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Urea derivatives of spirocyclic piperidines endowed with antibacterial activity

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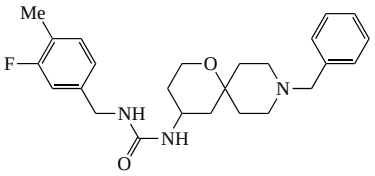
Antimycobacterial activity of certain ureas as well as spirocyclic piperidines described in the literature prompted us to synthesize and test a series of hybrids of spirocyclic piperidine with ureas. Surprisingly, no activity was detected against *Mycobacterium tuberculosis*. However, significant antibacterial activity was identified and confirmed against common gram-positive as well as gram-negative bacteria.

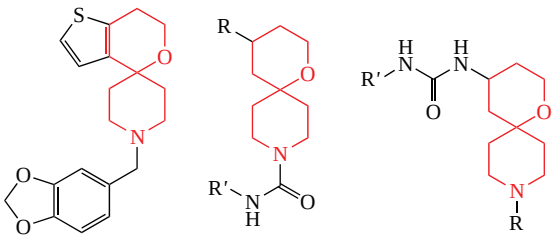


Broad-spectrum activity (MIC, μg ml^{-1}):
Enterococcus faecium – 38
Acinetobacter baumannii – 38
Klebsiella pneumoniae – 19
Staphylococcus aureus – 19
Enterobacter aerogenes – 38

Keywords: spirocyclic compounds, piperidines, ureas, epoxide hydrolase, membrane transporter Mmpl3, antimycobacterial activity, antibacterial activity.

Discovery of new biomolecules that are crucial for microorganisms' survival offers new opportunities to discover antibiotics with novel mechanisms of action and to combat emerging drug resistance.[1] Epoxide hydrolases (EHs) are important enzymes that catalyze hydrolytic opening of an epoxide to diol.[2] In humans and mammals in general, soluble epoxide hydrolase (sEH) is an enzyme that contributes importantly to metabolism of endogenous, biologically active lipids including epoxides of arachidonic acid.[3] Enzyme sEH has been targeted by various inhibitors to achieve therapeutic outcome in such areas as hypertension, diabetes, inflammation, pulmonary, kidney disease as well as neural pathologies.[4] These efforts resulted in numerous efficacious sEH inhibitors discovered[5] and some progressed into clinical development.[6]

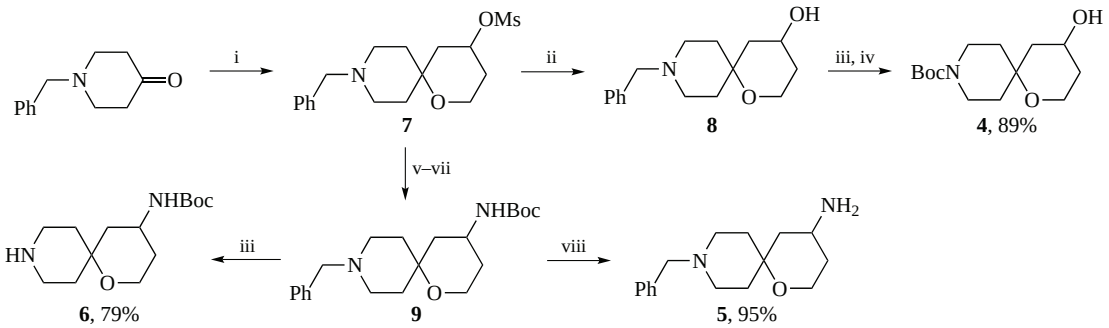
In the early 2000s, new epoxide hydrolases were discovered in mycobacteria and subsequently crystallized.[7] While no inhibitors for this enzyme were known at the time, co-crystallization with a small-molecule ligand was achieved for one of the human sEH inhibitors.[8] This stimulated search for selective inhibitors of the mycobacterial epoxide hydrolase. In 2011, urea lead compounds structurally similar to inhibitors of human sEH were identified *via* screening for antitubercular activity, thus establishing ureas as a new antibacterial chemotype.[9] At the same time, it was established that mycobacterial epoxide hydrolases had a non-essential character and the antimycobacterial properties of the urea compounds were primarily due to the inhibition of the membrane transporter Mmpl3, which was believed to play a major role in exporting mycolates to mycobacterial cell surface.[10] Over the following decade, a new spirocyclic piperidine antimycobacterial lead compound **1** emerged.[11] It was greatly reminiscent of the privileged[12] spirocyclic building blocks we employed in the design of anti-diabetic agents[13–15] as well as antibacterial nitrofurans[16] and fluoroquinolones.[17] Specifically, the presence of the 1-oxa-9-azaspiro[5.5]undecane moiety in structure **1** prompted us to synthesize spirocyclic piperidine ureas **2** and **3** and explore them as antibacterial agents. Herein, we report the results of this investigation.



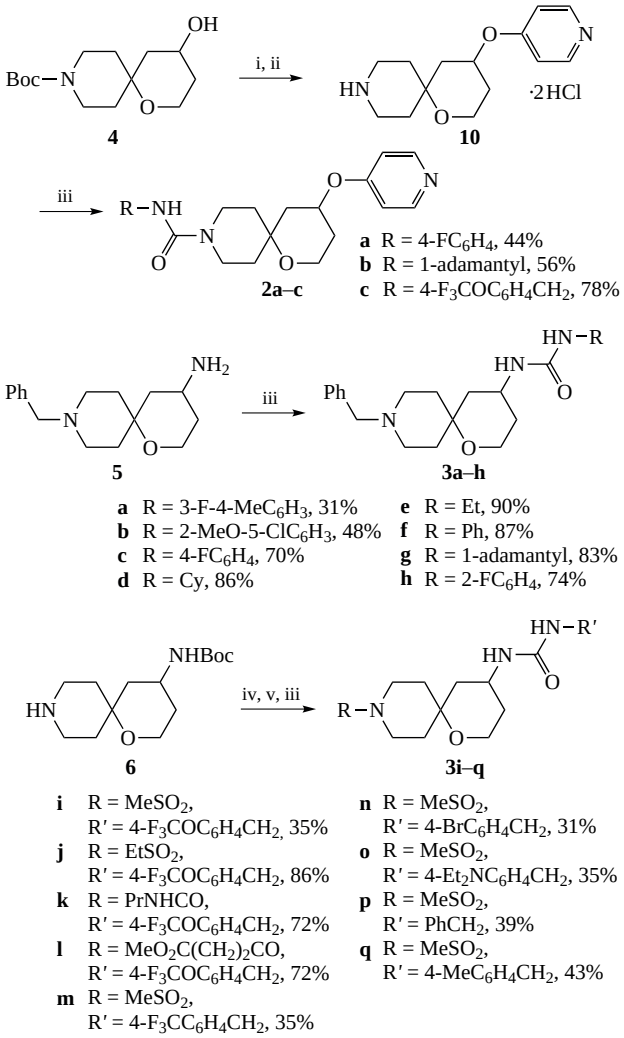
1 **2** **3**

The synthesis relied on the availability of multigram quantities of building blocks **4**,[13] **5**[18] and **6**[18] synthesized as described below (Scheme 1). Methanesulfonic acid-promoted Prins cyclization of homoallylic alcohol with *N*-benzyl-4-piperidone[19] gave mesylate **7**. Hydrolysis of the latter afforded alcohol **8**. Swapping benzyl group to Boc group afforded compound **4**. Displacement of the mesyl group in **7** with azide, subsequent reaction with triphenylphosphine and Boc-protection gave compound **9**. Finally, removal of the Boc-protection of the primary amino group in **9** or its hydrogenation over Pd/C furnished amines **5** and **6**, respectively (see Scheme 1).

Further modification of building blocks **4**–**6** is outlined in Scheme 2. Compound **4** was reacted with 4-chloropyridine in the presence of sodium hydride. Upon removal of the Boc group, the urea moiety was installed on intermediate **10** *via* the reaction with isocyanates to give compounds **2a**–**c** in moderate to good yields. Reaction of core building block **5** with isocyanates led to ureas **3a**–**h** in yields varying from modest to excellent. Finally,



Scheme 1 *Reagents and conditions*: i, CH_2=$CH(CH_2)_2OH$, MsOH, CH_2Cl_2, room temperature, 24 h (62%); ii, NaOH, aq. MeOH, room temperature, 6 h (98%); iii, HCO_2NH_4, Pd/C, EtOH, reflux, 10 h; iv, Boc_2O, Et_3N, CH_2Cl_2, room temperature, 12 h; v, NaN_3, DMF, 80 °C, 12 h; vi, PPh_3, aq. MeOH, reflux, 3 h; vii, Boc_2O, CH_2Cl_2, room temperature, 5 h (69% over 3 steps); viii, 4 M HCl in 1,4-dioxane, room temperature, 10 h.



Scheme 2 *Reagents and conditions*: i, NaH, DMF, 4-chloropyridine, 4 M HCl, 1,4-dioxane, 0→20 °C, 6 h; ii, TFA, CH_2Cl_2, 0 °C, then 4 M HCl in 1,4-dioxane (45%); iii, RNCO or R′NCO, Et_3N, Et_2O, room temperature, 18 h; iv, RCl, Et_3N, CH_2Cl_2, room temperature, 10 h; v, 4 M HCl in 1,4-dioxane, room temperature, 10 h.

sulfonylation or acylation of the secondary amino group in **6**, Boc removal and reaction with isocyanates gave ureas **3i**–**q** (see Scheme 2).

Racemic compounds **2a**–**c** and **3a**–**q** were screened for bactericidal activity against drug-sensitive *Mycobacterium tuberculosis* H37v strain. To our surprise, no activity whatsoever was detected. At the same time, the compounds were tested against six bacteria – common gram-positive *Enterococcus faecium* and *Staphylococcus aureus* as well as common gram-negative *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae* and *Enterobacter aerogenes* by the Kirby–Bauer disk diffusion test[20] (Table 1) under the Standard Operating Procedure of The European Committee on Antimicrobial Susceptibility Testing (EUCAST).[21] According to the data on the bacterial growth inhibition zone diameter (mm), a number of compounds tested truly displayed bactericidal activity.

Eight compounds (**2a**, **3a**,**b**, **3g**, **3i**, **3l**, **3o** and **3q**) that produced inhibition of more than one bacterial strain (see Table 1), were tested further in order to determine the minimum inhibitory concentration (MIC, μg ml^{-1}) using the serial broth dilutions method.[22] As it follows from the data presented in Table 2, the activity of the eight compounds was confirmed by the MIC values ranging from 19 to 150 mg ml^{-1}.

In conclusion, antimycobacterial activity of certain ureas as well as spirocyclic piperidines described in the literature prompted us to synthesize a series of spirocyclic piperidine ureas and test them against *Mycobacterium tuberculosis*. While no activity, surprisingly, was not detected in this case, we proceeded to test these compounds against common gram-positive as well as gram-negative bacteria. Here, primary single-dose testing by the Kirby–Bauer disk diffusion method identified several promising compounds which we confirmed to possess minimum inhibitory concentration ranging from 19 to 150 μg ml^{-1}. While this level of antibacterial activity is lower than that displayed by

Table 1 Bacterial growth inhibition zone diameter displayed by compounds **2a**–**c** and **3a**–**q** tested by the Kirby–Bauer disk diffusion method.

Compound	*E. faecium*	*P. aeruginosa*	*A. baumannii*	*K. pneumoniae*	*S. aureus*	*E. aerogenes*
2a	8	0	0	10	0	0
2b	0	0	0	0	0	0
2c	0	0	0	0	0	0
3a	9	0	9	11	11	9
3b	9	0	11	0	0	0
3c	0	0	0	0	0	0
3d	0	0	0	0	0	0
3e	0	0	0	0	0	0
3f	0	0	0	0	0	0
3g	0	11	9	10	0	0
3h	0	0	0	0	0	0
3i	10	0	11	12	0	0
3j	0	0	9	0	0	0
3k	0	0	0	8	0	0
3l	0	0	11	11	0	0
3m	0	0	8	0	0	0
3n	0	0	0	0	0	0
3o	12	0	9	0	0	0
3p	0	0	9	0	0	0
3q	0	0	9	10	0	0

Table 2 Minimum inhibitory concentration (MIC, mg ml^{-1}) determined for eight frontrunner compounds.

Compound	*E. faecium*	*P. aeruginosa*	*A. baumannii*	*K. pneumoniae*	*S. aureus*	*E. aerogenes*
2a	**75**	>150	>150	**38**	>150	150
3a	**38**	150	**38**	**19**	**19**	**38**
3b	**38**	>150	**19**	>150	>150	>150
3g	>150	**19**	**38**	**38**	>150	>150
3i	**38**	150	**19**	**19**	150	>150
3l	>150	>150	**19**	**19**	>150	>150
3o	**19**	>150	**38**	>150	>150	>150
3q	>150	>150	**38**	**38**	150	>150
Ciprofloxacin	2.0	0.125	0.25	0.03	0.5	0.03

broad-spectrum reference antibiotic ciprofloxacin (MIC 0.3 mg ml^{-1}), the specific antibacterial activity identified for the hitherto undescribed spirocyclic urea chemotype is evident and will be subject of further optimization.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2023.01.034.

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