A Rationally Designed Multifunctional Antibiotic for the Treatment of Drug-Resistant Acne

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Acne is a multifactorial skin disease, underpinned by colonization of *Propionibacterium acnes* and inflammation. The emergence of resistant *P. acnes* strains has affected the current acne treatment algorithm. This setback served as an impetus for rationally designing a library of next-generation antibiotics that exhibit a bactericidal effect on resistant *P. acnes* and exert an immunomodulatory function to reduce inflammation. In silico screening showed that one of the molecules, VCD-004, exhibits improved mode of binding to bacterial DNA gyrase. VCD-004 shows high potency against clinical isolates of resistant *P. acnes* and excellent efficacy in vivo. Furthermore, VCD-004 exhibits a superior mutant prevention index, suggesting that it impedes the development of resistance better than clindamycin. Additionally, it shows optimal skin penetration and has a potent anti-inflammatory effect via reduction of proinflammatory cytokines (IL-6) independent of its antibacterial action. VCD-004 affects *P. acnes*-induced nuclear accumulation of NF-κB in THP-1 cells. The in vitro viability of human keratinocytes in the presence of VCD-004 indicates a desirable therapeutic window for topical use. Such rationally designed bactericidal and immunomodulatory dual pharmacophore-based lipophilic molecule(s) can emerge as the next-generation topical therapy for acne with underlying resistant *P. acnes* etiology.

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INTRODUCTION

Acne vulgaris affects almost 9.4% of the world's population (Tan and Bhate, 2015). *Propionibacterium acnes,* a commensal human skin bacterium, is a prime pathogenic factor in the development of acne (Cunliffe et al., 1981). Topical and oral antibiotics play pivotal role in effective acne management. Clindamycin and erythromycin are commonly applied topical antibiotics, whereas minocycline and doxycycline are administered orally to patients with moderate to severe acne (Aslam et al., 2015). Although current therapy guidelines recommend judicious use of antibiotics in combination with other anti-acne drugs, like retinoids (Nast et al., 2012; Thiboutot et al., 2009), extensive use of currently approved antibiotics has led to generation of resistant *P. acnes* strains, which poses a major setback to the present treatment regimens (Leyden et al., 1983; Simonart and Dramaix, 2005). This necessitates development of drugs that can address the challenge of resistant *P. acnes*.

Not all antibiotics that exhibit efficacy against *P. acnes* are suitable for acne therapy (Sinha et al., 2016). Acne is a multifactorial disease with a significant inflammatory component (Leyden, 2003). As a result, we rationalized that the logical design of antibiotics against resistant *P. acnes* should incorporate pharmacophore(s) that can impart bactericidal activity against resistant strains of *P. acnes* and further confer an immunomodulatory activity to reduce acne-induced inflammation. We developed a library of molecules, to our knowledge previously unreported, with quinolone backbone and nitroheterocyclic motif arranged in different spatial orientations to satisfy the desired activities of a next-generation anti-acne drug. The currently used antibiotics, clindamycin and tetracyclines, are mostly bacteriostatic in nature (Tan, 2004). We chose a quinolone scaffold that can interfere in DNA supercoiling in DNA gyrase-DNA complex (Kampranis and Maxwell, 1998), resulting in a bactericidal effect. Nadifloxacin, a quinolone-based drug, is approved in parts of Europe and Japan for acne treatment (Jacobs and Appelbaum, 2006). Similarly, nitroheterocyclic molecules have been widely used as antibiotics and anti-inflammatory agents for specific indications (Bannatyne, 1999; Millikan, 2003; Shakir et al., 2011).

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Abbreviations: MIC, minimum inhibitory concentration; MTCC, The Microbial Type Culture Collection and Gene Bank; MPC, mutant prevention concentration; QBP, quinolone binding pocket

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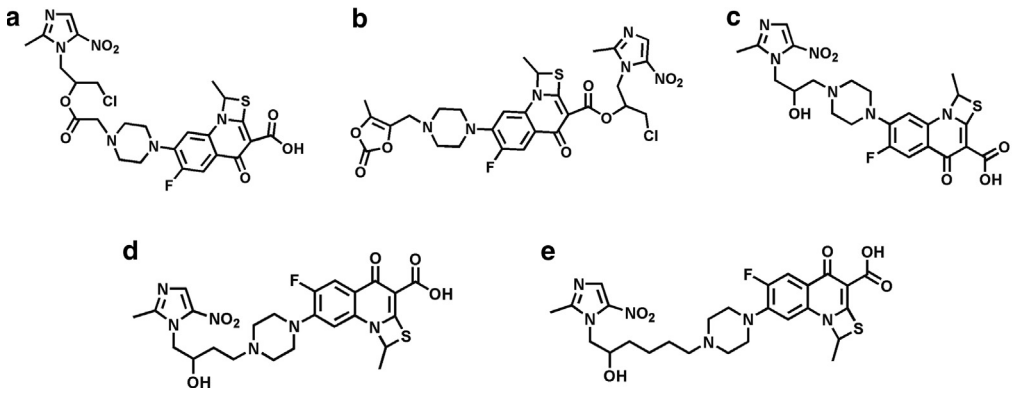

Figure 1. Molecular structures of the synthesized antibiotics. (**a**) VCD-002, (**b**) VCD-003, (**c**) VCD-004, (**d**) VCD 006, and (**e**) VCD-007.

Using in silico molecular docking, we structured an effective quinolone scaffold with optimum spatial orientation of the nitro-heterocyclic (3-[2-methyl-5-nitro-imidazolyl] propan-2-ol) motif to satisfy efficient binding at the quinolone binding pocket (QBP) of DNA gyrase-DNA complex. We report that one of these molecules, VCD-004, displays a different mode of binding at the QBP compared with fluoroquinolones. VCD-004 exhibits potent antimicrobial activity on clindamycin-susceptible and -resistant *P. acnes* strains and has a low mutant prevention index compared with clindamycin and nadifloxacin. VCD-004 exerts anti-inflammatory action, distinct from its bactericidal property, against dead *P. acnes*-induced inflammation both in vitro and in vivo, indicating a dual action on the bacteria and the host inflammation. VCD-004 was found to modulate the *P. acnes*-induced NF-κB signaling pathway. Furthermore, a topical 1% VCD-004 gel showed excellent skin penetration properties and superior efficacy in a *P. acnes* murine skin infection model. Finally, a favorable safety profile of VCD-004 in a human keratinocyte cell line connotes the emergence of such bi-pharmacophore molecules as a future therapy against acne, even with an underlying resistant *P. acnes* etiology.

RESULTS

Rational design and synthesis of VCD antibiotics

We used in silico-based design approach to synthesize five antibiotics, based on a strategy to incorporate a nitro-heterocyclic motif on a quinolone scaffold by varying tethering modes that confer different spatial conformations (Figure 1). These five antibiotics are, to the best of our knowledge, previously unreported. We wanted to investigate the preferred anchoring site between C-7 and C-3 of the quinolone scaffold for anchoring the nitro-heterocyclic motif to obtain better binding interactions toward DNA gyrase-DNA complex compared with other known fluoroquinolones. VCD-002, VCD-004, VCD-006, and VCD-007 were designed by incorporating nitro-imidazole moiety to the piperazine unit present at the C-7 position of the molecule, whereas in VCD-003, the C-3 carboxylic site of the quinolone scaffold was modified with a nitro-imidazole unit to affect the normal metal binding mode of quinolones with an Mg^{2+} ion present at the QBP.

Because the crystal structure of *P. acnes* gyrase is not reported, we performed molecular docking to analyze binding of the putative bi-pharmacophore structures in the QBP of DNA gyrase of a Gram-positive bacteria, *Staphylococcus aureus,* that has considerable amino acid sequence identity with respect to *P. acnes* (44.5% for gyrase A and 54.9% for gyrase B subunits). The residues of the two species involved in quinolone binding share significant sequence homology (see Supplementary Figure S1a and b online, highlighted in cyan).

For molecular docking, we used nadifloxacin and ciprofloxacin as reference ligands. Figure 2 shows energy-minimized binding poses of nadifloxacin and designed antibiotics (VCD-002, VCD-004, and VCD-007) bound to the QBP. Nadifloxacin (Figure 2a) binds like ciprofloxacin (see Supplementary Figure S2a online) near the nick of double-stranded DNA, where the aromatic ring of quinolone gains stacking interactions with nucleotide bases deoxycytosine and deoxyguanosine from top and deoxyadenosine and deoxythiamine from bottom. This intercalation between the bases orients the 3-carboxyl group of nadifloxacin toward Mg^{2+} and the OH-substituent of piperidine group at the C-7 position toward residues D437, R458, N476, and E477 of gyrase B.

In contrast, VCD molecules, depending on the spatial orientation of the nitro-heterocyclic group, prefer a different binding orientation in the QBP, where the position of the 3-carboxyl group and piperazine substituent of the structures are inverted compared with nadifloxacin/ciprofloxacin; thus, the functional moieties interacting with the amino acid residues of subunits gyrase A and gyrase B are switched (Figure 2b–d). Such inverse binding results in a co-ordination complex between Mg^{2+} (Mg^{2+} bridge) and the nitro group of VCDs, unlike the 3-carboxyl group in the case of nadifloxacin/ciprofloxacin. Among VCD molecules, VCD-004 showed the best docking pose, presenting maximum stacking interactions with the complex. The rigid tethering mode between piperazine and the nitro-heterocyclic motif in VCD-004 conferred maximum stacking interactions with DNA bases compared with other structures (Figure 2c). Moreover, we observed an increase in noncovalent interactions of VCD-004 in the QBP, including hydrogen bonding of the OH group (present in the linker) to deoxyguanosine and the 3-carboxyl group of the drug with R458, E1088, and D437 of DNA gyrase. In contrast, VCD-002 and VCD-007, with nitro-heterocyclic motifs approximately 6 Å away from C-7 piperazine motifs, show reduced stacking interactions, with DNA and hydrogen bonding interactions with the amino acid residues of the DNA-gyrase complex (Figure 2b and c). Similarly, VCD-003