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Steroids as Antibacterials

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ABSTRACT

The existence of steroid antibacterials has been studied since the golden age of antibiotics, although the literature on this topic is fragmented. To address this the current review seeks to bring these fragmented lines of enquiry together to assess the utility of steroidal compounds as antibacterials. In doing so this review will document the structural features, biosynthetic/synthetic origins, antibacterial toxicities, potential for fungal biotransformations, and catabolic pathways in the human host. Herein, we identify steroid antibacterials from fungal origins, synthetic steroids, and approved drugs that have the potential for future repurposing as antibacterials. Of particular note are the fusidane steroids of fungal origin, displaying low MIC values and being effective against a variety of Gram-positive and Gram-negative species. At present, fusidic acid is the only steroid that is prescribed as an antibacterial, despite many publications describing antibacterial activity in a range of well-known steroids. The impact of bacterial exposure to steroids naturally differs depending on the nature of the cell membranes, cell wall, and the ability of bacterial to import steroids for use as a carbon source, which is discussed herein. Also, this study explores the biotechnological potential of fungi for the production of antibacterial steroids, and how steroid drugs are metabolised in the human host. Together, this provides an assessment of the potential for steroids as future antibacterial drugs.

KEYWORDS

Steroid

Antibacterial

Cholesterol

Catabolism

Sapogenin

Fusidane

Repurposing

ABREVIATIONS

7-ACA: 7-amino cephalosporanic acid

AD: androst-4-ene-3,17-dione

ADR: adverse drug reactions

5 α -R: 5 α -reductase

CPC: cephalosporin C

CFU: colony forming units

3,4-DHSA: 3,4-dihydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione

DHT: dihydrotestosterone

DHPA: dihydroxyprogesterone acetophenide

4,9-DSHA: 4,5,9,10-diseco-3-hydroxy-5,9,17-trioxoandrosta-1(10), 2-diene-4-oic acid

FDA: federal drug administration

25-HC: 25-hydroxycholesterol

25-HCH: 25-hydroxycholesterol hydroxylase

HIP: 3 α -H-4 α (3'-propanoate)-7 α -methylhexahydro-1,5-indanedione

HRT: hormone replacement therapy

17 β HSD: 17 β -hydroxysteroid dehydrogenases

ICS: inhaled corticosteroids

IVDR: individual response in drug response

MDR: multi-drug resistant

MHRA: Medicines & Healthcare products Regulatory Agency

MIC: minimal inhibitory concentration

9OH-ADD - 9-hydroxy-1,4-androstadiene-3,17-dione

PDR: pan-drug resistant

TDR: totally drug resistant

UGT1A1: UDP glucuronosyltransferase 1

XDR: extensive drug resistant

CONTENTS

1. OVERVIEW

2. STEROIDS WITH KNOWN ANTIBACTERIAL ACTIVITY

- 2.1 Steroids as antibacterials
 - 2.1.1 Fungal antibacterials
 - 2.1.2 Hecogenin acetate
 - 2.1.3 Squalamine and Ceragenin CSA-13
 - 2.1.4 FDA-approved steroids
- 2.2 Antibacterial steroids and antifungals
- 2.3 Antibacterial steroids as antivirals

3. BACTERIAL METABOLISM OF STEROIDS

- 3.1 Environmental exposure of bacteria to steroids
- 3.2 Bacterial steroid biotransformations in industry
- 3.3 Bacterial steroid uptake
- 3.4 Bacterial steroid catabolism

4. FUNGAL BIOTRANSFORMATIONS – THEIR POSITIVE AND NEGATIVE ROLES

- 4.1 *De novo* formation of steroidal compounds
- 4.2 ‘Selection’ and selectivity of fungal biotransformations
- 4.3 The effect of fungal transformations of steroidal compounds
- 4.4 Metagenomic insight into steroid-induced mycobiome bioses
- 4.5 Production of steroids using fungal biocatalysts

5. METABOLIC FATES OF STEROID DRUGS

- 5.1 Primary modes of action for steroids with antimicrobial activity
- 5.2 Human metabolism of quineestrol
- 5.3 Human metabolism of fusidic acid
- 5.4 Breakdown of hecogenin acetate and squalamine in the body

6. FUTURE PERSPECTIVES AND CONCLUSIONS

- 6.1 Fungal antibacterials
- 6.2 Steroid detergents
- 6.3 Hecogenin acetate
- 6.4 FDA-approved steroid drugs
- 6.5 Fungi as a chassis for antibacterial steroid production
- 6.6 Conclusions

REFERENCES

1. OVERVIEW

The rise in antibacterial chemotherapeutic resistance is challenging current public health infrastructure, and this review seeks to explore the potential for steroid compounds as future antibacterials. The potential for microbes to develop resistance to antibiotics has been known about since the golden age of antibiotic discovery (Abraham and Chain, 1940), although many mechanisms have since emerged that confer resistance to many antibiotics across a wide range of bacteria (Nadeem *et al.*, 2020). Even more alarmingly many of these bacteria have acquired resistance to several classes of antibiotics (officially classed as multi-drug resistant [MDR]), or even to all but two categories of antibiotics (officially classed as pan- or totally resistant organisms or PDR/TDR). The ESKAPE pathogens - *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp., display a high degree of antibiotic resistance and continue to cause a significant global burden on human health (Miller and Arias, 2024). In addition, strains of *Mycobacterium tuberculosis*, (*M. tuberculosis*) have evolved extensive drug resistant (XDR) designation (Magiorakos *et al.*, 2012), are resistant to most of the antibiotics used to treat Tuberculosis (da Silva and Palomino, 2011). Recent estimations put the death toll as 5 million annual deaths caused by resistant bacterial infections (Murray *et al.*, 2022), highlighting the urgent need for alternative antibacterials.

Steroid drugs are commonly used as anti-inflammatories, or contraceptives but their utility as antibacterials has largely been overlooked, although some steroids have historically been used to treat bacterial infections and others have been shown to have antiviral properties (Lembo *et al.*, 2016; Marrugal-Lorenzo *et al.*, 2018; Dang *et al.*, 2024). During the period from 2000-2024 there were over 60 steroid compounds approved by the FDA for various medical applications, but not a single one was approved as an antibacterial. Many of these compounds were either contraceptives, anti-inflammatory, or for treatment of dermatological conditions (Singh and Bansal, 2025), and this review touches on whether some of these drugs can be repurposed as antibacterials.

The development of steroid antibacterials requires an understanding of the range of existing antibacterial steroids, the ability of microorganisms to metabolise steroids, with a view to chemical transformations of biotechnological value (i.e. for the production of new steroid drugs), as well as considering drug transit across pathogen membranes and potential intrinsic resistance to steroids via

microbial catabolic pathways. Hence, the capabilities of bacterial and fungal isolates to catalyse steroid transformations is analysed herein.

The final step in antibacterial drug discovery is administration to the patient, so it is of interest to understand what is known about how human metabolic pathways process steroids. These processes are discussed in the context of how these processes might impact upon the efficacy of future steroid drugs, and how issues with steroid drug delivery might be addressed in future.

2. STEROIDS WITH KNOWN ANTIBACTERIAL ACTIVITY

A review of the literature revealed 14 steroidal compounds that have been shown to exhibit antibacterial activity (Table 1 and Fig. 1). This section will introduce the antibacterial, antifungal and antiviral properties of these steroids.

2.1 Steroids as antibacterials

While some of the compounds in Table 1 are toxic to both bacteria and fungi, this section will cover the antibacterial activities. We will introduce the antibacterial activities of each compound in Table 1 and discuss in detail four of the compounds, each from a different category: Fungal antibacterials, steroidal sapogenins, steroidal detergents, and FDA-approved drugs.

Table 1. Steroids that have displayed antibacterial activity against microbial pathogens. Categories map to those in Fig. 1.

Steroid	Category	Reference	CAS Number-Pub Chem ID
Penijanthoids A & B	Fungal Antibacterial	(Guo <i>et al.</i> , 2019).	N/A - 156582124 & N/A - 156582125
Isocyclocitrinol A	Fungal Antibacterial	(Amagata <i>et al.</i> , 2003)	N/A – N/A
22-O-acetylisocyclocitrinol A	Fungal Antibacterial	(Amagata <i>et al.</i> , 2003)	N/A - 139587162
Fusidic acid	Fungal Antibacterial: Fusidane	(Godtfredsen <i>et al.</i> , 1962)	6990-06-3 - 3000226
Cephalosporin P ₁	Fungal Antibacterial: Fusidane	(Ritchie <i>et al.</i> , 1951)	13258-72-5 - 3037114
Helvolic acid	Fungal Antibacterial: Fusidane	(Waksman, Horning and Spencer, 1942)	29400-42-8 - 3002143
Squalamine	Steroid Detergent	(Moore <i>et al.</i> , 1993)	148717-90-2 - 72495
Ceragenin CSA-13	Steroid Detergent	(Li <i>et al.</i> , 1999)	N/A - 16678119
Hecogenin acetate	Steroid Sapogenin	(Araújo <i>et al.</i> , 2023)	915-35-5 - 101906
Quinestrol	FDA-approved	(Younis, AbdelKhalek, Abdelrahman S. Mayhoub, <i>et al.</i> , 2017)	152-43-2 - 9046
Prednicarbate	FDA-approved	(Younis, AbdelKhalek, Abdelrahman S. Mayhoub, <i>et al.</i> , 2017)	73771-04-7 - 6714002
Progesterone	FDA-approved	(Yotis and Stanke, 1966)	57-83-0 - 5994
Hydroxyprogesterone	FDA-approved	(Younis, AbdelKhalek, Abdelrahman S. Mayhoub, <i>et al.</i> , 2017)	68-96-2 - 6238
Algestone acetophenide	FDA-approved	(Younis, AbdelKhalek, Abdelrahman S. Mayhoub, <i>et al.</i> , 2017)	24356-94-3 - 5284538

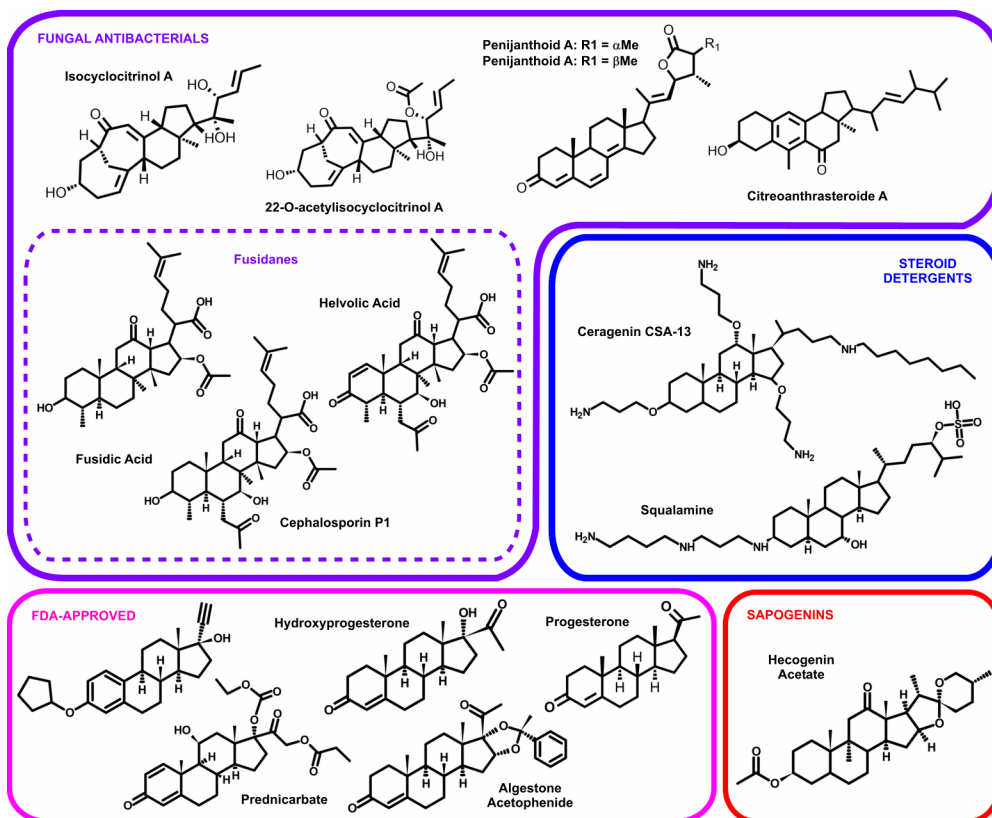


Fig. 1. Structures of the antibacterial steroidal compounds described in this work. The structures have been organised according to their categories in Table 1.

2.1.1 Fungal antibacterials

It is well known that fungi produce a large number of steroids, with *Penicillium* producing a total of 41 alone (Lindsay, Kinghorn and Rakotondraibe, 2023), including the antibacterials penijantheoids A and B, and potential antibacterial citreoanthrasteroid A (Fig. 1). The fungus *Penicillium janthinellum* produces the penijantheoids A and B, which were shown to exhibit only weak activity against three *Vibrio* spp. (MICs, 25.0–50.0 μ M) (Guo *et al.*, 2019). Another study used molecular docking to predict that citreoanthrasteroid A, produced by *Penicillium chrysogenum*, was likely to bind to the LasR protein of *P. aeruginosa*, a key component in quorum sensing for this bacterial pathogen. However, bacteriostatic/bactericidal effects of this isolated compound are yet to be confirmed, hence the omission from Table 1. The steroids isocyclocitrinol A and 22-O-acetylisocyclocitrinol A, produced by the marine fungus *Penicillium citrinum*, were reported to exhibit weak antibacterial activity against *S. epidermidis* (100 μ g/mL) and *Enterococcus durans* (100 μ g/mL) (Amagata *et al.*, 2003).

A subset of the fungal antibacterial category is the fusidane class of antibiotics (dashed box in Fig. 1), where the core structures are closely related to cholesterol. The class has 3 members, fusidic acid, Cephalosporin-P1 and helvolic acid. Firstly, we will focus on fusidic acid, which was first isolated from the yeast *Acremonium fusidioides* by (Godtfredsen *et al.*, 1962). In this study the authors examined the amount of fusidic acid required to inhibit the growth of bacterial strains by 50% (MIC₅₀). The compound was tested against growth of *Staphylococcus aureus* penicillin sensitive (six strains) [0.04-0.1 µg/ml] (0.7-0.19 µM), *Staphylococcus aureus* penicillin insensitive (fifteen strains) [0.05-0.2 µg/ml] (0.10-0.39 µM), *Streptococcus pyogenes* (2 strains) [4-20 µg/ml] (7.74-38.7 µM), *Neisseria gonorrhoeae* (3 strains) [0.4-0.8 µg/ml] (0.77-1.55 µM), *Corynebacterium diphtheriae* (2 strains) [0.01-0.02 µg/ml] (0.019-0.039 µM), *Escherichia coli* (1 strain) [>100 µg/ml] (>194 µM), *Klebsiella pneumoniae* (1 strain) [>100 µg/ml] (>194 µM), and others (Godtfredsen *et al.* (1962). Fusidic acid mainly targets Gram-positive bacteria via the inhibition of elongation factor G (EF-G) detachment from the ribosome during translation, and some Gram-negative bacteria, for example *N. gonorrhoeae*, also seemed to be sensitive in this initial study (Borg *et al.*, 2015). Since the discovery of fusidic acid, multiple bacteria have developed resistance mechanisms as described below (González-López and Selmer, 2025). One such mechanism in *S. aureus* is through the expression of FusB, which binds to the translational machinery and causes a conformational change in the fusidic acid binding site (O'Neill and Chopra, 2006). Further resistance mechanisms can arise when the ribosomal protein L6 acquires mutations that perturb the binding of fusidic acid (Norström, Lannergård and Hughes, 2007), and *S. aureus* can also express efflux transporters to remove fusidic acid from the cytoplasm. One of these transporters is the well-studied AcrAB-TolC tripartite transporter complex of *E. coli* (Tam *et al.*, 2020), which is also present in many Gram-negative bacteria (Oswald, Tam and Pos, 2016). In a study examining the effect of several mutations in the AcrB transporter subunit upon the fusidic acid-dependent growth inhibition of *E. coli* BW25113, the authors noted an MIC value of 128-256 µg/ml (247.7-495.4 µM) for cells expressing the WT transporter, 8-16 µg/ml (15.5-31.0 µM) for D407N, 256 µg/ml (495.4 µM) for I27A, 64-128 (123.9-247.7 µM) for L300A, 128 µg/ml (247.7 µM) for F332A, 64 µg/ml (123.9 µM) for V340A and others. While not all *acrB* mutations appear to elicit resistance to fusidic acid, MIC values for strains carrying several *arcB* mutations do support the hypothesis that fusidic acid is removed from the cytosol by the AcrA-AcrB-TolC efflux pump complex (González-López and Selmer, 2025). While there is emerging resistance to fusidic acid, it is still a useful therapeutic agent in a clinical

setting. However, despite its effectiveness and widespread use for the treatment of infections caused by various pathogenic bacteria, fusidic acid has never been approved by the FDA (Fernandes and Pereira, 2011).

The other two members of the fusidane class, helvolic acid and cephalosporin P1, have both been shown to have antibacterial properties. Helvolic acid was first isolated from *Aspergillus fumigatus* under the name Fumagicin (Waksman, Horning and Spencer, 1942). The next year the same authors published a study in which they reported the ability of helvolic acid (present in the day 6 extract of *A. fumigatus*) to inhibit growth of *Micrococcus luteus* (formerly *Sarcina lutea*) and *Bacillus mycoides* (Waksman, Horning and Spencer, 1943). The authors showed that if you incubated a culture of *M. luteus* in the presence and absence of 0.2 mg/ml (crystalline preparation) of helvolic acid CFUs were 710,000 and 7,000 respectively. For *B. mycoides* the CFU values were 40,000 and 6,700. In the same year, a study by Chain et al. (1943) examined growth inhibition using an unconventional drug dilution method to rank the sensitivity of various species to helvolic acid, which revealed the sensitivities of the following bacterial species in descending order: *C. diphtheriae* > *N. meningitidis* > *B. anthracis* > *S. aureus* > *S. pneumoniae* >>> *Salmonella typhi* = *E. coli*, and = *Pseudomonas aeruginosa*. Some of these results were confirmed when it was shown that helvolic acid is toxic towards *P. aeruginosa* and *S. aureus*, with MIC values of 4.6 and 5.8 µg/mL (8.09 and 10.2 µM), respectively (Yang et al., 2017). Taken together this suggests that helvolic acid seems to have similar activity to fusidic acid. More specifically the compound has generally been active against Gram-positive strains, but not as much against Gram-negative strains.

Cephalosporin P1 was first extracted from the organism *Acremonium chrysogenum* (in the published study *Cephalosporium*) in the 1950s (Burton and Abraham, 1950). A later study, utilising a dilution series of pure cephalosporin P1, investigated the bacteriostatic activity of the pure compound. For simplicity we will present the species (number of strains tested): culture dilution, cephalosporin P1 dilution. The authors found that in the species tested the following conditions lead to growth inhibition over 48 hours: *C. diphtheriae* > *N. gonorrhoeae* > *Mycobacterium smegmatis* > *S. aureus* >>> *S. haemolyticus* = *N. meningitidis* and = *Neisseria pharyngis*. The *in vitro* results suggest that the compound is most effective on Gram-positive bacteria, but the *Mycobacterial* species do not follow this pattern. In the same study a mouse model was used to investigate the impact of serum levels of cephalosporin P1 upon mouse survival during bacterial infection. The study revealed broad spectrum activity against multiple bacterial species, but

resistance by *Staphylococcus pneumoniae* strains. In a *S. aureus* mouse infection model where 90% of the mice died after 24 h, the administration of 5 mg/ml cephalosporin P1 resulted in survival of 50% of the mice for up to four days (Ritchie, Smith and Florey, 1951). A later study demonstrated an MIC₉₀ values of 0.5 µg/ml and 4 µg/ml (0.87 and 6.96 µM) against a strain of methicillin sensitive *S. aureus* and a strain of methicillin resistant *S. aureus* respectively (O'Neill *et al.*, 2002). In the same study the researchers found that deletion of the genes encoding AcrAB lead to increased sensitivity of *E. coli* to cephalosporin P1, a decrease of approximately 10-fold in MIC value.

2.1.2 Hecogenin acetate

The steroidal sapogenin hecogenin acetate is extracted from the leaves of *Agave sisalana* and in the *Agave* genus more generally. Initial investigation into the antibacterial activity of hecogenin acetate revealed that this sapogenin steroid displayed very high MIC values of 645 µg/ml and 512 µg/ml (1364.5 and 1083.1 µM) against *S. aureus* and *E. coli* respectively (Araújo *et al.*, 2023), but with no measurable inhibition against 3 other species. However, hecogenin acetate was shown to lower the MIC for gentamicin with a number of species: for *S. aureus* the MIC was 25.4 µg/ml (53.7 µM) and was reduced to 16 µg/ml (33.8 µM) in the presence of hecogenin acetate, and for *P. aeruginosa* the MIC for gentamycin was 40 µg/ml (84.6 µM) and was reduced to 16 µg/ml (33.8 µM) in the presence of hecogenin acetate. A similar observation was seen for *E. coli* where the MIC of norfloxacin alone was 322.5 µg/ml (682.3 µM), but it decreased to 161.3 µg/ml (341.2 µM) in the presence of hecogenic acetate. However, this effect was attenuated when mutants expressing efflux pumps were examined (Araújo *et al.*, 2023). A follow-up study expanded the bacterial species that were investigated for growth inhibition with the known antibiotics gentamicin, norfloxacin, and ampicillin (Araújo *et al.*, 2025). This study reported similar decreases in antibiotic MIC values with *P. aeruginosa* (gentamycin alone; 50.8 µg/ml (107.5 µM), gentamycin and hecogenin acetate; 16 µg/ml (33.8 µM), norfloxacin; 128 µg/ml (270.8 µM), norfloxacin and hecogenin acetate; 64 µg/ml (135.4 µM)). A similar effect was seen in a strain of *Enterococcus faecalis* (gentamycin alone; 101.6 µg/ml (214.9 µM), gentamycin and hecogenin acetate; 64 µg/ml (135.4 µM), norfloxacin alone; 256 µg/ml (541.6 µM), norfloxacin and hecogenin acetate; 128 µg/ml (270.8 µM) (Araújo *et al.* 2025). Hecogenin acetate was also shown to diminish biofilm growth against certain *S. aureus* strains, with hecogenin acetate reducing biofilm formation by up to 47% and also reducing *P. aeruginosa* biofilm growth

by up to 29% (Araújo *et al.*, 2024). Hecogenin acetate has been used for pain relief: in a mouse model it was shown to block an ATP-sensitive K⁺ channel leading to a reduced response to stimuli in the mouse tails (Gama *et al.*, 2013). This suggests that before using hecogenin acetate as a therapeutic careful consideration of off-target analgesic effects would be necessary.

2.1.3 Squalamine and Ceragenin CSA-13

Squalamine is a natural product from the stomach of the dog shark (*Squalus acanthias*). In a study where the substance was isolated and structurally characterized it was studied for antibiotic activity against various bacterial pathogens, including *E. coli* and *S. aureus* (Moore *et al.*, 1993). squalamine displayed MIC values in the range 1-10 µg/ml (1.59-15.9 µM) for all species tested with the exception of *Serratia marcescens*, where the activity was significantly lower (Moore *et al.*, 1993). The mechanism of action of squalamine was investigated in multiple studies. The most direct display of its mechanism was a dye release assay using a set of proteoliposomes, consisting of various lipids including phosphatidylserine, phosphatidylglycerol, cardiolipin, and phosphatidylcholine, containing 8-aminonaphthalene-1,3,6 trisulfonic acid (ANTS). ANTS release was observed from the proteoliposomes in the presence of only 1.8 µM squalamine. Interestingly, squalamine is more effective against anionic lipids, requiring three times less squalamine to elicit release than that required for cationic lipids such as phosphatidylcholine (Selinsky *et al.*, 1998). Another study used a combination of microscopy, growth assays, and ATP-release assays, which revealed that squalamine caused cell lysis in *S. aureus* and compromised membrane structural integrity in *P. aeruginosa* (Alhanout *et al.*, 2010 b). squalamine and its closely-related derivative ASD-2 were subsequently shown to reduce *S. aureus* load on the skin of mice when applied as a topical treatment (Djoughri-Bouktab *et al.*, 2011). In addition, squalamine has been shown to kill persistent/dormant *Acinetobacter baumannii* cells, diminishing cell viability 10-fold more compared to ciprofloxacin (Nicol *et al.*, 2019). Squalamine and a derivative known as Claramine A1 caused *P. aeruginosa* biofilm volumes to decrease by 45% and 60% respectively (Tareau *et al.*, 2025).

The antibacterial ceragenin CSA-13 was synthesised to be a more effective steroid detergent than squalamine and exhibited MICs ranging from 0.4-31 µg/ml (0.59-47.8 µM) against several microbial pathogens, including *E. coli*, *K. pneumoniae*, *E. faecalis*, *S. aureus*, and *C. albicans*. *C. albicans* exhibited the most resistance with an MIC of 31 µg/ml (Li *et al.*, 1999). As the synthesis of this compound was

inspired by the lipid-solubilisation properties of squalamine, it is likely to have a similar mechanism of action.

The therapeutic potential of squalamine is not limited to antibacterial function, as previous work has demonstrated neuroprotective effects in Alzheimer's Disease. Here, squalamine targets A β oligomers and removes them from the cell membrane (Gonçalves, Sodero and Cordeiro, 2024). Squalamine also appeared to prevent the growth of breast cancer tumours, partially by inhibiting angiogenesis (an effect that could be useful beyond cancer treatment) (Márquez-Garbán *et al.*, 2019).

2.1.4 FDA-approved steroids

One route to quickly utilising steroids as antibacterials is through repurposing already approved therapeutics. This process avoids the need for clinical trials of novel compounds and can greatly expedite the therapeutic development process (Parvathaneni *et al.*, 2019).

The bacteriostatic properties of progesterone have been known about for a long time, with 10-20 $\mu\text{g/mL}$ of progesterone being shown to modestly inhibit the growth of a range of Gram-positives but 11 α -hydroxyprogesterone, and 17 α -hydroxyprogesterone did not (Yotis and Stanke, 1966). In addition, Pregnenolone, 4-pregnen-20 β -ol-3-one, and 5 α -pregnane also modestly inhibited staphylococcal species, whereas pregnanolone, pregnandione, 11 α -hydroxyprogesterone, and 17 α -hydroxyprogesterone did not. However, many years later, hydroxyprogesterone was shown to diminish the growth of *Enterococcus faecium* with an MIC of 2 μM and the MIC for *S. aureus* was >16 μM (Younis, AbdelKhalek, Abdelrahman S Mayhoub, *et al.*, 2017). The same study identified four additional steroids with antibacterial activity (prednicarbate, quinestrol, hydroxyprogesterone, and algestone acetophenide) that are shown in Table 1 and Fig. 1. These compounds were screened against the ESKAPE pathogens and all four steroids inhibited growth of either vancomycin-resistant *Enterococcus* or Methicillin-resistant *S. aureus*, although only prednicarbate inhibited the growth of both organisms. (Younis, AbdelKhalek, Abdelrahman S. Mayhoub, *et al.*, 2017). A subsequent *in silico* analysis of the same FDA-approved drugs independently identified a series of FDA-approved steroids as likely inhibitors of cytochrome *bd* (Henry *et al.*, 2024), a bacterial respiratory complex that has attracted much interest as a drug target over the past decade (Borisov *et al.*, 2021; Friedrich, Wohlwend and Borisov, 2022; Saha *et al.*, 2024). *In vitro* evaluation of the docking results confirmed that quinestrol, a prodrug used as a therapeutic in hormone replacement, could inhibit the

cytochrome *bd* respiratory complex in *E. coli* and *S. aureus* membranes, inhibiting the growth of both species, and also eliciting a 10⁵-fold killing of *S. aureus* (LC₅₀ = 3.4 µg/mL) (9.33 µM) (Henry *et al.*, 2024). The ability to utilize such compounds for drug repurposing could lead to antibacterial therapeutics. These compounds would then add to the diversity of the antibacterial resources.

2.2 Antibacterial steroids as antifungals

There are many steroids that possess antifungal activities, although as this is a review on antibacterial steroids, exhaustive coverage of antifungals is beyond the current scope. Hence, coverage of this section focusses only on the antibacterial steroids in Table 1 that also have antifungal properties, followed by a brief commentary on how some well-known antifungals target steroid metabolism in fungi. Squalamine has previously been shown to exhibit a high level of antifungal activity against *Candida* spp., impairing growth with an MIC of 4-8 µg/ml (6.37-12.7 µM) (Moore *et al.*, 1993). This activity was verified in a later study using a growth inhibition screen of fungal clinical isolates from cystic fibrosis patients, including *C. albicans*, *A. fumigatus*, and *Candida krusei* (MIC values in the range 8-16 mg/L (12.7-25.5 µM) (Alhanout *et al.*, 2010 a). Ceragenin CSA-13 was also shown to elicit growth inhibition in *C. albicans* and other species of *Candida* with MIC values of 0.5-4 µg/ml and 0.5-64 µg/ml (0.74-5.91 µM and 0.74-94.5 µM) respectively (Bozkurt-Guzel, Hacıoglu and Savage, 2018). The same study measured the inhibition of biofilm formation by *C. albicans* and the other *Candida* species, with MIC concentrations of CSA-13 inhibiting biofilm growth at 48 h by over 40% compared to controls. While none of the steroidal detergents have been used in the clinic they do seem to be effective in their antifungal activities *in vitro*.

Currently, clinically used antifungal therapies do not include any steroid derivatives, as discussed below this is due to the ability of fungal species to metabolise steroidal compounds. However, the azole and polyene antifungals target the metabolism or function of the steroid ergosterol, an essential component of the fungal cell membrane. The azole antifungals are known to inhibit the conversion of lanosterol to ergosterol, resulting in a build-up of toxic lanosterol (Ghannoum and Rice, 1999), while polyenes such as amphotericin B are believed to remove ergosterol from the outer leaflet of yeast plasma membranes. It is currently thought that polyenes might intercalate at sites in the membrane containing ergosterol, and the polyenes then remove the ergosterol directly which leads to compromised membrane integrity (Ahmady *et al.*, 2024). The clear importance of ergosterol in fungal growth and survival and its structural similarity to

steroid drugs support the potential for steroid based compounds to target the synthesis, degradation or localisation of ergosterol in pathogenic fungi.

2.3 Clinical steroids as antivirals

Early work on steroids as antivirals discovered the ability of fusidic acid to prevent the infection of human erythroleukemia (HEL) cells by rhinovirus and Coxsackie virus (Acornley et al., 1967). The rhinovirus infection was also prevented by cephalosporin P1, although helvolic acid was ineffective. However, in the same study these *in vitro* observations were not mirrored in the human host, as volunteers taking a daily oral dose of 3000 mg of fusidic acid (100 mg/ml was detected in the volunteer's serum) were not protected against infection (measured by viral load in mucus and similar symptom severity). The authors also attempted to prevent viral infection by Coxsackie virus A21 in the lungs through inhalation of fusidic acid. Four volunteers took 50 mg/ml fusidate as a snuff and were infected with 30-fold TCD₅₀ of virus. The volunteers continued to take two doses of 50 mg/ml for four days. However, all volunteers developed colds and the inability of fusidic acid to prevent infections in human volunteers remains poorly understood. The oral administration showed that over 96 % of the fusidic acid was cleared from the human serum. The volunteers who nasally inhaled the fusidic acid were also infected with Coxsackie A21. This may suggest that the antiviral mechanism requires higher levels of fusidic acid *in vivo*. As of publication no studies have been conducted to confirm this or examine a mechanism of viral infection.

Another group of steroids that have been examined for their antiviral activity are the hydroxysterols, which are naturally occurring in host organisms, and best studied example is 25-hydroxycholesterol (25-HC) (Table 1 and Figure 1) (Lembo et al., 2016). Viral plaque assays were performed in which a set of HELA cells were preincubated in the presence or absence of 1 μ M 25-HC (Liu et al., 2013). The study decreases in plaque formation with Nipah virus (over 10-fold plaque forming unit decrease), Ebola virus (isolated in Zaire) (over 2-fold plaque forming unit decrease), and Rift Valley Fever virus (over 10-fold plaque forming unit decrease). To verify the effect of 25-HC *in vivo* the authors were able to generate a knock-out mouse that lacked 25-HCH, the hydroxylase enzyme that generates 25-HC. When examining the load of fluorescent mouse hepatitis virus in the spleen, the authors observed a 3.5-fold decrease in viral load of the mutant mouse compared to wild type mice at 9 days post-infection (Liu et al., 2013). Similar results were obtained in a study examining the infection of hepatoma cells and lung

carcinoma cells with hantavirus. The authors were able to demonstrate that in both of these cell lines when infected with hantavirus the level of 25-HCH was increased approximately 5-fold for each cell line as measured by qRT-PCR. At the same time the study examined the viral load (by GFP microscopy), virus RNA level (qRT-PCR) and the presence of nucleocapsid protein level (Western Blot) in increasing levels of 25-HCH expression. Each of these levels decreased significantly in the presence of 25-HCH in both cell lines. To verify that the effect is due to 25-HC the authors repeated the same measurements in the presence and absence of 25-HC and saw a similar decrease in RNA level, nucleocapsid protein and viral load in both cell lines. This then confirms that the effect on the hantavirus of interest was due to 25-HC levels (Dang *et al.*, 2024). In another study examining the viral infection of lung epithelial cells with SARS-Cov-2 Wang and co-workers observed (using the same techniques as the hantavirus study) the same effect of a change in the 25-HC level lead to a decrease of SARS-Cov-2, MERS-Cov and SARS-Cov viral load and decrease of viral RNA. The decrease in SARS-Cov-2 RNA level was also decreased in the presence of 25-HC in lung organoids (Wang *et al.*, 2020). There appears to be a well characterised relationship between 25-HC and viral infection, and a comprehensive review of this is available (Lembo *et al.*, 2016). In addition, a recent review on the physiological roles of 25-HC was written by (Mao *et al.*, 2022).

Mifepristone (Table 1 and Figure 1) is an FDA-approved steroid that is used as a hormonal therapeutic to induce abortion (Swica *et al.*, 2013). A recent study reported that mifepristone could lower the viral load in mouse adenovirus infection using a bioluminescence assay and quantitation of viral housekeeping genes (Marrugal-Lorenzo *et al.*, 2018). The authors also demonstrated using a plaque forming assay that 30 μ M mifepristone reduced infection by more than 50 %, and qPCR revealed that mifepristone induced a reduction in viral DNA replication. These results suggest that mifepristone may provide therapeutic opportunities against adenoviruses but if this is a generic or specific adenovirus response needs further studies. Again, this is a demonstration that drug repositioning may be a promising step in generating more antibacterial reagents.

Finally, a computational docking study identified five steroid compounds as potential inhibitors of the NSP-15 endoribonuclease from SARS-CoV2 (Dhanabalan *et al.*, 2023). Two of the compounds, dexamethasone and cortisone, are FDA-approved. The other compounds of interest were part of the ChemDiv Library (0449-0045, N001-0004, and N006-0008). These compounds were then further explored for binding using MD simulations with Gromacs v 5.1. These analyses predicted that the compounds of

interest would bind tightly in the active site of NSP-15, although no experimental confirmation was performed in this or other studies.

3. BACTERIAL METABOLISM OF STEROIDS

3.1 Environmental exposure of bacteria to steroids

Steroids are abundant in nature and accumulate in the environment as a result of human activity. This can be problematic as these compounds are often resistant to microbial degradation, largely due to the lack of functional groups and their low solubility in water. These ‘steroid pollutants’ include cholesterol from animals, ergosterol from yeast, and plant-derived phytosterols.

Of particular interest is the large amounts of cholesterol and its derivatives that can accumulate in the environment. Cholesterol is a precursor to Vitamin D₃, bile acids and steroid hormones (Fig. 2), the last two being excreted via human waste and industrial effluents. There has been increased interest in microbial cholesterol degradation, largely due to the role of steroid uptake and utilisation in persistent infections caused by *Mycobacterium tuberculosis* (Abuhammad, 2017). Indeed, strategies to disrupt these important catabolic processes have attracted much attention as potential antibacterial therapies.

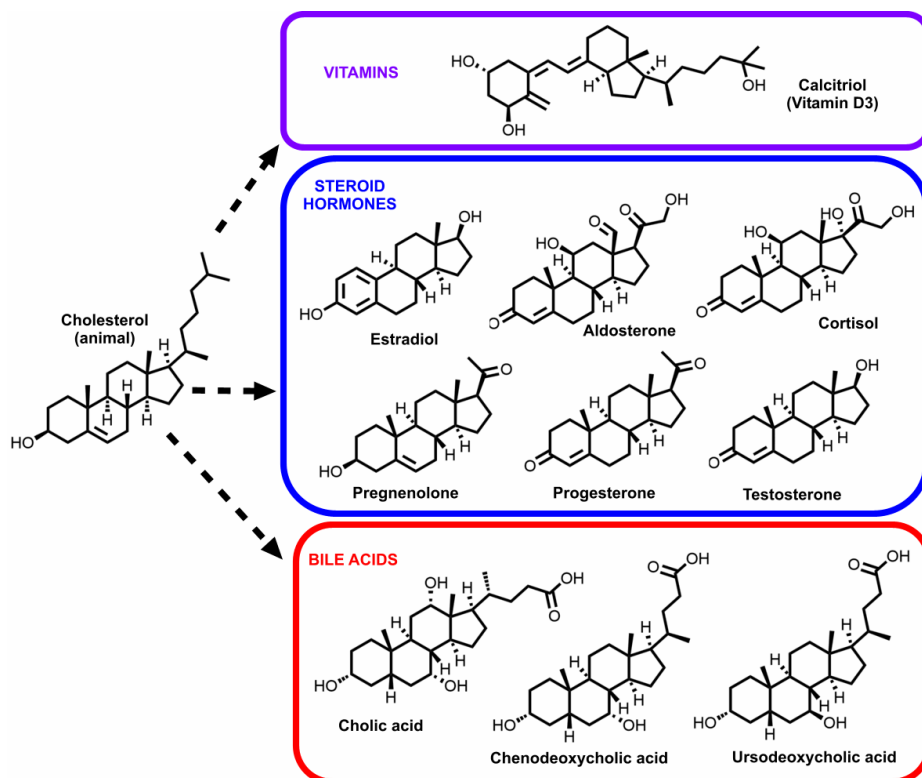


Fig. 2. Products of cholesterol metabolism in animals.

3.2 Bacterial steroid biotransformations in industry

Bacterial steroid catabolism has also garnered much attention for biotechnological applications, with enzyme-catalysed enantiomer-specific conversions offering greater structural specificities compared to chemical synthesis. This approach has previously been used for the industrial production of steroidal drugs using phytosterols as low-cost substrates. The potential for microorganisms to produce 17β -hydroxysteroids (e.g. testosterone and dihydrotestosterone (DHT)) via the action of 17β -hydroxysteroid dehydrogenases has been known about for many decades (Donova, Egorova and Nikolayeva, 2005), and there are a number of bacterial species that can naturally generate the precursor androst-4-ene-3,17-dione (AD) from low cost phytosteroid substrates. Recent work has reported the conversion of phytosterols to AD in *Mycolicibacterium neoaurum*, and then on to testosterone and DHT via the expression of recombinant enzymes from *Saccharomyces cerevisiae* and the spirochete *Treponema denticola* (Su *et al.*, 2025) (Fig. 3). Hence, a large literature has accumulated on steroid biotransformations to generate engineered strains that generate high-value products, which has advanced our understanding of steroid metabolism pathways in bacteria (Galán *et al.*, 2017).

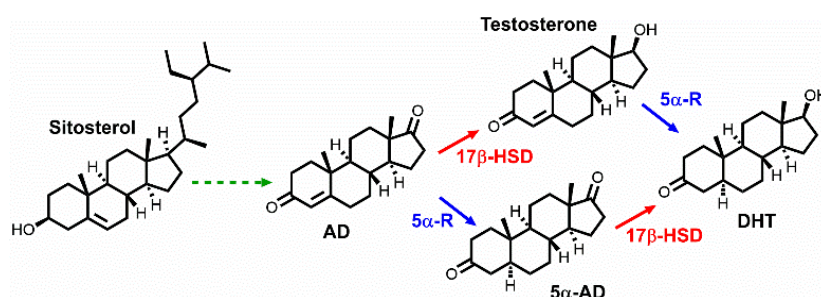


Fig. 3. Bacterial conversion of the phytosterol Sitosterol to 17β -hydroxysteroids. Bacterial species such as *Mycolicibacterium neoaurum* can naturally convert phytosterols such as sitosterol to androst-4-ene-3,17-dione (AD), and expression of recombinant enzymes 17β -hydroxysteroid dehydrogenases (17β HSD) and 5α -reductase (5α -R) generates testosterone and dihydrotestosterone (DHT).

3.3 Bacterial steroid uptake

The best characterised system for steroid import into bacteria is the ABC-like cholesterol import system of actinobacteria that is encoded by the *mce4* locus of *Rhodococcus jostii* RHA1, *M. tuberculosis* and *M. smegmatis* (Fig. 3). This process is clearly of great importance during *M. tuberculosis* pathogenesis,

where invading bacteria have easy access to cholesterol from the foamy macrophages within the granuloma of the TB lung, could potentially provide a route for the transport of steroid drugs into bacteria. The main transmembrane subunits of the Mce4 transporter are encoded by two *yrbE* genes, and the functions of the six ORFs that are downstream (*mce4A-F*) are thought to encode proteins involved in substrate transit through the membrane (Fig. 4A). The *mam4A-B* genes at the end of the operon are ‘Mce-associated membrane proteins’ along with a range of other orphaned ‘*omam*’ genes (5 in *M. tuberculosis*) that are important for cholesterol import but their precise roles are currently not known (Laura, E and Miriam, 2021). Other genes encoded elsewhere on the mycobacterial genome have also been shown to be required for cholesterol uptake in *M. tuberculosis*, including *mceG* that encodes an ATPase (Pandey and Sassetti, 2008; Fieweger *et al.*, 2023) and *lucA* that encodes an integral membrane protein (Nazarova *et al.*, 2017). Deletion of the *mce4* locus in *R. jostii* RHA1 was shown to impair cholesterol uptake (Van der Geize *et al.*, 2007) and this transporter was later shown to bind directly to cholesterol (Mohn *et al.*, 2008). Deletions of the *mce4* locus or the ATPase-encoding *mceG* gene of *M. tuberculosis* were subsequently shown to specifically abolish the use of cholesterol as a carbon source and impair growth in activated macrophages (Pandey and Sassetti, 2008). There is currently no published structure of the Mce4 transporter, although the CryoEM structure of the closely-related Mce1 lipid transporter from *M. smegmatis*, which shares the LucB and MceG subunits with Mce4, has been recently published (Chen *et al.*, 2023). The Mce1 structure was used as a model to infer the functional roles of the homologous Mce4 transporter subunits in cholesterol transport (Fig. 4B).

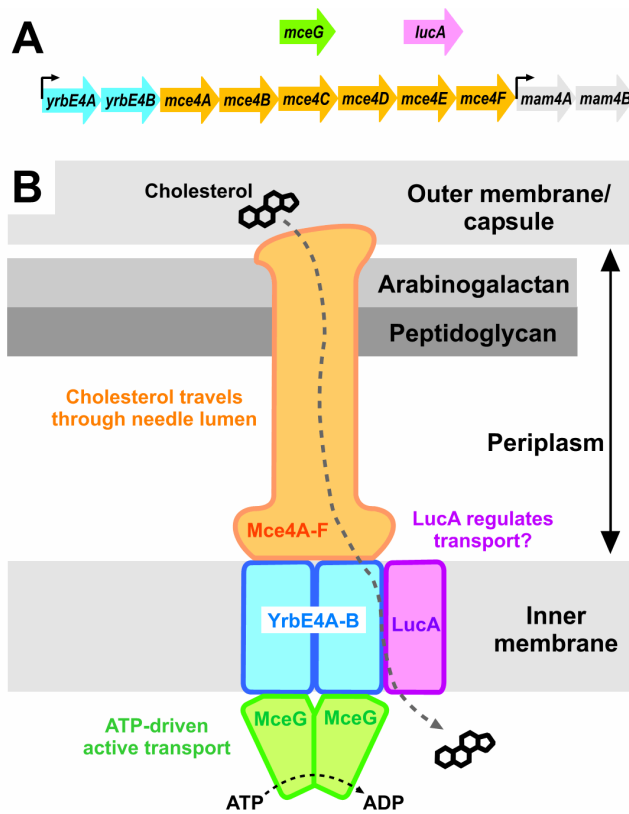


Fig. 4. Genes and protein subunits of the mycobacterial Mce4 cholesterol transporter. A) Operon structure of the *mce4* locus found in *M. tuberculosis* and *M. smegmatis*. The *lucB* and *mceG* genes are essential for cholesterol transport and are found elsewhere on the genome. B) Functional roles for the subunits of the mycobacterial Mce4 transporter complex.

3.4 Bacterial steroid catabolism

There is a long history of research into steroid catabolism across diverse bacterial species, with *Arthrobacter*, *Mycobacteria*, *Nocardia*, *Rhodococcus*, *Gordonia* and *Streptomyces* all reported to survive on cholesterol as the sole carbon source, as reviewed in (Wipperman, Sampson and Thomas, 2014). Much of this work has focussed on cholesterol catabolism in the Actinobacteria phylum, with a particular emphasis on the *Mycobacterium* and *Rhodococcus* genera. Given that this phylum contains a number of important bacterial pathogens, this topic is particularly pertinent for the current article that focusses on steroid antibacterials and may provide important insights into whether mycobacteria or other actinobacterial species are suitable or otherwise for targeting with steroid drugs. Bacterial cholesterol catabolism can

proceed via an aerobic pathway or via an anaerobic route, although understanding of the latter remains fragmented so herein we focus on the aerobic processes.

The best characterised bacterial steroid catabolism pathway is that of aerobic cholesterol breakdown, which provides useful insights into potential transformations that could be performed with a broader range of steroids (e.g. steroid antibacterials). Details of aerobic catabolic reactions and the enzymes that catalyse aerobic cholesterol breakdown have been pieced together over the years from investigations with a diverse selection of bacterial species. These studies and pathways have been reviewed extensively (Galán *et al.*, 2017; Horinouchi and Hayashi, 2025), so herein we aim to provide an overview of key processes and intermediates in the aerobic catabolic process (Fig. 5). Initially, ring A of cholesterol (Compound I, Fig. 4) is oxidised and isomerised and then cytochrome P450-mediated hydroxylation and oxidation of the sidechain generates a carboxyl moiety (Compound II, Fig. 5). Removal of the sidechain is then achieved via three steps of β -oxidation-type reactions to leave a keto group on the D ring (Compound V (AD), Fig. 4). This is then oxidised and hydroxylated to form 9OH-ADD (Compound VI, Fig. 5). Further hydroxylation and cleavage of ring B yields 3,4 DHSA (Compound VII, Fig. 5), which possess a catechol-like ring system that is subsequently cleave to produce 4,9-DHSA (Compound VIII, Fig. 5). This is then hydrolysed to generate HIP and 2-hydroxyhexa-2, 4-dienoic acid (Compounds IX and XI respectively, Fig. 5). These products are then thought to enter central metabolism, including the production of pyruvate from 2-hydroxyhexa-2, 4-dienoic acid (Kieslich, 1985; Horinouchi *et al.*, 2005).

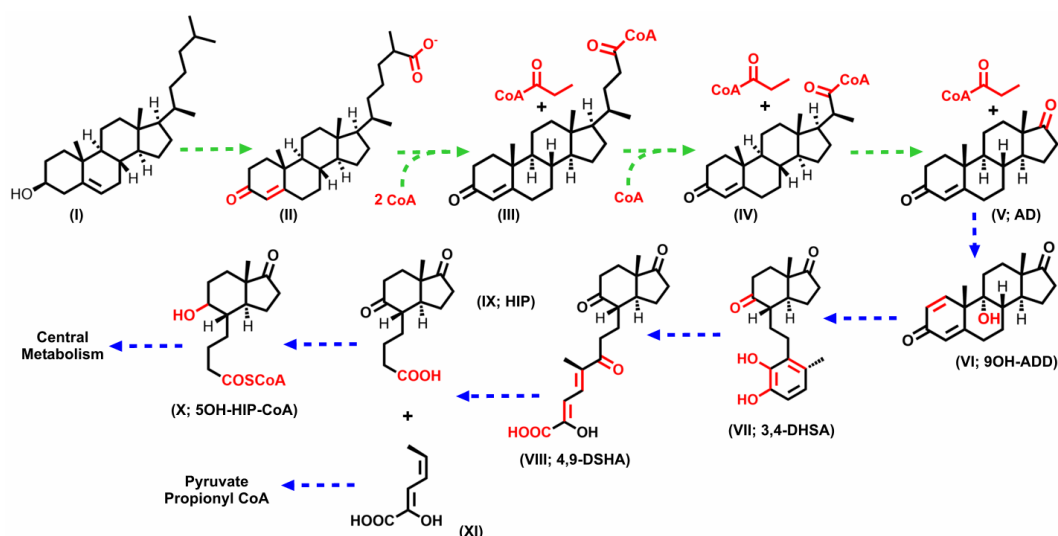


Fig. 5. Key intermediates in bacterial aerobic catabolism of cholesterol. Breakdown of the cholesterol sidechain is denoted by green arrows and the central/lower routes are linked by blue arrows. Intermediates: I, cholesterol; II, (25S)-3-oxocholest-4-en-26-oate; III, 3-oxochol-4-en-24-oyl-CoA; IV, 3-oxo-4-pregnene-20-carboxyl-CoA; V, 4-androstene-3,17-dione (AD); VI, 9-hydroxy-1,4-androstadiene-3,17-dione (9OH-ADD); VII, 3,4-dihydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione (3,4-DHSA); VIII, 4,5,9,10-diseco-3-hydroxy-5,9,17-trioxoandrosta-1(10), 2-diene-4-oic acid (4,9-DSHA); IX, 3 α -H-4 α -(3'-propanoate)-7 β -methylhexahydro-1,5-indanedione (HIP); X, 5OH-HIP-CoA; XI, 2-hydroxyhexa-2, 4-dienoic acid. N.B. For simplicity, not all intermediates are shown but chemical changes are shown in red.

4. FUNGAL BIOTRANSFORMATIONS – THEIR POSITIVE AND NEGATIVE ROLES

While some steroidal detergents have been shown to possess antifungal properties (Section 2.2), fungi are generally resistant to steroidal antibacterials. Furthermore, fungi produce ergosterol as an essential membrane component in addition to a variety of antibacterial steroids, therefore possessing diverse biosynthetic and catabolic capabilities in steroid metabolism. It is these characteristics that make fungal species valuable tools for the production of steroids of importance in biotechnology and potentially for antibacterial purposes.

Steroids represent one of the largest bioactive classes with suggested world markets in the region of US\$ 10 billion and the production exceeding 1,000,000 tons per year (Sultana, 2018). This suggests that the drivers for understanding their production, metabolism and biotransformation are a historic and continuing focus with modern analytics and biotechnology continually providing insight into utility, exposure and fate.

4.1. *De novo* formation of steroidal compounds

Ahead of considering the biotransformation of corticosteroids it must be recognised that steroids are, along with sphingolipids and glycerophospholipids, one of the three major components of the eukaryotic, including fungal, cellular membranes. Steroid biosynthesis is believed to have evolved *de novo* within eukaryotes although their structural similarity to bacterial hopanoids and the presence of putative steroid biosynthetic genes in bacteria may offer some evolutionary insight (Hoshino and Gaucher, 2021). It is not surprising therefore that *de novo* formation of a wide range of steroidal compounds is to be found

within the fungal kingdom with a recent publication summarising the 41 steroidal compounds that can be found within *Penicillium* alone (Lindsay, Kinghorn and Rakotondraibe, 2023). These range in activities and only antibacterial compounds, the main focus of the present review, are introduced in Section 2.1.1 and Figure 1.

Fusidane-type antibiotics such as helvolic acid, fusidic acid and cephalosporin P₁ are known to be synthesised *de novo* from fungi. Underpinning the ability to engineer and extend the biocatalysts able to produce steroids *de novo* work continues to extend the toolbox and approaches. Heterologous biosynthesis in *Aspergillus oryzae* NSAR1 has been demonstrated with novel biocatalytic steps, e.g. unusual C-4 demethylation, shown to potentiate the inhibitory effects of the antibacterials on *S. aureus* (Lv *et al.*, 2017).

Helvolic acid is nortriterpenoid produced by range of fungi including *Aspergillus*, *Fusarium* and *Metarhizium*. It has been shown to have antibacterial effects with metagenomic approaches extending this to population-based investigations such as its effect in bacteriostatic effect of insect cuticular microbiomes (Yanlei *et al.*, 2022). This study clearly shows helvolic acid was effective against a range of Gram-positive organisms (e.g. *Lactiplantibacillus plantarum*, *Corynebacterium nuroki*, *Enterococcus faecalis*, and *Leuconostoc mesenteroides*) whilst showing little or no effect against Gram-negatives. New antibacterial derivatives of helvolic acid continue to be discovered such as the sarocladiolactones A and B and sarocladic acid and from the endophytic fungus (*Sarocladium oryzae*) of wild rice (*Oryza rufipogon*). As with the parent compound these derivatives showed the most inhibition against *S. aureus* at a minimum of 1 µg/mL (Zhang *et al.*, 2021).

4.2 ‘Selection’ and selectivity of fungal biotransformations

Within the microbiome and the extended resident microflora on humans and animals the roles of bacteria, archaea, fungi, viruses and protozoa are many and varied. Whilst it might be argued that the bacteria dominate in terms of numbers of species and total abundance, the mycobiome has been revealed to play a major role in the biotransformation of endogenous and exogenous drugs. This has led to considering fate and dosing regime *in vivo* as well as informed personalised approaches based on metagenomics. Herein, we consider how our knowledge of fungal biotransformations impact upon the use and manufacture of steroids in humans and animals.

Fungi have a vast and diverse array of niche occupancies. They are almost exclusively non-motile and some may consider they rely on a more restricted array of detoxification mechanisms including reduced uptake, efflux pumping and sequestration/compartmentalisation. Additionally, as with many bioactive exposures, fungi also have an array of biotransformation mechanisms and it has long been established that a range of fungi are capable of transforming a range of steroidal compounds, both natural and synthetic. If one considers the diversity of compound classes either originating or synthesised naturally from a cholesterol precursor (e.g. steroid hormones, bile acids and vitamin D₃ as in Fig. 2) we can find examples of fungi that functionalise a range of the positions in the steroid nucleus. Herein we categorise the biotransformations on the basis of the numbering of the steroid nucleus shown in Fig. 6.

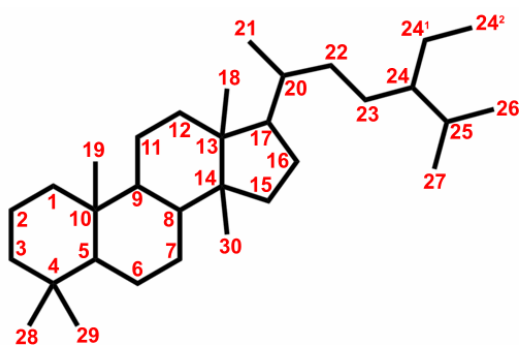


Fig. 6. Numbering of the steroid nucleus.

Of the historic zygomycota, *Mucor* and *Rhizopus* are commonly used in industry for the biotransformation of fungi. Indeed, the initial interest in fungal biotransformation of steroids centred on the 11 α -hydroxylation by *Rhizopus* spp. almost 70 years ago. Deficiency in chemical synthesis such as complex reaction conditions and regio-selectivity/specificity have underpinned much of the impetus in biotransformations with regio-specific hydroxylation, ring A aromatization, isomerisation and side-chain cleavage all being achieved with fungal catalysts under mild conditions. Fungal hydroxylases can hydroxylate steroids at almost all positions, including 1 β , 2 β , 4, 5 α , 6 β , 7 α , 7 β , 9 α , 10 β , 12 α , 12 β , 14, 15 α , 15 β , 17 α , 19, 22, and 26. However, the commercially important 11 α , 11 β and 16 α -hydroxylations have received much greater attention, principally to improve yield and the robustness of the biocatalyst (Nassiri-Koopaei and Faramarziize, 2015).

4.3 The effect of fungal transformations of steroidal compounds

Pharmacological antifungal activity is not extensively shown within the literature. It is clearly recognised that for ‘protection’, some fungi have the capability to transform and detoxify steroid hormones although this varies greatly in this diverse kingdom. As an example, within the ascomycetous fungi *Hortaea werneckii*, *Saccharomyces cerevisiae* and *Aspergillus oryzae* the antifungal effects of a range of related steroidal molecules were shown to differ (Cvelbar *et al.*, 2013). Dehydroepiandrosterone was shown to be the strongest inhibitor of growth in all three varieties of fungi followed by androstenedione and testosterone.

Indeed, the immunomodulatory effects of several corticosteroids have shown them to be advantageous to growth *in vivo* (Li and Denning, 2023). Co-formulation studies using topical corticosteroid treatments such as triamcinolone +/- econazole (with and without an antimicrobial) have further shown that skin dysbiosis, and skin barrier function, is not restored in atopic dermatitis (AD) when treated with corticosteroid (i.e. triamcinolone) alone (Tingting *et al.*, 2025). These studies suggest that these corticosteroids are broadly not antimicrobial and are therefore either not inhibitory or are biotransformed by the resident microflora.

4.4 Metagenomic insight into steroid-induced mycobiome bioses

It is recognised that our microbiome, and more specifically our mycobiome, play a role in our health and well-being with the gut as the major focus of current knowledge and understanding (Zhang *et al.*, 2022). This is largely underpinned by the abundance and diversity within this niche and the relative ease of sampling using faeces as a proxy. Microbial dysbioses and modulation of drug parameters due to *in vivo* exposure to bioactive molecules have become a current focus in pharmacology, giving rise to a dedicated branch of *omics*, namely *pharmacomicrobiomics*. The variation in individual microbiomes and their consequent effects are now attracting much interest as a leading cause of Individual Response in Drug Response (IVDR) and Adverse Drug Reactions (ADR) – [see (Zhao *et al.*, 2023)]. Within IVDR there exists great variation (30-80%) in efficacy rate across a wide range of therapeutic areas (Spear, Heath-Chiozzi and Huff, 2001) with corticosteroids used extensively in ‘some’ of the therapeutic areas such as asthma (60%). A small-scale study investigating the effect of inhaled corticosteroids (ICS) on the mycobiome of asthma patients suggest that species richness is reduced although no ICS modulation is shown (Huang *et al.*, 2022). It is recognised that the microbiome of an individual is only one of several

contributors to IVDR and even within this the mycobiome is a fraction thereof it is worth considering what contribution the mycobiome makes to steroid efficacy and its importance in IVDR thereto.

4.5 Production of steroids using fungal biocatalysts

The production of steroids has traditionally, and continues to be, achieved using predominantly fungal biocatalysts. This is largely achieved by traditional and innovative fermentation protocols deployed with selected, manipulated and engineered biocatalysts. If we focus on some of the examples shown in Figure 1 it is apparent that historic discovery of key steroids has provided the impetus for ongoing research and development to enhance their production and extend the diversity of bioactive class variants that are produced. As an example, fusidic acid is an antibacterial that is produced by the ascomycete, *Fusidium coccineum*. Since original production in 1960 there has continued to be interest in enhancing the bioproduction of fusidic acid from *Fusidium coccineum* although other sources such as *Cephalosporium lamellaecula* have been reported (Curbete and Salgado, 2016). A recent paper used a novel plasma based mutagenesis strategy to increase the yield of fusidic acid by 60% to 4.5 g.L⁻¹ in *F. coccineum* (Huang *et al.*, 2021), evidencing the continued focus on enhancement by mutation strategies. The value of fusidic acid continues to be recognised against an extending range of infectious agents. It has recently shown potent activity (approx. 10 µg/ml /19 µM) against *Mycoplasma genitalium*, a member of the Mollicute class of bacteria that cause Sexually Transmitted Infections (STI) in both men and women giving rise to diseases such as urethritis and pelvic inflammatory disease. Along with many other areas of science the drug discovery pipeline is currently being ‘augmented’ by Artificial Intelligence (AI) methods and it is interesting to note their use in the rational design of fusidic acid analogues extended the range of bioactivity by functionalisation at the 3-OH position (Wang *et al.*, 2025).

Cephalosporin P1 is a fusidane compound produced by *Acremonium* (formerly *Cephalosporium*) *chrysogenum*. First produced in 1948 the commercial production of the extensively used cephalosporins relies on the same, or closely related fungi for the production of the eponymous cephalosporin C (CPC) to enable the synthesis of the key intermediate 7-amino cephalosporanic acid (7-ACA) (Lin and Kück, 2022). As illustrated above the developmental focus of continuing work is centred on scale up and increasing the

titre of CPC, a complex precursor to many semisynthetic drugs, using *A. chrysogenum* (Lin and Kück, 2022).

As outlined herein, steroids have been produced *de novo* from a range of sources. The biosynthesis of steroids is further augmented using microbial biocatalysts to achieve regio and stereo-selective/specific biotransformations on existing steroidal precursor molecules. These are ordinarily very difficult, low-yielding, multi-step reactions to achieve chemically. The history, range and utility of biocatalysts used in steroid synthesis and modification have been excellently summarised in the historical perspective recently published by Prof. John Honour (Honour, 2025a). Of those presented originally, we reproduce an abridged version below showing the primary biotransformations that the fungi undertake within the gonane nucleus (Table 2 and Fig. 7).

Table 2. Reactions catalysed by fungal catalysts (adapted from (Honour, 2025a)).

Fungal catalyst	Reaction	Reference
<i>Rhizopus nigricans</i>	11 α -hydroxylation	(Breskvar, Cresnar and Hudnik-Plevnik, 1987)
<i>Cunninghamella blakesleeana</i>	11 α -hydroxylation	(Manosroi, Chisti and Manosroi, 2006)
<i>Apiocrea chrysosperma</i>	9 α - & 14 α hydroxylation;	(Smith, Latif and Kirk, 1988)
<i>Saccharomyces cerevisiae</i>	17-ketoreduction	(Cheng and Tsai, 2008)
<i>Mucor piriformis</i>	14 α hydroxylation	(Madyastha and Joseph, 1993)
<i>Sarocladium strictum</i>	15 α -hydroxylation	(Faramarzi, Yazdi and Amini, 2003)
<i>Penicillium decumbens</i>	5 α -reductase	(Holland, Dore and Xu, 1994)
<i>Aspergillus fumigatus</i>	11 α -; 15 β - and 7 α hydroxylation;	(Mukherjee, Banerjee and Mahato, 1982)

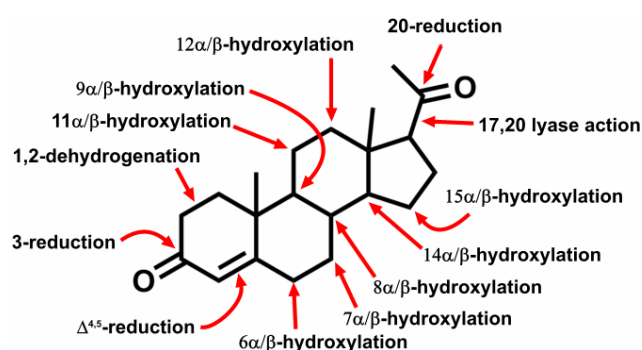


Fig. 7. Functionalisation of the steroid nucleus. Adapted from (Honour, 2025a).

In summary, diverse fungi are able to synthesise and transform a wide range of steroidal compounds. As key components in eukaryotic membranes *de novo* formation is not unexpected although the range of bioactive modified variants is growing and will continue to be discovered as bioprospecting and synthetic biology continues. The utility of fungi in the manufacture of corticosteroids is built primarily on their capability to regio- and stereo-specifically modify and functionalise the steroid nucleus under controlled *in vitro* conditions. Whilst some antimicrobial activity has been demonstrated *in vitro* it is clear that the immunomodulatory roles of corticosteroids themselves and the potential for the mycobiome (as components of the microbiome) can contribute to their suitability as antimicrobials *in vivo*.

5. METABOLIC FATES OF STEROID DRUGS

5.1 Primary actions of antibacterial steroids in humans

Of the antibacterial steroidal drugs reviewed herein, the FDA-approved drugs used in HRT (quinestrol), contraception (hydroxyprogesterone, algestone acetophenide) and as anti-inflammatories (prednicarbate) have well-characterised modes of action when used for their primary function which would represent inevitable off-target effects if the drugs were deployed as antibacterials. Quinestrol is a prodrug that is slowly metabolised to ethinyl estradiol, which binds to the estrogen receptors promoting the maintenance of the uterine lining, and can ultimately act to regulate the menstrual cycle in menopausal individuals. Hydroxyprogesterone is a progestin drug that can be used to manage menstrual cycles and prevent preterm births by binding to progesterone receptors (e.g. in the uterus and ovaries) to elicit an anti-inflammatory state thus maintaining the quiescent state in the uterus. Algestone acetophenide, also known more commonly as dihydroxyprogesterone acetophenide (DHPA), acts in the same way but is more often deployed as a contraceptive to suppress ovulation and maintain the endometrium in a state that is unsuitable for implantation. Finally, prednicarbate is a glucocorticoid receptor agonist that provides anti-inflammatory effects when used in topical steroid creams to treat Atopic Dermatitis caused by *S. aureus*, and it is active against a wide range of Gram-positive and Gram-negative pathogens.

While none of the fusidane class of antibacterials have been approved by the FDA, and their pharmacological consequences are generally poorly characterised, fusidic acid has been used widely outside the USA and is currently licenced in the UK by the MHRA for use in antibacterial topical cream and eye drops. Fusidic acid is not a repurposed drug so does not have any intentional targets outside the

bacterium, so the major pharmacological mechanism of this drug is targeting bacterial protein synthesis, as introduced in Section 2.1.1.

While there is substantial literature on the antibacterial properties of the remaining steroid drugs in Table 1, they are not approved for human use and the mechanisms for metabolic processing in the body are therefore poorly characterised. Furthermore, as these are all steroids many of these drugs are metabolised by the same liver enzymes. Hence, the scope of this section will be limited to the metabolism of quinestrol (as an example of the FDA-approved hormone drugs), fusidic acid (as an example of the fusidane class), along with Hecogenin acetate and squalamine.

5.2 Human metabolism of Quinestrol

Quinestrol is a cyclopentyl ether prodrug of ethinyl estradiol, differing from estradiol by the incorporation of a quaternary centre with an alkyne attached to the same carbon as an alcohol on the D ring (Fig. 8). It has been used in the past for hormone replacement therapy, as a hormonal birth control, for the treatment of menopause symptoms and for the treatment of some cancers such as breast and prostate. Quinestrol is a highly lipophilic molecule with a logP of 5.4 (Drugbank), which leads to distribution into adipose tissue (Williams *et al.*, 1967). Estrovis, the brand name for quinestrol, was administered as 100 µg/day for 1 week, then 100-200 µg/week, and following gastrointestinal absorption it is stored in the adipose tissue and slowly released and metabolised to the parent compound, ethinyl estradiol (Williams *et al.*, 1967).

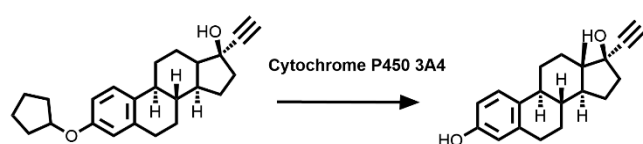


Fig. 8. Reaction scheme showing the conversion of quinestrol to ethinyl estradiol by cytochrome p450 enzymes in the liver.

On passage through the liver, quinestrol and mestranol are modified by Cytochrome P450 3A4 to liberate the biologically active metabolite, ethinyl estradiol (Fig. 8) (Schmider *et al.*, 1997; Ji *et al.*, 2024).

Following the cleavage of the cyclopentyl ether the ethinyl estradiol is then further susceptible to Cytochrome P450 enzymes, particularly 3A4, which further oxidises the steroidal core on both aromatic and aliphatic positions (Fig. 9) (Ball *et al.*, 1990; Yamazaki *et al.*, 1998). These oxidised species are then methylated by SAM-dependent catechol O-methyltransferase enzymes (Martucci and Fishman, 1993; Yager and Leih, 1996; Worda *et al.*, 2003). The metabolism of ethinyl estradiol has been thoroughly reviewed elsewhere (Zhang *et al.*, 2007)

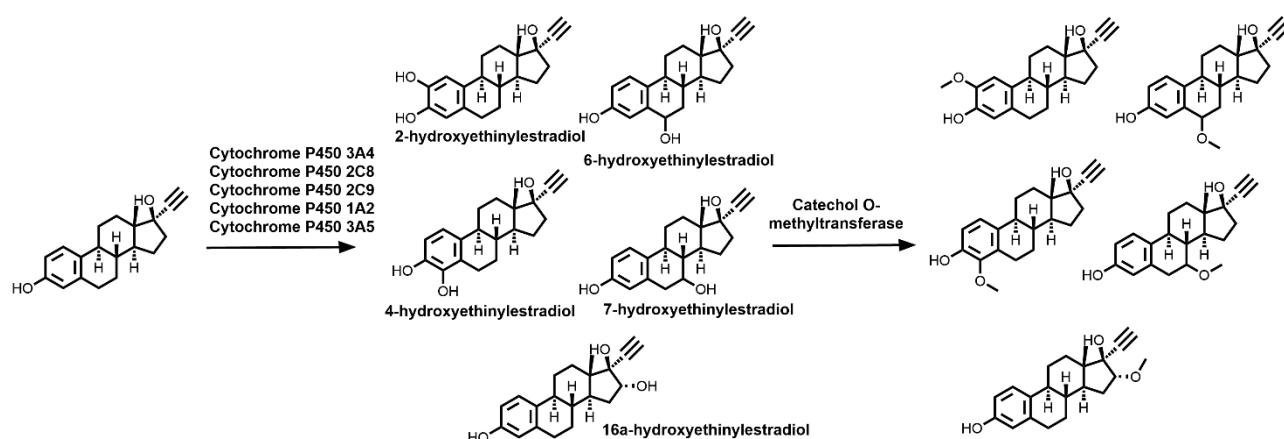


Fig. 9. Reaction schemes showing the oxidation and methylation of ethinyl estradiol by metabolic enzymes in the liver.

This active metabolite ethinyl estradiol, similar to estradiol and estrogen, as well as the P450 mediated oxidation products (Fig. 9), can then be cleared through phase II processes including glycosylation and sulfonylation of the A ring phenol, increasing polarity and enabling clearance from the body (Fig. 10) (Lépine *et al.*, 2004; Barbosa *et al.*, 2019).

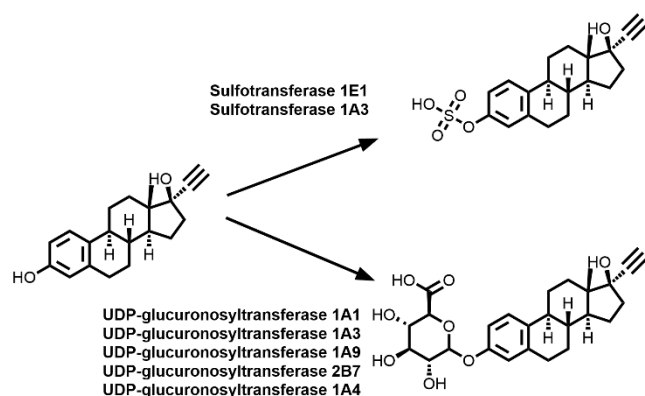


Fig 10. Sulfonation and glycosylation of the steroid core

It is important to note that there is evidence that ethinyl estradiol can, through the inhibition of Cytochrome P450s including 2C19 and 1A2, have potential drug to drug interaction issues (Shelepova *et al.*, 2005).

5.3 Human metabolism of fusidic acid

The natural product fusidic acid, derived from *Fusidium coccineum*, was developed as an antibacterial agent in the 1960's by Leo Pharma. It has an A, B, C and D ring structure common to steroid molecules, but is more polar than the estradiol-type molecules quineestrol and Mestranol with a logP of 4.42 (DrugBank).

Early investigations into the metabolism of fusidic acid by Godtfredson and Vangedal (Godtfredsen *et al.*, 1966) showed three main metabolites of which two were identified as the acyl glucuronide of fusidic acid (Fig. 11C) and the dicarboxylic acid (Fig. 11B). The final metabolite being postulated to be an oxidation product of the sidechain alkene (Fig. 11A).

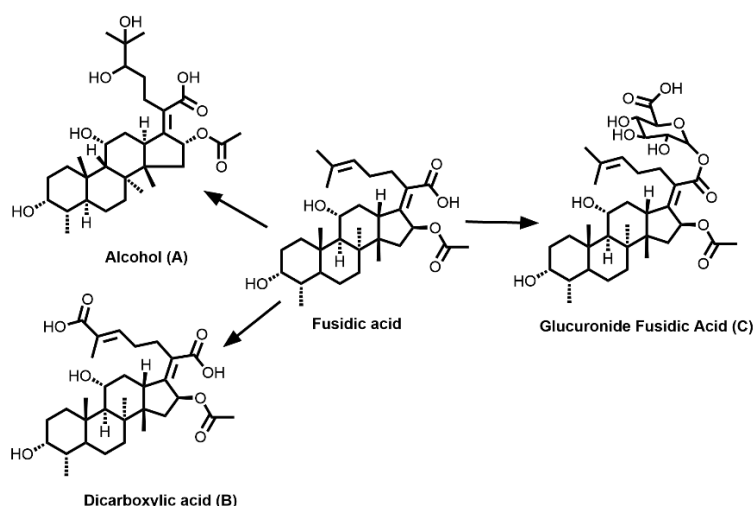


Fig. 11. Reaction schemes highlighting the metabolic modification of fusidic acid in the body.

In-vitro assessment of the metabolic clearance of fusidic acid showed that, like the estradiols, it is a substrate of Cytochrome P450 enzyme with 3A4 and 3A5 accounting for most of the metabolism (Gupta *et al.*, 2016). The phase 1 metabolism mediated by Cytochrome P450 presumably giving rise to the

oxidation products (A and B, Fig 12). Interestingly fusidic acid has also been shown to inhibit P450 enzymes responsible for its metabolism as well as others drug molecules including statins (Gupta *et al.*, 2016). Fusidic acid is also cleared through phase 2 metabolism processes with glucuronidation of the carboxylic acid being observed *in-vitro* through the UDP glucuronosyltransferase 1, UGT1A1 (Gupta *et al.*, 2016) (Fig. 11).

5.4 Breakdown of hecogenin acetate and squalamine in the body

Hecogenin is a steroidal sapogenin derived from the sisal plant (*Agave sisalana*) with a core steroid modified by fusion of the D ring to a spiroketal (Fig. 1). The ester form, hecogenin acetate, is produced by esterification of the secondary alcohol of the steroidal A ring (Kongkathip, Kongkathip and Noimai, 2006). There is little literature highlighting the metabolic stability of hecogenin acetate, but it could be expected that serum esterases would remove the acetate giving the parent hecogenin. The alcohol of hecogenin would be a potential site of phase 2 metabolism by glucuronidation, interestingly it has been utilised as an inhibitor of UGT1A4, a UDP-glucuronosyltransferase important for clearance of xenobiotics including statins (Al-Zoughool and Talaska, 2006).

Squalamine is an aminosterol bile salt isolated from dogfish sharks (*Squalus acanthias*) (Moore *et al.*, 1993). Structurally it has a core steroid ring system which is modified on the A ring with a spermidine moiety and a sulphated alcohol on C-27 of an aliphatic extension from the D ring (K S Moore *et al.*, 1993). Whilst investigations of the clearance of squalamine have been undertaken (Hao *et al.*, 2003) there is limited information about the molecular mechanisms that underpin the metabolism.

6. FUTURE PERSPECTIVES AND CONCLUSIONS

A range of antibacterial steroids were identified from a variety of sources, including fungal (e.g. fusidanes), plant (e.g. sapogenins), and several of synthetic origin. Potencies of key examples are reviewed and their suitability for future applications are discussed.

6.1 Fungal antibacterials

Of the fungal steroids identified, fusidic acid stood out as a potent antibacterial that is effective against a broad range of Gram-positive organisms (MIC values in low $\mu\text{g/mL}$ ranges) and is currently approved for human use in the UK by the MHRA. Activity against Gram-negatives was generally lower,

with the exception of *Neisseria gonorrhoeae* (MIC = 0.2-0.8 µg/mL) (0.39-1.55 µM). Helvolic acid, another fungal antibacterial, has been shown to be effective against a range of Gram-positives (e.g. MIC for *S. aureus* 5.8 µg/mL (10.2 µM) but has also been shown to diminish the growth of certain Gram-negatives (e.g. MIC for *P. aeruginosa* = 4.6 µg/mL) (8.09 µM).

Pharmacokinetic data following oral administration of Sodium Fusidate demonstrate the accumulation of antibacterial levels of fusidic acid in the plasma, with up to 33 µg/mL (63.9 µM) from a single dose of 550 mg, and all exposures were deemed safe and well tolerated by the human subjects (Still *et al.*, 2011). It is therefore unsurprising that there has been recent interest in deploying fusidic acid to treat infections caused by a range of bacterial pathogens (Phee *et al.*, 2015). The potency of fusidic acid against *Staphylococcus* spp. and low incidence of resistance is well-illustrated by a study on over 3000 clinical isolates collected in 13 European countries, where only 10% of these strains displayed MIC values higher than 2 µg/mL (Castanheira *et al.*, 2010). Other recent studies have highlighted the potential for fusidic acid/colistin combinations to combat *A. baumannii* (Phee *et al.*, 2015), and fusidic acid and C-3-hydroxy metabolic derivatives have been shown to be effective against *M. tuberculosis* with MIC₉₉ values in the low µM range (Njoroge *et al.*, 2019). Finally, MIC values for 98 enterococcal clinical isolates were ≤8 µg/mL (<15.5 µM), and enterococcal burden was significantly diminished by fusidic acid (Abdelmassih *et al.*, 2024).

6.2 Steroid detergents

The steroid detergent squalamine appears to be an effective antibacterial, with MIC values in the range 1-10 µg/mL (1.59-15.9 µM) for a variety of Gram-positives and Gram-negatives. Furthermore, the synthetic steroid detergent Ceragenin C13 is slightly more effective with MIC values against a range of Gram-positives and Gram-negatives ranging from 0.4-3.1 µg/mL (0.59-4.58 µM). While no clinical studies have been conducted to specifically investigate squalamine as an antibacterial, a pharmacokinetic study tested various dosages with a maximum of 538 mg/m²/day over 5 days that resulted in average plasma levels of 32 µg/mL (50.9 µM) (Bhargava *et al.*, 2001). While fatigue, nausea, anorexia, and hepatotoxicity toxicity were detected at these high concentrations, hepatotoxicity was undetectable at a dose rate of 192 mg/m²/day, which generated antibacterial concentrations of up to 11 µg/mL (16.3 µM) in plasma. While one might predict that the harsh mechanism of action involving membrane lipid disruption would be likely

to contribute to severe toxicity in the human host, plasma levels that significantly exceed the MIC for various pathogens are possible with relatively manageable side effects.

6.3 Hecogenin acetate

The sapogenin plant steroid hecogenin acetate appears to possess only weak antibacterial activity but is able to potentiate the action of various antibiotic classes (Section 2.1.2). This is interesting but suggests a generalised mechanism is in play, such as the permeation of the bacterial membrane or inhibition of efflux pumps. It would be useful to gain further understanding of these mechanisms before pursuing this as an antibacterial adjuvant.

6.4 FDA-approved steroid drugs

The modest bacteriostatic properties of progesterone have been known about for a long time (Yotis and Stanke, 1966), and a much later study highlights the potential of hydroxyprogesterone with an MIC of 2 μ M for *Enterococcus faecium* (Younis, AbdelKhalek, Abdelrahman S Mayhoub, *et al.*, 2017). The plasma levels of hydroxyprogesterone appear to be very low: weekly intramuscular injections of 250 mg 17-hydroxyprogesterone caproate, used to prevent spontaneous preterm births in pregnant women, was shown to elicit plasma concentrations in the range 2-21 ng/mL (Della Torre *et al.*, 2019). While this steroid has an MIC for *E. faecium* of 2 μ M (Younis, AbdelKhalek, Abdelrahman S Mayhoub, *et al.*, 2017), this drug is not broad acting, and these low levels are unlikely to be sufficient for antibacterial activity *in vivo*.

Prednicarbate has been shown to effectively inhibit the growth of *E. faecium* (MIC = 4 μ M) and *S. aureus* (MIC = 8 μ M) (Younis, AbdelKhalek, Abdelrahman S Mayhoub, *et al.*, 2017). This steroid is mainly deployed as a topical antibacterial, but plasma levels have been determined after oral administration, which revealed that this steroid is essentially undetectable in blood and is rapidly metabolised to prednisolone-17-ethylcarbonate (Barth *et al.*, 2009).

Algestone acetophenide, also known as dihydroxyprogesterone acetophenide (DHPA), was shown to effectively inhibit the growth of *E. faecium* (MIC = 16 μ M) (Younis, AbdelKhalek, Abdelrahman S Mayhoub, *et al.*, 2017). This drug is used as a long-acting contraceptive, which is injected into muscle tissue as oral administration is not possible. It is slowly absorbed into the bloodstream, and therefore is

unlikely to reach antibacterial concentrations, although precise plasma concentrations and half-life are elusive in publicly available literature.

The prodrug quinestrol, which was designed to be slowly metabolised to ethinylestradiol in the body, exerts its pharmacological activity against estrogen receptors as a HRT therapy or oral contraceptive. One thing to note is that quinestrol ($\log P = 5.4$) is stored in adipose tissue and ethinylestradiol slowly produced at pharmacologically active concentrations (Williams *et al.*, 1967; Quirk and Wendel, 2012). Detailed pharmacokinetics of quinestrol degradation are not freely available, although administration of 50 mg of ethinylestradiol as an oral contraceptive results in blood plasma levels of 400 pg/mL (Barnes and Levrant, 2007), which is well-below the MIC for any of the antibacterial steroids presented herein. Hence, <100 mg doses of quinestrol are unlikely to yield the requisite plasma concentrations to combat bacteremias. However, if quinestrol could be maintained in the inactive prodrug form and could be modified to avoid accumulation in the adipose, it might be possible to administer sufficient levels of quinestrol derivatives over multiple days to reach antibacterial levels. To preserve the pharmacologically inactive cyclopentyl ether moiety of quinestrol, the A ring could be modified to protect this drug from microsomal P450-mediated degradation, and lowering the hydrophobicity might reduce accumulation in the adipose tissue. Alternatively, as quinestrol is bactericidal against the cutaneous Gram-positive pathogen *S. aureus* (Henry *et al.*, 2024), this steroid might be better deployed as a topical antibacterial or as a component of antibacterial eye drops.

6.5 Fungi as a chassis for antibacterial steroid production

Current research continues to search for fungal species from which it is possible to derive more antibacterial compounds (Gomes *et al.*, 2021; Ordóñez-Enireb *et al.*, 2022). Over 50% of the antibacterial compounds approved for the clinic, between 1981-2019, were derived from microbial sources (Gomes *et al.*, 2021). As discussed previously several antibacterial compounds were derived from yeast. Most notably the fusidane class of antibiotics that show a high level of antibacterial activity, mainly against Gram-positives (Waksman, Horning and Spencer, 1942; Ritchie, Smith and Florey, 1951; Godtfredsen *et al.*, 1962). Furthermore, the genus *Penicillium* produces 41 unique steroidal compounds (Charmaine A. Lindsay, Kinghorn and Rakotondraibe, 2023) and several of these compounds have the potential to be antibacterial.

Given the diversity of naturally occurring steroidal compounds in fungi, it is unsurprising that it has been possible to engineer yeast to produce non-native steroids (Xu and Li, 2020; Gu et al., 2021). As discussed in Section 4.5, yeast enzymes are able to modify the steroid nucleus in a variety of ways (Table 2, Fig. 7). Indeed, several modifications of the steroid nucleus seen in the structures of the FDA-approved steroids in Fig. 1 could be performed using engineered yeast strains. For example, prednicarbate contains a double bond between carbons 1-2, and the enzymatic 1,2,-dehydrogenation reaction found in yeast could potentially be used to synthesise this as reported in Honour (2025). Also, prednicarbate has a hydroxyl group on carbon 11, which could be added with the 11 β hydroxylase that is native to *Cunninghamella blakesleeana* (J. Manosroi, Chisti and Manosroi, 2006). It would also be possible to synthesise prednicarbate from the precursor boldenone, which can be produced in *Komagataella pastoris* (formerly *P. pastoris*) (Xu and Li, 2020). As well as partial syntheses of prednicarbate, 17 α -hydroxyprogesterone could be produced from progesterone using a strain of *S. cerevisiae* transformed with a bovine cytochrome P450 with 17 α -hydroxylase activity (Degryse *et al.*, 1999). While it may be that it is possible to synthesise some of the steroid compounds *in vivo* in fungal cells presented above, these processes are less suitable for others such as the steroidal detergents that have been shown to possess antifungal activity.

6.6 Conclusions

The use of steroid drugs as antibacterials is an attractive prospect, although there are number of initial questions to consider, including: i) Is it broad acting enough? ii) Is it possible to attain antibacterial concentrations in the blood or would it be better deployed elsewhere (e.g. as a topical cream or in eye drops)? iii) are doses that yield antibacterial levels tolerable to the host? iv) Can it be easily produced using biotransformations or chemical approaches? v) Are there potential extensive off-target effects in the patient? Out of all of the steroids reviewed herein, fusidic acid appears to best satisfy all these criteria, although it is not used as a monotherapy for bloodstream infections due to limited activity against Gram-negatives, so is therefore used in combination therapies to treat Gram-positive bloodstream infections. On balance, steroids do appear to preferentially target Gram-positives and can be effective for external infections, so this would seem a more furtive short-term path to pursue for existing steroids with antimicrobial activities. Future derivatisations and drug encapsulation approaches can be used to improve

efficacy against Gram-negative pathogens and pave the way for the deployment of steroid antibacterials to treat systemic infections.

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