European Journal of Medicinal Chemistry 76 (2014) 20–30

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis, neuronal activity and mechanisms of action of halogenated enaminones

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ARTICLE INFO

Article history:
Received 12 August 2013
Received in revised form
22 January 2014
Accepted 3 February 2014
Available online 4 February 2014

Keywords:
Dihalogenated enaminones
Neuronal activity
Synthesis

ABSTRACT

Due to the excellent anticonvulsant activity of previously synthesized halogenated enaminones, more disubstituted analogs were synthesized and evaluated *in vitro*. The new enaminones either had no effect, depressed, or enhanced population spike (PS) amplitude in the rat hippocampus in a concentration-dependent manner. Structure–activity relationship (SAR) analysis indicated that compounds **21** and **25** (with dibromo substituents) were equipotent, and more potent than compound **2** (with dichloro substituents), with compound **25** being the most efficacious of all tested compounds. Both diiodo derivatives **30** and **31** tested produced no significant effect on PS. For PS depression, phenyl substitution on the cyclohexenone ring produced the most efficacious compound **25**. PS depressing analogues also depressed evoked excitatory postsynaptic current (EPSC) and action potential firing frequency. Removal of phenyl or methyl group from position 6 on the cyclohexenone ring of enaminone esters produced compound **28** which exhibited pro-convulsant effects. There was no direct correlation between C log *P* values and anticonvulsant activity of the halogenated enaminones. The mechanisms of anticonvulsant activity were the indirect suppression of excitatory synaptic transmission by enhancing extracellular GABA, and the direct suppression of action potential firing of the neurons.

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1. Introduction

Enaminones are synthetic compounds that consist of an amino group, joined through an alkene group to a ketone group [1–3]. Enaminones possess a range of pharmacological effects including antimalarial and anticonvulsant activities [1]. However, enaminones are largely devoid of neurotoxicity [4]. Halogenated enaminones have halogens such as fluoro, chloro, bromo, and iodo moieties incorporated in the molecules [5].

Epilepsy is a neurological disorder characterized by the onset of spontaneous convulsant and non-convulsant seizures that result from neuronal hyperexcitability and hypersynchronous neuronal firing [6]. About 50 million people worldwide are affected by epilepsy, and it is estimated that 30% of the patients suffer from therapy-resistant epilepsy. Resistance to antiepileptic drugs (AEDs) and the side effects associated with the current AEDs are the most serious problems in the treatment of epilepsy [7–9]. Therefore, there is an urgent need to design and synthesis novel anticonvulsants for the development of more effective and safer AEDs [6].

In light of the emerging importance of enaminones as potential AEDs [5], the rationale of the current study was to synthesize and evaluate disubstituted phenylamino enaminones. Thus, we assessed the effect of modification of 2,4-dihalogenation of the phenylamino moiety of the investigated enaminones. In our current study, nine new disubstituted phenylamino enaminones were synthesized, and their anticonvulsant activity and neurotoxicity evaluated to establish if some of these enaminones might become lead compounds for further development into new AEDs.

Abbreviations: aCSF, artificial cerebrospinal fluid; ADD, antiepileptic drug development; AED, antiepileptic drug; DMSO-d6, dimethyl sulfoxide-deuterated; EPSC, excitatory postsynaptic current; EPSP, excitatory postsynaptic potential; GABA, gamma aminobutyric acid; GABA-T, GABA transaminase; GAT, GABA transporters; GC–MS, gas chromatography–mass spectrometry; ND, not determined; NMR, nuclear magnetic resonance; mp, melting point; PS, population spike; SEM, standard error of the mean; TMS, tetramethylsilane; TTX, tetrodotoxin.

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0223-5234/$ – see front matter Published by Elsevier Masson SAS.
http://dx.doi.org/10.1016/j.ejmech.2014.02.002

We had reported that anticonvulsant enaminones are very stable compounds at room temperature [10,11], and previous investigations indicated that cyclized enaminones were more potent as anticonvulsants than the acyclic analogs. The NH proton was mandatory for anticonvulsant activity, and that enaminones were very promising as potential AEDs [12–20]. Initial evaluations of halogenated enaminones revealed some important analogs of which the most potent analog **20** was investigated for mechanisms of anticonvulsant activity [21–23]. We have investigated further in the current work the halogenated enaminones with more emphasis on disubstituted phenyl enaminones. We hereby report our findings that the 2,4-dibromophenyl enaminones are potent and efficacious anticonvulsant agents.

2. Chemistry

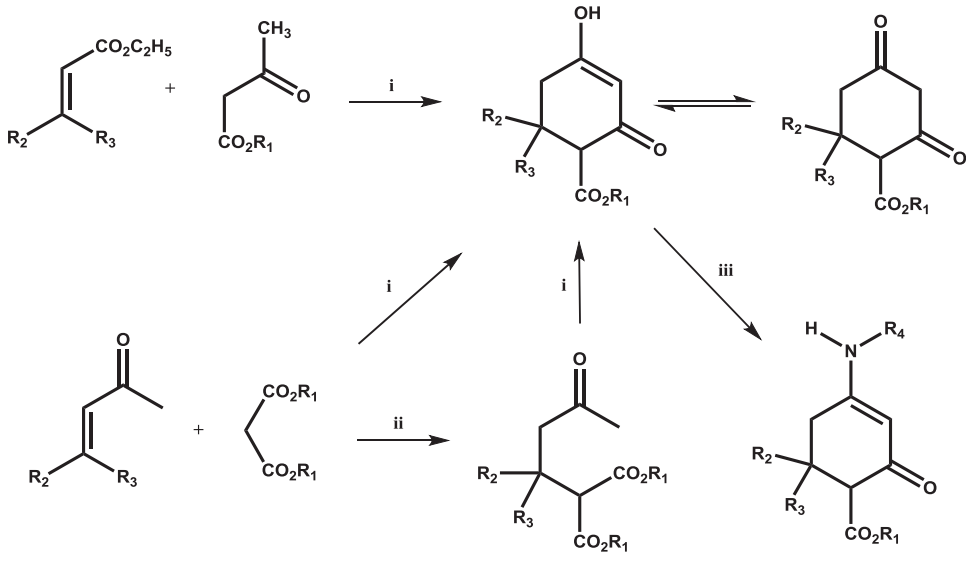
The general synthetic routes for the halogenated enaminones is shown in Scheme 1. Three independent routes of synthesis were employed in unequivocal synthesis of the cyclized 4-hydroxycyclohex-3-en-2-oxo-1-oates which were important intermediates for obtaining the desired halogenated enaminone esters. The alkyl acrylate was reacted with alkyl acetoacetate in the presence of freshly prepared sodium alkoxide to obtain the 4-hydroxycyclohex-3-en-2-oxo-1-oate which existed as tautomers. The second synthetic route involved the Michael addition reaction of the alkylidene ketone and dialkyl malonate in sodium alkoxide to give the 4-hydroxycyclohex-3-en-2-oxo-1-oate. The third route involved the reaction of the alkylidene ketone with dialkyl malonate under mild conditions to give the uncyclized Michael adduct which was cyclized in the presence of sodium alkoxide to 4-hydroxycyclohex-3-en-2-oxo-1-oate. The condensation of 4-hydroxycyclohex-3-en-2-oxo-1-oates with appropriate amino compounds yielded the halogenated enaminones as in Scheme 1, and in Table 1. The halogenated enaminone derivatives (**22**, **23**, **26** and **27**) lacking the ester functionality were prepared from the commercially available β-diketo compounds, namely, 4,4-dimethylcyclohexane-1,3-dione, 5,5-dimethylcyclohexane-1,3-dione, cyclohexane-1,3-dione and 5-methylcyclohexane-1,3-dione. The chiral enaminones were synthesized by non-stereoselective methods and evaluated pharmacologically as racemates. The synthesized compounds were stable solids and purified by recrystallization from suitable solvents. ^{1}H nuclear magnetic resonance (NMR) spectra of the synthesized enaminones were in $CDCl_3$ or DMSO-d_6 using TMS as an internal standard. The ^{13}C NMR spectra of representative compounds **2**, **21**, **25, 26** and **31**, were also performed. Elemental analyses were performed for all new enaminones (**21–22, 25–31**). Calculated log of partition coefficient (C log *P*) values for the new compounds were performed using ChemDraw® Ultra version 8.0, 2003, Cambridge Soft Corporation.

3. Pharmacology

All experiments in this study were carried out on male Sprague–Dawley rats (100–150 g). Coronal slices (350 μm thick) containing hippocampi were sliced in ice-cold artificial cerebrospinal fluid (aCSF). Slices were perfused fully submerged at 2–3 mL/min with aCSF (29–31 °C) that was bubbled with 95% O_2/5% CO_2). Tungsten bipolar stimulating electrodes were placed in the dendritic layer of area CA1 while recording glass electrodes were placed in the cell body layer to record field population spikes (PS) (Fig. 1). All drugs were applied by bath perfusion. PS magnitude was measured as the absolute amplitude from peak to trough and used as a measure of neuronal excitation. All values are presented as mean ± SEM and $P < 0.05$ was taken as being statistically significant.

4. Results and discussion

The halogenated enaminones **1–31** were synthesized from β-hydroxyketo starting materials, according to methods reported previously [1,3,5]. The general synthesis is shown in Scheme 1. The condensation of alkyl acrylate with alkyl acetoacetate, or alkyl vinyl ketone with dialkyl malonate gave the β-hydroxyketo intermediates, which were reacted with appropriate halogen substituted-phenylamines to obtain the halogenated enaminones. Since compound **2** was completely characterized in this study (mp, UV, IR, ^{1}H NMR, ^{13}C NMR, and MS), its synthesis was markedly different from reported literature [5].



R_1 = Me, Et; R_2 = H, Me, Ph; R_3 = H, Me; R_4 = Substituted phenyl

Scheme 1. Synthesis of halogenated enaminones. Reagents and conditions: (i) Na/MeOH for methyl esters; and Na/EtOH for ethyl esters, reflux, 2–4 h, (ii) Potassium carbonate, (iii) appropriate amino compound (NH_2R_4).