Asian Journal of Chemistry Vol. 18, No. 3 (2006), 1710–1718

Thermal Behaviour and Antimicrobial Activity of Novel Series of Benzoylthiourea Derivatives

HAKAN ARSLAN*†, NIZAM DURAN‡, NEFISE ÖZLEN ŞAHIN† and NEVZAT KÜLCÜ

Department of Chemistry, Faculty of Arts and Sciences, Mersin University Mersin 33343, Turkey

Fax: (90)(324)3415759; Tel: (90)(532)7073122

E-mail: arslanh@mersin.edu.tr

Thermal behaviour and antimicrobial activity was investigated for a series of thiourea derivatives. The thermal behaviour of the compounds on heating up to 1000 K in nitrogen atmosphere was examined by thermogravimetry and differential thermal analysis. The compounds decompose in one or two stages. GC-MS combined system was used to identify the products during pyrolytic decompositions. The kinetic analysis of the thermogravimetric data was performed using the Coats-Redfern and Horowitz-Metzger methods. The antibacterial activities of these thiourea derivatives were investigated for three Gram positive (*Enterococcus faecalis, Staphylocccus aureus* and *Staphylocccus epidermidis*) and two Gram negative (*Escherichia coli, Pseudomonas aeruginosa*) bacteria by employing broth microdilution method and subsequently, inhibitory activity against yeast-like fungi (*Candida albicans, Candida krusei, Candida glabrata* and *Candida parapsilosis*) was also determined. Only three of the compounds showed antimicrobial activity and that were at a moderate level. None of them exhibited sufficient antifungal activity to become a drug candidate.

Key Words: Thermal analysis, Thiourea, Antimicrobial activity, Benzoylthiourea derivative.

INTRODUCTION

Some thiourea derivatives are selectively analytical reagents, especially for the determination of transition metals in complex interfering matrices[1, 2]. The complexation capacity of some thiourea derivatives has been reported in several papers[3, 4]. N,N-dialkyl-N′-benzoylthioureas have been found to be useful ligands for the potential determination of traces of the transition metals by means of normal phase chromatography[2, 4]. The biological activities of this type of

†Department of Pharmaceutics, Faculty of Pharmacy, Mersin University, Mersin 33169, Turkey.

‡Department of Microbiology, Faculty of Medicine, Mustafa Kemal University, Hatay 31000, Turkey.

complexes have been successfully screened and some N-substituted-N′-carbonylthiourea derivatives have been used in commercial fungicides[5, 6].

Recently, Arslan *et al.*[7] reported the synthesis and characterization of N,N′-dialkyl-N′-benzoylthiourea derivatives and their metal complexes. Subsequently, such properties of these derivatives were examined[7-12] taking into account that thermal properties and antimicrobial efficacy of this type of benzoylthiourea derivatives, namely, N,N-dimethyl-N′-(2-chloro-benzoyl)thiourea (L^1), N,N-di-*n*-propyl-N′-(2-chloro-benzoyl) thiourea (L^2), N,N-di-*n*-propyl-N′-(4-chloro-benzoyl) thiourea (L^3), N,N-diphenyl-N′-(2-chloro-benzoyl)thiourea (L^4), N,N-diphenyl-N′-(4-chloro-benzoyl)thiourea (L^5), N,N-diphenyl-N′-(4-phenyl-benzoyl) thiourea (L^6) and N-pyrolidin-N′-(2-chloro-benzoyl) thiourea (L^7), have not been studied previously. In the present study, the thiourea derivatives were studied by DTA and TG and the mechanisms of thermal decomposition have been established. Furthermore, the decomposition kinetics and the outcome of the microbiological activity of these compounds is also reported.

EXPERIMENTAL

All chemicals used for the preparation of the compounds were of reagent grade quality. A GC-MS system, Finnigan model Mat Magnum, was used to identify pyrolysis products evolved during heating. The DTA and TG curves were obtained simultaneously with Shimadzu DT-40 model with DTA and TG apparatus. The measurements were performed by using a dynamic nitrogen atmosphere at a flow rate of 60 mL min^{-1} up to 1000 K. The heating rate was 10 K min^{-1} and the sample sizes ranged in mass from 8 to 13 mg contained in platinum crucible. α-Al_2O_3 was used as a reference material. Melting point determinations were performed with a digital melting point instrument from Electrothermal model 9200.

Synthesis of benzoylthiourea derivatives

The compounds were obtained in acetone using the reported method[7]. The substituted benzoylisothiocyanates were obtained from substituted benzoyl chloride (5×10^{-2} mol) and KSCN (5×10^{-2} mol) at 40°C (30 min). A solution of the amine was added to the mixture for 15 min at room temperature and stirred for 2 h. The solid organic phase was filtered and recrystallized from ethanol/dichloromethane. All synthesized derivatives were characterized with respect to melting point.

Antimicrobiological activity studies

Antibacterial activities of the compounds were tested against Gram (+) and Gram (–) bacteria such as *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus faecalis* (ATCC 29212), *Staphylocccus aureus* (ATCC 25923) and *Staphylocccus epidermidis* (ATCC 12228) and the antifungal activities of compounds against some yeast like fungi such as *Candida albicans* (ATCC 90028), *Candida krusei* (ATCC 6258), *Candida glabrata* (ATCC 32554) and *Candida parapsilosis* (ATCC 22019). Minimal inhibitory concentrations (MICs) were determined by broth microdilution methods following the procedures recommended by the National Committee for Clinical Laboratory Standards[13, 14]. Amikacin and flucanozole were used as reference compounds for bacteria and fungi, respectively.

Mueller-Hinton broth (Difco Laboratories, Detroit, USA) was used when testing bacterial strains. For Candida species, saboraud dextroz broth (Difco) was used. The inoculum densities were 1×10^6 cfu/mL for bacteria and fungi. The solutions in the test medium were furnished the required concentrations ranging from 1024–0.5 μg/mL. The microtiter plates were incubated at 35°C and read visually after 24 h. For Candida species, incubation period was 48 h. The minimum inhibitory concentration (MIC) values were recorded as the lowest concentrations of the substances that had no visible turbidity.

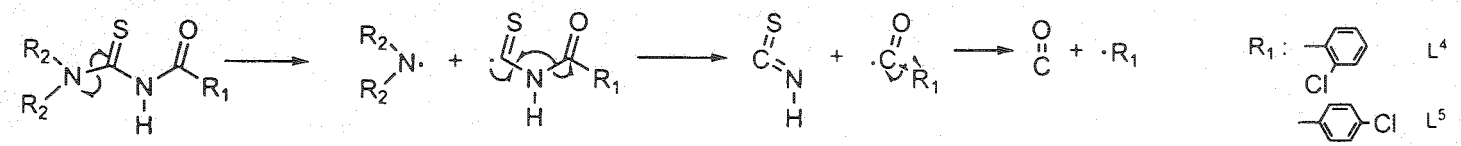
RESULTS AND DISCUSSION

Seven different derivatives were synthesized and characterized[7,8]. All the compounds were studied by thermogravimetric analysis from ambient temperature to 1000 K in nitrogen atmosphere. The TG, DTG and DTA curves of the compounds are presented in Fig. 1. The temperature ranges and percentage mass losses of the decomposition reaction are given in Table-1.

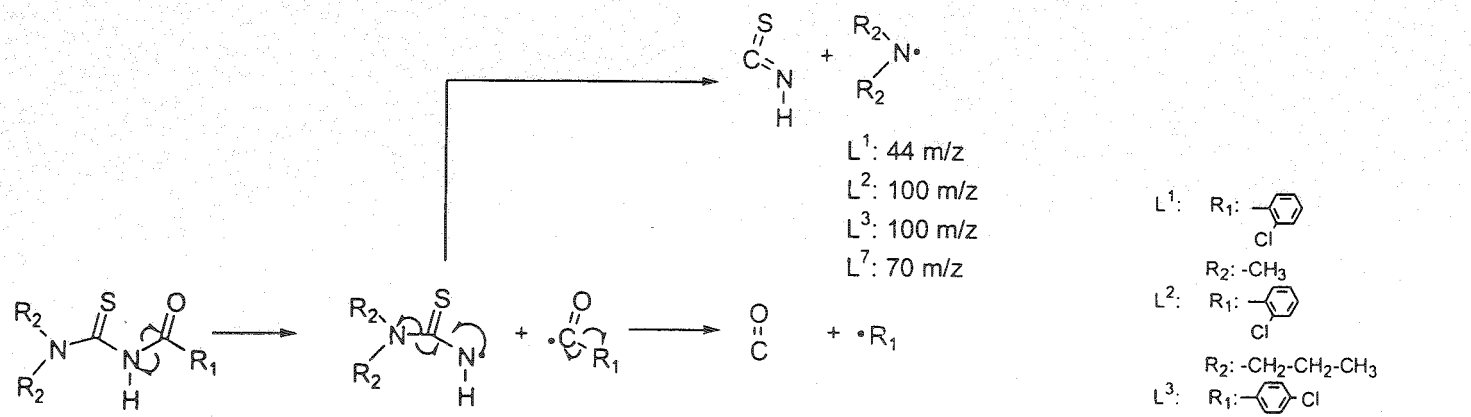
TABLE-1
THERMOANALYTICAL RESULTS FOR COMPOUNDS (L^1–L^7)

Compounds	Stage	Temperature of DTA peak maximum (K)	TG Temperature range (K)	Mass loss (%)		Evolved moiety
				TG	Theor.	
L^1	I	440	410–826	99.50	100.0	—
L^2	I	505	411–704	99.30	100.0	—
L^3	I	502	413–907	99.60	100.0	—
L^4	I	434	422–510	46.70	45.8	$\cdot N(C_6H_5)_2$
	II	482	510–848	53.30	54.2	—
L^5	I	438	428–519	46.64	45.9	$\cdot N(C_6H_5)_2$
	II	486	519–854	53.30	54.1	—
L^6	I	430	421–533	41.04	41.2	$\cdot N(C_6H_5)_2$
	II	480	533–830	58.70	58.8	—
L^7	I	455	449–770	99.70	100.0	—

The DTA profiles for L^2, L^3 and L^5 show one endothermic peak at 353 K, 380 K and 438 K. These peaks corresponding to the melting points of the compounds. L^1, L^4, L^6 and L^7 melt at 435 K, 423 K, 412 K and 446 K with spontaneous decomposition, respectively. The mechanisms of decomposition for the compounds are possible as shown by **Schemes 1** and **2**. The theoretical mechanisms dealt with in **Scheme 1** and **2** are confirmed by GC-MS data (Fig. 2 as an example). Observed MS peaks are responsible for evolved radical moiety. The decomposition mechanisms obtained from TG profiles are shown in Fig. 1. The theoretical and experimental per cent mass losses obtained from these decomposition stages are in good agreement. The mechanism of decomposition reaction for diphenylamine derivatives proceeds in two stages, the first of which is apparent in **Reaction-1**. The end product (**A**) of this stage undergoes pyrolysis in the second stage. The second decomposition mechanism for dialkylamine derivatives and pyrolidin derivative proceeds in one stage, which compounds undergo pyrolysis (**Scheme-2**).



Scheme-1



Scheme-2

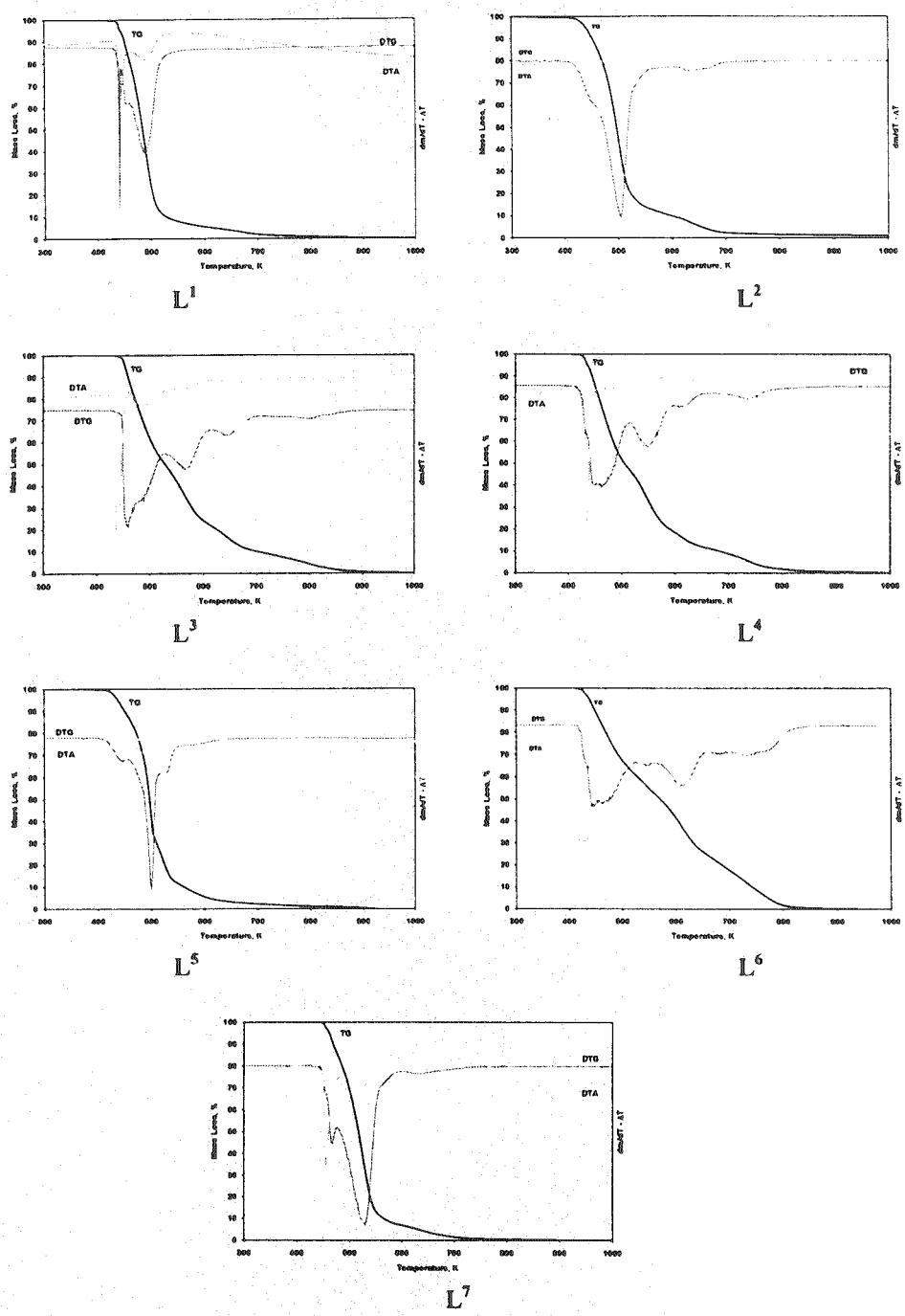
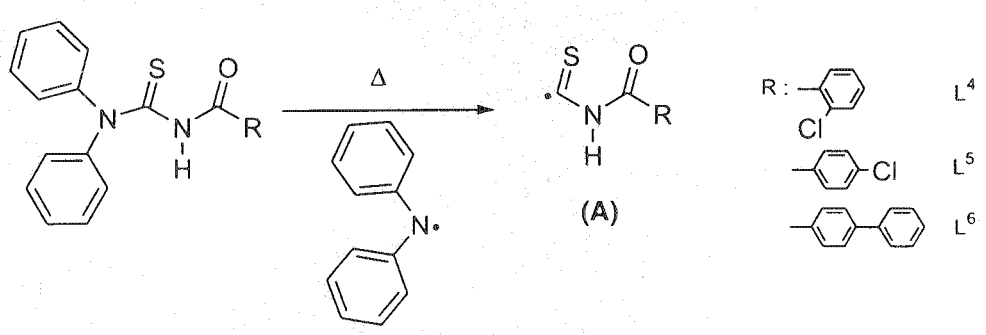


Fig. 1. DTA/TG/DTG curves for investigated compounds

In the study regarding decomposition kinetics, the integral method with the Coats-Redfern equation[15] and the approximation method with the Horowitz-Metzger equation[16] was used for calculation of kinetic parameters such as the activation energy, E^*, entropies, ΔS^*, and the pre-exponential factor, A, from a non-isothermal kinetic study[21, 22]. The decomposition kinetics parameters for L^4, L^5 and L^6 are presented in Table-2.



Reaction-1

TABLE-2
KINETIC DATA ON THE INVESTIGATED COMPOUNDS FOR STAGE I

Compounds	Parameters*	From Coats-Redfern eqn.	From Horowitz-Metzger eqn.
L^4	E^*	65.8	70.7
	A	1.02×10^5	3.59×10^5
	ΔS^*	−152.9	−142.5
	r	0.9991	0.9993
L^5	E^*	69.6	74.7
	A	1.68×10^5	6.06×10^5
	ΔS^*	−123.6	−138.3
	r	0.9993	0.9995
L^6	E^*	47.2	51.3
	A	4.89×10^2	14.6×10^2
	ΔS^*	−197.5	−188.4
	r	0.9941	0.9953

*Unit of parameters E^* in kJ mol^{-1}, A in s^{-1}, $n = 1.0$, ΔS^* in J mol^{-1} K^{-1}, r-correlation coefficient of the linear plot.

The linearization curves are shown in Figs. 3 and 4. The decomposition kinetics parameters found for the first decomposition stages of N,N-diphenyl-N′-(2-chloro-benzoyl)thiourea (L^4), N.N-diphenyl-N′-(4-chloro-benzoyl)thiourea (L^5) and N,N-diphenyl-N′-(4-phenyl-benzoyl)thiourea (L^6) are nearly equal to unity. As L^4 and L^5 possess similar structure (only the position of Cl atom is different), their activation energy values (L^4 = 70.7 and L^5 = 74.7 kJ mol^{-1}) are nearly equal for both methods used for calculation. However, L^6 has a phenyl group attached to the para position of benzoyl group of the molecule. Therefore, its activation energy is slightly different from those of L^4 and L^5. The kinetic data reached by both of the methods are in harmony with each other. These results comply with the structure of the compounds and decomposition mechanism.

The results obtained from the antibacterial and antifungal efficacy studies were given in Tables 3 and 4, respectively. It was determined that the benzoylthiourea derivatives under investigation inhibit the growth of some Gram (+) and Gram (−) bacteria and fungi at different levels. The lowest MIC values were obtained against *Staphylococcus epidermidis* with L^4, L^5 and L^6. The highest MIC value (512

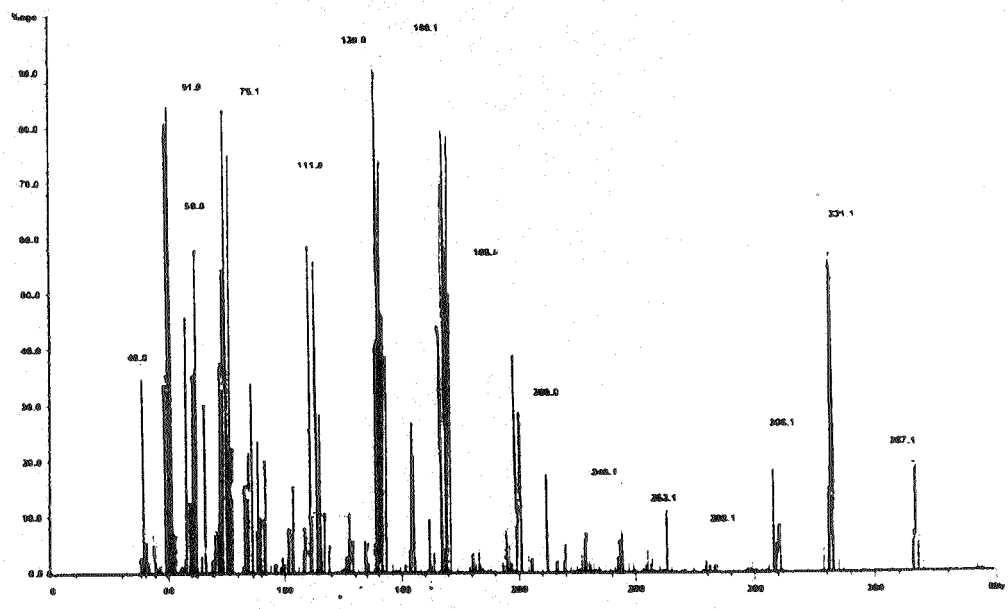


Fig. 2. MS spectrum of N,N-diphenyl-N′-(2-chloro-benzoyl)thiourea

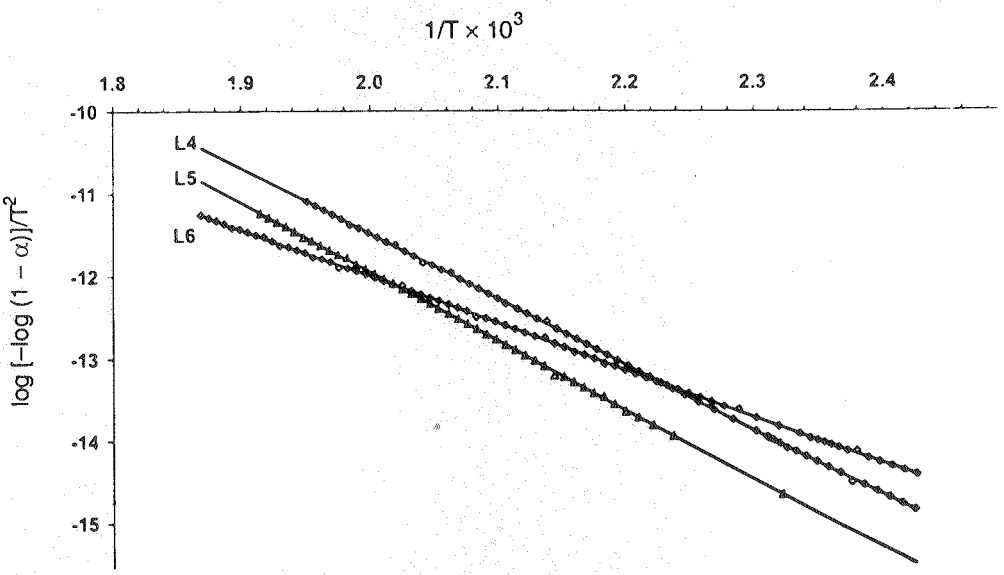


Fig. 3. Linearization curves of the first decomposition steps of Coats-Redfern methods for L^4, L^5 and L^6

μg/mL) was found against the growth of *Pseudomonas aureginosa* with L^2 and L^7. As evident from the data in Tables 3 and 4, antifungal efficacy of these compounds is very weak. Formulations of L^4, L^5 and L^6 possess moderate antibacterial activity only against *Staphylococcus epidermidis*.

In conclusion, this study indicates that these compounds cannot be used as drug substances with antibacterial and antifungal activities. L^4, L^5 and L^6 may be used as pharmaceutical excipients to provide microbiological stability. However, these three formulations need to be examined for such effect in gel or solution type pharmaceutical formulations. Such investigation has been carried out in our laboratory to ensure their use as an excipient with antibacterial efficacy. The data on

thermal behaviour of these compounds will be of great use upon proof of this assumption.

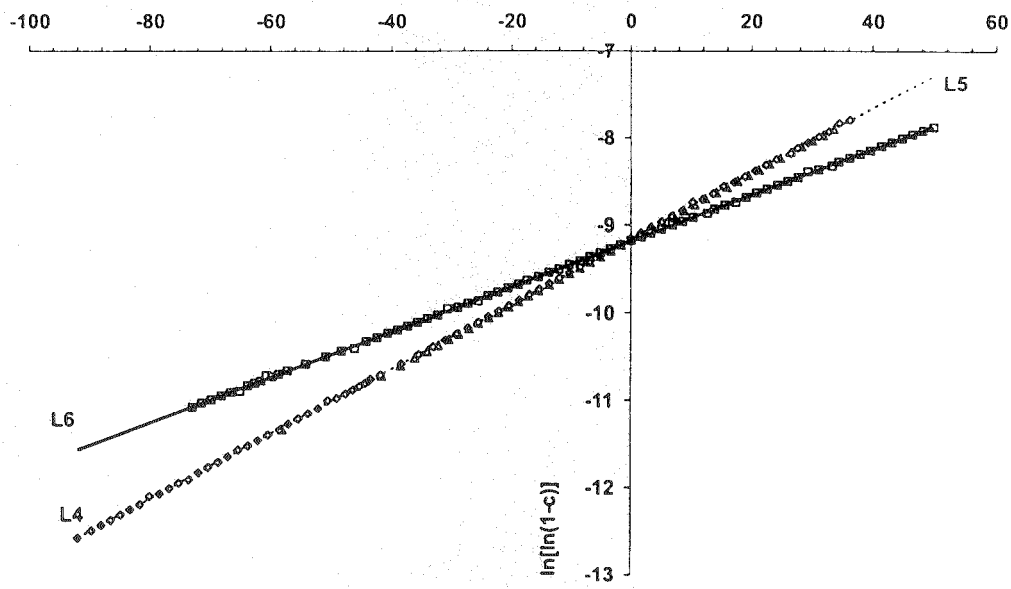


Fig. 4. Linearization curves of the first decomposition steps of Horowitz-Metzger methods for L^4, L^5 and L^6

TABLE-3
MIC VALUES (μg/mL) FOR ANTIBACTERIAL ACTIVITIES OF THE COMPOUNDS

Compds.	*Staphylococcus aureus* (ATCC 25923)	*Escherichia coli* (ATCC 25922)	*Staphylococcus epidermidis* (ATCC 12228)	*Pseudomonas aureginosa* (ATCC 27853)	*Enterococcus faecalis* (ATCC 29212)
L^1	128	128	128	256	128
L^2	128	128	128	512	128
L^3	256	128	128	256	128
L^4	256	256	64	256	128
L^5	128	256	64	128	128
L^6	128	256	64	128	128
L^7	256	128	128	512	128
Amikacin	4	1	4	2	32

TABLE-4
MIC VALUES (μg/mL) FOR ANTIFUNGAL ACTIVITIES OF THE COMPOUNDS

Compds.	*Candida albicans* (ATCC 90028)	*Candida krusei* (ATCC 6258)	*Candida paraphilosis* (ATCC 22019)	*Candida glabrata* (ATCC 32554)
L^1	512	512	256	512
L^2	512	512	256	512
L^3	512	512	256	256
L^4	512	256	512	256
L^5	512	256	512	512
L^6	256	256	512	512
L^7	512	256	256	512
Flucanazole	0.5	32	2	4

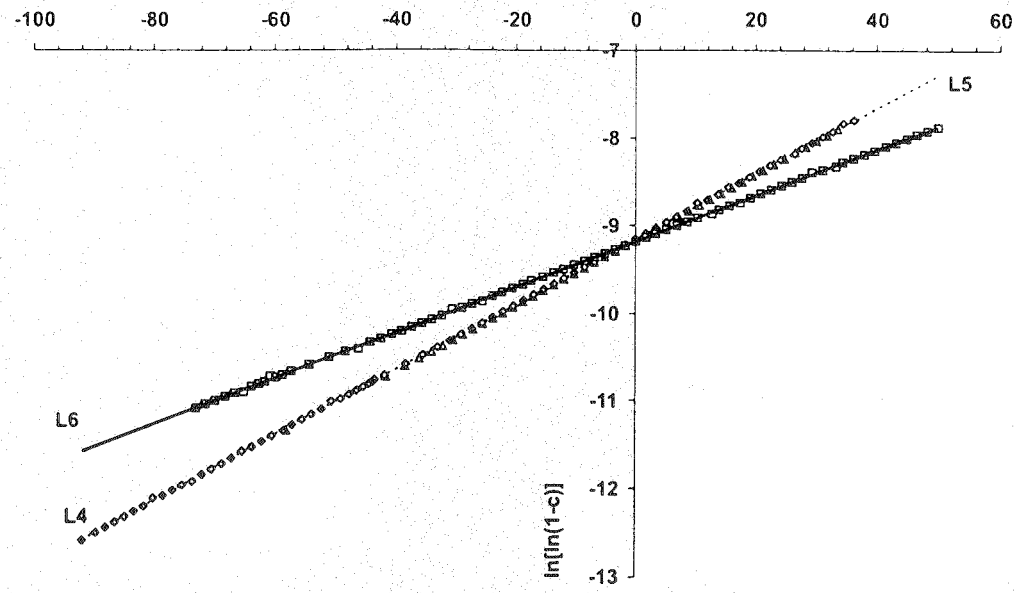


Fig. 4. Linearization curves of the first decomposition steps of Horowitz-Metzger methods for L^4, L^5 and L^6

ACKNOWLEDGEMENTS

The authors would like to thank Res. Assists. Gun Polat and M. Fatih Emen for their technical assistance and Dr. Gonul Arslan for providing facilities to conduct bioactivity tests.

REFERENCES

1. M. Schuster, *J. Anal. Chem.*, **342**, 791 (1992).
2. M. Merdivan, Ph. D. Thesis, Middle East Technical University, Ankara, Turkey (1994).
3. M. Schuster, B. Kugler and K.H. König, *J. Anal. Chem.*, **338**, 717 (1990).
4. K.H. König, M. Schuster, G. Schneeweiss and B. Steinbrech, *Fresenius Z. Anal. Chem.*, **319**, 66 (1984).
5. F.A. French, E.J. Blanz (Jr.), JR. DoAmaral and D.A. French, *J. Med. Chem.*, **13**, 1117 (1970).
6. F.A. French and E.J. Blanz (Jr.), *Cancer Res.*, **26**, 638 (1966).
7. H. Arslan, N. Külcü and U. Flörke, *Transition Met. Chem.*, **28**, 816 (2003).
8. G. Binzet, H. Arslan, U. Flörke, N. Nülcü and N. Duran, *J. Coord. Chem.* (2006) (in press).
9. H. Arslan, U. Flörke and N. Kulcu, *J. Chem. Crystallogr.*, **33**, 919 (2003).
10. F.M. Emen, U. Flörke, N. Kulcu and H. Arslan, *Acta Cryst.*, **E59**, o1159 (2003).
11. E. Kayhan, U. Flörke, N. Kulcu and H. Arslan, *Acta Cryst.*, **E59**, o1237 (2003).
12. H. Arslan, U. Flörke and N. Kulcu, *Acta Cryst.*, **E59**, o641 (2003).
13. Approved Standard, National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, M7-A4, Viallanova, PA (1997).
14. Approved Standard, National Committee for Clinical Laboratory Standards, Reference Methods for Broth Dilution Antifungal Susceptibility Testing Yeasts M27-A, Viallanova, PA (1997).
15. A.W. Coats and J.P. Redfern, *Nature*, **201**, 68 (1964).
16. H.H. Horowitz and G. Metzger, *Anal. Chem.*, **35**, 1464 (1963).

(*Received*: 20 April 2005; *Accepted*: 27 February 2006) AJC-4643