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Anticonvulsant evaluation and mechanism of action of benzylamino enaminones

Ivan O. Edafiogho,* Kethireddy V. V. Ananthalakshmi and Samuel B. Kombian

Faculty of Pharmacy, Kuwait University, PO Box 24923, Safat 13110, Kuwait

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Abstract—The mechanism of anticonvulsant action was evaluated for the benzylamino enaminones. The most potent enaminone in this series was the unsubstituted benzylamine analog (**30**; methyl 4-benzylamino-6-methyl-2-oxocyclohex-3-en-1-oate) which had an oral effective dose (ED_{50}) in rats of 27 mg/kg against maximal electroshock seizures, and a concentration 10-fold less than this dose depressed excitatory synaptic transmission, and action potential firing in the rat brain in vitro.

1. Introduction

Enaminones are known to possess a variety of medicinal properties including anticonvulsant, antimalarial, anti-inflammatory, and cardiovascular effects.[1] Aniline enaminones possess anticonvulsant properties which differ with *para*-substitutions on the phenyl moiety. Thus, the unique enaminone pharmacophore presents an excellent opportunity to develop anticonvulsant compounds that are devoid of neurotoxicity and yet possess wider margin of safety than conventional antiepileptic drugs such as valproate, carbamazepine, and phenytoin.[1]

In a continuing evaluation of the enaminones for use in seizure disorders, a comparison of enaminones from various unsubstituted and *para*-substituted benzylamino enaminones was undertaken with the aim of elucidating the essential structural parameters necessary for anticonvulsant activity. In addition, we tested the hypothesis that benzylamino enaminones interacted with glutamate and/or its receptors to produce anticonvulsant effects in rats and mice.[2,3] This hypothesis was based on the possible role of glutamate in the genesis of seizures[4,5] as well as computer modeling of enaminones and crystallographic studies, which suggest that anticonvulsant enaminones would fit a putative glutamate receptor site.[3,6] In this study, we hereby report a complete anticonvulsant evaluation of benzylamino enaminones, and the neurophysiological effects of a highly active analog (compound **30**), a moderately active analog (compound **31**), and an inactive analog (compound **21**) on glutamate-mediated synaptic responses and neuronal excitability in neurons of the nucleus accumbens (NAc).

2. Chemistry

Generally, the reaction of β-hydroxyketo compounds with benzylamine derivatives yielded the benzylamino enaminones **1**–**37** (Scheme 1 and Table 1). In this report, the benzylamino enaminones are displayed completely with their physicochemical, C log *P*, and in vivo anticonvulsant data on one table for ease of identifying the quantitative structure activity relationship (QSAR) of the analogs, as compiled from our previous reports.[2,7–9] This approach presents the benzylamino enaminones as a unique class of anticonvulsant enaminones in their own rights. Typical synthesis of the benzylamino enaminones involved the removal of water by Dean–Stark trap, and the use of excess amino compounds in the reactions to obtain the corresponding benzylamino enaminones with the amide group on the cyclohexenone ring.

The benzylamino enaminones consist of substituents in which R^1 = H, CH_3; R^2 = H, CH_3; R^3 = H, $CO_2C(CH_3)_3$, $CO_2C_2H_5$, CO_2CH_3, $CONHCH_2C_6H_5$, $CONHCH_2C_6H_4$(p-F), and R^4 = H, F, Cl, CN, CH_3, OCH_3, CO_2H, NO_2. The calculated partition coefficient

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* Corresponding author. Tel.: +965 498 6065; fax: +965 498 6840; e-mail: ivanoe@hsc.edu.kw

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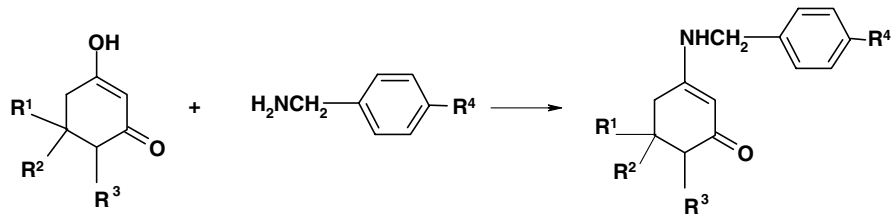
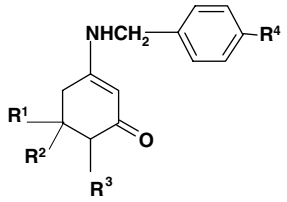


β-Hydroxyketo compounds Benzylamine derivatives Benzylamino enaminones

Scheme 1. General synthesis of benzylamino enaminones.

Table 1. Physicochemical, C log *P*, and anticonvulsant data for benzylamine enaminones[7–9]



Compound	R^1	R^2	R^3	R^4	Yield	Mp (°C)	C log *P*	ASP[a]
1	H	H	H	H	25	125–127	2.41	1
2	CH_3	H	H	H	25	137.5–139	2.93	1
3	CH_3	CH_3	H	H	15	124–127	3.45	1
4	H	H	H	Cl	48	170–172	3.12	4
5	CH_3	H	H	Cl	44	186–187	3.64	4
6	CH_3	CH_3	H	Cl	47	159–162	4.16	2
7	H	H	H	CH_3	39	153–155	2.91	2
8	CH_3	H	H	CH_3	32	146–148	3.43	3
9	CH_3	CH_3	H	CH_3	59	139–140	3.95	3
10	H	H	H	OCH_3	35	159–160	2.33	2
11	CH_3	H	H	OCH_3	68	160–162	2.85	3
12	CH_3	CH_3	H	OCH_3	82	159–160	3.37	3
13	H	H	H	CN	57	156–159	1.84	2
14	CH_3	H	H	CN	34	168–171	2.36	1
15	CH_3	CH_3	H	CN	43	215–217	2.90	3
16	CH_3	H	$CO_2C(CH_3)_3$	CN	37	172–175	3.46	3
17	CH_3	H	$CO_2C_2H_5$	CN	38	184–186	2.75	3
18	CH_3	H	CO_2CH_3	CN	44	204–208	2.22	3
19	CH_3	H	$CO_2C(CH_3)_3$	OCH_3	38	174–175	3.95	3
20	CH_3	H	$CO_2C_2H_5$	OCH_3	74	154–157	3.24	3
21	CH_3	H	CO_2CH_3	OCH_3	77	168.5–172	2.71	3
22	CH_3	H	$CO_2C(CH_3)_3$	CH_3	58	123–126	4.53	1
23	CH_3	H	$CO_2C_2H_5$	CH_3	76	134–135	3.82	3
24	CH_3	H	CO_2CH_3	CH_3	48	160–163	3.29	3
25	CH_3	H	$CO_2C(CH_3)_3$	Cl	47	182–185	4.74	3
26	CH_3	H	$CO_2C_2H_5$	Cl	69	169–172	4.03	3
27	CH_3	H	CO_2CH_3	Cl	64	173–174	2.79	2
28	CH_3	H	$CO_2C(CH_3)_3$	H	55	152–155	4.03	3
29	CH_3	H	$CO_2C_2H_5$	H	77	134–135	3.32	3
30	CH_3	H	CO_2CH_3	H	81	154–155	2.79	1
31	CH_3	H	CO_2CH_3	F	62	174–176	2.86	2
32	CH_3	H	CO_2CH_3	CO_2H	63	231–235 dec	2.46	3
33	CH_3	CH_3	CO_2CH_3	H	79	138–139	3.32	2
34	CH_3	H	CO_2CH_3	NO_2	66	174–176	2.46	3
35	CH_3	H	$CONHCH_2C_6H_4$(p-F)	F	8	183–184	3.94	3
36	CH_3	H	$CONHCH_2C_6H_5$	H	81	193–195	3.60	3
37	C_6H_5	H	$CONHCH_2C_6H_5$	H	22	219–220	4.49	3

[a] Anticonvulsant Screening Project (ASP) classifications in Phase 1 evaluation in mice against maximal electroshock seizures (MES) are as follows: (1) anticonvulsant activity at 100 mg/kg or less; (2) anticonvulsant activity at doses greater than 100 mg/kg; (3) inactive at doses of 300 mg/kg; (4) activity inconsistent.

(C log *P*) values were determined using the CS ChemDraw Ultra version 6.01.[10]

3. Anticonvulsant evaluations

The anticonvulsant evaluation of all 37 benzylamino enaminones was determined by in vivo methods,[2,7–9] and analogs **21**, **30**, and **31** were selected in the present work, and evaluated directly on neurons by in vitro methods.

3.1. In vivo anticonvulsant evaluation

Anticonvulsant evaluations were performed by the Anticonvulsant Screening Program at the Epilepsy Branch of the National Institutes of Neurological, Communicative Disorders, and Stroke (NINCDS) in Bethesda, USA, by methods that have been previously reported.[2,7] Phase 1 evaluation of the benzylamino enaminones in mice is shown in Table 1. To differentiate the results between different rodent species, the most active enaminone (**30**) was evaluated for oral (po) activity (Phase VIA) in the rat.

3.2. In vitro anticonvulsant evaluation

To examine the effects of these compounds on excitatory synaptic transmission and neuronal excitability, glutamate-mediated excitatory postsynaptic currents (EPSC) and action potentials (APs) were recorded in nucleus accumbens (NAc) cells in vitro for the most active analog (**30**), the moderately active analog (**31**), and the inactive analog (**21**) according to the methods of Kombian et al.[11]

4. Results and discussion

4.1. Chemistry

The physicochemical, C log *P*, and anticonvulsant data for benzylamine enaminones (**1**–**37**) are summarized in Table 1. These compounds were completely characterized, and the UV, IR, and NMR data confirmed the structures.[2,7–9] The detailed syntheses of typical unsubstituted benzylamino enaminone, substituted benzylamino ester, and benzylamino amide are described under Experimental section. The benzylamino enaminones contain one or two chiral centers; and the compounds with one chiral center would have racemic mixture of two diastereomers, while the compounds with two chiral centers would have racemic mixtures of four possible diastereomers. However, we were unable to separate the compounds into individual stereoisomers, hence the in vivo and in vitro studies were carried out on the racemic enaminones.

4.2. C log *P* data and anticonvulsant activity

When the C log *P* data of the benzylamino enaminones (**1**–**37**) were compared to in vivo anticonvulsant activity, there was no distinct correlation between lipophilicity (C log *P* value) and anticonvulsant effect. The C log *P* range for all 37 benzylamine enaminones was from 1.84 (for compound **13**) to 4.74 (for compound **25**). C log *P* measurements were for the protonated benzylamines, which predominated at physiological pH. A correlation could not be established between lipophilicity of the benzylamine enaminones and anticonvulsant activity. The C log *P* values of classes 1 and 2 anticonvulsants varied between 1.84 and 4.53, while the C log *P* values of the inactive (class 3) enaminones varied from 2.22 to 4.74. The main factor affecting the anticonvulsant effect of the benzylamino enaminones was the steric effect.[2,7] Generally however, the highly lipophilic compounds were inactive. In addition to steric effects, the lack of strong anticonvulsant activity in the *para*-substituted benzylamine series may be due to the lack of inductive effect with the *para*-substituents arising from the presence of the methylene bridge which effectively blocks this electronic contribution.

4.3. In vivo anticonvulsant evaluation

According to the anticonvulsant screening program (ASP),[7] six benzylamino enaminones **1**–**3**, **14**, **22**, and **30** were class 1 anticonvulsants, and they possessed significant protective effect against maximal electroshock seizures (MES) induced in mice. Seven benzylamino enaminones including **6**, **7**, **10**, **13**, **27**, **31**, and **33** were class 2 anticonvulsants and were moderately active. Twenty-one of these compounds (**8**, **9**, **11**, **12**, **15**–**21**, **23**–**26**, **28**, **29**, and **34**–**37**) were class 3 enaminones and were inactive. Two compounds **4** and **5** gave inconsistent results, and were grouped in Class 4. Although we reported certain aspects of the benzylamino enaminones in our previous work,[2,7–9] we hereby provide a more complete QSAR for the in vivo anticonvulsant activity for the compounds, and the possible mechanisms of action of benzylamino enaminones that may underlie their anticonvulsant activity.

4.3.1. Quantitative structure activity relationships (QSAR). The *para*-substitution pattern for this benzylamino enaminones included chloro ($+\sigma$, $+\pi$), methyl ($-\sigma$, $+\pi$), cyano ($+\sigma$, $-\pi$), and methoxy ($-\sigma$, $-\pi$) groups which were compared to the unsubstituted analog (0σ, 0π).[12] Class 4 benzylamines in Table 1 which included compounds **4** and **5** were found to be sparingly soluble and presented problems establishing a uniform dosage, thus accounting for inconsistent results. The enaminone ester with *para*-methyl substituent (**22**) possessed class 1 anticonvulsant activity, while one enaminone ester with *para*-chloro substituent (**27**) showed moderate, class 2 activity.[7]

Compound **30** (R^1, R^4 = H, R^2 = CH_3, R^3 = CO_2CH_3) was the most potent anticonvulsant benzylamino compound in this series, displaying an intraperitoneal (ip) ED_{50} in mice of 64.7 mg/kg, and a TD_{50} > 500 mg/kg and an oral (po) ED_{50} in rats of 26.8 mg/kg with no toxicity noted at dosages up to 500 mg/kg.[7] Given that bioavailability is often higher via a parenteral route as compared to the oral route, the current finding indicates that compound **30** is more potent in rats than in mice.

Furthermore, in corneal kindling model, compound **30** provided an ED_{50} of 78.3 mg/kg compared to phenytoin, 48.3 mg/kg, under the same conditions.[7] Kubicki et al.[13] in the X-ray analysis of compound **30** observed intermolecular N–H...O bonding, resulting in an extended 'sofa' conformation without intramolecular vinyl proton involvement. In determining the QSAR, it was found that in the same carbomethoxy series, *para*-substitutions led to less active or inactive compounds. However, the *p*-fluoro compound **31** ($R^1 = CH_3$, $R^2 = H$, $R^3 = CO_2CH_3$, $R^4 = F$) was active, providing an ip ED_{50} of 159 mg/kg in mice, and a po ED_{50} of 49.3 mg/kg and a $TD_{50} > 230$ mg/kg in rats. The activity of this analog is consistent with Topliss's findings that *p*-fluoro substitution produces a minimal change in σ and π effects compared to the unsubstituted compound.[12] The *p*-chloro (**27**, $R^1 = CH_3$, $R^2 = H$, $R^3 = CO_2CH_3$, $R^4 = Cl$; $+\sigma$, $+\pi$) provided spurious results, probably due to solubility problems, while the *p*-tolyl (**24**, $R^1 = CH_3$, $R^2 = H$, $R^3 = CO_2CH_3$, $R^4 = CH_3$; $-\sigma$, $+\pi$) and *p*-carboxy (**32**, $R^1 = CH_3$, $R^2 = H$, $R^3 = CO_2CH_3$, $R^4 = CO_2H$; $+\sigma$, $-\pi$) analogs were inactive. Further, in the dimedone series, the unsubstituted benzylamine compound (**3**, $R^1 = R^2 = CH_3$, $R^3 = H$, $R^4 = H$) was active with an ED_{50} of 53 mg/kg and a TD_{50} of 148 mg/kg in mice, and the comparable cyclohexenone derivative (**1**, $R^1 = R^2 = R^3 = H$, $R^4 = H$) also showed class 1 activity.

Within the 6-methyl series of the enaminones, the importance of the ester functionality in contributing to anticonvulsant activity was shown in compounds **28**, **29**, and **30**. Compound **30** (C log *P* 2.79) with a methyl ester group was highly active,[2,7] while changing the ester functionality to the ethyl ester **29** (C log *P* 3.32) or the *tert*-butyl ester **28** (C log *P* 4.03) completely abolished activity. None of the enaminone amides (**35**–**37**) was anticonvulsant probably due to their high lipophilicity and steric hindrance.

4.4. In vitro anticonvulsant evaluation

In our in vitro electrophysiology studies, 10 μM of compounds **21**, **30**, and **31** was tested on isolated EPSCs and on neuronal excitability. This concentration is 10 times less than the effective therapeutic dose of the most active compound **30**. Bath application of 10 μM of compound **21**, the methoxy-substituted benzylamine derivative, either did not affect EPSC amplitude or increased them in some cells resulting in an average change in EPSC amplitude of 19.5 ± 12.9% ($n = 4$; $p > 0.05$, paired Student's *t* test; Fig. 1). However, 10 μM of the unsubstituted benzylamine derivative compound **30**, or the fluoro-substituted derivative (compound **31**) which demonstrated excellent and intermediate in vivo anticonvulsant activity, respectively, reversibly depressed the evoked EPSC amplitude by −23.6 ± 5.9% ($n = 7$, $p < 0.05$, paired Student's *t* test, Fig. 1) and −30.8 ± 4.6% ($n = 5$, $p < 0.05$, paired Student's *t* test, Fig. 1C), respectively. Furthermore, both compounds **30** and **31**, at this same concentration, also reversibly reduced AP firing frequency. However, while the most active compound **30** depressed the AP firing frequency by

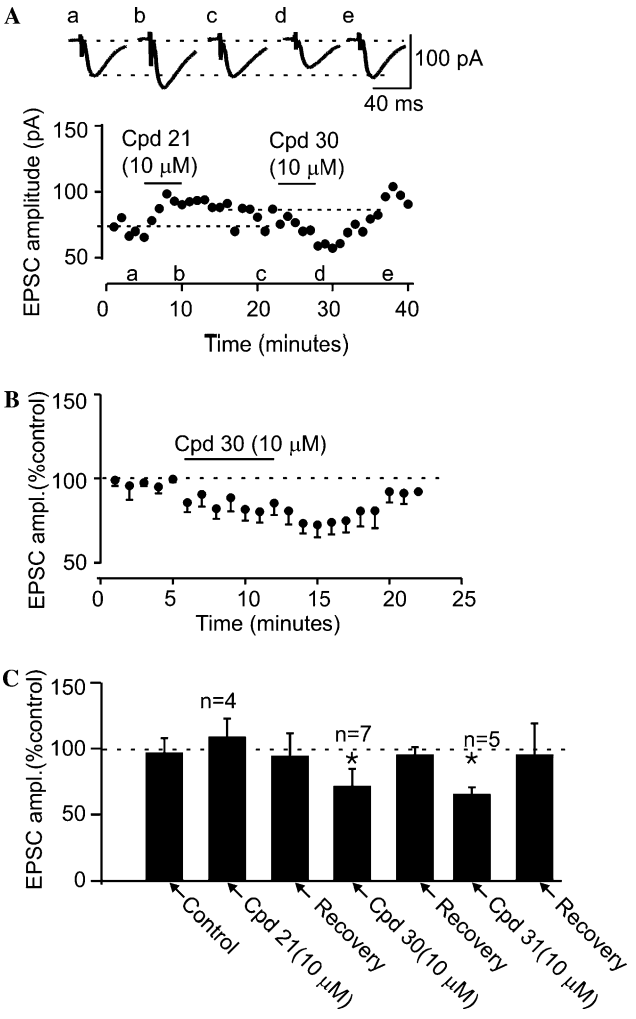


Figure 1. Effect of compounds **21**, **30**, and **31** on evoked EPSC amplitude. (A) A time-effect plot of the EPSC amplitude of a representative cell that was first exposed to compound **21** and subsequently compound **30** after recovery. Each compound was applied for 6 min. Inserts are sample synaptic responses taken at the times indicated by letters in the main graph. (B) An averaged time-effect graph showing the effect of compound **30** on EPSC amplitude in 7 cells. (C) Average bar graph summarizing the effects of compounds **21**, **30**, and **31** on EPSC amplitude. * represents statistical significance at $p < 0.05$.

−62.5 ± 14.7% ($n = 5$, $p < 0.05$, paired Student's *t* test, Fig. 2), the intermediately potent compound **31** only produced −33 ± 11.0% ($n = 5$, $p < 0.05$, paired Student's *t* test, Fig. 2B) suppression of AP firing frequency. Finally, compound **21**, the inactive analog had no significant effect on AP firing (−5.3 ± 7.5%, $n = 4$; $p > 0.05$; paired Student's *t* test). In both the EPSC and AP experiments, the onset of action was about 2 min with maximum effect observed at 5–6 min (Figs. 1 and 2). Recovery from the EPSC and action potential frequency depression was complete with responses returning to baseline levels in 8–15 min after the commencement of washout (Figs. 1 and 2). The effects of compounds **30** and **31** on EPSC and AP firing frequency at the tested concentration of 10 μM were without significant changes in holding current or membrane potential.

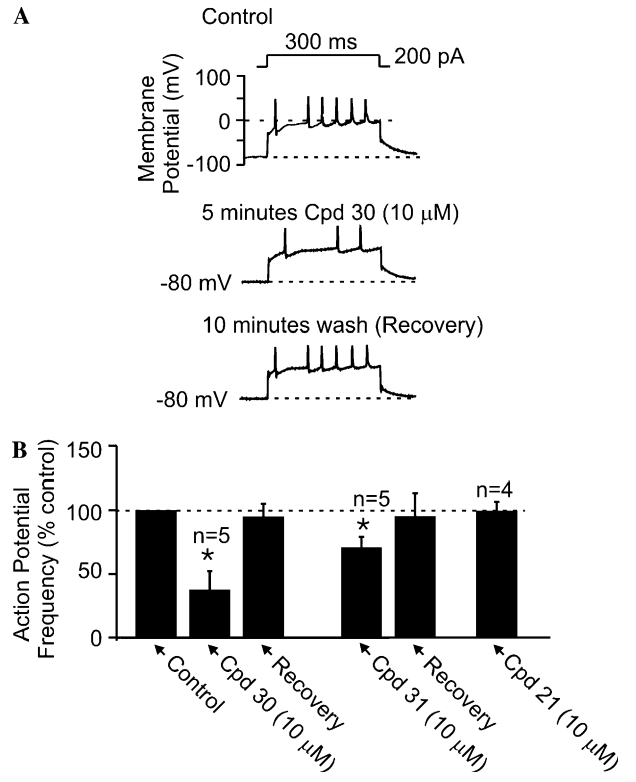
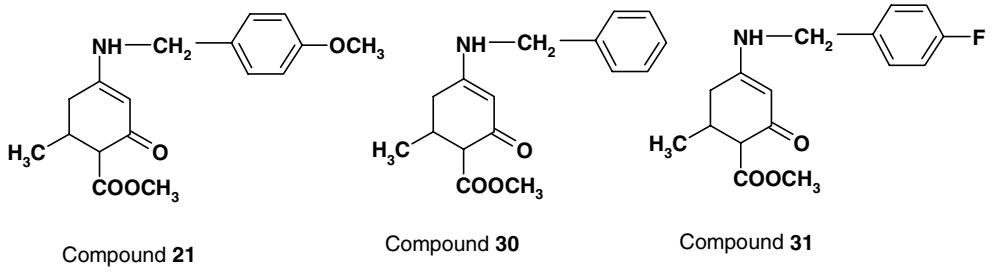


Figure 2. Compounds **30** and **31** decrease action potential firing frequency in neurons. (A) APs generated by a depolarizing current injection into a cell in control conditions (upper panel), 5 min after bath application of compound **30** (10 μM; middle panel), and 15 min after washing out compound **30** (lower panel). (B) Summary bar graph from five (5) neurons each treated with either compound **30** or **31** as in (A) showing the decrease and subsequent recovery in AP firing frequency induced by compounds **30** and **31**. Compound **21** did not affect the AP firing frequency in all four (4) cells tested. ∗ indicated statistical significance at $p < 0.05$.

4.4.1. Mechanisms of anticonvulsant action of benzylamino enaminones. In order to understand the mechanism(s) underlying the anticonvulsant actions of the benzylamine enaminones, their effects on neuronal physiology were investigated. The results of our in vitro study show that the most potent anticonvulsant enaminone (compound **30**) in the benzylamino series caused a significant depression of both glutamate-mediated excitatory synaptic transmission and action potential firing in cells of the central nervous system (CNS). Furthermore, it was observed that the intermediately potent analog, compound **31**, also produced a similar EPSC depression but lesser AP firing suppression than the more potent compound **30** (see Figs. 1C and 2B). Therefore, our in vitro data on synaptic transmission and neuronal excitability are consistent with the results of our in vivo studies which indicate that the unsubstituted benzylamino enaminone compound **30** is a potent (class 1) anticonvulsant agent, while the less potent fluoro-substituted compound **31** displayed less suppression of neuronal excitability. By comparison, the substituted analog compound **21** is inactive as an anticonvulsant, as it does not depress evoked EPSCs, nor reduce the AP firing frequency in the neurons of NAc.

Compound **21** is inactive, while compound **30** is anticonvulsant in vivo. The structural difference between these enaminones is that compound **21** has a *para*-substitution of an electron-donating substituent, while compound **30** has no *para*-substitution (Scheme 2). Accordingly, compound **30** is sterically less hindered to interact at the molecular level and this may be the optimum structural requirement for the benzylamino enaminones to depress excitatory synaptic transmission and neuronal excitation in the rat brain. Furthermore, this structure also enables this compound to suppress AP firing which could be via interaction with sodium channels. Both these effects may then contribute to or underlie its anticonvulsant property.

4.4.2. Electrophysiological data support mechanisms of action. We have performed initial molecular dynamic studies with a variety of common targets for the action of anticonvulsant compounds.[3,14] Out of these, the glutamate receptor (GLUR) best recognized the enaminones, and optimally distinguished between active and inactive enaminones. These molecular dynamic studies suggested that the observed anticonvulsant activity of benzylamine enaminone **27** may be due to inhibition of the glutamate machinery in the central nervous system. The in vitro electrophysiological studies seem to support the involvement of glutamate in the actions of enaminones to produce their anticonvulsant effects. The fact that compound **30**, the most potent anticonvulsant in the MES test, was found to also depress glutamate-mediated excitatory synaptic transmission suggests that an interaction with glutamate may underlie or contribute to its anticonvulsant activity. Recent neuropharmacological evidence in our laboratory suggests that this interaction may however be indirect through the enhancement of gamma amino butyric acid (GABA)



Scheme 2. Complete structures of compounds **21**, **30**, and **31**. The differences between the three compounds studied by electrophysiological recording are the methoxy (OCH_3) group in compound **21**, hydrogen (H) atom in compound **30**, and fluorine (F) atom in compound **31**.

level in rat brains.[11] Additional studies are required to determine if indeed this series of enaminones employ similar cellular mechanism(s) as those recently reported for other enaminones.[11] It is noted that the anilino enaminones whose mechanisms of anticonvulsant action were elucidated recently[11] are structurally different from the benzylamino enaminones which we are reporting in this work. However, the enaminone system is common to both types of moieties, and based on our current and previous data, we postulate that the enaminone pharmacophore existing in a sterically favored conformation is very important for excitatory synaptic depression and anticonvulsant activity.

5. Conclusions

Prior to our recent report,[11] the mechanism of anticonvulsant action of the enaminones was unknown.[20,21] Our evaluation of anticonvulsant benzylamino enaminones gave results which indicate that *para*-substitution of the benzylamine moiety with either carboxyl, chloro, cyano, fluoro, methoxy, methyl, or nitro groups decreases or abolishes the anticonvulsant activity of the enaminones. The most potent benzylamino enaminone was the unsubstituted benzylamine analog (**30**; R^1, $R^4 = H$, $R^2 = CH_3$, $R^3 = CO_2CH_3$). Furthermore, our in vitro electrophysiological data showed that the active benzylamino analogs (**30** and **31**) depressed glutamate-mediated excitation and action potential firing in neurons. These actions may contribute to their anticonvulsant activity.

6. Experimental

6.1. Chemical synthesis

Melting points of the enaminone compounds were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. Spectrometric (IR and NMR) data and elemental analyses showed the appropriate peaks and chemical shifts, and confirmed the molecular formulae for the benzylamino enaminones. Typical experiments illustrating the general synthesis of the enaminones are described below.

6.1.1. Synthesis of benzylamino enaminone with no ester group; 3-benzylaminocyclohex-2-en-1-one (1). To a solution of 1,3-cyclohexanedione (7.5 g, 67 mmol) in 75 mL of ethanol and 100 mL of benzene was added benzylamine (7.88 g, 70 mmol) and the mixture refluxed for 4 h. Evaporation of the reaction mixture yielded a dark brown oil which crystallized from diethyl ether. Two recrystallizations of the crude product from 2-propanol provided 3.4 g (25%) of greenish-yellow crystals of enaminone **1**, mp 125–127 °C (lit. 122–124 °C).[15]

6.1.2. Synthesis of benzylamino enaminone with ester group; methyl 4-benzylamino-6-methyl-2-oxocyclohex-3-en-1-oate (30). To a solution of methyl 4-hydroxyl-6-methyl-2-oxocyclohexen-3-en-1-oate (2 g, 10.9 mmol) in 100 mL of toluene was added benzylamine (1.61 g, 15 mmol) and the mixture refluxed for 3 h using a Dean–Stark water separator. During the reaction, 0.25 mL of water was collected. Evaporation of the mixture to dryness and two recrystallizations from ethyl acetate provided 1.6 g (81%) of enaminone **30**, mp 154–155 °C. Anal. (C, H, N).

6.1.3. Synthesis of benzylamino enaminone with amide group; 4-benzylamino-6-methyl-2-oxocyclohex-3-ene(*N*-benzyl) carboxamide (36). To a solution of methyl 4-hydroxyl-6-methyl-2-oxocyclohexen-3-en-1-oate (4 g, 22 mmol) in 100 mL of toluene was added benzylamine (6.4 g, 60 mmol) and the mixture refluxed for 6 h using a Dean–Stark water separator. During the reaction, 0.4 mL of water was collected. Evaporation of the reaction mixture to dryness and two recrystallizations from methanol provided 6.2 g (81%) of enaminone **36**, mp 193–195 °C. Anal. (C, H, and N).

6.2. In vivo anticonvulsant evaluations

Anticonvulsant evaluations of the benzylamino enaminones (**1**–**37**) were performed by the Antiepileptic Drug Development (ADD) Program, Epilepsy Branch, Neurological Disorders Program, National Institute of Neurological Disorders and Stroke, and included Phase I, II, VIA, and VIB test procedures which have been described.[16–18] These tests were performed in male Carworth Farms no. 1 (CF1) mice (Phases I and II) or male Sprague–Dawley rats (Phases VIA and VIB). Phase I and Phase VIA of the anticonvulsant evaluation comprised of maximal electroshock test, subcutaneous pentylenetetrazole (ScMet) test, and the rotorod test for neurological toxicity (Tox). Compounds were suspended in 0.5% aqueous methylcellulose and were administered at 30, 100, and 300 mg/kg dosage levels with anticonvulsant activity and motor impairment noted 30 min and 4 h after administration. Phase II and Phase VIB quantitated the anticonvulsant activity and motor impairment observed for the most promising enaminones in Phase I. Phase II quantitated data in CF1 mice by intraperitoneal (ip) administration, while Phase VIB quantitated oral rat data comparable to Phase II intraperitoneal (ip) data in mice. Data for the anticonvulsant evaluations are shown in Table 1.

6.3. Electrophysiology

Electrophysiological studies were performed with three compounds of this series, compounds **21**, **30**, and **31** (see Scheme 2) using in vitro patch-clamp recording techniques.[11] In brief, forebrain slices were prepared from male Sprague–Dawley rats using standard methods and pure glutamate-mediated excitatory postsynaptic currents (EPSCs) were recorded in voltage clamp mode as previously reported.[11] Pure glutamate-mediated responses were isolated by bath application of picrotoxin (50 μM) to eliminate the $GABA_A$ receptor-mediated component of synaptic responses (see Kombian et al.[19]). APs were recorded in current clamp mode and a series of hyperpolarizing and depolarizing currents were applied to the cells at their resting potential and the corresponding membrane responses were

captured. 10 mM stock solutions of compounds **21**, **30**, and **31** were made by dissolving them in DMSO. These were then aliquoted and stored frozen until application. They were bath perfused at the final concentration of 10 μM by dissolving the 10 mM stock solution in artificial cerebrospinal fluid resulting in a 1000-fold dilution of the DMSO (yielding <0.1% DMSO in the bath). This final solution was then applied for 5–6 min. A concentration of 10 μM of compounds **21**, **30**, and **31** was selected for this study because we have previously reported that other anticonvulsant enaminones of the aniline series produced robust synaptic depression at this concentration.[11] Furthermore, this concentration was calculated to be 10 times less than the equivalent oral dose that demonstrated in vivo anticonvulsant activity. Thus, if only 10% of administered compound **30** were absorbed following oral administration, and this entire amount reached the brain, it would be expected to produce the anticonvulsant effect.

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