Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 4431–4437
Bioorganic & Medicinal Chemistry

Synthesis and antibacterial activity of egonol derivatives

Safiye Emirdağ Öztürk, Yurdanur Akgül* and Hüseyin Anıl*

Ege University, Faculty of Science, Chemistry Department, 35100 Izmir, Turkey

Received 6 December 2007; revised 14 February 2008; accepted 19 February 2008
Available online 5 March 2008

Abstract—Synthesis of egonol derivatives, 5-(3″-chloropropyl)-7-methoxy-2-(3′,4′-methylenedioxyphenyl)benzofuran **1**, 5-(3″-bromopropyl)-7-methoxy-2-(3′,4′-methylenedioxyphenyl)benzofuran **2**, 3-[2-(1,3-benzodioxol-5-yl)-7-methoxy-1-benzofuran-5-yl]propanal **3**, 5-(3″-iodopropyl)-7-methoxy-2-(3′,4′-methylenedioxyphenyl)benzofuran **4**, 5-[3-(3″-bromopropyloxy) propyl]-7-methoxy-2-(3′,4′-methylenedioxyphenyl)benzofuran **5**, 3-[2-(1,3-benzodioxol-5-yl)-7-methoxy-1-benzofuran-5-yl]propylmethanoate **6**, 3-[2-(1,3-benzodioxol-5-yl)-7-methoxy-1-benzofuran-5-yl]propyloleate **7**, 5-[3″-hydroxypropyl]-6-bromo-7-methoxy-2-(3′, 4′-methylenedioxyphenyl)benzofuran **8**, 4-[2-(1,3-benzodioxol-5-yl)-7-methoxy-1-benzofuran-5-yl]butanenitrile **9**, 3-[2-(1,3-benzodioxol-5-yl)-7-methoxy-1-benzofuran-5-yl]propylbenzoate **10**, 5-[3″-hydroxypropyl]-7-methoxy-3-nitro-2-(3′,4′-methylenedioxyphenyl)benzofuran **11** and their antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans* and *Escherichia coli* are reported. The starting material egonol 5-[3″-(hydroxy)propyl]-7-methoxy-2-(3′, 4′methylenedioxyphenyl)benzofuran was isolated from seeds of *Styrax officinalis* L. The structural elucidication of these compounds (**1**–**11**) was established using 1D (^{1}H, ^{13}C), 2D NMR (HMBC, HMQC, COSY) and LCMS spectroscopic data. While egonol and some synthesised new compounds show similar antibacterial activity and MIC values against *S. aureus*, *B. subtilis*, *C. albicans* and *E. coli*, other new derivatives show different activity against *S. aureus*, *B. subtilis*, *C. albicans* and *E. coli*.

1. Introduction

Styrax officinalis L. is a member of the Styracaceae family, which is constituted of small trees and shrubs. It has been found from in Palestine to Italy. In Turkey, it grows mainly in north, south and western Anatolia.[4,10] Egonol is an aryl-benzofuran that contains a hydroxy group at C3″, which was first isolated in 1915 by Okada from the seed oil of *S. japonicum* and was formulated by Kawai as 5-[3″-(hydroxy)propyl]-7-methoxy-2-(3′,4′-methylenedioxyphenyl)benzofuran. Seeds of the fruit of *S. japonicum*, *S. obassia*, *S. formasanum*, *S. suberifolium* and *S. officinalis* are known to contain various egonol derivatives.[1,2,11,5] Egonol and its derivatives obtained from the seeds of *Styrax* species has attracted the attention of synthetic organic chemists, as a result of its activity against human leukaemic cells[8] in addition to anti-complement activity,[3] cytotoxic activity[9] and antifungal and antibacterial activity.[6] Here we report the synthesis and biological activity of eleven new egonol derivatives (see Fig. 1).

2. Results and discussion

The synthetic pathway in the present work is outlined in Schemes 1 and 2. Egonol isolated from the seeds of *S. officinalis* L. was used as the main starting material. The major product of the reaction of egonol with *p*-toluenesulfonylchloride in the presence of a small amount of pyridine was *5-[3″-chloropropyl]-7-metoxy-2-(3′,4′-methylenedioxyphenyl)benzofuran***1** (Scheme 1). Its positive ion-mode LSMS/APCI showed molecular ion peaks at *m*/*z* 347.1 and 345.1 $[M]^+$ consistent with a molecular formula of $C_{19}H_{17}ClO_4$. The ^{1}H and ^{13}C spectra of compound **1** exhibited peaks at δ 3.57 (H3″) and 44.46 (C3″). The corresponding signals for egonol were at δ 3.73 and 62.70.

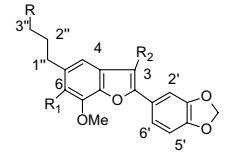
Reaction of egonol with $NaBr/H_2SO_4$ in water:acetone under reflux gave compound **2** (Scheme 1), whose LCMS–APCI gave ion peaks at *m*/*z* 391.0 and 393.0 $[M]^+$ suggesting a molecular formula of $C_{19}H_{17}BrO_4$. The ^{1}H NMR spectrum of compound **2** showed an H3″ upfield chemical shift at δ_H 3.43 instead of at δ_H 3.73 in egonol (Table 1). On the basis of the above results, compound **2** was deduced to be *5-(3″-bromopropyl)-7-methoxy-2-(3′,4′-methylenedioxyphenyl)benzofuran*.

Compound **3** was obtained from an oxidation reaction of egonol using PCC in acetone–CH_2Cl_2 (Scheme 1).

Keywords: *Styrax officinalis*; Styracaceae; Styrax; Antibacterial activity; Egonol; Benzofurans.
* Corresponding authors. Tel./fax: +90 232 3888264; e-mail addresses: yurdanur.akgul@ege.edu.tr; huseyin.anil@ege.edu.tr

0968-0896/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2008.02.057

	R	R_1	R_2
Egonol	OH	H	H
1	Cl	H	H
2	Br	H	H
4	I	H	H
5	$BrCH_2CH_2CH_2O$-	H	H
6	HCOO-	H	H
7	Oleate	H	H
8	OH	Br	H
9	CN	H	H
10	Benzoate	H	H
11	OH	H	NO_2



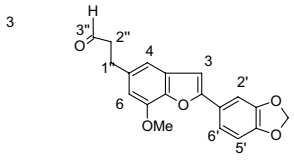
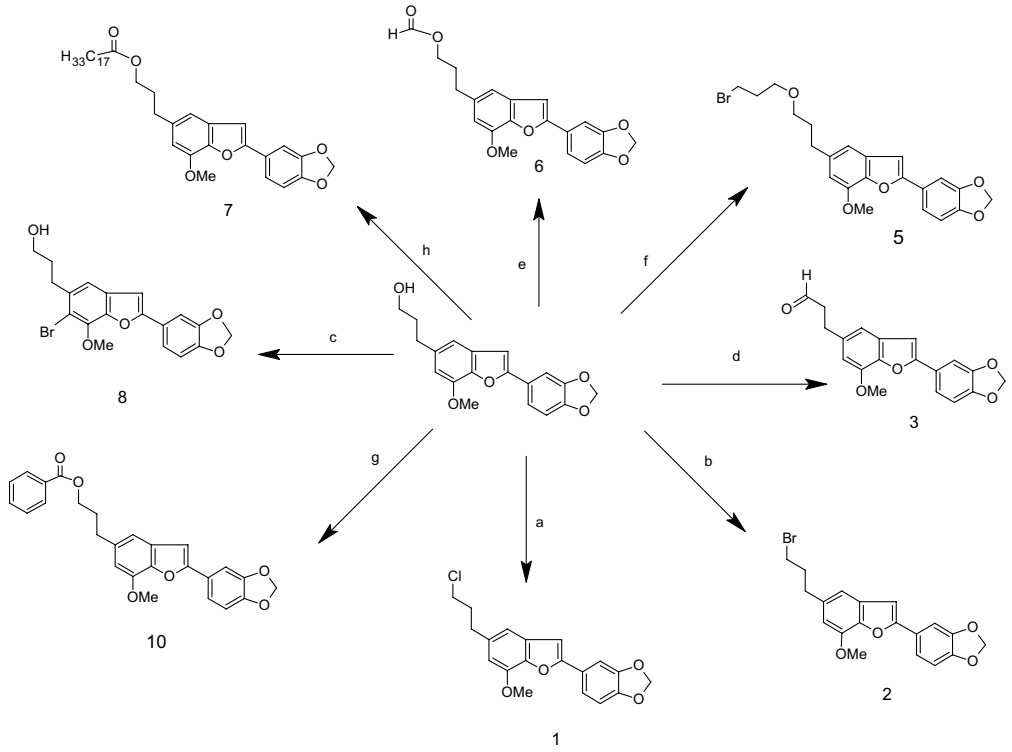


Figure 1. Structure of egonol and its derivatives.

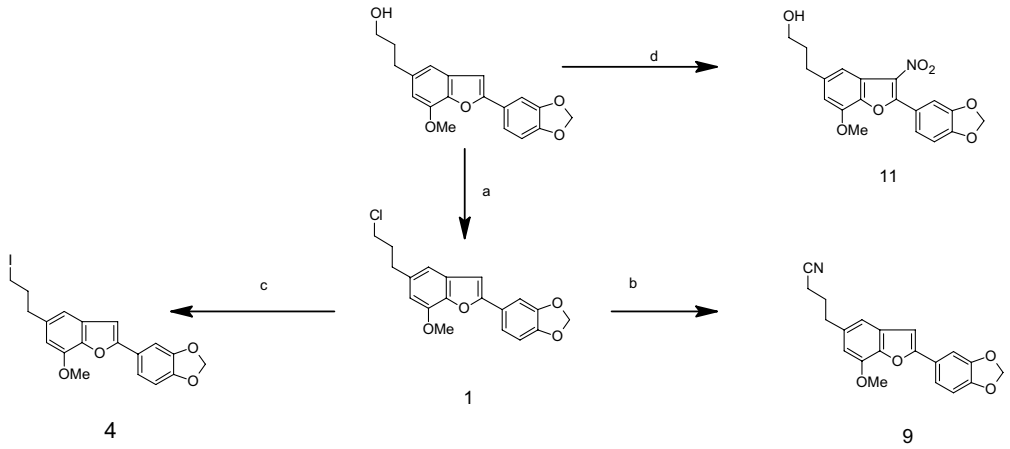
The ^{1}H NMR spectrum of compound **3** indicated two methylenes (δ_H 3.05, 2.85 instead of at δ_H 1.97, 2.85 in egonol), six aromatic methynes (δ_H 6.77, 6.96, 6.82, 7.32, 6.88, 7.41), one methylenedioxy (δ_H 6.00), one methoxy (4.04) and one aldehyde signal (δ_H 9.85). The main difference in the ^{1}H NMR spectrum of compound **3** with that of egonol is the presence of the aldehyde proton signal at δ_H 9.85 (1H, t) and the absence of a signal at δ_H 3.73 (2H, t) in egonol. Therefore, the structure of **3** was established as *3-[2-(1,3-benzodioxol-5-yl)-7-methoxy-1-benzofuran-5-yl]propanal.*

The product of the reaction of compound **1** with NaI was compound **4** (Scheme 2). ^{1}H and ^{13}C NMR analysis of compound **4** indicated the H3″/C3″ upfield chemical shift at δ_H 3.19/δ_C 6.72 instead of at δ_H 3.57/δ_C 44.46 in compound **1**. Therefore, the structure of compound **4** was established as *5-(3″-iodopropyl)-7-methoxy-2-(3′,4′-methylenedioxyphenyl)benzofuran.*

Compound **5** was obtained from the reaction of egonol with 1,3-dibromopropane and K_2CO_3 in DMF (Scheme 1). The molecular formula of **5** was assigned to be $C_{22}H_{23}BrO_5$ from the ion peaks at *m/z* 449.1 $[M]^+$ and 447.1 $[M]^+$ in the LCMS/APCI. The ^{1}H NMR spectrum of compound **5** showed a total of 23 proton signals, which included six methylenes (δ_H 3.56, 3.56, 3.48, 2.77, 2.12, 1.97), one methoxy (δ_H 4.03), one methylenedioxy (δ_H 6.00) and six aromatic methynes (δ_H 7.41, 7.32, 6.96, 6.87,6.79, 6.62). The ^{1}H spectrum of compound**5** indicated that the H-3″ (δ_H 3.48) was shifted upfield compared to that of H-3″ (δ_H 3.73) of egonol (Table 1). This suggested that compound **5** contained an ether bond. The ^{13}C NMR spectrum of **5** indicated a total of 22 carbons instead of 19 carbons, which is observed for egonol. Amongst the signals in the ^{13}C NMR spectrum were those for six methylene carbons (δ_C 70.33, 68.28, 32.85, 31.95, 32.16, 30.95), one methoxy carbon (δ_C 56.43), six aromatic methyne carbons (119.44, 112.62, 108.83, 107.83, 105.79, 100.61), one methylenedioxy carbon (δ_C 101.53) and eight quaternary carbons (156.29, 148.28, 148.21, 144.99, 142.72, 137.85, 131.25, 125.02). The corresponding carbons bearing the different protons were identified from the HMQC spectrum. In the COSY spectrum, the methylene proton at δ_H 2.12 (δ_C 30.95) showed vicinal coupling with the methylene protons at δ_H 3.56 (2H, m, δ_C 68.28; 2H, m, δ_C 31.95). The protons of another methylene δ_H 1.97 (δ_C 32.16) exhibited coupling with



Scheme 1. Synthesis of egonol derivatives (**1**–**3**, **5**–**8** and **10**). Reagents and conditions: (a) *p*-toluenesulfonylchloride/pyridine; (b) $NaBr/H_2SO_4$ in water, reflux; (c) NBS/CCl_4, reflux; (d) PCC in CH_2Cl_2; (e) DMF–KOH in THF; (f) 1,3-dibromopropane, K_2CO_3/DMF; (g) benzoylchloride in THF, $AlCl_3$; (h) oleic acid/*p*-toluenesulfonylchloride in pyridine.



Scheme 2. Synthesis of egonol derivatives (**4**, **9** and **11**). Reagents and conditions: (a) *p*-toluensulfonylchloride/pyridine; (b) NaCN in DMSO, reflux; (c) NaI in acetone; (d) KNO_3, H_2SO_4.

the methylene protons at δ_H 3.48 (2H, t, δ_C 70.33) and δ_H 2.77 (2H, t, δ_C 32.85). Thus, compound**5** was determined to be *5-[3-(3″-bromopropyloxy)propyl]-7-methoxy-2-(3′, 4′-methylenedioxyphenyl)benzofuran*.

Compound **6** was obtained from the reaction of egonol with KOH in DMF (Scheme 1) and gave a protonated ion peak at *m/z* 355.1 $[M+H]^+$ by LCMS–APCI, which suggested a molecular formula of $C_{20}H_{18}O_6$. The ^1H NMR spectrum of compound **6** showed that the resonance of H-3″ (δ_H 4.23) was shifted downfield compared to that of H-3″ of egonol (δ_H 3.73). The ^{13}C NMR spectrum of **6** exhibited 20 carbons instead of 19 carbons, which is observed for egonol (Table 2). The ^{13}C NMR spectrum of compound **6** showed three methylene carbons (63.49, 32.56, 30.82), one methoxy carbon (δ_C 56.44), six aromatic methyne carbons (δ_C 100.57, 112.61, 107.71, 105.79, 108.84, 119.48), one methylenedioxy carbon (δ_C 101.53), eight quaternary carbons (156.43, 148.30, 148.26, 145.10, 142.83, 136.84, 131.38, 124.93), and one carbonyl carbon (161.29). Thus, compound **6** was determined to be *3-[2-(1,3-benzodioxol-5-yl)-7-methoxy-1-benzofuran-5-yl]propylmethanoate*.

Compound **7** was obtained from the reaction of oleic acid and egonol (Scheme 1). The ^1H NMR spectrum of compound **7** showed that the resonance of H-3″ (δ_H 4.14) was shifted downfield compared to that of H-3″ of egonol (δ_H 3.71, Table 1). LCMS/APCI gave ion peaks at *m/z* 590.4, 591.3, 589.3 $[M]^+$. The ^{13}C NMR spectrum exhibited a total of 37 carbons (Table 2). Comparison of the ^1H and ^{13}C NMR spectral assignments for H-3″ and C3″ of **7** revealed downfield shifts of +0.41 and 1.11 ppm, respectively, suggesting that the C3″ was esterified in compound **7**. Therefore, compound **7** was established as 3-*[2-(1,3-benzodioxol-5-yl)-7-methoxy-1-benzofuran-5-yl]propyloleate*.

Compound **8** was obtained from the reaction of egonol with NBS in CCl_4 (Scheme 1). The molecular formula of **8**was assigned as $C_{19}H_{16}BrO_5$ from the ion peaks at *m/z* 405.0 $[M]^+$, 407.0 $[M]^+$ in the LCMS/APCI. The ^1H NMR spectrum of compound **8** exhibited one methylenedioxy (δ_H 6.01), three methylenes (δ_H 3.74, 2.88, 2.18), one methoxy (δ_H 4.04) and five aromatic protons (δ_H 7.41, 7.31, 6.85 (2H), 6.67), and indicated the disappearance of the H6 signal. The ^{13}C NMR spectra showed a total of 19 carbons, which included three methylenes (δ_C 62.39, 33.62, 32.32), one methoxy (δ_C 56.68), five aromatic methynes (δ_C 101.28, 109.15, 105.82,108.89, 119.71), one methylenedioxy (δ_C 102.099) and nine quaternary aromatic carbons (δ_C 156.68, 132.79, 136.18, 144.48, 142.13, 124.38, 148.57, 148.37, 105.70). The absence of a signal at δ_H 6.66/δ_C 107.86 (H5 in egonol) and the presence of a signal at δ_C 105.70 (instead at δ_C 100.76 in egonol) confirmed the presence of a C5 bromo derivative. Further, the correlation between δ_C 137.92/δ_H 6.66 in egonol was not observed in the HMQC spectrum of compound **8**. Although NBS was used for benzylic bromination, ^1H, ^{13}C, HMQC and HMBC NMR spectra indicated that the product was obtained by aromatic substitution (Scheme 1). Thus, compound **8** was identified as *5-[3″-hydroxypropyl]-6-bromo-7-methoxy-2-(3′,4′-methylenedioxyphenyl) benzofuran*.

The reaction of compound **1** and NaCN in DMSO afforded compound **9** (Scheme 2). Its structure was confirmed by ^1H and ^{13}C NMR spectra. The ^1H spectrum of compound **9** showed three methylene (δ_H 2.83, 2.33, 2.02), one methoxy (δ_H 4.03), one methylenedioxy (δ_H 5.99) and six aromatic methyne (δ_H 7.40, 7.32, 6.95, 6.87, 6.78, 6.60) protons. The ^{13}C NMR spectrum showed a total of 20 carbons, which included three methylene carbons (δ_C 34.87, 27.53, 16.54), one methoxy carbon (δ_C 56.68), one methylendioxy carbon (δ_C 101.63), six aromatic methyne carbons (δ_C 100.51, 112.78, 107.59, 105.82, 108.86, 119.51) and nine quaternary carbons (δ_C 156.59, 131.49, 135.60, 145.26, 142.97, 124.80, 148.34, 148.34, 119.81 (CN)). The ^1H and ^{13}C NMR spectra of compound **9** showed that the resonances of H3″ and C3″ (δ 2.83; 16.54) were shifted upfield compared to that of H3″ and C3″ (δ 3.57; 46.46) of compound**1**. Therefore, compound **9** was established as *4-[2-(1,3-benzodioxol-5-yl)-7-methoxy-1-benzofuran-5-yl]-butanenitrile*.

Table 1. ^{1}H NMR of egonol and its derivatives ($CDCl_3$, 400 MHz, 30 °C)

	Egonol	**1** δ ppm	**2** δ ppm	**3** δ ppm	**4** δ ppm	**5** δ ppm	**6** δ ppm	**7** δ ppm	**8** δ ppm	**9** δ ppm	**11** δ ppm
H3	6.81, s, 1H	6.77, s, 1H	6.77, s, 1H	6.77, s, 1H	6.79, s, 1H	6.79, s, 1H	6.78, s, 1H	6.78, s, 1H	6.67, s, 1H	6.78, s, 1H	—
H4	6.99, s, 1H	6.96, s, 1H	6.96, s, 1H	6.96, s, 1H	6.88, s, 1H	6.96, s, 1H	6.95, d, 1H, J = 1.6 Hz	6.95, d, 1H, J = 1.6 Hz	6.85, s, 1H	6.95, s, 1H	7.56, s, 1H
H6	6.66, s, 1H	6.62, s, 1H	6.62, s, 1H, J = 1.6	6.62, d, 1H, J=1.6	6.62, d, 1H, J = 0.8	6.62, s, 1H	6.60, d, 1H, J = 1.2	6.61, d, 1H J=1.2	—	6.60, s, 1H	6.77, s, 1H
H2′	7.34, d, 1H, J = 1.5	7.32, d, 1H, J = 4.0	7.32, d, 1H, J=4.0	7.32, d, 1H, J = 4.0	7.32, d, 1H, J = 1.6	7.32, d, 1H	7.32, d, 1H, J = 4.0	7.32d, 1H, J = 1.6 Hz	7.31, d, 1H, J=3.8	7.32, d, 1H, J = 1.2	7.49, d, 1H, J = 1.6
H5′	6.90, d, 1H, J = 8.1	6.88, d, 1H, J = 8.0	6.88, d, 1H, J = 8.0	6.88, d, 1H, J = 8.0	6.88, d, 1H, J = 8.4	6.87, d, 1H, J = 8.0	6.87, d, 1H, J = 8.0	6.88, d, 1H, J = 8.4	6.85, d, 1H, J = 8.0	6.87, d, 1H, J = 8.1	6.94, d, 1H, J = 8.4
H6′	7.43, dd, 1H, J = 1.6, 8.1	7.41, dd, 1H, J = 8.0, 2.0	7.41, dd, 1H, J = 1.6, 8.0	7.41, dd, H, J = 1.6, 8.0	7.39, dd, 1H, J = 1.2, 7.8	7.41, dd, H, J = 1.6, 8.0	7.40, dd, 1H, J = 1.6, 8.0	7.41, dd, H, J = 1.6, 8.3	7.41, dd, 1H, J = 1.6, 8.0	7.40, dd, 1H, J = 1.2, 7.8	7.63, d, 1H, J= 1.6, 8.4
OCH_2O	6.02, s, 2H	6.00, s, 2H	6.00, s, 2H	6.00, s, 2H	6.00, s, 2H	6.00, s, 2H	6.00, s, 2H	5.99, s, 2H	6.02, s, 2H	5.99, s, 2H	6.01, s, 2H
OMe	4.05, s, 3H	4.04, s, 3H	4.04, s, 3H	4.04, s, 3H	4.04, s, 3H	4.03, s, 3H	4.03, s, 3H	4.03, s, 3H	4.04, s, 3H	4.03, s, 3H	4.01, s, 3H
H1″	2.81, t, 2H, J = 7.5, 7.8	2.86, t, 2H, J = 6.8, 7.6	2.86, t, 2H, J = 7.6, 7.2	2.85, t, 2H, J = 1.2, 6.8	2.79, t, 2H, J = 7.2, 7.2	2.77, t, 2H, J = 7.6, 7.6	2.78, t, 2H, J = 7.5, 7.7	2.76, m, 2H	2.88, t, 2H, J = 7.5, 7.8	2.33, t, 2H, J = 7.2, 7.2	2.86, t, 2H, J = 7.2, 8.4
H2″	1.97, p, 2H	2.16, p, 2H	2.23, p, 2H	3.05, t, 2H, J = 8.0, 7.2	2.17, p, 2H	1.97, p, 2H	2.06, p, 2H	2.05m, 2H	2.18, p, 2H	2.02, p, 2H	2.00, p, 2H
H3″	3.73, t, 2H, J = 6.4, 6.4	3.57, t, 2H, J = 6.8, 6.4	3.43, t, 1H, J = 6.8, 6.0	9.85, t, 1H, J = 0.8, 1.6	3.19, t, 2H, J = 7.2, 6.8	3.48, t, 2H, J = 6.8, 6.0	4.23, t, 2H, J = 6.4, 6.2	4.14, t, 2H, J = 6.8, 6.4	3.74, t, 2H, J = 4.0, 1.6	2.83, t, 2H, J = 7.6, 7.2	3.74, t, 2H, J = 6.4, 6.4
*H*COO–						8.09, s, 1H					
Bromo-propyloxyoleate						3.56, m, 4H		5.36, 2H, m			
						2.12, p, 2H		2.76, 2H, m			
								2.32, 2H, t			
								J = 7.2, 7.2			
								2.05, 4H, m			
								1.63, m, 2H			
								1.31, 16H, br			
								s0.89, t, 3H			

Compound **10**, which was also isolated from *S. officinalis* L. as a minor compound, was obtained from the reaction of egonol with benzoylchloride and $AlCl_3$in THF (Scheme 1). Its structure was established by comparison of its ^{1}H NMR spectrum with literature data.[1]

Compound **11** was obtained from the reaction of egonol with KNO_3 (Scheme 2). Its structure was established by ^{1}H,^{13}C, HMQC and HMBC NMR spectra. The ^{1}H NMR spectrum of compound **11** exhibited one methylenedioxy (δ 6.01), three methylene (δ 3.74, 2.86, 2.00), one methoxy (δ 4.01) and five aromatic protons (7.63, 7.63, 7.56, 7.49, 6.94, 6.77), and indicated the disappearance of the H3 signal. The ^{13}C NMR spectra showed a total of 19 carbons, which included three methylenes (δ_C 62.32, 34.82, 32.87), one methoxy (δ_C 56.43), five aromatic methynes (δ_C 125.77, 112.78, 109.68, 110.13, 108.61), one methylenedioxy (δ_C 102.099) and nine quaternary aromatic carbons (δ_C 156.85, 123.71, 140.39, 141.06, 145.02, 145.02, 121.26, 150.79, 147.93). The absence of the signals at δ_H 6.81/δ_C 100.76 (H3 in egonol) and the presence of a signal at δ_C 123.71 (instead δ_C 100.76 in egonol) confirmed the presence of a C3 nitro derivative. Further, the correlation between δ_C 100.76 and δ_H 6.81 in the HMQC spectrum of egonol was not observed in the HMQC spectrum of compound **11**, and HMQC spectrum of compound **11** indicated that mononitration was obtained at C3 (Tables 1 and 2). Therefore, the structure of compound **11** was assigned as 5-[3″-hydroxypropyl]-7-methoxy-3-nitro-2-(3′,4′-methylenedioxyphenyl)benzofuran.

3. Conclusions

Egonol and synthesised compound were evaluated for antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans* and *Escherichia coli* (Table 3). Compounds **4**, **7**, **8**, **9**, **10** showed antibacterial activity towards *S. aureus*, *B. subtilis*, *C. albicans* and *E. coli* that were similar to egonol. This indicated that substitution of hydroxyl with I or CN at C3″; esterification of hydroxyl at C3″ with oleic acid or benzoic acid and aromatic substitution at C6 with Br or C3 with NO_3, did not result in increasing the activity of egonol. Only compounds **1**, **3** and **6** showed noticeable activities against *S. aureus*, *B. subtilis*, *C. albicans* and *E. coli*. Compounds **2** and **5** showed partial activities. In conclusion, compounds **1**–**11** showed partial activities.

4. Antibacterial activities

Egonol (10 mg), compound **1** (10 mg), compound **2** (8.8 mg), compound **3** (10 mg), compound 4 (10 mg), compound **5** (8.9 mg), compound **6** (10 mg), compound **7** (10 mg), compound **8** (10 mg), compound **9** (10 mg), compound **10** (10 mg) and compound **11** (10 mg) were evaluated for antibacterial activity by using the Muller Hilton Broth (MHB) method with *S. aureus*, *B. subtilis*, *C. albicans* and *E. coli*. Stock solution of all compounds were dissolved in 1 ml methanol and 0.3 ml dicholorom-

Table 2. ^{13}C NMR of compound **1**, **4**–**9**, **11** ($CDCl_3$, 100 MHz, 30 °C)

Egonol δ ppm	No.	**1** δ ppm	**4** δ ppm	**5** δ ppm	**6** δ ppm	**7** δ ppm	**8** δ ppm	**9** δ ppm	**11** δ ppm
156.51, q	C2	156.43	156.44	156.29	156.43	156.35	156.68	156.59	156.85
100.76, d	C3	100.56	100.56	100.61	100.57	100.58	101.28	100.51	123.71
131.46, q	C3a	131.38	131.37	131.25	131.38	131.31	132.79	131.49	140.39
112.72, q	C4	112.78	112.79	112.62	112.61	112.56	109.15	112.78	112.78
137.92, q	C5	136.58	136.28	137.85	136.84	137.72	136.18	135.60	141.06
107.86, d	C6	107.82	107.83	107.83	107.71	107.77	105.70	107.59	109.68
145.20.q	C7	145.27	145.10	144.99	145.10	145.05	144.48	145.26	145.02
142.88, q	C7a	142.84	145.10	142.72	142.83	142.75	142.13	142.97	145.02
125.12, q	C1′	124.92	124.91	125.02	124.93	124.98	124.38	124.80	121.26
105.95, d	C2′	105.78	105.79	105.79	105.79	105.79	105.82	105.82	110.13
148.46, q	C3′	148.32	148.31	148.28	148.30	148.29	148.57	148.34	150.79
148.39, q	C4′	148.27	148.27	148.21	148.26	148.22	148.37	148.34	147.93
109.02, d	C5′	108.84	108.85	108.83	108.84	108.83	108.89	108.86	108.61
119.62, d	C6′	119.47	119.47	119.44	119.48	119.46	119.71	119.51	125.77
32.83, t	C1″	33.27	36.67	31.95	30.82	30.98	32.32	34.87	34.82
35.08, t	C2″	34.68	35.50	32.16	32.56	32.76	33.62	27.53	32.87
62.70, t	C3″	44.46	6.72	68.28	63.49	63.81	62.39	16.54	62.32
56.57, s	OMe	56.4	56.45	56.43	56.44	56.48	56.68	56.48	56.43
101.71, t	OCH_2O	101.53	101.54	101.54	101.53	101.52	101.63	101.57	102.09
	Bromo-propyloxy			70.33, 30.95					
	CN							119.81	
	C=O				161.29	179.26			
	Oleate					130.23 (2C), 34.68, 25.24, 28.32(8C), 27.42 (2C), 31.74, 22.89, 14.31			

Table 3. Antibacterial activity of compound egonol and **1**–**11**

Compounds	*S. aureus*[a]	*B. subtilus*[a]	*C. albicans*[a]	*E. coli*[a]
Egonol	800	800	800	800
1	256	128	64	256
2	800	400	400	800
3	64	256	128	800
4	800	800	800	800
5	256	256	800	800
6	400	400	400	400
7	800	800	800	800
8	800	800	800	800
9	800	800	*	800
10	800	800	800	800
11	*	800	800	*

*Not active.
[a] MIC (μg/ml) = minimum inhibition concentration.

ethane separately and prepareted 1/2 Muller Hilton Broth (MHB) solution.

5. Experimental

5.1. General

The 1D NMR and 2D NMR spectra were measured in $CDCl_3$ at 400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR on a Varian AS-400 spectrometer. LCMS was recorded on a AGILENT 1100MSD spectrometer and FABMS was obtained using a zapSpec FAB (+) spectrometer. CC and PTLC were carried out on Si gel 60 (Merck 7734) and Merck 5554. TLC was carried out on Alugram (Sil G/UV 254, Merck 5554) using sulfuric acid at 120 °C.

5.2. Plant material

Fruits of *S. officinalis* L. were collected in September 2005 from Ephesus (Aydın, Turkey). A voucher specimen has been deposited in the herbarium of the Botanic Garden of Ege University.

5.3. Extraction

The shade dried and pulverised seed (241 g) of *S. officinalis* L. was exhaustively extracted with a *n*-hexane at room temperature. After removal of the solvent in vacuo, a crude yellow oily extract (113.72 g) was obtained. The hexane extract was hydrolysed with 33% KOH at 100 °C for 3 h. The reaction mixture was cooled at room temperature and extracted with CH_2Cl_2. The mixture was purified by CC using silica gel (Merck 7734) and CH_2Cl_2 as eluent to afford egonol (6 g), which was identified by ^1H and ^{13}C NMR.

5.4. Egonol

5-[3″-(hydroxy)propyl]-7-methoxy-2-(3′,4′-methylenedioxyphenyl)benzofuran. White powder. ^1H and ^{13}C NMR (Tables 1 and 2).

5.5. Synthesis

5.5.1. 5-(3″-Chloropropyl)-7-methoxy-2-(3′,4′-methylenedioxyphenyl) benzofuran (1), amorphous solid. To a mixture of egonol (472 mg, 1.45×10^{-3} mol) in pyridine (9 ml) was added *p*-toluenesulfonylchloride (286 mg, 2.61×10^{-3} mol) at room temperature and the mixture was stirred for 72 h. The reaction mixture was diluted with water (15 ml) and extracted with CH_2Cl_2 (3×

5 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was purified by PTLC (CH_2Cl_2). 33.2 mg compound was obtained (6.66%). Mp 120.0–121.7 °C. UV λ_{max} CH_2Cl_2: 229, 318 nm. IR spectrum ν_{max} CH_2Cl_2 cm^{-1}: 2919, 1615, 1596, 1443, 1229, 1033, 930, 847. ^1H and ^{13}C NMR: see Tables 1 and 2. LCMS/APCI *m/z* (rel. int.): 347.1 $[M]^+$ ((5.9), 345.1 $[M]^+$ (16.4), 282 $[M-(Cl-CH_2-CH_2)]^+$ (100), 283 $[M-(Cl-CH_2-CH_2)+H]^+$ (24.1).

5.5.2. 5-(3″-Bromopropyl)-7-methoxy-2-(3′,4′-methylenedioxyphenyl) benzofuran (2), viscous syrup. Concentrated H_2SO_4 (1.3 ml) was added slowly to egonol (50 mg, 1.53×10^{-4} mol) in CH_2Cl_2 (10 ml), followed by a slow addition of NaBr (15.8 mg) in 2 ml water. The mixture was then refluxed for 2 h. The reaction mixture was diluted with water (15 ml) and extracted with CH_2Cl_2 (3× 5 ml). The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue was purified by PTLC using CH_2Cl_2 as a solvent. 8.8 mg compound was obtained (12.80%). λ_{max} CH_2Cl_2: 229, 318 nm. IR spectrum ν_{max} CH_2Cl_2 cm^{-1}: 2917, 1596, 1473, 1360, 1229, 1146, 1039, 927, 760, 746. UV. ^1H NMR (Table 1). LCMS/APCI *m/z* (rel. int.): 391.0 $[M]^+$ (100), 389.0 $[M]^+$ (97.4), 309 $[M-Br]^+$ (22.9).

5.5.3. 3-[2-(1,3-Benzodioxol-5-yl)-7-methoxy-1-benzofuran-5-yl]propanal (3), viscous syrup. A mixture of egonol (50 mg, 1.53×10^{-4} mol) and acetone: CH_2Cl_2 (3:3 ml) was stirred at room temperature. PCC (146 mg) and molecular sieves (4A, 544 mg) were added in several portions and the mixture was stirred at room temperature for 24 h. Subsequently, the mixture was diluted with water (15 ml) and extracted with CH_2Cl_2 (3× 5 ml). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified on silica gel using hexane/EtOAc/H_2O: 6:4:0,5 as eluent to give the product (12.9 mg, 26.02%). UV λ_{max} CH_2Cl_2: 231, 318 nm. IR spectrum ν_{max} CH_2Cl_2 cm^{-1}: 2921, 2852, 1709, 1473, 1363, 1222, 1033, 927, 749. ^1H NMR (Table 1). LCMS/APCI *m/z* (rel. int.): 323.1 $[M-H]^+$(1.9), 282.2 $[M-CH_2CO]^+$ (100).

5.5.4. 5-(3″-Iodopropyl)-7-methoxy-2-(3′,4′-methylenedioxyphenyl) benzofuran (4), amorphous solid. To a mixture of compound **1** (40 mg, 1.16×10^{-4} mol) in acetone (5 ml) was added NaI (81 mg) with acetone (20 ml) in several portions at room temperature and the mixture was stirred for 120 h. The reaction mixture was diluted with water (15 ml) and extracted with CH_2Cl_2 (3× 5 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. 39 mg compound was obtained (100.36%). Mp 117.8–118.9 °C UV λ_{max} CH_2Cl_2: 230, 319 nm. IR spectrum ν_{max} CH_2Cl_2 cm^{-1}: 2906, 1722, 1622, 1497, 1475, 1363, 1231, 1031, 930, 740. ^1H and ^{13}C NMR (Tables 1 and 2). LCMS/APCI *m/z* (rel. int.): 437.0 $[M+H]^+$(28.1), 438.0 $[M+H]^+$ (6), 325.1 $[M-I]^+$ (30.2), 307.1 (100.0).

5.5.5. 5-[3-(7″-Bromopropyloxy)propyl]-7-mehtoxy-2-(3′,4′-methylenedioxyphenyl)benzofuran (5), amorphous solid. Egonol (100 mg, 3.07×10^{-4} mol) was added to a mixture of 1,3-dibromopropane (2 ml) and K_2CO_3 (500 mg) in DMF (5 ml). The mixture was stirred at room temperature for 72 h. It was diluted with CH_2Cl_2 (10 ml) and washed with water (3× 5 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was purified by PTLC (CH_2Cl_2) and 6.1 mg brown oily compound was obtained (4.46%). Mp 63.7–65.0 °C. UV λ_{max} CH_2Cl_2: 229, 318 nm. IR spectrum ν_{max} CH_2Cl_2 cm^{-1}: 2916, 2846, 1476, 1261, 1229, 1141, 1113, 1039, 950, 749. ^1H and ^{13}C NMR (Tables 1 and 2). LCMS/APCI *m/z* (rel. int.): 449.1 $[M]^+$ (100.0), 447.1 $[M]^+$ (96.9), 367.2 $[(M-Br)+H]^+$ (16.6), 355 $[M-(CH_2-Br)+H]^+$ (9.1), 327 $[M-(Br-CH_2-CH_2-CH_2)+H]^+$ (8.7).

5.5.6. 3-[2-(1,3-Benzodioxol-5-yl)-7-methoxy-1-benzofuran-5-yl]propylmethanoate (6), amorphous solid. A mixture of egonol (100 mg, 3.07×10^{-4} mol) and KOH (100 mg) in DMF (5 ml) was refluxed with stirring for 1 h. The reaction mixture was diluted with water (10 ml) and extracted with CH_2Cl_2 (3× 5 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was purified by PTLC using CH_2Cl_2 as a solvent to give the product as a white solid (20.6 mg, 18.97%). Mp 77.8–78.2 °C. UV λ_{max} CH_2Cl_2: 229, 318 nm. IR spectrum ν_{max} CH_2Cl_2 cm^{-1}: 2929, 2846, 1717, 1615, 1475, 1448, 1274, 1229, 1115, 1034, 921, 749, 710. ^1H and ^{13}C NMR (Tables 1 and 2). LCMS/APCI *m/z* (rel. int.): 355.1 $[M+H]^+$ (100), 356.1 $[M+2H]^+$ (22.1), 327.1 $[(M+2H)-CHO]^+$ (53.7).

5.5.7. 3-[2-(1,3-Benzodioxol-5-yl)-7-methoxy-1-benzofuran-5-yl]propyloleate (7), oily compound. To a stirred solution of egonol (472 mg, 1.45×10^{-3} mol) in pyridine (9 ml) was added oleic acid in CH_2Cl_2 (800 mg, 2.83×10^{-3} mol). The reaction mixture was refluxed for 8 h and was stirred for a further 72 h at room temperature. The reaction mixture was diluted with water (15 ml) and extracted with CH_2Cl_2 (3× 5 ml). The organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was purified by PTLC using CH_2Cl_2 as a solvent. 47.6 mg white solid compound was obtained (5.57%). UV λ_{max} CH_2Cl_2: 230, 318 nm. IR spectrum ν_{max} CH_2Cl_2 cm^{-1}: 2924, 2846, 1721, 1618, 1600, 1476, 1363, 1232, 1146, 1038, 929, 814, 735. ^1H and ^{13}C NMR (Tables 1 and 2). LCMS/APCI *m/z* (rel. int.): 589.3 $[M-H]^+$ (6.0), 590.4 $[M]^+$(3.0), 591.3 $[M+H]^+$(4.5), 282 (100).

5.5.8. 5-[3″-Hydroxypropyl]-6-bromo-7-methoxy-2-(3′,4′-methylenedioxyphenyl)benzofuran (8), brown amorphous solid. A mixture of egonol (100 mg, 3.07×10^{-4} mol), NBS (48 mg, 2.70×10^{-4} mmol), 3-chloroperoxybenzoic acid (10 mg, 5.79×10^{-5} mol) in CCl_4 (5 ml) was refluxed for 1 h. The reaction mixture was stirred at room temperature for 15 h. After addition of 10 ml water, the mixture was extracted with CH_2Cl_2 (3× 5 ml). The CH_2Cl_2 was dried with anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by PTLC using dichloromethane/methanol (80:4) as solvent system to give the product as a light brown solid (102 mg, 82.32%). Mp 137.3–145.0 °C. UV λ_{max} CH_2Cl_2: 229, 322 nm. IR spectrum ν_{max} CH_2Cl_2 cm^{-1}: 3296, 2925, 2857, 1733, 1599, 1476, 1363, 1232, 1141,

1039, 930, 749. ^{1}H and ^{13}C NMR (Tables 1 and 2). LCMS/APCI *m/z* (rel. int.): 405.0 $[M]^+$ (100), 407.0 $[M]^+$ (93.7), 326.1 $[M-Br]^+$ (46.8), $[(M-Br)+H]^+$ 327.1 (32.0).

5.5.9. 4-[2-(1,3-Benzodioxol-5-yl)-7-methoxy-1-benzofuran-5-yl]butanenitrile (9), amorphous solid. Sodium cyanide (10 mg, 2.09×10^{-4} mol) was dissolved in anhydrous dimethyl sulfoxide at 90 °C. Then compound **1** (50 mg, 1.39×10^{-4} mol) in DMSO was added in several portions. The mixture was stirred at room temperature for 1 day. After addition of 15 ml water, the mixture was extracted with CH_2Cl_2 (3× 15 ml). The CH_2Cl_2 was dried with anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified by PTLC using hexane/EtOAc/H_2O: 6:4:0.5 as a solvent to give the product as a yellow solid (48 mg, 103.08%). Mp 143.7–144.6 °C. UV λ_{max} CH_2Cl_2: 229,318 nm. IR spectrum $\nu_{max}CH_2Cl_2$ cm^{-1}: 2935, 2245, 1599, 1448, 1365, 1232, 1144, 1115, 1038, 929, 817, 742. ^{1}H NMR and ^{13}C NMR (Tables 1and 2). LCMS/APCI *m/z* (rel. int.): 336.1 $[M+H]^+$ (68.1), 309.1 $[M-CN]^+$ (22.0), 308.1 (100.0), 278.1 (18.5).

5.5.10. 3-[2-(1,3-Benzodioxol-5-yl)-7-methoxy-1-benzofuran-5-yl]propylbenzoate (10), amorphous solid. A mixture of $AlCl_3$ (30 mg), THF (7 ml) and benzoylchloride (28 mg, 1.99×10^{-3} mol) was stirred at ice bath temperature. Egonol (50 mg, 1.53×10^{-4} mol) in THF was added in several portions and the mixture was stirred at room temperature for 2 h. The mixture was poured into an ice-water mixture and extracted with CH_2Cl_2 (4× 5 ml). The extract was dried with anhydrous sodium sulfate, filtered and concentrated in vacuo. The residue was purified via column chromatography on silica gel using hexane/EtOAc: 15:1 as eluent to afford the product as a white solid (19.5 mg, 29.64%). Mp 71.9–74.5 °C UV λ_{max} CH_2Cl_2: 231, 318 nm. IR spectrum ν_{max} CH_2Cl_2 cm^{-1}: 2921, 1721, 1476, 1363, 1232, 1054, 1033, 930, 749. FABMS *m/z* (rel. int.): 430.0 $[M]^+$ (2), 405.1 (20), 391.1 (100), 149.9 (75).

5.5.11. 5-[3″-Hydroxypropyl]-7-methoxy-3-nitro-2-(3′,4′-methylenedioxyphenyl)benzofuran (11), yellow amorphous solid. Powdered KNO_3 (5.05×10^{3} mg, 0.050 mol) was treated with the appropriate amount of 96% H_2SO_4 (1 ml) and the mixture was stirred for 15 min at room temperature; CH_2Cl_2 (25 ml) was added to the homogeneous slurry so obtained and the mixture was cooled at 0 °C with vigorous stirring. A solution of egonol (50 mg, 1.53×10^{-4} mol) in CH_2Cl_2 was added dropwise and the stirring was continued at room temperature for the required time. The reaction mixture was then poured into 10% aqueous Na_2SO_4 and the separated organic phase was washed with 10% aqueous Na_2SO_4, dried over anhydrous Na_2SO_4, concentrated to dryness to afford the product[7] (46 mg, 81%).

Mp 140.7–142.7 °C. UV λ_{max} CH_2Cl_2: 228, 268, 384 nm. IR spectrum ν_{max} CH_2Cl_2 cm^{-1}:3379, 2922, 2851, 2483, 2132, 1605, 1473, 1338, 1056, 1033, 765, 743. ^{1}H and ^{13}C NMR (Tables 1 and 2). LCMS/APCI *m/z* (rel. int.): 372.1 $[M+H]^+$ (14.2), 282.2 (100.0).

Acknowledgments

This work was supported by a Grant 105T226 from TUBITAK. The authors thank Assoc. Prof. Stephan Thomas ASTLEY for proofing the manuscript.

References and notes

1. Akgul, Y. Y.; Anil, H. *Phytochemistry* **2003**, *63*, 939–943.
2. Anil, H. *Phytochemistry* **1980**, *19*, 2784–2786.
3. Byung-Sun, Min; Sei-Ryang, Oh; Kyung-Seop, Ahn; Jung-Hee, Kim; Joongku, Lee; Doo-Young, Kim; Eun-Hee, Kim; Hyeong-Kyu, Lee *Planta Med.* **2004**, *70*, 1210–1215.
4. Davis, P. H.. In *Flora of Turkey and the East Aegean Islands*; Edinburg University: Edinburg, 1972; Vol. 4, p 144.
5. Kyung-Jın, Yun; Byung-Sun, Min; Ji-Yeon, Kım; Kyung-Tae, Lee *Biol. Pharm. Bull.* **2007**, *30*, 139–144.
6. Pauletti, P. M.; Araujo, A. R; Young, M. C. M.; Giesbrecht, A. M.; Bolzani, V. *Phytochemistry* **2000**, *55*, 597–601.
7. Strazzolini, Paolo; Giumanini, Angelo G.; Runcio, Antonio *Tetrahedron Lett.* **2001**, *42*, 1387–1389.
8. Toshihiko, Hirano; Manabu, Gotoh; Kitaro, Oka *Life Sci.* **1994**, *55*, 1061–1069.
9. Qi-Lin, Li; Bo-Gang, Li; Hua-Yi, Qi; Xiao-Ping, Gao; Guo-Lin, Zhang *Planta Med.* **2005**, *71*, 847–851.
10. Vardar, Y.; Oflas, S. *Qual. Plant. Mater. Veg.* **1973**, *XXII*, 145–148.
11. Yinggang, Luo; Zhiheng, He; Hongjuan, Li *Fitoterapia* **2007**, *78*, 211–214.