Available online at www.sciencedirect.com
ScienceDirect
European Journal of Medicinal Chemistry 42 (2007) 729–733
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
http://www.elsevier.com/locate/ejmech

Laboratory note

Acid-catalyzed synthesis of oxathiolone fused chalcones. Comparison of their activity toward various microorganisms and human cancer cells line

Marek T. Konieczny [a,*], Wojciech Konieczny [a], Michał Sabisz [b], Andrzej Skladanowski [b], Roland Wakieć [b], Ewa Augustynowicz-Kopeć [c], Zofia Zwolska [c]

[a] Department of Organic Chemistry, Medical University of Gdańsk, Faculty of Pharmacy, 80-416 Gdańsk, Poland
[b] Department of Pharmaceutical Technology and Biochemistry, Gdansk University of Technology, 80-952 Gdańsk, Poland
[c] Department of Microbiology, National Research Institute of Tuberculosis and Lung Diseases, 01-138 Warsaw, Poland

Received 5 December 2006; accepted 14 December 2006
Available online 9 January 2007

Abstract

Substituted oxathiolone fused chalcones were prepared by condensation of 4-acetyl-5-methoxy-2-oxo-benz[1,3]oxathiole with benzaldehydes under acidic conditions. The compounds were tested for cytotoxic, antibacterial, antifungal and tuberculostatic activity. Three derivatives demonstrated weak activity against HeLa cells, two were slightly active against *Micrococcus luteus* and *Staphylococcus aureus*, and one was active against *Mycobacterium tuberculosis* $H_{37}Rv$.

Keywords: Chalcones; Oxathiolone; Cytotoxic; Antibacterial; Antifungal; Tuberculostatic

1. Introduction

Flavonoids exhibit a broad spectrum of activities [1–17], and therefore constitute a promising source of inspiration in search for a new medicine. Unfortunately, they are also known for the notorious lack of selectivity. One of the possible ways to overcome this problem could be careful, subtle modifications of the active molecules, resulting in desired changes in pharmacologically important parameters, such as their bulkiness, rigidity, lipophilicity, solubility, and so on. For this reason, we are interested in comparison of biological activity of compounds which comprise the flavonoid system **1** and possess similar substitution pattern, although they are based on different molecular frameworks (Fig. 1).

We have recently reported the synthesis of biologically active thioaurones (**2**) [18,19] and now we present the synthesis and properties of related chalcones (**3**) (Fig. 1). Both molecules **2** and **3** have identical substituents' pattern, significantly both involve masked *p*-hydroquinone system. However, structure of chalcone **3** is much more flexible and, contrary to the aurone **2**, the molecule does not need to be flat. It can be expected that these differences should significantly influence activity of chalcones [20].

2. Chemistry

By far the most popular way of synthesis of chalcones consists in base-catalyzed Claisen–Schmidt condensation of an appropriate acetophenone with benzaldehydes [21,22]. The reaction only seldom was done with acid catalysts [21,22], such as hydrochloride in absolute alcohols [23–25], acetic acid [24] or sulfuric acid in acetic acid [26]. Kinetics and mechanism of the last reaction were studied [26,27]. Comparative

* Corresponding author. Department of Organic Chemistry, Faculty of Pharmacy, Medical University of Gdańsk, 107 Gen. J. Hallera Street, 80-416 Gdańsk, Poland. Tel.: +48 58 349 3148; fax: +48 58 349 3206.
E-mail address: markon@amg.gda.pl (M.T. Konieczny).

0223-5234/$ - see front matter © 2007 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2006.12.014

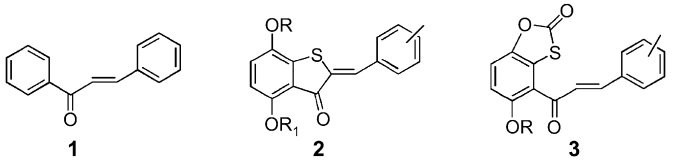


Fig. 1.

studies of chalcone synthesis under both acidic and alkaline conditions were done by Davey and Tivey [28]. It has recently been shown that chalcones could be prepared by toluenesulfonic acid-catalyzed, solvent-free reaction under microwave irradiation [29].

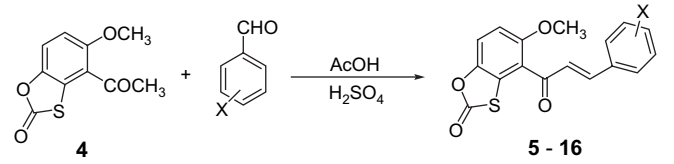
The desired chalcones **3** were prepared by condensation of substituted benzaldehydes with 4-acetyl-5-methoxy-2-oxobenz[1,3]oxathiole (**4**) [18] (Scheme 1).

The oxathiolone ring in both the starting material and products was alkali-sensitive and for this reason the standard, base-catalyzed Claisen–Schmidt condensation could not be used. However, satisfactory results were obtained for reactions carried out in acetic acid with catalytic amount of sulfuric acid. The reactions were almost quantitative, regardless of substituents, and the yields depended essentially on the yield of isolation (Table 1). It seems that the applied reaction conditions are suitable for synthesis of other hydroxychalcones with alkali-sensitive protective groups e.g. acetyl moieties.

3. Biological activity

The choice of rational screening strategy for new chemical entities constitutes a significant challenge for small academic organizations. The presently preferred target-oriented screening as a primary tool for evaluation of new compounds seems to be justified only if applied to dozens of different targets, as testing against a single target could result in a waste of precious potential of new chemical entities with potentially important biological properties. Unfortunately, multi-target screening is in most cases too costly for academia.

As a viable alternative to the target-oriented screening, to test short series of compounds as potential antitumor agents, we have chosen an approach based on the following principles: (1) preliminary screening should provide us with the information whether studied compounds exert any toxic effect toward living organisms or cells; (2) preliminary information on bioavailability and selectivity of action should be produced; (3) the tests should be easily accessible and inexpensive; (4) only compounds which are evaluated based on the above tests as ‘‘interesting’’ should be further tested using more advanced



Scheme 1.

Table 1
Activities of the synthesized compounds against tested microorganisms and cell lines

Compound	Substituent (yield %)	Antifungal activity	Bacteriostatic activity IC_{50} (μg/mL)					Cytostatic activity IC_{50} (μM) [(μg/mL)], HeLa	Tuberculostatic activity MIC (μg/mL)	
		IC_{50} value (μg/mL) *C. albicans*	*M. luteus*	*S. aureus*	*S. typhimurium*	*E. coli*	*P. vulgaris*		$H_{37}Rv$	*M. kansasii*
5	H (88)	>1000	>1000	>1000	>1000	>1000	>1000	8.4 [2.62]	>100	>100
6	4′-NO_2 (95)	>1000	>1000	>1000	>1000	>1000	>1000	—[a]	>100	>100
7	4′-OCH_3 (61)	>1000	>1000	>1000	>1000	>1000	>1000	—[a]	>100	>100
8	3′,4′-diOCH_3 (50)	>1000	>1000	>1000	>1000	>1000	>1000	14.1 [5.2]	>100	>100
9	4′-$N(CH_3)_2$ (60)	>1000	>1000	>1000	>1000	>1000	>1000	>100 [35]	>100	>100
10	4′-Br (87)	>1000	>1000	>1000	>1000	>1000	>1000	—[a]	>100	>100
11	4′-Cl (52)	62.5	15.6	7.8	>1000	>1000	>1000	5.2 [1.8]	100	>100
12	3′-Cl (37)	>1000	>1000	>1000	>1000	>1000	>1000	—[a]	25	100
13	2′-Cl (86)	>1000	>1000	>1000	>1000	>1000	>1000	—[a]	50	>100
14	4′-$OCH_2CH_2N(CH_3)_2$ (29)	125	15.6	15.6	250	125	125	8.3 [3.3]	100	100
15	4′-OCH_2CH_2morpholine (30)	250	31.2	31.2	250	125	125	—[a]	50	>100

[a] The value could not be determined as the compound precipitated out during incubation.

screening procedures. In the present studies, the prepared compounds were tested against various microorganisms (bacteria, fungi, tubercle) and one human cancer cell line.

The synthesized compounds (**5**–**15**) were screened for cytotoxic activity using HeLa cells, as well as for antibacterial activity against *Micrococcus luteus*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli*, and *Proteus vulgaris*, antifungal activity against *Candida albicans*, and tuberculostatic activity against *Mycobacterium tuberculosis* $H_{37}Rv$ and *Mycobacterium kansasii* strains. The results are given in Table 1.

Compounds **5**, **11** and **14** demonstrated weak activity against HeLa cells (Table 1), nevertheless, for all the compounds the activity was significantly higher than for analogous aurones **2** (R = OH, $R_1 = OCH_3$) [19], which suggested that loss of structural rigidity leads to increased cytotoxic activity. Among compounds which showed activity toward HeLa cells, derivative **5** demonstrated the highest selectivity as it did not exert any effect on the tested microorganisms.

Most of the prepared compounds were inactive against bacteria and fungi, only compounds **11** and **14** demonstrated moderate activity against *M. luteus* and *S. aureus*. One compound, the chloro derivative **12**, demonstrated week tuberculostatic activity against *M. tuberculosis* strain $H_{37}Rv$. The activity was selective, as **12** was inactive against HeLa cells, and did not affect the growth of bacteria or fungi.

4. Experimental

4.1. General

Melting points are uncorrected. Infrared spectra were obtained from KBr pellets on Thermo Mattson Satellite instrument. The ^1H and ^{13}C NMR spectra were recorded on 200 MHz (Varian Gemini) or 500 MHz (Varian Unity Plus) spectrometers. Elemental analyses were performed on Carlo-Erba 1108 instrument. TLC was carried out on Merck 0.2 mm silica gel 60 F_{254} aluminum plates.

4.2. General procedure for the condensation of 4-acetyl-5-methoxy-2-oxo-benz[1,3]oxathiole (*4*) with benzaldehydes

4-Acetyl-5-methoxy-2-oxo-benz[1,3]oxathiole (**1**) (224 mg, 1 mmol), suitable benzaldehyde (1.5 mmol) and conc. sulfuric acid (0.2 mL) in acetic acid (2 mL) were stirred at 60–100 °C until completion of the reaction (3–24 h). The cooled mixture was diluted with methanol (2–5 mL) and the precipitated solid was filtered off. The crude product was crystallized from 2-methoxyethanol, except for compounds **11**, **12**, **14** and **15**.

4.2.1. 5-Methoxy-4-(3-phenylacryolyl)benzo[d][1,3]-oxathiol-2-one (*5*)

Reaction with benzaldehyde, reaction temp. − 60 °C; time − 24 h. Orange solid, yield 88%, mp. 157–158 °C. Anal. Calcd for $C_{17}H_{12}O_4S$: C, 65.37; H, 3.87; S, 10.27. Found: C, 65.19; H, 3.84; S, 10.14. IR (KBr) (cm^{-1}): 1742, 1591, 1561, 1257, 1060. ^1H NMR (500 MHz, DMSO): δ 7.93 (d, 1H, $J = 15.6$ Hz, H-β), 7.74–7.82 (m, 4H, H-7, H-2′, H-6′, H-α), 7.47 (m, 3H, H-3′, H-4′, H-5′), 7.32 (d, 1H, $J = 9.3$ Hz, H-6), 4.04 (s, 3H, OCH_3).

4.2.2. 5-Methoxy-4-[3-(4′-nitrophenyl)acryolyl]benzo-[d][1,3]oxathiol-2-one (*6*)

Reaction with 4-nitrobenzaldehyde, reaction temp. − 60 °C; time − 24 h. Yellow solid, yield 95%, mp. 281–283 °C. Anal. Calcd for $C_{17}H_{11}NO_6S$: C, 57.14; H, 3.10; N, 3.92; S, 8.97. Found: C, 57.30; H, 3.01; N, 3.81; S, 8.74. IR (KBr) (cm^{-1}): 1739, 1596, 1511, 1340, 1057. ^1H NMR (500 MHz, DMSO): δ 8.28 (d, 2H, $J = 8.8$ Hz, H-3′, H-4′), 8.04 (d, 3H, $J = 8.8$ Hz, H-2′, H-6′), 8.05 (d, 1H, $J = 15.7$ Hz, H-β), 7.85 (d, 1H, $J = 15.7$ Hz, H-α), 7.79 (d, 1H, $J = 8.9$ Hz, H-7), 7.34 (d, 1H, $J = 8.9$ Hz, H-6), 4.04 (s, 3H, OCH_3).

4.2.3. 5-Methoxy-4-[3-(4′-methoxyphenyl)acryolyl]-benzo[d][1,3]oxathiol-2-one (*7*)

Reaction with 4-methoxybenzaldehyde, reaction temp. − 60 °C; time − 24 h. Orange solid, yield 61%, mp. 158–161 °C. Anal. Calcd for $C_{18}H_{14}O_5S$: C, 63.15; H, 4.12; S, 9.37. Found: C, 63.19; H, 4.03; S, 9.39. IR (KBr) (cm^{-1}): 1757, 1593, 1562, 1251, 1054. ^1H NMR (500 MHz, DMSO): δ 7.81 (d, 1H, $J = 15.8$ Hz, H-β), 7.76 (d, 1H, $J = 15.8$ Hz, H-α), 7.74 (m, 3H, H-2′, H-6′, H-7), 7.29 (d, 1H, $J = 9.2$ Hz, H-6), 7.02 (d, 2H, $J = 8.8$ Hz, H-3′, H-5′), 4.02 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3).

4.2.4. 5-Methoxy-4-[3-(3′,4′-dimethoxyphenyl)acryolyl]-benzo[d][1,3]oxathiol-2-one (*8*)

Reaction with 3,4-dimethoxybenzaldehyde, reaction temp. − 60 °C; time − 20 h. Yellow solid, yield 50%, mp. 184–187 °C. Anal. Calcd for $C_{19}H_{16}O_6S$: C, 61.28; H, 4.33; S, 8.61. Found: C, 61.12; H, 4.23; S, 8.49. IR (KBr) (cm^{-1}): 1740, 1593, 1509, 1264, 1062. ^1H NMR (200 MHz, $CDCl_3$): δ 7.87 (d, 1H, $J = 15.8$ Hz, H-β), 7.77 (d, 1H, $J = 15.8$ Hz, H-α), 7.40 (d, 1H, $J = 9.0$ Hz, H-7), 7.25 (dd, 1H, $J_1 = 8.0$ Hz, $J_2 = 2$ Hz, H-6′), 7.15 (d, 1H, $J = 2.0$ Hz, H-2′), 6.99 (d, 1H, $J = 9.0$ Hz, H-6), 6.91 (d, 1H, $J = 8.0$ Hz, H-5′), 4.03 (s, 3H, OCH_3), 3.95 (s, 6H, $2 \times OCH_3$).

4.2.5. 5-Methoxy-4-[3-(4′-dimethylaminophenyl)acryolyl]-benzo[d][1,3]oxathiol-2-one (*9*)

Reaction with 4-dimethylaminobenzaldehyde, reaction temp. − 60 °C; time − 24 h. Red solid, yield 50%, mp. 203–207 °C. Anal. Calcd for $C_{19}H_{17}NO_4S \cdot H_2O$: C, 61.10; H, 5.12; N, 3.75; S, 8.59. Found: C, 60.85; H, 5.21; N, 3.64; S, 8.49. IR (KBr) (cm^{-1}): 1740, 1520, 1258, 1062. ^1H NMR (500 MHz, DMSO): δ 7.77 (d, 1H, $J = 15.1$ Hz, H-β), 7.70 (m, 2H, H-α, H-7), 7.60 (d, 2H, $J = 8.8$ Hz, H-2′, H-6′), 7.29 (d, 1H, $J = 10.2$ Hz, H-6), 6.76 (d, 2H, $J = 8.8$ Hz, H-3′, H-5′), 4.02 (s, 3H, OCH_3), 3.01 (s, 6H, $N(CH_3)_2$).

*4.2.6. 5-Methoxy-4-[3-(4′-bromophenyl)acryolyl]benzo[d]-[1,3]oxathiol-2-one (**10**)*

Reaction with 4-bromobenzaldehyde, reaction temp. − 60 °C; time − 20 h. Yellow solid, yield 87%, mp. 193–196 °C. Anal. Calcd for $C_{17}H_{11}BrO_4S$: C, 52.19; H, 2.83; S, 8.20. Found: C, 52.13; H, 2.84; S, 8.19. IR (KBr) (cm^{-1}): 1749, 1643, 1462, 1256, 1055. ^1H NMR (500 MHz, DMSO): δ 7.92 (d, 1H, $J = 15.6$ Hz, H-β), 7.70–7.78 (m, 4H, H-α, H-7, H-2′, H-6′), 7.66 (d, 2H, $J = 8.3$ Hz, H-3′, H-5′), 7.31 (d, 1H, $J = 8.8$ Hz, H-6), 4.02 (s, 3H, OCH_3).

*4.2.7. 5-Methoxy-4-[3-(4′-chlorophenyl)acryolyl]benzo-[d][1,3]oxathiol-2-one (**11**)*

Reaction with 4-chlorobenzaldehyde, reaction temp. − 100 °C; time − 5 h. Yield of the crude product 280 mg (80%). The crude product was purified on silica gel column in methylene chloride–cyclohexane 2:1 solution to give 52% of **11** as a creamy solid, mp. 182–183 °C. Anal. Calcd for $C_{17}H_{11}ClO_4S$: C, 58.88; H, 3.20; S, 9.25. Found: C, 58.95; H, 3.04; S, 9.12. IR (KBr) (cm^{-1}): 1741, 1602, 1460, 1258, 1062. ^1H NMR (500 MHz, DMSO): δ 7.93 (d, 1H, $J = 15.6$ Hz, H-β), 7.75–7.84 (m, 4H, H-α, H-7, H-2′, H-6′), 7.54 (d, 2H, $J = 8.3$ Hz, H-3′, H-5′), 7.33 (d, 1H, $J = 9.2$ Hz, H-6), 4.04 (s, 3H, OCH_3).

*4.2.8. 5-Methoxy-4-[3-(3′-chlorophenyl)acryolyl]benzo-[d][1,3]oxathiol-2-one (**12**)*

Reaction with 3-chlorobenzaldehyde, reaction temp. − 100 °C; time − 3 h. Yield of the crude product 267 mg (77%). The crude product was purified on silica gel column in methylene chloride–cyclohexane 2:1 solution to give 37% of **12** as a creamy solid, mp. 172–173 °C. Anal. Calcd for $C_{17}H_{11}ClO_4S$: C, 58.88; H, 3.20; S, 9.25. Found: C, 58.70; H, 3.12; S, 9.11. IR (KBr) (cm^{-1}): 1756, 1597, 1465, 1258, 1056. ^1H NMR (500 MHz, DMSO): δ 7.93 (d, 1H, $J = 15.6$ Hz, H-β), 7.84 (bs, 1H, H-2′), 7.74–7.79 (m, 3H, H-α, H-7, H-6′), 7.47–7.55 (m, 2H, H-4′, H-5′), 7.32 (d, 1H, $J = 9.2$ Hz, H-6), 4.03 (s, 3H, OCH_3).

*4.2.9. 5-Methoxy-4-[3-(2′-chlorophenyl)acryolyl]benzo-[d][1,3]oxathiol-2-one (**13**)*

Reaction with 2-chlorobenzaldehyde, reaction temp. − 100 °C; time − 5 h. Yellow solid, yield 86%, mp. 210–211 °C. Anal. Calcd for $C_{17}H_{11}ClO_4S$: C, 58.88; H, 3.20; S, 9.25. Found: C, 58.81; H, 3.09; S, 9.19. IR (KBr) (cm^{-1}): 1752, 1598, 1462, 1256, 1055. ^1H NMR (500 MHz, DMSO): δ 8.04 (d, 1H, $J = 15.6$ Hz, H-β), 7.98 (dd, 1H, $J_1 = 7.8$ Hz, $J_2 = 2.0$ Hz, H-6′), 7.98 (d, 1H, $J = 15.6$ Hz, H-α), 7.79 (d, 1H, $J = 9.0$ Hz, H-7), 7.60 (dd, 1H, $J_1 = 7.8$ Hz, $J_2 = 1.4$ Hz, H-3′), 7.34 (d, 1H, $J = 9.0$ Hz, H-6), 4.04 (s, 3H, OCH_3).

*4.2.10. 5-Methoxy-4-{3-[4′-(2-dimethylaminoethoxy)-phenyl]acryolyl}benzo[d][1,3]oxathiol-2-one (**14**)*

Reaction with 4-(2-dimethylaminoethoxy)benzaldehyde, reaction temp. − 100 °C; time − 5 h. The reaction mixture was treated with ether to give an oily product. The oil was separated by decantation, treated with aqueous sodium bicarbonate and extracted with ethyl acetate. The organic layer was washed with water and brine, dried (sodium sulfate) and evaporated to dryness. The residue was dissolved in acetone, filtered through silica gel pad, evaporated, and crystallized from toluene–cyclohexane to give yellow solid, yield 29%, mp. 145–147 °C. Anal. Calcd for $C_{21}H_{21}NO_5S$: C, 63.14; H, 5.30; N, 3.51; S, 8.03. Found: C, 62.91; H, 5.15; N, 3.39; S, 7.88. IR (KBr) (cm^{-1}): 1742, 1592, 1556, 1251, 1066. ^1H NMR (500 MHz, DMSO): δ 7.83 (d, 1H, $J = 15.8$ Hz, H-β), 7.79 (d, 1H, $J = 15.8$ Hz, H-α), 7.71–7.77 (m, 3H, H-7, H-2′, H-6′), 7.32 (d, 1H, $J = 9.3$ Hz, H-6), 7.03 (d, 2H, $J = 8.3$ Hz, H-3′, H-5′), 4.12 (t, 2H, $J = 5.8$ Hz, O–CH_2), 4.04 (s, 3H, OCH_3), 2.64 (t, 2H, $J = 5.8$ Hz, N–CH_2), 2.20 (s, 6H, $N(CH_3)_2$).

*4.2.11. 5-Methoxy-4-{3-[4′-(2-morpholinoethoxy)phenyl]-acryolyl}benzo[d][1,3]oxathiol-2-one (**15**)*

Reaction with 4-(2-morpholinoethoxy)benzaldehyde, reaction temp. − 70 °C; time − 9 h. The cooled mixture was diluted with methanol (4 mL) and the solid was filtered off. The crude product was treated with aqueous sodium bicarbonate, filtered and dried. The crude product was crystallized from toluene to give yellow solid, yield 30%, mp. 184–185 °C. Anal. Calcd for $C_{23}H_{23}NO_6S$: C, 62.57; H, 5.25; N, 3.17; S, 7.26. Found: C, 62.35; H, 5.14; N, 3.29; S, 7.03. IR (KBr) (cm^{-1}): 1738, 1590, 1555, 1250, 1063. ^1H NMR (500 MHz, DMSO): δ 7.83 (d, 1H, $J = 15.6$ Hz, H-β), 7.79 (d, 1H, $J = 15.6$ Hz, H-α), 7.74–7.77 (m, 3H, H-7, H-2′, H-6′), 7.33 (d, 1H, $J = 9.3$ Hz, H-6), 7.05 (d, 2H, $J = 8.8$ Hz, H-3′, H-5′), 4.17 (t, 2H, $J = 5.8$ Hz, O–CH_2), 4.04 (s, 3H, OCH_3), 3.58 (4H, $J = 4.4$ Hz, $O(CH_2)_2$), 2.70 (t, 2H, $J = 5.8$ Hz, N–CH_2), 2.5 (under DMSO, $N(CH_2)_2$).

4.3. Cytotoxicity assays

Cell lines: Human cervix carcinoma HeLa S3 cells were maintained in high glucose DMEM medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine and antibiotics (100 units/mL penicillin and 100 μg/mL streptomycin) at 37 °C in 10% CO_2/air atmosphere. Cells were screened routinely for Mycoplasma by the PCR method with Mycoplasma *Plus* PCR Primer Set (Stratagene, La Jolla, CA).

The cytotoxicity was determined by the MTT assay. Briefly, exponentially growing cells were attached to 3×10^4 cells/well in a 24-well multiwell plates and the cellular viability was determined after 120 h of continuous exposure to different drug concentrations. Cells were incubated with the MTT tetrazolium salt for 4 h at 37 °C, and the formation of formazan was measured by a microplate reader. The concentrations required to inhibit cell growth by 50% compared to untreated controls were determined from the curves plotting survival as a function of dose by use of the Slide Write program. All values are averages of at least two independent experiments, each done in duplicate.

4.4. Determination of tuberculostatic activity

Tuberculostatic activity was tested with the test tube method of Youman's liquid medium containing 10% of bovine serum, toward *M. tuberculosis* $H_{37}Rv$ and *M. kansasii* strains with rifampicine (RMP) as a drug control. The minimum inhibiting concentration (MIC) for RMP was 6.2 μg/mL both for *M. tuberculosis* $H_{37}Rv$ and *M. kansasii*.

4.5. Determination of antibacterial and antifungal activities

Antibacterial and antifungal activities were determined by the serial twofold dilution microtiter plate method, in Nutrient Broth medium (Becton Dickinson/Difco), for antibacterial activity determination or in YEPG medium (1% yeast extract, 1% bacto-peptone, 2% glucose) for antifungal activity determination. Wells containing serially diluted examined compounds and inhibitor-free control were inoculated with overnight cultures of tested cells to the final concentration of 10^4 cells mL^{-1}. Plates were then incubated for 24 h at 37 °C (antibacterial activity determination) or for 48 h at 30 °C (antifungal activity determination). Microbial growth was quantified in each well by the measurement of an optical density at $\lambda = 595$ nm using the microplate reader (Labsystems, Multiscan Bichromatic). Drug concentrations causing 50% reduction of microbial growth in comparison to the drug-free control (IC_{50}) were read from the $A_{595} = f(\log c)$ curves, where A_{595} is the absorbance at $\lambda = 595$ nm and c is the concentration of a tested compound in μg/mL. The following microbial strains were used: (a) bacteria – *M. luteus* (clinical isolate from the collection of Medical University of Gdańsk), *S. aureus* ATCC 9144, *S. typhimurium* PCM 2180, *E. coli* ATCC 11775, *P. vulgaris* ATCC 6380; (b) fungi – *C. albicans* ATCC 10261.

Acknowledgements

We thank the Polish Ministry of Science and Higher Education for the grant no. 2 PO5F 055 28 and the Medical University of Gdańsk for the grant no. W-60.

References

[1] V. Cody, E. Middleton Jr., J.B. Harborne, Plant Flavonoids in Biology and Medicine. Biochemical, Pharmacological and Structure–Activity Relationships, Alan R. Liss Inc., New York, 1986.
[2] A. Das, J.H. Wang, E.J. Lien, in: E. Jucker (Ed.), Progress in Drug Research, vol. 42, 1994, pp. 133–166.
[3] E. Middleton Jr., C. Kandaswami, T.C. Theoharides, Pharmacol. Rev. 52 (2000) 673–751.
[4] P.-G. Pietta, J. Nat. Prod. 63 (2000) 1035–1042.
[5] A. Boumendjel, A. Di Pietro, C. Dumontet, D. Barron, Med. Res. Rev. 22 (2002) 512–529.
[6] P. Hodek, P. Trefil, M. Stiborova, Chem. Biol. Interact. 139 (2002) 1–21.
[7] A. Boumendjel, Curr. Med. Chem. 10 (2003) 2621–2630.
[8] C. Rice-Evans, L. Packer (Eds.), Flavonoids in Health and Disease, Marcel Dekker, New York, 2003.
[9] C. Rice-Evans, Free Radic. Biol. Med. 36 (2004) 827–828.
[10] T. Walle, Free Radic. Biol. Med. 36 (2004) 829–837.
[11] C. Manach, J.L. Donovan, Free Radic. Res. 38 (2004) 771–785.
[12] K.A. Youdim, B. ShukittHale, J.A. Joseph, Free Radic. Biol. Med. 37 (2004) 1683–1693.
[13] H.P. Kim, K.H. Son, H.W. Chang, S.S. Kang, J. Pharmacol. Sci. 96 (2004) 229–245.
[14] G. Williamson, D. Barron, K. Shimoi, J. Terao, Free Radic. Res. 39 (2005) 457–469.
[15] N.J. Lawrence, A.T. McGown, Curr. Pharm. Des. 11 (2005) 1679–1693.
[16] B. Botta, A. Vitali, P. Menendez, D. Misiti, G. DelleMonache, Curr. Med. Chem. 12 (2005) 713–739.
[17] L.M. Ni, C.Q. Meng, J.A. Sikorski, Expert Opin. Ther. Pat. 14 (2004) 1669–1691.
[18] M.T. Konieczny, W. Konieczny, S. Wolniewicz, K. Wierzba, Y. Suda, P. Sowiński, Tetrahedron 61 (2005) 8648–8655.
[19] M.T. Konieczny, W. Konieczny, S. Okabe, H. Tsujimoto, Y. Suda, K. Wierzba, Chem. Pharm. Bull. 54 (2006) 350–353.
[20] M.C. Wenlock, R.P. Austin, P. Barton, A.M. Davis, P.D. Leeson, J. Med. Chem. 46 (2003) 1250–1256.
[21] M.L. Go, X. Wu, X.L. Liu, Curr. Med. Chem. 12 (2005) 483–499.
[22] D.N. Dhar, The Chemistry of Chalcones and Related Systems, Willey, N.Y., 1981.
[23] R.E. Lyle, L.P. Paradis, J. Am. Chem. Soc. 77 (1955) 6667–6668.
[24] J.F. Miquel, Bull. Soc. Chim. Fr. (1961) 1369–1376.
[25] J.H. Adams, J. Org. Chem. 32 (1967) 3992–3998.
[26] D.S. Noyce, W.A. Pryor, J. Am. Chem. Soc. 77 (1955) 1397–1401.
[27] D.S. Noyce, W.A. Pryor, A.H. Bottini, J. Am. Chem. Soc. 77 (1955) 1402–1405.
[28] W. Davey, D.J. Tivey, J. Chem. Soc. (1958) 1230–1236.
[29] E.L. Gall, F. Texier-Boullet, J. Hamelin, Synth. Commun. 29 (1999) 3651–3657.