

In vitro Controlled Release from Solid Pharmaceutical Formulations of two new Adamantane Aminoethers with Antitubercular Activity (I).

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ABSTRACT

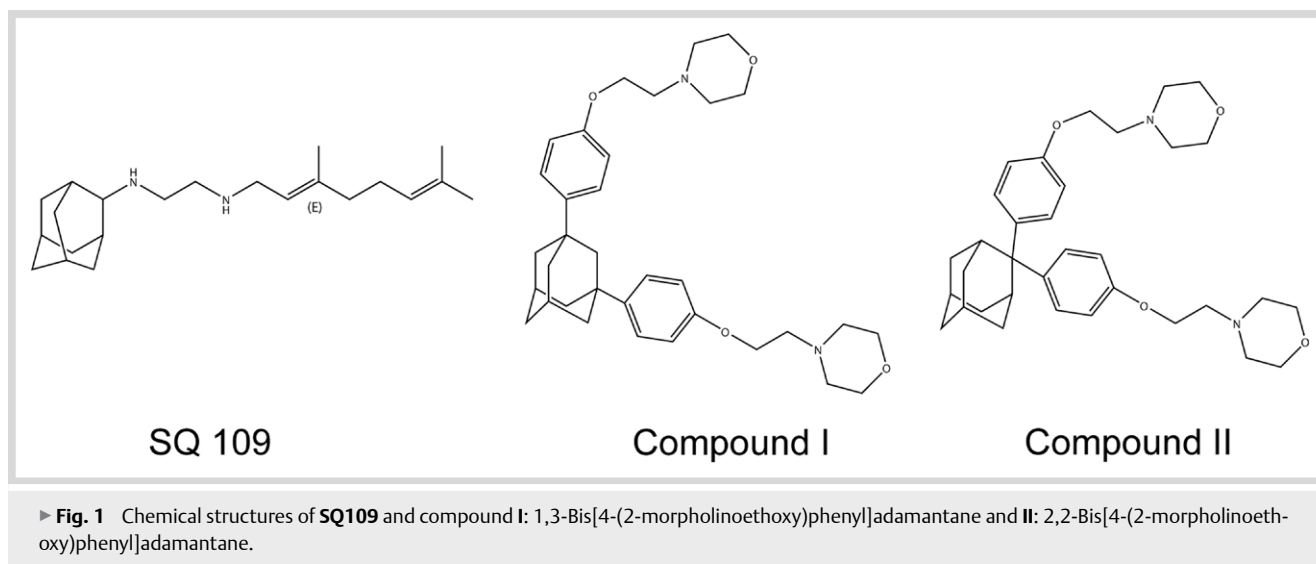
The aim of the present investigation was to develop matrix tablet formulations for the in vitro controlled release of 2 new tuberculocidal adamantane aminoethers (compounds **I** and **II**), congeneric to the adamantane derivative **SQ109**, which is in final clinical trials, using carefully selected excipients, such as polyvinylpyrrolidone, sodium alginate and lactose. The tablets were prepared using the direct compression method and dissolution experiments were conducted using the US Pharmacopoeia type II apparatus (paddle method) in gastric and intestinal fluids. The results confirm that both analogues, albeit more lipophilic than **SQ109**, showed satisfactory in vitro release characteristics from solid pharmaceutical formulations. In conclusion, these formulations merit further assessment by conducting in the future bioavailability in vivo studies.

Introduction

Tuberculosis (TB) is a disease usually caused by a bacterium called *Mycobacterium tuberculosis* (Mtb). Once rare in developed countries, tuberculosis infections began increasing in 1985, partly because of the emergence of HIV [1]. The bacteria usually attack the lungs, but Mtb bacteria can attack any part of the body, such as the kidney, spine, and brain. If not treated properly, TB disease can be fatal. Many strains of tuberculosis resist to the drugs mostly used to treat the disease and as a result new drugs and regimens are urgently needed to shorten the required duration of tuberculosis treatment [2–6]. *N*-Adamantan-2-yl-*N'*-[(*E*)-3,7-dimethylocta-2,6-dienyl]ethane-1,2-diamine (**SQ109**, ► **Fig. 1**), is a drug candidate that is active against both drug-susceptible and drug-resistant TB strains and affects cell wall synthesis [7]. It bears in its skeleton the active 1,2-ethylenediamine pharmacophore [8] of the most wide-

ly used antitubercular drug, ethambutol (EMB), and was found safe and well tolerated in Phase I and early Phase II clinical trials. Based on these findings and our previous experience, the diarylmethane moiety, which is reported in the literature [9], as active against *Mycobacterium tuberculosis*, was introduced into the adamantane skeleton. Moreover, 2 phenolic rings, functionalised with an aminoether side chain, were introduced onto C1, C3 (compound **I**) and for comparison purposes onto the C2 position of the adamantane ring (compound **II**).

Both of these compounds exhibit antitubercular activity [10]. Albeit the fact that the lipophilicities of compound (**I**) (clogP = 7.835) and (**II**) (clogP = 7.985) are substantially higher than that of **SQ109** (clogP = 6.205), they are, however, within the allowed limits for oral administration [11]. Therefore, it was intriguing to probe their oral absorption profile, because this information is of paramount impor-



tance for future in vivo studies and subsequent clinical trials on their potential as tuberculosis treating agents. To this end, dissolution studies, at pH values of 1.2 and 6.8, were conducted, as an initial attempt to decipher the release characteristics of these compounds from controlled release matrix tablets. Controlled release vs. immediate release was chosen as it is known to be more clinically useful in treating tuberculosis suffering patients compared with immediate release or conventional therapy [12].

Materials and Methods

Chemistry

The synthesis of compounds (**I**) and (**II**) involved the nucleophilic attack of the phenoxide, formed by adding sodium hydride in dry DMF to the corresponding phenolic derivative, to 4-(2-chloroethyl) morpholine, under heating [10].

Biological Evaluation

Bacterial strains and culture conditions Mycobacterium tuberculosis strains H37Rv, MmpL3 mutant and STR-starved 18b (SS18b) were grown at 37°C with shaking in 7H9 broth (Difco) supplemented with 10% albumin-dextrose-catalase (ADC) enrichment, 0.2% glycerol, 0.05% Tween 80. Streptomycin (STR) (50 µg/mL) was added to SS18b culture. Replicating strain H37Rv was generated as follow. H37Rv and MmpL3 mutant were grown to mid-logarithmic phase in medium. Non-replicating, SS18b was generated as follows: 18b was grown to mid-logarithmic phase in STR-containing medium and washed 3 times in phosphate-buffered saline containing 0.05% Tween 80 (PBST). Final bacterial pellets of H37Rv, MmpL3 mutant and SS18b strains were re-suspended in medium and frozen in 15% glycerol at -80°C in 0.5-milliliter aliquots (supplemented with 50 µg/mL STR for SS18b). When needed, one aliquot of H37Rv, MmpL3 mutant and SS18b was defrosted and inoculated in 7H9. SS18b culture was maintained at an optical density of 600 nm (OD600) between 0.2 and 0.5 for 2 weeks (with the addition of fresh medium if necessary), by which time they had stopped replicating. MmpL3 mutant was generated by Dr. Giovanna Riccardi in the

► **Table 1** Quantitative composition of the formulants used for the preparation of compounds (**I**) and (**II**) tablets.

Ingredients	Formulation 1 (mg)	Formulation 2 (mg)
Compound I or II	5	5
Sodium alginate	144	130
Magnesium stearate	2	2
Lactose monohydrate	49	–
PVP (M.W.: 55.000)	–	63
Total	200	200

University of Pavia. This strain has a mutation in mmpL3 genes and an amino acid change V681I. This strain is known to be resistant to BM212 and its derivatives. (<http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0056980>).

Antimicrobials Ethambutol (EMB) and rifampin (RIF) were purchased from Sigma-Aldrich. Adamantane aminoethers, EMB and RIF were dissolved in dimethyl sulfoxide (DMSO).

Resazurin reduction microplate assay (REMA) To determine the in vitro efficacy of compounds, a H37Rv culture (OD600 = 0.0001) and a 2 week old SS18b culture (OD600 = 0.1) were used in the REMA. 2-fold serial dilutions of each test compound were prepared in white 96-well plates containing the bacilli in a total volume of 100 µl and then were incubated for 6 days at 37°C before the addition of 10 µl of 0.025% resazurin. After overnight incubation, the fluorescence of the resazurin metabolite resorufin was determined (excitation, 560 nm; emission, 590 nm; gain, 70) by using a Tecan Infinite M200 microplate reader.

Statistical analysis Data were processed and graphs were constructed with Prism version 5.0 (GraphPad).

Materials used for the preparation of matrix tablets

Compounds **I** and **II** were provided by our colleagues in the Pharmaceutical Chemistry Division of our Department. Low viscosity Sodium Alginate and Polyvinylpyrrolidone (PVP, M.W.: 55.000) were purchased from SIGMA. Lactose monohydrate was purchased from Merck and Magnesium Stearate from Riedel-De Haen.

Tablets' preparation and dissolution experiments

The dissolution experiments involved flat tablets (10 mm diameter, 200 mg weight and 6–9 kp hardness). Each matrix tablet was comprised of compound (I) or compound (II) and combinations of the following excipients: low viscosity sodium alginate, lactose monohydrate, polyvinylpyrrolidone (M.W.: 55.000) and magnesium stearate (► **Table 1**). The tablets were stirred at 50 rpm in a USP XXIII dissolution apparatus II (Pharmatest, Hainerp, Germany) containing for the first 3 h 500 ml of gastric (pH 1.2) and for the next 5 h 1000 ml of intestinal fluids (pH 6.8) at $37 \pm 0.5^\circ\text{C}$. Samples (5 ml) were withdrawn at predetermined time intervals, filtered and analyzed at $\lambda_{\text{max}} = 223 \text{ nm}$ for compound (I) and $\lambda_{\text{max}} = 245 \text{ nm}$ for compound (II), using a Perkin-Elmer UV spectrophotometer (Norwalk, CT). All experiments were carried out in triplicate. The results obtained are graphically presented in ► **Fig. 2** and ► **Fig. 3**.

The structures of the new compounds were sketched in Maestro 10.2 [13] and prepared using the LigPrep 3.4 module [14]. They were minimized using the OPLS3 force field. Computer program QikProp [15] was used to predict the physically significant descriptors of the compounds.

Comparison indices, f_1 and f_2 , were also used to compare the dissolution profiles of compounds I and II [16, 17].

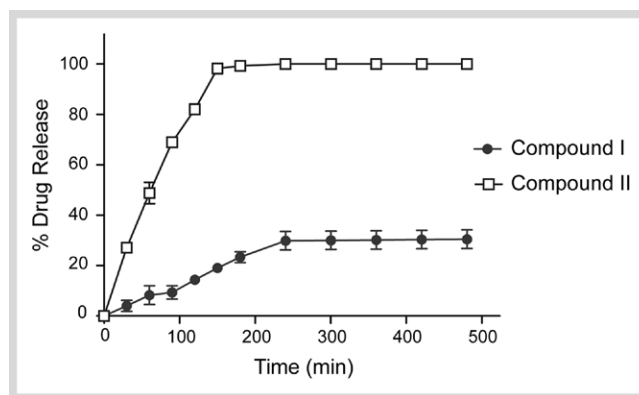
Results and Discussion

The 2 new adamantane isomers, 4,4'-[4,4'-[adamantane-1,3-diyl]bis(phenoxyethyl)]dimorpholine (I) and 4,4'-[4,4'-[adamantane-2,2-diyl]bis(phenoxyethyl)]dimorpholine (II), have shown different tuberculocidal potencies. The analogue (I) exhibited a significant activity in the REMA assay; the MIC value of (I) is $7.1 \mu\text{g/mL}$ against *M. tuberculosis* Mmpl3 mutant, which is at least 14-fold more potent than its congener (II). The same pattern was shown against the H37Rv strain, with derivative (I) having a MIC value of $24.1 \mu\text{g/mL}$, i. e., about 5-fold more active than (II).

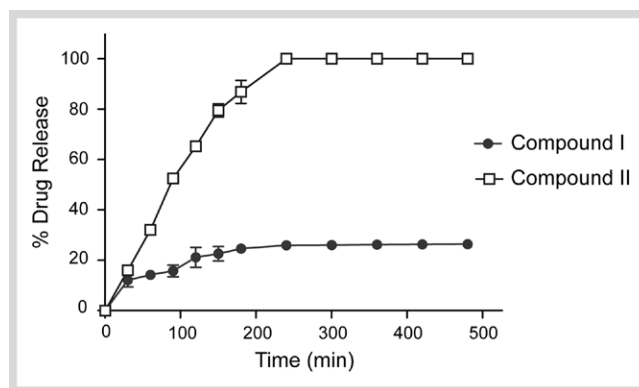
The solid pharmaceutical formulations include various excipients (► **Table 1**), which both in acidic (0–3 h, pH 1.2) and in neutral pH (3–8 h, pH 6.8) environment facilitate the controlled/extended release of these 2 new bioactive substances. It becomes apparent from the release curves of compounds I and II, shown in ► **Fig. 2, 3**, that the lactose formulant present in F1 tablets leads to a faster 100% release (180 min) than the PVP excipient in formulation F2 (240 min).

In all cases, the f_1 values are higher than 15 and the f_2 estimates are lower than 50%, implying a high degree of dissimilarity in the dissolution profiles of the compounds I and II (► **Table 2**). The difference in the overall release profiles observed between the compounds I and II (► **Fig. 2, 3**) is possibly related to their respective degrees of lipophilicity. These differences are also corroborated by the values of the f_1 and f_2 factors.

The theoretical clogP and QPlogPo/w values suggest that compound II is more lipophilic than its isomer I (► **Table 3**). This is possibly due to the fact that the 2 phenyl rings in I are in spatial vicinity, allowing them to form a π - π stacking complex (dotted line) (► **Fig. 4a**). Conversely, the respective phenyl rings in compound II are too far away to form this type of complex (► **Fig. 4b**). As a result more water molecules are trapped in the surrounding region of I and this justifies its higher solubilisation [16].



► **Fig. 2** Drug release (%) vs. time of Compounds I and II in Formulation 1.



► **Fig. 3** Drug release (%) vs. time of Compounds I and II in Formulation 2.

► **Table 2** f_1 and f_2 indices for Formulations 1 and 2.

Indices	Formulation 1	Formulation 2
f_1	81.49	68.42
f_2	12.40	17.32

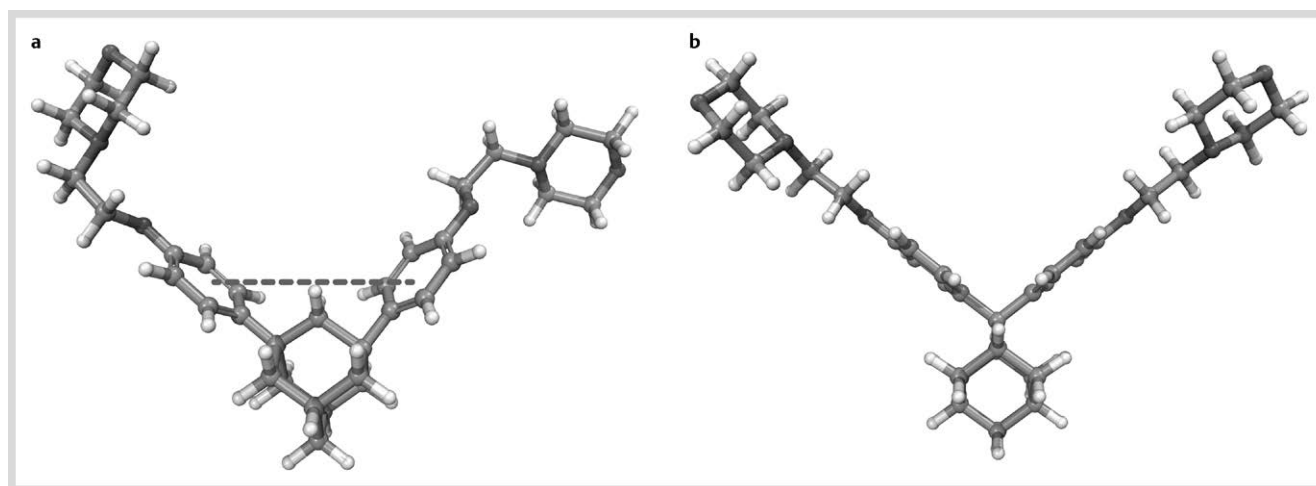
► **Table 3** Calculation of significant descriptors and pharmaceutically relevant properties for the compounds I and II.

Computed property	II	I
QPlogPo/w ¹	5.382	4.773
QPlogS ²	-5.271	-2.809
QPCCaco ³	566.843	548.164

¹ QPlogPo/w: Predicted octanol/water partition coefficient. Range or recommended values for 95% of known drugs: -2.0 – 6.5.

² QPlogS: Predicted aqueous solubility, log S. S in mol dm⁻³ is the concentration of the solute in a saturated solution that is in equilibrium with the crystalline solid. Range: -6.5 – 0.5.

³ QPCCaco: Predicted apparent Caco-2 cell permeability in nm/s. Caco-2 cells are a model for the gut-blood barrier. QikProp predictions are for non-active transport. <25 Poor; >500 Great.



► **Fig. 4** a Compound I: 1,3-Bis[4-(2-morpholinoethoxy)phenyl]adamantane; b Compound II: 2,2-Bis[4-(2-morpholinoethoxy)phenyl]adamantane

Conclusions

In conclusion, 2 new tuberculocidals adamantane aminoethers, although more lipophilic than **SQ109**, showed a satisfactory controlled release profile. Taking into account that the currently used antitubercular medicines have limited efficacy against the rising threat of drug-resistant Mtb, significant side effects, and must be given in combinations of 4–6 drugs for at least 6 months, for drug-sensitive Mtb, and up to 24 months for drug-resistant Mtb, new drug TB treatment with less frequent dosing and improved patient compliance, is very important. Information about the oral absorption profile of the molecules presented herein is very useful in future in vivo studies.

Conflict of interest

The authors have stated that they have no conflicts of interest to declare in the contents of this manuscript.

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