Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 76 (6) 823–830 (2011)
JSCS–4162
UDC 547.831+547.592+547.447:542.913
Original scientific paper

An efficient, microwave-assisted, one-pot synthesis of novel 5,6,7,8-tetrahydroquinoline-3-carbonitriles

DIPTI K. DODIYA*, HARESH K. RAM, AMIT R. TRIVEDI and VIRESH H. SHAH

Department of Chemistry, Saurashtra University, Rajkot-360 005, Gujarat, India

(Received 2 July 2010, revised 9 January 2011)

Abstract: An efficient, microwave-assisted synthesis of novel 2-alkoxy-5,6,7,8--tetrahydroquinoline-3-carbonitriles, which have not hitherto been reported, *via* reactions of cyclohexanone and arylidene malononitriles in the corresponding alcohols in presence of sodium is described. All the newly synthesized compounds were characterized by the IR, ^{1}H-NMR, ^{13}C-NMR and mass spectrescopic techniques and by elemental analyses. The newly synthesized compounds were evaluated for their antibacterial and antifungal activities.

Keywords: 2-alkoxytetrahydroquinoline-3-carbonitriles; quinoline-3-carbonitriles; one-pot synthesis; microwave-assisted synthesis; cyclohexanone; arylidene malononitriles.

INTRODUCTION

As a privileged fragment, quinoline is a ubiquitous subunit in many quinoline-containing natural products with remarkable biological activities. Members of this family have wide applications in medicinal chemistry, being used as antimalarial, anti-inflammatory, antiasthmatic, antibacterial, antihypertensive and tyrosine kinase inhibiting agents.[1] Because of their importance as substructures in a broad range of natural and designed products, significant efforts continue to be directed into the development of new quinoline-based structures.[2]

Among quinoline derivatives, tetrahydroquinolines are an important structural subunit of natural products and many tetrahydroquinoline derivatives exhibit interesting biological and pharmaceutical activities,[3] including anti-HIV,[4,5] anticancer,[6] antimalarial,[7] cholesteryl ester transfer protein inhibitors,[8] anti-diabetic,[9] *etc*. Consequently, synthetic methodologies for preparing tetrahydroquinoline derivatives have attracted considerable interest and several methods offering good results have been reported. However, most of them describe the synthesis of

* Corresponding author. E-mail: diptid85@gmail.com
doi: 10.2298/JSC100702071D

the 1,2,3,4-tetrahydroquinoline nucleus,[10–12] and concise methods to access usefully functionalized 5,6,7,8-tetrahydroquinolines are scarce in the literature.[13]

Recently, 5,6,7,8-tetrahydroquinolines have drawn considerable attention due to their interesting pharmacological applications as RET tyrosine kinase inhibitors,[14] anti-HIV,[15,16] anti-fungal,[17] anti-cancer[18] and C5a receptor antagonists agents.[19] The development of simple synthetic routes for widely used organic compounds from readily available reagents is one of the major tasks in organic synthesis. Application of microwave irradiation in achieving this task has been the focus of considerable attention in recent years and is becoming an increasingly popular technology.[20,21]

In view of these observations and as part of a continuing effort in our laboratory towards the development of new methods for the expeditious synthesis of biologically relevant heterocyclic compounds,[22] herein, a simple and efficient microwave-assisted synthesis of functionalized novel 2-alkoxy-5,6,7,8-tetrahydroquinoline-3-carbonitriles, which are also structurally relevant to recently reported bioactive 5,6,7,8-tetrahydroquinolines,[16–19] is described (Fig. 1).

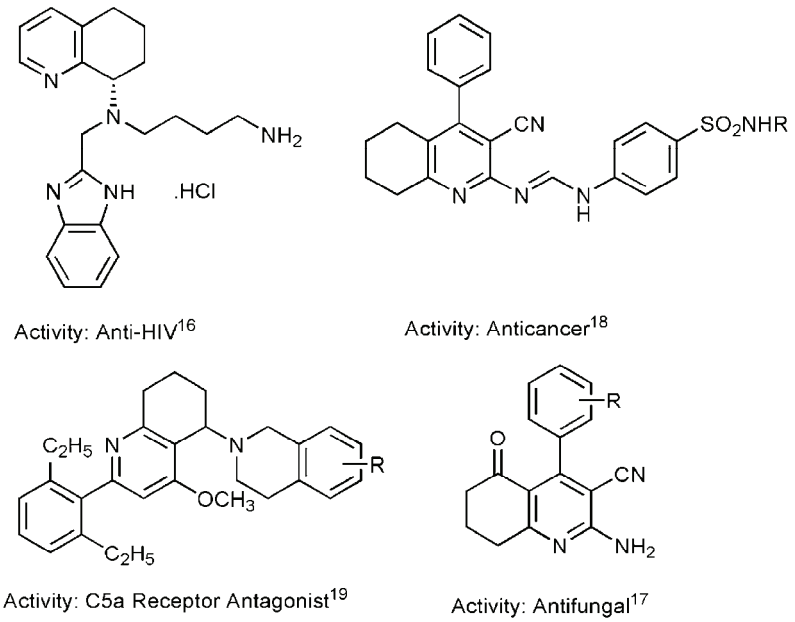


Fig. 1. Examples of some bioactive 5,6,7,8-tetrahydroquinolines structurally relevant to the synthesized 5,6,7,8-tetrahydroquinoline-3-carbonitriles.

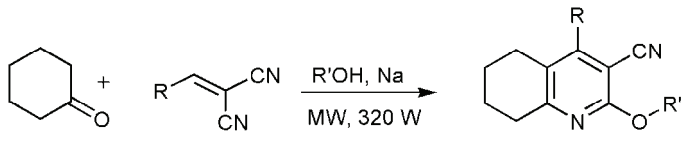
All the newly synthesized compounds **3a**–**j** were evaluated for their antibacterial and antifungal activity. The biological activity of the synthesized compounds was compared with reference standard drugs.

RESULTS AND DISCUSSION

Chemistry

There are very few reports in literature for the synthesis of 2-alkoxypyridine--3-carbonitriles,[23,24] but most of them do not describe the synthesis of fused pyridines or benzo-fused pyridines, *i.e.*, quinolines. During an extensive literature survey, only one such synthesis of benzothiepino-fused 2-alkoxy-pyridine-3-carbonitriles was found.[25] Herein, the efficient and rapid microwave-assisted one--pot synthesis of functionalized novel 2-alkoxy-5,6,7,8-tetrahydroquinoline-3--carbonitriles, which have not hitherto been reported, *via* the reactions of cyclohexanone, arylidene malononitriles in the corresponding alcohol in the presence of sodium is reported.

The arylidene malononitriles **2** were prepared following a reported literature procedure.[26] Treatment of cyclohexanone **1** with arylidene malononitriles **2** in the corresponding alcohol in the presence of sodium under microwave irradiation at 300 W afforded the 2-alkoxy-5,6,7,8-tetrahydroquinoline-3-carbonitriles **3a**–**j** in excellent yields (88–95 %) in a very short time (Scheme 1) as compared to conventional heating, which results in lower yields after longer reaction times (5––10 h).



1 2 3

3a R= phenyl,
3b R= 4-chlorophenyl,
3c R= 4-fluorophenyl,
3d R= 4-methoxyphenyl,
3e R= 4-hydroxyphenyl
3f R= phenyl,
3g R= 4-chlorophenyl,
3h R= 4-fluorophenyl,
3i R= 4-methoxyphenyl
3j R= 4-hydroxyphenyl

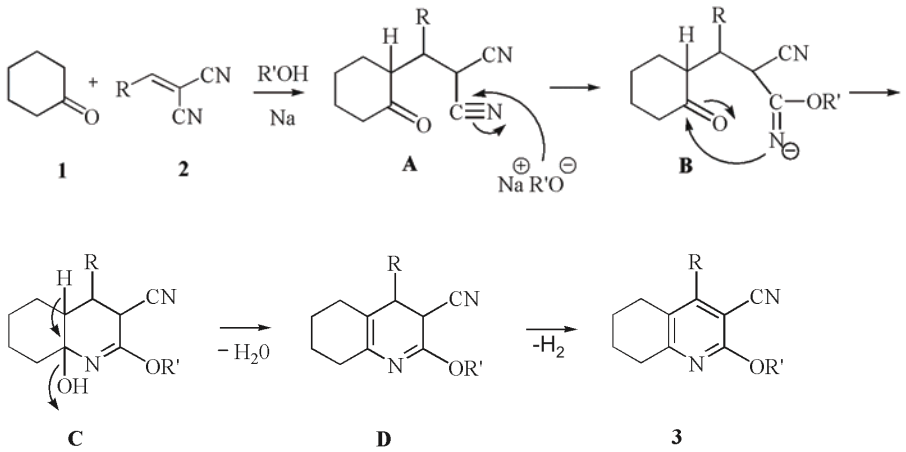
3a-e R' = methyl,
3f-j R' = ethyl

Scheme 1. Microwave-assisted synthesis of 2-alkoxy-5,6,7,8--tetrahydroquinoline-3-carbonitriles.

The proposed mechanism[27] involves Michael addition between **1** and **2** to generate intermediate **A**, followed by nucleophilic alkoxide attack at one of the nitrile groups of **A** with dehydration and subsequent dehydrogenation to give the tetrahydroquinoline **3** (Scheme 2).

All the newly synthesized 2-methoxy- and 2-ethoxy-5,6,7,8-tetrahydroquinolines **3a–j** were characterized by IR, ^{1}H-NMR, ^{13}C-NMR, mass spectroscopy

and elemental analyses (Supplementary material). The IR spectra of **3a–j** revealed the appearance of confirmatory bands characteristics of the stretching vibrations of the –CN and C=N groups at 2231–2225 cm^{-1} and 1635–1566 cm^{-1}, respectively. Furthermore, asymmetric and symmetric stretching vibration bands of the ether (C–O–C) linkage were also present in the IR spectra in the range of 1296–1263 cm^{-1} and 1097–1018 cm^{-1}, respectively, which confirmed the presence of 2-alkoxy groups. The ^{1}H-NMR spectra of compounds **3a–e** showed confirmatory signals in the δ range 3.75–3.80 ppm as a singlet for ($-OCH_3$), while the ^{1}H-NMR spectra of compounds **3f–j** revealed signals at δ 1.27–1.44 ppm as a triplet for the ($-CH_2-C\mathbf{H}_3$) and signals at δ 4.15–4.51 ppm as a quartet for ($-C\mathbf{H}_2-CH_3$) of the 2-ethoxy group in the title compounds, which confirmed the structure. The ^{13}C-NMR signals were also found to be in full agreement with the proposed structure.



Scheme 2. Proposed mechanism for synthesis of 2-alkoxy-5,6,7,8--tetrahydroquinoline-3-carbonitriles

Biological screening

All the synthesized compounds **3a–j** exhibited moderate to good antimicrobial activity. Compound **3i** showed the highest activity against all the bacteria and fungi. Compounds **3c** and **3g** also showed quite good antibacterial activity as compared to the standard drugs ampicillin and chloramphenicol. Compounds **3c**, **3g** and **3h** exhibited good antifungal activity against *A. niger* as compared to the standard griseofulvin.

EXPERIMENTAL

Chemistry

All chemicals were supplied either by E. Merck (Germany) or by S. D. Fine Chemicals (India). Melting points were determined in open capillaries and are uncorrected. The IR spec-

tra were recorded on a Shimadzu-FT-IR-8400 (Fourier transform infrared (FTIR)). The IR spectra were taken using KBr pellets. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AMX-400 spectrometer at 400 MHz and 100 MHz, respectively, in DMSO-d_6 with TMS as an internal standard. Gas chromatography–mass spectrometry (GC–MS) was performed on a Shimadzu-GCMS QP2010 series instrument and elemental analysis was performed using a Heraus CHN rapid analyzer. The microwave-assisted reactions were realised in a QPro-M microwave synthesizer.

*General procedure for the synthesis of 2-alkoxy-5,6,7,8-tetrahydroquinoline-3-carbonitriles (**3a–j**)*

A mixture of cyclohexanone **1** (2.5 mmol), arylidene malononitrile **2** (2.5 mmol) in the appropriate alcohol (15 mL) containing sodium (0.05 g) was irradiated under microwaves at 300 W for an appropriate time (Table I). The separated solid was collected, washed with water and then with methanol affording the corresponding **3a**–**j** in good purity. Further purification of the product was not required by recrystallization or any other method.

TABLE I. Microwave assisted synthesis of 2-alkoxy-5,6,7,8-tetrahydroquinoline-3-carbonitriles

Compound	R	R’	Yield, %	Time, min
3a	Phenyl	$–CH_3$	91	4
3b	4-Chlorophenyl	$–CH_3$	95	5
3c	4-Fluorophenyl	$–CH_3$	92	5
3d	4-Methoxyphenyl	$–CH_3$	89	5
3e	4-Hydroxyphenyl	$–CH_3$	94	7
3f	Phenyl	$–C_2H_5$	89	5
3g	4-Chlorophenyl	$–C_2H_5$	94	7
3h	4-Fluorophenyl	$–C_2H_5$	92	6
3i	4-Methoxyphenyl	$–C_2H_5$	92	6
3j	4-Hydroxyphenyl	$–C_2H_5$	88	8

Biological screening

Antimicrobial activity. All the title compounds **3a**–**j** were evaluated for their *in vitro* antimicrobial activity against two Gram-positive bacterial strains, *Streptococcus pyogenes*, *Staphylococcus aureus* and two Gram-negative bacterial strains *Escherichia coli*, *Bacillus subtilis* and one fungal strain *Aspergillus niger* at a concentration of 50 μg mL^{-1} in *N*,*N*-dimethylformamide using the cup plate method.[28] The solvent, *N*,*N*-dimethylformamide, showed no zone of inhibition. After 24 h of incubation at 37 °C, the zones of inhibition were measured in mm. The activities were compared with those of some known antibiotics, *i.e.*, ampicillin, chloramphenicol, ciprofloxacin, as well as griseofulvin at the same concentration. The results are summarized in Table II.

TABLE II. Antimicrobial activity of 2-alkoxy-5,6,7,8-tetrahydroquinoline-3-carbonitriles **3a**–**j**

No.	R	Zone of inhibition, mm				
		S. pyogenes	*S. aureus*	*E. coli*	*B. subtilis*	*A. niger*
3a	Phenyl	13	13	10	09	11
3b	4-Chlorophenyl	11	14	12	12	12
3c	4-Fluorophenyl	14	13	12	11	14

TABLE II. Continued

No.	R	Zone of inhibition, mm				
		S. pyogenes	*S. aureus*	*E. coli*	*B. subtilis*	*A. niger*
3d	4-Methoxyphenyl	13	13	10	10	12
3e	4-Hydroxyphenyl	9	12	10	10	12
3f	Phenyl	12	12	11	11	11
3g	4-Chlorophenyl	14	15	15	14	13
3h	4-Fluorophenyl	10	12	12	12	12
3i	4-Methoxyphenyl	14	13	13	16	18
3j	4-Hydroxyphenyl	13	14	10	10	10
Ampicillin		16	18	16	18	–
Chloramphenicol		18	16	19	16	–
Ciprofloxacin		23	17	20	19	–
Griseofulvin		–	–	–	–	20

CONCLUSIONS

A new and efficient microwave-assisted synthesis of novel functionalized 2-alkoxy-5,6,7,8-tetrahydroquinoline-3-carbonitriles from cyclohexanone and arylidene malononitriles has been reported. Considering the availability of the starting materials, the simple reaction procedure, simple work-up and robust nature, this chemical process provides a very straightforward route to construct various highly functionalized tetrahydroquinoline-3-carbonitriles.

The newly synthesized heterocycles exhibited moderate to promising antimicrobial activity against standard strains. These results make them interesting lead molecules for further synthetic and biological evaluation. It can be concluded that this class of compounds certainly hold great promise towards the pursuit of discovering novel classes of antimicrobial agents. Further studies to acquire more information concerning structure–activity relationships are in progress.

SUPPLEMENTARY MATERIAL

The results of IR, ^{1}H-NMR, ^{13}C-NMR, mass spectroscopy and elemental analyses of synthesized compounds are available electronically at http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

Acknowledgements. The authors are thankful to the Department of Chemistry, Saurashtra University for providing the laboratory facilities. Ms. Dipti K. Dodiya is thankful to UGC, New Delhi for providing financial support in the form of a Research Fellowship in Sciences for Meritorious Students.

И З В О Д

ЕФИКАСНА СИНТЕЗА 5,6,7,8-ТЕТРАХИДРОХИНОЛИН-3-КАРБОНИТРИЛА ОЗРАЧИВАЊЕМ МИКРОТАЛАСИМА

DIPTI K. DODIYA*, HARESH K. RAM, AMIT R. TRIVEDI и VIRESH H. SHAH

Department of Chemistry, Saurashtra University, Rajkot-360 005, Gujarat, India

Описана је ефикасна синтеза нових 2-алкокси-5,6,7,8-тетрахидрохинолин-3-карбонитрила, озрачивањем микроталасима, полазећи од циклохексанона и арилиден-малононитрила

у одговарајућем алкохолу, у присуству натријума. Сва нова једињења окарактерисана су IC, ^{1}H-NMR и ^{13}C-NMR спектроскопијом и масеном спектрометријом и елементалном анализом. Испитана је антибактеријска и антифунгална активност нових синтетисаних једињења.

(Примљено 2. јула 2010, ревидирано 9. јануара 2011)

REFERENCES

1. a) R. D. Larsen, E. G. Corley, A. O. King, J. D. Carrol, P. Davis, T. R. Verhoeven, P. J. Reider, M. Labelle, J. Y. Gauthier, Y. B. Xiang, R. Zamboni, *J. Org. Chem.* **61** (1996) 3398; b) Y. L. Chen, K. C. Fang, J. Y. Sheu, S. L. Hsu, C. C. Tzeng, *J. Med. Chem.* **44** (2001) 2374; c) M. D. Roma, G. Braccio, M. Chia, *Eur. J. Med. Chem.* **35** (2000) 1021; d) D. Doube, M. Bloun,; C. Brideau, C. Chan, S. Desmarais, D. Eithier, J. P. Falgueyeret, R. W. Friesen, M. Girad, Y. Girad,; J. Guay, P. Tagari, R. N. Yong, *Bioorg. Med. Chem. Lett.* **8** (1998) 1255; e) M. P. Maguire, K. R. Sheets, K. McVety, A. P. Spada, A. Zilberstein, *J. Med. Chem.* **37** (1994) 2129
2. M. Z. Hoemann, G. Kumaravel, R. L. Xie, R. F. Rossi, S. Meyer, A. Sidhu, G. D. Cuny, J. R. Hauske, *Bioorg. Med. Chem. Lett.* **10** (2000) 2675
3. a) M. P. Mertes, A. J. Lin, *J. Med. Chem.* **13** (1970) 276; b) M. Romesh, P. S. Moham, P. A. Shanmugam, *Tetrahedron* **40** (1984) 4041; c) N. B. Perry, J. W. Blunt, J. D. McCombs, M. H. Munro, *J. Org. Chem.* **51** (1986) 5476; d) N. M. Williamson, P. R. March, A. D. Ward, *Tetrahedron Lett.* **36** (1995) 7721; e) N. DeKempe, M. Keppens, *Tetrahedron* **52** (1996) 3705; f) A. Padwa, M. A. Brodney, B. Liu, K. Satake, T. A. Wu, *J. Org. Chem.* **64** (1999) 3595; g) R. Hiessbock, C. Wolf, E. Richter, M. Hitzler, P. Chiba, M. Kratzel, G. Ecker, *J. Med. Chem.* **42** (1999) 1921; h) D. B. Dimon, W. Robert, JP 2001163859 (2001); i) R. N. Asolkar, D. Schroder, R. Heckmann, S. Lang, I. Wagner--Dobler, H. Laatsch, , *J. Antibiotics* **57** (2004) 17
4. D. S. Su, A. J. Neville, J. J. Lim, E. Tinney, T. M. Williams, PCT Int. Appl. (2007)
5. R. T. Skerlj, G. J. Bridger, A. Kaller, E. J. McEachern, J. B. Crawford, Y. Zhou, B. Atsma, J. Langille, S. Nan, D. Veale, T. Wilson, C. Harwig, S. Hatse, K. Princen, E. De Clercq, D. Schols, *J. Med. Chem.* **53** (2010) 3376
6. a) E. Lukevics, T. Lapina, I. Segals, I. Augustane, V. N. Verovskii, *Khim.-Furm. Zh.* **22** (1988) 947; b) C. Ramesh, T. K. Nayak, R. Burai, M. K. Dennis, H. J. Hathaway, L. A. Sklar, E. R. Prossnitz, J. B. Arterburn, *J. Med. Chem.* **53** (2010) 1004
7. V. J. Bulbule, K. Rivas, C. L. Verlinde, W. C. Van-Voorhis, M. H. Gelb, *J. Med. Chem.* **51** (2008) 384
8. T. A. Rano, E. Sieber-McMaster, P. D. Pelton, M. Yang, K. T. Demarest, G. H. Kuo, *Bioorg. Med. Chem. Lett.* **19** (2009) 2456
9. C. Parmenon, J. Guillard, D.-H. Caignard, N. Hennuyer, B. Staels, V. Audinot-Bouchez, J. Boutin, C. Dacquet, A. Ktorza, M.-C. Viaud, *Bioorg. Med. Chem. Lett.* **18** (2008) 1617
10. a) S. Murahashi, Y. Imada, Y. Hirai, *Tetrahedron Lett.* **28** (1987) 77; b) J. N. Reed, J. Rotchford, D. Strickland, *Tetrahedron Lett.* **29** (1988) 5725; c) E. Borrione, M. Prato, G. Scorrano, M. Stivanello, V. Lucchini, G. Valle, *J. Chem. Soc., Perkin Trans. 1* (1989) 2245; d) L. Basolo, E. Beccalli, E. Borsini, G. Broggini, S. Pellegrino, *Tetrahedron* **64** (2008) 8182; e) V. Shridharan, C. Avendano, J. C. Menendez, *Synthesis* (2008) 1039; f) D. L. J. Clive, J. Peng, S. P. Fletcher, V. E. Ziffle, D. Wingert, *J. Org. Chem.* **73** (2008) 2330; g) S. V. Ryabukhin, A. S. Plaskon, D. M. Volochnyuk, S. E. Pipko, A. A. Tolmachev, *Synth. Commun.* **38** (2008) 3032; h) A. R. Jagdale, R. S. Reddy, A. Sudalai, *Org. Lett.* **11** (2009) 803; i) A. Patti, S. Pedotti, *Tetrahedron* **66** (2010) 5607; j) H.-G. Cheng,

C.-B. Chen, F. Tan, N.-J. Chang, J.-R. Chen, W.-J. Xiao, *Eur. J. Org. Chem.* **26** (2010) 4976
11. For leading references see: a) T. Nishio, M. Tabata, H. Koyama, M. Sakamoto, *Helv. Chim. Acta* **88** (2005) 78; b) A. R. Katritzky, S. Rachwal, B. Rachwal, *Tetrahedron* **52** (1996) 15031; c) G.-D. Isabelle, P. Gastaud, T. V. RajanBabu, *Org. Lett.* **3** (2001) 2053
12. a) V. Sriramurthy, O. Kwon, *Org. Lett.* 12 (2010) 1084; b) T. H. Babu, G. Shanthi, P. T. Perumal, *Tetrahedron Lett.* 50 (2009) 2881; c) D. L. J. Clive, J. Peng, S. P. Fletcher, V. E. Ziffle, D. Wingert, *J. Org. Chem.* **73** (2008) 2330
13. K. A. Skupinska, E. J. McEachern, R. T. Skerlj, G. J. Bridger, *J. Org. Chem.* **67** (2002) 7890
14. W. Brandt, L. Mologni, L. Preu, T. Lemcke, C. Gambacorti-Passerini, C. Kunick, *Eur. J. Med. Chem.* **45** (2010) 2919
15. K. S. Gudmundsson, S. D. Boggs, J. G. Catalano, A. Svolto, A. Spaltenstein, M. Thomson, P. Wheelan, S. Jenkinson, *Bioorg. Med. Chem. Lett.* **19** (2009) 6399
16. J. B. Crawford, G. Chen, D. Gauthier, T. Wilson, B. Carpenter, I. R. Baird, E. McEachern, A. Kaller, C. Harwig, B. Atsma, R. T. Skerlj, G. J. Bridger, *Org. Process Res. Dev.* **12** (2008) 823
17. A. R. Gholap, K. S. Toti, F. Shirazi, R. Kumari, M. Bhat, M. V. Deshpande, K. V. Srinivasan, *Bioorg. Med. Chem.* **15** (2007) 6705
18. M. M. Ghorab, F. A. Ragab, M. M. Hamed, *Eur. J. Med. Chem.* **44** (2009) 4211
19. J. K. Barbay, Y. Gong, M. Buntinx, J. Li, C. Claes, P. J. Hornby, G. V. Lommen, J. V. Wauweb, W. Hea, *Bioorg. Med. Chem. Lett.* **18** (2008) 2544
20. For recent reviews and books on microwave-assisted organic synthesis, see: a) C. Oliver Kappe, *Angew. Chem. Int. Ed.* **43** (2004) 6250; b) A. Loupy, *Microwaves in Organic Synthesis*, 2nd ed., Wiley-VCH, Weinheim, 2006; c) V. Polshettiwar, R. S. Varma, *Acc. Chem. Res.* **41** (2008) 629; d) P. Appukkuttan, E. V. Eycken, *Eur. J. Org. Chem.* (2008) 1133; e) A. Barge, S. Tagliapietra, L. Tei, P. Cintas, G. Cravotto, *Curr. Org. Chem.* **12** (2008) 1588; f) M. Pineiro, T. Pinho-e-Melo, *Eur. J. Org. Chem.* (2009) 5287
21. For recent examples of papers on microwave activation, see: a) E. M. Beccalli, G. Broggini, M. Martinelli, G. Paladino, E. Rossi, *Synthesis* (2006) 2404; b) H. Tsukamoto, T. Matsumoto, Y. Kondo, *J. Am. Chem. Soc.* **130** (2008) 388; c) M. Andaloussi, J. Lindh, J. Savmarker, P. J. R. Sjoberg, M. Larhed, *Chem. Eur. J.* **15** (2009) 13069; d) A. Reichelt, J. R. Falsey, R. M. Rzasa, O. R. Thiel, M. M. Achmatowicz, R. D. Larsen, D. Zhang, *Org. Lett.* **12** (2010) 792; e) B. Roberts, D. Liptrot, L. Alcaraz, *Org. Lett.* **12** (2010) 1264; f) E. M. Beccalli, A. Bernasconi, E. Borsini, G. Broggini, M. Rigamonti, G. Zecchi, *J. Org. Chem.* **75** (2010) 6923
22. a) D. K. Dodiya, A. R. Trivedi, S. H. Jarsania, S. J. Vaghasiya, V. H. Shah, *J. Serb. Chem. Soc.* **73** (2008) 683; b) A. R. Trivedi, A. B. Siddiqui, D. K. Dodiya, M. J. Soalnki, V. H. Shah, *J. Sulfur Chem.* **30** (2009) 590
23. F. E. Goda, A. A.-M. Abdel-Azizb, O. A. Attefc, *Bioorg. Med. Chem.* **12** (2004) 1845
24. J. N. Godhasra, M. C. Patel, N. N. Kansagara, B. P. Thanki, V. R. Shah, *Indian J. Heterocycl. Chem.* **18** (2009), 369
25. A. S. Girgis, N. Mishriky, M. Ellithey, H. M. Hosnia, H. Farag, *Bioorg. Med. Chem.* **15** (2007) 2403
26. N. M. Abd El-Rahman, A. A. El-Kateb, M. F. Mady, *Synth. Commun.* **37** (2007) 3961
27. O. Sangtae, J. H. In, A. M. Chan, S. Woon-Seob, L. Seokjoon, *Bioorg. Med. Chem.* **12** (2004) 3783
28. *Practical medical microbiology*, 14th ed., G. Collee, J. P. Duguid, A. G. Froser, B. P. Marmion, Eds., Churchill Livingstone, Edinburgh, UK, 1996.