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Synthesis, Binding and Structure–Affinity Studies of New Ligands for the Microsomal Anti-Estrogen Binding Site (AEBS)

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Abstract—New compounds have been synthesized based on the structure of the anti-tumoral drug tamoxifen and its diphenylmethane derivative, *N,N*-diethyl-2-[(4-phenyl-methyl)-phenoxy]-ethanamine, HCl (DPPE). These new compounds have no affinity for the estrogen receptor (ER) and bind with various affinity to the anti-estrogen binding site (AEBS). Compounds **2**, **10**, **12**, **13**, **20a**, **20b**, **23a, 23b**, **29** exhibited 1.1–69.5 higher affinity than DPPE, and compounds **23a** and **23b** have 1.2 and 3.5 higher affinity than tamoxifen. Three-dimensional structure analysis, performed using the intersection of the van der Waals volume occupied by tamoxifen in its crystallographic state and the van der Waals volume of these new compounds in their calculated minimal energy conformation, correlated well with their p*Ki* for AEBS ($r = 0.84$, $P < 0.0001$, $n = 18$). This is the first structure-affinity relationship (SAR) ever reported for AEBS ligands. Moreover in this study we have reported the synthesis of new compounds of higher affinity than the lead compounds and that are highly specific for AEBS. Since these compounds do not bind ER they will be helpful to study AEBS mediated cytotoxicity. Moreover our study shows that our strategy is a new useful guide to design high affinity and selective ligands for AEBS.

Tamoxifen is a triphenylethylenic antiestrogen (Fig. 1a) widely used around the world in the management and the prevention of breast cancers.[1] The rational for this application was the ability of tamoxifen to block the mitogenic activity of 17β-estradiol on the growth of breast cancer cells. This involves the competition of tamoxifen with estradiol on the estrogen receptor. However, tamoxifen act as a partial agonist of estradiol and induces beneficial hypolipemic effects on the patient, but increases the risk of development of endometrial cancer.[1] This prompted the development of pure antagonists, moreover, tamoxifen produced a cytotoxicity that is independent of ER[2,3] that might involve other cellular targets such as Protein kinase C,[4] calmoduline,[5] and a high affinity microsomal anti-estrogen binding site (AEBS).[6,7] Interestingly the development of ligands that have no affinity for ER and that are specific of AEBS has produced compounds of potential clinical value since they are cytotoxic against tumor cells[8–10] and display antiviral activities.[11] Such compounds induced cytotoxicity in a dose and time dependent way of tumoral cell lines of various origins[9,10] but are only cytostatic on primary culture of normal bovine endothelial aortic cells.[12] In particular, a diphenylmethane compound, the *N,N*-diethyl-2-[(4 -phenyl-methyl)-phenoxy]-ethanamine HCl (DPPE) (Fig. 1b), was the first specific and high affinity ligand for AEBS and no affinity for ER.[8] This compound has recently been successful in the treatment of chemotherapeutic refractive cancers in phase I and II clinical trials,[13–17] and is now into phase III trials. In vitro studies have shown that the potentiation of the chemotherapeutic index of cytotoxic drugs by tamoxifen and DPPE was mediated by AEBS.[18] Although DPPE's efficiency is moderate, it was tested on patients bearing different kinds of tumor independently of their hormonosensitivity (melanoma, prostate, ovarian…).[13–17] Since the affinity of DPPE for AEBS was 20 times lower than that of tamoxifen, it might be of particular interest to design higher affinity and selective ligands to target AEBS.

Structure–affinity studies revealed that AEBS exhibits high affinity for two different classes of compounds that are competitive inhibitors of tamoxifen on AEBS. The first class includes arylaminoethyl compounds, they are cationic and amphiphillic compounds such as triarylethylene,

AEBS: anti-estrogen binding site; ER: estrogen receptor; ATCC: American tissue culture collection; PBS: phosphate buffer saline; EDTA: ethylene-diamine-tetra-acetic acid; FCS: fetal calf serum

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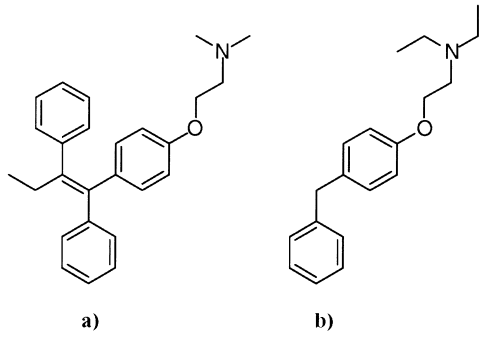


Figure 1. Chemical structure of AEBS ligands; (a) tamoxifen: (b) DPPE.

triarylpropenone, diarylbenzopyran, diarylbenzofuran and diphenylmethanes.[18,19] The second class includes neutral synthetic oxysterol derivatives restricted to 6- and 7-ketocholestanol.[20] Ketocholestanol, diarylbenzofuran and diphenylmethane compounds bound to AEBS with selectivity over ER, but the diphenylmethane series is the only series that have been demonstrated not to affect calmoduline,[5,21] or PKC dependent events.[22,23] In this paper we reported the synthesis of new compounds derived from the structure of the diphenylmethane compound DPPE; their affinity for AEBS and for the estrogen receptor were measured, and a structure–affinity study was done.

Results and Discussion

Synthesis

We explored the structure–affinity relationship of a series aryloxyethyl derivatives based on the structure of a tamoxifen derivative *N,N*-diethyl-2-[(4-phenyl-methyl)-phenoxy]-ethanamine HCl (DPPE) (Fig. 2). Compounds were synthesized according to Schemes 1 and 2.

Compounds **1–2**, **10–21b** and **23a–25** were prepared in high yield by reacting phenol with potassium carbonate as a base with the appropriate side-chain aminoethylchloride in a dimethylformamide:acetone (1:1) solution (Scheme 1). Compound **3** was similarly prepared and was subsequently transformed into the mesylate **4** by reaction of the compound **3** with methanesulfonylchloride (Scheme 2). Treatment of compound **3** with triphenylphosphine and tetrabromocarbon in methylenechloride produced

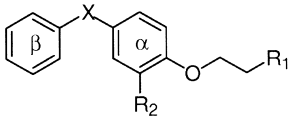
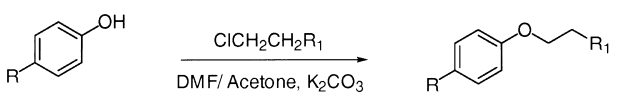
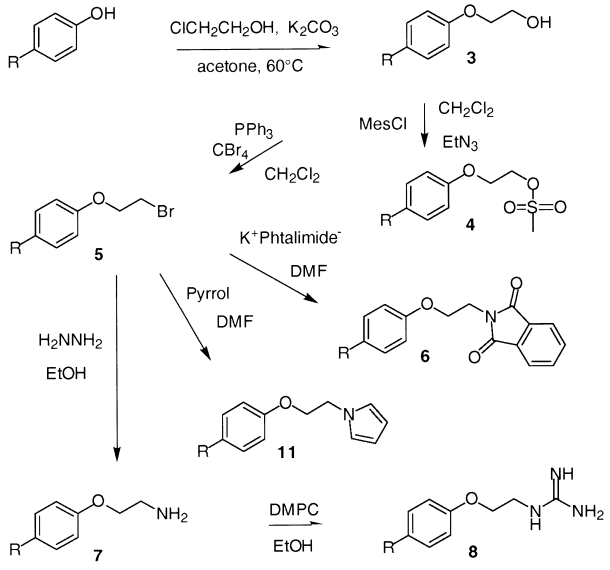


Figure 2. Structure–affinity relationship for the diphenylmethane based derivatives.



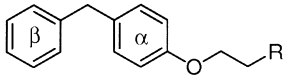
Scheme 1. Synthesis of compounds **1, 2, 10, 12–21b, 23a–25**; R=H, CH_3, Cl, Br, I, tBu, $PhCH_2$, PhCO, PhO, PhCH=CH, $PhCH_2CO$; R_1=$N(CH_3)_2$, $N(C_2H_5)_2$, c-$N(C_2H_4)_2$, c-$N(C_2H_4)CH_2$, c-$N(C_2H_4)O$.

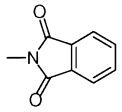


Scheme 2. Synthesis of compounds **3–8, 11**; R=$PhCH_2$.

compound **5** (Scheme 2). Reaction of compound **5** with potassium phtalimide gave compound **6**, and its hydrazinolysis gave compound **7** (Scheme 2). Treatment of compound **7** with 3,5-dimethylpyrazole-1-carboxamidine nitrate in methanol resulted in compound **8** (Scheme 2). Reaction of compound **2** in its free base state with methyliodide gave compound **9** (Table 1). Treatment of compound **5** with pyrrole gave compound **11** (Scheme

Table 1. Diphenylmethane derivatives of tamoxifen with altered basicity[a]

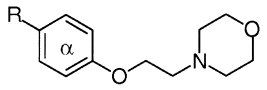


Compound	R	pK_a	Mp (°C)	Formula	K_i (nM)
Tamoxifen	$-N(CH_3)_2$	10.8	—		2.5±0.5
1 (DPPE)	$-N(CH_2CH_3)_2$	10.9	156–158	$C_{19}H_{25}NO$	55.6±2.2
2	$-N(CH_3)_2$	10.8	178–180	$C_{17}H_{21}NO$	42.3±3.1
3	$-OH$	—	70–72	$C_{15}H_{16}O_4$	N.M.
4	$-OSO_2CH_3$	—	58–60	$C_{16}H_{18}O_4S$	N.M.
5	$-Br$	—	60–62	$C_{15}H_{15}OBr$	N.M.
6		—	80–82	$C_{23}H_{19}NO_3$	N.M.
7	$-NH_2$	10.7	226–228	$C_{15}H_{18}NOCl$	924±18
8	$-NHC(=NH)NH_2$	13.65	102–104	$C_{16}H_{19}N_3O$	1530±26
9	$-N(CH_3)_3^+$	10.8	138–140	$C_{18}H_{24}NOI$	114±5.1
10	c-$N(C_4H_8)$	11.3	170–172	$C_{19}H_{23}NO$	8.8±0.9
11	c-$N(C_4H_4)$	—	oil	$C_{18}H_{19}NO$	N.M.
12	c-$N(C_5H_{10})$	11.2	180–182	$C_{20}H_{25}NO$	28.1±1.3
13	c-$N(C_4H_8)O$	8.7	185–186	$C_{19}H_{23}NO_2$	17.6±1.2

[a]Analysis for C, H, and N are within±0.4% of the theoretical values. Rat liver membranes were incubated with 3 nM of [^{3}H]tamoxifen and 12 concentrations of unlabeled test ligand ranging from 0.1 nM to 10 000 nM or 1 μM to 10 000 μM. Assays with [^{3}H]tamoxifen include 1 μM of 17ß-estradiol. IC_{50} values were determined using the iterative curve fitting program GraphPad prism. IC_{50} values were converted into the apparent *Ki* using the Cheng–Prusoff equation,[30] and the K_d values. Values are the average of three experiments, each carried out in triplicate. N.M. = not measurable.

2). Synthesis of compounds **27**–**29** has been reported previously.[4] Treatment of benzophenonic compounds **21a**, **21b** and **25** in CF_3COOH using $NaBH_4$[24] give benzhydrol derivatives **22a**, **22b** and **26** (Table 3). Compounds **30** to **32** were obtained by treatment of the aniline **28** in its free base state with the appropriated anhydride in dioxane (Table 4).

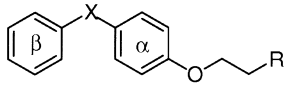
Table 2. 1-(2-*N*-morpholinoethoxy)benzenes[a]



Compound	R	Mp, °C	Formula	K_i (nM)
14	−H	189–190	$C_{12}H_{17}NO_2$	N.M.
15	−Cl	222–224	$C_{12}H_{16}ClNO_2$	N.M.
16	−Br	180–182	$C_{12}H_{16}BrNO_2$	3820±110
17	−I	178–180	$C_{12}H_{16}INO_2$	1630±47
18	$-CH_3$	208–210	$C_{13}H_{19}NO_2$	N.M.
19	−*t*Bu	190–192	$C_{16}H_{25}NO_2$	248±18

[a]Analysis for C, H, and N are within±0.4% of the theoretical values. K_i was determined as described in the caption of Table 1. N.M. = not measurable.

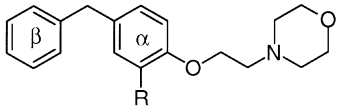
Table 3. Derivatives of DPPE with altered aromatic moieties[a]



Compound	R	X	Mp, °C	Formula	K_i (nM)
20a	*c*-N(C_4H_8)O	$-C(CH_3)_2$-	188–190	$C_{21}H_{27}NO_2$	48.1±2.5
20b	*c*-N(C_4H_8)	$-C(CH_3)_2$-	200–202	$C_{21}H_{27}NO$	10.1±1.2
21a	*c*-N(C_4H_8)O	−CO-	118–120	$C_{19}H_{21}NO_2$	102±4.5
21b	*c*-N(C_4H_8)	−CO-	128–130	$C_{19}H_{21}NO$	64.5±4.2
22a	*c*-N(C_4H_8)O	−CHOH-	84–86	$C_{19}H_{23}NO_3$	N.M.
22b	*c*-N(C_4H_8)	−CHOH-	106–108	$C_{19}H_{23}NO_2$	N.M.
23a	*c*-N(C_4H_8)O	−O-	170–172	$C_{18}H_{21}NO_3$	2.1±0.4
23b	*c*-N(C_4H_8)	−O-	166–168	$C_{18}H_{21}NO_2$	0.8±0.2
24	*c*-N(C_4H_8)O	−CH = CH-	216–218	$C_{20}H_{23}NO_2$	2010±11
25	*c*-N(C_4H_8)O	$-CH_2$-CO-	146–148	$C_{20}H_{23}NO_3$	850±12
26	*c*-N(C_4H_8)O	$-CH_2$-CHOH-	136–138	$C_{20}H_{25}NO_3$	N.M.

[a]Analysis for C, H, and N are within±0.4% of the theoretical values. K_i was determined as described in the caption of Table 1. N.M. = not measurable.

Table 4. *ortho*-Substituted 1-(2-*N*-morpholinylethoxy)benzylbenzene derivatives[a]



Compound	R	Mp, °C	Formula	K_i (nM)
27	$-NO_2$	111–112	$C_{19}H_{22}N_2O_4$	80±8.2
28	$-NH_2$	82–84*	$C_{19}H_{24}N_2O_2$	125±7.5
29	$-N_3$	122–123	$C_{19}H_{23}N_4O_2$	3±0.7
30	$-NH-CO-CH_3$	212–214	$C_{21}H_{26}N_2O_3$	730±12.5
31	$-NH-CO-(CH_2)_2-COOH$	150–151	$C_{23}H_{28}N_2O_5$	N.M.
32	$-NH-CO-(CH_2)_3-COOH$	171–172	$C_{24}H_{30}N_2O_5$	N.M.

[a]Analysis for C, H, N and O are within±0.4% of the theoretical values. *Free base. K_i was determined as described in the caption of Table 1. N.M. = not measurable.

Ligand binding

The synthesized compounds were evaluated for their binding affinity for the estrogen receptor (ER) extracted from MCF-7 cells by competing with [^{3}H]estradiol, and for binding to rat liver microsomal AEBS by competing with [^{3}H]tamoxifen. In this study tamoxifen displays a K_i of 2.4 nM for the estrogen receptor (ER) whereas DPPE and the new compounds prepared in this study have no affinity for ER. This indicates that the phenylpropene part of tamoxifen (Fig. 1) is required for estrogen binding affinity but not for binding to AEBS. We have looked at the consequence of structural modification of DPPE (Fig. 2) on the affinity for AEBS. The binding affinities for AEBS are expressed as K_i and are listed in Table 1 to 4.

Importance of the protonated side chain in the diphenylmethane series

Table 1 shows the consequences of the side chain modification in the diphenylmethane series on the binding to AEBS. Compound **1** is the DPPE and is the lead compound of the series. This compound has a 22-times weaker affinity than tamoxifen for AEBS. Compound **2** contains the dimethylaminoethoxy side chain and is the diphenylmethane homologue to tamoxifen. Compound **2** has 17-times less affinity than tamoxifen for AEBS, and a slight increase of affinity over DPPE. Elimination of *N*-alkyl substituant to give the primary amine **7** produced a 370-fold decrease in affinity for AEBS compared to tamoxifen. Replacement of the amine with an unprotonable substituant (**3**–**6**) at physiologic pH totally abolished the binding capacity for AEBS. Compound **8**, with a $pK_a > 12$, is the most basic compound of this series. Compound **8** had a very low affinity and produced a 612-times decrease of affinity compared with tamoxifen. The loss of affinity might be due to the delocalization of the positive charge and the higher hydration of the guanidinium group than the tertiary amine found the side chain of compounds **1**, **2**, **10**–**13**. The quarternarized amonium derivative **9** had a 46-fold decrease in affinity compared with tamoxifen. Compound **10** is a pyrrolidinic analogue of DPPE. This compound displayed a 3-times higher affinity than DPPE, showing that the cyclization of the aliphatic *N* substituant increases the affinity in the diphenylmethane series. Replacement of the pyrrolidine ring with the non-protonable pyrrol ring gave compound **11**, which has no detectable affinity for AEBS. The piperidine derivative **12** and the morpholinic derivative **13** have a little higher affinity than DPPE, and a slightly lower affinity than the pyrrolidinyl derivative (**10**). This illustrates that the ring part of the amine is important for high affinity. Compounds that are essentially non-basic in the Broensted–Lowry sense have no affinity for AEBS. The presence of a protonable amine is necessary but not sufficient for binding to AEBS as described for the triphenylethylenic series.[7,25,26] As reported for arylbenzofuran, triphenylethylene and other bulky aromatic derivatives, a cyclic amine such as pyrrolidine, cyclohexylamine and morpholine increased significantly the affinity for AEBS. Interestingly in the diphenylmethane series, the order

of affinity is not the same as in other series.[7,18,25,26] The highest affinity was obtained with the pyrrolidine derivative **10** followed by the morpholine derivative **13** with respectively 3.4 and 1.7-times more affinity than DPPE.

Replacement of the benzyl group in diphenylmethane backbone

Table 2 shows a series of 1(2-*N*-morpholinylethoxy) benzene derivatives used to determine the minimal structural requirements for binding to AEBS, keeping a high affinity morpholinylethoxy side chain. The analogue of compound **13** in which the benzyl group has been omitted (**14**) or replaced by a methyl group (**18**) or a chlorine atom (**15**) had no detectable affinity. When the benzyl group was replaced with a bulky halogen atom like bromine (**16**) and iodine (**17**) the affinity was partially restored but remained low. The tertiobutyl derivative **19** displayed a moderate affinity, which was 8-times lower than the DPPE. Increasing the bulk of the *para* substituent from the side chain on the α phenyl (Fig. 2) increased the affinity for AEBS. This showed that a bulky hydrophobic group in the *para* position from the side chain on the α phenyl is critical for high affinity binding to AEBS.

Modification of X between the two phenyl groups

Table 3 shows the structure and the affinity of various derivatives in which the aromatic moiety have been modified by replacing the benzylic methylene with various X groups. Replacement of the benzylic methylene with dimethylmethane group gives compounds **20a** and **20b** that have respectively 1.2 and 6-times higher affinity than DPPE. Compound **20a** has a 3-times lower affinity than its diphenylmethane counterparts compound **13**, and compound **20b** has the same affinity than its diphenylmethane counterpart compound **10**. This showed that cumyl derivatives **20a** and **20b** have a lower affinity than their diphenylmethane counterparts although the effect was weak. When X is carbonyl group (**21a,b**) the affinity for AEBS is lowered from 7- and 6-times respectively when compared with their diphenylmethane counterparts. The effect of this modification is a decrease of affinity and the order of magnitude is higher for benzophenonic compounds than for cumyl derivatives (**20a,b**). When X is a $-CH_2OH$- (**22a,b**) the affinity for AEBS is totally abolished independently of the structure of the amine side chain. Replacement of X with an oxygen gives compounds **23a** and **23b**. These compounds have 8 and 11-times higher affinity than their diphenylmethane homologues, 27 and 70-times higher affinity than DPPE and 1.2 and 3-times higher affinity than tamoxifen. The highest affinity was obtained with the less sterically hindered sp^3 atom, suggesting that the angle between the two planes defined by the benzene rings and their spatial positioning might be important. Among the compounds that have measurable affinities, pyrrolidinic compounds have higher affinity for AEBS than their morpholinic counterparts; however the order of magnitude in the change of affinity is not the same for a given X according to the structure of the amine side chain. The loss of affinity for AEBS for benzhydrol derivatives (**22a,b**) might be due to their lower hydrophobicity and/or the possible hydration at this position.

trans-Stylbene derivative (**24**) shows a 114.2 lower affinity than its diphenylmethane counterpart. One would have expected a higher affinity for this compound for AEBS because AEBS does not discriminate between the *cis* and *trans* isomers of tamoxifen.[7] It might be that in the case of compound **24**, the conformation of the stylbene moiety of the molecule is different from that of the *trans* isomer of tamoxifen. The methyl ketone derivative (**25**) has 8-times decreased in affinity than its benzophenonic counterpart (**21a**), and a 48-times decrease of affinity than its diphenylmethane counterpart. The methyl methanol derivative (**26**) have no measurable affinity for AEBS as observed for benzhydrol derivatives (**22a**) illustrating the drastic effect on AEBS affinity of an hydroxyl group on the benzylic position. Increasing the distance between the two phenyl-rings has drastic consequences on the affinity for AEBS. The same effect as observed for one atom X spaced derivatives was produced with the two atoms X spaced suggesting that the spatial orientation of the β phenyl ring (Fig. 2) and its distance from the ammonium side chain might be critical parameters for affinity.

ortho Substitution from the basic side chain on the α phenyl:

The presence of various groups in the *ortho* position on the α benzene ring from the side chain (Fig. 2) and its repercussion on the affinity for AEBS is shown in Table 4. These modifications were originally carried out with a view of developing compounds useful for affinity labeling[27,28] or affinity chromatography of AEBS. The presence of a nitro group (**27**) induced a 5-times decrease in affinity, and a 7-times decrease was observed with the amino group (**28**). Transformation into an azido group (**29**) produced an increase of affinity compared with compound **13**. Amidation of the compound **28** give the compound **30,** which had a 41-fold lower affinity than tamoxifen. Succinylation and glutamylation abolished the affinity for AEBS. Aromatic substitution might affect electronic density, which is important for stacking interactions, but other parameters such as steric hindrance, hydrophilicity and subsequently hydration might have occurred. Nitro- and amino-groups are opposite in term of electronic effect, but induced affinity decreases of the same order of magnitude, suggesting that this effect might be due to hydration. For compounds **31** and **32,** intramolecular electrostatic interaction with the amine side-chain might be involved in the loss of affinity.

Molecular modeling

The three-dimensional structure of tamoxifen has been determined.[29] A stick model constructed from the atomic coordinates is shown in Figure 3. Tamoxifen exhibits a propeller structure which is characteristic of triphenylethylenic compounds. The amine-aryl ether chain of tamoxifen is extended as in the calculated minimal energy conformation of the DPPE. However unlike

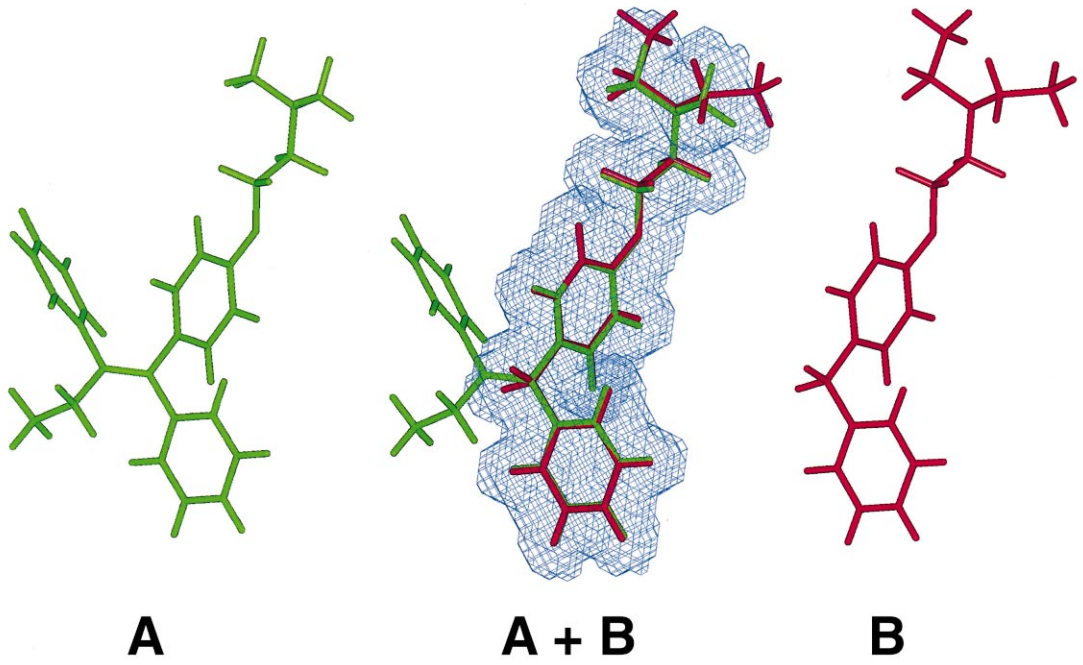


Figure 3. Molecular structure of tamoxifen and DPPE. Superimposition and intersection of their van der Waals volumes. On the left: three-dimensional structure of tamoxifen (A) depicted using coordinates from the X-ray crystallographic structure analysis by Précigoux et al.[29] On the right: minimum energy conformation of DPPE (B). On the center: superimposition and intersection of the van der Waals volume (A + B) of tamoxifen and DPPE.

the DPPE, the aromatic part of tamoxifen is not flexible: phenyl groups are conformationally restricted with a high-energy barrier of rotation. The van der Waals volume of tamoxifen is 321.95 Å^3. In its calculated minimum energy conformation, DPPE has a van der Waals volume of 207.63 Å^3. We superimposed the van der Waals volumes of the minimum energy conformation of the DPPE and the van der Waals volumes of the crystallographic structure of tamoxifen by superimposition of the following atoms: N–CH_2–CH_2–O–C_6H_4 (Fig. 3) as recently reported by our group for PBPE and tamoxifen.[10] The intersection of the van der Waals volume of DPPE and tamoxifen matches with the diphenylmethane and *N*,*N*-dimethylaminoethoxy moieties of tamoxifen. 80% of the volume of DPPE matches with the diphenylmethane and the basic side chain of the tamoxifen (Fig. 3). There is an excellent superimposition although the carbon bearing the phenyl ß is sp^3 in diphenylmethane and sp^2 in tamoxifen. Phenyl propene of tamoxifen, which is linked to a sp^2 carbon, introduces constraints that reduce the angle defined by the benzyllic carbon and the bonded phenyl rings.[29] In Table 5 we reported the van der Waals volume of each compound that binds AEBS as calculated when they are in their minimal

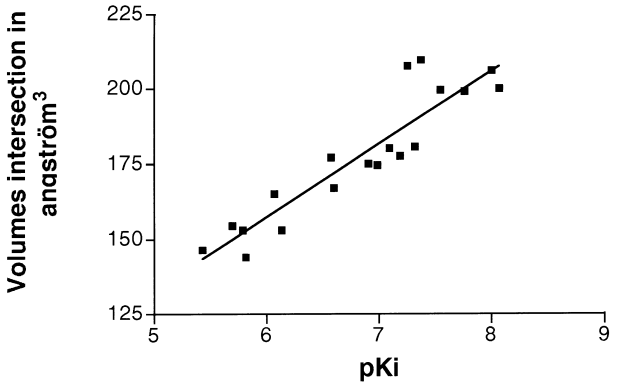


Figure 4. Relationship between van der Waals volume intersection and pK_i of the compounds with AEBS binding, a linear correlation ($r = 0.84$, $p < 0.0001$, $n = 18$) is drawn.

Table 5. Van der Waals volume intersection between new AEBS ligands and tamoxifen

Compound	Van der Waals volume in Å^3	Volume intersection in Å^3	K_i (nM)
Tamoxifen	321.95		2.5
1	250.75	207.63	55.6
2	221.64	209.49	42.3
7	192.21	177.26	270
8	222.94	144.12	1530
10	242.30	201.63	8.8
12	256.66	199.56	28.1
13	248.99	199.10	17.6
16	189.43	146.75	3820
17	199.83	153.31	1630
19	224.26	167.32	248
20a	279.61	180.44	48.1
20b	271.86	206.12	10.1
21a	248.35	174.72	102
21b	241.38	177.58	64.5
24	258.65	154.67	2010
25	265.71	165.29	850
27	267.46	180.12	80
28	259.51	175.80	125
30	290.25	153.27	730

energy conformation. We have reported the values of the intersection of their van der Waals volumes and those of tamoxifen in its crystallographic state. When the affinity for AEBS (pk_i) is plotted against the intersection of the van der Waals volume we obtain a linear correlation (Fig. 4) ($r = 0.84$, $P < 0.0001$, $n = 18$). This delineates for the first time that a linear quantitative structure-affinity relationship can be established between these two parameters. This illustrates that the rigid triphenylethylenic moiety of tamoxifen defines a spatial relationship driving the occupancy of the tamoxifen within AEBS and the binding of compounds from our series.

Compounds **23a** and **23b**, however, do not enter in this correlation. The spatial positioning of phenyl ß of these compounds in their minimal energy conformation does not match with the phenyl ß of tamoxifen and might define a more tightly interacting area in the binding site. Structure–affinity studies will be investigated further on the phenoxyphenol derivatives.

Conclusion

We have described the synthesis and AEBS binding affinities of a series of diphenyl methane based tamoxifen derivatives that are selective for AEBS over the estrogen receptor. None of the compounds that we prepared had detectable affinity for the estrogen receptor. Cyclic amines on the side chain, hydrophobic groups in the *para* position from this side chain on the phenyl ring, the spatial orientation and the distance between the two phenyl rings are critical for the affinity to AEBS. Three-dimensional analysis shows that a linear correlation can be established between the intersection of the van der Waals volume of tamoxifen and those of the new compounds. We have synthesized compounds with higher affinity for AEBS than DPPE, the lead compound from the diphenylmethane series, and two of them have higher affinity than tamoxifen. These compounds will help to characterize further AEBS-driven cytotoxicity and potentiation of the chemotherapeutic index of cytotoxic drugs.

Experimental

Materials and methods

^{1}H NMR were recorded at 200 MHz on a Brucker AC200 spectrometer. Chemical shifts are given in parts per million and tetramethylsilane was used as the internal standard for spectra obtained in DMSO-d_6 or $CDCl_3$. All J values are given in Hz. Mass spectra were recorded on a variant MAT 311 A mass spectrometer. Infrared spectra were recorded on a Perkin-Elmer 599 spectrophotometer. Elemental analysis was carried out by the microanalytical service laboratory of the Ecole Supérieure de Chimie of the Université Paul Sabatier, France. Reagents and solvents were used as obtained from commercial suppliers without further purification. Column chromatography was performed using silica gel, and reaction progress was determined by TLC analysis on silica gel coated aluminum plates. Visualization was with UV light (254 nM) or iodine. Melting points were uncorrected and measured on a Kofler apparatus.

Molecular structure analysis

Computational chemical calculations were performed on a Silicon Graphics Indigo workstation using INSIGHT II version 97.0 (Biosim/MSI). Minimum energy conformations were calculated using the Discover module (2.9.7/95.0/3.0.0) with CVFF forcefield. Van der Waals volumes and van der Waals volume intersections were determined using the Search-Compare module version 97.0 (Biosim/MSI).

Ligand binding assay (AEBS)

Anti-estrogen binding sites were labeled as previously described using rat liver membranes,[28] a rich source of AEBS, and [^{3}H]tamoxifen in the presence of 1 μM of 17ß-estradiol to mask residual estrogen receptors.[28] Assays were performed in 50 mM Tris–HCl, 1 mM EDTA, 12 mM thioglycerol at pH 7.4 for 18 h at 4 °C in a volume of 200 μL with 80 μg of microsomal proteins and 3 nM of [^{3}H]tamoxifen and 12 concentrations of unlabelled test ligand ranging from 0.1 nM to 10 000 nM. Assays with [^{3}H]tamoxifen included 1 μM of 17ß-estradiol. IC_{50} values were determined using the iterative curve fitting program GraphPad prism. IC_{50} values were converted into the apparent Ki using the Cheng–Prusoff equation,[30] and the K_d values. Non-specific binding was carried out with 2 μM of tamoxifen. Assays were terminated by loading 150 μL of the incubate on a 3.5 mL Sephadex LH-20 column equilibrated with 50 mM Tris–HCl, 1 mM EDTA, 12 mM thioglycerol at pH 7.4. Elutions were carried out by adding 1 mL fractions of 50 mM Tris–HCl, 1 mM EDTA, and 12 mM thioglycerol at pH 7.4. The eluates were collected and counted in ready safe scintillant (Beckman).

Ligand binding assay (estrogen receptor)

ER binding experiments were conducted using estrogen receptors extracted from MCF-7 cells as previously described.[31] Briefly MCF-7 (from ATCC) were grown to 80% confluency in DMEM medium (GiBCO BRL) supplemented with 10% FCS. Cells were then scraped, and washed twice with PBS. After centrifugation for 10 min at 1500 rpm, the cells were resuspended in TM buffer (20 mM Tris, HCl pH 7.4; sodium molybdate, 20 mM). Cells were broken by freeze-thaw lysis of the cell pellets in an equal volume of TM buffer. Cytosolic receptors were prepared by a 105 000 $g \times 60$ min centrifugation at 0 °C, and then stored at −80 °C. This cytosolic receptor solution was diluted to 60% in TM buffer, and then incubated with the corresponding ligand 18 h at 4 °C in a volume of 100 μL with 50 μg of protein and 2 nM of [^{3}H]estradiol and two concentrations (1 and 10 μM) of unlabeled test ligand. Assays were terminated by loading 65 μL of the incubate on a 1.2 mL Sephadex™ LH-20 column equilibrated with the TM buffer. The flow through was collected and counted for radioactivity in ready safe scintillant (Beckman).

Chemical synthesis

[2-(4-Benzyl-phenoxy)-ethyl]-diethyl-amine hydrochloride (DPPE) (1). 2-Diethylaminoethylchloride (0.50 g, 2.72 mmoles) was added to a solution of 4-benzylphenol (0.58 g, 2.72 mmoles) and K_2CO_3 (0.69 g, 5.44 mmoles) in 15 mL of a mixture of dry DMF:acetone (1:1). The reaction mixture was maintained for 18 h at 60 °C. The mixture was then filtered and poured into 100 mL of cold water and extracted twice with 100 mL of diethyl ether. The organic layer was washed 3 times with 10 mL of an aqueous solution of sodium hydroxide (0.1N) and then 3 times with 10 mL of brine. The etherous organic layer was then extracted with 5 mL of 12N hydrochloric acid. The aqueous layer was collected and evaporated. The white solid was then recrystalized in a mixture of isopropanol: acetone (3:1) to give 0.78 g (90%) of white crystals as the hydrochloride salt: mp 156–158 °C; ^{1}H NMR δ (DMSO-d_6) 7.1–7.6 (m, 9H, ArH), 3.91 (s, 2H, $ArCH_2$), 4.4 (t, J = 5.8 Hz, 2H, $ArOCH_2$), 2.82 (t, J = 5.8 Hz, 2H, CH_2N), 2.63 (q, 4H, NCH_2), 1.2 (t, 6H, CH_3). MS m/z 283 ($M+1$).

[2-(4-Benzyl-phenoxy)-ethyl]-dimethyl-amine hydrochloride (2). The same procedure was used as for compound **1** using the corresponding chloroethylamine. The white solid was then recrystalized in a mixture of isopropanol: acetone (3:1) to give 0.76 g (2.4 mmol, 88%) of white crystals as the hydrochloride salt: mp 178–180 °C, ^{1}H NMR δ (DMSO-d_6) 7.1–7.6 (m, 9H, ArH), 3.91 (s, 2H, $ArCH_2$), 4.4 (t, J = 5.8 Hz, 2H, $ArOCH_2$), 2.82 (t, J = 5.8 Hz, 2H, CH_2N), 2.62 (s, 6H, NCH_3). MS m/z 256 ($M+1$).

2-(4-Benzyl-phenoxy)-ethanol (3). The same procedure was used as for compound **1** with 2-chloro1ethanol instead of chloroethylamine. The reaction mixture was maintained for 18 h at 60 °C. The mixture was then filtered, poured into 100 mL of cold water and extracted twice with 100 mL of diethyl ether. The organic layer was washed 3 times with 10 mL of an aqueous solution of sodium hydroxide (0.1N) and then 3 times with 10 mL of brine. The final etheral solution was evaporated under reduced pressure and the white solid was then recrystalized in hexane. Yield 85%; mp 70–72 °C; ^{1}H NMR δ (DMSO-d_6) 7.1–7.6 (m, 9H, ArH), 4.01 (m, 4H, OCH_2CH_2O), 3.90 (s, 2H, $ArCH_2$); MS m/z 228 (M^+).

Methanesulfonic acid 2-(4-benzyl-phenoxy)-ethyl ester (4). 2 g (8.8 mmoles) of compound **3** was dissolved in 20 mL of CH_2Cl_2 containing a 50% excess of triethylamine (1.3 g, 13.1 mmoles) at 0 °C. 1.1 equivalents of methanesulfonylchloride was added dropwise over 10 min. The mixture was stirred for 15 min before being worked up 3 times with 10 mL of a cold 10% solution of HCl, then 3 times with 10 mL of a saturated solution of $NaHCO_3$, and finally 3 times with 10 mL of brine. The organic layer was then dried over $MgSO_4$ and evaporated to give 2.4 g of a white solid. Yield 89%, mp 60–62 °C, NMR δ (DMSO-d_6) 7.1–7.6 (m, 9H, ArH), 4.20 (t, 2H, $ArOCH_2$), 4.05 (t, 2H, CH_2OSO_2), 3.90 (s, 2H, $ArCH_2$), 3.69 (s, 3H, SO_2CH_3). MS m/z 306 (M^+).

2-(4-Benzyl-phenoxy)-ethylbromine (5). 2 g (8.8 mmoles) of compound **3** was dissolved in 20 mL of CH_2Cl_2 containing 1.1 equivalents of CBr_4 (1.3 g, 13.1 mmoles) at 0 °C. 1.2 equivalents of triphenylphosphine were then added. The mixture was then stirred for 1 h at 0 °C and then 1 h at room temperature. The solution was extracted 3 times with water and then dried over $MgSO_4$. Evaporation of CH_2Cl_2 and recrystalization in a minimum of methyl alcohol gave 2.05 g (69%) of a white solid: mp 60–62 °C; ^{1}H NMR δ (DMSO-d_6) 7.1–7.6 (m, 9H, ArH), 4.28 (t, 2H, $ArOCH_2$), 3.91 (s, 2H, $ArCH_2$), 3.62 (t, 2H, CH_2Br), MS m/z 291 (M^+).

2-[2-(4-Benzyl-phenoxy)-ethyl]-isoindole-1, 3-dione (6). 0.5 g of compound **5** (1.7 mmoles) and 1.1 equivalents of potassium phtalimide in 15 mL of dry DMF were mixed at 60 °C for 12 h. The mixture was poured into 100 mL of water and extracted twice with 25 mL of CH_2Cl_2. The organic layer was washed three times with 15 mL of NaOH 0.1N, then three times with 15 mL of brine. The organic layer was dried over $MgSO_4$ before being evaporated, and gave 0.58 g (95%) of a white solid, which was recrystalized in hexane. Mp 80–82 °C; ^{1}H NMR δ ($CDCl_3$) 7.1–8.2 (m, 13H, ArH), 3.86 (s, 2H, $ArCH_2$), 4.22 (t, J = 4.6 Hz, 2H, CH_2N), 4.05 (t, J = 4.6 Hz, 2H, $ArOCH_2$); MS m/z 357 (M^+).

2-(4-Benzyl-phenoxy)-ethylamine hydrochloride (7). A solution of 0.5 g of compound **6** (1.4 mmoles) and 5 equivalents of hydrazine hydrate in 10 mL of ethanol was heated to reflux for 3.5 h and then cooled. Removal of the solvent gave a yellow oil which was extracted with diethylether, washed 3 times with 10 mL of NaOH 0.1N, then three times with 10 mL of brine and finally was extracted with 5 mL of HCl 12N. The extract was evaporated and the recrystalized in isopropanol to give 0.3 g of white crystals as the hydrochloride salt: yield 81%; mp 226–228 °C; ^{1}H NMR δ (DMSO-d_6) 7.1–7.6 (m, 9H, ArH), 3.87 (s, 2H, $ArCH_2$), 3.82 (t, 2H, $ArOCH_2$), 2.85 (t, 2H, CH_2N), 2.15 (s, 2H, NH_2, exchangeable with D_2O); MS m/z 228 ($M+1$).

***N*-[2-(4-Benzyl-phenoxy)-ethyl]-guanidine (8).** A solution of 0.3 g (1.13 mmoles) of compound **7** and 3.5-dimethylpyrazole-1-carboxamidine nitrate in 5 mL of ethanol was heated to reflux for 4 h and then cooled. Removal of the solvent in vacuo and recrystalization of the residue from hexane gave the product **8** (350 mg, 93%). Mp 102–104 °C; ^{1}H NMR δ (DMSO-d_6) 7.1–7.6 (m, 9H, ArH), 3.86 (s, 2H, $ArCH_2$), 4.05 (t, J = 4.6 Hz, 2H, CH_2N), 3.12 (t, J = 4.6 Hz, 2H, $ArOCH_2$); MS m/z 270 ($M+1$).

[2-(4-Benzyl-phenoxy)-ethyl]-trimethyl-ammonium (9). 0.5 g of compound **2** was added in its free base state to an excess of methyl iodide in 10 mL of diethylether. The mixture was maintained under constant stirring overnight at room temperature. A precipitate appeared which was collected by filtration and washed extensively with diethylether. The white precipitate was recrystalized in isopropyl alcohol to give 0.55 g (82%) of compound **9**. Mp 138–140; ^{1}H NMR δ (DMSO-d_6) 7.1–8.2 (m, 9H, ArH), 3.92 (s, 2H, $ArCH_2$), 3.35 (t, J = 4.6 Hz, 2H, CH_2N), 4.05 (t, J = 4.6 Hz, 2H, $ArOCH_2$), 3.2 (s, 9H, NCH_3); MS m/z 358 (M^+).

1-[2-(4-Benzyl-phenoxy)-ethyl]-1*H*-pyrrole (10). A solution 0.5 g of compound **5** (1.7 mmoles) and 1.1 equivalents of pyrrole in 15 mL of dry DMF were mixed at 60 °C for 12 h. The mixture was poured into 100 mL of water and extracted twice with 25 mL of CH_2Cl_2. The organic layer was washed three times with 15 mL of NaOH 0.1N, then three times with 15 mL of brine. The organic layer was dried over $MgSO_4$ before being evaporated and gave 0.58 g (95%) of a yellow liquid. ^{1}H NMR δ (DMSO-d_6) 6.6–7.6 (m, 11H, ArH + pyrrol α-H), 6.2 (m, 2H pyrrol ß-H), 3.91 (s, 2H, $ArCH_2$), 4.06 (t, J = 5.8 Hz, 2H, $ArOCH_2$), 4.18 (t, J = 5.8 Hz, 2H, CH_2N); MS m/z 278 (M^+).

Compounds **11**–**21b** were prepared according to the method described above for compound **1**.

1-[2-(4-Benzyl-phenoxy)-ethyl]-pyrrolidine hydrochloride (11). Yield 72%; mp 170–172 °C; ^{1}H NMR δ (DMSO-d_6) 7.1–7.6 (m, 9H, ArH), 3.85 (s, 2H, $ArCH_2$), 4.05 (t, J = 5.8 Hz, 2H, $ArOCH_2$), 2.68 (t, J = 5.8 Hz, 2H, CH_2N), 2.52 (m, 4H, ring NCH_2), 1.7 (m, 4H ring CH_2 non-adjacent to N). MS m/z 282 (M + 1).

1-[2-(4-Benzyl-phenoxy)-ethyl]-piperidine hydrochloride (12). Yield 75%; mp 180–182 °C; ^{1}H NMR δ (DMSO-d_6) 7.1–7.6 (m, 9H, ArH), 3.96 (s, 2H, $ArCH_2$), 4.5 (t, J = 5.8 Hz, 2H, $ArOCH_2$), 2.51 (t, J = 5.8 Hz, 2H, CH_2N), 2.25 (t, 4H, CH_2 ring adjacent to N), 1.73 (m, 6H ring non adjacent to N); MS m/z 296 (M + 1).

4-[2-(4-Benzyl-phenoxy)-ethyl]-morpholine hydrochloride (13). Yield 78%; mp 185–186 °C; ^{1}H NMR δ (DMSO-d_6) 7.1–7.6 (m, 9H, ArH), 3.96 (s, 2H, $ArCH_2$), 4.2 (t, J = 5.8 Hz, 2H, $ArOCH_2$), 2.69 (t, J = 5.8 Hz, 2H, CH_2N), 3.95 (t, 4H, CH_2 ring adjacent to O), 2.25 (t, 4H, CH_2 ring adjacent to N); MS m/z 298 (M + 1).

4-(2-Phenoxy-ethyl)-morpholine hydrochloride (14). Yield 75%; mp 189–190 °C; ^{1}H NMR δ (DMSO-d_6) 6.8–7.2 (m, 5H, ArH), 4.01 (t, J = 5.8 Hz, 2H, $ArOCH_2$), 2,82 (t, J = 5.8 Hz, 2H, CH_2N), 3.65 (t, 4H, CH_2 ring adjacent to O), 2.30 (t, 4H, CH_2 ring adjacent to N); MS m/z 208 (M + 1).

4-[2-(4-Chloro-phenoxy)-ethyl]-morpholine hydrochloride (15). Yield 83%; mp 222–224 °C; ^{1}H NMR δ (DMSO-d_6) 6.8–7.2 (m, 4H, ArH), 4.01 (t, J = 5.8 Hz, 2H, $ArOCH_2$), 2,82 (t, J = 5.8 Hz, 2H, CH_2N), 3.65 (t, 4H, CH_2 ring adjacent to O), 2.30 (t, 4H, CH_2 ring adjacent to N). MS m/z 242 (M + 1).

4-[2-(4-Bromo-phenoxy)-ethyl]-morpholine hydrochloride (16). Yield 82%; mp 180–182 °C; NMR δ (DMSO-d_6) 6.8–7.3 (m, 4H, ArH), 4.01 (t, J = 5.8 Hz, 2H, $ArOCH_2$), 2,82 (t, J = 5.8 Hz, 2H, CH_2N), 3.65 (t, 4H, CH_2 ring adjacent to O), 2.30 (t, 4H, CH_2 ring adjacent to N); MS m/z 287 (M + 1).

4-[2-(4-Iodo-phenoxy)-ethyl]-morpholine hydrochloride (17). Yield 82%; mp 178–180 °C; ^{1}H NMR δ (DMSO-d_6) 6.6–7.5 (m, 4H, ArH), 4.01 (t, J = 5.8 Hz, 2H, $ArOCH_2$), 2,82 (t, J = 5.8 Hz, 2H, CH_2N), 3.65 (t, 4H, CH_2 ring adjacent to O), 2.30 (t, 4H, CH_2 ring adjacent to N); MS m/z 334 (M + 1).

4-(2-*p*-Tolyloxy-ethyl)-morpholine hydrochloride (18). Yield 80%; mp 208–210 °C; ^{1}H NMR δ (DMSO-d_6) 6.7–6.9 (m, 4H, ArH), 4.01 (t, J = 5.8 Hz, 2H, $ArOCH_2$), 2,82 (t, J = 5.8 Hz, 2H, CH_2N), 3.65 (t, 4H, CH_2 ring adjacent to O), 2.35 (s, 3H, $ArCH_3$), 2.30 (t, 4H, CH_2 ring adjacent to N); MS m/z 223 (M + 1).

4-[2-(4-*tert*-Butyl-phenoxy)-ethyl]-morpholine hydrochloride (19). Yield 78%; mp 190–192 °C; ^{1}H NMR δ (DMSO-d_6) 6.7–7.1 (m, 4H, ArH), 4.01 (t, J = 5.8 Hz, 2H, $ArOCH_2$), 2,82 (t, J = 5.8 Hz, 2H, CH_2N), 3.65 (t, 4H, CH_2 ring adjacent to O), 1.32 (s, 9H, CCH_3), 2.30 (t, 4H, CH_2 ring adjacent to N); MS m/z 264 (M + 1).

4-{2-[4-(1-Methyl-1-phenyl-ethyl)-phenoxy]-ethyl}-morpholine hydrochloride (20a). Yield 83%; mp 188–190 °C; ^{1}H NMR δ (DMSO-d_6) 6.89–7.28 (m, 9H, ArH), 4.39–4.44 (t, J = 5.8 Hz, 2H, $ArOCH_2$), 3.22 (t, J = 5.8 Hz, 2H, CH_2N), 3.85–3.91 (t, 4H, CH_2 ring adjacent to O), 3.43–3.51 (t, 4H, CH_2 ring adjacent to N), 1.63 (s, 6H, CCH_3); MS m/z 326 (M + 1).

1-{2-[4-(1-Methyl-1-phenyl-ethyl)-phenoxy]-ethyl}-pyrrolidine hydrochloride (20b). Yield 85%; mp 200–202 °C; ^{1}H NMR δ (DMSO-d_6) 6.89–7.31 (m, 9H, ArH), 4.29–4.34 (t, J = 5.8 Hz, 2H, $ArOCH_2$), 3.08–3.11 (t, J = 5.8 Hz, 2H, CH_2N), 3.53–3.57 (m, 4H, ring NCH_2), 1.89–1.97 (t, 4H, CH_2 ring non adjacent to N), 1.62 (s, 6H, CCH_3); MS m/z 309 (M + 1).

[4-(2-Morpholin-4-yl-ethoxy)-phenyl]-phenyl-methanone hydrochloride (21a). Yield 70%; mp 118–120 °C; ^{1}H NMR δ (DMSO-d_6) 6.9–7.71 (m, 9H, ArH), 4.02 (t, J = 5.8 Hz, 2H, $ArOCH_2$), 2,80 (t, J = 5.8 Hz, 2H, CH_2N), 3.67 (t, 4H, CH_2 ring adjacent to O), 2.38 (t, 4H, CH_2 ring adjacent to N); MS m/z 312 (M + 1).

Phenyl-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-methanone hydrochloride (21b). Yield 75%; mp 128–130 °C; ^{1}H NMR δ (DMSO-d_6) 6.9–7.71 (m, 9H, ArH), 4.05 (t, J = 5.8 Hz, 2H, $ArOCH_2$), 2,68 (t, J = 5.8 Hz, 2H, CH_2N), 2.28 (m, 4H, ring NCH_2), 1.6 (t, 4H, CH_2 ring non adjacent to N); MS m/z 296 (M + 1).

[4-(2-Morpholin-4-yl-ethoxy)-phenyl]-phenyl-carbinol hydrochloride (22a). To a mixture of 100 mg of compound **21a** (0.32 mmol) in 2.5 mL trifluoroacetic acid and 2.5 mL of CH_2Cl_2 was added 0.33 mmol of $NaBH_4$ at 0 °C The mixture was stirred at room temperature during 30 min until the starting material disappeared. The residue was hydrolyzed on ice, and the acid was neutralized with a solution of $NaHCO_3$. The aqueous phase was then extracted with 10 mL diethylether and the extract was washed 3 times with 5 mL of brine. The organic layer was dried over $MgSO_4$ and mixed with an etheral solution of 1N HCl, which produced the precipitation of compound **22a**. Yield 48%; mp 84–86 °C; ^{1}H NMR δ (DMSO-d_6) 7.1–7.6 (m, 9H, ArH), 5.8 (s, 1H, C*H*OH), 4.02 (t, J = 5.8 Hz, 2H, $ArOCH_2$), 2,80 (t, J = 5.8 Hz, 2H, CH_2N), 3.67 (t, 4H, CH_2 ring adjacent

to O), 2.38 (t, 4H, CH_2 ring adjacent to N); MS *m/z* 314 (M + 1).

[4-(2-Pyrrolidin-4-yl-ethoxy)-phenyl]-phenyl-methanol hydrochloride (22b). This compound was prepared according to the method described above for compound **22a**. Yield 52%; mp 106–108 °C; ^{1}H NMR δ (DMSO-d_6) 7.1–7.6 (m, 9H, ArH), 5.8 (s, 1H, C*H*OH), 4.05 (t, *J* = 5.8 Hz, 2H, $ArOCH_2$), 2,68 (t, *J* = 5.8 Hz, 2H, CH_2N), 2.28 (m, 4H, ring NCH_2), 1.6 (t, 4H, CH_2 ring non-adjacent to N); MS *m/z* 298 (M + 1).

Compounds **23a–25** were prepared according to the method described above for compound **1**.

1-[2-(4-Phenoxy-phenoxy)-ethyl]-morpholine hydrochloride (23a). Yield 82%; mp 170–172 °C; ^{1}H NMR δ (DMSO-d_6) 6.75–7.22 (m, 9H, ArH), 4.02 (t, *J* = 5.8 Hz, 2H, $ArOCH_2$), 2,80 (t, *J* = 5.8 Hz, 2H, CH_2N), 3.67 (t, 4H, CH_2 ring adjacent to O), 2.38(t, 4H, CH_2 ring adjacent to N); MS *m/z* 300 (M + 1).

1-[2-(4-Phenoxy-phenoxy)-ethyl]-pyrrolidine hydrochloride (23b). Yield 78%; mp 166–168 °C; ^{1}H NMR δ (DMSO-d_6) 6.75–7.22 (m, 9H, ArH), 4.05 (t, *J* = 5.8 Hz, 2H, $ArOCH_2$), 2,68 (t, *J* = 5.8 Hz, 2H, CH_2N), 2.28 (m, 4H, ring NCH_2), 1.6 (t, 4H, CH_2 ring non adjacent to N); MS *m/z* 284 (M + 1).

4-[2-(4-Styryl-phenoxy)-ethyl]-morpholine hydrochloride (24). Yield 83%; mp 216–218 °C; ^{1}H NMR δ (DMSO-d_6) 6.75–7.45 (m, 11H, ArH), 4.02 (t, *J* = 5.8 Hz, 2H, $ArOCH_2$), 2,80 (t, *J* = 5.8 Hz, 2H, CH_2N), 3.67 (t, 4H, CH_2 ring adjacent to O), 2.38 (t, 4H, CH_2 ring adjacent to N); MS *m/z* 310 (M + 1).

1-[4-(2-Morpholin-4-yl-ethoxy)-phenyl]-2-phenyl-ethanone hydrochloride (25). Yield 82%; mp 146–148 °C; ^{1}H NMR δ (DMSO-d_6) 6.89–7.77 (m, 9H, ArH), 4.02 (t, *J* = 5.8 Hz, 2H, $ArOCH_2$), 3.81 (s, 2H, $ArCH_2$), 2,80 (t, *J* = 5.8 Hz, 2H, CH_2N), 3.67 (t, 4H, CH_2 ring adjacent to O), 2.38 (t, 4H, CH_2 ring adjacent to N); MS *m/z* 326 (M + 1).

1-[4-(2-Morpholin-4-yl-ethoxy)-phenyl]-2-phenyl-ethanol hydrochloride (26). This compound was prepared according to the method described above for compound **22a**. Yield 52%; mp 136–138 °C; ^{1}H NMR δ (DMSO-d_6) 6.70–7.22 (m, 9H, ArH), 4.9 (d, 1H, C*H*OH), 4.02 (t, *J* = 5.8 Hz, 2H, $ArOCH_2$), 3.01 (t, 2H, $ArCH_2$), 2,80 (t, *J* = 5.8 Hz, 2H, CH_2N), 3.67 (t, 4H, CH_2 ring adjacent to O), 2.38 (t, 4H, CH_2 ring adjacent to N); MS *m/z* 328 (M + 1).

***N*-[5-Benzyl-2-(2-morpholin-4-yl-ethoxy)-phenyl]-acetamide hydrochloride (30).** Acetyl chloride (50 μL, 0.64 mmoles) was added to a solution of **28** (100 mg, 0.32 mmoles) in diethylether for 1 h under stirring. The white precipitate was collected and recrystalized in isopropanol: acetone (1:1) to give 240 mg of compound **30**. Yield 96%; mp 212–214 °C; ^{1}H NMR δ (DMSO-d_6) 7.1–7.7 (m, 8H, ArH), 4.02 (t, *J* = 5.8 Hz, 2H, $ArOCH_2$), 3.91 (s, 2H, $ArCH_2$), 2,80 (t, *J* = 5.8 Hz, 2H, CH_2N), 3.67 (t, 4H, CH_2 ring adjacent to O), 2.38 (t, 4H, CH_2 ring adjacent to N), 2.02 (s, 3H, NHCO-CH_3); MS *m/z* 355 (M + 1).

***N*-[5-Benzyl-2-(2-morpholin-4-yl-ethoxy)-phenyl]-succinamic acid (31).** Succinic anhydride (1 mmole) was added to a solution of 28 (200 mg, 0.64 mmole) in 5 mL of dioxane 1–4 for 1 h with stirring at room temperature. The white precipitate was collected, filtered and washed with diethylether to give 250 mg of compound **33**. Yield 95%, mp 150–151 °C; ^{1}H NMR δ (DMSO-d_6) 7.1–7.7 (m, 8H, ArH), 4.02 (t, *J* = 5.8 Hz, 2H, $ArOCH_2$), 3.91 (s, 2H, $ArCH_2$), 2,80 (t, *J* = 5.8 Hz, 2H, CH_2N), 3.67 (t, 4H, CH_2 ring adjacent to O), 2.38 (t, 4H, CH_2 ring adjacent to N), 2.60–2.70 (m, 4H, $NHCOCH_2CH_2COOH$); MS *m/z* 412 (M + 1).

4-[5-Benzyl-2-(2-morpholin-4-yl-ethoxy)-phenylcarbamoyl]-butyric acid (32). The same procedure as for compound **31** was used. Yield 98%; mp 171–172 °C; ^{1}H NMR δ (DMSO-d_6) 7.1–7.7 (m, 8H, ArH), 4.02 (t, *J* = 5.8 Hz, 2H, $ArOCH_2$), 3.91 (s, 2H, $ArCH_2$), 2,80 (t, *J* = 5.8 Hz, 2H, CH_2N), 3.67 (t, 4H, CH_2 ring adjacent to O), 2.38 (t, 4H, CH_2 ring adjacent to N), 1.95–2.22 (m, 6H, $NHCOCH_2CH_2CH_2COOH$); MS *m/z* 426 (M + 1).

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