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**Title:** Repurposing drugs for treatment of tuberculosis (TB): a role for non-steroidal anti-inflammatory drugs.

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**Running title:** Development of a new adjunct chemotherapy for TB.

## **Abstract**

### *Introduction*

The number of cases of drug-resistant *Mycobacterium tuberculosis*, the causative agent of tuberculosis (TB), has risen rapidly in recent years. This has led to the resurgence in repurposing existing drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs), for anti-TB treatment.

### *Sources of data*

Evidence from novel drug screening *in vitro*, *in vivo*, pharmacokinetic/pharmacodynamics analyses and clinical trials has been used for the preparation of this systematic review of the potential of NSAIDs for use as an adjunct in new TB chemotherapies.

### *Areas of agreement*

Certain NSAIDs have demonstrated inhibitory properties towards actively-replicating, dormant and drug-resistant clinical isolates of *M. tuberculosis* cells.

### *Areas of controversy*

NSAIDs are a diverse class of drugs, which have reported off-target activities and their endogenous antimicrobial mechanism(s) of action is still unclear.

### *Growing points*

It is essential that clinical trials of NSAIDs continue, in order to assess their suitability for addition to the current TB treatment regimen. Repurposing molecules such as NSAIDs is a vital, low-risk strategy to combat the trend of rapidly increasing antibiotic resistance.

**Keywords:** Tuberculosis, antimicrobial resistance, drug repurposing, NSAIDs, Carprofen, *Mycobacterium*.

## Introduction

Despite an effective tuberculosis (TB) treatment regimen being in place for several decades, the disease remains responsible for over 1.5 million deaths every year. Nearly a fifth is due to multi-drug resistant (MDR) strains that do not respond to the front-line drugs available for TB treatment.<sup>1</sup> This has provided the much needed impetus for the discovery and development of novel, more effective and safer anti-tubercular chemotherapeutics.<sup>2-4</sup> However, there is a high attrition rate of lead molecules passing from pre-clinical development to phase I clinical studies.<sup>5</sup> This has led to a shortage of novel molecules available for further development and incorporation into successful anti-tubercular therapy. While pharmaceutical companies are investing upwards of \$500million and 15 to 20 years' work in anti-infective research projects, the number of novel molecular entities approved by organisations such as the U.S. Food and Drug Administration (FDA), has been on a declining trend.<sup>6</sup> Workers in the field have identified an urgent need for new treatments and are shifting focus to repurpose drugs, either abandoned or still in use to treat other diseases, to combat the shortfall in productivity.<sup>7</sup> As a result, the current clinical trials pipeline for anti-tuberculosis therapy contains novel and pre-existing drugs that are being tested for their efficacy in combinatorial treatment regimens.<sup>8</sup>

Repurposing drugs involves discovering novel drug-target interactions of established drug treatments, with an aim to use them to treat different diseases. It is a strategy that has regained the interest of pharmaceutical companies and academic scientists alike for its potential to reduce investments, de-risk clinical activities and shorten the time required to market a drug for its new use. Many drugs have been successfully repurposed to-date.<sup>7</sup> Amongst these, thalidomide, first used as a morning sickness remedy with disastrous results, was approved for treatment of multiple myeloma and has since been repurposed for leprosy treatment.<sup>9</sup> It has also found use in managing severe treatment-associated paradoxical reactions in paediatric TB meningitis.<sup>10</sup>

The presence of gaps in drug-target interaction profiles is the most common barrier in repurposing molecular entities.<sup>11</sup> As discussed by Kinnings *et al.*, integrating the tools presented by computational and systems biology approaches will likely provide new insights, helping to de-mystify drug-interaction networks by successfully identifying possible off-targets of existing drugs.<sup>12</sup> They report that only 9 out of the 3,999 proteins encoded by *M. tuberculosis* are targets for drugs currently in use. Thus, building comprehensive drug-target interaction networks will reveal novel solutions to overcome the various resistance mechanisms developed by the causal pathogen.

As mentioned earlier, many anti-infectives previously used for other disease indications are being considered for, or are already in various phases of *in vitro/ in vivo*, as well as advanced clinical trial studies (Figure1).<sup>8, 13</sup> Several fluoroquinolone antibiotics and specific drugs such as clofazimine, linezolid, and metronidazole are a few examples and have been reviewed elsewhere.<sup>14, 15</sup> On the other hand, therapeutic agents originally intended to work upon various host-related systems have shown anti-infective properties specific to *M. tuberculosis*. Medications used to treat varied, unrelated human conditions such as psychoses and angina, serve to inhibit the multi-drug efflux pumps in *M. tuberculosis* thereby increasing the pathogen's susceptibility to other drugs.<sup>16, 17</sup> Several phosphodiesterase inhibitors have also shown promise as adjuvants for host-directed therapy.<sup>18</sup>

Of special interest are common, inexpensive non-steroidal anti-inflammatory drugs (NSAIDs) such as oxyphenbutazone and the 2-arylpropanoic acid family of drugs that have been found to be anti-tubercular. These drugs act upon replicating, dormant and also drug-resistant clinical isolates of *M. tuberculosis*.<sup>19, 20</sup> Here, we discuss the possibility of these NSAIDs, never previously considered for their anti-infective action, being repurposed as a part of new TB chemotherapy.

## **NSAIDs offer a promising TB treatment strategy**

The great potential for repurposing offered by NSAIDs in the context of anti-tuberculosis therapy has been recognised relatively recently.<sup>19-21</sup> NSAIDs have captured the attention of the scientific community, both for their biological activities and the ease with which these off-patent drugs can be repurposed. Many members of this class of molecules have shown selective anti-mycobacterial properties, along with possible pleotropic endogenous mechanisms of anti-tubercular action.<sup>19</sup>

NSAIDs are a group of molecules from chemically diverse families (Figure 2) that suppress inflammation by inhibiting the formation of prostaglandins, the mediators of inflammatory response. They achieve this by inhibiting cyclo-oxygenase enzymes, COX-1 and COX-2, involved in the synthesis of prostaglandins and other prostanoids. Classical NSAIDs block both enzymes without differentiation, however, newer COX-2 inhibitors are selective (e.g. celecoxib), and thus exhibit less gastric irritation as an adverse effect - increasing their popularity. There is significant evidence supporting the notion that some of these drugs also modulate immune responses via pathways independent of the cyclooxygenase-prostaglandin route.<sup>22, 23</sup>

Although originally utilised for anti-inflammatory, analgesic and antipyretic purposes, NSAIDs have been shown to have potential in the treatment of various cancers and neurodegenerative diseases such as Alzheimer's.<sup>24</sup> They have subsequently been found to possess inhibitory as well as bactericidal properties against a wide range of pathogens, both Gram-positive and Gram-negative, including *Enterococcus faecalis* and *Helicobacter pylori* respectively.<sup>25, 26</sup>

### *Diclofenac*

Diclofenac sodium was found to be bactericidal against *Escherichia coli*, *Listeria monocytogenes* as well as *M. tuberculosis*.<sup>27</sup> Encouraged by *in vitro* and *in vivo* studies

using the drug, investigations were extended and synergism was identified with streptomycin in murine tuberculosis.<sup>28</sup>

Diclofenac acid hydrazones and amides have also been shown to reduce lung and spleen bacillary loads by approximately 3.66 log<sub>10</sub> in mouse infection models at a dose of 25 mg/kg.<sup>29</sup> Inhibition of incorporation of thymidine, vital to DNA synthesis, has been reported as one of the likely mechanisms for the bactericidal action of diclofenac in *E. coli* and *Listeria* spp.<sup>30</sup>

### *Metal complexes*

Using metal complexes with active NSAIDs as ligands has proven to be a useful strategy in developing antimicrobials and has also been used to impart anti-tubercular properties to a selected group of NSAIDs that showed no such property prior to modification.<sup>31</sup> These organotin complexes of mefenamic, meclofenamic acid, indometacin, and tenoxicam showed *in vitro* MIC values of <1 µg/mL against *M. tuberculosis* and can be considered excellent lead molecules for development of a new class of anti-tuberculars.<sup>32</sup> However, the report failed to provide information regarding the bacteria-specific selectivity of the aforementioned drug complexes. The toxicity of organotin(IV) towards eukaryotic cells has raised concerns thereby limiting the immediate applications of these complexes in medicinal use.<sup>33</sup>

### *Oxyphenbutazone*

A high-throughput phenotypic screen revealed that oxyphenbutazone selectively inhibits the non-replicating subset of the *M. tuberculosis* pathogen whilst having no effect on the replicating bacteria.<sup>19</sup> One of the primary reasons for the lengthy duration of tuberculosis treatment is the need to eliminate non-replicating bacteria or 'persisters', that are difficult to treat due to their physiological status and unique endogenous metabolism.<sup>34</sup> In the Gold *et al.* model, the environment to which the drug was exposed (mildly acidic and high in reactive nitrogen intermediates) resulted in its hydroxylation; and the compound produced was

shown to be active against both, replicating and non-replicating bacilli in isolation. In addition, it was also found to be synergistic with oxidants and several conventional anti-tubercular drugs such as *p*-aminosalicylate.<sup>19</sup> The modified oxyphenbutazone served to deplete thiols and flavins, thereby potentially affecting a number of enzymatic reactions within the cell. The inability to generate spontaneous mutants further reinforces the argument that the endogenous mechanism of action of this drug may be multi-factorial.

However, in spite of being used regularly in veterinary medicine, its use in humans is restricted in the light of sporadic reports of fatal bone marrow depression caused as a side-effect of the medication.<sup>35</sup>

### *Celecoxib*

Among the NSAIDs promising fewer adverse effects, the COX-2 selective celecoxib was reported to reverse multidrug resistance of methicillin-resistant *Staphylococcus aureus* (MRSA) by inhibiting the bacterial efflux mechanism.<sup>36</sup> A similar effect was noted in *M. smegmatis* and its action is expected to be through an unknown protein that regulates the MDR-1 efflux pumps in bacteria.<sup>36</sup> This hypothesis is further substantiated by the fact that the drug exerts effects on COX-2 which in turn regulates the homologous MDR-1 pumps in humans.<sup>37</sup> Debilitating the extrusion mechanisms of bacteria is a powerful strategy to reverse resistance and tolerance seen in planktonic and bacterial biofilms. In the context of combination therapy, this introduces the possibility of reducing the dose or shortening the duration of treatment.

Based on the active pharmacophore of celecoxib, analogues that show potent inhibitory activity against *S. aureus* and *M. tuberculosis* have been synthesized and efforts to further optimise these compounds are ongoing.<sup>38</sup>



## *Aspirin*

Aspirin is a salicylate anti-inflammatory drug which in addition to primary use has shown to potentiate or act synergistically when used in conjunction with the front line anti-TB drug, pyrazinamide in a mouse-infection model study.<sup>39</sup> Gene expression profiling of *M. tuberculosis* in response to salicylate has shown to down-regulate genes involved in energy production. This could explain the synergy between the salicylate and pyrazinamide which is also known to deplete membrane energy and potential and thereby disrupt transport.

However, aspirin has also demonstrated modest antagonistic activity towards isoniazid raising the importance of evaluating at what time-point in the treatment regimen should NSAIDs be included in the therapy.<sup>40, 41</sup> A randomised study on the role of aspirin in TB meningitis suggested aspirin in combination with corticosteroids reduced the incidence of strokes and mortality.<sup>42</sup> A similar study on the role of aspirin as an adjunct with steroids for the treatment of HIV-negative adults with TB meningitis in Vietnam is still ongoing (clinical trials identifier: NCT02237365).

## *Ibuprofen*

Our whole-cell phenotypic evaluation studies using Spot culture growth inhibition assay (SPOTi),<sup>20, 43-45</sup> to screen a library of over-the-counter medicines, revealed commonly used NSAIDs ibuprofen and carprofen, and their analogues selectively inhibited the growth of replicating, non-replicating and even drug-resistant clinical isolates of the tubercle bacilli.<sup>20</sup> As some of the previously mentioned NSAIDs, ibuprofen also exhibits anti-fungal and anti-bacterial properties.<sup>46, 47</sup> Moreover, studies have demonstrated that it has a marked effect on reducing bacillary loads in the lung tissue of mouse infection models - in addition to exhibiting synergism with pyrazinamide.<sup>39, 48, 49</sup> It was shown to be effective at reducing TB burden in necrotising pulmonary granulomas, thereby conferring a level of protection from the disease. Eisen *et al.* postulate that this effect is not mediated by a direct anti-inflammatory mechanism, as this would require higher doses of the drug than the 80mg/kg

daily administrations.<sup>49</sup> They propose that ibuprofen acts via inhibiting tumour necrosis factor (TNF $\alpha$ -an inflammatory cytokine) to block granuloma formation.

The FDA-approved maximum daily intake for ibuprofen is 3200 mg, and on prolonged administration of the drug, a peak plasma concentration of up to 90.4  $\mu\text{g/mL}$  can be obtained.<sup>50</sup> This is well within the concentrations required to achieve anti-tubercular specific action *in vitro*.<sup>20</sup> Additionally, ibuprofen has been shown to be safe in various clinical trials,<sup>51</sup> with a low risk of irreversible liver damage more commonly associated with paracetamol and aspirin. Additionally, its pharmacokinetic properties include a short plasma half-life (under 3 hours), resulting in the drug being eliminated before it can form deleterious metabolites.<sup>52</sup> This short time for elimination also reduces the risk of gastrointestinal and renal damage in the long term.<sup>53</sup>

### *Carprofen*

Amongst the NSAIDs tested, carprofen was found to be the best candidate based on its low MIC of 40  $\mu\text{g/mL}$  and high selectivity index of 25 making it highly specific towards bacterial cells at concentrations non-toxic to eukaryotic cells.<sup>20</sup>

Carprofen was marketed for systemic human use as an analgesic, for nearly ten years since the 1980s. The human clinical trials of the drug reported only mild and transient side-effects to its use.<sup>54</sup> Possessing a higher affinity towards COX-2, it is expected to produce milder side-reactions than its non-selective counterparts. In healthy subjects, carprofen is absorbed rapidly and the peak plasma concentration is reached after an hour of its administration.<sup>55</sup> Crevosier *et al.* also reported that and the absolute bioavailability of the oral forms reached values of over 90%. Despite limited data implying that it has a good safety profile in humans when used at a range of 150-600 mg/day, carprofen was discontinued from the human market for commercial reasons.<sup>56</sup> Carprofen is a photosensitizing drug and reports of phototoxic and photo-allergic contact dermatitis may have precipitated the situation further.<sup>57, 58</sup> It was re-introduced in several parts of the world under various trade names (Rimadyl,

Norocarp, etc.) as pain-relieving medication for veterinary treatment. Pharmacokinetic studies of carprofen in mice and cows showed an extended half-life of the drug; however, in both cases the concentrations of carprofen administered was lower than the maximum approved dose.<sup>59, 60</sup>

The clinical implications of these findings are yet to be fully realised as there are no conclusive data on the effects of ibuprofen or carprofen on tuberculosis treatment outcomes. At present, WHO guidelines recommend incorporating NSAIDs into tuberculosis therapies, though this aimed at reducing the joint pain side effects caused by pyrazinamide, rather than as a means to treat the symptoms of tuberculosis per se.<sup>61</sup> A novel phase III trial (ClinicalTrials.gov Identifier: NCT02060006) to evaluate the feasibility and efficacy of using meloxicam, a cheap and widely available NSAID, as a preventive intervention for TB-immune reconstituted inflammatory syndrome (IRIS) is currently underway and results from the study are awaited.

### **Mechanism of action**

Developing modified molecules of these drugs with improved anti-mycobacterial efficacy requires knowledge of their mechanisms of action. Based on comparative bioinformatics investigation, initiation factor 2, a key player in protein translation initiation in bacteria,<sup>62</sup> appeared to be the likely target of this family of molecules.<sup>20</sup> Using transcriptomic analysis we have also identified significant modulation of a number of key metabolic pathways in *M. tuberculosis*.<sup>63</sup> Similar analyses also found aspirin to down-regulate the machinery required for transcription and translation in *M. tuberculosis*.<sup>64</sup> A recent study investigated the binding efficiencies of carprofen, bromfenac and vedaprofen to the DNA polymerase III  $\beta$  subunit (commonly known as the 'sliding clamp protein') of *E. coli*, to verify inhibition of DNA replication and repair as the bactericidal mechanism of action.<sup>65</sup> However the affinity of the drugs to the sliding clamp does not directly equate to their bactericidal property. Vedaprofen

has the highest affinity to *E. coli* sliding clamp yet the MIC of the drug against it is one of the highest (1,410 µg/mL). Carprofen exhibits an MIC of 680 µg/mL, one of the best performing NSAIDs chosen for the study, however its affinity to the *E. coli* sliding clamp is moderate at best. This lack of correlation between binding affinities and MIC could be due to differences in the membrane permeability of the drugs. The lower MIC of carprofen also serves as a strong argument in favour of the presence of other endogenous target(s) of the drug in *E. coli*.

Comparing the detailed crystal structures of the orthologues from *E. coli* and *M. tuberculosis* revealed marked differences in the secondary structures forming the key motifs and whether similar binding affinities will be observed with mycobacterial orthologues remains uncertain.<sup>66</sup> This warrants for a similar assay with the mycobacterial protein counterparts with the NSAIDs to confirm or disprove the presence of any association between the two.

Unveiling the anti-mycobacterial mechanism of action of the NSAIDs would help in identifying possible interactions with the other front-line drugs used for TB therapy. It is known that aspirin displays alternating effects when used with certain routine anti-TB drugs, potentiating some whereas debilitating others. Antagonism observed in the case of use of aspirin with isoniazid warrants investigation of the effects of adjunctive treatment of aspirin with other hydralazine drugs. Additionally, most NSAIDs have been shown to increase the adverse effects of para-aminosalicylic acid.<sup>67</sup> The other challenges in including NSAIDs in therapy would be to overcome the gastrointestinal effects of these drugs, however, these effects are modest compared to the hepatotoxicity exerted by the established anti-TB drugs. To better understand the role of NSAIDs in alleviating infection and its symptoms, the host-pathogen interaction and inflammatory responses of the host require in-depth research.

## **The role of immune-modulation in tuberculosis**

Inflammation, observed due to *M. tuberculosis* infection is a host immune-response to the pathogen itself in combination with the action of the cocktail of anti-TB drugs administered. Early inflammatory responses are beneficial to the host as they serve to kill or sequester the organism in highly structured granulomas. However, prolonged inflammatory responses are detrimental, leading to the formation of pathological lesions that play a fundamental role in the transmission of the disease and its exacerbation.<sup>68</sup> Therefore, therapy that is tailored to moderate the hypo- as well as hyper-responses could result in improvements in treatment outcome.<sup>69</sup>

Use of corticosteroids to control inflammation and reduce mortality from TB meningitis is already an established clinical practice and though they effect in very separate ways, this outcome supports the inclusion of NSAIDs in tuberculosis treatment regimens. When used as part of anti-tuberculosis therapy, NSAIDs are expected to influence both host- and pathogen-directed effects. However, the authors would like to add a caveat that though modulating macrophage response might prove useful to control the disease, the balance between cellular and chemokine responses is an area poorly understood in the context of host response to tuberculosis infection and vice versa.

### **Alternative modes and/or routes of delivery**

Recently, there has been an increase in the investigation of delivery routes that optimise anti-TB therapies. In particular, a growing number of researchers support delivery of anti-TB drugs via aerosol formulations. The inhalation of an anti-tubercular drug using either a nebuliser or a dry powder form, makes it is more likely to penetrate the alveoli and lung parenchyma thereby preventing establishment and progression of TB infection. Furthermore, delivering therapies in this way enables the primary site of the disease to be targeted directly, ensuring that the local concentration of the drug exceeds the MIC. Delivery of second line drug, capreomycin in a dry powder form has undergone testing in phase I clinical trials in a small number of healthy volunteers.<sup>70</sup> Promisingly, it was found to be well-tolerated.

Many other anti-tubercular compounds have been tested with similarly positive results, providing sufficient evidence to support pursuing further research in this area. Rifampicin loaded on chitosan particles is also proving to be an area of interest as these allow for a concentration-independent sustained release which could have significant impact on lowering the side-effects of chemotherapy as well as improving patient compliance as the dosing frequency would be reduced.<sup>71</sup>

A recent study suggests that the NSAID ibuprofen might be administered via the pulmonary route, by encapsulating it within gel micro-particles. However, although the authors found the drug retained 50% activity against *Candida albicans*, further testing is vital before the potential of this delivery method can be validated.<sup>72</sup> In short, the delivery of anti-tubercular drugs via the aerosol route represents an exciting area for improving the efficacy of current TB therapies.<sup>70</sup>

## **Conclusion**

While it becomes imperative to find new drugs to control TB it is also important to continually revise, redefine and perhaps, reclassify drugs that are already in use. The advantages offered by repurposing are manifold. It is crucial to understand not only their secondary targets but also the endogenous molecular mechanisms of action and how it would translate in a multi-drug combinatorial treatment regimen. Identifying how these drugs work would strengthen their case for inclusion in clinical trials as well as pave the way for designing more targeted drugs. As the search to find novel drugs to tackle antimicrobial resistance deepens, there is a need to evaluate the driving forces of resistance and widen our search to novel concepts as well to find a better cure for TB than what exists today.

NSAIDs have rightly been hailed for their anti-inflammatory properties but their anti-infective property needs to be investigated further as the combined effects promise a significant improvement in treatment outcomes. It is encouraging to note that structural modifications to

improve the antimicrobial activities of NSAIDs such as ibuprofen and carprofen are already underway.<sup>73</sup>

In a TB treatment setting, NSAIDs are primarily used to alleviate the symptoms that arise from the effects of this protracted disease and its therapy. These compounds have proven pharmacokinetic/ dynamic and toxicity profiles in basic animal models and there is reasonable evidence to justify their inclusion into early clinical trials. However, the best administration routes and the stage of infection at which treatment is administered (early or late infection) require critical consideration before initiation of any further investigation in a clinical setting.

### **Competing interests**

The authors declare that they have no competing interests.

### **Author's contributions**

SB conceived the project and along with AM prepared the first complete draft of the manuscript. All the other authors critically reviewed, revised, contributed significantly for its further improvement.

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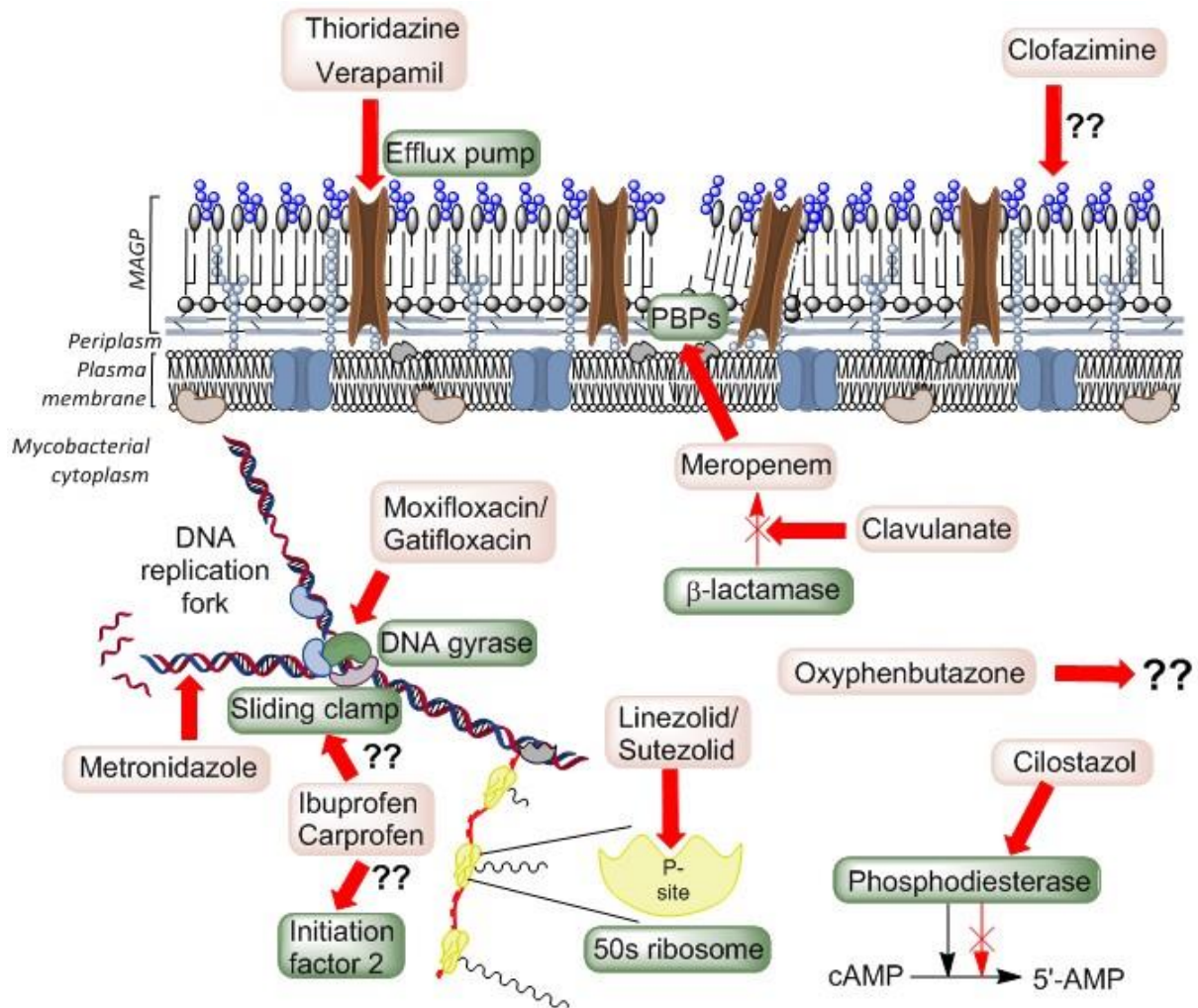
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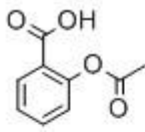
## Figure Legends

**Figure 1:** The possible endogenous mechanisms of action of repurposed drugs. The drugs and their targets are highlighted in the buff- and green-coloured boxes, respectively. The anagram MAGP is used to indicate the *mycolic acid-arabinogalactan-peptidoglycan* layer of the mycobacterial cell wall and PBP refers to the penicillin binding proteins responsible for the maturation of the cell wall peptidoglycan.

**Figure 2:** Chemical classes and the structures of the various NSAIDs under investigation for their antimicrobial properties

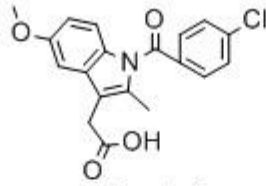


**Salicylic acid**

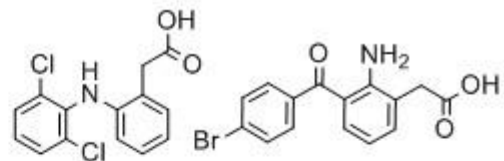


aspirin

**Acetic acid**



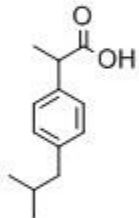
indometacin



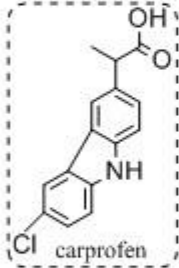
diclofenac

bromfenac

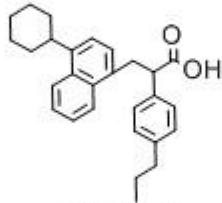
**Arylpropionic acid**



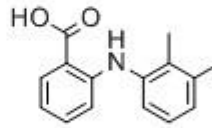
ibuprofen



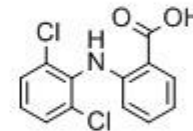
carprofen



vedaprofen

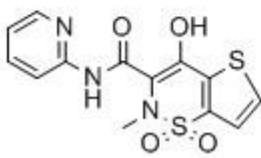


mefenamic acid

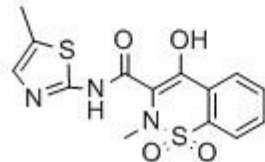


meclofenamic acid

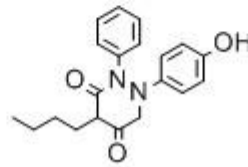
**Enolic acid**



tenoxicam

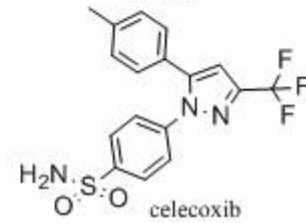


meloxicam



Oxyphenbutazone

**Coxib**



celecoxib