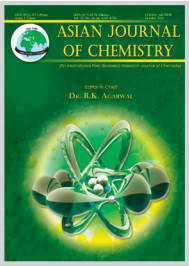
Asian Journal of Chemistry; Vol. 24, No. 11 (2012), 5305-5308

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



Synthesis and Biological Evaluation of Novel 2,4-Thiazolidinedione Derivatives as Antibacterial Agent

A.M. PATIL[1,*], A.S. SAYARE[1], S.S. CHITLANGE[3], A.P. KHADKE[2] and D.V. POKHARKAR[1]

[1]CAYMET's Siddhant College of Pharmacy, Sudumbre, Pune-412 109, India
[2]Yashoda Shikshan Prasarak Mandal, Yashoda Technical Campus, Faculty of Pharmacy, Satara-415 011, India
[3]Dr. D.Y. Patil Institute of Pharmaceutical Science and Research, Pune-411 018, India

*Corresponding author: Email : apkhadake@gmail.com

(*Received*: 31 October 2011; *Accepted*: 16 June 2012) AJC-11623

A novel series of 5-[1-substituted-3-(substituted phenyl)allylidine]thiazolidine-2,4-dione was synthesized. The target compounds **3(a-l)** were synthesized by azotropic removal of water by condensation of 2,4-thiazolidinedione with substituted benzylideneacetones/substituted cinnamaldehyde in presence of piperidine. The spectral characteristics (FT-IR, ^{1}H NMR spectroscopy) and physical properties (melting point) of the synthesized compounds were identified. The compounds synthesized were evaluated for their antibacterial activity by using cup plate method and minimum inhibitory concentration by two fold serial dilution method. The screening results of the synthesized compounds indicated that the compound **(3a), (3b), (3c), (3e), (3f), (3g), (3h), (3i), (3j), (3l)** exhibited significant antibacterial activity against gram positive and gram negative bacteria.

Key Words: Thiazolidine-2,4-dione, Antibacterial activity, Cinnamonaldehydes, Benzylideneacetones.

INTRODUCTION

Bacterial infections have increased dramatically in recent years. Bacteria have been the cause of some of the most deadly diseases and wide spread epidemics in human civilization. Bacterial diseases such as tuberculosis, typhus, plague, diphtheria, typhoid fever, cholera, dysentery and pneumonia have taken a high toll on humanity[1,2]. The introduction of antibiotics in the 1940s was thought to have eliminated the scourge of all infectious diseases. However, due to the wide spread use and misuse of antibiotics, bacterial resistance to antibiotics has become a serious public health problem. With the increase in resistance of bacteria to antibiotic treatment, attention has focused on developing novel approaches to antimicrobial therapy[3,4]. Literature survey has revealed that recently considerable interest has been focused on thiazolidinedione derivatives, which have been shown to possess a broad spectrum of biological activities. The most important of these are antibacterial, antifungal, antidiabetic, anticancer, euglycemic and hypolipidemic activity[5,6]. At the same time β-unsaturated aldehydes (cinnamaldehde) and ketones (dehydrozingerone and dehydrorheosmine) possess antibacterial, antiinflammatory and anticonvulsant activities. 2,4-Thiazolidinediones are the new class of antimicrobial agents with activity against Gram- positive and Gram negative pathogens[7,8]. So we synthesized a new series of 5-[1-substituted-3-(substituted phenyl)allylidine]thiazolidine-2,4-dione and evaluated for its antibacterial activity.

EXPERIMENTAL

All the chemicals used in the synthesis are of laboratory grade. The melting points determined on Veego electronic apparatus (model:-VMP-D). ^{1}H NMR spectra were recorded using NMR Varian-Mercury 300 MHz spectrometer. IR spectra recorded on-Shimadzu 8400-S FT-IR spectrophotometer. Antibacterial activity (zone of inhibition by cup plate method, minimum inhibitory concentration).

Step I: Synthesis of 2,4-thiazolidinedione[9]: A solution containing chloroacetic acid (11.3 g, 0.12 mol) in water (12 mL) and thiourea (9.2 g, 0.12 mol) dissolved in water (12 mL) was placed in a three-necked round bottom flask (250 mL). The mixture was stirred for 15 min to obtain a white precipitate, accompanied by considerable cooling. Concentrated hydrochloric acid (12 mL) was added slowly to the contents of the flask from a dropping funnel. The flask was then connected with a reflux condenser and gentle heat applied to effect complete dissolution, after which the reaction mixture was stirred and refluxed for 10 h at 100-110 ºC. On cooling, the contents of the flask solidified into a cluster of white needle shaped crystals. Thereafter the product was filtered, washed with water to remove traces of hydrochloric acid and dried. It was then recrystallized from hot water.

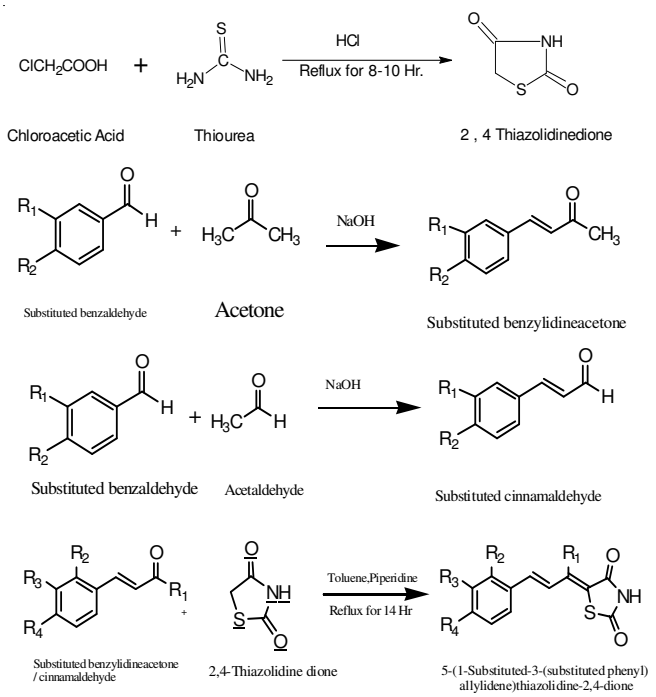
Step II: Synthesis of substituted benzylideneacetones[10]: Substituted benzaldehydes and acetone (40 mL, 0.56 mol) were added in 250 mL round bottom flask. The flask was swirled to dissolve the solid contents. Aqueous sodium hydroxide (40 mL, 10 % w/v) was added drop by drop. The flask was immediately stoppered and shaken vigorously to give a clear yellow solution, which gradually darkens to red. The mixture was allowed to stand at room temperature for 24 h. Hydrochloric acid (50 mL, 3 M) was added drop by drop to the reaction mixture. The flask was tightly stoppered and shaken vigorously to get yellow crystals. The product was filtered, washed with cold water and recrystallized from ethanol:water (50:50).

Synthesis of substituted cinnamaldehydes: 1 mol of substituted benzaldehydes and 10 mol of sodium hydroxide were dissolved in water (200 mL) at room temperature and the the mixture was cooled up to 5 ºC. 29 mL of aqueous solution of acetaldehyde (28 % w/v) was added drop by drop to this solution. Then the reaction solution was stirred for 1 h with addition of concentrated hydrochloric acid (65 mL) to get the crystals. The product was filtered and dispersed in water (50 mL) at 25 ºC, then it was dissolved by the addition of 9.5 mL aqueous solution sodium hydroxide (25 % w/v). Activated carbon (0.4 g) was added to the solution and the mixture was stirred for 1 h, and thereafter the activated carbon was filtered off. The aqueous solution obtained was neutralized by the addition concentrated hydrochloric acid (4.69 mL) to get the pure crystalline product.

Step III: Condensation of 2,4-thiazolidinedione with substituted benzylideneacetones/substituted cinnamaldehydes by *Knoevenagel* reaction: The target compound was synthesized by reacting the equimolar substituted benzylideneacetones/ substituted cinnamaldehydes and 2,4-thiazolidinedione, benzoic acid (0.5 g) in a 3-necked round-bottomed flask (250 mL) equipped with a Dean-Stark apparatus, suspended in dry toluene. To this a catalytic amount of piperidine (1 mL) was added. The mixture was refluxed for 14 h with stirring. The progress of reaction was monitored with TLC. After the complete azeotropic removal of water the reaction mixture was stirred for further 1 h. On cooling, the product was precipitated out from toluene. The compound was filtered and washed with cold toluene (**Scheme-I**). The product was air dried and recrystallized from toluene. The synthesized compounds represented in Table-1.

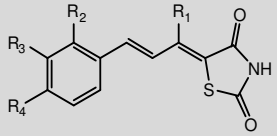
Antimicrobial screening: Evaluation of Antimicrobial activity was done against Gram +ve bacteria: *S. aureus, B. substilis* and Gram -ve bacteria: *E. coli, P. aeruginosa*.

Cup-plate method[5]: Gentamycin (5 µg/mL) was taken as standard drug for the comparison of the activity of the synthesized compounds. Each Petri dish containing Muller-Hinton agar medium was inoculated with one bacterial culture by spreading the suspension of the organism with a sterile glass rod with a bended tip. In each plate cups of 6 mm diameter were made at equal distances using sterile cork borer. Different concentrations of synthesized compounds were prepared and filled in cups. All plates were kept in the refrigerator for 30 min to allow the diffusion of sample to the surrounding agar medium. The Petri dishes were incubated at 37 ºC for 24 h. Diameter of the zone of inhibition was measured and the average diameter for each sample was calculated. The diameters obtained for the test sample were compared with that produced by standard gentamycin.



Scheme-I

TABLE-1
SYNTHESIS OF FINAL COMPOUND **3(a-l)**



S. No.	Compound code	R_1	R_2	R_3	R_4
1	3a	$-CH_3$	-H	-H	-H
2	3b	$-CH_3$	-H	-H	-OH
3	3c	$-CH_3$	-H	$-OCH_3$	-OH
4	3d	$-CH_3$	-H	-H	$-OCH_3$
5	3e	$-CH_3$	-H	-H	-Cl
6	3f	$-CH_3$	-H	-H	Br
7	3g	$-CH_3$	-Cl	-H	-H
8	3h	-H	-H	-H	-H
9	3i	-H	-H	-H	-OH
10	3j	-H	-H	$-OCH_3$	-OH
11	3k	-H	-OH	-H	-H
12	3l	-H	-H	-H	-Cl

Determination of MIC by tube dilution technique[11, 12]: All the synthesized compounds were dissolved separately to prepare a stock solution containing 100 µg/mL of DMSO. From stock solution take 5 mL and dilute to 5 mL of sterile nutrient broth to obtain 50 mg/mL. Thus successive concentrations like 25, 12.5, 6.25, 3.12, and 1.56 were prepared in a similar manner. The tubes were mixed well after each addition. All the tubes were inoculated with one loop full of one of the test organism. The process was repeated with different test organism. The tubes were incubated at 25 ºC for 24 h. The presence or absence of growth of microorganism was observed after incubation.

RESULTS AND DISCUSSION

FT-IR and ^{1}H-NMR spectral data for 2,4-thiazolidinedione derivatives **3(a-l)** was tabulated in Table-2. Concentration and zone of inhibition of synthesized compound by cup-plate method against *S. aureus, B. subtilis, E. coli* and *P. aeruginosa* was given in Table-3. Zone of inhibition against bacteria was presented in Fig. 1. The minimum inhibitory concentration was found using two fold dilution method and results were presented in Table-4. Minimum inhibitory concentration of synthesized compounds against bacteria was shown in Fig. 2. Many synthesized compound exhibit good antibacterial activity with minimum inhibitory concentration between 3.12 to 12.5 µg/mL. The reference compound used was gentamycin which exhibited MIC at 1.56 µg/mL. From the results depicted in table, it can be concluded that the synthesized compounds possess better antibacterial activity towards Gram positive than Gram negative bacteria. The synthesized compounds *viz.* **(3a), (3b), (3c), (3d), (3e), (3f), (3g), (3h), (3i), (3j), (3l)** exhibited good antibacterial activity against *S. aureus* and (**3k**) was proved to be less active against gram positive bacteria *S. aureus*.

TABLE-2
FT-IR AND ^{1}H-NMR SPECTRAL DATA FOR 2, 4-THIAZOLIDINEDIONE DERIVATIVES **3(a-l)**

Code	FT-IR spectral data	^{1}H-NMR spectral data
3a	3445 (N-H), 3062 (Ar-C-H), 2894 (Aliph-C-H), 1658 (C=O), 1650 (Aliph-CH=CH), 1541 (Ar-C=C), 749 (C-S-C)	2.480 (s, 3H, CH_3), 7.034-7.256 (m, 3H, Ar-H), 7.275-7.314 (m, 2H, Ar-H), 7.393-7.417 (d, 1H, C-H), 7.486-7.515 (d, 1H, C-H, 9.428 (s, 1H, NH)
3b	3425 (N-H), 3300 (Ar-OH), 3052 (Ar-C-H), 1744 (C=O), 1563 (Ar-C=C), 746 (C-S-C)	2.421 (s, 3H, CH_3), 6.040 (s, 1H, Ar-OH), 7.299.7.273 (d, 1H, C-H), 7.379-7.512 (m, 4H, Ar-H), 7.682-7.688 (d, 1H, C-H), 10.038 (s, 1H, NH)
3c	3552 (N-H), 3244 (Ar-OH), 3056 (Ar-OCH_3), 1678 (C=O), 1606 (Aliph-CH=CH), 1551 (Ar-C=C), 741 (C-S-C)	2.371 (s, 3H, CH_3), 3.935 (s, 3H, OCH_3), 6.060 (s, 1H, Ar-OH), 6.663.6.761 (d, 1H, Ar-C-H), 6.922-6.949 (d, 1H, C-H), 7.056-7.110 (m, 2H, Ar-C-H), 7.428-7.482 (d, 1H, C-H), 9.980 (s, 1H, NH)
3d	3173 (N-H), 3097 (Ar-C-H), 2979 (Ar-OCH_3), 1726 (C=O), 1580 (Ar-C=C), 753 (C-S-C)	2.187 (s, 3H, CH_3), 4.298 (s, 3H, Ar-OCH_3), 6.984-7.272 (m, 4H, Ar-H), 7.465-7.546 (d, 1H, C-H), 7.584-7.596 (d, 1H, C-H), 9.628 (s, 1H, NH)
3e	3333 (N-H), 3049 (Ar-C-H), 2957 (Aliph-C-H), 1722 (C=O), 1567 (Ar-C=C), 839 (-Cl), 749 (C-S-C)	2.186 (s, 3H, CH_3), 6.780-6.828 (d, 1H, C-H), 6.954-6.989 (d, 1H, C-H), 7.442-7.582 (m, 2H, Ar-H), 7.609-7.671 (m, 2H, Ar-H), 10.190 (s, 1H, NH)
3f	3290 (N-H), 3064 (Ar-C-H), 2953 (Aliph-C-H), 1720 (C=O), 1519 (Ar-C=C), 783 (-Br), 742 (C-S-C)	2.371 (s, 3H, CH_3), 6.563-6.617 (d, 1H, C-H), 6.717-6.762 (d, 1H, C-H), 6.903-7.067 (m, 2H, Ar-H), 7.428-7.482 (m, 2H, Ar-H), 9.980 (s, 1H, NH)
3g	3216 (N-H), 3052 (Ar-C-H), 1746 (C=O), 1587 (Ar-C=C), 815 (-Cl), 736 (C-S-C)	1.825 (s, 3H, CH_3), 6.653-6.821 (d, 1H, C-H), 6.892-7.098 (d, 1H, C-H), 7.352-7.566 (m, 4H, Ar-H), 10.486 (s, 1H, NH)
3h	3226 (N-H), 2923 (Ar-C-H), 1731 (C=O), 1681 (Aliph-CH=CH), 1532 (Ar-C=C), 731 (C-S-C)	6.812-6.986 (d, 1H, C-H), 7.054-7.198 (d, 1H, C-H), 7.464-7.516 (m, 1H, Ar-H), 7.581-7.624 (m, 2H, Ar-H), 7.760-7.784 (m, 2H, Ar-H), 8.024-8.065 (d, 1H, C-H), 9.980 (s, 1H, NH)
3i	3579 (N-H), 3280 (-OH), 3101 (Ar-C-H), 1731 (C=O), 1654 (Aliph-CH=CH), 1567 (Ar-C=C), 726 (C-S-C)	6.003 (s, 1H, Ar-OH), 6.655-6.675 (d, 1H, C-H), 7.003-7.097 (d, 2H, Ar-H), 7.386-7.405 (d, 1H, C-H), 7.531-7.569 (d, 2H, Ar-H), 7.811-7.839 (d, 1H, C-H), 9.859 (s, 1H, NH)
3j	3535 (N-H), 3264 (-OH), 3022 (-OCH_3), 1747 (C=O), 1605 (Aliph-CH=CH), 1562 (Ar-C=C), 743 (C-S-C)	3.811 (s, 3H, Ar-OCH_3), 5.944 (s, 1H, Ar-OH), 7.050-7.100 (m, 2H, Ar-H), 7.178-7.229 (m, 1H, Ar-H), 7.282-7.309 (d, 1H, C-H), 7.382-7.409 (d, 1H, C-H), 7.544-7.571 (d, 1H, C-H), 9.990 (s, 1H, NH)
3k	3214 (N-H), 3064 (-OH), 2955 (Ar-C-H), 1720 (C=O), 1642 (Aliph-CH=CH), 1567 (Ar-C=C), 754 (C-S-C)	5.856 (s, 1H, Ar-OH), 6.782-7.011 (d, 1H, C-H), 7.025-7.214 (d, 2H, C-H), 7.464-7.594 (m, 1H, Ar-H), 7.597-7.624 (m, 3H, Ar-H), 7.780-7.784 (d, 1H, C-H), 9.980 (s, 1H, NH)
3l	3436 (N-H), 3060 (Ar-C-H), 1745 (C=O), 1678 (Aliph-CH=CH), 833 (-Cl), 753 (C-S-C)	6.853-6.988 (d, 1H, C-H), 7.128-7.253 (d, 1H, C-H), 7.533-7.658 (m, 4H, Ar-H), 7.928-7.953 (d, 1H, C-H), 9.928 (s, 1H, NH)

TABLE-3
CONCENTRATION AND ZONE OF INHIBITION OF SYNTHESIZED COMPOUND AGAINST BACTERIA BY CUP-PLATE METHOD

S. No.	Code	*S. aureus*		*B. subtilis*		*E. coli*		*P. aeruginosa*	
		Conc. (µg/mL)	ZOI (mm)	Conc. (µg/mL)	ZOI (mm)	Conc. (µg/mL)	ZOI (mm)	Conc. (µg/mL)	ZOI (mm)
1	3a	30	16	25	17	35	18	30	17
2	3b	25	15	25	16	20	19	20	18
3	3c	35	18	35	19	45	15	45	16
4	3d	50	20	200	12	500	10	500	8
5	3e	10	17	10	22	5	15	10	17
6	3f	15	16	10	23	10	17	10	18
7	3g	15	19	15	20	20	20	20	21
8	3h	30	18	35	19	40	18	45	16
9	3i	25	20	25	21	30	19	45	17
10	3j	40	16	45	17	50	15	100	15
11	3k	50	14	100	14	200	14	200	12
12	3l	10	18	5	15	20	18	20	19
13	Gentamycin	5	19	5	22	5	18	5	22

The synthesized compound *viz.* **(3a), (3b), (3c), (3e), (3f), (3g), (3h), (3i), (3j), (3l)** exhibited good antibacterial activity against *B. substilis*. The synthesized compound *viz.* **(3b), (3e), (3f), (3g), (3i), (3l)** exhibited good antibacterial activity against *E. coli*. The synthesized compound *viz.* **(3a), (3b), (3e), (3f), (3g), (3l)** exhibited good antibacterial activity against *P. aeruginosa*. Thus it can be concluded that the compounds posses antibacterial activity in accordance to the chemical substitution as follows:

4-Cl > 2-Cl > 4-Br > 4-OH > 4-OH, 3-OCH_3 > 4-OCH_3 >2-OH

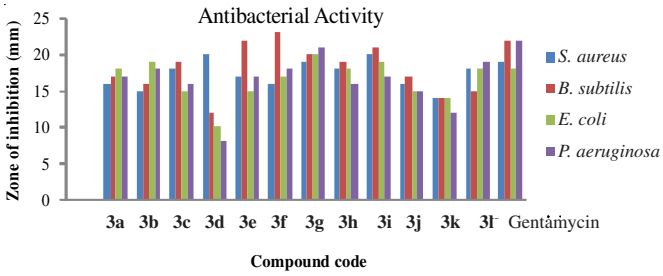


Fig. 1. Zone of inhibition against bacteria

TABLE-4
MIC OF SYNTHESIZED COMPOUNDS AGAINST BACTERIA

S. No.	Comp. code	*S. aureus* (µg/mL)	*B. subtilis* (µg/mL)	*E. coli* (µg/mL)	*P. aeruginosa* (µg/mL)
1	3a	12.5	12.5	25.0	12.5
2	3b	6.25	12.5	6.25	12.5
3	3c	12.5	12.5	25.0	25.0
4	3d	12.5	25.0	50.0	100.0
5	3e	6.25	6.25	3.12	6.25
6	3f	12.5	6.25	12.5	6.25
14	3g	6.25	6.25	6.25	6.25
7	3h	12.5	12.5	25.0	25.0
8	3i	12.5	12.5	12.5	25.0
9	3j	12.5	12.5	25.0	25.0
10	3k	25.0	25.0	25.0	50.0
12	3l	6.25	3.12	12.5	12.5
13	Gentamicin	1.56	1.56	3.12	3.12

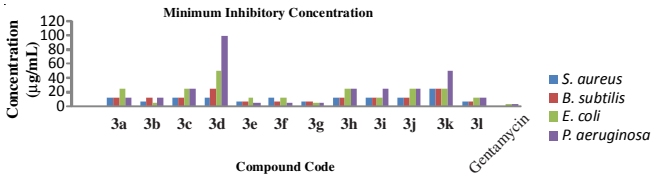


Fig. 2. Minimum inhibitory concentration of synthesized compounds against bacteria

The substitution of -OH group at *ortho* position showed less antibacterial activity but -OH at *para* position and -OH at *para* with -OCH_3 substitution on the ring exhibited better activity than only -OCH_3 substitution. The substitution of-OCH_3 at *para* position show least antibacterial activity against *E. coli* and *P. aeruginosa*. The synthesized compounds **(3e)** and **(3l)** with 4-Cl substitution possess potential to develop as antibacterial agent against gram positive and gram negative bacteria.

Conclusion

5-[1-Substituted-3-(substituted phenyl)allylidine]thiazolidine-2,4-dione derivatives were synthesized. The azotropic removal of water by condensation of 2,4-thia-zolidinedione with substituted benzylidene acetones or substituted cinnamaldehyde in presence of piperidine. The characterization of compounds was one by FT-IR, ^{1}H NMR spectroscopy and melting point. The compounds synthesized were evaluated for antibacterial activity by using cup plate method and minimum inhibitory concentration by two fold serial dilution method. The screening results of the synthesized compounds indicated that the compound **(3a), (3b), (3c), (3e), (3f), (3g), (3h), (3i), (3j), (3l)** exhibited significant antibacterial activity against gram positive and gram negative bacteria.

ACKNOWLEDGEMENTS

The authors are grateful to University of Pune for providing FTIR and ^{1}H NMR facilities.

REFERENCES

1. C. Prabhakar, G. Madhusudhan, K. Sahadev, Ch. Maheedhara Reddy, M.R. Sarma, G.O. Reddy, R. Chakrabarti, C.S. Rao, T.D. Kumar and R. Rajagopalan, *Bioorg. Med. Chem. Lett.*, **8**, 2725 (1998).
2. C.U. Meltem, B. Oya, A. Nurten and E. Rahmiye, *Turk. J. Chem.*, **30**, 355 (2006).
3. B. Oya, O. Ozgen, A. Mentes,e, N. Altanlar, O. Atli, E. Kendi and R. Ertan, *Bioorg. Med. Chem.*, **15**, 6012 (2007).
4. D.J. Funk and A. Kumar, *Crit. Care Clinics*, **27**, 53 (2011).
5. T. Tihomir, N. Zidar, M. Mueller-Premru, D. Kikelj and L.P. Masic, *Eur. J. Med. Chem.*, **45**, 1667 (2009).
6. F.L. Gouveia, R.M. de Oliveira, T.B. de Oliveira, I.M. da Silva, S.C. do Nascimento, K.X. de Sena and J.F. de Albuquerque, *Eur. J. Med. Chem.*, **44**, 2038 (2009).
7. X.F. Liu, C.J. Zheng, L.P. Sun, X.K. Liu and H.R. Piao, *Eur. J. Med. Chem.*, **46**, 3469 (2011).
8. Y.R. Prasad, P.P. Kumar, P.R. Kumar and S. Rao, *E.-J. Chem.*, **5**, 144 (2008).
9. T.B. Meral, B.D. Oya and A.K. Gulgun, *Farmaco*, **58**, 79 (2003).
10. G. Elias and M.N.A. Rao, *Eur. J. Med. Chem.*, **23**, 379 (1988).
11. B. Oya, A. Gulgun, T. Meral and E. Rahmiye, *Turk. J. Chem.*, **23**, 163 (1999).
12. V.K. Parihar, J. Dhawan, S. Kumar and S.N. Manjula, *Chem.-Biol. Int.*, **170**, 49 (2007).