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DESIGN OF DUAL ACTING ANTICONVULSANT-ANTIMUSCARINIC SUCCINIMIDE AND HYDANTOIN DERIVATIVES

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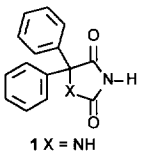
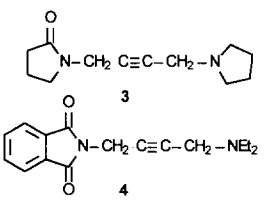
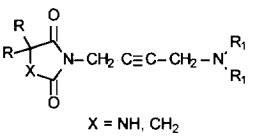
Abstract: A series of 4-dialkylamino-2-butynyl-succinimide and -hydantoin derivatives were designed containing pharmacophoric elements for dual acting anticonvulsant and antimuscarinic activity. Potent anticonvulsant agents were identified with an extremely rapid onset of protective activity. Succinimides **13** and **18** exhibited ED_{50} values of 20 and 9.6 mg/kg 10 minutes after administration in the MES seizure model and also exhibited good binding affinity for the M_1 muscarinic receptor. © 1997 Elsevier Science Ltd.

The key toxic lethal effect by poisoning with organophosphate cholinesterase inhibitors, such as pesticides or chemical warfare nerve agents, is the action of acetylcholine (ACh) in the brain, producing centrally mediated respiratory paralysis and violent convulsions.[1] Evidence indicates the seizures produced by these agents may not be related solely to the effects on central cholinergic activity. Centrally acting antimuscarinic agents are not effective in blocking organophosphate-induced seizures,[2] and acute organophosphate administration induces seizures when acetylcholinesterase (AChE) is already significantly inhibited.[3] Current therapy by pretreatment with physostigmine and atropine increases the 24-hour survival rate but does not inhibit the soman-induced convulsions.[4] Successful treatment requires concomitant administration of an anticonvulsant agent such as diazepam for these symptoms.[5,6] Although atropine is a potent antimuscarinic agent its usefulness as an antidote is limited by its poor central antimuscarinic activity when adminstered peripherally. Previous studies have focused on designing new centrally acting antimuscarinic agents as a replacement for atropine.[7] A drug molecule exhibiting a potent and rapid onset of anticonvulsant action and sufficient antimuscarinic activity to block the effects of ACh would be a valuable therapeutic agent for cholinesterase poisoning.







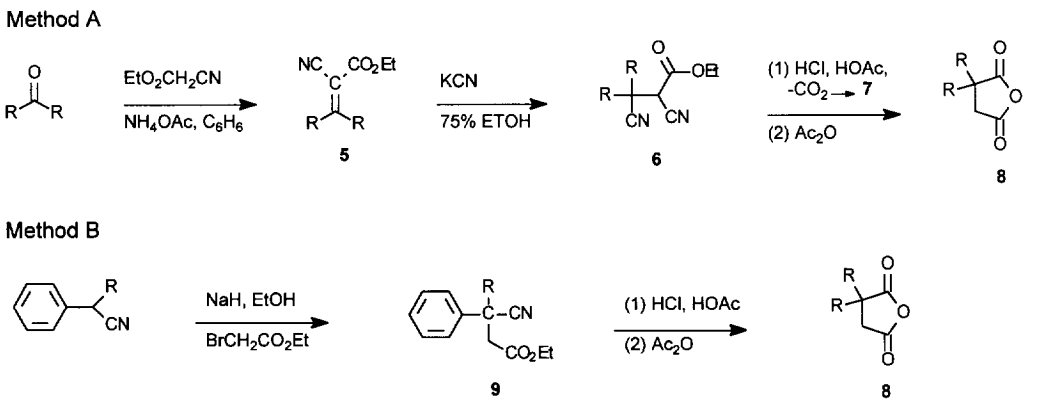
This paper reports on the design of a novel series of dual acting anticonvulsant/antimuscarinic succinimide and hydantoin derivatives based on the common pharmacophoric elements required for each activity. The prototype hydantoin, 5,5-diphenylhydantoin (phenytoin **1**), and the corresponding 3,3-diphenylsuccinimide (**2**) represent structural classes of compounds demonstrating potent anticonvulsant activity. The reported weak antagonist properties with the imide[8] analog of the competitive muscarinic

agonist oxotremorine[9] (**3**) and the good centrally mediated antimuscarinic activity of the phthalimide derivative **4**[10] suggested the oxotremorine-imide template may serve as a starting point to design centrally acting antimuscarinic agents with anticonvulsant properties. One of the critical determinants of antimuscarinic activity is the proper introduction of lipophilic groups into prototype competitive muscarinic agonists.[11] Therefore, a novel series of N-(4-dialkylamino-2-butynyl)-3,3-disubstituted succinimides and 3-(4-dialkylamino-2-butynyl)-5,5-disubstituted hydantoin derivatives were prepared and evaluated for anticonvulsant activity in the maximal electroshock seizure model[12] (MES) and for binding to M_1 and M_2 muscarinic receptor subtypes.

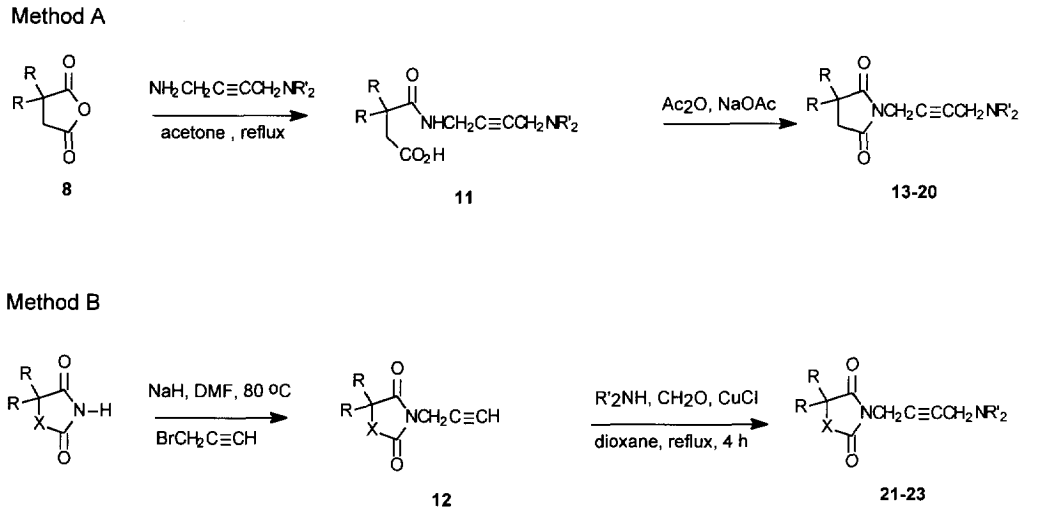
Chemistry

The target compounds were synthesized using standard literature methods as outlined in the schemes. The 4-dialkylamino-2-butynyl succinimides were prepared either from the corresponding succinic anhydrides (**8**) and a 4-dialkylamino-2-butynylamine (**10**) (method A) or from the imide (method B) as outlined in Scheme 2. Two general routes were employed to prepare the substituted succinic anhydride intermediates (Scheme 1). Method A utilizes a Knoevenagel condensation with ethyl cyanoacetate and a ketone to give intermediate **5**, which when treated with KCN in aqueous ethanol yields the crude dicyano esters **6**. Hydrolysis and decarboxylation to diacid **7**, followed by cyclization with acetic anhydride or acetyl chloride gives the corresponding anhydride intermediates **8**.[13] In method B, an appropriately disubstituted acetonitrile was alkylated with ethyl bromoacetate to give the cyano-esters **9**. Hydrolysis and ring closure as described in method A provides the anhydride intermediates **8**.[13] Treatment of the anhydrides **8** with an appropriate diamine **10**[10] at acetone reflux yields the succinamic acids **11** in high yield (Scheme 2, method A). The final succinimides **13-20** were formed in good yield using acetic anhydride and sodium acetate at 60-70 °C.[10] The hydantoin analogs were prepared as outlined in Scheme 2, method B. A hydantoin[13] or succinimide[14,15] was alkylated with propargyl bromide (NaH, DMF, 80 °C) to give the N-propynyl derivative **12**. The terminal alkyne was submitted to a Mannich reaction (secondary amine, formaldehyde, CuCl, dioxane reflux)[8,10] to give the target 3-(4-dialkylamino-2-butynyl)-5,5-disubstituted hydantoins **21-23**.[16]

Scheme 1.



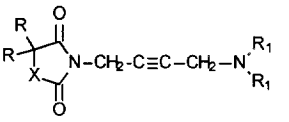
Scheme 2.



Results and Discussion

Compounds were evaluated for affinity at muscarinic M_1 sites by displacement of [^{3}H]pirenzepine from rat cortex homogenates and M_2 sites by displacement of [^{3}H](-)quinuclidinyl benzylate (QNB) from rat heart homogenates (Table 1).[7a,c] Phthalimide **4** exhibited K_i values of 30 and 100 nM for M_1 and M_2 sites, respectively. The succinimide analogs in this study showed some selectivity for binding to M_1 muscarinic receptors, as the analogs generally lacked affinity (> 10 μM) for M_2 sites. The exceptions, which showed moderate M_2 binding affinity, were the succinimide derivatives **13** (K_i = 380 nM), **14** (K_i = 440 nM) and hydantoin **21** (K_i = 950 nM). Diphenyl-succinimide **13** demonstrated the highest affinity (K_i = 120 nM) for M_1 sites while the isopropyl derivative **14** and the spiro dibenzosuberane **18** showed K_i values of 530 nM and 460 nM, respectively. Removal of one phenyl group in **13** resulted in **15** which showed weak muscarinic affinity. The spatial arrangement of the two aromatic rings is also important for M_1 affinity. The planar fluorene derivatives showed weak muscarinic binding affinity compared to the diphenyl substituted derivatives **13** and **14** or the more flexible tricyclic **18**. A requirement for nonplanarity in the binding of the aryl groups to the lipophilic region of the muscarinic receptor has also been observed with a series of classical α,α-disubstituted acetate type muscarinic antagonists,[16] suggesting common modes of binding with the two series. Hill values for the succinimide derivatives were near unity, indicating competitive binding to the M_1 site.

Table 1. Muscarinic M_1 and M_2 Binding Affinities of Hydantoin and Succinimide Analogs.



Compound	R	R	X	R1	K_i, μM M_1	M_2
13	Ph	Ph	CH	Et	0.12 ± 0.03	0.38 ± 0.05
14	Ph	Ph	CH	^{i}Pr	0.53 ± 0.12	0.44 ± 0.06
15	Ph	H	CH	Et	2.30 ± 0.34	>10
16	1-Indane		CH	Et	0.68 ± 0.30	>10
17	1-Indane		CH	C_4H_8	1.59 ± 0.02	>10
18	5-Dibenzosuberane		CH	Et	0.46 ± 0.02	2.09
19	9-Fluorene		CH	Et	1.63 ± 0.30	>10
20	9-Fluorene		CH	^{i}Pr	1.62 ± 0.22	>10
21	Ph	Ph	N	Et	0.38 ± 0.04	0.95 ± 0.04
22	Ph	Ph	N	C_4H_8	0.64 ± 0.05	>10
23	1-Indane		N	Et	1.05 ± 0.16	>10
4	(Phthalimide)			Et	0.03 ± 0.01	0.10 ± 0.07
R(-)QNB					0.00015	0.00003
pirenzepine					0.0052	0.267

Data are the mean ± SEM of at least three separate determinations performed in triplicate. Ligand conc. was 0.5 nM for (+)pirenzepine in rat cortical homogenates and 0.1 nM for [^{3}H]QNB binding in rat heart homogenates. Binding was performed as described previously.[7a,c]

The initial goal of the anticonvulsant study was to determine if the series demonstrated activity in the MES model, and then to assess an optimum time for peak protection for screening the additional compounds. Phenytoin was used as a control, which showed an ED_{50} of 16.2 mg/kg when administered 1 h prior to the MES challenge. Initial testing with hydantoin **21** showed 40% protection and high toxicity at 100 μM (41 mg/kg) 1 h after administration. The animals did not survive a higher dose of 300 μM (123 mg/kg). Succinimide **13** was then evaluated at an initial dose of 125 mg/kg (300 μM) administered 1 h prior to challenge and produced 100% protection in mice. Toxicity observed was sedation and slight ataxia. Animals were observed for 3 days after the challenge and showed 100% recovery with no deaths. A time course study for **13** at a lower dose (100 μM) continued to show 100% protection when administered 10, 20, 30, 60, and 120 min prior to challenge. This very rapid onset of anticonvulsant activity was unexpected and clearly a distinct advantage with this series. Therefore, ED_{50} values for the remaining compounds were determined 10 min after drug administration in the MES model.

The data in Table 2 reveals the compounds exhibit potent anticonvulsant activity in the MES assay at the 10 min time point. The spiro dibenzosuberane **18** was the most effective analog, demonstrating an ED_{50} of 9.6 mg/kg, while the diphenylsuccinimide derivatives **13** and **14** showed ED_{50} values of 20 and 17.5 mg/kg, respectively. The conformational flexibility of the aromatic rings does not appear critical for anticonvulsant activity as the planar fluorene-succinimide **19** was equally potent to the diphenyl derivative **13**. Removal of one phenyl ring reduced activity 3-fold with analog **15**, while indane **17** showed no protection up to 600 mg/kg. The phthalimide **3**, as opposed to the 3-phenylsuccinimide **15**, was inactive up to concentrations of 1000 mg/kg. In initial dosing studies the hydantoin derivatives were very toxic to the animals, causing immense ataxia and even death at higher doses. A comparison of ED_{50} values at 10 min indicates the hydantoin analogs were less active than the corresponding succinimides (compare the diphenyl-hydantoin **21**, ED_{50} = 84 mg/kg, with the diphenyl-succinimide **13**, ED_{50} = 20 mg/kg). The succinimide

analogs showed no toxicity at the 10 min ED_{50} doses and no ataxia up to 100 μM. Mild sedation was observed with **18** and **13** at 100 μM while no sedation was observed with **14** and **19** at this concentration. Compound **13** showed an apparent protective index (PI) of 5 for MES ED_{50}/concentration producing ataxia.

The compounds were also screened for anticonvulsant activity against pentylenetetrazole induced seizures. Most compounds were ineffective in this assay. The monophenyl-succinimide **15** gave 80% protection at a 150 μM dose, while **18** showed only partial protection (50%) at 50 μM and no activity at higher doses.

Table 2. Anticonvulsant Data for Hydantoin and Succinimide Analogs.

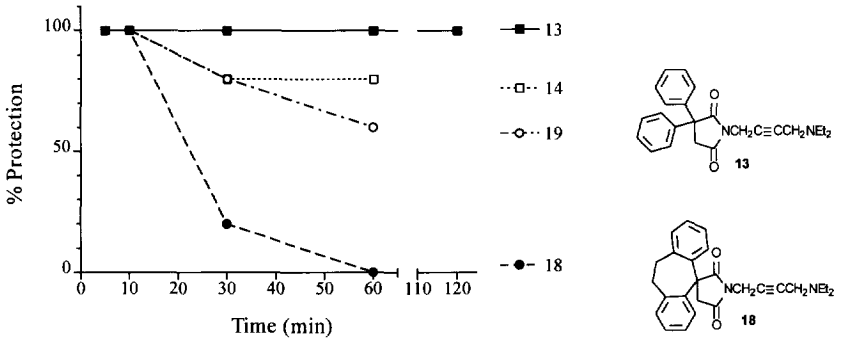
	----------MES ED_{50}----------	
Compound	mg/kg	μM/kg
13	20.1 (14.8 - 27.5)	49 (36 - 67)
14	17.5 (11.4 - 27.1)	40 (26 - 62)
15	48.9 (43.5 - 55.2)	126 (112 - 142)
17	> 600	
18	9.6 (7.4 - 13.1)	22 (17 - 30)
19	23.7 (15.5 - 36.0)	58 (38 - 88)
21	83.6 (66.7 - 105)	203 (162 - 255)
23	55.4 (42.3 - 72.7)	153 (117 - 201)
3	> 1000	
phenytoin	16.2 (10.6 - 24.8)[a]	
	9.5 (8.13 - 10.4)[b]	

Anticonvulsant studies were conducted in male BALB/c mice (5-10 animals per group). Maximal electroshock seizures were produced by 50 mA, 60 Hz, 0.2 sec current via ear clamps. MES challenge was 10 min after i.p. administration of test compound (as salts) in sterile water. ED_{50} values and confidence limits were calculated by log probit analysis using the computer program of Tallarida and Murray[17] (a) Determined at 1 h post administration. (b) Literature value 2 h post adminstration.[12a]

The more potent succinimide analogs at the 10 min time point (**13**, **14**, **18** and **19**) were further profiled in a time course study for protection (Figure 1). The compounds were tested at 2-times the 10 min ED_{50} dose for protection at times 20, 30, and 60 min. Compound **13**, which initially showed 100% protection from 10 min to 2 h prior to challenge, also protected at 5 min prior to the MES challenge at 41 mg/kg (100 μM). Compound **18**, the most potent analog at 10 min, showed only 20% protection at the 30 min time point and was ineffective at 1 h (22 mg/kg; 50 μM). Analog **14** (35 mg/kg; 100 μM) continued to exhibit good protection up to the 1 h time point, while the fluorene analog **19** (41 mg/kg; 100 μM) protected only 60% of the animals at the 60 min challenge. This study demonstrates all of the succinimide analogs tested appear to rapidly enter the CNS, although small structural changes affect the profile or continuance of protection.

The distinct advantage of this series is the very rapid onset of anticonvulsant activity. Phenytoin shows peak anticonvulsant activity at 2-3 h in mice[12a,c] whereas succinimide type anticonvulsants show peak activity 2 h after dosing. For example, 3,3-diphenylsuccinimide exhibits an ED_{50} of 45 mg/kg (180 μM/kg) when administered ip 2 h prior to the electroshock challenge.[13] The 4-diethylamino-2-butynyl derivative **13** described in this research showed an ED_{50} of 20 mg/kg at 10 min, and was at least 2 to 3-fold more potent at the 1-2 h time point. The structure-activity relationships of hydantoin or succinimide type anticonvulsants reveals that the greater the substitution on the imide nitrogen the greater the loss in anticonvulsant activity.[18] Incorporating basic alkylamino groups onto the chain further reduces or eliminates antiseizure properties. For example, N-(2-diethylaminoethyl)-3,3-diphenylsuccinimide lacks anticonvulsant activity.[13] The aminobutynes had not been previously evaluated. The tertiary amino group in the butynyl series reported here appears important for activity as the N-propynyl-5,5-diphenylhydantoin precursor to **20** was found inactive in the MES model.

Figure 1.



In summary, a novel series was designed to act as dual anticonvulsant/antimuscarinic agents. Novel compounds have been identified which show a potent, rapid onset of anticonvulsant activity, in addition to good M_1 binding affinity. Succinimides **18** and **13** exhibited ED_{50} values of 9.6 and 20 mg/kg at 10 min in the MES model, and M_1 binding affinity of 0.46 and 0.12 μM, respectively. A time-course study revealed the compounds display varying durations of anticonvulsant activity. Compound **18** exhibited a very short half-life, while compound **13** displayed protection from 5 min to 2 h after administration.

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References and Notes

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