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Steroid derivatives: a promising class of bacterial efflux pump inhibitors?

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UNIVERSITAS SCIENTIARUM SZEGEDIENSIS · SZEGEDI TUDOMÁNYEGYETEM

Steroid derivatives: a promising class of bacterial efflux pump inhibitors?

Natural Products

Natural Product derivatives

Synthetic compounds

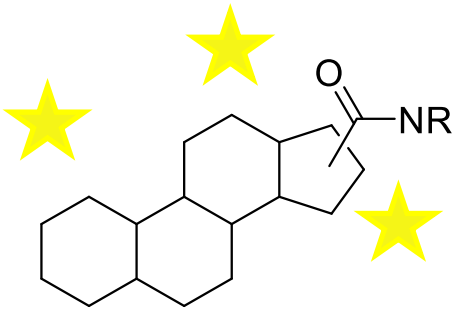
Screening

Antibacterial activity

Antifungal activity

Bacterial efflux pump inhibition

Synergy with antimicrobials



Abstract:

The quest for compounds capable of **circumventing antimicrobial resistance** is important and urgent. Current research has been focusing not only in the search for new antibiotics, but also for **"helper" compounds**, capable of **blocking resistance mechanisms** and, therefore, regaining the activity of currently used antibacterial drugs. In this scope, **bacterial efflux pump inhibitors** arise as interesting compounds, as they can block this resistance mechanism and lead to increased efficacy of antibiotics.

Our group has been studying the **potential of steroid derivatives**. One amide derivative was found to display promising activity in antibacterial and **in synergy assays**, as well as in the **efflux pump inhibition assays.**

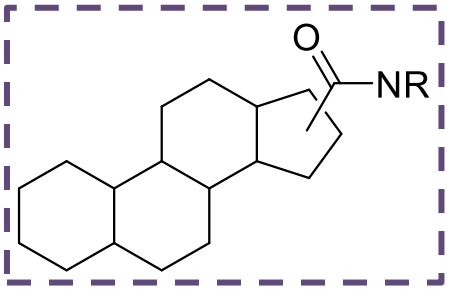
A screening for antimicrobial activity has been performed in Gram-positive and Gram-negative bacteria. Then, they were tested for their capability to modulate pump-mediated efflux. **The derivatives tested were able to increase the accumulation of ethidium bromide, which translates into efflux pump inhibition.**

Keywords: amides, antimicrobial, **bacterial efflux pumps**, **steroid derivatives**

Introduction

A screening of an in-house library of structurally diverse compounds was performed

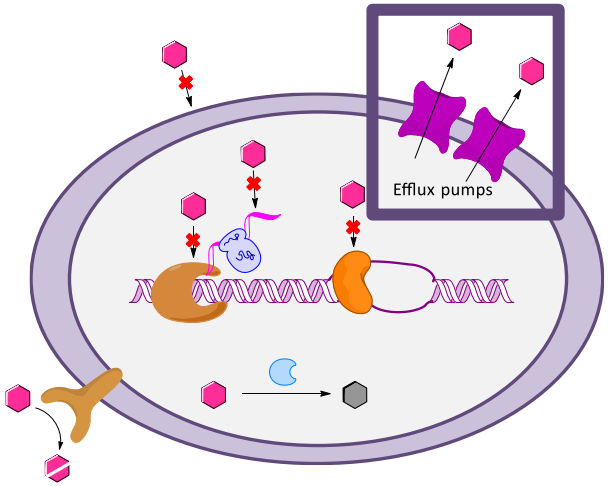
- Antimicrobial activity
- Antifungal activity
- Synergy with antimicrobial drugs



Four steroid derivatives emerged as promising

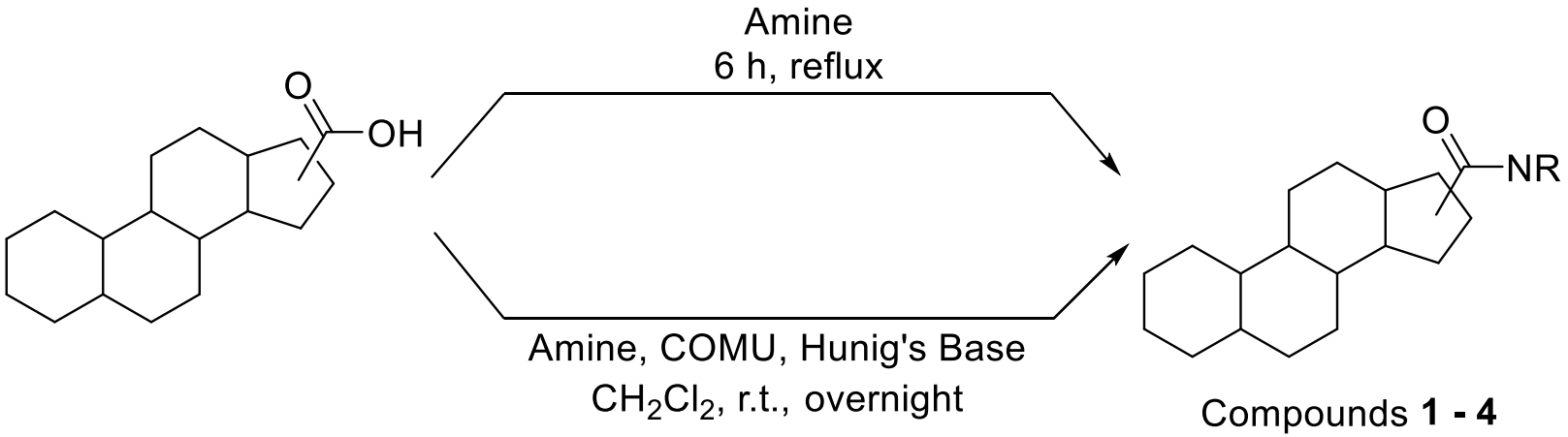
Compounds 1-4

Can they also influence resistance mechanisms?



Chemistry

The amide steroid derivatives were obtained through a coupling reaction with the desired amine



Antibacterial activity

Compounds 1-4 were tested against ATCC susceptible strains (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853 and *Enterococcus faecalis* ATCC 29212)

	E. coli ATCC 25922		*S. aureus* ATCC 29213		*P. aeruginosa* ATCC 27853		*E. faecalis* ATCC 29212	
	MIC [1]	MBC [2]	MIC	MBC	MIC	MBC	MIC	MBC
1	73-147	147	37	37	147	147	37	73
2	>139	ND [3]	>139	ND	>139	ND	>139	ND
3	>139	ND	>139	ND	>139	ND	>139	ND
4	>139	ND	>139	ND	>139	ND	70	ND

[1] Minimum Inhibitory Concentration (μM)
[2] Minimum Bactericidal Concentration (μM)
[3] Not determined

Antibacterial activity

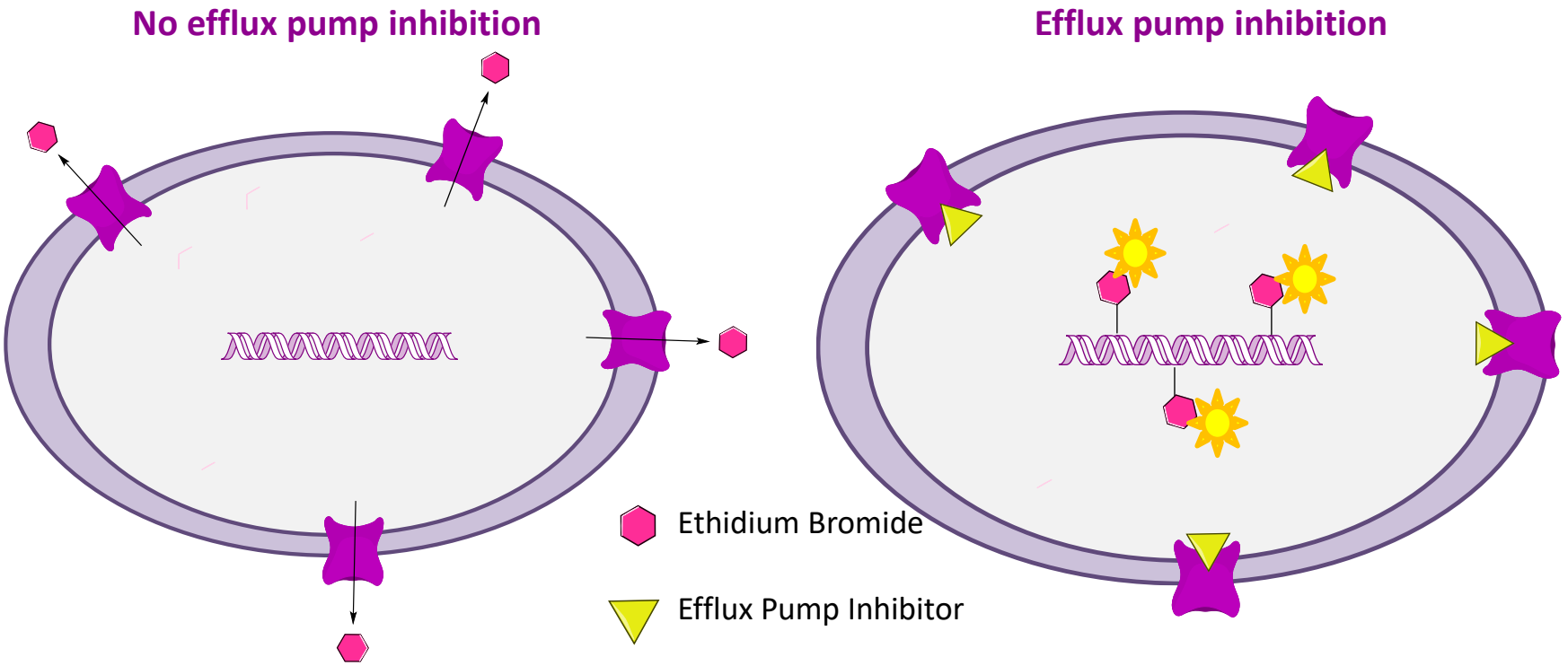
Compounds 1-4 were tested against resistant (*S. aureus* MRSA 272123) and a mutant strain with the *acrA* gene deleted (*Salmonella enterica* serovar Typhimurium SL1344), in order to access their capability of inhibiting bacterial efflux pumps

	S. aureus MRSA 272123	*S.* Typhimurium SL1344
	MIC [1]	MIC
1	12.5	6.25
2	>100	>100
3	>100	>100
4	>100	>100

[1] Minimum inhibitory concentration (μM)

Bacterial Efflux Pump Inhibition

Compounds 1-4 were assayed for their capability of inhibiting bacterial efflux pumps in *S. aureus* MRSA 272123 and *S. enterica* Typhimurium SL1344 through the accumulation of ethidium bromide, an efflux pump substrate, capable of increasing fluorescence



Bacterial Efflux Pump Inhibition

All the derivatives showed a higher relative fluorescence index (RFI) than reserpine (positive control) after 60 minutes of incubation for *S.* Typhimurium, and three derivatives led to a higher RFI than reserpine in *S. aureus*

$$RFI = \frac{RF_{treated} - RF_{untreated}}{RF_{untreated}}$$

	S. aureus **MRSA 272123**	***S.* Typhimurium** **SL1344**
Compound	RFI	
1	0.14	1.07
2	2.33	2.27
3	1.77	1.08
4	0.80	5.15
Reserpine	0.70	0.51

Antifungal activity

Compound 1 was also tested for its antifungal activity in strains susceptible (*Candida albicans* ATCC 10231, *Aspergillus fumigatus* ATCC 46645 and *Tricophyton rubrum* FF5) and resistant to azoles (*Candida krusei* ATCC 6258, *C. albicans* D5 and *A. fumigatus* C111)

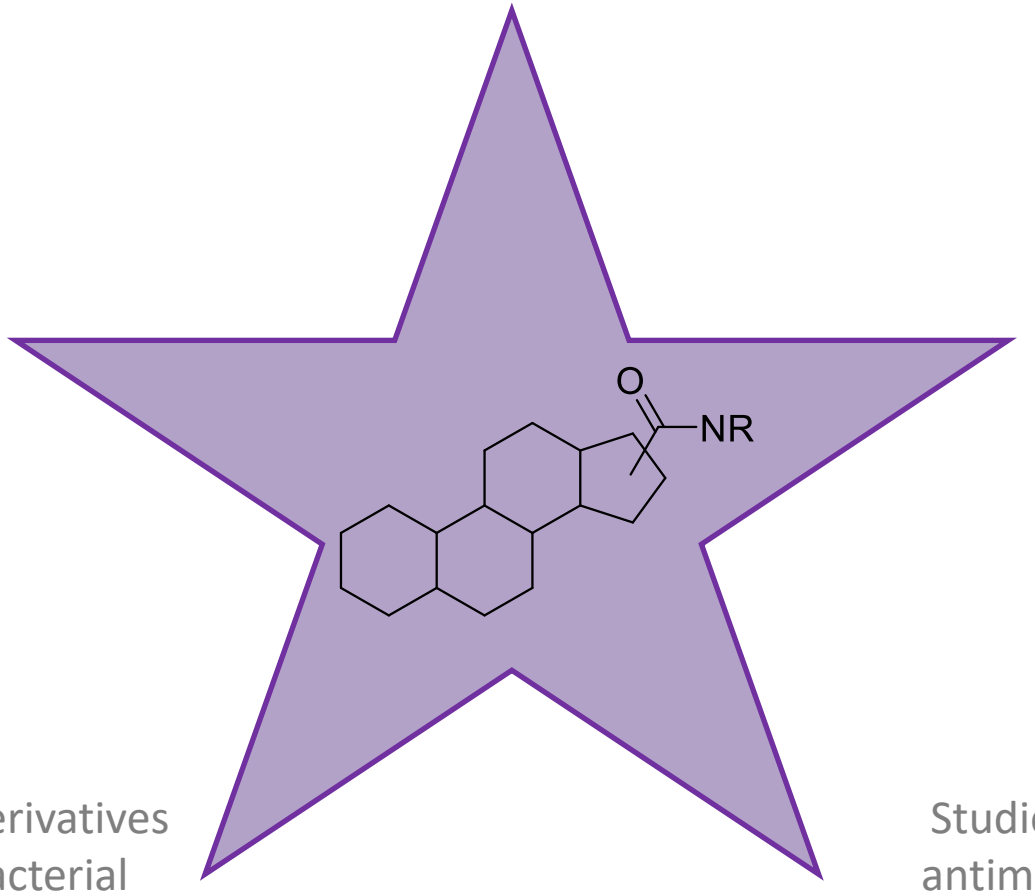
	C. albicans ATCC 10231		*C. albicans* D5		*C. krusei* ATCC 6258		*A. fumigatus* ATCC 46645		*A. fumigatus* C111		*T. rubrum* FF5	
	MIC	MFC [1]	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
1	147	147	147	147	147	147	294	>294	294	>294	147	147

[1] Minimum Fungicidal Concentration (µM)

Compound 1 was also able to inhibit the filamentation of *C. albicans*:
147 µM: 100%; 74 µM: 96.72%; 37 µM: 41.22%; 18 µM: 0%

Conclusions

A steroid derivative emerged as a hit compound against bacteria and fungi



Three amide derivatives were synthesized

Future work: Synthesis of derivatives for SAR studies

The four derivatives inhibit bacterial efflux pumps

Studies on other antimicrobial and resistance mechanisms

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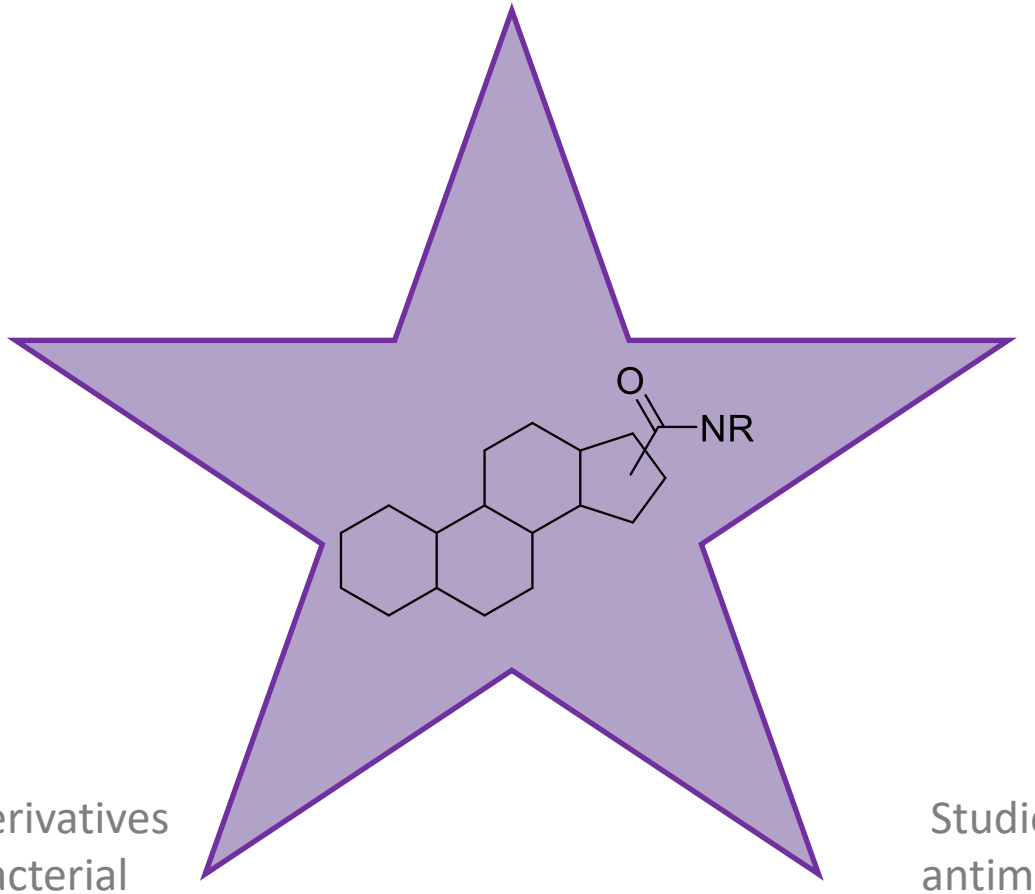

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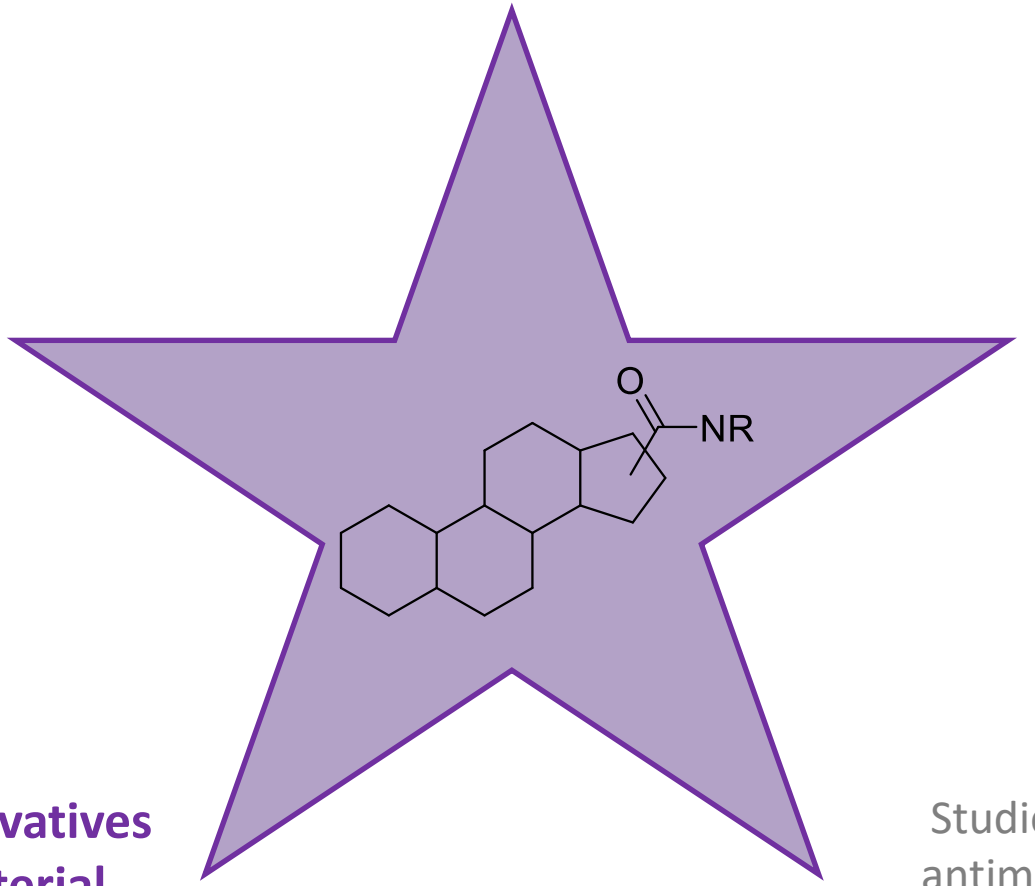

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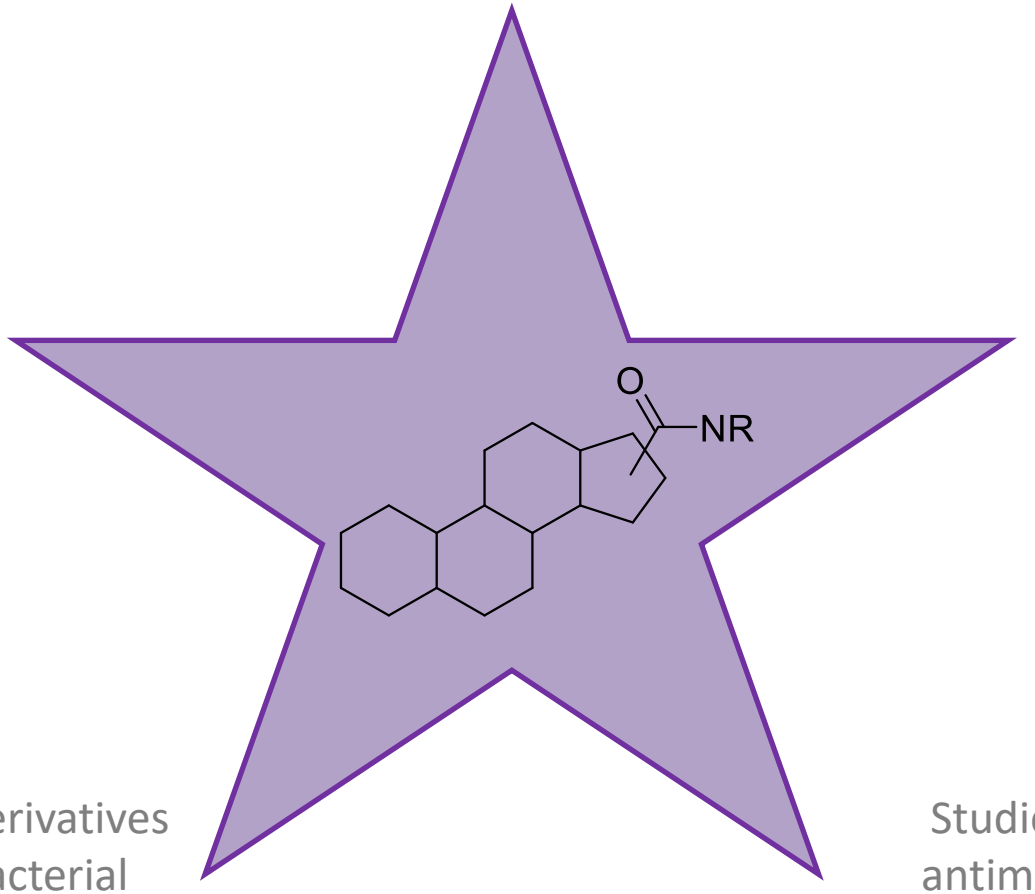

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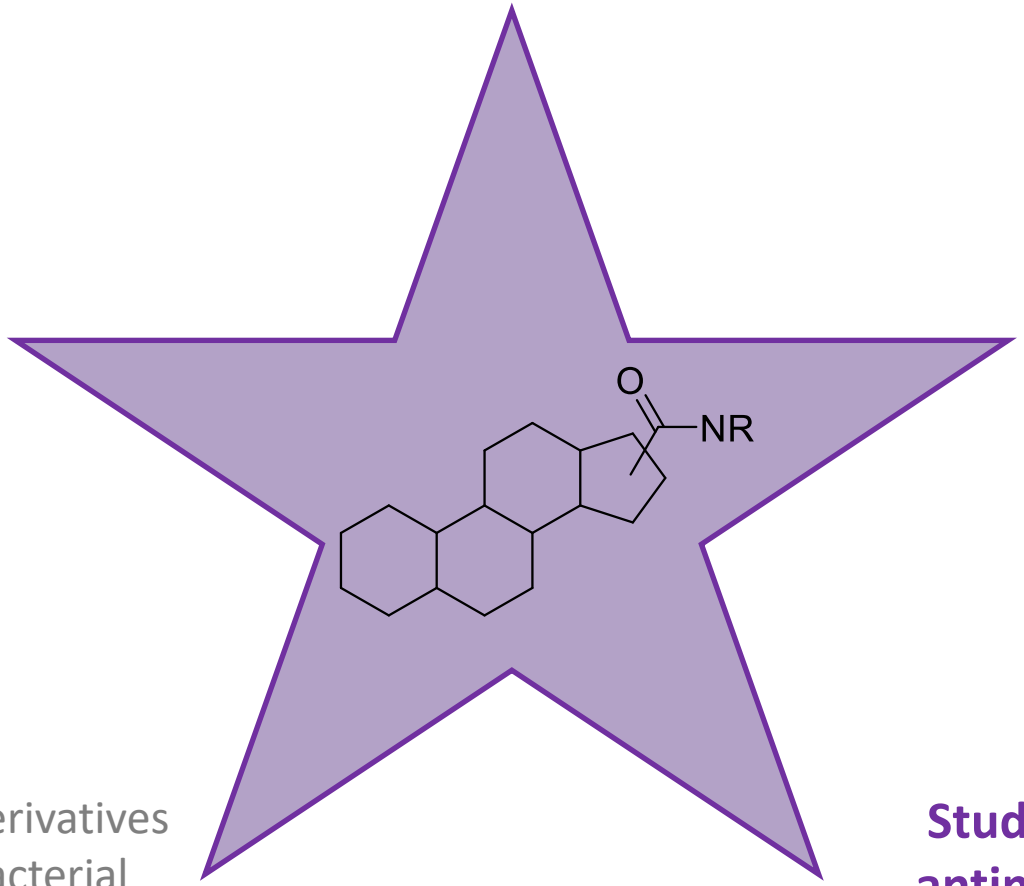

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Acknowledgments

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