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# Desirable drug–drug interactions or when a matter of concern becomes a renewed therapeutic strategy

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*Keywords:* drug–drug interactions; boosters; multi-drug resistance; drug combination; drug design; therapeutic strategy.

*Teaser:* Using drug–drug interactions to boost the efficiency of a therapeutic treatment appears to be a relevant strategy and is now a modern drug design approach.

## Author biographies

### Benjamin Guieu

Benjamin Guieu studied pharmacy at the University of Rennes 1 and received his PharmD in 2019. He obtained his PhD in chemistry in 2017 for his work on the development of gold-catalyzed cyclization for the synthesis of heterocycles and their applications to medicinal chemistry. Since 2018, he has been a postdoctoral researchers in medicinal chemistry in CERMN, at the University of Caen Normandie, working on the design of new drug candidates for neurodegenerative diseases.

### Nicolas Willand

Nicolas Willand received his PhD from the University of Lille in 2003. After a postdoctoral stay at the School of Pharmacy in Lille, in the laboratory of André Tartar, he became an assistant professor in 2004. In 2013, Nicolas became a full professor and project leader for the development of new antimicrobial strategies in a research unit headed by Benoit Deprez, in collaboration with industrial partners, such as Bioversys AG and GSK. His team works on small drug-like molecules or biomimetic compounds, driving their optimization and contributing to the successful development of novel anti-infectious drug candidates.

### Christophe Rochais

Christophe Rochais received his PhD in 2005 from the University of Caen. After a postdoctoral fellowship at the University of Nottingham, he was appointed lecturer in the School of Pharmacy of Caen in 2007 and, in 2014, he became a professor. His research interests include medicinal chemistry programs in the field of enzymatic inhibition and GPCR modulation to develop pharmacological tools and bioactive compounds. He leads a research group dedicated to the development of pleiotropic agents of interest for Alzheimer's disease. He was recently appointed as a member of the French National Academy of Pharmacy.

**Drug–drug interactions are sometimes considered to be detrimental and responsible for adverse effects. In some cases, however, some are stakeholders of the efficiency of the treatment and this combinatorial strategy is exploited by some drug associations, including levodopa (L-Dopa) and dopadecarboxylase inhibitors,  $\beta$ -lactam antibiotics and clavulanic acid, 5-fluorouracil (5-FU) and folinic acid, and penicillin and probenecid. More recently, some drug–drug combinations have been integrated in modern drug design strategies, aiming to enhance the efficiency of already marketed drugs with new compounds acting not only as synergistic associations, but also as real boosters of activity. In this review, we provide an update of examples of such strategies, with a special focus on microbiology and oncology.**

## Introduction

Administering more than one drug into a living organism often leads to drug–drug interactions, regardless of the reason for the association: simultaneous treatment of several disorders or research into additive or synergistic effects towards a multifactorial disease or a drug resistance. Drug–drug interactions, if not managed through, for example, dose adjustment, are sometimes considered to be detrimental and are often responsible for adverse effects [1]. In a few cases, however, some are stakeholders of the efficiency of the treatment and this strategy has been exploited using drug associations such as L-Dopa and dopadecarboxylase inhibitors,  $\beta$ -lactams and clavulanic acid,

5-FU and folinic acid, and penicillin and probenecid. More recently, some drug–drug combinations have been integrated in a modern drug design strategy where the association of an already marketed drug with usually one adjuvant molecule improves both its activity and potency. These small molecules, also called enhancers, boosters, or activators, have few or no direct therapeutic effect on their own. However, combined with an established therapeutic agent, they can improve its activity, resulting in better treatment outcomes. This forms part of a polypharmacology approach to disease treatment and can also be inspiring for the design of novel pleiotropic ‘autobooster’ drugs.

In this review, we provide overview of enhancer-based strategies in therapy by describing well-known examples, as well as recent research, with a focus on microbiology and oncology. We limit our scope to recently reported synergistic effects resulting from an interaction between drugs, excluding those resulting from independent actions of drugs concomitantly administered, either toward the same target, or toward several targets in the same pathology [2]. Three distinct boosting effects have been identified (Figure 1): targeting an identified mechanism of resistance; inhibiting a metabolic pathway to enhance the pharmacokinetic (PK) profile of the primary drug; and enhancing a pharmacological activity.

### Overcoming resistance

Drug resistance is a major concern because multidrug resistant (MDR) phenotypes have emerged in both pathogens and tumoral cells. Targeting the proteins responsible for this acquired resistance to maintain the activity of well-known agents is a promising strategy in drug discovery.

#### *β-lactamases inhibitors*

β-lactam antibiotics are among the most used antimicrobial agents because of their broad spectrum, efficacy, and safety. Four main classes are in clinical use: penicillins, cephalosporins, carbapenems, and monobactams. In a similar manner to other antibiotics, the emergence and spread of bacterial resistance occurred in response to the extensive use of β-lactams. Resistance to penicillin treatments was observed early during the 1940s [3], followed by the identification of β-lactamases or β-lactam-hydrolyzing enzymes [4]. The discovery of clavulanic acid (**1**, Table 1) [5] led to the development of one of the major strategies to overcome β-lactamase-mediated resistance: the combination of β-lactams with β-lactamase inhibitors, represented by amoxicillin–clavulanic acid, one of the most well-known drug associations [6].

The clinical use of each generation of β-lactam antibiotics was followed by the spread of various β-lactamases through not only mutations of existing enzymes, but also plasmid dissemination. Currently, ~2800 different β-lactamase subtypes have been documented [7], subdivided into four classes based on their amino-acid sequence: Class A, C, and D are all serine β-lactamases, whereas class B comprises metallo-β-lactamases (reviewed in [8,9]). Clavulanic acid, sulbactam, and tazobactam (first-generation inhibitors) are effective against class A β-lactamases, but have no benefit against strains that express multiple β-lactamases. Thus, the dissemination of MDR Gram-negative bacteria expressing extended-spectra-β-lactamases, carbapenemases, or multiple β-lactamases, and the threat they represent, highlights the need to identify new, pluripotent inhibitors.

New series of β-lactam inhibitors have been approved recently or are in the pipeline for clinical use. The first series comprises diazabicyclooctanes (DBOs). Among them, avibactam (**2**) has been approved in combination with ceftazidime for the treatment of complicated urinary track or intra-abdominal infections caused by MDR Gram-negative bacteria. Avibactam acts as an inhibitor of clinically relevant class A and class C β-lactamases, and some class D enzymes. Contrary to previous inhibitors acting as ‘suicide’ inhibitors [10], avibactam binds enzymes in a covalent but reversible way [11]. Other combinations including a DBO currently under development include: imipenem–relebactam, aztreonam–avibactam, and meropenem–nacubactam.

Boronic acid compounds have also been investigated because of their potential to inhibit serine proteases. The combination meropenem–vaborbactam (**3**) was approved for the treatment of MDR Gram-negative pathogens. Vaborbactam inhibits class A and C β-lactamases as well as clinically relevant carbapenemases [12]. It acts as a reversible inhibitor, forming a reversible covalent bond with the enzymes. Another combination, cefepime–taniborbactam (**4**) is under development [13] and several novel β-lactamase inhibitors are currently being studied [14].

These studies paved the way for safer treatments of infections with MDR pathogens. Unfortunately, the ability of bacteria to adapt to environmental constraints is significant: some mutants resistant to these new combinations have already been identified. A possible solution is a derived approach involving a combination of cefepime with zidebactam (**5**), a bicycloacyl hydrazide derived from DBO that is efficient against bacteria expressing all four classes of β-lactamase [15]. Although zidebactam inhibits certain class A and class C β-lactamases, it has no action against class B and class D carbapenemases. This combination has a new mechanism of action involving the concomitant inhibition of several penicillin-binding proteins. Therefore, zidebactam is considered to act as a β-lactam enhancer, boosting the activity of cefepime, and providing a new way to overcome β-lactamase-related resistance.

#### *Inhibition of P-gp and other efflux systems*

Efflux pumps are membrane proteins that occur in all living cells and have diverse biological roles. However, their overexpression has been associated with MDR in both bacteria and cancerous cells: they limit the intracellular accumulation of drugs by expelling them, consequently decreasing their activity. Several classes of efflux pump

have been described in prokaryotes (six classes, reviewed in [16]) and eukaryotes (five groups [17]). Among them, the ATP-Binding Cassette (ABC) class is the largest known family, subdivided into seven subclasses (A–G). The most studied ABC transporter involved in MDR is ABCB1, the first member of the ABCB family, also known as P-glycoprotein (P-gp). Since its discovery in 1976 [18], overexpression of P-gp has been associated with resistance to common antitumoral molecules, such as taxanes, vinca alkaloids, and anthracyclines [19]. Moreover, this mechanism is associated with resistance to antibiotics such as tetracyclines, erythromycin, and fluoroquinolones. Therefore, the identification of efflux pump inhibitors (EPIs) is a leading strategy to overcome resistance in both pathogens and tumors.

Several generations of P-gp inhibitors have been developed [20]: the first generation comprises compounds used for other indications, such as verapamil [21] (**6**), reserpine, tamoxifen, or cyclosporin A. However, the lack of efficacy and specificity of these compounds, requiring high concentrations to be active, result in high toxicity. Structural modification led to a second generation including dexverapamil and valsopodar (MSC833) with greater affinity for P-gp but unpredictable interactions because of the concomitant inhibition of other ABC transporters or cytochrome P450 enzymes. A third generation of selective and potent inhibitors, comprising lozuquidar, laniquidar, elacridar, and tariquidar, was developed. Although preclinical results were encouraging, these molecules have shown unexpected clinical limitations because of toxicity and lack of efficacy [22]. Encequidar (HM-30181, **7**), a tetrahydroisoquinoline derivative, has proved to be a potent and selective inhibitor of ABCB1 [23]. Several clinical trials are ongoing for Oraxol, a combination of oral paclitaxel with encequidar studied for the enhancement of the oral bioavailability and efficacy of paclitaxel. However, few EPIs have reached clinical trials, with often poor results regarding toxicity at active concentration, PK behavior, and *in vivo* efficacy. Given the predominant role of ABCB1 in MDR, the development of specific, nontoxic, and efficient P-gp inhibitors is still needed.

Several approaches concerning the development of a fourth generation have been developed, recently reviewed elsewhere [24]. Given the variety of the substrates of efflux pumps, the molecules in development comprise not only many different chemical moieties, such as tetrahydroquinolines, 1,4-dihydropyridines, flavonoids (e.g., flavones and chalcones), and indoles, but also peptidomimetics. Recently, several studies have reported that some antibiotics can reverse ABC transporter-mediated MDR in cancer. For example, in 2019, ciprofloxacin, a fluoroquinolone, was shown to reverse ABCB1-mediated MDR in cancer by inhibiting its efflux function at nontoxic concentrations (**8**) [25]. Some promising compounds also display dual inhibition activity, being able to act on both P-gp and other MDR mediators, such as Breast Cancer Resistant Protein (BCRP), an efflux pump expressed in certain resistant tumors. Other molecules, such as the flavonoid taxifolin (**9**), inhibit both the expression and activity of P-gp [26].

Investigations regarding the clinical safety and efficacy of the last generation of P-gp inhibitors are still needed. However, this strategy holds promise for the treatment of both resistant cancers and bacterial infections. Furthermore, although P-gp is the most studied efflux pump, other systems are also being investigated. In microbiology, the strategy relies on the identification of the multiple efflux pump systems expressed by a specific pathogen to develop an adapted approach. Recently, Lamut *et al.* reviewed current research concerning EPIs for some WHO priority pathogens [27].

#### *Inhibition of RND-type multidrug efflux pumps*

The overexpression of multidrug efflux pumps is an important mechanism of resistance in Gram-negative bacteria. Most Gram-negative bacteria express at least one efflux pump from the resistance-nodulation-division (RND) family of transporters that expel endogenous hydrophobic molecules. In addition, bacterial genomes encode alternative efflux pumps that are induced upon stress, or through mutations resulting in constitutive overexpression and resistance to antibiotics. For example, acquired drug resistance was described through upregulation of *acrAB-tolC* in *Escherichia coli* and *Klebsiella pneumoniae*, dysregulation of *mexAB-oprM*, *mexXY-oprM*, and *mexCD-oprJ* in *Pseudomonas aeruginosa* and overexpression of *adeABC*, *adeIJK*, and *adeFGH* in *Actinobacter baumannii* [28–30].

Several EPIs have been described in the literature; however, none have yet reached clinical use. The peptidomimetic PAbN and analogs were among the first EPI molecules developed [31]. They inhibit RND pumps in both *E. coli* and *P. aeruginosa*. The most potent and drug-like EPIs described to date are the pyranopyridines of the MBX series, developed by Microbiotix, which bind the hydrophobic trap of AcrB and prevent broad-spectrum efflux [32]. The most advanced compound, MBX-4191 (50 mg/kg, twice daily; **10**) enabled the rescue of minocycline against a resistant strain of *K. pneumoniae* in a murine sepsis model of infection [33].

#### *Thioamide boosters to treat MDR-TB*

TB remains the major cause of mortality worldwide from a single infectious agent. It was responsible for the death of 1.45 million people in 2018 according to the WHO and 10 million new cases are reported each year. Moreover, it is estimated that one-third of the worldwide population is infected by the latent form of TB, of whom 10% would ultimately develop the disease [34]. A range of treatments is available but the emergence of MDR-TB and extensively drug-resistant TB (XDR-TB) strains highlight the need for new therapeutic alternatives.

Thioamides [ethionamide (ETH) and prothionamide (PTH)] are second-line drugs widely used for the treatment of MDR-TB. They are prodrugs, similar to numerous antitubercular agents, given that they must undergo chemical conversion inside the bacteria to become active. ETH was first synthesized in 1956 and was shown later to be bioactivated by the Baeyer-Villiger monooxygenases (BVMO) EthA [35,36]; the concomitant NAD-adduct formed inhibits InhA and the biosynthesis of mycolic acids [37,38]. More recently it was shown that ETH might be also the

substrate of two other BVMOs: MymA [39] and Rv0565c [40]. EthA expression is negatively regulated by EthR, which belongs to the TetR/CamR family of transcriptional repressors [41]. Thus, the limited bioactivation of ETH, implies the use of high therapeutic doses, leading to significant adverse effects, such as dose-related hepatotoxicity and gastrointestinal distress [42]. The usefulness of inhibiting EthR with a small molecule was realized when a genetic inactivation of the repressor showed overexpression of EthA and a concomitant hypersensitivity of the bacteria to ETH [37]. The validation of EthR as a druggable target was confirmed when the two first X-ray structures of the protein were published [43,44].

**Structure-based drug design** Based on these data, Willand *et al.* elaborated a model of low-molecular-weight inhibitors, which can bind the ligand-binding domain of EthR. A focused library of 131 compounds was selected, from a large inhouse library, based on this pharmacophore [45]. The capacity of the compounds to inhibit the interaction between EthR and the DNA sequence, to which it binds, was evaluated using surface plasmon resonance (SPR) assays. A 1,2,4-oxadiazole family was then highlighted and the co-crystallization of BDM14500 with EthR confirmed binding to EthR as expected; more importantly, the stabilization of a conformation of the liganded EthR incompatible with DNA binding. Extensive structure-activity relationship (SAR) studies were then performed [46,47]. The analogs were evaluated by SPR, as well as by a phenotypic assay on *Mycobacterium tuberculosis* (*Mtb*; H37Rv-GFP strains)-infected macrophages. In the latter, the potency of the compounds to boost subactive doses of ETH (MIC<sub>99/10</sub> = 0.1 mg/ml) was measured and expressed as EC<sub>50</sub>. From a synthetic library of >500 molecules, BDM41906 (K<sub>D</sub> = 14.8 mM, IC<sub>50</sub> = 0.4 mM, EC<sub>50</sub> = 60 nM; **11**) showed a large improvement of potency and a suitable pharmacological and PK profile suitable for *in vivo* studies. Oral administration of BDM41906 in combination with ETH led to a drastic bacterial load reduction compared with ETH alone. BDM41906 (20 mg/kg) in combination with ETH (12.5 mg/kg) was as efficient as ETH 50 mg/kg given alone [48].

**Fragment-based lead discovery** A fragment-based approach was also implemented by Villemagne *et al.* to optimize a new chemical series of ETH boosters. Fragment-based lead discovery has undergone remarkable changes over the past 25 years, and it is now able to provide hits and lead compounds in numerous anti-infective programs [49]. It relies on the use of low-molecular-weight molecules called fragments, which exhibit better physicochemical properties and are more prone to cross the *M. tuberculosis* complex cell envelope [50]. BDM15048 (IC<sub>50</sub> = 160 mM) served as a good starting point for this approach [51]. Based on the X-ray structure of BDM15048 co-crystallized with EthR, growing, linking, and merging strategies were designed to improve boosting activities while keeping suitable physicochemical and PK properties. The fragment growing strategy, where the structure of the binding fragment is expanded towards unoccupied pockets, was initiated from the synthesis of a virtual library of 960 analogs. *In silico* docking led to the synthesis of ten compounds, of which one was active. Analoging led to BDM43266, with great affinity for EthR ( $\Delta T_m = 11.2^\circ\text{C}$ , IC<sub>50</sub> = 0.40 mM), and a potency to boost ETH in the low nanomolar range (EC<sub>50</sub> = 80 nM). This high potency was explained by the formation of two simultaneous H-bonds with Asn179 and Asn176. Finally, replacement of the methylthiazole by a metabolically more stable cyclopropyl-1,2,4-oxadiazole moiety led to the discovery of BDM71339 ( $\Delta T_m = 10.2^\circ\text{C}$ , EC<sub>50</sub> = 72 nM, **12**), which showed activation *in vivo* [52].

**Serendipity** New insights recently reinforced the search for ETH boosters with the discovery of a Small Molecule Aborting Resistance (SMART) compound, which reverts resistance to ETH by the expression of an alternative bioactivation pathway [53]. Previously described molecules were able to boost the known ETH bioactivation pathway, but were, by definition, inactive on EthA mutated strains. During the optimization process, synthesis of the spiranic analog SMART-420 (**13**) led to a compound with no affinity for EthR but that remained active as ETH booster with an EC<sub>50</sub> in the low nanomolar range (EC<sub>50</sub> = 50 nM). Transcriptomic studies on *Mycobacterium bovis* BCG led to the identification of two overexpressed genes encoding an oxidoreductase (rv0077c, called EthA2), and a transcription factor from the TetR family (rv0078, called EthR2). Direct binding of SMART-420 to EthR2 was confirmed by trial sequential analysis (TSA) and X-ray structure analysis [54]. Subsequent SMART-420 inhibition of EthR2 led to the expression of EthA2, which was shown to be involved in ETH bioactivation. Co-administration of SMART-420 with ETH boosted ETH activity on sensitive strains and, more importantly, circumvented the ETH resistance of EthA-mutated strains. Further studies on *Mtb* confirmed that boosting of ETH bioactivation with BDM41906 or SMART-420 resulted in the formation of similar metabolites [55]. Restoration of sensitivity to ETH by SMART-420 was evaluated in C57BL/6/J mice infected by aerosol with ETH-resistant *Mtb*. Mice treated with a combination of ETH and SMART-420 (both at 50 mg/kg) showed a striking reduction in bacterial load (4.6 log) in the lungs. The anti-TB activity was confirmed to result from restoration of the sensitivity of this resistant strain to ETH [53].

Another thioamide bioactivation pathway could be explored with the recently described MymA operon. MymA is a mycobacterial BVMO, and from the six BVMOs in *Mtb*, MymA and EthA share the greatest sequence homology. Grant *et al.* showed that resistance to ETH was conferred whenever loss of MymA function was induced [56]. Moreover, overexpression of MymA improved ETH MIC<sub>90</sub> twofold, suggesting that MymA has a role in the bioactivation of ETH. Complementary selection of EthA and MymA mutants led to significantly higher levels of resistance to ETH compared with loss of function of EthA alone or MymA alone. Given that MymA is under the control of VirS, triggering this transcription factor might allow the overexpression of MymA and the concomitant boosting of ETH in an EthA-independent manner.

A promising clinical candidate, BVL-GSK098 (**14**) [57], has completed GLP (Good Laboratory Practice) toxicology studies and is being prepared for first-in-human trials in late 2020. In combination with low doses of ETH, BVL-

GSK098 renders ETH quickly bactericidal while overcoming the development of resistance to ETH. It is expected that BVL-GSK098 might reduce the efficacious human oral dose of ETH threefold, enabling researchers to fully harness the potential of this drug. BVL-GSK098 could be the first example of bacterial transcriptional regulators to be assessed in clinical trials. This work has also paved the way to new strategies that might be applied to other prodrugs, and might provide the opportunity to change some second-line treatment drugs to first-line drugs. Thus, reprogramming the bioactivation pathway of prodrugs through the derepression of cryptic pathways is a promising tool to enhance current treatments.

#### *Other approaches in oncology*

Besides these strategies, the increasing understanding of tumoral cells biology has led to the identification of various targets to restore antitumoral activity.

The role of reactive oxygen species (ROS) in cancer progression and MDR has been evidenced, and both ROS and antioxidant enzymes levels are higher in tumoral cells. Thus, the modulation of ROS levels appears to be a strategy to target and sensitize tumoral cells, regardless of the mechanism of resistance. Given that ROS modulators display a proper specific activity on cancer cells, they cannot be considered as specific boosters of antitumoral agents. However, several key enzymes implied in ROS regulation are being targeted to restore anticancer drugs activity in resistant cells (reviewed in [58]).

The acidity of the tumor microenvironment is also a major player in tumor progression, dissemination, and chemoresistance, because the low pH compromises the efficacy of numerous anticancer agents. Intracellular proton pumps, in particular vacuolar type ATPases (V-ATPases), have an important role in the acidification of the extracellular medium [59]. Thus, several studies have demonstrated the use of proton pump inhibitors (PPIs) to overcome tumor resistance. As an example, He *et al.* reported promising results using esomeprazole to overcome YAP-related paclitaxel resistance in ovarian cancer (**15**) [60].

In EGFR-mutant nonsmall-cell lung cancer, *de novo* resistance to EGFR tyrosine kinase inhibitors (TKIs) is often associated with an epithelial–mesenchymal transition (EMT) phenotype [61]: Epithelial cells lose their characteristics, such as polarity and cell–cell interactions, to adopt a mesenchymal phenotype, leading to an increase in their invasive and resistance properties. TWIST1 is a transcription factor involved in the EMT process, and its expression has been associated with metastasis and therapeutic resistance through suppression of apoptosis. Yochum *et al.* demonstrated the benefit of targeting TWIST1 with the alkaloid harmine (**16**) to restore the activity of TKIs, such as osimertinib [62].

Antimetabolite-based DNA-damaging agents, such as cytarabine, hydroxyurea, or gemcitabine chemotherapeutics, can be potentiated by Checkpoint kinase 1 (Chk1) inhibitors [63]. Indeed, these stress-replicating drugs promote DNA damage or replication stress and trigger the ATR/Chk1 pathway. Chk1 mediates S and G<sub>2</sub> cell cycle checkpoints, leading to a transient delay in cell-cycle progression and initiation of DNA repair. Inhibition of Chk1 induces checkpoint failure, and cells enter mitosis with unrepaired DNA, resulting in cell death. Chk1 inhibitors induced sensitization to gemcitabine or hydroxyurea in cancer cell lines, particularly in tumor cells lacking functional p53 [64–66]. GDC-0425 (**17**), an orally bioavailable, small-molecule inhibitor of Chk1, was studied in combination with gemcitabine for the treatment of patients with refractory solid tumors in a Phase I trial [67]. In parallel to the determination of the safety and tolerability of GDC-0425 in combination with the cytotoxic drug, the authors studied the antitumor activity of this association. Preliminary signs of clinical activity were observed, especially in patients with TP53-mutant tumors. However, the authors highlighted the risk of increased toxicity. Oo *et al.* described reduced normal tissue toxicity whenever gemcitabine was replaced with hydroxyurea (HU), with equal efficacy to gemcitabine [68]. The chemopotential in p53 mutated tumor cells is also under investigation because the G<sub>1</sub> checkpoint is damaged in these cells. Thus, it is expected that the ability of tumour cells to repair DNA would be even more complex [69].

#### **Pharmacokinetics enhancers**

PK issues sometimes limit the efficacy of a treatment. Therefore, several strategies have been developed to circumvent this limitation by increasing exposure and, thus, enhancing the activity of a molecule. Here, we review several strategies involving metabolism or excretion inhibition.

#### *Probenecid*

As an historical example, the use of probenecid, an uricosuric agent (**18**; Table 2), in combination with penicillin, prevents the urinary excretion of antimicrobial agents, allowing the use of smaller doses [70]. More precisely, probenecid, a moderately lipophilic sulfamide, interacts in a competitive manner with penicillin for organic anion transporters in the kidney tubules, via which the antibiotic is excreted. Furthermore, probenecid also competes with penicillin for binding sites on albumin. It was originally developed during World War II to extend the limited supplies of penicillin. Subsequent studies showed that probenecid is also effective in decreasing the excretion of other antibiotics, such as quinolones [71].

#### *Levodopa metabolism in Parkinson's disease*

A well-known example of a PK enhancer is used for the treatment of Parkinson's disease (PD). L-Dopa was introduced for the treatment of PD during the 1960s. It is a precursor of dopamine and is able to cross the blood–brain barrier and enter the brain, where it is converted to dopamine. The major issue to using L-Dopa alone is its rapid degradation by peripheral enzymes, in particular dopa-decarboxylase (DDC), resulting in low systemic

exposure and very low brain delivery (only 1%). Thus, a crucial improvement of L-Dopa therapy was the discovery of DDC inhibitors [72], which do not enter the brain but act selectively on peripheral DDC. The combination of L-Dopa with carbidopa or benserazide [73] (**19** and **20**) has become systematic and leads to a significant reduction in the dose required to obtain maximal benefit, as well as a reduction in the adverse effects related to peripheral action, such as vomiting, nausea, or cardiac arrhythmia. However, L-Dopa treatment still needs to be improved to obtain continuous brain stimulation instead of the pulsatile delivery observed after oral intake. Thus, strategies, including cotherapies, are being developed to optimize the pharmacological effect of the L-Dopa/DDC inhibitor combination [74].

DDC inhibition shifted the peripheral metabolism to Catechol-O-Methyl-Transferase (COMT), an enzyme involved in the catabolism of catecholamine neurotransmitters. An increase in 3-O-Methyl-Dopamine (3-OMD) was observed and, given that it competes with L-Dopa for the transporter systems, COMT was identified during the 1990s as a target to improve L-Dopa PK [75]. Indeed, cotherapy with a COMT inhibitor improved the gastrointestinal absorption and brain delivery of L-Dopa. Additionally, it reduced fluctuating L-Dopa plasma levels and ameliorated motor complications (reviewed in [76]). Three COMT inhibitors are currently on the market: Entacapone and opicapone (**21** and **22**), which are peripheral-acting inhibitors, thus reducing systemic degradation of L-Dopa, and tolcapone (**23**), a central nervous system (CNS)-active inhibitor, allowing a reduction in the metabolism of both L-Dopa and dopamine. However, tolcapone causes serious hepatotoxicity and other adverse effects, limiting its use to patients who do not respond adequately to a peripherally acting inhibitor. One oral formulation, Stalevo®, combines L-Dopa, carbidopa, and entacapone to facilitate maintenance of patients with PD. Several hypotheses suggest that L-Dopa/DDC inhibitor treatments should be initiated in combination with COMT and MAO-B inhibitors to prevent fluctuations of the dopamine level [77]. MAO-B inhibitors, such as rasagiline or selegiline, have an effect on the oxidative metabolism of physiological dopamine in the brain, but cannot be classified as pharmacological enhancers because they show therapeutic activity on their own, and were not designed to improve a co-administered drug.

#### *CYP3A4 inhibitors in HIV treatment*

Ritonavir is a protease inhibitor that was initially developed as an HIV inhibitor, but its weak antiretroviral capacities and inadequate adverse effect profile at the required dosage limited this role. However, ritonavir is a strong inhibitor of CYP enzymes, especially CYP3A4, and also P-gp transporters. Thus, at lower doses, ritonavir is better tolerated and effective for enhancing the PK properties of other antiretroviral agents (**24**) [78], especially drugs such as lopinavir, atazanavir, or darunavir. The co-administration of ritonavir helps to maintain the therapeutic serum levels of the drugs, allowing a less frequent intake for the patient, as well as improved treatment efficacy. Ritonavir is also combined with paritaprevir for the treatment of hepatitis C, where it guarantees long-lasting plasma levels of the protease NS3-4A inhibitor.

Cobicistat (**25**) is a structural analog of ritonavir that does not have intrinsic inhibitory activity on the HIV protease. It was developed specifically as a PK enhancer [79] and is generally considered as an equipotent inhibitor of CYP3A4 and CYP2D6 to ritonavir; however, there are still important PK differences between the two agents, which could lead to clinically significant distinctions in drug interaction outcomes (reviewed in [80]). Cobicistat is marketed as a fixed-dose combination with protease inhibitors (i.e., atazanavir and darunavir) and the integrase inhibitor elvitegravir. A change from ritonavir to cobicistat-boosted regimens showed greater treatment satisfaction and minimal differences in adverse effects [81]. Although cobicistat was claimed to display almost no inhibitory activity on other CYP enzymes, it was recently demonstrated that there was no significant difference between cobicistat and ritonavir in selectivity regarding CYP enzymes including 2B6, 2C9, 2C19, and 2D6 [82].

The development of new pharmaco-enhancers for antiretroviral therapy remains a challenge. Several promising compounds were identified during the early 2010s, such as SPI-452 and TMC-558445, but their development appears to have been cancelled. Recently, Sevrioukova's group developed a pharmacophore model to design new CYP3A4-specific inhibitors [83]. This rational approach provided promising results in the development of inhibitors more potent than ritonavir [84].

#### *Dihydropyrimidine dehydrogenase inhibitors*

5-FU is an antimetabolite among the anticancer drugs most commonly used for solid cancers since 1957. It acts as a pyrimidine analog and disrupts RNA and DNA synthesis by inhibiting thymidylate synthase. However, >60% of the administered drug is metabolised and excreted in urine within 24 h [85]. 5-FU is degraded by dihydropyrimidine dehydrogenase (DPD), which is abundant in the gastrointestinal tract, liver, and peripheral blood mononuclear cells. Variability in the activity of this enzyme has been described, and is responsible for the interpatient variability in 5-FU PK and oral bioavailability. As a result, DPD inhibition was identified as an efficient approach to enhance the therapeutic effects of 5-FU. Several formulations have been developed [86]. First, a combination of uracil with tegafur, a 5-FU prodrug, in a 4:1 ratio (**26**): tegafur is metabolized into 5-FU by the hepatic mitochondrial system, and uracil is a natural substrate of DPD. Thus, the excess uracil acts as a competitive inhibitor, saturating DPD to inhibit 5-FU catabolism, resulting in higher plasma concentrations [87]. Another formulation, S-1, combined tegafur with gimeracil (5-chloro-2,4-dihydroxypyridine, **27**), a more potent DPD-inhibitor, and oteracil, a potassium oxonate, in a molar ratio of 1:0.4:1 [88]. Gimeracil favors the continuous release of 5-FU, whereas oxonate decreases the gastrointestinal toxicity of tegafur. By inhibiting the orotate phosphoribosyltransferase in intestinal cells

specifically, it decreases the conversion of 5-FU into cytotoxic metabolites, suppressing the toxic effects of 5-FU in the mucosal cells.

#### *Endosomal escape enhancers*

Targeted protein toxins, also called immunotoxins, represent a promising strategy for cancer treatment. They involve artificial proteins comprising a targeting moiety, in most of the cases an antibody-based domain, linked to a toxin specifically selected for the targeting of tumor cells. However, although many targeted toxins have been investigated over the past few decades, none have been approved for therapeutic use to date. In fact, targeting toxins is not enough to ensure their efficacy: in most of the cases, the protein toxins take effect within the cytosol of the cell. Then, one of the major issues is the lysosomal trapping and degradation of the toxins, which significantly limits their effectiveness and clinical applications. As a result, strategies to promote the endosomal escape of the immunotoxins have been developed. Endosomal escape enhancers (EEE) comprise not only small chemical molecules, but also peptides or proteins from viruses, bacteria or eukaryotes (reviewed in [89]). Here, we focus on chemical substances. Several chemical enhancers have been described to increase the efficacy of targeted toxins. As for all EEE, these molecules must not be toxic or induce the uptake of the toxin in regular cells. Furthermore, they must be degradable or excretable, and should not interfere with other metabolic processes in the organism. Subgroups have been identified based on their chemical structure and mechanism of action: lysosomotropic amines, carboxylic ionophores, and calcium channel antagonists.

Lysosomotropic amines were identified during the early 1980s. They act as proton reservoirs through their amine groups, increasing the pH of lysosomes, thus interrupting protein degradation by pH-dependent lysosomal enzymes. The first studied compounds were ammonium chloride and chloroquine (**28**), the effects of which were observed in the case of targeted toxins based on the ricin toxin A chain [90]. As an example, the immunotoxin T101-RTA was enhanced 6700-fold and 2500-fold by ammonium chloride and chloroquine, respectively. Other molecules were identified, such as amantadine, quinacrine, methylamines, and lipopolyamines.

Another successful family is that of carboxylic ionophores, which mediate the exchange of cations across the membrane, resulting in an increase in lysosomal pH. Monensin (**29**), the most-studied molecule, exchanges sodium ions and protons [91]. The cytotoxicity of several toxins containing the ricin toxin A chain is greatly enhanced by monensin, such as T101-RTA (50 000 fold) [90]. Grisorexin, lasalocid, and nigericin are other examples of carboxylic ionophores that have potentiated the cytotoxic effects of targeted toxins.

Calcium channels antagonists have also demonstrated enhancing effects on the cytotoxicity of targeted toxins. Verapamil and analogs were identified as enhancers for *Pseudomonas* exotoxins and also the ricin A chain (**30**) [92]. Within this group, the most potent enhancing effect was observed with perhexiline (**31**), which increases the cytotoxicity of two ricin chain A immunotoxins up to 2000-fold in leukemia cells. The enhancing activity is not directly related to the calcium-antagonistic activity, but appears to rely on the inhibition of lysosomal degradation. Concerning perhexilin and indolizines, the delay of the degradation might result from inhibition of the acid lysosomal sphingomyelinase, causing a shift in the membrane lipid composition of organelles, thus facilitating the release of the toxin in the cytosol [93].

Cyclosporin A (**32**), commonly used as an immunosuppressive agent, has shown enhancing activities with *Pseudomonas* exotoxin-based and ricin A chain immunotoxins *in vitro* and *in vivo* [94]. Cyclosporin A alone has very low activity on cell viability. This boosting effect probably results from a mechanism different from its immunosuppressive activity, because other immunosuppressors, such as tacrolimus and sirolimus, did not show any enhancing effect. Recently, Song *et al.* reported the cyclosporin A-enhancing effect on MAP30-S3, a fusion between MAP30, a ribosome-inactivating protein reported to have apoptotic effects on cancer cells, and S3, an epidermal growth factor receptor (EGFR)-targeting peptide, through endosomal escape [95].

Other organic compounds have demonstrated their ability to enhance the targeted toxin activity. However, because their mechanism of action is not yet elucidated, they cannot be considered as PK enhancers.

Despite several years of research and promising results, no immunotoxins have been approved for cancer treatment. One of the major concern is the low cytosolic uptake of the toxin, which requires an increase in dosage and, concomitantly, the toxicity. Moreover, EEEs often lack specificity, because they are not targeted to tumoral cells: thus, they can then enhance adverse effects on nontargeted cells. Finally, because the toxin and enhancer need to be at the interaction site simultaneously, PK parameters are often difficult to manage. Therefore, although targeting toxins specifically to tumoral cells appears promising, the development of EEEs remains a challenge in terms of improving the endosomal escape rate and accordingly decreasing the dosage of immunotoxins required.

#### **Pharmacological enhancers**

Some adjuvant molecules provide a synergistic effect through pharmacological enhancement of the established treatment. The mechanisms underlying the enhancing effects are not always fully understood, but open the way to the development of new therapeutic strategies.

#### *Adjuvant therapies for 5-FU treatment*

As discussed earlier, 5-FU remains one of the major therapeutic agents especially for the treatment of digestive cancers. In addition to PK enhancers, several adjuvants have been studied to improve the activity of 5-FU in surgically resected colon cancer treatment. The first identified adjuvant was levamisole (**33**), which reduced the mortality rate by 33% [96]. Levamisole was first used as an anthelmintic agent, but its immunomodulatory



activity attracted interest in oncology. Synergy with 5-FU was discovered empirically, and is still not fully understood. It might be linked to the increase in the expression of HLA class I molecules on tumor cells [97]. Subsequently, the combination of 5-FU with folinic acid (leucovorin, **34**), became the standard adjuvant chemotherapy for stage III colon cancer [98]. Folinic acid increases the affinity of 5-FU for thymidylate synthase, thus improving its inhibitory activity. However, it can also increase the adverse effects of 5-FU. Currently, the 5-FU/folinic acid association is combined with irinotecan and/or oxaliplatin for use in chemotherapy.

#### *Immunotoxin enhancers*

As developed earlier, some immunotoxins enhancers might act through mechanisms other than EEE. As an example, retinoic acid (**35**) has shown enhancing activity regarding ricin A chain-containing toxins, probably relying on an effect on the Golgi apparatus and vesicular routing [99]. Wortmannin, a fungi metabolite (**36**) known as a phosphatidylinositol-3-kinase (PI3K) inhibitor, enhances the cytotoxicity of saporin- and gelonin-based targeted toxins. This boosting effect might occur through an alternative mechanism not involving the PI3K inhibitory effect [100].

#### *Atovaquone and proguanil*

Atovaquone is a hydroxynaphthoquinone that is used as antimalarial agent. It selectively inhibits the parasitic mitochondrial electron transport, collapsing the mitochondrial transmembrane potential and, ultimately, disrupting nucleic acid synthesis. However, the use of atovaquone as a single agent against *Plasmodium falciparum* leads to the rapid development of resistance. To enhance its antimalarial activity, several other antimalarial compounds have been tested *in vitro* in combination with atovaquone. Among them, proguanil (**37**), a biguanide, significantly increased the ability of atovaquone to collapse mitochondrial *trans*-membrane potential [101]. Alone, the activity of proguanil is based on the primary metabolite cycloguanil, which is an inhibitor of the parasite dihydrofolate reductase, but it demonstrated weak activity *in vitro* and resistance is frequent.

Although the mechanism of interaction is not fully understood, the enhancement of atovaquone activity by proguanil appears to involve mechanisms other than the dihydrofolate reductase activity. This synergistic action results from the biguanide form, because cycloguanil and other dihydrofolate reductase inhibitors, such as pyrimethamine, did not show any enhancing effect. The good tolerability and similar PK profile of atovaquone and proguanil (reviewed in [102]) identified this combination as a reference for antimalarial prophylaxis, including for resistant strains.

#### *Recent research*

A few studies have identified new enhancer-based combinations over the past few years. In 2019, Kitabayashi *et al.* identified kenpaullone (**38**), an inhibitor of glycogen synthase kinase GSK3 $\beta$ , as an enhancer of the alkylating agent temozolomide in glioblastoma [103]. However, the mechanism involved is not fully understood. Through the inhibition of GSK3 $\beta$ , kenpaullone might attenuate the stem-cell properties of glioblastoma cells, thus increasing the apoptosis-inducing activity of temozolomide.

Recently, Perrone *et al.* reported the enhancing effect of Carba1, a microtubule-destabilizing carbazole derivative (**39**), on the activity of paclitaxel in tumoral cells [104]. Although their mechanisms of action are antagonistic, because paclitaxel stabilizes the microtubules, modulation of the dynamics of tubulin by Carba1 was demonstrated to favor the accumulation of paclitaxel in the microtubules, resulting in improved efficiency.

#### **Concluding remarks**

Drug–drug interactions for enhancer-based therapeutic strategies have been developed for several decades and are becoming increasingly relevant within the frame of a modern drug design approach, especially in oncology and microbiology. In that regard, the design of potent boosters recently allowed the renewal of old and safe drugs, the efficacy of which has been dramatically recovered. This useful strategy has sometimes benefited from facilitated access to large chemical databases annotated with biological activities, allowing crucial mining and integration of complex ligand–target relations. Such models, leading to a network multitarget pharmacology, appear as potent tools for the design of further drug associations [105–108].

However, this polymedication approach can sometimes have limitations, in particular linked to a possible enhancement of adverse effects or because of compliance issues. This is why it could evolve into the design of dual agents, which are unable to display a synergistic effect through their action on several targets, as observed with classical multitarget directed ligands, but that can act as genuine autoboosters. In this regard, examples of such prototypes have been recently reported with conjugates between antibiotics and siderophores and are considered as adequate vehicles for the transport of antimicrobials towards pathogenic microbes [109]. We have no doubt that this aut-booster approach will be widely developed in the near future.

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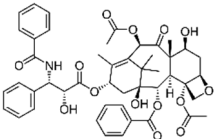
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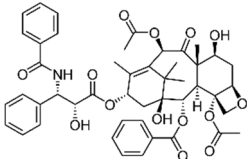
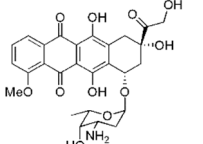
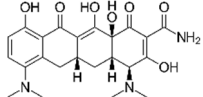
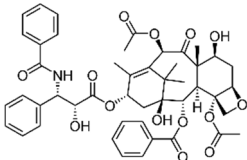
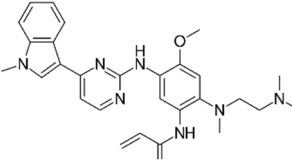
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**Figure 1.** Schematic representation of different strategies to display a boosted therapeutic activity through positive drug–drug interactions.

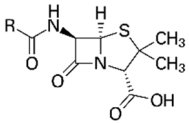
**Table 1. Drug combination to overcome a mechanism of resistance**

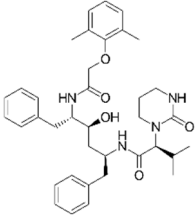
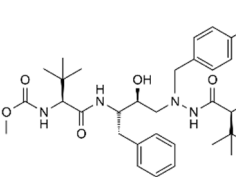
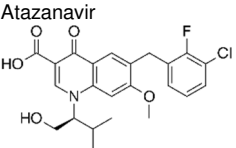
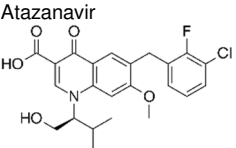
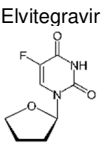
	Bioactive compound	Structure	Co-administered molecule	Inhibitor structure	Mechanism of action	Pathology	Current clinical status [97]	Refs
1	Amoxicillin		Clavulanic acid		Class A $\beta$ -lactamase inhibitor	Bacterial infections	Marketed	[6]
2	Ceftazidime		Avibactam		Class A and class C $\beta$ -lactamase inhibitor	Bacterial infections	Marketed	[11]
3	Meropenem		Vaborbactam		Class A and C $\beta$ -lactamase and carbapenemase inhibitor	Bacterial infections	Marketed	[12]
4	Cefepime		Taniborbactam		Broad spectrum $\beta$ -lactamases inhibitor	Bacterial infections	Phase III recruiting; [13] NCT03840148	
5	Cefepime		Zidebactam		Penicillin-binding proteins inhibitor	Bacterial infections	Phase I completed; [15] NCT02942810	
6	Antimicrobial agents Anticancer drugs		Verapamil		P-gp EPI	Bacterial infections and cancer	Phase I recruiting; [21] NCT03013933	
7	Paclitaxel		Encequidar (HM30181)		P-gp EPI	Breast cancer	Phase I recruiting; [23] NCT04168957	

8	Paclitaxel		Ciprofloxacin	P-gp EPI	MDR cancer overexpressing ABCB-1 transporters	Phase II recruiting; [25] NCT02773732
9	Doxorubicin		Taxifolin	P-gp EPI	MDR cancer	No clinical trial [26]
10	Minocycline		MBX-4191	AcrA/B-ToIC EPI	Bacterial infections	No clinical trial [33]
11	ETH		BDM41906	Mycobacterial transcription factor	MDR TB	No clinical trial [48]
12	ETH		BDM71339	Mycobacterial transcription factor	MDR TB	No clinical trial [52]
13	ETH		SMART-420	Mycobacterial transcription factor	MDR TB	No clinical trial [54]
14	ETH		BVL-GSK098	Mycobacterial transcription factor	MDR TB	Phase I [57]
15	Paclitaxel		Esomeprazole	Intracellular PPI	Epithelial ovarian cancer	No clinical trial [60]
16	Osimertinib		Harmine	Transcription factor TWIST1	EGFR-mutant non-small-cell lung cancer	No clinical trial [62]

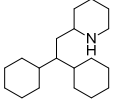
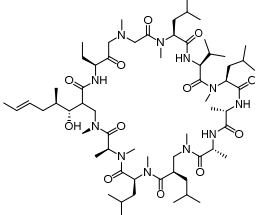
17	Gemcitabine	GDC-0425	Chk1 inhibitor	Refractory solid tumors	Phase I	[67]
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**Table 2. Drug combination to enhance the pharmacokinetic profile**

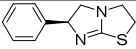
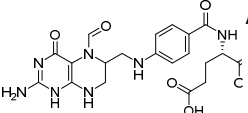
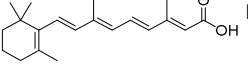
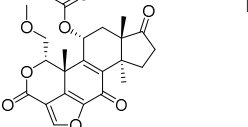
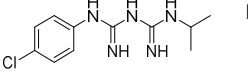
	Bioactive compound	Structure	Co-administered molecule	Structure	Mechanism of action	Pathology	Current clinical status [97]	Refs
18	Penicillin, quinolones		Probenecid		Urinary excretion inhibitor	Bacterial infections	Withdrawn	[70]
19	L-Dopa		Carbidopa		Dopa decarboxylase inhibitor	PD	Marketed	[73]
20	L-Dopa		Benserazide		Dopa decarboxylase inhibitor	PD	Marketed	[74]
21	L-Dopa		Entacapone		Peripheral COMT inhibition	PD	Marketed	[77]
22	L-Dopa		Opicapone		Peripheral COMT inhibition	PD	Phase III completed; NCT01227655	[77]
23	L-Dopa		Tolcapone		CNS COMT inhibition	PD	Marketed	[77]

24	Antiretroviral drugs (lopinavir, atazanavir, darunavir)		Ritonavir	CYP3A4 inhibitor	HIV	Marketed	[78]
		Lopinavir					
25	Protease inhibitors (atazanavir, darunavir); integrase inhibitors (elvitegravir)		Cobicistat	CYP3A4 inhibitor	HIV	Marketed	[79]
		Atazanavir					
			Elvitegravir				
26	Tegafur		Uracil	Dihydropyrimidine dehydrogenase (DDH) inhibitor	Solid tumors	Marketed	[87]
27	Tegafur		Gimeracil	DDH inhibitor	Solid cancers	Phase II recruiting; NCT04515615	[88]
28	Immunotoxins based on ricin A chain		Chloroquine (Lysosomotropic amines)	Endosomal escape enhancer	Cancer	No Clinical Trial	[90]
29	Immunotoxins. based on ricin A chain		Monensin (carboxylic ionophore)	Endosomal escape enhancer	Cancer	No clinical trial	[91]
30	Immunotoxins; Ricin A chain; <i>Pseudomonas</i> exotoxins		Verapamil and analogues	Endosomal escape enhancer	Cancer	No clinical trial	[92]



31	Immunotoxins; based on ricin A chain	Perhexilin		Endosomal escape enhancer	Cancer	No clinical trial	[93]
32	Immunotoxins; Ricin A chain; <i>Pseudomonas</i> exotoxins; MAP30-S3	Cyclosporin A		Endosomal escape enhancer	Cancer	No clinical trial	[94,95]

**Table 3. Drug combination to enhance the pharmacological activity**

Bioactive compound	Structure	Co-administered molecule	Structure	Mechanism of action	Pathology	Current clinical status [97]	Refs
33	5-FU	Levamisole		Adjuvant therapy	Colon cancer	No clinical trial	[97]
34	5-FU	Folinic acid (leucovorin)		Adjuvant therapy	Colon cancer	Phase II recruiting; NCT03044587	[98]
35	Immunotoxins based on ricin A chain	Retinoic acid		Pharmacological enhancer	Cancer	No clinical trial	[99]
36	Targeted toxins based on saponin and gelonin	Wortmannin		Pharmacological enhancer	Cancer	No clinical trial	[100]
37	Atovaquone	Proguanil		Pharmacological enhancer	Malaria prophylaxis	Marketed	[101]

38 Temozolomide

Kenpaullone

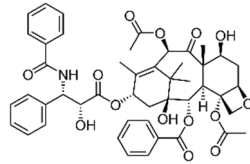
GSK3 $\beta$  inhibitor

Glioblastoma

No clinical trial

[103]

39 Paclitaxel



Carba-1

Microtubule-destabilizing agent

Cancer

No clinical trial

[104]

