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ANTIOXIDANT AND PROOXIDANT EFFECTS OF CURCUMIN, PIPERINE AND SEMI-SYNTHETIC AMIDES WITH ANTIMYCOBACTERIAL ACTIVITY ON THE LIPID OXIDATION PROCESS

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Abstract

Free radical oxidation is considered an important factor in the treatment of tuberculosis. Drugs or treatments with antioxidants are reflected in the literature but the choice of those used for tuberculosis is quite limited. Selected natural and synthetic compounds (hybrid molecules consisting of fenchane scaffold and cinnamoyl like residues attached through amide bond), possessing antimycobacterial activity were studied for their capacity to accelerate or decelerate lipid oxidation process. Three of the compounds under study showed prooxidant activity. To the best of our knowledge, this is the first report about the prooxidant activity of piperine under spontaneous autoxidation conditions. The presence of fenchane scaffold together with the 1,3-benzodioxole fragment, positively affects the activity but we can only speculate about the role of the amide part. Effect of equimolar binary mixtures with curcumin has also been studied. These new results may contribute to the search for efficient bio-antioxidant mixtures in the prevention and elimination of adverse reactions of antibacterial drugs.

Key words: fenchane amides, tuberculosis, antimycobacterial activity, prooxidant effect, lipid oxidation, bio-antioxidant mixtures

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Introduction. Tuberculosis is a classic chronic infectious disease, a type of recurrent infection that can affect any organ in the human body. The causative agent *Mycobacterium tuberculosis* (MTB) is not considered to be a natural part of the human microflora and its surroundings. Tuberculosis continues to be a serious medical and social problem. Each year, 10 million new patients are discovered and about 4 million die. Despite the efforts made to successfully treat, prevent and control the widespread circulation of resistant forms of mycobacterial strains, that are not susceptible to clinically available drugs, tuberculosis remains a serious health problem for both developed and developing countries [1].

Free radical oxidation, one of the most important bactericidal factors of phagocytes, is of particular importance in tuberculosis [2]. The stimulation of the process, as a protective reaction of the body, is aimed at destroying the pathogen. On the other hand, the capacity of MTB to counteract the bactericidal oxidative burst and to resist, contributes to the incompleteness of phagocytosis. Thus, the continued generation of free radicals in phagocytes, without causing harm to the MTB [3], causes destruction of the surrounding lung tissue. Drugs or treatments with antioxidants are reflected in the literature but the choice of those used for tuberculosis is quite limited [4].

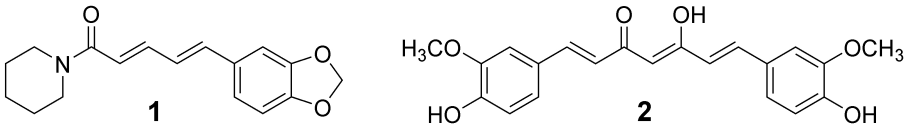


Fig. 1. Piperine (**1**) and curcumin (**2**)

Piperine (**1**) (Fig. 1) is a naturally occurring alkaloid and pungent principle isolated from the plants of *Piper* genus. Among the proven biological activities of piperine, making it a suitable and promising scaffold in drug design, is its protective efficacy against MTB [5]. However, its role as a bioenhancer is no less but even more intriguing from a research perspective. Piperine has been recognized as the world's first bioavailability enhancer in 1979 by Prasad et al. [6]. Six years later Zutshi et al. [7] reported that piperine enhanced bioavailability of rifampicin by about 60% during the course of treatment of tuberculosis. Bioenhancers reduce the dose and toxicity of the target chemical agents and even may shorten the treatment period [6, 8]. Curcumin (**2**) (Fig. 1) is also used as a bioenhancer for antimicrobials and anticancer drugs [9]. Its mechanism of action involves suppressing drug metabolizing enzymes (CYP3A4) in the liver as well as inducing changes in the drug transporter P-glycoprotein [9, 10].

The aim of the study is to assess the capacity of some natural and natural-like synthetic compounds with antimycobacterial activity to induce or combat the oxidation process as it is considered an important factor in the treatment of tuberculosis. To achieve this goal, we have chosen piperine and curcumin as well

as synthetic compounds consisting of fenchane scaffold and cinnamoyl like residues attached through amide bond.

Materials and methods. ***Synthesis of the compounds.*** Synthesis of the target compounds **7**, **8** [11] and **9** [12] (Fig. 2) and study of their in vitro antimycobacterial activity have already been described in our previous studies [11, 12]. Compound **8** was obtained after direct acylation of aminoalcohol **3** [11, 13] with cinnamoyl chloride **5** in basic conditions. Amides **7** and **9** were synthesized by coupling reaction between **3** and corresponding acids **4** and **6**, in presence of coupling reagent *O*-(Benzotriazol-1-yl)-N, N, N', N'-tetramethyluronium tetrafluoroborate (TBTU).

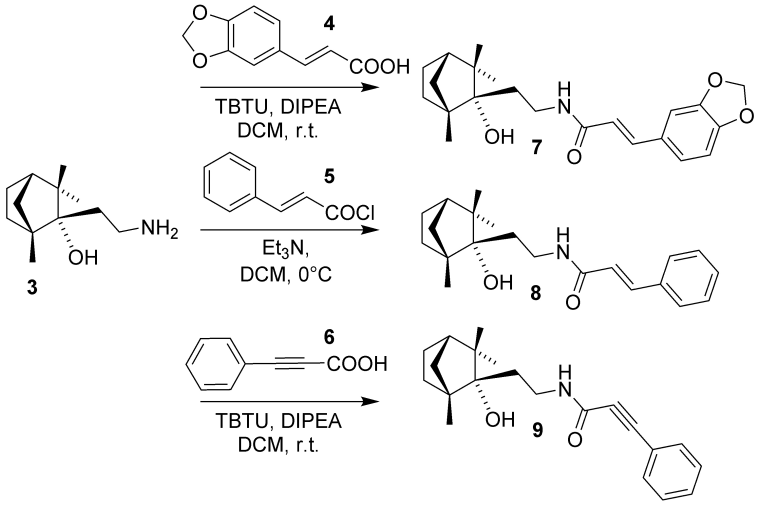


Fig. 2. Synthesis of compounds **7**–**9**

Chain-breaking antioxidant activity. Lipid samples containing various substances were prepared directly before use by adding aliquots of acetone solutions of the compounds under study to the lipid substrate (lard). More experimental details are given in [14]. The lipid autoxidation process was carried out in a thermostatic bath at $80 \pm 2\,^{\circ}\mathrm{C}$ by blowing air through the samples in special vessels. The oxidation process was monitored by withdrawing samples at measured time intervals and subjecting them to iodometric determination of lipid hydroperoxides expressed as peroxide value (PV, meq O_2/kg).

Determination of the main kinetic parameters. Induction periods (IP, hours) were determined as a cross point for the tangents to both parts of the kinetic curves. Oxidation rates were found by the tangents to the initial phase, i.e. the linear part.

Results and discussion. Kinetic curves of lipid autoxidation of the studied compounds at concentration of 0.1 mM are presented in Fig. 3. Kinetic parameters IP (induction period or the time in which the amount of antioxidant has been

fully consumed) as well as oxidation rates, obtained after processing the curves, are presented in Table 1. Protection factor PF, a measure of the capacity of a compound to increase the oxidation stability, is determined by the ratio of IP in presence and in absence of the compound. A value of PF = 1 corresponds to none antioxidant activity whereas PF < 1 indicates prooxidant activity. Inhibition degree ID or inhibition of the oxidation process which is a measure of antioxidant reactivity can be determined from the ratio of R_C, i.e. rate of the control sample, to rate in presence of a studied compound. Curcumin (***2***) showed the highest inhibition degree in this study (Fig. 3). Its antioxidant capacity highly increases with increasing its concentration, and this is well documented [15]. At 0.1 mM its IP is almost twice that of the control sample (Table 1). Piperine (***1***) and the synthesized compounds ***8*** and ***9***, with the exception of compound ***7***, showed prooxidant activity which is seen from the respective kinetic curves vs. that of the control sample.

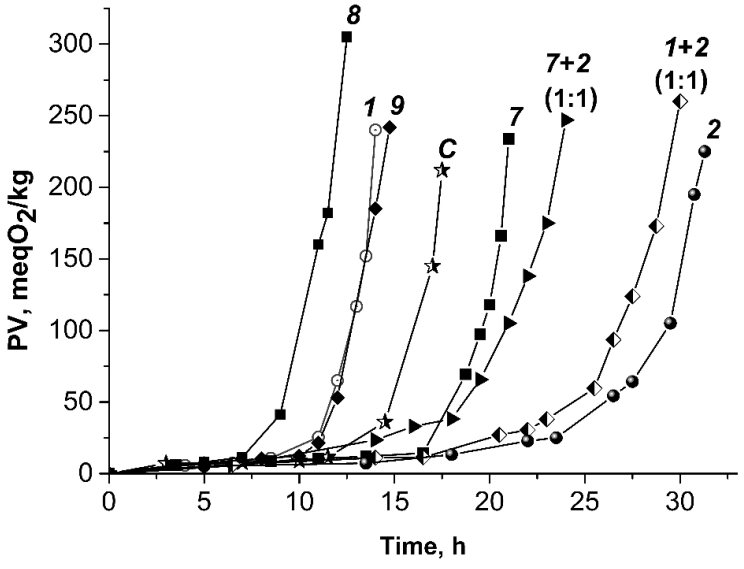


Fig. 3. Kinetic curves of accumulation of lipid peroxides (LOOH) during lipid autoxidation at 80 °C in the absence, i.e. control sample (C), and in the presence of 0.1 mM of compounds under study: piperine (***1***), curcumin (***2***) and compounds ***7***–***9*** (See Fig. 2)

There is no significant effect of replacing the double bond in the side chain with a triple bond in the structures of fenchone derivatives ***8*** and ***9***. Their IP values are within the standard deviations and similar rates of oxidation (R) in presence of ***8*** or ***9*** (at 0.1 mM) have also been observed (Table 1). Apparently, a substitution in the aromatic ring can neutralize and even reverse this effect turning it from *pro-* to weak *anti*-oxidant. This is shown by comparing the results

Table 1

Kinetic parameters of studied compounds at concentration 0.1 mM

Compounds (0.1 mM)	IP, h	PF IP_A/IP_C	R, 10^{-7} Ms^{-1}	ID (R_C/R_A)
1	13.0 ± 1.3	0.8	1.58 ± 0.10	0.8
2	29.5 ± 1.7	1.8	0.80 ± 0.06	1.7
7	20.0 ± 1.5	1.2	0.89 ± 0.07	1.5
8	11.0 ± 0.9	0.7	2.22 ± 0.11	0.6
9	12.5 ± 1.5	0.8	1.81 ± 0.09	0.7
Equimolar (1:1) binary mixtures				
1 + ***2***	26.5 ± 1.7	1.7	0.78 ± 0.07	1.7
7 + ***2***	21.5 ± 1.5	1.3	1.21 ± 0.09	1.1

Control sample: $IP_C = 16.0 \pm 1.5$ h; $R_C = 1.33\ 10^{-7}$ Ms^{-1}

for kinetic parameters of compound ***7*** (Table 1). The aromatic substitution of compounds ***1*** (piperine) and ***7*** (Fig. 4) is the same, both possess amide moiety in their structures and the presence of fenchane scaffold in ***7*** is the main structural difference. Fenchone itself exerts weak or moderate antiradical and antioxidant activities depending on its concentration [16, 17]. This gives us reason to consider that its presence in the molecule, as well as the presence of 1,3-benzodioxole fragment, positively affect the activity, but we can only speculate about the role of the amide part.

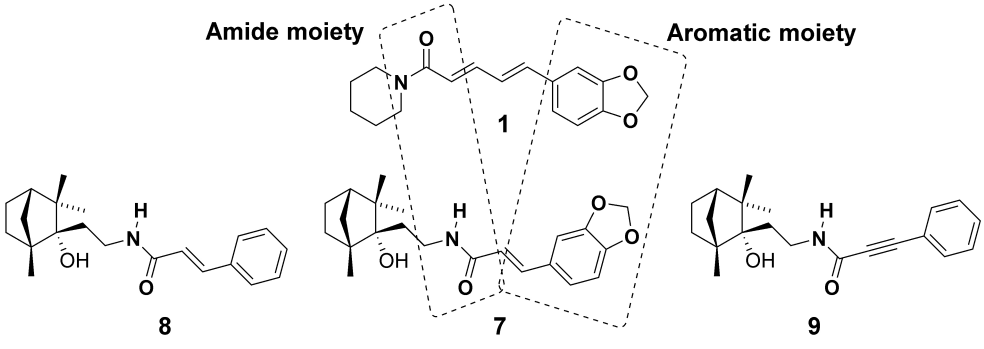


Fig. 4. Main scaffolds in the structures of piperine (**1**) and amides ***7***, ***8*** and ***9*** derived from (–)-fenchone

Compound ***1*** induces reactive oxygen species (ROS) generation and subsequent cell death has been studied in different types of cancer cells [18]. Its prooxidant activity has been shown to influence the redox state of cancer cells [19]. However, to the best of our knowledge, this is the first report about the prooxidant activity of piperine under spontaneous autoxidation conditions. Compound ***7***, whose antimycobacterial activity is comparable and even higher than that of the standard Ethambutol (EMB), demonstrates relatively high cytotoxicity onto

human embryonic cell lines from kidney HEK293 at the same time [12]. Therefore, it is important to investigate these compounds in combination with antioxidants. Non-toxicity of curcumin at high doses makes it suitable for inclusion in bio-antioxidant compositions.

If two or more antioxidants are added to oxidizing substrate, their effect can be synergistic, additive or antagonistic. The kinetic curves in Fig. 3 and the results summarized in Table 1 show that the activity of curcumin (***2***) is not changed significantly in the presence of piperine (***1***) in an equimolar binary mixture at 0.1 mM. In case of mixture of compound ***7*** with curcumin under the same conditions we observe antagonism. The latter takes place when the combined inhibiting effect of mixture is weaker than the sum of those of individual components [20]. In contrast, synergy occurs when the combined effect is higher than the effects of individual components. To make these comparisons the kinetic parameter IP is used: $IP_{2+7} < IP_2 + IP_7$.

Conclusions. Although very limited series of fenchane amides (***7***, ***8*** and ***9***) was investigated in this study, some preliminary conclusions may be proposed. Piperine and the synthesized fenchane derivatives ***8*** and ***9*** showed prooxidant activity, in contrast to compound ***7***. To the best of our knowledge, this is the first report about the prooxidant activity of piperine under spontaneous autoxidation conditions. There is no significant effect of replacing the double bond in the side chain with a triple bond in the structures of compounds ***8*** and ***9***. A substitution in the aromatic ring can neutralize and even reverse this effect turning it from *pro-* to weak *anti*-oxidant. This is shown by comparing the results for kinetic parameters of compound ***7***. The presence of fenchane scaffold in its molecule, together with the 1,3-benzodioxole fragment, positively affects the activity but we can only speculate about the role of the amide part. Antagonism is observed in equimolar binary mixture of ***7*** with curcumin. Therefore, the latter is not recommended to be used in combination with the antimycobacterial agent ***7***.

REFERENCES

[1] Manangan L. P., W. R. Jarvis (2000) Preventing Multidrug-resistant Tuberculosis and Errors in Tuberculosis Treatment around the Globe, Chest, **117**(3), 620–623.

[2] Shovkun L. A., D. A. Kudlay, N. Yu. Nikolenko, E. D. Kampos (2020) Characteristics of Certain Drugs with Antioxidant Activity and Their Use in Tuberculosis Treatment, Tuberculosis and Lung Diseases, **98**(4), 58–64.

[3] Piacenza L., M. Trujillo, R. Rafael (2019) Reactive Species and Pathogen Antioxidant Networks during Phagocitosys, J. Exp. Med., **216**(3), 501–516.

[4] Starostenko E. V., B. M. Dolzhanskii, A. M. Salpagarov, T. N. Levchenko (1991) Use of Antioxidants in Combination Treatment of Patients with Infiltrative Pulmonary Tuberculosis, Problemy Tuberkuleza, **69**(1), 9–11.

[5] Hegeto L. A., K. R. Caleffi-Ferracioli, J. Perez De Souza et al. (2019) Promising Antituberculosis Activity of Piperine Combined with Antimicrobials: A Systematic Review, Microbial Drug, **26**(1), 120–126.
[6] Prasad R., A. Singh, N. Gupta, C. Tarke (2016) Role of Bioenhancers in Tuberculosis, Int. J. Health Sci. Res., **6**(6), 307–313.
[7] Zutshi R. K., R. Singh, U. Zutshi et al. (1985) Influence of Piperine on Rifampicin Blood Levels in Patients of Pulmonary Tuberculosis, Journal of the Association of Physicians of India, **33**(3), 223–224.
[8] Zhang L., C. Virgous, H. Si (2019) Synergistic Anti-inflammatory Effects and Mechanisms of Combined Phytochemicals, J. Nutr. Biochem., **69**, 19–30.
[9] Ajazuddin, A. Alexander, A. Qureshi et al. (2014) Role of Herbal Bioactives as a Potential Bioavailability Enhancer for Active Pharmaceutical Ingredients, Fitoterapia, **97**, 1–14.
[10] Kumar V., V. Mittal, P. Jalwal (2015) Role of herbal bioenhancers (Piperine and Curcumin) on the oral bioavailability of Tamoxifen using experimental rats, J. Pharmacogn. Phytochem., **4**(2), 133–136.
[11] Dobrikov G. M., V. Valcheva, Y. Nikolova et al. (2014) Enantiopure Antituberculosis Candidates Synthesized from (−)-Fenchone, Eur. J. Med. Chem., **77**, 243–247.
[12] Slavchev I., G. M. Dobrikov, V. Valcheva et al. (2014) Antimycobacterial Activity Generated by the Amide Coupling of (−)-Fenchone derived Aminoalcohol with Cinnamic Acids and Analogues, Bioorg. Med. Chem. Lett., **24**(21), 5030–5033.
[13] Dimitrov V., G. Dobrikov, M. Genov (2001) Chiral β- and γ-Aminoalcohols derived from (+)-Camphor and (–)-Fenchone as Catalysts for the Enantioselective Addition of Diethylzinc to Benzaldehyde, Tetrahedron: Asymmetry, **12**(9), 1323–1329.
[14] Kancheva V. D., L. Saso, S. E. Angelova et al. (2012) Antiradical and Antioxidant Activity of New Bio-antioxidants, Biochimie, **94**(2), 403–415.
[15] Slavova-Kazakova A. K., S. E. Angelova, T. L. Veprintsev et al. (2015) Antioxidant Potential of Curcumin-related Compounds Studied by Chemiluminescence Kinetics, Chain-breaking Efficiencies, Scavenging Activity (ORAC) and DFT Calculations, Beilstein J. Org. Chem., **11**(11), 1398–1411.
[16] Das S., V. K. Sigh, A. K. Dwivedy et al. (2021) Exploration of some Potential Bioactive Essential Oil Components as Green Food Preservative, LWT – Food Science and Technology, **137**, 110498.
[17] El Omari N., A. Balahbib, S. Bakrim et al. (2023) Fenchone and Camphor: Main Natural Compounds from *Lavandula stoechas* L., expediting Multiple In vitro Biological Activities, Heliyon, **9**, e21222.
[18] Martín-Cordero C., A. J. León-González, J. M. Calderón-Montaño et al. (2012) Pro-oxidant Natural Products as Anticancer Agents, Current Drug Targets, **13**, 1006–1028.
[19] Yaffe P. B., C. D. Doucette, M. Walsh, D. W. Hoskin (2013) Piperine Impairs Cell Cycle Progression and Causes Reactive Oxygen Species-dependent Apoptosis in Rectal Cancer Cells, Exp. Mol. Pathol., **94**, 109–114.
[20] `https://oxystressmodulators.com/`

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