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Original article

Antileukotrienic *N*-arylethyl-2-arylacetamides in the treatment of ulcerative colitis

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Abstract

A series of arylacetic acid derivatives bearing methyl(arylethyl)amino groups were prepared and their antileukotrienic activities involving LTB_4 were evaluated. Regression analysis has shown a strong dependence of these activities on lipophilicity for both LTB_4 receptor binding and inhibition of LTB_4 biosynthesis; parabolic relationships were derived. The values of slopes of the ascending linear parts of these dependences indicate various types of hydrophobic binding at the site of ligand interaction with relevant biomacromolecules. The anti-inflammatory effect of the compounds under study was also evaluated in three animal models of inflammation and their possible utilization in the treatment of ulcerative colitis (UC) was followed. The importance of antileukotrienic activities for the anti-inflammatory effect, especially in the model of UC was discussed, but further experiments are necessary to confirm the respective relations.

Keywords: *N*-Phenethyl-2-arylacetamides; Antileukotrienic activity; QSAR; Regression analysis; Lipophilicity; Treatment of ulcerative colitis

1. Introduction

Inflammatory bowel diseases (IBD) such as ulcerative colitis (UC) and Crohn's disease (CD) are largely of unknown etiology and are among the most challenging human diseases [1]. There is evidence of an intense local immune response associated with recruitment of lymphocytes and macrophages followed by release of soluble cytokines. Excessive production of leukotrienes (LT) has been implicated in the pathogenesis of various immune and inflammatory diseases including IBD [2]. LTB_4 is a 5-lipoxygenase (5-LO) derived metabolite of arachidonic acid which is synthesized by a number of cell types, including eosinophils, neutrophils (PMNs), and macrophages [3]. It is a potent pro-inflammatory mediator playing a significant role in the amplification of many other inflammatory disease states [4,5] including psoriasis, gout, rheumatoid arthritis and asthma. In addition, LTB_4 is a mediator of inflammatory pain [6] and binds to peroxisome proliferator-activated receptor (PPARα), which could affect the duration of an inflammatory response to LTB_4 [7].

The majority of antileukotrienics share common structural features expressed by the following rules [8]: (i) a hydrophobic region in the proximity of a polar group is in the ''western'' part; (ii) the ''eastern'' part is a carrier of an acid group (carboxyl or equivalent); (iii) a flexible spacer of various lengths connecting both parts. The lipophilic fragments bearing phenethylamino [9–12] or ethyl(methyl)amino [13] groups are frequently used. It was found out that the presence of the ethyl(methyl)amino group has a positive influence on binding activity and oral efficiency in *in vivo* experiments [14]. The present work represents the continuation of our previous contributions [15–17] to the study of substituted arylalkanoic

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acids with antileukotrienic activities. Our effort was focused on the preparation of arylacetic acid derivatives bearing *N*-arylethyl-2-arylacetamido group through *N*-methyl-*N*-arylethylamides of (4-hydroxyphenyl)acetic acid as key intermediates. The series of new compounds **1–9** (Table 1) was generated, characterized by ^{1}H, ^{13}C NMR and elemental analysis and subjected to the evaluation of both *in vitro* antileukotrienic and *in vivo* anti-inflammatory activities including the treatment of ulcerative colitis. We have tried to elucidate the role of lipophilicity in antileukotrienic activities of the compounds studied using QSAR methodology [18,19].

2. Chemistry

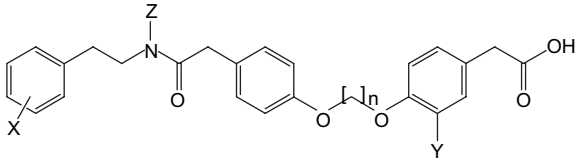
The series of compounds **1–9** was synthesized using the *N*-methyl-*N*-(arylethyl)amino group as lipophilic part of molecule and the arylacetic acid moiety as a carrier of carboxyl group. The structure of compounds was modified by the substitution on aromatic rings of the amine and acid and by the length of a spacer between both structure fragments. For the preparation of compounds **1–8** and **9**, respectively, two synthetic routes described in Schemes 1 and 2 were used. *N*-Arylethylamides of 4-methoxyphenylacetic acid **16–20** were prepared by the condensation of 4-methoxyphenylacetyl chloride with corresponding arylethylamine, and their demethylation afforded the key intermediates **21–25**. Methyl [4-(ω-bromoalkyloxy)aryl]acetates **30** and **31** were prepared as other synthons in the group of compounds **1–8**. The alkylation under the conditions of the Williamson reaction [20] gave esters **32–39**, which were hydrolysed to the corresponding acids **1–8**. The reverse technique of alkylation, where *N*-methyl-*N*-phenethylamide of [4-(4-bromoalkyloxy)phenyl] acetic acid **26** was used as an alkylating agent, was utilized for the preparation of acids **9**. From these intermediates, the esters **40a** and **40b** were prepared in higher yields and purity and in shorter reaction time. *N*-Methyl-*N*-phenethylamide of **13–15** was prepared from the corresponding 2-phenethylamines by formylation and subsequent reduction with $LiAlH_4$ according to Ref. [21]. Methyl esters of (3-chloro-4-hydroxyphenyl)-acetic acid (**27**), (4-hydroxyphenyl)acetic acid (**28**) and (4-hydroxy-3-methoxyphenyl)acetic acid (**29**) were prepared by the esterification of corresponding acids according to literature [22].

3. Results and discussion

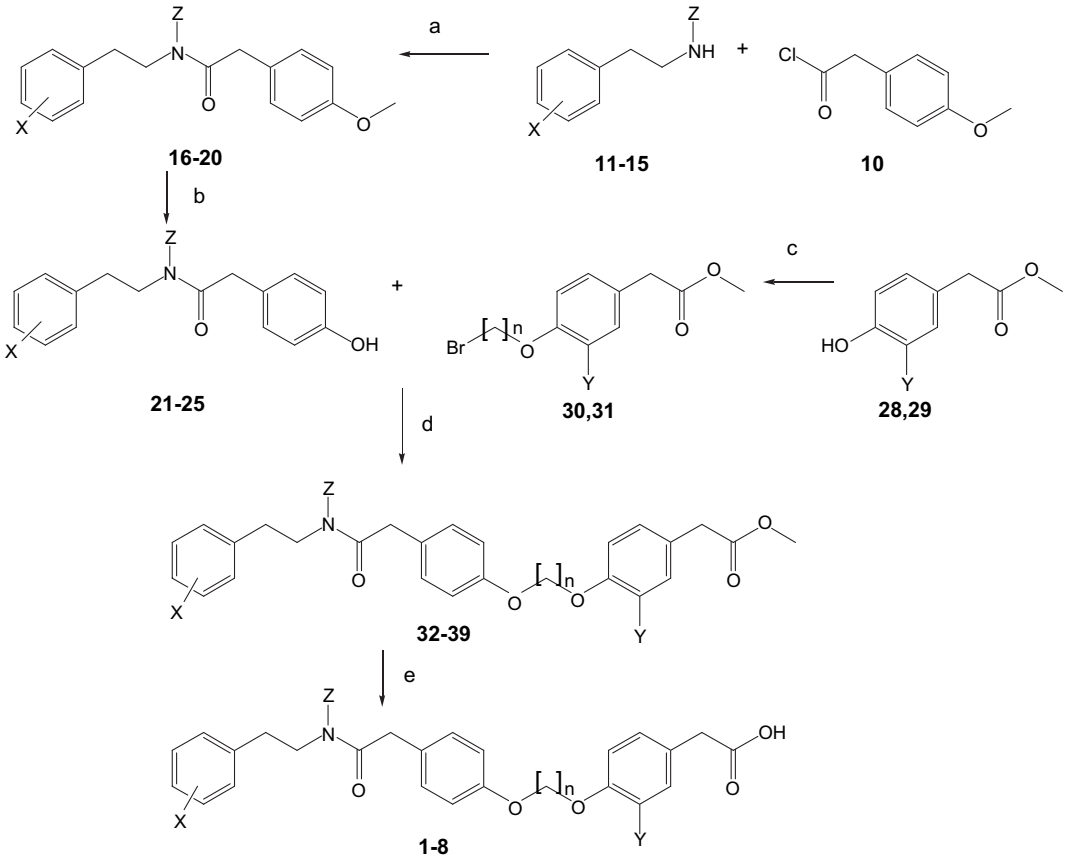
3.1. Biological activities

Compounds **1–9** were subjected to evaluation of antileukotrienic activity by testing *in vitro* the inhibition of LTB_4 biosynthesis as a criterion of 5-LO inhibition, and affinity to LTB_4 receptors, which is a prerequisite for antagonistic activity toward this leukotriene. Both antileukotrienic activities were expressed by the concentration IC_{50} causing 50% inhibition of LTB_4 biosynthesis or LTB_4 binding to receptors, respectively. The phenethylamino derivatives **1b** and **2b** are inactive in the inhibition of LTB_4 biosynthesis; the presence of tertiary nitrogen probably stimulates this antileukotrienic activity of the compounds under study. The compounds were also tested for their anti-inflammatory efficiency in two *in vivo* models of inflammation, i.e. carrageenan induced oedema in rats and arachidonic-acid-induced ear inflammation in mice. The effects were expressed as percentage inhibition in comparison to untreated control. Selected compounds were subjected to evaluation of anti-inflammatory activity in the *in vivo* model (mice) of ulcerative colitis (UC) induced by sodium dextrane sulfate. Sulfasalazine was used as the

Table 1
Characterization of compounds **1b–9b**



Acid	X	Y	Z	*n*
1b	H	H	H	4
2b	H	OCH_3	H	4
3a	H	H	CH_3	3
3b	H	H	CH_3	4
4a	H	OCH_3	CH_3	3
4b	H	OCH_3	CH_3	4
5b	3-CH_3	H	CH_3	4
6b	4-CH_3	H	CH_3	4
7b	4-Br	H	CH_3	4
8a	4-Br	OCH_3	CH_3	3
8b	4-Br	OCH_3	CH_3	4
9a	H	Cl	CH_3	3
9b	H	Cl	CH_3	4



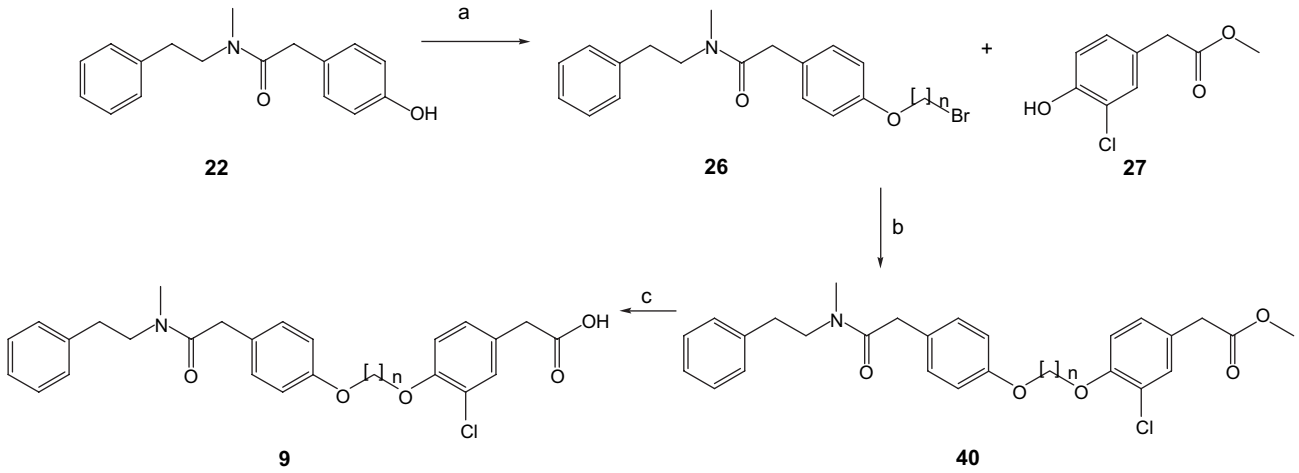
Scheme 1. Synthesis of compounds **1b**–**8b**. a. $(C_2H_5)_3N$, CH_2Cl_2, 20 °C; b. BBr_3, CH_2Cl_2, −72 to 20 °C; c. K_2CO_3, butan-2-one, 1,3-dibromopropane (1,4-dibromobutane), reflux; d. K_2CO_3, KI, butan-2-one, reflux; e. tetrahydrofuran–water 4:1, LiOH, 20 °C.

standard. UC was evaluated clinically (loss of weight, rectal prolaps, blood in stool, stool consistency), pathologically (colonic bleeding, length of colon), and histologically. The results of evaluation of antileukotrienic and anti-inflammatory efficiencies are summarized in Table 2; the values documenting the effect in the model of ulcerative colitis are shown in Table 3.

3.2. QSAR analysis

3.2.1. Parametrization of lipophilicity

To evaluate total lipophilicity of the compounds studied, values of log *P* (calculated in program KOWWIN, Version 1.63) were used. Lipophilicity was also evaluated using experimental values determined by reverse phase HPLC and



Scheme 2. Synthesis of compounds **9**: a. 18-crown-6, K_2CO_3, butan-2-one, 1,3-dibromopropane (1,4-dibromobutane), reflux; b. K_2CO_3, butan-2-one, reflux; c. tetrahydrofuran–water 4:1, LiOH, 20 °C.

Table 2
Antileukotrienic and anti-inflammatory activities of derivatives of phenethylcarbamoylmethyl–phenoxyalkyloxyphenylacetic acids **1–9**

Compound	Log *P*	Log *k*	LTB_4 biosynthesis		LTB_4 receptor binding		Carrageenan oedema % of inhibition	Ear lobe affection % of inhibition	
			IC_{50} (μM)	$Log(1/IC_{50})$	IC_{50} (μM)	$Log(1/IC_{50})$		Oedema	Hyperaemia
1b	5.44	0.089	>100[a]	–	n.d.	–	n.s.	23	0
2b	5.01	−0.079	>100[a]	–	n.d.	–	n.s.	23	7.7 (s)[b]
3a	5.16	0.166	40	4.398	18	4.745	39	20	n.s.
3b	5.65	0.287	20	4.699	4.7	5.328	30	45	n.s.
4a	4.73	0.013	>50[a]	–	67	4.174	n.s.	14	n.s.
4b	5.22	0.120	32	4.495	8.6	5.065	0	21	n.s.
5b	6.20	0.470	10	5.000	1.1	5.959	14	44	n.s.
6b	6.20	0.471	11	4.959	0.66	6.180	n.s.	20	24
7b	6.54	0.468	15	4.825	1.7	5.770	39	35	0
8a	5.62	0.193	n.d.	–	n.d.	–	3!	38	n.s.
8b	6.11	0.354	9.5	5.022	1.9	5.721	27	38	n.s.
9a	5.81	0.253	16	4.796	2.4	5.620	12	20	n.s.
9b	6.30	0.364	11	4.959	1.2	5.921	32	23	n.s.
Zileuton	n.d.	n.d.	0.01[a]	–	>100[a]	–	46	n.s.	13

n.d. = not determined; n.s. = not significant (the level of statistical significance $p > 0.05$).
[a] Not included in correlation.
[b] Stimulation.

expressed by values of log *k*, where *k* is capacity factor (cf. Section 4). Relations between the values of log *P* representing calculated values of logarithm of the partition coefficient in the system of *n*-octanol–water and experimental values of log *k* are expressed by the regression Eq. (1), where *n* is the number of compounds in correlation, *r*, *s* and *F* are the statistical criteria (cf. Section 4). Regression Eq. (1) did not show any systematic deviations from linearity. It can be stated that the intramolecular hydrophobic interactions of aromatic rings are not exerted in the chromatographic separation. The introduction of polar constants σ_1 of substituents on the phenethyl group and the indicator variable I_{NH} characterizing the presence of methyl on the *N*-atom of amide group to the Eq. (2) improves the relationship between both lipophilic parameters substantially. The reasons of frequent imperfect correlations between log *P* values and logarithm of retention factor log *k* have been extensively discussed [23–26].

$$\log k = 0.299(\pm 0.109)\log P + 1.457(\pm 0.625)$$
$$n = 13,\ r = 0.925,\ s = 0.068,\ F = 72.0,\ p = 0.01 \quad (1)$$

$$\log k = 0.273(\pm 0.075)\log P + 0.141(\pm 0.099)I_{NH} - 0.230(\pm 0.226)\sigma_1 - 1.422(\pm 0.404)$$
$$n = 13,\ r = 0.973,\ s = 0.041,\ F = 73.2,\ p = 0.01 \quad (2)$$

3.2.2. Regression analysis of antileukotrienic activities

Eqs. (3) and (4) were derived for inhibition of biosynthesis of LTB_4. The optimum value of lipophilicity, $\log P_{opt} = 6.24$ can be calculated from the parabolic dependence of Eq. (4). Similar dependences for lipophilicity, (Eqs. (5) and (6)), were obtained for LTB_4 receptor binding. The optimum value of lipophilicity calculated from Eq. (6), $\log P_{opt} = 6.57$, lies very close to the previous optimum. The inactive phenethylamino derivatives

Table 3
Activities of derivatives of phenethylcarbamoylmethyl–phenoxyalkyloxyphenylacetic acids **2–9** in model ulcerative colitis induced by dextran sodium sulfate (DSS)

Compound	Stool consistence[a]	Rectal prolaps[a]	Intestine bleeding[a]		Weight loss[b,c] (%)	Colon length[c] (cm)	Histology[c]
			Small	Large			
2b	2/5	0/5	0/5	4/5	80.0 ± 5.7	6.6 ± 0.3	1.6 ± 0.6
3b	3/5	0/5	0/5	2/5	86.8 ± 6.5	8.1 ± 0.8	1.0 ± 0.2
5b	4/5	0/5	0/5	3/5	91.8 ± 14.7	8.6 ± 0.4	1.2 ± 0.6
6b	2/5	0/5	0/5	3/5	78.1 ± 6.2	7.4 ± 0.9	1.1 ± 0.5
7b	1/5	0/5	0/5	1/5	96.1 ± 5.7	9.6 ± 1.1	0.8 ± 0.4
8a	2/5	0/5	0/5	3/5	91.4 ± 10.8	8.4 ± 0.5	0.9 ± 0.2
8b	0/5	0/5	0/5	0/5	91.8 ± 4.6	9.1 ± 0.5	0.6 ± 0.2
9b	5/5	0/5	0/5	5/5	74.2 ± 5.3	6.8 ± 1.0	1.9 ± 0.4
Sulfasalazine	4/10	0/10	0/10	7/10	87.2 ± 7.9	7.9 ± 0.3	1.0 ± 0.4
Control[d]	15/15	0/15	6/15	15/15	89.6 ± 11.0	8.4 ± 0.8	0.9 ± 0.2

[a] The ratio of diseased and total number of animals.
[b] Percent indicates body weight change at day 6 compared with day 1.
[c] The data are expressed as means ± SD at the level of significance $p < 0.05$ (unpaired two-tailed Student's *t*-test).
[d] DSS in drinking water, 0.5% aqueous solution of (carboxymethyl)cellulose.

1b and **2b** were not included in the regression analysis. The quantity IC_{50} represents concentration causing 50% inhibition of biosynthesis of LTB_4 in Eqs. (3) and (4) and concentration causing 50% inhibition of LTB_4 binding to receptors in Eqs. (5) and (6), respectively.

$$\log(1/IC_{50}) = 0.411(\pm 0.287)\log P + 2.369(\pm 1.703)$$
$$n = 9,\ r = 0.869,\ s = 0.112,\ F = 25.1,\ p = 0.01 \quad (3)$$

$$\log(1/IC_{50}) = 5.936(\pm 5.627)\log P - 0.476(\pm 0.471) \times (\log P)^2 - 13.549$$
$$n = 9,\ r = 0.954,\ s = 0.068,\ F = 41.1,\ p = 0.01 \quad (4)$$

$$\log(1/IC_{50}) = 0.993(\pm 0.429)\log P - 0.301$$
$$n = 10,\ r = 0.932,\ s = 0.226,\ F = 60.4,\ p = 0.01 \quad (5)$$

$$\log(1/IC_{50}) = 7.044(\pm 5.279)\log P - 0.536(\pm 0.467) \times (\log P)^2 - 17.199(\pm 14.785)$$
$$n = 10,\ r = 0.963,\ s = 0.169,\ F = 57.9,\ p = 0.05 \quad (6)$$

A comparison of quadratic relationships (4) and (6) indicates different character in the ascending part of parabola. We have evaluated the ascending linear parts of both the relationships for lipophilicity. Eq. (7) was obtained for the inhibition of biosynthesis of LTB_4 and Eq. (8) for the binding to the LTB_4 receptor. The slopes of these straight lines indicate that the different sites of action – as can be expected – operate in both models. The Gibbs energy of intermolecular hydrophobic interaction in both sites is obviously different.

$$\log(1/IC_{50}) = 0.604(\pm 0.064)\log P + 1.282(\pm 1.012)$$
$$n = 5,\ r = 0.991,\ s = 0.025,\ F = 213.2,\ p = 0.01 \quad (7)$$

$$\log(1/IC_{50}) = 1.184(\pm 0.370)\log P + 1.321(\pm 2.093)$$
$$n = 8,\ r = 0.976,\ s = 0.145,\ F = 140.8,\ p = 0.01 \quad (8)$$

The use of experimental values of $\log k$ afforded Eqs. (9) and (10) with lower total statistical significance in comparison with corresponding Eqs. (4) and (6). The quadratic members in Eqs. (9) and (10) have a markedly lower level of statistical significance ($p = 0.2$). This discrepancy can be probably explained by the fact that $\log k$ values do not fully characterize lipophilicity of compounds under study (cf. Eqs. (1) and (2)).

$$\log(1/IC_{50}) = 4.491(\pm 4.490)\log k - 4.912(\pm 4.411) \times (\log k)^2 + 3.930(\pm 0.960)$$
$$n = 9,\ r = 0.865,\ s = 0.113,\ F = 12.8 \quad (9)$$

$$\log(1/IC_{50}) = 6.467(\pm 6.398)\log k - 5.336(\pm 4.931) \times (\log k)^2 - 4.129(\pm 0.760)$$
$$n = 10,\ r = 0.936,\ s = 0.220,\ F = 32.7 \quad (10)$$

3.3. Conclusions

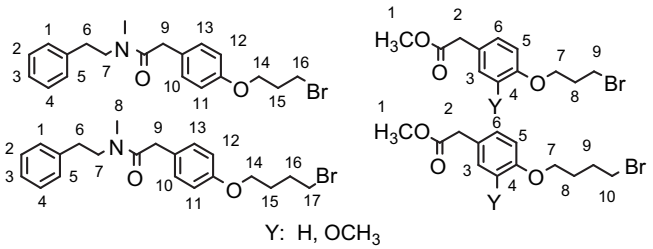
The relationships between the individual activities, especially when they are evaluated in various models *in vitro* and *in vivo*, can be remarkably affected by different transport through the biological system. In spite of that, at least in the case of the anti-inflammatory effect evaluated *in vivo*, it can be stated that the compounds active in both models of inflammatory oedema are also effective in the treatment of ulcerative colitis. This statement is valid for compounds **7b**, **8b** and **3b**. Unfortunately, compound **6b**, belonging also to the derivatives active in ulcerative colitis, does not correspond with this conclusion. With respect to close values of $\log P$, the different transport of these compounds to the site of action is obviously no reasonable explanation of the observed discrepancy. Some compounds prepared are active at 10^{-5} to 10^{-7} level of molar inhibitory concentration (IC_{50}) in both antileukotrienic activities. Three compounds, **6b**, **7b** and **8b**, significantly active in the inhibition of UC, are simultaneously distinguished by high antileukotrienic activities in both tests. Also in this case, exceptions from this rule exist, and two compounds, **5b** and **9b**, are not active in UC inhibition in spite of high antileukotrienic activities. The additional studies of relationships between the anti-inflammatory and antileukotrienic activities are in the preparation. Nevertheless, in the present relatively small group of compounds, five compounds manifest a higher (**8b**, **7b**) or an equal (**3b**, **6b**, **8a**) UC inhibitory activity in comparison with the standard sulfasalazine.

4. Experimental

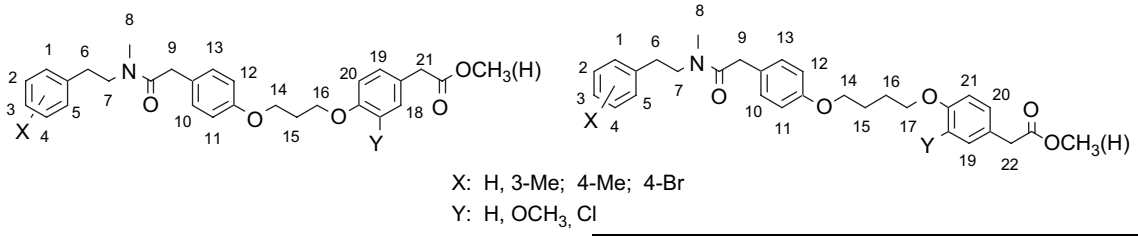
4.1. Chemistry

General: Melting points were determined on a Boeticus-type Kofler block and are not corrected. The ^1H NMR spectra of 6% solutions of the compounds in $CDCl_3$ (or in DMSO-d_6) containing TMS or 3-(trimethylsilyl)propanoic acid-d_4 as the internal standard and ^{13}C NMR of the compounds in DMSO-d_6 were measured on a Bruker-250-DXP (250 MHz). Chemical shifts are given in the δ-scale (ppm), coupling constants J in Hz. The following numbering was used for whole molecule of:

Bromoalkoxy derivatives **26**, **30**, **31**:



Substituted phenylacetic acids **1**–**9** and their methyl esters **32**–**40**:



The purity of compounds **1**–**9**, **16**–**26**, and **30**–**40** was evaluated by HPLC on an Alliance Waters 2695 liquid chromatograph (Waters Assoc., Milford, MA, USA) with UV detection (Waters 2487 dual detector) at 225 nm range. Cromasil C18 100A (300 mm × 4.6 mm) was obtained from Chromservis (Czech Republic). Gradient chromatography was performed with water (Q plus, Millipore, Germany) and acetonitrile (Merck, Darmstadt) with 0.1% of phosphoric acid (Merck, Darmstadt) as a mobile phase. The eluent flow-rate was 1 ml/min. Purity of compounds **1**–**9** was higher than 98.0%, purity of compounds **16**–**26** and **30**–**40** was higher than 95.0%.

*4.1.1. Synthesis of N-arylethylamides of (4-methoxyphenyl)-acetic acid **16**–**20***

To a mixture of appropriate arylethylamine (**11**–**15**) (0.164 mol) and triethylamine (24 ml) in dichloromethane (120 ml) was added 4-methoxyphenylacetyl chloride (**10**) (0.182 mol) in dichloromethane (110 ml) in 25 min. Mixture was stirred at ambient temperature in water bath and controlled by TLC, using *n*-hexane–ethyl acetate (3:2) as the eluent. 600 ml of dichloromethane was added when the absence of the starting compound was observed and the solution was washed with water (300 ml), 10% HCl (20 ml) and brine (300 ml). The organic layer was then dried with sodium sulfate and evaporated. These intermediates were used for subsequent *O*-demethylation without further purification. For microanalysis purpose, the solid form was recrystallized from EtOH or was purified by column chromatography.

*4.1.2. 2-(4-Methoxyphenyl)-N-phenethylacetamide **16***

Yellowish crystals (23.3 g, 53%); m.p. 92–95 °C (EtOH). Anal Calcd. for $C_{17}H_{19}NO_2$ (269.3): C, 75.81; H, 7.11; N, 5.20; Found: C, 75.71; H, 7.13; N, 5.17%. ^{1}H NMR ($CDCl_3$) δ: 2.72 (t, $J = 6.9$ Hz, 2H), 3.46 (m, 4H), 3.80 (s, 3H, OCH_3), 5.38 (bs, 1H, NH), 6.83 (m, 2H), 7.05 (m, 4H), 7.22 (m, 3H).

*4.1.3. 2-(4-Methoxyphenyl)-N-methyl-N-phenethylacetamide **17***

Yellowish oil (36.5 g, 83%). Anal Calcd. for $C_{18}H_{21}NO_2$ (283.4): C, 76.29; H, 7.47; N, 4.94; Found: C, 76.14; H, 7.50; N, 4.91%. ^{1}H NMR ($CDCl_3$) δ: 2.69 (t, $J = 7.3$ Hz, 1H), 2.82 (s, 1.5H, NCH_3), 2.83 (t, $J = 7.3$ Hz, 1H), 2.95 (s, 1.5H, NCH_3), 3.37 (s, 1H), 3.46 (t, $J = 7.3$ Hz, 1H), 3.51 (t, $J = 7.3$ Hz, 1H), 3.59 (s, 1H), 3.75 (s, 3 H, OCH_3), 6.81–6.83 (m, 2H), 7.00–7.34 (m, 7H).

*4.1.4. 2-(4-Methoxyphenyl)-N-methyl-N-(3-methylphenethyl) acetamide **18***

Yellowish oil (29.0 g, 66%). Anal Calcd. for $C_{19}H_{23}NO_2$ (297.4): C, 76.73; H, 7.80; N, 4.71; Found: C, 76.59; H, 7.83; N, 4.68%. ^{1}H NMR ($CDCl_3$) δ: 2.30 (s, 3H, CH_3), 2.68 (t, $J = 7.8$ Hz, 1H), 2.78 (t, $J = 7.6$ Hz, 1H), 2.85 (s, 1.5H, NCH_3), 2.96 (s, 1.5H, NCH_3), 3.42 (s, 1H), 3.48 (t, $J = 7.6$ Hz, 1H), 3.58 (t, $J = 7.6$ Hz, 1H), 3.61 (s, 1H), 3.77 (s, 3H, OCH_3), 6.78–6.92 (m, 3H), 6.93–7.25 (m, 5H).

*4.1.5. 2-(4-Methoxyphenyl)-N-methyl-N-(4-methylphenethyl) acetamide **19***

Yellowish oil (33.4 g, 76%). Anal Calcd. for $C_{19}H_{23}NO_2$ (297.4): C, 76.73; H, 7.80; N, 4.71; Found: C, 76.49; H, 7.91; N, 4.70%. ^{1}H NMR ($CDCl_3$) δ: 2.32 (s, 3H, CH_3), 2.68 (t, $J = 7.4$ Hz, 1H), 2.76 (t, $J = 7.3$ Hz, 1H), 2.81 (s, 1.5H, NCH_3), 2.93 (s, 1.5H, NCH_3), 3.39 (s, 1H), 3.42 (t, $J = 7.3$ Hz, 1H), 3.51 (t, $J = 7.3$ Hz, 1H), 3.59 (s, 1H), 3.73 (s, 3H, OCH_3), 6.70–6.72 (m, 2H), 6.92–7.08 (m, 6H).

*4.1.6. N-(4-Bromophenethyl)-2-(4-methoxyphenyl)-N-methylacetamide **20***

Yellowish oil (41.8 g, 95%). Anal Calcd. for $C_{18}H_{20}BrNO_2$ (362.3): C, 59.68; H, 5.56; Br, 22.06; N, 3.87; Found: C, 59.41; H, 5.50; Br, 21.89; N, 3.86%. ^{1}H NMR ($CDCl_3$) δ: 2.68 (t, $J = 7.3$ Hz, 1H), 2.79 (t, $J = 7.4$ Hz, 1H), 2.86 (s, 1.5H, NCH_3), 2.94 (s, 1.5H, NCH_3), 3.40 (s, 1H), 3.53 (t, $J = 7.5$ Hz, 1H), 3.59 (t, $J = 7.4$ Hz, 1H), 3.62 (s, 1H), 3.71 (s, 3H, OCH_3), 6.80–6.82 (m, 2H), 6.86–6.94 (m, 4H), 7.38 (m, 2H).

*4.1.7. Preparation of N-phenethylamides of (4-hydroxyphenyl) acetic acid **21**–**25***

The solution of appropriate amide (**16**–**20**) (0.052 mol) in dichloromethane (150 ml) was cooled to −72 °C and under N_2 atmosphere boron tribromide (0.097 mol) in dichloromethane (140 ml) was added drop wise. The mixture was then spontaneously heated to the ambient temperature and poured in ice (200 g). Water layer was washed by dichloromethane (50 ml) and combined organic layers were washed with 5% potassium carbonate (30 ml) and water (3× 20 ml), dried with magnesium sulfate and evaporated. The crude arylamides formed were recrystallized from appropriate solvents or were purified by column chromatography on silica gel.

*4.1.8. 2-(4-Hydroxyphenyl)-N-phenethylacetamide **21***

White crystals from 80% EtOH (6.5 g, 49%). Anal Calcd. for $C_{16}H_{17}NO_2$ (255.3): C, 75.27; H, 6.71; N, 5.49; Found: C, 75.13; H, 6.75; N, 5.47%. ^{1}H NMR ($CDCl_3$) δ: 2.72 (t, $J = 6.7$ Hz, 2H), 3.46 (m, 4H), 5.56 (bs, 1H), 6.77–6.79 (m, 2H), 6.97–6.99 (m, 2H), 7.00–7.02 (m, 2H), 7.15–7.29 (m, 3H).

*4.1.9. 2-(-4-Hydroxyphenyl)-N-methyl-N-phenethylacetamide **22***

White crystals (9.2 g, 66%); m.p. 138–141 °C (toluene). Anal Calcd. for $C_{17}H_{19}NO_2$ (269.3): C, 75.81; H, 7.11; N, 5.20; Found: C, 75.74; H, 7.13; N, 5.18%. ^{1}H NMR ($CDCl_3$) δ: 2.73 (t, $J = 7.4$ Hz, 1H), 2.81 (t, $J = 7.2$ Hz, 1H), 2.88 (s, 1.5H, NCH_3), 3.00 (s, 1.5H, NCH_3), 3.34 (s, 1H), 3.51 (t, $J = 7.3$ Hz, 1H), 3.57 (s, 1H), 3.59 (t, $J = 7.3$ Hz, 1H), 6.68 (m, 2H), 6.90 (d, $J = 10.5$, 10.5 Hz, 1H), 6.99 (dd, $J = 10.5$, 10.5, 1H), 7.10–7.32 (m, 5H).

*4.1.10. 2-(4-Hydroxyphenyl)-N-methyl-N-(3-methylphenethyl)-acetamide **23***

Yellowish oil (15.6 g, 95%). Anal Calcd. for $C_{18}H_{21}NO_2$ (283.4): C, 76.29; H, 7.47; N, 4.94; Found: C, 76.11; H, 7.48; N, 4.92%. ^{1}H NMR ($CDCl_3$) δ: 2.30 (s, 3H, CH_3), 2.69 (t, $J = 7.6$ Hz, 1H), 2.80 (t, $J = 7.5$ Hz, 1H), 2.85 (s, 1.5H, NCH_3), 2.93 (s, 1.5H, NCH_3), 3.42 (s, 1H), 3.49 (t, $J = 7.5$ Hz, 1H), 3.60 (t, $J = 7.4$ Hz, 1H), 3.61 (s, 1H), 6.69 (m, 2H), 6.90 (m, 3H), 7.09–7.24 (m, 3H).

*4.1.11. 2-(4-Hydroxyphenyl)-N-methyl-N-(4-methylphenethyl)-acetamide **24***

Yellowish oil (11.8 g, 72%). Anal Calcd. for $C_{18}H_{21}NO_2$ (283.4): C, 76.29; H, 7.47; N, 4.94; Found: C, 76.10; H, 7.50; N, 4.91%. ^{1}H NMR ($CDCl_3$) δ: 2.30 (s, 3H, CH_3), 2.70 (t, $J = 7.3$ Hz, 1H), 2.79 (t, $J = 7.4$ Hz, 1H), 2.82 (s, 1.5H, NCH_3), 2.93 (s, 1.5H, NCH_3), 3.38 (s, 1H), 3.43 (t, $J = 7.4$ Hz, 1H), 3.51 (t, $J = 7.3$ Hz, 1H), 3.61 (s, 1H), 6.73 (m, 2H), 6.82 (m, 2H), 6.95–7.14 (m, 4H).

*4.1.12. N-(4-Bromophenethyl)-2-(4-hydroxyphenyl)-N-methylacetamide **25***

Colourless glassy oil (15.6 g, 77%). Anal Calcd. for $C_{17}H_{18}BrNO_2$ (348.2): C, 58.63; H, 5.21; Br, 22.95; N, 4.02; Found: C, 58.42; H, 5.17; Br, 22.78; N, 4.00%. ^{1}H NMR ($CDCl_3$) δ: 2.68 (t, $J = 7.1$ Hz, 1H), 2.78 (t, $J = 7.3$ Hz, 1H), 2.88 (s, 1.5H, NCH_3), 2.97 (s, 1.5H, NCH_3), 3.40 (s, 1H), 3.41 (t, $J = 7.3$ Hz, 1H), 3.49 (t, $J = 7.4$ Hz, 1H), 3.61 (s, 1H), 6.71 (m, 2H), 6.84–7.04 (m, 4H), 7.38 (m, 2H).

*4.1.13. Synthesis of methyl [4-(ω-bromoalkoxy)phenyl] acetates **30a–31b***

To a solution of appropriate methyl 4-hydroxyaryl acetate **28**, **29** (0.1 mol; prepared by esterification [23]) in butan-2-one (300 ml), potassium carbonate (55.0 g) and ω,ω′-dibromoalkane (0.4 mol) were added. The mixture was refluxed and the reaction was stopped after the starting compound disappeared (TLC, chloroform–benzene–acetic acid 60:40:5). The solution was filtered and evaporated. The crude **30a–31b** were purified by crystallization from *n*-hexane.

*4.1.14. Methyl 4-(3-bromopropoxy)phenyl acetate **30a***

White crystals (20.7 g, 72%); m.p. 34–36 °C (*n*-hexane). Anal Calcd. for $C_{12}H_{15}BrO_3$ (287.2): C, 50.19; H, 5.27; Br, 27.83; Found: C, 49.98; H, 5.21; Br, 27.71%. ^{1}H NMR ($CDCl_3$) δ: 2.23 (m, 2H), 3.47 (t, $J = 6.3$ Hz, 2H), 3.52 (s, 2H), 3.63 (s, 3H, OCH_3), 4.02 (t, $J = 5.9$ Hz, 2H), 6.83 (m, 2H), 7.15 (m, 2H).

*4.1.15. Methyl [4-(4-bromobutoxy)phenyl]acetate **30b***

White crystals (21.7 g, 72%); m.p. 28–31 °C (*n*-hexane). Anal Calcd. for $C_{13}H_{17}BrO_3$ (301.2): C, 51.84; H, 5.69; Br, 26.53; Found: C, 51.67; H, 5.61; Br, 26.25%. ^{1}H NMR ($CDCl_3$) δ: 1.80–2.05 (m, 4H), 3.41 (t, $J = 6.4$ Hz, 2H), 3.51 (s, 2H), 3.62 (s, 3H, OCH_3), 3.90 (t, $J = 6.0$ Hz, 2H), 6.80 (m, 2H), 7.14 (m, 2H).

*4.1.16. Methyl [4-(3-bromopropoxy)-3-methoxyphenyl]acetate **31a***

White crystals (22.8 g, 72%), m.p. 35–38 °C (*n*-hexane). Anal Calcd. for $C_{13}H_{17}BrO_4$ (317.2): C, 49.23; H, 5.40; Br, 25.19; Found: C, 49.05; H, 5.36; Br, 24.93%. ^{1}H NMR ($CDCl_3$) δ: 2.19 (m, 2H), 3.46 (t, $J = 6.2$ Hz, 2H), 3.51 (s, 2H), 3.59 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.97 (t, $J = 6.2$ Hz, 2H), 6.75–6.92 (m, 3H).

*4.1.17. Methyl [4-(4-bromobutoxy)-3-methoxyphenyl]acetate **31b***

White crystals (23.5 g, 71%); m.p. 28–31 °C (*n*-hexane). Anal Calcd. for $C_{14}H_{19}BrO_4$ (331.2): C, 50.77; H, 5.78; Br, 24.13; Found: C, 50.59; H, 5.71; N, 23.97%. ^{1}H NMR ($CDCl_3$) δ: 1.87–2.13 (m, 4H), 3.48 (t, $J = 6.5$, 2H), 3.55 (s, 2H), 3.68 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 4.01 (t, $J = 6.2$ Hz, 2H), 6.74–6.85 (m, 3H).

*4.1.18. Synthesis of arylacetic acids **1b–8b***

To a mixture of **21–25** (0.010 mol), potassium carbonate (3.5 g), and potassium iodide (0.12 g) in butan-2-one (40 ml), **30–31** (0.011 mol) were added. The mixture was refluxed for 18 h and then filtered and evaporated. The crude esters **32–39** were purified either by crystallization or by column chromatography on silica gel. Their purity was evaluated by HPLC and structure confirmed by ^{1}H NMR spectra.

To a mixture of esters **32–39** (0.005 mol) in 20 ml of tetrahydrofuran–water 4:1, 1.80 g of lithium hydroxide was added. The mixture was stirred at ambient temperature overnight. After evaporation of tetrahydrofuran, the crude product was dissolved in water (50 ml) and acidified with acetic acid and filtered. The crude acids were purified by crystallization from appropriate solvents.

*4.1.19. 2-[4-(4-{4-[Phenethylcarbamoyl)-methyl]phenoxy}butoxy)phenyl]acetic acid **1b***

Hydrolysis of **32b** [yellowish crystals (3.3 g, 63%); m.p. 124–125 °C (EtOH)] afforded acid **1b** as white precipitate

(2.3 g, 92%); m.p. 159–161 °C ($CHCl_3$). Anal Calcd. for $C_{28}H_{31}NO_5$ (461.6): C, 72.86; H, 6.77; N, 3.03; Found: C, 73.05; H, 6.92; N, 3.29%. ^{1}H NMR (DMSO-d_6) δ: 1.87 (m, 4H), 2.75 (t, $J = 7.1$ Hz, 2H), 3.17 (t, $J = 7.0$ Hz, 2H), 3.27 (m, 2H), 3.34 (s, 2H), 4.03 (m, 4H), 6.80–6.81 (m, 4H), 7.11–7.26 (m, 9H), 7.46 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 26.0, 29.12, 33.23, 35.52, 38.41, 48.01, 50.78, 68.15, 114.78, 114.86, 115.07, 126.16, 126.41, 127.33, 127.81, 127.84, 128.44, 128.77, 130.22, 130.38, 139.89, 156.32, 157.87, 160.84, 170.66.

4.1.20. 3-Methoxy-2-[4-(4-{4-[(phenethylcarbamoyl)methyl]-phenoxy}butoxy)phenyl]acetic acid ***2b***

Hydrolysis of **33b** [yellowish crystals (3.3 g, 66%); m.p. 98–102 °C (4-methylpentan-2-one)] afforded pure acid **2b** as white crystals (2.1 g, 85%); m.p. 148–150 °C (EtOH). Anal Calcd. for $C_{29}H_{33}NO_6$ (491.6): C, 70.86; H, 6.77; N, 2.85; Found: C, 71.05; H, 6.62; N, 2.99%. ^{1}H NMR (DMSO-d_6) δ: 1.86 (m, 4H), 2.72 (t, $J = 7.2$ Hz, 2H), 3.22 (t, $J = 7.3$ Hz, 2H), 3.27 (s, 2H), 3.47 (s, 2H), 3.75 (s, 3H, OCH_3), 4.02–4.03 (m, 4H), 6.80–6.81 (m, 5H), 7.11–7.29 (m, 7H), 7.60 (s, 1H). ^{13}C NMR ($CDCl_3$) δ: 26.13, 26.33, 29.97, 35.66, 41.00, 41.23, 43.05, 56.23, 67.88, 68.96, 113.42, 113.51, 115.30, 121.89, 126.71, 127.00, 128.85, 128.98, 130.81, 138.87, 147.88, 149.73, 158.61, 172.30, 176.75.

4.1.21. 2-[4-(3-{4-[(N-Methyl-N-phenethylcarbamoyl)]methyl]-phenoxy}propoxy)phenyl]acetic acid ***3a***

Hydrolysis of **34a** [yellowish oil (2.4 g, 51%)] afforded pure acid **3a** as white crystals (2.1 g, 92%); m.p. 78–83 °C (aqueous EtOH). Anal Calcd. for $C_{28}H_{31}NO_5$ (461.6): C, 72.86; H, 6.76; N, 3.03; Found: C, 72.60; H, 6.89; N, 2.88%. ^{1}H NMR ($CDCl_3$) δ: 2.21 (m, 2H), 2.72 (t, $J = 7.2$ Hz, 1H), 2.82 (t, $J = 7.3$ Hz, 1H), 2.83 (s, 1.5H, NCH_3), 2.96 (s, 1.5H, NCH_3), 3.39 (s, 1H), 3.49 (t, $J = 7.4$ Hz, 1H), 3.56 (t, $J = 7.4$ Hz, 1H), 3.58 (s, 2H), 3.61 (s, 1H), 4.12–4.13 (m, 4H), 6.83–6.84 (m, 4H), 6.98–7.36 (m, 9H). ^{13}C NMR (DMSO-d_6) δ: 29.14, 33.38, 34.39, 36.08, 49.20, 51.54, 64.65, 114.71, 126.51, 126.76, 127.49, 128.11, 128.39, 128.73, 128.86, 129.12, 129.38, 130.40, 130.45, 130.81, 139.18, 139.63, 157.49, 157.72, 170.71, 173.39.

4.1.22. 2-[4-(4-{4-[(N-Methyl-N-phenethylcarbamoyl)methyl]-phenoxy}butoxy)phenyl]acetic acid ***3b***

Hydrolysis of **34b** [yellowish oil 2.6 g, 54%)] afforded pure **3b** as white crystals (1.3 g, 53%); m.p. 92–95 °C (aqueous EtOH). Anal Calcd. for $C_{29}H_{33}NO_5$ (475.6): C, 73.24; H, 7.00; N, 2.94; Found: C, 73.56; H, 7.25; N, 2.72%. ^{1}H NMR ($CDCl_3$) δ: 1.95 (m, 4H), 2.73 (t, $J = 7.1$ Hz, 1H), 2.83 (t, $J = 7.3$ Hz, 1H), 2.86 (s, 1.5H, NCH_3), 2.97 (s, 1.5H, NCH_3), 3.40 (s, 1H), 3.50 (t, $J = 7.3$ Hz, 1H), 3.53 (t, $J = 7.4$ Hz, 1H), 3.56 (s, 2H), 3.62 (s, 1H), 4.01–4.02 (m, 4H), 6.81–6.82 (m, 4H), 6.99–7.35 (m, 9H). ^{13}C NMR ($CDCl_3$) δ: 26.27, 33.87, 34.05, 35.00, 36.85, 39.87, 40.58, 50.50, 52.31, 67.77, 114.90, 115.01, 126.13, 126.57, 127.06, 127.33, 128.74, 129.06, 129.14, 130.03, 130.67, 138.42, 139.29, 158.13, 158.43, 172.13, 176.78.

4.1.23. 3-Methoxy-2-[4-(3-{4-[(N-methyl-N-phenethylcarbamoyl)methyl]phenoxy}propoxy) phenyl]acetic acid ***4a***

Hydrolysis of **35a** [yellowish oil (3.0 g, 59%)] afforded pure acid **4a** as white crystals (1.7 g, 69%); m.p. 122–123 °C (*i*-PrOH). Anal Calcd. for $C_{29}H_{33}NO_6$ (491.6): C, 70.86; H, 6.77; N, 2.85; Found: C, 70.58; H, 6.90; N, 2.81%. ^{1}H NMR ($CDCl_3$) δ: 2.21 (m, 2H), 2.72 (t, $J = 7.4$ Hz, 1H), 2.78 (t, $J = 7.4$ Hz, 1H), 2.81 (s, 1.5H, NCH_3), 2.94 (s, 1.5H, NCH_3), 3.40 (s, 1H), 3.50 (t, $J = 7.3$ Hz, 1H), 3.56 (t, $J = 7.4$ Hz, 1H), 3.56 (s, 2H), 3.58 (s, 1H), 3.78 (s, 3H, OCH_3), 4.15–4.16 (m, 4H), 6.78–6.79 (m, 5H), 6.91–7.34 (m, 7H). ^{13}C NMR ($CDCl_3$) δ: 29.54, 33.84, 34.97, 39.82, 41.04, 50.47, 56.20, 64.76, 66.05, 113.87, 115.02, 121.88, 126.56, 127.02, 127.32, 128.72, 129.01, 129.05, 129.11, 129.96, 139.23, 147.79, 149.75, 158.00, 172.02, 172.15, 176.32.

4.1.24. 3-Methoxy-2-[4-(4-{4-[(N-methyl-N-phenethylcarbamoyl)methyl]phenoxy}butoxy)phenyl] acetic acid ***4b***

Hydrolysis of **35b** [yellowish oil (3.7 g, 71%)] afforded pure acid **4b** as white crystals (2.2 g, 86%); m.p. 143–144 °C (*i*-PrOH). Anal Calcd. for $C_{30}H_{35}NO_6$ (505.6): C, 71.27; H, 6.98; N, 2.74; Found: C, 70.99; H, 7.06; N, 2.76%. ^{1}H NMR ($CDCl_3$) δ: 1.98 (m, 4H), 2.73 (t, $J = 7.3$ Hz, 1H), 2.84 (t, $J = 7.3$ Hz, 1H), 2.84 (s, 1.5H, NCH_3), 2.98 (s, 1.5H, NCH_3), 3.40 (s, 1H), 3.50 (t, $J = 7.4$ Hz, 1H), 3.56 (t, $J = 7.3$ Hz, 1H), 3.56 (s, 2H), 3.62 (s, 1H), 3.81 (s, 3H, OCH_3), 4.05–4.06 (m, 4H), 6.77–6.78 (m, 5H), 6.92–7.26 (m, 7H). ^{13}C NMR ($CDCl_3$) δ: 28.27, 28.44, 31.25, 34.29, 35.43, 44.89, 46.71, 50.62, 62.22, 63.62, 107.74, 107.89, 109.40, 116.26, 121.13, 121.64, 123.13, 123.43, 123.46, 123.53, 124.37, 132.80, 133.68, 144.12, 152.52, 156.30, 166.52, 176.58.

4.1.25. 2-{4-[4-(4-{[N-Methyl-N-(3-methylphenethyl)carbamoyl]methyl}phenoxy)butoxy]phenyl} acetic acid ***5b***

Hydrolysis of **36b** [yellowish oil (4.6 g, 92%)] afforded pure acid **5b** as white crystals (1.7 g, 68%); m.p. 87–90 °C (toluene–*n*-hexane, 1:1). Anal Calcd. for $C_{30}H_{35}NO_6$ (489.6): C, 72.26; H, 7.27; N, 2.80; Found: C, 72.09; H, 7.40; N, 2.56%. ^{1}H NMR ($CDCl_3$): 1.95 (m, 4H), 2.29 (s, 1.5H, CH_3), 2.33 (s, 1.5H, CH_3), 2.68 (t, $J = 7.3$ Hz, 1H), 2.78 (t, $J = 7.4$ Hz, 1H), 2.85 (s, 1.5H, NCH_3), 2.97 (s, 1.5H, NCH_3), 3.41 (s, 1H), 3.50 (t, $J = 7.4$ Hz, 1H), 3.55 (t, $J = 7.4$ Hz, 1H), 3.55 (s, 2H), 3.62 (s, 1H), 4.01–4.02 (m, 4H), 6.82–6.84 (m, 5H), 6.86–7.23 (m, 7H). ^{13}C NMR ($CDCl_3$) δ: 21.08, 25.75, 33.29, 33.33, 34.41, 36.21, 49.84, 51.70, 67.25, 114.22, 114.41, 125.57, 126.75, 127.07, 127.17, 128.09, 128.38, 129.34, 129.56, 130.11, 137.65, 137.93, 138.75, 157.53, 157.62, 170.91, 171.06, 183.56.

4.1.26. 2-{4-[4-(4-{[N-Methyl-N-(4-methylphenethyl)carbamoyl]methyl}phenoxy)butoxy]phenyl} acetic acid ***6b***

Hydrolysis of **37b** [yellowish oil (2.4 g, 48%)] afforded pure acid **6b** as white crystals (1.31 g, 54%); m.p. 98–102 °C (aqueous acetic acid). Anal Calcd. for $C_{30}H_{35}NO_6.2H_2O$ (539.7): C, 68.99; H, 7.66; N, 2.60; Found: C, 69.07; H, 7.78; N, 2.67%. ^{1}H

NMR ($CDCl_3$) δ: 1.95 (m, 4H), 2.29–2.30 (m, 3H, CH_3), 2.65 (t, $J = 7.6$ Hz, 1H), 2.74 (t, $J = 7.4$ Hz, 1H), 2.84 (s, 1.5H, NCH_3), 2.95 (s, 1.5H, NCH_3), 3.43 (s, 1H), 3.43 (t, $J = 7.4$ Hz, 1H), 3.48 (t, $J = 7.4$ Hz, 1H), 3.56 (s, 2H), 3.60 (s, 1H), 4.02–4.03 (m, 4H), 6.83–6.84 (m, 4H), 6.95–7.42 (m, 8H). ^{13}C NMR ($CDCl_3$) δ: 21.30, 26.32, 33.34, 33.96, 34.56, 36.77, 50.49, 52.37, 67.72, 114.75, 114.96, 127.13, 127.44, 128.92, 129.01, 129.42, 129.73, 130.04, 130.60, 135.32, 135.99, 136.19, 136.61, 158.11, 158.17, 171.94, 177.79.

4.1.27. 2-{4-[4-(4-{[N-(4-Bromophenethyl)-N-methylcarbamoyl]methyl}phenoxy)butoxy]phenyl acetic acid **7b**

Hydrolysis of **38b** [yellowish oil (3.6 g, 64%)] afforded pure acid **7b** as white crystals (2.0 g, 73%); m.p. 98–102 °C (EtOH). Anal Calcd. for $C_{29}H_{32}BrNO_5$ (554.5): C, 62.82; H, 5.82; Br, 14.41; N, 2.53; Found: C, 62.58; H, 5.70; Br, 14.14; N, 2.49%. ^{1}H NMR ($CDCl_3$) δ: 1.95 (m, 4H), 2.66 (t, $J = 7.7$ Hz, 1H), 2.78 (t, $J = 7.5$ Hz, 1H), 2.84 (s, 1.5H, NCH_3), 2.95 (s, 1.5H, NCH_3), 3.43 (s, 1H), 3.44 (t, $J = 7.5$ Hz, 1H), 3.48 (t, $J = 7.5$ Hz, 1H), 3.56 (s, 2H), 3.60 (s, 1H), 4.02–4.03 (m, 4H), 6.83–6.84 (m, 4H), 6.91–7.18 (m, 6H), 7.37–7.38 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 26.29, 33.25, 34.04, 34.43, 36.82, 50.01, 51.99, 67.81, 114.92, 115.03, 126.09, 126.88, 127.18, 128.75, 129.06, 129.15, 130.02, 130.67, 130.77, 130.92, 131.80, 132.16, 137.36, 138.20, 158.15, 158.45, 172.05, 176.74.

4.1.28. 3-Methoxy-2-{4-[3-(4-{[N-(4-bromophenethyl)-N-methylcarbamoyl]methyl}phenoxy) propoxy]phenyl} acetic acid **8a**

Hydrolysis of **39a** [yellowish oil (3.7 g, 63%) afforded pure acid **8a** as white crystals (2.9 g, 78%); m.p. 118–122 °C (toluene). Anal Calcd. for $C_{29}H_{32}BrNO_6$ (570.5): C, 61.06; H, 5.65; Br, 14.01; N, 2.46; Found: C, 61.31; H, 5.69; Br, 13.99; N, 2.46%. ^{1}H NMR ($CDCl_3$) δ: 2.25 (m, 2H), 2.65 (t, $J = 7.3$ Hz, 1H), 2.77 (t, $J = 7.4$ Hz, 1H), 2.83 (s, 1.5H, NCH_3), 2.96 (s, 1.5H, NCH_3), 3.42 (s, 1H), 3.44 (t, $J = 7.4$ Hz, 1H), 3.49 (t, $J = 7.4$ Hz, 1H), 3.56 (s, 2H), 3.60 (s, 1H), 3.82 (s, 3H, OCH_3), 4.16–4.17 (m, 4H), 6.81–6.82 (m, 5H), 6.90–7.09 (m, 4H), 7.35–7.36 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 29.48, 33.75, 34.90, 39.36, 39.54, 49.82, 50.68, 52.24, 67.60, 114.19, 114.97, 121.36, 126.71, 127.35, 127.39, 128.63, 129.09, 130.05, 130.39, 131.67, 139.54, 145.90, 150.31, 156.47, 170.79, 176.33.

4.1.29. 3-Methoxy-2-{4-[4-(4-{[N-(4-bromophenethyl)-N-methylcarbamoyl]methyl}phenoxy)butoxy]phenyl}acetic acid **8b**

Hydrolysis of **39b** [yellowish oil (4.4 g, 74%) afforded pure acid **8b** as white crystals (1.6 g, 54%); m.p. 143–144 °C (EtOH). Anal Calcd. for $C_{30}H_{34}BrNO_6$ (584.5): C, 61.65; H, 5.86; Br, 13.67; N, 2.40; Found: C, 61.37; H, 5.54; Br, 13.76; N, 2.43%. ^{1}H NMR ($CDCl_3$) δ: 1.98 (m, 4H), 2.65 (t, $J = 7.3$ Hz, 1H), 2.75 (t, $J = 7.4$ Hz, 1H), 2.84 (s, 1.5H, NCH_3), 2.98 (s, 1.5H, NCH_3), 3.40 (s, 1H), 3.41 (t, $J = 7.4$ Hz, 1H), 3.52 (t, $J = 7.3$ Hz, 1H), 3.56 (s, 2H), 3.62 (s, 1H), 3.81 (s, 3H, OCH_3), 4.05–4.06 (m, 4H), 6.78–6.79 (m, 5H), 6.89–7.08 (m, 4H), 7.36–7.37 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 26.02, 26.09, 33.18, 34.44, 34.93, 36.84, 49.47, 51.72, 67.93, 108.61, 108.98, 109.85, 116.37, 121.33, 121.75, 123.32, 123.74, 123.85, 123.89, 124.52, 132.32, 133.59, 144.42, 152.74, 156.41, 166.77, 176.37.

4.1.30. Synthesis of 2-[4-(ω-bromoalkoxy)phenyl]-N-methyl-N-phenethyl acetamides **26a**, **26b**

A mixture of **22** (56.0 g, 0.208 mol), potassium carbonate (145.9 g), 18-crown-6 (2.7 g) and appropriate dibromoalkane (1.04 mol) in butan-2-one (1000 ml) was refluxed. The reaction was stopped when the starting compound disappeared (TLC, chloroform–benzene–acetic acid 60:40:5), and was then cooled and filtered. The solution was evaporated and the crude product was purified by chromatography on silica gel using dichloromethane–ethyl acetate as eluent.

4.1.31. 2-[4-(3-Bromopropoxy)phenyl]-N-methyl-N-phenethyl acetamide **26a**

Yellowish oil (67.4 g, 83%). Anal Calcd. for $C_{19}H_{22}BrNO_2$ (376.3): C, 60.65; H, 5.89; Br, 21.23; N, 3.72; Found: C, 60.48; H, 5.84; Br, 20.99; N, 3.71%. ^{1}H NMR ($CDCl_3$) δ: 2.10 (m, 2H), 2.74 (t, $J = 7.4$ Hz, 1H), 2.85 (t, $J = 7.4$ Hz, 1H), 2.97 (s, 1.5H, NCH_3), 3.39 (s, 1.5H, NCH_3), 3.39 (s, 1H), 3.51 (t, $J = 7.3$ Hz, 1H), 3.58 (t, $J = 7.4$ Hz, 1H), 3.58–3.59 (m, 2H), 3.60 (s, 1H), 4.08 (m, 2H), 6.85–6.86 (m, 2H), 7.06–7.23 (m, 7H).

4.1.32. 2-[4-(4-Bromobutoxy)phenyl]-N-methyl-N-phenethyl acetamide **26b**

Yellowish oil (69.0 g, 85%). Anal Calcd. for $C_{20}H_{24}BrNO_2$ (390.3): C, 61.54; H, 6.20; Br, 20.47; N, 3.59; Found: C, 61.33; H, 6.17; Br, 20.19; N, 3.56%. ^{1}H NMR ($CDCl_3$) δ: 1.90 (m, 2H), 2.00 (m, 2H), 2.69 (t, $J = 7.3$ Hz, 1H), 2.78 (t, $J = 7.4$ Hz, 1H), 2.85 (s, 1.5H, NCH_3), 2.97 (s, 1.5H, NCH_3), 3.38 (s, 1H), 3.47 (m, 2H), 3.49 (t, $J = 7.2$, 1H), 3.59 (t, $J = 7.4$ Hz, 1H), 3.60 (s, 1H), 3.98 (m, 2H), 6.82–6.83 (m, 2H), 7.06–7.23 (m, 7H).

4.1.33. Synthesis of arylacetic acids **9a**, **9b**

To a mixture of **26** (0.0064 mol), potassium carbonate (4.5 g), and potassium iodide (0.8 g) in butan-2-one (30 ml), **27** (1.2 g, 0.064 mol) was added. The mixture was refluxed for 15 h and then filtered and evaporated. The crude esters were purified by column chromatography on silica gel. The purity of intermediate esters was evaluated by HPLC, and their structure confirmed by ^{1}H NMR and ^{13}C NMR spectra.

To a solution of ester **40** (0.005 mol) in 15 ml of MeOH, 20 ml of aqueous 0.5 M sodium hydroxide was added and the mixture was stirred at ambient temperature for 13 h. After evaporation of methanol, the crude product was dissolved in water (50 ml) and acidified with 15 ml of 2 M acetic acid. Water solution was washed with ethyl acetate (3 × 40 ml) and the organic layer once with brine, dried with anhydrous sodium sulfate and evaporated to dryness. The crude acids were purified by crystallization from appropriate solvents.

*4.1.34. 3-Chloro-2-[4-(3-{4-[(N-methyl-N-phenethylcarbamoyl)methyl]-phenoxy}propoxy)phenyl] acetic acid **9a***

Hydrolysis of **40a** [yellowish crystals (3.0 g, 92%); m.p. 68–71 °C (ethyl acetate)] afforded pure acid **9a** as white crystals (2.0 g, 82%), m.p. 116–117 °C (acetone–diethyl ether). Anal Calcd. for $C_{28}H_{30}ClNO_5$ (495.6): C, 67.80; H, 6.10; N, 2.82; Found: C, 67.78; H, 6.26; N, 2.73%. ^{1}H NMR ($CDCl_3$) δ: 2.24 (m, 2H), 2.73 (t, $J = 7.4$ Hz, 1H), 2.83 (t, $J = 7.2$ Hz, 1H), 2.84 (s, 1.5H, NCH_3), 2.98 (s, 1.5H, NCH_3), 3.39 (s, 1H), 3.50 (t, $J = 7.4$ Hz, 1H), 3.51 (m, 2H), 3.60 (t, $J = 7.3$ Hz, 1H), 3.61 (s, 1H), 4.21–4.23 (m, 4H), 6.79–6.80 (m, 3H), 6.96–7.09 (m, 4H), 7.14–7.30 (m, 5H). ^{13}C NMR ($CDCl_3$) δ: 29.52, 33.85, 34.98, 39.79, 40.27, 50.51, 52.33, 65.93, 113.87, 115.04, 123.20, 126.60, 127.09, 127.38, 127.43, 128.75, 129.04, 129.14, 130.03, 131.39, 138.37, 139.23, 153.80, 157.94, 172.15, 175.64.

*4.1.35. 3-Chloro-2-[4-(4-{4-[(N-methyl-N-phenethylcarbamoyl)methyl]phenoxy}butoxy)phenyl] acetic acid **9b***

Hydrolysis of **40b** [yellowish crystals (2.8 g, 84%), m.p. 50–52 °C (acetone)] afforded pure acid **9b** as white crystals (1.3 g, 52%), m.p. 90–93 °C (ethyl acetate). Anal Calcd. for $C_{29}H_{32}ClNO_5$ (510.0): C, 68.29; H, 6.32; N, 2.75; Found: C, 68.07; H, 6.24; N, 2.69%. ^{1}H NMR ($CDCl_3$) δ: 1.99 (m, 4H), 2.75 (t, $J = 7.4$ Hz, 1H), 2.82 (t, $J = 7.3$ Hz, 1H), 2.83 (s, 1.5H, NCH_3), 2.95 (s, 1.5H, NCH_3), 3.38 (s, 1H), 3.52 (m, 2H), 3.52 (t, $J = 7.3$ Hz, 1H), 3.59 (t, $J = 7.6$ Hz, 1H), 3.60 (s, 1H), 4.05 (m, 4H); 6.78–6.79 (m, 3H), 6.98–7.08 (m, 4H), 7.11–7.30 (m, 5H). ^{13}C NMR ($CDCl_3$) δ: 26.25, 26.37, 33.84, 34.86, 36.54, 40.70, 49.74, 51.92, 67.99, 114.49, 115.14, 121.83, 126.96, 127.23, 128.39, 129.18, 129.31, 129.83, 130.05, 130.81, 131.58, 139.64, 140.09, 153.43, 158.07, 171.21, 173.69.

4.2. Evaluation of lipophilicity

The values of log *P* were calculated using the KOWWIN program, version 1.63 (Syracuse Research Corp., U.S.A.).

Evaluation of lipophilicity by the HPLC method [16]. Experiments were carried out using a liquid chromatograph with an LCP 4100 pump (Ecom, Prague, Czech Republic), autosampler Waters 717 plus, UV detector Waters 486 (Waters Assoc., Milford, MA, U.S.A.) and data module CSW (DataApex, CR). Thermoquest Hypersil ODS 5 μm (Thermo Hypersil-Keystone, Asmoor Runcorn, GB) in a 250 × 4.6 mm I.D. column was used as a stationary phase and the mixture of acetonitrile and pH 5.75 buffer (1:1) was used as a mobile phase. Detection was performed by UV absorption at 233 nm. The retention time of sodium nitrate (0.02% solution) was taken as a t_0 and the capacity factor k was evaluated from the retention time of the solute, t_R, by the relationship: $k = (t_R - t_0)/t_0$.

4.3. Quantitative structure–activity analysis

The regression equations were calculated using Statgraphics Program, U.S. Version 4 (STSC Inc. U.S.A.).

The coefficients in the regression equations were calculated from experimental results by multiple regression analysis and their statistical significance was tested with the Student's *t*-test. Statistical significance of regression equations was tested by standard deviations (s), coefficients of multiple correlations (r) and the Fisher–Snedecor criterion (F). The statistical significance level p was better than 0.005 for both the whole equations and individual variables with the exceptions mentioned in the text.

4.4. Biological evaluation

The production of LTB_4 was determined in rat polymorphonuclear cells from the pleural exudate elicited by heat-inactivated rat serum [27,28]. The cells were stimulated with Ca^{2+} ionophore A23187 (Sigma) and incubated with various concentrations of the tested compounds. LTB_4 was determined using a commercial RIA kit (Amersham). The *in vitro* activity was expressed in concentration IC_{50} (μM) causing 50% inhibition of LTB_4 biosynthesis and calculated by linear regression. For the LTB_4 receptor binding studies, a slightly modified method of Cheng et al. [29] was used. The membrane fraction was prepared from male guinea pig spleen; 2 mg of the membranes was incubated with 0.3 nM ^{3}H-LTB_4 in 100 μl of the incubating mixture at 25 °C for 30 min. Non-specific binding was determined in the presence of 0.1 μM of LTB_4. The membranes were filtered through Whatman GF/C paper and washed with buffer three times. The radioactivity was measured by liquid scintillation spectrometry and the specific binding of ^{3}H-LTB_4 to the receptor was determined. The binding activity was expressed in concentration IC_{50} (μM), calculated by linear regression, causing 50% inhibition of LTB_4 binding to receptor. Six points at different concentrations were used for the calculation of C in both *in vitro* activities. All tests, including controls, were performed in duplicate with internal difference not higher than 10% and experimental data were analysed by program GraphPad Prism (U.S.A.).

Inhibition of carrageenan oedema was evaluated by the method of Winter [30]; the experimental conditions are described elsewhere [31]. The values of oedema were determined as mean ± SD not higher than 10% and expressed as a percentage inhibition after a dose of 100 mg/kg in comparison with untreated control. Arachidonic-acid-induced ear inflammation in mice was performed by the method of Opas [32], the ear pinna inflammation was induced by application of 20 μl of arachidonic acid solution in acetone (concentration 100 mg/ml). The compound was given orally (200 mg/kg) 1 h before oedema induction. The degree of ear hyperaemia and the weight of ear, evaluated 1 h after application of arachidonic acid, were determined as mean ± SD not higher than 10% and expressed as a percentage inhibition relative to untreated control. Ulcerative colitis in mice [33] was induced 7 days after the last dose of compound by the repeating addition of 3% dextran sodium sulfate (MW 40 000; ICN cat. No.160110) into drinking water for 7 days (days 21–28) to BALB/c mice of 250 g average weight. The compound was given in three doses of 250 mg/1 kg in 0.5% aqueous solution

of (carboxymethyl)cellulose in weekly intervals (days 0, 7 and 14). Colitis was evaluated clinically (loss of weight, rectal prolaps, blood in stool, stool consistency), pathologically (colonic bleeding, length of colon) as the ratio of diseased and total number of animals, and histologically.

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