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Synthesis and biological evaluation of novel *N*-substituted 1*H*-dibenzo[*a*,*c*]carbazole derivatives of dehydroabietic acid as potential antimicrobial agents

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ABSTRACT

A series of new *N*-substituted 1*H*-dibenzo[*a*,*c*]carbazole derivatives were synthesized from dehydroabietic acid, and their structures were characterized by IR, ^{1}H NMR and HRMS spectral data. All compounds were evaluated for their antibacterial and antifungal activities against four bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas fluorescens*) and three fungi (*Candida albicans*, *Candida tropicalis* and *Aspergillus niger*) by serial dilution technique. Some of the synthesized compounds displayed pronounced antimicrobial activity against tested strains with low MIC values ranging from 0.9 to 15.6 μg/ml. Among them, compounds **6j** and **6r** exhibited potent inhibitory activity comparable to reference drugs amikacin and ketoconazole.

The evolutionary adaptation of pathogenic microbes has led to the development of drug-resistant strains of bacteria and fungi. Despite many antibiotics and chemotherapeutics available, the emergence of multidrug resistant organisms in the last few decades has become a major public problem, which has made the management of infectious diseases more precarious.[1–3] This situation has stimulated an urgent need to develop more effective antimicrobial agents with novel chemical structures which are helpful for overcoming drug resistance and improving the antimicrobial potency. The heterocyclic carbazole motif is a privileged pharmacophore scaffold found in many biologically active compounds of diverse origins, including natural products and synthetic sources. Carbazole and its derivatives possess desirable electronic and charge-transport properties as well as large π-conjugated systems,[4–7] and their structures can be easily modified by introducing various functional groups. These characteristics result in the extensive potential applications of carbazole-based derivatives as industrially and pharmacologically important products.[8] Many recent literatures have reported that carbazole derivatives exhibit a variety of biological activities such as antimicrobial,[9–12] antiviral,[13] anticancer,[14,15] *anti*-inflammatory,[16] antimalarial and antiprion,[17] antipsychotic and anticonvulsant,[18] antidepressant,[19] antidiarrhoeal,[20] mosquitocidal,[21] immunosuppressive[22] and neuroprotective activities.[23]

Dehydroabietic acid (DHA, **1**), a natural occurring diterpene resin acid, can be extracted from *Pinus* rosin or commercial disproportionated rosin. Many derivatives of DHA have attracted great interest for their broad spectrum of biological activities including antimicrobial, antitumor, antiviral, antioxidant, gastroprotective and BK channel-opening activities.[24–29] In our previous studies, a series of 1*H*-dibenzo[*a*,*c*]carbazole derivatives were synthesized, some of which showed considerable antimicrobial activity.[30] Moreover, previous study has showed that some nitrogen containing moieties such as piperazine, azole moieties could modulate the physicochemical properties and thus increase the antimicrobial efficiency.[31] Especially, it is well known that azole moieties including imidazole and triazole nucleus as important pharmacophore appear extensively in various types of pharmaceutical agents. A number of azole drugs, for example, ketoconazole, itraconazole and fluconazole have been clinically used in the treatment of various infectious diseases with excellent therapeutic efficacy.[8]

In view of the above findings and as an extension of our study on the development of carbazole derivatives of DHA, we designed and synthesized a new series of *N*-substituted 1*H*-dibenzo[*a*,*c*]carbazole derivatives (**6a**–**6u**) with different amine or heterocycle groups on the alkyl side chain, so that the synergistic effect of these valuable moieties might result in antimicrobial molecules with better efficacy. The synthetic route for the novel *N*-substituted 1*H*-dibenzo[*a*,*c*]carbazole derivatives was outlined in Scheme 1. The key intermediate 2,3,4,4a,9,13*c*-hexahydro-7-isopropyl-1,4*a*-dimethyl-1*H*-dibenzo[*a*,*c*]carbazole-1-carboxylic acid methyl ester

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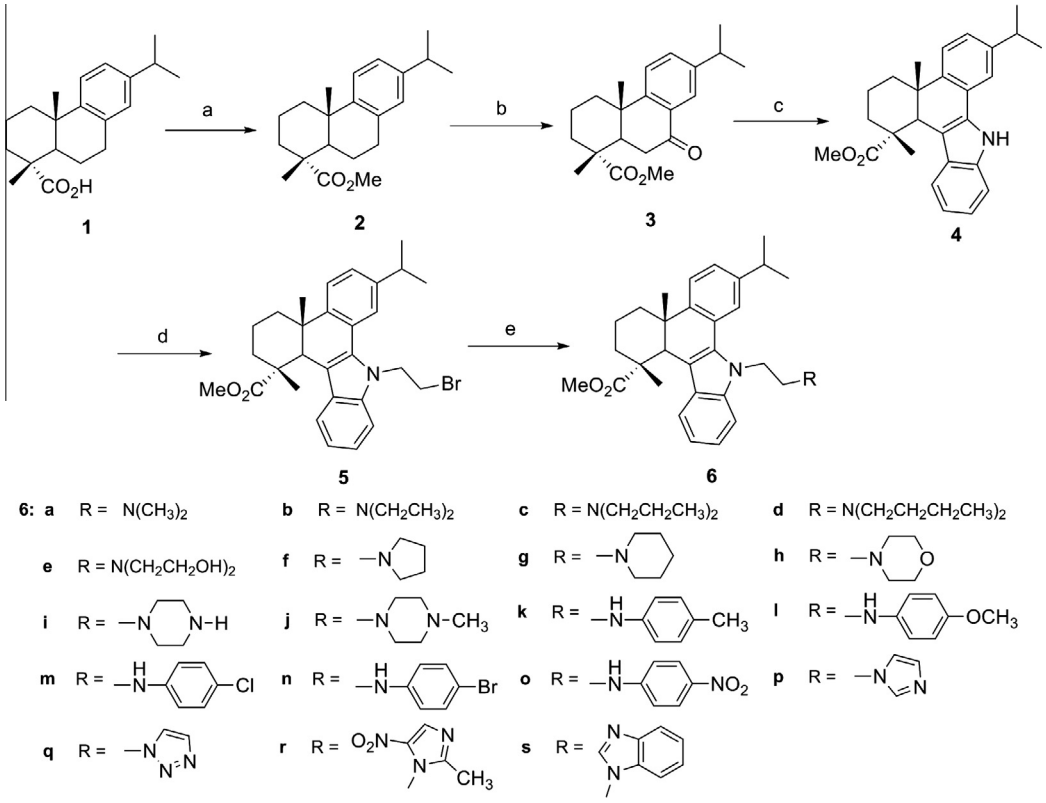
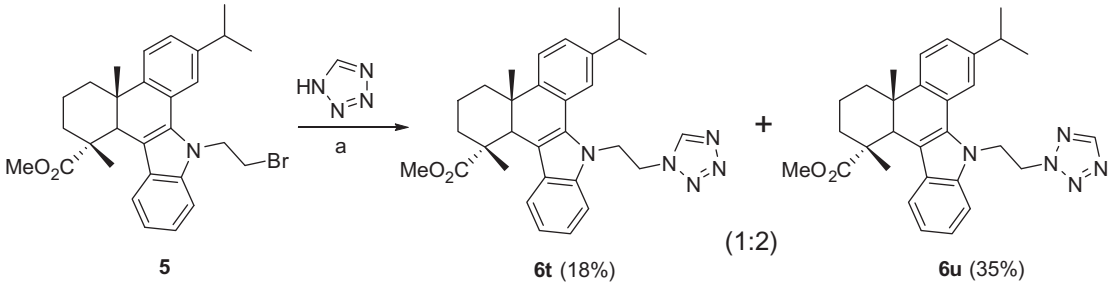


Scheme 1. Synthetic route of *N*-substituted carbazole derivatives (**6a–6s**) from dehydroabietic acid. Reagents and conditions: (a) (i) $SOCl_2$, benzene, reflux, 3 h, (ii) MeOH, reflux, 2 h; (b) CrO_3, AcOH, Ac_2O, 0 °C to rt, 12 h; (c) phenylhydrazine hydrochloride, EtOH, concd HCl, reflux, 3 h; (d) 1,2-dibromoethane, TBAB, NaOH, benzene, rt, 12 h; (e) respective amine, aniline or heterocycle, K_2CO_3, KI, MeCN, reflux, 8–12 h.

(**4**) was synthesized from dehydroabietic acid (**1**) according to the method described elsewhere.[30] Further *N*-alkylation of compound **4** was carried out at room temperature with excess of 1,2-dibromoethane in the presence of tetrabutylammonium bromide (TBAB) and NaOH, which afford the *N*-bromoethyl carbazole derivative **5** with 56% yield. The target carbazole derivatives **6a–6u** were synthesized in 18–67% yield by the reaction of **5** with different nucleophiles, including aliphatic amines, anilines and *N*-containing heterocycles, with the presence of K_2CO_3 and KI at reflux in MeCN. The structures of these products were characterized by their IR, ^{1}H NMR and HR ESIMS spectra. It is worth mentioning that the nucleophilic substitution of **5** with tetrazole afforded two regioisomers **6t** and **6u** with *N*-1 and *N*-2 alkylated tetrazole moiety, respectively, in a ca. 1/2 ratio in favor of the *N*-2 regioisomer (Scheme 2). Separation by flash chromatography over silica gel (petroether–acetone gradient) afforded *N*-1 and *N*-2 regioisomers as pure compounds. The ratio of the *N*-2/*N*-1 alkylation can be influenced by the electronic and steric effects as well as the solvent used in the reaction.[32,33] In some cases, the structural difference between the two regioisomers probably led to remarkable differentiation in their physiochemical properties and biological properties,[34] thus modulating the ratio of the regioisomers might be valuable to obtain more expected products. The two regioisomers were differentiated by their ^{1}H NMR data according to the literature. The signal of the N—CH—N proton for *N*-1-substituted isomer **6t** (δ 8.47 ppm) appeared at lower field than that of the corresponding *N*-2-substituted one **6u** (δ 8.13 ppm).[33]

The *in vitro* antimicrobial activities of all the synthesized compounds were evaluated for two Gram-positive bacteria viz. *Bacillus subtilis* CGMCC 1.1162, *Staphylococcus aureus* CGMCC 1.1361 and two Gram-negative organisms viz. *Escherichia coli* CGMCC 1.1571, *Pseudomonas fluorescens* CGMCC 1.1828, as well as three fungi viz. *Candida albicans* CGMCC 2.2086, *Candida tropicalis* CGMCC 2.3967 and *Aspergillus niger* CGMCC 3.316 by a modified twofold serial dilution method.[30,35] All target compounds were evaluated at the concentrations ranging from 0.45 to 250 μg/ml and scored for minimum inhibitory concentrations (MICs, μg/ml) that was defined as the lowest concentrations of the compound at which



Scheme 2. Preparation of (tetrazol-1-yl)- and (tetrazol-2-yl)-substituted isomers (**6t** and **6u**). Reagents and condition: (a) K_2CO_3, KI, MeCN, reflux, 8 h.

microbial growth was inhibited. Amikacin sulfate and ketoconazole were co-assayed as positive control against tested bacteria and fungi, respectively. The antibacterial and antifungal data were depicted in Table 1.

The results of antimicrobial screening data revealed that most of the synthesized compounds showed varying degrees of inhibition against both Gram-positive and Gram-negative bacteria. As seen in Table 1, the intermediates **3**–**5** did not show significant activity against all the tested organisms. For the tested aliphatic amines **6a**–**6j**, it was found that compounds **6h**–**6j** displayed valuable antibacterial activity with MIC values ranging from 1.9 to 7.8 μg/ml against Gram-positive bacteria (*B. subtilis* and *S. aureus*). Among them, the *N*-methylpiperazinyl substituted derivative **6j** showed excellent antibacterial potential against *S. aureus* with the MIC of 1.9 μg/ml, comparable to that of amikacin. The compound also showed significant activity against the two Gram-negative strains (*E. coli* and *P. fluorescens*) with the MICs of 15.6 and 7.8 μg/ml, respectively. Compounds **6b**, **6c**, **6d**, **6f** and **6g** exhibited moderate inhibitory activity against at least one of the four bacterial strains, while compounds **6a** and **6e** showed mild or no inhibition (>100 μg/ml) to the four bacteria. Among the aromatic amines and heterocycles **6k**–**6u**, compounds **6o**, **6p**, **6q**, **6r** and **6t** showed pronounced activity against the tested bacteria, while compounds **6k**–**6n**, **6s** and **6u** were found to have moderate or low activity against the four strains. It was worth noting that compound **6r** possessed the highest inhibition against two Gram-positive bacteria in the range of 0.9–1.9 μg/ml, which was equipotent to amikacin with MIC of 0.9 μg/ml against *B. subtilis*.

Similarly, the antifungal evaluation in vitro revealed that the synthesized compounds exhibited varying degrees of activity against three tested fungi. As shown in Table 1, compound **6j** was found to exerted prominent antifungal activity against three fungal strains with the MICs of 7.8 μg/ml, close to the reference drug ketoconazole. Noticeably, compound **6j** showed a broad antimicrobial spectrum exhibiting excellent inhibition against all the tested organisms. In addition, compounds **6c**, **6d**, **6i**, **6o** and **6r**–**6t** displayed moderate inhibitory activity against *C. albicans* and/or *C. tropicalis* with MICs of 31.2 μg/ml, while most compounds showed poor or no activity against *A. niger*.

From the *in vitro* antimicrobial activity data, preliminary structure–activity relationship (SAR) of the synthesized compounds **6a**–**6u** was studied. Generally, the nitrogen-containing heterocycles derivatives, for instance, compounds **6j** and **6r** with *N*-methyl piperazine and 2-methyl-5-nitroimidazole moiety, respectively, showed better antimicrobial activity against the tested organisms than those bearing aliphatic amine or substituted aniline side chains. Compounds **6c** and **6d** with dipropylamine and dibutylamine moiety were more active than their analogues (**6a** and **6b**) bearing smaller alkyl amine moiety, whereas compound **6e** with dihydroxyethyl amine moiety, a polar and medium size group, only exhibited mild antimicrobial activity. In addition, replacement of piperazine (**6i**) with *N*-methyl piperazine (**6j**) moiety led to substantial enhancement of antimicrobial activity. A possible explanation for this result was that the lipophilicity and size of the side chain of these derivatives played an important role in their antimicrobial activities. Among the derivatives containing aromatic ring side chains, compounds **6m** and **6o** bearing electron-withdrawing lipophilic groups such as –Cl and $-NO_2$ on the benzene ring showed stronger antibacterial activity than compounds with electron-releasing ones. Most derivatives with azole substituents exhibited notable antibacterial activities. Among them, compound **6r** endowed with 2-methyl-5-nitroimidazole moiety showed the strongest inhibitory activity. 5-Nitroimidazole compounds, such as metronidazole and tinidazole, are a class of clinically used antibiotics targeting a wide range of anaerobic microbes from protozoans including *Giardia lamblia*, *Trichomonas vaginalis* and *Entamoeba histolytica*, to bacteria such as *Helicobacter pylori*, *Clostridium difficile* and *Bacteroides fragilis*.[36] The mode of action of 5-nitroimidazoles in these organisms is mediated via bio-reduction of the nitro group to generate toxic, short-lived radical intermediates which will cause DNA damage of microbes and result in cell death.[37] However, compound **6r** displayed marked

Table 1
Antimirobial activity of novel carbazole derivatives (MIC values are in μg/ml)

Compd No.	Antibacterial activity				Antifungal activity		
	Gram-positive bacteria		Gram-negative bacteria				
	B. subtilis	*S. aureus*	*E. coli*	*P. fluorescens*	*A. niger*	*C. albicans*	*C. tropicalis*
3	31.2	31.2	>100	62.5	>100	>100	>100
4	31.2	62.5	>100	62.5	>100	>100	>100
5	31.2	62.5	>100	>100	>100	>100	>100
6a	62.5	>100	>100	62.5	>100	>100	>100
6b	31.2	62.5	>100	62.5	>100	62.5	>100
6c	15.6	31.2	31.2	31.2	>100	31.2	62.5
6d	15.6	15.6	31.2	15.6	62.5	31.2	31.2
6e	62.5	>100	62.5	62.5	>100	62.5	31.2
6f	31.2	62.5	>100	>100	>100	>100	>100
6g	15.6	62.5	>100	62.5	>100	>100	62.5
6h	15.6	7.8	31.2	31.2	62.5	62.5	31.2
6i	7.8	15.6	15.6	15.6	62.5	31.2	31.2
6j	3.9	1.9	15.6	7.8	7.8	7.8	7.8
6k	62.5	>100	>100	>100	>100	>100	>100
6l	>100	62.5	>100	>100	>100	>100	>100
6m	31.2	31.2	>100	62.5	>100	62.5	>100
6n	31.2	62.5	>100	>100	>100	>100	>100
6o	15.6	7.8	31.2	15.6	>100	31.2	62.5
6p	31.2	15.6	62.5	>100	>100	62.5	>100
6q	7.8	31.2	15.6	31.2	>100	>100	>100
6r	0.9	1.9	7.8	7.8	>100	31.2	31.2
6s	31.2	31.2	31.2	31.2	>100	31.2	62.5
6t	7.8	31.2	31.2	15.6	>100	31.2	62.5
6u	31.2	31.2	62.5	62.5	>100	>100	>100
Amikacin	0.9	0.9	1.9	0.9	–	–	–
Ketoconazole	–	–	–	–	7.8	3.9	3.9

activity against tested bacteria in aerobic conditions. The result indicated that the derivative might possess antimicrobial metabolisms other than the reduction of nitro group. In addition, the *N*-1-substituted tetrazole derivative **6t** exhibited pronounced antimicrobial activity against the bacterica *B. subtilis* and *P. fluorescens* and the fungus *C. albicans*, while its regioisomer **6u** merely showed moderate antibacterial activity and were inactive to the tested fungi. Azole-based derivatives with electron-rich heteroatomic ring systems can bind with enzymes or receptors in organisms through weak interactions such as coordination bonds, hydrogen bonds, ion–dipole, cation–π, π–π stacking and hydrophobic effect as well as van der Waals force, consequently exhibiting various bioactivities.[38] The structural variation of these azole derivatives, for example, the introduction of nitro group and the isomerization of tetrazole moiety, may probably cause significant change in their physiochemical properties such as lipophilicity, the cell membrane permeability and their abilities to bind with target enzymes or receptors in microorganisms.

In conclusion, a series of novel *N*-substituted 1*H*-dibenzo[*a*,*c*] carbazole derivatives (**6a**–**u**) were synthesized via an easy and efficient procedure from dehydroabietic acid. All the compounds were assayed for their *in vitro* antimicrobial activity against four bacteria and three fungi. As a result, derivatives bearing piperazine or azole heterocyclic moieties bridged by flexible ethyl chains showed broad antimicrobial spectrum and gave low inhibitory concentrations of 0.9–15.6 μg/ml. Among them, compound **6j** with *N*-methyl piperazine displayed good antimicrobial activity against all the tested strains. Compound **6r** containing 2-methyl-5-nitro-imizazole moiety exhibited excellent antibacterial activity against *B. subtilis* comparable to the reference drug amikacin. These derivatives showed their potential as new lead compounds in the study of antimicrobial chemotherapy. Further investigations, including the incorporation of different linkers and diverse heterocyclic moieties into this scaffold as well as various functional groups linked to the piperazine ring are currently in progress, and the findings are expected to give more information for the in-depth SAR study.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.11.009.

Reference and notes

1. Levy, S. B.; Marshall, B. *Nat. Med.* **2004**, *12*, 122.
2. Aksoy, D. Y.; Unal, S. *Clin. Microbiol. Infect.* **2008**, *14*, 411.
3. Demain, A. L.; Sanchez, S. *J. Antibiot.* **2009**, *62*, 5.
4. Zhang, F. F.; Zhou, C. H.; Yan, J. P. *Chin. J. Org. Chem.* **2010**, *30*, 783.
5. Huang, C. W.; Tsai, C. L.; Liu, C. Y.; Jen, T. H.; Yang, N. J.; Chen, S. A. *Macromolecules* **2012**, *45*, 1281.
6. Lee, C. W.; Yook, K. S.; Lee, J. Y. *Org. Electron.* **2013**, *14*, 1009.
7. Wakim, S.; Aich, B. R.; Tao, Y.; Leclerc, M. *Polym. Rev.* **2008**, *48*, 432.
8. Zhang, F. F.; Gan, L. L.; Zhou, C. H. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1881.
9. Karaburun, A. C.; Kaplancikli, Z. A.; Gundogdu-Karaburun, N.; Demirci, F. *Lett. Drug Des. Discovery* **2011**, *8*, 811.
10. Indumathi, T.; Ahamed, V. S. J.; Moon, S. S.; Fronczek, F. R.; Prasad, K. J. R. *Eur. J. Med. Chem.* **2011**, *46*, 5580.
11. Ryu, C. K.; Lee, S. Y.; Kim, N. Y.; Hong, J. A.; Yoon, J. H.; Kim, A. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 427.
12. Rajakumar, P.; Sekar, K.; Shanmugaiah, V.; Mathivanan, N. *Eur. J. Med. Chem.* **2009**, *44*, 3040.
13. Gudmundsson, K. S.; Boggs, S. D.; Sebahar, P. R.; Richardson, L. D.; Spaltenstein, A.; Golden, P.; Sethna, P. B.; Brown, K. W.; Moniri, K.; Harvey, R.; Romines, K. R. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4110.
14. Ferlin, M. G.; Gia, O.; Dalla Via, L. *ChemMedChem* **2011**, *6*, 1872.
15. Hajbi, Y.; Neagoie, C.; Biannic, B.; Chilloux, A.; Vedrenne, E.; Baldeyrou, B.; Bailly, C.; Merour, J. Y.; Rosca, S.; Routier, S.; Lansiaux, A. *Eur. J. Med. Chem.* **2010**, *45*, 5428.
16. Ho, F. M.; Kang, H. C.; Lee, S. T.; Chao, Y.; Chen, Y. C.; Huang, L. J.; Lin, W. W. *Biochem. Pharmacol.* **2007**, *74*, 298.
17. Thompson, M. J.; Louth, J. C.; Little, S. M.; Jackson, M. P.; Boursereau, Y.; Chen, B. N.; Coldham, I. *ChemMedChem* **2012**, *7*, 578.
18. Kaur, H.; Kumar, S.; Vishwakarma, P.; Sharma, M.; Saxena, K. K.; Kumar, A. *Eur. J. Med. Chem.* **2010**, *45*, 2777.
19. Bruhwyler, J.; Liegeois, J. F.; Geczy, J. *Pharmacol. Res.* **1997**, *36*, 23.
20. Mandal, S.; Nayak, A.; Kar, M.; Banerjee, S. K.; Das, A.; Upadhyay, S. N.; Singh, R. K.; Banerji, A.; Banerji, J. *Fitoterapia* **2010**, *81*, 72.
21. Ramsewak, R. S.; Nair, M. G.; Strasburg, G. M.; DeWitt, D. L.; Nitiss, J. L. *J. Agric. Food Chem.* **1999**, *47*, 444.
22. McAlpine, J. B.; Karwowski, J. P.; Jackson, M.; Mullally, M. M.; Hochlowski, J. E.; Premachandran, U.; Burres, N. S. *J. Antibiot.* **1994**, *47*, 281.
23. Miura, H.; Takano, K.; Kitao, Y.; Hibino, S.; Choshi, T.; Murakami, R.; Suzuki, H.; Yamada, M.; Ogawa, S.; Hori, O. *J. Pharmacol. Sci.* **2008**, *108*, 164.
24. Savluchinske-Feio, S.; Curto, M. J. M.; Gigante, B.; Roseiro, J. C. *Appl. Microbiol. Biotechnol.* **2006**, *72*, 430.
25. Xing, Y. H.; Zhang, W.; Song, J. J.; Zhang, Y. X.; Jiang, X. X.; Wang, R. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3868.
26. González, M. A.; Pérez-Guaita, D.; Correa-Royero, J.; Zapata, B.; Agudelo, L.; Mesa-Arango, A.; Betancur-Galvis, L. *Eur. J. Med. Chem.* **2010**, *45*, 811.
27. Lee, W. S.; Kim, J. R.; Han, J. M.; Jang, K. C.; Sok, D. E.; Jeong, T. S. *J. Agric. Food Chem.* **2006**, *54*, 5369.
28. Olate, V. R.; Pertino, M. W.; Theoduloz, C.; Yesilada, E.; Monsalve, F.; Gonzalez, P.; Droguett, D.; Richomme, P.; Hadi, A. H. A.; Schmeda-Hirschmann, G. *Planta Med.* **2012**, *78*, 362.
29. Cui, Y. M.; Yasutomi, E.; Otani, Y.; Ido, K.; Yoshinaga, T.; Sawada, K.; Ohwada, T. *Bioorg. Med. Chem.* **2010**, *18*, 8642.
30. Gu, W.; Wang, S. F. *Eur. J. Med. Chem.* **2010**, *45*, 4692.
31. Zhang, Y. Y.; Zhou, C. H. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4349.
32. Saper, R. J. *Aust. J. Chem.* **1984**, *37*, 2453.
33. Bia1ońska, A.; Bronisz, R. *Tetrahedron* **2008**, *64*, 9771.
34. Koyama, M.; Ohtani, N.; Kai, F.; Moriguchi, I.; Inouye, S. *J. Med. Chem.* **1987**, *30*, 552.
35. Mosaddik, M. A.; Banbury, L.; Forster, P.; Booth, R.; Markham, J.; Leach, D.; Waterman, P. G. *Phytomedicine* **2004**, *11*, 461.
36. Brook, I. *Expert Opin. Pharmacother.* **2011**, *12*, 1691.
37. Jorgensen, M. A.; Manos, J.; Mendz, G. L.; Hazell, S. L. *J. Antimicrob. Chemother.* **1998**, *41*, 67.
38. Peng, X. M.; Cai, G. X.; Zhou, C. H. *Curr. Top. Med. Chem.* **2013**, *13*, 1963.