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Research paper

Aminothiazoles: Hit to lead development to identify antileishmanial agents

Debnath Bhuniya [a], Rao Mukkavilli [a], Rahul Shivahare [b], Delphine Launay [c], Ravindra T. Dere [a], Anil Deshpande [a], Aditya Verma [b], Preeti Vishwakarma [b], Manjunath Moger [a], Ashok Pradhan [a], Hari Pati [a], Vadiraj S. Gopinath [a], Suman Gupta [b, *], Sunil K. Puri [b], Denis Martin [c]

[a] Advinus Therapeutics Ltd., Bengaluru 560 058, India
[b] Division of Parasitology, CSIR-Central Drug Research Institute, Lucknow 226 031, India
[c] Drugs for Neglected Diseases initiative (DNDi), 15 Chemin Louis Dunant, 1202 Geneva, Switzerland

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ABSTRACT

As part of Drugs for Neglected Diseases *initiative's* lead optimization program for the development of new chemical entities to treat visceral leishmaniasis (VL), a series of aminothiazoles were synthesized and screened for *in vitro* efficacy, solubility and microsomal stability. The primary aim of identifying a lead structure with sub-micromolar activity was achieved. Out of 43 compounds synthesized, 16 compounds showed *in vitro* activity at less than 1 μM against VL. Compound **32** showed excellent antileishmanial potency (IC_{50} = 3 nM) and had all the acceptable properties except for metabolic instability. Blocking the metabolic soft spots in compound **32**, where the 4-methoxy pyridine substituent was replaced by 5-ethoxy group, led to compound **36** (IC_{50} = 280 nM) with improved stability. To understand the disposition of **36**, *in vivo* pharmacokinetic study was conducted in a mouse model. Compound **36** showed high clearance (91 mL/min/kg); short half-life (0.48 h) after intravenous administration (1 mg/kg) and exposure ($AUC_{0\text{-}24}$) following oral administration was 362 ng h/mL with absolute bioavailability of 8%. To summarize, 43 analogs were synthesized out of which 15 compounds showed very potent sub-nanomolar efficacy in *in vitro* systems but the liability of metabolic instability seemed to be the major challenge for this chemical class and remains to be addressed.

1. Introduction

Neglected tropical diseases (NTDs) like visceral leishmaniasis (VL) represent the fourth most important communicable disease worldwide behind the respiratory infections, HIV/AIDS and diarrheal diseases. Tropical diseases like leishmaniasis affect the poor and powerless populations in rural and impoverished urban areas of developing countries and therefore are neglected from most drug development programs. Neglected tropical diseases are highly endemic with an ability to impair childhood growth (nearly one-half of the VL patients worldwide are children), intellectual development, as well as worker productivity [1,2].

Ironically, only 4% of approved drugs (and 1% of the new chemical entities approved) from 2000 to 2011 were for NTDs mainly due to low profitability margin for developers, poor health care policies and funding by governments, lack of drug discovery and development expertise [3]. To address the pressing need to develop drugs which are safe, affordable, easy to administer and efficacious, seven institutions from all over the world came together and founded in 2003 the Drugs for Neglected Diseases *initiative* (DND*i*) in Geneva, Switzerland.

Leishmaniasis is a parasitic illness caused by haemoflagellate protozoans belonging to the genus *Leishmania*. *Leishmania donovani* is the most common agent causing VL [4]. VL manifests as fever, hepatosplenomegaly and pancytopenia. If untreated, it is always fatal. VL is endemic in large geographical areas around the globe affecting 88 countries. An estimated 200,000 to 400,000 new cases of VL are reported worldwide each year with 20,000 to 30,000 deaths [5]. Over 90% of new cases are being reported from

* Corresponding author.
E-mail addresses: gupta_suman@yahoo.com, suman_gupta@cdri.res.in (S. Gupta).

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Bangladesh, Brazil, Ethiopia, India, Nepal and Sudan [6].

Currently, miltefosine is widely used as the only available oral drug for the treatment of VL but is teratogenic, has a long half-life, low therapeutic window and early signs of resistance have recently been reported [7,8]. A low cost injectable paromomycin has been registered in India for VL treatment but it presents cytotoxicity and hepatotoxicity [9,10]. Amphotericin B is administered parenterally, but it is very toxic in addition to being costly, and is typically used as a second line of treatment when antimonials fail. The liposomal formulation of Amphotericin B, AmBisome™ is less toxic but is also costly and still requires hospitalization [11,12].

The objective of DND*i* was to develop an effective and safe oral drug that would cost less than $10 per course. In order to achieve this ambitious aim, DND*i* has put together a team to undertake screening and lead optimization activities on VL and other neglected diseases in its portfolio.

The first ever high throughput screening (HTS) campaign against the intracellular form of *L. donovani* was completed at Institut Pasteur Korea (IPK) with a library of 200,000 compounds. From the hits identified, 10 scaffolds emerged and the one (aminothiazole) bearing the most appropriate drug-like features was selected for further optimization (Fig. 1). The aminothiazoles, fused aminothiazoles, and other aminothiazoles linked to various heterocyclic rings have recently gained attention for their chemotherapeutic activity for treatment of malaria [13], prion disease [14], anti-inflammatory/cancer [15] and tuberculosis [16].

The most active hits identified in the series were re-synthesized and their *in vitro* potency was confirmed in *L. donovani* amastigotes. Close analogs were then designed, synthesized and structure–activity relationships were established based on Lipinski and Veber rules (Table S1 and Fig. S1, see in supporting information) [17,18]. In addition to pharmacodynamic data, *in vitro* metabolism data in liver microsomes and mouse pharmacokinetic (PK) data helped the team in identifying leads with promising properties.

2. Results and discussion

2.1. Chemistry

All analogs of compound **1** (best hit selected through HTS) (Fig. 1) were synthesized using Scheme 1. Commercially available nitriles **2** were converted to acetophenones **3** by reacting with methyl magnesium bromide in THF at 0 °C over 2 h with 70–80% yield. Bromination of acetophenones **3** using HBr and bromine in acetic acid at 4–8 °C and further stirring at room temperature for 2 h afforded the corresponding α-bromoketones **4** with 90% yield. Substituted amines **5** were converted to the isothiocyanate intermediates by reacting with thiophosgene at ambient temperature, followed by reaction with ammonia gas at 0 °C over 10 min to give the corresponding thiourea **6** with 40–50% yield. Couplings of **4** with **6** were carried out under microwave irradiation in ethanol

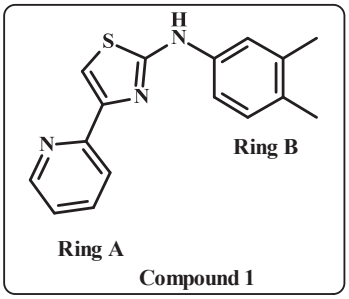

Fig. 1. Main subseries – Aminothiazoles (Hit 1).

over 3 min, the solid products **7**–**31** obtained were further isolated by filtration with yield consistently above 70%. To a solution of compound **7**–**31** in DMF, N-chlorosuccinimide was added at 0 °C and stirred at room temperature for 24 h. The reaction mixture was diluted with water and extracted with ethyl acetate to give compounds **32**–**48** in 40–50% yield.

2.2. Biological activity

All the synthesized compounds were tested methodically *in vitro* against the intracellular form of *L. donovani* and their cytotoxicity and ADME properties assessed.

2.2.1. First results on phenyl and pyridine analogs

Table 1 shows the SAR results observed with a pyridine (Py) and phenyl (Ph) as A-ring (Ar) modification on compound **1**. The *in vitro* screening results showed that pyridine ring substitution in most cases gives far better potency than the phenyl ring. We further investigated the position of the nitrogen in the pyridine ring and synthesized other analogs **16** & **17**. In A-ring, 2-pyridine was found to be optimal, and on closer examination of SAR, it was evident that electron-donating substituents like methyl and methoxy on B-ring were giving better potency (Table 1). Indeed, pyridine derivatives **1** (IC_{50} = 3.6 μM), **8** (IC_{50} = 2.6 μM), **9** (IC_{50} = 2.6 μM) showed better activity than all other compounds. It also seemed clear that the only suitable position of nitrogen in the A-ring was 2-position. The 2-pyrimidine analog **18** of compound **8** was also synthesized and screened but resulted in loss of potency. Before proceeding to explore further SAR and to understand the behavior of the series, *in vivo* pharmacokinetics of compound **9** (50 mg/kg) was evaluated following oral gavage administration in male Swiss Albino mice. The pharmacokinetic parameters are summarized in Table 2. Following oral suspension administration of compound **9**, the time to reach maximum plasma concentration (T_{max}) was 0.25 h. The concentration obtained at T_{max} was at least 6-fold lower than the expected efficacious level (IC_{50} = 670 ng/mL). Drug concentration was detected only till 3 h post dose (Fig. 2).

2.2.2. Improved potency by substitution in the B-ring

As compound **9** showed high *in vivo* clearance and the concentrations achieved were well below the desired IC_{50} value, further analogs **19**–**24** having different substitutions in the B-ring were synthesized, with A-ring being 2-pyridine, and evaluated against *L. donovani* amastigotes. Table 3 shows the SAR results observed with substitution in the phenyl B-ring. Compound **23** with a nanomolar potency (IC_{50} = 370 nM) was identified (Fig. 3).

2.2.3. Influence of pyridine instead of phenyl ring as B-ring

To further enhance the potency and expand the SAR, the phenyl ring (B-ring) of **9** was replaced with a pyridine or substituted pyridine ring and a series of analogs **25**–**31** were synthesized. Typically the increase in nitrogens in the structure makes the compound more hydrophilic. The potency results were very promising with almost all analogs showing nanomolar IC_{50} values, except for compound **25** (IC_{50} = 2.1 μM, Table 4). We were delighted to achieve targeted *in vitro* potency, however, none of the analogs had adequate metabolic stability as they were metabolized over 90% within 30 min of incubation with hamster and mouse liver microsomes (Table 4), even when pyridine as B-ring was substituted to reduce its metabolism.

To address the issue of metabolic instability of compounds in microsomes, we chose the most potent analog **30** (IC_{50} = 90 nM, Fig. 4) and blocked the potential metabolic hot spots with methoxy, ethoxy and triflurometyl substitutions in the A-ring **32**–**35**. Once again, potency was retained but none of the compounds were