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Microwave-assisted synthesis of some new coumarin–pyrazoline hybrids and their antimicrobial activity

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Abstract: A series of pyrazolines were synthesized by Michael addition of chalcones with hydrazine hydrate in the presence of sodium acetate under conventional heating and microwave irradiation. The structures of the newly synthesized chalcones and pyrazolines were established based on IR, ^{1}H- and ^{13}C--NMR and mass spectral data. All the synthesized compounds were screened for their antimicrobial activity. Some of the compounds showed very good activity compared to standard drugs against all pathogenic bacteria and fungi.

Keywords: coumarin; chalcones; Michael addition; microwave irradiation; pyrazolines.

INTRODUCTION

Coumarins are plant flavonoids widely distributed in nature. Coumarins are important oxygen-containing fused heterocycles used in drugs and dyes.[1] Natural coumarins are known to have antidiabetic activity,[2] anabolic, antioxidant and hepatoprotective activities.[3] Substituted coumarin derivatives were reported to have a variety of biological activities, such as anti-inflammatory,[4] antimicrobial,[5] antioxidant,[6] anticancer[7] and antiviral[8] activities. The potent antibiotics novobiocin and coumaromycin (Fig. 1) are coumarin derivatives. Compounds with a backbone of chalcones were reported to possess various biological activities, such as antimicrobial, anti-inflammatory, analgesic, antiplatelet, anti-ulcerative, antimalarial, anticancer,[9] antiviral, antileishmanial, antioxidant,[10] antitubercular,[11] antihyperglycemic, immunomodulatory, inhibition of the release of chemical mediators,[12] inhibition of leukotriene B4,[13] inhibition of tyrosinase[14] and inhibition of aldose reductase,[15] and estrogenic activities.[16] Licochalcone (Fig. 1) was found to exhibit antimalarial activity.[17] Chalcones are used as starting materials for the synthesis of various chemicals, including plastics, resins, pesti-

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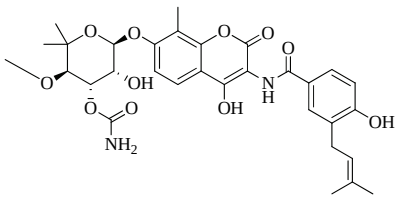
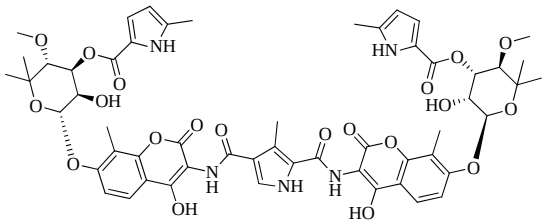
cides, dyes and pharmaceuticals.[18] Hence, the synthesis of chalcones has generated vast interest among organic as well as medicinal chemists.

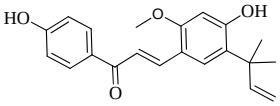


Novobiocin



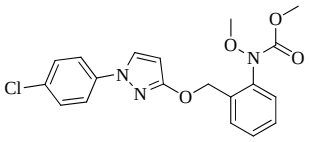
Coumermycin A_1

Coumarin core embedded drugs.



Licochalcone A

A drug based on chalcone motif



Pyraclostrobin

pyrazoline based antifungal agrochemical

Fig. 1. Biologically active compounds embedded with coumarin, chalcone and pyrazoline motifs.

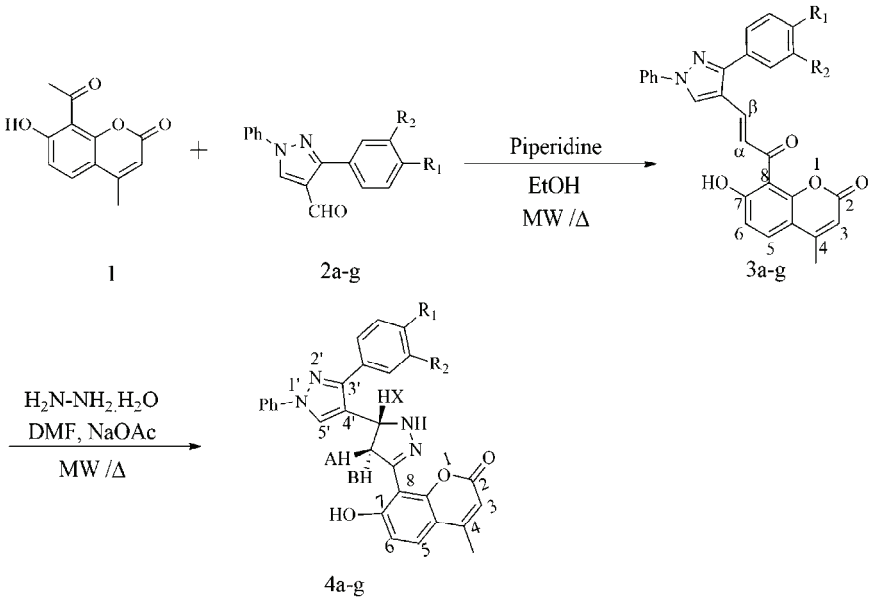
The existing literature is rich with progressive findings concerning the synthesis and pharmacological properties of pyrazolines. Pyrazolines were found to possess antimicrobial,[19] antibacterial,[20] anti-amoebic,[21,22] antidepressant,[23] anticonvulsant,[24] anti-inflammatory,[25,26] and antitumor activities. Pyraelestrobin (Fig. 1) is one of the most important fungicides on the active agrochemicals market.

Microwave irradiation has gained popularity in the past decade as a powerful tool for the rapid and efficient synthesis of a variety of compounds, resulting from the selective absorption of microwave energy by polar molecules.[27] The application of microwave irradiation provides enhanced reaction rates and improved product yields in organic synthesis and it is proving quite successful in the formation of a variety of carbon–heteroatom bonds. Recently, considerable efforts have been made in the design and realization of innovative synthetic protocols in organic synthesis, whereby a more eco-sustainable approach was adopted.[28–30] As part of an ongoing research program, the syntheses of new biologically active pyrazoline derivatives, substituted (*E*)-8-[3-(1,3-diphenyl-1*H*--pyrazol-4-yl)-1-oxo-2-propen-1-yl]-7-hydroxy-4-methyl-2*H*-1-benzopyran-2-one and substituted 8-(4′,5′-dihydro-1,3-diphenyl[4,5′-bi-1*H*-pyrazol]-3′-yl)-7-hyd-

roxy-4-methyl-2*H*-1-benzopyran-2-one, under conventional and non-conventional (microwave irradiation) methods, are reported herein.

RESULTS AND DISCUSSION

In this article, the syntheses are reported of new biologically active pyrazoline derivatives, substituted (*E*)-8-[3-(1,3-diphenyl-1*H*-pyrazol-4-yl)-1-oxo-2--propen-1-yl]-7-hydroxy-4-methyl-2*H*-1-benzopyran-2-one (**3a**–**g**) and substituted 8-(4′,5′-dihydro-1,3-diphenyl[4,5′-bi-1*H*-pyrazol]-3′-yl)-7-hydroxy-4-methyl-2*H*--1-benzopyran-2-one (**4a**–**g**), under conventional and non-conventional (microwave irradiation) methods. The synthesis of new derivatives of pyrazoline (**4a**–**g**) was realized as outlined in Scheme 1. The chalcones were prepared by reacting 8-acetyl-7-hydroxy-4-methylcoumarin (**1**) with substituted 1,3-diphenyl-1*H*-pyrazole-4-carboxaldehydes **2a**–**g** in presence of piperidine by conventional method as well as microwave irradiation using the Claisen–Schmidt condensation. The Michael addition of **3a**–**g** with hydrazine hydrate in DMF was realized by heating conventionally and irradiating with microwaves in the presence of sodium acetate affording the new pyrazoline derivatives **4a**–**g**.



Scheme 1. Synthetic route for the preparation of the coumarin–pyrazoline hybrids **4a**–**g**; **a**, R_1 = H, R_2 = H; **b**, R_1 = OCH_3, R_2 = H; **c**, R_1 = OCH_3, R_2 = OCH_3; **d**, R_1 = CH_3, R_2 = H; **e**, R_1 = F, R_2 = H; **f**, R_1 = Cl, R_2 = H; **g**, R_1 = Br, R_2 = H.

It was found that the synthesis of chalcones **3a**–**g** and pyrazolines **4a**–**g** by conventional method took a longer time and gave lower yields (Table I), when compared to the microwave irradiation technique in which the reaction proceeded

smoothly with excellent yields, within a few minutes for the pyrazolines **4a**–**g** and in 10–15 min for the chalcones **3a**–**g** (Table I).

TABLE I. Comparisons of the yields of the synthesized compounds **3a**–**g** and **4a**–**g**

Compd.	M.p., °C	Conventional method		MWI	
		t / h	Yield, %	*t* /min	Yield, %
3a	202	24	50	10	80
3b	242	20	51	15	80
3c	228	22	52	12	82
3d	206	24	56	14	86
3e	210	24	58	13	87
3f	215	20	50	14	86
3g	207	24	52	15	80
4a	233	2	52	1	80
4b	259	2.5	59	1.5	89
4c	256	3	52	2	80
4d	251	2	56	2	88
4e	253	2.5	56	1	88
4f	232	2	52	2	80
4g	226	3	54	2	86

Analytical and spectral data for compounds **3a**–**g** and **4a**–**g** are given in Supplementary material to this paper. The ^{1}H-NMR spectrum of chalcone **3a** showed characteristic signals at δ 8.04, 8.18 and 8.62 ppm corresponding to H_{α}, H_{β} and the pyrazole H, respectively. In the ^{13}C-NMR spectrum of chalcone **3a**, the carbonyl carbon appeared at δ 193.0 ppm. The mass spectra of **3a** showed the molecular ion peak at *m*/*z* 449 $[M+H]^+$. The ^{1}H-NMR spectrum of pyrazoline derivative **4a** displayed three characteristic signals due to the diastereotopic protons (H_A, H_B and H_X). The H_A proton, which is *cis* to H_X, resonated upfield at δ 3.78 ppm as a doublet of a doublet (*dd*), while the H_B proton, which is *trans* to H_X, resonated downfield at δ 4.09 ppm (*dd*). The H_X proton, which is vicinal to two methylene protons (H_A and H_B), was also observed as a doublet of a doublet at δ 5.14. The cyclization of chalcones into pyrazolines was further supported by the ^{13}C-NMR spectrum of **4a**, in which the C–H_A and C–H_X carbons of the pyrazoline ring resonated at δ 44.2 and 54.3 ppm, respectively. These values are in close agreement with the previously reported values for the pyrazoline carbons C–H_A and C–H_X.[31,32]. The combination of ^{1}H-NMR and ^{13}C-NMR provides strong evidence in support of structures assigned to pyrazoline derivatives. The mass spectrum of **4a** showed the molecular ion peak at *m*/*z* 463 $[M+H]^+$. These data were considered satisfactory for the structures assigned to compounds **3a**–**g** and **4a**–**g.**

Antibacterial activity

All the compounds were screened for their antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* using ampicillin as the standard drug. The activity was determined using cup plate agar diffusion method by measuring the zone of inhibition in mm. The compounds were screened at the concentration of 50 μg mL^{-1} in DMSO. From the screening studies (Table II), it is evident that the synthesized compounds **3b**, **3c**, **4a**, **4b**, **4c** and **4g** showed good antibacterial activity against all the tested organisms. It was further observed that the electron rich chalcone **3c**, with both aryl rings possessing a methoxy substituent, showed the best activity, and closely followed by **3b**, which has only one methoxy substituent. This leads to the conclusion that electron rich chalcones showed higher activity. Furthermore, changing the halogens from F to Cl or Br, did not provide any significant change in the levels of activity against bacteria. However, in the case of the pyrazoles derived from the chalcones, the activity does not depend much on the electronic nature of the compounds. This is evident from the fact that **4a**, **4b**, **4c** and **4g**, all have approximately the same level of activity.

TABLE II. Antibacterial activity (zone of inhibition, mm) of compounds **3a**–**g** and **4a**–**g**

Compd.	Gram-positive bacteria		Gram-negative bacteria	
	S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*
3a	16	4	5	12
3b	28	13	13	35
3c	30	12	9	32
3d	23	5	4	10
3e	22	7	8	25
3f	10	8	2	23
3g	22	10	7	22
4a	29	11	10	27
4b	27	10	8	28
4c	28	11	8	26
4d	20	7	5	22
4e	18	10	6	18
4f	15	9	8	21
4g	28	11	9	28
Ampicillin	30	12	10	30

Antifungal activity

All the compounds were screened for their antifungal activity against *Aspergillus niger*, *Penicillium italicum* and *Fusarium oxysporum* using griseofulvin as the standard drug. The activity was determined using the cup plate agar diffusion method by measuring the zone of inhibition in mm. The compounds were screened at a concentration of 50 μg mL^{-1} in DMSO. From the screening studies

(Table III), it was evident that the synthesized compounds **3b**, **3c**, **3e, 4a, 4c** and **4g** showed good antifungal activity against all the tested organisms. The fluorinated chalcone **3e** showed the highest activity, followed by the electron rich substrates **3b** and **3c**. When the fluorine atom was replaced by bromo or chloro substituents, the activity against fungi decreased. Among the pyrazoles, the unsubstituted compound **4a** showed the highest activity against the fungi. The highly electron rich pyrazole **4c** showed activity which was comparable to that of **4a,** but on the lower side, indicating that substituents on the pyrazole are detrimental to the observed activity. Among the halogen derivates, the bromo substituted compound **4g** showed significantly higher activity compared to those of the fluoro- and chloro-substituted compounds, **4e** and **4f**, respectively.

TABLE III. Antifungal activity (zone of inhibition, mm) of compounds **3a–g** and **4a–g**

Compd.	Fungus		
	A. niger	*P. italicum*	*F. oxysporum*
3a	8	16	22
3b	12	21	25
3c	11	20	23
3d	9	20	26
3e	12	24	28
3f	9	21	23
3g	6	18	23
4a	15	25	28
4b	8	14	19
4c	14	21	24
4d	7	15	18
4e	6	12	14
4f	8	10	10
4g	14	21	23
Griseofulvin	12	20	25

EXPERIMENTAL

Materials

All used materials were obtained commercially, mostly from Sigma–Aldrich, and were used without further purification.

Equipment

The melting points were determined in open capillaries and are uncorrected. The purity of the compounds was checked by TLC on silica gel 60 F_{254} (Merck). The ^{1}H-NMR and ^{13}C--NMR spectra were recorded on a Bruker Avance II 400 spectrometer using TMS as an internal standard. The IR spectra were recorded in KBr on a Shimadzu FTIR 8400S spectrophotometer. The mass spectra were obtained on a Shimadzu GCMS-QP 1000 mass spectrometer. The microwave reactions were performed in a Milestone multiSYNTH microwave system.

*General procedure for the synthesis of (*E*)-8-[3-(1,3-diphenyl-1*H*-pyrazol-4-yl)-1-oxo-2--propen-1-yl]-7-hydroxy-4-methyl-2*H*-1-benzopyran-2-one analogues **3a**–**g***

Conventional method. A mixture of 8-acetyl-7-hydroxy-4-methylcoumarin **1** (1 mmol), substituted 1,3-diphenyl-1*H*-pyrazole-4-carboxaldehydes **2a**–**g** (1 mmol) in ethanol (20 mL) and a few drops of piperidine was taken into a round bottomed flask and stirred at room temperature for 20–24 h. Progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was diluted with cold water and acidified with dil. HCl. The precipitate that formed was filtered, dried and recrystallized from ethanol to afford pure chalcones **3a**–**g**.

Microwave irradiation. A mixture of 8-acetyl-7-hydroxy-4-methylcoumarin **1** (1 mmol), substituted 1-(1,3-diphenyl-1*H*-pyrazole-4-carboxaldehydes **2a**–**g** (1 mmol) in ethanol (2 ml) and few drops of piperidine was taken in a glass vial equipped with a cap and then subjected to microwave irradiation at 100 W for 10 to 15 min. Progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was diluted with cold water and acidified with dil. HCl. The precipitate that formed was filtered, dried and recrystallized from ethanol to afford the pure chalcone.

*General procedure for the synthesis of substituted 8-(4′,5′-dihydro-1,3-diphenyl[4,5′-bi-1*H*--pyrazol]-3′-yl)-7-hydroxy-4-methyl-2*H*-1-benzopyran-2-one **4a**–**g***

Conventional method. To a solution of chalcone **3a**–**g** (1 mmol) in DMF (5 mL) containing sodium acetate (1 mmol), hydrazine hydrate (1 mmol) was added and the reaction mixture was heated at 80–90 °C for 2 to 3 h. The progress of the reaction was monitored by TLC. After the completion of the reaction, ice water was added. The solid product that separated was filtered, washed with water, dried and recrystallized from MeOH:$CHCl_3$ (1:1).

Microwave method. To a solution of chalcones **3a**–**g** (1 mmol) in DMF (1 mL) containing sodium acetate (1 mmol), in a 10 mL glass vial equipped with a cap, hydrazine hydrate (1 mmol) was added and the mixture was then irradiated for 1–2 min at an irradiation power of 180 W. The progress of the reaction was monitored by TLC. After completion of the reaction, ice water was added. The solid product that separated was filtered, washed with water, dried and recrystallized from MeOH:$CHCl_3$ (1:1).

Biological assays

Antibacterial activity, The novel synthesized compounds **3a**–**g** and **4a**–**g** were screened for their antibacterial activity against different types of bacterial strains, *i.e*., Gram-negative bacterial strains of *Pseudomonas aeruginosa* (9027) and *Escherichia coli* (ATCC-8739), Gram-positive bacterial strains of *Bacillus subtilis* (ATCC-11778) and *Staphylococcus aureus* (ATCC-9144) at a concentration of 50 μg mL^{-1}.

The cultures were diluted with 5 % autoclaved saline and the final volume was adjusted to a concentration of approximately 10^5–10^6 CFU mL^{-1}. The synthesized compounds were diluted with acetone for the antibacterial biological assays. For agar disc diffusion method,[33] the liquid form of the test compound was soaked on to a disc (5 mm) and then allowed to air dry, such that the disc became completely saturated with the test compound. The saturated chemical discs were introduced onto the upper layer of the medium evenly loaded with the bacteria and incubated at 37 °C for 24 to 48 h for better inhibition of the bacteria. The zones of inhibition were measured after 24 to 48 h. All the experiments were performed in triplicate and the results are expressed as zone of inhibition in mm. The zones of inhibition of synthesized compounds **3a**–**g** and **4a**–**g** were compared with the zone of inhibition of the standard antibiotic ampicillin (50 μg mL^{-1}).

Antifungal activity. The antifungal activity of synthesized compounds **3a**–**g** and **4a**–**g** was tested against three pathogenic fungi, namely *Fusarium oxysporum* (ATCC-7601), *Aspergillus niger* (ATCC-9029) and *Penicillium italicum* (ATCC-10454), by the poison plate technique at a concentration of 50 μg mL^{-1}. Three kinds of fungi were incubated in potato dextrose agar medium (PDA) at 25±1 °C for 5 days to obtain new mycelium for the antifungal assay and then mycelia as disks of approximately 0.45 cm diameter cut from the culture medium were picked up with a sterilized inoculation needle and inoculated into the centre of a PDA plate. The test compounds were dissolved in acetone (10 mL) then added to the PDA medium (90 mL). The final concentration of compounds in the medium was adjusted to 50 μg mL^{-1}. The inoculated plates were incubated at 25±1 °C for 5 days. Acetone was diluted with sterilized distilled water and used as the control, while griseofulvin (50 μg mL^{-1}) was used as the standard control for each treatment. Three replicates of the experiments were performed. The radial growth of the fungal colonies was measured on the sixth day.

CONCLUSIONS

Two new series of compounds **3a**–**g** and **4a**–**g** were synthesized under conventional and microwave irradiation conditions. In microwave irradiation method, the reactions were completed in shorter times with better yields compared to the conventional method. All the new compounds were screened for their antimicrobial activities. It was observed that compounds **3b**, **3c**, **4a**, **4b**, **4c** and **4g** exhibited broad spectrum of antibacterial activity, and compounds **3b**, **3c**, **3e, 4a, 4c** and **4g** showed a broad spectrum of antifungal activity against all the tested strains compared to the standard drugs at their respective concentrations.

SUPPLEMENTARY MATERIAL

Analytical and spectral data for compounds **3a**–**g** and **4a**–**g** are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

СИНТЕЗА НОВИХ КУМАРИН–ПИРАЗОЛИН ХИБРИДА ОЗРАЧИВАЊЕМ МИКРОТАЛАСИМА И ИСПИТИВАЊЕ ЊИХОВЕ АНТИМИКРОБНЕ АКТИВНОСТИ

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Синтетисана је серија деривата пиразолина **4a**–**g** Мајкловом реакцијом халкона **3a**–**g** са хидразин-хидратом у присуству натријум-ацетата загревањем класичним поступком или микроталасима. Структуре нових халкона **3a**–**g** и пиразолина **4a**–**g** утврђене су на основу IC, ^{1}H- и ^{13}C-NMR спектроскопије и масене спектрометрије. Свим синтетисаним једињењима испитана је антимикробна активност. Неки од деривата показују добре активности у поређењу са стандардним лековима према свим сојевима патогених бактерија и гљива.

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REFERENCES

1. S. Rajasekaran, G. K. Rao, S. P. N. Pai, A. Ranjan, *Int. J. ChemTech Res.* **3** (2011) 555
2. R. Sharma, V. Arya, *J. Chem. Pharm. Res.* **3** (2011) 204
3. R. D. H. Murrey, D. Medez, S. A. Brown, *The natural coumarins: occurrence, chemistry and biochemistry*, Wiley Interscience, New York, 1982, p. 1
4. A. I. Vogel, B. S. Furniss, A. J. Hannaford P. W. G. Smith, A. R. Tatchell, *Vogel's Textbook of Practical Organic Chemistry*, **5**th ed., Longman, Harlow, 1989, p. 1191
5. V. V. Mulwad, J. M. Shirodkhar, *Indian J. Heterocycl. Chem.* **11** (2002) 192
6. K. Manohar, G. Manjunath, K. Raviraj, *Indian J. Heterocycl. Chem.* **13** (2004) 201
7. V. Rajeswar Rao, K. Srimanth, P. Vijaya Kumar, *Indian J. Heterocycl. Chem.* **14** (2004) 141
8. Z. M. Nofal, M. I. El-Zahar, S. S Abd El-Karim, *J. Antimicrob. Agents Chemother.* **5** (2005) 483
9. H. N. Pati, H. L. Holt, R. L. Blanc Jr., J. Dickson, M. Stewart, T. Brown, *Med. Chem. Res.* **14** (2005) 19
10. C. L. Miranda, J. F. Stevens, V. Ivanov, M. McCall, B. Frei, M. L. Deinzer, *J. Agric. Food Chem.* **48** (2000) 3876
11. M. A. Ali, M. Shaharyar, A. A. Siddiqui, *Eur. J. Med. Chem.* **42** (2007) 268
12. H. H. Ko, L. T. Tsao, K. L. Yu, C. T. Liu, J. P. Wang, C. N. Lin, *Bioorg. Med. Chem.* **11** (2003) 105
13. A. M. Deshpande, N. P. Argade, A. A. Natu, J. Eckman, *Bioorg. Med. Chem.* **7** (1999) 1237
14. S. Khatib, O. Nerya, R. Musa, M. Shmnel, S. Tamir, J. Vaya, *Bioorg. Med. Chem.* **13** (2005) 433
15. F. Severi, S. Benvenuti, L. Costantino, G. Vampa, M. Melegari, L. Antolini, *Eur. J. Med. Chem.* **33** (1998) 859
16. Y. Kohno, S. Kitamura, S. Sanoh, K. Sugihara, N. Fujimoto, S. Ohta, *J. Pharmacol. Exp. Ther.* **33** (2005) 1115
17. B. Srinivasan, T. E. Johnson, R. Lad, C. Xing, *J. Med. Chem.* **52** (2009) 7228
18. D. L. Opdyke, *Food Cosmet. Toxicol.* **11** (1973) 1011
19. B. F. Abdel-Wahab, H. A. Abdel-Aziz, E. M. Ahmed, *Eur. J. Med. Chem.* **44** (2009) 2632
20. M. Abid, A. Azam, *Bioorg. Med. Chem. Lett.* **16** (2006) 2812
21. M. Abid, A. R. Bhat, F. Athar, A. Azam, *Eur. J. Med. Chem.* **44** (2009) 417
22. Y. R. Prasad, A. L. Rao, L. Prasoona, K. Murali, P. R. Kumar, *Bioorg. Med. Chem. Lett.* **15** (2005) 5030
23. Z. Ozdemir, H. B. Kandilci, B. Gumusel, U. Calıs, A. A. Bilgin, *Eur. J. Med. Chem.* **42** (2007) 373
24. I. G. Rathish, K. Javed, S. Ahmad, S. Bano, M. S. Alam, K. K. Pillai, S. Singh, V. Bagchi, *Bioorg. Med. Chem. Lett.* **19** (2009) 255
25. N. Gokhan-Kelekçi, S. Yabanoglu, E. Kupeli, U. Salgın, O. Ozgen, G. Ucar, E. Yeşilada, E. Kendi, A. Yeşilada, A. A. Bilgin, *Bioorg. Med. Chem.* **15** (2007) 5775
26. E. C. Taylor, H. H. Patel, *Tetrahedron* **48** (1992) 8089
27. C. O. Kappe, *Angew. Chem. Int. Ed. Engl.* **43** (2004) 6256
28. D. Ashok, A. Ganesh, *Indian J. Heterocycl. Chem.* **23** (2013) 7
29. D. Ashok, K. Sudershan, M. Khalilullah, *Green Chem. Lett. Rev.* **5** (2012) 121
30. D. Ashok, D. Shravani, *Tetrahedron Lett.* **49** (2008) 7227
31. R. Aggarwal, V. Kumar, S. P. Singh, *Indian J. Chem., B* **46** (2007) 1332
32. A. Ozdemir, G. Turan-Zitouni, Z. A. Kaplancikh, G. Revial, K. Guven, *Eur. J. Med. Chem.* **42** (2008) 403
33. H. J. Benson, *Microbiological Applications*, W. C. Brown Publications, Boston, MA, 1990.