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Adarotene-related retinoids as potential antimicrobial agents against multidrug-resistant gram-positive strains

Chaired by Dr. Alfredo Berzal-Herranz and Prof. Dr. Maria Emília Sousa

pharmaceuticals MDPI

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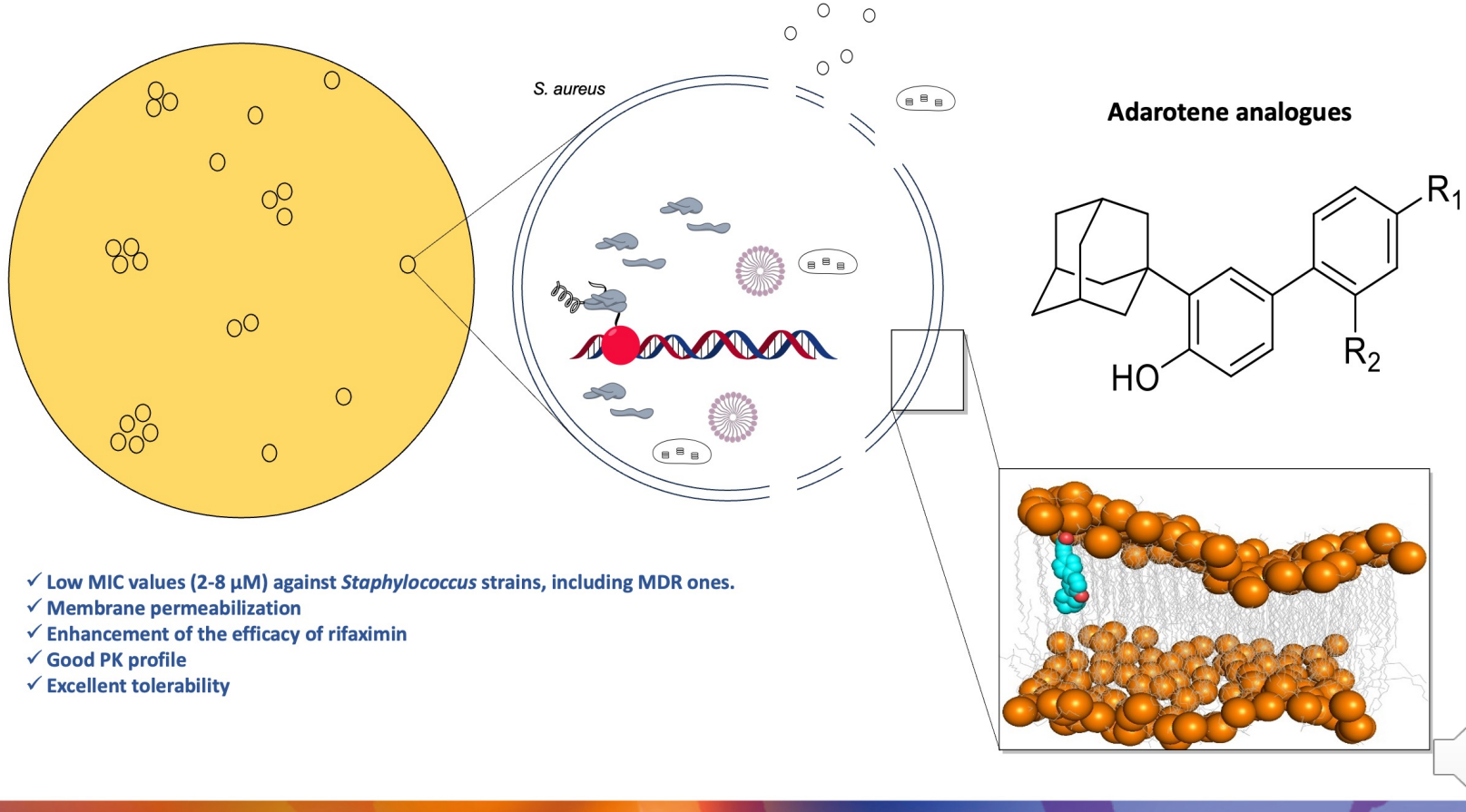
Adarotene-related retinoids as potential antimicrobial agents against multidrug-resistant gram-positive strains



- ✓ Low MIC values (2-8 µM) against *Staphylococcus* strains, including MDR ones.
- ✓ Membrane permeabilization
- ✓ Enhancement of the efficacy of rifaximin
- ✓ Good PK profile
- ✓ Excellent tolerability

Multidrug-resistant (MDR) pathogens are severely impacting our ability to successfully treat common infections. As a consequence, bacterial resistance to antimicrobial drugs represents one of the most impelling topics in medicinal chemistry. In recent years we have focused our efforts on the investigation of a panel of adarotene-related synthetic retinoids showing, together with favorable MICs, a detectable bactericidal effect on *S. aureus* and *E. faecalis* (including some MDR strains).[1] Based on these promising results, a small collection of adarotene related retinoids was prepared. Chemical modifications were performed on the carboxylic group and the double bond of the cinnamic portion, as well as polar substituents were introduced on ring A and ring B, in order to evaluate the potential structural determinants necessary to exert antibiotic activity (Figure 1). Overall, the results showed that compounds with a very good activity profile can be obtained by modulating the pattern of substitution on the adarotene moiety. Moreover, the shape and geometry of the molecules, together with the presence of key polar groups on the biphenyl backbone, could play a major role for the antimicrobial effect on resistant strains.

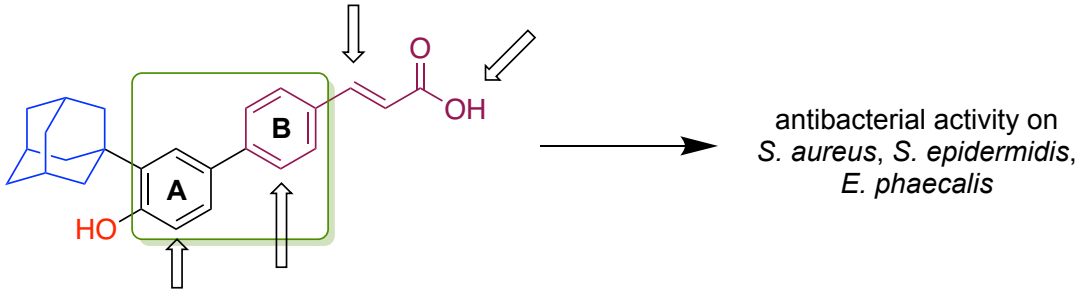
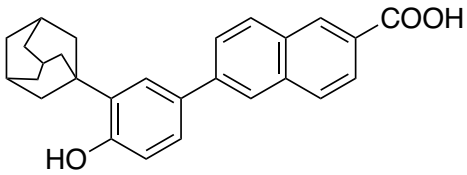


Figure 1: Structure of adarotene and suitable modifications on its scaffold.

References:

[1] S. Princiotto, S. Mazzini, L. Musso, F. Arena, S. Dallavalle, and C. Pisano, *Antibiotics* **2021**, *10*, 126.

Retinoid-related molecules (RRMs)



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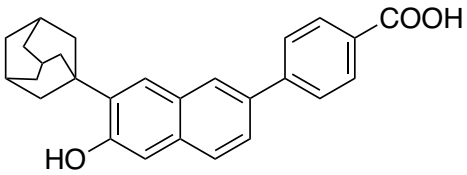

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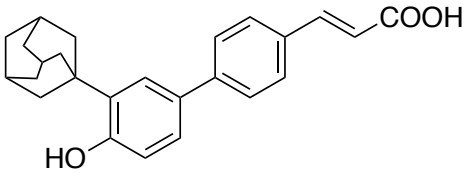
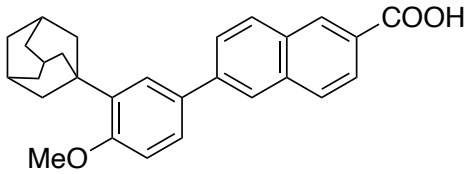


adarotene



adapalene

- Pro-apoptotic activity on solid tumors and leukemia
- Evidences of antimicrobial activity (Kim et al. *Nature* **556**, 103–107 (2018))

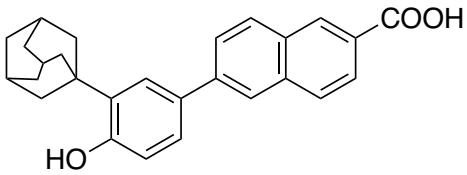
LETTER

doi:10.1038/nature26157

A new class of synthetic retinoid antibiotics effective against bacterial persisters

Wooseong Kim[1], Wenpeng Zhu[2], Gabriel Lambert Hendricks[1], Daria Van Tyne[3,4], Andrew D. Steele[5,6], Colleen E. Keohane[5,6], Nico Fricke[2], Annie L. Conery[7,8], Steven Shen[1], Wen Pan[1], Kiho Lee[1], Rajmohan Rajamuthiah[1], Beth Burgwyn Fuchs[1], Petia M. Vlahovska[9], William M. Wuest[5,6], Michael S. Gilmore[3,4], Huajian Gao[2], Frederick M. Ausubel[7,8] & Eleftherios Mylonakis[1]

Retinoid-related molecules (RRMs)



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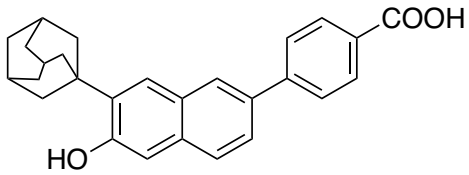

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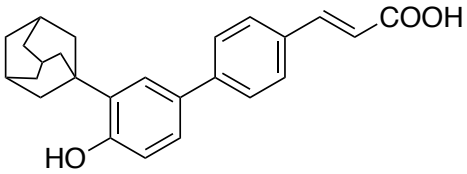
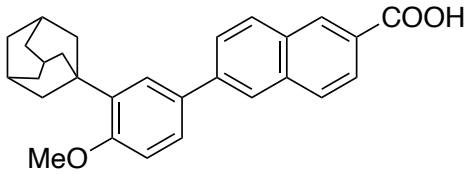
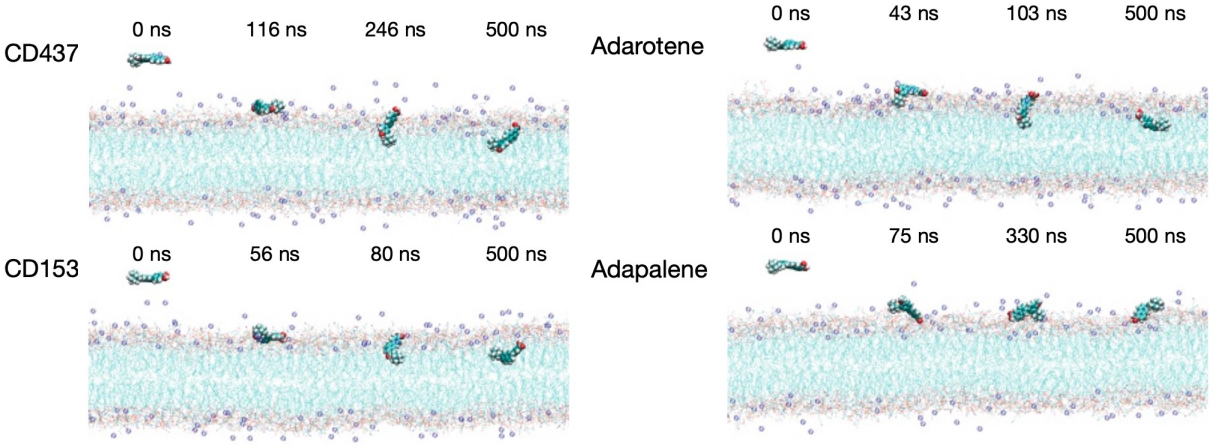


adarotene

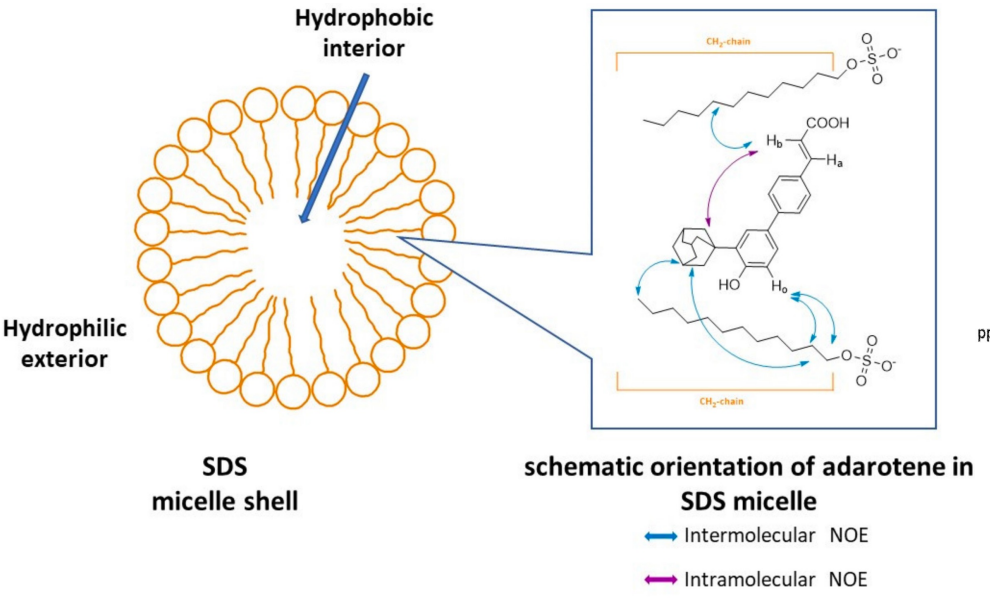


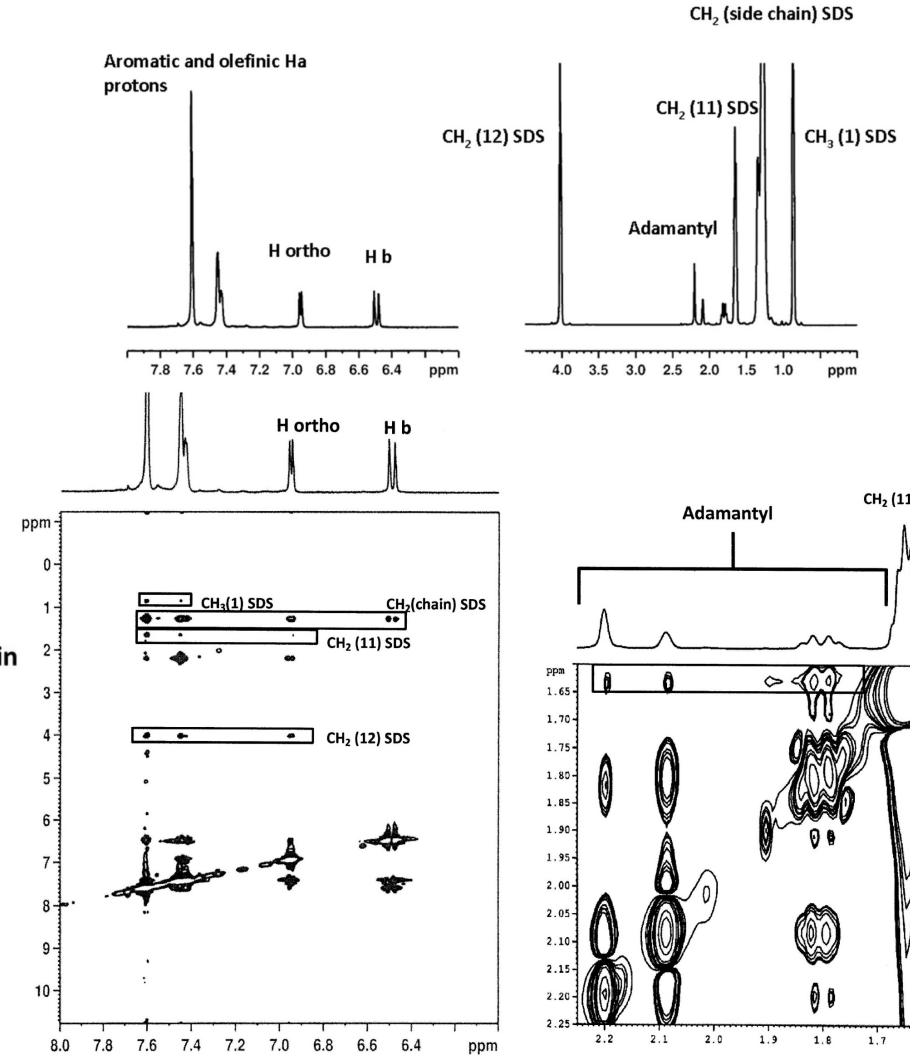
adapalene

- Pro-apoptotic activity on solid tumors and leukemia
- Evidences of antimicrobial activity (Kim et al. *Nature* **556**, 103–107 (2018))

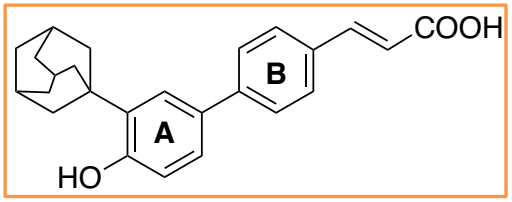


NMR studies on adarotene

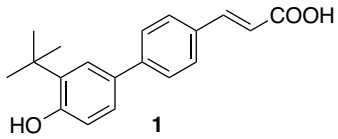




Princiotto et al. *Antibiotics*, **2021**, 126



In house library of adarotene analogues



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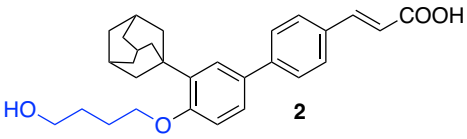

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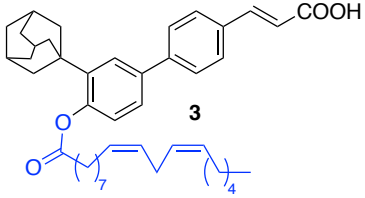

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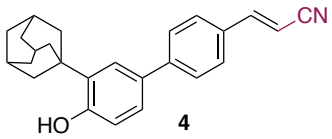

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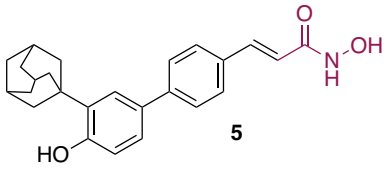

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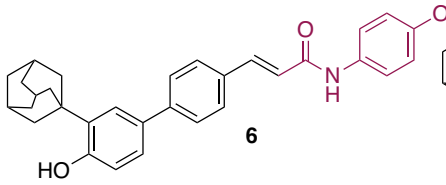

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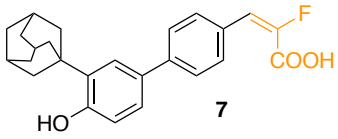

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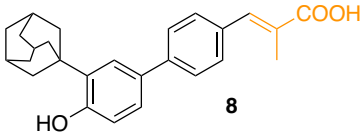

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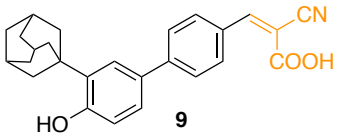

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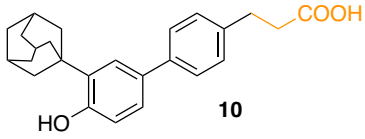

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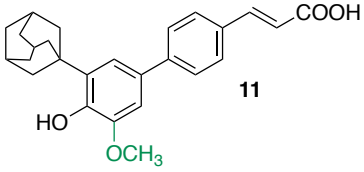

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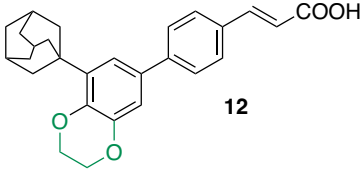

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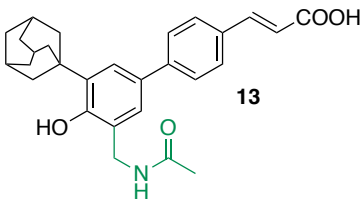

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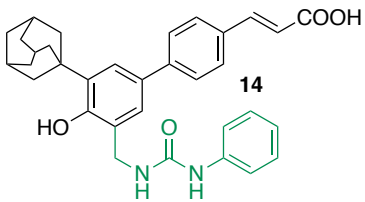

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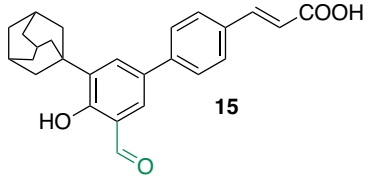

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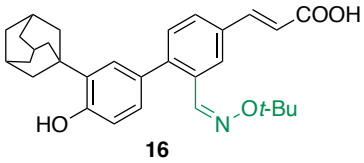

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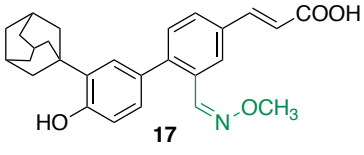

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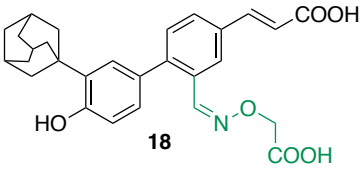

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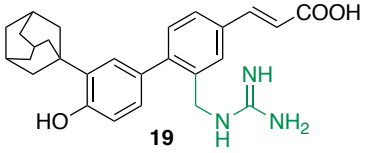

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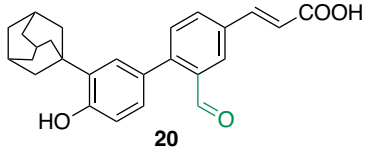

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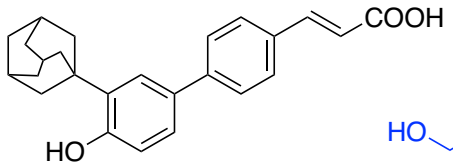
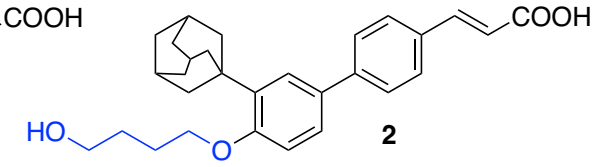
Biological activity evaluation

cpd	IC_{50} (μM)[a]	MIC (μg/mL) *S. aureus* Strain 1	MIC (μg/mL) *E. faecalis* Strain 1
Adarotene	0.23±0.08	8	1
1	30	64	>256
2	>10	256	128
3	7.8±0.7	32	128
4	8.3±1.4	128	32
5	1.1±0.6	64	>256
6	6.6±0.5	>256	>256
7	>3	4	4
8	7.19±1.27	2	1
9	>10	2	1
10	48.42±0.88	16	16
11	0.52±0.07	16	8
12	0.23±0.07	4	2
13	1.24±0.07	8	4
14	>10	8	4
15	1.64±0.03	64	64
16	>10	4	2
17	>10	4	8
18	>10	128	128
19	>10	32	32
20	3.2±0.2	2	8

[a] tested IGROV-1 cancer cell line



adarotene



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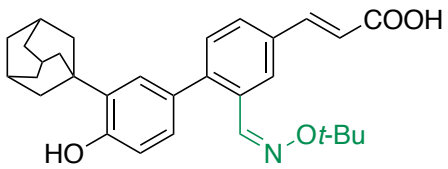

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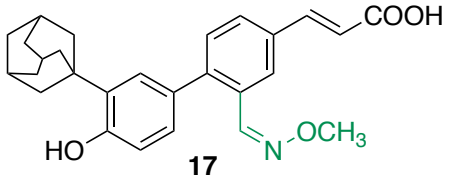

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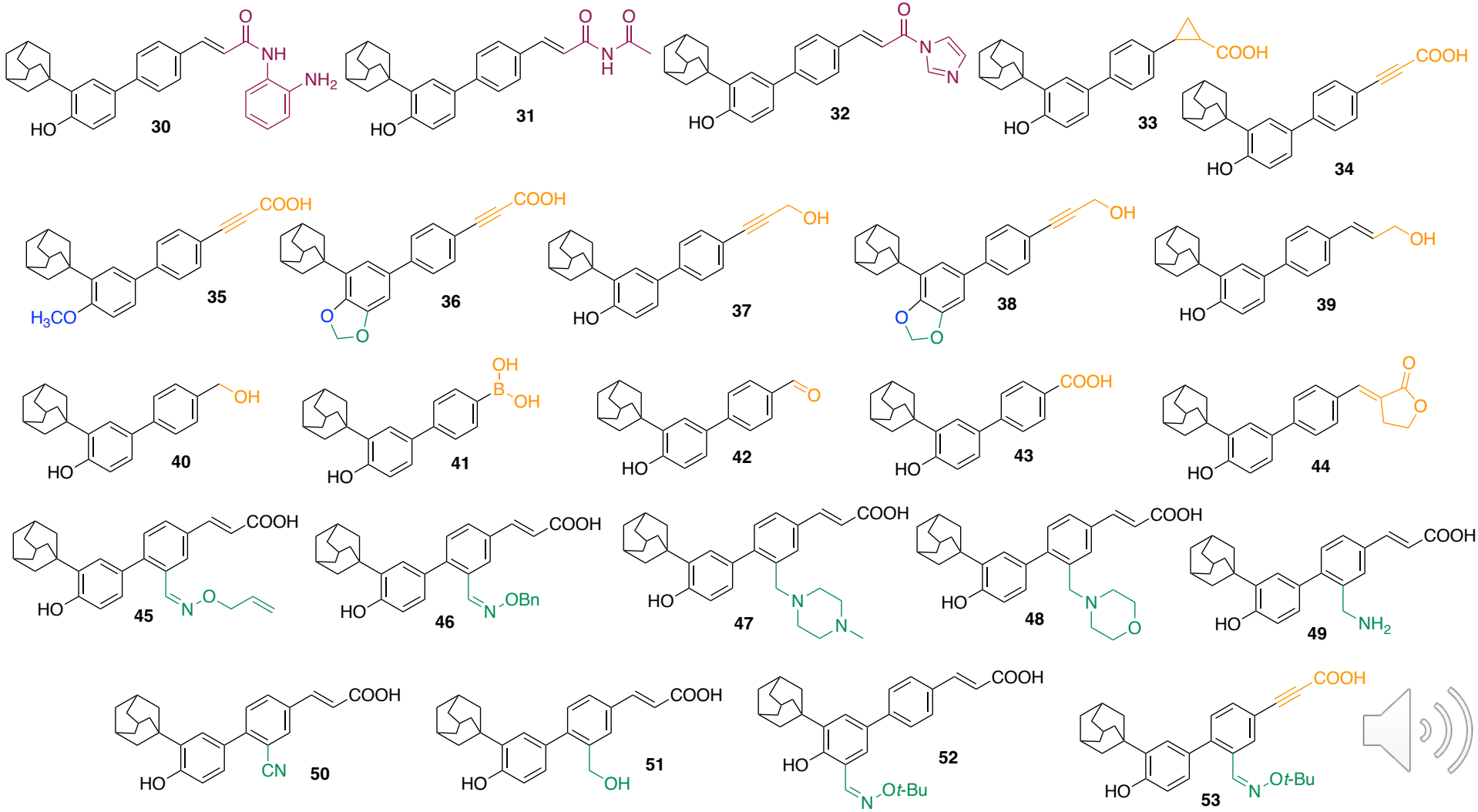
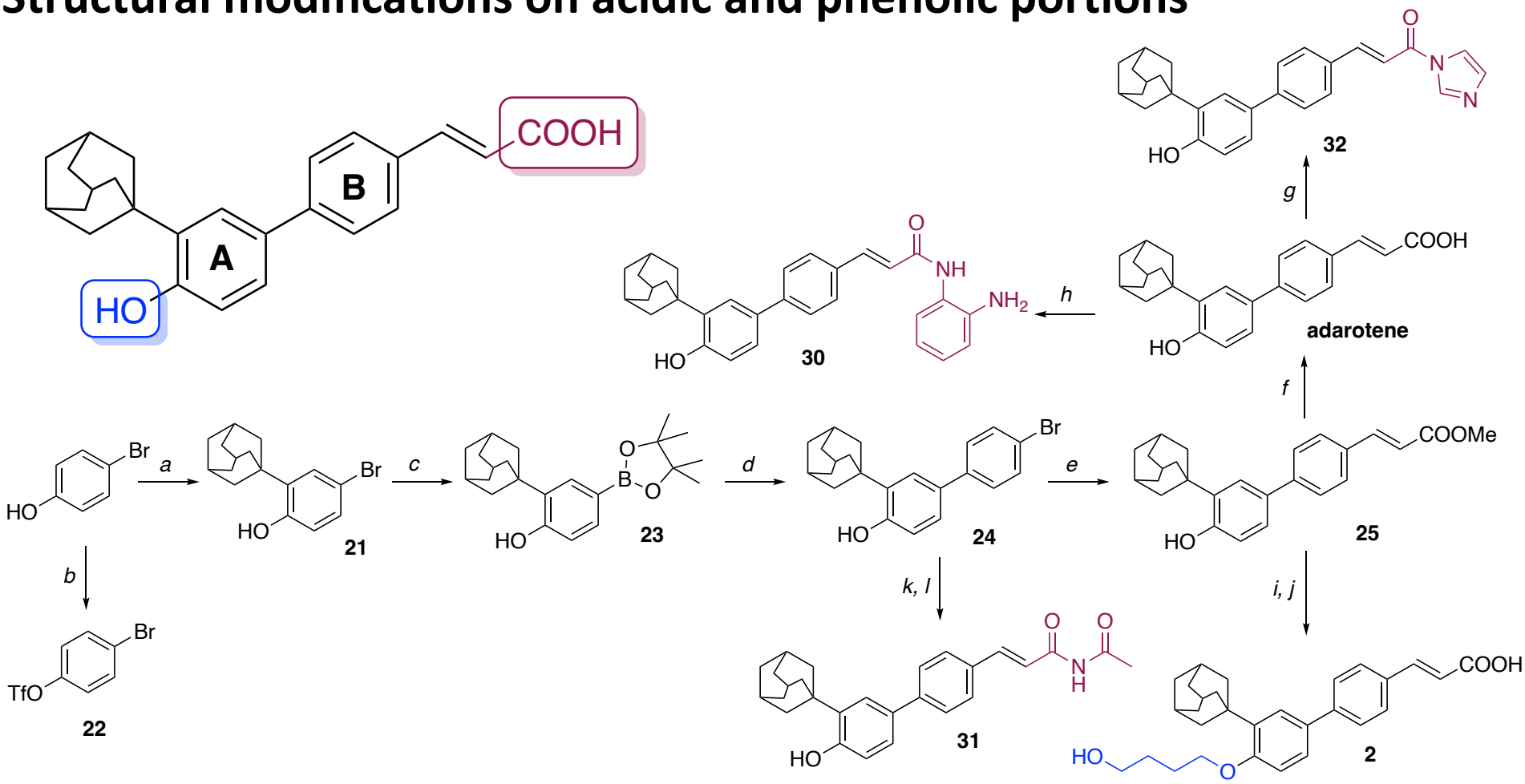
cpd	MIC (μg/mL) *S. aureus* ATCC 25923	*S. aureus* Strain 2	*E. faecalis ATCC* 51299	*E. faecalis* Strain 2
Adarotene	8	4	2	4
2	256	256	64	128
16	2	2	2	2
17	2	4	8	8

New adarotene analogues

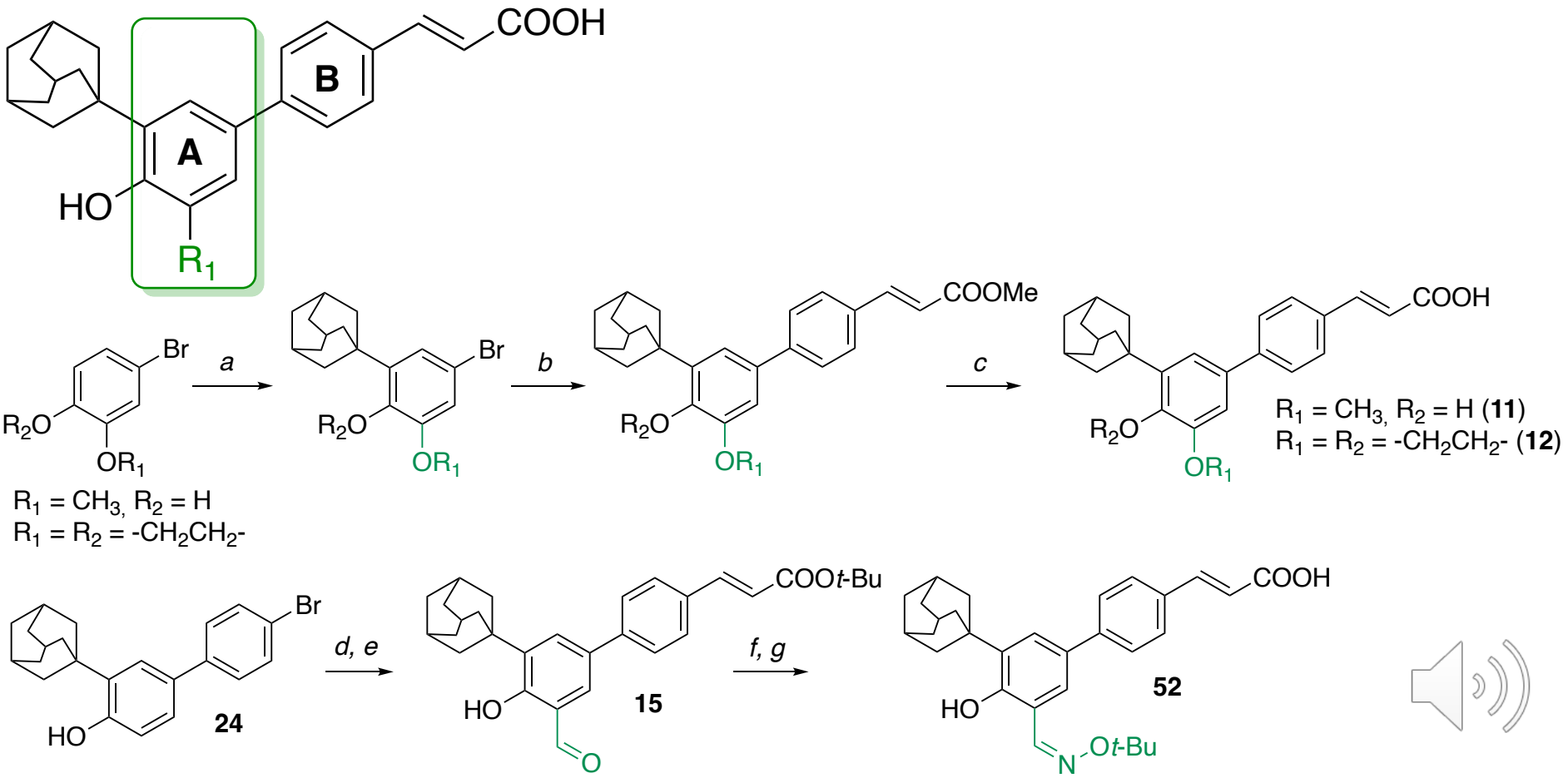


Structural modifications on acidic and phenolic portions



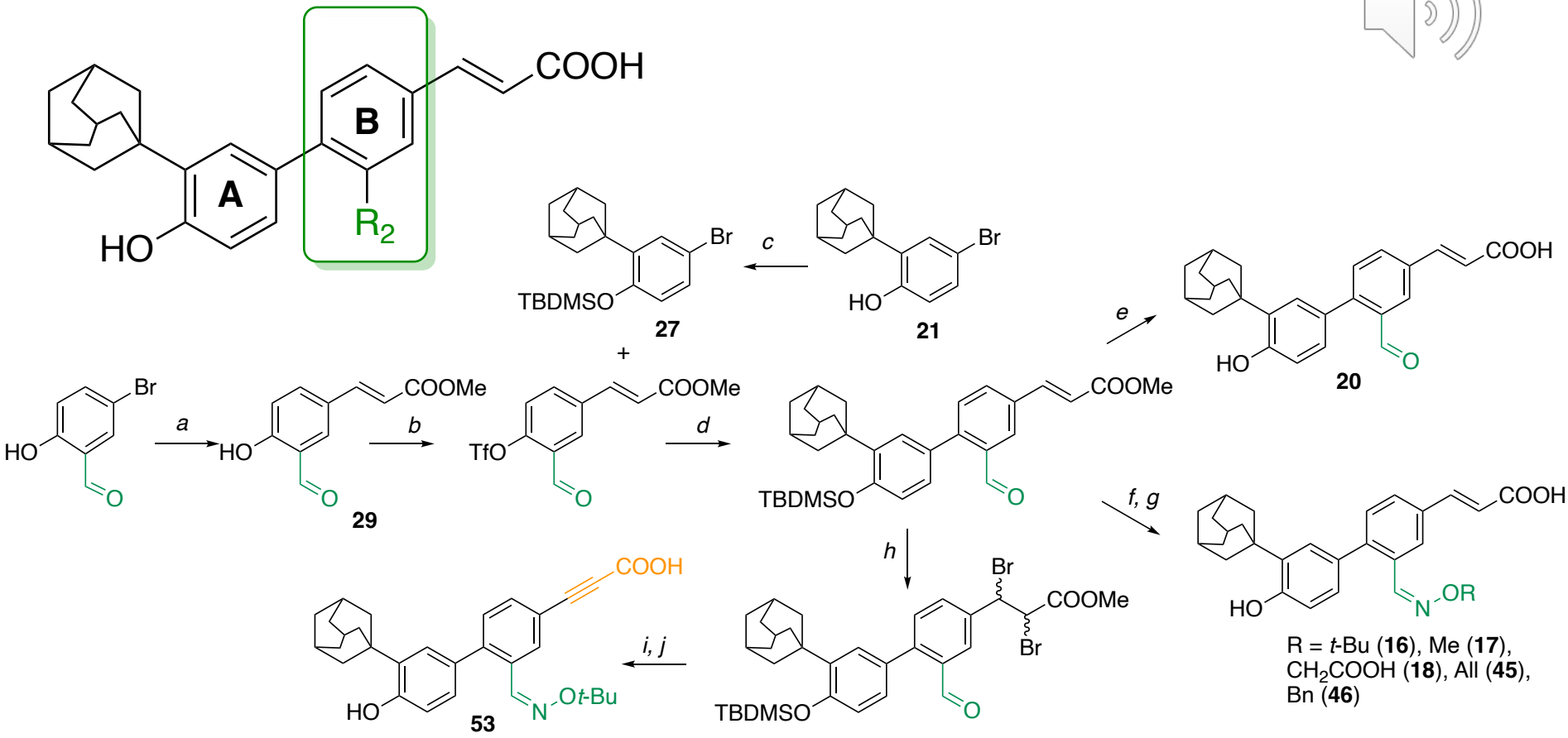
a) adamantan-1-ol, H_2SO_4/AcOH; b) Tf_2O, Py; c) bis(pinacolato)diboron, KOAc, $PdCl_2(dppf)$; d) **22**, $Pd(PPh_3)_4$, Na_2CO_3 2 M, DME/EtOH 9:1; e) methyl acrylate, TOTP, $Pd(OAc)_2$, Et_3N; f) LiOH H_2O, THF/H_2O; g) CDI, DMF; h) WSC, HOBT, 1,2-phenylenediamine, CH_3CN/THF; i) 4-bromobutyl acetate, K_2CO_3, DMF; j) i. 0.7N NaOH, CH_3OH, reflux; ii. 1M HCl; k) acrylonitrile, tri(*o*-tolyl)phosphine, $Pd(OAc)_2$; l) acetic anhydride, PTSA

Substitutions on ring A



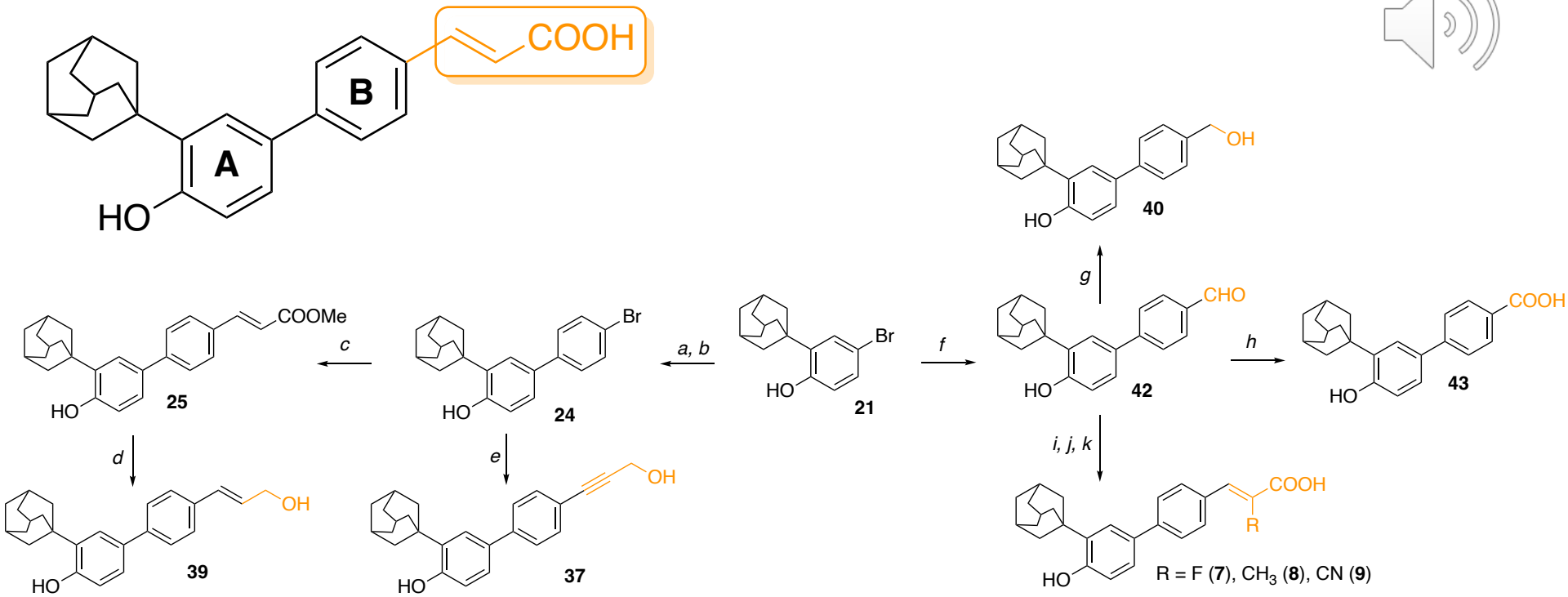
a) adamantan-1-ol, H_2SO_4/AcOH; b) bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, methyl *p*-bromocinnamate, Na_2CO_3 2 M and $PdCl_2$(dppf); c) LiOH THF/H_2O; d) $(CH_2O)_n$, $SnCl_4$, 2,6-lutidine, toluene; e) $Pd(OAc)_2$, tri(*o*-tolyl)phosphine, Et_3N, *tert*-butyl acrylate; f) *O*-*t*-butyl hydroxylamine hydrochloride, pyridine, EtOH, reflux; g) TFA, dry CH_2Cl_2

Substitutions on ring B



a) Methyl acrylate, $Pd(OAc)_2$, TOTP, TEA; b) $C_6H_5N(SO_2CF_3)_2$, Et_3N, CH_2Cl_2; c) TBDMSCl, Et_3N, DMAP, DMF; d) bis(pinacolato)diboron, KOAc, $PdCl_2(dppf)$, **27**, Na_2CO_3; e) LiOH H_2O, THF/H_2O, 1:1; f) *t*-$BuONH_2$·HCl (**16**) or $MeONH_2$·HCl (**17**) or NH_2OCH_2COOH·1/2 HCl (**18**) or $AllONH_2$·HCl (**45**) or $BnONH_2$·HCl (**46**) py, EtOH, reflux; g) LiOH·H_2O THF/H_2O 1:1; h) pyridinium tribromide, CH_2Cl_2; i) *t*-$BuONH_2$·HCl, EtOH, pyridine, reflux; j) KOH, isopropanol, reflux

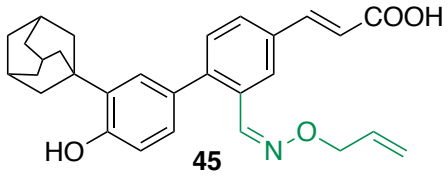
Substitutions on the cinnamic portion



a) $PdCl_2(dppf)\cdot CH_2Cl_2$, KOAc, diboro-bis-pinacolate, dioxane; b) **22**, $Pd(PPh_3)_4$, Na_2CO_3 2 M, DME/EtOH 9:1; c) methyl acrylate, tri(*o*-tolyl)phosphine, $Pd(OAc)_2$, Et_3N; d) $LiAlH_4$, THF; e) i. CuI, $PdCl_2(Ph_3P)_2$, diisopropylamine, Et_3N; ii. propargyl alcohol; f) 4-Formylbenzeneboronic acid, $Pd(Ph_3P)_4$, Na_2CO_3, toluene; g) $NaBH_4$ in MeOH; h) $KMnO_4$, H_2O/acetone; i) TBDMSCl, Et_3N, DMAP, DMF; j) $Ph_3PdC(CH_3)COOEt/CHCl_3$/BuLi/THF (**7**) or $EtOCOCHFPO(OEt)_2$/BuLi/THF (**8**) or methyl cyanoacetate, β-alanine, EtOH; k) LiOH H_2O, DMF

Biological activity evaluation

cpd	MIC (μM) *S. aureus* ATCC 25923	MIC (μM) *E. coli* ATCC 25922
Adarotene	21	-
16	**4**	>128
30	>128	>128
31	>128	>128
32	>128	>128
33	64	>128
34	16	>128
35	64	n.d.
36	16	>128
37	**4**	>128
38	**>128**	>128
39	16	>128
40	8	>128
41	**4**	>128
42	>128	>128
43	>128	>128
44	>128	>128
45	**4**	>128
46	**8**	>128
47	32	>128
48	32	>128
49	>128	>128
50	>128	>128
51	128	>128
52	>128	>128
53	**8**	>128



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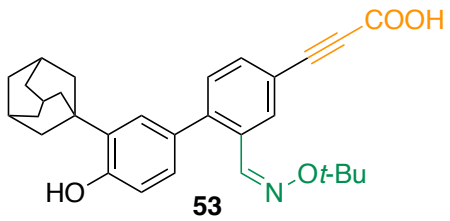

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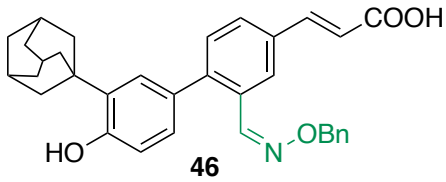

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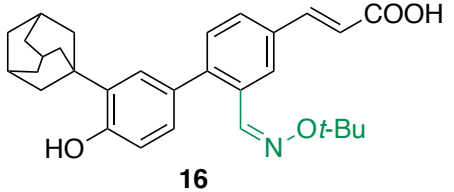

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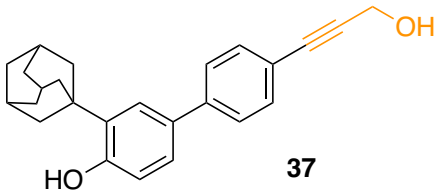
Strain	Resistance profile	MIC (μM)
***S. epidermidis* ATCC 12228**	-	4
***S. aureus* ATCC 43300**	MET - OXA	2
***S. aureus* #2**	BEN – CLI – DAP – ERI – LEV -OXA -VAN	4
***S. aureus* 13667073**	AZI – BEN – CIP – CLI – ERI – LEV – MOX - OXA	4
***S. aureus* 02216108**	BEN – CIP – LEV – MOX - OXA	2



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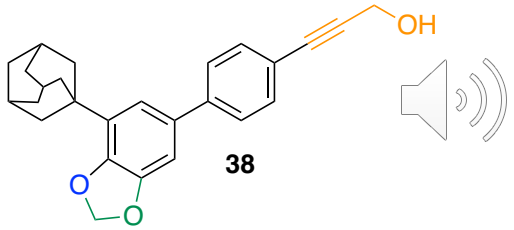

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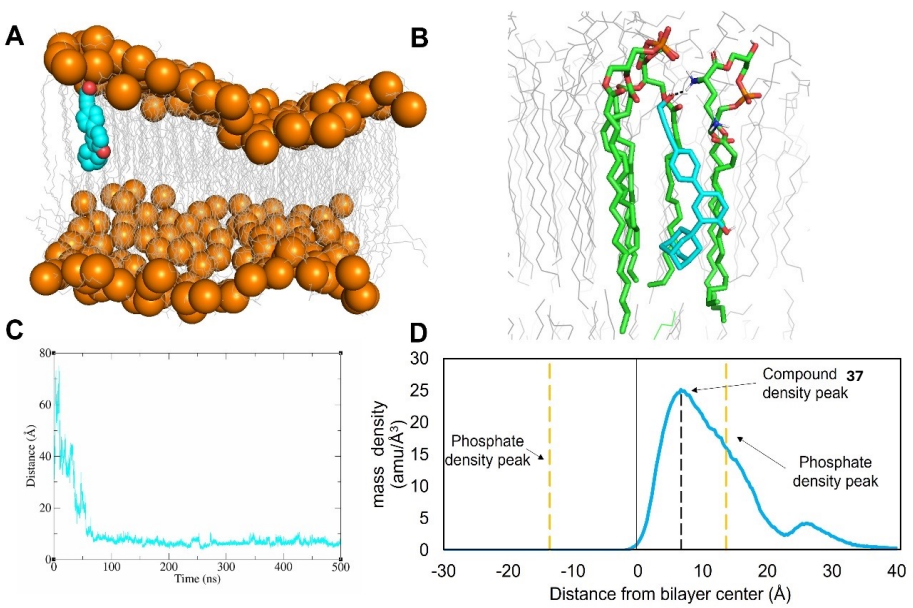
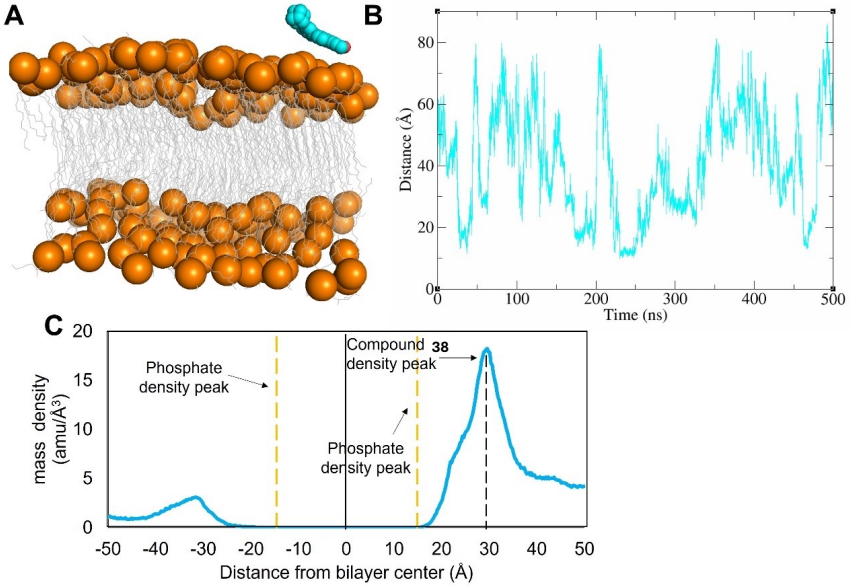
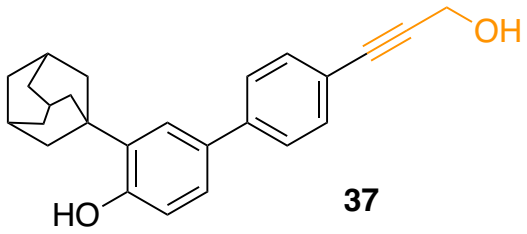
Molecular dynamic studies







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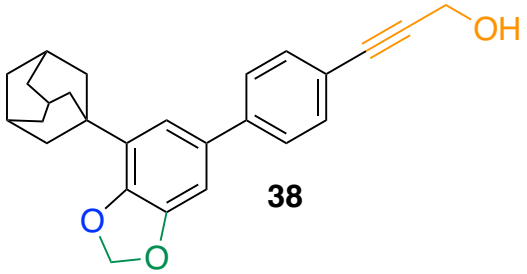

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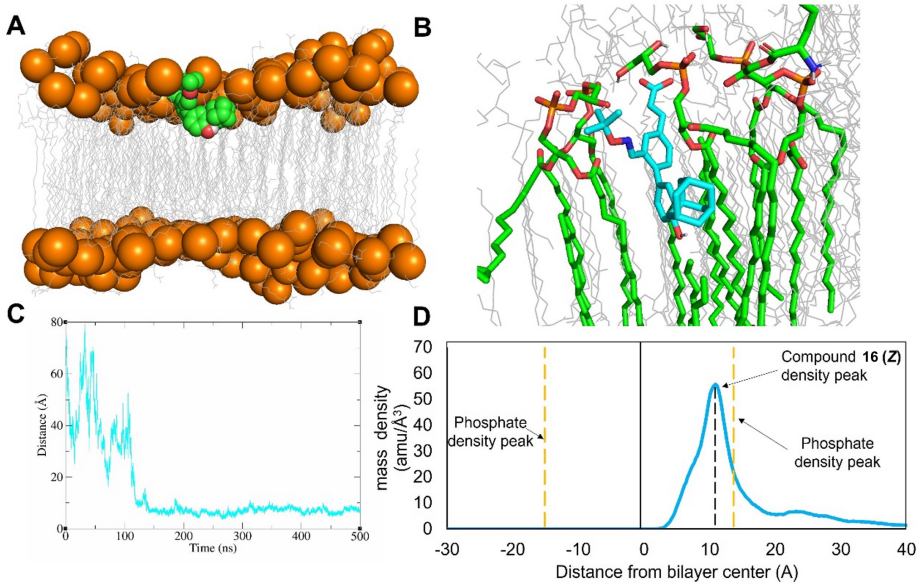
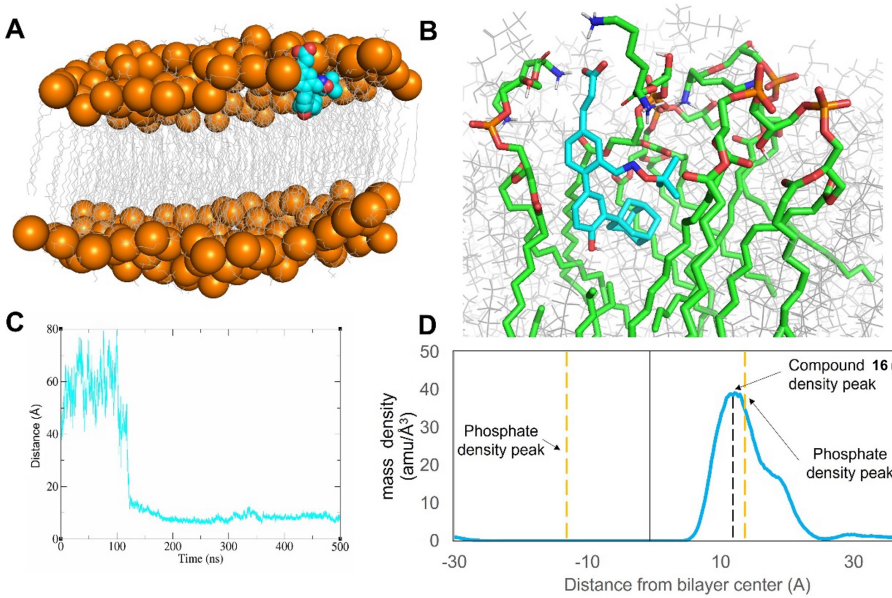
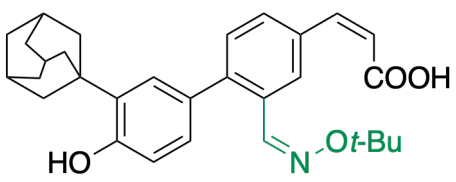
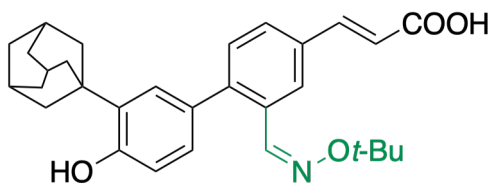
Molecular dynamic studies



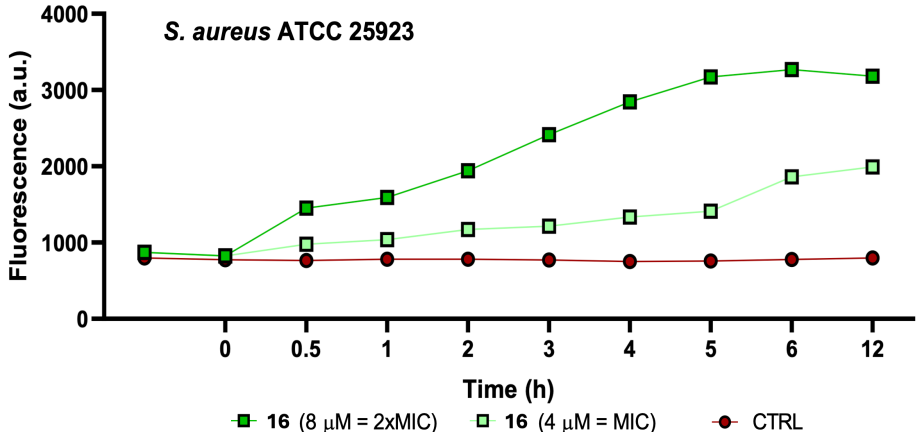




16 (*Z*)



16 (*E*)



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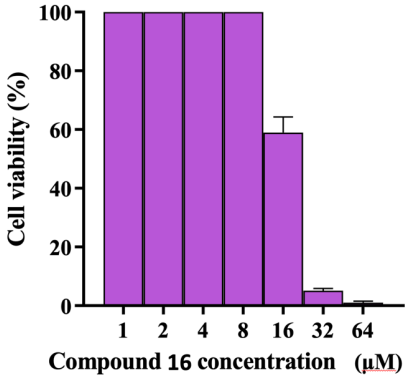
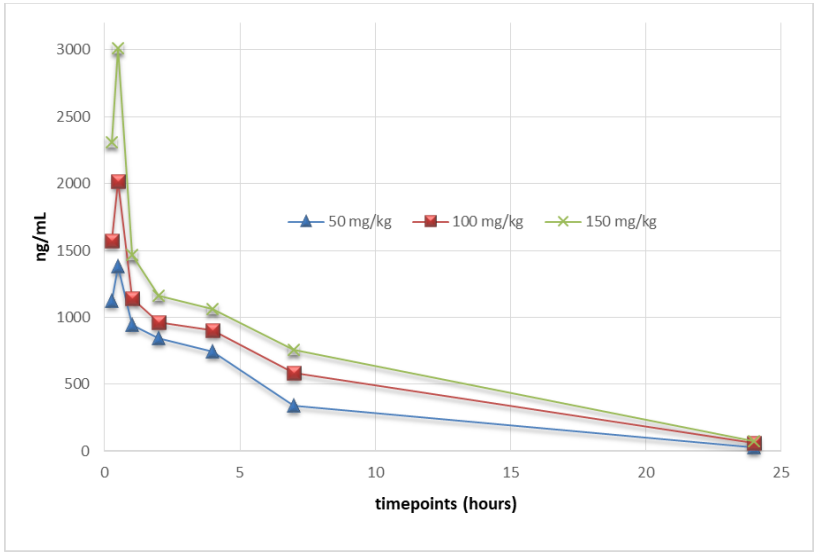
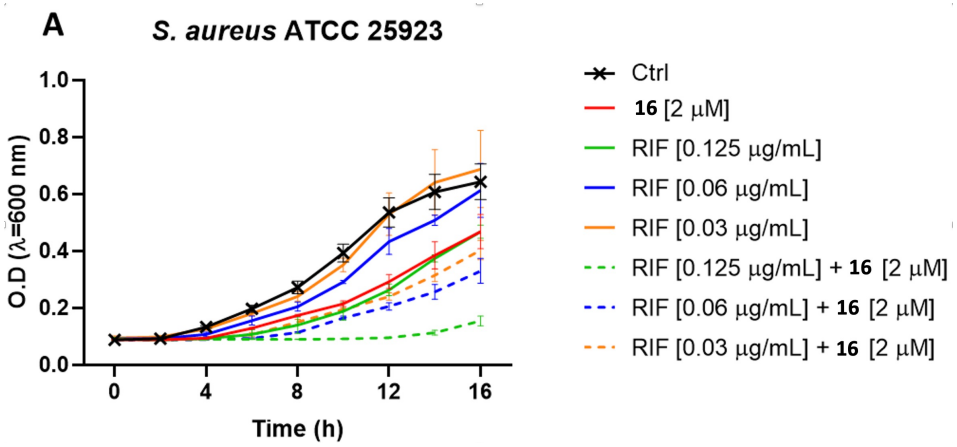
Pharmacokinetic studies and co-administration

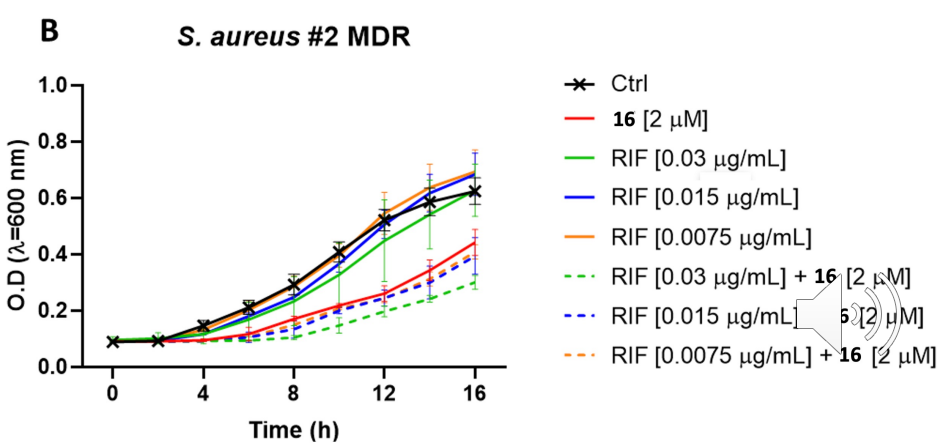
Compound **16**

Cell line	% Survival
HaCaT	109
Fibroblasts	88
AoSMC	99
HUVEC	88









Conclusions and future perspectives

- A collection of variously substituted adarotene derivatives has been prepared

- Preliminary structure-activity relationship studies indicated that:
  - the substitution of phenolic OH and carboxylic acid decreases the antimicrobial activity;
  - the functionalization of the cinnamic portion is tolerated;
  - the introduction of substituents on ring B considerably enhances the activity.

- Oxime **16** resulted as the most promising compound on several MDR strains of Gram + bacteria

- Suitable substituents are under study for the obtainment of compounds active on MDR Gram – bacteria

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