Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 43 (2008) 707–713
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Original article

Synthesis, physicochemical properties and antimicrobial evaluation of new (*E*)-chalcones

Z. Nowakowska [a,*], B. Kędzia [b], G. Schroeder [a]

[a] *Faculty of Chemistry, Adam Mickiewicz University, Grunwaldzka 6, 60-780 Poznań, Poland*
[b] *Institute of Medicinal Plants, Libelta 27, 61-707 Poznań, Poland*

Received 28 February 2007; received in revised form 18 May 2007; accepted 24 May 2007
Available online 2 June 2007

Abstract

In a wide search program towards new and efficient antimicrobial agents, a series of 40 substituted chalcones have been synthesized and tested for their in vitro antibacterial and antifungal activities. The structures of these compounds have been investigated by nuclear magnetic resonance spectroscopy and mass spectrometry. Among the (*E*)-4-aminoalkylthiochalcones and (*E*)-4-aminoalkoxychalcones tested, compounds **7**, **10**, **11**, **30** and **31** have exhibited good antibacterial property against *Staphylococcus aureus*, *Enterococcus faecalis* and *Bacillus subtilis*.

Keywords: Chalcone; Antibacterial activity; Antifungal activity

1. Introduction

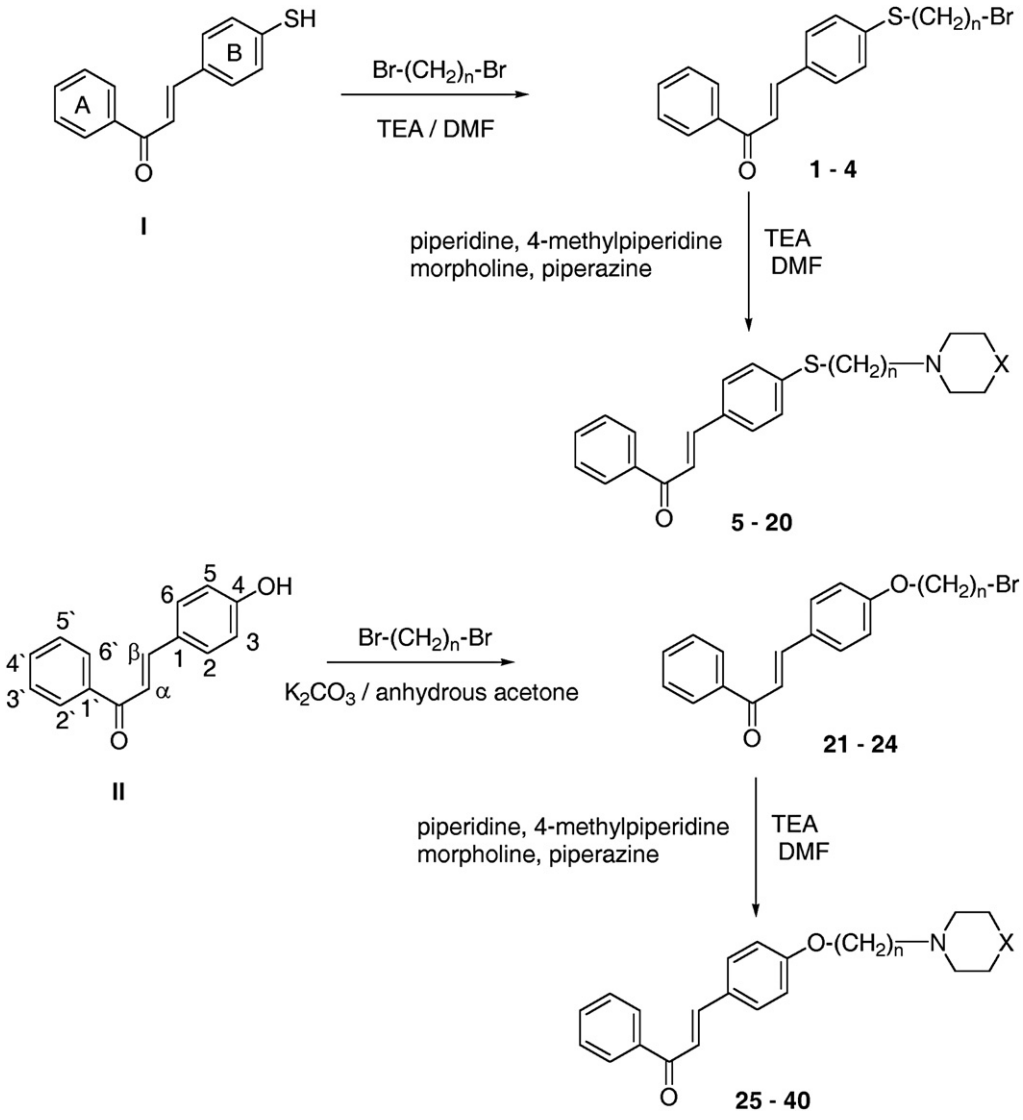
Chalcones represent an important group of natural compounds with a variety of biological activities including antibacterial and antifungal ones. They have found numerous applications as pesticides, photoprotectors in plastics, solar creams, food additives as well as anti-inflammatory and anticancer agents [1–5]. The oxygenated chalcone, licochalcone A, has been previously described as a moderately potent antibacterial compound with activity against Gram-positive bacteria. Rapid development of resistance to clinically important Gram-positive bacteria is a serious public health threat. *Staphylococcus aureus* can produce a number of diseases affecting humans and animals. Therefore, the search for novel bactericidal compounds is the object of continuous investigation [6–12]. Additionally, chalcones with basic amino functions have been reported to have enhanced selectivity and potency in biological properties [8,13]. To this date there have been no reports on the mercaptochalcones natural occurrence in plants, although a few sulfur-containing chalcone derivatives have been synthesized and tested as antibacterial agents and potential leukotriene receptor antagonists [14,15]. Thus, it is of interest to further elucidate the contribution of basic amino function to the biological activity of chalcones.

2. Chemistry

The aim of this investigation is to procure (*E*)-4-bromoalkylthiochalcones **1**–**4**, (*E*)-4-piperidino-*N*-alkylthiochalcones **5**–**8**, (*E*)-4-(4′-methylpiperidino)-*N*-alkylthiochalcones **9**–**12**, (*E*)-4-morpholino-*N*-alkylthiochalcones **13**–**16** and (*E*)-4-piperazino-*N*-alkylthiochalcones **17**–**20** from (*E*)-4-mercaptochalcone **I**, as well as (*E*)-4-bromoalkoxychalcones **21**–**24**, (*E*)-4-piperidino-*N*-alkoxychalcones **25**–**28**, (*E*)-4-(4′-methylpiperidino)-*N*-alkoxychalcones **29**–**32**, (*E*)-4-morpholino-*N*-alkoxychalcones **33**–**36**, and (*E*)-4-piperazino-*N*-alkoxychalcones **37**–**40** from (*E*)-4-hydroxychalcone **II** in the hope of obtaining new derivatives, which might show biological activity as antimicrobial and antifungal agents. The synthesis reaction is shown in Scheme 1 and the numbering

* Corresponding author. Tel.: +48 61 829 1002; fax: +48 61 865 8008.
E-mail address: zdzisian@amu.edu.pl (Z. Nowakowska).

0223-5234/$ - see front matter © 2007 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2007.05.006



Scheme 1. Scheme of synthesis of compounds **1**–**40**.

of the compounds is presented in Table 1. (*E*)-4-Mercaptochalcone **I** was alkylated with dibromoalkanes (1,4-dibromobutane, 1,5-dibromopentane, 1,6-dibromohexane and 1,10-dibromodecane) at room temperature in *N*,*N*-dimethylformamide in the presence of triethylamine as a base. The reactions were usually carried out for 3–4 h. Under these conditions, a chemoselective alkylation affording the corresponding (*E*)-4-bromoalkylthiochalcones, as convenient intermediates for the synthesis of the planned bridged chalcone derivatives, was possible. Yields of the products **1**–**4** were of the order of 76–88%. However, in the reaction of (*E*)-4-mercaptochalcone with dibromoalkanes with 4, 5 or 6 carbon atoms in the alkyl chain, an additional product was also detected and isolated in 12%, 8% and 5% yield. This minor compound was identified as bis-(4-chalconylthio)alkane.

(*E*)-4-Hydroxychalcone **II** was alkylated with dibromoalkanes in a boiling anhydrous acetone solution containing anhydrous potassium carbonate. Under these conditions, a chemoselective alkylation affording the corresponding (*E*)-4-bromoalkoxychalcones **21**–**24** was possible. The reactions were usually carried out for 5–6 h. During these reactions bis-(4-chalconyloxy)alkane with 4, 5 or 6 carbon atoms in the alkyl chain, was also obtained in the yield of 10%, 8% and 6%, respectively.

Table 1
Labeling of compounds **1**–**40**

Compd.	*n*	X	Compd.	*n*	X	Compd.	*n*	X
1	4	–	**15**	6	O	**29**	4	CH–CH_3
2	5	–	**16**	10	O	**30**	5	CH–CH_3
3	6	–	**17**	4	N–H	**31**	6	CH–CH_3
4	10	–	**18**	5	N–H	**32**	10	CH–CH_3
5	4	CH_2	**19**	6	N–H	**33**	4	O
6	5	CH_2	**20**	10	N–H	**34**	5	O
7	6	CH_2	**21**	4	–	**35**	6	O
8	10	CH_2	**22**	5	–	**36**	10	O
9	4	CH–CH_3	**23**	6	–	**37**	4	N–H
10	5	CH–CH_3	**24**	10	–	**38**	5	N–H
11	6	CH–CH_3	**25**	4	CH_2	**39**	6	N–H
12	10	CH–CH_3	**26**	5	CH_2	**40**	10	N–H
13	4	O	**27**	6	CH_2			
14	5	O	**28**	10	CH_2			

Another type of modification was the nucleophilic substitution of 1 mmol of (*E*)-4-bromoalkylthiochalcones **1**–**4** and (*E*)-4-bromoalkoxychalcones **21**–**24** with 2 mmol of piperidine, 4-methylpiperidine, morpholine or piperazine in DMF in the presence of 2 mmol equivalents of TEA. The corresponding (*E*)-4-aminoalkylthiochalcones **5**–**20** and (*E*)-4-aminoalkoxychalcones **25**–**40** were obtained.

3. Results and discussion

All compounds have been characterized on the basis of spectral studies (MS [16], ^{1}H NMR, ^{13}C NMR and IR) and elemental analysis. Assignments of the ^{1}H NMR and ^{13}C NMR resonances of these compounds were deduced on the basis of signal multiplicities, and by the concerted application of the two-dimensional NMR technique (HETCOR).

The ^{1}H and ^{13}C NMR data for the derivatives of (*E*)-4-mercaptochalcone **1**–**20** were nearly identical in the aromatic region of the spectra (Table 2). The spectra of these compounds showed two doublets at *δ*: 7.49–7.50 ppm and *δ*: 7.77–7.78 ppm ($J = 15.6$ Hz) integrating for one proton, which were assigned to the H-α and H-β protons, respectively. The *J* of 15.6 Hz between H-α and H-β proton is consistent with a *trans* relationship for the C=C double bond of the Ph–CO–CH=CH–Ph group. In the HETCOR experiment it was found that these protons correlated with the carbon atoms resonating at 120.97–121.17 ppm (C-α) and 144.01–144.31 ppm (C-β), respectively. The ^{13}C NMR signal at 32.23–32.76 ppm is assigned to the S–CH_2 carbon. This latter signal correlates with the proton triplet at 2.86–2.99 ppm. The spectra of **1**–**4** showed also signals attributed to the CH_2–Br protons (*δ*: 3.40–3.41 ppm, 2H, d) correlating with the carbon signal at 33.43–34.01 ppm.

The ^{1}H NMR spectra of **5**–**20** showed also a triplet at 2.22–2.89 ppm integrating for two protons and ascribed to the CH_2–N< protons of *N*-alkylthio chain linking (*E*)-chalcone and piperidine (4-methylpiperidine, morpholine or piperazine) rings, correlating in the HETCOR spectra with the carbon signal at 57.21–59.68 ppm. The presence of a doublet of protons of a methyl group in the range of 1.02–1.03 ppm in the ^{1}H NMR spectra of **9**–**12** proves the occurrence of the 4-methylpiperidine ring in the molecules of these compounds. The ^{1}H NMR spectra of **13**–**16** in the range of 3.71–3.72 ppm and 2.46–2.53 ppm showed two triplets of proton signals of the –CH_2–O–CH_2– and –CH_2–N–CH_2– groups of the morpholine ring. These signals indicate the occurrence of the morpholine ring in the molecules investigated. For **17**–**20** the signal observed at 2.40–2.44 ppm (with four proton integrals), corresponds to the –CH_2–N–CH_2– protons, whereas the four-proton signal at 2.90–2.95 ppm to the –CH_2–NH–CH_2– protons, of the piperazine moiety. The ^{13}C NMR spectra of these compounds show the relevant signals at 56.50–56.90 ppm and 45.88–46.18 ppm, respectively.

The NMR spectra of the derivatives of (*E*)-4-hydroxychalcone **21**–**40** show the presence of *trans*-olefinic protons [*δ*: 7.41–7.43 ppm (1H, d, $J = 15.65$ Hz, H-α); 7.78–7.79 ppm, (1H, d, $J = 15.65$ Hz, H-β)] correlated with the

Table 2
Chemical and physical data of compounds **1**–**20**

Compd.	M.p. (°C)	IR (KBr) [cm^{-1}]	^{13}C, ^{1}H NMR (*δ*), ppm								Elemental analyses, obs. (calc.)		
			C-α, H-α	C-β, H-β	C-1	C-2,6, H-2,6	C-3,5, H-3,5	C-4	C-2′,6′, H-2′,6′	C (%)	H (%)	N (%)
1	96–97[a]	1660, 1589, 1492, 1446, 1221, 992	121.17, 7.50	144.12, 7.77	131.89	128.78, 7.55	127.71, 7.29	140.72	128.43, 8.02	60.71 (60.80)	5.17 (5.10)	–
2	91–93[a]	1655, 1592, 1490, 1447, 1227, 989	121.15, 7.50	144.16, 7.77	131.88	128.84, 7.55	127.70, 7.30	140.78	128.42, 8.02	61.63 (61.70)	5.49 (5.44)	–
3	82–83[a]	1657, 1587, 1496, 1448, 1216, 986	121.11, 7.50	144.21, 7.78	131.78	128.81, 7.55	127.66, 7.29	140.89	128.39, 8.01	62.45 (62.53)	5.80 (5.75)	–
4	71–73[a]	1660, 1587, 1487, 1446, 1230, 985	121.09, 7.49	144.26, 7.77	131.70	128.80, 7.55	127.54, 7.29	141.29	128.43, 8.02	65.30 (65.35)	6.86 (6.80)	–
5	98–100[b]	1659, 1591, 1491, 1448, 1224, 988	121.09, 7.49	144.29, 7.77	131.60	128.70, 7.55	127.68, 7.29	140.86	128.30, 8.02	75.87 (75.95)	7.75 (7.70)	3.62 (3.69)
6	110–112[b]	1659, 1587, 1489, 1447, 1229, 988	121.06, 7.50	144.30, 7.78	131.63	128.75, 7.55	127.58, 7.29	141.05	128.36, 8.02	76.21 (76.29)	7.99 (7.94)	3.51 (3.56)
7	118–119[b]	1655, 1592, 1495, 1446, 1220, 980	121.04, 7.50	144.20, 7.77	131.70	128.78, 7.56	127.62, 7.30	141.16	128.37, 8.02	76.56 (76.61)	8.23 (8.16)	3.43 (3.44)
8	59–61[b]	1658, 1586, 1492, 1448, 1228, 989	121.06, 7.49	144.29, 7.77	131.67	128.79, 7.55	127.50, 7.29	141.34	128.43, 8.01	77.61 (77.70)	8.94 (8.91)	2.99 (3.02)
9	102–103[b]	1660, 1590, 1487, 1446, 1224, 986	121.49, 7.50	144.01, 7.77	131.99	128.83, 7.56	127.69, 7.30	140.81	128.33, 8.02	76.20 (76.29)	7.99 (7.94)	3.49 (3.56)
10	108–110[b]	1659, 1589, 1492, 1444, 1221, 992	120.99, 7.50	144.10, 7.78	131.86	128.80, 7.55	127.70, 7.29	140.93	128.39, 8.02	76.57 (76.61)	8.20 (8.16)	3.40 (3.44)
11	79–80[b]	1655, 1593, 1495, 1447, 1219, 980	121.02, 7.50	144.19, 7.77	131.80	128.82, 7.56	127.63, 7.29	140.99	128.34, 8.02	76.85 (76.91)	8.39 (8.37)	3.27 (3.32)
12	125–127[b]	1658, 1589, 1492, 1446, 1224, 988	120.97, 7.50	144.23, 7.77	131.55	128.75, 7.55	127.43, 7.30	141.25	128.35, 8.02	77.82 (77.94)	9.16 (9.07)	2.88 (2.93)
13	99–102[b]	1659, 1585, 1489, 1442, 1229, 1118, 990	120.99, 7.49	144.10, 7.78	131.68	128.68, 7.55	127.56, 7.29	141.20	128.38, 8.01	72.35 (72.40)	7.21 (7.13)	3.65 (3.67)
14	93–94[b]	1660, 1589, 1492, 1446, 1221, 1122, 992	121.11, 7.49	144.10, 7.77	131.64	128.69, 7.55	127.50, 7.29	141.27	128.28, 8.02	72.80 (72.87)	7.50 (7.39)	3.56 (3.54)
15	76–78[b]	1652, 1593, 1495, 1449, 1220, 1111, 980	121.07, 7.50	144.13, 7.78	131.60	128.72, 7.55	127.64, 7.29	140.82	128.30, 8.02	73.24 (73.31)	7.74 (7.63)	3.40 (3.42)
16	68–69[b]	1660, 1589, 1492, 1446, 1221, 1120, 992	120.99, 7.49	144.14, 7.77	131.58	128.70, 7.55	127.41, 7.30	141.18	128.33, 8.02	74.71 (74.79)	8.53 (8.44)	3.06 (3.01)
17	104–105[b]	3290, 1655, 1595, 1490, 1440, 1227, 988	121.13, 7.49	144.23, 7.77	131.89	128.72, 7.55	127.48, 7.29	141.23	128.27, 8.02	72.48 (72.59)	7.55 (7.42)	7.30 (7.36)
18	92–95[b]	3310, 1659, 1589, 1492, 1446, 1221, 986	121.09, 7.50	144.25, 7.78	131.80	128.74, 7.55	127.52, 7.30	141.20	128.28, 8.01	73.01 (73.06)	7.73 (7.66)	7.05 (7.10)
19	107–108[b]	3298, 1658, 1587, 1489, 1448, 1226, 989	121.07, 7.49	144.31, 7.78	131.85	128.75, 7.56	127.56, 7.30	141.30	128.31, 8.02	73.40 (73.49)	7.92 (7.89)	6.84 (6.86)
20	121–123[b]	3245, 1655, 1589, 1494, 1442, 1220, 985	120.99, 7.49	144.30, 7.78	131.87	128.82, 7.55	127.68, 7.29	141.32	128.38, 8.02	74.92 (74.95)	8.69 (8.68)	6.04 (6.03)

[a] Crystallization solvent: ethanol.
[b] Crystallization solvent: chloroform–ethanol.

119.44–119.79 C-α and 144.34–144.71 ppm C-β resonances. Additionally, the spectra display signals assigned to four aromatic protons showing the A_2B_2 pattern typical of a 4-substituted B-ring of chalcone (Table 3). For **21–40** the proton signal at 4.00–4.06 ppm (t, 2H) ascribed to O–CH_2 protons of alkoxy chain linking (*E*)-chalcone and bromo group or piperidine (4-methylpiperidine, morpholine or piperazine) ring is also observed. The correlation between the signal at 66.97 and 68.17 ppm with the ^{1}H NMR signal cited above allows the assignment of this signal to the O–CH_2 carbon.

For compounds **21–24**, the ^{13}C signal at 33.33–33.98 ppm was found to correlate, via ^1J (C,H) coupling, with the proton signal at 3.43–3.50 ppm, corresponding to the CH_2–Br group.

The ^{1}H NMR spectra of (*E*)-4-piperidinoalkoxychalcones **25–28** show a triplet at 2.95–2.96 ppm integrating for two protons. In the HETCOR spectra this signal correlates with the carbon resonating at 57.39–57.54 ppm (–CH_2–N< group). The presence of a doublet of protons of a methyl group in the range of 0.91–1.04 ppm in the ^{1}H NMR spectra of **29–32** proves the occurrence of the 4-methylpiperidine ring in the molecules of these compounds. This latter signal correlates with the carbon signal at 20.98–21.98 ppm. The spectra of compounds **33–36** having 4-morpholinoalkoxy substituent show signals at 2.36–2.43 ppm and 58.13–58.51 ppm assigned to a proton and a carbon from the group –CH_2–N<. The ^{1}H NMR spectra of these compounds show signals at 3.74–3.75 ppm and 2.46–2.48 ppm integrating for four protons and assigned to –CH_2–O–CH_2– and –CH_2–N–CH_2– protons of the morpholine moiety, respectively. These signals correlate with the carbon signals at 66.81–66.92 ppm and 53.63–53.75 ppm, respectively. These correlations allow the assignments of these signals to –CH_2–O–CH_2– and –CH_2–N–CH_2– carbons, respectively.

The IR spectra of **1–40** show strong absorption bands around 1647–1660 cm^{-1} for C=O, 1564–1571 cm^{-1} for C=C aromatic and 980–998 cm^{-1} for CH=CH *trans*. Additionally, the spectra of piperazine derivatives **17–20** and **37–40** display absorption bands ranging from 3245 to 3330 cm^{-1} for N–H and morpholine derivatives **13–16** and **33–36** display absorption bands ranging from 1111 to 1122 cm^{-1} for C–O.

4. Antimicrobial evaluation

The antibacterial activity was determined in vitro by the hole-plate agar diffusion method against Gram-positive bacteria including *S. aureus*, *Enterococcus faecalis* and *Bacillus subtilis*, and the Gram-negative bacteria including *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Yeast including *Candida albicans* and fungi including *Asperillus fumigatus* and *Microsporum gypseum* were used to test the antifungal activity. To evaluate the activity of chalcones **1–40** against food contaminant microorganisms, the minimal inhibitory concentrations (MICs) were determined (Table 4). MIC was determined as the lowest concentration of the compound tested that was able to inhibit visible growth of the bacteria or fungi. Known antibiotics like Chloramphenicol (the

Table 3
Chemical and physical data of compounds **21–40**

Compd.	M.p. (°C)	IR (KBr) [cm^{-1}]	^{13}C, ^{1}H NMR (δ), ppm							Elemental analyses, obs. (calc.)		
			C-α, H-α	C-β, H-β	C-1	C-2,6, H-2,6	C-3,5, H-3,5	C-4	C-2′,6′, H-2′,6′	C (%)	H (%)	N (%)
21	97–98[a]	1660, 1592, 1568, 1510, 1292, 1213, 1176, 993	119.75, 7.42	144.65, 7.78	127.62	130.23, 7.59	114.84, 6.92	160.93	128.39, 8.01	63.49 (63.52)	5.37 (5.33)	–
22	100–101[a]	1655, 1589, 1566, 1511, 1291, 1212, 1176, 986	119.65, 7.42	144.69, 7.79	127.48	130.19, 7.60	114.80, 6.93	161.01	128.36, 8.02	64.34 (64.35)	5.69 (5.67)	–
23	78–79[a]	1653, 1589, 1569, 1508, 1293, 1216, 1172, 985	119.62, 7.41	144.64, 7.79	127.55	130.18, 7.59	114.79, 6.92	160.90	128.30, 8.01	65.10 (65.12)	6.01 (5.99)	–
24	68–79[a]	1650, 1585, 1560, 1505, 1291, 1212, 1170, 985	119.59, 7.42	144.60, 7.78	127.50	130.20, 7.59	114.81, 6.92	160.88	128.32, 8.02	67.70 (67.72)	7.07 (7.05)	–
25	102–105[b]	1663, 1591, 1570, 1512, 1293, 1218, 1174, 985	119.48, 7.42	144.40, 7.79	127.40	130.09, 7.61	114.80, 6.91	161.01	128.29, 8.02	79.19 (79.30)	8.11 (8.04)	3.82 (3.85)
26	113–115[b]	1659, 1589, 1568, 1511, 1291, 1216, 1173, 993	119.53, 7.41	144.48, 7.78	127.36	130.15, 7.59	114.78, 6.91	160.97	128.28, 8.01	79.46 (79.54)	8.30 (8.28)	3.65 (3.71)
27	118–119[b]	1657, 1586, 1566, 1512, 1297, 1215, 1174, 997	119.49, 7.41	144.58, 7.78	127.30	130.10, 7.59	114.79, 6.92	160.88	128.24, 8.01	79.69 (79.76)	8.55 (8.50	3.61 (3.58)
28	134–135[b]	1660, 1588, 1567, 1511, 1297, 1214, 1174, 994	119.44, 7.41	144.63, 7.78	127.18	130.09, 7.59	114.80, 6.92	161.11	128.25, 8.01	80.35 (80.49)	8.30 (9.23)	3.14 (3.13)
29	92–93[b]	1658, 1590, 1568, 1516, 1295, 1219, 1175, 989	119.79, 7.43	144.34, 7.78	127.67	130.10, 7.60	114.73, 6.92	160.43	128.23, 8.02	79.45 (79.54)	8.33 (8.28)	3.64 (3.71)
30	103–104[b]	1655, 1586, 1567, 1511, 1295, 1214, 1176, 998	119.65, 7.42	144.48, 7.78	127.60	130.16, 7.59	114.78, 6.92	160.59	128.27, 8.02	79.68, (79.76)	8.61 (8.50)	3.67 (3.58)
31	92–94[b]	1658, 1589, 1569, 1513, 1295, 1216, 1176, 989	119.69, 7.41	144.54, 7.78	127.48	130.11, 7.59	114.80, 6.92	160.68	128.28, 8.02	79.95 (79.96)	8.72 (8.70)	3.45 (3.45)
32	83–84[b]	1652, 1588, 1570, 1511, 1291, 1217, 1174, 995	119.49, 7.42	144.67, 7.79	127.24	130.10, 7.59	114.82, 6.91	161.15	128.28, 8.01	80.63 (80.65)	9.45 (9.39)	3.02 (3.03)
33	83–85[b]	1649, 1590, 1569, 1513, 1295, 1215, 1178, 1113, 996	119.62, 7.42	144.71, 7.79	127.43	130.21, 7.59	114.84, 6.92	161.11	128.37, 8.02	75.57 (75.59)	7.48 (7.45)	3.80 (3.83)
34	78–81[b]	1657, 1591, 1577, 1512, 1298, 1212, 1174, 1113, 987	119.58, 7.42	144.59, 7.79	127.40	130.20, 7.60	114.86, 6.92	160.98	128.35, 8.02	75.86 (75.96)	7.78 (7.70)	3.69 (3.69)
35	98–100[b]	1652, 1591, 1567, 1513, 1295, 1216, 1179, 1111, 989	119.49, 7.42	144.68, 7.79	127.33	130.16, 7.60	114.81, 6.93	160.88	128.30, 8.02	76.29 (76.30)	7.96 (7.94)	3.58 (3.56)
36	90–92[b]	1652, 1598, 1571, 1512, 1291, 1216, 1175, 1115, 986	119.60, 7.42	144.62, 7.79	127.50	130.18, 7.60	114.82, 6.93	160.90	128.35, 8.02	77.40 (77.47)	8.79 (8.74)	3.11 (3.12)
37	115–116[b]	3260, 1650, 1590, 1565, 1511, 1296, 1212, 1174, 989	119.96, 7.41	144.59, 7.78	127.40	130.09, 7.60	114.84, 6.92	161.06	128.30, 8.02	75.68 (75.79)	7.79 (7.74)	7.60 (7.69)
38	123–125[b]	3275, 1652, 1588, 1571, 1511, 1294, 1216, 1175, 990	119.68, 7.42	144.65, 7.78	127.32	130.14, 7.59	114.80, 6.93	160.07	128.29, 8.02	76.10 (76.16)	8.11 (7.99)	7.42 (7.40)
39	128–129[b]	3330, 1647, 1588, 1565, 1512, 1295, 1216, 1174, 988	119.53, 7.41	144.64, 7.79	127.29	130.10, 7.59	114.81, 6.92	161.09	128.28, 8.01	76.48 (76.49)	8.25 (8.22)	7.15 (7.14)
40	138–140[b]	3280, 1649, 1590, 1564, 1512, 1295, 1213, 1175, 990	119.70, 7.42	144.70, 7.79	127.35	130.14, 7.59	114.79, 6.92	160.91	128.30, 8.01	77.60 (77.64)	9.11 (8.99)	6.20 (6.24)

[a] Crystallization solvent: ethanol.
[b] Crystallization solvent: chloroform–ethanol.

Table 4
Antimicrobial activity of the active compounds

Compd.	Minimum inhibitory concentration (MIC, μM)					
	S. aureus	*E. faecalis*	*B. subtilis*	*E. coli*	*C. albicans*	*M. gypseum*
1	267	–	–	–	–	–
2	–	–	–	–	–	193
5	26	264	26	–	–	264
6	254	254	254	254	127	254
7	25	25	25	–	<246	<246
8	216	–	–	–	–	216
9	25	254	254	–	–	191
10	18	25	25	–	184	184
11	24	24	18	–	<237	<237
14	–	–	–	–	–	189
15	–	–	–	–	–	<244
17	263	263	263	–	–	–
19	245	245	–	–	–	–
22	–	–	–	–	–	268
25	275	275	275	–	–	–
26	265	265	265	–	–	–
27	256	256	256	–	–	<256
28	21	209	209	–	–	–
29	133	265	265	–	–	265
30	19	26	128	–	–	256
31	25	25	19	–	–	123
33	–	–	–	–	–	274
34	–	–	–	–	–	264
35	–	–	–	–	–	254
A	15	15	15	15	–	–
B	–	–	–	–	0.5	11

MIC – the minimum inhibitory concentration is the lowest value of concentration of the investigated compound which breakes the evolution of the microorganism; A – Chloramphenicol; B – Amphotericin B.

reference antibacterial drug) and Amphotericin B (the reference antifungal drug) were used for comparison.

It is believed that the strong lipophilic character of the molecule plays an essential role in its producing the antibacterial effect. The permeability of the cell to the test compounds is one of the factors determining their antibacterial activity. In this context the presence of a hydrophobic moiety would be important for such activity. Basic amino functions (piperidine, 4-methylpiperidine, morpholine or piperazine) which are protonated at physiological pH, enhance the aqueous solubility of a generally hydrophobic chalcone template.

The lipophilicity of compounds, expressed as log *P* of the substituents in the B-ring, appears to be the main predictor for the activity. The octanol/water partition coefficient *C* log *P* being a measure of hydrophobicity/lipophilicity was calculated using ChemDraw Ultra 5.0 software integrated with CambridgeSoft Software Development Kit (CambridgeSoft Corporation) [17]. The results obtained are given in Table 5. The calculated values of log *P* for derivatives of (E)-4-mercaptochalcone were about 0.56 higher than for the corresponding compounds with an oxygen atom in the moiety. The lipophilic aptitude of a compound increased with increasing log *P*. The activity observed for compounds **1–20** was slightly higher than that of the oxygen analogues **21–40**. The piperidino and 4-methylpiperidino substituted derivatives of chalcone which had higher values of log *P* than the corresponding morpholino and piperazino substituted compounds were more active.

Regarding the correlation of the antimicrobial activity of substituted chalcones with the planarity of their molecules, the presence of a group in the *ortho*-position of the B-ring could introduce steric hindrance. The *para*-position appears to be of marginal steric effect. The molar refractivity (MR – which represents size and polarizability of a fragment or molecule) describing steric effects was calculated using ChemDraw Ultra 5.0 software (Table 5). Compounds holding electron withdrawing groups in the *para*-position improved the antimicrobial properties compared with the non-ring B-substituted chalcone.

4.1. Antibacterial activity

The antibacterial data indicated that among (*E*)-4-bromoalkylthiochalcones **1–4** and (*E*)-4-bromoalkoxychalcones **21–24**, only compound **1** showed appreciable antibacterial activity (MIC 267 μM) against *S. aureus*. Substitution of a piperidine group for a bromo group in **1–4** and **21–24** led to compounds **5–8** and **25–28**, respectively. These compounds exerted appreciable antibacterial activity against all the Gram-positive strains tested. Of these, **5** and **7** were found to be the most potent against *S. aureus* and *B. subtilis*, showing MICs of 26 and 25 μM, respectively. Compound **7** was also effective against *E. faecalis* with MIC of 25 μM. Additionally, compound **6** was found to be more active than the other derivatives with the MIC value of 254 μM against Gram-negative bacteria *E. coli*. (*E*)-4-(Piperidino-*N*-alkylthio)chalcones **5** and **7** showed higher antibacterial activity than (*E*)-4-(piperidino-*N*-alkoxy)chalcones **25** and **27**, respectively.

Replacement of the bromo group by a 4-methylpiperidine group brought an appreciable change in the activity. The

Table 5
Calculated *C* log *P* and MR values

Compd.	*C* log *P*	MR	Compd.	*C* log *P*	MR	Compd.	*C* log *P*	MR	Compd.	*C* log *P*	MR
1	5.41	101.6	**11**	6.48	133.2	**21**	4.85	95.2	**31**	5.91	126.7
2	5.83	106.2	**12**	8.15	151.6	**22**	5.26	99.8	**32**	7.58	145.2
3	6.25	110.8	**13**	4.18	116.4	**23**	5.68	104.4	**33**	3.62	109.9
4	7.92	129.2	**14**	4.60	121.0	**24**	7.35	122.8	**34**	4.04	114.5
5	5.32	119.4	**15**	5.02	125.6	**25**	4.75	113.0	**35**	4.45	119.2
6	5.73	124.1	**16**	6.69	144.0	**26**	5.17	117.6	**36**	6.12	137.6
7	6.15	128.6	**17**	3.96	118.1	**27**	5.58	122.2	**37**	3.40	111.6
8	7.82	147.1	**18**	4.38	122.7	**28**	7.25	140.6	**38**	3.81	116.2
9	5.65	124.0	**19**	4.80	127.2	**29**	5.08	117.6	**39**	4.23	120.8
10	6.06	128.6	**20**	6.47	145.7	**30**	5.50	122.2	**40**	5.90	139.2

MIC values of **9**–**11** and **29**–**31** indicated that the 4-methylpiperidinoalkylthio and 4-methylpiperidinoalkoxy derivatives of (*E*)-chalcone were active compounds against all the Gram-positive strains of the bacteria tested (MIC = 18–265 μM). Compounds with six carbon atoms in the alkyl chain (**11** and **31**) were the most potent against *B. subtilis* with MIC of 18 and 19 μM, respectively. The compounds with five carbon atoms in the alkyl chain, (**10** and **30**), were the most potent against *S. aureus* with MIC of 18 and 19 μM, respectively. Compounds **9**, **11**, and **31** were found to be active at MIC values of 24–25 μM against *S. aureus*. Compounds **10**, **11**, **30** and **31** showed a significant antibacterial activity against *E. faecalis* with MIC values of 24–26 μM.

Substitution of a morpholine group for a bromo group did not change the activity of **13**–**16** and **33**–**36** towards Gram-positive cocci and Gram-negative rods.

In the series of compounds with a piperazine group (**17**–**20** and **37**–**40**), only the derivatives with a sulfur atom and four or six methylene groups in the alkyl chain (**17** and **19**) were found to be active against Gram-positive strains of the bacteria tested.

All the compounds tested in this study displayed insignificant effect against the Gram-negative rods *E. coli*, *K. pneumoniae* and *P. aeruginosa*.

4.2. Antifungal activity

The in vitro antifungal activity of the substituted derivatives of (*E*)-4-mercaptochalcone **1**–**20** and (*E*)-4-hydroxychalcone **21**–**40** and of Amphotericin B as a reference drug on three fungi species is characterized in Table 4. Some of the compounds tested were endowed with a medium activity against *M. gypseum*. Of these, (*E*)-4-(4′-methylpiperidino)-*N*-hexylochalcone **31** was found to be most potent, showing MIC of 123 μM, whereas the MIC values for **2**, **9**, **10** and **14** were in the range of 184–193 μM or for **5**–**8**, **11**, **15**, **22**, **27**, **29**, **30**, and **33**–**35** they were in the range of 216–265 μM. Compounds with five or six carbon atoms in a piperidinoalkylthio or 4-methylpiperidinoalkylthio group (**6**, **7** and **10**, **11**) were also found to be potent against *C. albicans* with a medium activity. All the other compounds tested (**3**, **4**, **12**, **16**) exhibited no chemotherapeutical activity in vitro against the microorganisms tested.

5. Conclusion

In conclusion, several substituted (*E*)-chalcones were synthesized starting with (*E*)-4-mercaptochalcone or (*E*)-4-hydroxychalcone through the pathway involving bromoalkylation and amination. The microbiological study was undertaken to evaluate the effects of the substituted chalcones on the antibacterial and antifungal activities. As regards the structure–activity relationship of the derivatives of (*E*)-4-mercaptochalcone and (*E*)-4-hydroxychalcone the introduction of 4-alkylthio-*N*-piperidine, 4-alkylthio-*N*-4′-methylpiperidine or 4-alkoxy-*N*-4′-methylpiperidine group as a substituent in the *para* position of the (*E*)-4-mercaptochalcone or (*E*)-4-hydroxychalcone moiety, noticeably enhanced the antimicrobial activity displayed by the unsubstituted chalcones. The distance between the cyclic amino group (morpholine, piperidine, 4-methylpiperidine or piperazine) and the B-ring of the chalcone moiety was found to be of importance for the activity. An amino substituent spaced to the B-ring by four, five or six methylene groups increased the activity, whereas an amino substituent spaced by 10 methylene groups totally eliminated the activity. The most potent class of compounds has a piperidine and six carbon atoms in the alkyl chain (compound **7**) or 4-methylpiperidine and five (**10**, **30**) or six (**11**, **31**) carbon atoms in the alkyl side. The high activity of chalcones having the piperidine and 4-methylpiperidine groups (hydrophilic substituents) may reflect the fact that these compounds are more basic than the compounds with piperazine or morpholine group, and consequently have a higher degree of protonation in the assay.

6. Experimental section

6.1. Chemistry

6.1.1. General

The purity of all described compounds was checked by m.p., TLC and elemental analysis. Melting points (uncorrected) were determined on MEL-TEMPII apparatus. R_f values refer to TLC silica gel F_{254} TLC plates (Merck) developed with $CHCl_3$:MeOH (100:1) or $CHCl_3$:MeOH (10:1) and observed under UV light ($\lambda = 254$ and 366 nm). The IR spectra were recorded as KBr pellets using a Bruker IFS 113v FT-IR spectrometer. The ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Varian Mercury Spectrometer operating at 300.07 MHz (proton) or 75.46 MHz (carbon). The data were obtained from $CDCl_3$ solutions. Chemical shifts are given in the δ scale (ppm) using tetramethylsilane as an internal standard and coupling constants are expressed in Hz. ^{1}H NMR spectra were recorded with spectral width 9 kHz, acquisition time 2.0 s, pulse width 6 μs and double precision acquisition time. ^{13}C NMR spectra were recorded with spectral width 18.76 kHz, acquisition time 1.0 s, recycle delay 1.0 s and pulse width 15 μs. Heteronuclear 2D ^{13}CNMR–^{1}H NMR chemical shift correlation experiments were carried out using HETCOR spectra. The spectra were acquired with 2K data points, 256 increments and spectral width 19.63 kHz for ^{13}C NMR and 4.97 kHz for ^{1}H NMR. Elemental analyses were performed with a Vector Euro EA 3000 Analyzer. Analyses were within ±0.4% of the theoretical values. Piperidine, 4-methylpiperidine, piperazine and morpholine were purchased from Aldrich.

6.1.2. General synthesis of (*E*)-4-bromoalkylthiochalcones ***1–4***

To a stirred solution of a proper dibromoalkane (2 mmol) in *N*,*N*-dimethylformamide (5 ml) at room temperature, a solution of (*E*)-4-mercaptochalcone (0.240 g, 1 mmol) and triethylamine (0.32 ml, 2 mmol) in 10 ml of DMF was added in a drop-wise fashion. After stirring for 3.5–4 h, the obtained crude solid was filtered off (bis-(4-chalconylthio)alkanes were obtained). The reaction mixture was poured into ice-cold

water (50 ml) on continuous stirring, the crude product was precipitated. Purification of a (*E*)-4-bromoalkylthiochalcones **1–4** was accomplished by column chromatography (column – length 30 cm, diameter 2.0 cm) over silica gel (18 g – 63–100 mesh, Merck), eluting with chloroform. The fractions of 20 ml were collected and monitored by analytical TLC. The products desired were obtained from fractions 4–7. The product-containing fractions were collected and concentrated under reduced pressure to yield a yellow solid. Crystallization from ethanol gives yellow crystalline products.

6.1.3. General synthesis of (E)-4-aminoalkylthiochalcones ***5–20***

To a stirred solution of (*E*)-4-bromoalkylthiochalcone (1 mmol) in *N*,*N*-dimethylformamide (10 ml) at room temperature, triethylamine (0.28 ml, 2 mmol) and piperidine (4-methylpiperidine, morpholine or piperazine, 1 mmol) were added. After stirring for 3 days, the reaction mixture was added drop-wise to cold water. The crystalline precipitate was isolated by filtration and the precipitated solid was purified by column chromatography (column – length 30 cm, diameter 2.0 cm) on silica gel (15 g – 63–100 mesh, Merck). The column was eluted successively with the following solvents: chloroform [70 ml], chloroform–methanol mixtures [50:1 – 150 ml]. The fractions of 20 ml were collected and monitored by analytical TLC. The products desired were obtained from fractions 5–10. The isolated crude product was recrystallized from chloroform–ethanol.

6.1.4. General synthesis of (E)-4-bromoalkoxychalcones ***21–24***

In a 50 ml round-bottomed flask (*E*)-4-hydroxychalcone (1.12 g, 5 mmol), anhydrous potassium carbonate (3.8 g, 25 mmol), acetone (30.0 ml) and appropriate dibromoalkane (10 mmol) were placed. The mixture in the flask was refluxed at 50–55 °C for 5–6 h. After cooling, the inorganic salts were filtered off, the solvent was evaporated under reduced pressure, and the residue was purified by column chromatography. Crystallization from ethanol gives yellow crystalline products.

6.1.5. General synthesis of (E)-4-aminoalkoxychalcones ***25–40***

To a stirred solution of (*E*)-4-bromoalkoxychalcone (1 mmol) in *N*,*N*-dimethylformamide (10 ml) at room temperature, triethylamine (0.28 ml, 0.002 mmol) and piperidine (4-methylpiperidine, morpholine or piperazine, 1 mmol) were added. After stirring for 4 days, the reaction mixture was added drop-wise to cold water. The crystalline precipitate was isolated by filtration and the precipitated solid was purified by column chromatography (column – length 30 cm, diameter 2.0 cm) on silica gel (15 g – 63–100 mesh, Merck). The column was eluted successively with the following solvents: chloroform [80 ml], mixtures: chloroform–methanol [50:1 – 150 ml]. The fractions of 20 ml were collected and monitored by analytical TLC. The products desired were obtained from fractions 6–12. The isolated crude product was recrystallized from chloroform–ethanol.

6.2. Microbiology

The microorganisms used were supplied by the National Institute of Hygiene in Warsaw (*S. aureus* 209P FDA, *E. coli* PZH 026 B6, *C. albicans* PCM 1409 PZH), American type Culture Collection (*Streptococcus faecalis* ATCC 8040, *B. subtilis* ATCC 1633), Department of Microbiology, Poznań University of Medical Sciences (*K. pneumoniae* 231, *P. aeruginosa* SP1), Department of Medical Mycology, Poznań University of Medical Sciences (*A. fumigatus* C1, *M. gypseum* K1).

Compounds were dissolved using DMSO (Serva); concentration was 1000 μg ml^{-1}. The MIC values of the compounds were determined, with reference to standard microorganisms, by introducing 1 ml of the corresponding solutions at various concentrations into a series of tubes (each 12 × 100 mm), then 0.1 ml of a standardized 1:1000 diluted suspension of a microorganism was added. The MIC values were determined after 18 h of incubation at 37 °C. As a test medium for bacteria the fluid medium Penassay Broth (Difco) was used. In each assay both the bacterial culture sterility and standard bacterial growth were controlled. Sabouraud dextrose broth (Difco) was used as a test medium for fungi; MIC values were determined after 3–7 days of incubation at 25 °C. In all assays both fungi culture sterility and standard fungi growth were checked.

References

[1] J.R. Dimmock, D.W. Elias, M.A. Beazely, N.M. Kandepu, Curr. Med. Chem. 6 (1999) 1125–1149.
[2] M.L. Go, X. Wu, X.L. Liu, Curr. Med. Chem. 12 (2005) 483–499.
[3] Z. Nowakowska, Eur. J. Med. Chem. 42 (2007) 125–137.
[4] S. Iwata, T. Nishino, H. Inoue, N. Nagata, Y. Satomi, H. Nishino, S. Shibata, Biol. Pharm. Bull. 20 (1997) 1266–1270.
[5] R. Anto, K. Sukumaran, G. Kuttan, M. Rao, V. Subbaraju, R. Kuttar, Cancer Lett. 97 (1995) 33–37.
[6] S.F. Nielsen, T. Boesen, M. Larsen, K. Schønning, H. Kromann, Bioorg. Med. Chem. 12 (2004) 3047–3054.
[7] R.-J. Tsukiyama, H. Katsura, N. Tokuriki, M. Kobayashi, Antimicrob. Agents Chemother. 46 (2002) 1226–1230.
[8] S.F. Nielsen, M. Larsen, T. Boesen, K. Schønning, H. Kromann, J. Med. Chem. 48 (2005) 2667–2677.
[9] H. Haraguchi, K. Tanimoto, Y. Tamura, K. Mizutani, T. Kinoshita, Phytochemistry 48 (1998) 125–129.
[10] S.N. Lopez, M.V. Castelli, S.A. Zacchino, J.N. Dominguez, G. Lobo, J. Charris, J.C.G. Cortes, J.C. Ribas, C. Devia, A.M. Rodriguez, R.D. Enriz, Bioorg. Med. Chem. 9 (2001) 1999–2013.
[11] P. Boeck, P.C. Leal, R.A. Yunes, V.C. Filho, S.N. Lopez, M. Sortino, A. Escalante, R.L.E. Furlan, S.A. Zacchino, Arch. Pharm. Chem. Life Sci. 338 (2005) 87–95.
[12] L. Svetaz, A. Tapia, S.N. Lopez, R.L.E. Furlan, E. Petenatti, R. Pioli, G. Schmeda-Hirschmann, S.A. Zacchino, J. Agric. Food Chem. 52 (2004) 3297–3300.
[13] X. Liu, M.-L. Go, Bioorg. Med. Chem. 14 (2006) 153–163.
[14] K. Hirose, S. Ukai, T. Hattori, J. Pharm. Soc. Jpn. 91 (1971) 804–810.
[15] A. Levai, T. Patonay, Pharmazie 50 (1995) 429–430.
[16] Z. Nowakowska, Eur. J. Mass Spectrom. 12 (2006) 339–343.
[17] ChemDraw Ultra 5.0, CambridgeSoft Corporation, 100 Cambridge Park, Cambridge, MA 02140, USA.