บทความรับเชิญ

การออกแบบโมเลกุลของสารยับยั้งแบคทีเรียที่ออกฤทธิ์สามฤทธิ์ร่วมกันในโมเลกุลเดียว และสารโปร-ดรักส์

จอห์น บี เบรมเนอร์*

บทคัดย่อ

การเพิ่มขึ้นของเชื้อแบคทีเรียดื้อยาที่ก่อโรคในมนุษย์เป็นปัญหาด้านสุขภาพทั่วโลก ความท้าทายในการแก้ปัญหานี้ทำให้มีการเพิ่มความพยายามในการวิจัยแบบสหสาขาวิชามากขึ้น หนึ่งในความพยายามนั้นคือการพัฒนาโมเลกุลของสารยับยั้งเชื้อแบคทีเรียดื้อยาให้มีการออกฤทธิ์ยับยั้งเชื้อได้มากกว่าหนึ่งเป้าหมายในเซลล์ของแบคทีเรีย บทความนี้ได้กล่าวครอบคลุมถึงสิ่งที่ต้องพิจารณาในการออกแบบโมเลกุล ความเป็นไปได้ในการสังเคราะห์สารยับยั้งเชื้อแบคทีเรียที่ในโมเลกุลเดียวกันสามารถเกิดอันตรกิริยากับเป้าหมายในเซลล์ของแบคทีเรียได้ถึงสามเป้าหมายและออกฤทธิ์ร่วมกันอย่างมีประสิทธิภาพในการยับยั้งเชื้อแบคทีเรีย และกล่าวถึงสารโปร-ดรักส์ของสารยับยั้งเชื้อแบคทีเรียดังกล่าว โดยเฉพาะอย่างยิ่งการออกแบบและการสังเคราะห์สารยับยั้งเชื้อแบคทีเรียที่มีโครงสร้างของสารอัลคาลอยด์เบอร์เบอรีนเป็นองค์ประกอบ

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Molecular Design of Potential Triple-Action Antibacterial Agents and Related Pro-Drugs

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ABSTRACT

Increasing antibiotic resistance by human pathogenic bacteria is a global health concern. This health challenge calls for increased multi-disciplinary research efforts to address the issue and one such effort is concerned with the development of novel single molecule agents with more than one bacterial target site for action or interaction. This review covers molecular design considerations and some possible synthetic molecular entities for potential triple-action antibacterial agents and related pro-drugs, particularly those based on the quaternary alkaloid berberine.

Keywords: Antimicrobial agents, triple-action, pro-drugs, drug design

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Introduction

A number of recent health reports and papers [1-5] have highlighted the growing threat of increasing resistance to antibiotics of human pathogenic bacteria, especially some Gram-negative bacteria. This is a major health care issue worldwide and presents key multi-disciplinary research challenges. One strategy that has been adopted is the use of drug combinations. These can include two separate antibacterials with complementary activities [6] or one antibacterial with a second agent not necessarily antibacterial but which may enhance the activity of the other by blocking, for example, a resistance mechanism [7-9] or by aiding antibiotic penetration as in the case of the drug pentamidine which in Gram-negative bacteria disrupts the outer membrane and allows ingress by antibiotics to then attack the pathogenic bacteria [10]. More recently this has been extended to combinations of three separate drugs with some promising results [11-13]. Synergistic interactions have been observed with the combination of thymol, ethylendiaminetetraacetic acid (EDTA), and vancomycin, including a 16-fold enhancement in sensitivity of the Gram-negative pathogenic bacterium *Escherichia coli* (*E. coli*) [12]. The phenolic monoterpene thymol has antibacterial properties while the glycopeptide antibiotic vancomycin affects cell wall synthesis and is used for the treatment of infections caused by Gram-positive bacteria. EDTA, a chelating agent for Ca^{2+} and Mg^{2+} ions, was included as these ions are important in bacterial cell wall protection (particularly in Gram-negative bacteria) and it was thought that if this protection was compromised by chelation then other antibacterials might be more effective [12]. This did in fact seem to be the case. In other recent elegant work, Yeh and co-workers [11] have shown that the triple drug combination of ciprofloxacin, clindamycin and streptomycin revealed a lethal emergent antibacterial synergy against *E. coli,* although other triple antibiotic combinations did not. They have also developed [14] a very interesting framework to evaluate potentially therapeutically valuable synergies from three-way interactions of separate antibacterials using *E. coli.*

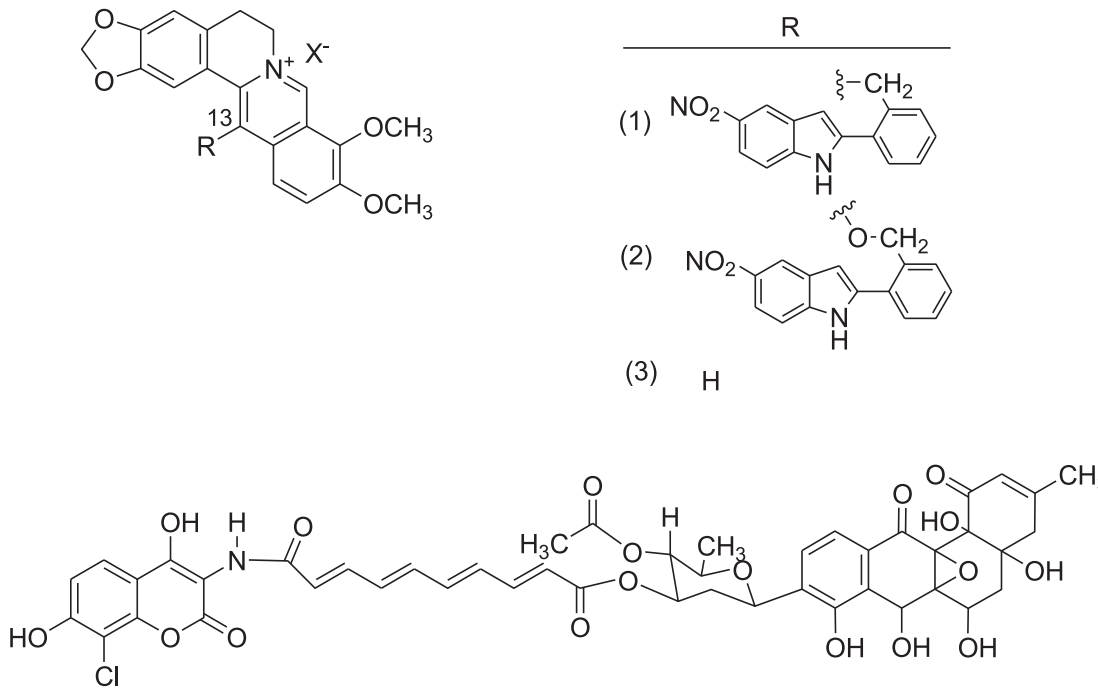
One significant potential problem, though, with drug combination antibacterial therapies is that *in vivo* the different drugs are likely to have different pharmacokinetic profiles which could compromise antibacterial potency with different concentrations of drugs being present at different times at the bacterial target sites. Also undesirable off-target effects are more likely to be an issue.

As an alternative to combinations, single compounds whose structures incorporate different molecular recognition features to enable targeting of a number of biological target sites can be an attractive option. This design paradigm is, along with multiple drug combinations that interact with different biological targets, part of the area referred to in general as polypharmacology [15, 16] (or as a multivalent approach [17]). These single molecules may be hybrids or agents

which stay intact at each target site, or single pro-drugs which can be cleaved selectively in or near the bacteria to release separate active components *in situ.* Work on such multi-action compounds to date has largely focused on the design, synthesis and microbiological evaluation of dual action, or potentially dual action, compounds. These have been extensively reviewed [8,18-21], but some examples include 13-substituted derivatives (1) and (2) of the antibacterial quaternary alkaloid berberine (3) in which the 13-substituent incorporates structural elements of the potent NorA bacterial efflux pump inhibitor INF55 (5-nitro-2-phenyl-1*H*-indole) with methylene [22-24] or methylene ether [25] linking groups to berberine. Berberine is a substrate for this pump which severely comprises its antibacterial activity in *Staphylococcus aureus* for example. The hybrid salts (1, X = Br) and (2, X = Br) show potent activity against *S. aureus.*

Interestingly, bifunctional antibiotics have also been found in nature as exemplified by the antibiotic simocyclinone D8 (4) [26]. This antibiotic, which was isolated from the soil microorganism *Streptomyces antibioticus* Tü 6040, is active against Gram-positive bacteria but not Gram-negatives, and is cytostatic against some human tumour cell lines. It is a very large molecule composed of a chlorinated aminocoumarin at one end and an angucyclic polyketide at the other, with a tetraene and deoxyhexose sugar unit linking the two. The molecule inhibits bacterial DNA gyrase by stopping DNA binding to the enzyme. Crystallographic analysis has revealed that (4) binds to the *E. coli* gyrase A subunit via two binding pockets that separately interact with the polyketide and aminocoumarin structural components [26]. These extra binding pockets, which each differ from the quinolone antibacterial binding site, may offer some new avenues for antibacterial drug development.

Less, however, has been done with single molecule triple-action antibacterials although the potential here for such agents or related pro-drug entities is significant and multifaceted. Acting at three bacterial target sites synchronously or near synchronously could result in very potent antibacterial activity and greatly hinder resistance development. In this article, some general design parameters for potential single molecule triple action antibacterials are discussed, together with some specific known or suggested molecular expressions of these agents.



Simocyclinone D8 (4)

Discussion

Design Parameters

The general design parameters are similar to those involved with antibacterial dual-acting hybrids [8, 19, 21] and pro-drugs [8], although there are added possibilities when three separate action modes with respect to the biological targets are considered. In addition to direct antibacterial targets like interfering with cell wall formation, or bacterial DNA function or protein synthesis, the indirect activities could include a range of combinations including dislocation of quorum sensing [27], toxin blockade, impacts on the uptake of essential nutrients, like iron, or the inhibition or attenuation of bacterial efflux pump activity. Efflux pumps represent an important, although not the only, resistance mechanism for bacteria to counteract antibacterials by pumping them out of the cell thus reducing their concentrations to sub-effective levels [28, 29]. In addition, efflux pumps also have a number of other functions, such as extrusion of endogenous metabolites like the siderophore, enterobactin, or the efflux of toxic endogenous metabolic intermediates [28]. So inhibiting efflux pumps could have other deleterious bacterial effects. Efflux pump inhibitors have been shown to decrease the emergence of resistance as well as reducing biofilm formation and bacterial virulence [29]. Clearly many direct and indirect action combinations are possible, but it is essential in the design of such singe molecule agents to consider possibilities for potentiation or synergism, rather than antagonistic

interactions or just additive interactions [30]. The evaluation protocols elaborated by Yeh and co-workers [14] for combinations of three agents will be useful in defining activity groupings in triple action agent design.

Chemically, the new designed agents need to be stable and be relatively readily accessible synthetically, with syntheses scalable to enable larger quantities for more extensive biological testing if required. In addition physicochemical properties like molecular weight, water solubility, lipophilicity, and numbers of H-bond donors and acceptors need to be considered, amongst others, for potential oral administration, although intravenous administration is an alternative if this is not possible. Potentially toxic structural moieties should also be avoided together with functionality likely to be a problem metabolically. Biologically, the potential antibacterial activity spectrum, potency (particularly against drug resistant strains), the assessment of rapid or slow resistance development to the new agents, and possible off target effects need to be considered.

There are a number of other considerations but the focus in this review is on the basic chemical design side as explored below.

Molecular Expression of Single Molecule Agents

a. Non-cleavable-Triple Action Agents

Many combinations and permutations are possible within a single molecule with respect to recognition entities and their bacterial target sites. This results in synthetic challenges as well as efficacy challenges particularly as binding at one target, for example by molecular recognition unit A, may be negatively impacted by the presence of B and/or C. The effect of the other recognition units and linker groups on interaction with each target site, as well as the effective concentration required at each site, are important aspects to consider

While molecular expressions of triple action designs can be grouped in many different ways, in this article the non-cleavable designs have been sub-divided into six types (i. to vi.) involving structural recognition elements generalized for simplicity by the letters A, B, and C. These elements could involve a range of functional groups embedded in one or more rings or not in ring systems but attached to them or to acyclic units.

General Non-Cleavable Types

In the general types i to iii outlined below, dashed lines are used to indicate variations in the nature of the linking groups between the recognition elements as many different linkage architectures are possible affording a range of opportunities for different relative dispositions of the three recognition element regions generalized as A, B, and C. The recognition elements

represent the structural motifs likely to interact with the specific bacterial target sites. These are general design motifs and in each case other possible variations with different ordering of A, B and C is assumed. With all these structural types, the design needs to be such as to resist chemical or enzymatic cleavage so they reach their target biological interaction sites intact.

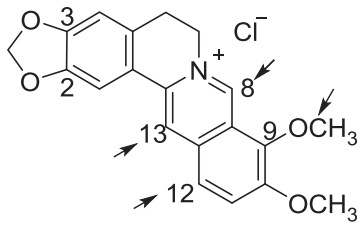
Type i. A - - B - - C

With this type, two linking groups are involved and these links could include acyclic (linear or branched) groups with one or more atoms and/or one or more cyclic groups including spirocyclic ones. Incorporating such rings in the linkers offers the prospect of different dispositions of the motifs A, B and C and spirocyclic systems are an important part of modern drug design [31]. With this general type, the whole assemblage or parts thereof could also be embedded in one or more ring systems, with up to three linking groups (e.g. if A was joined to C by another linking group) or units present.

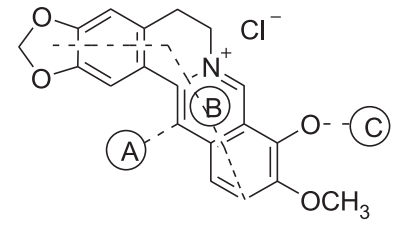
The biologically active quaternary alkaloid berberine (3) (shown below as its chloride salt) provides considerable scope for the design of potential triple action agents of general type i. It is available relatively cheaply commercially, and possesses a number of sites for chemical manipulation and introduction of different groups. Reaction opportunities include nucleophilic addition at C8 and electrophilic substitution at C13 (after conversion to dihydroberberine or 8-substituted dihydroberberines and subsequent re-conversion to the quaternary salt structure in this case [22, 32-34]) and electrophilic substitution at C12 on berberine itself or derivatives [35]. At each of these positions substituents incorporating different biological target motifs, A, or C (5), for example, could be incorporated. The heterocyclic skeleton in berberine could act as a recognition element in itself (shown as B in (5)) for DNA binding [36] or inhibition of assembly of the protein FtsZ, vital for cell division [37, 38].

Berberine, and substituted berberines, can also be converted in good yield to the corresponding 9-hydroxyberberine (the alkaloid berberrubine) by simple thermolysis [39] providing a handle for the introduction of further groups by reaction at the 9-OH site produced. The 9-hydroxyl group could possibly also be used to direct further nucleophilic attack, for example by boronic acids via a Petasis type reaction [40], to give 8-substituted derivatives of type (6) or (7). In the case of (7), the recognition element C could then be incorporated by standard ‘click chemistry’ [41] using an appropriate azide derivative to give (8).

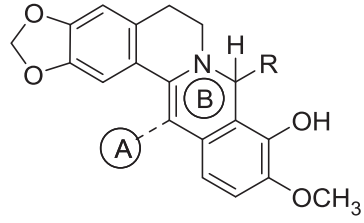
In addition to the above sites for the attachment of substituents, the C2,3-fused methylenedioxy group in berberine can be selectively ring opened to afford a 2,3-diol (demethyleneberberine) on reaction with boron tribromide under mild reaction conditions [42] and these groups in turn could then serve as points of attachment for other groups although regioselectivity issues may be a problem.

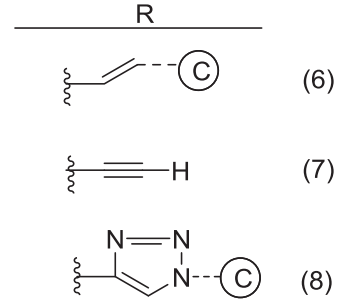


Berberine chloride (3)



(5)

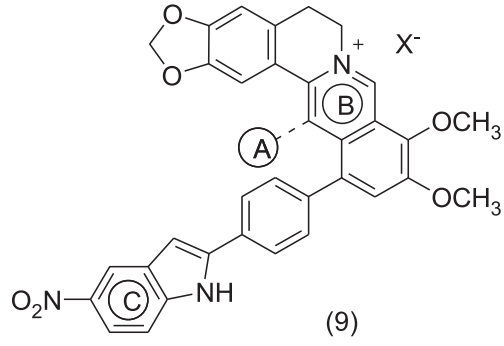




(6) (7) (8)

Type ii. A - - - BC

With this type only one multi-atom linking group is present, with the other two recognition units being directly attached via a common atom or bond. Using berberine as the starting point one could envisage, for example, attaching the NorA efflux pump blocking moiety, the 5-nitro-2-phenyl-1*H*-indole unit (C), directly to berberine B to which was also attached to another unit A via some appropriate linker group to give (9).



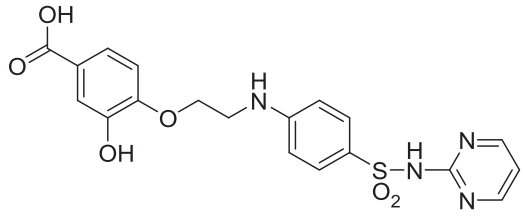
(9)

Type iii. A - - B/C

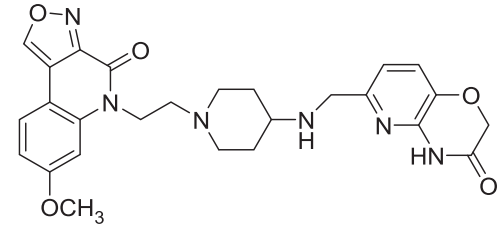
This design type represents an extension of type ii where B and C are partly merged or even completely merged. In this latter case if all the atoms overlap then B and C would be equivalent but able to interact separately in different binding modes to two different biological targets and different from the target of A. Thus it would still be a triple-action agent. This design

has been realized molecularly with the antibacterial designated PD-2b (10) [43]. This compound was designed from a linked combination of a protocatechuic acid unit at one end and a sulfadiazine unit at the other. From computer–based docking studies, the hybrid compound was shown to target both DNA gyrase subunit B and topoisomerase IV subunit B (of the Gram-negative pathogen *Pseudomonas aeruginosa*) as well as dihydrofolate reductase enzymes and is a potential antibacterial lead structure for further development.

The novel bacterial topoisomerase inhibitor REDX 06213 (11) can also be considered under this general structural type [44]. This compound, together with others in this group, has an isoxazoloquinolone group at one end which interacts with DNA (A), a linking group, and then a pyrido-oxazinone motif (B/C) at the other end which binds to bacterial gyrase and to topoisomerase IV enzymes. The compound showed good antibacterial activity against multi-drug resistant strains of *E. coli* and *Acinebacter baumannii* with low potential for the development of resistance.

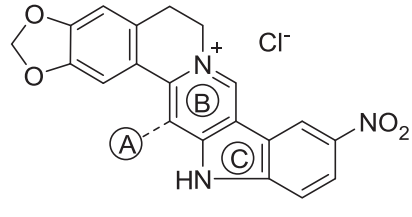


PD-2b (10)

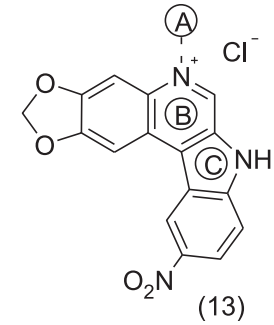


REDX 06213 (11)

Extensions of the type iii triple action agent design to berberine-based analogues might potentially include structures like (12) and (13) with the heterocyclic core represented by B binding to bacterial DNA and the recognition unit incorporated in the linked group A in each case interacting with a gyrase or topoisomerase enzyme. The fused 5-nitroindolic unit C could act to interfere with efflux of the whole quaternary salt by the NorA efflux pump. The proposed structure (13) involves a greater overlap of the rings in the original berberine, and the four-ring fused heteroaromatic skeleton (a 7*H*-indolo[2,3-*c*]quinoline) is isomeric with those present in the bioactive indoloquinoline alkaloids [45].



(12)



(13)

Type iv. ABC

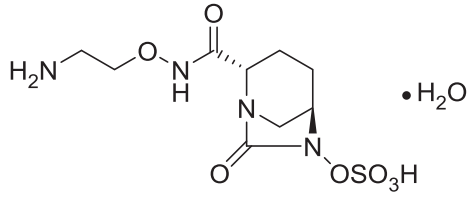
Characteristic of this design type, and of types v. and vi. below, is the absence of any extra linking groups. So for type iv, the three recognition elements could each be joined directly via one or two common atoms and one or two common bonds. As far as can be ascertained, there are no clear examples of this general type of triple action antibacterial agent in the literature, but possibilities for the future based on berberine can be envisaged where A and C could be directly attached to berberine (recognition unit B) perhaps at positions 8 and 12, 8 and 13, or 12 and 13.

Type v. AB/C

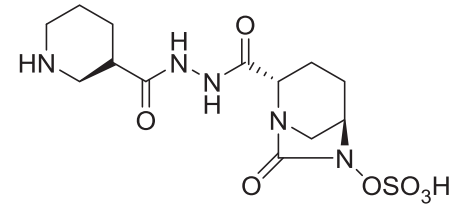
This type would have no linking groups but would include partial or complete structural overlaps of two of the recognition elements, and if the latter then with B equivalent to C two biological targets could be envisaged as noted for type iii. Direct attachment of A to B/C in structures (12) or (13) could be possible candidates for compounds of type v.

Type vi. A/B/C

With this final general type, a partial or complete overlap of the recognition elements could be envisaged, although it is less likely to be complete overlap of all three units to have one molecule interacting with similar binding strengths at three different biological targets with the same recognition element. Literature examples here include the diazabicyclooctane derivatives OP0595 (14) [46, 47], and Zidebactam (15) [48], plus the modified fluoroquinolone derivative Levonadifloxacin (16) and its oral aminoacid ester prodrug. [49] The core diazabicyclooctanone unit in (14) and (15) acts like a ß-lactam unit; OP0595 inhibits class A and C ß-lactamases as well has having good antibacterial activity against a number of Gram-negative bacteria through strong binding to the penicillin-binding protein PBP2. Furthermore, (14) acts as what is referred to [46] as an “enhancer” of the antibacterial activity of ß−lactams which bind to other penicillin-binding proteins, with this effect being independent of the blocking of the ß-lactamases [46]. Zidebactam (15) is also a triple action agent with very good activity against some Gram-negative bacterial strains [48].

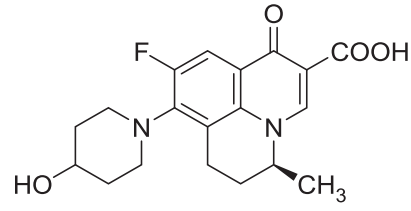


OP0595 (14)



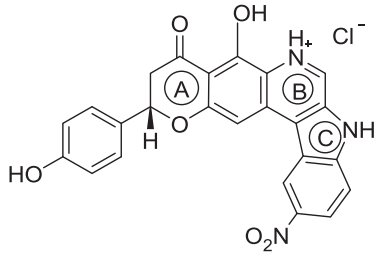
Zidebactam (15)

Levonadifloxacin (16) belongs to the benzoquinolizine fluoroquinolone group and is a bactericidal agent active against quinolone resistant MRSA (Methicillin-resistant *Staphylococcus aureus*). Compound (16) preferentially targets the bacterial enzyme DNA gyrase along with Topoisomerase IV. In addition, (16) inhibits the NorA efflux pump in *S. aureus* [49], which is another resistance mechanism implicated in fluoroquinolones together with resistance developing through enzyme mutation.

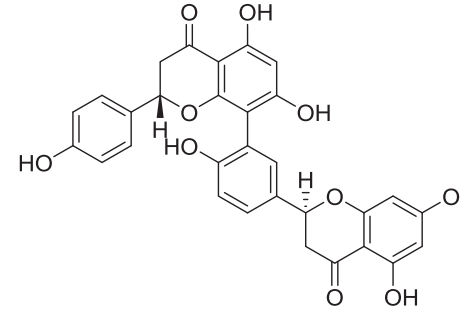


Levonadifloxacin (16)

While not described as yet, one could also envisage other compounds of type vi. with partial structural overlaps and with potentially a triple action profile. For example, part of the structure (13) could be merged with a unit A as in the structure (17). The merged unit A reflects part of the flavonoid structure present in the dimeric flavanone, tetrahydroamentoflavone (18) [50]. Compounds of this type are present in an extract from the Brazilian peppertree, in which the flavone rich extract showed inhibitory activity against the accessory gene regulator (agr) alleles in *S. aureus* without growth inhibition [50]. It thus suppresses quorum sensor expression and quorum sensing controlled virulence factors. The extract showed very promising activity *in vivo* in the treatment of dermonecrosis caused by a virulent strain of MRSA. While the assessment of pure components does not appear to be reported as yet, compound (18) is a one of the significant components in the active extract, and it is thus conceivable that compound (17) could also display quorum quenching activity. With (17) then there would be a DNA focus for two of the interactions (A/B) while C might interfere with the NorA pump-mediated efflux of the molecule in *S. aureus.*



(17)



(18)

b. Cleavable–Triple action Pro-drugs.

Similar general structural motifs can be envisaged for the potential triple action pro-drugs as noted for the triple action agent designs above but with the incorporation of a cleavable group or groups. However, in order to reduce potential negative off target effects *in vivo,* the cleavage reaction should be triggered only when at or in the bacterium. Triggers could include bacterially specific enzymes like ß–lactamases [51] or peptide deformylase [52], the latter being involved in the hydrolysis of a terminal *N*-formyl group from peptides. While considerable work has been done on inhibitors of this enzyme as antibacterial agents [52] there is potential for use of this enzyme in the pro-drug context. Other biorthogonal approaches to pro-drug cleavage [53] involving non-biological, physical or chemical triggers to release the active component or components from the pro-drug could also be useful if the pro-drug can be concentrated in bacteria or on their surface.

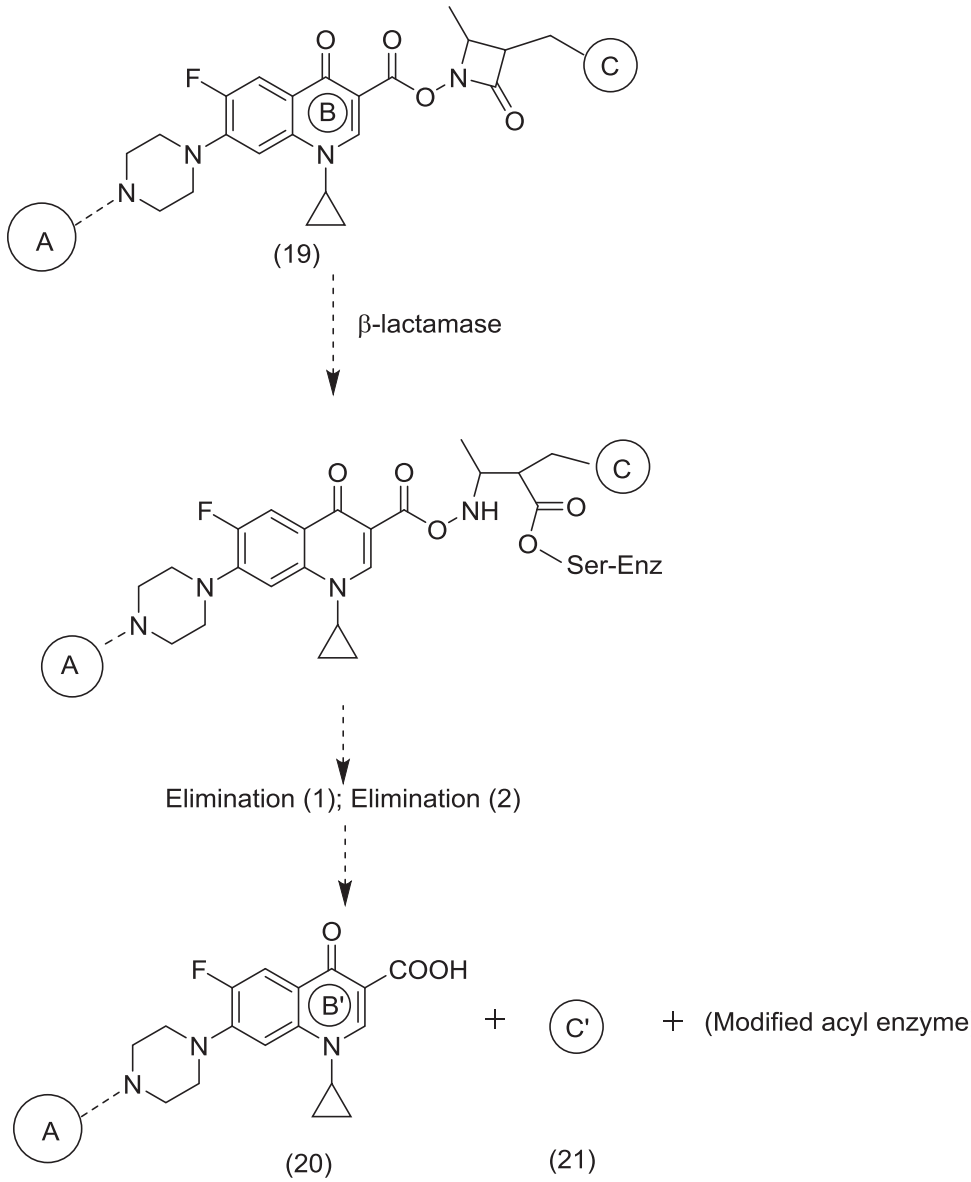
General Cleavable Types

In these general types, the descriptors A′, B′ , or C′ are used after pro-drug activation and bond cleavage since other atoms may still be attached at these sites so they would be like A, B and C and still have the respective key recognition features. In each type below there could be variations in which linking group is cleaved and the order of A, B, and C.

Cleavable Type i. $\mathbf{A - - B - - C \longrightarrow A - - B' + C'}$

With this general type of pro-drug design, cleavage of one of the linking groups could reveal a dual action hybrid (A - - B′) together with another molecule C′ capable of interacting with a third separate target involved with the bacterium, the advantage being that the active components would be released in or near the bacterium. While the initial molecule may have some or no antibacterial activity in itself, the aim would be to expose active components in high concentration on reaching the bacterium utilizing bacterially specific enzymes. One possible molecular expression of this type is illustrated by (19). In this case it is proposed that a monocyclic-lactam triggering unit, to which C is attached, be linked to a fluoroquinolone core B also incorporating a further recognition moiety A (Scheme 1). If the ß-lactam in (19) was cleavable by a ß-lactamase enzyme, then, by analogy with some *N*-sulfonyloxy-ß-lactam inhibitor studies [54, 55], a subsequent elimination (1) might ensue to give the fluoroquinolone (ciprofloxacin) derivative (20) in which the key 3-carboxylic acid would be present. The iminium group produced in the remaining acyl enzyme moiety (or the ketone hydrolysis product [54]) could then serve to activate a second elimination (2) to give C′ (21) if the attached fragment C had an appropriately positioned electron acceptor group. While (20) and (21) could potentially be

separately prepared and then used in combination, the pro-drug strategy suggested would provide a means to help overcome any pharmacokinetic issues when used *in vivo* to treat resistant bacterial infections. From a synthetic viewpoint, the *N*-acyloxy substituted ß-lactam unit in (19) should be accessible based on the carbodiimide and Mitsunobu reaction methodology for other mono ß-lactams [55].



Scheme 1. Possible fragmentation pathway of the type i pro-drug (19) after ß-lactamase activation.

Cleavable Type ii. $\mathbf{A - - B - - C \longrightarrow \longrightarrow A' + B' + C'}$

In this case sequential cleavage of each linking group would lead to the release of three separate compounds A′, B′ and C′. As an illustrative example, molecule (20) above might include a feature in the linking group to A which was susceptible to cleavage by a later trigger to give A′ and perhaps the antibacterial ciprofloxacin as B′.

Cleavable Type iii. $\mathbf{A - - B - - C - - X \longrightarrow A - - B - - C' + X'}$

With this third design type, selective cleavage of one linking group might result in the fully active triple action agent being exposed near the biological target sites. In theory, fragment X′ might also be designed to have a fourth biological action but in this article the focus is on triple action considerations. By way of example of this type, the cytoplasmic enzyme peptide deformylase might be considered for cleavage of the C - - X linkage [52]. This enzyme does appear to have some substrate tolerance and the intermediate or final amine generated in the formamide hydrolysis process could then serve as a trigger to release a triple action antibacterial agent in high concentration intracellularly.

Conclusion

There are many possibilities for the design and development of radically new, triple-action antibacterials and, while the medicinal chemical and other disciplinary challenges are major, such antibacterial agents could ultimately help to meet the antibiotic resistance threat and improve health care outcomes in the future.

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