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Design and synthesis of macrocyclic scaffolds for compounds with potential antituberculosis/antibacterial activity and improved CYP450 properties

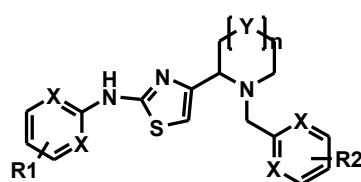
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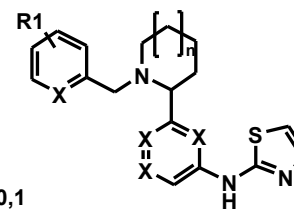
Design and synthesis of macrocyclic scaffolds for compounds with potential antituberculosis/antibacterial activity and improved CYP450 properties

Graphical Abstract

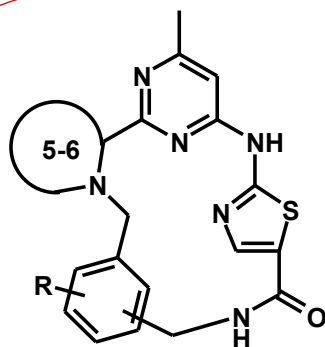


A

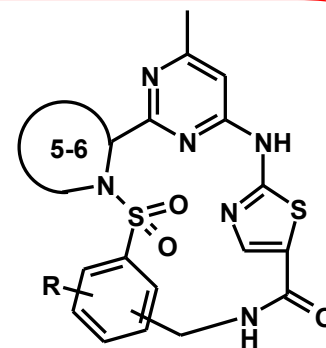
$X = C, N, n = 0, 1$



B



C



D



Abstract:

Based on an earlier suggested general pharmacophore approach, compounds active against both tuberculosis virulent strain *H37Rv* and *Staphylococcus Aureus* were obtained. The main drawback of these compounds was an almost complete CYP 3A4 inhibition, which presumably could be overcome by macrocyclization. Two 15-16-membered macrocyclic scaffolds with pharmacophore groups located in appropriate positions were designed and synthetic schemes for their achievement were elaborated. Testing of these compounds against CYP450 enzymes confirmed generally lower inhibition of key cytochromes CYP 3A4 and CYP2D6 by suggested macrocyclic compounds compared to acyclic prototypes. New macrocyclic compounds retained the ability to inhibit *Staphylococcus aureus* growth. The best compound XI showed low CYP450 inhibition and significant (77.2%) inhibition of *Staphylococcus aureus* growth.

Keywords: antibacterial, pharmacophore, Serine/Threonine kinase, cytochrome



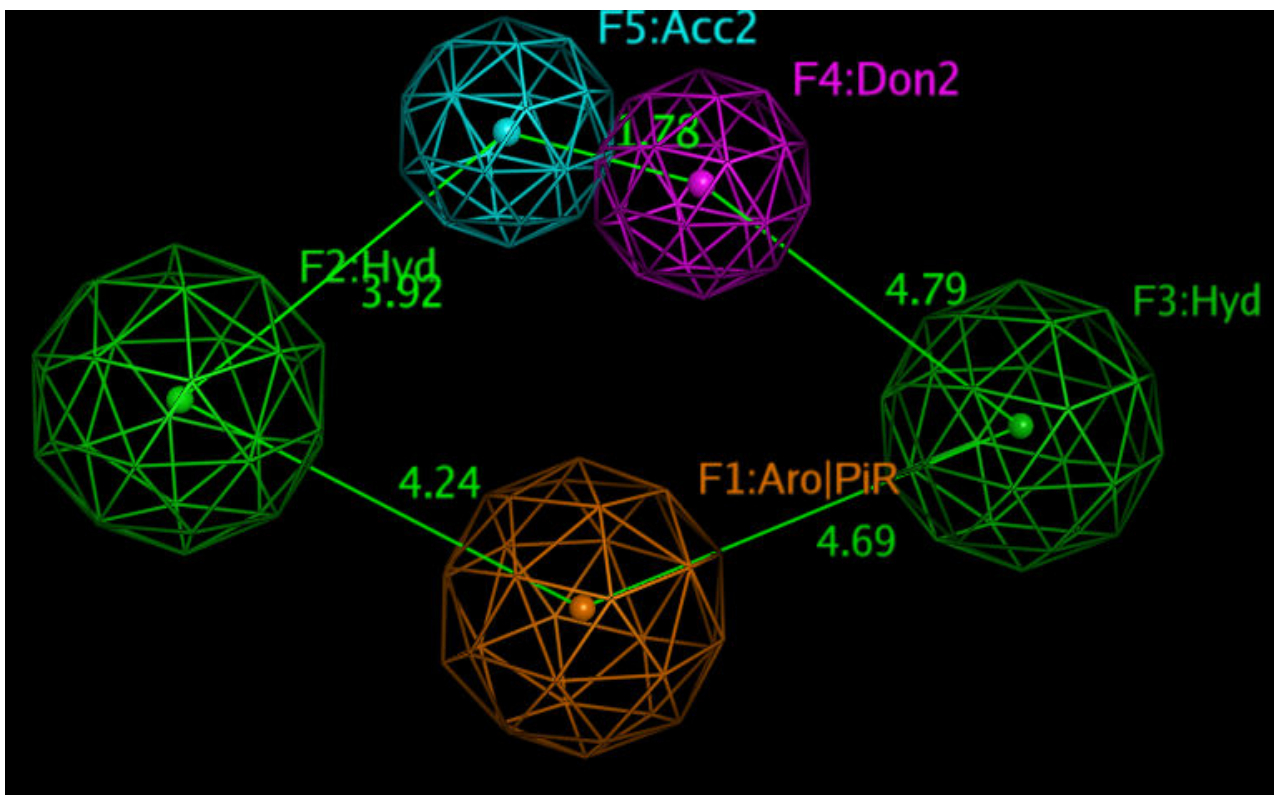
Introduction. Serine/Threonine kinases

- Serine/Threonine kinases are found in both eukaryotes and prokaryotes
- Among 500+ human protein kinases at least 125 of them are serine/threonine kinases (STK).
- Eukaryotic-like serine and threonine kinases (eSTKs) were found in *Bacillus subtilis*, *Staphylococcus aureus*, *Corynebacterium glutamicum*, *Streptomyces coelicolor*, *Mycobacterium tuberculosis* and *Streptococcus pneumoniae*



Introduction. General pharmacophore for Ser/Thr kinase inhibitors

Previously a concept of general pharmacophore for Ser/Thr kinase inhibitors was developed (N. I. Vasilevich, E. A. Aksenova, D.N. Kazyulkin, and I.I. Afanasyev, *Chem.Biology & Drug Design*, DOI: 10.1111/cbdd.12733, (2016)).

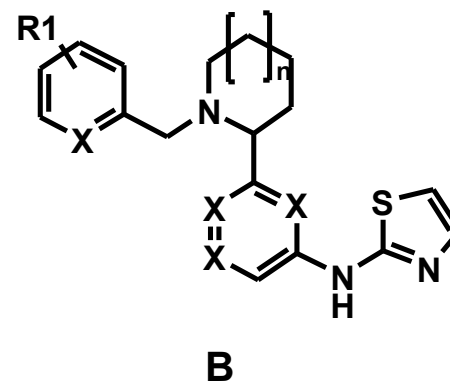
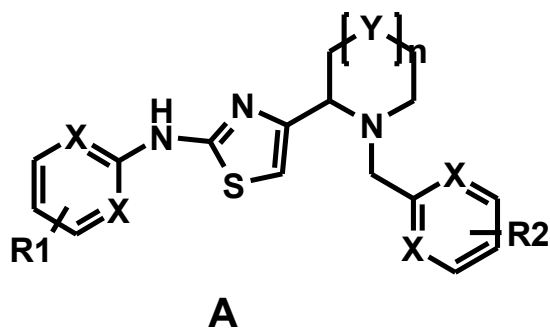


Hydrophobic areas are shown in green, aromatic cycle – in brown, projection of hydrogen bonds acceptor – in blue, and projection of hydrogen bonds donor – in pink



Results and discussion. Optimization of the general pharmacophore for bacterial PknB kinases

Based on general pharmacophore approach two scaffolds were selected

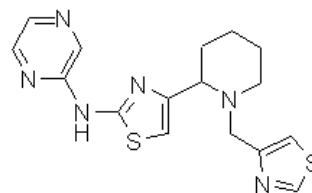


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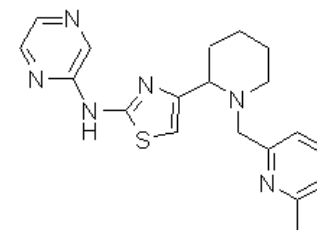
Results and discussion. Activity of selected compounds

- Compounds of both scaffolds showed activity against *H37Rv* and *Staphylococcus Aureus*
- Main drawback of these compounds is almost complete inhibition of key cytochrome CYP3A4
- Macrocyclization often helps to improve ADME parameters such as solubility, permeability, selectivity, CYP450 inhibition etc. (*Future Med. Chem.*, 2012, 4(11), 1409–1438; *J. Med. Chem.*, 2011, 54, 1961–2004; *J Comput Aided Mol Des*, 2012, 26,437–450)
- Possible way to improve CYP3A4 is closing compounds of scaffolds A and B into macrocycle



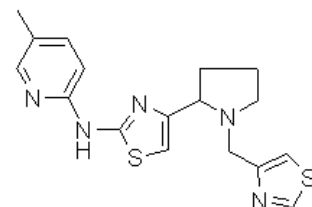
I

H37Rv: MIC 2 μM
S.aureus: growth -2,8% at 100 μM
HeLa: inhibition 20% at 20 μM
CYP3A4: inhibition 95% at 10 μM



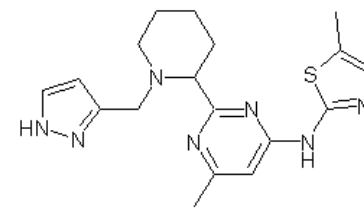
II

H37Rv: MIC 3 μM
S.aureus: growth -2,5% at 100 μM
HeLa: inhibition 60% at 20 μM
CYP3A4: inhibition 95% at 10 μM



III

H37Rv: MIC 8 μM
S.aureus: growth -2,0% at 100 μM
HeLa: inhibition 16% at 20 μM
CYP3A4: inhibition 94% at 10 μM



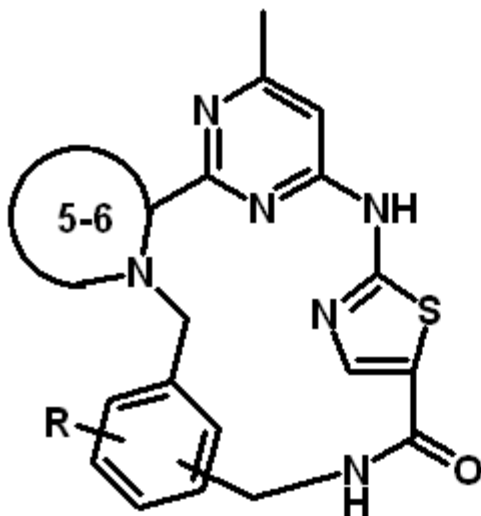
IV

H37Rv: MIC 1 μM
S.aureus: growth -0,4% at 100 μM
HeLa: inhibition 80% at 20 μM
CYP3A4: 97% at 10 μM

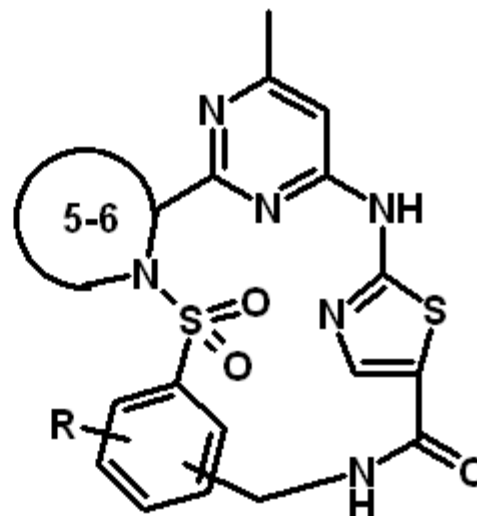


Results and discussion. Design of macrocyclic scaffolds

- Geometry of scaffolds A and B allows to close them to 15-16 macrocycle
- Scaffolds C and D contain all pharmacophore elements including three aromatic cycles and donor of hydrogen bond in proper position



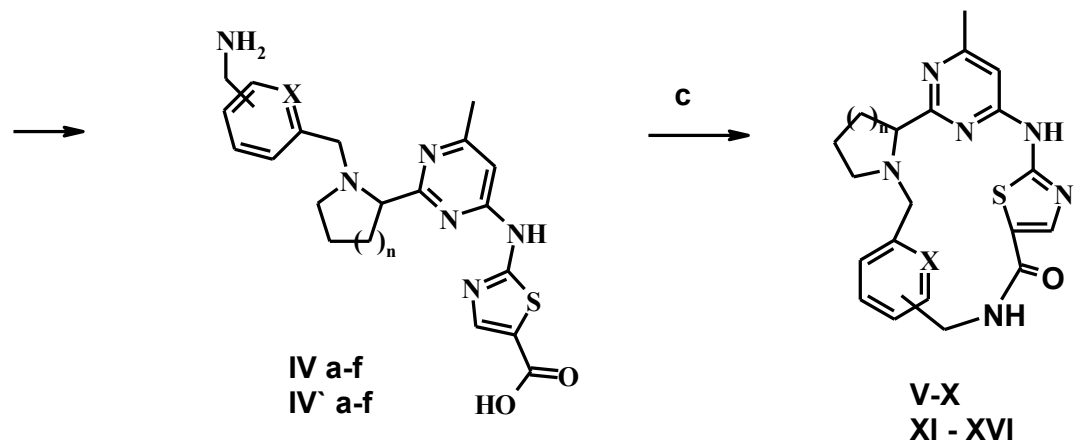
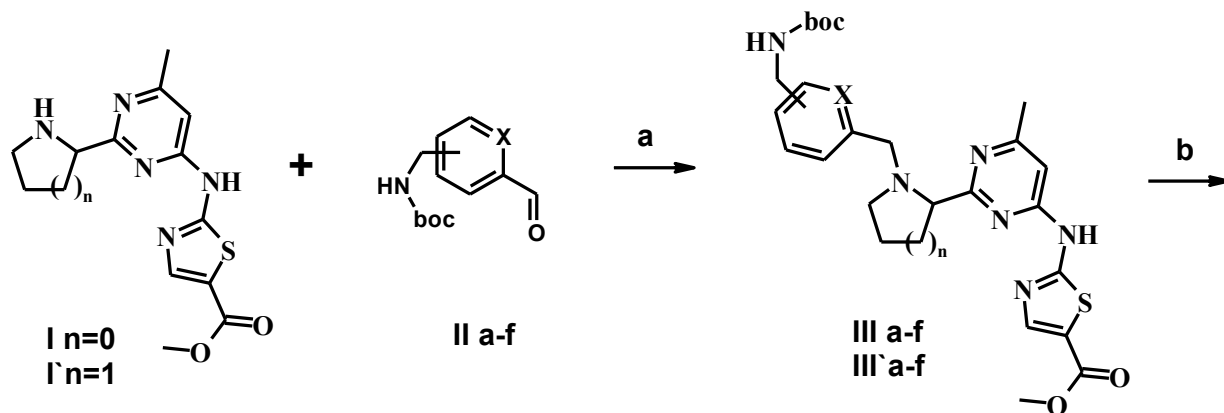
Scaffold C



Scaffold D



Results and discussion. Synthesis of compounds of Scaffold C

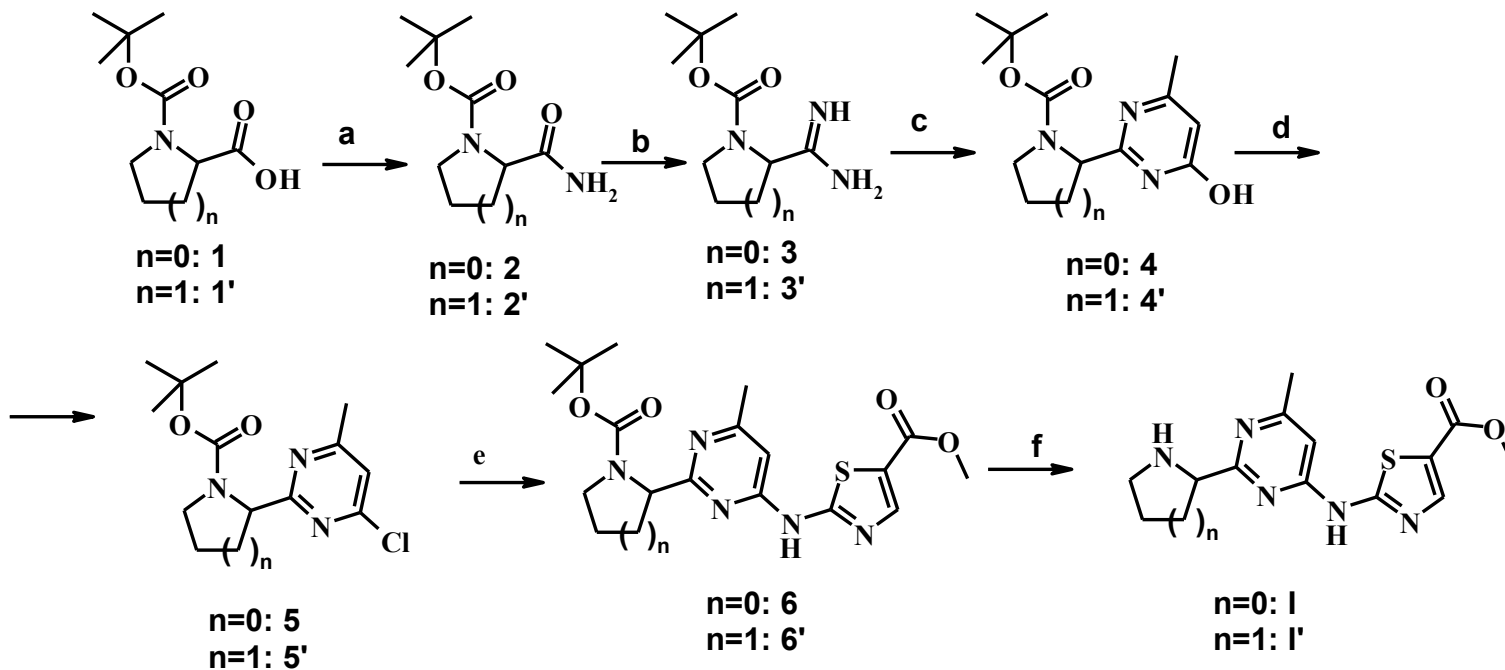


$X = C, N$

- a) $NaBH(OAc)_3$; CH_2Cl_2 , r.t.,
 b) HCl ; $NaOH$, $H_2O-MeOH$; r.t.;
 c) $TBTU$, Et_3N , r.t.



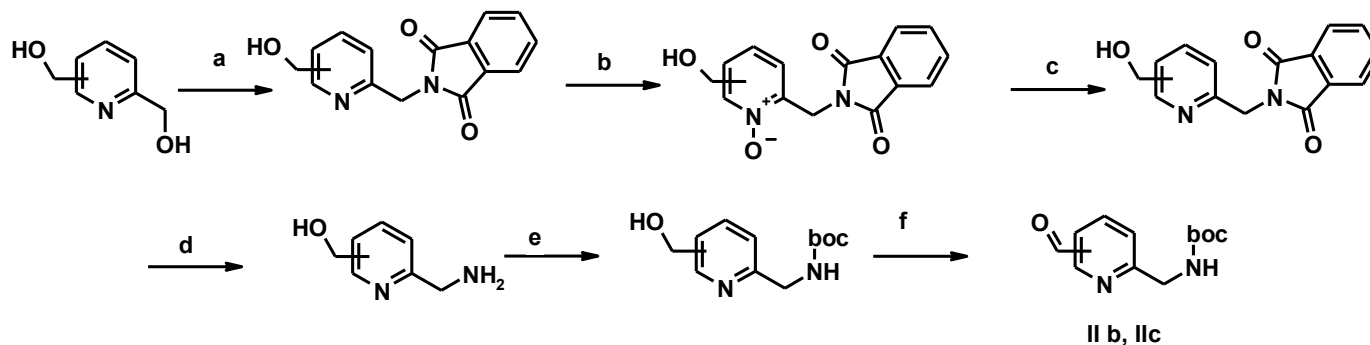
Results and discussion. Synthesis of Intermediate I



- CDI in acetonitrile, then $(\text{NH}_4)_2\text{CO}_3$ and $(\text{CH}_3)_3\text{N}$, r.t.
- Triethyloxonium tetrafluoroborate in CH_2Cl_2 , then 10% NH_3/MeOH , r.t.
- t*-BuONa/EtOH, then ethyl acetoacetate, reflux
- POCl_3 , *N,N*-dimethylaniline, toluene, 60°C
- 2-amino-5-carbomethoxythiazole, 9,9 dimethyl-4,5-bis(diphenylphosphino)xanthene, tris(dibenzylidenacetone)dipalladium (0)-chloroform, Na_2CO_3 microwave 140°C
- HCl/dioxane



Results and discussion. Synthesis of Aldehydes II

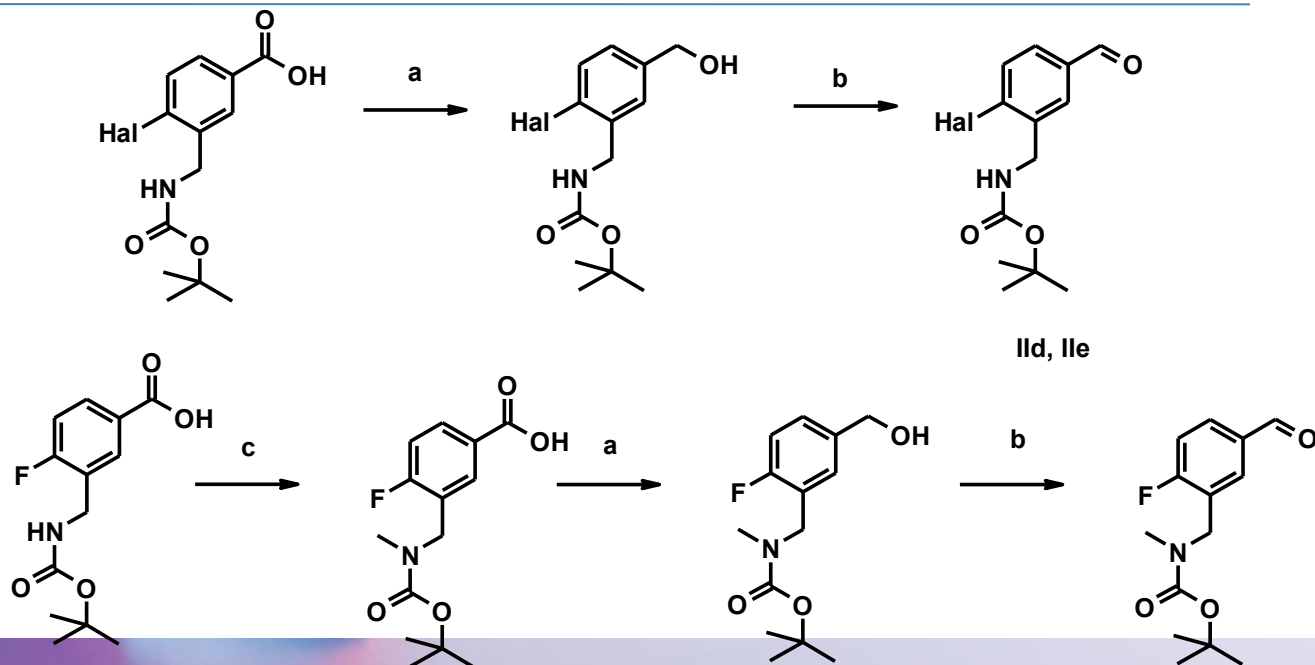


a) DIAD, Ph_3P , phthalimide, $Tf\Phi$, r.t.;
 b) *m*-CPBA, CH_2Cl_2 , r.t.;
 c) TFAA, CH_2Cl_2 , r.t.;
 d) $N_2H_4 \times H_2O$, ethanol, reflux;
 e) Boc_2O , CH_2Cl_2 , r.t.;
 f) Dess-Martin reagent, CH_2Cl_2 , r.t.

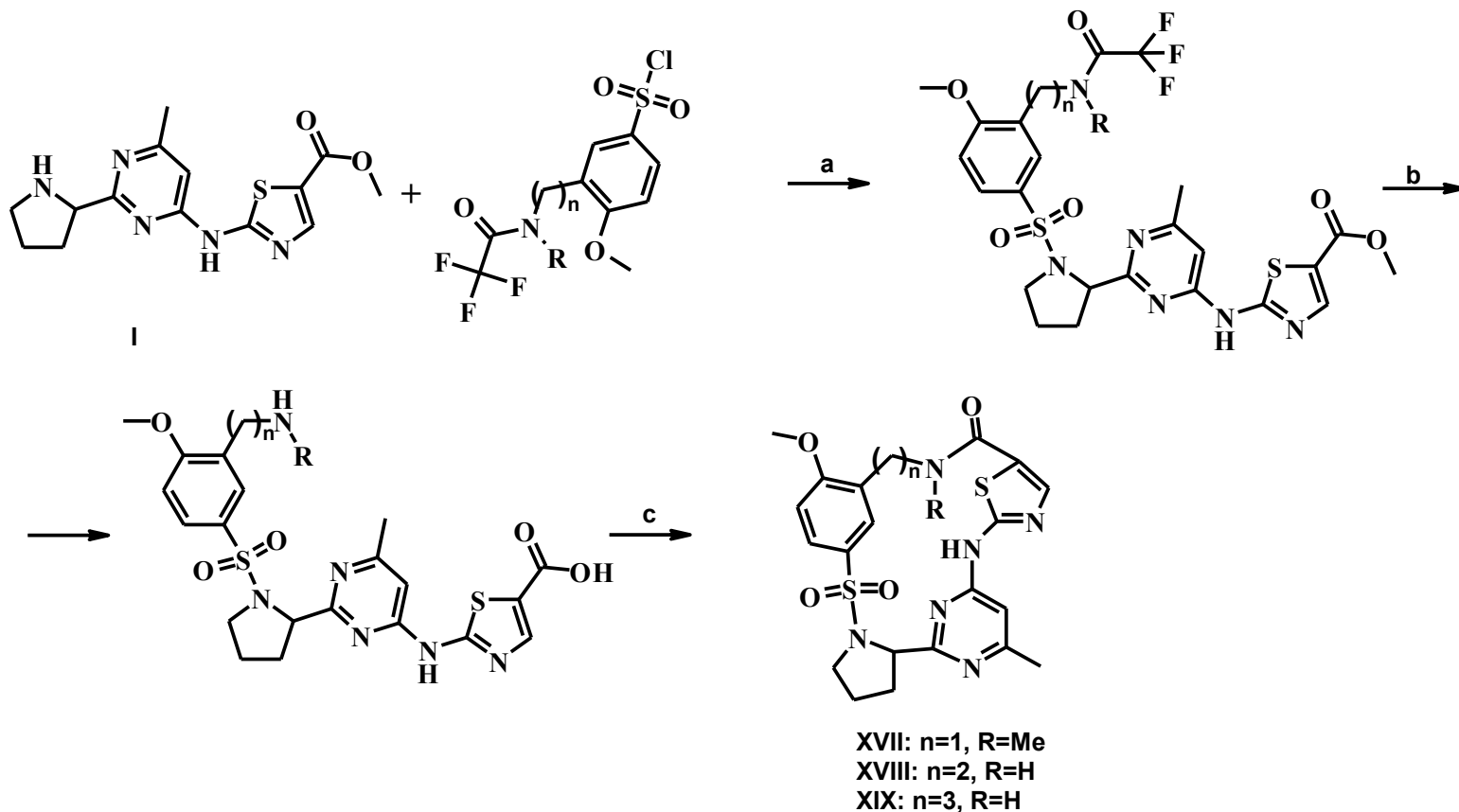
a) *i*-butyl chloroformate, THF, then $NaBH_4$, EtOH;

b) pyridinium chlorochromate, CH_2Cl_2 , r.t.;

c) CH_3I , NaH, THF



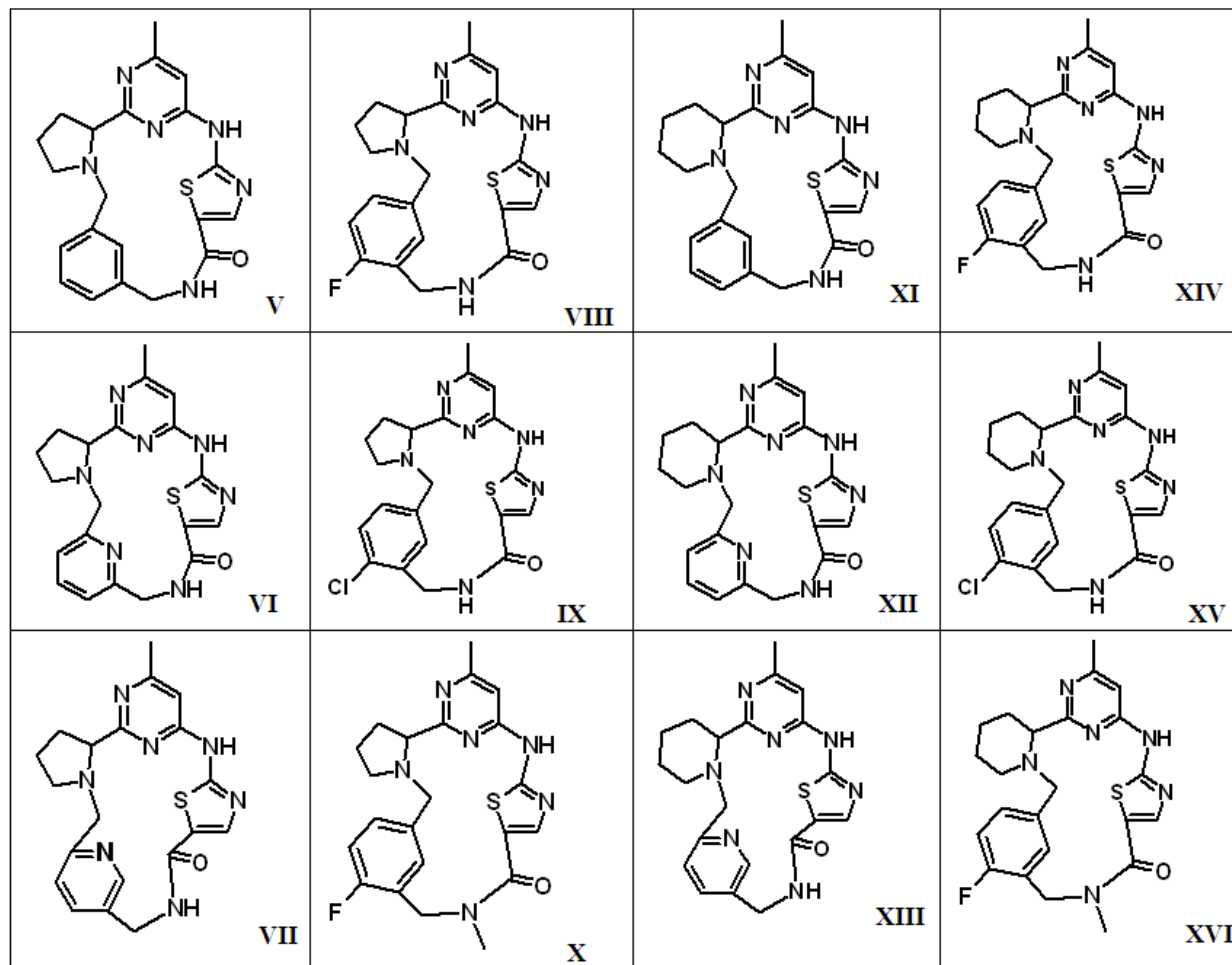
Results and discussion. Synthesis of compounds of Scaffold D



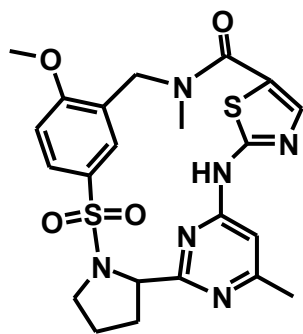
a) Me_3N , acetonitrile, r.t.; b) sat. $NaOH/CH_3OH$; c) TBTU, Me_3N , THF



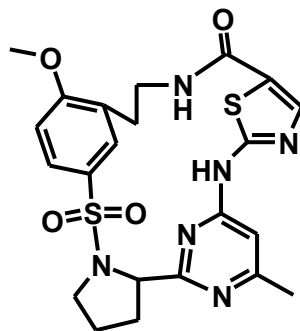
Results and discussion. Synthesized compounds of Scaffold C



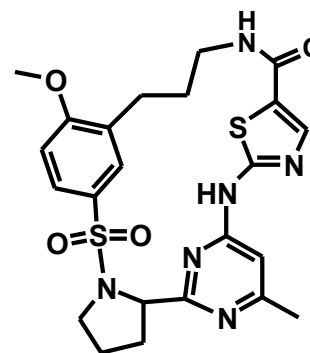
Results and discussion. Synthesized compounds of Scaffold D



XVII



XVIII



XIV



Results and discussion. CYP450 inhibition and growth of *S.Aureus*

	CYP 3A4, % inh at 10 μM (IC ₅₀)	CYP 1A2, % inh at 10 μM (IC ₅₀)	CYP 2C9, % inh at 10 μM (IC ₅₀)	CYP 2D6, % inh at 10 μM (IC ₅₀)	CYP 2C19, % inh at 10 μM (IC ₅₀)	<u>S.Aureus</u> , growth at 100 μM
I	95 (0.55 μM)	38	65	83 (1.54 μM)	71 (3.70 μM)	-2.8
II	95 (0.63 μM)	21	38	69	35	-2.5
III	94 (0.81 μM)	38	51	92 (1.49 μM)	54	-2.0
IV	96 (0.723)	33	30	82 (2.13 μM)	29	-0.4
V	87%	-7	14	4	14	16.7
VI	97% (0.37 μM)	40	26	46	9	48.8
VII	73%	16	12	-1	-5	25.0
VIII	62%	13	50	24	70 (4.18 μM)	22.5
IX	76%	28	19	5	76 (3.16 μM)	49.2
X	88%	53	24	56	26	75.2
XI	58%	3	4	3	5	22.8
XII	75%	2	17	-4	84 (1.63 μM)	67.2
XIII	62%	7	1	80% (1.23 μM)	14	24.3
XIV	99% (0.38 μM)	71% (1.56 μM)	80% (3.69 μM)	49	68	18.0
XV	92% (0.58 μM)	11	39	69	18	4.1
XVI	65%	9	14	1	3	63.9
XVII	86	15	83 (1.61 μM)	10	49	87.4
XVIII	59	3	18	-4	97 (0.15 μM)	86.6
XIX	98 (0.51 μM)	78	89 (1.88 μM)	87 (0.15 μM)	78	88.3

Parent acyclic compounds
 Compounds of Scaffold C
 Compounds of Scaffold D



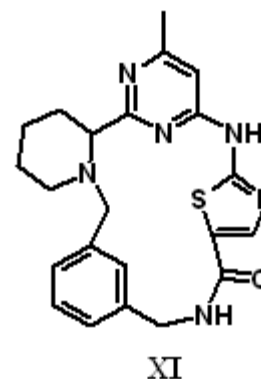
Results and discussion. CYP450 inhibition and growth of *S.Aureus*

- In most cases macrocyclization improved the inhibition of key cytochromes CYP3A4 and CYP2D6.
- Scaffold **C** seems to be preferable compared to Scaffold **D** in terms of CYP 450 inhibition, however, the difference is not essential.
- Macrocyclic compounds were able to inhibit the growth of *Staphylococcus aureus* though with reduced potency.
- Scaffold **C** is preferable compared to Scaffold **D** in terms of *Staphylococcus aureus* growth inhibition



Conclusions

- Two macrocyclic scaffolds for compounds with potential antituberculosis/antibacterial activity were design
- Synthetic schemes aimed at 15-16 member macrocyclic compounds belonging to these scaffolds were developed
- New macrocyclic compounds demonstrated improved CYP3A4 and CYP2D6 inhibition simultaneously retaining the ability to inhibit *Staphylococcus aureus* growth.
- The best compound XI showed low CYP450 inhibition and significant (77.2%) inhibition of *Staphylococcus aureus* growth.
- Further optimization of designed macrocyclic compounds is required to get safe compounds with high antibacterial activity.



Acknowledgments

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