THE IN VITRO EVALUATION OF ortho-/meta-/para-ALKOXYPHENYLCARBAMIC ACID ESTERS BEARING 4-(2-FLUORO-/2-METHYLPHENYL)PIPERAZIN-1-YL MOIETY AGAINST MYCOBACTERIUM TUBERCULOSIS H₃, R₅ STRAIN

Ivan Malík⁴, Rodney Govender, Eva Sedlárová, Jozef Csöllei, Josef Jampílek, Aidan Coffey, Jim O’Mahony

Address(es): Dr. Ivan Malík,
1Comenius University in Bratislava, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Odbojárov 10, 832 32 Bratislava, Slovak Republic. Phone number: +421-2-50-117-226.
2Cork Institute of Technology, Department of Biological Sciences, Rossa Avenue, Bishopstown, Cork, Ireland.
3University of Veterinary and Pharmaceutical Sciences, Faculty of Pharmacy, Department of Chemical Drugs, Palackého 1/3, 612 42 Brno, Czech Republic.

*Corresponding author: malikivan001@gmail.com

ARTICLE INFO

Received 4. 11. 2013
Revised 19. 11. 2013
Accepted 21. 11. 2013
Published 1. 2. 2014

Regular article

OPEN ACCESS

ABSTRACT

In a continual research of ortho-/meta-/para-alkoxyphenylcarbamoylacid esters as antimycobacterially active compounds, the purpose of current paper was to in vitro screen the potency of the molecules containing incorporated substituted N-phenylpiperazin-1-yl moiety against attenuated M. tuberculosis H₃,R₅ strain which was grown in Middlebrook broth, supplemented with Oleic-Albumin-Dextrose-Catalase supplement and mycobactin J (2 µg/mL) as well. The susceptibility of concerned mycobacterial strain was investigated in a 96-well plate format, the plates were incubated at 37°C for 7 days. According to minimum inhibitory concentrations, as the lowest concentrations which prevented a visual colour change, it was observed that positional ortho-/meta-/para-alkoxy side chain isomerism as well as high lipophilicity did not decisively influenced the effectiveness of inspected compounds. Moreover, qualitatively different substitution within basic compartment in terms of electronic properties and possible hydrophobic interactions did not lead to the improvement in the activity.

Keywords: Mycobacterium tuberculosis H₃,R₅, N-aryl piperazines, alkoxyphenylcarbamates

INTRODUCTION

Tuberculosis (TB), an infectious disease caused by highly virulent bacilli from the Mycobacterium (M.) strain, remains a major global health problem despite the availability of chemotherapy and bacille Calmette-Guérin vaccine. According to the report of World Health Organization (WHO, 2013), such threat causes ill-health among millions of people each year and ranks as the second leading cause of death from an infectious disease worldwide, after the human immunodeficiency virus (HIV). It was reported that there were 8.6 million new TB cases in 2012 and 1.3 million TB deaths (just under 1.0 million among HIV-negative people and 0.3 million HIV-associated TB deaths). Most of these TB cases and deaths occur among men, but the burden of disease among women is also high. In 2012, there were an estimated 2.9 million cases and 410 000 TB deaths among women, as well as an estimated 530 000 cases and 74 000 deaths among children worldwide (WHO, 2013). A very brief insight into the history revealed that the M. tuberculosis strain H₃ was originally isolated in 1905 and gained attention for its noted virulence in animal model, a distinctive characteristics used in the classification of “human tuberculosis” in the early 1900s. According to the ability to establish the infection, in 1934, the H₃ strain was dissociated into “virulent” (R₅) strain and its “avirulent” counterpart (R₆), as reported in the scientific papers of Steenkyn et al. (1934, 1946). The distinguishing characteristics of H₃,R₅ and H₃,R₆ are maintained indefinitely on subculture, suggesting that these two strains differed genetically (Gonzalo-Assensio et al., 2008; Zheng et al., 2008). In the last decade, attenuated M. tuberculosis H₃,R₅ successively became one of the most commonly used controls for M. tuberculosis identification and investigation of its virulence and genetic properties (Chen et al., 2006; Soto et al., 2002; Wang et al., 2005; Zheng et al., 2008). Synthesis and physicochemical parameters determination of ortho-/meta-/para-alkoxyphenylcarbamoylacid esters has been at the heart of the research at the Department of Pharmaceutical Chemistry of Faculty of Pharmacy in Bratislava. Introduced structures were primarily investigated in terms of their very notable local anesthetic potency by the research groups of professor Csöllei (Čizmárík, 1976; Čizmárík et al., 1978) who synthesized and studied especially monobasic esters. In addition, besides these compounds, the research team of professor Csöllei was later focused on preparation and analysis of monobasic and dibasic derivatives as well (Csöllei et al., 1988; Csöllei et al., 1993). Concerned molecules consisted of some fundamental parts: lipophilic moiety, polar carbamoyloxy group, (branched) connecting chain and basic fragment. Described model was very similar to the chemical structure of very well-known local anaesthetics, lidocaine (Figure 1), which was discovered from systematic investigations at the Institute of Chemistry at Stockholm University (Erdtman and Löfgren, 1937; Gird, 1949). As referred research papers concluded, the intensity of local anesthetic efficiency of the basic esters was strongly dependent on the modification of all their essential compartments. A few years ago, inspecting ortho-/meta-/para-alkoxyphenylcarbamoylacid esters as antimycobacterially active agents, it was found out that some of them, which contained la substituted N-phenylpiperazin fragment, have shown promising activity against M. tuberculosis H₃,R₅ (Waissler et al., 2007). It was suggested that the presence of sterically bulky substituent (with electron-withdrawing effect) attached to phenyl ring within their basic compartment might be regarded as favorable in terms of the activity against given virulent strain of mycobacteria (Waissler et al., 2007). However, none of those alkoxyphenylcarbamoyl acid-based compounds has been in vitro screened for its potency against attenuated M. tuberculosis H₃,R₅ strain yet. Following mentioned, the objective of current research would be to in vitro investigate and to reveal some structural and physicochemical features of the substances 1-8 (Figure 1, Table 1) which might appear to be essential for the potency maintenance (or even enhancement) of concerned compounds and to contribute to knowledge for further complex structure – antimycobacterial activity relationships study as well.
Figure 1 The molecule of lidocaine (A) and general chemical structure of currently antimycobacterially investigated compounds (B)
efficiency of currently in vitro screened compounds against avirulent *M. tuberculosis* H₃⁷Rv. In contrast, it should be clearly noticed that applied standard INH was the most effective within entire set of tested compounds and it has shown the value of MIC=0.50 µg/mL, as listed in Table 1.

**CONCLUSION**

In conclusion, the results of current research revealed that positional ortho-/meta-/para-isomerism of alkoy group directly attached to lipophilic part within substituted alkoxyphenylcarbamic acid derivatives as well as their high lipophilicity were not regarded as essential factors for their potency against avirulent *M. tuberculosis* H₃⁷Rv. Moreover, assuming the presence of basic, varyingly substituted *N*-phenylpiperazin-1-yl moiety, electronic effects and possible hydrophobic interactions, which were induced by such qualitatively different substitution, were not reflected in improved activity. For further drug development within considered class of the compounds it could be suggested that the presence of two protonated basic centers instead of protonated (substituted) *N*-phenylpiperazin-1-yl fragment would lead to more promising molecules. In addition, it might be not necessary to take into account the integration of aromatic system within their basic part.

**Acknowledgments:** The authors are very grateful to Slovak Grant Agency for Science for supporting by the VEGA Grant Projects No. 1/0039/12 and No. 1/0055/11 as well as by the grant project of the Irish Department of Agriculture Fisheries and Food (FIRM): Refs 08RDC1601 and 08RDC1617. The authors thank the anonymous reviewers for their valuable comments and helpful revision suggestions.

**REFERENCES**


CLSI. 2013. Clinical and Laboratory Standards Institute. Standards and guidelines regarding clinical and laboratory testing for use within the healthcare and medical testing communities.


