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Antimycobacterial and herbicidal activity of ring-substituted 1-hydroxynaphthalene-2-carboxanilides

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ABSTRACT

In this study, a series of 22 ring-substituted 1-hydroxynaphthalene-2-carboxanilides were prepared and characterized. Primary in vitro screening of the synthesized compounds was performed against *Mycobacterium marinum*, *Mycobacterium kansasii* and *Mycobacterium smegmatis*. The compounds were also tested for their activity related to inhibition of photosynthetic electron transport (PET) in spinach (*Spinacia oleracea* L.) chloroplasts. Most of tested compounds showed the antimycobacterial activity against the three strains comparable or higher than the standard isoniazid. *N*-(3-Fluorophenyl)-1-hydroxynaphthalene-2-carboxamide showed the highest biological activity (MIC = 28.4 μmol/L) against *M. marinum*, *N*-(4-fluorophenyl)-1-hydroxynaphthalene-2-carboxamide showed the highest biological activity (MIC = 14.2 μmol/L) against *M. kansasii*, and *N*-(4-bromophenyl)-1-hydroxynaphthalene-2-carboxamide expressed the highest biological activity (MIC = 46.7 μmol/L) against *M. smegmatis*. This compound and 1-hydroxy-*N*-(3-methylphenyl)naphthalene-2-carboxamide were the most active compounds against all three tested strains. The PET inhibition expressed by IC_{50} value of the most active compound 1-hydroxy-*N*-(3-trifluoromethylphenyl)naphthalene-2-carboxamide was 5.3 μmol/L. The most effective compounds demonstrated insignificant toxicity against the human monocytic leukemia THP-1 cell line. For all compounds, structure–activity relationships are discussed.

1. Introduction

Tuberculosis (TB) is more prevalent in the world today than at any other time. *Mycobacterium tuberculosis*, the pathogen responsible for TB, uses diverse strategies to survive in a variety of host lesions and to evade immune surveillance.[1] In 2011, there were an estimated 8.7 million new cases of TB (13% co-infected with HIV), and 1.4 million people died from TB, including almost one million deaths among HIV-negative individuals and 430,000 among people who were HIV-positive. The number of cases of multi-drug-resistant strains of *M. tuberculosis* (MDR-TB) notified in 27 high MDR-TB burden countries is increasing and reached almost 60,000 worldwide in 2011, this is only one in five (19%) of the notified TB patients estimated to have MDR-TB.[2] Nontuberculous mycobacteria (NTM) are important environmental pathogens that can cause a broad spectrum of diseases, such as pulmonary disease, lymphadenitis, skin and soft tissue disease, gastrointestinal and skeletal infections. The number of infections that can be associated with specific species as well as the number of new species as etiological agents has exploded the last few years due to the increase of the number of immunocompromised patients.[3] The emergence of MDR-TB makes the discovery of new molecular scaffolds a priority, and the current situation even necessitates the re-engineering and repositioning of some old drug families to achieve effective control. Also more effective drugs are needed for the treatment of NTM.[1,3]

Salicylanilides (*N*-substituted hydroxybenzamides) are compounds with a wide range of pharmacological activities, whereas antibacterial/antimycobacterial[4–10] and antifungal[6,7] effects become more and more important. They also showed herbicidal[6–9] activity. Their mechanism of action is still under investigation; they also serve as inhibitors of protein kinase epidermal growth factor receptor (EGFR PTK).[11] They are generally designed to compete with ATP for binding in catalytic domain of tyrosine kinase.[12] The latest studies specified them also as selective inhibitors of production of interleukin-12p40 that plays a specific role in initiation, expansion and control of cellular response to tuberculosis.[13]

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The presence of an amide group is characteristic not only in various antimycobacterial drugs[14] but also in a number of herbicides acting as photosynthesis inhibitors.[6–9,15–18] Over 50% of commercially available herbicides act by reversible binding to photosystem II (PS II), a membrane–protein complex in the thylakoid membranes, which catalyses the oxidation of water and the reduction of plastoquinone,[19] and thereby inhibit photosynthesis.[20–22]

Both pharmaceuticals and pesticides are designed to target particular biological functions, and in some cases these functions overlap in their molecular target sites or they target similar processes or molecules. However, the consequences of inhibition of the overlapping target site can be completely different for plants and animals. Therefore a compound that has lethal action on plants may be beneficial for mammals.[23] Taking into consideration that herbicides may also have molecular sites of action in mammals, until recently most pharmaceutical companies had pesticide divisions, sometimes with a different name. All compounds generated by either division of the company were evaluated for both pesticide and pharmaceutical uses. In the past, some leading pesticides have become pharmaceuticals and vice versa. However, little information of this type was published and must usually be deduced from patent literature. One of the exceptions is fluconazole, a fungicide product discovered by the pharmaceutical sector that is now used both as a pharmaceutical and also patented as a chemical with applications in crop production.[23–25] Moreover, good correlation between antimicrobial activities and herbicidal effects was found [6–9,26 and refs. therein].

Promising results of antimicrobial and herbicidal screening of some salicylanilides-like compounds,[5–10] styrylhydroxyquinolines and hydroxyquinolinecarboxamides[16,27,28] inspired us to prepare and evaluate ring-substituted 1-hydroxynaphthalene-2-carboxanilides. The design of these *N*-substituted 1-hydroxynaphthalene-2-carboxanilides is based on the principle of bioisosterism and ring analogy with salicylanilides and quinoline-like compounds, see Figure 1. Thus in the context of the previously-published results,[5–9,16,17,27,28] primary in vitro screening of the synthesized compounds was performed against *Mycobacterium marinum*, *Mycobacterium kansasii* and *Mycobacterium smegmatis*. A series of 22 compounds was also tested for their inhibitory effect on photosynthetic electron transport in spinach chloroplasts (*Spinacia oleracea* L.). As many low-molecular-weight drugs cross biological membranes through passive transport, which strongly depends on their lipophilicity, the experimental log*k* values were determined by means of the HPLC method. The structure–activity relationships between the chemical structure, physical properties and in vitro biological activities of all the evaluated compounds are discussed.

2. Results and discussion

2.1. Chemistry

There are many methods for the preparation of *o*-hydroxy-carboxanilides. The discussed compounds were synthesized by using modified microwave-assisted synthesis,[8,9] thus synthesis of the target compounds was carried out only in one step with satisfactory yields. All the studied compounds were prepared according to Scheme 1. The condensation of commercially available 1-hydroxy-2-naphthoic acid and ring-substituted aniline using phosphorus trichloride in chlorobenzene under microwawe conditions yielded a series of 22 *N*-substituted 1-hydroxynaphthalene-2-carboxamides **1**–**8c**.

2.2. Lipophilicity

Lipophilicity is a property that has a major effect on absorption, distribution, metabolism, excretion and toxicity properties as well as pharmacological activity, because drugs cross biological membranes through passive transport, which strongly depends on their lipophilicity. Lipophilicity has been studied and applied as an important drug property for decades.

Lipophilicity of studied compounds **1**–**8c** was predicted as log*P* using ACD/Percepta software and determined by RP-HPLC as capacity factor logarithm (log*k*). The procedure was performed under isocratic conditions with methanol as an organic modifier in the mobile phase using an end-capped nonpolar C_{18} stationary RP column. The results are shown in Table 1 and illustrated in Figure 2.

The results obtained with all the ring-substituted 1-hydroxynaphthalene-2-carboxanilides show that the experimentally-determined lipophilicity (log*k*) of the discussed compounds poorly correlates with the calcd values of compounds **1**–**8c** as shown in Figure 2, contrary to ring-substituted 3-hydroxynaphthalene-2-carboxanilides investigated by Kos et al.[8] or chlorosalicylanilide derivatives.[7] Figure 2A illustrates the match of log*k* to log*P* of the *ortho*-substituted derivatives, while Figure 2B illustrates the match of log*k* to log*P* of the *meta*- and *para*-substituted compounds. Similar poor correlation between experimentally and predicted lipophilicity values was described by Gonec et al.[9] and Musiol et al.[16] where also a phenolic moiety was in the α-position of the aromatic ring and a carboxamide group in the β-position. Different properties of 1-OH and 3-OH moieties resulting in different behaviour of the whole molecules within lipophilicity measurement are related to the different position of the β-ring of naphthalene towards phenolic moieties. Different electron densities in position 1 or 3 are connected with this fact, resulting in different acidity and reactivity of individual 1-OH and 3-OH moieties,[29–32] therefore the α-position of the hydroxyl moiety in the naphthalene scaffold is responsible for stronger intramolecular interactions than in the case of ring-substituted 3-hydroxynaphthalene-2-carboxanilides, and these interactions were not involved in prediction by the used software.

When compared calcd log*P* with experimentally determined log*k* (see Table 1 and Fig. 2), the most significant deviations can be found for nitro and methoxy moieties. Nevertheless it is important to note that the software was not able to distinguish lipophilicity values of methyl derivatives **3a**–**c**. 1-Hydroxy-*N*-(2-nitrophenyl)naphthalene-2-carboxamide (**8a**) showed the lowest lipophilicity, while 1-hydroxy-*N*-(2-methoxyphenyl)naphthalene-

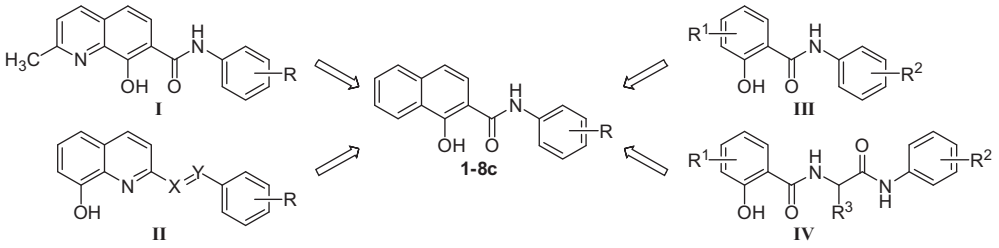

Figure 1. Design of compounds **1**–**8c** on the basis of structural analogy/bioisosterism to hydroxyquinolinecarboxamides (I)[16] styrylhydroxyquinolines (II)[27,28] and salicylanilides (III and IV).[5–7]