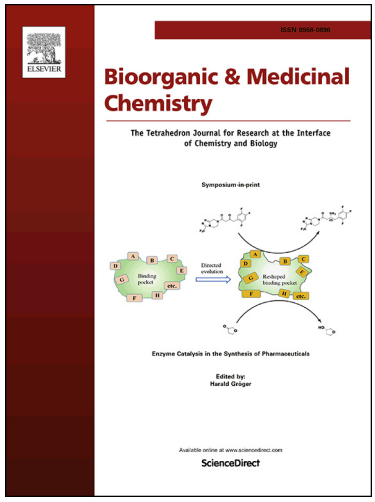

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Structure-Activity Relationship of Novel (Benzoylaminophenoxy)phenol Derivatives as Anti-prostate Cancer Agents

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Abstract

The androgen receptor (AR) is a ligand-inducible transcription factor belonging to the nuclear receptor superfamily, and is a target molecule for development of drugs to treat prostate cancer. However, AR antagonists in clinical use, such as flutamide (**3a**) and bicalutamide (**4**), encounter resistance after several years of hormone therapy, predominantly due to mutations of AR. Thus, although some new-generation AR antagonists have been developed, novel types of AR antagonists are still required to treat drug-resistant prostate cancer. We previously reported a novel (benzoylaminophenoxy)phenol derivative **10a**, which is structurally distinct from conventional AR antagonists. Here, we systematically examined the structure-activity relationship of (benzoylaminophenoxy)phenol derivatives on the inhibitory activity on the prostate cancer cell proliferations. We found that the 4-[4-(benzoylamino)phenoxy]phenol backbone is important for anti-prostate cancer activity. Introduction of a small substituent at the 2 position of the central benzene ring (B ring) increases the activity. Among the synthesized compounds, **19a** and **19b** exhibited the most potent inhibitory activity toward dihydrotestosterone-induced proliferation of several androgen-dependent cell lines, SC-3 (wild-type AR), LNCaP (T877A AR), and 22Rv1 (H874Y AR), but interestingly also inhibited proliferation of AR-independent PC-3 cells. These compounds, which have a different pharmacophore from conventional AR antagonists, are promising drug candidates for the treatment of prostate cancer.

Keywords

prostate cancer, cell proliferation, phenoxyphenol, pharmacophore

1. Introduction

Androgen receptor (AR) is a ligand-inducible transcription factor belonging to the nuclear receptor superfamily.[1,2] The endogenous ligands (androgens) of AR, testosterone (**1**) and its active metabolite dihydrotestosterone (DHT, **2**) (Figure 1), regulate various physiological phenomena, including differentiation, growth, and maintenance of male reproductive organs.[3,4] Since androgen plays an important role in progression of prostate cancer,[5] various AR antagonists have been developed for clinical use. In general, non-steroidal AR antagonists are preferred, because steroidal compounds are considered to have greater potential for off-target activities. Flutamide (**3a**)[6] and bicalutamide (**4**, Figure 1)[7] are typical nonsteroidal AR antagonists in clinical use to treat prostate cancer, but the development of resistance after several years of hormone therapy, so-called castration-resistant prostate cancer (CRPC), is significant problem.[8,9] Mutations of AR, such as T877A, are the major cause of CRPC.[10-12] Hydroxyflutamide (**3b**), the active metabolite of **3a**, can activate T877A AR, and exacerbate the prostate cancer.[13]

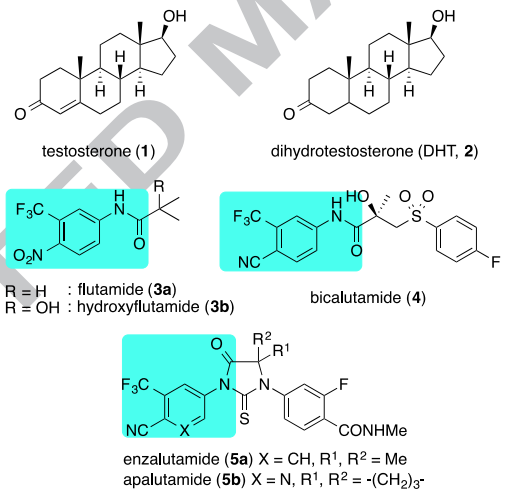


Figure 1. Structures of testosterones and typical nonsteroidal AR antagonists.

Some second-generation AR antagonists that are effective against CRPC, such as enzalutamide (**5a**) and apalutamide (**5b**), have recently been developed.[14] However, most of the nonsteroidal AR antagonists so far known have been developed from flutamide (**3a**) and bicalutamide (**4**) as lead compounds, and have the common pharmacophore of an anilide structure with electron-withdrawing functional groups, such as nitro, cyano, or trifluoromethyl groups, on the phenyl ring, as shown in blue in Figure 1. Compounds **5a** and **5b** contain essentially the same pharmacophore, although in these compounds, the bulky aryl ring on the heterocyclic linker is important for the antagonism. On the other hand, some AR antagonists bearing a different skeleton or pharmacophore have also been reported, such as

DIMN (**6**), isoxazolone derivative **7** and (2*H*)-phthalazinone derivative **8** (Figure 2).[15-17]

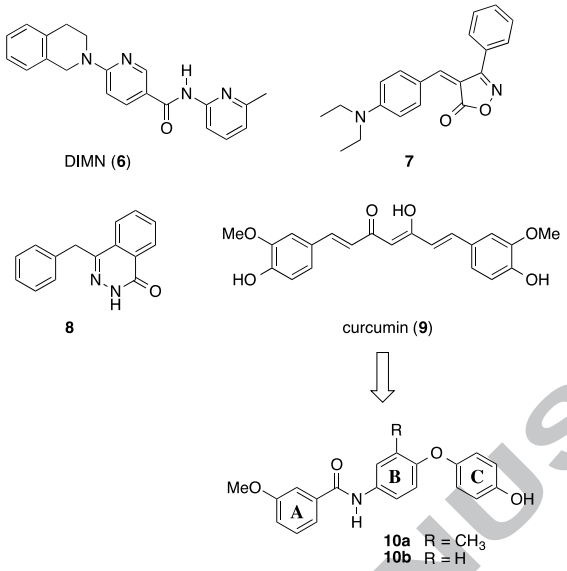


Figure 2. Structures of AR antagonists with a different pharmacophore from flutamide (**3a**) or bicalutamide (**4**)

We have developed and synthesized novel AR antagonists based on curcumin (**9**) as a lead compound.[18] Curcumin (**9**) is major pigment in turmeric, and exhibits various biological activities, including anticancer, antioxidant and anti-inflammatory activities. Further, some curcumin derivatives show antiandrogenic activities in AR-positive cell lines, as well as cytotoxic activity toward AR-negative PC-3 cells.[19] Curcumin (**9**) consists of two phenolic groups linked by an conjugated 1,3-diketone (or its enol form). To reduce the chemical and metabolic reactivity of curcumin (**9**), we designed AR antagonist candidates containing 4-(4-benzoylaminophenoxy)phenol as stable aromatic core structure. Among the resulting compounds, **10a** showed AR antagonistic activities in SC-3 cells (wild-type AR), LNCaP cells (T877A-mutated AR), and 22Rv1 cells (H874Y-mutated AR).[19] Thus, it was considered a promising lead compound for antiandrogen therapy of prostate cancer. Therefore, in the present study, we synthesized various derivatives of **10a**, and examined the structure-activity relationship in detail.

2. Results and discussion

2.1. Chemistry

Compound **10a** contains three benzene rings named A, B, and C from the left, as shown in Figure 2, linked by an amide bond (A–B rings) and an oxygen atom (B–C rings). Our previous work showed that loss of the phenolic hydroxyl group on the C ring (Figure 2) caused a significant decrease of AR antagonistic activity in SC-3 cell assay.[18] The substituent on the A ring also affected the potency, and a 3-methoxy or 3-dimethylamino group was

effective, while compounds with a nitro group were inactive. Based on these results, we synthesized compounds **11** – **19**, and examined their AR antagonistic activities. The substituents on the terminal benzene rings are limited to methoxy or dimethylamino for the A ring and hydroxyl for the C ring, and the positions of the terminal hydroxyl group (compounds **11** and **14**), ether linkage (compounds **12** and **13**), amide moiety (compounds **15** and **16**), and substituents on the central B ring (compounds **17** – **19**) were modified.

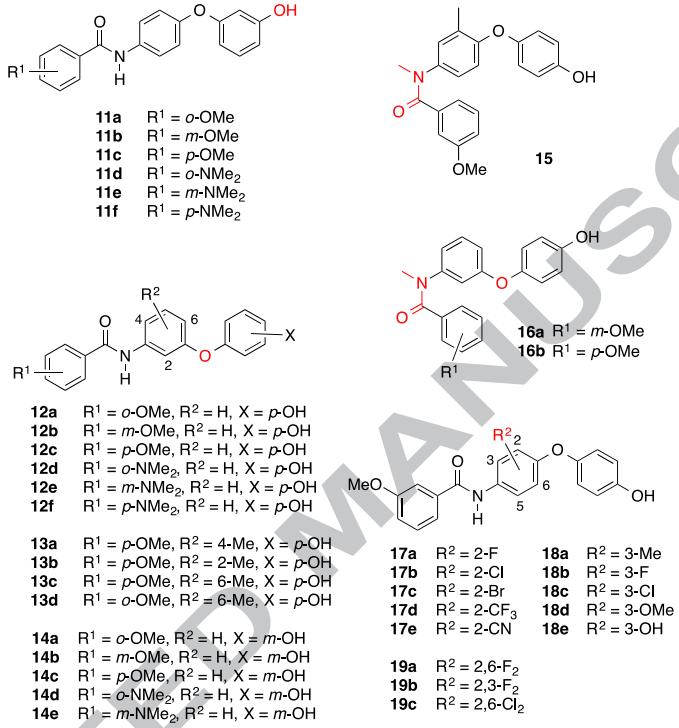
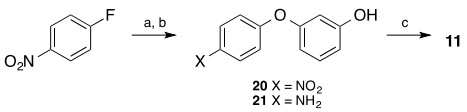


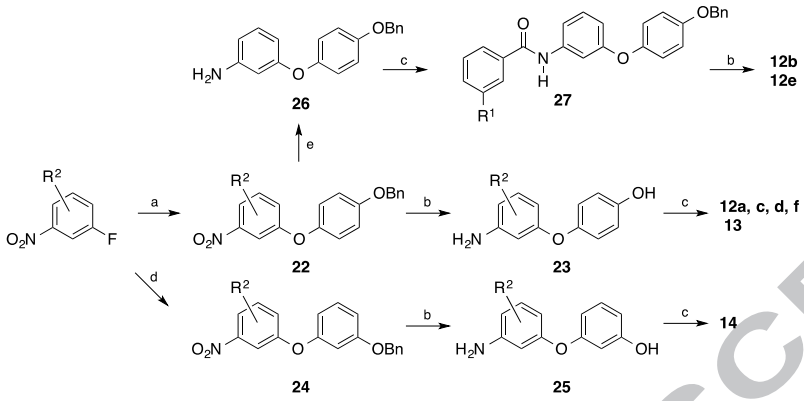
Figure 3. Structures of 4-(4-benzoylaminophenoxy)phenol derivatives

Compounds **11** – **19** were synthesized according to Schemes 1 – 4. Reaction of *p*-fluoronitrobenzene with resorcinol under basic conditions afforded diphenylether **20**, which was hydrogenated to afford amine **21** (Scheme 1). Compounds **11** were obtained by the coupling reaction of **21** with various benzoyl chloride derivatives. In the synthesis of compounds **12** – **14** (Scheme 2), *m*-fluoronitrobenzenes were reacted with monoprotected hydroquinone or resorcinol to improve the yields of the 1:1 adducts. The obtained diphenylethers **22** and **24** were hydrogenated, followed by condensation with various benzoyl chlorides to afford compounds **12** – **14**. Compound **22** was reduced with sodium borohydride in the presence of copper (II) sulfate to afford amine **26** with a benzyloxy group on the C ring; this was reacted with benzoyl chloride, followed by debenzylation to afford **12b** or **12e**.



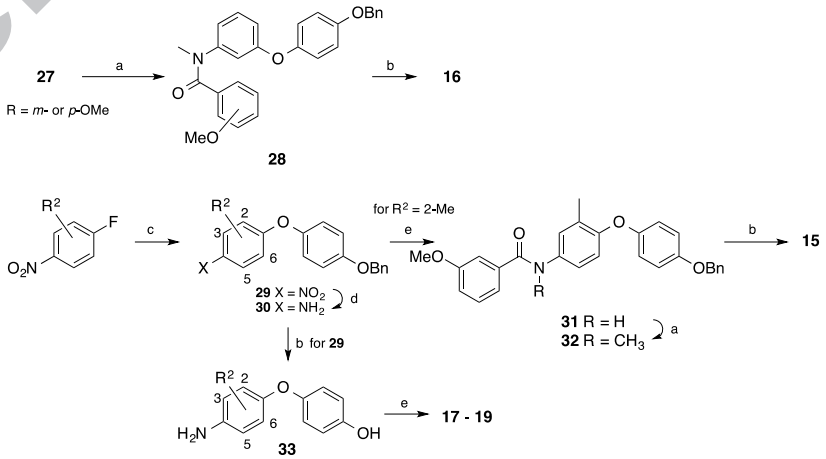
Scheme 1. Synthesis of compounds **11**: (a) resorcinol, NaOH, DMSO; (b) H_2, Pd-C, MeOH;

(c) R^1-PhCOCl, THF



Scheme 2. Synthesis of compounds **12 - 14**: (a) *O*-Monobenzylhydroquinone, K_2CO_3, DMF; (b) H_2, $Pd(OH)_2$, MeOH/THF; (c) R-PhCOCl, THF; (d) *O*-monobenzylresorcinol, K_2CO_3, DMF; (e) $CuSO_4$, $NaBH_4$

We have shown that aromatic secondary amides such as benzanilide generally exist in trans form, while the *N*-methylated amides exist in folded cis form both in the crystal and in various solvents.[20] In order to clarify the relationship between the amide conformation and the activity, *N*-methylated amide derivatives **15** and **16** were synthesized by *N*-methylation of *O*-benzyl-protected (benzoylaminophenoxy)phenols **31** and **27**, respectively, followed by debenzylation (Scheme 3). In the ^{1}H NMR spectra of compounds **15** and **16**, the chemical shifts of aromatic protons were shifted to higher field, compared to those of the corresponding secondary amides **10a**, **12b**, and **12c**, as observed in the cases of benzanilide and *N*-methylbenzanilide, indicating that these compounds existed mainly in cis form in solution. Finally, we synthesized compounds **17** – **19** bearing various substituents on the central B ring from **29** (Scheme 3).



Scheme 3. Synthesis of compounds **15 - 19**: (a) NaH, DMF; CH_3I; (b) H_2, $Pd(OH)_2$, THF ; (c) *O*-Monobenzylhydroquinone, NaOH, DMSO; (d) $CuSO_4$, $NaBH_4$; (e) *m*-MeOPhCOCl, THF.

2.2. Inhibitory activity on prostate cancer cell proliferations

The biological activities of the synthesized compounds were first evaluated in terms of growth-inhibitory activity toward androgen-dependent SC-3 cells bearing wt AR.[21] Compounds **11** – **19** alone did not affect the proliferation of SC-3, i.e., they did not act as AR agonists. The inhibitory activity of the test compounds was examined in terms of their effect on 1 nM DHT-dependent proliferation of SC-3 cells, and the IC_{50} values are shown in Table 1.

Table 1. AR antagonistic activity of compounds **11** – **19** in SC-3 cell assay[a]

Compound[b]	IC_{50} (μM)	Compound[b]	IC_{50} (μM)	Compound[b]	IC_{50} (μM)
10a	0.75	**13a**	>10 [d]	**17a**	0.40
11a	inactive[c]	**13b**	>10 [d]	**17b**	0.48
11b	>10 [d]	**13c**	>10 [d]	**17c**	5.7
11c	inactive[c]	**13d**	5.3	**17d**	4.3
11d	inactive[c]	**14a**	inactive[c]	**17e**	inactive[c]
11e	inactive[c]	**14b**	>10 [d]	**18a**	3.0
11f	3.2	**14c**	>10 [d]	**18b**	0.65
12a	>10 [d]	**14d**	inactive[c]	**18c**	1.7
12b	inactive[c]	**14e**	>10 [c]	**18d**	3.4
12c	>10 [d]	**15**	7.6	**18e**	inactive[c]
12d	6.3	**16a**	inactive[c]	**19a**	0.51
12e	inactive[c]	**16b**	inactive[c]	**19b**	0.53
12f	7.40			**19c**	0.48

[a] Inhibitory activity of test compounds toward DHT-induced proliferation of SC-3 cells was examined.
[b] Structures: see Figure 3.
[c]'Inactive' means that test compound did not inhibit the proliferation of SC-3 cells at concentrations up to 10 μM.
[d]'>10' means that test compound at 10 μM inhibited the proliferation of SC-3 cells by less than 50% versus control cells.

Compound **10a**, the lead compound in this study, inhibited the DHT-induced proliferation of SC-3 cells with the IC_{50} value of 0.75 μM. Here, we found that compounds **11** bearing a *m*-hydroxyl group on the C ring were inactive or showed very weak activity, compared to **10a**. Only compound **11f** bearing a *p*-dimethylamino group on the A ring exhibited significant potency (IC_{50}: 3.2 μM). Since compound **10b** without the 2-methyl group on the B ring also exhibited significant potency (IC_{50}: 2.0 μM), the decrease of the activity in compounds **11** should be due to the inappropriate position of the hydroxyl group on the C ring. Similarly, a change in the position of the ether linkage, that is, meta to the amide nitrogen atom in compounds **12 - 14**, decreased the activity, independently of the substituents on the A and B rings. Compounds **15** and **16** bearing an *N*-methyl amide moiety lacked

activity. These compounds have *cis* conformation of the amide structure, indicating that the conformation of the amide bond is critical for the activity. Thus, inhibitory activity towards SC-3 cell proliferation was observed only with compounds having the 4-[4-(benzoylamino)phenoxy]phenol backbone among the synthesized compounds. Finally, we examined the effect of the substituent on the B ring of compound **10**. The 2-methyl group on the B ring of **10a** is important, since its removal resulted in lower activity. Among compounds bearing a 2-substituent, **17a** (IC_{50}: 0.40 μM) with a fluorine atom and **17b** (IC_{50}: 0.48 μM) with a chlorine atom showed potent activity. Introduction of a larger substituent such as a bromine atom or trifluoromethyl group was not effective. Among compounds with a 3-substituent on the B ring, only **18b** (IC_{50}: 0.65 μM) with a 3-fluorine atom was as active as **10a**. In this case, the introduction of a chlorine atom decreased the activity. Compounds bearing multiple substituents on the B ring also exhibited potent activity, and the activities of **19a - c** bearing two halogen atoms on the B ring were comparable to those of **17a** and **17b** with one halogen atom. Thus, a small 2-substituent, such as methyl, fluorine, or chloride, increased the activity, and **17a** was the most potent of the synthesized compounds.

Next, we examined the activity of selected compounds towards other cell lines. LNCaP[22,23] and 22Rv1[24] cells are androgen-dependent, having mutated T877A and H874Y ARs, respectively, while PC-3 shows androgen-independent cell growth. Bicalutamide (**4**) inhibited the proliferation of LNCaP cells, but not 22Rv1 or PC-3 cells, in the concentration range examined. Selected compounds inhibited all three cell lines dose-dependently (Figure 4), in accordance with our previous finding that **10a** inhibits the proliferation of these three cell lines. Here, we found that **19a** and **19b** showed more potent inhibitory activity than the lead compound **10a**, especially in 22RV1 and PC-3 cell assays. From these results, our developed compounds would act as AR antagonists toward both wild-type and mutated ARs, and also that some mechanism of cell growth inhibition other than AR antagonism is involved. Further structural optimization and the detailed mechanistic studies including AR specificity are on going.

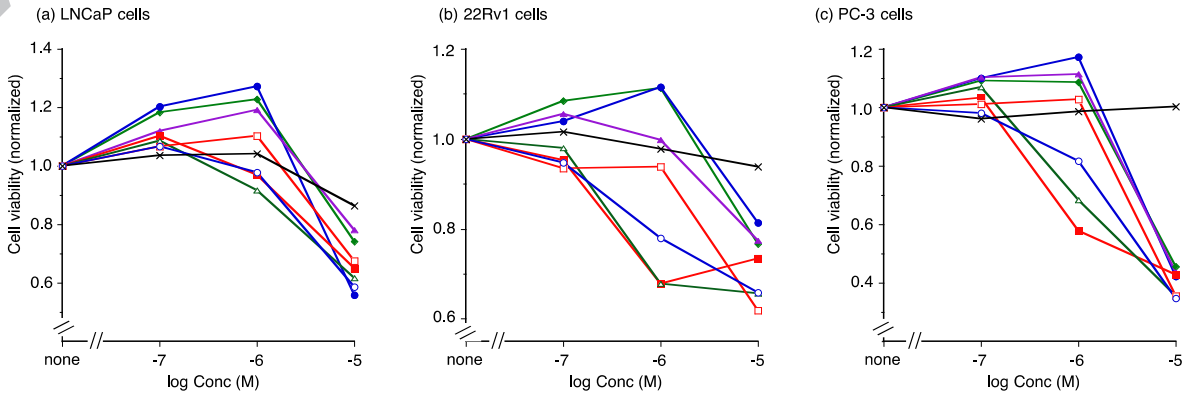


Figure 4. Inhibitory activities of selected derivatives on the proliferation of three human prostate cancer cells. Bicalutamide (×), **10a** (○), **17a** (◆), **17b** (●), **18b** (▲), **19a** (■), **19b** (△), and **19c** (□). Cell viability was determined by the WST method and normalized to vehicle-treated cells as 1.

3. Conclusion

We synthesized a series of (benzoylaminophenoxy)phenol derivatives based on the lead compound **10a** and examined their structure-activity relationship as anti-prostate cancer agents. The 4-[4-(benzoylamino)phenoxy]phenol backbone is important for anti-prostate cancer activity in this series. Introduction of a small substituent at the 2-position of the B-ring increased the activity. Among the synthesized compounds, **19a** and **19b** exhibited the most potent growth-inhibitory activity toward androgen-dependent carcinoma cell lines SC-3 (wild-type AR), LNCaP (T877A AR), 22Rv1 (H874Y AR), and also inhibited the growth of AR-independent PC-3 cells. Since these compounds have a different pharmacophore from conventional AR antagonists based on flutamide (**3a**) and bicalutamide (**4**), they may be promising candidates for treatment of prostate cancer.

4. Experimental

4.1. Chemistry

All reagents were purchased from Sigma-Aldrich Chemical Co., Tokyo Kasei Kogyo Co., Wako Pure Chemical Industries, or Kanto Kagaku Co., Inc. Silica gel for column chromatography was purchased from Kanto Kagaku Co., Inc. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL ECA 600, or Bruker 600 spectrometer. Mass spectral data was obtained on a Bruker Daltonics microTOF-2focus, Thermo Scientific Q-Exactive or Waters Q-TOF Premier in the positive ion detection mode. Melting points were determined on a RFS-30 melting point apparatus (Round Science).

4.2. Synthesis of 11a (General procedure for compounds 11, Scheme 1)

A 5 M aqueous solution of sodium hydroxide (12.5 ml, 62.5 mmol) was added to DMSO (35 ml) at room temperature under an Ar atmosphere. After 30 min, resorcinol (4.13 g, 37.5 mmol) was added, and the mixture was stirred at 50°C for 30 min. Then, a solution of 4-fluoronitrobenzene (3.53 g, 25.0 mmol) in DMSO (5 ml) was added slowly to the reaction mixture, and stirring was continued at 50°C for 2.5 h. The reaction mixture was poured into ice water and filtered. The aqueous layer was acidified with 2 M HCl, and extracted with ethyl acetate. The organic layer was dried with magnesium sulfate, and evaporated to give **20** (3.82 g, 66%).

20: ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.21 (d, J = 9.3 Hz, 2 H), 7.28 (t, J = 8.2 Hz, 1 H), 7.04 (d, J = 7.1 Hz, 2 H), 6.71 (ddd, J = 8.0, 2.3, 0.7 Hz, 1 H), 6.66 (ddd, J = 8.2, 2.2, 0.8 Hz, 1 H), 6.59 (t, J = 2.3 Hz, 1 H), 4.96 (br, 1 H), ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.01, 157.17,

155.99, 142.82, 130.98, 125.93, 117.40, 112.63, 112.41, 107.87.

A mixture of **20** (2.31 g, 10 mmol) and 10% palladium on carbon (234 mg) in methanol (100 ml) was stirred under a hydrogen atmosphere for 2 h. The reaction mixture was filtered over Celite, and the filtrate was evaporated. The residue was purified by silica-gel column chromatography (hexane/ethyl acetate 1:1) to afford **21** (1.93 g, 96%).
21: ^{1}H NMR (600 MHz, CD_3OD) δ 7.09 (t, *J* = 8.2 Hz, 1 H), 6.84 (d, *J* = 8.6 Hz, 2 H), 6.76 (d, *J* = 9.8 Hz, 2 H), 6.46 (dt, *J* = 8.0, 1.3 Hz, 1 H), 6.38 (dt, *J* = 8.7, 1.0 Hz, 1 H), 6.34 (td, *J* = 0.5, 2.2 Hz, 1 H), ^{13}C NMR (150 MHz, CD_3OD) δ 162.04, 160.09, 150.02, 145.31, 131.24, 122.32, 118.02, 110.42, 109.52, 105.58.

21 (101 mg, 0.50 mmol) was added to a solution of *o*-anisoyl chloride (271 mg, 1.59 mmol) in tetrahydrofuran (15 ml). The mixture was stirred at room temperature for 30 min, then poured into water, and extracted with ethyl acetate. The organic layer was washed successively with saturated sodium hydrogen carbonate, 2 M HCl, water, and brine, dried over magnesium sulfate, and evaporated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate 3:1 to 2:1) to afford **11a** (130 mg, 77%).
11a: Colorless prisms (methanol); mp 176.2°C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.95 (dd, *J* = 7.8, 1.8 Hz, 1 H), 7.70 (dd, *J* = 6.6, 1.8 Hz, 2 H), 7.57 (td, *J* = 8.9, 1.7 Hz, 1 H), 7.23 (d, *J* = 8.4 Hz, 1 H), 7.17 (t, *J* = 8.4 Hz, 1 H), 7.14 (t, *J* = 7.2 Hz, 1 H), 7.05 (dd, *J* = 6.6, 1.8 Hz, 2 H), 6.45 (dd, *J* = 7.8, 1.8 Hz, 1 H), 6.57 (dd, *J* = 8.2, 2.2 Hz, 1 H), 6.45 (t, *J* =2.1 Hz, 1 H), 4.07 (s, 3 H); ^{13}C NMR (150 MHz, CD_3OD) δ 166.87, 160.54, 160.34, 159.15, 155.41, 135.47, 134.52, 132.24, 131.52, 124.43, 123.87, 122.41, 120.81, 113.38, 111.64, 110.76, 107.00, 57.03; HRMS Calcd. for $C_{20}H_{18}NO_4$ $[M+H]^+$ 336.1230; Found 336.1231.

Compound **11b** – **11f** were synthesized similarly.
11b: Colorless prisms (chloroform); mp 112.4°C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.69 (d, *J* = 8.9 Hz, 2 H), 7.53 (m, 2 H), 7.44 (t, *J* = 8.0 Hz, 1 H), 7.17 (m, 2 H), 7.04 (dt, *J* = 8.9, 2.0 Hz, 2 H), 6.57 (dd, *J* = 8.2, 1.7 Hz, 1 H), 6.48 (dd, *J* = 8.2, 1.9 Hz, 1 H), 6.46 (t, *J* = 7.3 Hz, 1 H), 3.89 (s, 3 H) ; ^{13}C NMR (150 MHz, CD_3OD) δ 168.90, 161.64, 160.48, 160.30, 155.52, 137.87, 135.56, 131.52, 130.99, 124.43, 120.98, 120.67, 119.00, 114.25, 111.65, 110.80, 107.03, 56.24; HRMS Calcd. for $C_{20}H_{18}NO_4$ $[M+H]^+$ 336.1230; Found 336.1234.

11c: Colorless prisms (chloroform/ethyl acetate); mp 183.3°C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.96 (d, *J* = 8.4 Hz, 2 H), 7.68 (d, *J* = 9.0 Hz, 2 H), 7.17 (t, *J* = 7.8 Hz, 1 H), 7.07 (d, *J* = 8.4 Hz, 2 H), 7.04 (d, *J* = 8.4 Hz, 2 H), 6.56 (dd, *J* = 8.1, 2.2 Hz, 1 H), 6.48 (dd, *J* = 8.0, 2.2 Hz, 1 H), 6.45 (t, *J* = 1.6, Hz 1 H), 3.91 (s, 3 H); ^{13}C NMR (150 MHz, CD_3OD) δ 168.69, 164.48, 160.56, 160.31, 115.36, 135.79, 131.51, 130.81, 128.49, 124.42, 120.71, 115.16, 111.62,

110.76, 106.99, 56.30; HRMS Calcd. for $C_{20}H_{18}NO_4$ $[M+H]^+$ 336.1230; found 336.1236.

11d: Pale brown prisms (methylene chloride/hexane); mp 103.7°C; ^{1}H NMR (600 MHz, CD_3OD) δ 8.01 (d, *J* = 7.8 Hz, 1 H), 7.71 (d, *J* = 8.4 Hz, 2 H), 7.55 (t, *J* = 8.4 Hz, 1 H), 7.41(d, *J* = 8.4 Hz, 1 H), 7.25 (t, *J* = 7.2 Hz, 1 H), 7.17 (t, *J* = 8.4 Hz, 1 H), 7.06 (d, *J* = 8.4 Hz, 2 H), 6.57 (dd, *J* = 8.4, 1.2 Hz, 1 H), 6.46 (dd, *J* = 8.4, 1.2 Hz, 1 H), 6.45 (t, *J* = 2.1 Hz, 1 H), 2.88 (s, 6 H); ^{13}C NMR (150 MHz, CD_3OD) δ 167.66, 160.57, 160.32, 155.16, 154.02, 135.74, 133.90, 131.97, 131.54, 128.85, 125.14, 123.33, 121.52, 121.01, 111.55, 110.64, 106.87, 45.52; HRMS Calcd. for $C_{21}H_{21}N_2O_3$ $[M+H]^+$ 349.1547; found 349.1536.

11e: Pale yellow prisms (chloroform); mp 171.0°C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.69 (d, *J* = 8.6 Hz, 2 H), 7.37, (t, *J* = 9.0 Hz, 1 H), 7.34 (s, 1 H), 7.28 (d, *J* = 6.9 Hz, 1 H), 7.17 (t, *J* = 8.2 Hz, 1 H), 7.04 (d, *J* = 8.4 Hz, 2 H), 7.02 (d, *J* = 9.7 Hz, 1 H), 6.57 (dd, *J* = 8.2, 1.7 Hz, 1 H), 6.48 (dd, *J* = 8.2, 1.7 Hz, 1 H), 6.45 (t, *J* = 2.2 Hz, 1 H), 3.05 (s, 6 H); ^{13}C NMR (150 MHz, CD_3OD) δ 170.02, 160.52, 160.32, 155.38, 152.46, 137.16, 135.72, 131.54, 130.54, 124.42, 120.70, 117.43, 116.90, 113.05, 111.58, 110.71, 106.91, 41.18; HRMS Calcd. for $C_{21}H_{21}N_2O_3$ $[M+H]^+$ 349.1547; found 349.1543.

11f: Pale yellow prisms (chloroform); mp 163.9°C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.88 (dt, *J* = 7.0, 2.0 Hz, 2 H), 7.66 (dt, *J* = 6.8, 2.2 Hz, 2 H), 7.16 (t, *J* = 8.4 Hz, 1 H), 7.03 (dt, *J* = 7.0, 2.0 Hz, 2 H), 6.82 (dt, *J* = 7.0, 2.0 Hz, 2 H), 6.56 (ddd, *J*= 8.2, 2.3, 0.7 Hz, 1 H), 6.48 (ddd, *J*= 8.0, 2.3, 0.7 Hz, 1 H), 6.45 (t, *J* = 2.3 Hz, 1 H), 3.10 (s, 6 H) ; ^{13}CNMR (150 MHz, CD_3OD) δ 169.26, 160.65, 160.27, 155.09, 154.84, 136.09, 131.50, 130.45, 124.39, 122.55, 120.73, 112.52, 111.54, 110.71, 106.91, 40.53; HRMS Calcd. for $C_{21}H_{21}N_2O_3$ $[M+H]^+$ 349.1547; found 349.1547.

4.3. Synthesis of 12a (General procedure for compounds 12 and 13, Scheme 2)

A mixture of *p*-(benzyloxy)phenol (220 mg, 1.10 mmol), *m*-fluoronitrobenzene (149 mg, 1.05 mmol), and potassium carbonate (173 mg, 1.25 mmol) in DMF (1.5 ml) was stirred at 150°C for 3 h, and at 80°C for 24 h. It was allowed to cool to room temperature, then 1 M HCl was added, and the mixture was extracted with ethyl acetate. The organic layer was dried over sodium sulfate, and evaporated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate 19:1) to afford **22a** (237 mg, 70%)

22a: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.89 (ddd, *J* = 7.8, 2.4, 0.6 Hz, 1 H), 7.72 (t, *J* = 2.4 Hz, 1 H), 7.43 (m, 5 H), 7.35 (t, *J* = 5.4 Hz, 1 H), 7.28 (ddd, *J* = 8.4, 2.4, 0.6 Hz, 1 H), 7.01 (s, 4 H), 5.08 (s, 2 H).

A mixture of **22a** (160 mg, 0.5 mmol) and palladium hydroxide on carbon (17 mg) in

tetrahydrofuran (5 ml) and methanol (5 ml) was stirred under a hydrogen atmosphere for 18 h. The reaction mixture was filtered over Celite, and the filtrate was evaporated to afford **23a** (106 mg, quant) as a pale brown oil.

23a: ^{1}H NMR (600 MHz, CD_3OD) δ 7.05 (t, *J* = 8.4 Hz, 1 H), 6.88 (dt, *J* = 9.0, 2.4 Hz, 2 H), 6.80 (dt, *J* = 9.0, 2.4 Hz, 2 H), 6.46 (ddd, *J* = 7.8, 1.8, 0.6 Hz, 1 H), 6.53 (t, *J* = 2.4 Hz, 1 H), 6.29 (ddd, *J* = 8.4, 2.4, 0.6 Hz, 1 H).

23a (89 mg, 0.44 mmol) was added to a solution of *o*-anisoyl chloride (228 mg, 1.34 mmol) in tetrahydrofuran (15 ml). The mixture was stirred at room temperature for 30 min, then poured into water, and extracted with ethyl acetate. The organic layer was washed successively with saturated sodium hydrogen carbonate, 2 M HCl, water, and brine, dried over magnesium sulfate, and evaporated. The residue was purified by silica gel column chromatography (chloroform/ethyl acetate 4:1) to afford **12a** (63 mg, 43%).

12a: Colorless prisms (chloroform/ethyl acetate); mp 189.4°C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.89 (dd, *J*= 7.7, 1.6 Hz, 1 H), 7.56 (td, *J* = 7.8, 1.6 Hz, 1 H), 7.48 (t, *J* = 1.0 Hz, 1 H), 7.29 (m, 2 H), 7.21 (d, *J* = 8.4 Hz, 1 H), 7.12 (t, *J* = 7.4 Hz, 1 H), 6.94 (dt, *J* = 8.9, 2.2 Hz, 2 H), 6.84 (dt, *J* = 8.8, 2.1 Hz, 2 H), 6.70 (m, 1 H), 4.04 (s, 3 H); ^{13}C NMR (150 MHz, CD_3OD) δ 167.01, 161.06, 159.09, 155.34, 150.72, 141.26, 134.52, 132.17, 131.04, 124.54, 122.40, 122.34, 117.55, 115.85, 114.51, 113.38, 111.33, 57.02; HRMS Calcd. for $C_{20}H_{18}NO_4$ $[M+H]^+$ 336.1230; found 336.1226.

Compound **12c, d, f** and **13** were synthesized similarly.

12c: Colorless prisms (ethyl acetate); mp 163.6°C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.92 (d, *J* = 8.9 Hz, 2 H), 7.40 (t, *J* = 2.2 Hz, 1 H), 7.37 (ddd, *J* = 7.9, 1.7, 0.7 Hz, 1 H), 7.29 (t, *J* = 8.1 Hz, 1 H), 7.05 (d, *J* = 8.9 Hz, 2 H), 6.94 (d, *J* = 8.9 Hz, 2 H), 6.83 (d, *J* = 8.9 Hz, 2 H), 6.71 (ddd, *J* = 8.1, 2.3, 0.8 Hz, 1 H), 3.90 (s, 3 H); ^{13}C NMR (150 MHz, CD_3OD) δ 168.75, 164.50, 160.99, 155.33, 150.70, 141.68, 130.90, 130.85, 128.50, 122.39, 117.54, 116.26, 115.12, 114.46, 111.58, 56.27; HRMS Calcd. for $C_{20}H_{18}NO_4$ $[M+H]^+$ 336.1230; found 336.1224.

12d: Pale brown prisms (methanol/ ethyl acetate); mp 196.4°C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.96 (dd, *J* = 7.9, 1.5 Hz, 1 H), 7.54 (td, *J* = 8.6, 1.6 Hz, 1 H), 7.48 (t, *J* = 2.0 Hz, 1 H), 7.40 (d, *J* = 8.1 Hz, 1 H), 7.31 (m, 1 H), 7.24 (m, 2 H), 6.95 (d, *J* = 8.9 Hz, 2 H), 6.84 (d, *J* = 8.8 Hz, 2 H), 6.69 (dd, *J* = 8.2, 2.2 Hz, 1 H), 2.85 (s, 6H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 165.56, 159.33, 154.43, 151.62, 148.22, 141.14, 131.98, 130.34, 128.02, 121.99, 121.60, 11 9.20, 116.75, 113.87, 112.27, 108.67, 44.30; HRMS Calcd. for $C_{21}H_{21}N_2O_3$ $[M+H]^+$ 349.1547; found 349.1544.

12f: Pale yellow prisms (ethyl acetate); mp 205.8°C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.87 (d,

J = 8.9 Hz, 2 H), 7.43 (t, *J* = 2.2 Hz, 1 H), 7.39 (d, *J* = 8.0 Hz, 1 H), 7.31 (t, *J* = 8.2 Hz, 1 H), 6.97 (d, *J* = 8.9 Hz, 2 H), 6.86 (d, *J* = 8.9 Hz, 2 H), 6.83 (d, *J* = 9.0 Hz, 2 H), 6.71 (dd, *J* = 8.1, 2.0 Hz, 1 H), 3.10 (s, 6 H); ^{13}C NMR (150 MHz, CD_3OD) δ 169.29, 160.93, 155.27, 154.86, 150.78, 141.97, 130.83, 130.50, 122.55, 122.35, 117.53, 116.26, 114.17, 112.47, 111.60, 40.50; HRMS Calcd. for $C_{21}H_{21}N_2O_3$ $[M+H]^+$ 349.1547; found 349.1544.

13a: Pale orange prisms (methanol); mp 189.5-190°C; ^{1}H NMR (600 MHz, acetone-d_6) δ 8.83 (s, 1 H), 8.34 (s, 1 H), 7.95 (d, *J* = 8.9 Hz, 1 H), 7.34 (dd, *J* = 11.0, 2.1 Hz, 2 H), 7.17 (d, *J* = 8.2 Hz, 1 H), 7.01 (dt, *J* = 8.9, 2.0 Hz, 2 H), 6.89 (dt, *J* = 9.0, 2.1 Hz, 2 H), 6.83 (dt, *J* = 8.9, 2.7 Hz, 2 H), 6.67 (dd, *J* = 8.3, 2.8 Hz, 1 H), 3.86 (s, 3 H), 3.28 (s, 3 H); ^{13}C NMR (150 MHz, acetone-d_6) δ 165.42, 163.33, 157.84, 154.55, 150.21, 138.73, 131.80, 131.69, 130.18, 128.04, 126.32, 121.54, 116.99, 116.90, 114.90, 114.63, 114.52, 114.44, 55.80, 17.440; HRMS Calcd. for $C_{21}H_{20}NO_4$ $[M+H]^+$ 350.1387; found 350.1384.

13b: Pale brown prisms (methanol); mp 203-204°C; ^{1}H NMR (600 MHz, acetone-d_6) δ 9.03 (s,1 H), 8.01 (dt, *J* = 9.0, 2.1 Hz, 2 H), 7.33 (d, *J* = 8.2 Hz, 1 H), 7.13 (t, *J* = 8.3 Hz, 1 H), 7.04 (dt, *J* = 9.0, 2.1 Hz, 2 H), 6.83 (s, 1 H), 6.64 (d, *J* = 7.5 Hz, 1 H), 3.87 (s, 3 H), 2.21 (s, 3 H); ^{13}C NMR (150 MHz, acetone-d_6) δ 165.61, 163.32, 157.29, 154.16, 150.90, 139.27, 130.23, 126.87, 121.12, 120.37, 116.97, 116.88, 115.57, 114.45, 55.81, 11.23; HRMS Calcd. for $C_{21}H_{20}NO_4$ $[M+H]^+$ 350.1387; found 350.1383.

13c: Colorless prisms (chloroform/ethyl acetate); mp 179.0-179.5°C; ^{1}H NMR (600 MHz, acetone-d_6) δ 9.35 (br, 1 H), 8.21 (br, 1 H), 7.91 (d, J = 8.3 Hz, 2 H), 7.54 (dt, J = 8.2, 2.8 Hz, 1 H), 7.29 (t, J = 2.1 Hz, 1 H), 7.19 (d, 8.3 Hz, 1 H), 6.99 (dt, J = 9.0, 2.7 Hz, 2 H), 6.85 (m, 4 H), 3.85 (s, 3 H), 2.22 (s, 3 H); ^{13}C NMR (150 MHz, acetone-d_6) δ 165.47, 163.21, 157.09, 154.19, 150.64, 139.59, 131.72, 130.09, 128.26, 123.93, 120.66, 116.99, 116.90, 115.26, 114.33, 110.14, 55.76, 15.81; HRMS Calcd. for $C_{21}H_{20}NO_4$ $[M+H]^+$ 350.1387; found 350.1383.

13d: Colorless needles (methanol); mp 190.0-191.5°C; ^{1}H NMR (600 MHz, acetone-d_6) δ 9.81 (br, 1 H), 8.19 (s, 1 H), 7.98 (dd, *J* = 8.3, 2.1 Hz, 1 H), 7.51 (ddd, *J* = 8.2, 6.9, 1.4 Hz, 1 H), 7.48 (d, *J* = 2.7 Hz, 1 H), 7.36 (dd, *J* = 8.3, 2.1 Hz, 1 H), 7.21 (d, *J* = 8.2 Hz, 1 H), 7.18 (d, *J* = 8.2 Hz, 1 H), 7.08 (td, *J* = 7.6, 1.3 Hz, 1 H), 6.84 (s, 4 H), 4.03 (s, 3 H), 2.21 (s, 3H); ^{13}C NMR (150 MHz, CD_3OD) δ 166.65, 158.68, 157.52, 154.37, 151.23, 138.59, 134.12, 132.17, 131.74, 125.87, 124.25, 122.01, 120.60, 117.16, 116.22, 112.92, 111.51, 56.60, 15.93; HRMS Calcd. for $C_{21}H_{20}NO_4$ $[M+H]^+$ 350.1387; found 350.1385.

4.4. Synthesis of 12b (Scheme 2)

A solution of copper (II) sulfate· $5H_2O$ (267 mg, 1.07 mmol) in methanol (4 ml) was added to a solution of sodium borohydride (111 mg, 2.94 mmol) and **22** (165 mg, 0.5 mmol) in methylene chloride (5 ml), and the mixture was stirred at 0°C for 3 h, and at room temperature for 2 h. The mixture was poured into water, and extracted with methylene chloride. The organic layer was washed with water and brine, dried over sodium sulfate, and evaporated. The residue was dissolved in methylene chloride, and 1 M hydrochloric acid in ether was added to it. The resulting precipitate was collected to afford **26** (108 mg, 63%).
26: Pale yellow powder; ^{1}H NMR (600 MHz, CD_3OD) δ 7.43 (d, *J* = 7.6 Hz, 2 H), 7.36 (t, *J* = 6.9 Hz, 2 H), 7.29 (t, *J* = 6.9 Hz, 1 H), 7.26 (d, *J* = 2.0 Hz, 1 H), 7.12 (dd, *J* = 8.9, 2.8 Hz, 1 H), 7.01 (dt, *J* = 9.6, 2.8 Hz), 6.90 (dt, *J* = 8.9, 2.0 Hz, 2 H), 6.80 (d, *J* = 8.9 Hz, 1 H), 5.06 (s, 2 H), 2.32 (s, 3 H); ^{13}C NMR (150 MHz, CD_3OD) δ 158.08, 156.78, 151.44, 138.65, 131.96, 129.52, 128.92, 128.58, 126.62, 126.08, 122.65, 121.20, 119.00, 117.32, 71.50, 16.31.

m-Anisoyl chloride (161 mg, 0.18 mmol) was added to a solution of **26** (103 mg, 0.49 mmol) in pyridine (5 ml). The mixture was stirred at room temperature for 45 min, then poured into water, and extracted with ethyl acetate. The organic layer was successively washed with saturated sodium hydrogen carbonate, 2 M hydrochloric acid, water and brine, dried over magnesium sulfate, and evaporated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate 2:1) to give **27a** (R^1 = OMe, 122 mg, 90%).
27a: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.74 (br, 1 H), 7.44 (d, *J* = 7.6 Hz, 2 H), 7.33-7.41 (m, 7 H), 7.29 (t, *J* = 8.3 Hz, 1 H), 7.01 (ddd, *J* = 7.6, 2.8, 1.4 Hz, 1 H), 7.01 (dt, *J* = 8.9, 2.7 Hz, 2 H), 6.97 (dt, *J* = 9.6, 2.7 Hz, 2 H), 6.75 (ddd, *J* = 8.3, 2.0, 1.4 Hz, 1 H), 5.05 (s, 2 H), 3.86 (s, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.44, 160.01, 159.15, 155.29, 149.99, 139.13, 136.97, 136.33, 130.06, 129.80, 128.60, 128.00, 127.53, 121.01, 118.58, 118.12, 115.9597, 114.08, 113.73, 112.46, 109.35, 70.52, 55.50.

A mixture of **27a** (74 mg, 0.17 mmol) and palladium hydroxide on carbon (5 mg) in tetrahydrofuran (5 ml) and methanol (2 ml) was stirred at 45°C under a hydrogen atmosphere for 41 h. The reaction mixture was filtered over Celite, and the filtrate was evaporated. The residue was purified by silica gel column chromatography (hexane /ethyl acetate 3:2), followed by recrystallization from chloroform/ethyl acetate to give **12b** (33 mg, 57 %).
12b: Colorless prisms (chloroform/ethyl acetate); mp 144.6°C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.50 (t, *J* = 7.7 Hz, 1 H), 7.48 (d, *J* = 2.1 Hz, 1 H), 7.43 (t, *J* = 8.0 Hz, 1 H), 7.42 (d, *J* = 2.5 Hz, 1 H), 7.39 (d, *J* = 8.1 Hz, 1 H), 7.30 (t, *J* = 8.2 Hz, 1 H), 7.16 (ddd, *J* = 8.0, 2.2, 0.5 Hz, 1 H), 6.94 (d, *J* = 8.9 Hz, 2 H), 6.84 (d, *J* = 8.9 Hz, 2 H), 6.72 (dd, *J* = 8.1, 1.7 Hz, 1 H), 3.89 (s, 3 H); ^{13}C NMR(150 MHz, CD_3OD) δ 169.01, 161.64, 161.00, 155.35, 150.67, 141.49, 137.90, 130.97, 130.94, 122.40, 121.03, 119.12, 117.55, 116.30, 114.66, 114.21, 111.63, 56.25; HRMS Calcd. for $C_{20}H_{18}NO_4$ $[M+H]^+$ 336.1230; found 336.1226.

Compound **12e** were synthesized similarly.

12e: Colorless prisms; mp 167.2°C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.43 (t, J = 1.8 Hz, 1 H), 7.38 (d, J = 7.6 Hz, 1 H), 7.34 (t, J = 7.9 Hz, 1 H), 7.30 (t, J = 8.2 Hz, 1 H), 7.28 (s, 1 H), 7.22 (d, J = 7.6 Hz, 1 H), 6.98 (ddd, J = 8.2, 2.6, 0.5 Hz, 1 H), 6.94 (d, J = 8.9 Hz, 2 H), 6.83 (d, J = 8.9 Hz, 2 H), 6.72 (ddd, J = 7.4, 2.6, 1.6 Hz, 1 H), 3.03 (s, 6 H); ^{13}C NMR (150 MHz, CD_3OD) δ 170.17, 161.00, 155.37, 152.55, 150.56, 141.62, 137.14, 130.92, 130.48, 122.46, 117.51, 117.32, 116.70, 116.21, 114.46, 112.90, 111.49, 41.06; HRMS Calcd. for $C_{21}H_{21}N_2O_3$ $[M+H]^+$ 349.1547; found 349.1544.

4.5. Synthesis of 14a (General procedure for compounds 14, Scheme 2)

O-Monobenzylresorcinol (220 mg, 1.10 mmol) was added to a solution of *m*-fluoronitrobenzene (149 mg, 1.05 mmol) and potassium carbonate (173 mg, 1.25 mmol) in DMF (1.5 ml) under an argon atmosphere, and the mixture was stirred at 150°C for 3 h and at 80°C for 24 h. It was allowed to cool to room temperature, then poured into 1 M HCl, and extracted with ethyl acetate. The organic layer was dried over sodium sulfate, and evaporated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate 19:1) to afford **24a** (273 mg, 80%).

24a: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.94 (ddd, *J* = 8.4, 2.4, 0/6 Hz, 1 H), 7.81 (t, *J* = 2.4 Hz, 1 H), 7.48 (t, *J* = 7.8 Hz, 1 H), 7.36 (m, 7 H), 6.84 (dd, *J* = 8.4, 2.4 Hz, 1 H), 6.67 (m, 2 H), 5.06 (s, 2 H).

A mixture of **24a** (166 mg, 0.5 mmol) and palladium hydroxide on carbon (20 mg) in THF (5 ml) and methanol (5 ml) was stirred under a hydrogen atmosphere for 14 h. The reaction mixture was filtered over Celite, and evaporated to give **25a** (107 mg, quant), which was used for the next reaction without further purification. **25a** (115 mg, 0.57 mmol) was added to a solution of *o*-anisoyl chloride (295 mg, 1.73 mmol) in tetrahydrofuran (15 ml), and the mixture was stirred at room temperature for 30 min, then poured into water, and extracted with ethyl acetate. The organic layer was washed successively with saturated sodium hydrogen carbonate, 2 M hydrochloric acid, water and brine, dried over magnesium sulfate, and evaporated. The residue was washed with dichloromethane to give **14a** (122 mg, 71%), which was purified by recrystallization from chloroform.

14a: pale brown prisms (chloroform); mp 166.0°C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.90 (dd, *J* = 7.7, 1.7 Hz, 1 H), 7.56 (m, 2 H), 7.37 (m, 2 H), 7.21 (d, *J* = 8.3 Hz, 1 H), 7.18 (t, *J* = 8.1 Hz, 1 H), 6.80 (ddd, *J* = 7.9, 3.3, 1.0 Hz, 1 H), 6.59 (ddd, *J* = 8.3, 2.2, 0.7 Hz, 1 H), 6.52 (dd, *J* = 8.2, 1.7 Hz, 1 H), 6.48 (t, *J* = 2.3 Hz, 1 H), 4.04 (s, 3 H) ; ^{13}C NMR (150 MHz, CD_3OD) δ 167.10, 160.36, 160.01, 159.35, 159.04, 141.42, 134.56, 132.12, 131.57, 131.19, 124.50, 122.34, 116.72, 116.04, 113.27, 112.76, 111.85, 111.14, 107.35, 56.95; HRMS; Calcd. for

$C_{20}H_{18}NO_4$ $[M+H]^+$ 336.1230; found 336.1228.

Compound **14b** – **14e** were synthesized similarly.

14b: Pale brown prisms (chloroform); mp 129.5-130.5°C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.40 (m, 5 H), 7.33 (m, 1 H), 7.13 (m, 2 H), 6.77 (dd, *J* = 8.3, 2.1 Hz, 1 H), 6.54 (dd, *J* = 8.3, 2.7 Hz, 1 H), 6.48 (dd, *J* = 8.2, 2.0 Hz, 1 H), 6.44 (s, 1 H), 3.85 (s, 3 H); ^{13}C NMR (150 MHz, CD_3OD) δ 168.72, 161.30, 160.07, 159.65, 159.03, 141.36, 137.52, 131.29, 130.81, 130.71, 120.73, 118.84, 116.83, 115.83, 113.82, 112.73, 111.58, 110.93, 107.14, 55.90; HRMS Calcd. for $C_{20}H_{18}NO_4$ $[M+H]^+$ 336.1230; found 336.1228.

14c: Pale brown prisms; mp 132-132.5°C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.89 (dd, *J* = 6.8, 2.0 Hz, 2 H), 7.45 (t, *J* = 2.0 Hz, 1 H), 7.42 (d, *J*= 9.0 Hz, 1 H), 7.31 (t, *J* = 8.2 Hz, 1 H), 7.13 (t, *J* = 8.2 Hz, 1 H), 7.01 (d, *J* = 8.9 Hz, 2 H), 6.75 (dd, *J* = 8.6, 2.7 Hz, 1 H), 6.54 (dd, *J* = 8.6, 2.7 Hz, 1 H), 6.48 (dd, *J* = 8.3, 2.1 Hz, 1 H), 6.44 (t, *J* = 2.4 Hz, 1 H), 3.86 (s, 3 H); ^{13}C NMR (150 MHz, CD_3OD) δ 168.76, 164.51, 160.34, 159.97, 159.30, 141.84, 131.59, 131.08, 130.91, 128.36, 117.11, 115.94, 115.11, 113.01, 111.85, 111.22, 107.43, 56.27; HRMS Calcd. for $C_{20}H_{18}NO_4$ $[M+H]^+$ 336.1230; found 336.1228.

14d: pale yellow prisms (chloroform); mp 164.8°C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.97 (d, *J* = 7.2 Hz, 1 H), 7.58 (s, 1 H), 7.55 (t, *J* = 8.1 Hz, 1 H), 7.38 (m, 3 H), 7.24 (t, *J* =7.6 Hz, 1 H), 7.19 (t, *J* = 8.1 Hz, 1 H), 6.79 (dt, *J* = 6. 8, 2.3 Hz, 1 H), 6.59 (dd, *J* = 8.2, 2.3 Hz, 1 H), 6.53 (dd, *J* = 8.2, 2.2 Hz, 1 H), 6.49 (t, *J* = 2.2 Hz, 1 H), 2.86 (s, 6 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.83, 160.38, 159.95, 159.50, 154.02, 141.64, 134.00, 131.99, 131.58, 131.32, 128.78, 125.15, 121.54, 116.33, 115.81, 112.35, 111.90, 111.20, 107.42, 45.50; HRMS Calcd. for $C_{20}H_{18}NO_4$ $[M+H]^+$ 349.1547; found 349.1544.

14e: Pale brown prisms; mp 179.3°C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.51 (t, *J* = 2.3 Hz, 1 H), 7.47 (ddd, *J* = 8.2, 1.7, 0.8 Hz, 1 H), 7.36 (m. 2 H), 7.30 (t, *J* = 1.9 Hz, 1 H), 7.23 (dd, *J* = 6.9, 1.3 Hz, 1 H), 7.18 (t, *J* = 8.1 Hz, 1 H), 6.99 (dd, *J* = 6.9, 1.3 Hz, 1 H), 7.18 (t, *J* = 8.1 Hz, 1 H), 6.99 (dd, *J* = 8.0, 2.6 Hz, 1 H), 6.81 (ddd, *J* = 8.2, 2.3, 0.7 Hz, 1 H), 6.58 (ddd, *J* = 8.2, 2.2, 0.7 Hz, 1 H), 6.53 (ddd, *J* = 8.3, 2.3, 0.7 Hz, 1 H), 6.49 (t, *J*= 2.3 Hz, 1 H), 3.04 (s, 6 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.11, 160.27, 159.98, 159.27, 152.56, 141.71, 137.09, 131.54, 131.03, 130.46, 117.36, 117.27, 116.68, 116.07, 113.20, 112.93, 111.89, 111.26, 107.48, 41.04. HRMS Calcd. for $C_{21}H_{21}N_2O_3$ $[M+H]^+$ 349.1547; found 349.1542.

4.6. Synthesis of 15 (Scheme 3)

An aqueous solution of 5 M sodium hydroxide (2.0 ml, 10 mmol) was added to DMSO (8 ml) at room temperature under an Ar atmosphere. After 10 min, *O*-monobenzylhydroquinone (961

mg, 4.8 mmol) was added to it. The mixture was stirred at 50°C for 15 min, then 2-fluoro-5-nitrotoluene (620 mg, 4.0 mmol) was added, and stirring was continued at 50°C for 5.5 h, and at room temperature for 15 h. The reaction mixture was then poured into ice water and the precipitate was collected by filtration to give **29a** (R^2 = 2-Me, 1.04 g, 65%).
29a (R^2 = 2-Me): Yellow powder; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.13 (d, *J* = 2.1 Hz, 1 H), 7.96 (dd, *J* = 8.9, 2.7 Hz, 1 H), 7.36 (m, 5 H), 7.02 (dt, *J* = 9.6, 2.8 Hz, 2 H), 6.99 (dt, *J* = 8.9, 2.7 Hz, 2 H), 6.69 (d, *J* = 8.9 Hz, 1 H), 5.08 (s, 2 H), 2.42 (s, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.21, 156.04, 148.51, 142.06, 136.67, 128.65, 128.12, 127.48, 126.64, 123.14, 121.50, 116.24, 114.47.

A solution of copper (II) sulfate·$5H_2O$ (502 mg, 2.0 mmol) in methanol (4 ml) was added to a solution of sodium borohydride (189 mg, 5.0 mmol) and **29a** (R^2 = 2-Me, 234 mg, 0.70 mmol) in methylene chloride (5 ml), and the mixture was stirred at 0°C for 2.5 h. Sodium borohydride (76 mg, 2.0 mmol) was added to it, and stirring was continued at 0°C for 2 h. Sodium borohydride (76 mg, 2.0 mmol) was added, and the reaction mixture was stirred at 0°C for 1 h, then poured into water, and extracted with methylene chloride. The organic layer was washed with water and brine, dried over sodium sulfate, and evaporated. The residue was dissolved in ether, and 1 M hydrochloric acid in ether was added to the solution. The resulting precipitate was collected to afford **30** (205 mg, 86%).
30: Pale brown powder; ^{1}H NMR (600 MHz, CD_3OD) δ 7.43 (d, *J* = 7.6 Hz, 2 H), 7.33 (m, 3 H), 7.26 (d, *J* = 2.0 Hz, 1 H), 7.12 (dd, *J* = 8.9, 2.8 Hz, 1 H), 7.01 (dt, *J* = 9.6, 2.8 Hz, 2 H), 6.90 (dt, *J* = 8.9, 2.0 Hz, 2 H), 6.80 (d, *J* = 8.9 Hz, 1 H), 5.06 (s, 2 H), 2.33 (s, 3 H) ; ^{13}C NMR (150 MHz, CD_3OD) δ 158.08, 156.78, 151.44, 138.65, 131.96, 129.52, 128.92, 128.58, 126.62, 126.08, 122.65, 121.20, 119.00, 117.32, 71.50, 16.31.

m-Anisoyl chloride (61 mg, 0.36 mmol) was added to a solution of **30** (102 mg, 0.30 mmol) in pyridine (5 ml). The mixture was stirred at room temperature for 30 min, then poured into water, and extracted with ethyl acetate. The organic layer was washed successively with saturated sodium hydrogen carbonate, 1 M hydrochloric acid, water and brine, dried over sodium sulfate, and evaporated to give **31** (113 mg, 86%).
31: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.74 (s, 1 H), 7.54 (d, *J* = 2.7 Hz, 1 H), 7.39 (m, 8 H), 7.08 (dt, *J* = 6.9, 2.7 Hz, 1 H), 6.93 (dt, *J* = 9.6, 2.7 Hz, 2 H), 6.75 (dt, *J* = 9.0, 2.4 Hz, 2 H), 6.56 (d, *J* = 9.0 Hz, 1 H), 5.04 (s, 2 H), 3.87 (s, 3 H), 2.28 (s, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.41, 159.97, 154.37, 152.25, 151.58, 137.03, 136.43, 133.14, 130.19, 129.77, 128.57, 127.96, 127.49,123.37, 119.10, 118.77, 118.55, 117.94, 115.85, 112.45, 70.56, 55.48, 16.38.

Sodium hydride (8.0 mg, 0.30 mmol) was washed with *n*-hexane twice. A solution of **31** (44 mg, 0.10 mmol) in dry DMF (0.5 ml) was added to a suspension of sodium hydride in dry

DMF (0.3 ml) at 0°C, and the mixture was stirred at room temperature for 30 min. Iodomethane (43 mg, 0.30 mmol) in dry DMF (0.3 ml) was added at 0°C, and stirring was continued at room temperature for 30 min. Remaining iodomethane was removed *in vacuo*. The residue was poured into water, and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over sodium sulfate, and evaporated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate 2:1 to 3:2) to afford **32** (38 mg, 86%).

32: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37 (m, 5 H), 7.08 (t, *J* = 7.9 Hz, 1 H), 6.95 (br, 1 H), 6.90 (m, 3 H), 6.86 (br d, *J* = 7.5 Hz, 1 H), 6.80 (m, 3 H), 6.75 (br d, *J* = 7.5 Hz, 1 H), 6.61 (d, *J* = 9.0 Hz, 1 H), 5.02 (s, 2 H), 3.69 (s, 3 H), 3.46 (s, 3 H), 2.17 (s, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.33, 158.85, 154.63, 154.07, 150.82, 139.72, 137.20, 136.90, 129.39, 128.68, 128.55, 127.96, 127.41, 119.21, 118.25, 115.85, 115.81, 70.50, 55.14, 38.51, 16.15.

A mixture of **32** (38 mg, 0.086 mmol) and palladium hydroxide on carbon (5 mg) in THF (2 ml) was stirred under a hydrogen atmosphere for 21 h. The reaction mixture was filtered over Celite, and the filtrate was evaporated. The crude product was recrystallized from methanol to give **15** (9 mg, 30%).

15: Colorless prisms (methanol); mp 172-173°C; ^{1}H NMR (600 MHz, acetone-d_6) δ 7.12 (t, J = 7.5 Hz, 2 H), 6.90 (br dd, *J* = 9.0, 2.8 Hz, 1 H), 6.87 (d, *J* = 7.6 Hz, 2 H), 6.85 (m, 1 H), 6.80 (m, 3 H), 6.74 (dt, *J* = 9.0, 2.1 Hz, 2 H), 6.60 (d, *J* = 8.9 Hz, 1 H), 3.66 (s, 3 H) , 3.37 (s, 3 H) , 2.13 (s, 3 H); ^{13}C NMR (150 MHz, acetone- d_6) δ 170.16, 159.80, 155.29, 154.27, 154.17, 150.54, 140.88, 138.99, 130.67, 130.11, 129.47, 126.74, 121.65, 120.35, 118.53, 118.53, 117.00, 115.95, 114.62, 55.45, 38.37, 16.18; HRMS Calcd. for $C_{21}H_{20}NO_4$ $[M+H]^+$ 350.1387; found 350.1384.

4.7. Synthesis of 16a (Scheme 3)

Sodium hydride (8.0 mg, 0.30 mmol) was washed with *n*-hexane twice. A solution of **27a** (R^1 = *m*-OMe, 44 mg, 0.10 mmol) in dry DMF (0.7 ml) was added to a suspension of sodium hydride in dry DMF (0.3 ml) at 0°C, and the mixture was stirred at room temperature for 30 min. Iodomethane (47 mg, 0.33 mmol) was added at 0°C, and stirring was continued at room temperature for 40 min. Remaining iodomethane was removed *in vacuo*, then the residue was poured into water, and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over sodium sulfate, and evaporated. The residue was purified by silica gel column chromatography (hexane/ethyl 2:1) to give **28a** (37 mg, 82%).

28a: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.44 (d, *J* = 6.9 Hz, 2 H), 7.4 (t, *J* = 7.6 Hz, 2 H), 7.34 (t, *J* = 7.6 Hz, 1 H), 7.20 (t, *J* = 8.3 Hz, 1 H), 7.06 (t, *J* = 8.2 Hz, 1 H), 6.84 (m, 5 H), 6.78 (m, 2 H), 6.48 (dt, *J* = 8.9, 2.1 Hz, 2 H), 6.45 (t, *J* = 2.1 Hz, 1 H), 5.04 (s, 2 H), 3.67 (s, 3 H), 3.45 (s, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.31, 159.02, 158.62, 155.16, 149.40, 145.99, 137.02,

136.85, 130.09, 128.77, 128.59, 128.01, 127.41, 121.03, 120.47, 120.11, 116.32, 116.22, 115.98, 115.86, 113.36, 70.41, 55.14, 38.09.

A mixture of **28a** (37 mg, 0.085 mmol) and palladium hydroxide on carbon (5 mg) in tetrahydrofuran (2.5 ml) was stirred under a hydrogen atmosphere for 18.5 h. The reaction mixture was filtered over Celite, and evaporated. The crude product was recrystallized from chloroform to give **16a** (19 mg, 64%).

16a: Colorless prisms (chloroform); mp 160-160.5°C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.21 (t, *J* = 8.3 Hz, 1H), 7.09 (dt, *J* = 7.5, 1.4 Hz, 1 H), 6.84 (m, 3 H), 6.78 (m, 2 H), 6.72 (dt, *J* = 9.0, 2.1 Hz, 2 H), 6.59 (dt, *J* = 9.0, 2.1 Hz, 2 H), 6.42 (br, 1 H), 5.16 (s 1 H), 3.69 (s, 3 H), 3.46 (s, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.55, 159.07, 158.84, 152.24, 149.01, 145.89, 136.94, 130.14, 128.82, 121.03, 120.80, 119.97, 116.38, 116.33, 116.15, 115.99, 113.36, 55.19, 38.19 HRMS Calcd. for $C_{21}H_{20}NO_4$ $[M+H]^+$ 350.1387; found 350.1385.

Compound **16b** was synthesized similarly.

16b: Colorless needles; mp 107.5-108.5 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.24 (dt, *J* = 8.9, 2.1 Hz, 2 H), 7.21 (t, *J* = 8.2 Hz, 1 H), 6.81 (dd, *J* = 6.9, 2.1 Hz, 1 H), 6.78(ddd, *J* = 8.3, 2.7, 1.4 Hz, 1 H), 6.72 (m, 4 H), 6.63 (dt, *J* = 8.9, 2.1 Hz, 2 H), 6.47 (t, *J* = 2.1 Hz, 1 H), 5.34 (br d, *J* = 10.3 Hz, 1 H), 3.79 (s, 3 H) , 3.46 (s, 3 H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.45, 160.70, 158.80, 152.30, 149.11, 146.39, 130.79, 130.14, 127.74, 120.71, 120.15, 116.37, 116.29, 115.78, 113.08, 55.27, 38.42; HRMS Calcd. for $C_{21}H_{20}NO_4$ $[M+H]^+$ 350.1387; found 350.1383.

4.8. Synthesis of 17a (General procedure for compounds 17 - 19, Scheme 3)

A mixture of **29b** (R^2 = 2-F, 153 mg, 0.80 mmol) and palladium hydroxide on carbon (36 mg) in THF (4 ml) and methanol (4 ml) was stirred under a hydrogen atmosphere for 20 h, then filtered over Celite. The filtrate evaporated to give **33a** (R^2 = 2-F, 115 mg, 67%).

33a (R^2 = 2-F): red brown solid; ^{1}H NMR (600 MHz, CD_3OD) δ 6.78 (t, *J* = 8.9 Hz, 2 H), 6.70 (m, 4 H), 6.53 (dd, *J* = 12.4, 2.8 Hz, 1 H), 6.44 (ddd, *J* = 8.9, 2.8, 1.4 Hz, 1 H); ^{13}C NMR (150 MHz, CD_3OD) δ 156.37 (d, *J* = 242.73 Hz), 153.59, 153.14, 146.86 (d, *J* = 8.63 Hz), 136.46 (d, *J* = 12.92 Hz), 123.95, 118.27, 116.80, 112.04, 104.48 (d, *J* = 21.54 Hz).

m-Anisoyl chloride (97 mg, 0.18 mmol) was added to a solution of **33a** (R^2 = 2-F, 104 mg, 0.47 mmol) in THF (10 ml). The mixture was stirred at room temperature for 50 min, then poured into water, and extracted with ethyl acetate. The organic layer was washed successively with saturated sodium hydrogen carbonate, 1 M hydrochloric acid, water and brine, dried over sodium sulfate, and evaporated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate 2:1 to 1:1) to give **17a** (134 mg, 80%).

17a: Colorless prisms (chloroform); mp 160-161°C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.73 (dd, *J* = 13.0, 2.8 Hz, 1 H), 7.49 (dd, *J* = 7.6, 1.4 Hz, 1 H), 7.47 (t, *J* = 2.1 Hz, 1 H), 7.41 (t, *J* = 7.9 Hz, 1 H), 7.36 (dt, *J* = 8.9, 1.7 Hz, 1 H), 7.14 (ddd, *J* = 8.3, 2.7, 1.3 Hz, 1 H), 6.95 (t, *J* = 9.0 Hz, 1 H), 6.68 (dt, *J* = 8.9, 2.1 Hz, 2 H), 6.76 (dt, *J* = 8.9, 2.4 Hz, 2 H), 3.86 (s, 3 H); ^{13}C NMR (150 MHz, CD_3OD) δ 168.55, 161.33, 154.71 (d, *J* = 244.16 Hz), 154.67, 151.33, 142.94 (d, *J* = 11.49 Hz), 137.34, 136.04 (d, *J* = 10.065 Hz), 130.75, 121.52, 120.70, 120.01, 118.81, 118.12, 117.10, 113.89, 111.09 (d, *J* = 22.98 Hz), 55.91; HRMS Calcd for $C_{20}H_{17}NO_4F$ $[M+H]^+$: 354.1142. Found 354.1142.

Compounds **17b** – **e**, **18**, and **19** were synthesized similarly.

17b: Colorless needles (hexane/ethyl acetate); mp: 187-188 °C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.92 (d, *J* = 2.8 Hz, 1 H), 7.48 (m, 3 H), 7.41 (t, *J* = 7.5 Hz, 1 H), 7.13 (dd, *J* = 8.2, 2.7 Hz, 1 H), 6.87 (d, *J* = 9.0 Hz, 1 H), 6.82 (dt, *J* = 9.0, 2.1 Hz, 2 H), 6.77 (dt, *J* = 8.9, 2.7 Hz, 2 H), 3.86 (s, 3 H); ^{13}C NMR (150 MHz, CD_3OD) δ 167.22, 160.00, 153.58, 150.41, 149.43, 135.97, 134.39, 129.41, 124.24, 122.93, 120.56, 119.37, 118.88, 117.49, 115.87, 112.54, 54.58; HRMS Calcd for $C_{20}H_{17}NO_4Cl$ $[M+H]^+$: 370.0846. Found 370.0844.

17c: Colorless prisms (hexane/ethyl acetate); mp 192-193°C; ^{1}H NMR (600 MHz, CD_3OD) δ 8.07 (d, *J* = 2.2 Hz, 1 H), 7.55 (dd, *J* = 8.9, 2.8 Hz, 1 H), 7.48 (d, *J* = 7.5 Hz, 1 H), 7.46 (t, *J* = 2.1 Hz, 1 H), 7.41 (t, *J* = 7.6 Hz, 1 H), 7.13 (dq, *J* = 6.9, 1.4 Hz, 1 H), 6.83 (m, 3 H), 6.77 (dt, *J* = 9.0, 2.8 Hz, 2 H), 3.86 (s, 3 H); ^{13}C NMR (150 MHz, CD3OD) δ 168.56, 161.34, 154.96, 152.99, 150.75, 137.31, 135.91, 130.76, 127.29, 122.64, 120.86, 120.72, 119.91, 118.84, 117.22, 114.35, 113.88, 55.92; HRMS Calcd for $C_{20}H_{17}NO_4Br$ $[M+H]^+$: 414.0341. Found 414.0342.

17d: Colorless prisms (hexane/ethyl acetate); mp 189°C; ^{1}H NMR (600 MHz, CD_3OD) δ 8.09 (d, *J* = 2.8 Hz, 2 H), 7.78 (dd, *J* = 8.9, 2.8 Hz, 1 H), 7.50 (dt, *J* = 8.9, 1.4 Hz, 1 H), 7.47 (dd, *J* = 2.8, 1.2 Hz, 1 H), 7.41 (t, *J* = 8.2 Hz, 1 H), 7.13 (dd, *J* = 8.3, 2.8 Hz, 1 H), 6.89 (dt, *J* = 8.9, 2.1 Hz, 2 H), 6.86 (d, *J* = 8.9 Hz, 1 H), 6.50 (dt, *J* = 8.9, 2.0 Hz, 2 H), 3.86 (s, 3 H); ^{13}C NMR (150 MHz, CD_3OD) δ 168.61, 161.35, 155.57, 154.43, 149.98, 137.19, 134.37, 130.77, 127.24, 124.82 (q, *J* = 270.00 Hz), 121.98, 121.24 (d, *J* = 31.59 Hz), 120.87 (d, *J* = 5.75 Hz), 119.25, 118.91, 117.37, 113.87, 55.92; HRMS Calcd for $C_{21}H_{17}NO_4F_3$ $[M+H]^+$: 404.1110. Found 404.1108.

17e: Colorless prisms (acetonitrile); mp 229-230°C; ^{1}H NMR (600 MHz, acetone-d_6) δ 9.74 (br, 1 H), 8.29 (t, *J* = 3.1 Hz, 1 H), 7.94 (dt, *J* = 8.9, 2.8 Hz, 1 H), 7.55(dd, *J* = 6.9, 2.1 Hz, 1 H), 7.41 (t, *J* = 8.2 Hz, 1 H), 7.14 (ddd, *J* = 8.2, 2.8, 1.4 Hz, 1 H), 7.01 (dt, *J* = 9.0, 2.1 Hz, 1 H), 6.91 (dt, *J* = 9.0, 2.8 Hz, 2 H), 6.87 (d, *J* = 9.0 Hz, 1 H), 3.85 (s, 3 H); ^{13}C NMR (150

MHz, DMSO-d_6) δ 165.29, 159.21, 156.02, 154.67, 146.87, 135.66, 133.99, 129.67, 127.34, 124.67, 121.07, 119.84, 117.55, 116.02, 112.93, 101.50, 55.37; HRMS: Calcd for $C_{21}H_{17}N_2O_4$ [M+H]+: 361.1188. Found 361.1187.

18a: Colorless prisms (chloroform); mp 78.5-79.5°C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.52 (d, *J* = 7.5 Hz, 1 H), 7.50 (d, *J* = 2.6 Hz, 1 H), 7.41 (t, *J* = 7.9 Hz, 1 H), 7.21 (d, *J* = 8.2 Hz, 1 H), 7.14 (dd, *J* = 8.3, 2.1 Hz, 1 H), 6.87 (dt, *J* = 9.2, 2.7 Hz, 2 H), 6.83 (d, *J* = 2.8 Hz, 1 H), 6.78 (dt, *J* = 8.9, 2.1 Hz, 2 H), 6.75 (dd, *J* = 8.3, 2.8 Hz, 1 H), 3.86 (s, 3 H); ^{13}C NMR (150 MHz, CD_3OD) δ 169.24, 161.34, 158.98, 155.01, 150.45, 137.69, 136.96, 131.29, 130.75, 129.33, 122.01, 120.72, 120.04, 117.18, 116.06, 113.89, 55.89, 18.35; HRMS Calcd for $C_{21}H_{20}NO_4$ $[M+H]^+$: 350.1387. Found 350.1813.

18b: Pale brown prisms (hexane/ethyl acetate); mp: 156-157°C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.51 (d, *J* = 2.7 Hz, 1 H), 7.50 (s, 1 H), 7.48 (d, *J* = 2.8 Hz, 1 H), 7.41 (t, *J* = 7.9 Hz, 1 H), 6.91 (dt, *J* = 8.9, 2.1 Hz, 2 H), 6.81 (dt, *J* = 8.9, 2.4 Hz, 2 H), 6.73 (m, 2 H) 3.86 (s, 3 H); ^{13}C NMR (150 MHz, CD_3OD) δ 169.06, 161.29, 159.59 (d, *J* = 10.05 Hz), 159.06, 157.42, 155.50, 149.61, 136.57, 130.83, 129.03, 122.42, 120.83, 120.52 (d, *J* = 12.93 Hz), 119.07, 117.40, 113.90, 113.51, 105.84 (d, *J* = 22.98 Hz), 55.95; HRMS Calcd for C_{20} H_{17} NO_4F $[M+H]^+$: 354.1142. Found 354.1141.

18c: Colorless prisms (chloroform); mp 176-176.5°C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.54 (d, *J* = 9.2 Hz, 1 H), 7.53 (d, *J* = 7.6 Hz, 1 H), 7.50(br, 1 H), 7.42(t, *J* = 7.7 Hz, 1 H), 7.15 (dd, *J* = 8.2, 2.7 Hz, 1 H), 7.00 (d, *J* = 2.8 Hz, 1 H), 6.92 (dt, *J* = 9.0, 2.8 Hz, 2 H), 6.90 (dd, *J* = 8.9, 2.8 Hz, 1 H), 6.82 (dt, *J* = 8.9, 2.8 Hz, 2 H), 3.87 (s, 3 H); ^{13}C NMR (150 MHz, CD_3OD) δ 167.65, 160.04, 158.04, 154.34, 148.22, 135.36, 130.68, 129.48, 128.69, 121.059, 119.40, 117.70, 117.47, 116.06, 115.63, 112.57, 54.56; HRMS Calcd for $C_{20}H_{17}NO_4Cl$ $[M+H]^+$: 370.0846. Found 370.0844.

18d: Colorless prisms (hexane/ethyl acetate); mp 161-161.5°C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.41 (s, 1 H), 9.34 (s, 1 H), 7.49 (m, 3 H), 7.40 (t, *J* = 7.5 Hz, 1 H), 7.12 (dd, *J* = 8.2, 2.1 Hz, 1 H), 6.90 (dt, *J* = 9.0, 2.1Hz, 2 H), 6.73 (dt, *J* = 9.0, 2.1 Hz, 2 H), 6.72 (d, *J* = 2.1 Hz, 1 H), 6.38 (dd, *J* = 8.9, 2.7 Hz, 1 H), 3.80 (s, 3 H), 3.75 (s, 3 H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 164.82, 159.22, 156.65, 153.80, 153.54, 148.02, 135.92, 129.57, 126.43, 121.19, 120.75, 119.65, 117.37, 116.22, 112.64, 107.72, 101.70, 55.79, 55.30; HRMS Calcd for $C_{21}H_{20}NO_5$ $[M+H]^+$: 366.1341. Found 366.1332.

18e: Pale pink prisms (hexane/ethyl acetate); mp 147-148°C; ^{1}H NMR (600 MHz, acetone-d_6) δ 9.45 (br, 1 H), 8.40 (br, 1 H), 7.57 (m, 3 H), 7.43 (t, *J* = 8.2 Hz, 1 H), 7.15(dd, *J* = 7.6, 2.7

Hz, 2 H), 6.91 (dt, *J* = 9.0, 2.8 Hz, 2 H), 6.85 (dt, *J* = 8.9, 2.8 Hz, 2 H), 6.50 (d, *J* = 2.7 Hz, 1 H), 6.45 (dd, *J* = 9.0, 2.8 Hz, 1 H), 3.86 (s, 3 H); ^{13}C NMR (150 MHz, CD_3OD) δ 168.41, 161.40, 158.76, 155.02, 151.85, 150.38, 137.11, 130.83, 125.73, 122.09, 121.47, 120.47, 118.83, 117.13, 113.81, 109.25, 106.15, 55.90; HRMS Calcd for $C_{20}H_{18}NO_5$ $[M+H]^+$: 352.1185. Found 352.1182.

19a: Colorless prisms (chloroform); mp 169 °C; ^{1}H NMR (600 MHz, acetone-d_6) δ 7.71 (d, *J* = 10.3 Hz, 1 H), 7.54 (dd, *J* = 7.6, 2.7 Hz, 1 H), 7.51(t, J = 2.1 Hz, 1 H), 7.43 (t, J = 7.9 Hz, 1 H), 6.95 (m, 4 H), 3.00 (s, 3 H); ^{13}C NMR (150 MHz, CD_3OD) δ 168.61, 161.36, 158.19 (d, *J* = 7.19 Hz), 156.53 (d, *J* = 5.75 Hz), 154.08, 152.75, 137.48 (t, *J* = 12.93 Hz), 137.09, 130.81, 129.44 (t, *J* = 14.37 Hz), 120.74, 119.00, 117.00 (d, *J* = 37.35 Hz), 113.96, 105.64 (d, *J* = 27.29 Hz), 55.94; HRMS Calcd for $C_{20}H_{16}NO_4F_2$ $[M+H]^+$: 372.1047. Found 372.1046.

19b: Pale brown prisms (hexane/ethyl acetate); mp 141-141.5°C; ^{1}H NMR (600 MHz, CD_3OD) δ 7.52 (dd, *J* = 8.2, 1.2 Hz, 1 H), 7.49 (t, *J* = 2.1 Hz, 1 H), 7.42 (t, *J* = 8.2 Hz, 1 H), 7.28 (td, *J* = 8.9, 2.1 Hz, 1 H), 7.15 (dd, *J* = 8.2, 2.7 Hz, 1 H), 6.91 (dt, *J* = 8.9, 2.1 Hz, 2 H), 6.79 (dt, *J* = 8.9, 3.1 Hz, 2 H), 6.73 (td, *J* = 8.9, 2.8 Hz, 1 H), 3.86 (s, 3 H); ^{13}C NMR (150 MHz, acetone-d_6) δ 166.25 (d, *J* = 10.05 Hz), 160.70, 155.11, 146.00 (dt, *J* = 247.04, 10.82 Hz), 144.99, 143.29 (dd, *J* = 245.58, 12.93 Hz), 136.39, 130.45, 123.20 (t, *J* = 10.07 Hz), 120.87 (d, *J* = 15.80 Hz), 120.69, 120.53, 118.63, 117.08 (d, *J* = 12.92 Hz), 114.26, 113.58, 55.72; HRMS Calcd for $C_{20}H_{16}NO_4F_2$ $[M+H]^+$: 372.1047. Found 372.1058.

19c: Colorless prisms (chloroform); mp 168-169°C; ^{1}H NMR (600 MHz, acetone-d_6) δ 9.78 (br, 1 H), 8.22 (br, 1 H), 8.97 (d, *J* = 2.0 Hz, 2 H), 7.57 (d, *J* = 7.5 Hz, 1 H), 7.53 (t, *J* = 2.1 Hz, 1 H), 7.43 (t, *J* = 7.9 Hz, 1 H), 7.16 (dd, *J* = 8.3, 2.8 Hz, 1 H), 6.78 (dt, *J* = 9.0, 2.1 Hz, 2 H), 6.70 (dt, *J* = 9.6, 2.8 Hz, 2 H), 3.87 (s, 3 H); ^{13}C NMR (150 MHz, CD_3OD) δ 168.62, 161.37, 153.73, 151.73, 145.13, 138.16, 137.02, 130.82, 122.28, 120.79, 119.07, 116.89, 116.73, 113.98, 55.95; HRMS Calcd for $C_{20}H_{16}NO_4Cl_2$ $[M+H]^+$:404.0456. Found 404.0459.

4.9. SC-3 Growth Inhibition Assay[18]

SC-3 cells were cultured in MEMα (Wako Co.) supplemented with 2% FBS (fetal bovine serum) and 1 nM DHT at 37°C in an incubator under an atmosphere of 5% CO_2 in humidified air. All experiments were performed in triplicate or more. Cells were trypsinized and diluted to 20,000 cells/mL with MEMα supplemented with 2% charcoal-stripped FBS. This cell suspension was seeded in 96-well plates at a volume of 100 μL/well and the plates were incubated for 24 h. Then, 10 μL of medium was removed from each well, and replaced with 10 μL of drug solution supplemented with serial dilutions of test compound or DMSO as a dilution control in the presence of 1 nM DHT. The plates were incubated at 37°C under 5%

CO_2 in air for 3 days, and the cell number was determined using a Cell Counting Kit-8 (Dojindo). A 10 μL aliquot of WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt] was added to each well, then the cells were incubated for 2 h, and the absorbance at 450 nm was measured with a microplate reader. This parameter is proportional to the number of living cells in the culture. Initially we tested the activity of compounds at the concentration of 10 μM, and if the inhibition exceeded 50% at the concentration of 10 μM, the IC_{50} value was determined in the concentration range of 0.01 to 10 μM. IC_{50} (Table 1) is the concentration of test compound that reduces DHT-induced cell growth to 50% of the control.

4.10. LNCaP cell proliferation assay[18]

The human prostate adenocarcinoma cell line LNCaP was routinely cultivated in RPMI-1640 supplemented with 10% FBS at 37°C in an incubator under an atmosphere of 5% CO_2 in humidified air. All experiments were performed in triplicate or more. Cells were trypsinized and diluted to 20,000 cells/mL with RPMI-1640 supplemented with 10% charcoal-stripped FBS. This cell suspension was seeded in 96-well plates at a volume of 100 μL/well and the plates were incubated at 24 h. Then, 10 μL of medium was removed from each well, and replaced with 10 μL of the drug solution supplemented with serial dilutions of the test compounds or DMSO as a dilution control in the presence or absence of 10 nM DHT. The final concentrations of compounds were 10 nM to 10 μM. Cells were incubated for 6 days, and half of the medium was removed and replaced once after 3 days with medium containing test compound or DMSO as a dilution control in the presence or absence of 10 nM DHT. At the end of the incubation, proliferation was evaluated by adding 10 μM WST-8 to microcultures and incubating the cells for 2 h. The absorbance at 450 nm was measured. This parameter is proportional to the number of living cells in the culture.

4.11. 22Rv1 growth inhibition assay[18]

The human prostate cancer cell line 22Rv1 was routinely cultivated in RPMI-1640 supplemented with 10% FBS at 37 °C in an incubator under an atmosphere of 5% CO_2 in humidified air. All experiments were performed in triplicate or more. Cells were trypsinized and diluted to 20,000 cells/mL with RPMI-1640 supplemented with 10% charcoal-stripped FBS. This cell suspension was seeded in 96-well plates at a volume of 100 μL/well and the plates were incubated at 24 h. Then, 10 μL of medium was removed from each well, and replaced with 10 μL of drug solution supplemented with serial dilutions of test compound or DMSO as a dilution control in the presence of 1 nM DHT in triplicate microcultures. After incubation for 3 days, cell proliferation was evaluated by adding 10 μM WST-8 to microcultures and incubating the cells for 2 h. The absorbance at 450 nm was measured. This parameter is proportional to the number of living cells in the culture.

4.12. PC-3 growth inhibition assay[18]

The human prostate cancer cell line PC-3 was routinely cultivated in RPMI-1640 medium supplemented with 10% FBS at 37°C in an incubator under an atmosphere of 5% CO_2 in humidified air. Cells were trypsinized and diluted to 20,000 cells/mL with RPMI-1640 supplemented with 10% charcoal-stripped FBS. This cell suspension was seeded in 96-well plates at a volume of 100 μL/well and incubated at 24 h. Then, 10 μL of medium was removed from each well, and replaced with 10 μL of drug solution supplemented with serial dilutions of test compound or DMSO as a dilution control in the presence of 1 nM DHT. Cells were incubated for 3 days, and at the end of the incubation time, proliferation was evaluated by adding 10 μM WST-8 to the microcultures and incubating the cells for 2 h. The absorbance at 450 nm was measured. This parameter is proportional to the number of living cells in the culture.

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Graphical Abstract

Structure-Activity Relationship of Novel (Benzoylaminophenoxy)phenol Derivatives as Anti-prostate Cancer Agents

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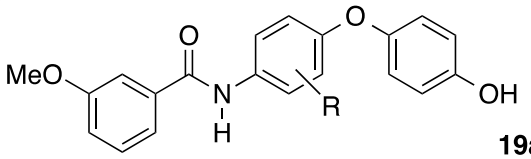

IC_{50} in SC-3 cell assay

19a R = 2,6-F_2 0.51 μM
19b R = 2,3-F_2 0.53 μM