International Journal of Antimicrobial Agents 40 (2012) 463–466

Contents lists available at SciVerse ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag

Short communication

Evaluation of the in vitro and intracellular efficacy of new monosubstituted sulfonylureas against extensively drug-resistant tuberculosis

Di Wang[a,1], Li Pan[b,1], Gang Cao[b], Hong Lei[a], Xianghong Meng[a], Jufang He[a], Mei Dong[a,*], Zhengming Li[b,**], Zhen Liu[c,***]

[a] Department of Clinical Laboratory, The 309th Hospital of Chinese People's Liberation Army, Beijing 100091, China
[b] State Key Laboratory of Elemento-Organic Chemistry, National Pesticide Engineering Research Center (Tianjin), Nankai University, Tianjin 300071, China
[c] The 309th Hospital of Chinese People's Liberation Army, Beijing 100091, China

ARTICLE INFO

Article history:
Received 7 March 2012
Accepted 15 June 2012

Keywords:
Sulfonylurea
Mycobacterium tuberculosis
Antitubercular activity
Extensively drug-resistant

ABSTRACT

Acetohydroxyacid synthase (AHAS) has been regarded as a potential drug target against *Mycobacterium tuberculosis* as it catalyses the first step in the pathway for biosynthesis of branched-chain amino acids. In our previous work, several monosubstituted sulfonylureas that are inhibitors of AHAS showed obvious in vitro activity against *M. tuberculosis*. In this study, further exploration of the antitubercular activity of newly synthesised monosubstituted sulfonylureas was conducted. A series of new compounds were identified that exhibit significant activity against in vitro and intracellular extensively drug-resistant *M. tuberculosis*. These results provide a further insight into the structural requirements for targeting AHAS to develop potential new agents to combat tuberculosis.

1. Introduction

The emergence of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) seriously threatens the control of TB globally [1–4]. To combat these MDR/XDR-TB infections, new and effective pharmaceuticals are urgently needed [5,6]. Recently, acetohydroxyacid synthase (AHAS; EC 2.2.1.6) has been identified as an attractive target for designing a new generation of anti-TB agents [7–9]. AHAS is a key enzyme in the biosynthesis of branched-chain amino acids (leucine, isoleucine and valine) by higher plants, algae, fungi and bacteria, and no homologous enzyme has been observed in humans or animals [10]. Sulfonylureas, with the general features of a central sulfonylurea bridge with an *o*-substituted aromatic ring attached to the sulphur atom and a heterocyclic ring attached to the nitrogen atom, have been recorded as inhibitors of AHAS [8]. Preliminary studies indicated that some sulfonylurea compounds, such as sulfometuron-methyl (SM), chlorimuron-methyl and metsulfuron-methyl, displayed antitubercular activity. A common feature of all the above compounds is that they are meta-substituted in both meta-positions [7,8].

Our team have reported that some monosubstituted sulfonylureas in which the heterocyclic ring is substituted with only one substituent at the meta-position also exhibited potent activity against TB in vitro, even against clinical MDR and XDR cases [11,12]. Experimental data demonstrated that these compounds are potential lead structures for the development of novel antimycobacterial agents. Further structural optimisation is required to find more effective anti-TB agents. In addition, because TB is an intracellular infection, demonstration of in vitro activity must be followed by evaluation of the compound's killing ability against intracellular organisms. In previous studies, a branched-chain amino acid auxotrophic mycobacterial strain failed to proliferate because of its inability to use amino acids from the host [13], indicating that inhibitors of branched-chain amino acid biosynthesis could kill the infectious *Mycobacterium* despite amino acids being freely available from the host. Several reports have confirmed that sulfonylurea compounds are effective against TB in cultured macrophages [8] and in mice [7]. Here we report a series of new monosubstituted sulfonylureas and determined their inhibitory activity against intracellular XDR-TB isolates.

2. Materials and methods

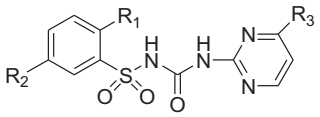
2.1. Compounds and strains

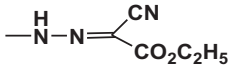
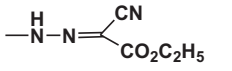
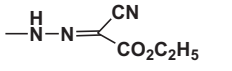
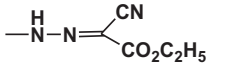
Synthesis and determination of the in vitro activity of monosubstituted sulfonylurea derivative compounds **30–33** were as described in our previous report [11]. Synthesis of compounds **1–12**

* Corresponding author. Tel.: +86 10 6677 5937; fax: +86 10 6677 5937.
** Corresponding author. Tel.: +86 22 2350 3732; fax: +86 22 2350 5948.
*** Corresponding author. Tel.: +86 10 5152 0725.
E-mail addresses: dongmei309@yahoo.com.cn (M. Dong), nkzml@vip.163.com (Z. Li), lzddm329@126.com (Z. Liu).
[1] These two authors contributed equally to this work.

0924-8579/$ – see front matter © 2012 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.ijantimicag.2012.06.012

Table 1
Antimycobacterial activities of title sulfonylurea compounds.



Compound	R_1	R_2	R_3	MIC for H37Rv (mg/L)	MIC range for XDR-TB isolates (mg/L)[a]
1	$COOC_2H_5$	NO_2	CH_3	>80	N/D
2	$COOCH_3$	Cl	CH_3	40	40
3	$COOC_2H_5$	Cl	CH_3	10	5–10
4	$COOC_2H_5$	Cl	OCH_3	>80	N/D
5	$COOCH_3$	Br	CH_3	40	40–80
6	$COOCH_3$	Br	OCH_3	>80	N/D
7	$COOCH_3$		CH_3	>80	N/D
8	$COOCH_3$		OCH_3	>80	N/D
9	$COOC_2H_5$		CH_3	>80	N/D
10	$COOC_2H_5$		OCH_3	>80	N/D
11	$COOCH_3$	$NHSO_2CH_3$	CH_3	>80	N/D
12	$COOCH_3$	$NHPO(OC_2H_5)_2$	CH_3	>80	N/D
13	$COOCH_3$	$COOC_2H_5$	CH_3	>80	N/D
14	Br	$COOCH_3$	OCH_3	>80	N/D
15	Br	$COOCH(CH_3)_2$	CH_3	10	10–20
16	Br	$CONH_2$	CH_3	>80	N/D
17	Br	$CONHC_2H_5$	OCH_3	>80	N/D
18	Br	$CONHCH(CH_3)_2$	OCH_3	>80	N/D
19	$COOCH_3$	$NHCOCH_2Cl$	CH_3	>80	N/D
20	$COOCH_3$	$NHCOCHCl_2$	CH_3	20	10–20
21	$COOCH_3$	$NHCOCCl_3$	CH_3	80	40–80
22	$COOCH_3$	$NHCOCH_2Cl$	OCH_3	>80	N/D
23	$COOCH_3$	$NHCOCHCl_2$	OCH_3	40	20–40
24	$COOCH_3$	$NHCOCCl_3$	OCH_3	>80	N/D
25	$COOC_2H_5$	$NHCOCH_2Cl$	CH_3	>80	N/D
26	$COOC_2H_5$	$NHCOCHCl_2$	CH_3	80	80
27	$COOC_2H_5$	$NHCOCH_2Cl$	OCH_3	>80	N/D
28	$COOCH(CH_3)_2$	$NHCOCH_2Cl$	CH_3	>80	N/D
29	$COOCH(CH_3)_2$	$NHCOCH_2Cl$	OCH_3	>80	N/D
30[b]	Cl	$NHCOCH_2Cl$	CH_3	10	5–10
31[b]	Cl	$NHCOCH_2Cl$	OCH_3	10	5–10
32[b]	Cl	$NHCOCH{=}CH_2$	CH_3	20	20
33[b]	Cl	$NHCOCH{=}CH_2$	OCH_3	40	20–40

MIC, minimum inhibitory concentration; XDR-TB, extensively drug-resistant *Mycobacterium tuberculosis*; N/D, not determined.
[a] In total, 16 XDR-TB strains isolated from The 309th Hospital of Chinese People's Liberation Army (Beijing, China).
[b] Reference [12].

was as reported in the literature [14]. Other compounds reported in this paper were first synthesised as follows: 1,8-diazabicyclo [5.4.0] undec-7-ene was added to 10 mL of acetonitrile containing sulfonamide (2 mmol) and phenyl 4-substituted pyrimidin-2-ylcarbamate (2 mmol). After 8–24 h of stirring at room temperature, the solution was slowly adjusted to pH 1. The resulting precipitate was collected, washed with water and dried to obtain the final product, which was recrystallised (petroleum ether and acetone) or purified by flash chromatography on silica gel (petroleum ether/acetone).

Standard *M. tuberculosis* strain H37Rv (ATCC 27294), which is susceptible to all the anti-TB drugs, was purchased from the Beijing Institute for Tuberculosis Control. XDR-TB refers to strains that are resistant to at least: (i) isoniazid (INH) and rifampicin (RIF); (ii) one of the three injectable second-line drugs amikacin (AMK), kanamycin (KAN) or capreomycin; and (iii) one of the fluoroquinolones. In total, 16 clinical XDR-TB isolates identified from The 309th Hospital of Chinese People's Liberation Army (PLA) (Beijing, China) were used in this work [15].

2.2. Determination of in vitro activity

The detailed protocol to determine the in vitro activity of the compounds against *M. tuberculosis* has been described previously [11,12]. Briefly, each compound was diluted two-fold on Middlebrook 7H10 agar media supplemented with OADC (oleic acid–albumin–dextrose–catalase). Prepared *M. tuberculosis* strains were added to the plates, incubated at 36.5 °C and cultures were examined for bacterial growth 4 weeks later. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of a compound that inhibited visible bacterial growth of *M. tuberculosis*.