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Microwave-assisted synthesis and antimicrobial activities of flavonoid derivatives

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Abstract—Eleven flavonoid derivatives were synthesised using a modified Baker–Venkataraman rearrangement, and subsequent microwave-assisted closure of the heterocyclic ring. All of the synthetic compounds displayed antifungal activity against *Aspergillus niger* and *Fusarium oxysporium*, and two of the synthetic flavonoid analogues exhibited significant activity against methicillin-resistant *Staphylococcus aureus*.

The ability of bacteria to acquire resistance to existing chemotherapies is a major concern and a critical challenge for medicine in the 21st century. Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a serious problem in hospitals and is considered to be a growing threat to community health.[1] Perhaps even more worrying is the emergence of bacterial strains that have resistance to the drug vancomycin, which has been a mainstay of MRSA therapy.[2] Fungal infections also present a serious risk to human health, particularly in individuals with compromised immune systems.[3] In addition, plant pathogenic fungi can cause serious damage to crops.[4] The discovery of new classes of selective antibacterial and antifungal agents should therefore become a priority.

Flavonoids are a major class of oxygen-containing heterocyclic natural products that are widespread in green plants.[5] Flavonoids have been recognised to have a protective effect in plants towards microbial invasion by plant pathogens,[6,7] and flavonoid rich plant extracts have been used for centuries to treat human disease.[8] Isolated flavonoids have been shown to possess a host of important biological activities, and the potential of naturally occurring flavonoids as anti-infective agents has been recognised.[6,9–11] Some natural flavonoids and synthetic flavonoid derivatives have shown promising antibacterial activity against drug resistant strains, including MRSA, as well as antifungal activities.[8,10–15] Herein we describe a short synthesis of a series of simple flavonoid derivatives, and describe some preliminary antimicrobial activity in MRSA and fungi.

Flavones **3a**–**k** were synthesised from 2-hydroxyacetophenones using the Baker–Venkataraman rearrangement,[16] followed by microwave-assisted condensation to close the heterocyclic ring (Scheme 1, Table 1).[17] Thus, solutions of the appropriately substituted *o*-hydroxyacetophenones **1a**–**k** in pyridine were treated with a slight excess of the selected acid chlorides and two equivalents of DBU, then heated at 80 °C for 16 h. In several cases the resulting 1,3-diketones **2a–c** were purified by column chromatography, but in general it was convenient to use the crude products **2d**–**k** in the cyclisation reaction.

Microwave-assisted cyclisation of 1-(2-hydroxyaryl)propane-1,3-diones has been reported to proceed efficiently in the presence of $CuCl_2$, or under acidic conditions (H_2SO_4/AcOH) or in ionic liquid ($[EtNH_3]NO_3$) to

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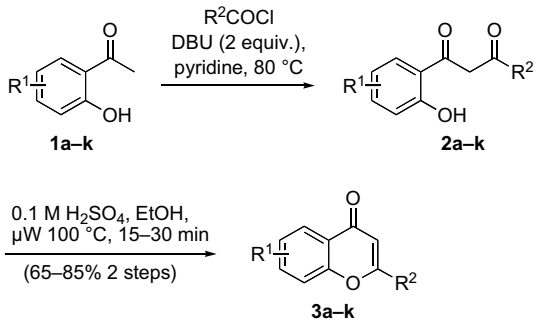


Scheme 1. Microwave-assisted synthesis of flavonoid derivatives **3a**–**k**.

afford flavones and chromones.[18] The latter two procedures were developed using modified domestic microwave ovens,[18b,c] which do not offer the level of control available from modern purpose-built microwave synthesizers.[17] We required a simple and controlled method to effect the cyclisation of compounds **2a**–**k** in high yield. Furthermore, we wished to avoid the use of copper salts in the preparation of compounds for use in antibacterial and antifungal assays as copper can be deliterious to the growth of these organisms.[19] Conventionaly, cyclisation is carried out in hot acetic acid containing sulfuric acid, and although this proved to be effective, the removal of large amounts of acetic acid was inconvenient. We found that a practical and economical method for microwave-assisted cyclisation of the diones **2a**–**k** was achieved by simply using EtOH containing a small amount of concentrated H_2SO_4 (100:1 by volume).[20,21]

Preliminary assessment of the antibacterial activities of synthetic flavonoids **3a**–**k** was undertaken using the filter paper disc diffusion method.[22] Two of the compounds, both carrying cyclohexyl groups at their 2-positions, inhibited the growth of methicillin-resistant *S. aureus* (MRSA, NCTC 10442) whilst none of them displayed any activity against *Escherichia coli* (Gram negative). This lack of antibacterial activity in *E. coli* is consistent with the findings of Göker et al. for a series of amidinobenzimidazole functionalised 2-phenyl and 2-methyl-4H-1-benzopyran-4-ones.[12] The active compounds were subjected to further evaluation in broth media fortified with different concentrations (400, 200, 100 and 50 μg/mL) of **3j** and **3k** in DMSO. Gentamycin was used as a positive control at the same concentrations in sterile water, and a negative control was introduced by applying the solvent (DMSO) used to dissolve the compounds. Bacterial growth was assessed using the Bradford method for analysis of protein content after 3 h incubation at 37 °C (Fig. 1),[23] and IC_{50} values were calculated for gentamycin (25.0 μg/mL), **3j** (69.6 μg/mL) and **3k** (12 mg/mL). It is interesting to note that a simple cyclohexyl derivative **3j**, prepared in two steps from commercial materials, has almost half the potency of the clinical antibacterial agent gentamycin.

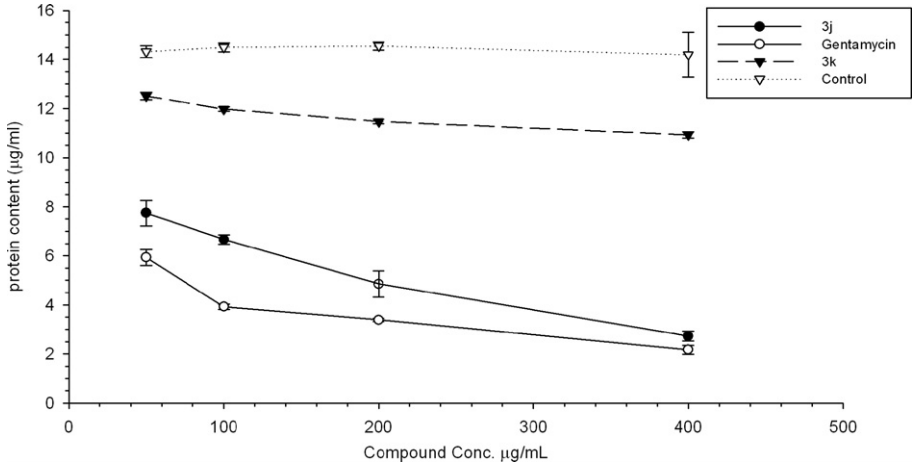


Figure 1. Activity of compounds against MRSA: protein content after treatment versus concentration of the tested compounds.

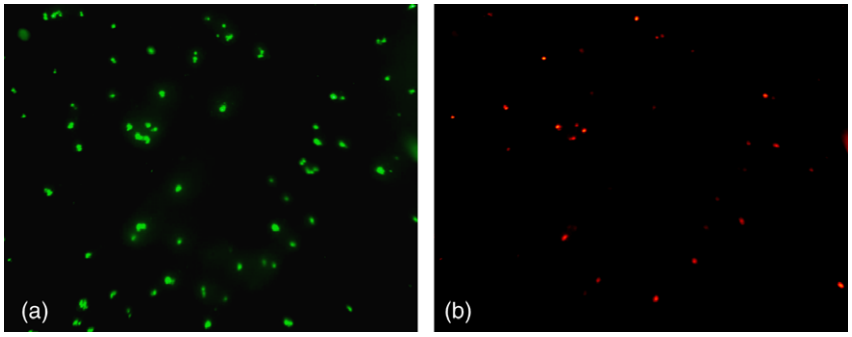
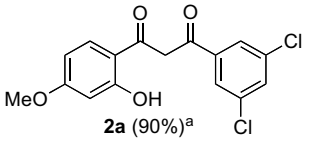
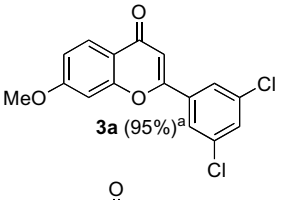
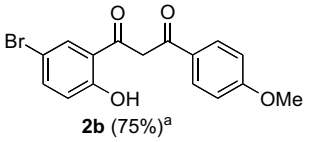
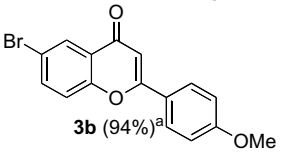
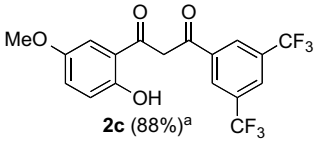
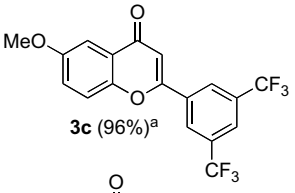
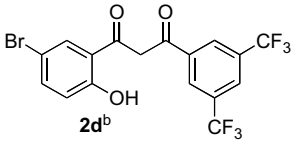
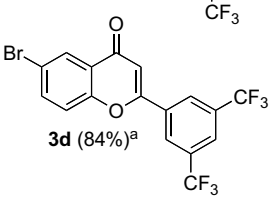
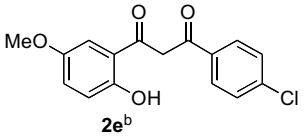
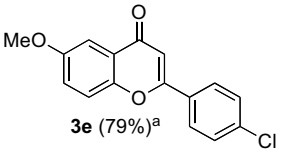
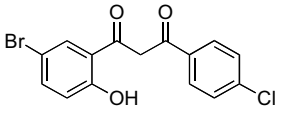
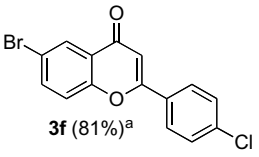
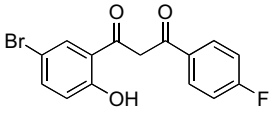
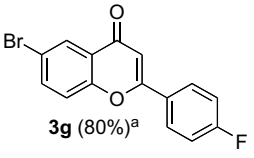
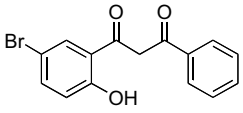
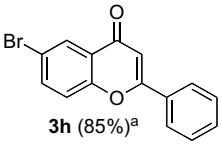
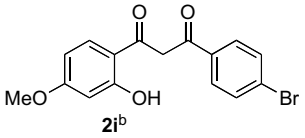
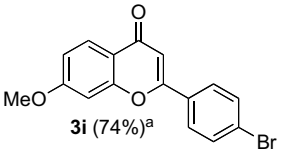
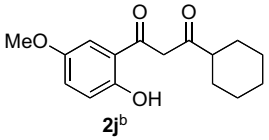
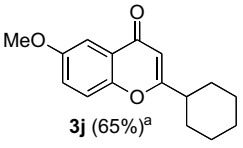


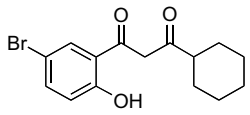
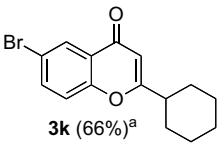
Figure 2. Baclight images of MRSA after exposure to **3j**. (a) Total cells with Syto9 stain. (b) Dead cells stained with propidium iodide.

Table 1. Structures and yields for synthetic flavone analogues **3a**–**k**

Entry	1-(2-Hydroxyaryl)propane-1,3-diones	Flavone analogues	Yield (%)[c]
1	 **2a** (90%)[a]	 **3a** (95%)[a]	85
2	 **2b** (75%)[a]	 **3b** (94%)[a]	70
3	 **2c** (88%)[a]	 **3c** (96%)[a]	84
4	 **2d**[b]	 **3d** (84%)[a]	84
5	 **2e**[b]	 **3e** (79%)[a]	79
6	 **2f**[b]	 **3f** (81%)[a]	81
7	 **2g**[b]	 **3g** (80%)[a]	80
8	 **2h**[b]	 **3h** (85%)[a]	85
9	 **2i**[b]	 **3i** (74%)[a]	74
10	 **2j**[b]	 **3j** (65%)[a]	65

(*continued on next page*)

Table 1 (*continued*)

Entry	1-(2-Hydroxyaryl)propane-1,3-diones	Flavone analogues	Yield (%)[c]
11	 **2k**[b]	 **3k** (66%)[a]	66

[a]Yield refers to analytically pure material.
[b]Crude material used in next step.
[c]Yield over two steps.

Table 2. Antifungal activity of flavonoids **3a–k** against *Aspergillus niger*-NCTC 275 and *Fusarium oxysporium*

Compound	*A.n.*[a] IC_{50}[b] (μg/mL)	*F.o.*[c] IC_{50}[b] (μg/mL)
3a	177.6	10592.6
3b	83.4	2249.1
3c	93.4	177.4
3d	60.3	65.2
3e	41.9	1428.9
3f	157.4	281.8
3g	141.8	682.3
3h	77.1	26915.3
3i	28.2	175.8
3j	58.9	116.7
3k	90.4	106.7
Mycostatin	3.2	—

[a] *Aspergillus niger*.
[b] Values are means of replicates.
[c] *Fusarium oxysporium*.

To gain some insight into the mode of action of the flavonoid **3j**, a viability assay method (Baclight)[24] was carried out which utilises a dual staining technique to identify live and dead cells, deemed by intrusion of the fluorescent dye propidium iodide through damaged cell membranes (Fig. 2). This experiment clearly shows the loss of membrane integrity in some of the cells, indicating that cyclohexyl derivative **3j** is bactericidal.

Antifungal activity of the synthetic flavonoids **3a–k** was examined against *Aspergillus niger*-NCTC 275 and *Fusarium oxysporium*. Assays were performed in agar media with final concentrations of 200, 100, 50 and 10 μg/mL.[25] A disc of the fungal mycelium was set in the middle of the Petri dish and the plates were incubated at 37 °C until full growth of the control plates (4 days), then the inhibition diameter was measured and IC_{50} values were obtained (Table 2). All of the flavonoids showed significant inhibition of fungal growth against *Aspergillus* and about half of them were effective in inhibiting *Fusarium* growth. The most potent synthetic compound **3i** against *Aspergillus* was about one order of magnitude less potent than the polyene antifungal antibiotic mycostatin, again highlighting the potential of these readily accessible structures for optimisation as anti-infective agents.

In summary, we have synthesised a small collection of flavonoid derivatives using a microwave-assisted heterocyclisation step. In our preliminary studies, most of the compounds displayed antifungal activity, and one of them showed significant antibacterial activity against MRSA. Given the simplicity of the compounds prepared, there is excellent potential for optimisation to produce potent antibacterial and antifungal agents and studies towards these ends are in progress.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.11.081.

References and notes

1. Hryniewicz, W. *Infection* **1999**, *27*, S13; Payne, D.; Tomasz, A. *Curr. Opin. Microbiol.* **2004**, *7*, 435; Walsh, F. M.; Amyes, S. G. B. *Curr. Opin. Microbiol.* **2004**, *7*, 439.
2. Goldstein, F. W. *Clin. Microbiol. Infect.* **2007**, *13*, 2.
3. (a) Chignard, M.; Balloy, V.; Sallenave, J.-M.; Si-Tahar, M. *Clin. Immunol.* **2007**, *124*, 238; (b) Vandewoude, K. H.; Blot, S. I.; Benoit, D.; Colardyn, F. *J. Hosp. Infect.* **2004**, *56*, 269; (c) Soubani, A. O.; Chandrasekar, P. H. *Chest* **2002**, *121*, 1988.
4. Logrieco, A.; Moretti, A.; Perrone, G.; Mulè, G. *Int. J. Food Microbiol.* **2007**, *119*, 11.
5. Bohm, B. A. *Introduction to Flavonoids*; Gordon & Breach: Amsterdam, Netherlands, 1998; *The Handbook of Natural Flavonoids*; Harborne, J. B., Baxter, H., Eds.; Wiley: Chichester, UK, 1999; Vols. 1 and 2.
6. Harborne, J. B.; Williams, C. A. *Phytochemistry* **2000**, *55*, 481.
7. Treutter, D. *Environ. Chem. Lett.* **2006**, *4*, 147.
8. Cushnie, T. P. T.; Lamb, A. J. *Int. J. Antimicrob. Agents* **2005**, *26*, 343.
9. Havsteen, B. H. *Pharmacol. Ther.* **2002**, *96*, 67.
10. Cushnie, T. P. T.; Hamilton, V. E. S.; Lamb, A. J. *Microbiol. Res.* **2003**, *158*, 281.
11. Pretorius, J. C. *Curr. Med. Chem.* **2003**, *2*, 335.
12. Göker, H.; Boykin, D. W.; Yildiz, S. *Bioorg. Med. Chem.* **2005**, *13*, 1707.

13. Wei, D. G.; Yang, G. F.; Wan, J.; Zhan, C. G. *J. Agric. Food. Chem.* **2005**, *53*, 1604.
14. Alcaráz, L. E.; Blanco, S. E.; Puig, O. N.; Tomás, F.; Ferretti, F. H. *J. Theor. Biol.* **2000**, *205*, 231.
15. Céspedes, C. L.; Avila, J. G.; Martínez, A.; Serrato, B.; Calderón-Mugica, J. C.; Salgado-Garciglia, R. *J. Agric. Food. Chem.* **2006**, *54*, 3521.
16. Baker, W. *J. Chem. Soc.* **1933**, 1381; Mahal, H. S.; Venkataraman, K. *J. Chem. Soc.* **1934**, 1767; Wagner, H.; Farkas, L. In *The Flavonoids*; Harborne, J. B., Mabry, T. J., Mabry, H., Eds.; Chapman Hall: London, 1975.
17. For some recent reviews of microwave-assisted synthesis: *Microwave Assisted Organic Synthesis*; Tierney, J. P., Lidstroem, P., Eds.; Blackwell: Oxford, UK, 2005; Kappe, C. O. *Angew. Chem. Int. Ed.* **2004**, *43*, 6250; Hayes, B. L. *Aldrichim. Acta* **2004**, *37*, 66; *Microwaves in Organic Synthesis*; Loupy, A., Ed.; Wiley-VCH: Weinheim, Germany, 2002; Lidstrom, P.; Tierney, J.; Wathey, B.; Westman, J. *Tetrahedron* **2001**, *57*, 9225.
18. (a) Kabalka, G. W.; Mereddy, A. R. *Tetrahedron Lett.* **2005**, *46*, 6315; (b) Sarda, S. R.; Pathan, M. Y.; Paike, V. V.; Pachmase, P. R.; Jadhav, W. N.; Pawar, R. P. *Arkivoc* **2006**, 43; (c) Tsukayama, M.; Kawamura, Y.; Ishizuka, T.; Hayashi, S. B.; Torii, F. *Heterocycles* **2003**, *60*, 2775.
19. Borkow, G.; Gabbay, J. *Curr. Med. Chem.* **2005**, *12*, 2163; Domek, M. J.; LeChevallier, M. W.; Cameron, S. C.; McFeters, G. A. *Appl. Environ. Microbiol.* **1984**, *48*, 289; Noyce, J. O.; Michels, H.; Keevil, C. W. *Appl. Environ. Microbiol.* **2006**, *72*, 4239.
20. *Preparation of 2-cyclohexyl-6-methoxy-4H-chromen-4-one (**3j**)*. To a solution of 2-hydroxy-4-methoxyacetophenone (400 mg, 2.4 mmol) in pyridine (10 mL) was added cyclohexanecarbonyl chloride (422 mg, 2.88 mmol) followed by DBU (0.7 mL, 5.28 mmol). The reaction mixture was stirred at 80 °C for 16 h, then cooled to rt and poured onto a mixture of ice and 2 N HCl (aq). The mixture was extracted with EtOAc (3× 20 mL). The combined organic extract was washed with brine and dried ($MgSO_4$), then the solvents were removed under reduced pressure to give the crude diketone **2j** as yellow oil that was used directly in the next reaction. Microwave tubes charged with a mixture of the crude diketone **2j** in EtOH (25 mL) and concentrated H_2SO_4 (0.25 mL) were placed in a microwave synthesizer (CEM, Discover) and subjected to microwave irradiation at 100 °C (power 100 W) for 30 min. The tubes were removed and the mixture was poured onto iced water. The product was extracted with EtOAc (2× 20 mL), the combined organic extract was dried ($MgSO_4$), and the solvent was removed under reduced pressure. Purification by column chromatography (silica gel, ether/hexane, 2:98–1:1) gave the title flavone **3j** as a buff coloured solid (402 mg, 1.56 mmol, 65% over two steps). Mp 71–72 °C; ν_{max} (film, cm^{-1}) 2928 (s), 2853 (s), 1645 (s), 1616 (s), 1578 (s), 1483 (s), 1435 (s), 1377 (s), 1354 (s), 1298 (s), 1277 (s), 1203 (s); ^1H NMR (300 MHz, $CDCl_3$) δ: 7.75 (1H, d, J = 3.0 Hz), 7.31 (1H, d, J = 9.0 Hz), 7.18 (1H, dd, J = 3.0, 9.0 Hz), 6.12 (1H, s), 3.84 (3H, s), 2.48 (1H, m), 2.01–1.70 (5H, m), 1.49–1.20 (5H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 178.5 (C), 173.2 (C), 156.7 (C), 151.4 (C), 124.4 (C), 123.4 (CH), 119.3 (CH), 107.2 (CH), 104.8 (CH), 55.9 (CH_3), 42.8 (CH), 30.5 ($2CH_2$), 25.9 ($3CH_2$); MS (ES^+) *m/z* 259 $[M+H]^+$.
21. All compounds tested were purified by silica gel column chromatography. Copies of the ^1H NMR and ^{13}C NMR spectra are available as supporting information.
22. *Goodman and Gilman's The Pharmacological Basis of Therapeutics*; Brunton, L. L., Lazo, J. S., Parker, K. L., Eds., 11th ed.; McGraw-Hill: USA, 2006.
23. Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248.
24. Boulos, L.; Prevost, M.; Barbeau, B.; Coallier, J.; Desjardins, R. *J. Microbiol. Methods* **1999**, *37*, 77.
25. Hammer, K. A.; Carson, C. F.; Riley, T. V. *J. Appl. Microbiol.* **1999**, *86*, 446.