RESEARCH ARTICLE

The potential for new and resilient anti-cancer drugs based upon minor groove binders for DNA

A prospective view based on research at the University of Strathclyde.

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Abstract
Anti-infective and anticancer drugs share the serious problem that over time resistance develops to their effects leading to clinical obsolescence. Research at the University of Strathclyde has discovered a platform of anti-infective drugs based upon minor groove binders for DNA that have exceptional resilience to the development of resistance in their target organisms (bacteria, fungi, and parasites). This property is associated with the fact that the Strathclyde minor groove binders (S-MGBs) act at more than one discrete molecular target. One of the compounds has successfully completed a phase IIa clinical trial for the treatment of *Clostridioides difficile* infections. Several other compounds have shown activity against a number of cancer cell lines *in vitro* with indications of *in vivo* activity in a mouse model of lung cancer. This paper places these discoveries in the context of previous studies of minor groove binders as anticancer agents and considers whether the benefits of multitargeting successfully demonstrated in anti-infective applications can be translated to anticancer applications.

1. Drug resistance and resilience in infectious disease and in cancer

It is hard to imagine, unless you have had the experience yourself, the dismay in finding that your cancer is no longer responding to the drug treatment regimen.[1] Similarly, it must be distressing to hear from your clinician that your infection is untreatable with available drugs.[2] Therefore, both cancer and infectious disease treatment share the limitation of resistance to available drugs, inherent or acquired. And both share some underlying biological mechanisms that are consequences of evolutionary pressures on the cancer cell or the pathogen by the drug. From the point of view of this discussion, structural mutation of the drug target is the most relevant, although increased drug metabolism and efflux play major roles in practice. It is clearly important that drug discovery in both cancer and anti-infective therapy should take account of these challenges; however, there are two substantial contextual differences between the two. Firstly, infectious disease, as is so obvious from the COVID-19 pandemic, is a public health issue. Secondly, the standard commercial reward model for new drugs in infectious disease is broken. Both factors conspire to make anti-infective drug discovery, in particular the effects of drug resistance, a more difficult field to navigate. Nonetheless, there are underlying principles emerging as a result of these challenges that we should take cognisance of within anticancer drug discovery to develop approaches for new drugs to be resilient to resistance. With the challenges of emerging resistance to available medicines, this article provides a short review of minor groove binders for DNA as anticancer drugs and then explores how research into anti-infective and anticancer drugs at the University of Strathclyde offers a way forward.

Most of the studies at the University of Strathclyde have concerned infectious disease but because of the common underlying biological background, it is reasonable to suggest that what has been learned in discovering novel anti-infective treatments can cross over to anticancer therapy. Research at Strathclyde has indeed led to interesting anticancer compounds as well as highly active anti-infective compounds, as will be discussed later in the Strathclyde Minor Groove Binder (S-MGB) program.

2. What can be done?

It has been common in drug discovery to develop a molecule with high selectivity to a specific biological target, most commonly a protein. In principle this is thought to lead to predictable therapeutic outcomes and straightforward development giving a simple story to satisfy the regulators. This ‘single drug – single target – single effect’ discovery paradigm is becoming less dominant, not least because it is unrealistic; many drugs with a primary target turn out to act through multiple mechanisms. It is, nevertheless, entirely reasonable to attribute the effectiveness of a drug to its interaction with its primary target because if this is lost, so is the therapeutic benefit. A second reason to consider more than one target for a drug is that the disease may genuinely require multiple interventions to be successfully treated, which is the principle of polypharmacology approaches. The first research at Strathclyde into multi-targeted drugs took up this challenge more that 20 years ago in the case of schizophrenia for which the therapeutic rationale required a compound capable of acting as a 5-HT_7 antagonist and an M_4 agonist. The approach was successful at least to the extent of effectiveness in an animal model of the disease.[3]

Whilst the Strathclyde schizophrenia research was an advance, the compounds themselves were in fact little more than two separate drugs acting at one of the target receptors linked by a very short chain, an amidine. Linking two molecules active at

separate but relevant targets has become standard, known as a conjugation approach, in designing drugs for complex diseases, including cancer.[4,5] Beyond simple conjugation, the ideal multi-targeted drug is a single molecule with the recognition elements in itself without auxiliary linked components – a hybrid approach. This is not fanciful and whilst perhaps not recognised at the time of its original design, the highly successful anticancer drug, pemetrexed, is an outstanding example (Chemical structures of compounds mentioned in the text are collected in the Appendix).[6] Pemetrexed interacts with several enzymes that take part in the folate-mediated synthesis of nucleotide components for DNA, thymidylate synthase, dihydrofolate reductase, and glycinamide ribonucleotide formyltransferase. It is now a very significant contributor to lung cancer chemotherapy.

In cancer chemotherapy in general, it has been argued that many drugs designed to target specific proteins actually exert their therapeutic effect through off-target action.[7] For example, PF-3758309 is the first p21-activated kinase (PAK) inhibitor to enter clinical trials for Adult T-cell leukemia/lymphoma.[8] However, Lin et al. have recently demonstrated that this drug's cell killing effects are not significantly compromised in cancer cells lacking its putative target, suggesting its ability to block cancer cell growth must be through an off-target effect.[7]

Whether the polypharmocological effect is intentional or serendipitous in the drug design, it illustrates a common factor: there is a recognition element for the drug on the target proteins that is shared by the targets, a pharmacophore for several proteins. Such common features would seem to be relevant when considering the design of a new multi-targeted drug. Multitargeting is therefore a valid alternative for the discovery of new drugs. The important consequence of this is that such drugs should not only benefit in terms of efficacy from engaging with two or more relevant targets but should also prove resilient to mutation of one of the targets. This is not to say that multitargeting is the only appropriate current approach to the design of anticancer drugs. It is an alternative. Indeed, in a current anticancer project at Strathclyde, inhibitors of IKKα for prostate cancer treatment, exquisite sensitivity for the target is required to mitigate the risk of debilitating side effects for the patient.[9]

In the discovery of any novel therapeutic agent consideration of toxicity and selectivity is central. Whilst this is true for the more 'traditional' protein drug targets that have dominated drug discovery, it is perhaps a greater concern for drugs that target DNA. Indeed, instinctively, drugs that target DNA and its functions might be considered to be risky prospects, but the evidence from both anticancer and anti-infective drug fields that sufficient selectivity and safety can be obtained is extensive. The operative word here is 'sufficient' and it is certainly the case that clinicians would welcome compounds with improved safety margins in cancer therapy and that patients would applaud reductions in side effects. Building a drug discovery project on DNA binding compounds is therefore a risk, but a balanced risk if the experimental programme is sensitive to the requirements for a new medicine.

3. Minor Groove Binders in cancer chemotherapy

The designation 'minor groove binder' (MGB) refers to the mode of binding of a small molecule to DNA and not to a specific family of compounds. Indeed, there are several classes of MGB that have already featured in cancer therapy that interact with DNA by forming a covalent bond with one of the nucleobases (usually guanine). For example,

the duocarmycins are highly potent minor groove binding alkylating agents that are too intrinsically toxic for clinical use.[10,11] They consist of a DNA binding component and an alkylating component that operates by an elegant chemical mechanism in which the actual alkylating functional group is latent in the duocarmycin molecule itself. Their high potency, however, makes them suitable for investigation as antibody conjugates targeted to cancer cells, and this is an area of current investigation. The pyrrolobenzodiazepines (PBDs) are a second class of alkylating anticancer MGB that have been extensively studied in clinical trials.[12,13] Although PBDs have been investigated for activity against solid tumours and haematological malignancies, current clinical investigations also feature antibody conjugates. Trabectidin is a totally different case of a minor groove binding anticancer compound.[14] Unlike duocarmycins and PBDs, it is hard to see intuitively how trabectidin binds to DNA. Yet binding to the minor groove DNA is one part of its mechanism of action. Trabectidin (Yondelis®) is available clinically for the treatment of soft-tissue sarcomas and ovarian cancer. It has been approved by the US FDA and EU EMA as an orphan drug for these indications.

The Strathclyde family of minor groove binders (S-MGBs) is structurally based upon the natural products, distamycin and netropsin. These natural product templates, which are oligoamides of pyrrole amino acids, have been studied previously as the basis for anticancer compounds. Tallimustine combined the alkylating substructure of a typical nitrogen mustard with the DNA-binding ability of a pyrrole oligoamide.[15] It has been evaluated in clinical trials up to Phase II for soft tissue sarcoma but was found to have dose limiting myelotoxicity. Brostacillin has a similar basic design, an alkylating agent attached to a DNA binding oligoamide, but the alkylating agent is latent, requiring intracellular activation.[16] It too has reached Phase II clinical trials but showed insufficient efficacy combined with limiting toxicity for further development.[17]

It would seem from these extensive studies with other minor groove binders that compounds whose primary mechanism of attack is DNA alkylation are likely to have insufficient selectivity and too great toxicity for development as anticancer drugs. The question can then be asked whether minor groove binders without alkylating properties can be effective in anticancer applications. This is one of the challenges that has been put to S-MGBs, which intentionally lack DNA alkylating capabilities. In the context of DNA, S-MGBs are small molecule ligands based upon the distamycin and netropsin templates with flexibility for pharmacological manipulation. Most of the compounds for which biological activity has been established (see section 4) bind to AT rich sequences (Figure 1) with a range of preferences but not with absolute selectivity, as might be required in a kinase inhibitor, for example. There are many similar short sites in genomic DNA and consequently small changes in the structure of such DNA are unlikely to prevent effective binding of an S-MGB. In this sense, S-MGBs are intrinsically multitargeted, a property that militates against the emergence of resistance. These two properties of controllable toxicity through the avoidance of alkylating properties together with a strategy that promotes resilience to resistance are at the heart of successful and promising applications of S-MGBs in both anti-infective and anticancer applications. Before describing what has been discovered for S-MGBs in anticancer applications, including lung cancer, we summarise what has been achieved with anti-infective S-MGBs to identify those features that can be translated into anticancer applications.

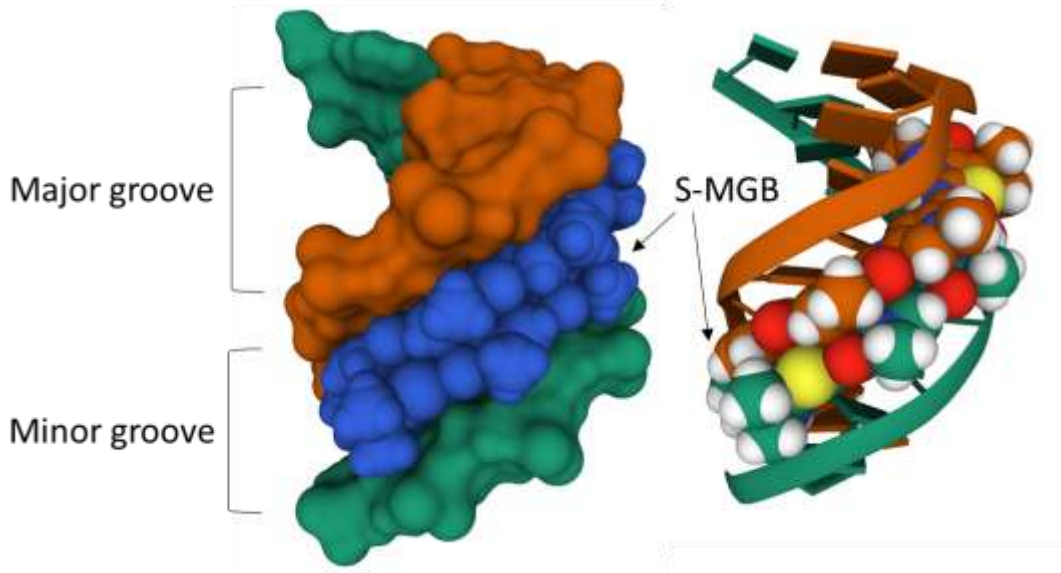

Figure 1: S-MGB-54 binding to the minor groove of the dsDNA oligomer, d(CGACTAGTCG)$_2$. Two molecules of S-MGB bind within the minor groove. Left hand image presents DNA as a molecular surface, with each chain coloured red or green, and the S-MGB is presented as a space filling model, coloured blue. Right hand image presents DNA as a cartoon model, with each chain coloured red or green, and the S-MGB is presented as a space filling model, coloured based on element type. The images are prepared from the PDB entry 2MNE.

4. What have we achieved with anti-infective S-MGBs

As can readily be understood from the above, DNA is a special drug target. Since all organisms use DNA, in principle, if toxicity can be avoided, it should be possible to treat infections caused by a wide range of pathogens, bacteria, fungi, parasites, and viruses, to which, of course, cancer can be added. S-MGBs have been shown to be effective not only in the clinical trial of MGB-BP-3 for *C. difficile* infections but also in several animal models of different infectious diseases. Such animal models serve as proof-of-concept experiments that validate the compounds for lead optimisation and preclinical studies. The proof-of-concept study is usually the end point of the academic drug discovery phase although mechanism of action research continues. In summary, S-MGBs have been shown to be effective in treating the following infections; points of interest that could be significant in anticancer therapy are noted.

a. In *in vivo* studies at the University of Capetown, S-MGB-364 successfully reduced the bacterial load in a mouse model of tuberculosis using the multidrug resistant HN878.[18,19,20] There was also a favourable immunological response in which a reduction in the recruitment of eosinophils was especially significant. Dosing was intranasal and the active compounds could also be formulated in non-ionic surfactant vesicles (NIVs).
b. The fungal disease, aspergillosis, caused by *Aspergillus fumigatus* has been studied in collaboration with the University of Manchester.[18,21] Aspergillosis is a disease that affects especially immune-compromised patients, including those undertaking cancer chemotherapy. S-MGB-363 was found to provide a

substantial prolongation of life in the mouse comparable to that afforded by the clinically used posaconazole. There is strong evidence from fluorescence microscopy that the effectiveness of an S-MGB in antifungal applications depends greatly upon its uptake into the fungal cell before engaging with the nucleus, a feature that is probably significant in obtaining high selectivity with respect to mammalian cells. S-MGB-363 is active against drug resistant strains of fungi, including the emergent *Candida auris*, and also retains its activity after multiple passages of the fungus.

c. S-MGBS have not only been shown to be effective against human pathogens, but also against animal pathogens. Studies of Animal African Trypanosomiasis with the University of Glasgow and the Swiss Tropical and Public Health Institute have shown that S-MGB-234, and S-MGB-235 are able to cure trypanosomiasis caused by several species of the trypanosome parasite in mouse models.[22] The activity against several species of trypanosome is in itself unusual and to an extent justifies the design strategy of S-MGBs. These S-MGBs are also active against parasite strains resistant to existing drugs (diminazene and pentamidine, for example) and show high resilience to the development of resistance. It has been found that S-MGBs are also active *in vitro* against Leishmaniasis and malaria parasites.[23]
d. The most advanced S-MGB is MGB-BP-3, which has been developed to be phase III ready for the treatment of *Clostridioides difficile* infections by our partner company, MGB Biopharma.[24] Its development builds directly upon the laboratory chemical and microbiological results from Strathclyde through preclinical research and chemical synthesis scale up. In 2020 MGB-BP-3 successfully completed a phase IIa clinical trial in which it was shown to be safe, well-tolerated, and curative in patients with recurrent infection. Dosing was oral using a gastric capsule formulation. Mechanism of action studies have shown that MGB-BP-3 engages with DNA at *pdnaD* and *pmraY* loci in the model Gram positive bacterium, *Staphylococcus aureus* which blocks the action of a number of essential genes.[25] It also inhibits bacterial topoisomerases and gyrases (unpublished results). A contribution to MGB-BP-3's high selectivity in the clinic is its minimal absorption from the GIT leading to strong physical targeting of the drug to the site of the disease. The kill is so rapid that *C. difficile* is unable to adapt to sporulate which leads to essentially absence of disease recurrence. Lastly, it has not so far been possible to produce a resistant strain of *S. aureus in vitro* despite 80 passages with MGB-BP-3. In short, MGB-BP-3 does exactly what the design says it should: safe to use in patients, rapid action, and resilient to resistance.

From the above summaries, S-MGBs are clearly effective and developable in the anti-infective context. They are also multitargeted in that they engage with DNA at several sites and interfere with DNA processing, factors that must contribute to resilience to resistance. Now can these properties be translated to cancer and lung cancer in particular? Safety and selectivity are largely obtained by manipulating the physicochemical properties of the S-MGB through parts of the structure that do not interfere with DNA binding. Toxicity is generally consistent with standard medicinal chemical expectations that the more lipophilic the S-MGB is, the more likely it is to be toxic. Whilst the trends can be spotted, the specific requirements for each application must be found by experiment. This is as true for anticancer activity as it is for anti-infective activity.

5. Translating the field: S-MGBs in cancer

An important pointer to the potential utility of S-MGBs is their non-alkylating properties. There have been relatively few studies of non-alkylating MGBs as anticancer agents based upon distamycin and none has yet reached preclinical development. Yet taking the properties of anti-infective S-MGBs together with cell-based studies of anticancer properties of distamycin-based MGBs it is possible to see a strong way ahead for the discovery of anticancer MGBs. Such studies have come from groups at Strathclyde and Huddersfield, and an International Group of scientists with contributions from Austria, Holland, UK and USA.

Scientists from the International Group were interested in MGBs that interfered with *TOP2A* gene expression, which encodes for topoisomerase IIα.[26,27] Having found MGBs with the required basic activity (ref. 27, compound **3**) research turned to improving the properties of this compound with respect to binding affinity for a specific DNA target site (5'-TACGAT-3') which is close to the ICB2 site, a CCAAT box, in the *TOP2A* gene promoter. Interestingly and generally, for an MGB to have an effect on the binding of a transcription factor, it is better for it to bind close to but not at the transcription factor's binding site alone; in this way, the conformation of DNA is distorted to prevent transcription factor binding but there is no direct competition with the transcription factor itself. A series of variations in structure (ref. 27, compound **3**) was made to investigate the effects of small changes in molecular shape and improved compounds for binding were indeed found with (ref. 27, compound **6b 6e**) being the most sequence selective. The analogue with the greatest binding affinity (ref. 27, compound **6a**), however, was unable to enter cells and had no effect on the expression of the *TOP2A* gene in cell-based experiments, despite its activity extracellularly.

These results are an important demonstration that strong and selective binding to a specific DNA sequence are not in themselves sufficient criteria for the discovery of a potential anticancer MGB. Cellular and subsequently nuclear uptake are critical. With these points, experience at Strathclyde would concur. There is, however, one very significant difference. The International Group's MGBs have been designed to target a specific DNA sequence and the function of a specific gene. In other words, they are single target MGBs. If that target should mutate, for example to remove G, a large part of the affinity and therefore potential effectiveness could be lost. Such a problem has not yet been demonstrated but it is for this reason that S-MGBs are designed to target general AT rich sequences of DNA. Evidence for the benefits of this strategy have been outlined for anti-infective properties in the previous section. Nevertheless, it is fair to point out that S-MGBs with similarly tight DNA-binding selectivity have been obtained taking advantage of binding to GC base pairs and some of these have significant anticancer activity.[28,29]

Studies of S-MGBs in anticancer applications have investigated *in vitro* activity against a number of cancers including a human colon carcinoma (HCT116), a cisplatin sensitive and cisplatin resistant ovarian cell line (A2780, A2780cis) and melanoma cancer (B16-F10) in a model of lung cancer. In the study of the first three, carried out in collaboration with scientists at the University of Huddersfield activity against the cancer cell lines was compared a human non-cancerous retinal epithelial cell line (ARPE19).[29] The central points of this study were to establish that S-MGBs are effective and selective as anticancer agents, are active against cell lines resistant to standard therapy (cisplatin in this case), and do not act by an alkylation

mechanism. Several S-MGBs (4, 74, and 317) were identified with activities comparable to cisplatin and carboplatin *in vitro* but with better selectivity indices [Table 1]. It was also found that by comparing the effects of S-MGBs in cisplatin sensitive and insensitive cell lines that there was no cross-resistance suggesting a different mechanism of action from alkylation. This was supported by evidence that S-MGB 4 does not induce DNA double strand breaks through the DNA damage stress protein sensor, p53. Comparing the physicochemical properties of the S-MGBs studied suggested that for such cellular activity, there was indeed a sweet spot in terms of the lipophilicity of the compounds in terms of the selectivity indices with a logD $_{7.4}$ value of about 2.5.

Table 1. Anticancer activity of selected S-MGBs against ovarian and colon cancer cell lines. IC_{50} (μM ± SD)

Compound	A2780 ovarian	A2780 resistant	HCT116 colon	ARPE19 non-cancer	$LogD_{7.4}$
S-MGB-4	0.89 ± 0.20	1.12 ± 0.27	1.33 ± 0.12	> 10	2.48
S-MGB-74	0.16 ± 0.01	1.51 ± 0.17	> 10	> 10	1.05
S-MGB-317	1.46 ± 0.01	1.08 ± 0.05	2.12 ± 0.75	>10	2.42
cisplatin	1.47 ± 0.04	10.27 ± 1.77	3.26 ± 0.38	6.41 ± 0.95	

In the other study of S-MGBs from an exploratory set of 47 structurally diverse compounds, five were found to be significantly active, comparable or better to that of a standard therapy, gemcitabine.[30] [Table 2] As noted above, the target cell line was B16-f10-luc. The best compound, S-MGB 176, had an activity of about 70-fold greater than that of gemcitabine, which when combined with an exceptionally favourable selectivity index of 125, makes S-MGB-176 a significant starting point for lead optimization. All the high activity compounds had $logD_{7.4}$ values between 3.5 and 5 perhaps suggesting a necessary minimum lipophilicity required for activity. In support of this, S-MGB-176 was found to be very stable metabolically in rat hepatocytes with only 1% of oxidized metabolites being detectable. At first sight, therefore, S-MGB-176 is a promising lead for the discovery of a new range of treatments for lung cancer.

Table 2. Activity of selected S-MGBs against B16-f10-luc cells

compound	IC_{50} (μM ± SD)	$logD_{7.4}$
S-MGB-176	0.16 ± 0.01	4.85
S-MGB-196	0.81 ± 0.08	3.51
S-MGB-201	1.1 ± 0.51	4.50
S-MGB-222	10.4 ± 0.5	2.95
S-MGB-269	2.2 ± 0.22	5.45
gemcitabine	11.0 ± 1.7	-

6. Conclusions: from anti-infective S-MGBs to anticancer drugs

In summary, the anti-infective studies have clearly established that S-MGBs are druggable, disease targetable, and when appropriately developed and formulated, safe and effective in patients. Oral, intraperitoneal, and intranasal administration routes have all been used with formulations including gastric tablets, non-ionic surfactant vesicles, and solution. Importantly, in each of antibacterial, antifungal and antiparasitic applications, activity against strains resistant to currently used drugs has been found together with resilience to the development of resistance to the S-MGBs themselves. Multitargeting, as has been established in the case of MGB-BP-3, pays off. S-MGBs do just what they were designed to do. With respect to intrinsic i*n vitro* activity against several types of cancer cells, exemplar S-MGBs were found to have activities strongly competitive with those of the established drugs, cisplatin and gemcitabine. S-MGBs with significantly better selectivity indices than the established drugs were identified.

For the discovery of anticancer drugs, S-MGBs and similar compounds such as those studied by the International Group have significant advantages over other classes of minor groove binder. There is a wide range of structural variation possible to obtain the required profile. The binding affinity to DNA of S-MGBs is much lower than that of MGBs such as the PBDs and duocarmycins, which bind to DNA covalently, but they are much less toxic. Moreover covalent binding distamycin analogues, which are more closely related to the structure of S-MGBs, such as tallimustine and brostallicin, have not succeeded in the clinic. The non-covalent interaction of S-MGBs with DNA could therefore be a substantial advantage in therapeutic terms. Indeed, in other fields of medicinal chemistry, with the exception of some protease inhibitors, there has often been a reluctance to develop compounds that act by covalent bonding because of concerns about toxicity.

In terms of resilience of new anti-cancer compounds, the multiple mechanisms of action of S-MGBs, established for antibacterial activity and indicated in other anti-infective applications, could transfer the benefits of combination therapy through one compound, an S-MGB, engaging targets associated with DNA function including DNA itself just as pemetrexed has done acting in the folate field inhibiting the biosynthesis of nucleobases and nucleotides.

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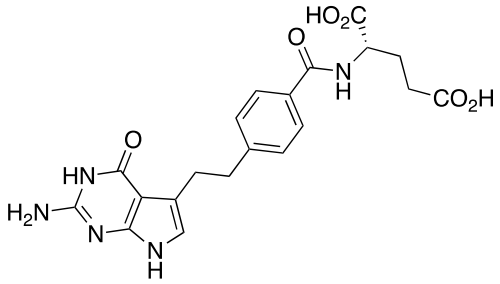
APPENDIX

Structures of compounds mentioned in the text



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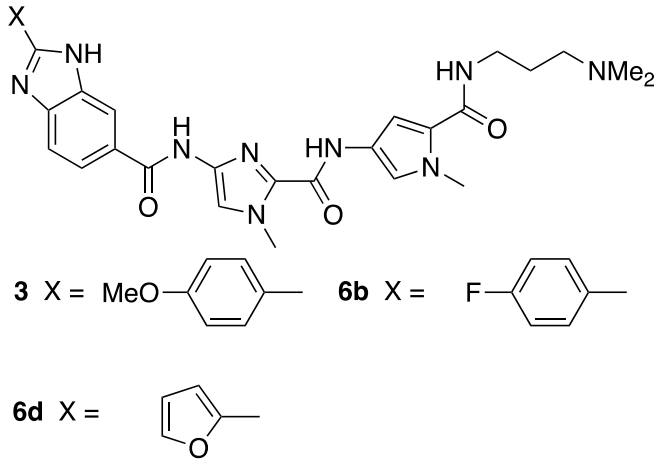


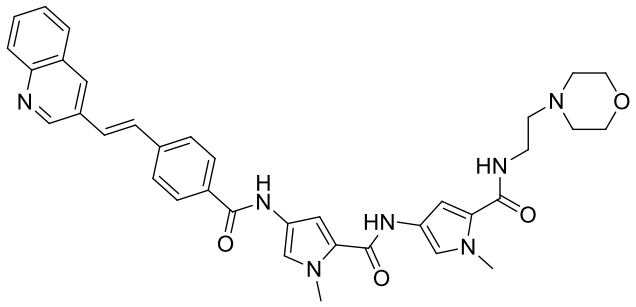
3 X = MeO **6b** X = F

6d X =

Anticancer MGBs from the International Group

STRATHCLYDE S-MGBS

MGB-BP-3



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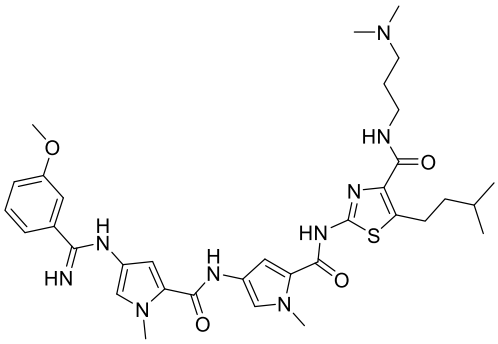

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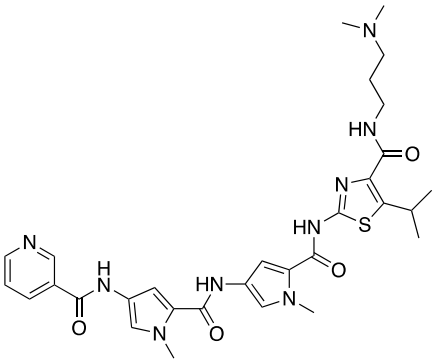

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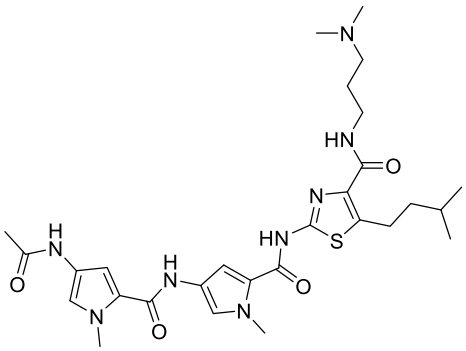

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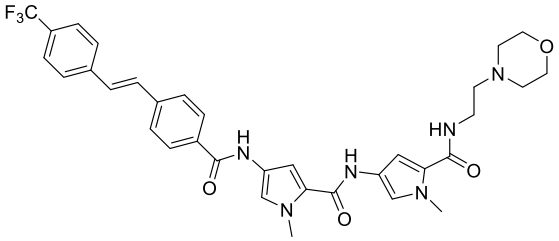

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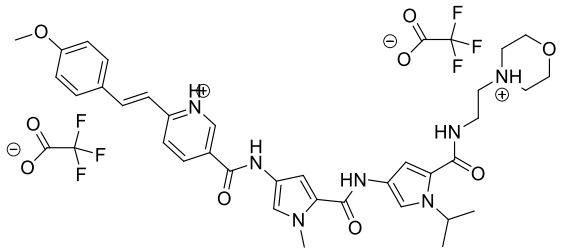

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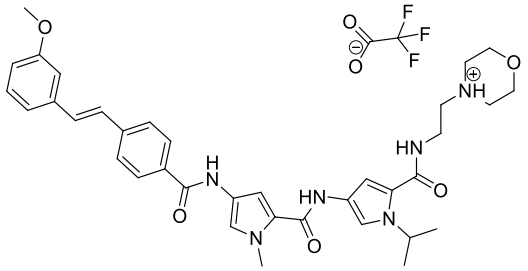

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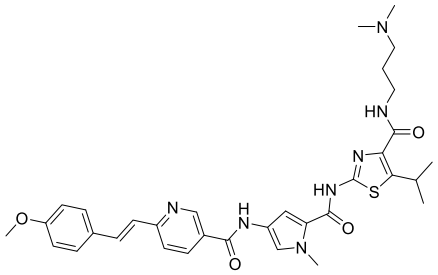

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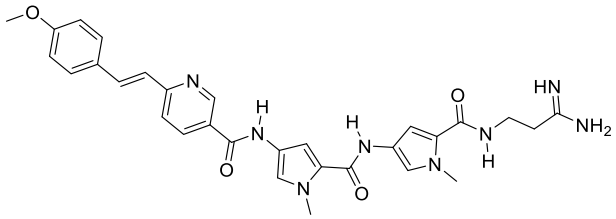

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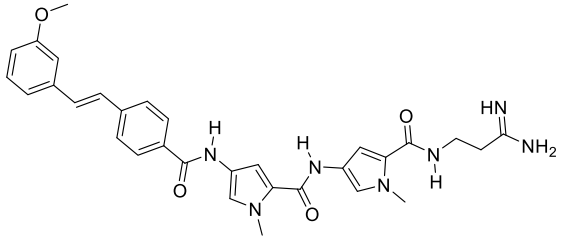

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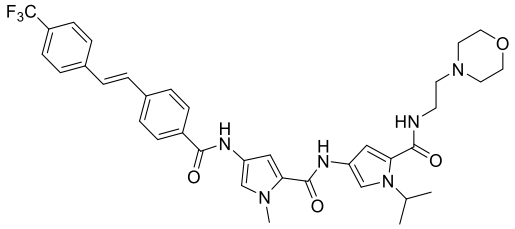

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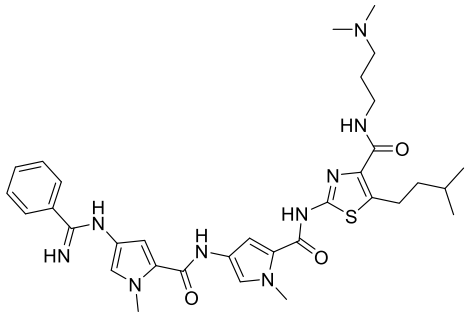

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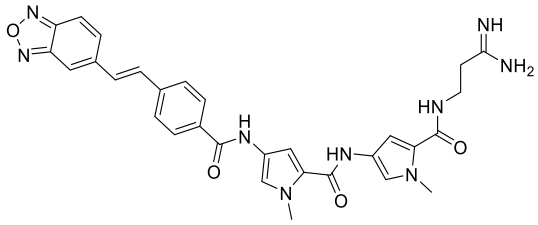

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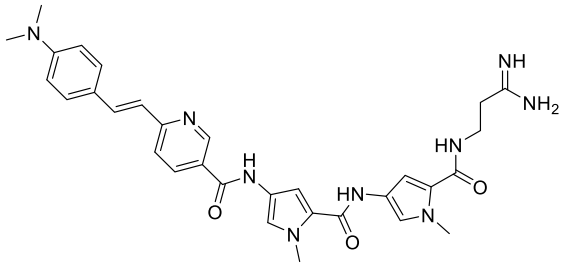

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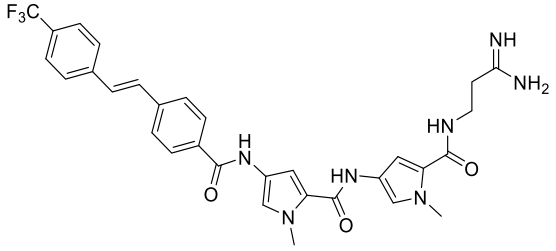
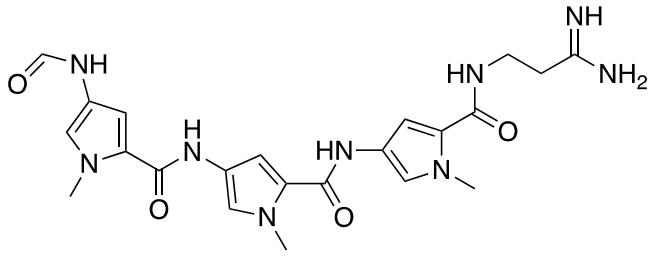


MINOR GROOVE BINDERS MENTIONED BY NAME IN THE TEXT



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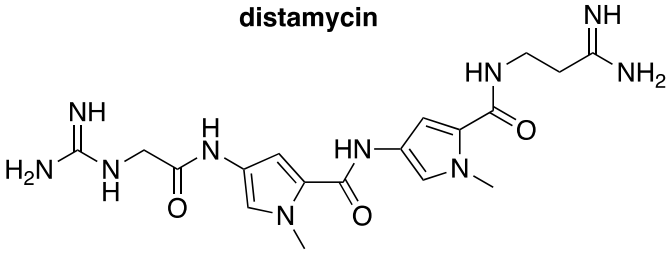

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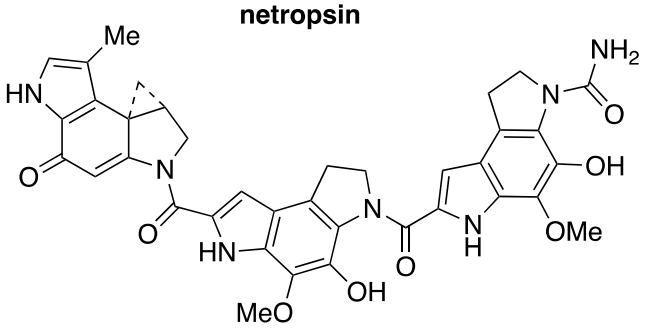

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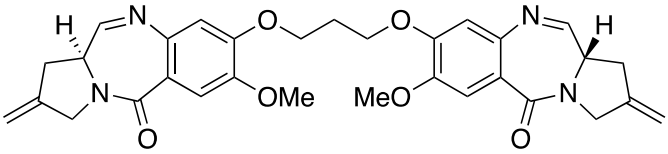
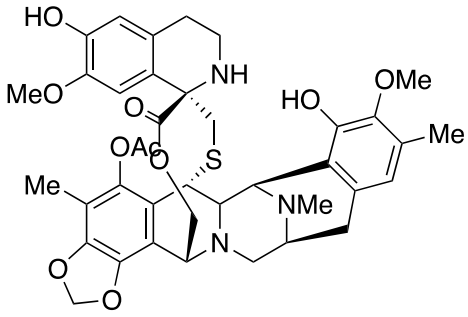


A linked dimeric pyrrolobenzodiazepine (PBD) SJG136



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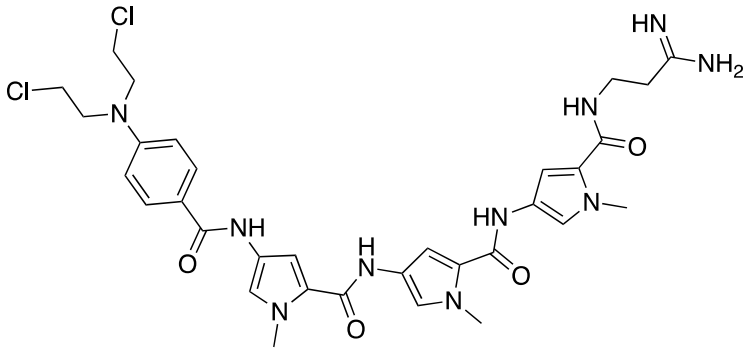

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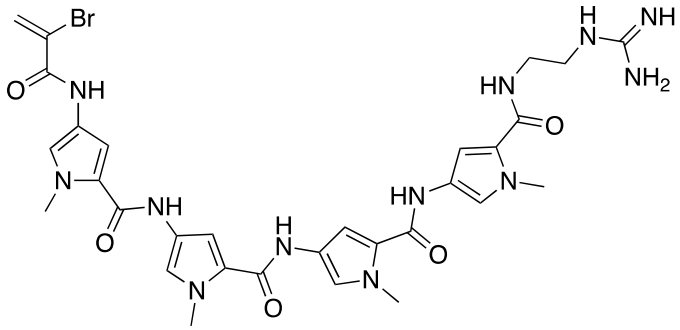
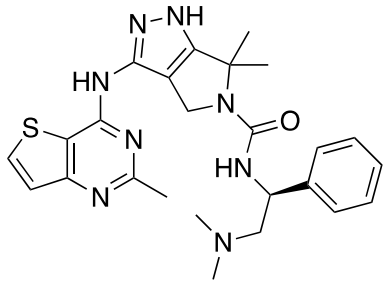


Brostallicin



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