FABAD J. Pharm. Sci., 18, 57-61, 1993

Some S-Aminocarbonylmethyl N-Substituted Dithiocarbamate Derivatives with Antispasmodic Activities in Isolated Rat and Rabbit Ileum

Erhan PALASKA*, Cihat ŞAFAK*, Hakkı ERDOĞAN*, Kevser EROL**, R. Serdar ALPAN**

Summary : Sixteen new dithiocarbamate derivatives were synthesized by the reaction of potassium salt of appropriate dithiocarbamate and N-chloroacetylpyrrolidine and/or N-chloroacetylmorpholine in this paper. Their structures were elucidated by UV, IR, ^{1}H-NMR spectra and elemental analysis. The compounds were investigated for their activities on the acetylcholine -and histamine- induced contractions by the 10^{-4} M concentration with percentage inhibitions ranging from 38-70 %. Also the compounds 4 and 16 are more active than the others in terms of their inhibitory activities on the isolated rat and rabbit ileum.

Received : 5.1.1993
Accepted : 2.4.1993

Keywords : Dithiocarbamates, synthesis, acetylcholine and histamine, rat ileum, rabbit ileum, smooth muscle.

Bazı S - Aminokarbonilmetil N-Sübstitüe Ditiyokarbamat Türevleri, İzole Sıçan ve Tavşan İleumu Üzerindeki Antispazmodik Aktiviteleri

Özet : Bu çalışmada, 16 yeni ditiyokarbamat türevi bileşik uygun ditiyokarbamat potasyum tuzları ile N-kloroasetilpirolidin ve/veya N-kloroasetilmorfolinin reaksiyonuyla elde edilmişlerdir. Bileşiklerin yapıları UV, IR, ^{1}H-NMR ve elementer analizleri ile aydınlatılmıştır. Sentezi yapılan bileşiklerin asetilkolin ve histamin ile oluşturulan sıçan ve tavşan ileumu kasılmaları üzerine etkileri araştırılmıştır. Bileşikler histamin ile oluşturulan kasılmalara karşı 10^{-4} M konsantrasyonda % 38-70 arasında ümitvar bir etkinlik göstermekte, ayrıca elde edilen sonuçlar, bileşik 4 ve 16'nın izole sıçan ve tavşan ileumundaki etkileri bakımından diğerlerine göre daha aktif olduğunu göstermiştir.

Anahtar sözcükler : Ditiyokarbamatlar, sentez, asetilkolin ve histamin, sıçan ileumu, tavşan ileumu, düz kas.

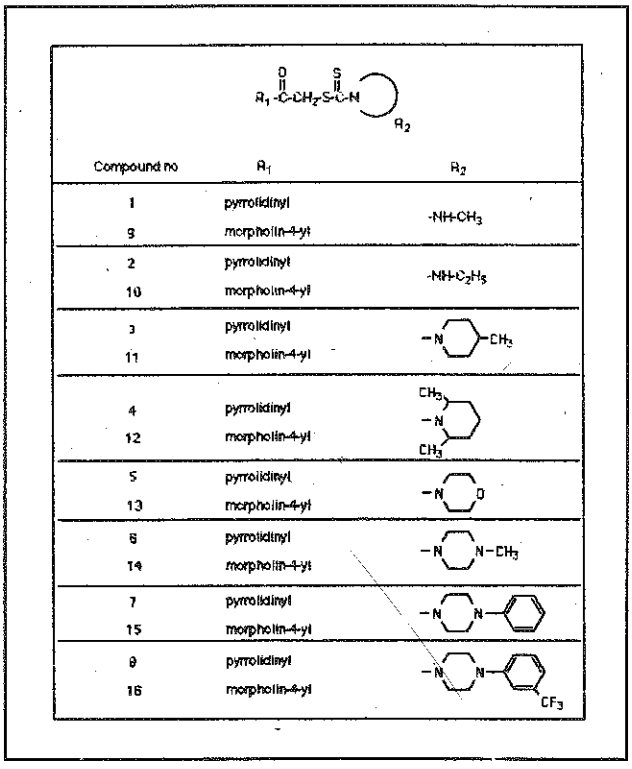
Introduction

It is well known that, dithiocarbamate derivatives have pharmacological activities such as antimicrobial[1-9], antiparkinson[10], anticholinergic and antihistaminic[11-14]. In our previous studies, we synthesized some substituted dithiocarbamate derivatives and investigated their antimicrobial, anticholinergic and antihistaminic activity[7-9,11-14]. In this study, we aimed to synthesize some new S-pyrrolidinyl and S-morpholinyl esters of N-mono- and/or N,N-disubstituted dithiocarbamate derivatives and investigate for their activities on the contractile responses of rat and rabbit ileum to acetylcholine and histamine.

The synthesized compounds are shown below.

(*) Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hacettepe University, 06100 Ankara, Turkey.

(**) Department of Pharmacology, Faculty of Medicine, Anadolu University, Eskişehir, Turkey.



Material and Methods

Chemistry

All chemicals were supplied from Aldrich (Steinheim, Germany). Melting points were determined with a Thomas Hoover Capillary melting point apparatus and were uncorrected. UV spectra were recorded using Shimadzu UV-160A UV-Visible Recording Spectrophotometer (10^{-5}M, methanol). IR spectra were measured on a Perkin Elmer 457 Infrared Spectrophotemeter. ^{1}H-NMR spectra were recorded on a Bruker Ac 80 MHz spectrometer using tetramethylsilane as internal standard (DMSO-d_6). All chemical shifts were reported as δ (ppm) vales. Microanalyses were performed by TÜBİTAK Research Institute (Gebze, Turkey) and within $\pm$ 0.4 % of calculated values. The purity of the compounds were checked by thin layer chromatography on silica gel HF 254+366 nm (Merck).

N - Chloroacetylpyrrolidine and/or morpholine

A solution of 0.2 M pyrrolidine and/or morpholine in 100 ml dry ether was treated dropwise at -5°C with stirring with 0.1 M chloroacetyl chloride in 100 ml ether. After the mixture was stirred 3 h. at 20°C pyrrolidin and/or morpholin hydrochloride filtered off, and the filtrate distillated to obtain N-chloroacetylpyrrolidine and/or morpholine.

S - aminocarbonylmethyl N-substituted dithiocarbamates

A mixture of potassium salt of appropriate dithiocarbamate (0.01 M) and appropriate acyl derivative (0.01 M) was refluxed in 20 ml acetone for 3 h. The mixture was poured into crushed ice. The resulting product was filtered, washed succesively with water and dried to give crude product which was recrystallized from cyclohexane-benzene. Empiric formula, molecular weight, melting point and percentage yield of the compounds were given in Table 1.

Table 1. Empiric formula, molecular weight, melting point and percentage yield of the compounds

Compound	Empiric formula	M.w.	m.p. (°C)	Yield %
1	$C_8H_{14}N_2OS_2$	218.3	128	58
2	$C_9H_{16}N_2OS_2$	232.3	88	62
3	$C_{13}H_{22}N_2OS_2$	286.4	96	76
4	$C_{14}H_{24}N_2OS_2$	300.5	90	67
5	$C_{11}H_{18}N_2O_2S_2$	274.4	120	74
6	$C_{12}H_{21}N_3OS_2$	287.4	112	86
7	$C_{17}H_{23}N_3OS_2$	349.5	132	80
8	$C_{18}H_{22}F_3N_3OS_2$	417.5	149	92
9	$C_8H_{14}N_2O_2S_2$	234.3	70	65
10	$C_9H_{16}N_2O_2S_2$	248.3	64	70
11	$C_{13}H_{22}N_2O_2S_2$	302.5	71	63
12	$C_{14}H_{24}N_2O_2S_2$	316.5	127	84
13	$C_{11}H_{18}N_2O_3S_2$	290.4	114	77
14	$C_{12}H_{21}N_3O_2S_2$	303.4	119	79
15	$C_{17}H_{23}N_3O_2S_2$	365.5	202	88
16	$C_{18}H_{22}F_3N_3O_2S_2$	433.5	134	94

Pharmacology

Albino rats of either sex, weighing 200-250 g, for the analysis of anticholinergic activity and albino rabbits (New Zeland) weighing 1.5-2.0 kg for the analysis of antihistaminic activity were used in the present study. Animals used for the test were fasted overnight. After animals were sacrificed by cervical dislocation, the ileum (10-15 cm of terminal portion) was immediately removed, discarding

the 5-8 segment proximal to the ileo-caecal junction. Segments of 1.5-2 cm long were mounted vertically in a 10 ml organ bath containing Krebs-Henseleit solution of the following composition (mM); NaCl: 116, KCl: 5.9, $CaCl_2$: 2.5, $MgSO_4$: 1.2, NaH_2PO_4: 1.2, $NaHCO_3$: 25.5, glucose: 11. The bath contents were maintained at 37°C and aerated by 95 % O_2 and 5 % CO_2.

A tension of 2 g was applied and isometric recording was made using an isometric transducer (TB - 651T) coupled to a Nikon-Kohden Recorder system. The preparations were allowed to equilibrate for at least 60 min. with regular washes every 15 min. Then rat ileum contracted by acetylcholine (Ach) (10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}M) and rabbit ileum was contracted by histamine (10^{-5}, 10^{-4}, 10^{-3}M) with a contact time of 1 min. Exposure to each Ach and histamine concentration was followed by three consecutive washes and 15 min. rest before the next administration. Maximum contractions were elicited by the concentration of 10^{-3}M Ach and 10^{-4}M histamine. The compounds tested were used as antagonists at 10^{-4}M concentration. All compounds were diluted in dimethylsulfoxide and added to the bath fluid in volumes of 0.1 ml. No change in colour and precipitation was observed during bioassay.

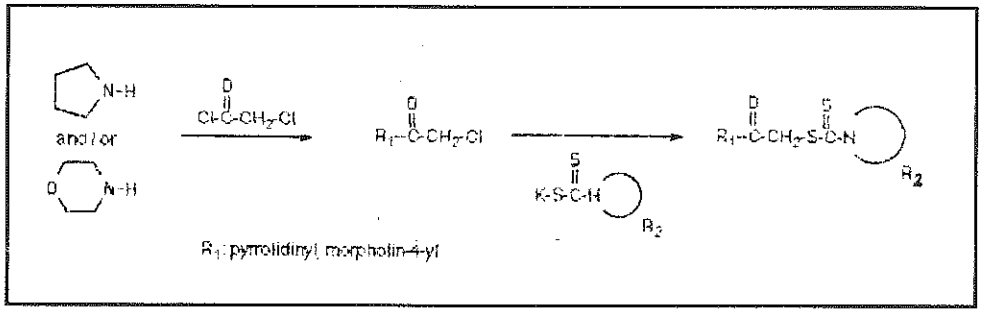
The preparations were incubated with each compounds (10^{-4}M) for 5 min. and then the responses to Ach (10^{-4}M) or histamine (10^{-4}M) were recorded.

The data were expressed on means ± S. E. mean. n refers to the number of experiments. Statistical evaluation was made by Student's t-test; p values less than 0.01 were considered to be statistically significant. The responses to Ach and antagonists were compared with those to atropine (10^{-4}M) and the responses of histamine and antagonists were compared with (those responses) to dimenhydrinate.

Results and Discussion

Some physical and spectral properties of the compounds are given in Tables 1 and 2.

S-aminocarbonylmethyl N-substituted dithiocarbamates were synthesized by refluxing N-chloroacetylpyrrolidine and/or N-chloroacetylmorpholine, prepared by the reaction of chloroacetylchloride and pyrrolidine and/or morpholine, with N-substituted dithiocarbamate derivatives in acetone (Scheme 1).



Scheme 1: Synthesis of the compounds.

Potassium salts of N-subtituted dithiocarbamate derivatives were synthesized by the reaction of appropriate amines with carbon disulfide and potassium hydroxide.

All spectral data are in accordance with assumed structures. UV spectra have three absorption bands at about 220, 250 and 280 nm. The IR spectra of the compounds showed C = 0 and C = S stretching bands at 1680-1665 and 1260-1230 cm^{-1} respectively. In ^{1}H-NMR spectra, -CH_2-S- protons appeared at 4.80- 4.92 ppm. All protons belonging to aliphatic and cyclic amine moiety of the compounds showed the expected chemical shift and integral values.

The pharmacological activity data of the compounds are seen in Table 3.

Table 2. Spectral and Elemental Analysis of the Compounds.

Comp. No	^{1}H-NMR (DMSO-d_6) δ(ppm)	IR (KBr) (cm-1)		Calc.	Found
1	1.68 (4H, m, pyr*H_3, H_4), 3.45 (3H, d, CH_3NH-), 3.63-4.07 (4H, m, pyr H_2, H_5), 4.85 (2H, s, -CH_2-S-)	3280 (N-H) 1670 (C=0) 1250 (C=S)	C : H : N :	44.01 6.46 12.83	44.42 6.38 12.76
2.	1.36 (3H, d, CH_3CH_2NH-), 1.70 (4H, m, pyr H_3, H_4), 3.60-4.10 (6H, m, pyr H_2, H_5, CH_3CH_2NH-), 4.80 (2H, s, -CH_2-S-)	3300 (N-H) 1665 (C=0) 1260 (C=S)		46.52 6.94 12.06	46.78 6.90 11.85
3.	1.42 (3H, d, CH_3-), 1.70-1.95 (9H, m, pyrH_3, H_4, pip H_3-H_5), 3.65-4.10 (8H, m, pyr H_2, H_5, pip H_2, H_6), 4.85 (2H, s, -CH_2-S-)	1680 (C=0) 1235 (C=S)		54.51 7.74 9.78	54.39 7.66 9.92
4.	1.46 (16H, m, $(CH_3)_2$, pyr H_3, H_4, pip H_3-H_5), 3.62-4.05 (4H, pyr H_2, H6), 4.45 (1H, q, pip H_2), 4.90 (2H, s, -CH_2-S-), 5.60 (1H, q, pip H_6)	1675 (C=0) 1230 (C=S)		55.96 8.05 9.32	56.03 8.08 9.22
5.	1.67 (4H, m, pyr H_3, H_4), 3.62-4.56 (12 H, m, morpholine protons, pyr H_2, H_5), 4.85 (2H, s, -CH_2-S-)	1675 (C=0) 1230 (C=S)		48.15 6.61 10.21	48.07 6.41 10.39
6.	1.70 (4H, m, pyr H_3, H_4), 2.76 (3H, s, N-CH_3), 3.60-4.55 (12H, m, pyr H_2, H_5, piperazine protons), 4.86 (2H, s, -CH_2-S-)	1680 (C=0) 1235 (C=S)		50.14 7.36 14.62	50.34 7.32 14.57
7.	1.68 (4H, pyr H_3, H_4), 3.62-4.50 (12 H, m, pyr H_2, H_5, piperazine protons), 4.88 (2H, s, -CH_2-S-), 6.95-7.80 (5H, m, aromatic protons)	1675 (C=0 1235 (c=0)		58.42 6.63 12.02	58.66 6.49 11.89
8.	1.70 (4H, m, pyr H_3, H_4), 3.58-4.56 (12 H, m, pyr H_2, H_5,piperazine protons), 4.85 (2H, s, -CH_2-S-), 6.90-7.75 (4H, m, aromatic protons)	1675 (C=0) 1240 (C=S)		51.78 5.31 10.06	51.76 5.42 10.12
9.	3.43 (3H, d, CH_3NH-), 3.75 (4H, t, mor H_3, H_5), 3.82-4.58 (4H, broad d, morpholine H_2, H_6), 4.85 (2H, s, -CH_2-S-)	3285 (N-H) 1675 (C=0) 1240 (C=S)		41.00 6.02 11.95	40.93 6.16 11.96
10.	1.38 (3H, d, CH_3CH_2NH-), 3.65-4.60 (10H, m, CH3, CH_2NH-, morpholine protons), 4.85 (2H, s, -CH_2-S-)	3290 (N-H) 1670 (C=0) 1260 (C=S)		43.52 6.49 11.28	43.55 6.61 11.31
11.	1.42 (3H, d, CH_3-), 1.98 (5H, t, pip H_3, H_5), 3.78-4.60 (12H, pip H_2, H_6, morpholine protons), 4.85 (2H, s, -CH_2-S-)	1675 (C=0) 1230 (C=S)		51.63 7.33 9.26	51.46 7.27 9.33
12.	1.46-2.03 (12H, m, $(CH_3)_2$, pip H_3-H_5), 3.76-4.55 (9H, m, pip H_2, morpholine protons), 4.92 (2H, s, -CH_2-S-), 5.65 (1H, q, pip H_6)	1675 (C=0) 1235 (C=S)		53.13 7.64 8.85	53.31 7.72 8.86
13.	3.74 - 4.56 (16H, m, morpholine protons), 4.86 (2H, s, -CH_2-S-)	1680 (C=0) 1240 (C=S)		45.50 6.25 9.65	45.49 6.32 9.78
14.	2.75 (3H, s, N-CH_3), 3.62-4.58 (16H, m, piperazine and morpholine protons), 4.88 (2H, s, -CH_2-S-)	1685 (C=0) 1235 (C=S)		47.50 6.98 13.85	47.34 7.03 13.68
15.	3.60 - 4.55 (16H, m, piperazine and morpholine protons), 4.86 (2H, s, -CH_2-S-), 6.90-7.80 (5H, m, aromatic protons)	1680 (C=0) 1230 (C=S)		55.86 6.34 11.50	55.58 6.49 11.37
16.	3.55 - 4.53(16H, m, piperazine and morpholine protons), 4.85 (2H, s, -CH_2-S-), 6.95-7.80 (4H, m, aromatic protons)	1675 (C=0) 1235 (C=S)		49.87 5.12 9.69	50.02 5.14 9.73

(*) pyr. : pyrrolidine, pip.: piperidine, s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet.

The antispasmodic activities of the compounds were tested on rat ileum and rabit ileum using atropine sulfate and dimenhydrinate as test references. As can be seen in Table 3, among the compounds tested, compounds 4 and 16 may have potent antispasmodic activities. The compounds carrying open chain amine portion in their structures (compounds 1, 2, 9 and 10) showed lower activity against acetylchloline-induced contractions than the compounds having cyclic amine portion. Pyrrolidinyl derivatives (compounds 1-8) showed higher activity against acetylcholine-induced contractions than their morpholinyl analogs except compound 16. It can be said that all the compounds have been more potent antispasmodic derivatives against histamine-induced contractions compared with their acetylcholine-induced contractions.

Table 3. The inhibition of maximum contractions (n=7)

Compound (10^{-4} M)	% inhibition	
	Ach (10^{-4} M)	Histamine (10^{-4} M)
1	31.6 ± 5.5	57.7 ± 9.0
2	19.5 ± 4.9	67.7 ± 6.1
3	41.5 ± 9.9	58.1 ± 7.9
4	55.0 ± 9.8	70.8 ± 6.4
5	43.9 ± 7.7	37.9 ± 8.6
6	22.2 ± 3.3	63.3 ± 3.5
7	45.7 ± 7.6	49.8 ± 6.3
8	45.3 ± 2.4	49.5 ± 4.5
9	12.1 ± 3.8	41.8 ± 8.2
10	8.9 ± 2.8	59.5 ± 9.6
11	11.3 ± 3.2	48.8 ± 6.5
12	36.1 ± 8.0	47.1 ± 5.5
13	22.2 ± 2.1	62.5 ± 7.5
14	29.8 ± 5.8	49.4 ± 6.5
15	36.5 ± 4.8	69.1 ± 5.7
16	56.8 ± 10.9	61.7 ± 6.1
Atropine sulfate	100.0	—
Dimenhydrinate	—	100.0

p values < 0.01

References

1. Hidaka, H., Matsumato, I., Nakagawa, K., Horiuchi, K., "Pyrrole Dithiocarbamates", Japan Kokai 73 99, 160 (C1. 16 E331), 15 Dec 1973; ref. C. A. 80, 95723b, 1974.
2. Chabrio, P., Maillard, G., Quevauviller, A., "Relation Between Chemical Structure and Antibacterial and Antifungal Activity of Esters of N-Substituted Dithiocarbamic Acid", *Ann. Pharm. Franc.* 14, 720-8, 1956.
3. Gupta, S. P., Garg, D. M. L., "Potential Fungicidal Compounds II. Some Bis (Halonitrophenyl) and (-Naphthyl) Esters of Piperazino bis (Dithiocarbamic Acid)", *J. Indian Chem. Soc.* 42 (6), 412-4, 1965.
4. Zsolnai, T., "Die Antimikrobielle Wirkung von Potentiellen Isothiocyanate-Bildern 4.", *Arzneim. Forsch./Drug Res.* 18, 1319-21, 1968.
5. Zsolnai, T., "Die Antimikrobielle Wirkung von Thiocyanaten Isothiocyanaten und Potentiellen Isothiocyanat Bildern", *Arzneim. Forsch./Drug Res.* 21, 121-7, 1971.
6. Kumar, B. V., Reddy, V. M., "Synthesis and Biological Activities of Some New S-(Benzimidazole-2-ylmethyl) N-Substituteddithiocarbamates and N1-Substituted N4- (Benzimidazole-2-ylmethyl) Sulfanilamides", *Indian J. Chem.* 24B, 1298-1301, 1985.
7. Şafak, C., Erdoğan, H., Ertan, M., Yuluğ, N., "The Synthesis of Some Substituted Carbamodithioic Acid Esters and Their Antimicrobial Activities", *J. Chem. Soc Pak.*, 12 (4), 296-301, 1990.
8. Şafak, C., Erdoğan, H., Palaska, E., Saraç, S., Yuluğ, N., "Synthesis and Antimicrobial Activities of Some 3-Methyl-6-[2-(N, N-disubstituted thiocarbonylthio) propionyl]-2-benzoxazolones, *FABAD J. Pharm. Sci.*, 16, 81-7, 1991.
9. Erdoğan, H., Şafak, C., Balkan A., Palaska, E., Yuluğ, N., "Studies on Some S- (3-Methylbenzoxazolone-6-yl) Acetyl/Propionyl 4-Substituted Piperazinocarbamodithioic Acid Derivatives", *H. Ü. J. Fac. Pharm*, 11 (1), 13-20, 1991.
10. Pandey, V. K., Lohani, H. C., "Synthesis of Possible Antiparkinsonism Compounds", Indian Chem. J., 33-5, 1980.
11. Şafak, C., Erdoğan, H., Ertan, M., Sunal, R., "Synthesis of Some Carbamodithioic Acid Esters and Their Anticholinergic Properties", *Arch. Pharm. (Weinheim)*, 321, 859-61, 1988.
12. Erdoğan, H., Şafak, C., Palaska, E., Ertan, M., Sunal, R., "Some New Carbamodithioic Acid Esters", *Arch. Pharm. (Weinheim)*, 321, 945-8, 1988.
13. Şafak, C., Erdoğan, H., Yeşilada, A., Erol, K., Cimgi, I., "Synthesis and Pharmacology of Some New Carbamodithioic Acid Esters", *Arzneim. Forsch./Drug Res.*, 42 (I), 123-6, 1992.
14. Palaska, E., Saraç, S., Şafak, C., Erdoğan, H., Erol, K., Alpan, R. S., "Studies on Some 10-[2-(N, N-Disubstituted thiocarbamoylthio)-acetyl] phenothiazine Derivatives", *Arzneim. Forsch./Drug Res.* 42 (II), 1453-5, 1992.