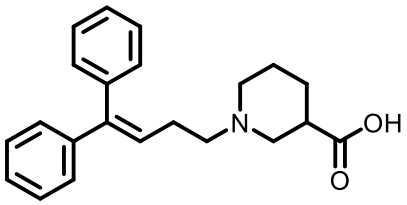
## 2.1. NIPECOTIC ACID: AN *IN VITRO* GABA REUPTAKE INHIBITOR

Nipecotic acid possesses GABA reuptake inhibitory activity. It has been reported that nipecotic acid binds to the GABA transporters and thereby blocking GABA uptake into presynaptic neurons and permitting more GABA to be available for receptor binding on the surfaces of post-synaptic cells [Krogsgaard-Larsen, 1980; Krogsgaard-Larsen and Johnston, 1975]. Several such studies have been reported to confirm the potential GABA reuptake inhibition by nipecotic acid and its derivatives. In a study, (-) enantiomer of nipecotic acid was found to be more potent for the transporter systems than the S(+) enantiomer [Johnston *et al*., 1976]. Also, a comparison between (RS)-nipecotic acid and GABA transport was done in cultured astrocytes and it was found that (RS)-nipecotic acid requires atleast two sodium ions per molecule for its uptake in the astrocytes [Larsson and Schousboe, 1981]. It has been reported that intraocular injections of nipecotic acid preferentially inhibits neuronal uptake of $^{3}$H-GABA and causes a dose-dependent decrease in $^{14}$C-GABA uptake in the inner plexiform layer of adult rabbit retina [Madtes and Redburn, 1983]. In another investigation with pressure ejection of GABA onto voltage-clamped skate retinal horizontal cells, it was revealed that nipecotic acid initiate a current similar to that produced by GABA in standard skate retinal cells which suggests that it may acts as a partial agonist for GABA transport [Malchow and Ripps, 1990]. Moreover, In a study of molecular characterization of different GABA transporters it was reported that amongst the four transporters of GABA, GAT1 and GAT4 have neural specific gene expression and both were more sensitive to inhibition by nipecotic acid [Liu *et al*., 1993]. In another study of GABA uptake and release by a mammalian cell line (1F9) it was revealed that in 1F9 cells, external GABA and nipecotic acid increases the efflux of preloaded $^{3}$H-GABA [Corey *et al*., 1994].

In a research nipecotic acid was tested against pharmacosensitive and pharmacoresistant type of epilepsy induced by lowering the concentration of extracellular $Mg^{2+}$ and it was concluded that at high concentrations (1-5 mM) nipecotic acid suppressed all forms of epileptic discharges [Pfeiffer *et al.*, 1996]. A computer aided molecular modeling was done on nipecotic acid to establish the conformation by which transport takes place. It was found that R (-)-nipecotic acid shows partially extended conformation which was found necessary for the transport to occur. Also, nipecotic acid was found to increase the passive release of $^{3}$H-GABA in rat brain slices [Chebib and Johnston, 1997]. It has also been reported that nipecotic acid (100 µM) blocked bicuculline induced epileptiform activity in the rat neocortical slices evoked by threshold stimulation (45 µA) [Sutor and Luhmann, 1998]. It has been demonstrated in a study that intracerebral perfusions of nipecotic acid increases extracellular concentration of GABA [Del Arco *et al.*, 1998]. In a study a spontaneous bursting activity on the retina of an immature turtle has been reported that nipecotic acid (10 µM) inhibited the GABA uptake and thereby decreases the burst rate [Sernagor and Grzywacz, 1999]. Another breakthrough suggests that nipecotic acid, which was used as GABA uptake inhibitor can directly activates $GABA_A$ receptors justifying its agonist properties on GABAA receptors [Barrett-Jolley, 2001].

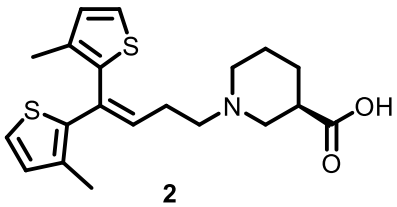
## 2.2. PIPERIDINE-3-CARBOXYLIC ACID DERIVATIVES AS ANTICONVULSANTS

Younger *et al*., have reported a new GABA reuptake inhibitor SKF 89976-A by attaching a 4,4-diphenyl-3-butenyl group to the amine of nipecotic acid. Results of the *in vitro* studies on rat brain synaptosomes revealed that SKF 89976-A **(1)** showed 20 times more potent GABA uptake inhibitory activity than the parent compound. The reported derivative also demonstrated anticonvulsant activity in bicuculline and PTZ-induced convulsion model in rats and mice respectively [Yunger *et al.*, 1984].




1

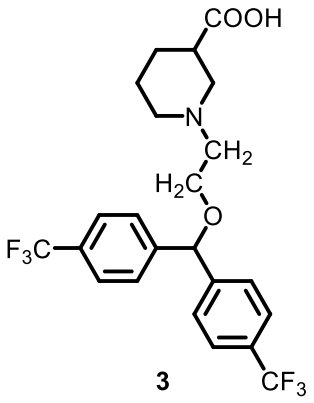
Braestrup had synthesized (R)-N-[4,4-Bis(3-methyl-2-thienyl)-but-3-en-Iyllnipecotic acid (NO 328) which is now marketed as tiagabine. The novel derivative **(2)** has been reported for its anticonvulsant potential in rodent models of epilepsy [Braestrup 1987]. The molecular mechanism of this revolutionary compound was again established by Braestrup *et al.* They have reported the $^{3}$H-GABA uptake inhibitory potential of NO 328 in a rat forebrain synaptosomal preparation ($IC_{50}$= 67 nM) and in primary cultures of neurons and astrocytes [Braestrup *et al.*, 1990]. The anticonvulsant activity of tiagabine was further established against pentylenetetrazol (PTZ), bicuculline and methyl 6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate (DMCM) induced seizures in rodents [Nielsen *et al.*, 1991]. Extensive clinical studies of tiagabine have also been performed to prove its efficacy for the management of epilepsy [Gustavson and Mengel, 1995].




2

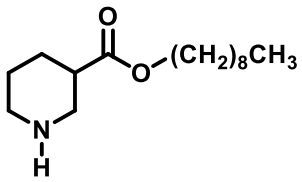
The findings have attracted scientist community to further explore piperidine-3-carboxylic acid, resulting in the discovery of N-(benzhydryl ethyl ether) derivatives. The lipophilic side chains were introduced on the ring nitrogen of piperidine-3-carboxylic acid to enhance the lipophilicity of the parent compound. The synthesized derivatives exhibit *in vitro* inhibition of GABA uptake. The selected lead **(3)** in this study was found to be effective in rodent models of epilepsy after oral administration

and the study was progressed to Phase 1 clinical trials. Unfortunately due to serious neurological and psychological side effects after the administration of single dose to humans, further clinical evaluation has been stopped [Pavia *et al*., 1992].




**3**

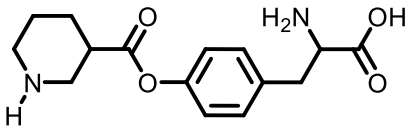
In the sequence, alkyl and phenyl derivatives of piperidine-3-carboxylic acid have been synthesized by Hinko *et al.* in 1996. The synthesized compounds were tested against maximal electroshock (MES) induced convulsions in mice. Nonyl ester with longer chain **(4)** exhibit protection against MES induced seizures without producing any cholinergic side effects. Other derivatives have minimal anticonvulsant efficacy, while some derivatives also produce cholinergic side effect [Hinko *et al*., 1996].




**4**

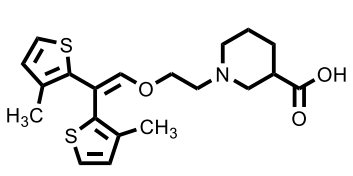
In another investigation Bonina *et al.* have synthesized four new nipecotic acid esters by chemical conjugation with glucose, galactose, and tyrosine as prodrugs. The synthesized compounds were subjected to *in vitro* enzyme hydrolysis studies. The findings of the hydrolysis studies suggest that the esters were found stable in a buffer solution (pH 7.4) at different temperatures. However, all the esters were found susceptible to cleavage by porcine esterase. The results of *in vivo* anticonvulsant

activity of the synthesized prodrugs in Diluted Brown Agouti (DBA)/2 mice revealed that only nipecotic tyrosine ester **(5)** was found to exhibit a considerable dose-dependent anticonvulsant activity, while the other compounds failed to protect the mice from audiogenic seizures [Bonina *et al*., 1999].



**5**

Later, in another investigation Knutsen *et al.* have synthesized Diaryl/ hetroaryl oxime and diaryl/ hetroaryl vinyl ether derivatives of piperidine-3-carboxylic acid. Compound **6** with a five atom linker connecting the diaryl moiety with piperidine-3-carboxylic acid demonstrate better *in vitro* $^{3}$H-GABA uptake inhibition when compared to the standard compounds tiagabine and SKF 89976-A. The increased *in vitro* potency was due to the insertion of ether oxygen in the connecting linker. The $ED_{50}$ values of the oxime derivatives, **7** & **8** and the vinyl ether derivatives **6** & **9-12** in a DMCM induced convulsion model in mice was found to be less than 1 which was significantly less than the $ED_{50}$ of tiagabine and SKF 89976-A. The findings of *in vivo* investigation revealed better protection of seizures by the some of the synthesized derivatives as compared to the standard compounds [Knutsen *et al*., 1999].

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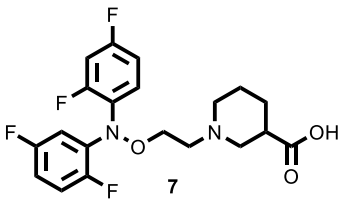


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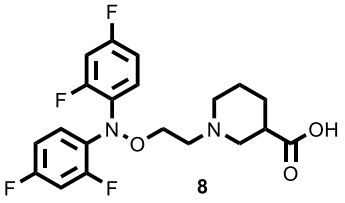


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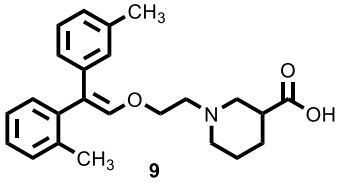


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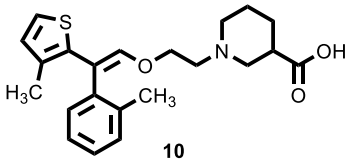


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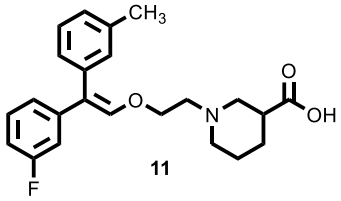


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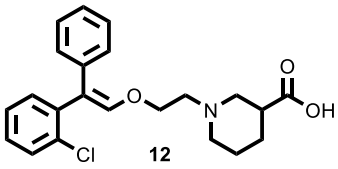
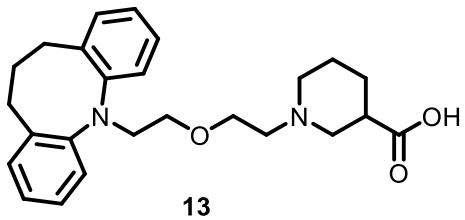



12

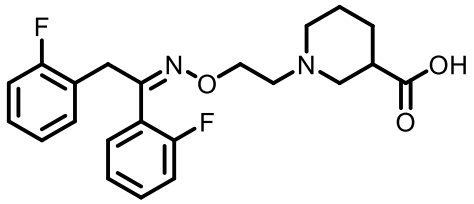
In an effort to increase the BBB permeability, Andersen *et al.* have synthesized tricyclic derivatives nipecotic acid by the substitution of lipophilic moieties like (10,11-dihydro-5H-dibenz[b,f]azepin-5-yl)alkoxyalkyl or (10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)alkoxyalkyl on the ring nitrogen of piperidine-3-carboxylic acid. Some of the synthesized compounds were reported to exhibit significant *in vitro* inhibition of GABA uptake in rat synaptosomes and *in vivo* anticonvulsant activity in DMCM induced convulsion model in mice. One derivative, (R)-1-(2-(2-(10,11-dihydro-5H-dibenz[b,f]azepin-5-yl)-ethoxy)ethyl)-3-piperidinecarboxylic acid **(13)** exhibited comparable or somewhat enhanced protective index than that of tiagabine **(2)** [Andersen *et al*., 2001a].




13

Furthermore, Andersen *et al.* have synthesized asymmetrical analogues of nipecotic acid with some modifications in the lipophilic portion. In this series,

Alkylene, alkyl or cycloalkylene moieties have been introduced by replacing one of the aryl groups of the previously synthesized derivatives. Two general strategies were used for the preparation of the compounds. One strategy was via N-alkylation of the nipecotic acid, with the appropriate halogenide or mesylate. The second strategy was via O-alkylation of an oxime or an alcohol with (R)-1-(2-bromoethyl)-3-piperidinecarboxylic acid ethyl ester. Many derivatives demonstrated considerable *in vitro* GABA uptake inhibition as well as *in vivo* anticonvulsant activity against DMCM induced convulsions. Compounds **14, 15** & **16** exhibited comparable *in vivo* anticonvulsant activity and protective index with that of tiagabine [Andersen *et al*., 2001].

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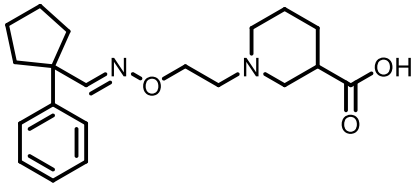


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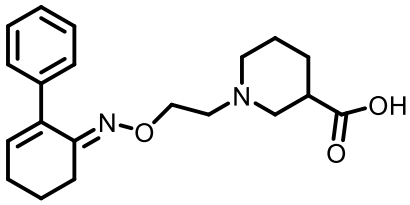
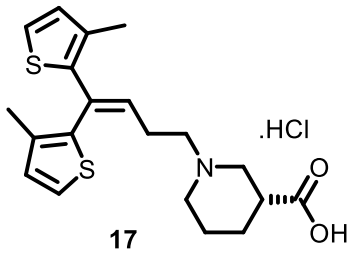



**16**

In another research, Hoesl *et al*. have synthesized for the first time, 6-substituted derivatives of piperidine-3-carboxylic acid. Pure isomeric forms of 6-(4,4-diphenylbutyl) piperidine-3-carboxylic acid and 6-(4,4-diphenylbutenyl) piperidine-3-carboxylic acid were successfully synthesized and evaluated for *in vitro* GABA reuptake inhibitory activity at GAT-1 and GAT-3 transporters. However, the derivatives failed to exhibit significant potency [Hoesl *et al*., 2004].

Zhang *et al.* have reported the synthesis of 4, 4-diaryl/diheteroaryl-3-butenyl derivatives of piperidine-3-carboxylic acid as inhibitors of GABA transport. The outcome of *in vitro* reuptake bioassay in cultured cells revealed that compounds **17-20** exhibits considerable $^{3}$H-GABA inhibitory activity [Zhang *et al*., 2005].

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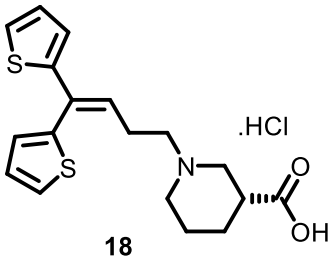


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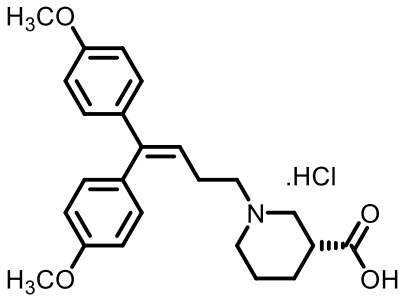


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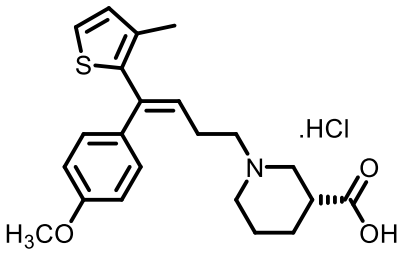
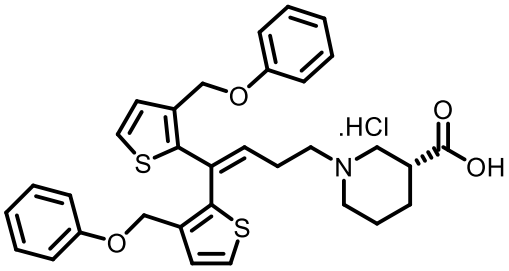


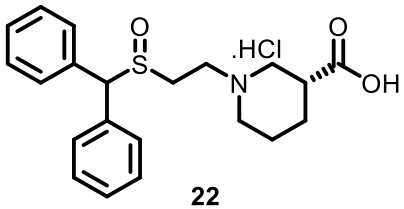

**20**

Zheng *et al.*, have reported the synthesis of N-diarylalkenyl-piperidinecarboxylic acid derivatives. The synthesized compounds were tested for GABA transport inhibition on GAT-1 GABA transporter protein. It was found that R-1-[4,4-bis(3-phenoxymethyl-2-thienyl)-3-butenyl]-3-piperidinecarboxylic hydrochloride **(21)** showed comparable GAT-1 inhibition with that of tiagabine. The enhancement of GAT-1 inhibition as compared to the parent compound corresponds to the attachment of bulky scaffolds such as phenoxymethyl and benzyloxymethyl at the thiophene ring [Zheng *et al*., 2006].



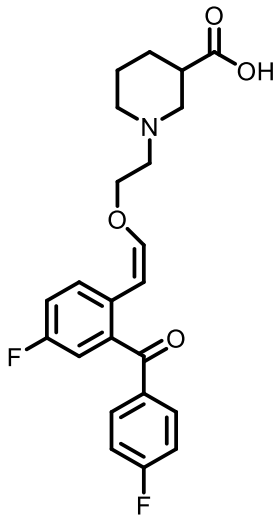

**21**

Zhang *et al.* have synthesized a new series of (R)-1-(2-diarylmethylthio/sulfinyl) ethyl-piperidine-3-carboxylic acid hydrochlorides and (R)-1-(3-diarylmethylthio) propyl-piperidine-3-carboxylic acid hydrochlorides. Results of biological evaluation revealed that the compounds with diarylmethylsulfinyl ethyl side chain exhibits significant inhibition of GAT-1. Compound **22** was reported to be the most promising compound with *in vitro* GAT-1 inhibitory activity as high as 496 times in comparison to nipecotic acid [Zhang *et al*., 2007].




**22**

In pursuance to develop novel GABA transport inhibitors Quandt *et al.* have reported the synthesis of novel *N*-substituted derivatives of piperidine-3-carboxylic acid with a vinyl ether linker and fluorine substituted unsymmetrical biaryl residue. The compounds were subjected to evaluation of GABA uptake inhibition against four murine transporter subtypes i.e. mGAT1-mGAT4 on HEK cell lines. The results revealed that the several compounds showed potential *in vitro* $^{3}$H-GABA uptake inhibition with some *Z*-isomers **(23-26)** showing high selectivity towards mGAT-1 [Quandt *et al*., 2013].



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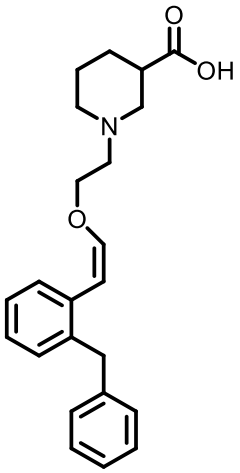

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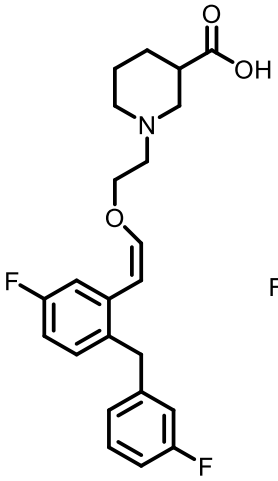

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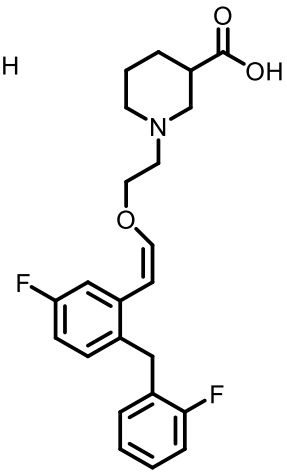
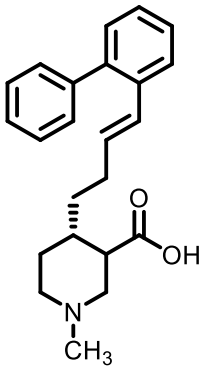


26

In another investigation, Hellenbrand *et al.* have synthesized 4-substituted derivatives of piperidine-3-carboxylic acid as GABA transport inhibitors. Docking studies revealed that the synthesized compounds acquire same binding pose as that of parent compound i.e.piperidine-3-carboxylic acid which a reported inhibitor of mGAT-1. Biaryl moieties were attached at fourth position via Alkenyl- or alkynyl linkers. Compound **27** was reported to show reasonable inhibition on mGAT-1, while compound **28** demonstrates plausible mGAT-4 inhibitory activity. Other compounds exhibited insignificant inhibition of murine transporter subtypes [Hellenbrand *et al.*, 2016].



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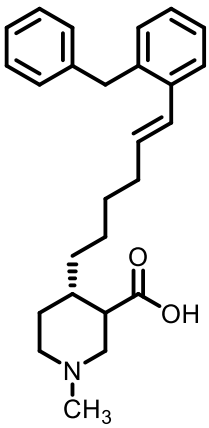
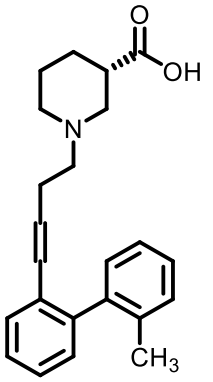


28

In a recent study, Lutz *et al.* have reported the synthesis of novel *N*-substituted derivatives of nipecotic acid as potent inhibitors of GAT-1. The derivatives were synthesized by introducing aromatic moieties to the ring nitrogen of nipecotic acid via alkyne-type spacer. The findings revealed the importance of biphenyl moiety over 2-benzylphenyl moiety in term of biological activity. The results also suggested the significance of the length of but-3-inyl and $C_5$ spacer with respect to affinity of compounds towards mGAT-1. It was found that compounds **29-32** were most potent inhibitors of mGAT-1 along with high selectivity as compared to other compounds [Lutz *et al.*, 2017].

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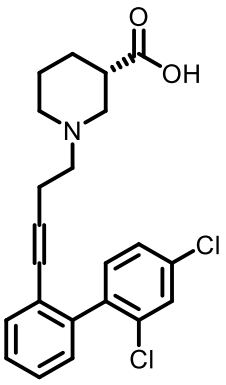


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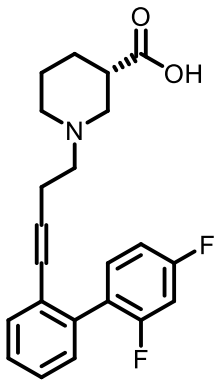


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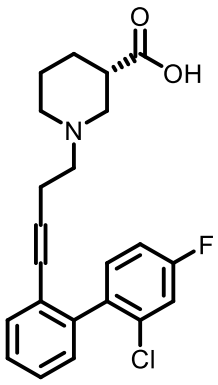
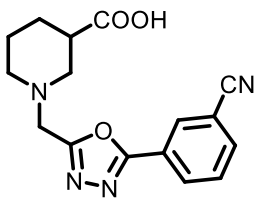



**32**

In a quest to search for novel compounds with antiepileptic potential Ravi *et al.* have synthesized novel *N*-substituted derivatives of piperidine-3-carboxylic acid by attaching a heterocyclic aryl substituted 1,3,4-oxadiazole moiety. Anticonvulsant activity of the synthesized compounds has been evaluated against PTZ induced convulsions in mice. It was revealed that compounds **33-37** showed significant protection against chemical induced seizures [Singh *et al.*, 2018].

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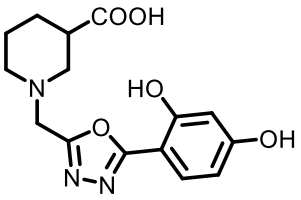


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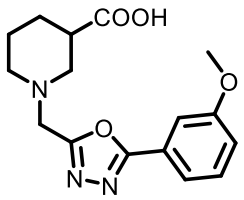


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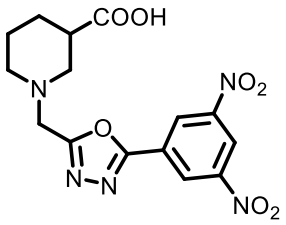


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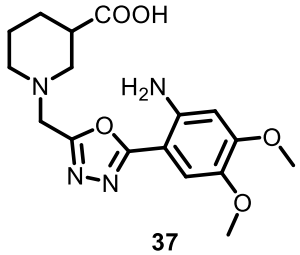
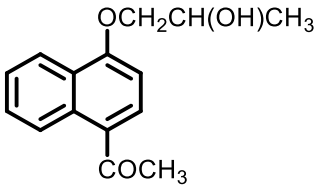



37

## 2.3. NAPTHALENE DERIVATIVES AS ANTICONVULSANTS

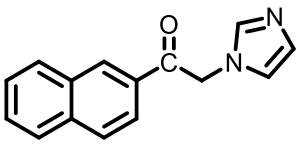
Hunter *et al.* have reported synthesis and evaluation of series of 4-acyl-1-naphthoxy alcohols and ketones derivatives for anti convulsant activity using strychnine, pentylenetetrazole and electroshock induced convulsions in rats and mice. Out of all the synthesized compounds, 1-(4-Acetyl-l-naphthoxy)-2-propanol **(38)** was found to be most effective in strychnine as well as electroshock induced convulsions in mice and rats against. Also, the compound **38** was reported to be devoid of any toxic manifestations [Hunter *et al.*, 1964].




38

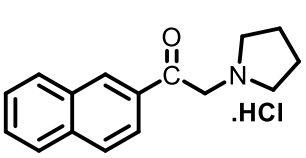
Walker *et al.* have synthesized 1-(naphthoylalky1)-1H-imidazoles derivatives and screened them through MES induced seizures model for anti convulsant activity. They observed that 1-(naphthoylalky1)-1H-imidazoles derivatives containing a variety of functional groups in the alkylene bridge demonstrate potent anticonvulsant activity. Findings on QSAR of the study also revealed that anticonvulsant activity is

intrinsic property of 1-(naphthylalky1) imidazole derivatives that can be extended to the derivatives containing ketones, cyclic ketals, cyclic and acyclic thioketals, alcohols, ethers, esters, and homologues, but not sulfoxide or sulfone. On the basis of pharmacological studies compound **39** was selected for further evaluation [Walker *et al*., 1981].




**39**

Nafimidone, 1-(2-naphthyl)-2-(1-imidazolyl)ethanone is an anticonvulsant drug having naphthyl ring and imidazole side chain. Calis *et al.* had explored the significance of imidazole ring in the anticonvulsant activity of this drug. Out of 18 compounds only three (**40, 41 & 42**) were found to be effective against MES induced convulsions. Results indicated that presence of imidazole ring is necessary for activity in MES model. Eight compounds (**41-48**) were found active in subcutaneous metrazole (s.c.MET) induced seizure model. The findings suggested that replacement of imidazole ring with other moieties leads to anticonvulsant activity against s.c.MET test [Çaliş *et al*., 1988].

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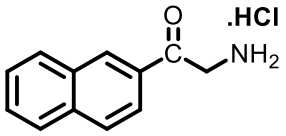


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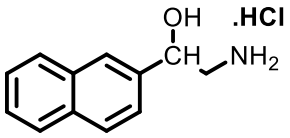


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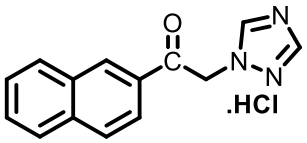


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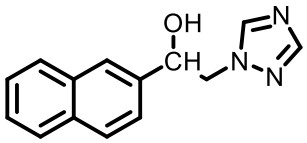


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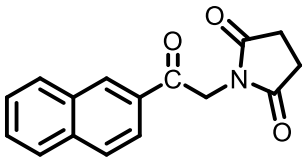


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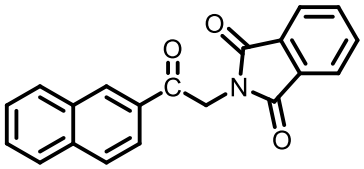


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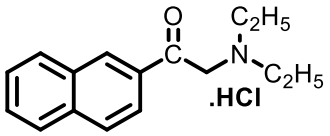


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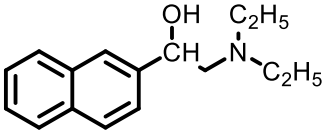
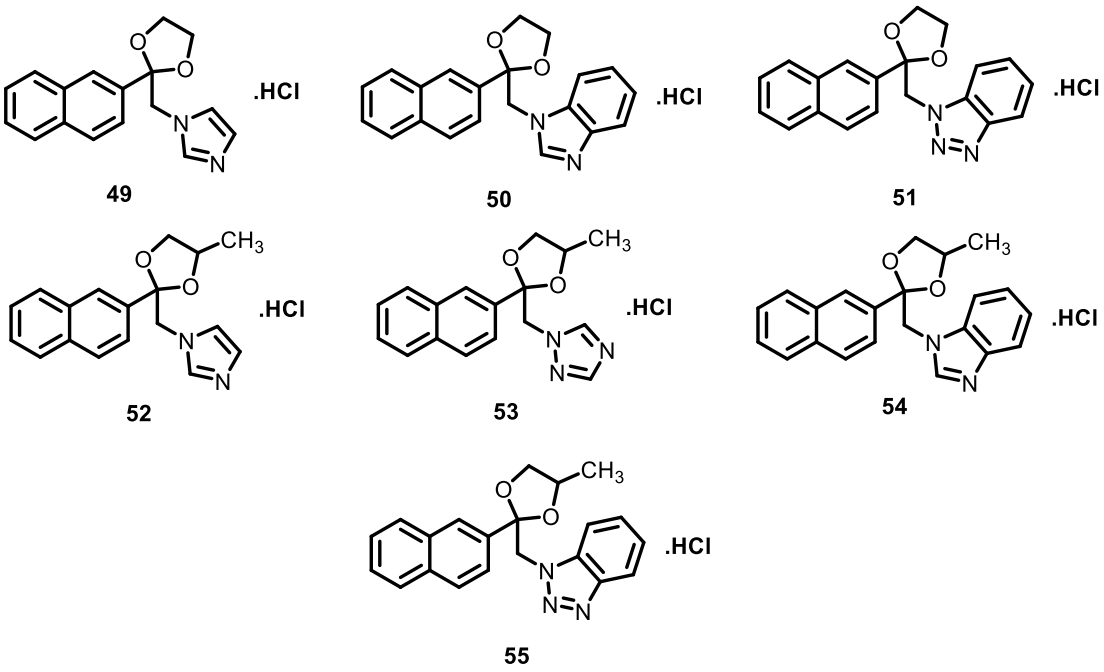
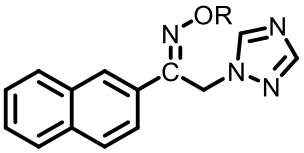



48

Özkanlı *et al.* have synthesized and evaluated dioxolane derivatives of 2-acetylnaphthalene for their anticonvulsant activity. MES test and s.c.MET test were used to assess the anti convulsant activities of the test compounds. Out of ten compounds synthesized, compounds, **49-54** were found protective against MES induced seizures and **52-55** were found protective against s.c.Met induced seizures. It was also revealed that the replacement of imidazole ring with other azoles does not influence the anticonvulsant activity of the synthesized compounds [Özkanlı *et al*., 2003].



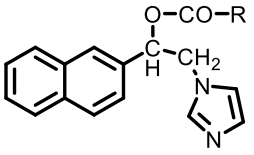

Oxime and oxime ether derivatives of 1-(2-naphthyl)-2-(1,2,4-triazol-1-yl)ethanone were reported by Karakurt *et al*. as potential anticonvulsant compounds MES and s.c.MET tests in mice and rats were used to assess the anticonvulsant activity. Results revealed that compounds **56-60** were active against both the models of epilepsy [Karakurt *et al*., 2006].



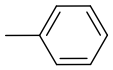
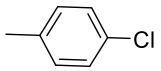

| Comp. | R |
|---|---|
| **56** | $-CH_3$ |
| **57** | $-C_2H_5$ |
| **58** | $-C_3H_7$ (*n*) |
| **59** | $-C_3H_7$ (*i*) |
| **60** | $-CH_2CH=CH_2$ |

Later on Karakurt *et al.* in continuation of their previous work to explore more naphthyl derivatives having anticonvulsant activity and synthesized a new series of 2-acetylnaphthalene derivatives. The synthesised compunds were based on the structural modification of nafimidone 1-(2-naphthyl)-2-(imidazol-1-yl)ethanone. Alkyl chain between naphthalene and imidazole ring was the main site for substitution to create new compounds. MES and s.c.MET seizure tests were used to assess anticonvulsant

activity of the compounds. The findings of the study indicated that all the ester derivatives of nafimidone alcohol (**61-68**) were active in tested models. However ester derivatives without imidazole ring failed to show protection against MES and scMET induced convulsions [Karakurt *et al*., 2010].

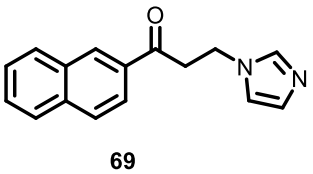



.HCl

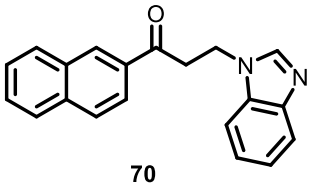
| Comp. | R | Comp. | R |
|---|---|---|---|
| 61 | $-CH_2CH_2CH_3$ | 66 | $-(CH_2)_3-NH-COOC(CH_3)_3$ |
| 62 | $-CH(CH_3)_2$ | | |
| 63 | $-CH_2(CH_2)_2CH_3$ | 67 |  |
| 64 | $-CH_2CH(CH_3)_2$ | | |
| 65 | $-CH(C_3H_7)_2$ | 68 |  |



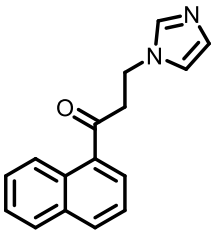
Kumar and Pathak have synthesized a series of acetyl naphthalene and substituted acetyl naphtahlene derivatives having imidazole, benzoimidazole, piperidine and piperazine ring and evaluated them for anti convulsant activity using pentylenetetrazole (scPTZ) test. The results showed that some of the acetyl naphthalene and substituted acetyl naphthalene derivatives of heterocyclic compounds (**69-74**) were significantly active as anticonvulsant [Kumar and Pathak, 2013].




**69**




**70**

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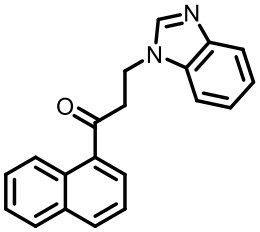


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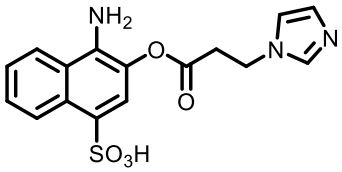


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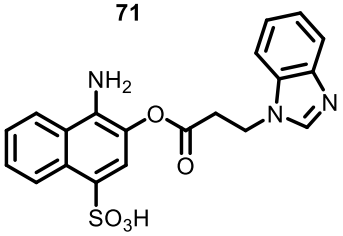


**74**