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SYNTHESIS AND ANTIMYCOBACTERIAL ASSAY OF SOME XANTHONE DERIVATIVES

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Abstract: A series of some derivatives of 2-xanthone was synthesized and evaluated for their activity against *M. tuberculosis* in primary and/or secondary microbiological assays. The cytotoxic activity of some compounds was also evaluated. The most active compounds were: [**I**] 2-(2-(4-(2-(4-chloro-3-methylphenoxy)ethyl)piperazin-1-yl)ethoxy)-9H-xanthen-9-one, [**III**] 2-((4-(2-(4-chlor-3-methylphenoxy)ethyl)piperazin-1-yl)methyl)-9*H*-xanthen-9-one dihydrochloride and [**XVIII**] ethyl 4-(2-hydroxy-3-(9-oxo-9H-xanthen-2-yloxy)propyl) piperazine-1-carboxylate, which displayed 98%, 98% and 94% inhibition of *M. tuberculosis* growth, respectively. Furthermore, compounds **III** and **XVIII** revealed their cytotoxic activity (SI < 1). Other structures varied greatly in their anti *M. tuberculosis* activity, however, several trends in their structure in relation to their antituberculous activity have been observed.

Keywords: mycobacterium, tuberculosis, xanthone derivatives, synthesis
Abbreviations: DMSO – dimethyl sulfoxide; MABA – Microplate Alamar Blue Assay; MDR-TB – Multidrug-resistant TB; SI – sensitivity index; TMS – tetramethyl silane; TB – tuberculosis

Although a vaccine and effective chemotherapy against tuberculosis (TB) have been available for more than half a century, TB was declared by the World Health Organization (WHO) a global emergency in 1993 (1, 2). The data of the WHO show that in 2004 the number of infected persons in the global population was almost 9 millions and about 1,7 million people died of TB that year. Both the highest number of deaths and the highest mortality per capita are in the WHO Africa region, where HIV has led to rapid growth of TB epidemic, and increased the likelihood of dying from TB. It accounts for about 13% of AIDS deaths worldwide (3).

The recommended modern therapy for TB consists of two phases. First-line antituberculous medications embrace: isoniazid, rifampin, pyrazinamide and either ethambutol or streptomycin given for approximately two months. Due to resistance, several variations in this strategy have been introduced and sometimes more toxic alternative drugs including ethionamide, aminosalycylic acid and ofloxacin are used. The continuation phase lasts for about three months and includes rifampin and isoniazid therapy (4).

Reasons behind the failure to reduce the number of TB cases globally has been attributed to both serious side effects (hepatitis, gastrointestinal intolerance, renal failure, dermatological, hematological and neurological reactions (5-7) of currently available antituberculous drugs and widespread trends in resistance to these drugs (8).

In 1997, the World Health Organization and the International Union Against Tuberculosis and Lung Disease found resistance to the first-line drugs in every country under investigation (9). Multidrug resistant TB (MDR-TB) is defined as the resistance to at least isoniazid and rifampin with or without resistance to other drugs. Nearly three per cent of all newly diagnosed patients have MDR-TB globally. Throughout the world there is an ongoing campaign aimed at searching for new potentially antimycobacterial compounds that will help stop the progression of the disease. Already several lead compounds as well as available drugs' derivatives have been found. Potentially antituberculous active compounds include analogues of thiolactomycin (10), 1,2-diamine analogues of ethambutol (11, 12), cyclic secondary amine substituted phenyl and ben-

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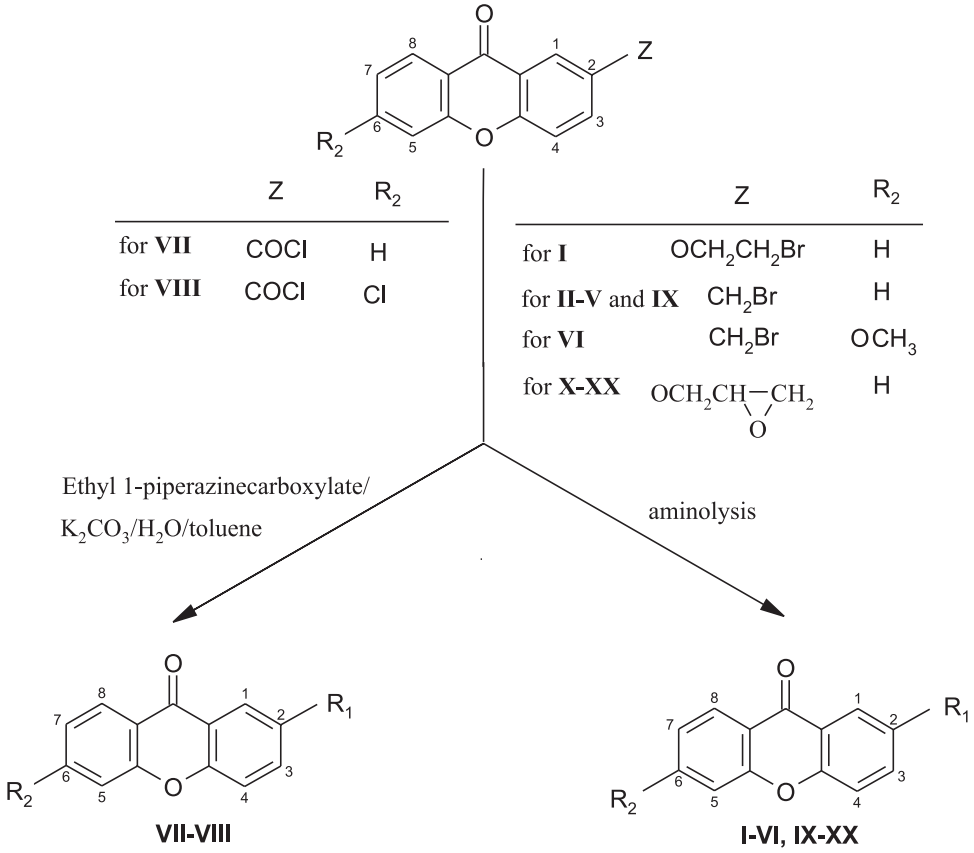


Figure 1. Synthesis of the tested compounds [**I-XX**].

zyl nitrofuranyl amides (13) and 1-(4-fluorophenyl)-3-(4-(1-((pyridine-4-carbonyl)-hydrazono)ethyl)-phenyl)thiourea (14).

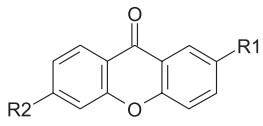
Recently great attention is also paid to the antimycobacterial activity of some naturally occurring (15-17) and synthetic xanthone derivatives (18-20). Thus, herein are reported the results of study aimed at evaluating the potential antimycobacterial activity of several xanthone derivatives. The most promising results of the *in vitro* evaluation of antituberculosis activity were previously reported as a short communication (21). Furthermore, some of the aminoalkanolic derivatives of presented herein compounds were also formerly reported for their circulatory and/ or anticonvulsant activity (22-24).

EXPERIMENTAL

Chemical methods and materials

Melting points were determined using a Büchi SMP-20 apparatus. Microanalyses were performed on an Elementar Vario EL III (Elementar Analysensysteme, Hanau, Germany) in the Department of Pharmaceutical Chemistry, Medical College, the Jagiellonian University. All the results were within an acceptable range. Theoretical values of logP combined (partition coefficient) were estimated with the Pallas 3.1.1.2. program. The IR spectra (ν_{max} in cm^{-1}) were recorded on a Perkin Elmer spectrometer, the samples were prepared as KBr pellets. The ^1H NMR spectra were performed with a Varian-Mercury spectrometer at 300 MHz, using signal from TMS in $CDCl_3$ as an internal standard or on a Bruker AMX spectrometer at 500.13 MHz and 125.17 MHz, using a signal from DMSO in DMSO-d_6 and TMS in $CDCl_3$ as internal standard. The results are presented in the following format: chemical shift d (ppm), multiplicity, number of protons, *J* values in Hertz (Hz), proton's position. Multiplicities are showed as the abbreviations: s (singlet), brs (broad singlet), bb (broad bond), d (doublet), dd (doublet of doublets), ddd (double doublet of doublets), t (triplet), dt (doublet of triplets), q (quartet), m (multiplet). TLC was performed on silica gel Kieselgel 60 F_{254} precoated plates (Merck), with an appropriate developing sys-

Table 1. Chemical structures, log P_{comb}. and some antimycobacterial data of the tested compounds [**I-XX**].



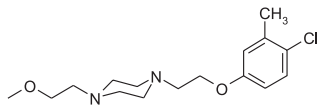
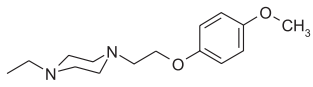
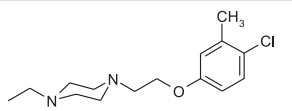
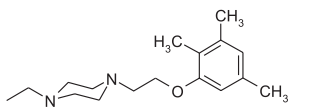
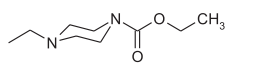
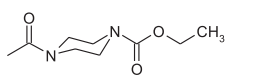
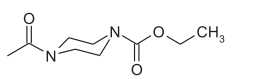
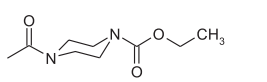
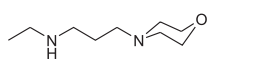
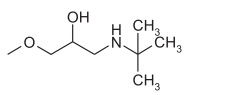
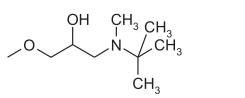
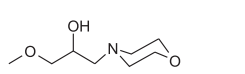
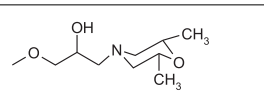
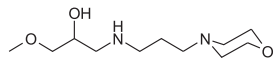
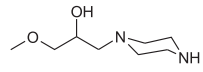
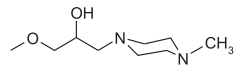
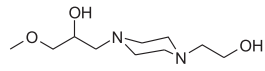
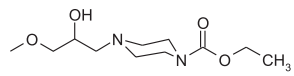
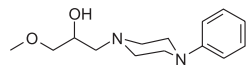
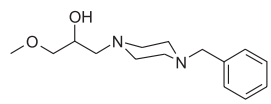
Compd.	R1	R2 Pallas	$LogP_{comb.}$† (mg/mL)	**MIC**‡ (%)	Inhibition§
I	 × 2 HCl	H	4.42	< .25	98
II	 × 2 HCl	H	3.32	> .25	65
III	 × 2 HCl	H	4.55	< 2.5	98
IV		H	4.82	> .25	11
V		H	2.79	> .25	0
VI		OCH_3	2.62	> .25	0
VII		H	2.24	> .25	0
VIII		Cl	2.72	> .25	4
IX	 × 2 HCl	H	2.47	> 2.5	9
X	 × HCl	H	3.20	> 2.5	35
XI	 × HCl	H	3.58	> 2.5	32
XII	 × HCl	H	1.47	> 2.5	35
XIII	 × HCl	H	2.59	> 2.5	34

Table 1 cont.

Compd.	R1	R2 Pallas	LogP$_{comb.}$† (mg/mL)	**MIC**‡ (%)	Inhibition§
XIV	 × HCl	H	1.60	> 2.5	63
XV		H	1.57	> 2.5	3
XVI	 × 2 HCl	H	2.08	> 2.5	25
XVII	 × 2 HCl	H	1.37	> 2.5	14
XVIII		H	2.21	< 2.5	94
XIX		H	4.16	> 2.5	24
XX	 × 2 HCl	H	3.24	> 2.5	59

†Pallas 3.1.1.2 [available online www.compudrug.com]. The predictions of logPcomb. values for compounds: 1-3, 9-16 and 19-20 were determined for appropriate bases.

‡Minimal inhibitory concentration against *Mycobacterium tuberculosis* H37Rv.

§MIC Rifampin = 0.25 μg mlL^{1} (98 % inhibition) vs. M.tuberculosis

tem of ethanol/ethyl acetate (1:1, v/v), chloroform /methanol (1:2, v/v), toluene/acetone (5:3, v/v) or toluene. Spots were visualized in UV light. Reagents and solvents were commercially available materials of reagent grade.

Preparation of starting materials

2-(2-Bromoethoxy)-9H-xanthen-9-one was obtained from 2-hydroxy-9H-xanthen-9-one (m.p. 231°C) (25) (m.p. 236-238°C) (24). First step of this procedure was the reaction between the parent compound with redistilled 2-chloroethanol in the presence of anhydrous potassium carbonate in acetone. The mixture was refluxed for 48 h and hot-filtered. The solvent was then evaporated and to the residue was added water and 5% sodium hydroxide solution. The mixture was stirred, then unsoluble precipitate was filtered off and washed with water. The resulting solid was crystallized from ethanol. The next step was bromination with phosphorus tribromide in chloroform according to well-known procedures (26).

2-(2-Hydroxyethoxy)-9H-xanthen-9-one

M.p. 151-153°C; Analysis: calcd. for $C_{15}H_{12}O_4$ m.w. 256.25: %C 70.30; %H 4.72. Found: %C 69.97; %H 5.02; IR (KBr, cm^{-1}): 3430, 2947, 1616, 1488, 1271, 1250, 1232, 1150; ^{1}H NMR 500.13 MHz (δ_H ppm): 3.78 (dt, *J* = 4.4, *J* = 5.4, 2H, C$\underline{H}_2$-OH), 4.12 (t, *J* = 4.4, 2H, Ar-O-CH$_2$), 4.94 (t, *J* = 5.4, 1H, OH), 7.47 (ddd, *J* = 1.1 Hz, *J* = 7.1 Hz, *J* = 8.0 Hz, 1H, H-7), 7.48 (dd, *J* = 3.1 Hz, *J* = 9.1 Hz, 1H, H-3), 7.55 (dd, *J* = 0.5 Hz, *J* = 3.1 Hz, 1H, H-1), 7.62 (dd, *J* = 0.5 Hz, *J* = 9.1 Hz, 1H, H-4) 7.64 (ddd, *J* = 0.5 Hz, *J* = 1.1 Hz, *J* = 8.5 Hz, 1H, H-5), 7.86 (ddd, *J* = 1.8 Hz, *J* = 7.1 Hz, *J* = 8.9 Hz, 1H, H-6), 8.19 (ddd, *J* = 0.5 Hz, *J* = 1.8 Hz, *J* = 8.0 Hz, 1H, H-8); R_F = 0.53 (toluene/acetone (5:3, v/v)).

2-(2-Bromoethoxy)-9H-xanthen-9-one

M.p. 183-185°C; Analysis: calcd. for $C_{15}H_{11}O_3Br$ m.w. 319.14: %C 56.40; %H 3.47. Found: %C 56.69; %H 3.43; IR (KBr, cm^{-1}): 1646, 1616, 1459, 1317, 1265, 1213, 1145; ^{1}H NMR

500.13 MHz (δ_H ppm): 3.84 (t, *J* = 5.4, 2H, CH_2-Br), 4.48 (t, *J* = 5.4, 2H, Ar-O-CH_2), 7.48 (ddd, *J* = 1.1 Hz, *J* = 7.1 Hz, *J* = 8.0 Hz, 1H, H-7), 7.53 (dd, *J* = 3.2 Hz, *J* = 9.1 Hz, 1H, H-3), 7.62 (dd, *J* = 0.5 Hz, *J* = 3.2 Hz, 1H, H-1), 7.65 (dd, *J* = 0.5 Hz, *J* = 9.1 Hz, 1H, H-4) 7.65 (ddd, *J* = 0.5 Hz, *J* = 1.1 Hz, *J* = 8.5 Hz, 1H, H-5), 7.87 (ddd, *J* = 1.7 Hz, *J* = 7.1 Hz, *J* = 8.9 Hz, 1H, H-6), 8.22 (ddd, *J* = 0.5 Hz, *J* = 1.7 Hz, *J* = 8.0 Hz, 1H, H-8); R_F = 0.86 (toluene/acetone (5:3, v/v)).

General procedure for the synthesis of **I-VI** and **IX** (see scheme in Figure 1): To a mixture of 2-(2-bromoethoxy)-9H-xanthen-9-one (for **I**) (3.19 g, 10 mmol) or 2-(bromomethyl)-9H-xanthen-9-one (for **II-V** and **IX**) (2.89 g, 10 mmol) or 2-(bromomethyl)-6-methoxy-9H-xanthen-9-one (for **VI**) (3.20 g, 10 mmol) and anhydrous potassium carbonate (1.38 g, 10 mmol) in toluene (40 mL) the appropriate amine (12 mmol) was added. The mixture was refluxed for 4-5 h and then the solvent was evaporated. The residue was dissolved in the appropriate amount of hot 2% HCl and purified with charcoal. From the cooled filtrate the precipitate was separated by addition of 10% NaOH. The separated solid was dried and recrystallized from toluene. Four bases were converted into hydrochloride salts (**I-III** and **IX**) in propanol/acetone (4:1, v/v) with an excess of ethanol saturated with HCl.

Synthesis and properties of the parent compounds of **II-VI** were previously reported (23, 27). The appropriate phenoxyethyl piperazines necessary to obtained of **I-IV** were synthesized according to the procedure described formerly in literature (26). Synthesis of **VII-VIII** was carried out by N-acylation of ethyl 1-piperazinecarboxylate with 9-oxo-9*H*-xanthene-2-carbonyl chloride (for **VII**) or 6-chloro-9-oxo-9*H*-xanthene-2-carbonyl chloride (for **VIII**) (28) in toluene in the presence of K_2CO_3 according to the well known procedure.

Compounds **X-XX** were prepared earlier by amination of 2-(2,3-epoxypropoxy-9*H*-xanthen-9-one) with appropriate amines in n-propanol according to the earlier published procedures (24).

Physicochemical data of the tested compounds

2-(2-(4-(2-(4-Chloro-3-methylphenoxy)ethyl)piperazin-1-yl)ethoxy)-9H-xanthen-9-one dihydrochloride (**I**)

M.p. 249-251°C; Analysis: calcd. for $C_{28}H_{31}N_2O_4Cl_3$ m.w. 565.84: %C 59.42; %H 5.52; %N 4.95. Found: %C 59.32; %H 5.62; %N 4.89; IR (KBr, cm^{-1}): 2988, 2954, 1657, 1619, 1592, 1274, 1245, 1226, 1120; ^{1}H NMR (base) 300 MHz (δ_H ppm): 2.28 (s, 3H, CH_3-Ar), 3.2-4.0 (m, 12H, CH_2-N), 4.4 (brs, 2H, CH_2-O), 4.55 (brs, 2H, CH_2-O), 6.85-8.18 (m, 10H, H-arom. (phenyl, xanthone)).

2-((4-(2-(4-Methoxyphenoxy)ethyl)piperazin-1-yl)methyl)-9*H*-xanthen-9-one dihydrochloride (**II**)

M.p. 278-280°C; (m.p. base 117-119°C); Analysis: calcd. for $C_{27}H_{30}N_2O_4Cl_2 \times 1/2\ H_2O$ m.w. 526,52: %C 61.58; %H 5.93; %N 5.32. Found: %C 61.27; %H 5.76; %N 5.34; IR (base) (KBr, cm^{-1}): 2933, 2804, 1661, 1619, 1609, 1263, 1252, 1233, 1120; ^{1}H NMR (base) 500.13 MHz (δ_H ppm): 2.36-2.58 (m, 8H, CH_2 (pip.)), 2.66 (t, *J* = 6.0 Hz, 2H, CH_2-N), 3.59 (s, 2H, Ar-CH_2-N), 3.69 (s, 3H, O-CH_3), 3.98 (t, *J* = 6.0 Hz, 2H, CH_2-O-Ar), 6.82-6.87 (m, 4H, H-arom. (phenyl)), 7.48 (ddd, *J* = 0.5 Hz, *J* = 1.0 Hz, *J* = 7.1 Hz, 1H, H-7), 7.62 (d, *J* = 8.7 Hz, 1H, H-4), 7.66 (ddd, *J* = 0.5 Hz, *J* = 1.0 Hz, *J* = 8.4 Hz, 1H, H-5), 7.79 (dd, *J* = 2.2 Hz, *J* = 8.7 Hz, 1H, H-3), 7.88 (ddd, *J* = 1.71 Hz, *J* = 7.1 Hz, *J* = 8.4 Hz, 1H, H-6), 8.10 (d, *J* = 2.2 Hz, 1H, H-1), 8.20 (dd, *J* = 1.7 Hz, *J* = 7.9 Hz, 1H, H-8); R_F = 0.17 (toluene/acetone (5:3, v/v)).

2-((4-(2-(4-Chlor-3-methylphenoxy)ethyl)piperazin-1-yl)methyl)-9*H*-xanthen-9-one dihydrochloride (**III**)

M.p. 291-293°C; (m.p. base 116-118°C); Analysis: calcd. for $C_{27}H_{29}N_2O_3Cl_3$ m.w. 534.88: %C 60.52; %H 5.45; %N 5.22. Found: %C 60.81; %H 5.20; %N 5.32; IR (base) (KBr, cm^{-1}): 3035, 2947, 2811,1651, 1620, 1592, 1464, 1230, 1134; ^{1}H NMR (base) 500.13 MHz (δ_H ppm): 2.27 (s, 3H, CH_3), 2.37-2.55 (m, 4H, CH_2 (pip.(e))), 2.68 (t, *J* = 5.9 Hz, 2H, CH_2-N), 3.38-3.45 (m, 4H, CH_2 (pip.(a))), 3.60 (s, 2H, Ar-CH_2-N), 4.04 (t, *J* = 5.9 Hz, 3H, O-CH_3), 6.78 (dd, *J* = 3.0 Hz, *J* = 8.8 Hz, 1H, H-6 (phenyl)), 6.94 (d, *J* = 3.0 Hz, 1H, H-2 (phenyl)), 7.26 (d, *J* = 8.8 Hz, 1H, H-5 (phenyl)), 7.49 (ddd, *J* = 1.0 Hz, *J* = 7.1 Hz, *J* = 8.0 Hz, 1H, H-7), 7.64 (d, *J* = 8.5 Hz, 1H, H-5), 7.67 (dd, *J* = 0.7 Hz, *J* = 8.4 Hz, 1H, H-4), 7.80 (dd, *J* = 2.2 Hz, *J* = 8.6 Hz, 1H, H-6), 7.88 (ddd, *J* = 1.7 Hz, *J* = 7.1 Hz, *J* = 8.6 Hz, 1H, H-8), 8.09 (d, *J* = 2.0 Hz, 1H, H-3), 8.20 (dd, *J* = 1.6 Hz, *J* = 7.9 Hz, 1H, H-1); R_F = 0.31 (toluene/acetone (5:3, v/v)).

2-((4-(2-(2,3,5-Trimethylphenoxy)ethyl)piperazin-1-yl)methyl)-9*H*-xanthen-9-one (**IV**)

M.p. 134-136°C; Analysis: calcd. for $C_{29}H_{37}N_2O_3$ m.w. 456.56: %C 76.28; %H 7.06; %N 6.13. Found: %C 75.88; %H 7.03; %N 6.33; IR (KBr, cm^{-1}): 2930, 2809, 1665, 1611, 1586, 1491, 1323, 1246, 1216, 1113; ^{1}H NMR 500.13 MHz (δ_H

ppm): 2.01 (s, 3H, CH_3-Ar), 2.14 (s, 3H, CH_3-Ar), 2.20 (s, 3H, CH_3-Ar), 2.41-2.46 (m, 4H, CH_2 (pip.(e))), 2.51-2.57 (m, 4H, CH_2 (pip.(a))), 2.71 (t, *J* = 5.9 Hz, 2H, CH_2-N), 3.60 (s, 2H, Ar-CH_2-N), 4.01 (t, *J* = 5.9 Hz, 2H, CH_2-O), 6.55 (brs, 1H, H-4 (phenyl)), 6.59 (brs, 1H, H-6 (phenyl)), 7.47 (ddd, *J* = 1.0 Hz, *J* = 7.1 Hz, *J* = 8.0 Hz, 1H, H-7), 7.61 (dd, *J* = 0.5 Hz, *J* = 8.5 Hz, 1H, H-4), 7.64 (ddd, *J* = 0.5 Hz, *J* = 1.0 Hz, *J* = 8.4 Hz, 1H, H-5), 7.79 (dd, *J* = 2.2 Hz, *J* = 8.5, 1H, H-3), 7.86 (ddd, *J* = 1.7 Hz, *J* = 7.1 Hz, *J* = 8.4 Hz, 1H, H-6), 8.09 (dd, *J* = 0.5 Hz, *J* = 2.2 Hz, 1H, H-1), 8.20 (ddd, *J* = 0.5 Hz, *J* = 1.7 Hz, *J* = 7.9 Hz, 1H, H-8); R_F = 0.35 (toluene).

Ethyl 4-((9-oxo-9*H*-xanthen-2-yl)methyl)piperazine-1-carboxylate (**V**)

M.p. 107-109°C; Analysis: calcd. for $C_{21}H_{22}N_2O_4$ m.w. 366.39: %C 68.83; %H 6.05; %N 7.65. Found: %C 68.24; %H 5.72; %N 7.25; IR (KBr, cm^{-1}): 2981, 2955, 1693, 1655, 1619, 1609, 1466, 1248, 1130; ^{1}H NMR 500.13 MHz (δ_H ppm): 1.19 (t, *J* = 7.0 Hz, 3H, CH_3), 2.32-2.43 (m, 4H, CH_2 (pip. (e))), 3.32-3.45 (m, 4H, CH_2 (pip.(a))), 3.62 (s, 2H, Ar-CH_2-N), 4.04 (q, *J* = 7.0 Hz, 2H, O-CH_2), 7.48 (ddd, *J* = 1.0 Hz, *J* = 7.1 Hz, *J* = 8.0 Hz, 1H, H-arom), 7.60 (d, *J* = 8.5 Hz, 1H, H-arom), 7.64 (dd, *J* = 0.6 Hz, *J* = 8.6 Hz, 1H, H-arom.), 7.79 (dd, *J* = 0.6 Hz, *J* = 8.6 Hz, 1H, H-arom), 7.87 (ddd, *J* = 1.7 Hz, *J* = 7.1 Hz, *J* = 8.6 Hz, 1H, H-arom), 8.08 (d, *J* = 1.9 Hz, 1H, H-arom), 8.19 (dd, *J* = 1.7 Hz, *J* = 7.9 Hz, 1H, H-arom); R_F = 0.54 (toluene/acetone (5:3, v/v)).

Ethyl 4-((6-methoxy-9-oxo-9*H*-xanthen-2-yl)methyl)piperazine-1-carboxylate (**VI**)

M.p. 166-168°C; Analysis: calcd. for $C_{22}H_{24}N_2O_5$ m.w. 396.44: %C 66.65; %H 6.10; %N 7.07. Found: %C 66.60; %H 5.97; %N 6.88; IR (KBr, cm^{-1}): 2774, 1688, 1645, 1618, 1588, 1432, 1242, 1120; ^{1}H NMR 500.13 MHz (δ_H ppm): 1.17 (t, *J* = 7.0 Hz, 3H, CH_3), 2.34-2.42 (m, 4H, CH_2 (pip. (e))), 3.35.-3.42 (m, 4H, CH_2 (pip. (a))), 3.63 (s, 2H, CH_2-Ar), 3.94 (s, 3H, O-CH_3), 4.03 (q, *J* = 7.0 Hz, 2H, CH_2), 7.06 (dd, *J* = 2.4 Hz, *J* = 8.7 Hz, 1H, H-7), 7.16 (d, *J* = 2.4 Hz, 1H, H-5), 7.60 (d, *J* = 8.6 Hz, 1H, H-4), 7.78 (dd, *J* = 2.2 Hz, *J* = 8.6 Hz, 1H, H-3), 8.08 (d, *J* = 2.2 Hz, 1H, H-1), 8.11 (d, *J* = 8.7 Hz, 1H, H-8), R_F = 0.57 (toluene/acetone (5:3, v/v)).

Ethyl 4-(9-oxo-9*H*-xanthene-2-carbonyl)piperazine-1-carboxylate (**VII**)

M.p. 143-145°C; Analysis: calcd. for $C_{21}H_{20}N_2O_5$ m.w. 380.37: %C 66.30; %H 5.30; %N 7.36. Found: %C 65.52; %H 4.80; %N 7.65; IR (KBr, cm^{-1}): 2970, 1700, 1666, 1624, 1458, 1289, 1264,1130; ^{1}H NMR 500.13 MHz (δ_H ppm): 1.20 (t, *J* = 7.2 Hz, 3H, CH_3), 3.30-3.90 (m, 8H, CH_2 (pip.)), 4.07 (q, *J* = 7.2 Hz, 2H, O-CH_2), 7.52 (ddd, *J* = 1.8 Hz, *J* = 7.1 Hz, *J* = 8.5 Hz, 1H, H-7), 7.70 (ddd, *J* = 0.5 Hz, *J* = 1.0 Hz, *J* = 8.5 Hz, 1H, H-5), 7.75 (dd, *J* = 2.2 Hz, *J* = 8.6 Hz, 1H, H-4), 7.91 (dd, *J* = 0.5 Hz, *J* = 8.6 Hz, 1H, H-6), 7.93 (ddd, *J* = 1.0 Hz, *J* = 7.1 Hz, *J* = 7.9 Hz, 1H, H-8), 8.21 (dd, *J* = 0.5 Hz, *J* = 2.2 Hz, 1H, H-3), 8.21 (ddd, *J* = 0.5 Hz, *J* = 1.7 Hz, *J* = 7.9 Hz, 1H, H-1); R_F = 0.41 (toluene/acetone (5:3, v/v)).

Ethyl 4-(6-chloro-9-oxo-9*H*-xanthene-2-carbonyl) piperazine-1-carboxylate (**VIII**)

M.p. 197-199°C; Analysis: calcd. for $C_{21}H_{19}N_2O_5Cl$ m.w. 414.84: %C 60.80; %H 4.62; %N 6.75. Found: %C 61.06; %H 4.65; %N 6.82; IR (KBr, cm^{-1}): 2980, 1689, 1666, 1627, 1613, 1439, 1244; ^{1}H NMR 500.13 MHz (δ_H ppm): 1.17 (t, *J* = 7.1 Hz, 1H, CH_3), 2.34-2.42 (m, 4H, CH_2 (pip.(e))), 3.33-3.42 (m, 4H, CH_2 (pip.(a))), 3.63 (s, 2H, CH_2-Ar), 4.03 (q, *J* = 7.1 Hz, 2H, CH_2), 7.51 (dd, *J* = 2.0 Hz, *J* = 8.4 Hz, 1H, H-7), 7.61 (d, *J* = 8.6 Hz, 1H, H-4), 7.81 (d, *J* = 2.0 Hz, 1H, H-5), 7.81 (dd, *J* = 2.2 Hz, *J* = 8.6 Hz, 1H, H-3), 8.07 (d, *J* = 2.2 Hz, 1h, H-1), 8.17 (d, *J* = 8.4 Hz, 1H, H-8); R_F = 0.6 (toluene/acetone (5:3, v/v)).

The physicochemical properties of compounds **IX-XX** were formerly reported (24).

Biological tests

In vitro evaluation of antimycobacterial activity against *M. tuberculosis* $H_{37}Rv$.

Primary screening was conducted at doses 12.5 or 6.25 μg/mL against *M. tuberculosis* H_{37}Rv (ATCC 27294; American Type Culture Collection, Rockville, MD) in BACTEC 12B medium. Compounds exhibiting fluorescence were tested in the BACTEC 460-radiometric system (29). Compounds demonstrating at least 90% inhibition were tested against *M. tuberculosis* H_{37}Rv at lower concentration to determine the actual minimum inhibitory concentration (MIC) in the Microplate Alamar Blue Assay (MABA). The MIC was defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls. Rifampin (Sigma Chemical Company, St. Louis, MO) was included as a positive drug control. Two compounds effecting > 90% inhibition in the primary screening were additionally tested against *M. avium* (ATCC 25291) in the MABA. Clarithromycin was included as a positive drug control.

Cytotoxic activity

Compounds were tested for overt cytotoxicity (IC_{50}) in VERO cells. After 72 h exposure, viability

was assayed on the basis of cellular conversion of MTT into a formazan product using the Promega CellTiter 96 Non-radioactive Cell Proliferation Assay. IC_{50} value divided by MIC equals SI coefficient, which describes cytotoxicity of compounds.

RESULTS AND DISCUSSION

Some 2-substituted xanthones were assayed for their inhibition of *M. tuberculosis* activity. Additionally, compounds expressing the highest activity were also examined both for their *M. avium* activity as well as their cytotoxity was evaluated. The results of the *in vitro* evaluation of antituberculosis activity are reported in Table 1. The highest level of activity against *M. tuberculosis* was observed for compounds **III** and **XVIII**, 98% and 94%, respectively (21). Thus, both compounds were also examined for their anti *M. avium* activity as well as their cytotoxicity. Both of them revealed cytotoxic activity (SI < 1), whereas only compound **III** showed significant anti *M. avium* activity (88% inhibition, MIC > 12,5 μg/mL). Taking these facts into account another group of derivatives was synthesized. Some of the presented structures are new (**I, II, IV, XV** and **XIX**). Among the new group of compounds **I** revealed the same anti *M. tuberculosis* activity as its parent compound **III** (98%). In addition, it was also observed that the other analogues of **III** (**II** and **IV**) showed lower antimycobacterial activity. It was also noticed that within the group of 2-piperazinylmethylxanthone derivatives a lack of the phenoxy moiety resulted in the loss of activity against *M. tuberculosis*. The same was observed for 2-piperazinocarbonylxanthone. In the group of the 2-(3-N-piperazino-2-hydroxy-1-propoxy)-xanthone the presence of phenoxy moiety was not necessary for antituberculous activity. In this group the highest activity was observed for compound **XVIII** which possesses the ethoxycarbonyl group. Hydrolysis of this structure resulted in significant decrease in activity (3% for compound **XV**). In the morpholine derivatives, their activity was between 9% [**IX**] and 63% [**XIV**]). It can be stated that the longer was the chain, the higher activity was observed.

Because the cells of mycobacteria are hydrophobic and possess very high lipid content of the cell envelope, constituting up to 40% of their dry weight (30), it was of interest to compare Log P_{comb} values calculated for the bases of the examined compounds (computer programs perform calculations only for bases). In our own experience in experimental determining of lipophilic parameters, lipophilicity of hydrochlorides and appropriate bases does not vary considerably. The calculated values of Log P_{comb} varied significantly from 1.37 for compound **XVII** to 4.82 for compound **IV**. The comparison of the lipophilic properties indicates that values of log P for the structures containing phenyl moiety are higher than values of log P for the other derivatives, what seems to be correlated with microbiological effects. However, no exact relation between log P and antimycobacterial activity of the tested compounds was found. It was observed that two out of three compounds exhibiting the highest activity against *M. tuberculosis* (**I** and **III**) possess high log P values (more than 4.4), which was not, however, seen for compound **XVIII** (log P = 2.21).

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