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Short communication

# Design, synthesis and evaluation of novel polypharmacological antichlamydial agents

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ABSTRACT

Discovery of new polypharmacological antibacterial agents with multiple modes of actions can be an alternative to combination therapy and also a possibility to slow development of antibiotic resistance. In support to this hypothesis, we synthesized 16 compounds by combining the pharmacophores of *Chlamydia trachomatis* inhibitors and inhibitors of type III secretion (T3S) in gram-negative bacteria. In this study we have developed salicylidene acylhydrazide sulfonamides (**11c** & **11d**) as new antichlamydial agents that also inhibit T3S in *Yersinia pseudotuberculosis*.



## 1. Introduction

*Chlamydia trachomatis* is a common sexually transmitted pathogen that can cause infertility and *Chlamydia pneumoniae* causes respiratory infections and pneumonia [1]. Each year millions of people are infected by *C. trachomatis* especially below the age of twenty five [2]. These infections are treated with broad spectrum antibiotics which affect pathogens as well as the normal endogenous microflora and thus select for antibiotic resistance in both populations [3]. Antibiotic resistant *C. trachomatis* can easily be generated *in vitro*, but so far only limited amount of resistance has been detected in patients [4]. Development of antibiotic resistance has been suggested to be considerably slower when using multiple antibiotics simultaneously [5], but this is not feasible in most clinical situations with common infections, where patient compliance is often questionable [3,6]. However, targeting multiple mechanisms of the disease with a single molecule would not endanger patient compliance and could offer benefits in slowing development of antibiotic resistance. Modern drug discovery has been strongly focused on the discovery and development of potent and selective drugs that act on a specific target. Polypharmacological approaches based on single molecules that act on multiple targets of relevance for one or several pathways have been suggested as an alternative strategy to find new treatments of diseases [7]. Unintended polypharmacology is generally associated with side-effects and successful design of polypharmacological drugs is one of the major challenges in drug development. For many drug molecules beneficial or undesired polypharmacological effects are discovered late and may be not even until the drug has reached the market.

We have previously shown that salicylidene acylhydrazides such as **I** (Fig. 1) are potent antichlamydial compounds with putative mechanisms including type (III) secretion system inhibition [8], iron chelation [9], and effects on HemG in heme biosynthesis [10].

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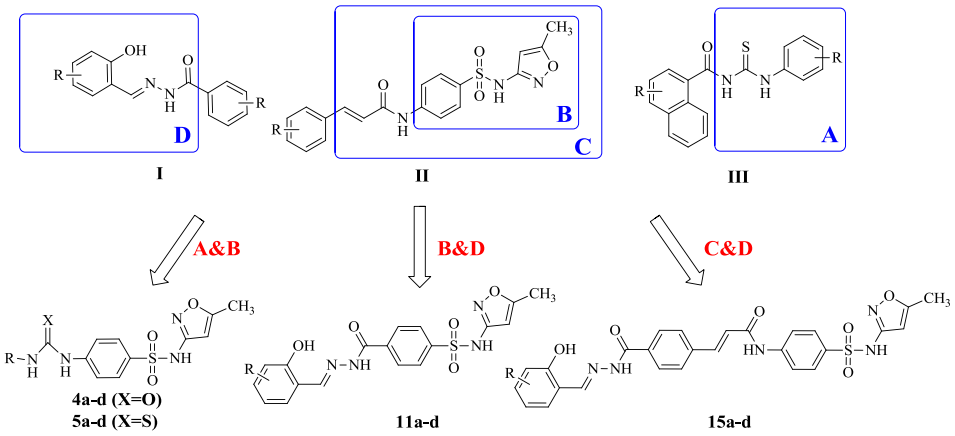



**Fig. 1.** Structures of *C. trachomatis* inhibitors and synthesized compounds.

Target identification studies in *Escherichia coli* O157 have suggested additional targets for this compound class [11]. Recently, we used phenotypic high content screening and identified acylated sulfonamides exemplified by **II** (Fig. 1) and N-(phenylcarbamothioyl)-1-naphthamides **III** (Fig. 1) as potent inhibitors of *Chlamydiae* [12]. In addition, recent literature describe urea and thiourea derivatives as inhibitors of gram-negative bacteria [13–16]. In this study we explore the possibility to develop novel antichlamydial agents by combination of the identified pharmacophores from the different classes of anti-chlamydial compounds and T3S inhibitors. With this hypothesis we designed and synthesized ureido-sulfonamides **4a**–**d**, thioureido-sulfonamides **5a**–**d**, salicylidene acylhydrazide sulfonamides **11a**–**d** and salicylidene acylhydrazide-cinnamoyl sulfonamides **15a**–**d** (Fig. 1) and evaluated them for their biological activities against *Chlamydiae* and T3S.

## 2. Results and discussion

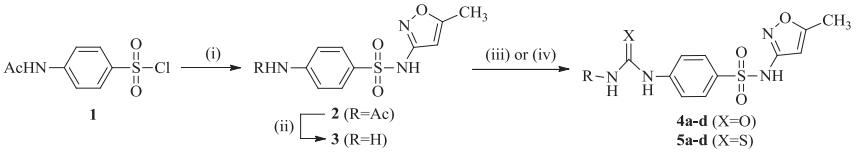
### 2.1. Chemistry

In compounds **4a**–**d** and **5a**–**d** we combine the ureido and thioureido motif A from the screening hit **III** with the core element B of antichlamydial compounds **II** (Fig. 1) [12] to explore if the antichlamydial activity could be improved. The salicylidene acylhydrazide pharmacophore D in compound **I** is responsible for both iron chelation [9,17] and T3S inhibition [18] and by combination with the core and extended scaffolds B and C in the antichlamydial compounds **II** (Fig. 1) [12] we envisioned that the resulting compounds **11a**–**d** and **15a**–**d** would maintain all three biological activities. The specific building blocks and substitution patterns as well as the positions for merging the different structural elements were selected based on previous structure–activity relationships the identified positions that allow substantial structural variation [12,18,19].

The ureido and thioureido compounds **4a**–**d** and **5a**–**d** respectively were synthesized as outlined in Scheme 1. The intermediate *N*-(4-(*N*-(5-methylisoxazol-3-yl)sulfamoyl)phenyl)acetamide **2** (59%) was obtained by reacting 4-acetamidobenzene-1-sulfonyl chloride **1** with 5-methylisoxazol-3-amine in pyridine, which was subjected for deacetylation with NaOH to achieve 4-amino-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide **3** in 93% yield [12]. Compound **3** was then reacted with triphosgene to form the corresponding isocyanate that subsequently was combined with different amines to obtain the urea compounds **4a**–**d** in 16–30% yields. To synthesize the thioureido derivatives **5a**–**d**, compound **3** was reacted with thiophosgene according to a modified procedure [20] to form the isothiocyanate which was then treated with the respective amines to achieve thioureido derivatives in 14–45% yields.

Scheme 2 describes the synthetic pathway leading to the salicylidene acylhydrazide sulfonamide hybrids **11a**–**d**. Compound **7** (74%) was prepared by reacting 4-bromobenzenesulfonyl chloride **6** with 5-methylisoxazol-3-amine with catalytic amount of DMAP in pyridine. Pd-catalyzed cyanation was performed to convert **7** to 4-cyano-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide **8** by using $Zn(CN)_2$ as a CN source [21] in 36% yield and the cyano group was then hydrolyzed to acid with KOH [22] in nearly quantitative yield. The resulting carboxylic acid was coupled with Boc-hydrazide in presence of EDC·HCl and DMAP to obtain the intermediate **10a** in 75% yield [23]. N-Boc deprotection was performed by using 4 M HCl in 1,4-dioxane to obtain hydrazide hydrochloride salt (**10b**) in 83% yield. The hydrazide was subjected to condensation with selected salicylaldehydes [11,18] to obtain salicylidene acylhydrazide sulfonamides (**11a**–**d**) in 39–55% yields.

The salicylidene acylhydrazide sulfonamides based on the extended scaffold i.e. **15a**–**d** were synthesized as summarized in Scheme 3. N-Acylation of 4-amino-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide **3** with acryloyl chloride and NaH gave **12** in 47% yield and coupling of Boc-hydrazide to 4-bromobenzoic acid in presence of EDC·HCl and DMAP [23] yielded 4-bromobenzene-Boc-




**Scheme 1.** Reagents and conditions: (i) 5-methylisoxazol-3-amine, pyridine, DMAP, rt, 12 h; (ii) NaOH, $H_2O$, reflux, 4 h; (iii) Triphosgene, DIPEA, $RNH_2$, THF, 0 °C–rt, 15 h (for **4a**–**d**); (iv) a: Thiophosgene, THF, 0 °C–rt, overnight; b: $Et_3N$, $RNH_2$, 1,4-dioxane, reflux, 12 h (for **5a**–**d**).