Research Article

Design, Synthesis and Antimycobacterial Evaluation Of *N*-٤-bromoacetyl norfloxacin.

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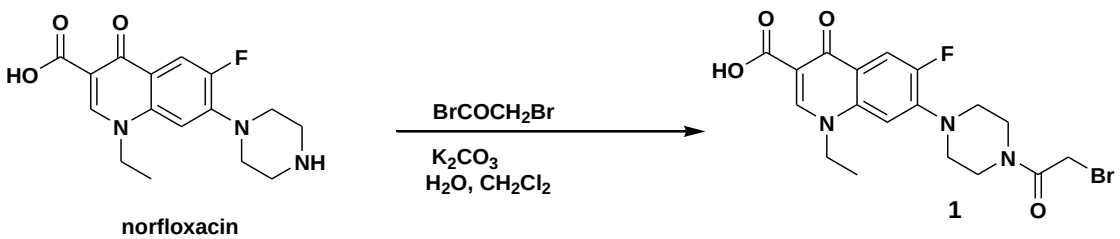
Abstract
A new derivative of norfloxacin; ٧-(٤-(٢-bromoacetyl)piperazin-١-yl)-١-ethyl-٦-fluoro-١,٤ dihydro-٤-oxoquinoline-٣-carboxylic acid; was synthesized, characterized by different spectroscopic techniques and evaluated against *Mycobacterium tuberculosis* H٣٧Rv. The prepared compound exhibited more potent antitubercular activity than norfloxacin.
Key words: Norfloxacin, *Mycobacterium tuberculosis*

Introduction
Tuberculosis (TB) is considered one of the most chronic bacterial infectious diseases all over the world and the second cause of death due to infectious disease after AIDS.[١,٢] The most widely used chemotherapy for treatment of uncomplicated TB cases is a combination of isoniazid, rifampicin, pyrazinamide, ethambutol or streptomycin.[٣] The emergence of multi-drug resistant tuberculosis (MDR-TB) and extensively drug resistant tuberculosis(XDR-TB)strain[٣,٤] has added significant difficulty in managing this serious disease. Thus the development of a new potent anti TB drug active against resistance strains has become of paramount importance.[٥] Currently, fluroquinolones as moxifloxacin and ciprofloxacin were approved by WHO as second-line agents in treatment of TB patients and considered one of the main tools to combat resistant TB strains.[٥] Herein, we report synthesis, characterization and evaluation of *N*-acetyl norfloxacin against *Mycobacterium tuberculosis* H٣٧Rv.

Results and discussion
Chemistry
The target compound ١ was prepared *via* Acylation of norfloxacin with bromoacetyl bromide (**Scheme ١**). The structure of the target compound was confirmed by IR, ^{١}H-NMR, ^{١٣}C-NMR and elemental microanalysis. IR spectrum of target compound ١ showed appearance of new peak at ١٦٥١ related to that of amidic carbonyl(NCO).[١] H-NMR spectrum showed the appearance of singlet signal at ٤.٢٠ ppm ,[١٣]C-NMR showed the appearance of new signal at ٢٨.٢٩ ppm related to (Br<u>C</u>H$_{٢}$)and the elemental micro analysis also, confirm the structure of the target compound.



Scheme١: Synthesis of norfloxacin derivative ١.

Biology

Results of the anti-TB revealed that the target compound show slightly higher potency against pathogenic *M. tuberculosis* H٣٧Rv with MIC; ٧.١μM than norfloxacin which has MIC; ٩.٨μM which might be explained by such increased lipophilicity could enhance the penetration through the lipid rich cell wall of *Mycobacterium*, and lipophilicity is an important consideration in the design and activity of newer antitubecular agents.[٦]

Experimental

Chemistry

Melting points were determined on Stuart electro-thermal melting point apparatus and are uncorrected.IR spectra were recorded on Nicolet iS٥ FT-IR spectrometer at Minia University.[١] H NMR spectra were carried out using Bruker apparatus ٤٠٠ MHz spectrometer, using TMS as internal reference at Sohag University. Elemental analysis was carried out in Al-Azhar University at the regional center for mycology and biotechnology. Reactions were routinely monitored by thin-layer chromatography (TLC) using Merck ٩٣٨٥ pre-coated aluminum plate silica gel (Kieselgel ٦٠) ٥ x ٢٠ cm plates with a layer thickness of ٠.٢ mm, and spots were visualized by exposure to UV-lamp at λ = ٢٥٤ nm. Materials: Chemicals and solvents used in the preparation of the target compound are of commercial grade, and purchased from Aldrich, Merck, and El-Nasr pharmaceutical Chemicals Companies.

Synthesis of the target compound ١.

To a stirred solution of norfloxacin (٠.٣١٩ g, ١.٠ mmol) in dichloromethane (٥٠mL) was added a solution of potassium carbonate (٠.١٥٢ g, ١.١ mmol) in distilled water (٥٠ml) at ٠-٥°C. Then, bromoacetyl bromide (٠.٢٢g, ١.١ mmol) in dichloromethane (٢٥ml) was slowly added over a period of ٣٠ min. Stirring was continued for ٢ h at ٠-٥ °C, then at room temperature for additional ١٢ h. The whole mixture was then transferred to a separatory funnel where it was extracted with dichloromethane and washed successively with ١*N*HCl and water. The organic layer was separated, dried over anhydrous sodium sulphate, filtered and the solvent was evaporated under reduced pressure to give compound **١.**

White powder; yield: ٠.٣٧٠ g (٨٤٪); mp: ٢٤٧-٢٤٩ ℃; ^{١}H-NMR (٤٠٠ MHz, DMSO-$d_٦$): ١.٤٥ (٣H, t, *J* = ٧.٦ Hz, *N*$CH_٢\underline{CH_٣}$), ٣.٣٨-٣.٤٢ (٤H, m, piperazinyl-H), ٣.٧٠-٣.٧٤ (٤H, m, piperazinyl-H), ٤.٢٠ (٢H, s, $Br\underline{CH_٢}$), ٤.٥٩ (٢H, q, *J* =٧.٦ Hz, *N*$\underline{CH_٢}CH_٣$), ٧.٢٢ (١H, d,$J_{H\text{-}F}$ = ٧.٦ Hz, H٨), ٧.٩٦ (١H, d, $J_{H\text{-}F}$ =١٣.٦ Hz, H٥), ٨.٩٤ (١H, s, H٢), ١٥.٢٢ (١H, s, COOH).^{١٣}C-NMR (١٠٠ MHz, DMSO-$d_٦$): ١٤.٧٦, ٢٨.٢٩, ٤١.٦١, ٤٦.١١, ٤٩.٥٦, ١٠٦.٨٦, ١٠٧.٥٨, ١١١.٧٣ (*J* = ٢٣ Hz), ١٢٠.١٠, ١٣٧.٦٢, ١٤٥.٥٦ (*J* = ١١ Hz), ١٤٩.١٤, ١٥٣.٢٩(*J* = ٢٤٨ Hz), ١٦٥.٤٩, ١٦٦.٥٧ and ١٧٦.٦٧; Anal. Calcd for $C_{١٨}H_{١٩}BrFN_٣O_٤$: C, ٤٩.١١; H, ٤.٣٥; N, ٩.٥٤. Found: C, ٤٩.٣٤; H, ٤.٣١; N, ٩.٧٨.

Biology

Anti TB activity

Briefly, the inoculum was prepared from fresh LJ medium re-suspended in ٧H٩-S medium (٧H٩ broth, ٠.١٪ casitone, ٠.٥٪ glycerol, supplemented oleic acid, albumin, dextrose, and catalase [OADC]), adjusted to a McFarland tube No. ١, and diluted ١:٢٠; ١٠٠ µl was used as inoculum. The compound ١ stock solution was thawed and diluted in ٧H٩-S at four-fold the final highest concentration tested. Serial two-fold dilutions of each drug were prepared directly in a sterile ٩٦-well microtiter plate using ١٠٠ µl ٧H٩-S. A growth control containing no antibiotic and a sterile control were also prepared on each plate. Sterile water was added to all perimetre wells to avoid evaporation during the incubation. The plate was covered, sealed in plastic bags and incubated at ٣٧°C in normal atmosphere. After ٧ days incubation, ٣٠ ml of alamar blue solution was added to each well, and the plate was re-incubated overnight. A change in colour from blue (oxidised state) to pink (reduced) indicated the growth of bacteria, and the MIC was defined as the lowest concentration of drug that prevented this change in colour.

Conclusion

A novel norfloxacin derivative was synthesized, characterized by different spectroscopic and elemental microanalysis techniques and evaluated against (H٣٧Rv).

Result revealed that new compound ١ has more potent activity than norfloxacin against ($H^{37}Rv$).

Acknowledgments

Sincere thanks are expressed to prof. **Dr. Sriram**, Chair Professor, Department of Pharmacy, Birla Institute of Technology & Science-Pilani, Hyderabad Campus, Jawahar Nagar, Hyderabad for his great help in performing anti TB screening.

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