Access to new antimicrobial 4-methylumbelliferone derivatives

MARWA ZAYANE^a, ANIS ROMDHANE^{a,*}, MEJDA DAAMI-REMADI^b and
HICHEM BEN JANNET^a

^aLaboratoire de Chimie Hétérocyclique, Produits Naturels et Réactivité, Equipe: Chimie Médicinale et Produits Naturels, Faculté des Sciences de Monastir, Université de Monastir, Avenue de l'Environnement, 5019 Monastir, Tunisie

^bUR13AGR09, Production Horticole Intégrée au Centre Est Tunisien, Centre Régional des Recherches en Horticulture et Agriculture Biologique de Chott-Mariem, Université de Sousse, 4042, Chott-Mariem, Tunisie
e-mail: anis_romdhane@yahoo.fr

MS received 4 April 2015; revised 16 June 2015; accepted 21 June 2015

Abstract. Synthesis of some novel coumarin esters has been accomplished through iodine-catalyzed method using 4-methylumbelliferone as the starting material. Condensation of hydrazide, which was obtained in two steps from 4-methylumbelliferone, with some arylaldehydes provided hydrazone derivatives, while the reaction with phenylthioisocyanate leads to the thiosemicarbazide that evolved into two new compounds. Finally, condensation reaction of hydrazide with three diketones afforded new pyrrole and pyrazole derivatives. Structures of all synthesized compounds were established on the basis of spectroscopic methods including ¹H NMR, ¹³C NMR and ES-HRMS. They were finally tested for their antimicrobial activity and the structure-activity relationship was discussed.

Keywords. 7-hydroxy-4-methyl-coumarin; (4-methylumbelliferone); imine; triazole; thiadiazole; pyrazole; pyrrole; antimicrobial activity.

1. Introduction

Coumarins occupy an important place in the realm of natural products and organic synthesis. They constitute an important class of oxygen heterocycles and belong to the family of lactones having benzopyrane system.¹ The coumarin skeleton is found in many natural products and is also used as an intermediate for the synthesis of specific and promising heterocyclic compounds, possessing a wide spectrum of biological activities such as anti-inflammatory,² anti-tubercular,³ antioxidant,⁴ antimicrobial⁵ and anticoagulant agents.⁶ Many methods for the preparation of coumarin-based compounds have been reported.^{7–9} One of the main representative of this class is the 4-methylumbelliferone, used as a useful precursor for the preparation of such compounds.^{10–12}

The study of nitrogen heterocyclic compounds has led a considerable development due to the revelation of their varied effects in diverse domains. In this context, pyrroles, pyrazoles, thiadiazoles and triazoles, and their derivatives are very attractive targets. They have been the subject of many chemical and biological studies on account of their pharmacological activity, such as

antimicrobial,¹³ anticancer,¹⁴ antitubercular,¹⁵ antioxidant and anti-inflammatory agents.¹⁶

In view of the above observations and as a continuation of our previous work on the synthesis of new coumarin derivatives,¹⁷ we report here the synthesis of a precursor **2** from the 4-methylumbelliferone **1** and its use as building blocks in the preparation of some novel coumarin esters **4** and other new derivatives **6–13** incorporating the coumarin and pyrrole, pyrazole, thiadiazole or triazole moieties and their antibacterial and antifungal activity.

2. Experimental

2.1 General information

All reactions were monitored by TLC using aluminium sheets of Merck silica gel 60 F₂₅₄, 0.2 mm thickness. Melting temperatures were determined on an electrothermal 9002 apparatus and were reported as uncorrected. NMR spectra were recorded on a Bruker AC-300 spectrometer at 300 MHz (¹H) and 75 MHz (¹³C). All chemical shifts are reported as δ values (ppm) relative to residual non-deuterated solvent. Mass spectra were obtained with ESI-TOF (LCT, Waters) using the reflectron mode in the positive ion mode.

*For correspondence

2.2 Synthesis

2.2a Preparation of ethyl 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetate 2: Equimolar solution (15 mmol) of 7-hydroxy-4-methylcoumarin **1**, anhydrous potassium carbonate and ethyl chloroacetate was refluxed in dry DMF (40 mL) with continuous stirring for 5 h under argon atmosphere. After addition of ice water, the solid formed was collected by filtration, dried and recrystallized from ethanol to give compound **2**.

2.2b Preparation of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetic acid 3: A solution of compound **2** (15 mmol) and NaOH 5% (15 mL) was refluxed in ethanol (30 mL) for 2 h in open air. After cooling, the mixture was acidified with HCl (6M). The precipitate obtained was filtered, washed several times with cold water and recrystallized from ethanol to give the acid **3**.

2.2c General procedure for preparation of esters 4: A mixture of compound **3** (1 mmol) and the appropriate alcohol (10 mL) was refluxed in the presence of a catalytic amount of iodine (I₂) for 1h 30 min under argon atmosphere. After removal of the excess of alcohol, the residue was extracted with diethyl ether and the organic phase was washed with a sodium thiosulfate solution (0.1 M) and distilled water. After decantation, the organic phase was dried over sodium sulfate and concentrated. The residue obtained was purified by silica gel column to obtain the esters **4a-f**.

2.2d Preparation of 2-(4-methyl-2-Oxo-2H-chromen-7-yloxy) acetohydrazide 5: Compound **2** (15 mmol) was dissolved in a solution containing ethanol (30 mL) and hydrazine hydrate (5 mL) and the mixture was left under reflux for 2 h in open air. The precipitate formed during the reaction was filtered hot and then recrystallized in ethanol/water (90/10) to give compound **5**.

2.2e General procedure for preparation of imines 6: Equimolar solution (1.2 mmol) of 2-(4-methyl-2-Oxo-2H-chromen-7-yloxy)acetohydrazide **5** and the aromatic aldehyde was refluxed in dry ethanol (15 mL) for 1 h under argon atmosphere. The white precipitate formed during the reaction was filtered hot and then recrystallized in ethanol to afford compounds **6a-c**.

2.2f Preparation of 2-(2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetyl)-N-phenylhydrazinecarbothioamide 7:

A mixture of compound **5** (1 mmol) and phenyl isothiocyanate (1 mmol) in dry dioxane (15 mL) was heated to reflux for 24 h under argon atmosphere. The crude product thus obtained was filtered to give compound **7**.

2.2g Preparation of 4-methyl-7-((4-phenyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl) methoxy)-2H-chromen-2-one 8: A mixture of **7** (1 mmol) and a 2% aq. solution of NaOH (6 mL) was heated to reflux for 4 h in open air. Then, the solution was neutralized with AcOH, extracted by AcOEt and purified by silica gel column to finally produce compound **8**.

2.2h Preparation of 4-methyl-7-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)methoxy)-2H-chromen-2-one 9: A solution of **7** (1 mmol) in conc. H₂SO₄ (4 mL) was kept at room temperature for one day in open air. After neutralization of the solution with diluted NH₄OH, the solid product was filtered off and dried to give compound **9**.

2.2i General procedure for preparation of compounds 10-13: Equimolar solution of compound **5** (1.2 mmol) and diketone was refluxed in dry ethanol (15 mL) for 1h 30 min under argon atmosphere. The precipitate formed during the reaction was filtered and then purified by silica gel column to finally produce the compounds **10-13**.

2.3 Biological activity

2.3a Antibacterial activity: The purified products were screened for their antibacterial activity by the agar disc diffusion method.¹⁸ NA medium cooled at 45°C was supplemented with a bacterial suspension (10⁶ CFU/mL) and poured into Petri plates. After solidification, sterile Whatman paper discs (diameter 6 mm) were placed at the surface of the culture medium and 20 µL (1000 µg/mL) of the product dissolved in DMSO was dropped onto each disc. The negative control plates had no product added to the filter paper whereas in the positive control plates, discs were impregnated with the same volume of ampicillin solution (5 mg/mL). The treated Petri dishes were incubated at 25°C for 48 h. The antibacterial activity was evaluated by measuring the diameter of the inhibitory zones formed around the discs. The experiment was replicated twice.

2.3b Antifungal activity: *Aspergillus flavus*, *Aspergillus niger* and *Penicillium italicum* were used for the screening of antifungal activity of the products tested by using

the disc diffusion method.¹⁹ A conidial suspension of the tested fungi was prepared (10^4 – 10^5 CFU/mL) and added to PDA medium cooled at 45°C and poured uniformly into Petri plates (diameter 90 mm). Sterilized paper discs (6 mm, Whatman No. 1 filter paper) were impregnated with 20 μ L (1000 μ g/mL) of the product dissolved in DMSO and placed on the culture plates whereas the negative control plates had no product added to the filter paper. In the positive control plates, discs were imbibed with the same volume of a carbendazim suspension (0.5 mg/mL). The diameter of the inhibition zone (mm) around the disc was measured after incubation at 25°C for 4 days and compared with control. The test was performed in triplicate.

3. Results and Discussion

3.1 Chemistry

According to the literature,²⁰ the reaction of 4-methylumbelliferone **1** with ethyl chloroacetate upon refluxing in dry DMF using potassium carbonate afforded the key intermediate **2** which was treated with a solution of NaOH (5%) in ethanol and followed by protonation,²¹ to give 2-(4-methyl-2-oxo-2H-chromen-7-yloxy)-acetic acid **3**. The structure of this compound was identified by ¹H and ¹³C NMR spectra. Esterification of compound **3** with a series of alcohols using iodine as catalyst,^{22,23} led to the formation of new esters **4a-f** (scheme 1).

The structures of compounds **4a-f** were characterized by ¹H NMR showing new signals in the region (0.85–4.97 ppm) attributed to protons introduced by the used alcohol. Further, the ¹³C NMR spectra of these

compounds exhibited the presence of new signals at (9.6–78.9 ppm) relative to the carbons also introduced by the used alcohol.

Mechanistically, being Lewis acid, I⁺ makes a strong covalent bond with carbonyl function of acid **3** and then, the non-isolable intermediate **3'** is subjected to nucleophilic attack by the alcohol used on the electron-deficient carbon, followed by an intramolecular rearrangement to give new coumarin esters **4a-f** along with regeneration of the catalyst (scheme 2).

Our approach to the target coumarin compounds **6-13** was started with the synthesis of the precursor **5** via the condensation between the coumarin ester **2** and an excess of hydrazine hydrate under reflux of ethanol.²⁴

The above-mentioned precursor **5** which contains the hydrazide moiety represents a class of intermediates

Scheme 2. Proposed mechanism for iodine catalyzed esterification.

Scheme 1. Synthetic route of esters **4a-f**.

which are known to be highly reactive and used for the synthesis of heterocyclic compounds which incorporate five- and six-membered rings containing nitrogen.²⁵ The ¹H NMR spectrum of compound **5** showed the disappearance of the signals relative to the alkoxy protons of the ester and the appearance of new signals, attributable to the NH and NH₂ protons at 9.45 and 4.38 ppm, respectively.

Firstly, refluxing **5** with different aromatic aldehydes in ethanol²⁶ was conducted until TLC indicated that the starting materials have been completely converted into products **6a-c** which were confirmed by their ¹H and ¹³C NMR data (scheme 3).

Compounds **6a-c** were established by their ¹H NMR spectra showing the disappearance of NH₂ protons at 4.38 ppm and the appearance of a new singlet at 8.50-8.58 ppm attributable to the N=CH proton in addition to the signals relative to protons introduced by the coumarin hydrazide **5** and the used aromatic aldehydes.

The ¹³C NMR spectra of compounds **6a-c** displayed in particular a new signal at 144.1-146.8 ppm attributable to the iminic carbon CH=N in addition to the new signals at 109.2-153.3 ppm relative to the aromatic carbons introduced by the used aldehydes.

According to the literature,^{26,27} reaction of compound **5** with phenylisothiocyanate in refluxing dioxane gave the thiosemicarbazide **7** (scheme 4).

The ¹H NMR spectrum of the compound **7** exhibited essentially two new signals due to -NHCSNH- at 9.69-10.33 ppm. Further, the ¹³C NMR spectrum of this compound showed the presence of new signals corresponding to the aromatic carbons introduced by the phenylisothiocyanate and a signal at 181.1 ppm assigned to the C=S carbon.

A survey of the literature indicates that the reaction of hydrazides with isothiocyanates is a typical procedure applied for the preparation of 1,2,4-triazole-3-thiones or 1,3,4-thiadiazoles.¹⁹ The two-step reactions led initially to the thiosemicarbazide **7**, which subsequently undergoes cyclocondensation under acidic or basic reaction conditions.²⁷ Using these methods, the intermediate **7** was converted into triazolthione **8** (35%) by heating in aqueous NaOH solution and into thiadiazole **9** (82%) by stirring in the presence of a solution of conc. H₂SO₄ at room temperature (scheme 4).

The ¹H NMR spectra of compounds **8** and **9** exhibited a singlet relative to NH proton at 7.01 and 4.03 ppm, respectively, and these signals were in good agreement with the expected cyclization reaction. The observation of a new signal at 166.9 ppm in the ¹³C NMR spectrum of compound **8** attributable to the thiocarbonyl function added to the disappearance of those corresponding to the amide and thiosemicarbazide carbonyl functions in the precursor **7** confirmed the identified structure.

The disappearance of the amide carbonyl and the thiocarbonyl signals from the ¹³C NMR spectrum of compound **9** was in concordance with the proposed structure.

With the aim to continue the study of the reactivity of the hydrazide **5**, we reacted it with a series of diketones (pentane-2,4-dione, hexane-2,5-dione and 1-phenyl-1,3-butanedione) in refluxing ethanol according to the literature¹³ to produce the corresponding pyrrole **10** and pyrazole **11-13** derivatives, respectively. Despite the low yield (50%) of the formation of the 5-hydroxy-3,5-dimethyl-4,5-dihydro-1*H*-pyrazole **11**, we did not notice its dehydration that normally leads to the corresponding 3,5-dimethyl-1*H*-pyrazol. This finding can be

Scheme 3. Synthetic route of compounds **6-7**.

Scheme 4. Synthetic route of triazolethione **8** and thiadiazole **9**.

Scheme 5. Synthetic route of compounds **10-13**.

explained by the hydrogen bond between the OH group and the carbonyl function leading to the formation of a stable pseudo-six-membered ring.

On the other hand, the treatment of the hydrazide **5** with 1-phenyl-1,3-butanedione led to primarily the 5-hydroxy-3,5-dimethyl-4,5-dihydro-1H-pyrazole **12** together with its di-dehydrated analogue **13**. This result can be explained by the presence of the phenyl group which causes a steric hindrance inhibiting the formation of a hydrogen bond and stabilizes the molecule by the extensive conjugation with the pyrazole system (scheme 5).

The structure of compound **11** was evidenced via its ^1H NMR spectrum showing the appearance of a new signal attributable to the OH proton at 4.26 ppm which provides no correlation in the CHcorr spectrum and two doublets at 2.82 and 3.03 ppm ($J = 18.6$ Hz) related

to CH_2 group ($\text{H}_{6'}$) and the disappearance of the signals related to the NH and NH_2 protons at 9.45 and 4.38 ppm, respectively. The ^{13}C NMR spectrum, the same compound showed the appearance of new signals related to carbons $\text{C}_{6'}$ and $\text{C}_{7'}$ at 51.2 and 144.1 ppm, respectively.

Similarly, the ^1H NMR spectrum of compound **10** showed the appearance of a singlet related to the NH proton at 8.69 ppm and the disappearance of the signal related to the NH_2 protons at 4.38 ppm. Analysis of ^{13}C NMR spectrum of this compound showed the appearance of new signals attributable to the magnetically equivalent carbons ($\text{C}_{6'} + \text{C}_{7'}$) at 120.6 ppm.

The ^1H NMR spectrum of compound **12** showed the disappearance of the signals of the NH and NH_2 protons at 9.45 and 4.38 ppm, respectively and the appearance of a singlet assigned to the OH proton at 7.03 ppm

and two doublets in the region 3.03–3.18 ppm corresponding to the non-equivalent methylene protons ($H_{6'a}$, $H_{6'b}$). The ^{13}C NMR spectrum of this compound displayed new signals at 55.2 and 143.4 ppm attributable to $C_{6'}$ and $C_{7'}$, respectively. Compound **13** obtained after dehydration of pyrazole **12** was confirmed by its 1H NMR spectrum showing the disappearance of the singlet relative to the OH proton at 7.03 ppm and the appearance of a new singlet at 6.97 ppm relative to $H_{6'}$. Further, the ^{13}C NMR spectrum of the pyrazole **13** showed essentially two new signals at 108.2 and 144.5 ppm attributable to the carbons $C_{6'}$ and $C_{7'}$, respectively.

The ESI-HRMS of all the synthesized compounds showed the correct protonated molecular ion peaks $[M+H]^+$ which are compatible with the proposed structures.

3.2 Biological activity

All the synthesized compounds have been evaluated for their antibacterial and antifungal activities.

3.2a Antibacterial activity: The antibacterial activity of these compounds was performed against a panel of microorganisms: *Pseudomonas savatanoi*, *Pseudomonas huttiensis* and *Agrobacterium tumefaciens*. The results given in table 1 shows that most compounds have displayed acceptable antibacterial activity against

P. savatanoi. Thus, compounds **4b** (IZ=11 mm) and **4e** (IZ=13 mm) were most active against this strain. We noticed that the presence of two free hydroxyl groups in compound **4a** has not improved this activity. The results showed that only compounds **6a**, **6b** and **6c** exhibited activity against *P. huttiensis* with IZ values of 11, 9 and 8.5 mm, respectively. This finding shows that the presence of the dimethylamino group in para position of the aromatic system introduced by the aldehyde used seems at the origin of this relatively high activity.

It was found that the conversion of compound **7** into triazole thione **8** and thiadiazole **9** via the thiosemicarbazide function did not improve its activity towards *P. savatanoi* (IZ=11, 9 and 9.5 mm, respectively). The thiosemicarbazide function in compound **7** could contribute to its activity. On the other hand, these compounds did not show any activity against *P. huttiensis* and only compound **8** exhibited a low activity towards *A. tumefaciens* (IZ=7.5 mm).

Pyrazole and pyrrole derivatives **10–12** were found to be active towards *A. tumefaciens*. The activity of compounds **11** and **12** could be explained by the presence in each one of the tertiary alcohol and of the asymmetric center C_8 , whereas the activity of compound **10** against the same strain (IZ=9 mm) could be due to the pyrrole and the acetamide moieties. The presence of the pyrazole and the extent of its conjugation with the phenyl group in compound **13** could explain the loss in activity against the same bacterium. The activity of compounds **12** (IZ=10.5 mm) and **13** (IZ=9.5 mm) against *P. savatanoi* could be due to the presence of the common *N*-2-oxoethoxy and phenyl systems (table 1).

3.2b Antifungal activity: The antifungal activity of the synthesized compounds was tested against three fungal strains: *Aspergillus flavus*, *A. niger* and *Penicillium italicum*. According to the results given in table 2, most synthesized compounds showed variable degrees of inhibition against *A. niger*. However, compounds **4a–d** showed practically the most relatively high activity against this fungus (IZ=10–13 mm). This finding could be explained by the nature of the alcohols used in the preparation of these esters comparatively to **4f** (IZ=9.5 mm) and **4e** (not active). Similarly, the activity of compounds **6a–c** against the same fungus varies depending on the aryl group introduced by each aldehyde used in their preparation.

The results mentioned in table 2 showed that compound **7** and its derivative **8** (triazole thione) display the same activity (IZ=9.5 mm) against *A. niger*, whereas compound **9** (thiadiazole) was found to be inactive. This result could be explained by the loss of the thiosemicarbazide skeleton in this compound but

Table 1. Antibacterial activity of the synthesized compounds.

Product	Inhibition zone diameter (mm)		
	<i>Pseudomonas savatanoi</i>	<i>Pseudomonas huttiensis</i>	<i>Agrobacterium tumefaciens</i>
4a	8.5	–	–
4b	11	–	10
4c	10	–	–
4d	–	–	12
4e	13	–	–
4f	10	–	–
6a	–	11	–
6b	12	9	–
6c	–	8.5	–
7	11	–	–
8	9	–	7.5
9	9.5	–	–
10	–	–	9
11	–	–	10.5
12	10.5	–	11
13	9.5	–	–
Ampicilin	22	18	25
DMSO	–	–	–

– no inhibition zone observed.

Table 2. Antifungal activity of the synthesized compounds.

Product	Inhibition zone diameter (mm)		
	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Penicillium italicum</i>
4a	9	12	7.5
4b	–	10	–
4c	–	13	10
4d	9	12	–
4e	10	–	–
4f	9	9.5	–
6a	–	9	–
6b	–	10.5	–
6c	–	11	9.5
7	7	9.5	–
8	7.5	9.5	–
9	–	–	–
10	–	10	–
11	–	9.5	–
12	–	11	–
13	7.5	11	–
FONG	37	32.5	40.5
DMSO	–	–	–

FONG: Carbendazime 0.5 mg/mL

– no inhibition zone observed.

another side is kept in compound **8** in a cyclized form. Compounds **10**, **11**, **12** and **13** displayed activity towards *A. niger* with IZ values of 10, 9.5, 11 and 11 mm, respectively. However, the pyrrole and the pyrazol moieties have certainly contributed to this activity. *P. italicum* did not show any sensitivity against most tested compounds and certain of these (**4a**, **4d-f**, **7**, **8**, **13**) exhibited moderate to low activity towards *A. flavus*.

4. Conclusion

In conclusion, this work reports the synthesis of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetic acid **3** in two steps starting from 4-methylumbelliferone, and its use as a key intermediate to access new coumarin esters **4a-f**. In the second part of this work, we described the successful synthesis of new coumarin hydrazones **6a-c**, sulfur compounds **7-9** and new pyrrole and pyrazole compounds **10-13**, via the condensation reaction of 2-(4-methyl-2-oxo-2H-chromen-7-yloxy) acetohydrazide **5**, previously prepared from 4-methylumbelliferone, with arylaldehyde, phenylthioisocyanate and three diketones, respectively. The antimicrobial activity of the newly synthesized compounds was evaluated.

Supplementary Information

¹H and ¹³C NMR spectra and analytical data for all new synthesized compounds are available at www.ias.ac.in/chemsci.

Acknowledgements

The authors are grateful to Miss Amna Benzarti, NMR service at the Faculty of Monastir, University of Monastir for the NMR analysis and to the Ministry of Higher Education and Scientific Research of Tunisia for financial support.

References

- Curini M, Cravotto G, Epifano F and Giannone G 2006 *Curr. Med. Chem.* **13** 199
- Bansal Y, Sethi P and Bansal G 2013 *Med. Chem. Res.* **22** 3049
- Kawate T, Iwase N, Shimizu M, Stanley S A, Wellington S, Kazyanskaya E and Hung D T 2013 *Bioorg. Med. Chem. Lett.* **23** 6052
- Gacche R N and Jadhav S G 2012 *J. Exp. Clin. Med.* **4** 165
- Nagamallu R and Kariyappa A K 2013 *Bioorg. Med. Chem. Lett.* **23** 6406
- Weigt S, Huebler N, Strecker R, Braunbeck T and Broschard T H 2012 *Reprod. Toxicol.* **33** 133
- Gammon D W, Hunter R and Wilson S A 2005 *Tetrahedron* **61** 10683
- Santana L, Uriarte E, González-Díaz H, Zagotto G, Soto-Otero R and Méndez-Alvarez E 2006 *J. Med. Chem.* **49** 1149
- Messaoudi S, Peyrat J F, Brion J D and Alami M 2010 *Adv. Synt. Catal.* **352** 1677
- Ramesh P, Das A T, Mohandass P and Nagasathya R 2008 *Indian J. Chem.* **47** 1447
- Ganesh R K, Prarthana V R, Sandeep G, Saurabh A, Arunima V, Atin K and Suresh T 2010 *Lett. Drug. Des. Discov.* **7** 46
- Kumar T, Dewangan D, Alexander A, Nagori K and Tripathi D K 2011 *Asian J. Biochem. Pharm. Res.* **2** 2231
- Refat H M and Fadda A A 2013 *Eur. J. Med. Chem.* **70** 419
- Al-Tel T H 2010 *Eur. J. Med. Chem.* **10** 4615
- Joshi S D, Vagdevi H M, Vaidya V P and Gadaginamath G S 2008 *Eur. J. Med. Chem.* **43** 1989
- Bhalgat C M, Ali M I, Ramesh B and Ramu G 2014 *Arabian. J. Chem.* **7** 986
- Zghab I, Trimeche B, Touboul D and Ben Jannet H 2014 *C. R. Chimie.* **17** 171
- Marmonier A 1987 In *Antibiotiques Technique de diffusion en gélose méthode des disques. Bactériologie médicale Techniques usuelles* (SIMEP SA-Paris, France) pp. 4, 237–243
- Barry A L and Thornberry C 1991 In *Manual of Clinical Microbiology* A H Ballows, W J Jr. Hausler, K L Herrman, H J Isenberg and Shadomy (Eds.) 5th ed. (American Society for Microbiology Press: Washington, DC) pp. 1117–1125

20. Hamdi N, Al-Ayed A S, Said R B and Fabienne A 2012 *Molecules* **17** 9321
21. Al-Amiery A, Musa A Y, Kadhum A A H and Mohamad A B 2011 *Molecules* **16** 6833
22. Ramalinga K, Vijayalakshmi P and Kaimal T N B 2002 *Tetrahedron Lett.* **43** 879
23. Vrazic J D and Zupan M 2009 *Tetrahedron Lett.* **50** 2347
24. Abdel-Aziz H A, Hamdy N A, Farag A M and Fakhr I M I 2007 *J. Chin. Chem. Soc.* **54** 1573
25. Yassin F A 2010 *J. Microbiol. Antimicrob.* **2** 93
26. Cacic M, Trkovnik M, Cacic F and Has-Schon E 2006 *Molecules* **11** 134
27. Šarkanj B, Molnar M, Cacic M and Gille L 2013 *Food Chem.* **139** 488