:1, v/v to afford **8** (228 mg, 15%) as a colorless solid. MS (EI) *m/z*: 401 (M^+). ^1H NMR (DMSO-d_6) δ: 1.35–1.52 (1H, m), 1.42 (9H, s), 1.75–1.85 (1H, m), 2.04, (6H, s), 2.09, (3H, s), 2.50–2.55 (1H, m), 3.12–3.22 (1H, m), 3.35–3.55 (3H, m), 7.05–7.55 (5H, m), 12.16 (1H, br s). IR (ATR): 3241, 2969, 2813, 2765, 2204, 1602, 1565, 1455 cm^{-1}. Anal. Calcd for $C_{25}H_{31}N_5$: C, 74.78; H, 7.78; N, 17.44. Found: C, 74.76; H, 7.85; N, 17.28. $[\alpha]_D^{25}$ +152 (*c* 0.169, $CHCl_3$).

5.2. In vitro antifungal activity

In vitro antifungal activity was evaluated by the serial dilution methods in 96-well microtest plates using RPMI 1640 with 0.165 M MOPS buffer or YPD [1% yeast extract (Difco, Detroit, MI), 2% peptone (Difco, Detroit, MI), and 20% glucose] as the medium.[10–13,20–22] In the experiments using RPMI 1640 media, alamar blue (Biosource, Camarillo, CA) was added to accurately define its MIC-2 (the lowest drug concentration showing 50% growth inhibition compared to the control without drug). Test organisms were purchased from the American Type Culture Collection (Rockville, MD), the Institute for Fermentation Osaka (Osaka, Japan), or Teikyo Institute of Medical Mycology (Tokyo, Japan). Initial cell densities (1×10^3–1×10^4 cells/mL) and incubation times (18–72 h) were decided for each strain in consideration of their growth speed. The fungi were incubated at 30 °C (RPMI-1640) or 37 °C (YPD). Following incubation, OD_{570} was measured with a Wallac 1420 ARVOsx multi-label counter (Wallac, Tokyo, Japan), and MIC-2s (50% growth inhibition) were calculated. OD_{600} was measured and MIC-1s (80% growth inhibition) were calculated. Compounds were tested at different concentrations ranging from 0.004 to 16 μg/mL.

5.3. Incorporation study with growing cells using [^{14}C]-glucose

An incorporation study with growing cells was conducted based on the methods described by Kitamura et al.[10] Exponentially growing cells of *C. albicans* ATCC 90028 were suspended in RPMI 1640 medium to give ~0.7 of absorbance at 595 nm. After the drug solution and radioactive [^{14}C]-glucose were added, the reaction tubes were incubated at 30 °C with occasional shaking. After 3 h of incubation, samples were taken and crude fractions of β-1,3-glucan, β-1,6-glucan, chitin, and mannan were prepared as follows. The harvested cells were suspended in 3% NaOH aq and heated at 80 °C for 1 h. Mannan fractions were prepared from the supernatant using Fehling's reaction. Insoluble material was washed and digested with Zymolyase 100T (Seikagaku-kougyou) overnight. After digestion, the insoluble material was harvested as a chitin fraction. The supernatants were taken as glucan fractions

(β-1,3-glucan + β-1,6-glucan fraction) and were dialyzed overnight. After dialysis, samples were taken as a β-1,6-glucan fraction. The radioactivity of each fraction was counted with a toluene scintillator. The radioactivities of the β-1,3-glucan fraction were calculated by subtracting that β-1,6-glucan fraction from the glucan fraction.

5.4. Distribution coefficient (Log *D*)

The distribution coefficients (Log *D*) were determined by the shake-flask method. Four hundred μM of compound solution of each compound in a 2 mL *n*-octanol–2 mL BPS solution was placed on a shaker for 30 min at pH 7.4. After centrifuging each solution separately at 3000 rpm for 10 min, an LC/MS method was used to assay each layer. The LC/MS system consisted of an 1100 Series LC/MSD (Agilent) and an X Terra® MSC18 3.5 μm, 3.0 × 30 mm column (Waters). The mobile phase was a 10 mM ammonium acetate buffer (pH 4.5)/0.05% (v/v) acetic acid mixture in acetonitrile with a gradient condition (95:5–10:90). Analyst software program (version 1.4, Applied BioSystems) was used to calculate the Log *D*.

5.5. Metabolic stability

Compounds (final 1 μM) were incubated with human liver microsomes in sodium phosphate buffer (pH 7.4) for 20 min at 37 °C. The microsomal protein concentration in the assay was 0.5 mg/mL. Reaction was started by the addition of NADPH at 37 °C and stopped by the addition of MeOH after 30 min. After centrifuging each solution separately at 3500 rpm for 10 min at 4 °C, the corresponding loss of parent compound was determined by LC/MS/MS.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.08.044. These data include MOL files and InChiKeys of the most important compounds described in this article.

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