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Design, Synthesis and Anticonvulsant Activity of Some Derivatives of Xanthone with Aminoalkanol Moieties

Anna M. Waszkielewicz[1*], Karolina Słoczyńska[2], Elżbieta Pękala[2], Paweł Żmudzki[3],Agata Siwek[4], Anna Gryboś[4], and Henryk Marona[1]

[1] Department of Bioorganic Chemistry, Chair of Organic Chemistry, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-688 Krakow, Poland

[2] Department of Pharmaceutical Biochemistry, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-688 Krakow, Poland

[3] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-688 Krakow, Poland

[4] Department of Pharmacobiology, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-688 Krakow

* corresponding author: awaszkie@cm-uj.krakow.pl

Abstract

A series of new xanthone derivatives have been synthesized and evaluated for their anticonvulsant properties in the maximal electroshock (MES), subcutaneous metrazole (ScMet) tests as well as for neurotoxicity in the rotarod (TOX) in mice, *i.p.* and rats, *p.o.* Compound **9**: *R,S*-2-{2-[(1-hydroxybutan-2-yl]amino)ethoxy}-9*H*-xanthen-9-one and compound **12**: *R,S*-2-{3-[(1-hydroxybutan-2-yl)amino]propoxy}-9*H*-xanthen-9-one exerted activity in rats, *p.o.* 2 and 4 h after administration, respectively. Therefore, metabolic stability of the compounds was evaluated with use of rats microsomes, resulting in half-life $t_{1/2}$ 136 and 108 min, respectively, indicating that either the metabolites are very active, or the parent compounds exert other ADME properties other than metabolism which influence the late onset of activity.

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Epilepsy constitutes a set of neurological disorders which are characterized by recurrent seizures. Its incidence in overall population reaches 50/100 000/year, prevalence is in the range 5-10/1000 and remission is observed in 70% of cases. Moreover, epilepsy carries an increased risk of premature death especially in patients with chronic epilepsy. In spite of large progress in pharmacotherapy, 30% of patients experience pharmacoresistant seizures (1). According to the above, premises for drug discovery efforts in this field exist.

One of the premises for search of new drugs in the group of aminoalkanol derivatives is activity of cardiovascular drugs on the central nervous system in terms of stabilizing cell membrane potential and thus preventing seizures. Among such drugs one can find propranolol, which is currently used for some seizures in children (2). On the other hand, the antiarrhythmic drug mexiletine, prevents seizures, but simultaneously lowers seizure threshold and increases patients' susceptibility towards seizures (3, 4). It is worth mentioning that unlike propranolol, the structure of mexiletine constitutes aroxyalkylamine and it does not contain aminoalkanol moiety. The properties of these two drugs combined have been premises for our interest in aroxyalkylaminoalkanols in terms of potential drug candidates for the treatment of seizures (5).

Aminoalkanol derivatives and their anticonvulsant activity have been subject of our research for many years, with variously substituted aroxyalkyl or aroxyacyl moiety. Such aroxy group formerly constituted 2,3-dimethyl-, 2,4-dimethyl, 2,5-dimethyl- 2,6-dimethyl-, as well as 2-chloro-5-methyl-, 4-chloro-2-methyl, 4-chloro-3-methyl, or 2-chloro-6-methylphenol (6-9). Among the published results, the most active compounds were found (*R*)-2-{[2-(2,6-dimethylphenoxy)ethyl]amino}propan-1-ol (I) (6) and (*S*)-2-{[2-(2,6-dimethylphenoxy)ethyl]amino}butan-1-ol hydrochloride (II) (Fig. 1) (10).

We also have published derivatives of xanthone exhibiting anticonvulsant activity in maximal electroshock MES seizures in mice or rats (11), with aminoalkanol moieties such as *R,S*-6-chloro-2-{[(1-hydroxypropan-2-yl)amino]methyl}-9*H*-xanthen-9-one (III) (12), (*S*)-6-chloro-2-{[(1-hydroxybutan-2-yl)amino]methyl}-9*H*-xanthen-9-one (IV) (12), as well as without aminoalkanol moieties, such as 2-[(4-benzylpiperazin-1-yl)methyl]-6-methoxy-9*H*-xanthen-9-one (V) (Fig. 1) (13). So far, according to our experience, position of substituents in the

xanthone rings plays crucial role and the most active compounds III-V contain substituents at positions C2 and C6 of the xanthone structure. Since propranolol contains vast naphthoxy moiety, the use of xanthonoxy moiety as aroxy part of the structures was the next step of structure design within our research.

Due to the above premises, the aim of this study was to synthesize and perform pharmacological examination of anticonvulsant activity of aroxyalkylaminoalkanol derivatives, where aroxy moiety constituted xanthonoxy group, and the position of oxygen substituent in the xanthone ring is C2, C3, or C4, expecting that position C2 might be favorable.

During the research, in case of compounds **9** and **12** administered to rats, where activity appeared 2 and 4 h after administration, respectively, the pharmacological results drew our attention also to possible activity of a metabolite. Therefore, as a consequence we examined potential metabolism of the compounds with use of rat microsomes.

During our former studies we showed some binding to serotonergic receptors for xanthone derivatives exhibiting anticonvulsant activity (13), therefore we evaluated this mechanism of action for a promising compounds **8a**, **9**, and **12**.

Methods and Materials

Chemistry

The aminoalkanols used for synthesis of compounds **8**-**10**, **12**-**13**, **15**, and **17** were racemic (Table 1). 1-Aminobutan-2-ol was achieved according to formerly published procedures (10). Other reagents were purchased from Alfa Aesar GmbH&Co KG (Karlsruhe, Germany) and Merck Sp. z o.o. (Warszawa, Poland) and solvents were commercially available materials of reagent grade.

Melting points (mp) were determined using a Büchi SMP-20 apparatus and are uncorrected. Analyses of C, H, N were within ± 0.4% of the theoretical values. Analytical TLC was carried out on precoated plates (silica gel, 60 F-254 Merck). Spots were visualized with UV light.

The theoretical values of partition coefficient LogP was calculated with programs ChemBioDraw Ultra 12.0 (CambridgeSoft) and with Molinspiration online toolkit (14) for

base forms (Table 1). ^{1}H NMR spectra for compounds **1**-**2**, **4**-**7**, **11**-**12**, and **16** were recorded at Faculty of Pharmacy, Jagiellonian University Medical College (Krakow, Poland) with a Varian Mercury-VX 300 NMR spectrometer at 29˚C. Chemical shifts were referenced against solvent lock signal. Standard Varian pulse sequences were used for 2D experiments. ^{1}H NMR and ^{13}C NMR spectra for compounds **3**, **8**-**10, 13**-**15**, **16a**, and **17** were recorded at Faculty of Chemistry, Jagiellonian University (Krakow, Poland), on a Bruker AVANCE III 600 (resonance frequencies 600.20 MHz for ^{1}H and 150.94 MHz for ^{13}C) equipped a 5-mm probehead: PABBO with z-gradient or TBI with XYZ gradients. The ^{1}H spectra were recorded with 16 scans, 1 s relaxation delay, 4 s acquisition time, 128 kW FID size, with 16234 Hz spectral width. The ^{13}C spectra were recorded with WALTZ-16 ^{1}H broadband decoupling, a few thousands scans, 2 s relaxation delay, 0.9 s acquisition time, 64 kW FID size, 36 057 Hz spectral width. Standard pulse sequences from Bruker library were used for 2D spectra. Gradient enhanced sequences were used for the homo- and heteronuclear 2D experiments. All processing and analysis were performed using Bruker's TopSpin 3.0 software suite.

Results are presented in the following format: chemical shift δ (ppm), multiplicity, *J* values in Hertz (Hz), number of protons, protons' position. Multiplicities are showed as the abbreviations: s (singlet), bs (broad singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublets of doublets), t (triplet), qn (quintet), m (multiplet).

The IR spectra were recorded on a Jasco FT/IR 410 spectrometer (KBr pellets). For mass spectrometry analysis samples were prepared in acetonitrile/water (10/90 v/v) mixture. The LC/MS system consisted of a Waters Acquity UPLC, coupled to a Waters TQD mass spectrometer (electrospray ionization mode ESI-tandem quadrupole). All the analyses were carried out using an Acquity UPLC BEH C18, 1.7 lm, 2.1 x 100 mm column. A flow rate of 0.3 mL/min and a gradient of (5–95)% B over 10 min and then 100% B over 2 min was used. Eluent A: water/0.1% HCO_2H; eluent B: acetonitrile/0.1% HCO_2H. LC/MS data were obtained by scanning the first quadrupole in 0.5 s in a mass range from 50 to 1000 Da; eight scans were summed up to produce the final spectrum.

The title 2*N*-[(xanthonoxy)alkyl]aminoalkan-1-ols are presented in Table 1.

General procedures for preparation of the starting materials

The following starting materials have been achieved according to formerly published procedures (15) (Scheme 1) and their properties were published as well: 2-(2-hydroxyethoxy)-9*H*-xanthen-9-one and 4-(2-hydroxyethoxy)-9*H*-xanthen-9-one (15), 2-(2-chloroethoxy)-9*H*-xanthen-9-one (16), 2-(3-hydroxypropoxy)-9*H*-xanthen-9-one (17), 4-(3-hydroxypropoxy)-9*H*-xanthen-9-one (18).

2-Hydroxyxanthone was obtained by Ullmann's (19) condensation of *o*-chlorobenzoic acid with *p*-methoxyphenol in the form of sodium salts (catalytic amount of Cu/Cu_2O) in liquid paraffin (*ca.* 210 °C). The obtained 2-carboxy-4'-methoxydifenyl ether was subject to cyclization and demethylation with 80% H_2SO_4 (10-fold excess) for 3 h on a boiling water bath. After pouring into ice, filtration and washing with water and 10% $NaHCO_3$, the crude product was dissolved in 10% NaOH, and the dissolved product was 2-hydroxy-9*H*-xanthen-9-one (yield 85-90%). Some remaining portion of 2-methoxy-9*H*-xanthen-9-one which was not soluble in NaOH, was subject to demethylation with 80% H_2SO_4. The final product was recrystallized from xylene resulting in white powder, mp 236-238 °C (235-236 °C (20).

The same method was used for achievement of 4-hydroxy-9*H*-xanthen-9-one using *o*-chlorobenzoic acid and *o*-methoxyphenol as starting materials. The resulting product was recrystallized from xylene and achieved as white powder, mp 239-241 °C (230-233 °C (21), 234-236 °C (22).

3-Hydroxy-9*H*-xanthen-9-one (23) necessary for synthesis of **3** was obtained by methanolysis of 3-chloro-9*H*-xanthen-9-one to 3-methoxy-9*H*-xanthen-9-one and subsequent demethylation of the product using 80% H_2SO_4. The product was recrystallized from xylene resulting in white powder, mp 238-240 °C (241-242 °C (24)).

The starting materials **1**-**2**, **4**, **6**-**7** were achieved according to two methods A and B. In method A, reaction of appropriate 2-, 3-, or 4-hydroxy-9*H*-xanthen-9-one with chloroalkanol was performed (resulting in *e.g.* **3**) (16), and the product was subject to chlorination using $SOCl_2$. Alternative method B constitutes reaction between respective 2-, 3-, or 4-hydroxy-9*H*-xanthen-9-one and 2-bromo-1-chloroethane or 3-bromo-1-chloropropane (Scheme 1).

Method A

2-(2-Chloroethoxy)-9*H*-xanthen-9-one (**1**) was obtained from 2-(2-hydroxyethoxy)-9*H*-xanthen-9-one (mp 151-153 °C) (15). The first step was the reaction between 2-

hydroxyxanthone with redistilled 2-chloroethanol in acetone in the presence of anhydrous K_2CO_3 and catalytic amount of TEBA and KI. The mixture was refluxed for 48 h and filtered hot. Then the solvent was evaporated. Water and 5% NaOH was added to the residue and the mixture was stirred. The insoluble precipitate was filtered off and washed with water. The resulting solid was recrystallized from EtOH. The next step was chlorination with $SOCl_2$ in toluene under reflux. The crystallization of the product was performed with use of EtOH or n-hexene/toluene 3:1.

Additionally, 3-(2-bromoethoxy)-9*H*-xanthen-9-one (**5**) was achieved by bromination of 3-(2-hydroxyethoxy)-9*H*-xanthen-9-one (**3**) with PBr_3 in $CHCl_3$ solution as an alternative alkylating agent to **1**. After distillation of solvent and washing with water, crystallization was performed from EtOH.

Method B

2-(2-Chloroethoxy)-9*H*-xanthen-9-one (**1**) was obtained also alternatively by condensation of 2-hydroxy-9*H*-xanthen-9-one with 2-bromo-1-chloroethane in acetone in the presence of anhydrous K_2CO_3 and catalytic amount of TEBA. The mixture was refluxed for 24 h and filtered hot. Then the solvent was evaporated to dryness. Water and 5% NaOH was added to the residue and the mixture was stirred. The insoluble precipitate was filtered off and washed with water. The product was crystallized with use of EtOH or n-hexane/toluene 1:1 and was used for synthesis of compounds **8**-**10**.

The same methods were used for the formation of compounds **2**, **4**, and **6**-**7** and in case of **3** the compound was result of the condensation of 3-hydroxy-9*H*-xanthen-9-one with 2-chloroethanol. The product was crystallized with use of EtOH or n-hexane/toluene 1:1.

General procedures for preparation of the title compounds

Compounds **8**-**17** were obtained by aminolysis of appropriate compounds **1**-**7** in toluene in the presence of K_2CO_3, according to previously published procedures (25) or in DMF in presence of TEA and catalytic amount of KI. The achieved bases were recrystallized from n-heptane/toluene (3:1). Some portions of amines were converted to hydrochlorides with use of EtOH saturated with gaseous HCl in EtOH solution and the achieved products were recrystallized from mixture acetone/EtOH 1:3, resulting in compounds **8a-9a**, and **14a-17a**.

2-(2-chloroethoxy)-9*H*-xanthen-9-one (1)

$C_{15}H_{11}O_3Cl$; M=274.70; mp 180-182 °C; R_f=0.89 (CH_3OH/ethyl acetate 1/1); $N^{calc}/_{found}=^{0.00}/_{0.00}$, $C^{calc}/_{found}=^{65.58}/_{65.43}$, $H^{calc}/_{found}=^{4.04}/_{3.90}$; ^{1}H NMR ($CDCl_3$): δ (ppm) 8.34 (dd, *J*=8.2, *J*=1.8, 1H, Ar-H6), 7.76-7.68 (m, 2H, Ar-H5, Ar-H8), 7.52-7.44 (m, 2H, Ar-H7, Ar-H3), 7.42-7.34 (m, 2H, Ar-H4, Ar-H1), 4.39-4.33 (m, 2H, -O-CH_2-), 3.87 (t, *J*=5.9, 2H, -CH_2-Cl); LC-MS $[M+H]^+$ m/z: 275.18, 100%.

2-(3-chloropropoxy)-9*H*-xanthen-9-one (2)

$C_{16}H_{13}O_3Cl$; M=288.73; mp 136-138 °C; R_f=0.91 (CH_3OH/ethyl acetate 1/1); $N^{calc}/_{found}=^{0.00}/_{0.00}$, $C^{calc}/_{found}=^{66.56}/_{66.89}$, $H^{calc}/_{found}=^{4.54}/_{4.45}$; ^{1}H NMR ($CDCl_3$): δ (ppm) 8.35 (dd, *J*=8.1, *J*=1.7, 1H, Ar-H6), 7.75-7.68 (m, 2H, Ar-H5, Ar-H8), 7.51-7.30 (m, 4H, Ar-H7, Ar-H3, Ar-H4, Ar-H1), 4.24 (t, *J*=5.9, 2H, -O-CH_2-), 3.77 (t, *J*=6.4, 2H, -CH_2-Cl), 2.29 (qn, *J*=6.1, 2H, -CH_2-CH_2-CH_2); LC-MS $[M+H]^+$ m/z: 289.09, 98.43%.

3-(2-hydroxyethoxy)-9*H*-xanthen-9-one (3)

$C_{15}H_{12}O_4$; M=256.26; mp 158-160 °C (mp 236-238 °C (26)); R_f=0.84 (CH_3OH/ethyl acetate 1/1); $N^{calc}/_{found}=^{0.00}/_{0.00}$, $C^{calc}/_{found}=^{70.31}/_{70.52}$, $H^{calc}/_{found}=^{4.72}/_{4.68}$; ^{1}H NMR (DMSO-$d_6$): δ (ppm) 8.17 (ddd, *J*=7.9, *J*=1.7, *J*=0.4, 1H, H-8), 8.10 (d, *J*=8.9, 1H, H-1), 7.85 (ddd, *J*=8.5, *J*=7.1, *J*=1.7, 1H, H-6), 7.62 (ddd, *J*=8.5, *J*=1.0, *J*=0.4, 1H, H-5), 7.47 (ddd, *J*=7.9, *J*=7.1, *J*=1.0, 1H, H-7), 7.15 (d, *J*=2.4, 1H, H-4), 7.06 (dd, *J*=8.9, *J*=2.4, 1H, H-2), 4.97 (bs, 1H, -OH), 4.19 (t, *J*=4.8, 2H, Ar-O-CH_2-), 3.78 (t, *J*=4.8, 2H, -CH_2-OH); ^{13}C NMR: 174.51 (C=O), 164.09 (C3), 157.16 (C4a), 155.25 (C4b), 134.65 (C6), 127.20 (C1), 125.51 (C8), 123.94 (C7), 120.86 (C8a), 117.53 (C5), 114.50 (C8b), 113.63 (C2), 100.68 (C4), 70,22 (Ar-O-CH_2-), 58,95 (-CH_2-OH); LC-MS $[M+H]^+$ m/z: 257.24, 97.88%.

3-(2-chloroethoxy)-9*H*-xanthen-9-one (4)

$C_{15}H_{11}O_3Cl$; M=274.70; mp 163-164 °C; R_f=0.88 (CH_3OH/ethyl acetate 1/1); $C^{calc}/_{found}=^{65.59}/_{65.88}$; $H^{calc}/_{found}=^{4.04}/_{3.95}$; $N^{calc}/_{found}=^{0.00}/_{0.00}$; ^{1}H NMR ($CDCl_3$): δ (ppm) 8.31 (dd, *J*=7.6, *J*=1.8, 1H, Ar-H6), 8.25 (d, *J*=8.8, 1H, Ar-H1), 7.73-7.65 (m, 1H, Ar-H8), 7.44 (d, *J*=8.2, 1H, Ar-H5), 7.40-7.33 (m, 1H, Ar-H7), 6.95 (dd, *J*=8.8, *J*=2.3, 1H, Ar-H2), 6.87 (d, *J*=2.3, 1H, Ar-H4), 4.34 (t, *J*=5.9, 2H, -O-CH_2-), 3.87 (t, *J*=5.9, 2H, -CH_2-Cl); LC-MS $[M+H]^+$ m/z: 275.18, 100%.

3-(2-bromoethoxy)-9*H*-xanthen-9-one (5)

$C_{15}H_{11}O_3Br$, M=319.15, mp 172-174 °C; R_f=0.84 (CH_3OH/ethyl acetate 1/1); ^1H NMR ($CDCl_3$): δ (ppm) 8.32 (dd, *J*=8.2, 1.8 Hz, 1H, Ar-H6), 8.29 - 8.24 (m, 1H, Ar-H1), 7.73 - 7.65 (m, 1H, Ar-H8), 7.45 (d, *J*=8.2 Hz, 1H, Ar-H5), 7.41 - 7.33 (m, 1H, Ar-H7), 6.96 (dd, *J*=9.1, 2.6 Hz, 1H, Ar-H2), 6.89 (d, *J*=2.3 Hz, 1H, Ar-H4), 4.45 - 4.38 (m, 2H, Ar-O-CH_2-), 3.70 (t, *J*=6.2 Hz, 2H, -CH_2-Br); LC-MS $[M+H]^+$ m/z: 320.99, 96.78%.

4-(2-chloroethoxy)-9*H*-xanthen-9-one (6)

$C_{15}H_{11}O_3Cl$; M=274.70; mp 149-150 °C (no mp (16)); R_f=0.91 (CH_3OH/ethyl acetate 1/1); $N^{calc}/_{found}=^{0.00}/_{0.00}$, $C^{calc}/_{found}=^{65.59}/_{65.39}$, $H^{calc}/_{found}=^{4.04}/_{3.91}$; ^1H NMR ($CDCl_3$): δ (ppm) 8.33 (dd, *J*=8.0, *J*=1.8, 1H, Ar-H6), 8.00-7.93 (m, 1H, Ar-H8), 7.78-7.70 (m, 1H, Ar-H5), 7.59 (dd, *J*=8.5, *J*=0.5, 1H, Ar-H2), 7.43-7.35 (m, 1H, Ar-H1), 7.30-7.25 (m, 2H, Ar-H3, Ar-H8), 4.43 (t, *J*=5.9, 2H, -O-CH_2-), 3.95 (t, *J*=5.9, 2H, -CH_2-Cl); LC-MS $[M+H]^+$ m/z: 275.25, 100%.

4-(3-chloropropoxy)-9*H*-xanthen-9-one (7)

$C_{16}H_{13}O_3Cl$; M=288.73; mp 117-119 °C; R_f=0.88 (CH_3OH/ethyl acetate 1/1); $N^{calc}/_{found}=^{0.00}/_{0.00}$, $C^{calc}/_{found}=^{66.56}/_{66.93}$, $H^{calc}/_{found}=^{4.54}/_{4.40}$; ^1H NMR ($CDCl_3$): δ (ppm) 8.34 (dd, *J*=7.6, *J*=1.8, 1H, Ar-H6), 7.96-7.89 (m, 1H, Ar-H8), 7.77-7.69 (m, 1H, Ar-H5), 7.60-7.55 (m, 1H, Ar-H1), 7.42-7.35 (m, 1H, Ar-H2), 7.30-7.25 (m, 2H, Ar-H3, Ar-H7), 4.32 (t, *J*=5.9, 2H, Ar-O-CH_2-), 3.92-3.85 (m, 2H, -CH_2-Cl), 2.39 (quin, *J*=6.0, 2H, -CH_2-CH_2-CH_2-); LC-MS $[M+H]^+$ m/z: 289.21, 100%.

The title compounds

***R,S*-2-{2-[(2-hydroxybutyl)amino]ethoxy}-9*H*-xanthen-9-one hydrochloride (8a)**

$C_{19}H_{22}NO_4Cl$; M=363.84; mp 216-218 °C (**8** - base mp 86-88 °C); base R_f=0.90 (CH_3OH/ethyl acetate 1/1); (base $C_{19}H_{21}NO_4$; M=327.38 $N^{calc}/_{found}=^{4.28}/_{3.93}$, $C^{calc}/_{found}=^{69.70}/_{69.54}$, $H^{calc}/_{found}=^{6.47}/_{6.12}$); ^1H NMR (DMSO-d_6): δ (ppm) 9.00 (bs, 2H, NH_2^+), 8.21 (ddd, *J*=8.0, *J*=1.7, *J*=0.5, 1H, H-8), 7.89 (ddd, *J*=8.5, *J*=7.1, *J*=1.7, 1H, H-6), 7.71 (dd, *J*=9.1, *J*=0.4, 1H, H-4), 7.68 (ddd, *J*=8.5, *J*=1.0, 0.5, 1H, H-5), 7.65 (dd, *J*=3.1, *J*=0.4, 1H, H-1), 7.60 (dd, *J*=9.4, *J*=3.1, 1H, H-3), 7.49 (ddd, *J*=8.0, *J*=7.1, *J*=1.0, 1H, H-7), 5.36 (d, *J*=5.2, 1H, -OH), 4.45 (ddd, *J*=11.0, *J*=5.2, *J*=5.0, 2H, Ar-O-CH_2-), 3.81-3.75 (m, 1H, >CH-), 3.43 (dd, *J*=5.2, *J*=5.0, 2H, -CH_2-N), 3.13 (dd, *J*=12.8, *J*=3.0, 1H, N-CHH-CH), 2.88 (dd,

J=12.8, *J*=9.7, 1H, N-CHH-CH), 1.51-1.36 (m, 2H, -CH_2-CH_3), 0.91 (t, *J*=7.4, 3H, -CH_3); ^{13}C NMR: 175.68 (C=O), 155.44 (C-4b), 154.11 (C-2), 150.50 (C4a), 135.42 (C-6), 125.85 (C-8), 124.89 (C-3), 124.23 (C-7), 121.39 (C-8b), 120.38 (C-8a), 119.86 (C-4), 118.12 (C-5), 107.19 (C-1), 67.02 (>CH-), 63.82 (Ar-O-CH_2-), 52.22 (-N-CH_2-CH<), 45.82 (-CH_2-N), 27.54 (-CH_2- (Et)), 9.48 (-CH_3); LC-MS $[M+H]^+$ m/z: 328.36, 100%.

_R,S_-2-{2-[(1-hydroxybutan-2-yl]amino)ethoxy}-9_H_-xanthen-9-one (9)

$C_{19}H_{21}NO_4$; M=327.38; mp 105-106 °C (**9a** – hydrochloride mp 211-213 °C); R_f=0.39 (CH_3OH/ethyl acetate 1/1); ^{1}H NMR (DMSO-d_6): δ (ppm) 8.20 (dd, *J*=8.0; J=1.7, 1H, H-8); 7.87 (ddd, *J*=8.7; *J*=7.1; *J*= 1.7, 1H, H-6); 7.65 (dd, *J*=8.7; *J*=1.0, 1H, H-5); 7.64 (d, *J*=9.1, 1H, H-4); 7.57 (d, *J*=3.1, 1H, H-1); 7.49 (dd, *J*=9.1; *J*=3.1, 1H, H-3); 7.48 (ddd, *J*=8.0; *J*=7.1; *J*=1.0, 1H, H-7); 4.45 (bs, 1H, -OH); 4.17-4.10 (m, 2H, Ar-O-CH_2-); 3.40 (dd, *J*=10.8; *J*= 4.5, 1H, -CHH-OH); 3.28 (dd, *J*=10.8; *J*=6.3, 1H, -CHH-OH); 2.95 (t, *J*=5.7, 2H, -CH_2-N); 2.49-2.43 (m, 1H, >CH-); 1.83 (bs, 1H, -NH-); 1.44-1.31 (m, 2H, -CH_2- (Et)); 0.86 (t, *J*=7.5, 3H, -CH_3); ^{13}C NMR: 175.66 (C=O); 155.42 (C-4b); 154.98 (C-2); 150.13 (C4a); 135.25 (C-6); 125.83 (C-8); 124.86 (C-3); 124.09 (C-7); 121.40 (C-8b); 120.39 (C-8a); 119.68 (C-4); 118.07 (C-5); 106.50 (C-1); 68.64 (Ar-O-CH_2-); 62.64 (-CH_2-OH); 60.01 (>CH); 45.46 (-CH_2-N); 23.55 (-CH_2- (Et)); 9.92 (-CH_3); LC-MS $[M+H]^+$ m/z: 328.36, 98.91%.

_R,S_-2-{2-[(2-hydroxy-2-phenylethyl)amino]ethoxy}-9_H_-xanthen-9-one (10)

$C_{23}H_{21}NO_4$; M=375.42; mp 158-160 °C; R_f=0.47 (CH_3OH); $N^{calc}/_{found}=^{3,73}/_{3,60}$, $C^{calc}/_{found}=^{73,58}/_{73,78}$, $H^{calc}/_{found}=^{5,64}/_{5,61}$; IR (KBr, cm^{-1}): 3966, 3869, 3444, 3301, 3078, 2939, 2867, 2371, 2347, 1929, 1894, 1751, 1699, 1648, 1218; ^{1}H NMR (DMSO-d_6): δ (ppm) 8.21 (ddd, *J*=8.0, *J*=1.7, *J*=0.5, 1H, H-8), 7.88 (ddd, *J*=8.5, *J*=7.2, *J*=1.7, 1H, H-6), 7.49 (d, *J*=9.4, 1H, H-4), 7.67 (ddd, *J*=8.5, *J*=1.1, *J*=0.5, 1H, H-5), 7.65 (d, *J*=3.1, 1H, H-1), 7.60 (dd, *J*=9.4, *J*=3.1, 1H, H-3), 7.47 (ddd, *J*=8.0, *J*=7.2, *J*=1.1, 1H, H-7), 7.37-7.35 (m, 2H, Ar'-H2), 7.33-7.29 (m, 2H, Ar'-H3), 7.24-7.21 (m, 1H, Ar'-H4), 5.29 (d, *J*=5.2, 1H, -OH), 4.64 (ddd, *J*=8.1, *J*=5.2, *J*=4.7, 1H, >CH-), 4.19-4.10 (m, 2H, Ar-O-CH_2-), 2.97 (t, *J*=5.5, 2H, -CH_2-N), 2.72 (ddd, *J*=12.0, *J*=8.1, 1H, N-CHH-CH), 2.71 (ddd, *J*=12.0, *J*=4.7, 1H, N-CHH-CH), 2.01 (bs, 1H, NH); ^{13}C NMR: 175.68 (C=O), 155.43 (C-4b), 154.98 (C-2), 150.16 (C-4a), 144.48 (C-1'), 135.28 (C-6), 127.81 (C-3', C-5'), 126.66 (C-4'), 125.85 (C-8), 125.80 (C-2', C-6'), 124.88 (C-3), 124.12 (C-7), 121.42 (C-8b), 120.41 (C-8a), 119.71 (C-4), 118.08 (C-5), 106.51 (C-1), 71.53 (>CH-), 68.28 (Ar-O-CH_2-), 57.48 (-N-CH_2-CH<), 47.78 (-CH_2-N); LC-MS $[M+H]^+$ m/z: 376.36, 96.69%.

2-{3-[(1-hydroxy-2-methylpropan-2-yl)amino]propoxy}-9*H*-xanthen-9-one (11)

$C_{20}H_{23}NO_4$; M=341.40; mp 127-129 °C; R_f=0.28 (CH_3OH/ethyl acetate 1/1); ^1H NMR (DMSO-d_6): δ (ppm) 8.18 (dd, *J*=7.9, *J*=1.5, 1H, Ar-H6), 7.90-7.81 (m, 1H, Ar-H8), 7.67-7.58 (m, 2H, Ar-H5, Ar-H7), 7.54 (d, *J*=2.9, 1H, Ar-H3), 7.42 - 7.49 (m, 2H, Ar-H1, Ar-H4), 4.33 - 4.53 (m, 1H, NH), 4.13 (t, *J*=6.4, 2H, -O-CH_2-CH_2-), 3.30 (s, 1H, OH), 3.15 (d, *J*=4.1, 2H, -CH_2-OH), 2.61 (t, *J*=6.7, 2H, -CH_2-NH-), 1.82 (qn, *J*=6.4, 2H, -CH_2-CH_2-CH_2-), 0.91 (s, 6H, >C(-CH_3)$_2$); LC-MS $[M+H]^+$ m/z: 342.46, 98.88 %.

***R,S*-2-{3-[(1-hydroxybutan-2-yl)amino]propoxy}-9*H*-xanthen-9-one (12)**

$C_{20}H_{23}NO_4$, M=341.40; mp 110-112 °C; R_f=0.25 (CH_3OH/ethyl acetate 1/1); ^1H NMR ($CDCl_3$): δ (ppm) 8.35 (dd, *J*=8.0, *J*=1.5, 1H, Ar-H), 7.75-7.68 (m, 2H, Ar-H), 7.51-7.30 (m, 4H, Ar-H), 4.18 (t, *J*=6.2, 2H, -O-CH_2), 3.64 (dd, *J*=10.6, *J*=4.0, 1H, -CHH-OH), 3.30 (dd, *J*=10.5, *J*=6.7, 1H, -CHH-OH), 2.97-2.74 (m, 2H, -CH_2-NH-), 2.64-2.52 (m, 1H, NH-CH-), 2.01 (qn, *J*=6.4, 2H, -CH_2-CH_2-CH_2-), 1.62-1.34 (m, 2H, -CH_2-CH_3), 0.99-0.86 (m, 3H, -CH_2-CH_3); LC-MS $[M+H]^+$ m/z: 342.39, 100%**.**

***R,S*-3-{2-[(1-hydroxybutan-2-yl)amino]ethoxy}-9*H*-xanthen-9-one (13)**

$C_{19}H_{21}NO_4$; M=327.38; mp 140-142 °C; R_f=0.36 (CH_3OH); $N^{calc}/_{found}=^{4,28}/_{4,30}$, $C^{calc}/_{found}=^{69,71}/_{69,58}$, $H^{calc}/_{found}=^{6,47}/_{6,41}$; IR (KBr, cm^{-1}): 3427, 3290, 3148, 2964, 2930, 2857, 1658, 1605, 1598, 1497, 1474, 753; ^1H NMR (DMSO-d_6): δ (ppm) 8.17 (ddd, *J*=7.9, *J*= 1.7, *J*=0.4, 1H, H-8), 8.10 (d, *J*=8.9, 1H, H-1), 7.84 (ddd, *J*=8.5, *J*=7.1, *J*=1.7, 1H, H-6), 7.62 (ddd, *J*=8.5, *J*=1.0, *J*=0.4, 1H, H-5), 7.47 (ddd, *J*=7.9, *J*=7.1, *J*=1.0, 1H, H-7), 7.15 (d, *J*=2.4, 1H, H-4), 7.06 (dd, *J*=8.9, *J*=2.4, 1H, H-2), 4.58 (bs, 1H, -OH), 4.19 (m, 2H, Ar-O-CH_2-), 3.40 (dd, *J*=10.8, *J*=4.6, 1H, -CHH-OH), 3.30 (bs, 1H, NH (+H_2O), 3.28 (dd, *J*=10.8, *J*=6.3, 1H, -CHH-OH), 2.95 (t, *J*=5.7, 2H, -CH_2-N), 2.49-2.43 (m, 1H, >CH-), 2.28 (s, 3H, Ar-CH_3), 1.96 (bs, 1H, -NH-), 1.44-1.31 (m, 2H, -CH_2- (Et)), 0.85 (t, *J*=7.5, 3H, -CH_3); ^{13}C NMR: 174.97 (C=O), 164.28 (C3), 157.44 (C4a), 155.50 (C4b), 135.04 (C6), 127.53 (C1), 125.77 (C8), 124.29 (C7), 121.02 (C8a), 117.82 (C5), 114.74 (C8b), 113.95 (C2), 100.89 (C4), 68.79 (Ar-O-CH_2-), 62.47 (-CH_2-OH), 59.91 (CH), 45.16 (-CH_2-N), 23.39 (-CH_2- (Et)), 9.91 (-CH_3); LC-MS $[M+H]^+$ m/z: 328.36, 100%.

4-{2-[N-(2-hydroxyethyl)-N-(methyl)amino]ethoxy}-9*H*-xanthen-9-one (14a)

$C_{18}H_{20}NO_4Cl$; M=349.81; mp 210-212 °C (**14** – base mp 79-81 °C); R_f=0.26 (CH_3OH/ethyl acetate 1/1); $N^{calc}/_{found}=^{4.00}/_{4.03}$, $C^{calc}/_{found}=^{61.80}/_{61.71}$, $H^{calc}/_{found}=^{5.76}/_{5.80}$; ^{1}H NMR (DMSO-$d_6$): δ (ppm) 10.92 (bs, 1H, NH^+), 8.19 (ddd, *J*=8.0, *J*=1.8, *J*=0.5, H-8), 7.90 (ddd, *J*=8.5, *J*=7.1, *J*=1.8, H-6), 7.78 (dd, *J*=8.0, *J*=1.5, H-1), 7.70 (ddd, *J*=8.5, *J*=7.1, *J*=1.8, H-5), 7.61 (dd, *J*=8.0, *J*=1.5, H-3), 7.50 (ddd, *J*=8.0, *J*=7.1, *J*=1.0, H-7), 7.41 (dd, *J*=8.0, *J*=8.0, H-2), 5.46 (bs, 1H, OH), 4.68 (t, *J*=5.0, 2H, Ar-O-CH_2), 3.92 (t, *J*=5.5, 2H, CH_2-OH), 3.74 (bs, 2H, N-CH_2-CH_2-O-Ar), 3.45 (bs, 2H, N-CH_2-CH_2-OH), 3.04 (s, 3H, CH_3-N); (base $C_{18}H_{19}NO_4$; M=313.35, LC-MS $[M+H]^+$ m/z: 314.08, 99.02%).

***R,S*-4-{2-[(1-hydroxypropan-2-yl)amino]ethoxy}-9*H*-xanthen-9-one hydrochloride (15a)**

$C_{18}H_{20}NO_4Cl$; M=349.81; mp 221-223 °C (**15** – base mp 136-138 °C); R_f=0.20 (CH_3OH/ethyl acetate 1/1); $N^{calc}/_{found}=^{4.00}/_{3.89}$, $C^{calc}/_{found}=^{61.80}/_{61.66}$, $H^{calc}/_{found}=^{5.76}/_{5.42}$; (base IR (KBr, cm^{-1}): 3276, 3144, 3099, 2971, 2947, 2929, 2870, 1653, 1496, 1346, 1285, 1230, 1076, 755); ^{1}H NMR (DMSO-d_6): δ (ppm) 9.20, 8.95, 8.89 (bs, 2H, NH^+, NH), 8.21 (dd, *J*=8.0, *J*=1.7, 1H, H-8), 7.92 (ddd, *J*=8.5, *J*=7.1, *J*=1.7, 1H, H-6), 7.81 (dd, *J*=8.1, *J*=1.5, 1H, H-1), 7.71 (dd, *J*=8.5, *J*=1.1, 1H, H-5), 7.61 (dd, *J*=8.0, *J*=1.5, 1H, H-3), 7.52 (ddd, *J*=8.0, *J*=7.1, *J*=1.1, 1H, H-7), 7.43 (dd, *J*=8.1, *J*=8.0, 1H, H-2), 5.45 (t, *J*=4.9, 1H, OH), 4.56 (t, *J*=5.4, 2H, CH_2-O-Ar), 3.79-3.72 (m, 1H, CHH-OH), 3.66-3.59 (m, CHH-OH), 3.57-3.42 (m, 3H, CH_2-N, CH), 1.33 (d, *J*=4.4, 3H, CH_3); ^{13}C NMR: 175.79 (C=O), 155.21 (C-4b), 146.92 (C-4), 146.81 (C-4a), 146.01 (C-6), 135.53 (C-8), 125.90 (C-7), 124.51 (C-2), 123.93 (C-8b), 122.03 (C-8a), 120.86 (C-5), 118.27 (C-3), 117.51 (C-1), 65.31 (Ar-O-CH_2-), 61.01 (-CH_2OH), 55.03 (>CH-), 42.88 (-CH_2-N), 13.21 (-CH_3); LC-MS $[M+H]^+$ m/z: 314.08, 100%; (base $C_{18}H_{19}NO_4$; M=313.35; LC-MS $[M+H]^+$ m/z: 314.08, 97.99%).

4-{2-[(1-hydroxy-2-methylpropan-2-yl)amino]ethoxy}-9*H*-xanthen-9-one hydrochloride (16a)

$C_{19}H_{22}NO_4Cl$; M=363.84; mp 252-254 °C (**16** – base mp 156-158 °C); R_f=0.22 (CH_3OH/ethyl acetate 1/1); $N^{calc}/_{found}=^{3.85}/_{3.75}$, $C^{calc}/_{found}=^{62.72}/_{62.14}$, $H^{calc}/_{found}=^{6.09}/_{6.34}$; (base $C_{19}H_{21}NO_4$; M=327.38; $N^{calc}/_{found}=^{4,28}/_{4,23}$, $C^{calc}/_{found}=^{69,71}/_{69,52}$, $H^{calc}/_{found}=^{6,47}/_{6,42}$); IR (KBr, cm^{-1}): 3404, 3276, 3138, 3100, 2971, 2947, 2929, 2872, 2706, 1651, 1496, 1285, 1076, 756; IR base (KBr, cm^{-1}): 3276, 3139, 2972, 2947, 2930, 2871, 1654, 1496, 1285, 1076, 755; base ^{1}H NMR (DMSO-d_6): δ (ppm) 8.18 (dd, *J*=7.9, 1.5 Hz, 1H, Ar-H6), 7.87 (ddd, *J*=8.6, 7.2, 1.8

Hz, 1H, Ar-H8), 7.64 - 7.74 (m, 2H, Ar-H5, Ar-H1), 7.44 - 7.55 (m, 2H, Ar-H2, Ar-H7), 7.36 (t, *J*=7.9 Hz, 1H, Ar-H3), 4.54 (s, 1H, OH), 4.19 (t, *J*=5.9 Hz, 2H, Ar-O-CH_2-), 3.20 (d, *J*=5.3 Hz, 2H, -CH_2-OH), 2.93 (t, *J*=5.9 Hz, 2H, -CH_2-NH-), 1.77 - 1.92 (m, 1H, NH), 0.98 (s, 6H, >C-$(CH_3)_2$); ^{1}H NMR (DMSO-d_6): δ (ppm) 8.96 (bs, 2H, NH_2^+), 8.22 (ddd, *J*=8.0, *J*=1.7, *J*=0.4, 1H, H-8), 7.92 (ddd, *J*=8.7, *J*=7.1, *J*=1.7, 1H, H-6), 7.81 (dd, *J*=8.0, *J*=1.5, 1H, H-1), 7.69 (ddd, *J*=8.7, *J*=1.0, *J*=0.4, 1H, H-5), 7.61 (dd, *J*=8.1, *J*=1.5, 1H, H-3), 7.52 (ddd, *J*=8.0, *J*=7.1, *J*=1.0, 1H, H-7), 7.43 (dd, *J*=8.1, *J*=8.0, 1H, H-2), 5.65 (t, *J*=5.2, 1H, OH), 4.53 (t, *J*=5.6, 2H, Ar-O-CH_2-), 3.55 (d, *J*=5.2, 2H, CH_2-OH), 3.45 (bs, 2H, CH_2-NH_2^+), 1.34 (s, 6H, 2x -CH_3); ^{13}C NMR: 175.82 (C=O), 155.20 (C-4b), 146.92 (C-4), 146.01 (C-4a), 135.54 (C-6), 125.90 (C-8), 124.50 (C-7), 123.94 (C-2), 122.00 (C-8b), 120.84 (C-8a), 118.28 (C-5), 118.17 (C-3), 117.39 (C-1), 65.13 (Ar-O-CH_2-), 64.58 (-CH_2OH), 59.75 (C_q), 40.10 (-CH_2-N), 20.30 (2x -CH_3); LC-MS $[M+H]^+$ m/z: 328.36, 97.49%; (base $C_{19}H_{21}NO_4$; M=327.38, LC-MS $[M+H]^+$ m/z: 328.36, 97.49%).

***R,S*-4-{2-[(1-hydroxybutan-2-yl)amino]ethoxy}-9*H*-xanthen-9-one hydrochloride (17a)**

$C_{19}H_{22}NO_4Cl$; M=363.84; mp 183-185 °C (**17** – base mp 125-127 °C); R_f=0.27 (CH_3OH/ethyl acetate 1/1); base $C_{19}H_{21}NO_4$; M=327.38, $N^{calc}/_{found}=^{4,28}/_{4,16}$, $C^{calc}/_{found}=^{69,71}/_{69,62}$, $H=^{calc}/_{found}{}^{6,47}/_{6,27}$; IR (KBr, cm^{-1}): 3381, 3166, 2975, 2811, 2466, 1649, 1594, 1496, 1278, 753; base ^{1}H NMR (DMSO-d_6): δ (ppm) 8.20 (ddd, *J*=8.0, *J*=1.8, *J*=0.5, 1H, H-8), 7.89 (ddd, *J*=8.5, *J*=7.1, *J*=1.7, 1H, H6), 7.74 (dd, *J*=8.0, *J*=1.4, 1H, H-1), 7.67 (ddd, *J*=8.5, *J*=1.1, *J*=0.6, 1H, H-5), 7.54 (dd, *J*=8.0, *J*=1.4, 1H, H-3), 7.49 (ddd, *J*=8.0, *J*=7.1, *J*=1.1, 1H, H-7), 7.38 (dd, *J*=8.0, *J*=8.0, 1H, H-2), 4.46 (t, *J*=5.3, 1H, OH), 4.29-4.20 (m, 2H, Ar-O-CH_2), 3.46-3.40 (m, 1H, CHH-OH), 3.35-3.29 (m, 1H, CHH-OH), 3.03 (t, *J*=5.9, 2H, CH_2-N), 2.56-2.51 (m, 1H, CH), 1.92 (bs, 1H, NH), 1.46-1.35 (m, 2H, CH_2-R), 0.89 (t, *J*=7.4, 3H, CH_3); LC-MS $[M+H]^+$ m/z: 328.36, 98.29%.

Pharmacology

Antiepileptic activity and neurotoxicity assays were carried out within the Antiepileptic Drug Development Program, Epilepsy Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institute of Health in Rockville, USA (27). Compounds were injected as suspensions in 0.5% methylcellulose at doses 30, 100 and 300 mg/kg b.w. intraperitoneally (*i.p.*) into mice. The preliminary evaluation was a qualitative assay which used small groups of animals (1-8) and included three tests: maximal electroshock seizure (MES), subcutaneous pentylenetetrazol (ScMet), and neurotoxicity

(rotarod), noted at 30 min and 4 h after administration (28).

The MES were elicited by 60 Hz alternating current at 50 mA (mice) or 150 mA (rats) delivered for 0.2 s *via* corneal electrodes. A drop of 0.9% NaCl solution was placed into each eye prior to applying the electrodes. Protection in the MES test was defined as the abolition of the hindlimb tonic extension component of the seizure.

The ScMet test was performed by administration 85 mg/kg of pentylenetetrazole in 0.9% NaCl solution into the posterior midline of mice. Seizures were awaited at minimal time of 30 min subsequent to subcutaneous administration of pentylenetetrazole. A failure to observe even a threshold seizure (a single episode of clonic spasm of at least 5 s in duration) was regarded as protection.

TOX defined as neurological deficit was measured in mice (*i.p.*) or rats (*p.o.*) by the rotarod test. The animal was placed on a 1 inch diameter knurled plastic rod rotating at 6 rpm. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the three trials. In rats, neurological deficit was indicated by ataxia and loss of placing response and muscle tone.

The pilocarpine test involves determination whether the investigated compound can halt acute pilocarpine-induced status. A challenge dose of pilocarpine (50 mg/kg) is administered *i.p.* and the rats are observed until the first convulsive (*e.g.* stage 3, 4, or 5) seizure (time zero). The seizure severity is determined using the Racine scale (29) At this point a minimally toxic dose of the candidate drug is administered to a group of 8 male albino Sprague Dawley rats (150-180 g) *i.p.* Efficacy is defined by the ability of an investigational drug to halt the further expression of pilocarpine induced convulsive seizures (*e.g.* stage 3, 4, or 5).

Receptor binding studies

Binding experiments were conducted in 96-well microplates in a total volume of 250 μL of appropriate buffers. Reaction mix included 50 μL solution of test compound, 50 μL of radioligand and 150 μL of diluted membranes or the tissue suspension. Specific assay conditions for each receptors are shown in Table 2. Recombinant human proteins were used for 5-HT_{1A}, 5-HT_6, and 5-HT_7 receptors. The radioactivity was measured in MicroBeta2 scintillation counter (PerkinElmer, USA). Radioligand binding data were analyzed using iterative curve fitting routines (GraphPAD/Prism, Version 5.0 – San Diego, CA, USA). K_i values were calculated from the Cheng and Prusoff equation. The concentrations of analyzed compounds ranged from 10^{-10} to 10^{-5} M.

Metabolic stability

Metabolism prediction was performed with use of MetaSite 5.1.1. Mass 3.2.1 by Molecular Discovery Ltd. (Germany) (30).

Sprague-Dawley rat liver microsomes (RLMs), glucose-6-phosphate, NADP, glucose-6-phosphate dehydrogenase and levallorphan were procured from Sigma Aldrich.

*In vitro metabolism of compounds **9** and **12** using rat liver microsomes*

Reaction samples consisted of RLMs (0.2 mg microsomal protein/mL), substrate (20 μM), and NADPH-regenerating system (NADP, glucose-6-phosphate, glucose-6-phosphate dehydrogenase and 100 mM potassium phosphate buffer) in the presence of potassium phosphate buffer (100 mM, pH 7.4). At first, the mixtures containing microsomes, test compound and buffer were preincubated at 37 °C for 15 min before NADPH-regenerating system was added to commence the metabolic reaction. The mixture was then incubated at various time points ranging from 5 to 120 min. Subsequently, an internal standard (levallorphan, 20 μM) was added, and the samples were quenched by addition of perchloric acid. The reaction mixtures were centrifuged and supernatants were collected for LC-MS/MS analysis (UPLC/MS, Waters Corporation, Milford, MA, USA). In control samples NADPH-regenerating system was replaced with phosphate buffer (31-33). Microsomal incubations were conducted in duplicate. The *in vitro* half times ($t_{1/2}$) and intrinsic clearance (Cl_{int}) of compounds **9** and **12** in liver microsomes were determined according to procedures previously described (34).

Results

Chemistry

Calculated parameters for **8**-**17** such as LogP, topological polar surface area TPSA, molecular volume have been performed by means of Molinspiration online toolkit (14) and are presented in Table 1.

Compounds **8**-**17** were obtained by *N*-alkylation of appropriate aminoalkanols using (2-xanthonoxy)ethyl-, (2-xanthonoxy)propyl-, (3-xanthonoxy)ethyl-, (4-xanthonoxy)ethyl-, or 4-(xanthonoxy)propyl chloride, or (3-xanthonoxy)ethyl bromide (respectively). The reaction was performed in the presence of K_2CO_3 as a proton acceptor in toluene solution. The yield of alkylation was in the range 45-70%. Appropriate xanthonoxyalkyl chlorides were achieved

by chlorination of appropriate xanthonoxyethanol or appropriate (xanthonoxy)propanol with use of $SOCl_2$ or by reaction of 3-chloro-1-bromopropan or 2-chloro-1-bromoethan with appropriate hydroxyxanthone in acetone in presence of K_2CO_3 as a proton acceptor as well as TEBA, KI. 3-(2-Bromoethoxy)-9*H*-xanthen-9-one **5** was achieved for the reason of confirmation of method since its precursor 3-(2-hydroxyethoxy)-9*H*-xanthen-9-one **3** was achieved with mp different from reference (26).

The amine products **8-9** and **14-17** were converted to hydrochlorides with EtOH saturated with HCl in EtOH. The synthesis is shown in Scheme 1.

Pharmacology

Compounds **8a**-**17a** were evaluated in preliminary anticonvulsant screening in MES, ScMet and TOX tests in mice, *i.p.* Compounds **8a**-**9** and **12**-**13** are classified as class 1 ASP due to their activity at 100 mg/kg b.w. or less. Simultaneously, the active compounds **8a-9** and **12** exhibit neurotoxicity at the dose 100 mg/kg b.w. 0.5 h after administration. Moreover, in case of these compounds some activity lasted until 4 h after administration without neurotoxicity, which may lead to presumption that metabolism may produce active and non-toxic compounds. The results are presented in Table 3.

Compounds **9**, **12**-**13** were advanced within the screening program to the next phase for oral administration (*p.o.*) to rats and the results of activity in MES and neurotoxicity were observed until 4 h after administration. It is interesting that the compounds are inactive at the dose 30 mg/kg b.w. until 2, 4, or 1 h after administration, respectively. The results of **9** and **12** suggest activity rather not of the compound itself, but of a metabolite. These results vary significantly from phenoxyalkylaminoalkanols, which exhibit activity immediately after administration and time of peak effect is usually 0.25 h or 0.5 h after administration (6). The results are presented in Table 4. None of the compounds are neurotoxic at the used dose.

Compound **12** was also evaluated in the next stage of ASP – the pilocarpine test for verification of activity in a rodent model of status epilepticus. The activity was observed at 300 mg/kg b.w. The results are presented in Table 5. It is coherent with the results of MES (rats, *p.o.*) (Table 4) that in spite of lack of activity for 2 h after administration, some activity appears after 4 h. Death observed at 200 mg/kg of experiment might probably be related to the risk of status epilepticus itself.

Due to our former results of receptor binding of xanthone derivatives, compounds **8a**, **9** and **12** were subject to preliminary serotonergic receptor evaluation. They did not exhibit satisfying binding. The results are presented in Table 6.

The results of activity of compounds **9** and **12** in rats, *i.p.* and *p.o.* drew our attention towards metabolism of these compounds by rat liver microsomes. For the purpose of this study, prediction of metabolism was performed with use of MetaSite 5.1.1. Mass 3.2.1. The results of proposed three most probable metabolites with their molecular mass and calculated LogP are presented in Figure 2. Then the compounds were subject to microsomal metabolism and the results were compared.

Three metabolites of compound **9** (M1-M3) and five metabolites of compound **12** (M1-M5) were identified in rat liver microsomes (Table 7, Figure 3). In case of both compounds the major and most abundant metabolite, M1 was the product of oxidation of alcohol moiety in the parent compounds, whereas M2 metabolites of both compounds were the products of dealkylation (Figure 4). MetaSite prediction of oxidation of alcohol groups of both xanthone derivatives as the most probable pathway of biotransformation was confirmed by *in vitro* testing and MS/MS spectra. Both compounds demonstrated Cl_{int} values lying in the medium category range (26 and 32 μL/mg/min) (Table 8).

Discussion

For preliminary estimation whether the relatively large xanthonoxy moiety improves activity in the synthesized group of compounds, a coherent group of 2-aminoalkan-1-ols was chosen, among so far the optimal 2-aminopropan-1-ol, 2-aminobutan-1-ol, as well as their simple modifications.

Calculation of several physicochemical parameters by means of Molinspiration online toolkit was an important step in the research (14). Such calculations are useful at early stage of drug discovery process in terms of predicting essential parameters and facilitating rational drug design. The parameters include LogP, topological polar surface area TPSA, molecular volume, as well as violations from the Lipinski rule of five (Table 1). The first calculated parameter, LogP (with use of ChemBioDraw) was in the range 2.76-3.68, and miLogP

(Molinspiration toolkit), was found in the range 2.98-4.34, while in the literature optimum value for CNS active compounds is about 2-3 (37). The most beneficial value of TPSA should be <120 $Å^2$ for orally administered drugs and <60-70 $Å^2$ for compounds designed to penetrate blood-brain barrier. The proposed structures are consistent with the rule for oral drugs and they are on the edge of the rule for CNS drugs (calculated values are in the range 62.91-91.93 $Å^2$). The volumes range 285.78-340.62. None of the compounds exhibited any violations from the Lipinski rule of five (38), making them potentially promising drug-like agents.

As it was stated in the Introduction, the pharmacological evaluation of anticonvulsant activity of the achieved compounds verified that the optimal position of substituents in the xanthone ring was expected to be C2 and on the basis of twelve presented compounds the assumptions were proved. Three out of four most active compounds are 2-xanthonoxy derivatives, *i.e.* **8a-9**, and **12**. Compound **13** is the derivative of xanthone substituted in position C3. Substitutions in position 4 did not provide activity below 300 mg/kg b.w. (mice, *i.p.*). The observed most probable metabolites M1 look rather hydrophilic and are larger than the parent compound, therefore, they may not be likely to cross blood-brain barrier.

The aminoalkanol moiety optimal in the studied group of compounds was proved to be aminobutanol – either 2-aminobutan-1-ol (as in **9**, **12**-**13**, **17a**) which is coherent with reference structures II and IV or 1-aminobutan-2-ol (**8a**).

As long as the linker between xanthonoxy and aminoalkanol moiety is concerned, among the used ethylene and propylene – propylene is more beneficial, on the basis of comparison between compounds **9** and **12**. This result is another surprise contrary to our findings in other aroxyalkylaminoalkanol derivatives, where ethylene is superior to propylene (structures I and II) (6).

The results of activity of compounds **9** and **12** in rats, *i.p.* and *p.o.* where activity was observed 2 and/or 4 h after administration, became premises for study of metabolism of these compounds by rat liver microsomes. The achieved values of Cl_{int} (Table 8) were lower than those reported previously for imipramine (36). Basing on the data for **9** and **12** it can be concluded that both compounds are relatively stable metabolically. Considering the activity of compounds **9** and **12** in rats (Tables 3-4) either it may be a very active metabolite (possibly M1 in both cases) which appears late, or it may be other ADME factors other than metabolism, *i.e.* absorption or distribution, that may influence time of onset of activity of

compounds **9** and **12**.

Conclusions

As a conclusion, according to our former studies in terms of structure-activity relationship of xanthone derivatives and phenoxyalkylaminoalkanols, synthesis of the chosen group of *N*-[(xanthonoxy)alkyl]aminoalkanols where 2-, 3- or 4-hydroxyxanthone is used in place of phenol, was a logical choice.

The assumptions were promising, especially with use of xanthone and expectations regarding position C2 of substituents, as well as choice of aminoalkanols. Moreover, in spite of the choice of very active reference structures, their modification and design with consistence of the Lipinski rule described in the Introduction, calculations consistent with literature premises, the experiments showed that the calculation of parameters did not fulfill expectations for rational finding of an active molecule *in vivo*.

On the other hand, research regarding metabolism of active compounds provides wide view regarding the metabolic pathway in the organism and its products.

Having completed screening documentation regarding the title group of compounds, one can suggest further possible modifications in the other xanthone ring, which might enhance anticonvulsant activity. Moreover, when a larger group of derivatives results from our research, in order to compare derivatives in a full manner, it may be reasonable to synthesize enantiomers of chiral active derivatives.

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K/ZDS/005487.

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Scheme 1: Synthesis of the starting materials (**1**-**7**) and the title compounds (**8**-**17**).

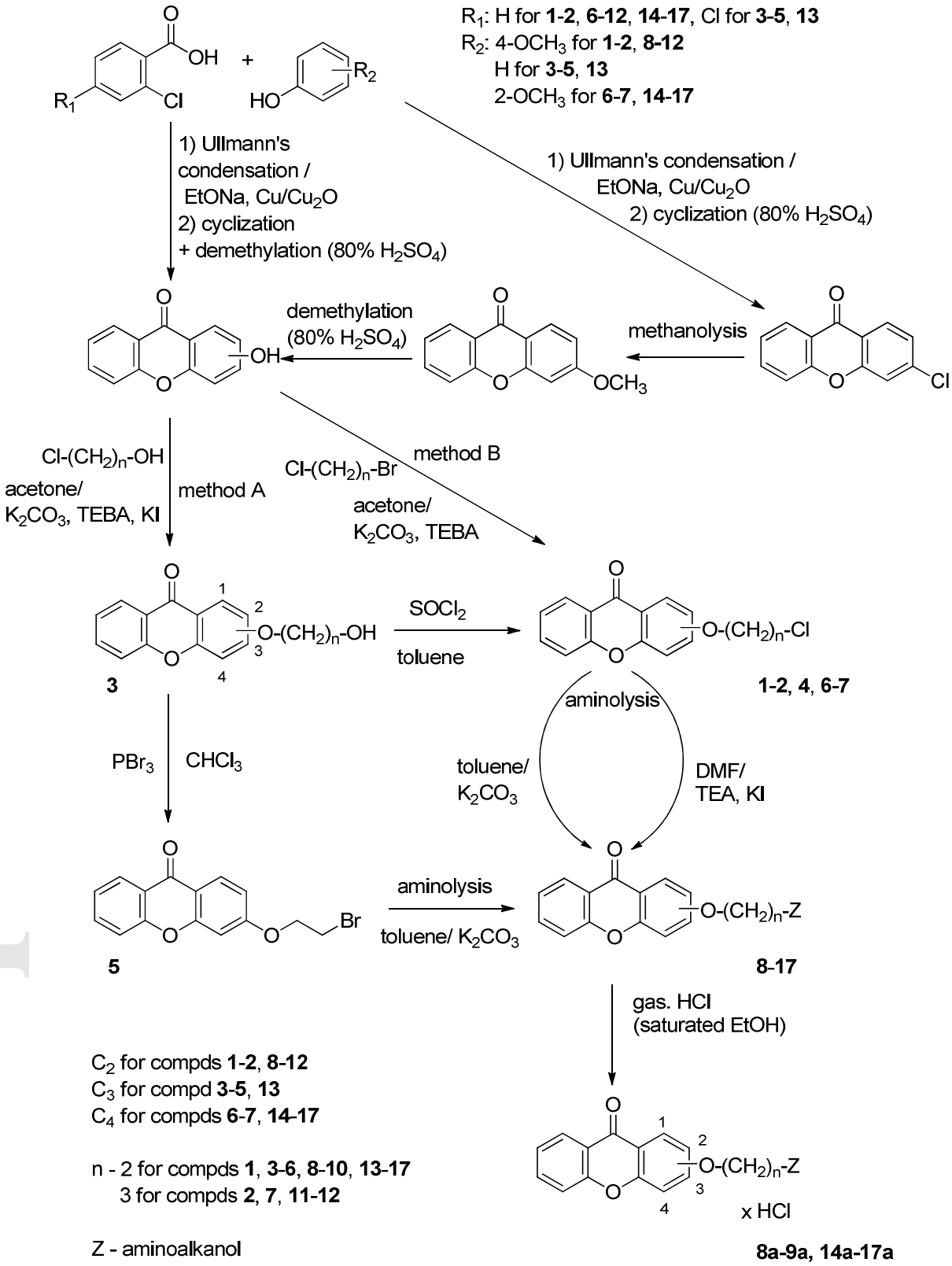
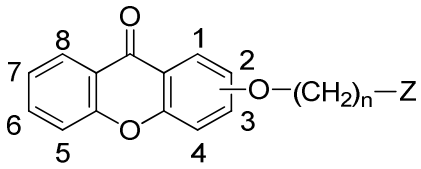
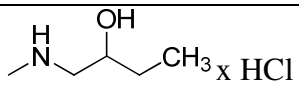
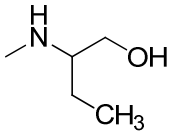
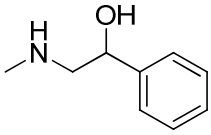
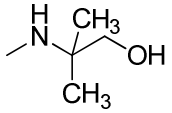
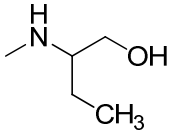
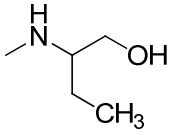
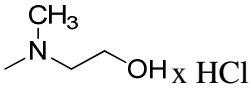
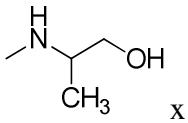
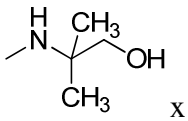
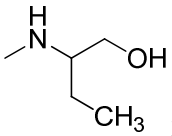


Table 1: Structures and logP of the title compounds (**8a**-**17a**) (mice, *i.p.*).



Compd.	Position	n	Z	Config.	$LogP_{calc}$ [a)]	miLogP [b)]	TPSA [Å^2]	Volume [Å^3]
8a	C2	2	 x HCl	*R,S*	2.95	3.27	71.70	302.58
9				*R,S*	2.95	3.62	71.70	302.58
10				*R,S*	3.68	4.34	71.70	340.62
11		3		-	3.19	3.84	71.70	318.82
12				*R,S*	3.32	3.89	71.70	319.38
13	C3	2		*R,S*	2.95	3.62	71.70	302.58
14a	C4	2	 x HCl	-	2.76	2.98	62.91	286.13
15a			 x HCl	*R,S*	2.42	3.06	71.70	285.78
16a			 x HCl	-	2.82	3.54	71.70	302.01
17a			 x HCl	*R,S*	2.95	3.60	71.70	302.58

Calculations were performed with use of [a)] ChemBioDraw Ultra 12.0 (CambridgeSoft) [b)] Molinspiration online toolkit (14) for base forms.

Table 2: Preliminary data on anticonvulsant activity and neurotoxicity of the title compounds (mice, *i.p.*).

Compd	Dose	MES [a]		ScMet [a]		TOX [b]		ASP class [c]
		0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	
8a	3	–				–		1
	10	–				–		
	30	1/1	–	–	–	–	–	
	100	3/3	1/3	–	–	7/8	–	
	300					4/4		
9	3	–				–		1
	10	–				–		
	30	1/1	–	–	–	–	–	
	100	3/3	3/3	–	–	7/8	–	
	300	1/1	1/1	–	–	4/4	–	
10	30	–	–	–	–	–	–	3
	100	–	–	–	–	–	–	
	300	–	–	–	–	–	–	
11	30	–	–	–	–	–	–	
	100	–	–	–	–	5/8	–	
	300					4/4	–	
12	30	–	–	–	–	–	–	1
	100	3/3	2/3	–	–	6/8	–	
	300					4/4		
13	30	–	–	–	–	–	–	1
	100	1/3	–	–	–	–	–	
	300	1/1	–	–	–	–	–	
14a	30	–	–	–	–	–	–	3
	100	–	–	–	–	8/8	–	
	300	–	–	–	–	4/4	–	
15a	30	–	–	–	–	–	–	2
	100	–	–	–	–	–	–	
	300	1/1	–	–	–	4/4	–	
16a	30	–	–	–	–	–	–	2
	100	–	–	–	–	–	–	
	300	1/1	–	–	–	4/4	1/2	
17a	30	–	–	–	–	2/4	–	4

100	–	–	–	–	6/8	–
300	1/1	–	|	|	4/4	|

[a)] Number of animals protected / number of animals tested; [b)] number of animals exhibiting toxicity / number of animals tested in the rotorod test; [c)] ASP classification: 1 – anticonvulsant activity at doses 100 mg/kg or less; 2 – anticonvulsant activity at doses greater than 100 mg/kg; 3 – compound inactive at 300 mg/kg; 4 – compound either active or inactive but toxic at doses of 30 mg/kg; – indicates that the compound was not active or toxic in the particular case; | indicates that the compound was not tested in the particular case.

Table 3: Anticonvulsant activity and neurotoxicity of the tested compounds (rats, *p.o.*).

Compound	Test	Dose	Time [h]				
		[mg/kg b.w.]	0.25	0.5	1.0	2.0	4.0
9	MES[a)]	30	–	–	–	1/4	–
	TOX[b)]	30	–	–	–	–	–
12	MES	30	–	–	–	–	1/4
	TOX	30	–	–	–	–	–
13	MES	30	–	–	1/4	–	–
	TOX	30	–	–	–	–	–

[a)] Number of animals protected / number of animals tested; [b)] number of animals exhibiting toxicity / number of animals tested in the rotorod test; – indicates that the compound was not active or toxic in the particular case.

Table 4: Pilocarpine induced status epilepticus results (rats, *i.p.*).

Compd.	Dose	Time [h] [a)]					No. of
	[mg/kg b.w.]	0.25	0.5	1.0	2.0	4.0	deaths
12	30	–	–	–	–	–	7/7
	100	–	–	–	–	1/2	at dose 200
	300	2/2	2/2	2/2	2/2	2/2	

[a)] Number of animals protected / number of animals tested.

Table 5: Results of binding to serotoninergic receptors of reference and tested compounds.

Compd.	K_i [nM]		
	5-HT_{1A}	5-HT_6	5-HT_{7b}
9a	2136	16240	8301
buspiron	12	-	-
clozapine	143	4	18

Inhibition constants (K_i) were calculated according to the equation of Cheng and Prusoff (36). Radioligand binding assays to rats brain tissues using [^{3}H]8-OH-DPAT for 5-HT_{1A}, [^{3}H]-LSD for 5-HT_6, [^{3}H]-5-CT for 5-HT_7.

Table 6: Results of binding to serotoninergic receptors of reference and tested compounds.

Compound	K_i [nM]		
	5-HT_{1A}	5-HT_6	5-HT_7
	[^{3}H]-8-OH-DPAT	[^{3}H]-LSD	[^{3}H]-LSD
8a	320.0 ± 11.3	-	-
9	659.0 ± 21.4	-	-
12	> 5000	-	-
serotonin	1.2 ± 0.05	-	-
methiothepin	-	1.0 ± 0.1	0.4 ± 0.02

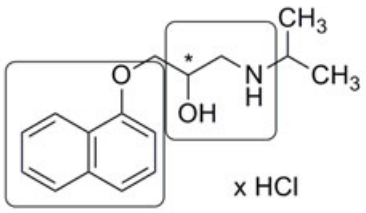
Inhibition constants (K_i) were calculated according to the equation of Cheng and Prusoff (35).

Table 7: Half life ($t_{1/2}$) and intrinsic clearance (Cl_{int}) data for compounds **9** and **12** in rat liver microsomal system.

Compound	$t_{1/2}$ [min]	Cl_{int} [µL/mg/min]
9	136	26
12	108	32
Imipramine (37)	5	302

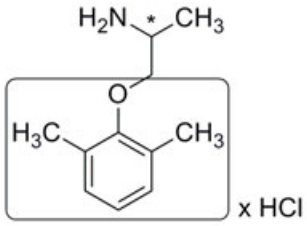
Table 8: Half life ($t_{1/2}$) and intrinsic clearance (Cl_{int}) data for compounds **9** and **12** in rat liver microsomal system.

Compound	$t_{1/2}$ [min]	Cl_{int} [µL/mg/min]
9	136	26
12	108	32
Imipramine (36)	5	302



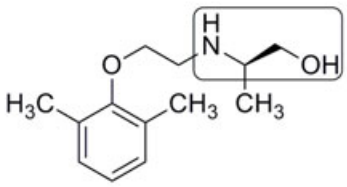
Propranolol

ED_{50}=15.2 mg/kg (mice, *i.p.*)



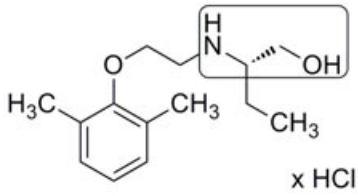
Mexiletine

ED_{50}=20 mg/kg (mice, *i.p.*)



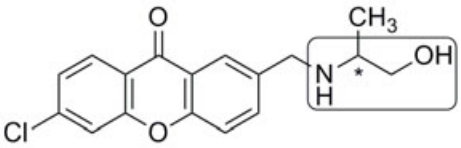
I

ED_{50}=5.34 mg/kg (mice, *i.p.*)
TD_{50}=29.48 mg/kg (mice, *i.p.*)



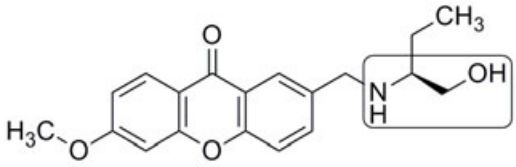
II

ED_{50}=7.57 mg/kg (mice, *i.p.*)
TD_{50}=34.45 mg/kg (mice, *i.p.*)



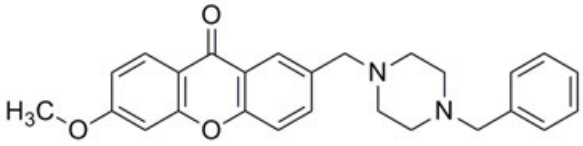
III

ED_{50} = 56.2 mg/kg (mice, *i.p.*)



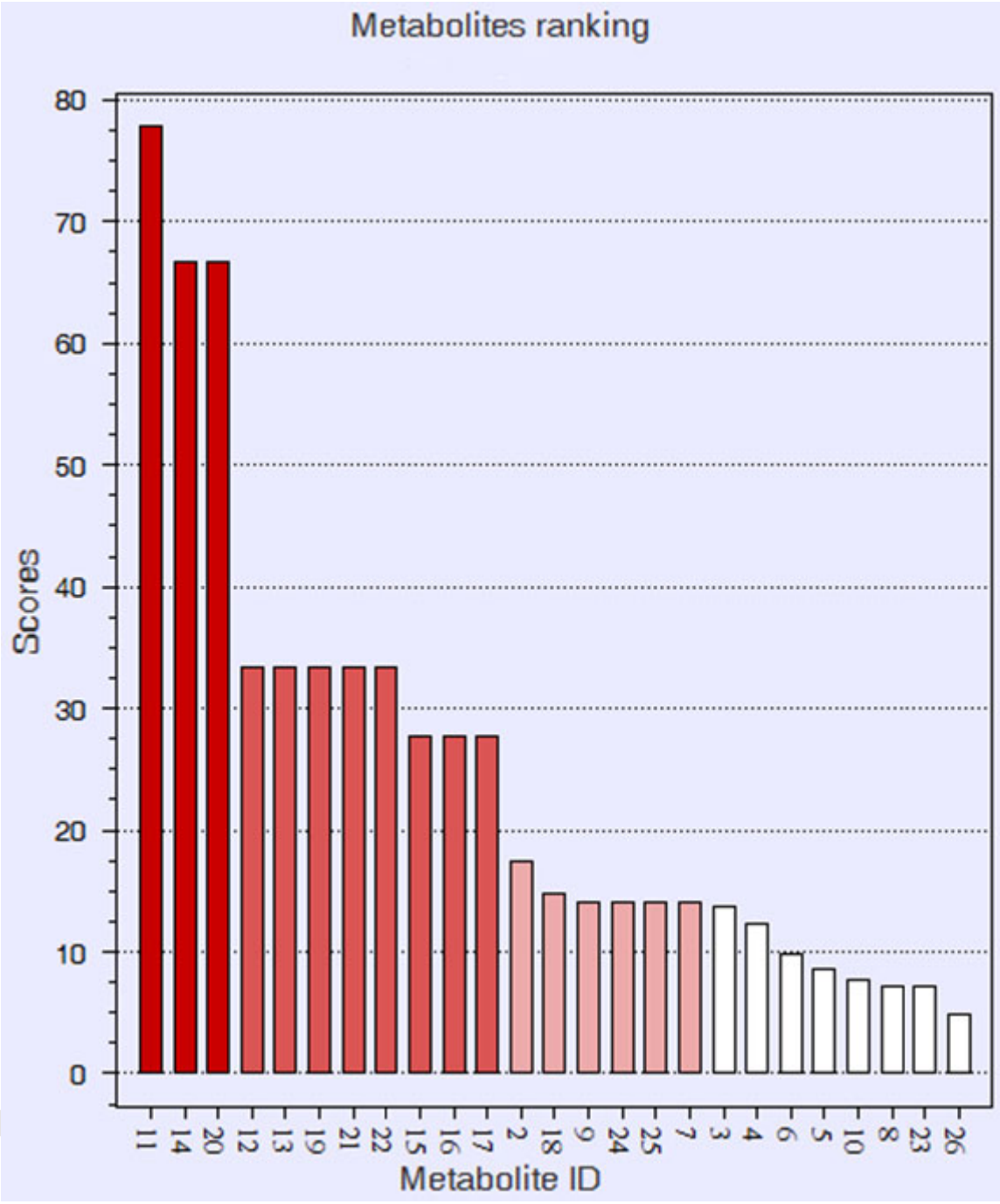
IV

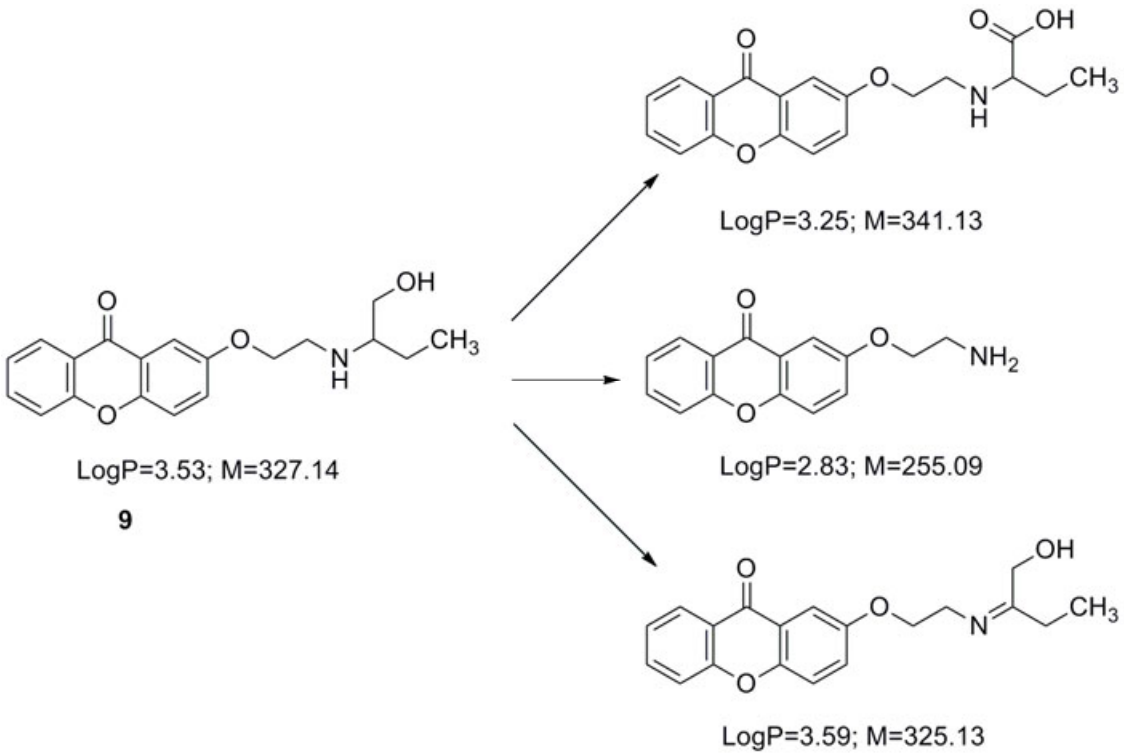
ED_{50} = 47.57 mg/kg (mice, *i.p.*)
TD_{50} > 400 mg/kg (mice, *i.p.*)



V

ED_{50} = 105 mg/kg (rats, *p.o.*)
TD_{50} > 300 mg/kg (rats, *p.o.*)





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