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RESEARCH ARTICLE

Synthesis of Some New N,N-Disubstituted Dithiocarbamic Acid 2-(6-Arylhexahydropyrimidine-2,4-dion-3-yl)ethyl Esters and In Vitro Evaluation of Antimicrobial Activities

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Synthesis of Some New N, N-Disubstituted Dithiocarbamic Acid 2-(6-Arylhexahydropyrimidine-2,4-dion-3-yl)ethyl Esters and In Vitro Evaluation of Antimicrobial Activities

Summary

*In this study, a number of novel dithiocarbamate derivatives were synthesized for the evaluation of their antimicrobial activities. These compounds were originally prepared by the reaction of potassium salts of N,N-disubstituted dithiocarbamic acids with 3-(2-chloroethyl)-6-arylhexahydropyrimidine-2,4-diones. The structures of the synthesized compounds were confirmed by the spectral data (IR, ^{1}H-NMR) and elemental analysis. The antimicrobial activities of all of the compounds were investigated by microdilution broth method using two Gram-positive (Staphylococcus aureus ATCC 29213, Enterococcus faecalis ATCC 29212) and two Gram-negative (Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853) bacteria and three fungi (Candida albicans ATCC 90028, Candida krusei ATCC6258, Candida parapsilosis ATCC 22019). Among the compounds tested 2-[6-(3-chlorophenyl)hexahydropyrimidine-2,4-dione-3-yl]ethyl morpholine-4-carbodithioate (**compound 7c**) showed the most favorable antibacterial activity (MIC: 16 μg/mL).*

Key Words : *Hexahydropyrimidine-2,4-diones, 6-arylhexahydropyrimidine-2,4-diones, N,N-disubstituted carbamodithioic acid 3-ethyl-6-arylhexahydropyrimidine-2,4-dione esters, carbamodithioic acid esters, synthesis, antimicrobial activity, antibacterial activity, antifungal activity.*

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Bazı Yeni N,N-Disübstitüe Ditiyokarbamik asit 2-(6-Arilhekzahidropirimidin-2,4-dion-3-il)etil Esterleri ve In Vitro Antimikrobiyal Aktivitelerinin Değerlendirilmesi

Özet

*Bu çalışmada, antimikrobial aktivitenin değerlendirilmesi için bir grup yeni ditiyokarbamat türevleri sentezlenmiştir. Bu bileşikler, 3-(2-kloroetil)-6-arilhekzahidropirimidin-2,4-dionlar ile N,N-disübstitüe ditiyokarbamatların potasyum tuzlarının reaksiyonu sonucu hazırlanmıştır. Sentezlenen bileşiklerin yapıları IR, ^{1}H-NMR ve elementel analiz ile aydınlatılmıştır. Bileşiklerin antimikrobiyal aktiviteleri ise ikisi Gram-pozitif (Staphylococcus aureus ATCC 29213, Enterococcus faecalis ATCC 29212) ve ikisi Gram-negatif (Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853) olmak üzere dört çeşit bakteri ve üç mantar (Candida albicans ATCC 90028, Candida krusei ATCC 6258, Candida parapsilosis ATCC 22019) kullanılarak mikrodilüsyon yöntemi ile değerlendirilmiştir. Deneye alınan bileşikler içerisinde; 2-[6-(3-klorofenil)hekzahidropirimidin-2,4-dion-3-il]etil morfolin-4-karboditiyoat (**bileşik 7c**) en yüksek antibakteriyel aktiviteyi (MİK: 16 μg/mL) göstermiştir.*

Anahtar kelimeler : *Hekzahidropirimidin-2,4-dionlar, 6-arilhekzahidropidimidin-2,4-dionlar, N,N-disübstitüe karbamoditiyoik asit 3-etil-6-arilhekzahidropirimidin-2,4-dion esterleri, karbamoditiyoik asit esterleri, sentez, antimikrobiyal aktivite, antibakteriyal aktivite, antifungal aktivite.*

INTRODUCTION

The targeting compounds have both dithiocarbamate and pyrimidine structure as can be seen in **Scheme 1**. In the literature, pyrimidine derivatives have been shown to possess a broad spectrum of biological activities such as diuretic[1,2], antihypertensive[1,2], antiinflammatory[1], anticancer[3,4], antiepileptic[5,6] and antimicrobial[2,7-9]. On the other hand, numerous studies regarding dithiocarbamate derivatives, have demonstrated that these compounds have potential anticholinergic[10-12], tuberculostatic[13], herbicidal[14]

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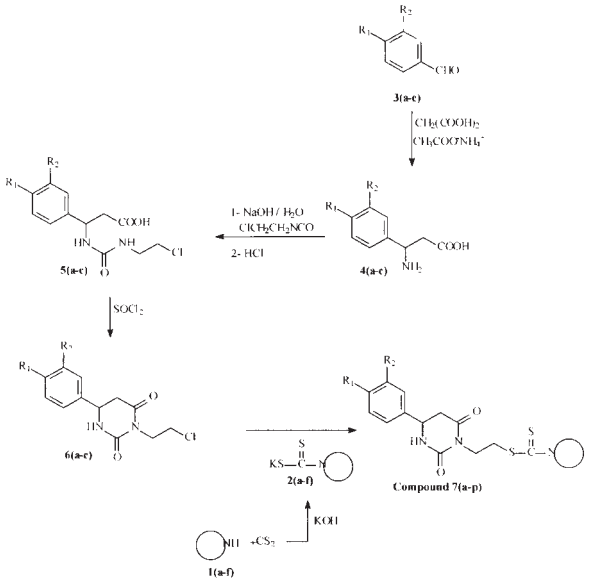


For 1 and 2 : **a :** morpholine, **b :** pyrrolidine, **c :** piperidine, **d :** N-phenylpiperazine, **e :** 4-methylpiperidine, **f :** 3-methylpiperidine
For 3, 4, 5 and 6 : **a :** benzaldehyde, **b :** 4-chlorobenzaldehyde, **c :** 3-chlorobenzaldehyde

Scheme 1: The synthesis pathway of the tested compounds.

and antimicrobial[15-19] activity. In our previous studies, we synthesized some new hexahydropyrimidine-2,4-dione and dithiocarbamate derivatives and investigated their antimicrobial activities[9,20]. These previous studies led us to synthesize several derivatives of 3-substituted-6-arylhexahydropyrimidine-2,4-dione bearing a dithiocarbamate functional group at position 3 of hexahydropyrimidine-2,4-dione ring, **compounds 7a-7l** (**Table 1**).

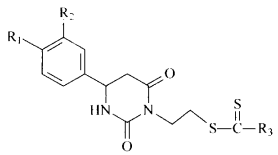
In this study, the antimicrobial activities of the 16 compounds in **Table 1** were tested; 12 of these compounds (**compounds 7a-7l**) were synthesized previously to evaluate their anticonvulsant activities[21]. These compounds (**7a-7l**) have been synthesized again to check their antimicrobial activities, according to the method used previously[21], while four of the compounds (**compounds 7m-7p**) were synthesized specifically for this study as shown in **Scheme 1**. The 16 dithiocarbamate derivatives used in this study had not been evaluated previously from the microbiological aspect in the literature. All compounds were tested for their antimicrobial activities against two Gram-positive (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212) and two Gram-negative (Escherichia coli ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) bacteria and also three fungi (*Candida albicans* ATCC 90028, *Candida krusei* ATCC 6258, *Candida parapsilosis* ATCC 22019) using microdilution broth method.

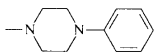
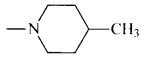
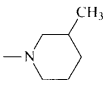
MATERIALS and METHODS

Chemistry

All chemicals used in this study were purchased from Aldrich Chemical Co. (Steinheim, Germany), Merck (Hohenbrunn, Germany) and Riedel-de Haën (Seelze, Germany). Melting points were determined on a Thomas Hoover apparatus (Philadelphia, PA,

Table 1. Structures of the tested compounds



Compound No.	R_1	R_2	R_3
7a	H	H	
7b	Cl	H	
7c	H	Cl	
7d	H	H	
7e	Cl	H	
7f	H	Cl	
7g	H	H	
7h	Cl	H	
7i	H	Cl	
7j	H	H	
7k	Cl	H	
7l	H	Cl	
7m	H	H	
7n	H	Cl	
7o	H	H	
7p	H	Cl	

USA) and were uncorrected. IR spectra were recorded on a Perkin Elmer FT-IR spectrometer 1720 X (Überlingen, Germany) as KBr disc (γ, cm^{-1}). ^{1}H-NMR spectra in dimethylsulfoxide-d_6 were obtained on a Bruker AC 80 MHz spectrophotometer (Karlsruhe, Germany). Elemental analyses were performed with a Perkin Elmer Model 240 C and Leco CHNS-932 (St. Joseph, MI, USA), at the Scientific and Technical Research Council of Turkey. The purity of the compounds was assessed by TLC on Kieselgel 60 F254 (Merck, Darmstadt, Germany).

Four new compounds were synthesized according to the method described previously[20,21] as shown in **Scheme 1**. The first step of the synthetic pathway in **Scheme 1** involved the Rodionow-Johnson reaction[22-25] using malonic acid and ammonium acetate in an ethanolic solution of starting material (**3a,3b**). Compounds **4a,4b** were treated with an equivalent amount of 2-chloroethylisocyanate in the presence of an aqueous sodium hydroxide solution at room temperature to yield the sodium salts of the 3-[3N'-(2-chloroethyl)ureido]-3-arylpropanoic acids (**5a, 5b**), which remained in solution; upon addition of a mineral acid, compounds **5a,5b** were precipitated. The latter was refluxed in thionyl chloride to give, after removal of the solvent, the attempted 3-(2-chloroethyl)-6-arylhexahydropyrimidine-2,4-diones (**6a,6b**). N,N-Disubstituted dithiocarbamate potassium derivatives (**2e,2f**) were prepared by the reaction of the appropriate amines (**1e,1f**) with CS_2 and KOH[12,20,21]. Potassium salts of N,N-disubstituted dithiocarbamoic acids (**2e,2f**) were reacted with 3-(2-chloroethyl)-6-arylhexahydropyrimidine-2,4-diones (**6a,6b**) to obtain compounds **7m-p**.

Microbiology

Minimal inhibitory concentrations (MICs) were determined by broth microdilution method following the procedures recommended by the National Committee for Clinical Laboratory Standards (NCCLS)[26,27]. Fluconazole and ceftazidime were used as the reference drugs for fungi and bacteria, respectively. Two Gram-positive (*S. aureus* ATCC 29213, *E. faecalis* ATCC 29212) and two Gram-negative (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853) bacteria were used as quality control strains. For testing antifungal activities of the compounds, three fungi were tested: *C. albicans* ATCC 90028, *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019. Reference drugs were dissolved in sterile distilled water. The stock solutions of the compounds were prepared in dimethylsulfoxide (DMSO). The dilutions in the test medium were prepared at the required concentration of 512-0.5 μg/mL and for the reference drugs; 64-0.0625 μg/mL. The final inoculum densities were 5 x 10^5 cfu/mL for bacteria and 0.5-2.5 x 10^3 cfu/mL for fungi. MIC was defined as the lowest concentration of the compound that inhibited visible growth of microorganisms. It was established that DMSO lacked antimicrobial activity against any of the test microorganisms.

Antibacterial activity assay

The cultures were grown on Mueller-Hinton agar (MHA) (BBL, MD, USA) for all bacteria after 18-24 h of incubation at 35°C. Before the assay, all of the bacteria were grown in Mueller-Hinton broth (MHB) for 2-6 h. Then the bacterial suspensions were adjusted to 0.5 McFarland turbidity (1 x 10^8 cfu/mL). The microtiter plates were incubated at 35°C and inspected visually after 18-24 h. The MIC values were recorded as the lowest concentrations of the substances that had no visible turbidity.

Antifungal activity assay

All fungi were cultivated in Sabouraud dextrose agar (Merck). Roswell Park Memorial Institute (RPMI)-1640 medium (ICN-Flow, Aurora, OH, USA) with L-glutamine, buffered with 3-(N-morpholino)propanesulfonic acid (MOPS) (Buffer-ICN-Flow, Aurora, OH-USA) at pH=7.4 was used as the test medium. The microtiter plates were incubated at 35°C and evaluated visually after 48 h. The MIC values were recorded as the lowest concentrations of the substances that had no visible turbidity.

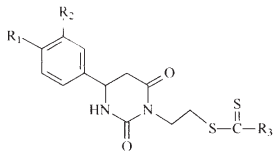
RESULTS and DISCUSSION

Chemistry

N,N-Disubstituted carbamodithioic acid 3-ethyl-6-arylhexahydropyrimidine-2,4-dione ester derivatives (**7a,7p**) (**Table 1**) were obtained as outlined in

Scheme 1. Empirical formula, molecular weight, melting point, yield and elemental analysis properties of the four compounds (**7m-7p**) which were synthesized specifically for this study are presented in **Table 2**. The structures of the synthesized compounds were proved by IR, ^{1}H-NMR and elemental analysis. All spectral data were in accordance with the assumed structures. The results of elemental analysis were within ± 0.4% of theoretical values. In the IR spectra of the compounds, the N-H stretching bands of hexahydropyrimidine structure were seen at about 3290-3236 cm^{-1}, the two C=O stretching bands at about 1725-1724 and 1681-1680 cm^{-1}, respectively, and the C=S stretching bands of the dithiocarbamate function at about 1234-1224 cm^{-1}. In the ^{1}H-NMR spectra, CH_3- protons at piperidine were seen at 0.95 ppm, protons of piperidine at about 1.40-1.80 ppm, the neighboring methylene protons to N atom and S atom, in alkyl chain between hexahydropyrimidine and dithiocarbamate structures, at 2.80-2.95 and 3.10-3.95 ppm, respectively. The methylene protons at the 5-position and the -CH- proton at the 6-position in the hexahydropyrimidine were seen at about 2.95-3.20 and 4.60-4.80 ppm, respectively. Aromatic protons were observed at about 7.20-7.40 ppm and –NH protons at 8.20-8.30 ppm (**Table 3**). We also proved the formation of synthesized compounds with ^{1}H-NMR parameters (chemical shifts) in our previous paper[21].

Table 2. Empirical formulas, molecular weights, melting points and yields of the synthesized compounds.



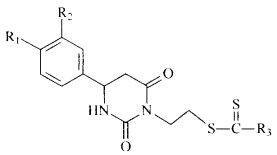
Compound No.	Empirical Formula	Molecular Weight	Melting Point (°C)a	Yield (%)b	Analysisc
7m	$C_{19}H_{25}N_3O_2S_2$	391.55	156-8	42.54	C,H,N,S
7n	$C_{19}H_{24}ClN_3O_2S_2$	425.99	137-8	75.68	C,H,N,S
7o	$C_{19}H_{25}N_3O_2S_2$	391.55	155-7	58.94	C,H,N,S
7p	$C_{19}H_{24}ClN_3O_2S_2$	425.99	140-1	82.46	C,H,N,S

a Melting points were determined on a Thomas Hoover apparatus and are uncorrected.
b Yields are of the products obtained from first crystallization.
c C, H, N, S were performed by the Scientific and Technical Research Council of Turkey Instrumental Analysis Laboratories (Ankara, Turkey). The results of elemental analysis were within ± 0.4% of theoretical values.

Microbiology

A range of compounds were screened for their *in vitro* antimicrobial activity by microdilution broth method. Moreover, their antibacterial and antifungal activities were determined as MIC values. Fluconazole and ceftazidime were used as the standard antifungal and antibacterial drug, respectively. The results of the studies are reported in **Table 4**. According to the values, antibacterial and antifungal activities of the compounds were not similar to that of ceftazidime and fluconazole, which were used as control agents. The antibacterial activity of 2-[6-(3-

Table 3. Spectral data of the synthesized compounds.



Compound No.	IR (cm^{-1})	NMR (DMSO-d_6, δ, ppm)
7m	3259 (NH st) 1724 (C=O st) 1680 (C=O st) 1224 (C=S st)	0.95 (3H; d; CH_3-), 1.40 (8H, m, piperidine), 2.80 (2H, t, -N-CH_2-), 3.20 (2H, d, -CH_2-), 3.90 (2H, t, -CH_2-S-), 4.80 (1H, t, -CH-), 7.20 (5H, s, aromatic), 8.20 (1H, s, -NH)
7n	3236 (NH st) 1724 (C=O st) 1680 (C=O st) 1224 (C=S st)	0.95 (3H; d; CH_3-), 1.60-1.80 (8H, m, piperidine), 2.95 (2H, t, -N-CH_2-), 3.05 (2H, d, -CH2), 3.95 (2H, t, -CH_2-S-), 4.60 (1H, t, -CH-), 7.30-7.40 (4H, m, aromatic), 8.30 (1H, s, -NH)
7o	3290 (NH st) 1725 (C=O st) 1680 (C=O st) 1234 (C=S st)	0.95 (3H; d; CH_3-), 1.20-1.60 (8H, m, piperidine), 2.80 (2H, t, -N-CH_2-), 2.95 (2H, d, -CH_2), 3.10 (2H, t, -CH_2S-), 4.60 (1H, t, -CH-), 7.20 (5H, s, aromatic), 8.20 (1H, s, -NH)
7p	3266 (NH st) 1726 (C=O st) 1681 (C=O st) 1234 (C=S st)	0.95 (3H; d; CH_3-), 1.70 (8H, m, piperidine), 2.80 (2H, t, -N-CH_2-), 2.95 (2H, d, -CH_2-), 3.10 (2H, t, -CH_2-S-), 4.60 (1H, t, -CH-), 7.20-7.30 (4H, m, aromatic), 8.30 (1H, s, -NH)

Table 4. Antibacterial and antifungal activities of the tested compounds (MIC in μg/mL)

Compound Number	Bacteria (MIC-μg/mL)				Fungi (MIC-μg/mL)		
	S. aureus	*E. faecalis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*C. krusei*	*C. parapsilosis*
7a	>512	>512	>512	>512	256	512	512
7b	128	128	256	128	128	128	64
7c	16	256	256	128	128	128	128
7d	512	>512	256	256	>512	>512	>512
7e	256	128	256	128	64	64	128
7f	64	128	256	128	128	128	128
7g	>512	>512	>512	256	256	512	>512
7h	256	128	256	128	64	128	128
7i	>512	512	>512	512	512	512	512
7j	512	256	256	>512	>512	>512	>512
7k	256	>512	512	>512	>512	>512	>512
7l	>512	>512	>512	>512	>512	>512	>512
7m	128	>512	>512	256	256	512	256
7n	>512	>512	>512	>512	>512	>512	>512
7o	128	128	>512	512	256	256	256
7p	>512	128	256	128	128	128	128
Ceftazidime	16	-	0.5	4			
Fluconazole					1	16	8

Ceftazidime was used as a control for bacteria and **Fluconazole** as a control for fungi.

chlorophenyl)hexahydropyrimidine-2,4-dione-3-yl]ethyl morpholine-4-carbodithioate (**7c**) and 2-[6-(3-chlorophenyl)hexahydropyrimidine-2,4-dione-3-yl]ethyl pyrrolidine-4-carbodithioate (**7f**) against *S. aureus* (MIC: 16 μg/mL and 64 μg/mL respectively) were found comparable to ceftazidime. The antifungal activities of 2-[6-(4-chlorophenyl)hexahydropyrimidine-2,4-dione-3-yl]ethyl morpholine-4-carbodithioate (**7b**) against *C. parapsilosis* (MIC: 64 μg/mL), of 2-[6-(4-chlorophenyl)hexahydropyrimidine-2,4-dione-3-yl]ethyl pyrrolidine-4-carbodithioate (**7e**) against *C. albicans* (MIC: 64 μg/mL) and *C. krusei* (MIC: 64 μg/mL) and of 2-[6-(4-chlorophenyl)hexahydropyrimidine-2,4-dione-3-yl]ethyl piperidine-4-carbodithioate (**7h**) against *C. albicans* (MIC: 64 μg/mL) were found higher than the other tested compounds.

CONCLUSION

In the tested N,N-disubstituted carbamodithioic acid 3-ethyl-6-arylhexahydropyrimidine-2,4-dione ester derivatives which contain heterocyclic rings in their structure as morpholine (**7a-7c**), pyrrolidine (**7d-7f**) and nonsubstituted piperidine (**7g-7i**), the antibacterial and antifungal activities were observed whereas in the N,N-disubstituted carbamodithioic acid 3-ethyl-6-arylhexahydropyrimidine-2,4-dione ester derivatives which contain substituted heterocyclic rings including 3-methylpiperidine (**7o-7p**), 4-methylpiperidine (**7m-7n**) and N-phenylpiperazine (**7j-7l**) showed no antifungal and antibacterial activities. Among the tested comppunds, N,N-disubstituted carbamodithioic acid 3-ethyl-6-arylhexahydropyrimidine-2,4-dione esters, which have nonsubstituted heterocyclic rings and 6-(4-chlorophenyl) derivatives (**7b, 7e** and **7h**) showed antifungal activity whereas 6-(3-chlorophenyl) derivatives except **7i**, showed antibacterial activity.

Finally, among the tested N,N-disubstituted carbamodithioic acid 3-ethyl-6-arylhexahydropyrimidine-2,4-dione ester derivatives, 2-[6-(3-chlorophenyl)-hexahydropyrimidine-2,4-dione-3-yl]ethyl morpholine-4-carbodithioate (**7c**) effect was found to be similar to ceftazidime against *S. aureus* ATCC 29213 (MIC: 16 μg/mL). As a result it could be concluded that **compound 7c** was the most important compound in this series regarding its antibacterial activity.

Acknowledgement

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