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Anti-protozoal and plasmodial FabI enzyme inhibiting metabolites of *Scrophularia lepidota* roots ☆

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Abstract

The ethanolic root extract of *Scrophularia lepidota*, an endemic plant of the Turkish flora, has been investigated for its anti-protozoal and inhibitory effect towards plasmodial enoyl-ACP reductase (FabI), a key enzyme of fatty acid biosynthesis in *Plasmodium falciparum*. Chromatographic separation of the extract yielded 10 iridoids (**1**–**10**), two of which are new, and a known phenylethanoid glycoside (**11**). The structures of the new compounds were determined as 3,4-dihydro-methylcatalpol (**8**) and 6-*O*-[4″-*O*-*trans*-(3,4-dimethoxycinnamoyl)-α-L-rhamnopyranosyl]aucubin (scrolepidoside, **9**) by spectroscopic means. The remaining metabolites were characterized as catalpol (**1**), 6-*O*-methylcatalpol (**2**), aucubin (**3**), 6-*O*-α-L-rhamnopyranosyl-aucubin (sinuatol, **4**), 6-*O*-β-D-xylopyranosylaucubin (**5**), ajugol (**6**), ajugoside (**7**), an iridoid-related aglycone (**10**) and angoroside C (**11**). Nine isolates were active against *Leishmania donovani*, with the new compound **9** being most potent (IC_{50} 6.1 μg/ml). Except for **4**, all pure compounds revealed some trypanocidal potential against *Trypanosoma brucei rhodesiense* (IC_{50} values 29.3–73.0 μg/ml). Only compound **10** showed moderate anti-plasmodial (IC_{50} 40.6 μg/ml) and FabI enzyme inhibitory activity (IC_{50} 100 μg/ml). **10** is the second natural product inhibiting the fatty acid biosynthesis of *Plasmodium falciparum*.

Keywords: *Scrophularia lepidota*; Scrophulariaceae; Iridoid; Phenylethanoid glycoside; *Plasmodium*; *Trypanosoma*; *Leishmania*; Enoyl-acyl carrier protein reductase (FabI)

1. Introduction

Malaria, trypanosomiasis and leishmaniasis belong to the most widespread and poorly controlled parasitic diseases in the world. Approximately 40% of the world's population is at risk of malaria with more than 300 million new cases and 1 million deaths annually (WHO, 2000). Trypanosomiasis, caused by both *Trypanosoma brucei* and *T. cruzi,* threatens millions of people living in tropical regions. Visceral leishmaniasis, caused by *Leishmania donovani*, is endemic in many parts of the world and affects an estimated 15 million people worldwide (Ashford et al., 1992). Anti-protozoal drugs, on the other hand, are inadequate due to their toxicity, lack of efficacy and inability to eliminate all life cycles stages of these parasites from the host. Furthermore, there is an

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escalating problem of widespread resistance to commonly used chemotherapeutic agents. Thus, new anti-protozoal agents with novel targets are urgently needed.

Fatty acids (FAs) are crucial for all living organisms and the enzymes involved in their biosynthesis are organized in two distinct ways. Higher eukaryotes and fungi accomplish FA biosynthesis by a large, multifunctional protein, which is classified as type I fatty acid synthase (FAS-I) (Smith, 1994), while plants and most prokaryotes perform the same enzymatic steps using separate enzymes (type II fatty acid synthase, FAS-II) (Harwood, 1996). It has been shown that a number of obligate endoparasites, including *P. falciparum* and *T. brucei* synthesize their own FAs using a type II pathway (Waller et al., 1998; Gardner et al., 1998; Morita et al., 2000). As type II FAS is absent in humans, this pathway shows great potential as a target for the development of anti-protozoal agents. The enoyl-ACP reductase enzyme (also known as FabI) is one of the most important enzymes in FA biosynthesis of *P. falciparum* and commits the final reduction step in chain elongation (Waller et al., 1998). Therefore, it is an excellent target for anti-malarial drug discovery.

In the course of our search for new anti-protozoal lead compounds from endemic Turkish plants, we investigated the roots of *Scrophularia lepidota* Boiss. (Scrophulariaceae), which showed trypanocidal, leishmanicidal, anti-malarial and plasmodial FabI enzyme inhibiting properties in initial screenings. Chromatographic separation of the crude extract afforded 9 iridoid glycosides, two of which (**8** and **9**) are new to the literature, an iridoid-related aglycone (**10**) and a known phenylethanoid glycoside, angoroside C (**11**). The current study presents the isolation, structure elucidation and the biological profile of compounds **1**–**11**.

2. Results and discussion

In this study, the ethanolic root extract of *S. lepidota*, on which no chemical or biological study had been performed before, has been investigated. In preliminary screening (Table 2), the crude extract was inactive against *T. cruzi*, but it showed activity against *T. brucei rhodesiense* (IC_{50} 38.4 μg/ml), *L. donovani* (IC_{50} 26 μg/ml) and multidrug-resistant (K1) strain of *P. falciparum* (IC_{50} 17.5 μg/ml). The latter activity prompted us to investigate the ability of the crude extract to inhibit the purified *P. falciparum* FabI enzyme (IC_{50} 80 μg/ml). By employing a combination of C18-VLC, C18-MPLC and SiO_2 column chromatography (CC), eleven compounds were isolated. The detailed procedure for purification of the compounds **1**–**11** is elaborated in Section 4.

Based on the spectroscopic data, compounds **1** and **2** were readily recognized as catalpol (**1**) and 6-*O*-methylcatalpol (**2**), very common iridoid glucosides of the family Scrophulariaceae (El-Naggar and Beal, 1980; Boros and Stermitz, 1990). Again from the 1D and 2D NMR, MS and $[\alpha]_D$ data, compounds **3**–**7** were identified as known iridoid glycosides, aucubin (**3**), 6-*O*-α-L-rhamnopyranosyl-aucubin (sinuatol, **4**), 6-*O*-β-D-xylopyranosylaucubin (**5**), ajugol (**6**), ajugoside (**7**) (El-Naggar and Beal, 1980; Boros and Stermitz, 1990) and angoroside C (**11**), a phenylethanoid glycoside previously reported from several *Scrophularia* species (Çalış et al., 1988; de Santos et al., 2000).

Compound **8** was isolated as an amorphous powder, which was conclusively identified as 3,4-dihydro-methylcatalpol on the basis of extensive 1D and 2D NMR experiments (HSQC, DQF-COSY, HMBC and ROESY). It was assigned the molecular formula $C_{16}H_{26}O_{10}$, determined by ESIMS and HR-MALDIMS. The ^{13}C NMR spectrum contained 16 signals, six of which belonged to a β-glucose unit (Table 1). The remaining 10 signals were sorted out as a methoxyl, three methylenes, three oxymethines, two methines and a fully substituted C atom. Comparison of the 1D NMR data of **8** with those of 6-*O*-methylcatalpol (**2**) suggested many similarities. The most striking exception was the absence of the typical olefinic signals ($\Delta^{3,4}$) of the cyclopentane-pyran iridoid aglycone in **8**. Instead, these protons were replaced by two pairs of non-equivalent methylene signals; one pair of aliphatic (H-4a δ 1.56, H-4b δ 1.77) and one pair of oxygenated (H-3a δ 3.53; H-3b δ 3.89). In the HSQC spectrum, these CH_2 carbons resonated at δ 24.2 (C-4, *t*) and δ 63.0 (C-3, *t*), suggesting that the olefinic bond ($\Delta^{3,4}$) was saturated. These observations were in good agreement with the molecular formula, UV and the IR data. Further proof for the 3,4-dihydro-iridoid structure came from the DQF-COSY and the HMBC data (Table 1). The relative stereochemistry of **8** was established by a ROESY experiment. The ROE cross peaks between H-6/H-7, H-6/H-1, H-7/H-1, H-5/H-9 and H-1/H-1′ indicated the relative configuration of **8** to be the same as that of **2**. Hence, **8** is 3,4-dihydro-methylcatalpol.

Compound **9**, named as scrolepidoside, was also obtained as pale yellow amorphous powder. The ESIMS and HRMALDI-MS revealed the molecular formula $C_{32}H_{42}O_{16}$, requiring 12 double bond equivalents (DBE). The UV spectrum of **9** exhibited absorption bands at λ_{max} 214, 240, 280(sh) and 316 nm, characteristic for an iridoid enol–ether system and a cinnamoyl chromophore. The IR displayed intense absorption bands due to hydroxyl (3374 cm^{-1}), conjugated carbonyl (1705 cm^{-1}), conjugated double bond (1632 cm^{-1}) and aromatic ring (1599, 1514, 1453 cm^{-1}). The ^1H NMR spectrum of **9** was very similar to that of **4**, except for the presence of additional signals due to a *trans* -3,4-dimethoxycinnamic acid moiety [δ 6.45 (*d*, H-α) and δ 7.67 (*d*, H-β); δ 6.97 (*d*, H-5‴); δ 7.19 (*dd*,