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Design, synthesis and structure–affinity relationships of aryloxyanilide derivatives as novel peripheral benzodiazepine receptor ligands

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Abstract—Since the peripheral benzodiazepine receptor (PBR) has been primarily found as a high-affinity binding site for diazepam in rat kidney, numerous studies of it have been performed. However, the physiological role and functions of PBR have not been fully elucidated. Currently, we presented the pharmacological profile of two high and selective PBR ligands, *N*-(2,5-dimethoxybenzyl)-*N*-(4-fluoro-2-phenoxyphenyl)acetamide (**7-096**, DAA1106) (PBR: $IC_{50}=0.28$ nM) and *N*-(4-chloro-2-phenoxyphenyl)-*N*-(2-isopropoxybenzyl)acetamide (**7-099**, DAA1097) (PBR: $IC_{50}=0.92$ nM). The compounds are aryloxyanilide derivatives, and identified with known PBR ligands such as benzodiazepine (**1**, Ro5-4864), isoquinoline (**2**, PK11195), imidazopyridine (**3**, Alpidem), and indole (**5**, FGIN-1-27) derivatives. The aryloxyanilide derivatives, which have been derived by opening the diazepine ring of **1**, are a novel class as PBR ligands and have exhibited high and selective affinity for peripheral benzodiazepine receptors (PBRs). These novel derivatives would be useful for exploring the functions of PBR. In this paper, the design, synthesis and structure–affinity relationships of aryloxyanilide derivatives are described.

1. Introduction

The peripheral benzodiazepine receptor (PBR) has been found primarily as a high-affinity binding site for diazepam in rat kidney.[1] In contrast to the central benzodiazepine receptor (CBR), which is associated with γ-aminobutyric $acid_A$ ($GABA_A$)-regulated ion channels[2] in the central nervous system,[3,4] PBR lacks coupling to $GABA_A$ receptors.

PBR has been found in many peripheral tissues,[5–7] in blood cells[7,8] and in glial cells in the brain.[7,9,10] Its primary localization has been reported to be mainly in the mitochondrial outer membranes in many tissues,[11–14] although PBR is located on the inner membrane of the rat lung mitochondria.[13] Furthermore, PBR was also found on plasma membranes,[7,8] which lack mitochondria. Plasma membrane PBR has been described in heart, liver, adrenal, and testis and on hematopoietic cells.[7]

PBR is composed of at least three subunits, an isoquinoline binding subunit with a molecular mass of 18 kDa, a voltage-dependent anion channel (VDAC) with a molecular mass of 32 kDa and an adenine nucleotide carrier with a molecular mass of 30 kDa.[15] cDNA encoding PBR has been cloned from humans,[16] bovines,[17] rats[18] and mice.[19] PBR plays roles in cell proliferation,[20] steroidogenesis,[21] calcium flow,[22] cellular respiration,[23] cellular immunity,[24] and malignancy.[25]

As endogenous ligands for peripheral benzodiazepine receptors (PBRs), anthraline, diazepam-binding inhibitor (DBI) and proptoporphyrin IV have been reported. Anthraline, 16 kDa protein, binds to both PBR and the dihydropyridine binding sites.[26] DBI, a 104 amino acid neuropeptide,[27] has been found in human brain, and DBI-like immunoreactivity has been found in the cerebrospinal fluid of human volunteers.[28] DBI has also been found in peripheral tissues rich in PBRs, such as adrenal gland, testis and kidney.[29] The major physiological porphyrins, protoporphyrin IX and heme, have been labeled PBR with nanomolar affinity, and their

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affinity has been 1000 times higher for PBRs than for central benzodiazepine receptors (CBRs).[30]

PBR has exhibited different specificities for ligands. Compounds **1** (Ro5-4864) and **2** (PK11195) exhibited high affinity for PBRs but not for CBRs,[9,31,32] whereas compound **3** (clonazepam) exhibited low affinity for PBRs and high affinity for CBRs.[33] Interestingly, in contrast to the highly species-dependent interaction of compound **1** with PBRs,[34–37] compound **2** exhibited high affinity for PBRs from both humans and bovines.[32] As other PBR ligands, compounds **4** (alpidem) and **5** (FGIN-1) have been reported. Compound **4** is an imidazopyridine derivative and binds with high affinity to both PBRs and CBRs.[38] Compound **5** is a 2-aryl-3-indoleacetamide derivative and exhibits high affinity for PBRs with high selectivity over CBRs (Chart 1).[39,40]

The physiological functions of PBR have not been fully elucidated, due in part to the lack of potent and selective ligands for PBRs. Our interest was concentrated on opening the diazepine ring of **1** for discovering new PBR ligand since **1** has more rigid structure than other PBR ligands such as **2**, **4** and **5**. We have presented potent and selective PBR ligands **7-096** (DAA1106) (PBR: $IC_{50} = 0.28$ nM, CBR: $IC_{50} > 1000$ nM) and **7-099** (DAA1097) (PBR: $IC_{50} = 0.92$ nM, CBR: $IC_{50} > 1000$ nM),[41–43] compounds which were novel aryloxyanilide derivatives designed by opening the diazepine ring of **1** (Fig. 1). Compound **7-096** is potent and selective ligand for PBRs since the binding of [^{3}H]**7-096** has not been affected by several neurotransmitter-related compounds, including adrenoceptor, γ-aminobutylic acid, dopamine, 5-hydroxytrypyamine, acetylcholine, histamine, glutamate and CBR ligands even at a concentration of 10 μM.[42] Compounds **7-096** and **7-099** showed potent anxiolytic-like properties in laboratory animals.[41] Furthermore, it has been suggested that (1) **7-096** and **7-099** binding sites on PBR share common domain with that of PK11195, but also contain motif that do not interact efficiently with PK11195; (2) these additional sites are par of the PBR molecular, since similar result are found using cells or recombinant PBR; (3) the binding of **7-099** to PBR induces changes in the receptor similar to that triggered by PK11195, allowing steroidogenesis activation; (4) the fact that **7-096** dose not activate steroidogenesis despite its high affinity for PBR suggests that its binding on PBR leads to conformational changes that do not permit or antagonize PBR steroidogenic function.[43] Thus, aryloxyanilide derivatives are unique PBR ligands for studying the structure–function relationship of PBR.

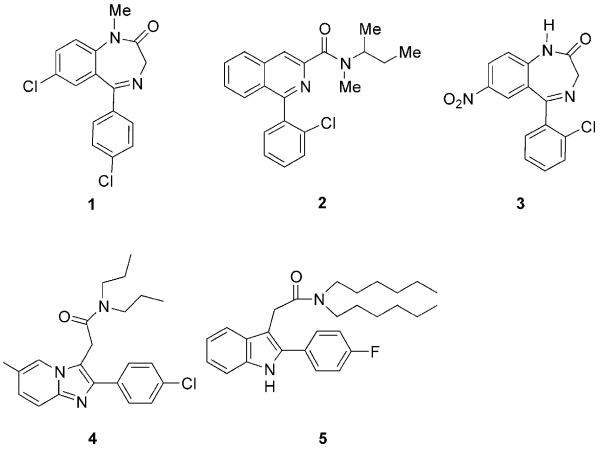


Chart 1.

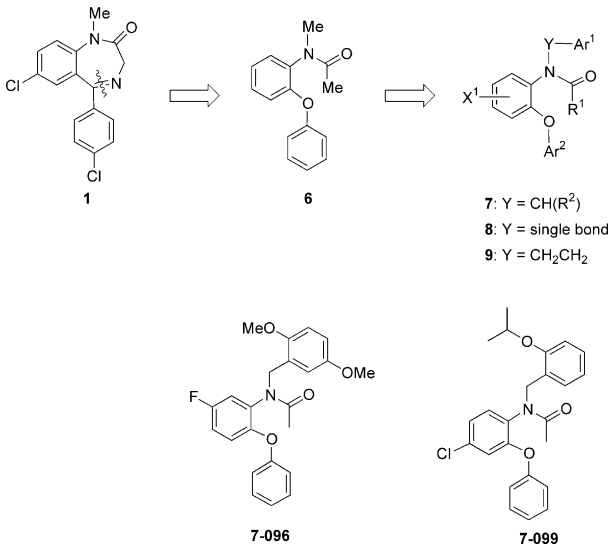


Figure 1.

In this paper, the design, synthesis and structure–affinity relationships of aryloxyanilide derivatives, which are novel PBR ligands, are presented.

2. Chemistry

The syntheses of derivatives **6**, **7**, **8** and **9** are shown in Schemes 1–8.

General synthetic methods for aryloxyanilide derivative **7** are indicated in Scheme 1.

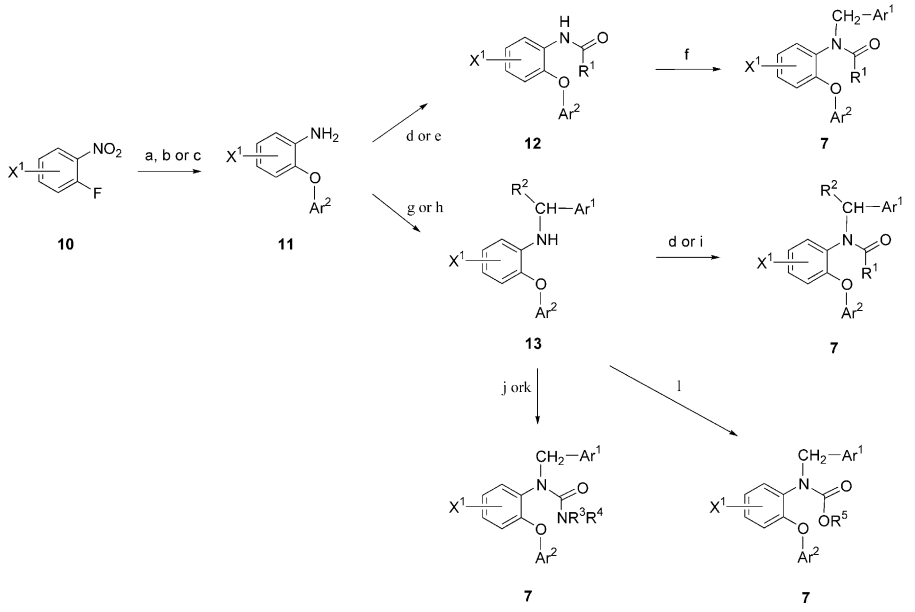
Noncommercial 2-aryloxyaniline **11** was prepared by treatment of 2-halonitrobenzene **10** with hydroxyaryl compounds under basic conditions followed by hydrogenolysis (Method A) or reduction utilizing powdered Fe (Method B) (Table 1).

The 2-aryloxyaniline **11** was treated with acyl chloride under basic conditions followed by arylmethylchloride in the presence of sodium hydride to afford derivative **7** (Method C). Formyl compound **7-061** was synthesized by benzylation utilizing 2-methoxybenzylchloride in the presence of sodium hydride after treatment of compound **11** with formic acid (Method D). Furthermore, derivative **7** was prepared by reductive alkylation of 2-aryloxyaniline **11** with the corresponding carbonyl compound utilizing sodium borohydride followed by acylation using acyl chloride (Method E) or by reductive alkylation of 2-aryloxyaniline **11** with the corresponding carbonyl compound utilizing hydrogenation followed by acylation using acid anhydride (Method F).

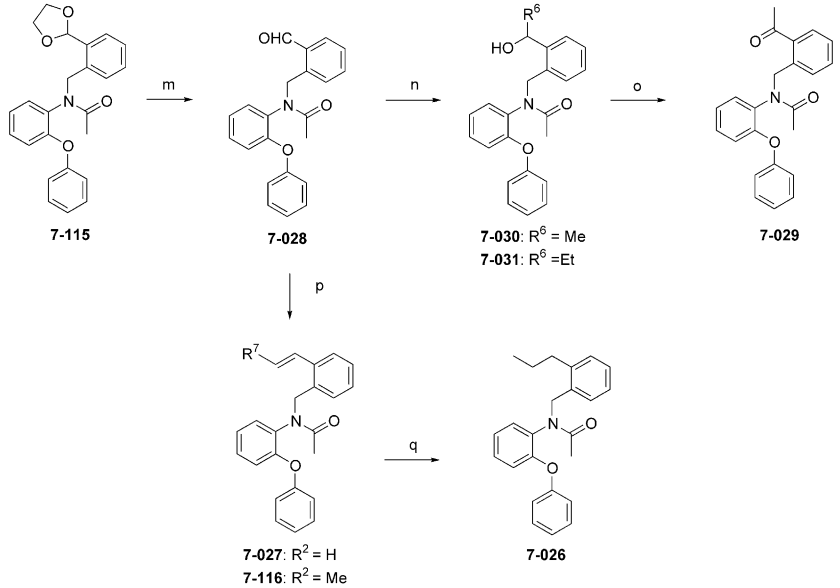
The methods of synthesis of urea and carbamate compounds are shown in Scheme 1. Urea compounds **7-078**, **7-079**, **7-112**-**7-114** were obtained by reaction of *N*-benzylaryloxyaniline **13** with triphosgen followed by addition of the corresponding amines (Method G). Urea compound **7-077** was obtained by treatment of *N*-benzylaryloxyaniline **13** with KNCO (Method H). Carbamate compounds **7-080** and **7-081** were prepared by treatment of *N*-benzylaryloxyaniline **13** with triphosgen followed by the corresponding sodium alkoxides (Method I).

Acetal compound **7-115**, which was synthesized by Method C, was deprotected under acidic conditions to yield aldehyde **7-028** (Method J) (Scheme 2).

The method of synthesis of derivative **7** via aldehyde **7-028** as a key intermidiate is shown in Scheme 2. Aldehyde **7-028** was treated with Grignard reagent or Wittig reagent to yield alcohol **7-030** and **7-31** or vinyl phenyl compounds **7-027** and **7-116**, respectively (Method K or Method M). In compound **7-113**, the 2-propenyl group was a mixture of *E* and *Z* isomers, and its hydrogena-



Scheme 1. Reagents and conditions: (a) Ar^2OH, K_2CO_3, DMF; (b) PtO_2, H_2, MeOH; (c) Fe, NH_4Cl, EtOH, H_2O; (d) R^1COCl, Et_3N, CH_2Cl_2; (e) HCO_2H, toluene; (f) NaH, Ar^1CH_2Cl, DMF; (g) $Ar^1CH_2C{=}O(R^2)$, $NaBH_4$, MeOH; (h) $Ar^1CH_2C{=}O(R^2)$ H_2, PtO_2, MeOH; (i) $(R^1CO)_2O$, pyridine; (j) triphosgen, CH_2Cl_2 and then R^3R^4NH (k) KNCO, H_2O; (l) triphosgen, CH_2Cl_2 and then R^5ONa. Method A: a, b; Method B: a, c; Method C: d, f; Method D: e,f; Method E: g, d; Method F: h, i; Method G: g, i; Method H: g, k; Method I: g, l.



Scheme 2. Reagents and conditions: (m) TsOH–H_2O, acetone; (n) R^6MgBr, THF; (o) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2; (p) R^7 $CH{=}PPh_3$, THF; (q) H_2, PtO_2, MeOH. Method J: m; Method K: n; Method L: o; Method M: p; Method N: q.

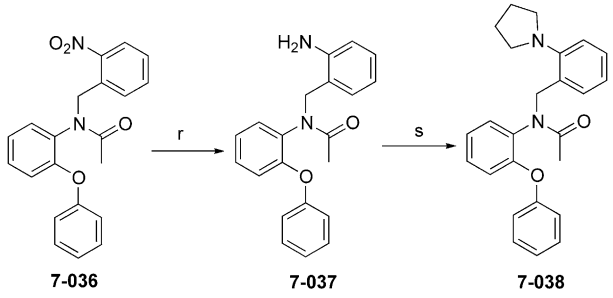
tion yielded propyl compound **7-026** (Method N). Swern oxidation of alcohol **7-030** afforded keto compound **7-29** (Method L).

The 2-nirobenzyl compound **7-036**, which was synthesized by Method E, was hydrogenalated on PtO_2 to yield aniline compound **7-037** (Method O). By treatment of **7-037** with 1,4-dibromobutane, pyrrolidine compound **7-038** was formed (Method P) (Scheme 3).

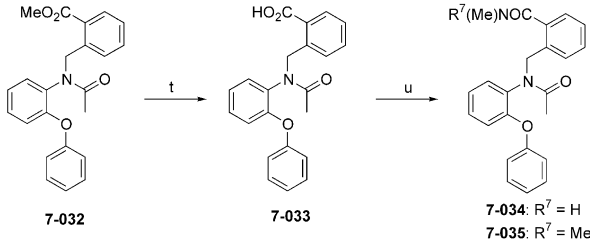
Ester compound **7-032** was hydrolyzed under basic conditions to yield the acid compound **7-033** (Method Q), which was amidated with corresponding amines via acyl chloride (Method R) (Scheme 4).

Introduction of a hydroxy group and amino group at the terminal end of an acetyl group (R^1 = Me) **7-005** was performed via chloroacetyl compound **7-072** (Scheme 5). *N*-Chloroacetyl-2-phenoxyanilide **7-072**, which was synthesized by Method B, was converted to **7-073** by treatment with AcONa in the presence of phase transfer catalysis (Method S), and then hydrolysis of the terminal acetyl group of **7-073** then yielded the hydroxyacetyl compound **7-074** (Method T). An azido group was introduced by treatment of **7-072** with NaN_3 to yield the azido compound **7-075** (Method U). Hydrogenation of **7-075** gave aminoacetyl compound **7-073** (Method V).

The hydroxyl phenyl compound **7-022** and hydroxycarbonylmethoxy compound **7-020** were synthesized from compound **7-117**, which was synthesized by Method C, in Scheme 6. Hydrolysis of the acetyl group of **7-117** yielded **7-022** (Method W). *O*-Alkylation of **7-022** with ethyl bromoacetate under basic conditions followed by hydrolysis of its acetate yielded **7-020** (Method X).



Scheme 3. Reagents and conditions: (r) H_2, PtO_2, MeOH; (s) 1,4-dibromobutane, K_2CO_3, KI, DMF; Method O: r; Method P: s.



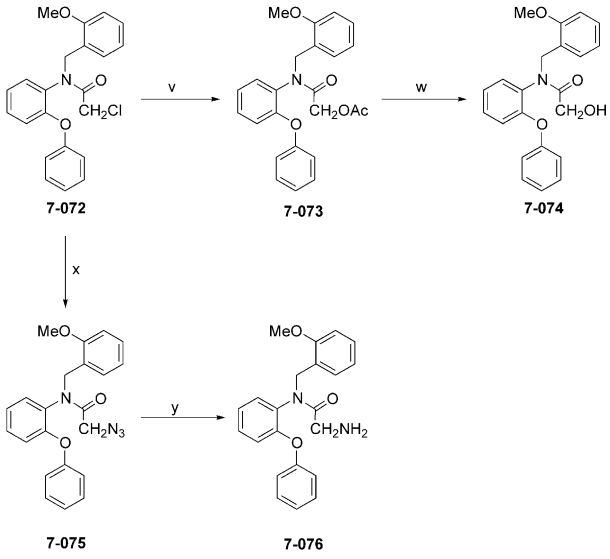
Scheme. 4. Reagents and conditions: (t) aq KOH, MeOH; (u) $SOCl_2$, HMPA, THF then aq R^7 MeNH; Method Q: t; Method R: u.

The synthesis of **8** is described in Scheme 7. Compound **8** was synthesized from *N*-acetyl-2-phenoxyaniline **12-001** by Ulmann reaction utilizing CuBr and copper powder (Method Y).

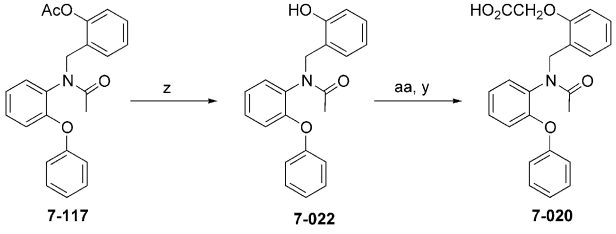
Scheme 8 shows the method of synthesis of **9**, which has a 2-phenylethyl group on the nitrogen atom. *N*-acylation of 2-phenoxyaniline **11-003** with 2-methoxyphenylacetylchloride and reduction with $LiAlH_4$ gave **14**. Acetylation of **14** with acetylchloride yielded the desired product **9** (Method Z).

3. Results and discussion

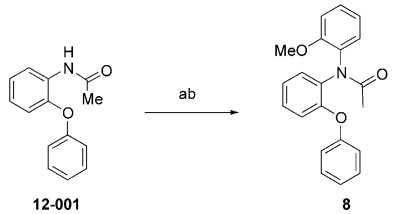
Compounds **1**, **2**, **5**, **6**, **7-001**–**7-114**, **7-118**, **8** and **9** were evaluated for PBRs binding affinities in mitochondria prepared from rat cerebral cortex against radioligand



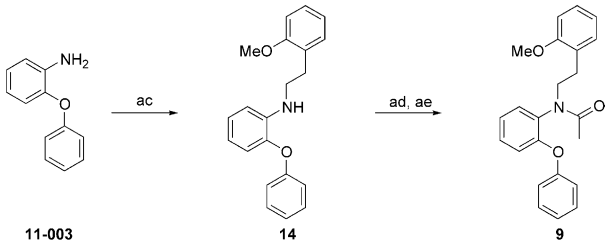
Scheme 5. Reagents and conditions: (v) AcONa, *n*-Bu_4NBr, PhH; (w) K_2CO_3, MeOH; (x) NaN_3, DMF; (y) H_2, PtO_2, MeOH; Method S: v; Method T: w; Method U: x; Method V: y.



Scheme 6. Reagents and conditions: (z) aq KOH, MeOH; (aa) $BrCH_2CO_2Me$, NaH, DMF; Method W: z; Method X: aa, z.



Scheme 7. Reagents and conditions: (ab) 2-Methoxyiodobenzene, Cu, CuBr, K_2CO_3, $PhNO_2$; Method Y: ab.



Scheme 8. Reagents and conditions: (ac) 2-methoxyphenylacetylchloride, Et_3N, CH_2Cl_2; (ad) $LiAlH_4$, THF; (ae) AcCl, Et_3N, CH_2Cl_2; Method Z: ac–ae.

[^3H]-PK11195,[41] and the obtained IC_{50} values are shown in Table 1. High-PBR-affinity compounds (IC_{50} < 100 nM) among compounds **6**, **7-001**–**7-114**, **7-118**, **8** and **9** did not exhibit significant CBR binding affinity (IC_{50} > 1000 nM) in membranes prepared from rat cerebral cortex against radioligand [^3H]flunitrazepam.[41]

Compound **6**, which was designed by opening the diazepine ring of **1** (Fig. 1), demonstrated high affinity for PBRs, with an IC_{50} value of 6.7 nM. Furthermore, our effort at chemical modification of *N*-methyl group of **6** yielded the *N*-benzyl compound **7-001** as the best compound in points of affinity and selectivity for PBRs over CBRs (PBR: IC_{50} = 2.9 nM, CBR: IC_{50} > 1000 nM). Based on the data, we concentrated our interest on chemical modification of Ar^1, Ar^2, R^1, X^1 and Y.

3.1. Chemical modification of Ar^1

Introduction of a methoxy group or chloro atom onto the phenyl ring of the *N*-benzyl group in **7-001** tended to increase binding affinity (**7-006**–**7-010** versus **7-001**). Among these, 2-mthoxy compound **7-005** and 2-chloro compound **7-008** exhibited much higher binding affinity for PBRs, with IC_{50} values of 0.15 and 0.49 nM, respectively. This tendency was observed for pyridine compounds **7-002–7-004** and **7-011–7-013** too, as well. Conversion of the phenyl group of the benzyl group with a pyridine ring resulted in significant decrease in affinity for PBRs (**7-002–7-004** versus **7-001**). Introduction of a methoxy group at the ortho position from the methylene group of the pyridylmethyl group increased affinity for PBRs markedly (**7-002** versus **7-011, 7-003** versus **7-012** and **7-013**). These results suggest that conformation of the aromatic ring is important in obtaining high affinity for PBRs.

Next, the methoxy group of **7-005** was converted with various substituents. 2-Fluoro (**7-023**), 2-bromo (**7-024**), 2-methyl (**7-025**) and nitro (**7-036**) compounds exhibited high affinity for PBRs, but the affinities were respectively 44-, 23-, 28- and 31-fold lower than that of **7-005**. The size (C_{1-5}) of the alkoxy group did not significantly affect affinity for PBRs (**7-005** versus **7-014–7-018**). 2-Methylthio compound **7-019** exhibited the same PBR affinity as the corresponding methoxy compound **7-005**. 2-Propyl (**7-026**), 2-vinyl (**7-027**), formyl (**7-028**), acyl (**7-029**) and methoxycarbonyl (**7-032**) compounds had the same or slightly increased binding affinity for PBRs compared with methoxy compound **7-005**. These findings suggest the following: (1) the electron density on the benzene ring of the benzyl group might not affect interaction between PBR and ligand, and (2) the length of the substituent on the benzene or the pyridine ring is more important in yielding high PBR affinity than the steric bulk of the substituent.

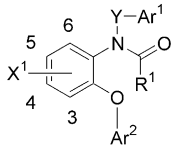
In contrast, the hydroxycarbonylmethoxy compound **7-033** did not exhibit significant affinity for PBRs, and compounds substituted with an alkyl-containing hydroxyl group (**7-030** and **7-031**) exhibited 16- and 19-fold lower affinities for PBRs, respectively, than keto compound **2-029**. The dimethylaminoethoxy compound **7-021** exhibited moderate affinity for PBRs, its affinity was much lower than that of isopentyl compound **7-018** but higher than that of acid compound **7-20**. Amidation of the carboxylic acid of **7-033** with monomethylamine (**7-034**) or dimethylamine (**7-035**) increased PBR affinity compared with **7-033**. More interestingly, amino compound **7-037** did not exhibit significant PBR affinity but pylloridino compound **7-038** and nitro compound **7-036** exhibited high affinities for PBRs. In light of the evidence, it is clear that a hydrophilic substituent including an acidic proton such as carboxylic, phenolic, alcoholic or amino proton is unsuitable for high PBR affinity. This phenomenon might be described below: (1) the acidic proton might hinder the relationship between PBR and ligand or (2) the acidic proton might change the molecular conformation of the corresponding original ligand.

Among dimethoxybenzyl compounds (**7-039–7-043**), 2,5-dimethoxy compound **7-041** exhibited the highest affinity for PBRs (IC_{50} = 0.085 nM), with affinity slightly higher than that of 2-methoxy compound **7-006**. 2,3-, 2,4- and 3,5-dimethoxy compounds, **7-039**, **7-040** and **7-043**, exhibited slightly lower affinity for PBRs than 2-methoxy compound **7-006**, but 2,6-dimethoxy compound **7-042** did not exhibit significant affinity for PBRs. Furthermore, 2,4,6-trimethoxy compound **7-044** did not exhibit significant affinity for PBRs. These findings suggest that the methoxy group plays the role of controller in creating suitable conformation of the benzene ring for interaction with PBR.

3.2. Chemical modification of Ar^2

Conversion of the phenyl group (Ar^2) with a naphthyl (**7-045**) or pyridyl (**7-046–7-048**) group decreased affinity for PBRs. In particular, pyridyl compounds **7-046–7-048** did not exhibit significant affinity for PBRs (IC_{50} > 100 nM). Next, utilizing methoxy, methyl and fluoro groups, the relationship between substituted position on the phenyl group and PBR affinity was investigated. On the whole, the order of suitable position for PBR affinity was 4-position ≥3-position ≥2-position. However, the better compounds among them, **7-051** (IC_{50} = 0.16 nM), **7-052** (IC_{50} = 0.11 nM), **7-054** (IC_{50} = 0.12 nM) and **7-057** (IC_{50} = 0.29 nM) exhibited almost the same PBR affinity as non-substituted compound **7-005** (IC_{50} = 0.15 nM). Furthermore, methylthio (**7-058**: IC_{50} = 2.2 nM), chloro (**7-059**: IC_{50} = 8.9 nM) and bromo (**7-060**: IC_{50} = 13 nM) compounds exhibited lower affinity for PBRs than the corresponding methoxy

Table 1. Aryloxyanilide derivatives: physical and binding data



Compd	Method[a]	Ar^1–Y	Ar^2	R^1CO	X^1	Analysis[b]	Mp (°C)[c]	IC_{50} (nM)[d,e]
1								3.1
2								1.1
5								5.5
6		Me	Ph	Me–CO	H	$C_{15}H_{15}NO_2$	Oil	6.7
7-001	C	Ph–CH_2	Ph	Me–CO	H	$C_{21}H_{19}NO_2$	80.5–81.0 (Et_2O)	2.9
7-002	C	2-Py–CH_2	Ph	Me–CO	H	$C_{20}H_{18}N_2O_2$	86.5–87.5 (Et_2O)	28
7-003	C	3-Py–CH_2	Ph	Me–CO	H	$C_{20}H_{18}N_2O_2$	83.5–80.0 (Et_2O)	>100
7-004	C	4-Py–CH_2	Ph	Me–CO	H	$C_{20}H_{18}N_2O_2$	114.5–115.0 (Et_2O)	83
7-005	C	2-MeO–Ph–CH_2	Ph	Me–CO	H	$C_{22}H_{21}NO_3$	83–84 (acetone-hex)	0.15
7-006	C	3-MeO–Ph–CH_2	Ph	Me–CO	H	$C_{22}H_{21}NO_3$	Oil	1.4
7-007	C	4-MeO–Ph–CH_2	Ph	Me–CO	H	$C_{22}H_{21}NO_3$	99.5–100.0	0.66
7-008	C	2-Cl–Ph–CH_2	Ph	Me–CO	H	$C_{21}H_{18}ClNO_2$	Oil	0.49
7-009	C	3-Cl–Ph–CH_2	Ph	Me–CO	H	$C_{21}H_{18}ClNO_2$	Oil	2.0
7-010	C	4-Cl–Ph–CH_2	Ph	Me–CO	H	$C_{21}H_{18}ClNO_2$	92.0–93.0 (hex–Et_2O)	1.2
7-011	E	3-MeO–2–Py–CH_2	Ph	Me–CO	H	$C_{21}H_{20}N_2O_3$	95.0–96.0 (Standed)	1.0
7-012	E	2-MeO–3–Py–CH_2	Ph	Me–CO	H	$C_{21}H_{20}N_2O_3$	78.5–79.0 (IPE–hex)	0.50
7-013	E	4-MeO–3–Py–CH_2	Ph	Me–CO	H	$C_{21}H_{20}N_2O_3$	90.5-91.0 (IPE–hex)	1.1
7-014	E	2-EtO–Ph–CH_2	Ph	Me–CO	H	$C_{23}H_{23}NO_3$	Oil	0.12
7-015	E	2-*n*-PrO–Ph–CH_2	Ph	Me–CO	H	$C_{24}H_{25}NO_3$	Oil	0.12
7-016	E	2-*i*-PrO–Ph–CH_2	Ph	Me–CO	H	$C_{24}H_{25}NO_3$	Oil	0.098
7-017	E	2-*n*-PenO–Ph–CH_2	Ph	Me–CO	H	$C_{26}H_{29}NO_3$	Oil	0.15
7-018	E	2-*i*-PenO–Ph–CH_2	Ph	Me–CO	H	$C_{26}H_{29}NO_3$	Oil	0.16
7-019	C	2-MeS–Ph–CH_2	Ph	Me–CO	H	$C_{22}H_{21}NO_2S$	Oil	0.18
7-020	X	2-HO_2CCH_2O–Ph–CH_2	Ph	Me–CO	H	$C_{23}H_{21}NO_5$	156.5–157.0 (IPE)	>100
7-021	E	2-$Me_2NCH_2CH_2O$–Ph–CH_2	Ph	Me–CO	H	$C_{25}H_{28}N_2O_3$	Oil	33
7-022	W	2-HO–Ph–CH_2	Ph	Me–CO	H	$C_{21}H_{19}NO_2$	123.0–124.5 (IPE)	3.2
7-023	C	2-F–Ph–CH_2	Ph	Me–CO	H	$C_{21}H_{18}FNO_2$	90.0–90.5 (IPE)	6.7
7-024	C	2-Br–Ph–CH_2	Ph	Me–CO	H	$C_{21}H_{18}BrNO_2$	84.0–84.5 (IPE)	3.5
7-025	E	2-Me–Ph–CH_2	Ph	Me–CO	H	$C_{22}H_{21}NO_2$	83.5–84.0 (IPE)	4.2
7-026	N	2-*n*–Pr–Ph–CH_2	Ph	Me–CO	H	$C_{24}H_{25}NO_2$	Oil	0.22
7-027	M	2-(CH_2=CH)–Ph–CH_2	Ph	Me–CO	H	$C_{23}H_{21}NO_2$	Oil	0.20
7-028	J	2-OHC–Ph–CH_2	Ph	Me–CO	H	$C_{22}H_{19}NO_3$	114.0–117.0 (Standed)	0.38
7-029	L	2-MeCO–Ph–CH_2	Ph	Me–CO	H	$C_{23}H_{21}NO_3$	110.0–110.5 (AcOEt–hex)	0.27
7-030	K	2-(MeC(OH)H)–Ph–CH_2	Ph	Me–CO	H	$C_{23}H_{23}NO_3$	Oil	4.4
7-031	K	2-(EtC(OH)H)–Ph–CH_2	Ph	Me–CO	H	$C_{24}H_{25}NO_3$	Oil	5.2
7-032	F	2-MeO_2C–Ph–CH_2	Ph	Me–CO	H	$C_{23}H_{21}NO_4$	76.0–78.0 (IPE)	0.064
7-033	Q	2-HO_2C–Ph–CH_2	Ph	Me–CO	H	$C_{22}H_{19}NO_4$	Oil	63
7-034	R	2-MeHNCO–Ph–CH_2	Ph	Me–CO	H	$C_{23}H_{22}N_2O_3$	Oil	2.9
7-035	R	2-Me_2NCO–Ph–CH_2	Ph	Me–CO	H	$C_{24}H_{24}N_2O_3$	Oil	3.7
7-036	E	2-NO_2–Ph–CH_2	Ph	Me–CO	H	$C_{21}H_{18}N_2O_4$	96.0–96.5 (AcOEt–hex)	4.6
7-037	O	2-NH_2–Ph–CH_2	Ph	Me–CO	H	$C_{21}H_{20}N_2O_2$	155.5-156.0 (MeOH)	>100
7-038	P	2-Pyrrolidino–Ph–CH_2	Ph	Me–CO	H	$C_{25}H_{26}N_2O_2$·HCl	110.0-112.5 (AcOEt–Et_2O)	1.3
7-039	C	2,3-$(MeO)_2$–Ph–CH_2	Ph	Me–CO	H	$C_{23}H_{23}NO_4$	Oil	0.29
7-040	F	2,4-$(MeO)_2$–Ph–CH_2	Ph	Me–CO	H	$C_{23}H_{23}NO_4$	Oil	0.72
7-041	C	2,5-$(MeO)_2$–Ph–CH_2	Ph	Me–CO	H	$C_{23}H_{23}NO_4$	Oil	0.085
7-042	F	2,6-$(MeO)_2$–Ph–CH_2	Ph	Me–CO	H	$C_{23}H_{23}NO_4$	Oil	>100
7-043	C	3,5-$(MeO)_2$–Ph–CH_2	Ph	Me–CO	H	$C_{23}H_{23}NO_4$	Oil	0.26
7-044	C	2,4,6-$(MeO)_3$–Ph–CH_2	Ph	Me–CO	H	$C_{24}H_{25}NO_5$	Oil	>100
7-045	F	2-MeO–Ph–CH_2	1-Nap	Me–CO	H	$C_{26}H_{23}NO_3$	66.0–68.0 (AcOEt–hex)	11
7-046	F	2-MeO–Ph–CH_2	2-Py	Me–CO	H	$C_{21}H_{20}N_2O_3$	81.0–82.0 (Et_2O)	>100
7-047	F	2-MeO–Ph–CH_2	3-Py	Me–CO	H	$C_{21}H_{20}N_2O_3$	64.0–65.0 (AcOEt–hex)	>100
7-048	F	2-MeO–Ph–CH_2	4-Py	Me–CO	H	$C_{21}H_{20}N_2O_3$	93.0–94.0 (Et_2O)	>100
7-049	F	2-MeO–Ph–CH_2	2-MeO–Ph	Me–CO	H	$C_{23}H_{23}NO_4$	Oil	0.72
7-050	F	2-MeO–Ph–CH_2	3-MeO–Ph	Me–CO	H	$C_{23}H_{23}NO_4$	Oil	0.34
7-051	F	2-MeO–Ph–CH_2	4-MeO–Ph	Me–CO	H	$C_{23}H_{23}NO_4$	Oil	0.16
7-052	F	2-MeO–Ph–CH_2	2-Me–Ph	Me–CO	H	$C_{23}H_{23}NO_3$	Oil	0.11
7-053	F	2-MeO–Ph–CH_2	3-Me–Ph	Me–CO	H	$C_{23}H_{23}NO_3$	Oil	0.29
7-054	F	2-MeO–Ph–CH_2	4-Me–Ph	Me–CO	H	$C_{23}H_{23}NO_3$	79.0–80.0 (AcOEt–hex)	0.12
7-055	F	2-MeO–Ph–CH_2	2-F–Ph	Me–CO	H	$C_{22}H_{20}FNO_3$	104.0–105.0 (AcOEt–hex)	39
7-056	F	2-MeO–Ph–CH_2	3-F–Ph	Me–CO	H	$C_{22}H_{20}FNO_3$	54.0–55.0 (AcOEt–hex)	0.41
7-057	F	2-MeO–Ph–CH_2	4-F–Ph	Me–CO	H	$C_{22}H_{20}FNO_3$	Oil	0.29
7-058	F	2-MeO–Ph–CH_2	4-MeS–Ph	Me–CO	H	$C_{23}H_{23}NO_3S$	97.0–98.0 (Et_2O–hex)	2.2
7-059	F	2-MeO–Ph–CH_2	4-Cl–Ph	Me–CO	H	$C_{22}H_{20}ClNO_3$	62.0–63.0 (Et_2O–hex)	8.90

(*continued on next page*)

Table 1 (*continued*)

Compd	Method[a]	Ar^1–Y	Ar^2	R^1CO	X^1	Analysis[b]	Mp (°C)[c]	IC_{50} (nM)[d,e]
7-060	F	2-MeO–Ph–CH_2	4-Br–Ph	Me–CO	H	$C_{22}H_{20}BrNO_3$	116.0–117.0 (AcOEt–hex)	13
7-061	C	2-MeO–Ph–CH_2	Ph	H–CO	H	$C_{21}H_{19}NO_3$	Oil	0.57
7-062	C	2-MeO–Ph–CH_2	Ph	Et–CO	H	$C_{23}H_{23}NO_3$	Oil	0.093
7-063	E	2-MeO–Ph–CH_2	Ph	*n*-Pr–CO	H	$C_{24}H_{25}NO_3$	Oil	1.3
7-064	E	2-MeO–Ph–CH_2	Ph	*i*-Pr–CO	H	$C_{24}H_{25}NO_3$	94.5–95.0 (Et_2O)	3.5
7-065	E	2-MeO–Ph–CH_2	Ph	*n*-Bu–CO	H	$C_{25}H_{27}NO_3$	Oil	1.5
7-066	E	2-MeO–Ph–CH_2	Ph	*n*–Pen–CO	H	$C_{26}H_{29}NO_3$	Oil	1.8
7-067	E	2-MeO–Ph–CH_2	Ph	*c*-Pr–CO	H	$C_{24}H_{23}NO_3$	73.0–74.0 (Et_2O–hex)	1.0
7-068	E	2-MeO–Ph–CH_2	Ph	*c*-Bu–CO	H	$C_{25}H_{25}NO_3$	85.0-86.0 (Et_2O–hex)	5.6
7-069	E	2-MeO–Ph–CH_2	Ph	*c*-Pen–CO	H	$C_{26}H_{27}NO_3$	92.5–93.5 (Et_2O–hex)	24
7-070	E	2-MeO–Ph–CH_2	Ph	Ph–CO	H	$C_{27}H_{23}NO_3$	125.5–127.0 (AcOEt–hex)	4.6
7-071	E	2-MeO–Ph–CH_2	Ph	CF_3–CO	H	$C_{22}H_{18}F_3NO_3$	Oil	4.6
7-072	E	2-MeO–Ph–CH_2	Ph	$ClCH_2$–CO	H	$C_{22}H_{20}ClNO_3$	83.0–83.5 (Standed)	0.34
7-073	S	2-MeO–Ph–CH_2	Ph	$MeCO_2CH_2$–CO	H	$C_{24}H_{23}NO_5$	Oil	8.9
7-074	T	2-MeO–Ph–CH_2	Ph	$HOCH_2$–CO	H	$C_{22}H_{21}NO_4$	70.0–71.0 (Standed)	3.9
7-075	U	2-MeO–Ph–CH_2	Ph	N_3CH_2–CO	H	$C_{22}H_{20}N_4O_3$	Oil	11
7-076	V	2-MeO–Ph–CH_2	Ph	H_2NCH_2–CO	H	$C_{22}H_{22}N_2O_3$	85.0–86.0 (AcOEt–IPE)	>100
7-077	H	2-MeO–Ph–CH_2	Ph	NH_2–CO	H	$C_{21}H_{20}N_2O_3$	89.5–90.0 (AcOEt)	3.5
7-078	G	2-MeO–Ph–CH_2	Ph	Me(H)N–CO	H	$C_{22}H_{22}N_2O_3$	133.0–134.0 (AcOEt)	4.6
7-079	G	2-MeO–Ph–CH_2	Ph	Me_2N–CO	H	$C_{23}H_{24}N_2O_3$	94.5–95.0 (standed)	100
7-080	I	2-MeO–Ph–CH_2	Ph	MeO–CO	H	$C_{22}H_{21}NO_4$	Oil	0.45
7-081	I	2-MeO–Ph–CH_2	Ph	EtO–CO	H	$C_{23}H_{23}NO_4$	Oil	0.87
7-082	C	2-MeO–Ph–CH_2	Ph	Me–CO	4-F	$C_{22}H_{20}FNO_3$	83.5–84.0 (AcOEt–hex)	0.34
7-083	C	2-MeO–Ph–CH_2	Ph	Me–CO	5-F	$C_{22}H_{20}FNO_3$	91.5–92.0 (AcOEt–hex)	0.79
7-084	C	2-MeO–Ph–CH_2	Ph	Me–CO	6-F	$C_{22}H_{20}FNO_3$	Oil	0.50
7-085	C	2-MeO–Ph–CH_2	Ph	Me–CO	3-Cl	$C_{22}H_{20}ClNO_3$	105.5–106.5 (AcOEt–hex)	48
7-086	C	2-MeO–Ph–CH_2	Ph	Me–CO	4-Cl	$C_{22}H_{20}ClNO_3$	113.0–114.5 (AcOEt–hex)	0.20
7-087	C	2-MeO–Ph–CH_2	Ph	Me–CO	5-Cl	$C_{22}H_{20}ClNO_3$	109.0–109.5 (AcOEt–hex)	2.0
7-088	C	2-MeO–Ph–CH_2	Ph	Me–CO	3-Me	$C_{23}H_{23}NO_3$	84.5–85.5 (AcOEt–hex)	63
7-089	C	2-MeO–Ph–CH_2	Ph	Me–CO	4-Me	$C_{23}H_{23}NO_3$	107.5–108.0 (AcOEt–hex)	0.87
7-090	C	2-MeO–Ph–CH_2	Ph	Me–CO	5-Me	$C_{23}H_{23}NO_3$	81.5–82.0 (AcOEt–hex)	0.34
7-091	C	2-MeO–Ph–CH_2	Ph	Me–CO	5-CF_3	$C_{23}H_{20}F_3NO_3$	113.0–113.5 (AcOEt–hex)	2.2
7-092	C	2-MeO–Ph–CH_2	Ph	Me–CO	5-MeO	$C_{23}H_{23}NO_4$	125.5–126.0 (AcOEt–hex)	8.1
7-093	C	2-MeO–Ph–CH_2	Ph	Me–CO	5-H_2NCO	$C_{23}H_{22}N_2O_4$	250.0–251.0 (Standed)	>100
7-094	C	2-MeO–Ph–CH_2	Ph	Me–CO	5-H_2NSO_2	$C_{22}H_{22}N_2SO_5$	192.5–193.5 (Standed)	>100
7-095	C	2,5-$(MeO)_2$–Ph–CH_2	Ph	Me–CO	4-F	$C_{23}H_{22}FNO_4$	Oil	1.3
7-096	C	2,5-$(MeO)_2$–Ph–CH_2	Ph	Me–CO	5-F	$C_{23}H_{22}FNO_4$	92.5–93.5 (IPE)	0.28
7-097	C	2-EtO–Ph–CH_2	Ph	Me–CO	4-Cl	$C_{23}H_{22}ClNO_3$	103.5–104.0 (hex)	0.79
7-098	C	2-*n*-PrO–Ph–CH_2	Ph	Me–CO	4-Cl	$C_{24}H_{24}ClNO_3$	95.5–96.0 (hex)	0.68
7-099	C	2-*i*-PrO–Ph–CH_2	Ph	Me–CO	4-Cl	$C_{24}H_{24}ClNO_3$	108.0–108.5 (hex)	0.92
7-100	C	2,5-$(MeO)_2$–Ph–CH_2	Ph	Me–CO	4-Cl	$C_{23}H_{22}ClNO_4$	103.5–105.0 (IPE)	0.18
7-101	C	2-MeO–Ph–CH_2	Ph	Et–CO	4-Cl	$C_{23}H_{22}ClNO_3$	Oil	6.6
7-102	C	2-EtO–Ph–CH_2	Ph	Et–CO	4-Cl	$C_{24}H_{24}ClNO_3$	81.5–83.0 (hex)	0.93
7-103	C	2-*n*-PrO–Ph–CH_2	Ph	Et–CO	4-Cl	$C_{25}H_{26}ClNO_3$	87.5–88.0 (hex)	1.1
7-104	C	2-*i*-PrO–Ph–CH_2	Ph	Et–CO	4-Cl	$C_{25}H_{26}ClNO_3$	Oil	13
7-105	C	2,5-$(MeO)_2$–Ph–CH_2	Ph	Et–CO	4-Cl	$C_{24}H_{24}ClNO_4$	Oil	6.9
7-106	F	2-*i*-PrO–Ph–CH_2	4-Me–Ph	Me–CO	4-Cl	$C_{25}H_{26}ClNO_3$	95.0–96.0 (Et_2O–hep)	17
7-107	F	2-*i*-PrO–Ph–CH_2	4-MeO–Ph	Me–CO	4-Cl	$C_{25}H_{26}ClNO_4$	53.0–56.0 (Et_2O–hep)	11
7-108	F	2-*i*-PrO–Ph–CH_2	4-F–Ph	Me–CO	4-Cl	$C_{24}H_{23}ClFNO_3$	82.0–83.0 (Et_2O–hep)	6.1
7-109	F	2,5-$(MeO)_2$–Ph–CH_2	4-Me–Ph	Me–CO	4-Cl	$C_{24}H_{24}ClNO_4$	109.0–110.0 (Et_2O–hep)	3.2
7-110	F	2,5-$(MeO)_2$–Ph–CH_2	4-MeO–Ph	Me–CO	4-Cl	$C_{24}H_{24}ClNO_5$	121.0–122.0 (Et_2O–hep)	6.7
7-111	F	2,5-$(MeO)_2$–Ph–CH_2	4-F–Ph	Me–CO	4-Cl	$C_{234}H_{21}ClFNO_4$	102.0–103.0 (Et_2O–hep)	3.3
7-112	G	2,5-$(MeO)_2$–Ph–CH_2	4-Me–Ph	Me(H)N–CO	4-Cl	$C_{24}H_{25}ClN_2O_4$	130.0–131.0 (Et_2O)	2.2
7-113	G	2,5-$(MeO)_2$–Ph–CH_2	4-MeO–Ph	Me(H)N–CO	4-Cl	$C_{24}H_{25}ClN_2O_5$	153.0–154.0 (Et_2O)	4.3
7-114	G	2,5-$(MeO)_2$–Ph–CH_2	4-F–Ph	Me(H)N–CO	4-Cl	$C_{23}H_{22}ClFN_2O_4$	127.0–128.0 (Et_2O)	2.2
7-115	C	2-(OCH_2CH_2O)CH–Ph–CH_2	Ph	Me–CO	H	$C_{24}H_{23}NO_4$	Oil	NT
7-116	M	2-(MeCH=CH)–Ph–CH_2	Ph	Me–CO	H		Oil	NT
7-117	E	2-CH_3CO_2–Ph–CH_2	Ph	Me–CO	H	$C_{23}H_{21}NO_4$	Oil	NT
7-118	F	2-MeO–Ph–CH(Me)	Ph	Me–CO	H	$C_{23}H_{23}NO_3$	96.5–97.0 (Standed)	2.2
8	Y	2-MeO–Ph	Ph	Me–CO	H	$C_{21}H_{19}NO_3$	Oil	9.8
9	Z	2-MeO–Ph–$(CH_2)_2$	Ph	Me–CO	H	$C_{23}H_{23}NO_3$	79.0–80.0 (hex)	0.45

[a] Methods are described in the text.
[b] Elemental analyses for all compounds were within ±0.4% of the theoretical values for the indicated formula.
[c] Stand, compounds were allowed to stand at room temperature to crystallize; Hex, hexane; IPE, diisopropylether; Hep, heptane.
[d] IC_{50} values represent the means of 1–3 separate experiments obtained from 5–10 concentrations of each compound, run in duplicate. Variation between experiments was less than 25%. NT, not test.
[e] High-PBR-affinity compounds (IC_{50} < 100 nM) among compounds **6**, **7-001**–**7-114**, **7-118**, **8** and **9** did not exhibit significant CBR binding affinity (IC_{50} > 1000 nM) in membranes prepared from rat cerebral cortex against radioligand [^{3}H]flunitrazepam.

(**7-051**: $IC_{50} = 0.16$ nM) and fluoro (**7-057**: $IC_{50} = 0.29$ nM) compounds. These findings suggest that introduction of a substituent onto the benzene ring does not increase affinity for PBRs.

3.3. Chemical modification of R^1

In R^1, exchange of the methyl group of the acetyl group in **7-005** with a proton slightly decreased PBR affinity (**7-005** versus **7-061**), but prolongation of methyl to an ethyl group slightly increased PBR affinity, with an IC_{50} value of 0.093 nM, (**7-005** versus **7-062**). Compounds **7-063–7-069**, compounds which had alkyl groups longer or larger than ethyl group, exhibited lower affinity for PBRs than methyl (**7-005**) and ethyl (**7-062**) compounds, as phenyl (**7-070**) and trifluoromethyl (**7-071**) compounds exhibited lower affinity for PBRs than methyl (**7-005**) and ethyl (**7-062**) compounds. More interestingly, bulky alkyl groups such as isopropyl and cyclic alkyl compounds yielded lower PBR affinity than straight-chain alkyl compounds (**7-064** versus **7-063**, **7-068** versus **7-065**, **7-069** versus **7-066**). Furthermore, cyclic propyl compound **7-067** exhibited higher affinity for PBRs than isopropyl compound **7-064**. These findings suggest that R^1 exhibits a steric restriction and that methyl and ethyl groups yield high affinity for PBRs.

Similarly, compounds substituted with chloro- (**7-072**), acetyloxy- (**7-073**), hydroxyl- (**7-074**), azido- (**7-075**) or amino- (**7-076**) methyl group for R^1 exhibited lower affinity for PBRs than the original compound **7-005**. Among them, aminomethyl compound **7-076** decreased affinity for PBRs markedly. It is possible that this marked decrease depends on the basicity of amino group, but this is unclear, since other amino compounds have not been synthesized and evaluated.

Urea compounds **7-077–7-079** exhibited lower affinity for PBRs than corresponding linear acyl compounds **7-005**, **7-062** and **7-064**, respectively. Among them, compounds **7-077** and **7-078** exhibited good affinity for PBRs, with IC_{50} values of 3.5 and 4.6 nM, respectively. In contrast, urea compound **7-079** exhibited lower affinity ($IC_{50} = 100$ nM) than **7-078**. This low affinity results from more bulky dimethylamino group of **7-079** than monomethylamino group of **7-078**, as isopropyl compound **7-064** exhibits about 38 times lower affinity for PBRs than ethyl compound **7-062**.

Carbamate compounds **7-080** and **7-081** exhibited high affinity for PBRs ($IC_{50} = 0.45$ and 0.87 nM, respectively), as the corresponding linear acyl compounds **7-062** and **7-063** exhibited high affinity for PBRs. The PBR affinity of carbamate compound 2 was higher than that of the corresponding urea compound **7-078**.

3.4. Chemical modification of X^1

Introduction of a fluoro, chloro, methyl, trifluoromethyl, carbamoyl or sulfamoyl group onto benzene ring decreased PBR affinity compared with the original compound **7-005**. Introduction of a fluoro (**7-082–7-084**), chloro (**7-086**, **7-087**) or methyl (**7-089**, **7-090**) group onto the 4-, 5- or 6-position produced a comparatively slight decrease in PBR affinity. However, introduction of a chloro (**7-085**: $IC_{50} = 48$ nM) or methyl group (**7-088**: $IC_{50} = 63$ nM) onto the 3-position marked decreased the affinity of **7-005** for PBRs. These findings suggest that the substituent at the 3-position produce steric hindrance to interaction with PBR or to negatively affect the conformation of Ar^1 for interaction with PBR.

Among substituents at the 5-position, the order of PBR affinity was methyl (**7-090**: $IC_{50} = 0.34$ nM) $\geq$ fluoro (**7-083**: $IC_{50} = 0.79$ nM) $\geq$ chloro (**7-087**: $IC_{50} = 2.0$ nM) = 5-trifluoromethyl (**7-091**: $IC_{50} = 2.2$ nM) > 5-methoxy (**7-092**: $IC_{50} = 8.1$ nM) > > carbamoyl (**7-093**: $IC_{50} > 100$ nM) = sulfamoyl (**7-094**: $IC_{50} > 100$ nM) compound. These findings suggest that the sterically acceptable space on the benzene ring for interaction with PBR is very narrow, and in particular that interaction between PBR and ligand is hindered by hydrophilic substituents.

3.5. Combined chemical modification

Compound **7-095** ($IC_{50} = 1.3$ nM), in which a fluorine atom was introduced onto the 4-position (X^1) of 2,5-dimethoxybenzyl compound **7-041**, exhibited 15-fold lower affinity for PBRs than **7-041** ($IC_{50} = 0.085$ nM). In contrast, the corresponding 5-fluoro compound **7-096** (DAA1106) exhibited high affinity for PBRs with an IC_{50} value of 0.28 nM. The reason for this difference in PBR affinity is unclear, since 4-fluoro compound **7-082** ($IC_{50} = 0.34$ nM) exhibited slightly higher affinity than the 5-fluoro compound **7-083** ($IC_{50} = 0.79$ nM), in case of the 2-methoxy compound (Ar^1), unlike the 2,5-dimethoxybenzyl compounds **7-095** and **7-096**.

Next, Ar^1 and R^1 of the 4-chloro compound **7-086** were chemically modified. Conversion of the 2-methoxybenzyl group (Ar^1) in the 4-chloro compound **7-086** with 2-ethoxybenzyl (**7-097**), 2-propoxybenzyl (**7-098**), 2-isopropoxybenzyl (**7-099**, DAA1097) and 2,5-dimethoxybenzyl (**7-100**) groups did not markedly change PBR affinity, compared with **7-086**. The propanoyl ($R^1 = Et$) compound **7-101** ($IC_{50} = 6.6$ nM) exhibited 33-fold lower affinity for PBRs than acetyl compound **7-086** ($IC_{50} = 0.20$ nM). Similarly, exchange of the 2-methoxybenzyl group (Ar^1) in the 4-chloro compound **7-101** with 2-isopropoxybenzyl (**7-104**, $IC_{50} = 13$ nM) and 2,5-dimethoxybenzyl (**7-105**, $IC_{50} = 6.9$ nM) groups resulted in remarked decrease of PBR affinity compared with the corresponding acetyl compounds **7-099** ($IC_{50} = 0.92$ nM) and **7-100** ($IC_{50} = 0.18$ nM), respectively. However, 2-ethoxybenzyl (**7-102**, $IC_{50} = 0.93$ nM) and 2-propoxybenzyl (**7-103**, $IC_{50} = 1.1$ nM) groups slightly decreased PBR affinity compared with the corresponding acetyl compounds **7-097** ($IC_{50} = 0.79$ nM) and **7-098** ($IC_{50} = 0.68$ nM), respectively. This variability might depend principally on the deference of whole molecular conformation organized by substituent of Ar^1 and R^1. Furthermore, this decrease in PBR affinity might be slightly increased in magnitude by steric hindrance by the chloro substituent, since introduction of a chlorine atom onto the benzene ring slightly decreased PBR affinity (**7-005** versus **7-086**).

Methyl, methoxy and fluoro groups were introduced onto the 4-position of phenyl group (Ar^2) of compounds **7-099** and **7-100**, with the expectation that they would exhibit the same PBR affinity as the original two compounds, since 4-methylphenyl (**7-054**, $IC_{50} = 0.12$ nM), 4-methoxyphenyl (**7-051**, $IC_{50} = 0.16$ nM) and 4-fluorophenyl (**7-057**, $IC_{50} = 0.29$ nM) compounds exhibited the same affinity for PBRs as phenyl compound **7-005** ($IC_{50} = 0.15$ nM). However, the PBR affinities of 2-isopropoxy compounds **7-106–7-108** and 2,5-dimethoxy compounds **7-109–7-111** were much lower than those of the original 2-isopropoxy compound **7-099** and 2,5-dimethoxy compound **7-100**, respectively. Furthermore, translation from the acetyl group (R^1) of compounds **7-109–7-111** ($IC_{50} = 3.2$, 6.7 and 3.3 nM, respectively) to a *N*-methylcarbamoyl group (**7-112–7-114**, $IC_{50} = 2.2$, 4.3 and 2.2 nM, respectively) yielded no significant change in PBR affinity in sharp contrast to the difference in PBR affinity between acety compound **7-005** ($IC_{50} = 0.15$ nM) and *N*-methylcarbamoyl **7-078** ($IC_{50} = 4.6$ nM). These findings suggest that PBR affinity is affected by overall molecular conformation organized by the substituents of Ar^1 and R^1 and that substituents of Ar^2 and X^1 respond to conformations acceptable for interaction with PBR.

3.6. Chemical modification of Y

Introduction of a methyl group onto the methylene group and conversion of methylene length (Y) of **7-005** was studied. Racemic compound **7-118**, a compound in which a methyl group was introduced onto the methylene group of **7-005**, exhibited 15-fold lower affinity than **7-005**. This negative finding might have resulted from steric hindrance of the introduced methyl group with interaction with PBR or effects on molecular conformation.

Furthermore, the shortened compound **8** and prolonged compound **9** exhibited lower affinity than the methylene compound **7-005**, with IC_{50} values of 9.8 and 0.45 nM, respectively. These findings suggest that the non-substituted methylene group is better than methylmethylene, shortened and prolonged high-affinity ligands for PBRs.

4. Conclusions

In this paper, we have reported the synthesis and SARs of 2-aryloxyanilide derivatives, which were obtained by ring-opening of Ro5-4864, as PBR ligands. Many 2-aryloxyanilide derivatives exhibited remarkably high affinity and selectivity for PBRs over CBRs. This successful design will have significant effects on the design of new PBR ligands. Furthermore, 2-aryloxyanilide derivatives can be used to probe the physiological functional roles of PBR. Interesting results of pharmacological studies utilizing typical compounds **7-096** (DAA1106) and **7-099** (DAA1097) have already been published.[41–43] Therefore, **7-096**, **7-099** and other high-affinity compounds reported herein are useful for examining the physiological functions of PBR.

5. Experimental

Melting points were determined on a Yanaco MP-500D melting point apparatus and were uncorrected. Proton nuclear magnetic resonance (NMR) spectra were obtained using a Varian Gemini 2000 (200 MHz). Chemical shifts are reported in parts per million relative to tetramethylsilane as an internal standard. Several amide compounds were a mixture of *E* and *Z* isomers caused by the amide bond on NMR spectra. The ratio of *E* and *Z* isomers is reported as following: 3.63 (3H×3/4, s), 3.78 (3H×1/4, s). Mass spectra (MS) were obtained on a Shimadzu Profile (EI and CI), JEOL JMS-SX102 (FAB) or Micromass Platform LC (IonSpray and ES). Elemental analyses were performed by a Perkin–Elmer 2400 (carbon, hydrogen and nitrogen) or Yokogawa IC7000P (halogen and sulfur). Analytical thin-layer chromatography was conducted on precoated silica gel 60 F_{254} plates (Merck). Silica gel [C-200, 100–200 mesh (Wako Pure Chemical)] was used for column chromatography, using the solvent systems (volume ratios) indicated below.

5.1. Method A: 5-fluoro-2-phenoxyaniline (11-001)

A mixture of 2,5-difluoronitrobenzene (46.4 g, 292 mmol), phenol (28.8 g, 306 mmol) and K_2CO_3 (44.3 g, 321 mmol) in DMF (150 mL) was stirred at 75 °C for 3 h. The mixture was concentrated in vacuo, and the residue was partitioned between AcOEt and water. The separated organic phase was washed with 1 M aqueous NaOH, 1 M aqueous HCl, saturated aqueous $NaHCO_3$ and saturated brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/EtOAc 15:1) to obtain 5-fluoro-2-phenoxynitrobenzene (67.3 g, 99% yield) as yellow oil: NMR ($CDCl_3$) δ 6.91–7.50 (7H, m), 7.71 (1H, dd, $J = 3.1$, 7.7 Hz); MS (EI) m/z 233 (M^+), 186 ($M^+ - 47$, 100%).

A mixture of 5-fluoro-2-phenoxynitrobenzene (23.3 g, 99.9 mmol) and PtO_2 (200 mg) in MeOH (100 mL) was stirred at 50 °C for 4.5 h under a hydrogen atmosphere. The mixture was filtered through Celite. The filtrate was concentrated in vacuo and concentrated to yield **11-001** (19.6 g, 97% yield) as colorless oil: NMR ($CDCl_3$) δ 3.88 (2H, br s), 6.40 (1H, ddd, $J = 3.1$, 8.6, 8.6 Hz), 6.53 (1H, dd, $J = 3.1$, 9.9 Hz), 6.84 (1H, dd, $J = 5.5$, 8.6 Hz), 6.86–7.13 (3H, m), 7.21–7.40 (2H, m); MS (EI) m/z 203 (M^+, 100%).

This product was used in the next step without further purification.

5.2. Method B: 4-chloro-2-phenoxyaniline (11-002)

In a manner similar to the preparation of 5-fluoro-2-phenoxynitrobenzene in Method A, 4-chloro-2-phenoxynitrobenzene (3.84 g, 51% yield) was obtained from 2,4-dichloronitrobenzene (5.76 g, 30.0 mmol), 2-phenol (2.83 g, 30.1 mmol) and K_2CO_3 (4.56 g, 33.0 mol) in DMF (30 mL) as a light yellow crystal: mp 84.0–84.5 °C (hexane/Et_2O); NMR ($CDCl_3$) δ 6.95 (1H, d, $J = 2.2$

Hz), 7.02–7.33 (4H, m), 7.34–7.52 (2H, m), 7.94 (1H, d, $J=8.8$ Hz); MS CI m/z 252 ($M^+ + 2$), 250 (M^+, 100%).

A mixture of 4-chloro-2-phenoxynitrobenzene (3.70 g, 14.8 mmol), powdered Fe (2.70 g, 48.3 atom) and NH_4Cl (340 mg, 6.36 mmol) in a mixture of EtOH (20 mL) and water (8 mL) was stirred at 85 °C for 1 h. The mixture was extracted with AcOEt, dried over Na_2SO_4, filtered and concentrated to yield **11-002** (3.09 g, 95% yield) as colorless oil: NMR ($CDCl_3$) δ 3.84 (2H, br s), 6.74 (1H, d, $J=8.6$ Hz), 6.83 (1H, d, $J=2.4$ Hz), 6.88–7.46 (6H, m); MS (EI) m/z 221 ($M^+ + 2$), 219 (M^+, 100%).

This product was used in the next step without further purification.

5.3. Method C: *N*-acetyl-*N*-(2-chlorobenzyl)-2-phenoxyaniline (7-008)

To a solution of 2-phenoxyaniline **11-003** (28.5 g, 154 mmol) and Et_3N (25.8 mL, 185 mmol) in CH_2Cl_2 was added dropwise AcCl (11.5 mL 162 mmol) under cooling in an ice bath. After stirring at room temperature for 1.5H, the reaction mixture was concentrated in vacuo, and the residue was poured into water and extracted with AcOEt three times. The combined organic layer was washed with 0.5 M aqueous HCl, saturated aqueous $NaHCO_3$ and saturated brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/EtOAc 4:1) to obtained *N*-acetyl-2-phenoxyaniline **12-001** (33.7 g, 96% yield); mp 86–87 °C; ^1H NMR ($CDCl_3$) δ 2.17 (3H, s), 6.84 (1H, dd, $J=1.6$, 7.9 Hz), 6.90–7.26 (5H, m), 7.27–7.49 (2H, m), 7.74 (1H, br s), 8.44 (1H, dd, $J=1.3$, 8.0 Hz); MS (ESI) m/z 250 ($M^+ + Na$, 100%).

To a suspension of NaH (60% dispersion in mineral oil, 400 mg, 10.0 mmol) in dry DMF (30 mL) was added **12-001** above (2.00 g, 8.80 mmol) and the mixture was stirred for 0.5 h at room temperature. To the mixture was added 2-chlorobenzylchloride (1.64 g, 10.2 mmol), and the reaction mixture was stirred for 0.5 h at room temperature. The mixture was poured into ice water and extracted with Et_2O three times. The combined organic layer was washed with 0.5 M aqueous HCl, saturated aqueous $NaHCO_3$ and saturated brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/AcOEt 4:1) to obtain **7-008** (2.92 g, 94% yield) as an colorless oil; NMR ($CDCl_3$) δ 1.97 (3H, s), 4.81 (1H, d, $J=15.0$ Hz), 5.29 (1H, d, $J=15.0$ Hz), 6.84–7.53 (13H, m) ; MS (EI) m/z 353 ($M^+ + 2$), 351 (M^+), 316 ($M^+ - 35$, 100%). Anal. ($C_{21}H_{18}ClNO_2$) C, H, N.

5.4. Method D: *N*-formyl-*N*-(2-methoxybenzyl)-2-phenoxyaniline (7-061)

A solution of **11-001** (10.1 g, 54.6 mmol) and formic acid (6.1 mL, 107 mmol) in toluene (30 mL) was heated at reflux for 8 h. The reaction mixture was partitioned between AcOEt and 0.5 M aqueous HCl. The separated organic layer was washed with saturated aqueous $NaHCO_3$ and saturated brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/AcOEt 4:1) to obtain *N*-formyl-2-phenoxyaniline **12-002** (9.35 g, 80%) as a pale brown crystal: mp 98–99 °C; NMR (DMDO-d_6) δ 6.82-7.50 (8H, m), 8.20–8.62 (2H, m); MS (EI) m/z 213 (M^+, 100%).

To a cooled solution of **12-002** above (753 mg, 3.53 mmol) in dry DMF (13 mL) in an ice bath was added NaH (60% dispersion in mineral oil, 169 mg, 4.23 mmol), and the mixture was stirred for 10 min. To the solution was added dropwise 2-methoxybenzylchloride (663 mg, 4.23 mmol). The mixture was stirred for 40 min at room temperature, poured into ice water and then extracted with AcOEt three times. The combined organic layer was washed with 0.5 M aqueous HCl and saturated brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/AcOEt 4 : 1) to obtain **7-061** as a colorless oil (823 mg, 70%): NMR ($CDCl_3$) δ 3.63 (3H×3/4, s), 3.78 (3H×1/4, s), 4.75 (2H×1/4, s), 4.99 (2H×3/4, s), 6.72–7.40 (13H, m), 8.35 (1H×3/4, s), 8.47 (3H×1/4, s); MS (CI) m/z 334 ($M^+ + 1$), 121 ($M^+ - 212$, 100%). Anal. ($C_{21}H_{19}NO_3$) C, H, N.

5.5. Method E: *N*-acetyl-*N*-(2-isopropoxybenzyl)-2-phenoxyaniline (7-016)

A solution of 2-isopropoxybenzaldehyde (1.85 g, 11.3 mmol) and 2-phenoxyaniline **11-003** (1.85 g, 9.99 mmol) in MeOH (10 mL) was stirred at room temperature for 0.5H, followed by cooling in an ice bath. To the cooled mixture was added $NaBH_4$ (1.50 g, 39.7 mol) in several parts. The reaction mixture was stirred for 0.5 h with cooling in an ice bath followed by stirring at room temperature for 0.5 h. After adding dropwise 5% aqueous AcOH (32 mL), the mixture was stirred at room temperature for 10 min followed by extraction with AcOEt three times. The combined organic layer was washed with saturated aqueous $NaHCO_3$ and saturated brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/AcOEt 25:1) to obtain *N*-(2-isopropoxybenzyl)-2-phenoxyanilne **13-001** (2.65 g, 80% yield) as an oil: NMR ($CDCl_3$) δ 1.29 (6H, d, $J=6.1$ Hz), 4.30–4.42 (2H, m), 4.43–4.63 (1H, m), 4.65–4.92 (1H, m), 6.55–6.70 (1H, m), 6.72–7.39 (12H, m); MS (ESI) m/z ($M^+ + Na$, 100%).

To a solution of **13-001** above (2.65 g, 7.95 mmol) and Et_3N (1.5 mL, 10.8 mmol) in THF (30 mL) was added AcCl (0.80 mL, 11.3 mmol). After stirring at room temperature for 0.5H, the reaction mixture was poured into water and extracted with AcOEt three times. The combined organic layer was washed with 0.5 M aqueous HCl, saturated aqueous $NaHCO_3$ and saturated brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/AcOEt 4:1) to obtain **7-016** (2.65 g, 89% yield) as an oil; NMR ($CDCl_3$) δ 1.09 (3H, d, $J=7.0$ Hz), 1.17 (3H, d, $J=7.0$ Hz), 1.93 (3H, s), 4.26–4.45 (1H, m), 4.69 (1H, d,

$J = 14.5$ Hz), 5.17 (1H, d, $J = 14.5$ Hz), 6.71–7.40 (13H, m) ; MS (EI) m/z 376 ($M^+ + 1$), 149 (M^+ - 226, 100%). Anal. ($C_{24}H_{25}NO_3$) C, H, N.

5.6. Method F: *N*-acetyl-*N*-(2,4-dimethoxybenzyl)-2-phenoxyaniline (7-040)

A suspension of 2-phenoxyaniline **11-003** (3.70 g, 20.0 mmol), 2,4-dimethoxybenzaldehyde (3.70 g, 22.3 mmol) and PtO_2 (70 mg) in MeOH (60 mL) was stirred overnight under a hydrogen atmosphere. The resulting precipitate was dissolved by addition of $CHCl_3$ (30 mL) and then the catalyst was removed by filtration through Celite. The filtrate was concentrated in vacuo. The residual solid was recrystallized from MeOH to obtain *N*-(2,4-dimethoxybenzyl)-2-phenoxyaniline **13-002** (5.06 g, 76% yield) as a colorless amorphous: NMR ($CDCl_3$) δ 3.69 (3H, m), 3.78 (3H, m), 4.29 (2H, d, $J = 6.2$ Hz), 4.64 (1H, br t, $J = 6.2$ Hz), 6.35–6.44 (2H, m), 6.56–6.69 (1H, m), 6.73–7.36 (9H, m); MS (ESI) m/z 358 ($M^+ + Na$, 100%).

To a solution of **13-002** above (1.00 g, 2.98 mmol) in pyridine (1.18 g, 14.9 mmol) was added acetic anhydride (0.76 g, 7.44 mmol). After stirring at room temperature overnight, the reaction mixture was poured into water and extracted with AcOEt three times. The combined organic layer was washed with 0.5 M aqueous HCl, saturated aqueous $NaHCO_3$ and saturated brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/AcOEt 3:1) to obtain **7-040** (1.09 g, 97%) as a colorless oil: NMR ($CDCl_3$) δ 1.92 (3H, s), 3.52 (3H, s), 3.76 (3H, s), 4.65 (1H, d, $J = 14.4$ Hz), 5.09 (1H, d, $J = 14.4$ Hz), 6.28 (1H, d, $J = 2.4$ Hz), 6.35 (1H, dd, $J = 2.4$, 8.4 Hz), 6.83–7.36 (10H, m); MS (EI) m/z 377 (M^+), 151 ($M^+ - 226$, 100%). Anal. ($C_{23}H_{23}NO_4$) C, H, N.

5.7. Method G: *N*-(methoxybenzyl)-*N*-(methylaminocarbonyl)-2-phenoxyaniline (7-078)

In a manner similar to the preparation of **13-001** in Method E, *N*-(2-methoxybenzyl)-2-phenoxyanilne **13-003** (13.4 g, 80% yield) was obtained from 2-methobenzaldehyde (7.44 g, 54.7 mmol), 2-phenoxyaniline **11-003** (10.14 g, 54.7 mmol) and $NaBH_4$ (2.07 g, 54.7 mmol) as a colorless crystal: mp 48–49 °C; NMR ($CDCl_3$) δ 3.74 (3H, s), 4.37 (2H, s), 4.73 (1H, brs), 6.52–7.39 (13H, m); MS (ESI) m/z 328 ($M^+ + Na$, 100%).

To a solution of triphosgene (751 mg, 2.53mmol) in CH_2Cl_2 (14 mL) was added dropwise a solution of **13-003** above (2.03 g, 6.65 mmol) and diisopropylethylamine (1.03 g, 7.98 mmol) in CH_2Cl_2 (25 mL), and the mixture was stirred at room temperature for 5 min. Into the mixture was blown an excess amount of methylamine, and the resulting mixture was stirred at room temperature for 5 min. After concentration in vacuo, the reaction mixture was concentrated in vacuo and partitioned between AcOEt and water. The separated organic layer was washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$ and saturated brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residual solid was recrystallized from AcOEt to obtain **7-078** as a colorless crystal: mp 133.0–134.0 °C; NMR ($CDCl_3$) δ 2.74 (3H, s), 3.60 (3H, s), 4.30 (1H, brs), 4.89 (2H, s), 6.68–7.50 (13H, m); MS (CI) m/z 363 ($M^+ + 1$, 100%). Anal. ($C_{22}H_{22}N_2O_3$) C, H, N.

5.8. Method H: *N*-(aminocarbonyl)-*N*-(2-methoxybenzyl)-2-phenoxyaniline (7-077)

To a solution of **13-003** (1.54 g, 5.04 mmol) in HOAc (20 mL) was added dropwise a solution of KNCO (1.23 g, 15.1 mmol) in water (10 mL), and the mixture was stirred at room temperature for 2.5 h. The reaction mixture was poured into water and extracted with AcOEt three times. The combined organic layer was washed with saturated aqueous $NaHCO_3$ and saturated brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/AcOEt 3:1) followed by recrystallization from AcOEt to obtain **7-077** (1.69 g, 96%): mp 89.5–90.0 °C; NMR ($CDCl_3$) δ 3.63 (3H, s), 4.44 (2H, brs), 4.90 (2H, brs), 6.74–7.42 (13H, m); MS (EI) m/z 348 (M^+), 121 ($M^+ - 227$, 100%). Anal. ($C_{21}H_{20}N_2O_3$) C, H, N.

5.9. Method I: *N*-(2-methoxybenzyl)-*N*-methoxycarbonyl-2-phenoxyaniline (7-080)

To a solution of triphosgene (775 mg, 2.61 mmol) in CH_2Cl_2 (14 mL) was added dropwise a solution of **13-003** (2.16 g, 7.07 mmol) and diisopropylethylamine (1.10 g, 8.48 mmol) in CH_2Cl_2 (25 mL), and the mixture was stirred at room temperature for 15 min. The reaction mixture was concentrated in vacuo, and the residue was partitioned between AcOEt and 5% aqueous HCl. The separated organic layer was washed with saturated aqueous $NaHCO_3$ and saturated brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/AcOEt 15:1) to obtain *N*-chlorocarbonyl-*N*-(2-methoxybenzyl)-2-phenoxyaniline (2.57 g, 99%) as a colorless solid: NMR ($CDCl_3$) δ 3.63 (3H, s), 4.75 (1H, d, $J = 14.0$ Hz), 5.15 (1H, d, $J = 14.0$ Hz), 6.71–7.46 (13H, m); MS (FAB) m/z 368 ($M^+ + 1$), 121 ($M^+ - 246$, 100%).

To a solution of sodium methoxide (215 mg, 3.98 mmol) in THF (5 mL) was added dropwise a solution of *N*-chlorocarbonyl-*N*-(2-methoxybenzyl)-2-phenoxyaniline (1.22 g, 3.32 mmol) in THF (5 mL) with cooling in an ice bath, and the mixture was stirred at room temperature for 20 min. The mixture was concentrated in vacuo and the residue was partitioned between AcOEt and 5% aqueous HCl. The separated organic layer was washed with saturated aqueous $NaHCO_3$ and saturated brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/AcOEt 6:1) to obtain **7-080** (1.18 g, 98%) as a colorless oil: NMR ($CDCl_3$) δ 3.58 (3H, s), 3.63 (3H, s), 4.82 (2H, brs), 6.75–7.42 (13H, m); MS (EI) m/z 363 (M^+), 121 ($M^+ - 242$, 100%). Anal. ($C_{22}H_{21}NO_4$) C, H, N.

5.10. Method J: *N*-acetyl-*N*-(2-formylbenzyl)-2-phenoxyaniline (7-028)

In a manner similar to the preparation of **7-008** in

Method C, *N*-acetyl-*N*-[2-(1,3-dioxolanyl)benzyl]-2-phenoxyaniline (**7-115**) (2.94 g, 77%) was obtained from 2-([1,3]dioxolan-2-yl)benzylchloride (1.94 g, 9.77 mmol), *N*-acetyl-2-phenoxyaniline (2.20 g, 9.77 mmol) and NaH (60% dispersion in mineral oil, 430 mg, 10.8 mmol) as a light yellow oil: NMR ($CDCl_3$) δ 1.94 (3H, s), 3.90–4.14 (4H, m), 4.61 (1H, d, $J=14.7$ Hz), 5.55 (1H, d, $J=14.7$ Hz), 5.87 (1H, s), 6.91–6.99 (5H, m), 7.08–7.40 (7H, m), 7.50–7.62 (1H, m),; MS (FAB) *m/z* 390 ($M^+ + 1$), 286 ($M^+ - 103$, 100%) . Anal. ($C_{24}H_{23}NO_4$) C, H, N.

A solution of *N*-acetyl-*N*-[2-(1,3-dioxolan-2-yl)benzyl]-2-phenoxyaniline (2.75 g, 7.06 mmol) and *p*-toluenesulfonic acid monohydrate (0.10 g, 0.53 mmol) in acetone (40 mL) was stirred at room temperature for 6 h. To the mixture was added saturated aqueous $NaHCO_3$ solution, and the mixture was concentrated in vacuo to remove acetone. The residual mixture was extracted with AcOEt three times. The combined organic layer was washed with water and saturated brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/AcOEt 3:1) to obtain **7-028** (2.12 g, 85%) as a pale yellow crystal: mp 114.0–117.0 °C; NMR ($CDCl_3$) δ 1.99 (3H, s), 5.31 (1H, d, $J=15.6$ Hz), 5.45 (1H, d, $J=15.6$ Hz), 6.73–7.88 (13H, m), 10.12 (1H, s); MS (FAB) *m/z* 346 ($M^+ + 1$, 100%) . Anal. ($C_{22}H_{19}NO_3$) C, H, N.

5.11. Method K: *N*-acetyl-*N*-[2-(1-hydroxyethyl)benzyl]-2-phenoxyaniline (7-030)

To a 0.26 M solution of methyl magnesium bromide in THF (20.3 mL) was added a solution of **7-028** (1.20 g, 3.47 mmol) in THF (7.0 mL). After stirring at room temperature for 1H, saturated aqueous NH_4Cl was added to the reaction mixture with cooling in an ice bath, and the mixture was extracted with AcOEt. The combined organic layer was washed with saturated brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/AcOEt 1:1) to obtain **7-030** (1.19 g, 95%) as an light yellow oil: NMR ($CDCl_3$) δ 1.34 (3H×1/2, d, $J=6.2$ Hz), 1.40 (3H×1/2, d, $J=6.2$ Hz), 1.93 (3H×1/2, s), 1.97 (3H×1/2, s), 4.87 (1H×1/2, d, $J=13.0$ Hz), 4.967 (1H×1/2, d, $J=13.0$ Hz), 5.10 (1H×1/2, d, $J=13.0$ Hz), 519 (1H×1/2, d, $J=13.0$ Hz), 6.60–7.51 (13H, m) ; MS (EI) *m/z* 361 (M^+), 117 ($M^+ - 244$, 100%). Anal. ($C_{23}H_{239}NO_3$) C, H, N.

5.12. Method L: *N*-acetyl-*N*-(2-acetylbenzyl)-2-phenoxyaniline (7-029)

A solution of oxalyl chloride (0.64 mL, 7.3 mmol) in dry CH_2Cl_2 (40 mL) was cooled to-78 °C. To the solution was added dropwise a solution of DMSO (0.78 mL, 11.0 mmol) in dry CH_2Cl_2 (7.6 mL). The mixture was stirred at −78 °C for 10 min, and then to the solution was added dropwise a solution of **7-030** (1.35 g, 3.74mmol) in dry CH_2Cl_2 (13 mL). After stirring at −78 °C for 15 min, the temperature of the reaction mixture was gradually increased to −45 °C, followed by stirring at that temperature for 1 h. To the mixture was added dropwise Et_3N (3.9 mL, 28.0 mmol) at −45 °C and the mixture was stirred at 0 °C for 20 min. After the addition of saturated aqueous NH_4Cl, the mixture was extracted with EtOAc three times. The combined organic layer was washed with saturated brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/AcOEt 1:1) and recrystallized from AcOEt/hexane to obtain **7-029** (911 mg, 68%): mp 110.0–110.50 °C; NMR ($CDCl_3$) δ 1.99 (3H, s), 2.35 (3H, s), 5.13 (1H, d, $J=15.6$ Hz), 5.38 (1H, d, $J=15.6$ Hz), 6.80–7.75 (13H, m); MS (EI) *m/z* 359 (M^+), 316 ($M^+ - 43$, 100%). Anal. ($C_{23}H_{21}NO_3$) C, H, N.

5.13. Method M: *N*-acetyl-*N*-(2-vinylbenzyl)-2-phenoxyaniline (7-027)

Under a nitrogen atmosphere, to a suspension of methyltriphenylphosphonium bromide (2.54 g, 7.11 mmol) in dry THF (10 mL) cooled to −15 °C 1.63 M *n*-butyllithium in hexane (3.60 mL, 5.93 mmol) was added dropwise to maintain the reaction temperature at −15 to −10 °C. The mixture was warmed to room temperature gradually, and stirred for 20 min. To the mixture was added dropwise a solution of **7-028** (820 mg, 2.37 mmol) in THF (5 mL), and the mixture was stirred at room temperature for 1 h. To the mixture was added saturated aqueous NH_4Cl, and the mixture obtained was extracted with AcOEt three times. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/AcOEt 4:1) to obtain **7-027** (697 mg, 86%) as a colorless oil: NMR ($CDCl_3$) δ 1.93 (3H, s), 4.67 (1H, d, $J=14.5$ Hz), 5.22 (1H, dd, $J=1.5$, 10.8 Hz), 5.35 (1H, d, $J=14.5$ Hz), 5.51 (1H, dd, $J=1.5$, 17.4 Hz), 6.80–7.44 (14H, m); MS (EI) *m/z* 343 (M^+), 117 ($M^+ - 226$, 100%). Anal. ($C_{23}H_{21}NO_2$) C, H, N.

5.14. Method N: *N*-acetyl-*N*-(2-propylbenzyl)-2-phenoxyaniline (7-026)

In a manner similar to the preparation of **7-027** in Method M, *N*-acetyl-*N*-(2-(1-prop-1-enyl)benzyl)-2-phenoxyanilne (**7-116**) (859 mg, 83%) was obtained by treatment of **7-028** (1.01 g, 2.92 mmol) with triphenyl-propylidene-λ^5-phosphane prepared by treatment of ethyltriphenyl-phosphonium bromide (4.34 g, 11.7 mmol) with 1.63 M *n*-butyllithium in hexane (6.6 mL, 10.8 mmol) as a light yellow oil. This product was a mixture of geometrical isomers (the ratio 3:2) and was used in the next step without further purification on silica gel chromatography.

5.14.1. Crude 7-116. NMR ($CDCl_3$) δ 1.50–1.63 (3H×3/5, m), 1.75–1.84 (3H×2/5, m), 1.90–1.993 (3H, m), 4.57 (1H×3/5, d, $J=14.5$ Hz), 4.62 (1H×2/5, d, $J=14.5$ Hz), 5.25 (1H×3/5, d, $J=14.5$ Hz), 5.33 (1H×2/5, d, $J=14.5$ Hz), 5.62–6.05 (1H, m), 6.22–6.38 (1H×3/5, m), 6.47–6.62 (1H×2/5, m), 6.73–7.40 (13H, m); MS (ESI) *m/z* 380 ($M^+ + Na$, 100%).

A suspension of the above crude mixture of geometrical isomers (the ratio 3:2) **7-116** (757 mg, 2.12 mmol) and PtO_2 (15 mg, 0.066 mmol) in EtOH (7.0 mL) was stirred

under a hydrogen atmosphere at room temperature for 3 h. The catalyst was removed by filtration through Celite, and the filtrate was concentrated in vacuo. The residue was chromatographed on silica gel (hexane/AcOEt 4:1) to obtain **7-026** (647 mg, 85%) as a colorless oil: NMR ($CDCl_3$) δ 0.89 (3H, t, $J=7.3$ Hz), 1.37–1.55 (2H, m), 1.96 (3H, s), 2.42–2.51 (2H, m), 4.68 (1H, d, $J=14.5$ Hz), 5.20 (1H, d, $J=14.5$ Hz), 6.81–7.37 (13H, m); MS (SI) m/z 360 ($M^+ + 1$, 100%). Anal. ($C_{24}H_{25}NO_2$) C, H, N.

5.15. Method O: *N*-acetyl-*N*-(2-aminobenzyl)-2-phenoxyaniline (7-037)

In a manner similar to the preparation of **7-016** in Method C, *N*-acetyl-*N*-(2-nitrobenzyl)-2-phenoxyanilne (**7-036**) (9.09 g, 71% yield) was obtained by treatment of **12-001** (8.00 g, 35.2 mmol), which was yielded in Method E, with 2-nitrobenzylchloride (11.4 g, 52.8 mmol) in the presence of NaH (60% dispersion in mineral oil, 2.12 g, 53.0 mmol) in dry DMF (80 mL), as a yellow crystal: mp 96.0–96.5 °C; NMR ($CDCl_3$) δ 2.04 (3H, s), 5.18 (1H, d, $J=16.2$ Hz), 5.39 (1H, d, $J=16.2$ Hz), 6.82–7.56 (11H, m), 7.73–7.96 (2H, m); MS (ESI) m/z 385($M^+ + 23$, 100%). Anal. ($C_{21}H_{18}N_2O_4$) C, H, N.

A suspension of **7-036** (8.00 g, 22.1 mmol) and PtO_2 (66 mg, 0.29 mmol) was stirred under a hydrogen atmosphere at room temperature overnight. After dissolving the precipitate by addition of $CHCl_3$ (40 mL), the catalyst was removed by filtration. The filtrate was concentrated in vacuo and the residual solid was recrystallized from MeOH to obtain **7-037** (6.88 g, 94%): mp 155.5–156 °C; NMR ($CDCl_3$) δ 1.94 (3H, s), 4.62 (1H, d, $J=14.5$ Hz), 4.40–4.80 (2H, m), 6.36–7.42 (13H, m); MS (ESI) m/z 355 ($M^+ + Na$, 100%). Anal. ($C_{21}H_{20}N_2O_2$) C, H, N.

5.16. Method P: *N*-acetyl-*N*-(2-pyrrolidinobenzyl)-2-phenoxyanilne hydrochloride (7-038)

A mixture of **7-037** (1.00 g, 3.01 mmol), 1,4-dibromobutane (680 mg, 3.15 mmol), K_2CO_3 (1.03 g, 7.45 mmol) and KI (50 mg, 0.30 mmol) in DMF (10 mL) was heated at 70 °C for 3 days. The reaction mixture was partitioned between AcOEt and water. The separated organic layer was washed with water and saturated brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/AcOEt 3:1), and the purified product was treated with 4 M HCl in AcOEt (0.9 mL) in Et_2O (5 mL) followed by recrystallization from AcOEt/Et_2O to obtain **7-038** (490 mg, 39%): mp 110.0–112.5 °C; NMR ($CDCl_3$) δ 2.30 (3H, m), 2.18–2.52 (4H, m), 3.22–4.32 (4H, m), 5.23 (1H, d, $J=15.6$ Hz), 5.46 (1H, d, $J=15.6$ Hz), 6.72–6.90 (3H, m), 7.06–7.61 (9H, m), 7.70–7.83 (1H, m), 12.98 (1H, brs); MS (ESI) m/z 409 ($M^+ + Na$, 100%). Anal. ($C_{25}H_{26}N_2O_2$·HCl) C, H, N.

5.17. Method Q: *N*-acetyl-*N*-(2-carboxybenzyl)-2-phenoxyanilne (7-033)

In a manner similar to the preparation of **7-040** in Method F, *N*-acetyl-*N*-(2-methoxycarbonylbenzyl)-2-phenoxyaniline (**7-032**) (2.44 g, 92% yield) was obtained by acetylation of *N*-(2-methoxycarbonylbenzyl)-2-phenoxyanilne (2.34 g, 7.02 mmol) yielded (3.75 g, 64% yield) by treatment of 2-methoxycarbonylbenzaldehyde (2.91 g, 17.7 mmol) and 2-phenoxyaniline **11-003** (3.28 g, 17.7 mmol) on PtO_2 (60 mg) under a hydrogen atmosphere, as a colorless crystal: mp 76.0–78.0 °C; NMR ($CDCl_3$) δ 1.98 (3H, s), 3.71 (3H, s), 5.19 (1H, d, $J=15.4$ Hz), 5.51 (1H, d, $J=15.4$ Hz), 6.83–7.43 (11H, m), 7.64 (1H, dd, $J=0.9$, 7.7 Hz), 7.79 (1H, dd, $J=1.4$, 7.7 Hz); MS (EI) m/z 375 (M^+), ($333^+ - 42$, 100%). Anal. ($C_{23}H_{21}NO_4$) C, H, N.

A mixture of **7-032** (2.26 g, 6.02 mmol) and 2 M aqueous KOH (3.6 mL, 7.2 mmol) in MeOH (23 mL) was heated at 60 °C for 1 h. The reaction mixture was concentrated in vacuo, and the residue was chromatographed on silica gel (hexane/AcOEt 1:1) to obtain **7-033** (2.01 g, 92%) as a colorless oil: NMR ($CDCl_3$) δ 1.99 (3H, s), 4.93 (1H, d, $J=15.4$ Hz), 5.29 (1H, d, $J=15.4$ Hz), 6.92–7.47 (11H, m), 7.56–7.60 (1H, m), 7.82–7.87 (1H, m); MS (FAB) m/z 362 ($M^+ + 1$, 100%). Anal. ($C_{22}H_{19}NO_4$) C, H, N.

5.18. Method R: *N*-acetyl-*N*-(2-dimethylaminocarbonylbenzyl)-2-phenoxyanilne (7-035)

To a solution of **7-033** (500 mg, 1.39 mmol) and HMPA (0.1 mL, 0.57 mmol) in THF (10 mL) was added $SOCl_2$ (0.2 mL, 2.74 mmol), and the mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated in vacuo, and the residue was dissolved in THF (10.0 mL). To the solution was added dropwise 50% aqueous Me_2NH (2.0 mL, 22.2 mmol), and the mixture was stirred at room temperature for 1 h. The reaction mixture was partitioned between AcOEt and 1 M aqueous HCl, and the separated water layer was extracted with AcOEt twice. The combined organic layer was washed with saturated brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/AcOEt 3:1) to obtain **7-035** (490 mg, 91%) as an oil: NMR ($CDCl_3$) δ 1.96 (3H, s), 2.62 (3H, s), 3.01 (3H, s), 4.57 (1H, d, $J=15.0$ Hz), 5.26 (1H, d, $J=15.0$ Hz), 6.79–7.48 (13H, m); MS (EI) m/z 388 (M^+), 300 ($M^+ - 88$, 100%). Anal. ($C_{24}H_{24}N_2O_3$) C, H, N.

5.19. Method S: *N*-acetoxyacetyl-*N*-(2-methoxybenzyl)-2-phenoxyanilne (7-073)

In a manner similar to the preparation of **7-016** in Method E, *N*-chloroacetyl-*N*-(2-methoxybenzyl)-2-phenoxyanilne (**7-072**) (2.96 g, 77% yield) was obtained by treatment of *N*-(2-methoxybenzyl)-2-phenoxyanilne **13-003** (3.05 g, 10.0 mmol) with chloroacetyl chloride (3.42 g, 20 mmol) in pyridine (10 mL, 123 mmol) as a colorless crystal: mp 83.0–83.5 °C; NMR ($CDCl_3$) δ 3.57 (3H, s), 3.97 (2H, s), 4.76 (1H, d, $J=14.3$ Hz), 5.20 (1H, d, $J=14.3$ Hz), 6.66–7.43 (13H, m); MS (EI) m/z 381 (M^+), 91 ($M^+ - 290$, 100%). Anal. ($C_{22}H_{20}ClNO_3$) C, H, N.

A mixture of **7-072** (1.01 g, 2.64 mmol), AcONa (1.30 g,

15.8 mmol) and tetrabutyl ammonium bromide (170 mg, 0.527 mmol) in benzene (10 mL) was heated at 80 °C for 5 h. The reaction mixture was partitioned between AcOEt and water, and the separated organic layer was washed with water and saturated brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/AcOEt 3:1) to obtain **7-073** (1.03 g, 96%) as a colorless oil: NMR ($CDCl_3$) δ 2.15 (3H, s), 3.58 (3H, s), 4.46 (1H, d, J = 14.8 Hz), 4.59 (1H, d, J = 14.8 Hz), 4.74 (1H, d, J = 14.5 Hz), 5.16 (1H, d, J = 14.5 Hz), 6.71–7.38 (13H, m); MS (EI) m/z 405 (M^+), 121 ($M^+ - 284$, 100%). Anal. ($C_{24}H_{23}NO_5$) C, H, N.

5.20. Method T: *N*-Hydroxyacethyl-*N*-(2-methoxybenzyl)-2-phenoxyaniline (7-074)

A mixture of **7-073** (525 mg, 1.30 mmol), K_2CO_3 (537 mg, 3.89 mmol) in MeOH (6.0 mL) was stirred at 50 °C for 7 h. The reaction mixture was poured into water and extracted with AcOEt three times. The combined organic layer was washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$ and saturated brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/AcOEt 3:1) to obtain **7-074** (450 mg, 96%) as a colorless crystal: mp 70.0–71.0 °C; NMR ($CDCl_3$) δ 3.41 (1H, t, J = 4.8 Hz), 3.59 (3H, s), 3.89–3.95 (2H, m), 4.76 (1H, d, J = 14.3 Hz), 5.24 (1H, d, J = 14.3 Hz), 6.73–7.39 (13H, m); MS (FAB) m/z 364 ($M^+ + 1$), 121 ($M^+ - 243$, 100%). Anal. ($C_{22}H_{21}NO_4$) C, H, N.

5.21. Method U: *N*-azidoacetyl-*N*-(2-methoxybenzyl)-2-phenoxyaniline (7-075)

A mixture of **7-072** (1.51 g, 3.95 mmol) and sodium azide (770 mg, 11.9 mmol) in DMF (10 mL) was stirred at room temperature overnight. The reaction mixture was poured into water and extracted with AcOEt three times. The combined organic layer was washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$ and saturated brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/AcOEt 3:1) to obtain **7-075** (1.55 g, 100%) as an colorless oil: NMR ($CDCl_3$) δ 3.56 (3H, s), 3.64 (1H, d, J = 15.9 Hz), 3.76 (1H, d, J = 15.9 Hz), 4.78 (1H, d, J = 14.2 Hz), 5.18 (1H, d, J = 14.2 Hz), 6.71–7.40 (13H, m); MS (EI) m/z 388 (M^+), 121 ($M^+ - 267$, 100%). Anal. ($C_{22}H_{20}N_4O_3$) C, H, N.

5.22. Method V: *N*-aminoacetyl-*N*-(2-methoxybenzyl)-2-phenoxyanilne (7-076)

A mixture of **7-075** (647 mg, 1.67 mmol) and PtO_2 (20 mg) in MeOH (7.0 mL) was stirred under a hydrogen atmosphere at room temperature overnight. The mixture was filtered through Celite, and the filtrate was concentrated in vacuo. The residue was chromatographed on silica gel (hexane/AcOEt 6:1) and recrystallized from AcOEt diisopropylether to obtain **7-076** (240 mg, 40%) as a colorless crystal: mp 85.0–86.0 °C; NMR ($CDCl_3$) δ 3.21 (2H, s), 3.58 (3H, s), 4.73 (1H, d, J = 14.4 Hz), 5.20 (1H, d, J = 14.4 Hz), 6.70–7.41 (13H, m); MS (FAB) m/z 363 ($M^+ + 1$, 100%). Anal. ($C_{22}H_{22}N_2O_3$) C, H, N.

5.23. Method W: *N*-acetyl-*N*-(2-hydroxybenzyl)-2-phenoxyaniline (7-022)

Unpurified *N*-acetyl-*N*-(2-acetoxybenzyl)-2-phenoxyaniline (**7-117**), which was obtained by treatment of a mixture of 2-acetoxybenzaldehyde (1.74 g, 10.6 mmol) and 2-phenoxyaniline **11-003** (1.85 g, 10.0 mmol) with $NaBH_4$ (3.00 g, 79.3 mmol) in MeOH (30 mL) followed by acetylchloride (2.00 mL, 28.1 mmol) in the presence of Et_3N (4.0 mL, 28.7 mmol) in CH_2Cl_2 (50 mL) in a manner similar to the preparation of **7-016** in Method C, was treated with 5% aqueous KOH (14 mL) in MeOH (40 mL) at room temperature for 1 h. The mixture was concentrated in vacuo, poured into water and extracted with AcOEt three times. The combined organic layer was washed with 0.5 M aqueous HCl, saturated aqueous $NaHCO_3$ and saturated brine, dried over Na_2SO_4, filtered, concentrated in vacuo and crystallized from diisopropylether to obtain **7-022** (1.86 g, 56%) as a light yellow crystal: mp 123.0–124.5 °C; NMR ($CDCl_3$) δ 1.95 (3H, s), 4.50 (1H, d, J = 14.5 Hz), 4.98 (1H, d, J = 14.5 Hz), 6.60–7.42 (13H, m), 9.54 (1H, s); MS (FAB) m/z 334 ($M^+ + 1$, 100%). Anal. ($C_{21}H_{19}NO_2$) C, H, N.

5.24. Method X: *N*-acetyl-*N*-(2-carboxymethoxybenzyl)-2-phenoxyaniline (7-020)

To a solution of **7-022** (666 mg, 2.00 mol) in dry DMF (10 mL) was added NaH (60% dispersion in mineral oil, 80 mg, 2.00 mmol), and the mixture was stirred at room temperature for 0.5 h. To the mixture was added methyl bromoacetate (0.30 mL, 3.20 mmol), and the mixture was stirred at room temperature for 0.5 h. The reaction mixture was poured into 0.5 M aqueous HCl solution, and extracted with AcOEt three times. The combined organic layer was washed with saturated aqueous $NaHCO_3$ and saturated brine, dried over Na_2SO_4, filtered and concentrated in vacuo.

A mixture of the above residue and 5% aqueous KOH (5.0 mL) in MeOH (10.0 mL) was stirred at room temperature for 1 h. The reaction mixture was acidified by addition of 2 M aqueous HCl (pH 2) and extracted with AcOEt. The combined organic layer was washed with saturated brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was crystallized from diisopropylether to obtain **7-020** (745 mg, 95%): mp 156.5–157.0 °C; NMR ($CDCl_3$) δ 1.96 (3H, s), 4.35 (1H, d, J = 14.5 Hz), 4.57 (1H, d, J = 14.5 Hz), 4.84 (1H, d, J = 14.0 Hz), 5.22 (1H, d, J = 14.0 Hz), 6.52–6.88 (6H, m), 6.93–7.35 (7H, m); MS (FAB) m/z 392 ($M^+ + 1$, 100%). Anal. ($C_{23}H_{21}NO_5$) C, H, N.

5.25. Method Y: *N*-acetyl-*N*-(2-methoxyphenyl)-2-phenoxyaniline (8)

A mixture of **12-001** (2.27 g, 10.0 mmol), 2-methoxyiodobenzene (1.3 mL, 10.0 mmol), K_2CO_3 (1.38 g, 10.0 mmol), copper powder (133 mg, 2.09 mmol) and CuBr

(200 mg, 1.39 mmol) in nitrobenzene (20 mL) was heated at reflux for 8 h. After addition of EtOAc, the precipitate was filtered off and the filtrate was washed with 0.5 M aqueous HCl, saturated aqueous $NaHCO_3$ and saturated brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/AcOEt 4:1) to obtain **8** (660 mg, 23%) as an light yellow oil: NMR ($CDCl_3$) δ 1.97 (3H×2/3, s), 2.13 (3H×1/3, s), 3.98 (3H, s), 6.80–7.62 (13H, m) ; MS (EI) *m*/*z* 333 (M^+), 291 (M^+-42, 100%). Anal. ($C_{21}H_{19}NO_3$) C, H, N.

5.26. Method Z: *N*-acetyl-*N*-[2-(2-methoxyphenyl)ethyl]-2-phenoxyaniline (9)

To a solution of 2-methoxyphenylacetic acid (4.98 g, 30.0 mmol) and DMF (0.5 mL) in toluene (30 mL) was added $SOCl_2$ (4.00 mL, 54.8 mmol), and the mixture was stirred at 70 °C for 1 h. The mixture was concentrated in vacuo and the residue was dissolved in CH_2Cl_2 (20 mL). The solution was added dropwise to a solution of 2-phenoxyaniline **11-003** (5.55 g, 30.0 mmol) and triethylamine (4.6 mL, 33.0 mmol) in CH_2Cl_2 (30 mL). The reaction mixture was stirred at room temperature for 1 h and concentrated in vacuo. The residue was partitioned between ice water AcOEt. The separated water phase was extracted with AcOEt twice. The combined organic phase was washed with 0.5M aqueous HCl, saturated aqueous $NaHCO_3$ and saturated brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was dissolved in dry THF (40 mL) and the solution was added dropwise to a suspension of $LiAlH_4$ (1.70 g, 44.8 mmol) in dry THF (40 mL). The mixture was heated at reflux for 0.5 h and then cooled in an ice bath. To the cooled mixture was added dropwise saturated aqueous Na_2SO_4, and the resulting precipitate was filtered through $MgSO_4$ plate prepared from its powdered anhydrate. The filtrate was concentrated in vacuo and chromatographed on silica gel (hexane/AcOEt 10:1) to obtain *N*-[2-(2-methoxyphenyl)ethyl]-2-phenoxyaniline **14** (8.23 g, 86%) as an oil: NMR ($CDCl_3$) δ 2.83–3.02 (2H, m), 3.26–3.49 (2H, m), 3.77 (3H, s), 4.49 (1H, brs), 6.51–7.40 (13H, m); MS (ESI) *m*/*z* 320 ($M^+ + 1$), 135 ($M^+ - 184$, 100%).

To a solution of **14** (3.19 g, 10.0 mmol) and Et_3N (1.5 mL, 10.8 mmol) in CH_2Cl_2 (30 mL) was added AcCl (0.80 mL, 11.3 mmol) with cooling in an ice bath. After stirring at room temperature for 0.5H, the reaction mixture was concentrated in vacuo. The residue was partitioned between Et_2O water and the separated water layer was extracted with Et_2O twice. The combined organic layer was washed with 0.5 M aqueous HCl, saturated aqueous $NaHCO_3$ and saturated brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/AcOEt 4:1) and crystallized from hexane to obtain **9** (2.64 g, 73%) as a colorless crystal: mp 79.0–80.0 °C; NMR ($CDCl_3$) δ 1.90 (3H, s), 2.80–3.05 (2H, m), 3.56–3.87 (1H, m), 3.71 (3H, s), 3.97–4.16 (1H, m), 6.73–7.42 (13H, m),; MS (SI) *m*/*z* 362 ($M^+ + 1$, 100%). Anal. ($C_{23}H_{23}NO_3$) C, H, N.

5.27. Binding study

5.27.1. PBR. Preparation of mitochondria was as follows: Rata were decapitated, the whole brain rapidly removed and the cerebral cortex homogenized in 10 volumes of 10 mM HEPES buffer (pH 7.4) containing 0.32 M sucrose, using a Teflon homogenizer, then centrifuged at 900*g* for 5 min. The supernatant was centrifuged at 12,000*g* for 10 min. The pellet (crude mitochondrial fraction) was washed with 50 mM HEPES buffer (pH 7.4) once, and suspended in 50 mM HEPES buffer (pH 7.4) at the protein concentration of 0.3 mg/mL. The crude mitochondrial preparation (1 mL) was incubated with [^3H]-PK11195 for 90 min at 4 °C, and the reaction was stopped by rapid filtration through a GF/B glass filter presoaked with 0.3% polyethyleneimine, after which the filters were washed three times with 3 mL of the buffer. The radioactivity was quantified in a liquid scintillation spectrometer. Nonspecific binding was determined in the presence of 10 μM PK11195. The specific binding was determined by subtracting nonspecific from total binding.

5.27.2. CBR. Rata were decapitated, the whole brain rapidly removed and the cerebral cortex was homogenized with 50 mM potassium phosphate buffer (pH 7.4), then centrifuged at 500*g* for 5 min. The supernatant was centrifuged at 48,000*g* for 20 min, and the pellet was washed once with the buffer. The final pellet, suspended in 50 mM potassium phosphate buffer (pH 7.4) at the protein concentration of 0.4 mg/mL was used as a crude membrane preparation. The membrane preparation (1 mL) was incubated with [^3H]flunitrazepam (2 nM) for 1 h at 4 °C, and the reaction was stopped by rapid filtration through a GF/B glass filter presoaked with 0.3% polyethyleneimine, after which the filters were washed three times with 3 mL of the buffer. The radioactivity was quantified in a liquid scintillation spectrometer. Nonspecific binding was determined in the presence of 10 μM diazepam. The specific binding was determined by subtracting nonspecific from total binding.

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