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Synthesis and evaluation of a novel series of pseudo-cinnamic derivatives as antituberculosis agents

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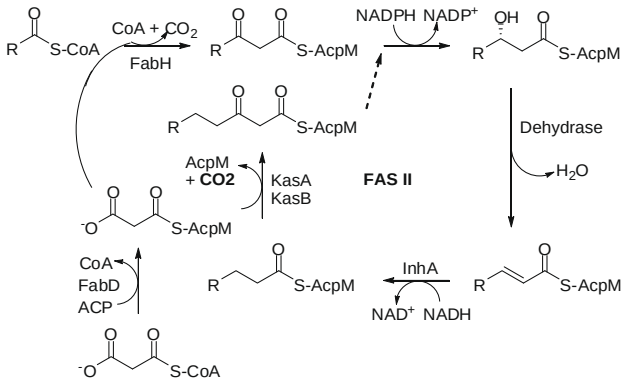
ABSTRACT

In an effort to develop potent new antituberculous drugs effective against *Mycobacterium tuberculosis*, we have prepared series of cinnamic derivatives (thioesters and amides) with 4-hydroxy and 4-alkoxy groups and investigated the in vitro activities of these compounds. Among them some displayed a good in vitro antibacterial activity, such as (*E*)-*N*-(2-acetamidoethyl)-3-{4-[(*E*)-3,7-dimethylocta-2,6-dienyloxy]phenyl}acrylamide **4b** that showed a minimum inhibitory concentration of 0.1 μg/mL (0.26 μM) against *M. tuberculosis* H37Rv.

Mycobacterium tuberculosis, the causative agent of tuberculosis, is the greatest single infectious cause of mortality worldwide, killing roughly two million people annually.[1] In addition, human immunodeficiency virus infection has been a major contributing factor in the actual resurgence of tuberculosis.[2] The emergence of multidrug-resistant (MDR) strains of *M. tuberculosis* that are resistant to the two most effective drugs, isonicotinic acid hydrazide (INH)[3] and rifampicin[4] have reaffirmed tuberculosis as a primary public health threat. Moreover, strains that are even more resistant than MDR, the so-called extensively drug resistant (XDR), have recently been described.[5] So there is a pressing need for new chemotherapeutic agents to combat the emergence of resistance and shorten the duration of treatment to improve patient compliance.[6]

Mycobacteria produce a wide array of complex fatty acids such as mycolic acids, very long chain (C-60-90) α-branched and β-hydroxylated fatty acids, not found in mammalian cells, whose biosynthesis involved the fatty acid synthase type II (FAS-II) system.[7] FAS-II is an acyl carrier protein (ACP)-dependent fatty acid synthase system found in plants, bacteria, parasites, and mitochondria where it usually performs de novo biosynthesis.[7] The FAS-II system is however unique in mycobacteria in that it elongates long chain fatty acids produced by the FAS-I system to yield the very long meromycolic acyl chain of mycolates. Genetic knockout and knock down experiments have shown that the individual enzymes of the FAS-II pathway are essential for mycobacterial cell survival.[8–11] One of the four steps of the elongation cycles of the growing long chain fatty acids (Scheme 1) catalyzed by the enoyl reductase InhA, an NADH-dependent enzyme, has proven to be the target of both



Scheme 1. General scheme of FAS-II system involved in mycolic acids biosynthesis.

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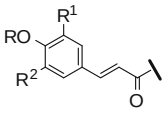
the front-line antituberculous drug INH and ethionamide.[12] Therefore, the FAS-II biosynthesis pathway represents a validated and yet promising target for drug discovery.[13] Among families of compounds that have been explored as inhibitors of the FAS-II system, we can mention diphenyl ether systems interacting with the enzyme-cofactor binary complex, the compounds that covalently bind to the cofactor enzyme site, but also indols, benzofuran and more recently cinnamic acid based derivatives.[14,15] Herein we present our preliminary results on the synthesis and evaluation of the antimycobacterial activities of some cinnamic acid derivatives. It is envisaged from structural similarities, that these compounds may act on the biosynthesis of mycolic acids and more specifically on FAS-II, which uses α,β-enoyl systems as substrates (Scheme 1).[16]

The compounds synthesized, present the following general characteristics. They possess all cinnamoyl main frames namely the aromatic moiety adjacent to the enoyl function. The aromatic group possesses the different substitutions of the cinnamic acids namely ferulic acid (4-OH, 3-OMe), sinapic acid (4-OH, 3,5-OMe), or coumaric acid (4-OH). Compounds lipophilicity was modulated by different 4-alkoxy substitutions, such as methoxy, isopentenyloxy, geranyloxy, or farnesyloxy groups introduced only on para-coumaric derivatives. The carbonyl function of the enoyl system was functionalized with *N*-acetylcysteamine, the smallest frame of ACP or its amide analogue (first family of compounds). Aromatic systems like thiophenyl or aminopyridine have also been introduced into the α-position from the carbonyl (second family). Finally, tryptamine, a frame with linear side chain and aromatic ring has been used as coupling reagent. These different patterns have been introduced in order to estimate the impact on activity of functionalised carbonyl group addressed to the substrate's or cofactor's active site.

All synthesis started from acid derivatives. 4-Methoxycinnamic, coumaric, ferulic, and sinapic acids are commercially available; acids **1a–c** were obtained in three steps starting from the coumaric acid. Firstly the corresponding methyl ester was obtained by refluxing the acid for 24 h in methanol in presence of H_2SO_4 and 3 Å molecular sieves (86% yield after silica gel purification).[17] Then we proceeded to alkylation of the 4-OH phenolic group. All reactions were carried out by refluxing the convenient alkyl bromide (isopentenyl, geranyl, or farnesyl) in dry acetone in presence of K_2CO_3 and KI.[18] The reactions were monitored by TLC until completion. The corresponding phenoxy ethers obtained in good yield (86–95%) after silica gel purification, were then converted quantitatively into the corresponding acids **1a–c** under reflux and basic conditions (K_2CO_3 in methanol).

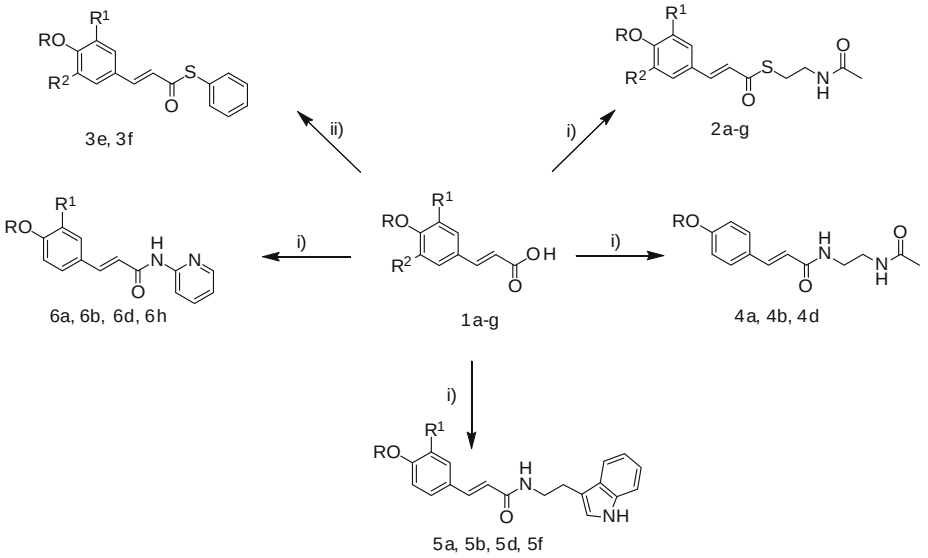
Acids **1a–c** thus prepared and the commercially available ones were used as starting materials for the preparation of all thioesters and amides mentioned in Scheme 2. Coupling of acid **1a–g** with thiols (*N*-acetylcysteamine) or various amines (2-aminopyridine, acetylethylenediamine, or tryptamine) using EDC in the presence of DMAP, in CH_2Cl_2 for 24 h afforded the respective thioesters **2a–g** and amides derivatives **4a, 4b, 4d–f, 5a–b, 5d, 5f, 6a–b, 6d** in good to excellent yield (62–96%).[19] Only thiophenyl esters **3e–f** were synthesized differently through coupling reaction between

Table 1
Structure, MIC, and solubility data of cinnamic acid derivatives



Compound	R	R^1	R^2	TB MIC (μg/mL)	*C*Log*P*[a]
2a	Isopentenyl	H	H	32	3.46
2b	Geranyl	H	H	0.6	5.75
2c	Farnesyl	H	H	0.6	8.54
2d	CH_3	H	H	63	1.65
2e	H	H	H	63	1.09
2f	H	OCH_3	H	125	0.91
2g	H	OCH_3	OCH_3	125	0.74
3e	H	H	H	8	4.10
3f	H	OCH_3	H	8	3.92
4a	Isopentenyl	H	H	63	2.78
4b	Geranyl	H	H	0.1	4.81
4d	CH_3	H	H	>500	1.08
5a	Isopentenyl	H	H	63	5.43
5b	Geranyl	H	H	32	7.54
5d	CH_3	H	H	500	3.63
5f	H	OCH_3	H	8	2.89
6a	Isopentenyl	H	H	16	4.34
6b	Geranyl	H	H	1	6.65
6d	CH_3	H	H	63	2.54
6h	CH_3	OCH_3	H	125	2.10

[a] *C*Log*P* was calculated using the ChemDraw Ultra, version 10.0, software by Cambridge Soft.



Scheme 2. Reagents and conditions: (i) DMAP, EDC, *N*-acetylcysteamine, 4-acetylethylenediamine, tryptamine, or 2-aminopyridine CH_2Cl_2, rt, 24 h; (ii) $(COCl)_2$, DMF, CH_2Cl_2, 0 °C, 1 h, then thiophenol, BuLi, THF, −30 °C, 2 h.

the thiophenate lithium salt and the corresponding carboxymethylene iminium chlorides prepared by the reaction of carboxylic acids with *N,N*-dimethylchloromethylene iminium chloride.[20] Compounds were purified by flash silica gel chromatography (60–70%) before being tested on *M. tuberculosis*.

Susceptibility of *M. tuberculosis* (strain H37Rv) to the synthetic compounds was tested by determining the minimal inhibitory concentration (MIC) (Table 1). We used a colorimetric microassay based on the reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma) to formazan by metabolically active cells.[21,22] Briefly, serial twofold dilutions of each compound were prepared in 7H9 broth (Middlebrook 7H9 broth base, Difco) using 96-well microtiter plates and 100 μL of *M. tuberculosis* suspension in 7H9 broth were added to each well. After 6 days incubation, MTT was added (50 μL, 1 mg/mL). After one day incubation, solubilization buffer was added to each well. The optical densities were measured at 570 nm. The MIC was determined as the lowest concentration of compound that inhibited bacterial growth (absorbance from untreated bacilli was taken as a control for growth). In these conditions, ethambutol, an effective and clinically used drug, was also tested exhibited a MIC of 2–4 μg/mL, in agreement with published data, validating thus the method used in the present study.

Concerning the first family of compounds **2a–g** bearing the linear *N*-acetylcysteamine frame, MIC results indicate that introduction of alkene chains increase the activity. The best result, also in connection with an acceptable lipophilic factor, was found when geranyl chain (**2b**, 0.6 μg/mL, 1.5 μM) was introduced. Activity was increased 100-fold in comparison with the compound **2d** (0.6 vs 63 μg/mL, respectively).

The same trends were also observed when *N*-acetylethylenediamine was used as coupling partner of the enoyl system of amides possessing only 4-alkoxy substitution (compounds **4a**, **4b**, **4d**). While amides **4a**, **4d**, are less active than the corresponding thioester counterparts, the derivative **4b** showed a very potent activity (0.1 μg/mL). It is noteworthy, that **4b** has *C*Log*P* less than 5, which means that it is likely to have greater oral bioavailability by Lipinski's rules[23] than **2b** (as well as MIC, Table 1).

Regarding the second family of compounds tested, two aromatic systems are reported here: thiophenyl and aminopyridine derivatives. While **3e**, **3f** aromatic thioesters, present weak activities against *M. tuberculosis*, their MIC values are found to be eightfold lower than those of the corresponding linear thioesters **2e** and **2f**, indicating a better activity for aromatic thioesters. Their high lipophilicity, and the fragility of the C(O)–SAr bond towards hydrolysis will probably prevent these compounds from being investigated by further modification.

The pyridyl amides **6a**, **6d** synthesized showed the same trends as the corresponding linear amides in terms of activity vs substitution pattern on the 4-hydroxy position. The best result was obtained for the geranylated derivative **6b** (1 μg/mL, 2.6 μM). However, **6b** in comparison to **4b** showed higher lipophilicity as well as MIC.

Finally, amides possessing the mixed linear and aromatic frame found in tryptamine were tested. Tryptamine derivatives seem to behave as weaker inhibitors in comparison to the corresponding aromatic and linear ones.

In conclusion, this preliminary study shows that cinnamic derivatives may be regarded as interesting leads in the design and synthesis of *M. tuberculosis* inhibitors. While the 2-aminopyridine derivative **6b** presented a good MIC value against *M. tuberculosis*, the linear thioester **2b** and especially the amide **4b** showed the best inhibitory activity of this explored series. Taking into account our preliminary results on the families of compounds evaluated, our efforts are now focused on the understanding of their activities on the FAS-II system. Efforts are also undertaken towards elaboration of new families possessing the functionalized cinnamoyl frames.

Acknowledgments

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