Received: 8 September 2018 | Revised: 16 October 2018 | Accepted: 17 October 2018
DOI: 10.1002/ardp.201800269

FULL PAPER

DPhG ARCH PHARM
Archiv der Pharmazie

Synthesis, molecular modeling studies, and anticonvulsant evaluation of novel 1-((2-hydroxyethyl)(aryl)-amino)-*N*-substituted cycloalkanecarboxamides and their acetate esters

Mohamed N. Aboul-Enein[1] | Aida A. El-Azzouny[1] | Kamilia M. Amin[2] | Mona E. Aboutabl[3] | Mai I. Abo-Elmagd[1]

[1] Department of Medicinal and Pharmaceutical Chemistry, Medicinal Chemistry Group, Pharmaceutical and Drug Industries Research Division, National Research Centre (ID: 60014618), Dokki, Giza, Egypt

[2] Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Cairo University, Cairo, Egypt

[3] Department of Medicinal and Pharmaceutical Chemistry, Pharmacology Group, Pharmaceutical and Drug Industries Research Division, National Research Centre (ID: 60014618), Dokki, Giza, Egypt

Correspondence
Prof. Dr. Mohamed N. Aboul-Enein, Department of Medicinal and Pharmaceutical Chemistry, Medicinal Chemistry Group, Pharmaceutical and Drug Industries Research Division, National Research Centre (ID: 60014618), 33 El Bohouth St., P.O. 12622, Dokki, Giza, Egypt.
Email: mnaboulenein@yahoo.com

Funding information
National Research Centre (ID: 60014618), Dokki, Giza, Egypt, Grant number: 11010311

Abstract

A series of 1-((2-hydroxyethyl)(aryl)amino)-*N*-substituted cycloalkanecarboxamides **IXa–l** and their acetate esters **Xa–l** were designed and synthesized as new anticovulsant agents. The evaluation of the anticonvulsant effect was performed *in vivo* by subcutaneous pentylenetetrazole (scPTZ) and maximal electroshock (MES) tests in mice. Further, neurotoxicity, hepatotoxicity, and acute toxicity were determined. All the new candidates displayed 100% anticonvulsant activity in the scPTZ screen in the dose range of 0.0057–0.283 mmol/kg. The most potent compounds in the scPTZ screen were **Xh** (ED_{50} = 0.0012 mmol/kg), **Xd** (ED_{50} = 0.002 mmol/kg), **Xf** (ED_{50} = 0.004 mmol/kg), **IXj** (ED_{50} = 0.0047 mmol/kg), **Xl** (ED_{50} = 0.0076 mmol/kg), and **Xi** (ED_{50} = 0.008 mmol/kg). They exhibited higher fold activity in the anticonvulsant potential than the gold standards, phenobarbital and ethosuximide. Compound **Xf** was active in both scPTZ and MES screens. It showed ED_{50} of 0.016 mmol/kg in MES screen. In the neurotoxicity screens, none of the test compounds displayed any minimal motor impairment at the maximum administered dose. The 3D pharmacophore model using Biova 1 Discovery Studio 2016 programs exhibited high fit value. The anticonvulsant evaluation results were compatible with the molecular modeling study.

KEYWORDS
1-[(2-hydroxyethyl)(aryl)amino]-*N*-substituted cycloalkanecarboxamides, acetate esters, anticonvulsants, epilepsy, molecular modeling studies

1 | INTRODUCTION

Epilepsy is a common neurologic affection characterized predominantly by periodic and sudden occurrence of seizures that are caused by abnormal excessive or synchronous cerebral neurons discharge. Epilepsy affects about 70 million people worldwide.[1,2] Actually, epilepsy is considered as the third most frequent neurological disease in the elderly after cerebrovascular diseases and dementia.[3] Presently, the chronic administration of antiepileptic drugs (AEDs) is the main treatment for epilepsy. Despite the great marketed

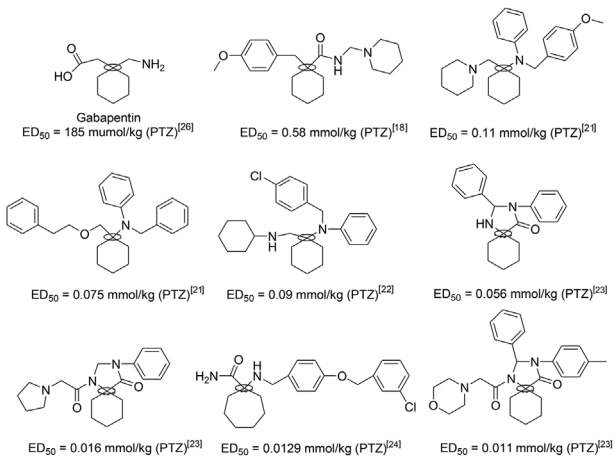


FIGURE 1 Structures of gabapentin and certain anticonvulsants bearing the 1,1-disubstituted cycloalkane moiety

therapeutic arsenal of old and new AEDs, about 30% of patients are not seizure-free.[4] Moreover, many AEDs are effective toward only 60% of patients besides inducing some undesirable chronic side-effects, which seriously cause devastating outcomes on patient's quality and style of life.[5] Therefore, there is an enormous demand for the development of novel AEDs with more escalating efficacy and fewer side effects.[6]

1,1-Disubstituted cycloalkanes were reported to display a broad spectrum of biological activities[7–16] among which the anticonvulsant one[17–24] and the antiepileptic one such as gabapentin[25,26] as depicted in Figure 1.

Moreover, several investigations have reported that ethanolamine-*O*-sulfate (EOS) was active against audiogenic seizures by irreversibly inhibiting the GABA-transaminase (4-aminobutyrate-2-oxoglutarate) which is the major catabolic enzyme for GABA in mammalian brain, consequently raises the brain GABA concentration.[27] Also, the ethanolamine moiety is proven to improve and enhance drug penetration to the central nervous system as in the prodrug ester of dexibuprofen which is used in the management of neurodegenerative disorders (Figure 2).[28]

Besides, it was previously disclosed the anticonvulsant potential of certain diazaspiroalkanediones **1** (Figure 3).[29] Accordingly, it was found of interest to design and synthesize a set of novel 1-((2-hydroxyethyl)(aryl)amino)-*N*-substituted cycloalkanecarboxamides (**IXa–l**). Also, the acetate esters of the latter, namely, 2-((1-substituted carbamoyl)cycloalkyl)(aryl)amino)ethyl acetates (**Xa–l**) were prepared to increase the lipophilicity and BBB penetration consequently the anticonvulsant profile. Noteworthy, the structural framework of compounds **IXa–l** and **Xa–l** comprise the following pharmacophoric moieties (Figure 3): (A) 1,1-disubstituted cycloalkanes; (B) selected substituents present in the molecule of the active diazaspiroalkanediones; (C) presence of the ethanol amine entity; (D) the acetate fragment, thus aiming to reach new candidates exhibiting pronounced anticonvulsant potential. Also, molecular modeling studies and anticonvulsant evaluation of the novel target compounds were performed. The anticonvulsant activity was assessed via MES and scPTZ methods in addition to neuro- and hepatotoxicity. The structure of the designed compounds was confirmed by elemental analysis, IR, ^{13}C NMR, ^{1}H NMR, and MS sperctral data as well as X-ray crystallographic analysis of **IXg**. Additionally, the target compounds contain the essential pharmacophoric features that are proposed to be necessary for a molecule to display anticonvulsant activity.[30]

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

Synthesis of the target compounds **IXa–l** and **Xa–l** is illustrated in Scheme 1. The cycloalkanone **II** was subjected to Strecker synthesis using either toluidine or anisidine in the presence of potassium cyanide and glacial acetic acid to give the respective nitriles **IIIa–d**.[31] Partial hydrolysis of **III** using sulfuric acid at room temperature yielded the respective amide derivatives **IVa–d**.[32] The cyano methyl group was introduced using paraformaldehyde and potassium cyanide in glacial acetic acid to afford compounds **Va–d**[33] which were subjected to hydrolysis using sodium hydroxide in ethanol to furnish the corresponding acids **VIa–d**.[33] Cyclization was accomplished using 4 N hydrochloric acid and ethylenediamine in dioxane to obtain the diketopiperazine derivatives **VIIa–d**. Subsequent alkylation using either benzyl chloride or phenethylbromide in the presence of potassium carbonate and catalytic amount of tetrabutylammoniun bromide gave compounds **VIIIa–f**.[33] The new intermediates **IIIf**, **Ve**,**f**, **VIe**,**f**, **VIIe**,**f**, and **VIIIi–l** possessing the cycloheptane nucleus were synthesized as their previously mentioned congeners. The target compounds **IXa–l** were reached through reductive cleavage of the cyclic imide ring of **VIIIa–l** using sodium borohydride in ethanol. Their structures were confirmed through elemental analysis, IR, ^{13}C NMR,

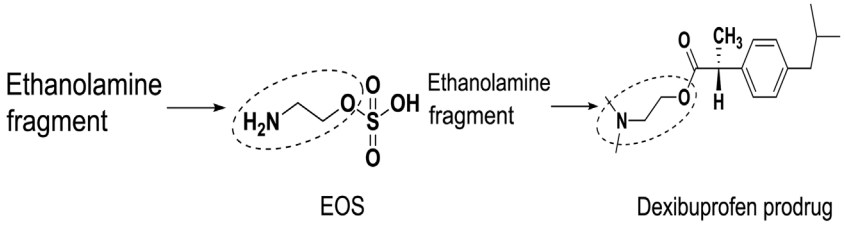


FIGURE 2 Structures of ethanolamine-*O*-sulfate (EOS) and dexibuprofen prodrug

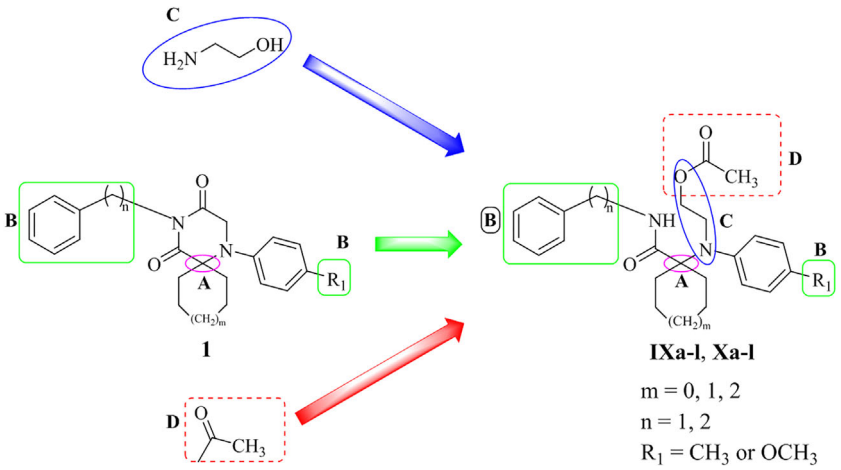
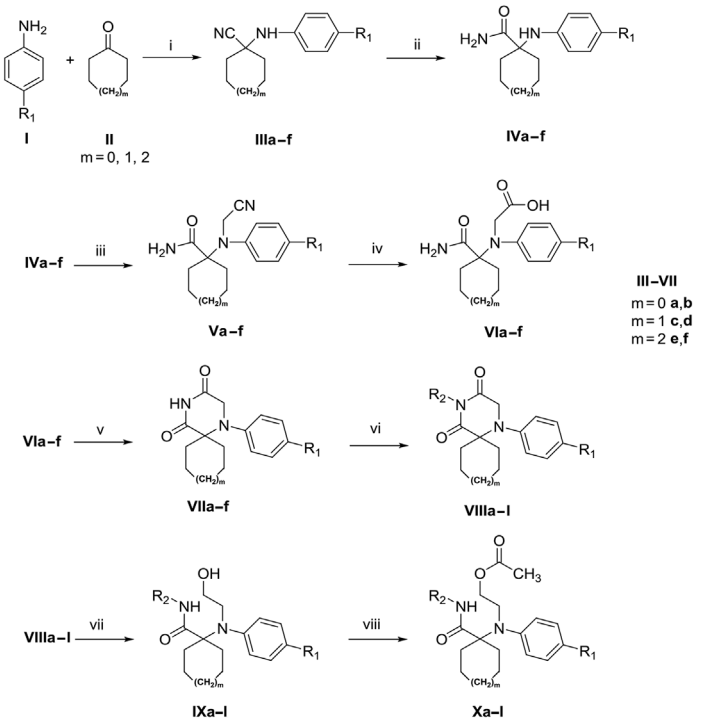


FIGURE 3 Pharmacophoric structural fragments of title compounds. (A) 1,1-Disubstituted cycloalkanes (purple), (B) selected substituent present in the active diazaspiroalkanediones (green), (C) the ethanol amine entity (blue), (D) the acetate fragment (red)



No.	M	R_1	R_2	No.	m	R_1	R_2
VIIIa, IXa, Xa	0	4-CH_3	-CH_2Ph	**VIIIg, IXg, Xg**	1	4-OCH_3	-CH_2Ph
VIIIb, IXb, Xb	0	4-CH_3	-CH_2 CH_2 Ph	**VIIIh, IXh, Xh**	1	4-OCH_3	-CH_2 CH_2 Ph
VIIIc, IXc, Xc	0	4-OCH_3	-CH_2Ph	**VIIIi, IXi, Xi**	2	4-CH_3	-CH_2Ph
VIIId, IXd, Xd	0	4-OCH_3	-CH_2 CH_2 Ph	**VIIIj, IXj, Xj**	2	4-CH_3	-CH_2 CH_2 Ph
VIIIe, IXe, Xe	1	4-CH_3	-CH_2Ph	**VIIIk, IXk, Xk**	2	4-OCH_3	-CH_2Ph
VIIIf, IXf, Xf	1	4-CH_3	-CH_2CH_2Ph	**VIIIl, IXl, Xl**	2	4-OCH_3	-CH_2CH_2Ph

SCHEME 1 Synthesis of compounds **IXa–l** and **Xa–l**. Reagents and conditions: (i) KCN, glacial acetic acid, 24 h, r.t.; (ii) conc. H_2SO_4, 48 h, r.t.; (iii) glacial acetic acid, paraformaldehyde, KCN, 37% formaldehyde; (iv) 50% aqueous ethanol, NaOH, 24 h, reflux; (v) dioxane, 4 N HCl, ethylenediamine, 24 h, reflux; (vi) $ClCH_2C_6H_5$ or $BrCH_2CH_2C_6H_5$, acetone, K_2CO_3, tetrabutylammoniumbromide, 7 h, reflux; (vii) $NaBH_4$, ethanol, 24 h, r.t.; (viii) acetic anhydride, dichloromethane, triethylamine, DMAP, 24 h, r.t.

^{1}H NMR, and MS spectral data as well as X-ray crystallographic analysis of **IXg** as a representative example that showed the cleavage of the cyclic imide (Figure 4). The IR showed broad band at 3500–3000 for the OH group, ^{1}H NMR exhibited exchangeable NH peak, in addition to the absence of one carbonyl cyclic imide peak in ^{13}C NMR. The acetate esters **Xa–l** were achieved using acetic anhydride, triethylamine and catalytic amount of 4-dimethylaminopyridine and were confirmed by the presence of both amidic and ester carbonyl peaks in IR and ^{13}C NMR.

2.2 | X-ray structure determination

The study of the title structure **IXg** was undertaken to establish its three-dimensional structure. Single crystals of **IXg** (CCDC#: 1854009) compatible for X-ray diffraction were achieved by slow evaporation of ethanol from ethanolic solution of the compound to give cubic colorless crystals with the following crystallographic parameters: a = 10.2710(4) Å, b = 19.4956(9) Å, c =10.5602(6) Å, α = 90.00°, β = 102.771(3)°, γ = 90.00°, V = 2062.3(2) Å^3, (Z) = 4, goodness-of-fit on F^2 = 1.146 (Table 1). The crystal structure of **IXg** is monoclinic with a space group of P2$_1$/n. All diagrams and calculations were performed using maXus. The X-ray crystal structure of **IXg** is shown in Figure 4.

2.3 | Anticonvulsant activity

2.3.1 | Phase I anticonvulsant evaluation

The initial pharmacological evaluation (phase 1 screening) was adopted using the standard procedure of Antiepileptic Drug Development (ADD) program of the National Institute of Neurological Disorders and Stroke (NINIDS), Epilepsy section.[34] This protocol consists of using the "gold standards" screens, namely subcutaneous pentylenetetrazole (scPTZ) screen and the maximal electroshock seizure (MES) screen. The anticonvulsant activity of the newly synthesized hydroxyethylamino derivatives **IXa–l** and their acetates **Xa–l** was evaluated using the scPTZ and MES tests and was expressed as % protection besides their neurotoxicity are presented in Tables 2 and 3. It was reported that PTZ test represents a valid model for generalized myoclonic seizures. By contrast, the MES test is a predictor of possible therapeutic efficacy against generalized tonic-clonic seizures.[35] The obtained data showed that all the compounds were effective in scPTZ test while most of them were effective against MES test except compounds **IXj**, **Xj**, and **Xk**.

All the scPTZ screening tests of the hydroxyethylamino derivatives **IXa–l** were more potent than the reference drugs (phenobarbital and ethosuximide) as they showed the same antiseizure profile (100% protection) at lower doses on molecular bases except compound **IXa** was less potent than phenobarbital. Compounds **IXa–l** dispalyed 100% protection against scPTZ-induced seizures at dose levels of 0.0253–0.283 mmol/kg ≡ 10–100 mg/kg as compared with phenobarbital (0.13 mmol/kg ≡ 30 mg/kg) and ethosuximide (1.06 mmol/kg ≡ 150 mg/kg) where compound **IXj** possessing the cycloheptane scaffold was the most active congener. It exhibited 11.9- and 196.8-fold more potent anticonvulsant effect than phenobarbital and ethosuximide, respectively. Concerning the MES test of **IXa–l**, the dose which displayed 100% anticonvulsant activity in the scPTZ screening or higher doses have been selected. In this screening test, compounds **IXa**, **IXe–g**, **IXi**, **IXk**, and **IXl** gave protection in half or more of the tested mice after 0.5 h post-administration. Meanwhile compounds **IXb**, **IXd**, and **IXh** exhibited 33% protection. On the other hand, only compound **IXj** did not exert protection against electro-induced seizures in MES test. In the neurotoxicity screen, all the target compounds did not show any minimal motor impairment in the maximum dose administered (Table 2).

Regarding the scPTZ screening of the amino ethyl acetate derivatives **Xa–l**, all the compounds were more potent than the reference drugs, except **Xa** and **Xk**. Moreover, all the compounds evoked 100% protection against scPTZ-induced seizures at dose levels

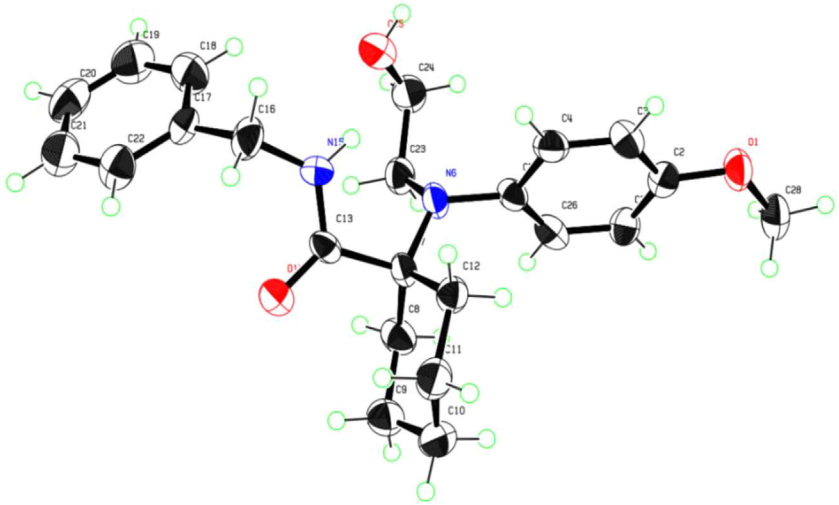


FIGURE 4 ORTEP diagram of **IXg** with thermal ellipsoids drawn at 50% probability

TABLE 1 Crystal data and structure refinement for **IXg**

CCDC number	1854009
Empirical formula	$C_{23}H_{30}N_2O_3$
Molecular weight	382.5
Temperature	298 K
Wavelength	0.71073
Crystal system	Monoclinic
Space group	$P2_1/n$
Cell dimensions	a = 10.2710 (4) Å
	b = 19.4956 (9) Å
	c = 10.5602 (6) Å
	α = 90.00°
	β = 102.771 (3)°
	γ = 90.00°
Volume	2062.26 (17) Å^3
Z	4
Density	1.232 Mg/m^3
Crystal size	0.200 × 0.3400.480 × mm
Absorption coefficient	0.081 mm
F000	824
Theta range for data collection	2.916–31.988°
Index range	$-14 \le h \le 14$, $0 \le k \le 27$, $0 \le l \le 15$
Absorption correction	Multi scan
Max. and min. transmission	0.98 and 0.61
Refinement method	Full matrix least square on F^2
No. of parameters	253
No. of restraints	0
Goodness-of-fit on F^2	1.146
R (reflections)	0.048 (2079)
wR2 (reflections)	0.15 (2079)
Data completeness	0.432

of 0.0057–0.2 mmol/kg ≡ 2.5–80 mg/kg as compared with the reference standards (Table 3). The most active congeners were compounds **Xh**, **Xd**, **Xf**, **Xl**, and **Xi**. They possessed more potent activities which reached 46.7-, 28-, 14-, 7.3-, and 7-fold than phenobarbital, and 770.8-, 462.5-, 231.3-, 121.7-, and 115.6-fold than ethosuximide, respectively.

MES test is considered a unique and effective screen test in detecting drugs among which is phenytoin that blocks human generalized tonic–clonic seizures and whose mechanism of action is reported to be via blocking the sodium channels. Interestingly, phenytoin acts via this mechanism of action and is reported to be active in MES test.[36] In this study, compound **Xf** displayed 100% activity in MES test at dose level 0.094 mmol/kg with ED_{50} = 0.0166 mmol/kg which is 2.3-fold more potent than the reference drug phenytoin. Meanwhile, compounds **Xa**,**b**, **Xg–i**, and **Xl** showed protection in half or more of the tested mice after 0.5 h post-administration. Moreover, compounds **Xc–e** exhibited 33% protection while compounds **Xj–k** displayed no activity in MES test. Therefore, it is suggested that the compounds that demonstrated activity in MES test could have a mode of action through inhibiting the voltage gated sodium channels.

Furthermore, the generalized myoclonic seizures induced subcutaneously by PTZ resemble generalized myoclonic seizures in human.[37] Previous studies have reported that scPTZ-induced seizures were prevented by drugs that enhance $GABA_A$ receptor-mediated inhibitory transmission such as phenobarbital[38] and also by drugs that inhibit T-type Ca^{2+} currents such as ethosuximide.[36,37,39] Accordingly, the findings of this study suggest that compounds **IXa–l** and **Xa–l** possibly exhibited their anticonvulsant potential through inhibiting or attenuating the PTZ-induced seizures in mice by enhancing the GABA-ergic neurotransmission and/or inhibiting Ca^{2+} channel.

In the neurotoxicity screen, all the test compounds did not show any minimal motor impairment in the maximum dose administered.

2.3.2 | Phase II anticonvulsant evaluation

In this study, we found that the acetate derivatives **Xa–l** were more active than their corresponding alcohol ones **IXa–l**. The promising results of phase I anticonvulsant activity encouraged us to undergo phase II anticonvulsant evaluation. The protective index (as a ratio of TD_{50} and ED_{50}) values for the compounds **Xa–l** was calculated. The most active compounds namely **Xd**, **Xf**, **Xh**, **Xi**, and **Xl** with the lowest ED_{50} showed protective index ranging from >5.68 to >2.89 using the dose that demonstrated 100% protection in the scPTZ test and showed no neurotoxicity. Also, for compound **Xf**, the PI was calculated according to the dose that showed 100% protection and devoid of neurotoxicity in the MES test. Compound **Xf** demonstrated similar PI index in both scPTZ and MES test.

Additionally, among the active acetate derivatives, we selected the most active compounds namely **Xd**, **Xf**, **Xh**, **Xi**, and **Xl** to investigate the possible hepatotoxic effect. Hepatotoxicity is considered as one of the most serious side effects of antiepileptic drugs. The serum enzyme activity assay was carried out for the most active compounds **Xd**, **Xf**, **Xh**, **Xi**, and **Xl**. Elevated liver enzymes are considered as specific markers of liver dysfunction. The data of liver enzymes analysis including: ALT, AST, and ALP, as well as albumin and total protein were represented as mean ± SEM in Table 4. The nominated compounds did not exhibit any significant change in serum enzymes level value as well as albumin and total protein as compared to control. This result demonstrated that there are no signs of hepatotoxicity. Moreover, all animals were devoid of acute toxicity signs at the tested dose range during the observation period.

2.3.3 | Structure–activity relationship (SAR)

As a result of the *in vivo* studies, the anticonvulsant effect of the synthesized compounds **IXa–l** and **Xa–l** in the scPTZ test is mostly attributed to the electron donating properties of $-CH_3$ or $-OCH_3$ groups at the four position of the anilino ring, the benzyl or phenethyl

TABLE 2 Anticonvulsant and neurotoxicity evaluation of compounds **IXa–l** as well as the reference drugs against subcutaneous pentylenetertrazole and electro-induced seizures in mice, as well as their fit values

	scPTZ		MES				
Compound no.	Dose[a] mg/kg (mmol/kg)	Max. % protection	Dose mg/kg (mmol/kg)	Max. % protection	Neurotoxicity[b]	ED_{50} mg/kg (confidence limits)[c] ED_{50} mmol/kg (confidence limits)[c]	Fit value
IXa	100 (0.283)	100	100 (0.283)	50	0/6	35.14 (44.39–26.42)	2.990
						0.099 (0.125–0.074)	
IXb	20 (0.054)	100	20 (0.054)	–	0/6	3.723 (4.122–3.367)	2.995
			40 (0.1)	33.3	0/6	0.01 (0.011–0.009)	
IXc	40 (0.1)	100	40 (0.1)	16.7	0/6	7.462 (8.277–6.734)	2.993
						0.02 (0.022–0.018)	
IXd	40 (0.1)	100	40 (0.1)	–	0/6	8.958 (11.45–6.872)	2.978
			80 (0.2)	33.3	0/6	0.023 (0.029–0.017)	
IXe	20 (0.054)	100	20 (0.054)	50	0/6	4.482 (5.588–3.346)	2.998
						0.012 (0.015–0.009)	
IXf	80 (0.21)	100	80 (0.21)	50	0/6	12.37 (12.4–12.33)	2.996
					0/6	0.032 (0.0325–0.0324)	
IXg	100 (0.26)	100	100 (0.26)	50	1/6	14.47 (17.15–12.05)	2.981
						0.037 (0.044–0.031)	
IXh	40 (0.1)	100	40 (0.1)	–	0/6	8.953 (12.21–6.334)	2.988
			100 (0.252)	33.3	0/6	0.022 (0.03–0.015)	
IXi	20 (0.052)	100	20 (0.052)	50	0/6	3.319 (3.622–3.035)	2.995
						0.0087 (0.009–0.0079)	
IXj	10 (0.0253)	100	10 (0.0253)	–	0/6	1.862 (2.061–1.683)	2.988
						0.0047 (0.005–0.0042)	
IXk	40 (0.1)	100	40 (0.1)	33.3	0/6	7.446 (8.243–6.734)	2.980
			120 (0.3)	66.7	0/6	0.018 (0.02–0.0169)	
IXl	40 (0.097)	100	40 (0.097)	66.7	0/6	16.14 (20.39–12.26)	2.980
						0.039 (0.049–0.0298)	
Phenobarbital	30 (0.13)	100	–	–	nd	13.2 (15.90–6.80)	1.980
						0.056 (0.068–0.029)	
Ethosuximide	150 (1.06)	100	–	–	nd	130.55 (177.87–98.78)	–
						0.925 (1.260–0.699)	
Phenytoin	–	–	45 (0.16)	100	nd	9.50 (10.96–7.24)	–
						0.0376 (0.0434–0.0287)	

– Indicates absence of activity. nd: not determined.

[a]The minimal dose which exhibited the maximum anticonvulsant activity in scPTZ test.

[b]Rotarod test: number of mice exhibiting neurotoxicity (falling of the rotarod)/number of mice tested. Control group demonstrated 0/6 in the neurotoxicity test and were devoid of anticonvulsant activity.

[c]ED_{50}: median effective dose in scPTZ test.

carboxamide side chain, in addition to the cycloalkyl ring size scaffold (cyclopentyl, cyclohexyl, or cycloheptyl), Tables 2 and 3, respectively.

In the hydroxyethyl amino derivatives linked to cyclopentane ring **IXa–d**, it was found that **IXb** was the most active congener. It contains the cyclopentyl ring linked to both electron donating $-CH_3$ group at the four position of the anilino moiety, and phenethyl carboxamide side chain. In **IXc** and **IXd** when $-OCH_3$ as electron releasing group replaced the $-CH_3$ at the four position of the anilino moiety in the presence of either benzyl or phenethyl carboxamide side chains, respectively, nearly equipotent anticonvulsant activity was observed. The least anticonvulsant activity was observed with both 4-methyl anilino and benzyl carboxamide substituent in compound **IXa**. In the cyclohexane series **IXe–h**, the most active congeners were **IXe** having the 4-methyl anilino and the benzyl carboxamide side chain,

TABLE 3 Anticonvulsant and neurotoxicity evaluation of compounds **Xa–l** as well as the reference drugs against subcutaneous pentylenetetrazole and electro-induced seizures in mice, as well as their fit values

	scPTZ		MES						
Compound no.	Dose[a] mg/kg (mmol/kg)	Max. % protection	Dose mg/kg (mmol/kg)	Max. % protection	Neurotoxicity[b]	ED_{50} mg/kg (confidence limits)[d] ED_{50} mmol/kg (confidence limits)[d]	TD_{50}	PI[e]	Fit value
Xa	80 (0.2)	100	80 (0.2)	66.7	0/6	40.0 (45.27–24.97)	>80	>2.00 (scPTZ)	2.998
						0.101 (0.115–0.063)			
Xb	20 (0.048)	100	20 (0.097)	66.7	0/6	4.341 (5.174–3.640)	>20	>4.61 (scPTZ)	2.992
						0.011 (0.013–0.009)			
Xc	40 (0.097)	100	40 (0.097)	33.3	0/6	14.140 (14.250–14.020)	>40	>2.83 (scPTZ)	2.993
						0.034 (0.035–0.0.34)			
Xd*	5 (0.011)	100	5 (0.011)	–	0/6	1.083 (1.429–0.785)	>5	>4.62 (scPTZ)	2.9991
			40 (0.094)	33.3	0/6	0.0025 (0.0033–0.0018)			
Xe	20 (0.048)	100	20 (0.048)	33.3	0/6	6.162 (6.284–6.043)	>20	>3.25 (scPTZ)	2.997
					0/6	0.0151 (0.0154–0.0148)			
Xf*	10 (0.023)	100	10 (0.023)	66.7	0/6	7.05 (6.792–7.315)[c]	>40 (MES)	>5.67 (MES)	2.992
			40 (0.094)	100	0/6	0.0166 (0.016–0.017)[c]	>10	>5.68 (scPTZ)	
						1.762 (1.829–1.698)			
						0.0042 (0.0043–0.0040)			
Xg	20 (0.047)	100	20 (0.047)	50	1/6	4.934 (5.298–4.597)	>20	>4.05 (scPTZ)	2.98
						0.011 (0.012–0.011)			
Xh*	2.5 (0.0057)	100	2.5 (0.0057)	–	0/6	0.542 (0.776–0.358)	>2.5	>4.61 (scPTZ)	2.993
			10 (0.022)	50	0/6	0.0012 (0.0018–0.0008)			
Xi*	10 (0.023)	100	10 (0.023)	50	0/6	3.459 (3.832–3.105)	>10	>2.89 (scPTZ)	2.61
					0/6	0.008 (0.009–0.007)			
Xj	40 (0.091)	100	40 (0.091)	–	0/6	16.12 (16.82–15.43)	>40	>2.48 (scPTZ)	2.992
			80 (0.183)	–	0/6	0.036 (0.038–0.035)			
Xk	80 (0.182)	100	80 (0.182)	–	0/6	32.23 (33.65–30.85)	>80	>2.48 (scPTZ)	2.996
					0/6	0.073 (0.076–0.070)			
Xl*	10 (0.022)	100	10 (0.022)	–	0/6	3.459 (3.832–3.105)	>10	>2.89 (scPTZ)	2.987
			40 (0.088)	50	0/6	0.0076 (0.0084–0.0068)			
Phenobarbital	30 (0.13)	100	–	–	nd	13.2 (15.90–6.80)	nd	nd	1.98
						0.056 (0.068–0.029)			

(Continues)

TABLE 3 (Continued)

Compound no.	scPTZ		MES						
	Dose[a] mg/kg (mmol/kg)	Max. % protection	Dose mg/kg (mmol/kg)	Max. % protection	Neurotoxicity[b]	ED_{50} mg/kg (confidence limits)[d] ED_{50} mmol/kg (confidence limits)[d]	TD_{50}	PI[e]	Fit value
Ethosuximide	150 (1.06)	100	-	-	nd	130.55 (177.87–98.78)	350.10 (346.20–268.90)	2.68	-
						0.925 (1.260–0.699)			
Phenytoin	-	-	45 (0.16)	100	nd	9.50 (10.96–7.24)	62.64 (69.82–50.52)	6.59	-
						0.0376 (0.0434–0.0287)			

– Indicates absence of activity. nd: not determined.
[a]The minimal dose which exhibited the maximum anticonvulsant activity in scPTZ test.
[b]Rotarod test: number of mice exhibiting neurotoxicity (falling of the rotarod)/number of mice tested. Control group demonstrated 0/6 in the neurotoxicity test and were devoid of anticonvulsant activity.
[c]ED_{50}: median effective dose in MES test.
[d]ED_{50}: median effective dose in scPTZ test.
[e]Protective index (TD_{50}/ED_{50}).
*Most active compounds.

then **IXh** having the 4-methoxyanilino and the phenethyl carboxamide side chain. By increasing the cycloalkane ring size as in the cycloheptane series **IXi–l**, compound **IXj** was the most active congener where the cycloheptane ring is linked to both the 4-methyl anilino and the phenethyl carboxamide moieties. The different congeners of **IXa–l** exhibited anticonvulsant potential in the following decreasing order: **IXj** > **IXi** > **IXb** = **IXe** > **IXk** > **IXc** = **IXd** = **IXh** > **IXf** > **IXg** = **IXl** > **IXa**.

Regarding the scPTZ screening of the amino ethyl acetate derivatives, it was found that compound **Xd** was the most acive congener among **Xa–d** where the cyclopentane ring was attached to the phenethyl carboxamide side chain and the anilino group having at the 4-position the electron releasing OCH_3 group. Moreover, **Xd** showed the highest fit value among **Xa–l** in the 3D pharmacophore model. Meanwhile, replacement of the methoxy group with the electron donor $-CH_3$ group decreased the anticonvulsant activity as in **Xb** and **Xc** regardless whether the side chain was benzyl or phenethyl. In compounds **Xe–h** bearing the cyclohexane scaffold, the most active congeners were **Xh** then **Xf** where the cyclohexyl ring was linked to the phenethyl carboxamide side chain and either electron donor 4-OCH_3 or 4-CH_3 anilino groups, respectively. Also, the anticonvulsant effect was reduced in **Xe** and **Xg** on linking the cyclohexyl scaffold with the benzyl carboxamide side chain and with either the 4-CH_3 or 4-OCH_3 anilino groups, respectively. Increasing the size of the cycloalkane ring to cycloheptane **(Xi–l)** produced two of the most active congeners **Xl** having 4-OCH_3 anilino and phenethyl carboxamide moieties, then **Xi** possessing 4-CH_3 anilino and benzyl carboxamide moieties, where compound **Xl** possessed higher fit value than **Xi** in the 3D pharmacophore model. Surprisingly, compounds possessing the cycloheptane nucleus showed less anticonvulsant potentiality than their congeners in the cyclopentane and cyclohexane series where compounds **Xj–l** were less potent than their analogues in the cyclopentyl and cyclohexyl series, **Xb–d** and **Xf–h**, respectively. One explanation of this reduced activity might be due to the low solubility of the more lipophilic analogs in the aqueous plasma where they easily bind to plasma proteins which may lead to lower drug concentrations available for diffusion into the CNS.[40] Hence, delivering a drug via the circulatory system for treatment of CNS diseases requires a balance between BBB permeability and plasma solubility. The different congeners of the acetate series showed anticonvulsant potential in the following decreasing order: **Xh** > **Xd** > **Xf** > **Xl** = **Xi** > **Xb** = **Xg** = **Xe** > **Xc** = **Xj** > **Xk** > **Xa**. The results of their fit values were consistent with the anticonvulsant activity in the scPTZ test.

Generally, the acetate derivatives **Xa–l** showed better anticonvulsant activity than the precursor hydroxyethylamino derivatives **IXa–l**. Also, the occurrence of electron donating group such as $-OCH_3$ or $-CH_3$ at position 4 of the anilino moiety improves the anticonvulsant activity. The phenethyl carboxamide side chain as a substituent of the cycloalkyl ring is preferable than the benzyl carboxamide one. The presence of both 4-OCH_3 anilino and phenethyl carboxamide fragments in one molecule augment the activity. The cyclopentyl and cyclohexyl ring size may be preferable than the cycloheptyl one in the acetate derivatives.

TABLE 4 Determination of liver enzyme, albumin and total protein with the most active compounds **Xd**, **Xf**, **Xh**, **Xi**, and **Xl**

Compound no.	ALT (U/L)	AST (U/L)	ALP (U/L)	Albumin (g/dL)	Total protein (g/dL)
Control	23.00 ± 2.51	70.00 ± 7.76	12.00 ± 0.64	3.14 ± 0.34	7.30 ± 0.06
Xd	20.18 ± 3.15	58.67 ± 2.25	13.95 ± 1.25	3.41 ± 0.07	7.78 ± 0.33
Xf	18.46 ± 2.93	57.00 ± 4.58	10.76 ± 2.86	3.28 ± 0.14	8.21 ± 0.64
Xh	29.42 ± 0.95	74.33 ± 10.38	11.06 ± 0.48	3.35 ± 0.12	7.71 ± 0.31
Xi	22.95 ± 4.46	85.78 ± 7.15	12.35 ± 0.74	3.85 ± 0.07	8.11 ± 0.08
Xl	20.65 ± 0.38	81.67 ± 4.25	10.35 ± 1.61	3.50 ± 0.07	9.00 ± 0.38

Data are represented as mean ± SEM and were analyzed by ANOVA followed by Student Newman Keul test, $n = 8$. ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase.

2.4 | Molecular modeling study

The molecular modeling study was accomplished aiming to analyze and investigate the anticonvulsant activity of compounds **IXa–l** and **Xa–l**. This was done by running the common feature pharmacophore model protocol in Discovery Studio 2016Biova 1 software. The essential pharmacophoric elements of antiepileptic drugs and numerous anticonvulsant compounds include the occurrence of ring aromatic (RA), hydrogen bond acceptor (HBA), and hydrophobic moiety (HY). The used training sets containing the essential features to generate the pharmacophore model were demonstrated in Figure 5. In addition, the generated hypothetical pharmacophore model was presented in Figure 6, which might be responsible for interface with the receptor active site. Distances and angles between the pharmacophoric features of the training sets (A, HBA, HY) are shown in Table 5.[41–44]

The validation of the generated pharmacophore was based on full mapping of all essential features and high fit values with the used training sets (compounds **2–11**, Figure 7). Validating retrospectively the latter simulated fit values of test set compounds **IXa–l** and **Xa–l** with the given hypothesis were more consistent with the experimental values than other hypotheses. The fit values of test compounds **IXa–l** and **Xa–l** are shown in Tables 2 and 3. Mapping of anticonvulsants pharmacophoric features of the most potent acetate ester candidates according to their ED_{50} values, **Xh**, **Xd**, **Xf**, **Xi**, and **Xl**, and phenobarbital is illustrated in Figure 8.

3 | CONCLUSION

The anticonvulsant potential of certain 1-((2-hydroxyethyl)(aryl) amino)-*N*-substituted cycloalkanecarboxamides **IXa–l** and 2-((1-substituted carbamoyl)cycloalkyl)(aryl)amino)ethyl acetates **Xa–l** was investigated. All the compounds displayed 100% anticonvulsant activity in the scPTZ screen at a dose range from 0.0057 to 0.283 mmol/kg. It could be concluded that the acetate esters were more potent than their starting alcohols due to expected higher lipophilicity. The most potent compounds in the scPTZ screen were **Xh** (ED_{50} = 0.0012 mmol/kg), **Xd** (ED_{50} = 0.002 mmol/kg), **Xf** (ED_{50} = 0.004 mmol/kg), **IXj** (ED_{50} = 0.0047 mmol/kg), **Xl** (ED_{50} = 0.0076 mmol/kg), and **Xi** (ED_{50} = 0.008 mmol/kg). They possessed more potent activities which reached 46.7-, 28-, 14-, 11.9-, 7.3-, and 7-fold than phenobarbital, and 770.8-, 462.5-, 231.3-, 196.8-, 121.7-, and 115.6-fold than ethosuximide, respectively. It is noteworthy to mention that compound **Xf** was active in both scPTZ and MES screens. It showed ED_{50} of 0.016 mmol/kg in MES screens which is 2.3-fold more potent than phenytoin. In the neurotoxicity screen, none of the test compounds evoked any minimal motor impairment at the maximum administered dose. Furthermore, the achieved molecular simulation fitting to the anticonvulsant 3D-pharmacophore model exhibited high-fit values. The determined fitting scores were correlated with the scPTZ experimental results.

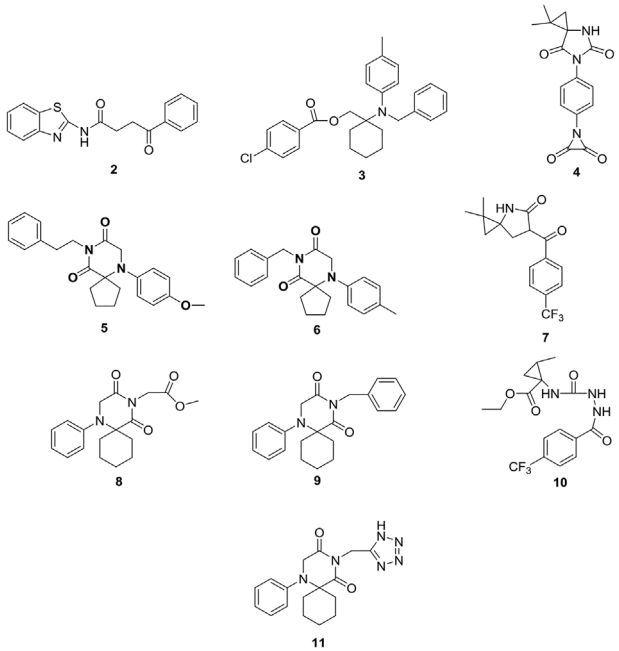


FIGURE 5 Representative examples of anticonvulsants (training set) with their ED_{50} values in scPTZ test. **2**: ED_{50} = 0.49 mmol/kg[42]; **3**: ED_{50} = 0.0056 mmol/kg[44]; **4**: ED_{50} = 0.45 mmol/kg[1]; **5**: ED_{50} = 0.011 mmol/kg[29]; **6**: ED_{50} = 0.017 mmol/kg[29]; **7**: ED_{50} = 0.19 mmol/kg[1]; **8**: ED_{50} = 0.012 mmol/kg[29]; **9**: ED_{50} = 0.071 mmol/kg[29]; **10**: ED_{50} = 0.15 mmol/kg[41]; **11**: ED_{50} = 0.019 mmol/kg[29]

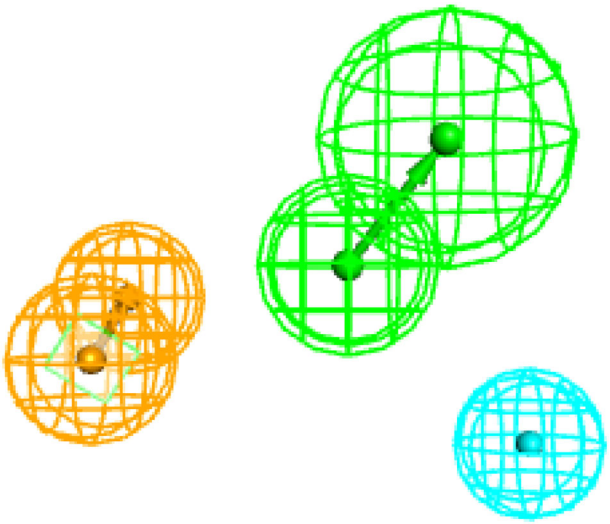


FIGURE 6 Pharmacophore model of anticonvulsants generated by Discovery Studio 2016 Biova 1 software. Pharmacophore features are colored: green for hydrogen bond acceptor (HBA), orange for aromatic region (RA), and light blue for hydrophobic region (HY)

Interestingly, these compounds could be considered as promising antiepileptic candidates.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

Mass spectra were run on Jeol JMS-AX500 mass spectrometer. Elemental analysis were carried out in Microanalytical Units at National Research Centre and the results were within ±0.4% of the theoretical value. Infrared (IR) spectra were recorded as thin film (for oils) in KBr discs or as KBr pellets (for solids) with Jasco IR and FTIR-6100 spectrometer and values are represented in cm^{-1}. ^1H NMR and ^{13}C NMR spectra were carried out on Jeol ECA 500 MHz or Bruker Avance 300 spectrometers using TMS as internal standard. Chemical shift values are recorded in ppm δ scale. The ^1H NMR data were represented as follows: chemical shifts, multiplicity (s. singlet, br.s. broad singlet, d. doublet, t. triplet, and m. multiplet), number of protons, and type of protons. ^{13}C NMR data are represented as chemical shifts and type of carbon atoms.

The X-ray was performed using maXus (Bruker Nonius, Delft & MacScience, Japan) at the X-ray Unit, National Research Centre. Purification was performed using column chromatography and solvent system petroleum ether (40–60)/ethyl acetate (7:3) for compounds **VIIIa–f** and dichloromethane/ethyl acetate (9:1) for compounds **IXa–l** and **Xa–l**. Also, aluminum oxide 60G F_{254} neutral plates for TLC from Merck were used for thin layer chromatography. Visualization was performed by illumination with UV-light source (254 nm).

Compounds **IIIa–e**, **IVa–f**, **Va–d**, **VIa–d**, **VIIa–d**, **VIIIa–f** were prepared according to adopted procedures.[17,29,31,32] Please also see the Supporting Information for further details.

The InChI codes of the investigated compounds together with some biological activity data are provided as Supporting Information.

TABLE 5 The constraint distances and angles between the features of the generated anticonvulsant pharmacophore model

Dimensions	Features of the given anticonvulsant model
Constraint distances (Å) between feature	HBA-RA: 5.53
	HBA-HY: 8.55
	RA-HY: 10.02
Constraint angles between features	HBA-HY-RA: 32.38
	HBA-RA vector: 64.73
	HY-HBA vector: 106.73
	HY-RA vector: 87.59

4.1.2 | Synthesis of 1-[(4-methoxyphenyl)amino]-cycloheptanecarbonitrile (IIIf)

Compound **IIIf** was synthesized according to the reported procedure.[31] Yellow solid, m.p. 58°C, yield 73.68%. IR (KBr, cm^{-1}): 2221 (nitrile group); MS (EI) *m/z* (%): $C_{15}H_{20}N_2O$, 244 (M^+, 39), 122 (100).

4.1.3 | Synthesis of 1-[(aryl)(cyanomethyl)amino]-cycloheptanecarboxamides Ve,f

Compounds **Ve**,**f** were synthesized according to the reported procedure.[33]

1-[(Cyanomethyl)(4-methylphenyl)amino]-cycloheptanecarboxamide (Ve)

Buff solid, m.p. 84°C, yield 82%. IR (KBr, cm^{-1}): 2220 (nitrile group), 1671 (amide carbonyl group); MS (EI) *m/z* (%): $C_{17}H_{23}N_3O$, 285 (M^+, 19), 57 (100); ^1H NMR ($CDCl_3$) δ ppm 1.46–2.2 (m, 12H, 6 x CH_2, cycloheptyl), 2.27 (s, 3H, CH_3), 3.9 (s, 2H, CH_2-CN), 6 (s, 2H, NH_2), 6.5–7 (m, 4H, $H_{ar.}$); ^{13}C NMR ($CDCl_3$) δ ppm 20.51 (CH_3), 22.92, 29.9, 36.2 (6 x CH_2, cycloheptyl), 41 ($\underline{CH_2}$-CN), 63.68 (Cq), 116 (CN), 116.09, 129.68 ($CH_{ar.}$), 128.03, 142.26 (2 x $C_{ar.}$), 180.34 (C═O).

1-[(Cyanomethyl)(4-methoxyphenyl)amino]-cycloheptanecarboxamide (Vf)

Brown solid, m.p. 84–85°C, yield 95%.MS (EI) *m/z* (%): $C_{17}H_{23}N_3O_2$, 301 (M^+, 10), 218 (100); ^1H NMR ($CDCl_3$) δ ppm

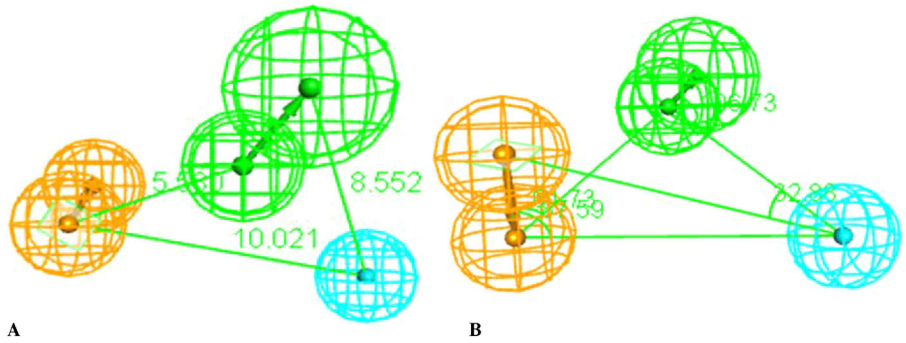


FIGURE 7 (A and B) Constraint distances and angels for pharmacophore model of the training set (**2–11**), test set (**IXa–l** and **Xa–l**) and phenobarbital. The chemical features colored green, orange, and light blue represent hydrogen bonding acceptor (HBA), ring aromatic (RA), and hydrophobic features (HY), respectively

1.2–2 (m, 12H, 6 x CH_2, cycloheptyl), 3.7 (s, 3H, OCH_3), 4.1 (s, 2H, CH_2-CN), 6.3 (s, 2H, NH_2), 6.6–7 (m, 4H, $H_{ar.}$); ^{13}C NMR ($CDCl_3$) δ ppm 22.9, 29.9, 34.2 (6 x CH_2, cycloheptyl), 41.4 ($\underline{CH_2}$-CN), 55.4 (OCH_3), 64.6 (Cq), 116 (CN), 114.59, 128.6 ($CH_{ar.}$), 141.2, 157.8 (2 x $C_{ar.}$), 180 (C═O).

4.1.4 | Synthesis of [(aryl)(1-carbamoylcycloheptyl)-amino]acetic acids VIe,f

Compounds **VIe**,**f** were synthesized according to the reported procedure.[33]

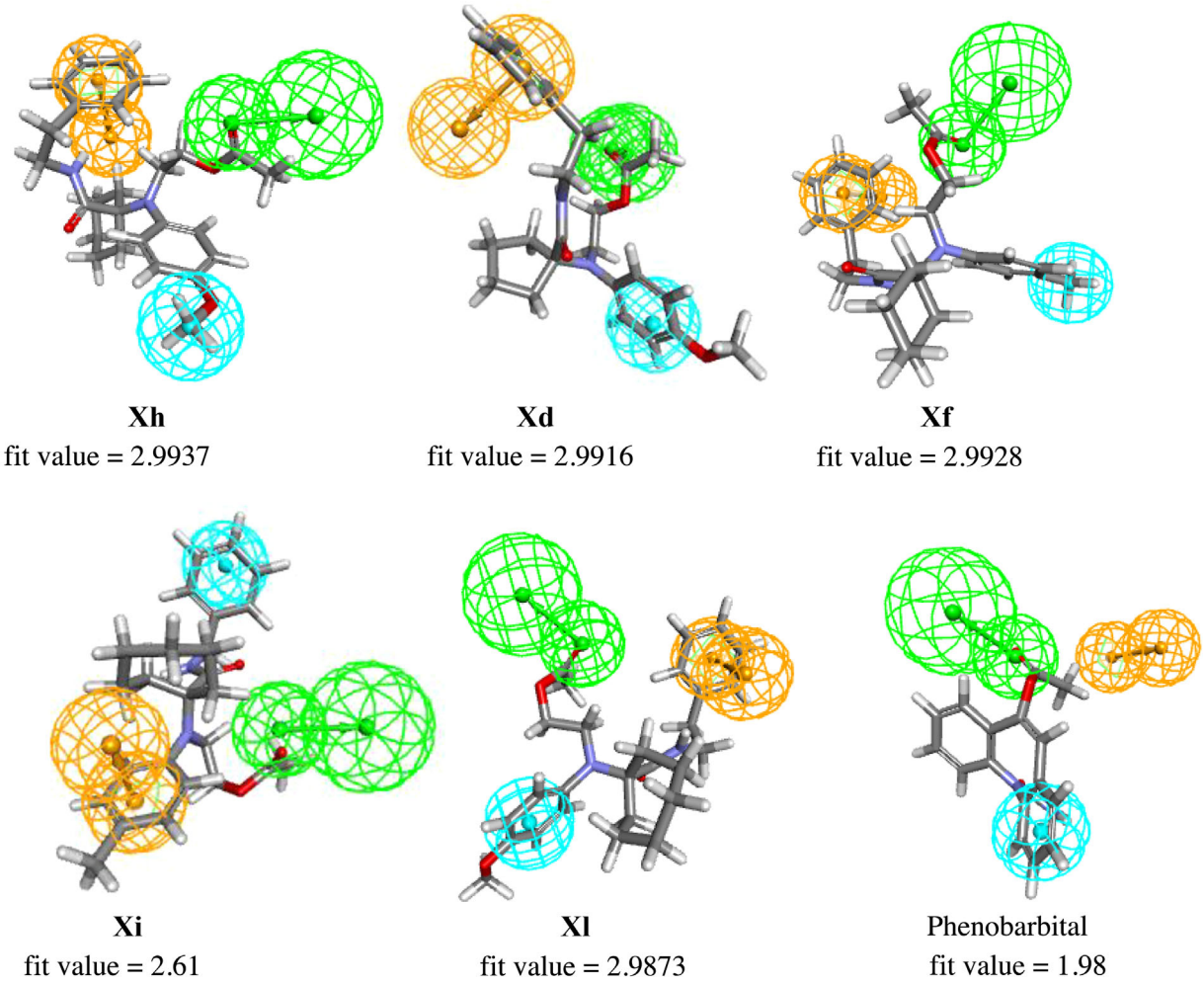


FIGURE 8 Mapping of anticonvulsants pharmacophore fragments of the most active test compounds **Xh**, **Xd**, **Xf**, **Xl**, **Xi**, and phenobarbital

[(1-Carbamoylcycloheptyl)(4-methylphenyl)amino]acetic acid (VIe)

Yellow solid, m.p. 80–82°C, yield 70%. MS (EI) *m/z* (%): $C_{17}H_{24}N_2O_3$, 304 (M^+, 21), 203 (100); ^{1}H NMR ($CDCl_3$) δ ppm 1.52–2.2 (m, 12H, 6 x CH_2, cycloheptyl), 2.29 (s, 3H, CH_3), 4.1 (s, 2H, $\underline{CH_2}$-COOH), 6.7–7 (m, 4H, $H_{ar.}$), 7.08 (s, 2H, NH_2), 8.6 (s, H, OH); ^{13}C NMR ($CDCl_3$) δ ppm 20.52 (CH_3), 23.87, 30.3, 35.93 (6 x CH_2, cycloheptyl), 50.06 ($\underline{C}H_2$-COOH), 69.4 (Cq), 119.8, 129 ($CH_{ar.}$), 129.9, 143.24 (2 x $C_{ar.}$), 177.61, 183.64 (2 x C═O).

[(1-Carbamoylcycloheptyl)(4-methoxyphenyl)amino]acetic acid (VIf)

Brown viscous oil, yield 84%. IR (KBr, cm^{-1}): 3431 (COOH), 1658 (C═O, amide), 1720 (C═O, carboxylic acid); MS (EI) *m/z* (%): $C_{17}H_{24}N_2O_4$, 320 (M^+, 6), 218 (100); ^{1}H NMR ($CDCl_3$) δ ppm 1.46–2.04 (m, 12H, 6 x CH_2, cycloheptyl), 3.73 (s, 3H, OCH_3), 3.9 (s, 2H, $\underline{CH_2}$-COOH), 6.5–6.8 (m, 4H, $H_{ar.}$), 7.06 (s, 2H, NH_2), 8.3 (s, H, OH); ^{13}C NMR ($CDCl_3$) δ ppm 23.78, 29.7, 35.7 (6 x CH_2, cycloheptyl), 52 ($\underline{C}H_2$-COOH), 55.5 (OCH_3), 70.18 (Cq), 114.1, 125 ($CH_{ar.}$), 139.15, 155.81 (2 x $C_{ar.}$), 176.5, 182.8 (2 x C═O).

4.1.5 | Synthesis of 1-aryl-1,4-diazaspiro[5.5]-dodecane-3,5-diones VIIe,f

Compounds **VIIe**,**f** were synthesized according to the reported procedure.[33]

1-(4-Methylphenyl)-1,4-diazaspiro(5,6)dodecane-3,5-dione (VIIe)

White solid, m.p. 120°C, yield 60%. MS (EI) *m/z* (%): $C_{17}H_{22}N_2O_2$, 286 (M^+, 40), 105 (100); ^{1}H NMR ($CDCl_3$) δ ppm 1.462–2.024 (m, 12H, 6 x CH_2, cycloheptyl), 2.213 (s, 3H, CH_3), 3.92 (s, 2H, O═C-$C\underline{H_2}$-N), 6.893 (d, 2H, *J* = 10 Hz, $H_{ar.}$), 6.998 (d, 2H, *J* = 10 Hz, $H_{ar.}$), 8.85 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ ppm 20.9 (CH_3) 22.31, 29.55, 34.11 (6 x CH_2, cycloheptyl), 54.43 (O═C-$\underline{C}H_2$-N), 64.64 (Cq), 126.95, 129.95 ($CH_{ar.}$), 135.85, 145.8 (2 x $C_{ar.}$), 172.25, 177.44 (2 x C═O).

1-(4-Methoxyphenyl)-1,4-diazaspiro(5,6)dodecane-3,5-dione (VIIf)

Buff solid, m.p. 92°C, yield 60%. IR (KBr, cm^{-1}): 3426 (NH) and 1675, 1641 (C═O, imide carbonyl); MS (EI) *m/z* (%):$C_{17}H_{22}N_2O_3$, 302 (M^+, 100); ^{1}H NMR ($CDCl_3$) δ ppm 1.23–2.05 (m, 12H, 6 x CH_2, cycloheptyl), 3.7 (s, 3H, OCH_3), 3.97 (s, 2H, O═C-$\underline{CH_2}$-N), 6.78 (d, 2H, *J* = 8.6 Hz, $H_{ar.}$), 7 (d, 2H, *J* = 8.6 Hz, $H_{ar.}$), 8.3 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ ppm 22.29, 29.73, 34.12 (6 x CH_2, cycloheptyl), 54.41 (OC-$\underline{C}H_2$-NH_2), 55.49 (OCH_3), 65.06 (Cq), 114.51, 128.37 ($CH_{ar.}$), 141.16, 157.78 (2 x $C_{ar.}$), 171.96, 177.15 (2 x C═O).

4.1.6 | Synthesis of 1-aryl-4-substituted-1,4-diazaspiro[5.5]-undecane-3,5-diones VIIIi–l

Compounds **VIIIi–l** were synthesized according to the reported procedure.[29]

4-Benzyl-1-(4-methylphenyl)-1,4-diazaspiro(5.6)dodecane-3,5-dione (VIIIi)

Yellow viscous oil; yield (67%). IR (KBr, cm^{-1}): 1725, 1675 (C═O, imide carbonyls); MS (EI) *m/z* (%): $C_{24}H_{28}N_2O_2$, 376.33 (M^+, 93.62), 91 (100); ^{1}H NMR ($CDCl_3$) δ ppm 1.27–2.02 (m, 12H, 6 x CH_2, cycloheptyl), 2.26 (s, 3H, CH_3), 4.08 (s, 2H, O═C-$C\underline{H_2}$-N), 5.04 (s, 2H, $C\underline{H_2}$-C_6H_6), 6.7 (d, 2H, *J* = 8.6 Hz, $H_{ar.}$), 6.9 (d, 2H, *J* = 8.6 Hz, $H_{ar.}$), 7.3–7.4 (m, 5H, $H_{ar.}$); ^{13}C NMR ($CDCl_3$) δ ppm 22.36, 29.83, 34.8 (6 x CH_2, cycloheptyl), 32.05 (CH_3), 42.7 ($\underline{CH_2}$-C_6H_5), 55.04 (O═C-$\underline{CH_2}$-NH_2), 64.9 (Cq), 127.11, 127.6, 128.5, 129.2, 129.8 ($CH_{ar.}$), 135.7, 137.4, 145.8 (3 x $C_{ar.}$), 171.07, 176.6 (2 x C═O).

1-(4-Methylphenyl)-4-(2-phenethyl)-1,4-diazaspiro(5.6)-dodecane-3,5-dione (VIIIj)

Yellow viscous oil, yield 61%. IR (KBr, cm^{-1}): 1728, 1677 (C═O, imide carbonyls); MS (EI) *m/z* (%): $C_{25}H_{30}N_2O_2$, 390.05 (M^+, 26.69), 271 (91.52), 91 (100); ^{1}H NMR ($CDCl_3$) δ ppm 1.494–2.026 (m, 12H, 6 x CH_2, cycloheptyl), 2.285 (s, 3H, CH_3), 2.88 (t, *J* = 10 Hz, 2H, $C\underline{H_2}$-C_6H_5), 4.03 (s, 2H, O═C-$C\underline{H_2}$-N), 4.1 (t, *J* = 10 Hz, 2H, $C\underline{H_2}$-CH_2-C_6H_5), 6.8 (d, 2H, *J* = 8 Hz, $H_{ar.}$), 7.02 (d, 2H, *J* = 8 Hz, $H_{ar.}$), 7.203–7.296 (m, 5H, $H_{ar.}$); ^{13}C NMR ($CDCl_3$) δ ppm 20.89 (CH_3) 22.39, 29.63, 34.63 (6 x CH_2, cycloheptyl), 33.98 ($\underline{C}H_2$-C_6H_5), 40.55 ($\underline{C}H_2$-CH_2-C_6H_6), 54.84 (O═C-$\underline{C}H_2$-N), 64.83 (Cq), 126.53, 126.94, 128.47, 129.07, 129.88 ($CH_{ar.}$), 135.68, 138.4, 145.94 (3 x $C_{ar.}$), 170.87, 176.6 (2 x C═O).

4-Benzyl-1-(4-methoxyphenyl)-1,4-diazaspiro[5.6]dodecane-3,5-dione (VIIIk)

Yellow viscous oil, yield 80%. IR (KBr, cm^{-1}): 1720, 1673 (C═O, imide carbonyl); MS (EI) *m/z* (%): 392.25 (M^+, 100), 91 (100); ^{1}H NMR ($CDCl_3$) δ ppm 1.53–2.033 (m, 12H, 6 x CH_2, cycloheptyl), 3.7 (s, 3H, OCH_3), 4.04 (s, 2H, O═C-$C\underline{H_2}$-N), 5.02 (s, 2H, $C\underline{H_2}$-C_6H_5), 6.6 (d, 2H, *J* = 8.6 Hz, $H_{ar.}$), 6.77 (d, 2H, *J* = 8.6 Hz, $H_{ar.}$), 7.3–7.39 (m, 5H, $H_{ar.}$); ^{13}C NMR ($CDCl_3$) δ ppm 22.28, 29.63, 34.7 (6 x CH_2, cycloheptyl), 42.6 ($\underline{C}H_2$-C_6H_5), 54.9 (OC-$\underline{C}H_2$-N), 55.42 (OCH_3), 65.25 (Cq), 114.307, 127.66, 128.44, 128.5, 129.2 ($CH_{ar.}$), 136.95, 141.2, 157.6 (3 x $C_{ar.}$), 171.1, 176.5 (2 x C═O).

1-(4-Methoxyphenyl)-4-(2-phenylethyl)-1,4-diazaspiro[5.6]-dodecane-3,5-dione (VIIIl)

Yellow viscous oil, yield 60%. IR (KBr, cm^{-1}): 1722, 1673 (C═O, imide carbonyl); MS (EI) *m/z* (%): $C_{25}H_{30}N_2O_3$, 406.26 (M^+, 100); ^{1}H NMR ($CDCl_3$) δ pp m 1.4–1.97 (m, 12H, 6 x CH_2, cycloheptyl), 2.89 (t, *J* = 7.6 Hz, 2H, $C\underline{H_2}$-C_6H_5), 3.75 (s, 3H, OCH_3), 4 (s, 2H, O═C-$C\underline{H_2}$-N), 4.08 (t, *J* = 7.6 Hz, 2H, $C\underline{H_2}$-CH_2-C_6H_5), 6.7 (d, 2H, *J* = 9.6 Hz, $H_{ar.}$), 6.9 (d, 2H, *J* = 9.6 Hz, $H_{ar.}$), 7.22–7.29 (m, 5H, $H_{ar.}$); ^{13}C NMR ($CDCl_3$) δ ppm 22.37, 29.82, 34.62 (6 x CH_2, cycloheptyl), 34 ($\underline{C}H_2$-C_6H_5), 40.5 ($\underline{C}H_2$-CH_2-C_6H_6), 54.6 (O═C-$\underline{C}H_2$-N), 55.4 (OCH_3), 65.1 (Cq), 114.4, 126.6, 128.3, 128.5, 129.1 ($CH_{ar.}$), 138.4, 141.2, 157.6 (3 x $C_{ar.}$), 171.07, 176.64 (2 x C═O).

4.1.7 | General procedure for the synthesis of 1-((2-hydroxyethyl)(aryl)amino)-*N*-substituted cycloalkane carboxamides IXa–l

Sodium borohydride (0.02 mol) was added portionwise during 5–10 min to the solution of the appropriate substituted diazaspiroalkanedione (**VIIIa–l**) (0.01 mol) in ethanol (30 mL) at room temperature. The mixture was stirred for 24 h, then the solvent was removed in vacuum. Water (150 mL) was added and the mixture extracted with dichloromethane (3 × 50 mL). The extracts were dried with anhydrous sodium sulfate. Evaporation of the solvent gave the title compounds.

N-Benzyl-1-[(2-hydroxyethyl)(4-methylphenyl)amino]-cyclopentane carboxamide (IXa)

Brown solid, m.p. 115–117°C, yield 80%. IR (KBr, cm^{-1}): 3432 (OH), 3305 (NH) and 1644 (C═O, amide); MS (EI) *m/z* (%): 353 (M^++1, 21), 91 (100), 218 (29); ^{1}H NMR ($CDCl_3$) δ ppm 1.68–2.1 (m, 8H, 4 x CH_2, cyclopentyl), 2.28 (s, 3H, CH_3), 3.2 (t, *J* = 4.5 Hz, 2H, C$\underline{H_2}$-CH_2-OH), 3.4 (q, *J* = 5 Hz, 2H, C$\underline{H_2}$-OH), 4.4 (d,*J* = 5.7 Hz, 2H, C$\underline{H_2}$-C_6H_5), 6.9 (d, 2H, *J* = 8.4 Hz, $H_{ar.}$), 7.02 (d, 2H, *J* = 8.4 Hz, $H_{ar.}$), 7.25–7.28 (m, 5H, $H_{ar.}$), 8.2 (s, 1H, NH, D_2O exchangeable), OH (spread over the chart); ^{13}C NMR ($CDCl_3$) δ ppm 20.83 (CH_3), 24.75, 35.11 (4 x CH_2, cyclopentyl), 43.59 (C$\underline{H_2}$-C_6H_5), 51.2 (C$\underline{H_2}$-CH_2-OH), 60.1 (C$\underline{H_2}$-OH), 76.93 (Cq), 125.07, 127.21, 127.77, 128.61, 129.42 ($CH_{ar.}$), 133.01, 139.17, 143.03 (3 x $C_{ar.}$), 177.77 (CO). Anal. calcd. for $C_{22}H_{28}N_2O_2$: C, 74.97; H, 8.01; N, 7.95. Found: C, 74.5; H, 7.88; N, 7.7.

1-[(2-Hydroxyethyl)(4-methylphenyl)amino]-*N*-(2-phenylethyl)-cyclopentane carboxamide (IXb)

Yellow viscous oil, yield 86%. IR (KBr, cm^{-1}): 3432 (OH), 3298 (NH) and 1642 (C═O, amide); MS (EI) *m/z* (%): 376 (M^++1, 21), 218 (100); ^{1}H NMR ($CDCl_3$) δ ppm 1.64–2.04 (m, 8H, 4 x CH_2, cyclopentyl), 2.27 (s, 3H, CH_3), 2.79 (t, *J* = 6.7 Hz, 2H, C$\underline{H_2}$-C_6H_5), 3.1 (t, *J* = 6.7 Hz, 2H, C$\underline{H_2}$-CH_2-C_6H_5), 3.3 (t, *J* = 4.8 Hz, 2H, C$\underline{H_2}$-CH_2-OH), 3.5 (q, *J* = 6.7 Hz, 2H, C$\underline{H_2}$-OH), 6.9 (d, 2H, *J* = 8.6 Hz, $H_{ar.}$), 7.03 (d, 2H, *J* = 8.6 Hz, $H_{ar.}$), 7.13–7.27 (m, 5H, $H_{ar.}$), 7.91 (s, 1H, NH, D_2O exchangeable), OH (spread over the chart); ^{13}C NMR ($CDCl_3$) δ ppm 20.86 (CH_3), 24.76, 35.17 (4 x CH_2, cyclopentyl), 35.64 (C$\underline{H_2}$-C_6H_5), 40.76 (C$\underline{H_2}$-CH_2-C_6H_5), 50.68 (C$\underline{H_2}$-CH_2-OH), 60.04 (C$\underline{H_2}$-OH), 76.95 (Cq), 124.42, 128.51, 129.06, 129.13, 129.35 ($CH_{ar.}$), 133, 139.53, 143.03 (3 x $C_{ar.}$), 177.8 (C═O). Anal. calcd. for $C_{23}H_{30}N_2O_2$: C, 75.37; H, 8.25; N, 7.64. Found: C, 74.9; H, 7.8; N, 7.3.

N-Benzyl-1-[(2-hydroxyethyl)(4-methoxyphenyl)amino]-cyclopentane carboxamide (IXc)

White solid, m.p. 106–108°C, yield 80%. IR (KBr, cm^{-1}): 3461 (OH), 3308 (NH) and 1644 (C═O, amide); MS (EI) *m/z* (%): 369 (M^++1, 27), 234 (100); ^{1}H NMR ($CDCl_3$) δ ppm 1.64–2.07 (m, 8H, 4 x CH_2, cyclopentyl), 3.07 (t, *J* = 4.75, 2H, C$\underline{H_2}$-CH_2-OH), 3.3 (q, *J* = 5.7 Hz, 2H, C$\underline{H_2}$-OH), 3.7 (s, 3H, OCH_3), 4.43 (d, *J* = 5.75, 2H, C$\underline{H_2}$-C_6H_5), 6.7 (d, 2H, *J* = 8.6 Hz, $H_{ar.}$), 7.03 (d, 2H, *J* = 8.6 Hz, $H_{ar.}$), 7.23–7.3 (m, 5H, $H_{ar.}$), 8.2 (s, 1H, NH, D_2O exchangeable), OH (spread over the chart); ^{13}C NMR ($CDCl_3$) δ ppm 24.96, 34.7 (4 x CH_2, cyclopentyl), 43.56 (C$\underline{H_2}$-C_6H_5), 52.67 (C$\underline{H_2}$-CH_2-OH), 55.49 (OCH_3), 60.034 (C$\underline{H_2}$-OH), 76.92 (Cq), 113.97, 127.29, 127.87, 128.67, 129.02 ($CH_{ar.}$), 138.4, 139.26, 156.91 (3 x $C_{ar.}$), 177.71 (C═O). Anal. calcd. for $C_{22}H_{28}N_2O_3$: C, 71.71; H, 7.66; N, 7.6. Found: C, 71.45; H, 7.7; N, 7.3.

1-[(2-Hydroxyethyl)(4-methoxyphenyl)amino]-*N*-(2-phenylethyl)cyclopentane carboxamide (IXd)

Yellow viscous oil, yield 89%. IR (KBr, cm^{-1}): 3422 (OH), 3282 (NH) and 1639 (C═O, amide); MS (EI) *m/z* (%): 383 (M^++1, 33), 234 (100); ^{1}H NMR ($CDCl_3$) δ ppm 1.63–2.07 (m, 8H, 4 x CH_2, cyclopentyl), 2.81 (t, *J* = 6.7 Hz, 2H, C$\underline{H_2}$-C_6H_5), 3.1 (t, *J* = 5.7 Hz, 2H, C$\underline{H_2}$-CH_2-C_6H_5), 3 (t, *J* = 4.75 2H, C$\underline{H_2}$-CH_2-OH), 3.3 (q, *J* = 5.7 Hz, 2H, C$\underline{H_2}$-OH), 3.7 (s, 3H, OCH_3), 6.77 (d, 2H, *J* = 8.6 Hz, $H_{ar.}$), 7.03 (d, 2H, *J* = 8.6 Hz, $H_{ar.}$), 7.23–7.3 (m, 5H, $H_{ar.}$), 8.2 (s, 1H, NH, D_2O exchangeable), OH (spread over the chart); ^{13}C NMR ($CDCl_3$) δ ppm 24.96, 34.7 (4 x CH_2, cyclopentyl), 43.56 (C$\underline{H_2}$-C_6H_5), 48 (C$\underline{H_2}$-CH_2-C_6H_5), 52.67 (C$\underline{H_2}$-CH_2-OH), 55.49 (OCH_3), 60.034 (C$\underline{H_2}$-OH), 76.92 (Cq), 113.97, 127.29, 127.87, 128.67, 129.7 ($CH_{ar.}$), 138.4, 139.26, 156.91 (3 x $C_{ar.}$), 177.71 (C═O). Anal. calcd. for $C_{23}H_{30}N_2O_3$: C, 72.22; H, 7.91; N, 7.32. Found: C, 72.5; H, 7.8; N, 7.2.

N-Benzyl-1-[(2-hydroxyethyl)(4-methylphenyl)amino]-cyclohexanecarboxamide (IXe)

Yellow solid, m.p. 140°C, yield 90%. IR (KBr, cm^{-1}): 3429 (OH), 3313 (NH) and 1631 (C═O, amide); MS (EI) *m/z* (%): 367 (M^++1, 21), 232 (100); ^{1}H NMR ($CDCl_3$) δ ppm 1.45–1.95 (m, 10H, 5 x CH_2, cyclohexyl), 2.3 (s, 3H, CH_3), 3.17 (t, *J* = 5.7 Hz, 2H, C$\underline{H_2}$-CH_2-OH), 3.26 (q, *J* = 6.5 Hz, 2H, C$\underline{H_2}$-OH), 4.4 (d, *J* = 5.7 Hz, 2H, C$\underline{H_2}$-C_6H_5), 6.94 (d, 2H, *J* = 8.4 Hz, $H_{ar.}$), 7.07 (d, 2H, *J* = 8.4 Hz, $H_{ar.}$), 7.23–7.35 (m, 5H, $H_{ar.}$), 7.43 (s, 1H, NH, D_2O exchangeable), OH (spread over the chart); ^{13}C NMR ($CDCl_3$) δ ppm 20.86, 22.7, 25.44 (5 x CH_2, cyclohexyl), 33.04 (CH_3), 35.56 (C$\underline{H_2}$-C_6H_5), 50.25 (C$\underline{H_2}$-CH_2-OH), 59.8 (C$\underline{H_2}$-OH), 66.18 (Cq), 126.37, 127.72, 128.52, 128.91, 129.14 ($CH_{ar.}$), 134.15, 139.46, 142.29 (3 x $C_{ar.}$), 176.54 (C═O). Anal. calcd. for $C_{23}H_{30}N_2O_2$: C, 75.37; H, 8.25; N, 7.64. Found: C, 75.8; H, 8.01; N, 7.21.

1-[(2-Hydroxyethyl)(4-methylphenyl)amino]-*N*-(2-phenylethyl)-cyclohexanecarboxamide (IXf)

Yellow viscous oil, yield 82%. IR (KBr, cm^{-1}): 3432 (OH), 3282 (NH) and 1639 (C═O, amide); MS (EI) *m/z* (%): 381 (M^++1, 21), 232 (100), 91 (76); ^{1}H NMR ($CDCl_3$) δ ppm 1.45–1.95 (m, 10H, 5 x CH_2, cyclohexyl), 2.32 (s, 3H, CH_3), 2.87 (t, *J* = 10 Hz, 2H, C$\underline{H_2}$-C_6H_5), 3.27 (t, *J* = 10 Hz, 2H, C$\underline{H_2}$-CH_2-C_6H_5), 3.33 (t, *J* = 5 Hz, 2H, C$\underline{H_2}$-CH_2-OH), 3.55 (q, *J* = 10 Hz, 2H, C$\underline{H_2}$-OH), 6.94 (d, 2H, *J* = 10 Hz, $H_{ar.}$), 7.07 (d, 2H, *J* = 10 Hz, $H_{ar.}$), 7.23–7.35 (m, 5H, $H_{ar.}$), 7.43 (s, 1H, NH, D_2O exchangeable), OH (spread over the chart); ^{13}C NMR ($CDCl_3$) δ ppm 20.86, 22.7, 25.44 (5 x CH_2, cyclohexyl), 33.04 (CH_3), 35.56 (C$\underline{H_2}$-C_6H_5), 40.47 (C$\underline{H_2}$-CH_2-C_6H_5), 50.25 (C$\underline{H_2}$-CH_2-OH), 59.8 (C$\underline{H_2}$-OH), 66.18 (Cq), 126.37, 127.72, 128.52, 128.91, 129.14 ($CH_{ar.}$), 134.15, 139.46, 142.29 (3 x $C_{ar.}$), 176.54 (C═O). Anal. calcd. for $C_{24}H_{32}N_2O_2$: C, 75.75; H, 8.48; N, 7.36. Found: C, 75.5; H, 8.8; N, 7.3.

N-Benzyl-1-[(2-hydroxyethyl)(4-methoxyphenyl)amino]-cyclohexanecarboxamide (IXg)

White solid, m.p. 100°C, yield 88%. IR (KBr, cm^{-1}): 3432 (OH), 3282 (NH) and 1636 (C═O, amide); MS (EI) *m/z* (%): 382 (M^++1, 10), 248 (100), 91 (55); ^{1}H NMR ($CDCl_3$) δ ppm 1.42–2.1 (m, 10H, 5 x CH_2, cyclohexyl), 3.1 (t, *J* = 6.5 Hz, 2H, CH_2-CH_2-OH), 3.28 (q, *J* = 6.5 Hz, 2H, CH_2-OH), 3.75 (s, 3H, OCH_3), 4.4 (d, *J* = 6 Hz 2H, CH_2-C_6H_5), 6.74 (d, 2H, *J* = 11 Hz, $H_{ar.}$), 7.02 (d, 2H, *J* = 11 Hz, $H_{ar.}$), 7.29–7.313 (m, 5H, $H_{ar.}$), 7.6 (s, 1H, NH, D_2O exchangeable), OH (spread over the chart); ^{13}C NMR ($CDCl_3$) δ ppm 22.89, 25.47, 33.09 (5 x CH_2, cyclohexyl), 43.41 (CH_2-C_6H_5), 51.02 (CH_2-CH_2-OH), 55.36 (OCH_3), 59.8 (CH_2-OH), 66.35 (Cq), 113.7, 127.28, 127.95, 128.62, 129.8 ($CH_{ar.}$), 137.66, 139.04, 157.05 (3 x $C_{ar.}$), 176.23 (C═O). Anal. calcd. for $C_{23}H_{30}N_2O_3$: C, 72.22; H, 7.91; N, 7.32. Found: C, 71.78; H, 8.01; N, 7.21.

1-[(2-Hydroxyethyl)(4-methoxyphenyl)amino]-*N*-(2-phenylethyl)-cyclohexanecarboxamide (IXh)

Yellow viscous oil, yield 80%. IR (KBr, cm^{-1}): 3419 (OH), 3282 (NH) and 1631 (C═O, amide); MS (EI) *m/z* (%): 397 (M^++1, 21), 248 (100); ^{1}H NMR ($CDCl_3$) δ ppm 1.23–1.859 (m, 10H, 5 x CH_2, cyclohexyl), 2.84 (t, *J* = 6.7 Hz, 2H, CH_2-C_6H_5), 3.13 (t, *J* = 6.5 Hz, 2H, CH_2-CH_2-C_6H_5), 3.23 (t, *J* = 6.7 Hz, 2H, CH_2-CH_2-OH), 3.52 (q, *J* = 6.7 Hz, 2H, CH_2-OH), 3.77 (s, 3H, OCH_3), 6.77 (d, 2H, *J* = 8.4 Hz, $H_{ar.}$), 6.96 (d, 2H, *J* = 8.4 Hz, $H_{ar.}$), 7.202–7.293 (m, 5H, $H_{ar.}$), 7.4 (s, 1H, NH, D_2O exchangeable), OH (spread over the chart); ^{13}C NMR ($CDCl_3$) δ ppm 22.87, 25.51, 33.15 (5 x CH_2, cyclohexyl), 35.59 (CH_2-C_6H_5), 40.5 (CH_2-CH_2-C_6H_5), 50.84 (CH_2-CH_2-OH), 55.46 (OCH_3), 59.83 (CH_2-OH), 66.29 (Cq), 114.37, 128.62, 128.52, 128.95, 129.78 ($CH_{ar.}$), 137.7, 139.49, 157.1 (3 x $C_{ar.}$), 176.28 (C═O). Anal. calcd. for $C_{24}H_{32}N_2O_3$: C, 72.7; H, 8.13; N, 7.06. Found: C, 72.94; H, 8.2; N, 7.21.

N-Benzyl-1-[(2-hydroxyethyl)(4-methylphenyl)amino]-cycloheptanecarboxamide (IXi)

Yellow solid, m.p. 108°C, yield 80%. IR (KBr, cm^{-1}): 3389 (OH), 3286 (NH) and 1641 (C═O, amide); MS (EI) *m/z* (%): 381 (M^++1, 21), 98 (100), 246 (77); ^{1}H NMR (DMSO) δ ppm 1.31–2.01 (m, 12H, 6 x CH_2, cycloheptyl), 2.22 (s, 3H, CH_3), 3 (t, *J* = 4.5 Hz, 2H, CH_2-CH_2-OH), 3.2 (q, *J* = 5 Hz, 2H, CH_2-OH), 4.2 (d, *J* = 5.75 Hz, 2H, CH_2-C_6H_5), 7.04 (d, 2H, *J* = 8.4 Hz, $H_{ar.}$), 7.11 (d, 2H, *J* = 8.4 Hz, $H_{ar.}$), 7.2–7.25 (m, 5H, $H_{ar.}$), 8.6 (s, 1H, NH, D_2O exchangeable), OH (spread over the chart); ^{13}C NMR ($CDCl_3$) δ ppm 20.93 (CH_3), 23.82, 30.58, 34.86 (6 x CH_2, cycloheptyl), 42.77 (CH_2-C_6H_5), 52.06 (CH_2-CH_2-OH), 59.13 (CH_2-OH), 70.18 (Cq), 127.08, 127.48, 127.89, 128.7, 129.41 ($CH_{ar.}$), 133.65, 140.46, 144 (3 x $C_{ar.}$), 177.44 (C═O). Anal. calcd. for $C_{24}H_{32}N_2O_2$: C, 75.75; H, 8.48; N, 7.36. Found: C, 75.9; H, 8.52; N, 7.5.

1-[(2-Hydroxyethyl)(4-methylphenyl)amino]-*N*-(2-phenylethyl)-cycloheptanecarboxamide (IXj)

White solid, m.p. 78–80°C, yield 90%. IR (KBr, cm^{-1}): 3432 (OH), 3319 (NH) and 1645 (C═O, amide); MS (EI) *m/z* (%): 395.2 (M^++1, 21), 98 (100); ^{1}H NMR ($CDCl_3$) δ ppm 1.4–2.01 (m, 12H, 6 x CH_2, cycloheptyl), 2.29 (s, 3H, CH_3), 2.84 (t, *J* = 5 Hz, 2H, CH_2-C_6H_5), 3 (t, *J* = 5.5 Hz, 2H, CH_2-CH_2-C_6H_5), 3.22 (t, *J* = 5.5 Hz, 2H, CH_2-CH_2-OH), 3.5 (q, *J* = 6.5 Hz, 2H, CH_2-OH), 6.99 (d, 2H, *J* = 5 Hz, $H_{ar.}$), 7.03 (d, 2H, *J* = 5 Hz, $H_{ar.}$), 7.2–7.29 (m, 5H, $H_{ar.}$), 7.86 (s, 1H, NH, D_2O exchangeable), OH (spread over the chart); ^{13}C NMR ($CDCl_3$) δ ppm 20.93 (CH_3), 23.83, 30.8, 35.18 (6 x CH_2, cycloheptyl), 35.53 (CH_2-C_6H_5), 40.65 (CH_2-CH_2-C_6H_5), 51.65 (CH_2-CH_2-OH), 59.78 (CH_2-OH), 70.38 (Cq), 126.34, 127.73, 128.33, 128.94, 129.32 ($CH_{ar.}$), 134.81, 139.6, 142.83 (3 x $C_{ar.}$), 178.17 (C═O). Anal. calcd. for $C_{25}H_{34}N_2O_2$: C, 76.1; H, 8.69; N, 7.1. Found: C, 75.84; H, 8.9; N, 6.92.

N-Benzyl-1-[(2-hydroxyethyl)(4-methoxyphenyl)amino]-cycloheptanecarboxamide (IXk)

White solid, m.p. 73–76°C, yield 80%. IR (KBr, cm^{-1}): 3389 (OH), 3286 (NH) and 1641 (C═O, amide); MS (EI) *m/z* (%): 397 (M^++1, 21), 263 (100); ^{1}H NMR ($CDCl_3$) δ ppm 1.26–2.12 (m, 12H, 6 x CH_2, cycloheptyl), 2.9 (t, *J* = 5.7 Hz, 2H, CH_2-CH_2-OH), 3.2 (q, *J* = 4.8 Hz, 2H, CH_2-OH), 3.7 (s, 3H, OCH_3), 4.4 (d, *J* = 6.7 Hz, 2H, CH_2-C_6H_5), 6.8 (d, 2H, *J* = 8.6 Hz, $H_{ar.}$), 7.1 (d, 2H, *J* = 8.6 Hz, $H_{ar.}$), 7.2–7.3 (m, 5H, $H_{ar.}$), 8 (s, 1H, NH, D_2O exchangeable), OH (spread over the chart); ^{13}C NMR ($CDCl_3$) δ ppm 23.82, 30.58, 34.86 (6 x CH_2, cycloheptyl), 43 (CH_2-C_6H_5), 52.06 (CH_2-CH_2-OH), 55.4 (OCH_3), 59.8 (CH_2-OH), 70.18 (Cq), 113.6, 128.4, 128.6, 129.01, 129.1 ($CH_{ar.}$), 138.2., 139.7, 157.5 (3 x $C_{ar.}$), 178.17 (C═O). Anal. calcd. for $C_{24}H_{32}N_2O_3$: C, 72.7; H, 8.13; N, 7.06. Found: C, 72.49; H, 7.98; N, 6.9.

1-[(2-Hydroxyethyl)(4-methoxyphenyl)amino]-*N*-(2-phenylethyl)-cycloheptanecarboxamide (IXl)

Colourless viscous oil, yield 85%. IR (KBr, cm^{-1}): 3440 (OH), 3282 (NH) and 1636 (NH-C═O, amide); MS (EI) *m/z* (%): 411 (M^++1, 25), 98 (100); ^{1}H NMR ($CDCl_3$) δ ppm 1.4–2.01 (m, 12H, 6 x CH_2, cycloheptyl), 2.86 (t, *J* = 6.7 Hz, 2H, CH_2-C_6H_5), 2.91 (t, *J* = 6.5 Hz, 2H, CH_2-CH_2-C_6H_5), 3.17 (t, *J* = 6.7 Hz, 2H, CH_2-CH_2-OH), 3.51 (q, *J* = 6.7 Hz, 2H, CH_2-OH), 3.77 (s, 3H, OCH_3), 6.77 (d, 2H, *J* = 8.6 Hz, $H_{ar.}$), 7.04 (d, 2H, *J* = 8.6 Hz, $H_{ar.}$), 7.22–7.3 (m, 5H, $H_{ar.}$), 7.8 (s, 1H, NH, D_2O exchangeable), OH (spread over the chart); ^{13}C NMR ($CDCl_3$) δ ppm 23.91, 31.03, 35.15 (6 x CH_2, cycloheptyl), 35.56 (CH_2-C_6H_5), 40.71 (CH_2-CH_2-C_6H_5), 52.21 (CH_2-CH_2-OH), 55.45 (OCH_3), 59.81 (CH_2-OH), 70 (Cq), 113.88, 128.58, 128.64, 129.04, 129.09 ($CH_{ar.}$), 138.17, 139.73, 157.43 (3 x $C_{ar.}$), 178.14 (C═O). Anal. calcd. for $C_{25}H_{34}N_2O_3$: C, 73.14; H, 8.35; N, 6.82. Found: C, 73.48; H, 8.19; N, 6.68.

4.1.8 | General procedure for the synthesis of 2-((1-substituted carbamoyl)cycloalkyl)(aryl)amino)ethyl acetates Xa–l

A solution of the hydroxyethyl amino derivative (0.071 mol), triethylamine (194.6 mmol, 19.7 g), dimethylaminopyridine (1.22 mmol, 0.15 g), acetic anhydride (142.03 mmol, 14.5 g), and dichloromethane (2.34 mol, 150 mL) was stirred overnight at about 20–25°C. To the resulting solution water was added followed by addition of HCl till pH of the resulting solution was 1–2. The solvent was distilled out completely. Ethyl acetate was added to the resulting residue, washed with water, dried using anhydrous Na_2SO_4 and the organic layer was evaporated under reduced pressure to give the target compounds **Xa–l**.

Purified by column chromatography using a mixture of methylene chloride/ethyl acetate (7:3) as a mobile phase.

2-{[1-(Benzylcarbamoyl)cyclopentyl](4-methylphenyl)amino}-ethyl acetate (Xa)

Yellow solid, m.p. 80–82°C, yield 88%. IR (KBr, cm^{-1}): 3350 (NH), 1735 (C═O, ester carbonyl), 1652 (C═O, amide carbonyl); MS (EI) *m/z* (%): 395.3 (M^++1, 38.5), 260 (100); ^{1}H NMR ($CDCl_3$) δ ppm 1.33–2.08 (m, 8H, 4 x CH_2, cyclopentyl), 1.7 (s, 3H, O═C-CH_3), 2.26 (s, 3H, CH_3), 3.2 (t, *J* = 5.75 Hz, 2H, CH_2-CH_2-O), 3.82 (t, *J* = 5.75 Hz, 2H, CH_2-O), 4.4 (d, *J* = 5.75 Hz, 2H, CH_2-C_6H_5), 6.85 (d, 2H, *J* = 8.4 Hz, $H_{ar.}$), 7.04 (d, 2H, *J* = 8.4 Hz, $H_{ar.}$), 7.13–7.27 (m, 5H, $H_{ar.}$), 7.5 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR ($CDCl_3$) δ ppm 20.84 (CH_3), 20.8 (O═C-CH_3), 25.19, 34.78 (4 x CH_2, cyclopentyl), 42.3 (CH_2-C_6H_5), 48.68 (CH_2-CH_2-O), 62.64 (CH_2-O), 76.94 (Cq), 125.41, 126.53, 128.64, 128.92, 129.54 ($CH_{ar.}$), 133.87, 139.27, 143 (3 x $C_{ar.}$), 170.67 (O═C-CH_3), 177.35 (C═O). Anal. calcd. for $C_{24}H_{30}N_2O_3$: C, 73.07; H, 7.67; N, 7.1. Found: C, 73.46; H, 7.78; N, 7.21.

2-{(4-Methylphenyl)[1-((2-phenylethyl)carbamoyl)cyclopentyl]-amino}ethyl acetate (Xb)

Yellow viscous oil, yield 88%. IR (KBr, cm^{-1}): 3389 (NH), 1738 (CO, ester carbonyl), 1652 (C═O, amide carbonyl); MS (EI) *m/z* (%): 409 (M^++1, 21), 260 (100); ^{1}H NMR ($CDCl_3$) δ ppm 1.35–2.06 (m, 8H, 4 x CH_2, cyclopentyl), 1.86 (s, 3H, O═C-CH_3), 2.28 (s, 3H, CH_3), 2.79 (t, *J* = 5.75 Hz, 2H, CH_2-C_6H_5), 3.1 (t, *J* = 5.75 Hz, 2H, CH_2-CH_2-O), 3.52 (q, *J* = 5.75 Hz, 2H, CH_2-CH_2-C_6H_5), 3.82 (t, *J* = 5.75 Hz, 2H, CH_2-O), 6.85 (d, 2H, *J* = 8.4 Hz, $H_{ar.}$), 7.04 (d, 2H, *J* = 8.4 Hz, $H_{ar.}$), 7.13–7.27 (m, 5H, $H_{ar.}$), 7.5 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR ($CDCl_3$) δ ppm 20.84 (CH_3), 20.8 (O═C-CH_3), 25.19, 34.78 (4 x CH_2, cyclopentyl), 35.94 (CH_2-C_6H_5), 40.82 (CH_2-CH_2-C_6H_5), 48.68 (CH_2-CH_2-O), 62.64 (CH_2-O), 76.94 (Cq), 125.41, 126.53, 128.64, 128.92, 129.54 ($CH_{ar.}$), 133.87, 139.27, 143 (3 x $C_{ar.}$), 170.67 (O═C-CH_3), 177.35 (C═O). Anal. calcd. for $C_{25}H_{32}N_2O_3$: C, 73.5; H, 7.9; N, 6.86. Found: C, 73.75; H, 7.79; N, 6.605.

2-{[1-(Benzylcarbamoyl)cyclopentyl](4-methoxyphenyl)amino}-ethyl acetate (Xc)

Yellow viscous oil, yield 66%. IR (KBr, cm^{-1}): 3372 (NH), 1738 (C═O, ester carbonyl), 1662 (C═O, amide carbonyl); MS (EI) *m/z* (%): 411.32 (M^++1, 66), 276.05 (100); ^{1}H NMR ($CDCl_3$) δ ppm 1.35–2.08 (m, 8H, 4 x CH_2, cyclopentyl), 1.73 (s, 3H, O═C-CH_3), 3.16 (t, *J* = 6.7 Hz, 2H, CH_2-CH_2-O), 3.75 (s, OCH_3), 3.8 (t, *J* = 6.7 Hz, 2H, CH_2-O), 4.4 (d, *J* = 5.75 Hz, 2H, CH_2-C_6H_5), 6.8 (d, 2H, *J* = 8.4 Hz, $H_{ar.}$), 7.02 (d, 2H, *J* = 8.4 Hz, $H_{ar.}$), 7.23–7.3 (m, 5H, $H_{ar.}$), 7.9 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR ($CDCl_3$) δ ppm 20.5 (O═C-CH_3), 25.21, 34.37 (4 x CH_2, cyclopentyl), 43.59 (CH_2-C_6H_5), 49.78 (CH_2-CH_2-O), 55.46 (OCH_3), 62.47 (CH_2-O), 76.94 (Cq), 114.11, 127.41, 127.67, 128.75, 128.81 ($CH_{ar.}$), 138.1, 139.04, 157.36 (3 x $C_{ar.}$), 170.76 (OC-CH_3), 177.54 (C═O). Anal. calcd. for $C_{24}H_{30}N_2O_4$: C, 70.22; H, 7.37; N, 6.82. Found: C, 70.06; H, 7.5; N, 6.74.

2-{(4-Methoxyphenyl)[1-((2-phenylethyl)carbamoyl)cyclopentyl]-amino}ethyl acetate (Xd)

Yellow viscous oil, yield 78.4%. IR (KBr, cm^{-1}): 3350 (NH), 1735 (C═O, ester carbonyl), 1652 (CO, amide carbonyl); MS (EI) *m/z* (%): 425.29 (M^++1, 38.5), 276.14 (100); ^{1}H NMR ($CDCl_3$) δ ppm 1.3–2.04 (m, 8H, 4 x CH_2, cyclopentyl), 1.84 (s, 3H, O═C-CH_3), 2.81 (t, *J* = 6.7 Hz, 2H, CH_2-C_6H_5), 3.06 (t, *J* = 5.75 Hz, 2H,CH_2-CH_2-O), 3.53 (q, *J* = 6.7 Hz, 2H, CH_2-CH_2-C_6H_5), 3.72 (t, *J* = 5.75 Hz, 2H, CH_2-OH), 3.76 (s, 3H, OCH_3), 6.77 (d, 2H, *J* = 8.4 Hz, $H_{ar.}$), 6.92 (d, 2H, *J* = 8.4 Hz, $H_{ar.}$), 7.17–7.28 (m, 5H, $H_{ar.}$), 7.7 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR ($CDCl_3$) δ ppm 20.82 (O═C-CH_3), 25.25, 34.38 (4 x CH_2, cyclopentyl), 35.93 (CH_2-C_6H_5), 40.81 (CH_2-CH_2-C_6H_5), 49.55 (CH_2-CH_2-O), 55.46 (OCH_3), 62.51 (CH_2-O), 76.96 (Cq), 114.06, 126.53, 128.66, 128.69, 128.93 ($CH_{ar.}$), 138.09, 139.26, 157.28 (3 x $C_{ar.}$), 170.7 (O═C-CH_3), 177.68 (C═O). Anal. calcd. for $C_{25}H_{32}N_2O_4$: C, 70.73; H, 7.6; N, 6.6. Found: C, 70.47; H, 7.52; N, 6.57.

2-{[1-(Benzylcarbamoyl)cyclohexyl](4-methylphenyl)amino}ethyl acetate (Xe)

White solid, m.p. 76–78°C, yield 90%. IR (KBr, cm^{-1}): 3344 (NH), 1733 (C═O, ester carbonyl), 1648 (C═O, amide carbonyl); MS (EI) *m/z* (%): 409.5 (M^++1, 21), 98 (100); ^{1}H NMR ($CDCl_3$) δ ppm 1.33–1.97 (m, 10H, 5 x CH_2, cyclohexyl), 1.75 (s, 3H, O═C-CH_3), 2.3 (s, 3H, CH_3), 3.2 (t, *J* = 5.75 Hz, 2H, CH_2-CH_2-O), 3.7 (t, *J* = 5.75 Hz, 2H, CH_2-O), 4.48 (d, *J* = 5.75 Hz, 2H, CH_2-C_6H_5), 6.9 (d, 2H, *J* = 8.6 Hz, $H_{ar.}$), 7 (d, 2H, *J* = 8.6 Hz, $H_{ar.}$), 7.25–7.33 (m, 5H, $H_{ar.}$), 7.4 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR ($CDCl_3$) δ ppm 20.66 (CH_3), 20.97 (CH_3-C═O), 23.04, 25.48, 32.95 (5 x CH_2, cyclohexyl), 43.39 (CH_2-C_6H_5), 47.93 (CH_2-CH_2-O), 62.81 (CH_2-O), 66.83 (Cq), 127.43, 127.71, 128.76, 128.87, 129.37 ($CH_{ar.}$), 135.4, 139.04, 142.24 (3 x $C_{ar.}$), 170.76 (O═C-CH_3), 176.41 (NH-C═O). Anal. calcd. for $C_{25}H_{32}N_2O_3$: C, 73.5; H, 7.9; N, 6.86. Found: C, 73.82; H, 7.79; N, 6.82.

2-{(4-Methylphenyl)[1-((2-phenylethyl)carbamoyl)cyclohexyl]-amino}ethyl acetate (Xf)

White solid, m.p. 45°C, yield 90%. IR (KBr, cm^{-1}): 3344 (NH), 1735 (C═O, ester carbonyl), 1646 (C═O, amide carbonyl); MS (EI) *m/z* (%): 423 (M^++1, 25.3), 274 (100); ^{1}H NMR ($CDCl_3$) δ ppm 1.47–1.89 (m, 10H, 5 x CH_2, cyclohexyl), 1.8 (s, 3H, O═C-CH_3), 2.3 (s, 3H, CH_3), 2.8 (t, *J* = 6.7 Hz, 2H, CH_2-C_6H_5), 3.2 (t, *J* = 6.7 Hz, 2H, CH_2-CH_2-O), 3.5 (q, *J* = 6.7 Hz, 2H, CH_2-CH_2-C_6H_5), 3.7 (t, *J* = 6.7 Hz, 2H, CH_2-O), 6.9 (d, 2H, *J* = 8.6 Hz, $H_{ar.}$), 7 (d, 2H, *J* = 8.6 Hz, $H_{ar.}$), 7.16–7.25 (m, 5H, $H_{ar.}$), 7.31 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR ($CDCl_3$) δ ppm 20.86 (CH_3), 20.98 (CH_3-C═O), 22.96, 25.46, 32.78 (5 x CH_2, cyclohexyl), 36.01 (CH_2-1C_6H_5), 40.54 (CH_2-CH_2-C_6H_5), 47.83 (CH_2-CH_2-O), 62.83 (CH_2-O), 66.67 (Cq), 126.56, 127.72, 128.68, 128.85, 129.33 ($CH_{ar.}$), 135.42, 139.25, 142.35 (3 x $C_{ar.}$), 170.67 (O═C-CH_3), 176.36 (NH-C═O). Anal. calcd. for $C_{26}H_{34}N_2O_3$: C, 73.9; H, 8.11; N, 6.63. Found: C, 73.65; H, 8.04; N, 6.58.

2-{[1-(Benzylcarbamoyl)cyclohexyl](4-methoxyphenyl)amino}-ethyl acetate (Xg)

Yellow viscous oil, yield 80%. IR (KBr, cm^{-1}): 3384 (NH), 1734 (C═O, ester carbonyl), 1648 (C═O, amide carbonyl); MS (EI) *m/z* (%): 425 (M^++1, 11), 290 (100); ^{1}H NMR ($CDCl_3$) δ ppm 1.2–2 (m, 10H, 5 x CH_2, cyclohexyl), 1.5 (s, 1H, NH, D_2O exchangeable), 1.87 (s, 3H, O═C-CH_3), 3.2 (t, *J* = 5.75 Hz, 2H, CH_2-CH_2-O), 3.7 (t, *J* = 5.75 Hz, 2H, CH_2-O), 3.81 (s, 3H, OCH_3), 4.5 (d, *J* = 7.5 Hz, 2H, CH_2-C_6H_5), 6.8 (d, 2H, *J* = 11 Hz, $H_{ar.}$), 7 (d, 2H, *J* = 11 Hz, $H_{ar.}$), 7.29–7.238 (m, 5H, $H_{ar.}$); ^{13}C NMR ($CDCl_3$) δ ppm 20.7, 25.51, 32.92 (5 x CH_2, cyclohexyl), 23.06 (CH_3-C═O), 43.38 (CH_2-C_6H_5), 48.22 (CH_2-CH_2-O), 55.44 (OCH_3), 62.79 (CH_2-O), 66.84 (Cq), 113.86, 127.44, 127.73, 128.77, 130.37 ($CH_{ar.}$), 137.58, 139.03, 157.64 (3 x $C_{ar.}$), 170.78 (O═C-CH_3), 176.3 (NH-C═O). Anal. calcd. for $C_{25}H_{32}N_2O_4$: C, 70.73; H, 7.6; N, 6.6. Found: C, 70.49; H, 7.73; N, 6.58.

2-{(4-Methoxyphenyl)[1-((2-phenylethyl)carbamoyl)cyclohexyl]-amino}ethyl acetate (Xh)

Light brown solid, m.p. 76–78°C, yield 90%. IR (KBr, cm^{-1}): 3335 (NH), 1732 (C═O, ester carbonyl), 1647 (C═O, amide carbonyl); MS (EI) *m/z* (%): 439.3 (M^++1, 15), 290 (100); ^{1}H NMR ($CDCl_3$) δ ppm 0.9 (s, 1H, NH, D_2O exchangeable), 1.2–1.83 (m, 10H, 5 x CH_2, cyclohexyl), 1.87 (s, 3H, O═C-CH_3), 2.8 (t, *J* = 6.7 Hz, 2H, CH_2-C_6H_5), 3.16 (t, *J* = 5.75 Hz, 2H, CH_2-CH_2-O), 3.5 (q, *J* = 5.75 Hz, 2H, CH_2-CH_2-C_6H_5), 3.7 (t, *J* = 5.75 Hz, 2H, CH_2-O), 3.8 (s, 3H, OCH_3), 6.7 (d, 2H, *J* = 8.6 Hz, $H_{ar.}$), 6.9 (d, 2H, *J* = 8.6 Hz, $H_{ar.}$), 7.2–7.31 (m, 5H, $H_{ar.}$); ^{13}C NMR ($CDCl_3$) δ ppm 20.88, 25.49, 32.9 (5 x CH_2, cyclohexyl), 22.99 (CH_3-CO), 36 (CH_2-C_6H_5), 40.5 (CH_2-CH_2-C_6H_5), 48.14 (CH_2-CH_2-O), 55.44 (OCH_3), 62.82 (CH_2-O), 66.69 (Cq), 113.84, 126.57, 128.69, 128.86, 130.34 ($CH_{ar.}$), 137.69, 139.25, 157.6 (3 x $C_{ar.}$), 170.7 (O═C-CH_3), 176.26 (NH-C═O). Anal. calcd. for $C_{26}H_{34}N_2O_4$: C, 71.21; H, 7.81; N, 6.39. Found: C, 70.9; H, 8.21; N, 5.923.

2-{[1-(Benzylcarbamoyl)cycloheptyl](4-methylphenyl)amino}-ethyl acetate (Xi)

White solid, m.p. 88°C, yield 78%. IR (KBr, cm^{-1}): 3338 (NH), 1732 (C═O, ester carbonyl), 1647 (C═O, amide carbonyl); MS (EI) *m/z* (%): 423 (5) (M^++1), 288 (100); ^{1}H NMR ($CDCl_3$) δ ppm 1.72 (s, 3H, O═C-CH_3), 1.42–2.15 (m, 12H, 6 x CH_2, cycloheptyl), 2.3 (s, 3H, CH_3), 3.1 (t, *J* = 6.7 Hz, 2H, CH_2-CH_2-O), 3.72 (t, *J* = 6.7 Hz, 2H, CH_2-O), 4.4 (d, *J* = 5.75 Hz, 2H, CH_2-C_6H_5), 6.98 (d, 2H, *J* = 8.4 Hz, $H_{ar.}$), 7.05 (d, 2H, *J* = 8.4 Hz, $H_{ar.}$), 7.2–7.3 (m, 5H, $H_{ar.}$), 7.8 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR ($CDCl_3$) δ ppm 20.6 (CH_3), 21.02 (O═C-CH_3), 23.97, 30.95, 34.99 (6 x CH_2, cycloheptyl), 43.53 (CH_2-C_6H_5), 48.97 (CH_2-CH_2-O), 62.4 (CH_2-O), 71.01 (Cq), 126.55, 128.65, 128.71, 128.9, 129.52 ($CH_{ar.}$), 135.77, 139.12, 142.55 (3 x $C_{ar.}$), 170.72 (O═C-CH_3), 177.7 (NH-C═O). Anal. calcd. for $C_{26}H_{34}N_2O_3$: C, 73.9; H, 8.11; N, 6.63. Found: C, 73.6; H, 8.01; N, 6.42.

2-{(4-Methylphenyl)[1-((2-phenylethyl)carbamoyl)cycloheptyl]-amino}ethyl acetate (Xj)

White solid, m.p. 110°C, yield 88.8%. IR (KBr, cm^{-1}): 3331 (NH), 1734 (C═O, ester carbonyl), 1641 (C═O, amide carbonyl); MS (EI) *m/z* (%): 437 (M^++1, 3), 288 (100); ^{1}H NMR ($CDCl_3$) δ ppm 1.4–2.07 (m, 12H, 6 x CH_2, cycloheptyl), 1.85 (s, 3H, O═C-CH_3), 2.29 (s, 3H, CH_3), 2.83 (t, *J* = 6.7 Hz, 2H, CH_2-C_6H_5), 3.06 (t, *J* = 5.75 Hz, 2H,CH_2-CH_2-O), 3.53 (q, *J* = 6.65 Hz, 2H, CH_2-CH_2-C_6H_5), 3.67 (t, *J* = 5.75 Hz, 2H, CH_2-O), 6.98 (d, 2H, *J* = 8.4 Hz, $H_{ar.}$), 7.05 (d, 2H, *J* = 8.4 Hz, $H_{ar.}$), 7.2–7.28 (m, 5H, $H_{ar.}$), 7.5 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR ($CDCl_3$) δ ppm 20.84 (CH_3), 21.02 (O═C-CH_3), 23.93, 30.92, 34.91 (6 x CH_2, cycloheptyl), 36.06 (CH_2-C_6H_5), 40.78 (CH_2-CH_2-C_6H_5), 48.85 (CH_2-CH_2-O), 62.44 (CH_2-O), 70.1 (Cq), 126.55, 128.65, 128.71, 128.9, 129.52 ($CH_{ar.}$), 135.53, 139.36, 142.63 (3 x $C_{ar.}$), 170.61 (O═C-CH_3), 177.63 (NH-C═O). Anal. calcd. for $C_{27}H_{36}N_2O_3$: C, 74.28; H, 8.31; N, 6.42. Found: C, 73.8; H, 7.94; N, 6.22.

2-{[1-(Benzylcarbamoyl)cycloheptyl](4-methoxyphenyl)amino}-ethyl acetate (Xk)

White solid, m.p. 80°C, yield 90%. IR (KBr, cm^{-1}): 3388 (NH), 1725 (C═O, ester carbonyl), 1673 (C═O, amide carbonyl); MS (EI) *m/z* (%): 439 (M^++1, 3.17), 304 (100); ^{1}H NMR ($CDCl_3$) δ ppm 1.4–2.16 (m, 12H, 6 x CH_2, cycloheptyl), 1.75 (s, 3H, O═C-CH_3), 3.1 (t, *J* = 5.7 Hz, 2H, CH_2-CH_2-O), 3.7 (t, *J* = 5.7 Hz, 2H, CH_2-O), 3.77 (s, 3H, OCH_3), 4.4 (d, *J* = 6.7 Hz, 2H, CH_2-C_6H_5), 6.78 (d, 2H, *J* = 8.6 Hz, $H_{ar.}$), 7 (d, 2H, *J* = 8.6 Hz, $H_{ar.}$), 7.24–7.31 (m, 5H, $H_{ar.}$), 7.84 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR ($CDCl_3$) δ ppm 20.61 (OC-CH_3), 23.95, 30.98, 34.93 (6 x CH_2, cycloheptyl), 43.51 (CH_2-C_6H_5), 49.27 (CH_2-CH_2-O), 55.44 (OCH_3), 62.34 (CH_2-O), 71.05 (Cq), 114.04, 127.4, 127.62, 128.75, 130.21 ($CH_{ar.}$), 137.82, 139.14, 157.83 (3 x $C_{ar.}$), 170.69 (O═C-CH_3), 177.66 (NH-C═O). Anal. calcd. for $C_{26}H_{34}N_2O_4$: C, 71.21; H, 7.81; N, 6.39. Found: C, 71.56; H, 7.93; N, 6.16.

2-{(4-(Methoxyphenyl)[(1-((2-phenylethyl)carbamoyl)-cycloheptyl]amino}ethyl acetate (Xl)

White solid, m.p. 90°C, yield 90%. IR (KBr, cm^{-1}): 3334 (NH), 1734 (C═O, ester carbonyl), 1643 (C═O, amide carbonyl); MS (EI) *m/z* (%): 453 (M^++1, 0.39), 304 (100); ^{1}H NMR ($CDCl_3$) δ ppm 1.23–2.05 (m, 12H, 6 x CH_2, cycloheptyl), 1.85 (s, 3H, O═C-CH_3), 2.8 (t, *J* = 6.7 Hz, 2H, CH_2-C_6H_5), 3 (t, *J* = 5.75 Hz, 2H, CH_2-CH_2-O), 3.5 (q, *J* = 6.7 Hz, 2H, CH_2-CH_2-C_6H_5), 3.6 (t, *J* = 5.75 Hz, 2H, CH_2-O), 3.77 (s, 3H, OCH_3), 6.7 (d, 2H, *J* = 8.6 Hz, $H_{ar.}$), 7 (d, 2H, *J* = 8.6 Hz, $H_{ar.}$), 7.2–7.31 (m, 5H, $H_{ar.}$), 7.5 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR ($CDCl_3$) δ ppm 20.83 (O═C-CH_3), 23.92, 30.94, 34.85 (6 x CH_2, cycloheptyl), 36.06 (CH_2-C_6H_5), 40.75 (CH_2-CH_2-C_6H_5), 49.16 (CH_2-CH_2-O), 55.43 (OCH_3), 62.39 (CH_2-O), 70.87 (Cq), 114, 126.56, 128.65, 128.89, 130.18 ($CH_{ar.}$), 137.91, 139.35, 157.77 (3 x $C_{ar.}$), 170.59 (O═C-CH_3), 177.6 (NH-C═O). Anal. calcd. for $C_{27}H_{36}N_2O_4$: C, 71.65; H, 8.02; N, 6.19. Found: C, 71.25; H, 8.42; N, 5.915.

4.2 | Anticonvulsant activity

4.2.1 | Animals and chemicals

The anticonvulsant activity of the compounds under investigation **IXa–l** and **Xa–l** was tested on adult male albino mice weighing 19–25 g.

All experimental procedures involving animals were performed according to the guidelines of the ethical committee of the National Research Centre for Experimental Animal Use. The mice were obtained from Animals House Colony of the National Research Centre, Cairo, Egypt and were housed in polypropylene cages under the standard conditions of light (12 h light/dark cycle) and temperature (23 ± 2°C). The mice were allowed free access to water and standard chow and were allowed to acclimatize to laboratory conditions before the start of the experiment.

The animals were randomly assigned to control, reference and tested experimental groups, each consisting of six mice. Each mouse was used only once and all tests were performed between 09:00 a.m. and 04:00 p.m. All the tested compounds were suspended in 7% Tween 80 as a vehicle. All chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA).

4.2.2 | scPTZ-induced seizures test[45]

scPTZ test was performed to access the production of threshold or minimal (clonic) seizures. PTZ, prepared as an aqueous solution, was administered subcutaneously in the loose fold of the skin on the back of the mice neck at a dose of 85 mg/kg (0.61 mmol/kg)[46] which is known to produce clonic seizures that last for a period of at least 5 s in 97% (CD97) of the animals tested. Six mice were used in both the control and the experimental groups. The control experiments were performed using the solvent alone. The other groups, each of which received individually the reference drugs, ethosuximide (150 mg/kg ≡ 1.06 mmol/kg)[47] or phenobarbital[48] (30 mg/kg ≡ 0.13 mmol/kg) or one of the test compounds in graded doses, **IXa–I** and **Xa–I** (2.5–100 mg/kg, *i.p.*).

The mice were observed for 30 min after PTZ administration for the occurrence of seizures. A threshold convulsion is defined as one episode of clonic convulsions which persist for at least 5 s. Failure to observe such threshold seizure indicates the ability of the test compound to abolish PTZ induced seizure threshold and is considered as protection.[49,50]

4.2.3 | MES test[51]

MES is a model used to identify generalized tonic-clonic seizures. Animals were randomly assigned to groups each of six mice. The first group served as the control group. The second group received phenytoin (45 mg/kg) as a reference drug and the other groups of mice received the test compounds individually by *i.p.* with the dose, which induces 100% protection in pentylenetetrazole test, or higher. Thirty minutes later, an electric current of fixed current intensity of 25 mA and 0.2 s stimulus duration was produced via Rodent Shocker generator (constant-current stimulator Type 221, Hugo Sachs Elektronik, Freiburg, Germany) and delivered via ear-clip electrode producing electroconvulsions in mice. The ability of the test compound to overcome the tonic hind limb extension (i.e., 120° outstretching of the mouse hind limb to the plane of the body axis) indicates its capability to inhibit MES-induced seizure spread.[52]

4.2.4 | Neurotoxicity screen

Neurotoxicity is determined using rotarod test which is well-established test for the detection of minimal neurological deficit. The mice were trained to maintain equilibrium on a rotating 1-inch-diameter knurled plastic rod at a speed of 10 rpm for at least 1 min in each of three trials using a rotarod device (UGO Basile, 47600, Varese, Italy). Only animals that fulfill this criterion were included into the experiment. The selected trained mice were classified into control group and experimental groups. The animals in the experimental groups were given the reference drug or one of the test compounds via *i.p.* route at dose which exerted 100% protection in PTZ test; meanwhile, the control group received the vehicle. Thirty minutes after the administration of the test compound or vehicle, the mice were placed again on the rotating rod and the neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min.[53]

4.2.5 | Serum liver enzyme activity

Mice were divided into six groups ($n = 6$). Group 1 served as control group and received the vehicle (7% aqueous suspension of Tween 80) only; Groups 2–6 received compound **Xd**, **Xf**, **Xh**, **Xi**, and **Xl** at doses (5, 10, 2.5, 10, and 10 mg/kg, *i.p.*), the doses that caused 100% protection in the scPTZ test, dissolved in vehicle. The treatments were continued out for a period of 7 days. Twenty-four hours after the last administration of the compounds, the animals were anaesthetized. The blood samples were collected by cardiac puncture followed by centrifugation at 3000 rpm for 10 min for the separation of sera. The serum samples obtained were used for the analyses of the liver enzymes AST, ALT, and ALP, as well as total protein and albumin were determined using commercially available kits (Biodiagnostic, Egypt).

4.2.6 | Acute toxicity test

Various doses of the test compounds ranging from 10 to 300 mg/kg dissolved in 7% tween 80 were given via *i.p.* to different groups of healthy adult mice.[54] After the administration of the compounds, mice were observed continuously for the first 2 h for any gross behavioral changes and deaths, and occasionally for 4 h, then intermittently for the next 24 h and for the onset of any delayed effects. The behavior of the animal and any other toxic symptoms were also observed for 72 h and then the animals were kept on observation for 14 days.[55]

4.3 | Molecular modeling studies

The hypothesis was generated using common feature pharmacophore model protocol in Discovery Studio 2016 Biova 1 software. The test sets (lead compounds) **2–13** (Figure 5) which were reported to have anticonvulsant activity were used to generate the anticonvulsant pharmacophore. A set of conformational models of each structure of the lead compounds was performed and used to generate the common feature hypotheses, where ten hypotheses were generated (Supporting Information).

4.4 | Single X-ray crystallography

Single crystals of suitable dimensions were chosen for X-ray diffraction studies. The X-ray intensity data were collected at a temperature of 298 K. All diagrams and calculations were performed using maXus.[56] The structure of title compound was refined using *SHELXL*-97.

ACKNOWLEDGMENTS

The authors would like to thank the National Research Centre (ID: 60014618), Dokki, Giza, Egypt, for the support of this research. The authors thank Dr. Walaa Hamada Abd-Allah for her assistance in performing the molecular modeling study.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

ORCID

Mohamed N. Aboul-Enein http://orcid.org/0000-0001-8749-0556

REFERENCES

[1] X. He, M. Zhong, T. Zhang, W. Wu, Z. Wu, J. Yang, Y. Xiao, Y. Pan, G. Qiu, X. Hu, *Eur. J. Med. Chem.* **2010**, *45*, 5870.
[2] S. Botros, N. A. Khalil, B. H. Naguib, Y. El-Dash, *Eur. J. Med. Chem.* **2013**, *60*, 57.
[3] F. Azam, I. A. Alkskas, S. L. Khokra, O. Prakash, *Eur. J. Med. Chem.* **2009**, *44*, 203.
[4] E. Perucca, J. French, M. Bialer, *Lancet Neurol.* **2007**, *6*, 793.
[5] G. Zaccara, D. Franciotta, E. Perucca, *Epilepsia* **2007**, *48*, 1223.
[6] P. Kwan, M. J. Brodie, *N. Engl. J. Med.* **2000**, *342*, 314.
[7] G. C. Crawley, J. M. Girodeau, US5350754, **1994**.
[8] J. Poudrel, P. Hullot, J. Vidal, J. Girard, J. Rossi, A. Muller, C. Bonne, *Bioorg. Med. Chem.* **1996**, *6*, 2349.
[9] M. Aboul-Enein, A. El-Azzouny, N. Abdallah, A. Hegazy, M. Ebeid, *Sci. Pharm.* **1990**, *58*, 273.
[10] P. Karak, K. Kumar, K. Mazumdar, M. Mookerjee, S. G. Dastidar, *Indian J. Med. Res.* **2003**, *118*, 192.
[11] M. N. Aboul-Enein, A. A. El-Azzouny, Y. A. Maklad, M. I. Attia, *Sci. Pharm.* **2001**, *69*, 329.
[12] T. R. Williams, L. M. Sirvio, US4089897, **1978**.
[13] K. J. McClure, M. Maher, N. Wu, S. R. Chaplan, W. A. Eckert, D. H. Lee, A. D. Wickenden, M. Hermann, B. Allison, N. Hawryluk, *Bioorg. Med. Chem.* **2011**, *21*, 5197.
[14] A. Markosyan, S. Gabrielyan, F. Arsenyan, R. Sukasyan, *Pharm. Chem. J.* **2015**, *48*, 783.
[15] G. Tamura, S. Suzuki, A. Takatsuki, K. Ando, K. Arima, *J. Antibiot.* **1968**, *21*, 539.
[16] M. G. Bogdanov, M. D. Palamareva, B. T. Gocheva, D. B. Dimitrova, *J. Heterocyclic Chem.* **2007**, *44*, 673.
[17] M. N. Aboul-Enein, A. El-Azzouny, *Acta Pharm. Suec.* **1986**, *23*, 107.
[18] S. Mukhopadhyay, S. Bhattacharya, Y. Rao, P. Das, *Indian J. Pharmacol.* **1982**, *14*, 191.
[19] J. D. Leander, *Epilepsy Res.* **1989**, *4*, 28.
[20] M. N. Aboul-Enein, A. El-Azzouny, F. Ragab, W. Soliman, Y. Maklad, *Sci. Pharm.* **2006**, *74*, 1.
[21] M. N. Aboul-Enein, A. A. El-Azzouny, A. A. Makhlouf, Y. A. Makld, *Egypt Pharmaceut. J.* **2004**, *3*, 19.
[22] M. N. Aboul-Enein, A. El-Azzouny, F. Ragab, M. S. Abdel-Maksoud, Y. Maklad, *Egypt Pharmaceut. J.* **2014**, *13*, 1.
[23] M. N. Aboul-Enein, A. A. El-Azzouny, O. A. Saleh, K. M. Amin, Y. A. Maklad, R. M. Hassan, *Arch. Pharm.* **2015**, *348*, 575.
[24] M. N. Aboul-Enein, A. A. El-Azzouny, Y. A. Maklad, M. A. Ismail, N. S. Ismail, R. M. Hassan, *Res. Chem. Intermediat.* **2015**, *41*, 3767.
[25] K. Fink, D. J. Dooley, W. P. Meder, N. Suman-Chauhan, S. Duffy, H. Clusmann, M. Göthert, *Neuropharmacology* **2002**, *42*, 229.
[26] N. O. Dalby, E. B. Nielsen, *Epilepsy Res.* **1997**, *28*, 63.
[27] R. W. Horton, G. M. Anlezark, M. C. B. Sawaya, B. S. Meldrum, *Eur. J. Pharmacol.* **1977**, *41*, 387.
[28] X. Zhang, X. Liu, T. Gong, X. Sun, Z. Zhang, *Acta Pharmacol. Sin.* **2012**, *33*, 279.
[29] M. N. Aboul-Enein, A. A. El-Azzouny, M. I. Attia, Y. A. Maklad, M. E. Aboutabl, F. Ragab, W. H. El-Hamid, *Int. J. Mol. Sci.* **2014**, *15*, 16911.
[30] K. Unverferth, J. Engel, N. Höfgen, A. Rostock, R. Günther, H. Lankau, M. Menzer, A. Rolfs, J. Liebscher, B. Müller, *J. Med. Chem.* **1998**, *41*, 63.
[31] H. Henecka, P. Kurz, E. Müller, *Methoden der Organischen Chemie. 4 Auflage, Stuttgart* **1952**, *3*, 427.
[32] P. R. Desai, D. R. Desai, J. S. Saharia, B. R. Saharia, *J. Indian Chem. Soc.* **1963**, *40*, 525.
[33] M. N. Aboul-Enein, A. A. El-Azzouny, *Acta. Pharm. Suec.* **1986**, *23*, 461.
[34] R. J. Porter, J. J. Cereghino, G. D. Gladding, B. J. Hessie, H. J. Kupferberg, B. Scoville, B. G. White, *Cleve. Clin. Q.* **1984**, *51*, 293.
[35] W. Löscher, D. Schmidt, *Epilepsy Res.* **1988**, *2*, 145.
[36] J. Obniska, A. Rapacz, S. Rybka, B. Powroźnik, E. Pękala, B. Filipek, P. Żmudzki, K. Kamiński, *Eur. J. Med. Chem.* **2015**, *102*, 14.
[37] M. Bialer, H. S. White, *Nat. Rev. Drug Discov.* **2010**, *9*, 68.
[38] M. Smith, K. S. Wilcox, H. S. White, *Neurotherapeutics* **2007**, *4*, 12.
[39] J. M. Rho, R. Sankar, *Epilepsia* **1999**, *40*, 1471.
[40] A. Misra, S. Ganesh, A. Shahiwala, S. P. Shah, *J. Pharm. Sci.* **2003**, *6*, 252.
[41] X. He, M. Zhong, T. Zhang, W. Wu, Z. Wu, Y. Xiao, X. Hu, *Eur. J. Med. Chem.* **2012**, *54*, 542.
[42] M. Z. Hassan, S. A. Khan, M. Amir, *Eur. J. Med. Chem.* **2012**, *58*, 206.
[43] A. P. Nikalje, A. Ansari, S. Bari, V. Ugale, *Arch. Pharm.* **2015**, *348*, 433.
[44] W. H. Abd-Allah, M. E. Aboutabl, M. N. Aboul-Enein, A. A. El-Azzouny, *Bioorg. Chem.* **2017**, *71*, 135.
[45] C. R. Clark, M. J. Wells, R. T. Sansom, G. N. Norris, R. C. Dockens, W. R. Ravis, *J. Med. Chem.* **1984**, *27*, 779.
[46] R. Fariello, R. McArthur, A. Bonsignori, M. Cervini, R. Maj, P. Marrari, P. Pevarello, H. Wolf, J. Woodhead, H. White, *J. Pharamcol. Exper. Therap.* **1998**, *285*, 397.
[47] M. Sayyah, A. Mandgary, *Iran. Biomed. J.* **2003**, *7*, 139.
[48] M. N. Aboul-Enein, A. A. El-Azzouny, M. I. Attia, Y. A. Maklad, K. M. Amin, M. Abdel-Rehim, M. F. El-Behairy, *Eur. J. Med. Chem.* **2012**, *47*, 360.
[49] O. Alam, P. Mullick, S. Verma, S. J. Gilani, S. A. Khan, N. Siddiqui, W. Ahsan, *Eur. J. Med. Chem.* **2010**, *45*, 2467.
[50] J. P. Stables, H. J. Kupferberg, *Molecular and Cellular Targets for Anti-Epileptic Drugs* (Eds: G. Avanzini, G. Regesta, P. Tanganelli, M. Avoli) Vol. 12, John Libbey & Company Ltd, England **1997**, Chapter 16, pp. 191–198.
[51] J. Luszczki, M. Czuczwar, P. Gawlik, G. Sawiniec-Pozniak, K. Czuczwar, S. Czuczwar, *J. Neural Transm.* **2006**, *113*, 1157.
[52] H. White, M. Johnson, H. Wolf, H. Kupferberg, *Ital. J. Neurol. Sci.* **1995**, *16*, 73.
[53] N. Dunham, T. Miya, *J. Pharm. Sci.* **1957**, *46*, 208.
[54] R. Cao, Q. Chen, X. Hou, H. Chen, H. Guan, Y. Ma, W. Peng, A. Xu, *Bioorg. Med. Chem.* **2004**, *12*, 4613.
[55] N. Pahari, D. Saha, V. K. Jain, B. Jain, D. Mridha, *Int. J. Pharm. Sci. Res.* **2010**, *1*, 399.

[56] S. Mackay, C. J. Gilmore, C. Edwards, N. Stewart, K. Shankland, *maXus Computer Program for the Solution and Refinement of Crystal Structures*, Bruker Nonius, The Netherlands, MacScience, Japan and The University of Glasgow **1999**.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Aboul-Enein MN, El-Azzouny AA, Amin KM, Aboutabl ME, Abo-Elmagd MI. Synthesis, molecular modeling studies, and anticonvulsant evaluation of novel 1-((2-hydroxyethyl)(aryl)amino)-*N*-substituted cycloalkanecarboxamides and their acetate esters. *Arch Pharm Chem Life Sci*. 2018;1–19. https://doi.org/10.1002/ardp.201800269