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Abstract

Photoantimicrobial - i.e. light-activated - antimicrobial agents constitute a subset of compounds from a variety of dye classes, mainly synthetic. However, in terms of clinical acceptance, this identification as ‘dye’ is disadvantageous. The following is an attempt, via rationalisation and precedent, to put the case for the medical use of photoantimicrobials, at a time of an accepted need for alternative approaches to infection control, beside that of conventional antimicrobial drugs. Note: the term antibiotic is employed here with the everyday, rather than the scientific, meaning, i.e. rather than antibacterial.

Keywords	Antimicrobial resistance; Biocide; Dyes in medicine; Infection control; Infectious Disease; Photoantimicrobial.
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Highlights

Photosensitising dyes and light can be effective antimicrobials

Photoantimicrobials are effective against all microbial types

Effective clinical work is increasingly reported

Efficacy against drug-resistant microbial pathogens is particularly important

The problem with dyes in infection control

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Abstract

Photoantimicrobial - i.e. light-activated - antimicrobial agents constitute a subset of compounds from a variety of dye classes, mainly synthetic. However, in terms of clinical acceptance, this identification as 'dye' is disadvantageous. The following is an attempt, via rationalisation and precedent, to put the case for the medical use of photoantimicrobials, at a time of an accepted need for alternative approaches to infection control, beside that of conventional antimicrobial drugs.

Note: the term *antibiotic* is employed here with the everyday, rather than the scientific, meaning, i.e. rather than *antibacterial*.

Keywords

Antimicrobial resistance; Biocide; Dyes in medicine; Infection control; Infectious Disease; Photoantimicrobial.

1. Introduction

Infection control is a diverse area of healthcare which evolved most rapidly in the middle of the last century, mainly to the benefit of *Homo sapiens*. In the modern consciousness it is not an area associated with dyes. Consider the following two situations.

Contemporary view

In 2017, a businesswoman attending her GP with suspected tonsillitis is expecting to be prescribed an antibiotic, to have the prescription filled at a local pharmacy and then to begin the self-administered therapy at home. The GP, having examined the patient's neck glands externally and the back of her throat with a light and a tongue suppressor, suspects bacterial infection and prescribes the penicillin derivative, amoxicillin.

The woman has the prescription filled at the pharmacy and is supplied with a seven-day course of capsules. These are taken for the first three days, by which time she no longer has a sore throat or swollen glands and, having many other important matters to deal with at the office, forgets about the situation with her throat and so discontinues the course.

Pre-antibiotic view

In 1932, a Sheffield (UK) steelworks' foreman presents at a local hospital with a burn wound to his left forearm. The wound is cleaned and then dressed with a greasy formulation containing the bright yellow dye acriflavine. The foreman returns to work straight from the hospital. As the wound eventually heals, there is no follow-up.

The first scenario described is not untypical. However, in terms of the fight against antimicrobial drug resistance (AMR) the discontinuation of therapy is a cause for concern which has, unfortunately, been with us since the general availability of antibiotics following the Second World War. Moreover, it is only one of a *number* of causes for concern.

The second scenario, again, does not represent an unusual occurrence. Because it is set in the pre-antibiotic era, the conventional approach to local infection would often involve the use of an antimicrobial dye. Methylene blue had been used in malaria (though probably not in Sheffield) for over forty years by this time; "Flavine therapy" - usually employing acriflavine, proflavine or brilliant green - had saved countless lives in the base hospitals in France during World War I; acriflavine, brilliant green and crystal (gentian) violet continued to be used in healthcare in controlling infection. Furthermore, occurrences in the industrial screening of derivatives of these dyes would shortly usher in the above-mentioned antibiotic era via the azoic dye Prontosil and the consequent sulphonamide 'Gold Rush' of the late 1930s [1].

While both situations describe effective infection control, there are obviously potential downsides in each case. The former describes potentially sub-lethal dosing, which is accepted as bad practice, potentially leading to drug resistance development among the patient's internal microbiota [2]. The latter approach was not always successful and, clearly, produced staining of the wound and, presumably, the surrounding tissue. The application of acriflavine also required medical assistance.

Of the two approaches, antibiotic therapy has enjoyed generally unchallenged use since the mid-1940s rapidly eclipsing the dyes which had been in widespread use in infection control for the previous 30 years.

1.1. Antimicrobial resistance

There can be little doubt that straightforward dosing using antibiotic capsules or suspensions has allowed simple control of a high percentage of bacterial infections and that this control has required very little in terms of medical supervision. Such end-user independence in the face of the pathogenic threat is logically highly desirable, and an aspirational hallmark of highly evolved, affluent civilisation. A similar situation pertains to the food animal stock required by such a society.

However, such has been our over- and mis-use of antibiotics – against self-limiting or non-bacterial infection in humans, or as growth-promoters in livestock, for example – that bacterial drug resistance has now attained dangerous levels and without a productive pipeline of new antibiotics is now cited as a threat to civilisation in the same breath as global warming and international terrorism [3-5].

In terms of modern alternatives to antibiotics, the main coverage is given to vaccines, bacteriophages and other biological approaches [6]. The use of dyes in this respect seems to be promoted only by those working in the field of photoantimicrobials.

2. Dyes and photoantimicrobials

But why not use dyes in infection control? As noted above, flavine therapy was not always successful, but the modern, targeted use of such - or related - dyes *in conjunction with targeted light* provides highly effective microbial killing via the intermediacy of reactive oxygen species (Figure 1), whether of penicillin-sensitive streptococci, meticillin-resistant *Staphylococcus aureus* or ESBL-expressing *Klebsiella pneumoniae* [7]. Given that several articles have appeared recently reporting the apparent imperviousness of strains of the latter bacterium against *any* antibiotic [8], the photoantimicrobial approach (Figure 1) offers considerable benefit.

[Figure 1]

So why aren't dyes – and particularly photoantimicrobial examples – being introduced to support the conservation of our essential antibiotic arsenal?

The answer may lie a considerable way back, in the early part of the last century.

The purpose of flavine therapy was to stain selectively and thus inactivate the microbes present in the target tissue. The application of sufficient quantities of dye to facilitate this effect inevitably led to staining of the tissue surrounding the target area. Were this process to be carried out with a modern, colourless biocide, such as chlorhexidine, such staining - although present - would, of course, be invisible. The comparison of dye and biocide action is covered below. Tissue staining, or discolouration, is unpopular with patients, especially when visible in public, or when garments become stained. In the period before sulphonamide introduction, when brilliant green was a common antibacterial, often used in obstetrics, the famous medic L.P. Garrod wrote of complaints from patients concerning the dye: "It is objected to on account of its staining propensities; whether stained linen or death from septicaemia is the greater evil is a question which seems to admit of only one answer." [9]. This comment was made in the "pre-antibiotic" period, so often referenced by today's media.

It is also well-known that Alexander Fleming was disparaging about the use of dyes in infectious disease. He wrote in a 1917 Lancet article that "… the theoretical basis for the use of dyes [as antimicrobials] is thoroughly unsound" [10]. It should be noted that Fleming's argument was based on *in vitro* laboratory work, rather than Browning's successful clinical use of acriflavine and brilliant green.

In order to minimise the staining problem, the Australian chemist Adrien Albert carried out an enormous amount of acridine synthesis during the 1930s and 40s, developing possibly the first

properly organised molecular structure-activity relationships and delivering, among others, the non-staining antibacterial drugs aminacrine and diflavine (9-amino and 2,6-diaminoacridine, respectively), as well as the (yellow) antimalarial mepacrine [11].

2.1. Methylene blue and malaria

It is of little surprise that the conventional drugs derived from medical dyes in the mid-20th Century – such as the sulphonamides or chloroquine - were colourless but, as our supply of effective, colourless contemporary drugs dwindles, can we really use a distaste for staining as a reason not to use effective, coloured alternatives? And there is a modern, 21st Century precedent.

Drug resistance is not a new phenomenon. Monotherapy of malaria produced significant levels of chloroquine-resistant parasites (plasmodia) by the early 1960s and in sub-Saharan Africa by the following decade [12]. This was, and is, a scourge, particularly among the young. As a response, methylene blue was introduced – as a conventional antimalarial, rather than a photoantimicrobial - for the treatment of juvenile malaria in Burkina Faso in 2005 [13]. This represents the systemic administration of an intensely blue substance which leads to colouring of the urine and stool, as well as clothing and intimate apparel. Furthermore, the population being treated belongs to highly structured and regulated tribal systems where a child producing strangely coloured waste might otherwise be ostracised. This has been avoided by extended discussions with tribal elders prior to the commencement of therapy [14].

Such an approach might be seen by those in affluent societies with easy access to high-tech healthcare to be a retrograde step. It is not. Rather it represents the logical use of an effective, relatively inexpensive drug, taking into account an insignificant side-effect, in the face of widespread treatment failures with conventional therapeutics.

The argument for the use of methylene blue - or another of the approved medical dyes which are also photosensitisers (e.g. toluidine blue or crystal violet) - as a photoantimicrobial in modern healthcare is very similar, save for the fact that treatment would be localised, rather than systemic. There is an understandable assumption that treatment using this approach must be limited to topical therapy. This is not the case since, given access to endoscopic techniques and fibre optic technology, most regions of the body are accessible, both to the local delivery of a photoantimicrobial and also of light.

2.2. Advantages of the photoantimicrobial approach

In addition, one of the major strengths of photoantimicrobials is their broad-spectrum, truly antimicrobial action (i.e. against bacteria, viruses, fungi and protozoa), regardless of conventional resistance status. As noted, 21st Century resistance, for example to antibacterial drugs, is increasingly difficult – and expensive - to treat with other conventional agents (Figure 2).

[Figure 2]

3. Photoantimicrobial use in the clinic

Thus we have a combination of highly –effective and rapid antimicrobial action which works best against a localised infection, regardless of microbial type. How might this be used positively in modern infection control?

Tonsillitis is a very common illness which may have a bacterial or viral aetiology. Its treatment is often given as a good example of bad practice, *viz.* the prescription amoxicillin (typically, as noted above) by physicians before this aetiology is established, often leading to pointless – and ultimately dangerous – antibiotic exposure of the patient's microbiota. The application of a photoantimicrobial, such as methylene blue, to the tonsils, followed by a short illumination – about 30 seconds – with a light probe should provide sufficient bacterial kill locally, with no effect further on the alimentary tract, or systemically. Any photoantimicrobial swallowed during the procedure would have no effect, as only the illuminated area would be activated. Such a situation can be assumed for most local infections, in each case allowing the removal of conventional antimicrobials from the treatment protocol, and this would be possible regardless of the resistance status of the infecting microbes.

Photoantimicrobial application in this way could be of major impact if the infection is already difficult to treat using conventional agents – for example in drug-resistant cases or where a drug cocktail is required, as in pulmonary tuberculosis [18,19]. Other presentations include diabetic foot ulcers, which have been shown to be responsive to this approach (Figure 3) in cases where the standard option is amputation [20]. Even without the spectre of infection by multiple-drug resistant bacteria, this would be of enormous benefit to the patients involved, as well as offering enormous cost savings in terms of surgical procedures, rehabilitation and onward care.

[Figure 3]

The same approach is currently in use in patient decolonisation in Canada. The effect of light-activated methylene blue against meticillin-resistant *Staphylococcus aureus* (MRSA) is well established [21] and this has been applied to the decolonisation of elective surgical patients in a

Vancouver hospital, with subsequent decreases reported in post-op MRSA infection rates [22]. In such cases photoantimicrobials conserve the standard prophylactic drugs normally employed in addition to those required in an anti-MRSA capacity, post-op. There is no reason why the prophylactic route cannot be applied to 'lesser' infections which commonly precede highly dangerous ones, such as pneumonia, meningitis and sepsis, thus blocking the progression from, for example, tonsillitis, otitis media or sinusitis to these high mortality-associated diseases.

3.1. Using directed light for therapeutic activation.

Clearly, effective photoantimicrobial action is only achieved with an efficient light source – i.e. of the correct wavelength range and of sufficient power output – and this may be another perceived hurdle to clinical acceptance.

An undoubted strength, in theory, of modern antibiotic use is that in most cases the drugs are self-administered, usually via the oral route. Ideally, the involvement of the clinician is solely in examining the sufferer and prescribing the requisite drug.

The addition of light activation to the therapeutic equation may require medical supervision or operation. There is, of course, a parallel - and absolutely routine - situation in many dermatology departments in the treatment of psoriasis, vitiligo and other skin disorders with psoralens, activated by ultraviolet-A radiation (PUVA therapy). The dangers of UV-A are well documented, while there are none with red light [23], but the fact remains that the proper direction of illumination, as well as providing the correct light fluence (i.e. how much, for how long), might require trained personnel. This would certainly be the case for procedures requiring administration and optical fibre use inside the body.

Should the requirement for trained medical staff – typically general practice nurses are envisaged – be a sufficient reason to discount the approach? Again, it is emphasised that photoantimicrobials

are effective against resistant bacteria, so a one-off treatment – e.g. again for an Ear, Nose & Throat/Upper Respiratory Tract infection - would be sufficient, regardless of resistance status. Surely the initial expense in training and equipment would far outweigh both the multiple treatments required for resistant disease and the deleterious effects on patients' microbiota? Obviously, other alternative therapies, such as vaccines and bacteriophages would also require medical administration.

3.2. Photoantimicrobials vs. biocidal agents

What is the difference between a photoantimicrobial agent and a biocide? Aside from the requirement for light activation, both types have multiple sites of action. However, for biocides, such as bisguanides (e.g. chlorhexidine gluconate) or quaternary ammonium salts (e.g. benzalkonium chloride) this is mainly due to the extremely high concentration in which they are administered – usually at tens or hundreds of times the minimum inhibitory concentration for the target organism. While this is acceptable externally in terms of host toxicity, such concentration at internal sites could be dangerous [24]. As can be seen from Figure 4, cationic photoantimicrobials are active at much lower concentrations on illumination, and these concentrations fall far below safe levels for known vital stains, such as methylene blue, when used systemically – usually in 1 % w/v solution, equivalent to 32000 μmol L^{-1}.

[Figure 4]

4. Conclusion and ways forward

Various clinicians in the Pacific North-West and in Brazil are using methylene blue for local photodisinfection, for the most part in dental applications. This successful approach allows the

conservation of antibiotics and should be both a clear demonstration of the utility of the approach and a strong argument for its wider introduction, both in local disinfection and in prophylaxis. The approach is particularly relevant in the current – and likely lasting - period of widespread decreasing antibiotic efficacy and it now requires active participation from those with influence in both the healthcare and pharmaceutical/biotech lobbies in order to realise this. In addition, the introduction of protocols requiring the administration of photoantimicrobials – and other, non-conventional approaches - must engender a new way of thinking about infection control. As a modern society this should not be beyond possibility, and we have, of course, done it before.

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Figure legends

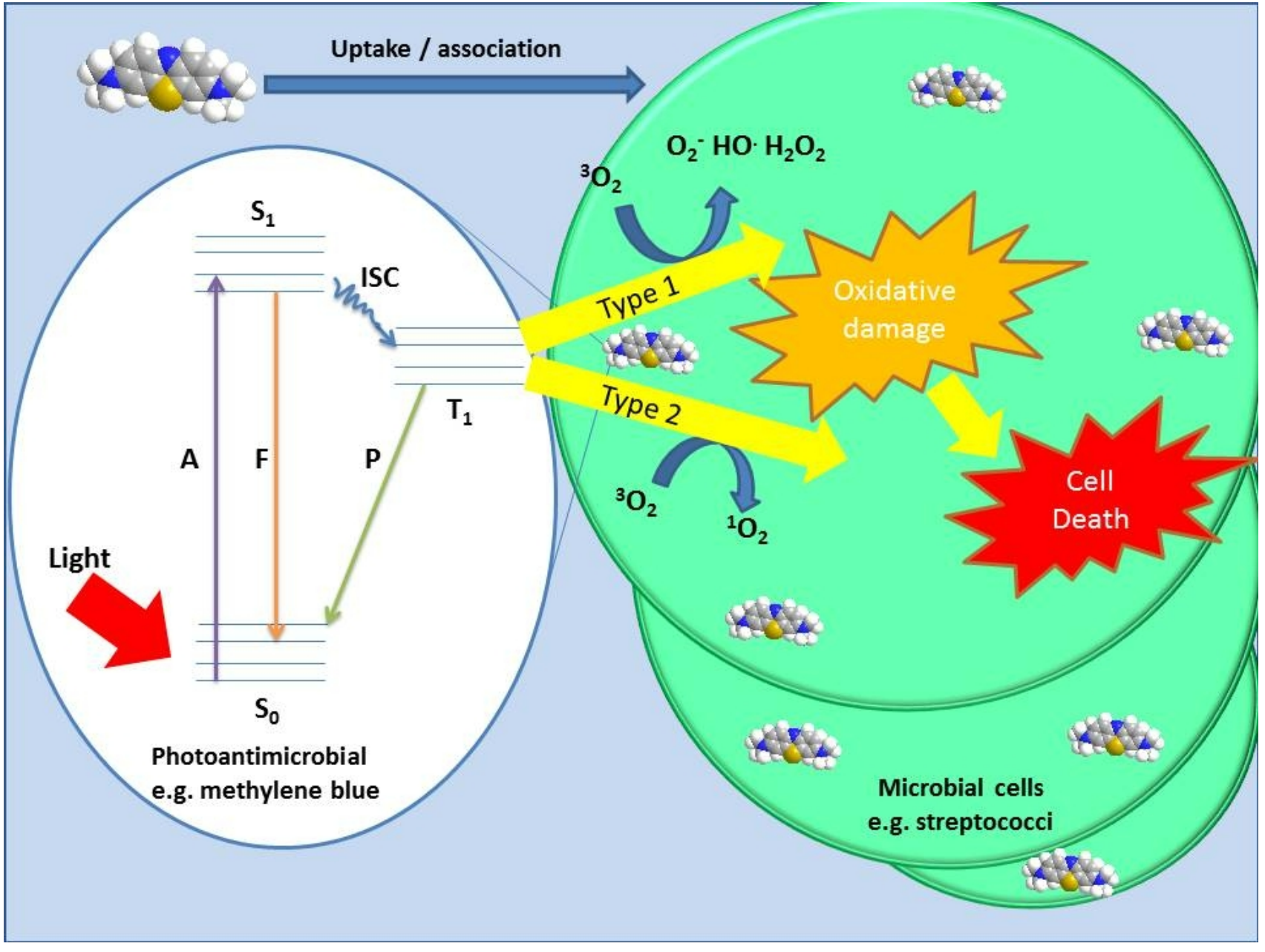
Figure 1. Adapted Jablonski diagram for photoantimicrobial action. Key: S_0 – singlet electronic ground state of photoantimicrobial molecule; S_1 – singlet excited state; T_1 – triplet excited state; A – photon absorption; F- relaxation by fluorescence; ISC – intersystem crossing; P – phosphorescence; $^3O_2$ – ground-state, triplet oxygen. Reactive oxygen species: $^1O_2$ – excited-state, singlet oxygen; O_2^- - superoxide anion; $HO^\cdot$ – hydroxyl radical; H_2O_2 – hydrogen peroxide.

Figure 2. Minimum bactericidal or fungicidal concentrations (MBC or MFC, respectively, in micromoles) of standard antimicrobial agents and photoantimicrobials against: (a) *Pseudomonas aeruginosa*, (b) methicillin-resistant *Staphylococcus aureus*, (c) *Propionibacterium acnes* and (d) *Candida albicans in vitro*. Light activation 660 nm LED array, light fluence = 6 J cm^{-2}. Photoantimicrobial activity is shown by pale grey bars, black bars indicate dark activity, maximum concentration tested = 100 μM. Drug key: Levo – levofloxacin; fluclox – flucloxacillin; vanc – vancomycin; BPO – benzoyl peroxide; flucon – fluconazole. Exemplar photoantimicrobial structures are given above [15-17].

Figure 3. Successful photoantimicrobial treatment of the diabetic foot. (a) Initial presentation; (b) 90 days' post-treatment with methylene blue/toluidine blue/light (Courtesy of Dr J.P. Tardivo, Fundação Medicina ABC, Santo André, SP, Brazil).

Figure 4. Minimum bactericidal concentrations (MBC, in micromoles) of standard biocidal agents (CTAB = cetyl trimethylammonium bromide) and photoantimicrobials against *Staphylococcus aureus*

(pale grey bars) and *Escherichia coli* (dark grey bars) *in vitro*. Light activation 660 nm LED array, light fluence = 6 J cm^{-2}. Black bars indicate dark activity, maximum concentration tested = 100 μM. Photosensitiser structures are provided in Figure 2, above [15-17].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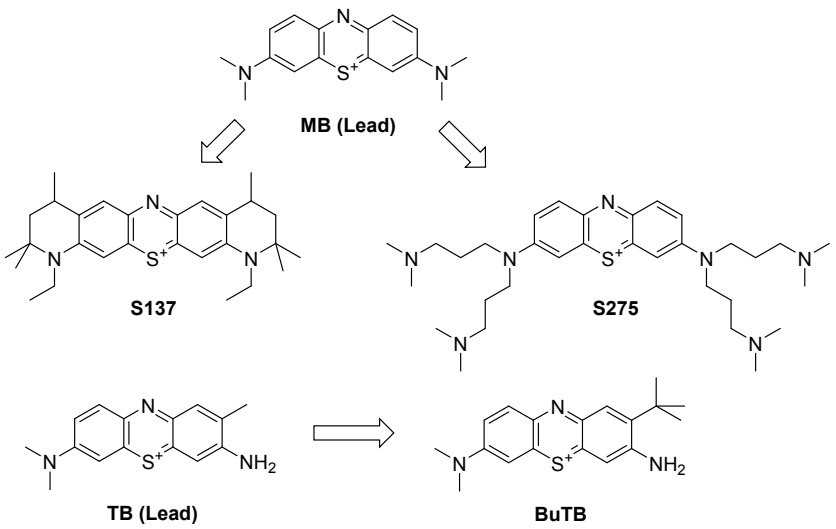
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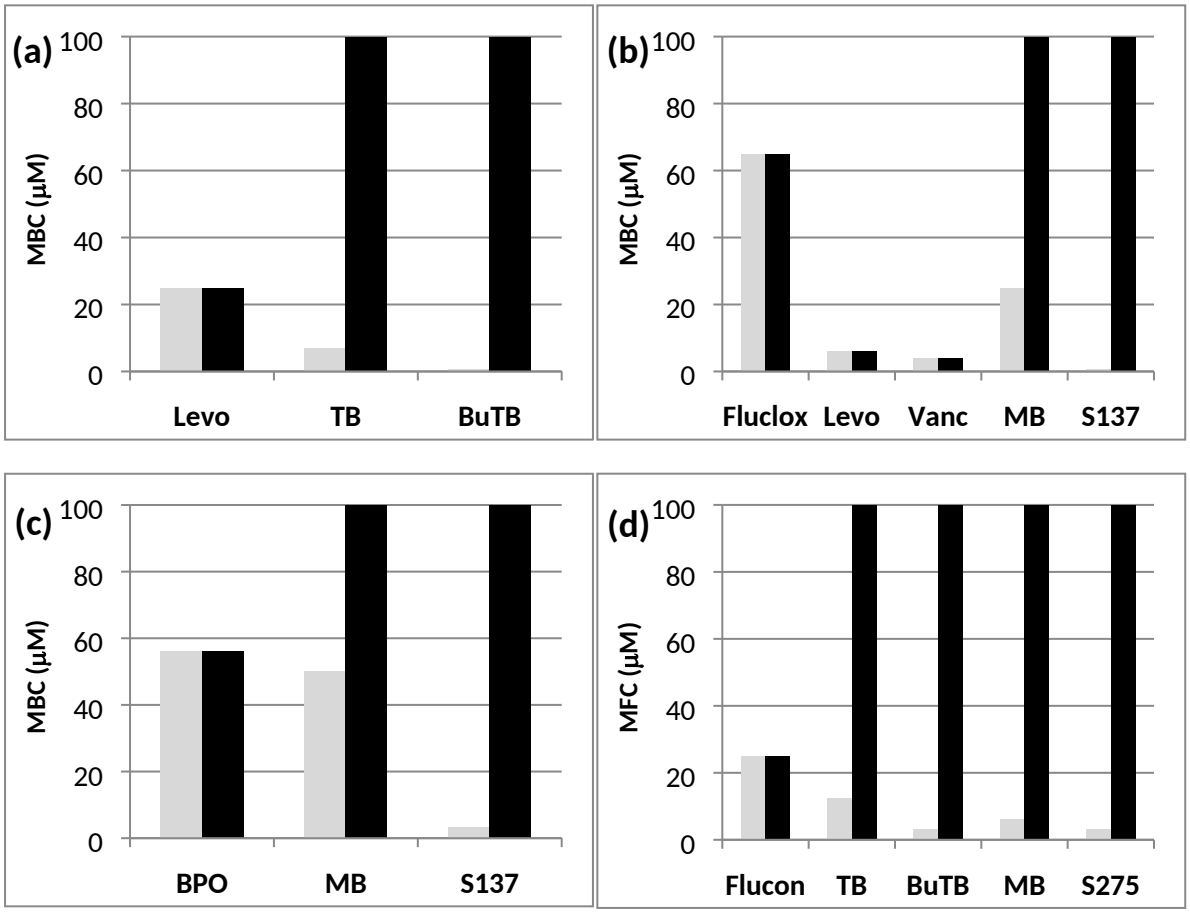
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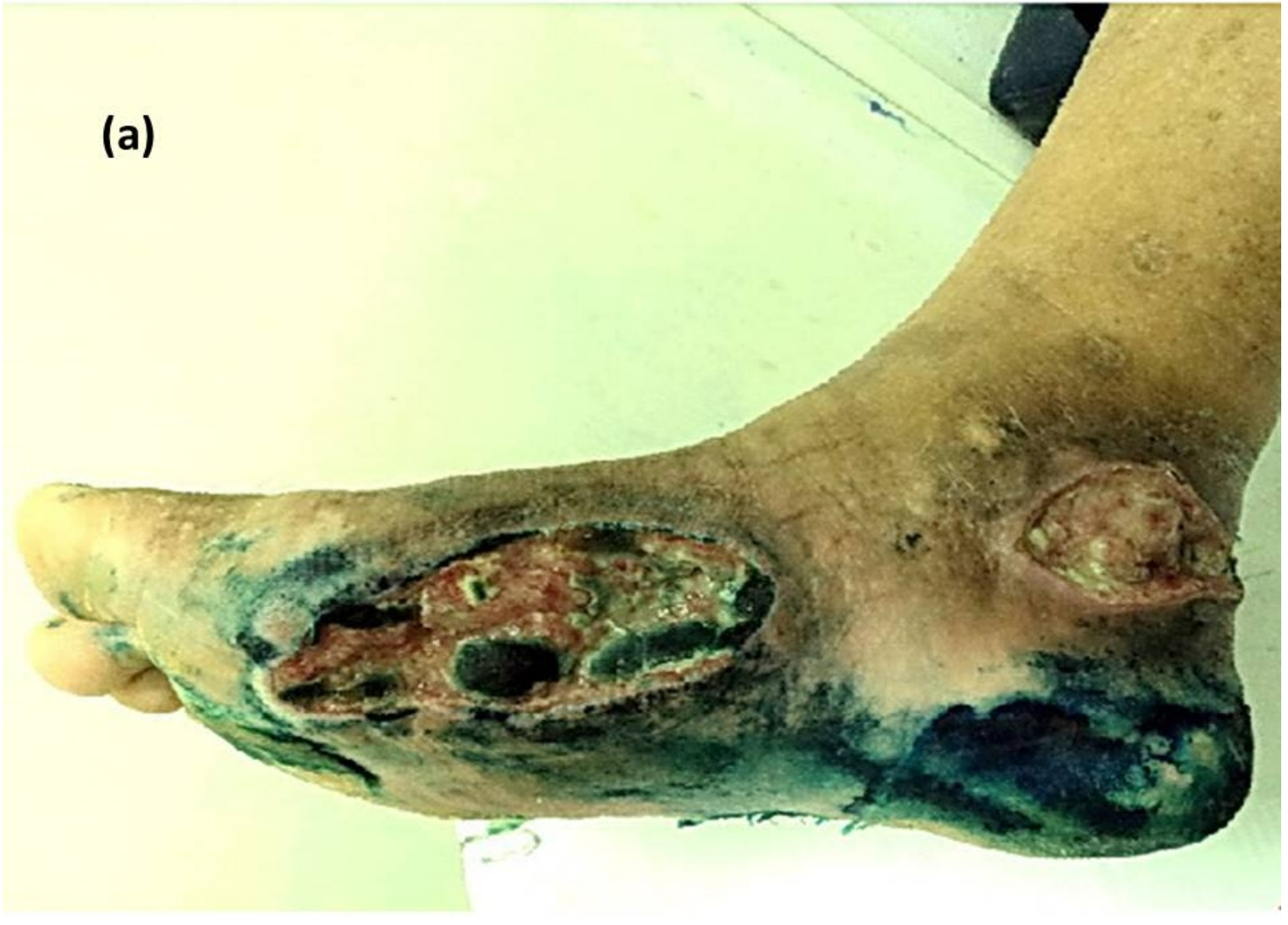
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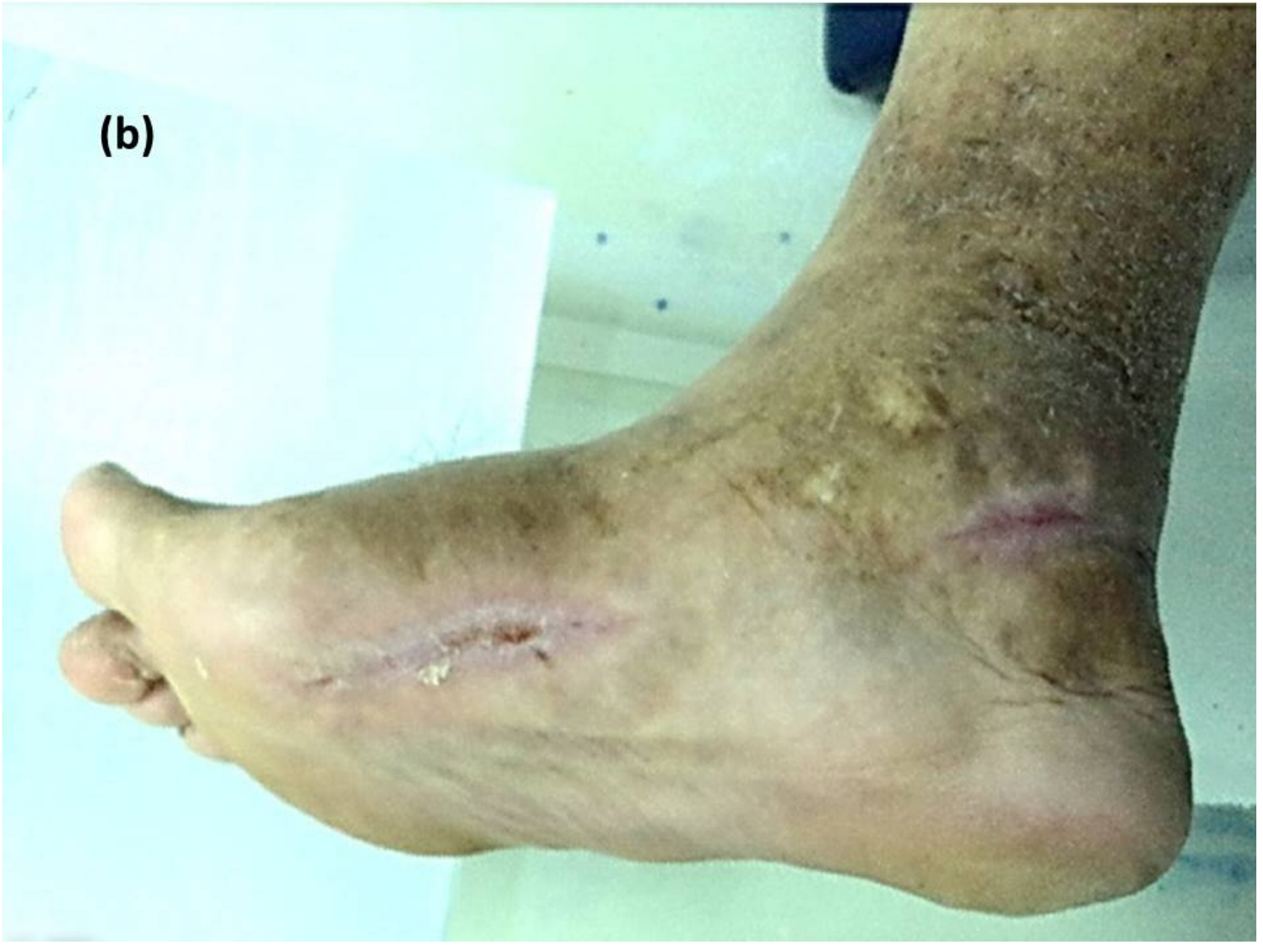
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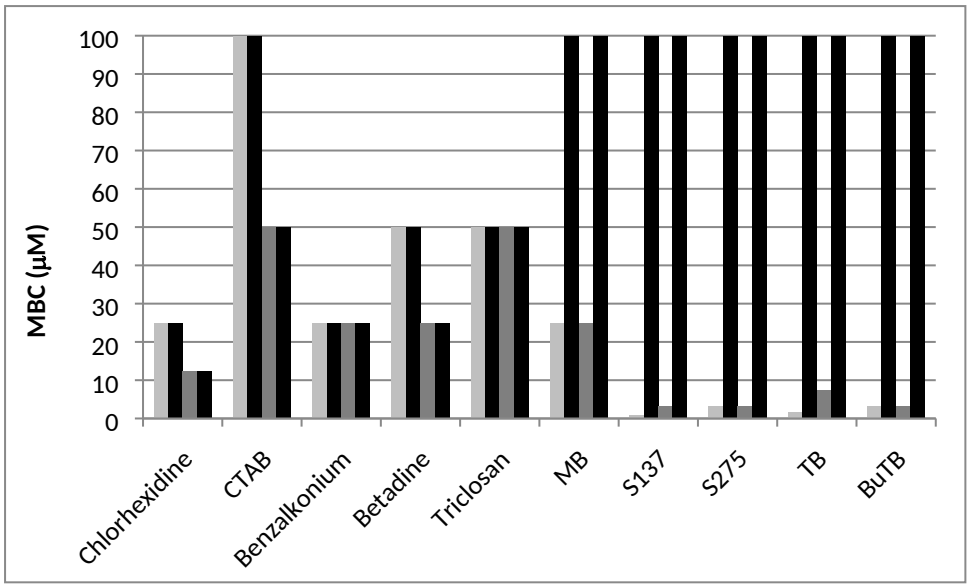
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