The Role of Structure Activity Relationship Studies in the Search for New GABA Uptake Inhibitors

Katarzyna Kulig* and Marta Szwaczkiewicz

Department of Physicochemical Drug Analysis, Chair of Pharmaceutical Chemistry, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-688 Kraków, Poland

Abstract: γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system (CNS). A decrease in GABAergic neurotransmission seems to be involved in neurological pathologies such as epilepsy, anxiety and pain. This review is focus on structure activity relationship studies aiming the search for new GABA uptake inhibitors.

Key Words: γ-Aminobutyric acid, GABA, GABA uptake antagonist, GABA transporter, GAT.

INTRODUCTION

γ-Aminobutyric Acid (GABA) is well recognized as the principal inhibitory neurotransmitter in the cerebral cortex. GABA is formed within GABAergic axon terminals by transamination of α-ketoglutarate to glutamic acid, which is then decarboxylated by glutamic acid decarboxylase (GAD) to GABA. It is realised into the synapse and then acts one of three types of GABA receptors: $GABA_A$, $GABA_B$, and $GABA_C$. After release from the presynaptic axon terminals, GABA is rapidly removed by uptake into both glial and presynaptic nerve terminals and then is catabolized by GABA transaminase to succinic semialdehyde. Succinic semialdehyde is converted to succinic acid by succinic acid semialdehyde dehydrogenase and then enters the Krebs cycle [1].

As is given above a GABA synapse contains several important regulatory points such as the synthesizing enzyme (GAD), the vesicular GABA transporters (VGAT) the release mechanism the GABA receptors, the plasma membrane GABA transporters (GATs) and the GABA catabolic enzyme GABA transaminase (GABA-T). Each one of these entities constitutes a potential drug targets but so far only GABA receptors, GATs and GABA-T have proven therapeutically relevant target [2].

This review is focus on presentation of compounds that have been evaluated as GABA uptake inhibitors in neuronal and glial uptake assays. These compounds have been primary investigated in relation to epilepsy. More recent studies showed that they could be also useful in therapy of other CNS-related disorders such as neuropathic pain and schizophrenia, where GABA mediated neurotransmissions is believed to be involved [3-5].

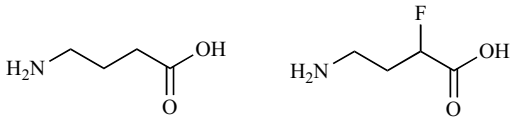
Up to date four different GABA uptake transporters have been described GAT-1, GAT-2, GAT-3 and betaine BGT-1. Their nomenclature is spices dependent and mGAT1 – GAT4 in mouse correspond to rGAt-1, rBGT-1, rGAT-2 and rGAT-3 in rat, respectively. Rats nomenclature is also used for the human subtypes, but the human analogue of mGAT3 has not been cloned (Table **1**) [2].

Table 1. Nomenclature of the Cloned GABA Transporters from Mouse, Rat and Human

Species	Nomenclature			
Mouse	GAT1	GAT2	GAT3	GAT4
Rat	GAT-1	BGT-1	GAT-2	GAT-3
Human	GAT-1	BGT-1	not cloned	GAT-3

ACYCLIC GABA UPTAKE INHIBITORS

Finding GABA (**1**) itself is a potent inhibitor of [^{3}H]-GABA uptake inhibitor both glial and neuronal cells (mGAT1 IC_{50}=2.8 μM, mGAT2 IC_{50}=14 μM, mGAT3 IC_{50}=3.9 μM and mGAT4 IC_{50}=3.4 μM) become a starting point of search for new GAT inhibitors. Therefore, GABA receptor antagonist have been tested as GABA uptake inhibitor. The most potent GAT inhibitor among its close analogues is 2-fluoro-GABA (**2**), which IC_{50} value of neuronal GABA uptake into brain slice equals 35 μM.



GABA (**1**) 2-Fluoro-GABA (**2**)

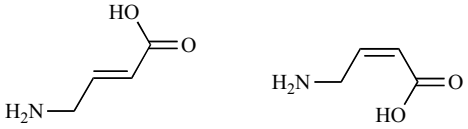
Fig. (1).

Introduction of double bond in GABA molecule resulted in 4-aminocrotonic acids. Its *trans* diastereomer (TACA) (**3**) displayed a similar high potency for three transporters mGAT1, mGAT3 and mGAT4 (IC_{50} 3.3, 7.2 and 5.1 μM)

*Address correspondence to this author at the Department of Physicochemical Drug Analysis, Chair of Pharmaceutical Chemistry, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-688 Kraków, Poland; E-mail: mfkkulig@cyf-kr.edu.pl

1389-5575/08 $55.00+.00

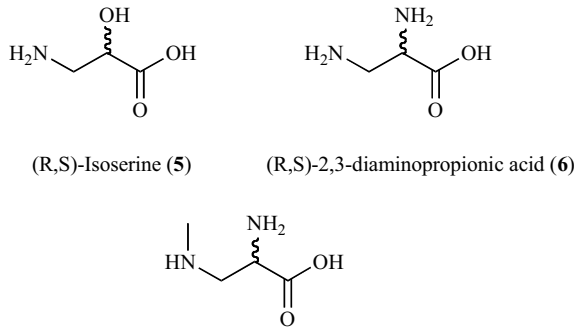
and 10 times lower for mGAT2 (IC_{50} 64 μM). While the subtype selectivity of *cis* diastereomer (CACA) (**4**) appeared to be noticeably different, exhibiting a marked and almost identical potency at mGAT3 and mGAT4 (IC_{50} 9.5 and 9.2 μM) and a far lower activity at mGAT1 and mGAT2 (IC_{50} 280 μM both) [7].



TACA (**3**) CACA (**4**)

Fig. (2).

The next acyclic compounds identified as a new GABA uptake inhibitor is (*R*, *S*)-Isoserine (**5**). This compound displays a high potency at mGAT3 and mGAT4 (IC_{50} 4.3, 5.9 μM) associated with high preference for these two transporters (IC_{50} 2500 (mGAT1) 230 (mGAT2) μM). Similarly, (*R*, *S*)-2,3-diaminopropionic acid (**6**) was an inhibitor of mGAT3 and mGAT4 (IC_{50} 11 and 5.6 μM). It is also worth to note its potency and subtype selectivity could be slightly reduced by the addition of *N*-methyl group (**7**) (IC_{50} 260 (mGAT1), 160 (mGAT2)35 (mGAT3) and 20 (mGAT4) μM) [7].



(R,S)-Isoserine (**5**) (R,S)-2,3-diaminopropionic acid (**6**)

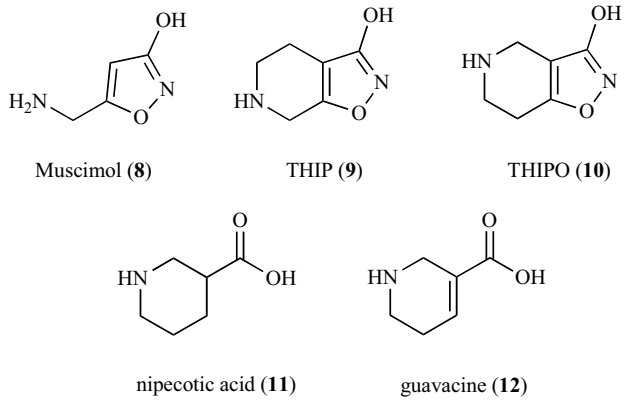
(R,S)-2-amino-3-methyl-aminopropionic acid (**7**)

Fig. (3).

CYCLIC GABA UPTAKE INHIBITORS

Knowing GABA can exist in a wide variety of conformations due to comparative freedom of rotation about the single bonds. Extensive structure activity studies on conformationally restricted analogues of GABA were undertaken.

Muscimol (5-aminomethyl-3-isoxazolol) (**8**), a constituent of the fly agaric mushroom (*Amonita muscaria*), is a GABA analogues that acts as a bioisosteric substitute for the carbocyclic acid and induced conformational restrictions on the carbon backbone of GABA. It was found muscimol interact with $GABA_A$ receptor (IC_{50} 0.006 μM) and transporters (31% at 0.5 μM) as well as GABA-T [8]. Further conformational restrictions of muscimol by an ethylene linker gave 4,5,6,7-tetrahydroizoxazolo[4,5-c]pyridine-3-ol (THIP) (**9**). This compound selective activates receptors (IC_{50} 0.13 μM) without any apparent effects on GABA transport. THIP does not display the high affinity of muscimol in a symptomal GABA binding assay, but is currently known as a potent super-agonist at δ-subunit containing $GABA_A$ receptor [9]. Moving the basic nitrogen atom in the piperidine ring led to the β-alanine analogue THPO (**10**). This compound display essentially no receptor affinity but improved uptake inhibitory properties (IC_{50} 462 μM). The obtained results led to discovery of two very selective and potent transport inhibitors nipecotic acid (**11**) (IC_{50} 9 μM) and guvacine (**12**) (IC_{50} 8 μM), which are cyclic amino acid parents of THPO. These compounds are complementary devoid of measurable receptor activity and therefore highly selective GABA transport inhibitors [9].



Muscimol (**8**) THIP (**9**) THIPO (**10**)

nipecotic acid (**11**) guvacine (**12**)

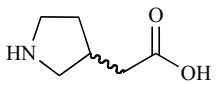
Fig. (4).

The structure of THPO was also redesigned by moving its basic nitrogen atom to an exocyclic position giving (*R, S*)-3-hydroxy-4-amino-4,5,6,7-tetrahydro-1,2-benzisoxazole (*exo*-THPO) (**13**) (IC_{50} 1000 (mGAT1), 3000 (mGAT2), >3000 (mGAT3) and >3000 (mGAT4) μM). In a series of *N*-alkylated *exo*-THPO derivatives, the *N*-methylated compound *N*-Me-*exo*-THPO (**14**) (IC_{50} 450 (mGAT1), >3000 (mGAT2), >3000 (mGAT3) and >3000 (mGAT4) μM) turned out to be the most glia – selective GABA transport inhibitor reported yet [10].

exo-THPO (**13**) N-Me-exo-THPO (**14**)

Fig. (5).

Further structure-activity studies on chiral GABA analogues were performed. It was found (R,S) *homo*-β-proline (**15**) is an inhibitor of $GABA_A$ (IC_{50} 0.2 μM) and $GABA_B$



Homo-β-proline (**15**)

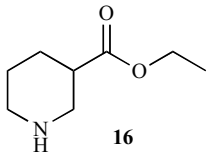
Fig. (6).

(IC_{50} 0.03 μM) receptor binding and of GABA uptake (IC_{50} 2.5 μM), and could be regarded as conformationally restricted GABA analogue [11].

LIPOPHILIC GABA UPTAKE INHIBITORS

Homo-β-proline (**15**), guvacine (**12**) and nipecotic acid (**11**) were the most potent inhibitors of GABA uptake, however these compounds do not easily enter the CNS of animals in pharmacologically significant amounts following peripheral administration, presumably due to their hydrophilic character, so the affords to obtain their orally active analogues were undertaken.

One strategy for introducing pharmacologically significant concentration of GABA-uptake inhibitors into CNS is to administrate their esters. Being more lipophilic compounds esters can cross the blood-brain barrier, and in the CNS they may be hydrolyzed to the parent compounds. It was found peripheral administration of ethyl ester of nipecotic acid (**16**) elevates GABA levels in synaptosomes (52 % (100 μM) and display anticonvulsant activity in animals [12].



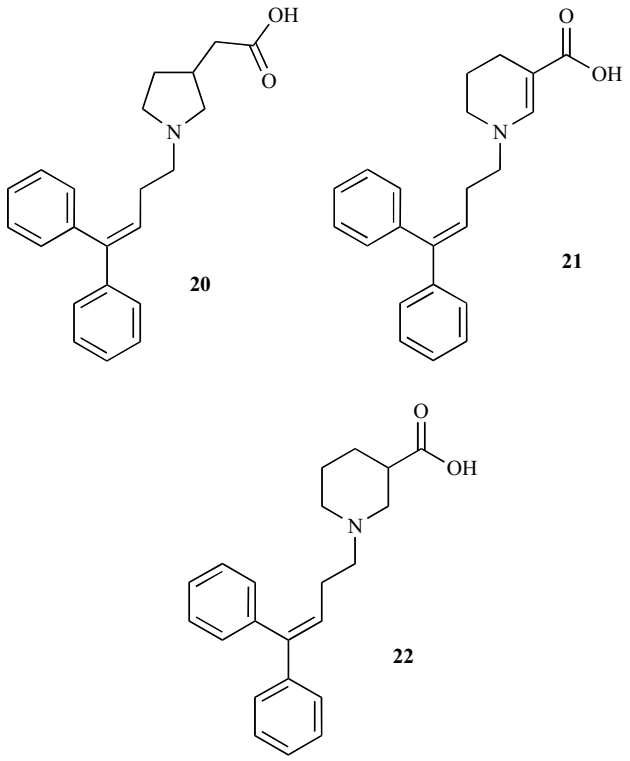
16

Fig. (7).

The next strategy to discover orally active GABA-uptake inhibitors was identification and alkylation position of bulk tolerance on known GABA uptake inhibitors such as for example GABA, nipecotic acid and guvacine with lipophilic group.

Introduction of methyl moiety into amino group of GABA resulted in compound (**17**), which characterized lower then GABA affinity for both GABA uptake (IC_{50} 65 μM) into synaptosomes, and for $GABA_A$ receptor (IC_{50} 67 μM). Similar effect was observed when amine group of GABA was blocked by bulky 4,4-diphenyl-3-butenyl rest (**18**). The obtained compound (**18**) posses six fold lower synaptosomes GABA uptake activity (IC_{50} 18 μM) then GABA itself. Conversion the primary amino group of GABA into tertiary one by both *N*-methylation and *N*-4,4-diphenyl-3-butenylation resulted in compounds (**19**) showing GABA uptake (IC_{50} 4.0 μM) affinities comparable with that of GABA [13].

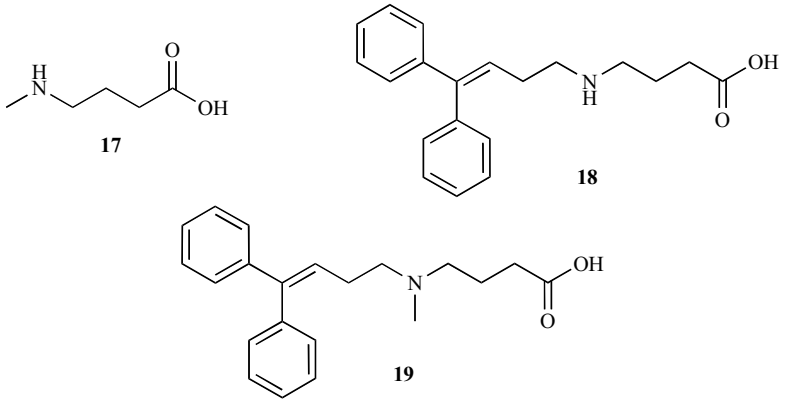
The 4,4-phenyl-3-butenyl rest was also introduced into *homo*-β-proline (**20**) (IC_{50} 0.12 μM), guvacine (**21**) (IC_{50} 0.20 μM), nipecotic acid (**22**) (SKF 89976-A) (IC_{50} 0.20 μM) and (R)- nipecotic acid ((R)-**22**) (IC_{50} 0.11 μM). The key observation to be drawn from performed research is that the obtained compounds (**20 - 22**) were respectively 12-, 19-, 24- and 17- fold more potent that their parent structure and had IC_{50} ranging 120 to 260 nM [13].



20 **21** **22**

Fig. (9).

The structure-activity relationship of *N*-substitutent on compound (**22**) were explored to determine what features of the diphenylbutenyl group contribute to its high inhibitory potency and to develop more potent GABA-uptake inhibitors. It was shown that reduction of the olefinic double bond



17 **18** **19**

Fig. (8).

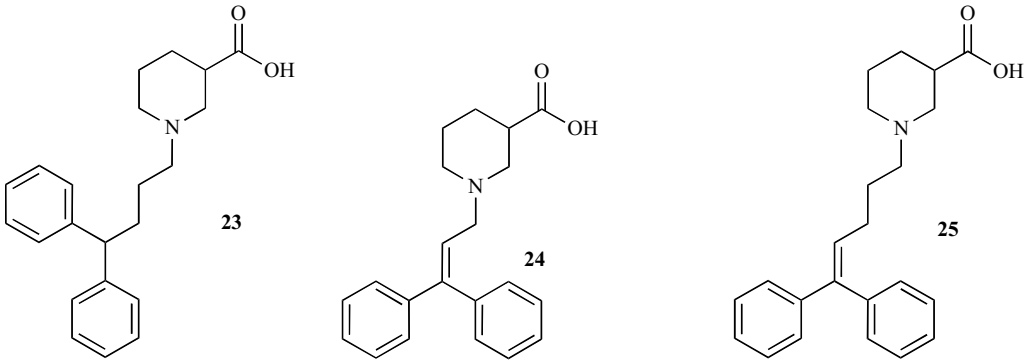


Fig. (10).

(**23**) (27 % (10 μM)) or shortening of the ethylene bridge (**24**) (5 % (10 μM)) resulted in dramatic decrease in potency. In addition increasing the length of the ethylene bridge (**25**) (31% (1 μM)) resulted in a smaller but still significant decrease in potency [13].

To identifying novel GABA uptake inhibitors with improved potency and selectivity, a large range of 4,4-disubstituted-3-butenyl nipecotic acid derivatives containing both aryl and heteroaryl groups have been prepared. When, compared to reference GABA uptake such as SKF 89976A compound comprising of simple thiophene/phenyl (**26**) (IC_{50} 290 nM) isosteric replacement showed no significant improvements in potency over parent compounds [14].

Incorporation of two phenyl groups in SKF 89976A into a tricyclic system resulted in compound ((R)-**27**) that was only weakly active as GABA uptake inhibitor (IC_{50} 1920 nM).

Replacement of two phenyl groups with thiophenes (**28**) (IC_{50} 256 nM) resulted in compounds having increased potency *in vitro* as inhibitor of GABA uptake, especially interesting were compounds with a substituents located in an "ortho" position on one ((R)-**29**) (IC_{50} 61 nM) or both ((R)-**30**) (IC_{50} 67 nM) of the heteroaromatic moieties. The same tendency was observed with the introduction of two o-methyl-groups in SKF 89976A (**31**) (IC_{50} 82 nM) resulting in 4-fold increase in *in vivo* potency. One compound from the above series (R)-1-[4,4-bis-(3-methyl-2-thienyl)-3-butenyl]-3-piperidine carboxylic acid (NNC-05-0328) ((R)-**30**) tiagabine was introduced for the treatment of epilepsy [14-16]. Tiagabine is a selective GABA reuptake inhibitor, increase synaptic GABA activity *via* inhibition of the GAT-1 transpoter on presynaptic neurons and glial cells. Tiagabine is an effective drug for the adjunctive treatment of partial seizures. Recent investigations suggest also that this compound may be beneficial in other psychiatric disorders and drug dependence [15].

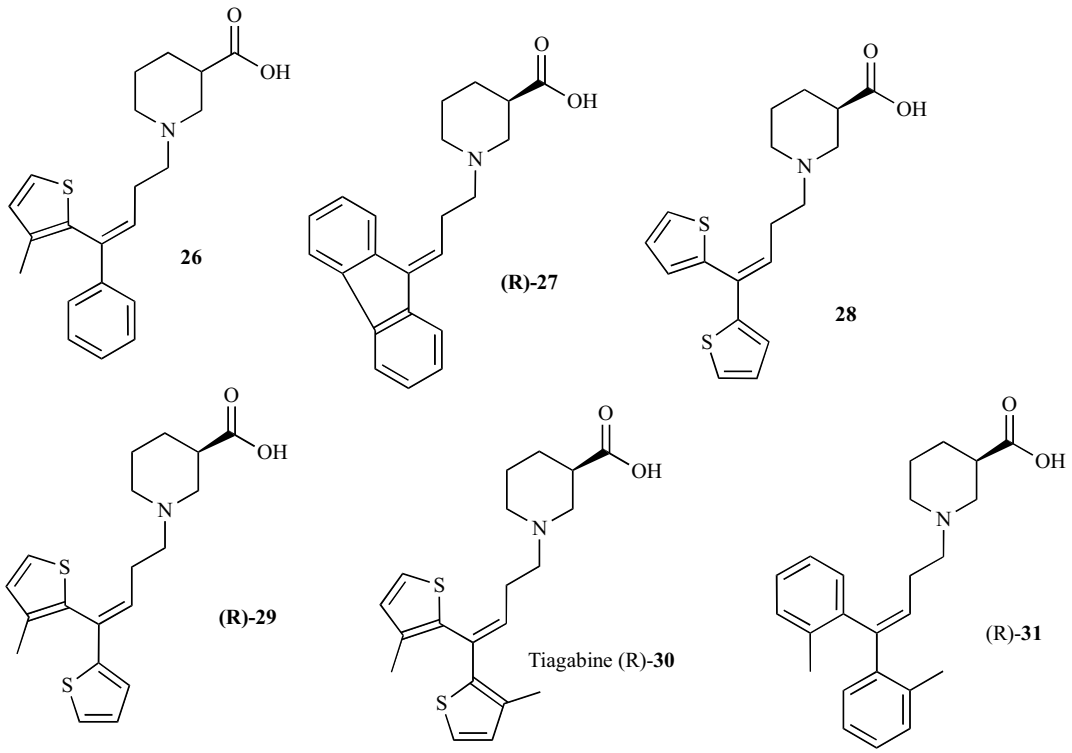
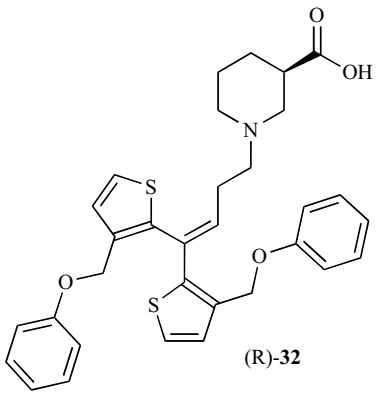


Fig. (11).

The three-dimensional quantitative structure activity relationship studies on *N*-diarylalkenyl-piperidinecarboxylic acid suggested that either one or two of the aryl rings substituted with bulky group in the *ortho* position may improve the GAT1 inhibitory activity. The performed biological assay showed that the most potent compound (R)-1-[4,4-bis(3-phenoxymethyl-2-thienyl)-butenyl]-3-piperidinecarboxylic acid (R)-(**32**) (IC_{50} = 0.34 μM) possess GAT1 inhibitory activity close to tiagabine. This observation proved that the proper steric effect in *ortho* position on thiophene ring may play a critical role for potential GAT1 inhibitory activity [17].



(R)-**32**

Fig. (12).

Being a lead structure in the search for new GABA uptake inhibitors, SKF 89976A was subject of several further structure activity relationship studies. It was found replacement of double bond in this molecule by -CH-O- group was resulted in potent GABA uptake inhibitor (**33**) (IC_{50} = 3.4 μM), which showed anticonvulsant activity after systematic administration in the kindled rat model of partial complex seizures. While, the lengthening the side chain (**34**) (IC_{50} = 12 μM) or replacing the oxygen atom with a sulphur one (**35**) (IC_{50} = 60 μM) cause a loss of GABA uptake activity. The best activity profile was reach for compound having two phenyl ring that in the 4-position are substituted by chloride atoms (**36**) (IC_{50} = 0.41 μM). It was found the (S)-(**36**) (IC_{50} = 14 μM) enantiomer was approximately 50 times less potent then (R)-(**36**) (IC_{50} = 0.18 μM) one. To obtain a potent GABA inhibitor that contains no chiral center, its unsaturated analogue was obtained (**37**). Compound (**37**) was a potent GABA uptake inhibitor (IC_{50} = 0.41 μM), additionally *in vivo* evaluation revelled anticonvulsant activity ED_{50} = 17 mg/kg, but ataxia at only slightly higher doses was the reason that its further evaluation was terminated. The lowering of this undesirable effect was achieved by replacing the chloride atoms into two triflouromethyl groups (**38**). Compound CI-966 (**38**) was also potent GABA uptake inhibitor (IC_{50} = 0.34 μM) producing a concentration related blockade of Na dependent, high-affinity [^{3}H]GABA uptake into rat hippocampal slices. Its 10-20 times increment in *in vitro* potency might result from several factors including, the hydrogen bonding ability of the side chain oxygen atom, a loss of electron delocalization by olefin removal, or change in conformational constrains a diphenyl conformation that better matches the three dimensional-topography of the GABA uptake inhibitors. The promising results of both *in vitro* and *in vivo* tests let compound to enter preliminary tolerance and

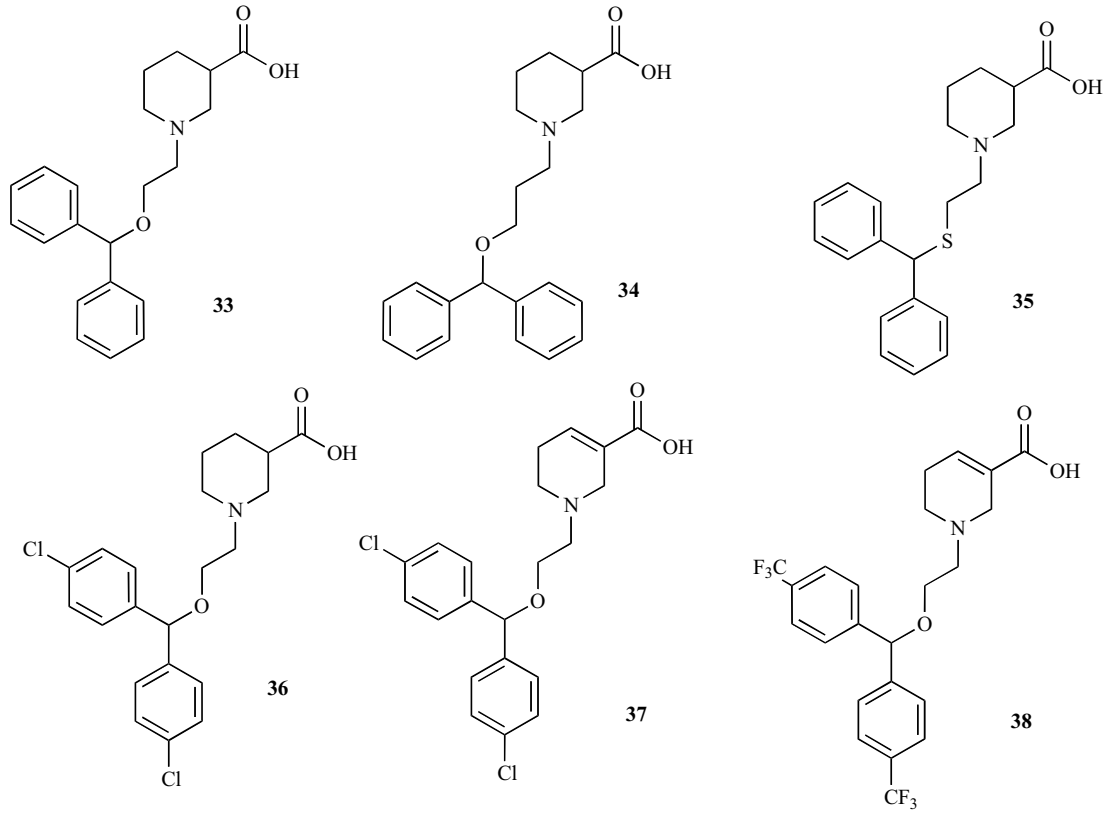


Fig. (13).

safety study on the healthy volunteers, but its highest dose administrated serve neurological and psychological side effects. Further clinical tests of this compound was terminated [18].

The double bond in SKF 89976A was also replaced by the isosteric >N-CH_2- moiety (**39**) (IC_{50} = >9000 nM), but this direct replacement is not allowed as potency is completely lost when compare to the patent compound. However, successive elongation of the side chain in compound leads to improved potency. For compound (**40**) containing seven carbon atoms, potency has been totally recovered (IC_{50} = 232 nM). It was also found that the placement of the oxygen atom and the length of the chain is very critical. The most beneficial result was obtained for compound (**41**) (IC_{50} = 90 nM) [19].

The analogues of SKF 89976A in which chain an oxygen atom was added were obtained. The resulting compound (**42**) displayed similar to parent compound GABA uptake activity (IC_{50} = 258 nM). The influence of substituents in the phenyl ring was also tested. It was shown an *ortho*-methyl- substitution on both phenyl groups is beneficial for potency (**43**) (IC_{50} = 114 nM). As these methyl substituents only allow the out of plane conformations of the aryl groups in this compound, they may introduce the preferred conformations of the aryl groups in the binding site. It was also proved one *ortho*-methyl substituent (**44**) (IC_{50} = 236 nM) seems not to create enough strain in the aryl moiety of this compounds to improve potency compared to the parent compound. The most potent GABA uptake inhibitor in these series was compound (**45**) (IC_{50} = 75 nM) [19].

In the series of hydrogenated ether derivatives (**46 - 48**), a very different structure activity relationship is observed compared to above described compounds (**42 - 45**). Opposite to the observation described above, one *ortho*-methyl substituents (**46**) (IC_{50} = 86 nM) lowers the potency to some extent, but *ortho*-methyl substituents on both phenyl groups lower (**47**) (IC_{50} = 358 nM) the potency in comparison to unsubstituted compound (**48**) (IC_{50} = 55 nM). The explanation of the missing ortho effect may be the change in the sp^2 system in the allyl ethers to a less strained sp^3 system in the hydrogenated analogues. In this case, the aryl groups are easily oriented in the preferred conformations due to the flexible sp^3 one [19].

The GABA uptake inhibitor activity was also displayed by diaryl/ heteroaryl- oxime and vinyl ether moiety joined by an aliphatic "linker" to a cyclic amino acid. Among the synthesized heretoaryl-oximes compounds containing two 3-methyl-2-thienyl- (**49**) (IC_{50} = 41 nM), two phenyl groups

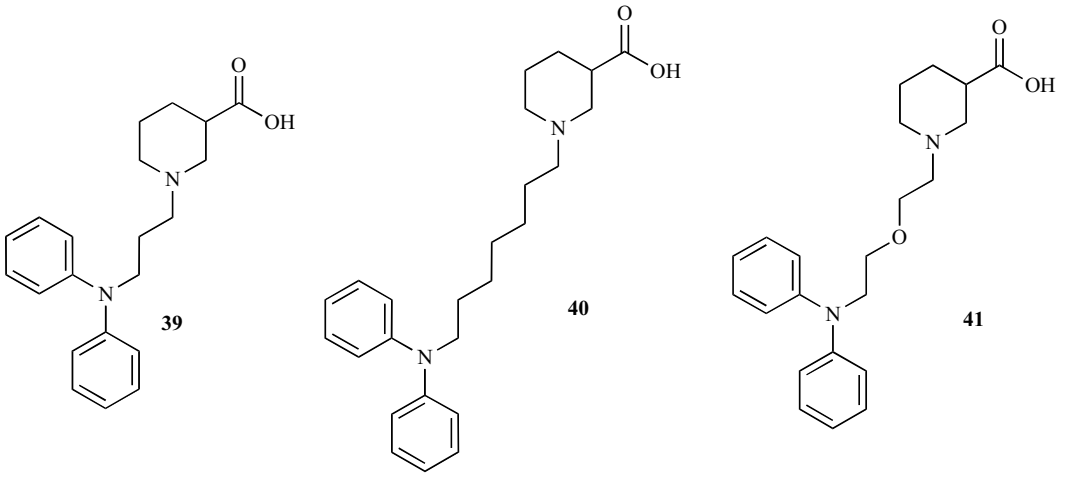


Fig. (14).

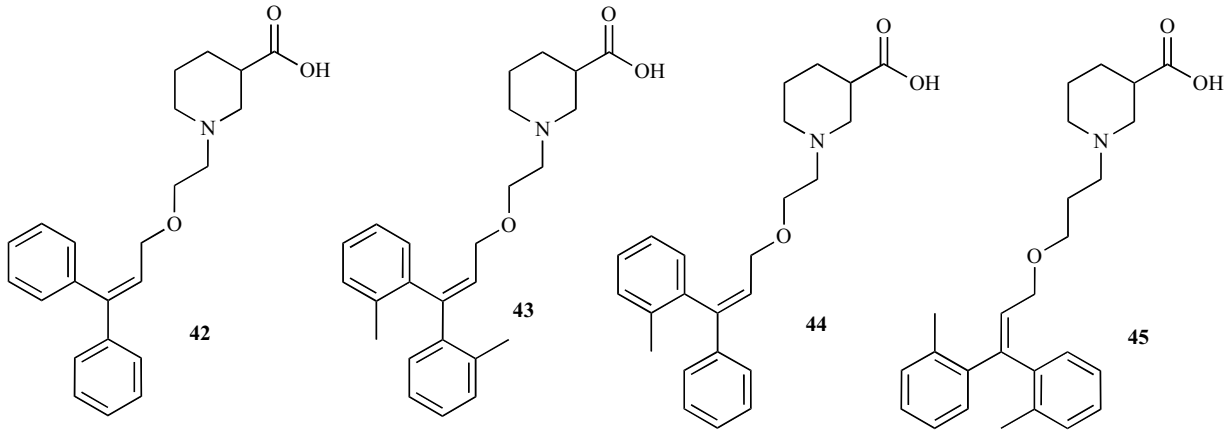


Fig. (15).

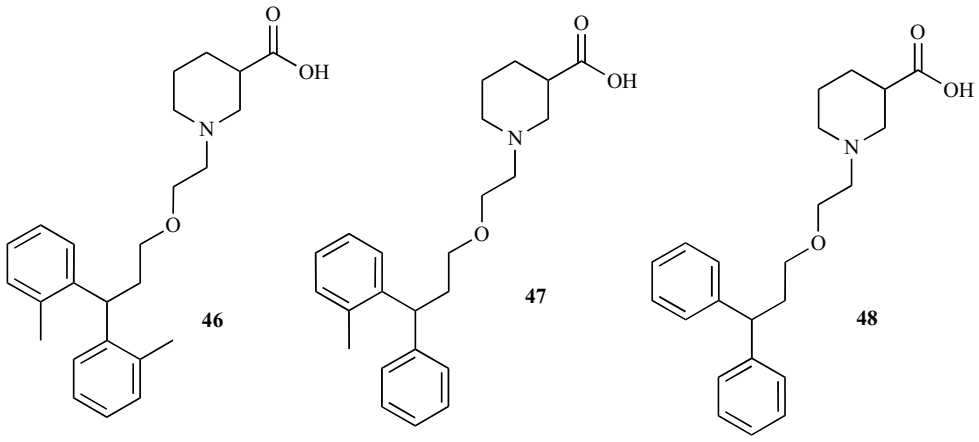


Fig. (16).

(**50**) (IC_{50} = 81 nM) and di-*ortho*-methyl-phenyl groups (**51**) (IC_{50} = 31 nM) were the most active ones. Significant improve in the activity was observed for their vinyl ether analogues (**52 -54**) (IC_{50} = 14, 86 and 19 nM). The interesting GABA uptake inhibiting activity (IC_{50} = 12 nM) was also displayed for compound (**55**) that has both *ortho*-methyl-phenyl and *ortho*-chlorophenyl substituents. These highly potent GABA uptake inhibitors may have been potential in the treatment of epilepsy in humans [20].

New GABA uptake inhibitors was also search among compounds in which the diaryl moiety of earlier described compounds was transformed into various tricyclic ring systems. The most interesting compounds in this series were phenothiazine (**56**) (IC_{50} = 308 nM), 10,11-dihydro-5H-dibenzo[b, f]azepine (**57**) (IC_{50} = 184 nM), and mono- or di-chloro- substituted 10,11-dihydro-5H-dibenzo[b, f]azepines (**58, 59**) (IC_{50} = 74, 68 nM) derivatives [21].

The design of selective GAT-2, GAT-3 or BGT-1 inhibitors was based on comparisons structures of EGYT-3886 (IC_{50} 26 (hGAT-1), 30 (rGAT-2), 46 (hGAT-3) and 39 (hBGT-1) μM) with GAT-1 inhibitor CI-966 (IC_{50} 0.26 (hGAT-1), 1280 (rGAT-2) 333 (hGAT-3) and 300 (hBGT-1) μM).

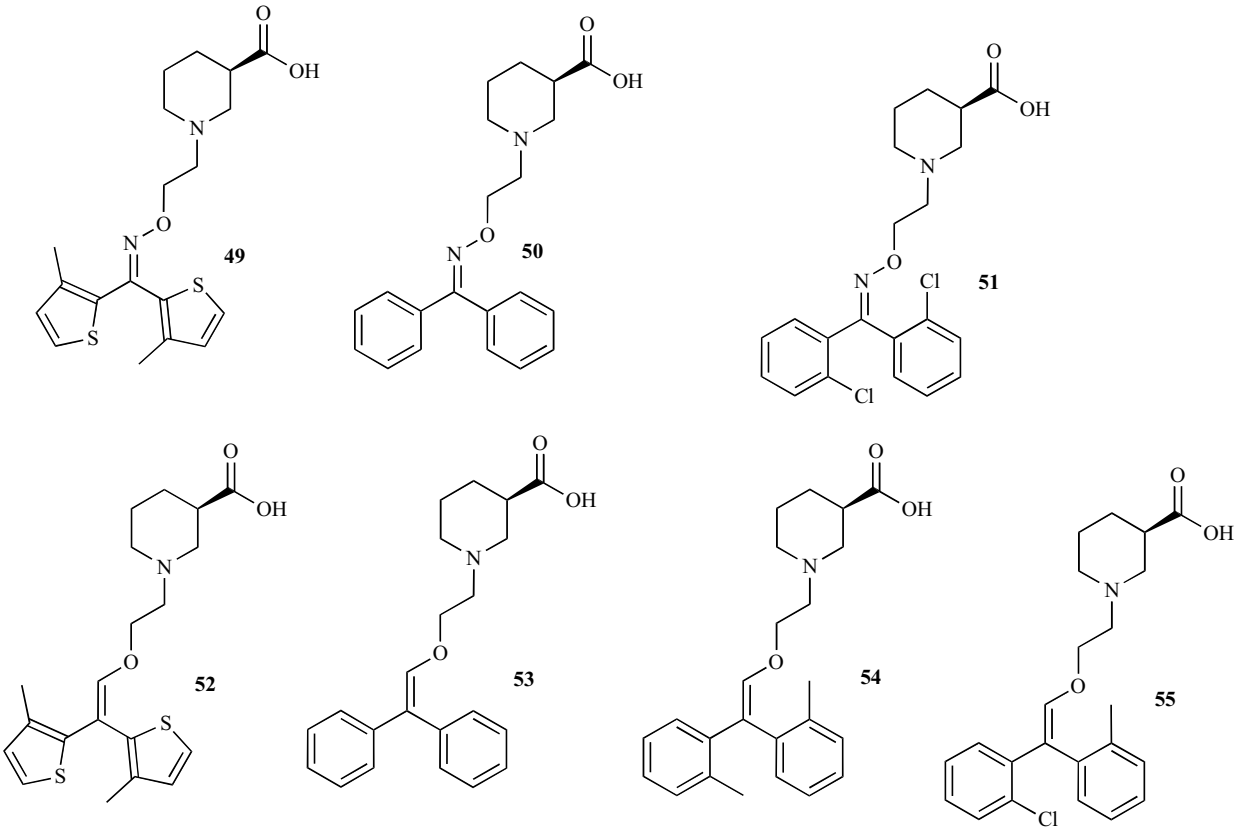


Fig. (17).

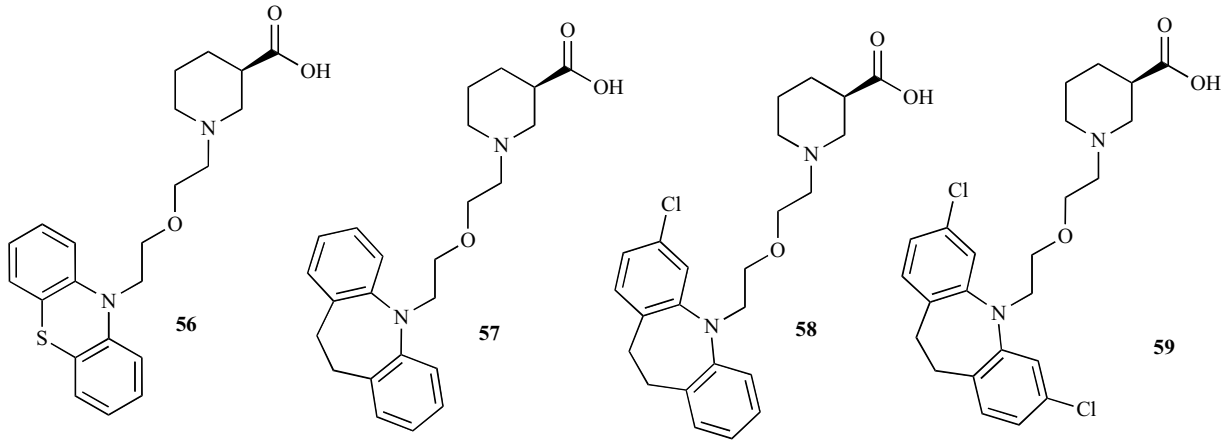


Fig. (18).

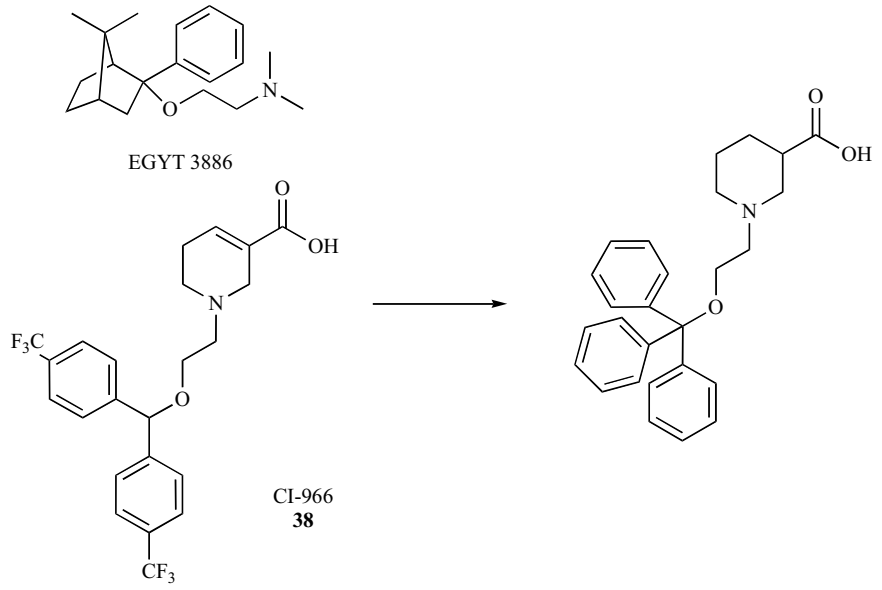


Fig. (19).

It was revealed that:

- both have an aminoethanol unit in common;

- the oxygen atom of EGYT-3886 is linked to a quaternary carbon atom, whereas that of CI-966 is linked to a tertiary carbon atom;

- CI-966 is a guvacine derivative, whereas EGYT-3886 is a dimethylamino-etanol one.

Based on above a series of nipecotic derivatives, which mimic key structural features of EGYT-3886 and CI-966 were design. From the novel series of triaryl substituted (S)-nipecotic acid derivatives (**60**), compound showed moderate affinity and selectivity for cloned human GABA transporter hGAT-3 (IC_{50} 388 (hGAT-1), 21 (rGAT-2), 5 (hGAT-3) and 140 (hBGT-1) μM). Compound (**60**) could be useful in further GAT investigations [22].

The subtype selective GABA uptake inhibitor were search among *N*-methyl-*exo*-THPO derivatives. The most interesting results were obtained for EF1502 (**61**) that was more potent then GABA itself at both GAT-1 and GAT-2 (IC_{50} 7 (GAT1), 26 (GAT2)μM) without significant effects at GAT3 and GAT4 (IC_{50} >300 (GAT3), >300 (GAT4) μM). This interesting profile of the racemic unexpectedly turned

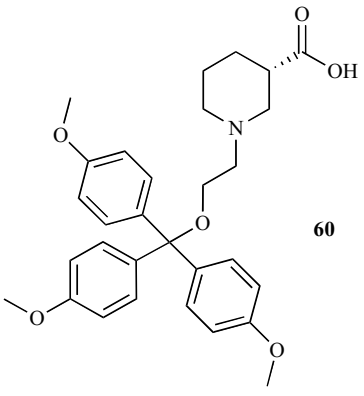


Fig. (20).

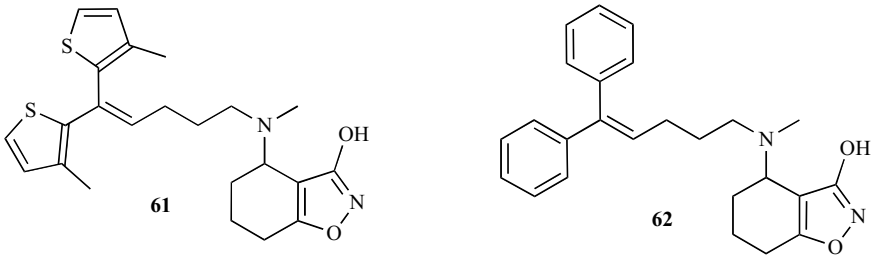


Fig. (21).

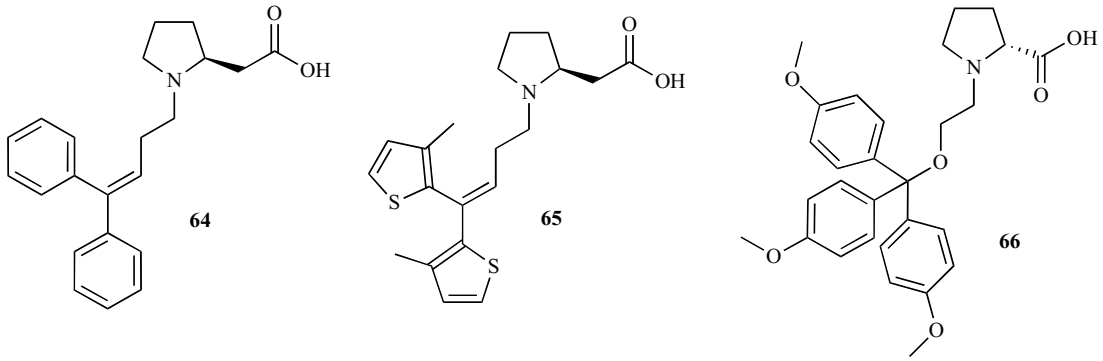


Fig. (22).

out to emerge from very different profiles of enantiomers. GAT1 activity could be assigned primary to (R) enantiomer (IC_{50} 4 (GAT1), 22 (GAT2) μM), whereas enantiomers (S) (IC_{50} 120 (GAT1), 34 (GAT2)μM) and (R) contributed to the potency of racemates at GAT2. *N*-methyl-exo-THPO derivative (**62**) was GAT1 selective GABA uptake inhibitor (IC_{50} 2 (GAT1), 200 (GAT2), >100 (GAT3), >100 (GAT4) μM) [23-25].

To search for subtype selective GABA uptake inhibitors several pyrrolidine derivatives were synthesizes. It was found compounds (**64**), (**65**), (**66**) were potent and selective GAT-1 and GAT-3 inhibitors (IC_{50} 0.39 (GAT-1), 64.8 (GAT-3) μM) (**64**) (IC_{50} 0.34 (GAT-1), 26.6 (GAT-3) μM) (**65**) and (IC_{50} 67.8 (GAT1), 3.10 (GAT3) μM) (**66**). It was also observed that in comparison to the corresponding 4-unsubstited compounds the 4-hydroxypyrrolidine-2-carboxylic acid showed a significant decrease in the inhibitory potency [26, 27].

NATURAL PRODUCTS

The compounds for the study of the GABAergic system, which could be a potential source for new anticonvulsant drugs are search among natural product. *Parawixia bistriata* (Araneidae, Araneae) is a South American social orb weaver spider. *Parawixia bistriata* crude venom inhibit by 73% the GABA uptake in synaptosomes from cerebral cortex. Among the isolated polyamine toxin FrPbAII (174 Da) blocked epileptic seizures induced by many chemical convulsants. Experiments using synaptosomes from rat cerebral cortices show that it inhibits GABA and glycine uptakes, with no effects on ion channels, GABAergic relase or binding at its specific receptors [28, 29].

SUMMARY

This review describes different structures design and tested as potential GABA uptake inhibitors. Among them, tiagabine represent an unique antiepileptic drug, which inhibit GABA uptake into synaptosomal membranes, neurones, and gial cells. Knowing, the GABA system is the most successful target for the rational design of novel antiepileptic compounds, one possible way for search new active molecules are GABA uptake inhibitors.

ACKNOWLEDGEMENTS

This study was supported from by the Polish Ministry of Sciences and Higher Education grant DAAD/54/2006. The authors would like to thanks Prof. Dr. Hab Barbara Malawska for her helpful discussion.

REFERENCES

[1] Treiman, D. M. *Epilepsia,* **2001**,*42*(Suppl. 3), 8.
[2] Sarup, A.; Miller, O.; Schousboe, A. *Curr. Drug Targets –CNS Neurol. Dis.,* **2003**, *2,* 269.
[3] Conti, F.; Minelli, A.; Melone, M. *Brain Res. Rev.,* **2004**, *45*, 196.
[4] Iversen, L. *Biochem. Pharmacol.*, **2004**, *68*, 1537.
[5] Volk, D. W.; Lewis, David A. *Curr. Neuropharmacol.,* **2005**, *3,* 45.
[6] Høg, S.; Greenwood, J. R.; Madsen, K. B.; Larsson, O. M.; Frølund, B.; Schousboe, A.; Krogsgaard-Larsen, P.; Clausen, R. P. *Curr. Topics Med. Chem.,* **2006**, *6,* 1861.
[7] Kragler, A.; Höfner, G.; Wanner, K. T. *Eur. J. Pharmacol.,* **2005**, *519,* 43.
[8] Krogsgaard-Larsen, P.; Johnston, G*J. Neurochem.*, **1975**, *25,* 797.
[9] Adkins, C. E.; Pillai, . V.; Kerby, J.; Bonnert, T. P.; Haldon, C.; McKernan, R. M.; Gonzalez, J. E.; Oades, K.; Whiting, P. J.; Simpson, P. B. *J. Biol. Chem*., **2001**, *276*, 38934.
[10] Falch, E.; Perregaard, J.; Frølund, B.; Søkilde, B.; Buur, A.; Hansen, L. M.; Frydenvang, K.; Brehm, L.; Bolvig, T.; Larsson, O. M.; Sanchez, C.; White, H. S.; Schousboe, A.; Krogsgaard-Larsen, P. *J. Med. Chem.,* **1999**, *42*, 5402.
[11] Nielsen, L.; Brehm, L.; Krogsgaard-Larsen P. *J. Med. Chem.,* **1990**, *33,* 71.
[12] Ali, F. E.; Bondinell, W. E.; Dandridge, P. A.; Frazee, J. S.; Garvey, E.; Girard, G. R.; Kaiser, C.; Ku, T. W.; Lafferty, J. J.; Moonsammy, G. I.; Oh, H.-J.; Rush, J. A.; Setler, P. E.; Stringer, O. D.; Venslavsky, J. W.; Volpe, B. W.; Yunger, L. M.; Zirkle C. L. *J. Med. Chem.,* **1985**, *28,* 653.

[13] Falch, E.; Krogsgaard-Larsen P. *Eur. J. Med. Chem.,* **1991**, *26*, 69.
[14] Andersen, K. E.; Braestrup, C.; Groenwald, F. C.; Joergensen, A. S.; Nielsen, E. B.; Sonnewald, U.; Soerensen, P. O.; Suzdak, P. D.; Knutsen L. J. S. *J. Med. Chem.,* **1993**, *36,* 1716.
[15] Bialer, M.; Johannessen, S. I.; Kupferberg, H. J.; Levy, R. H.; Perucca, E.; Tomson, T. *Epilepsy Res.*, **2007**,*73*, 1.
[16] Kwan, P.; Sills, G.J.; Brodie M. J. *Pharmacol. Ther.,* **2001**, *90*, 21.
[17] Zheng, J.; Wen, R.; Luo, X.; Lin, Zhang, G. J.; Xu, L.; Guo, L.; Jiang, H. *Bioorg. Med. Chem. Lett.,* **2006**, *16*, 225.
[18] Pavia, M. R.; Lobbestael, S. J.; Nugiel, D.; Mayhugh, D. R.; Gregor, V. E.; Taylor, C. P.; Schwarz, R. D.; Brahce, L.; Vartanian, Mark G. *J. Med. Chem*., **1992**, *35,* 4238.
[19] Andersen, K. E.; Sørensen, J. L.; Huusfeldt, P. O.; Knutsen, L. J. S.; Lau, J.; Lundt, B. F.; Petersen, H.; Suzdak, P. D.; Swedberg, M. D. B. *J. Med. Chem.,* **1999**, *42,* 4281.
[20] Knutsen, L. J.S., Andersen, K. E.; Lau, J.; Lundt, B. F.; Henry, R. F.; Morton, H. E.; Naerum, L.; Petersen, H.; Stephensen, H.; Suzdak, P. D.; Swedberg, M. D. B.; Thomsen, C.; Sørensen, P. O. *J. Med. Chem.,* **1999**, *42*, 3447.
[21] Andersen, K. E.; SØrensen, J. L.; Lau, J.; Lundt, B. F.; Petersen, H.; Huusfeldt, P. O.; Suzdak ,P. D.; Swedberg, Michael D. B. *J. Med. Chem.,* **2001**, *13,* 2152.
[22] Dhar, T. G. M.; Borden, L. A.; Tyagarajan, S.; Smith, K. E.; Branchek, T. A.; Weinshank, R. L.; Gluchowski, C. *J. Med. Chem.,* **1994**, *37*, 2334.
[23] Sarup, A.; Larsson, O. M.; Bolvig, T.; Frølund, B.; Krogsgaard-Larsen, P.; Schousboe, A. *Neurochem. Int.,* **2003**,*43*, 445.
[24] Clausen, R. P.; Frølund, B.; Larsson, O. M.; Schousboe, A.; Krogsgaard-Larsen, P.; White H. S. *Neurochem. Int.,* **2006**, *48,* 637.
[25] Clausen, R. P.; Moltzen, E. K.; Perregaard, J.; Lenz, S. M.; Sanchez, C.; Falch, E.; Frølund, B.; Bolving, T.; Sarup, A.; Larsson, O. M.; Schousboe, A.; Krogsgaard-Larsen, P. *Bioorg. Med. Chem.,* **2005**, *13,* 895.
[26] Zhao, X.; Hoesl, C. E.; Höfner, G. C.; Wanner, Klaus T. *Eur. J. Med. Chem.,* **2005**, *40*, 231.
[27] Fülep, G. H.; Hoesl, C. E.; Höfner, G.; Wanner, Klaus T. *Eur. J. Med. Chem.,* **2006***, 41*, 809.
[28] Cairrão, M.A.R., Ribeiro, A.M., Pizzo, A.B., Fontana, A.C.K., Beleboni, R.O.. Coutinho-Netto, J., Miranda, A., Santos, W. F. *Pharmaceut. Biol.,* **2002**, *40*, 472.
[29] Liberato, J. L.; Siqueira Cunha, A. O.; Mortari, M. R.; Gelfuso, É. A.; de Oliveira Beleboni, R.; Coutinho-Netto, J.; Ferreira dos Santos W. *Brain Res.*, **2006**, *1124*, 19.

Received: 22 April, 2008 **Revised: 02 July, 2008** **Accepted: 02 July, 2008**