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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and biological evaluation of N^1,N^2-bis-[4-(*t*-amino)-2-butynyl]phthalamides as oxotremorine and acetylcholine antagonists

Zuhair Muhi-eldeen · Elham Al-Kaissi · Hanan Ghantous

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Abstract A series of N^1,N^2-bis-[4-(*t*-amino)-2-butynyl] phthalamides have been synthesized and investigated for the blocking of the motor effect of oxotremorine in intact mice and for their antagonistic activity towards acetylcholine on isolated guinea pig ileum preparations. All N^1,N^2-bis-[4-(*t*-amino)-2-butynyl]phthalamides showed more selectivity as oxotremorine antagonists than atropine and less potent than atropine on peripheral cholinergic antagonistic activity.

Keywords Acetylcholine antagonist · Aminoacetylenic derivatives · Aminoacetylenic phthalamides derivatives · Oxotremorine antagonist

Introduction

Tremorine **1**, and oxotremorine **2**, were very active muscarinic agent when tested in vivo or in isolated organs and comparable to acetylcholine in potency (Cho *et al.*, 1962). They are relatively specific in producing central, as opposed to peripheral cholinergic effects (Inch *et al.*, 1973). The observations of Parkinson-like effect exerted by tremorine and more intense by oxotremorine have initiated intensive research on antiparkinsonian agents, in particular from their central cholinergic role in reducing tremor (Muhi-Eldeen *et al.*, 1980). Several series of acetylenic compounds which are structurally related to oxotremorine with specific structural changes to afford the antagonistic activity have been synthesized. These include amides, esters, cyclic imides (Muhi-Eldeen *et al.*, 1980; Ringdhal *et el.*, 1979a; Karlen, 1970; Schulte and Rucker, 1970; Muhi-Eldeen *et al.*, 1982; Dahlbom *et al.*, 1974; Ringdhal *et el.*, 1979b; Eicholzer and Orgen, 1977). Previously we have synthesized in our laboratories a series of *N*-[4-(*t*-amino)-2-butynyl]phthalimides (Muhi-Eldeen *et al.*, 1980), and investigated for their oxotremorine and peripheral acetylcholine antagonistic activity. All compounds except one showed oxotremorine antagonistic activity and all showed peripheral acetylcholine antagonistic activity as compared to atropine (Muhi-Eldeen *et al.*, 1980). In reviewing the above mentioned structural modifications, we were interested in inserting two aminoacetylenic moiety in amide form namely N^1,N^2-bis-aminoacetylenic phthalamides **13–16** to verify whether the presences of bis-aminoacetylenic moiety in phthalamide may yield a more effective oxotremorine antagonists as seen in tacrine dimer in treatment of Alzheimer (Scutara *et al.*, 2011), and in bivalent bendamustine and melphalan in cancer treatment (Bolognesi *et al.*, 2007). The new aminoacetylenic phthalamides were generated through the hydrazinolysis of corresponding *N*-[4-(*t*-amino)-2-butynyl]phthalimides **5–8** as illustrated in the experimental part. The IR, ^{1}HNMR, ^{13}CNMR and elemental analysis were consistent with the assigned structures. All synthesized compounds showed greater selectivity as oxotremorine antagonist than peripheral

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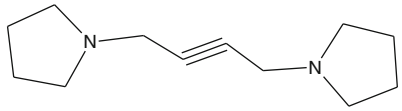
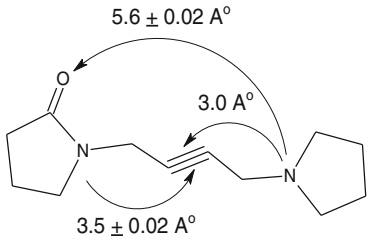
cholinergic antagonistic activity except compound **15** in which the cyclic amine is 2,6-dimethylpiperidine was inactive as oxotremorine antagonist.



1-[4-(1-pyrrolidinyl)-2-butynyl]pyrrolidine

Tremonine



1-[4-(1-pyrrolidinyl)-2-butynyl]-2-pyrrolidinone

Oxotremonine

Materials and method

Chemical methods

Melting points were measured using Fischer–Johns melting point apparatus. Infrared spectra (IR) were recorded on a Nicolet Impact-400 FT-IR spectrophotometer. ^{1}H-NMR was recorded with the aid of Bruker-DPX300 MHz spectrometers. Elemental analysis was performed in the laboratories of Dr. Bernhardt, Mulheim, Germany. The results obtained had a maximum deviation of ±0.04 % from the theoretical value.

N-propargylphthalimide **4**

This compound was prepared in 65 % yield according to the method described in the literature of *Cho et al.*, 1962 mp 150–151 °C.

N-[4-(*t*-amino)-2-butynyl]phthalimides **5–8**

These compounds were prepared in 65–86 % yield as described in reference (Muhi-Eldeen *et al.*, 1980), mp (85–86 °C), (105–107 °C), (132–134 °C), (75–77 °C). The IR, ^{1}HNMR spectra and elemental analysis were consistent with the assigned structures.

N^1,N^2-bis-[4-(*t*-amino)-2-butynyl] phthalamides **13–16**

A mixture of *N*-[4-(*t*-amino)-2-butynyl]phthalimide (0.025 mol) hydrazine hydrate (0.03 mol) and ethanol 95 % (60 ml) were refluxed for 1.5 h. The mixture was allowed to cool and (4 ml) of concentrated hydrochloric acid was added drop wise, stirred for 1.5 h, and filtered, the precipitate was washed with (30 ml) of water and filtered through section on Celite. The solvent was removed under reduced pressure afforded a mixture of two products, the corresponding hydrochloride salt of aminoacetylene amine and [N^1,N^2-bis-[4-(*t*-amino)-2-butynyl]phthalamide. The free amine was generated through treatment of HCl salt with (K_2Co_3/ether). The ether was dried with anhydrous $MgSO_4$ and concentrated in vacuum affording the corresponding [4-(*t*-(amino)-2-butynyI]amine **9–12**, the boiling points of the aminoacetylenic amines are listed in (Table 1), 1R(neat,cm^{-1}). 3500–3300 (broad, NH_2, Stretch), 2850–2800 (CH_2, Stretch), 2120 (C≡C, Stretch), 1420, 1330 (CH_2, bending); ^{1}HNMR (DMSO-d6)δ, 1.8–1.6(m, CH_2 of cyclic protons), 2.6 (t, –CH_2–*N*-cyclic amine), 2.85 (m, 2H, NH_2–CH_2–C≡), 4.0–4.5(m, 2H, NH_2–C).

N^1,N^2-bis-[4-(*t*-amino)-2-butynyl] phthalamides **13**

The title compound was prepared according to the general procedure for the synthesis of N^1,N^2-bis-[4-(*t*-amino)-2-butynyl]phthalamides Scheme 1. The yield %, mp, and elemental analysis were shown in (Table 2). IR (KBr,Cm^{-1}): 3200–3150 (NH, stretch), 2950–2845 (ArH, stretch), 2230 (C≡C, stretch), 1665 (C=O, stretch, amide), 1612, 1458 (Ar, C=C, stretch), 1030, 948 (Ar, C=C, bending). ^{1}HNMR (DMSO-d_6): δ, 1.53 (m, 2H, $^{11}CH_{2'}$), 1.59 (m, 4H, $^{10}CH_2$–$^{10'}CH_2$), 1.64 (m, 4H, $^{9}CH_2$–$^{9'}CH_2$), 3.39 (t, 2H, $J = 2.4$ Hz, $^{8}CH_2$–N), 3.66 (t, CH_2, $J = 2.4$ Hz, $^{5}CH_{2'}$), 7.4 (d, 1H, $J = 4.2$ Hz, Ar^{3}H), 7.8 (d, 1H, $J = 4.2$ Hz, Ar^{1}H). ^{11}CNMR (DMSO-d_6): δ, 24.3 (^{13}C), 25.8 ($^{10,10'}$C), 27.1 ($^{9,9'}$C), 52.9 (^{8}C), 66.6 (^{5}C), 74.2 (^{7}C), 79.3 (^{6}C), 123.8 (^{3}C), 131.9 (^{1}C), 135.2 (^{2}C), 167.0 (^{4}C).

N^1,N^2-bis-[4-(2-methyl-1-piperidinyl)-2-butynyl]phthalamide **14**

Compound **14** was prepared according to the procedure listed for compounds **13–16**. The yield %, mp, and elemental analysis were shown in (Table 2). IR (KBr,Cm^{-1}): 3200–3150 (NH, stretch), 2900–2820 (ArH, stretch), 2220 (C≡C, stretch), 1670 (C=O, stretch, amide), 1620, 14560 (Ar, C=C, stretch), 1030, 948 (Ar, C=C, bending). ^{1}HNMR (DMSO-d_6): δ, 1.12 (d, 3H, $J = 4.2$ Hz, $^{12}CH_3$), 1.5 (m, 6H, $^{10}CH_2$–$^{10'}CH_2$–$^{11}CH_2$), 1.64(m, 2H, ^{9}CH), 1.64 (m,

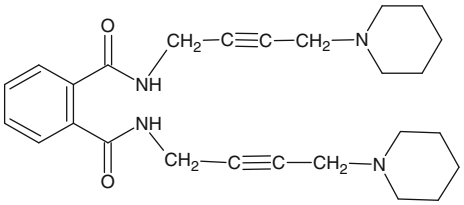
Table 1 Physical data for 4-*tert*-amino-2-butynylamines (**9–12**)

Cyclic amine	Comp. no.	R_2	R_2	*n**	Yield (%)	Formula	B.P. (°C) (mmHg)
Piperidine	**9**	H	H	1	78	$C_9H_{14}N_2$	75–77 (0.7)
2-Methyl-piperidine	**10**	CH_3	H	1	61	$C_{10}H_{18}N_2$	78–79 (0.7)
2,6-Dimethyl piperidine	**11**	CH_3	CH_3	1	43	$C_{11}H_{20}N_2$	79–81 (0.7)
Perhydro-Azocine	**12**	H	H	3	40	$C_{11}H_{20}N_2$	81–82 (0.7)

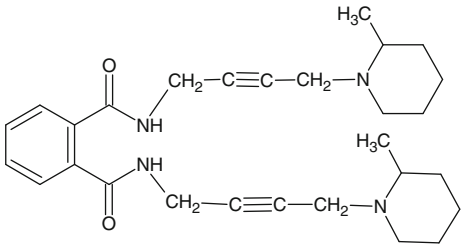
* *n* Number of carbon

Scheme 1 The synthesis N^1,N^2-bis-[4-(*t*-amino)-2-butynyl]phthalamides

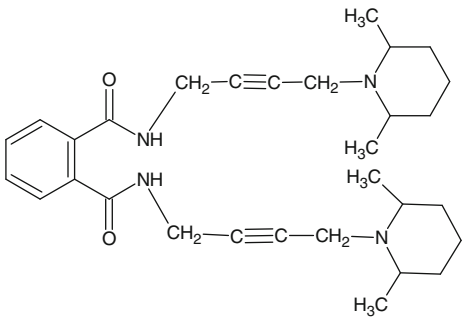
The synthesis *N*^{1},*N*^{2}-bis-[4-(t-amino)-2-butynyl]phthalamides



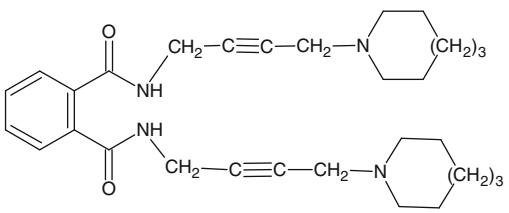
***N*^{1},*N*^{2}-bis-[4-(1-piperidinyl)-2-butynyl]phthalamide (13)**



***N*^{1},*N*^{2}-bis-[4-(2-methyl-1-piperidinyl)-2-butynyl]phthalamide (14)**



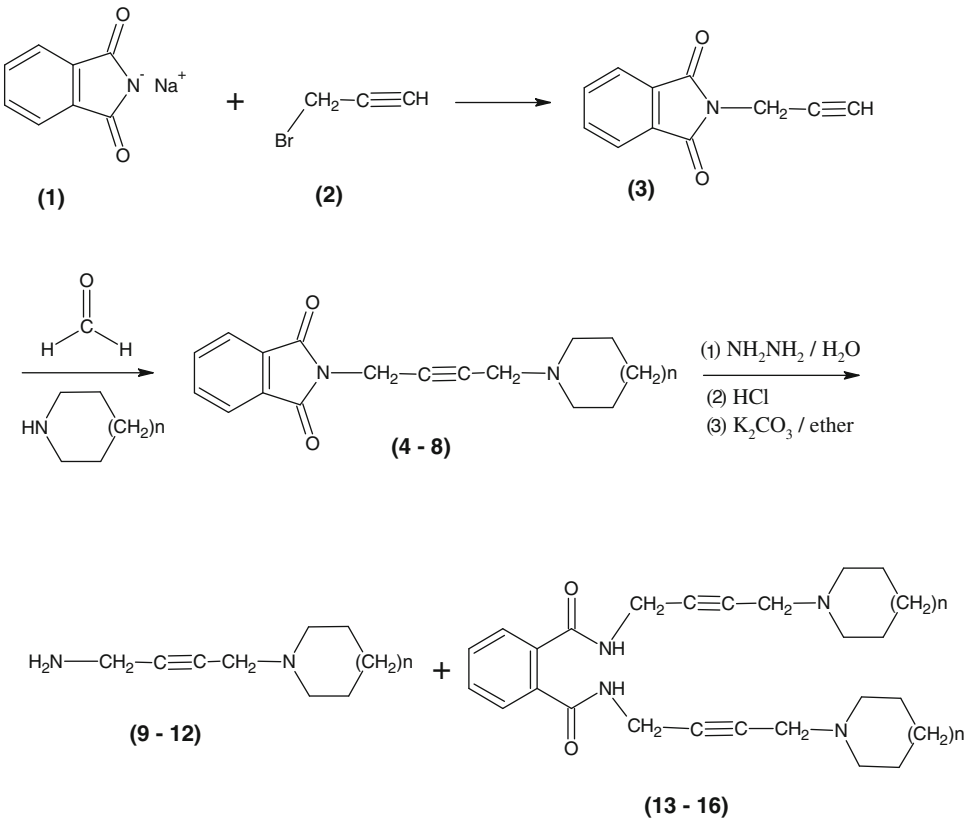
***N*^{1},*N*^{2}-bis-[4-(2,6-dimethyl-1-piperidinyl)-2-butynyl]phthalamide (15)**



***N*^{1},*N*^{2}-bis-[4-(perhydro-1-azapinyl)-2-butynyl]phthalamide (16)**

1H, $^{9'}$CH), 3.05 (t, $^8CH_2$, J = 2.4 Hz), 3.66 (t, 2H, J = 2.4 Hz, $^5CH_2$), 7.4 (d, 1H, J = 4.2 Hz, Ar^{3}H) 7.8 (d, 1H, J = 4.2 Hz, Ar^{1}H). ^{13}CNMR (DMSO-d_6): δ, 20.0 (^{12}C), 20 ($^{11'}$C), 24 (^{11}C), 26.2 ($^{10'}$C), 27.4 (^{10}C), 43.8($^{9'}$C), 52.9 (^{8}C), 54.5 (^{5}C), 78.2 (^{7}C), 79.5 (^{6}C), 123.8 (^{3}C), 131.9 (^{1}C), 135.2 (^{2}C), 167.2 (^{4}C).

Scheme 1 continued



Scheme of synthesis

where n= 1,3

Table 2 Physical data for N^1,N^2-bis-[4-(*t*-amino)-2-butynyl]phthalamides (**13**–**16**)

Cyclic amine	Comp. no.	R_1	R_2	*n**	Yield (%)	Formula	mp* (°C)	Calc (%)			Found (%)		
								C	H	N	C	H	N
Piperidine	13	H	H	1	14.0	$C_{26}H_{34}N_4O_2$	140–142	71.65	7.85	12.91	71.63	7.82	12.92
2-Methyl-Piperidine	14	CH_3	H	I	14.1	$C_{28}H_{38}N_4O_2$	151–152	72.31	8.22	12.12	72.30	8.23	12.15
2,6-Dimethyl Piperidine	15**	CH_3	CH_3	1	16.6	$C_{30}H_{42}N_4O_2$	158–160	73.64	8.57	11.42	73.66	8.60	11.43
Perhydro-azocine	16	H	H	3	14.7	$C_{30}H_{42}N_4O_2$	132–134	73.64	8.57	11.42	73.61	8.60	11.43

* All derivatives were recrystallized from methanol; Microanalyses were performed in the laboratories of Dr. Bernhardt, Mulheim, Germany

** The 2,6-dimethyl in **11** and **15** are cis(diequatorial)

N^1,N^2-bis-[4-(2,6-dimethyl-1-piperidinyl)-2-butynyl]phthalamide **15**

The title compound (**15**) was prepared according to the method describe for compounds **13**–**16**. The yield %, mp, and elemental analysis were described in (Table 2). IR (KBr,Cm^{-1}): 3180–3150 (NH, stretch, amide), 3030–29301 (ArH, stretch), 2250 (C≡C, stretch), 1660–1640 (C=O, stretch, amide), 1612, 14565, 1390 (Ar, C=C, stretch), 1100, 10485, 940 (Ar, C=C, bending). ^{1}HNMR (DMSO-d_6): δ, 1.14 (d, 3H, $J = 4.4$ Hz, $^{12}CH_3$), 1.14 (d, 3H, $J = 4.2$ Hz, $^{12'}CH_3$), 1.5 (m, 6H, $^{10}CH_2$–$^{11}CH_2$–$^{10'}CH_2$), 1.8 (m, 2H, $^{9'}$CH–$^{9'}$CH), 3.4 (t, 2H, $J = 2.4$ Hz, $^8CH_2$), 3.68 (t, 2H, $J = 2.4$ Hz, $^5CH_2$), 7.4 (d, 1H, $J = 4.2$ Hz, Ar^{3}H) 7.8 (d, 1H, $J = 4.2$ Hz, Ar^{1}H). ^{13}CNMR (DMSO-d_6): δ, 22.02 (^{12}C), 24.03 ($^{11'}$C), 25.8 ($^{10,10'}$C), 27.1 ($^{9,9'}$C), 52.9 ^{8}C), 66.6 (^{5}C), 74.2 (^{7}C), 79.3 (^{6}C), 128.8 (^{3}C), 131.9 (^{1}C), 135.2 (^{2}C), 167.0 (^{4}C).

N^1,N^2-bis-[4-(perhydro-1-azapinyl)-2-butynyl]phthalamide **16**

The title compound was synthesized according to the general procedure for the preparation of N^1,N^2-bis-[4-(*t*-amino)-2-butynyl]phthalamides. The yield %, mp, and elemental analysis were shown in (Table 2). IR (KBr,Cm^{-1}): 3210–2950 (NH, stretch), 2924, 2831 (Ar, C=C, stretch), 2220 (C ≡ C, stretch), 1662, 1640 (C=O, stretch, amide), 840, 794, 717 (Ar, C=C, stretch, bending). ^{1}HNMR (DMSO-d_6): δ, 1.2–1.4 (m, 10H, $^{10}CH_2$–$^{10'}CH_2$–$^{11}CH_2$–$^{12}CH_2$–$^{13}CH_2$), 1.6 (m, 4H, $^9CH_2$–$^{9'}CH_2$), 3.03 (t, 2H, $J = 2.4$ Hz, $^8CH_2$), 3.66 (t, 2H, $J = 2.4$ Hz, $^5CH_{2'}$), 7.4 (d, 1H, $J = 4.2$ Hz, Ar^3H), 7.8 (d, 1H, $J = 4.2$ Hz, Ar^1H). ^{13}CNMR (DMSO-d_6): δ, 26.8 (^{11}C,^{12}C,^{13}C), 27.4 ($^{10,10'}$C), 28.15 ($^{9,9'}$C), 47.7 (^{8}C), 54.7 (^{7}C), 78.5 (^{6}C), 80.8 (^{6}C), 123.8 (^{3}C), 131.9 (^{1}C), 135.1 (^{2}C), 167.2 (^{4}C).

Pharmacology

Male mice, averaging 20–25 g, were used throughout the experiments. Distilled water was used for reagent preparation; oxotremorine was purchased from Fluka AG, Chemische Fabrik, Switzerland.

Each test compound was administered intraperitoneally in geometrically spaced doses to groups of five mice in volume not exceeding 10 ml/kg. After 15–20 min oxotremorine was injected intraperitoneally in a dose of 40 0μg/kg. 15–20 min later the intensity of the motor effects was graded visually by a three point system previously described (Eicholzer and Ogren, 1977).

The tremorolytic dose was estimated by visual interpolation as the dose in mol/kg., which reduced the mean tremor response by one point relative to a control group receiving oxotremorine only.

Guinea pigs of either sex, weighing 350–550 g were sacrificed by a sharp blow on the head.

Terminal portions (2- to 3-cm long) of the ileum were removed in close proximity to the ileo-cecal junction, and cleared of their contents by gently flushing with warm Krebs solution. Two of such segments were mounted in separate 50 ml jacketed muscle chambers, containing Krebs solution at 37 °C and bubbled with a mixture of oxygen (95 %) and CO_2 (5 %). After mounting, the tissue was allowed to stabilize for 1 h prior to the addition of test compounds. Cumulative dose response curves were obtained using acetylcholine only.

The test compound was added after 15-min intervals at the lowest concentration and then after another 15 min, a dose response curve of acetylcholine was obtained in the presence of test compound. This procedure was repeated after at least three concentrations of the test compound increasing the ratio 1/3, 1/10, 1/30. All compounds were dissolved in 0.1 N HCl on the same day of the biological evaluation. Contractions of the ileum were recorded on a physiograph using an isotonic lever exerting I gm tension. From the results obtained, a graphic estimate was made of the concentrations. For non-competitive antagonist, the effective concentration was recorded as that which reduces the maximum response to acetylcholine by 50 % and the PD_2 value which is defined as the non-competitive antagonist which reduces the maximum response to one half was estimated (Arunlanksana and Schild, 1959).

$$PD_2 = -\log B_{50}$$

Results and discussion

The Mannich reaction of *N*-propargylphthalimide **4** with paraformaldehyde and various selected cyclic amines in peroxide-free dioxan in the presence of catalytic amount of cuprous chloride yielded 65-86 % of *N*-[4-(*t*-amino)-2-butynyl]phthalimides **5**–**8**. Reaction of compounds **5**–**8** with hydrazine hydrate followed by the addition of hydrochloric acid yielded two products, the hydrochloride salts of 4-*tert*-amino-2-butynylamines. The free base **9**–**12** (Table 1) was generated through treatment with potassium carbonate (ether) in 40-60 % yield. The second products were the N^1,N^2-bis-[4-(*t*-amino)-2-butynyl]phthalamides **13**–**16** (Table 2). The elemental analysis, IR, ^{H1}NMR and ^{13}CNMR were consistent with assigned structures as shown in the experimental part.

The new N^1,N^2-bis-[4-(*t*-amino)-2-butynyl]phthalamides (Table 3) were tested for their blocking action on the motor effects of oxotremorine in intact mice and for their antagonistic activity towards acetylcholine on isolated guinea pig ileal preparations. The methods used were described in the experimental part. The pharmacological results are summarized in (Table 3) and include atropine as a reference compound. Three of the aminoacetylenic phthalamide derivatives **13**, **14**, **16** antagonize the tremorgenic effects of oxotremorine, they were more potent than atropine in their central effects. Compound **15** in which the cyclic amine is 2,6-dimethylpiperidine was inactive as oxotremorine antagonist.

These observation were in agreement with our previous finding with the inactivity of *N*-[4-(2,6-dimethylpiperidino)-2-butynyl]phthalimides **7** (Table 4). The inactivity may be attributed to the steric factors around the basic nitrogen in phthalimide and phthalamide derivatives. Furthermore, the aminoacetylenic phthalamides were more potent than the aminoacetylenic phthalimides as oxotremorine antagonist, this may be attributed to either bis-aminoacetylenic functional groups or due to the N^1,N^2-bis-aminoacetylenic moiety are appropriately located in phthalamide to block oxotremorine activity. All compounds listed in (Table 3)

Table 3 Pharmacological data for N^1,N^2-bis-[4-(*t*-amino)-2-butynyl]phthalamides (**13–16**)

Compound no.	In vitro dose (mol/kg) in mice required to produce oxotremorine blockade[a]	Concentration (mol/L) to antagonize acetylcholine on isolated guinea pig ileum[b]	Acetylcholine antagonism on isolated guinea pig ileum (PD_2)[c]
13	8.0×10^{-6}	1.6×10^{-6}	5.6
14	4.2×10^{-7}	1.2×10^{-6}	5.7
15	Inactive	3.0×10^{-6}	5.8
16	3.2×10^{-8}	5.0×10^{-6}	5.9
Atropine	2.8×10^{-6}	3.7×10^{-9}	8.4

[a] Dose of the test compound required to block the dose of oxotremorine (400 μg/kg) inducing a predetermined tremor in mice

[b] Concentration of the test compound required to reduce the maximum response to acetylcholine by 50 %

[c] The value of the antagonist, which reduces the maximum response of acetylcholine to one half

Table 4 Pharmacological data for compounds *N*-[4-(*t*-amino)-2-butynyl]pthalimides (**5–8**)

Compound* no.	In vivo dose (mol/kg) in mice required to produce oxotremorine blockade	Concentration (mol/L) to antagonize acetylcholine on isolated guinea pig ileum	Acetylcholine antagonism on isolated guinea pig ileum (PD_2)
5	7.6×10^{-4}	1.6×10^{-6}	5.8
6	9.6×10^{-6}	1.2×10^{-6}	5.9
7	Inactive	2.0×10^{-6}	5.7
8	2.4×10^{-6}	8.0×10^{-7}	6.1
Atropine	2.8×10^{-6}	3.7×10^{-9}	8.4

* As reported in the study of Cho *et al.*, 1962

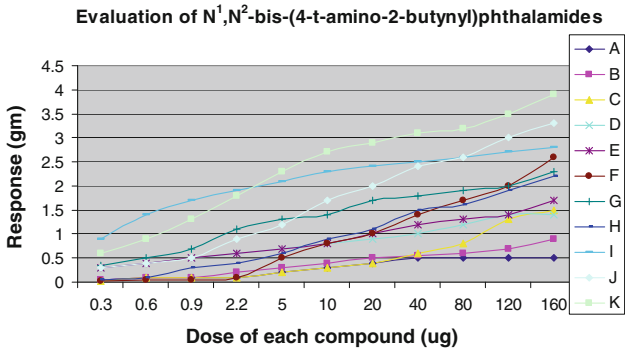


Fig. 1 Effect of compounds 13-16 and atropine on acetylcholine dose–response curves in guinea pig ileum *A* Mean control in the presence of 2 μg of compound **13**. *B* Mean control in the presence of 6 μg of compound **13**. *C* Mean control in the presence of 20 μg of compound **13**. *D* Mean control in the presence of 2 μg of compound **14**. *E* Mean control in the presence of 6 μg of compound **14**. *F* Mean control in the presence of 20 μg of compound **14**. *G* Mean control in the presence of 6 μg of compound **15**. *H* Mean control in the presence of 20 μg of compound **15**. *I* Mean control in the presence of 20 μg of compound **16**. *J* Mean control in the presence of 20 μg of atropine. *K* Mean control in the presence of 60 μg of atropine

possessed a competitive antagonistic effect to acetylcholine at low concentration but behaved as non-competitive antagonists at higher concentration. Compounds **13–16** were less active than atropine as peripheral cholinergic antagonists, this in line with our objective to have specific central antagonistic effect to oxotremorine over peripheral cholinergic effects. The dose response curves obtained on isolated guinea pig ileal preparations of compounds **13–16** and atropine are shown in Fig. 1. As the concentration of the compounds is increased, the curve is shifted to the right and at the highest concentration used a reduction in the maximum response was observed, the negative logarithm of the molar concentration of the antagonist that caused 50 % reduction of the maximum response due to agonist is estimated (Table 3). It is evidence from (Table 3) that introduction of one methyl group around the basic nitrogen in compound **14** or increase the size of the cyclic amine to azocine in **16** resulted in an increase in the tremorolytic and peripheral antagonistic activity to acetylcholine, may indicate that the higher hydrophobic factors in the absence of steric factors around the basic nitrogen may enhance the tremolytic and peripheral acetylcholine antagonistic activity. Further work is necessary to confirm the various points suggested in this article.

Conclusion

In conclusion, the new series of bis-aminoacetylenic phthalamides showed more potent selective central oxotremorine antagonistic activity and lower peripheral

acetylcholine activity than atropine. Further investigation in progress to find out more selective compounds to be more effective in treatment Parkinson's disease.

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