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Original Article

Predictive and Anti-Acne Activity of Minocycline Hybrids

Mansi Shah[1,2,*], Bhanubhai Suhagia[2], Sunita Goswami[3], Arpit Patwari[4]

[1]Faculty of Pharmacy, Dharamsinh Desai University, Nadiad, Gujarat, INDIA.
[2]Department of Pharmacology, L.J. Institute of Pharmacy, LJ University, Ahmedabad, Gujarat, INDIA.
[3]L.M. College of Pharmacy, Ahmedabad, Gujarat, INDIA.
[4]Pharmace Research Laboratory, Gandhinagar, Gujarat, INDIA.

ABSTRACT

Background: The *Propionibacterium acnes* (*P. acnes*) has been the most abundant acne causing bacteria on human skin. Tetracyclines and macrolides have been used against this bacterium. A need to develop newer antibiotics has been aroused due to point mutations in the genes of *P. acnes*. This work emphases our efforts to discover novel minocycline hybrids that fight against *P. acnes*. Nine hybrid compounds were synthesized using minocycline and naturally occurring aldehydes and ketones available from plant sources. **Materials and Methods:** In this study the antibacterial activity of the proposed minocycline hybrids was ascertained using web-application "antiBac-Pred" and *in vitro* antibacterial activity was processed using standards protocols against *P. acnes*. The zone of inhibition and minimum inhibitory concentration of synthesized compounds was determined by agar cup diffusion method and broth micro dilution method respectively and compared with standard drugs minocycline, chloramphenicol, ciprofloxacin and ampicillin. **Results:** The "antiBac-Pred" web application allowed us to predict that the designed minocycline hybrid can inhibit the growth of *P. acnes* based on the score of each compound expressed as confidence in its activity. The *in vitro* antibacterial activity showed promising results, with compound 2 demonstrating almost 4 times lower Minimum Inhibitory Concentration (MIC) for *P. acnes* and approximately 2 times lesser than minocycline, chloramphenicol, ciprofloxacin and ampicillin against *P. acnes*. **Conclusion:** Novel minocycline hybrids may serve as promising scaffolds which can be further optimized in terms of their Absorption Distribution Metabolism Excretion and Toxicity (ADMET) and be clinically utilized as an alternative for the treatment of bacterial infections due to *P. acnes*.

Keywords: Antibiotic resistance, "antiBac-Pred", *in vitro* antibacterial activity, *Propionibacterium acnes*, Minocycline hybrids.

Correspondence:
Ms. Mansi Shah[1,2]
[1]Faculty of Pharmacy, Dharamsinh Desai University, Nadiad-387001, Gujarat, INDIA.
[2]Department of Pharmacology, L.J. Institute of Pharmacy, LJ University, Ahmedabad, Gujarat, INDIA.
Email: mansi.shah2809@gmail.com

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INTRODUCTION

The *Propionibacterium acnes* (*P. acnes*), a gram-positive bacterium is an age old bacteria which is involved in the pathogenesis of acne. It is a chronic condition affecting almost 85% of adolescents and adults of 18 years or above.[1] Hyperseborrhea, changed sebum composition, follicular hyperkeratinization, microbial flora abnormalities, inflammation and immunological responses are the key factors that contribute to the complicated aetiology of acne.[2] In pre-teens acne occurs due to fluctuation in the hormone levels, however in adults hormonal fluctuation occurs during menstruation and pregnancy. Though acne is not life threatening it severely affect the patient psychologically. Apart from the dermatological effect, it is considered as the opportunistic pathogen affecting the patients post surgeries.[3] Since, *P. acnes* plays a key role in acne associated inflammation it triggers release of various proinflammatory cytokines like Interleukin (IL)-1β, IL-6, IL-8 and Tumour Necrosis Factor- α (TNF-α) which causes inflammation.[4] There are a number of antibiotics like Tetracyclines (minocycline, doxycycline), Macrolides, Clindamycin, Penicillin, Cephalosporins, Quinolones used to treat acne.[5] Tetracyclines have antibacterial and anti-inflammatory properties which make them effective in treating acne. Antimicrobial efficacy involves inhibiting protein synthesis in bacterial cells. Tetracyclines reversibly bind to the bacterial ribosome's 30S subunit, preventing acyl-transfer RNA from binding. Protein synthesis stops, halting bacterial growth and multiplication, resulting in a bacteriostatic effect. The tetracyclines also reduces the generation and secretion of proinflammatory cytokines such IL-1β, IL-8 and TNF-α. Inhibiting these cytokines prevents neutrophil chemotaxis and activation. Tetracyclines reduce Reactive Oxygen Species (ROS) and prevent oxidative damage to biological macromolecules. Tetracyclines inhibit proteinases, including matrix metalloproteinases, which can cause acne. Thus, minocycline a Second-generation tetracycline, an effective

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broad spectrum antibiotic exerts bacteriostatic effect on *P. acnes*.[6] However, due to the resistance which has developed especially against tetracycline and macrolides there is an urgent need to develop newer drugs against *P. acnes*.[7]

Among the various approaches, one of the approaches is to synthesize hybridized antibiotic molecules which broadens the spectrum of antibacterial activity, delays emergence of resistance, gives protection against specific resistance determinants, achieves synergistic activities and enhances penetration into gram positive and gram-negative organisms.[8-10]

As per the data obtained from various studies, it have been reported that modification at the 9th position of minocycline structure leading to the development of 9-aminominocycline increases the efficacy and potency of antibiotic against various resistant species and enhances the antimicrobial effect.[11-13] Also among the various phytochemicals, natural as well as synthetic aldehydes/ketones have also been proven to exert the antimicrobial activity by destroying the outer bacterial cell membrane.[14-18]

Herein, we have predicted the antibacterial activity of designed novel 9 minocycline hybrids against *P. acnes* using a web-application "antiBac-Pred", synthesized and evaluated these compounds for their anti-bacterial activity *in vitro.*

MATERIALS AND METHODS

Compound Test Preparation

ChemDraw Ultra 12.0 has been used to create visual representations of the chemical structures of minocycline-hybrid molecules. Then these visual structures have been converted to the SMILES format (which is a textual representation) which has been used for further software based chemical analysis.

Prediction For Antibacterial Activity Against *P. acnes* Using "antiBac-Pred"

The prediction of anti-bacterial activity for 9 novel minocycline hybrids against the *P. acnes* had been done using the web-application antiBac-Pred. The antiBac-Pred has been the first web-based tool that allows user to assess the inhibitory effect of chemical compounds against as many as 353 bacteria, including both resistant and non-resistant ones in concentration below 10000 nM.

The web-application expresses the score of each compound as confidence in its activity, which exhibits the difference between probabilities for chemical compound to inhibit/do not inhibit the growth of particular bacteria. The higher the confidence means the higher chance of positive prediction to be true. Only the activities with Pa>Pi (confidence>0) has to be considered as possible for a particular compound.[19]

To make prediction, each minocycline hybrid chemical structures had been drawn using ChemDraw Ultra 12 and copied as SMILE structure, the input of this SMILE structure had been done in antiBac-Pred and clicked on "predict" button, which gives the antibacterial activity against *P. acnes* based on confidence value.

Synthesis of Minocycline Hybrids

The SAR studies of minocycline have reported that modification at the 9th position enhances the therapeutic activity of minocycline. Nitration and subsequent reduction at the 9th position of minocycline have been reported.[12] Herein; we have followed the protocol by Zang *et al.*[20] with slight modifications wherein various aldehydes and ketones have been covalently linked with 9-amino minocycline through conventional reductive amination reaction to generate a set of 9-minocycline secondary amine hybrids (Figure 1). These hybrids were characterised using different analytical techniques. Synthesis and characterisation data has been reported in our unpublished article (article *in-press*).[21]

In vitro Antibacterial Activity

All the synthesized hybrids were evaluated to check their antibacterial potential against gram positive *P. acnes* MTCC 1951. *In vitro* antibacterial studies were performed by using agar cup diffusion method and broth micro dilution method.

Agar Cup Diffusion Method

In this method Mueller-Hinton agar was utilized for the antibacterial screening of synthesized 9 hybrid compounds. Initially stock solution of 2000 µg/mL was prepared by dissolving 20 mg test compound in 10 mL DMSO as a solvent. Further step-down dilutions were made of 1000 µg/mL, 500 µg/mL and 250 µg/mL in DMSO and the solutions were evaluated for primary screening. All the compounds which showed Zone of Inhibition (ZOI) at strength 250 µg/mL were further evaluated at lower strengths of 100 µg/mL, 50 µg/mL, 25 µg/mL and 5 µg/mL. The process of preparation of culture started by inoculating the required bacteria (*P. acnes* MTCC 1951) in 10 mL of nutrient agar and incubated for 24 hr. Afterwards the prepared culture was centrifuged and then was compared with McFarland 0.5 to get required microbial count 10^8 CFU/mL. Subsequently, in a petriplate inoculated prepared agar culture was poured to form a uniform layer of 5mm and allowed to solidify. Using a sterile cork borer a cavity was made at the centre of the agar petriplate and 0.2 mL of prepared solution of tests compound was added and allowed to stabilize at room temperature for 1hr. Then the agar plates were incubated at 37°C for 18 hr and anti-microbial activity in terms of circular zone of inhibition was observed around the cavity. Zone of inhibition was measured by using a calibrated vernier calliper. This method was performed in triplicate for all test compounds and standard drugs i.e. minocycline, chlorpamphenicol, ciprofloxacin and ampicillin; average readings were recorded in millimetres (mm) as ZOI.

MIC Determination By Broth Micro Dilution Method

In this method, Muller-Hinton broth was used for antimicrobial susceptibility testing. Initially, the required bacterial culture was prepared by inoculating the bacteria into 10 mL of sterile broth, incubated for 24 hr and then centrifuged. Then the density of cultured bacteria was compared to 0.5 McFarland to get required microbial count 10^8 CFU/mL. In a test tube, prepared sterile 9.9 mL of Muller Hinton broth was added followed by addition of 0.1 mL of above prepared bacterial suspension. In primary screening the solution of 1000 µg/mL, 500 µg/mL and 250 µg/mL concentrations of the test drugs were taken from the prepared stock solution 2000 µg/mL (20 mg of synthesized hybrid compound/standard drug dissolved in 10 mL of DMSO). After adding respective concentration of test drugs in the test tube, it was then allowed to stabilize for 10-15 min at room temperature and incubated at 37°C for 24 hr. After incubation each tube was observed for visible microbial growth. The lowest concentration inhibiting growth of the organism is recorded as the MIC. From the results obtained in primary screening, the secondary screening was done by making serial dilutions and establishing the MIC values. Same procedure was repeated for standard drugs. The above procedure was performed in triplicate and average results have been reported.

Table 1: Antibacterial prediction against *Propionibacterium acnes* based on web-application "antiBac-Pred".

Compound No.	Confidence (Resistant)	Confidence (non-resistant)
1.	0.6435	0.0840
2.	0.6126	0.0533
3.	0.6046	0.0487
4.	0.5854	0.1239
5.	0.5705	0.1265
6.	0.6182	0.0857
7.	0.6396	0.0610
8.	0.5935	0.0974
9.	0.6588	0.0483

RESULTS

Data From "antiBac-Pred" Web-Application

Web-application "antiBac-Pred" predicts antibacterial of a compound against *P. acnes* with an interactive table which shows the details of compound and values of confidence in the presence of a growth inhibitory effect. All the tested compounds showed prediction of antibacterial activity against resistant and non-resistant *P. acnes* and the confidence values have been represented in the Table 1 mentioned below.

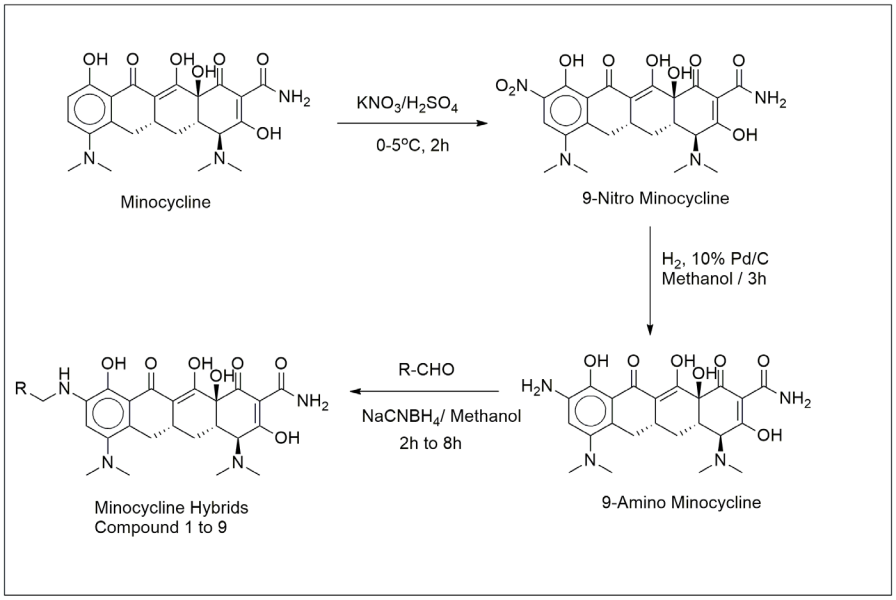
In vitro Antibacterial Activity

The zone of inhibition and MIC values has been calculated for all compounds and the results have been summarised in Table 2 and Table 3 respectively. The results of the Minimum Inhibitory Concentration (MIC) tests, which measure the lowest concentration of a compound needed to inhibit the growth of bacteria revealed that:

The compound 2 demonstrated the highest potency among all the hybrids tested. It was found to be more potent than the

Table 2: Zone of Inhibition (mm) against *Propionobacterium acnes*.

Propionobacterium acnes (MTCC1951)						
Conc. (µg/mL)	5	25	50	100	250	500
Compound No.	(Average Zone of inhibition in mm)					
1	NZ	NZ	NZ	12	14	16
2	NZ	19	21	22	22	24
3	NZ	NZ	NZ	12	13	15
4	NZ	NZ	NZ	14	16	17
5	NZ	NZ	NZ	13	16	17
6	NZ	NZ	15	15	16	20
7	NZ	NZ	NZ	NZ	13	13
8	NZ	NZ	13	14	16	19
9	NZ	NZ	NZ	13	15	17
Minocycline	NZ	NZ	NZ	14	15	20
Chloramphenicol	NZ	NZ	14	19	20	21
Ciprofloxacin	NZ	NZ	19	21	22	22
Ampicillin	NZ	NZ	13	14	16	18



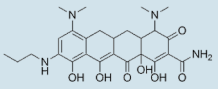
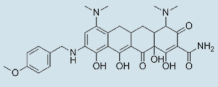
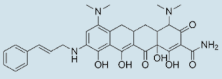
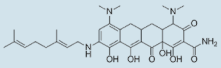
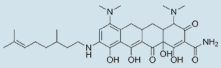
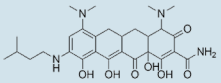
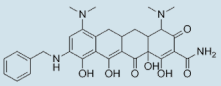
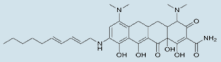
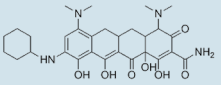
Compound No.	Aldehyde/Ketone Used	Product Structure
1.	Propionaldehyde	
2.	Anisaldehyde	
3.	Cinnamaldehyde	
4.	Citral	
5.	Citronellal	
6.	Isovaleraldehyde	
7.	Benzaldehyde	
8.	Decadienal	
9.	Cyclohexanone	

Figure 1: Synthetic Scheme for Minocycline Hybrids.

standard antibiotics used as controls, including minocycline (the parent compound), chloramphenicol, ciprofloxacin and ampicillin. The compound 2 exhibited almost 4 times lesser MIC values as compared to minocycline while 2 times lesser MIC as compared chloramphenicol and ciprofloxacin. Compounds 6 and 8 exhibited antibacterial activity comparable to that of chloramphenicol and ciprofloxacin. Compounds 1, 4 and 9 showed stronger antibacterial activity than Minocycline. This suggests that synthesized compounds could be a particularly promising candidate for further development as an antibacterial agent against *P. acnes*. The antibacterial activity based on MIC of compounds 3 and 5 was found to be equivalent to that of minocycline. While they did not surpass minocycline in potency,

Table 3: MIC values of hybrids against *Propionobacterium acnes*.

Propionobacterium acnes (MTCC 1951)	
Compound No.	**MIC value (µg/mL)**
1	62.5
2	25
3	100
4	62.5
5	100
6	50
7	125
8	50
9	62.5
Minocycline	100
Chloramphenicol	50
Ciprofloxacin	50
Ampicillin	40

they still retained the ability to inhibit *P. acnes* effectively, making them viable alternatives or complements to minocycline.

DISCUSSION

The study investigated the antibacterial activity of nine minocycline hybrids focussing their efficacy against *P. acnes,* a bacterium commonly associated with acne. The target prediction for these compounds suggested potential effectiveness against *P. acnes* which was subsequently validated through *in vitro* antibacterial assays. Altering the modifications at the 9th position of minocycline with various aldehydes as secondary amines in all the cases either enhanced the anti-bacterial activity or almost retained it similar to minocycline, indicating the potential to explore several other secondary amines to be synthesized and validated. Bulky aromatic aldehydes like p-anisaldehyde derived hybrids displayed maximum activity to contrary benzaldehyde derived hybrid had poor anti-bacterial profile; suggesting the importance and role of various functional group substituted phenyl rings for better activity. Aliphatic aldehydes derived hybrids also increased the potential by almost 2 folds. The chain length, branching and degree of unsaturation for further enhancing the potential shall be established with more studies over the time. Cyclic aliphatic derived agents also showed potential with clear increase in the activity against *P. acnes*.

Results indicated that based on MIC, compound 2 was found to be 4 times more potent as compared to minocycline, 2 times more potent as compared to ciprofloxacin and chloramphenicol and almost 1.5 times more potent as compared to ampicillin. Compound 6 and compound 8 exhibits equivalent potency as that of chloramphenicol and ciprofloxacin.

CONCLUSION

The present work establishes clearly a newer set of minocycline hybrids with superior profile against *P. acnes*. These molecules have the potential to replace the age-old molecules in future and can affect thousands of lives across the globe generating a ray of hope for better treatment against acnes. A detailed and thorough screening of more such designed hybrids against several other pathogenic bacteria can always be explored as a tool for development of novel antibiotic drug discoveries.

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DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ABBREVIATIONS

NZ: No Zone; **MIC:** Minimum Inhibitory Concentration; **ADMET:** Absorption, distribution, metabolism, excretion and toxicity; **ZOI:** Zone of Inhibition; **NZ:** No zone; **MIC:** Minimum Inhibitory Concentration.

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