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Research Article

Innovative Hybrid Compounds Targeting Tuberculosis: Development, Characterization and Bioefficacy Analysis of 6-substituted-2-Chloroquinoline-3-Carbaldehyde Hydrazide Ester Derivatives

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ABSTRACT

This study delves into the biological activity of ester compounds obtained from analogs of 6-substituted-2-chloroquinoline-3-carbaldehyde hydrazide, aiming to exploit the combined antitubercular properties of quinoline and hydrazide to create innovative hybrid compounds. The molecules underwent a meticulous multi-step synthesis process, followed by purification through recrystallization. Methodologies such as proton nuclear magnetic resonance (^{1}H-NMR), carbon-13 nuclear magnetic resonance (^{13}C-NMR), fourier-transform infrared (FTIR) and mass spectrometry were used to confirm the molecular structures of developed derivatives. SWISSADME, an online tool, was utilized to predict the ADME properties, shedding light on their pharmacokinetic profiles. Evaluation of *in-vitro* antitubercular activity employing the Alamar blue method highlighted compounds 4a and 4f, exhibiting noteworthy efficacy, achieving threshold concentrations of 6.25 µg/mL for *Mycobacterium tuberculosis* inhibition. These findings suggest the possibility of novel quinoline scaffold as a potential molecule for TB treatment, contributing to ongoing endeavors in TB drug discovery and potentially laying the groundwork to develop effective antitubercular therapies.

Introduction

Tuberculosis (TB) persists because of *Mycobacterium tuberculosis* still exists as a formidable global health threat characterized by high morbidity and mortality rates. Despite the availability of effective treatment protocols, TB control faces obstacles such as the rise of drug-resistant strains, prolonged treatment periods, and its intersection with the human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) epidemic. These challenges underscore the pressing need for innovative anti-TB agents that offer improved efficacy, shorter treatment durations, and effectiveness against drug-resistant TB strains.[1-5]

The pursuit of novel anti-TB compounds has been a focal point in the battle against tuberculosis. Among various chemical entities, chloroquinoline-3-carbaldehyde scaffolds have garnered interest because of their potent antimicrobial properties.

Quinoline, a heterocyclic aromatic organic compound, has been a cornerstone in the synthesis of antimalarial, antibacterial, antifungal and many more therapeutic agents, reflecting its versatile therapeutic potential.[6-34] Furthermore, hydrazide analogs have been explored for their pharmacological activities, including anti-TB effects, providing a solid foundation for their use in drug discovery.[35,36]

The integration of chloroquinoline and hydrazide frameworks to develop hybrid molecules represents a novel approach in TB drug discovery. These compounds combine the antimicrobial efficacy of both parent molecules, aiming to enhance activity against *M. tuberculosis* while potentially reducing the likelihood of

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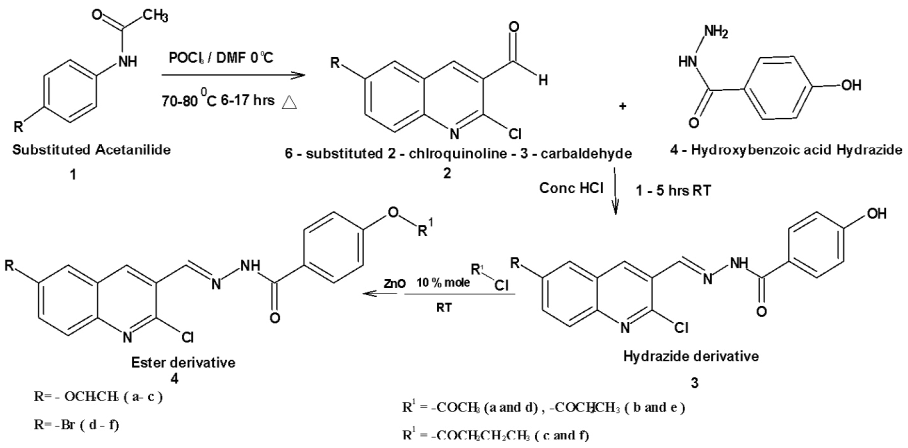

Fig. 1: Scheme for synthesis of ester derivatives of 6-substituted-2-chloroquinoline-3-carbaldehyde hydrazide derivatives

resistance development. Moreover, the synthesis of Schiff base adds to the molecular complexity, possibly offering unique mechanisms of action against TB bacteria.

However, the endeavor to discover new anti-TB agents is fraught with challenges. The process of designing, synthesizing, assessing the biological activity of these substances demands careful consideration of their pharmacokinetic profiles, safety, and effectiveness. The integration of advanced computational tools for predicting ADME properties, alongside traditional assessments like *in-vivo* and *in-vitro* evaluations, plays a crucial role in the early stages of drug development. [37,38]

This study seeks to contribute to the ongoing endeavors in TB drug discovery by exploring the development, characterization, and bioefficacy assessment of ester derivatives of 6-substituted-2-chloroquinoline-3-carbaldehyde hydrazide analogs. The ADME characteristics of the synthesized molecules were predicted by employing SwissADME, a web-based platform. By harnessing the well-established antitubercular properties of quinoline and hydrazide, combined with the innovative design of novel hybrid molecules, we aim to lay the groundwork for the development of fresh and efficacious antitubercular therapies.

Materials And Methods

Chemicals

In the laboratory, 6-substituted-2-chloroquinoline-3-carbaldehyde and its derivatives were synthesized following methods outlined in the literature. Analytical grade chemicals were employed in the synthesis, procured from Alfa Aesar, S.D. Fine chemicals, and Spectrochem Limited. An electrical open-capillary tube equipped melting point apparatus was used to ascertain melting points and are reported without correction. The synthesis progress was tracked using silica gel-G plates, 0.5 mm thick, for thin-layer chromatography (TLC) with spot visualization achieved using iodine vapors and ultraviolet light. Purification of all compounds was carried out via recrystallization using suitable organic solvents. Infrared (IR) spectra were acquired using an FT/IR-4100 instrument employing the KBr pellet technique. Mass spectra were captured using a Bruker ESI-MS device.

The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Avance Neo 500 MHz NMR spectrometer (Bruker, USA), with deuterated chloroform ($CDCl_3$) as the solvent and tetramethyl silane serving as the reference standard.

Synthetic Procedures

A general synthetic protocol for 2-chloroquinoline-3-carbaldehyde analogues (2a, 2d)[39] (Fig. 1)

Drying tube-equipped flask was used to cool 0.125 mol (9.13 g, 9.6 mL) of N, N-dimethylformamide to 0°C. Gradually, through a dropping funnel 0.35 mol (53.7 g, 32.2 mL) of phosphorous oxychloride was added dropwise with constant stirring. Subsequently, 0.05 mol of substituted acetanilide was introduced gradually and the flask was refluxed for an appropriate time duration (6–17 hours) at 70 to 80°C, and the reaction status was tracked using TLC. After the reaction was accomplished and the mixture had reached ambient temperature, it was introduced into ice-cold water (200–300 mL) dropwise. The crude product was filtered, dried, and then recrystallized using ethyl acetate.

Characterization data of 6-substituted-2-chloroquinoline-3-carbaldehyde (2a, 2d)

- *2-Chloro-6-ethoxyquinoline-3-carbaldehyde (2a)*

IR υ (cm^{-1}): 2850 (C-H Aldehyde), 1690 (C=O), 1450–1649 (C-C Aromatic), 1590 (C=N), 720 (C-Cl)

^{1}HNMR (500 MHz, $CDCl_3$: δ 9.73 (s, 1H), 7.24–7.90 (Aromatic, 4H), 4.06 (m, 2H) 1.34(t, 3H)

^{13}C-NMR: δ 14.7, 64.2, 106.4, 121.2, 126.3, 127.6, 130.9, 138.5, 146.5, 147.6, 156.2, 189.3.

2-Chloro-6-bromoquinoline-3-carbaldehyde (2d)

IR υ (cm^{-1}): 2800 (CH Aldehyde), 1590 (C=O), 1350–1480

(C-C Aromatic), 1520 (C=N), 750 (C-Cl), 700 (C-Br)
^{1}H-NMR (500 MHz, $CDCl_3$: δ 9.86 (s, 1H), 8.14-9.04 (Aromatic, 4H)
^{13}C-NMR: δ 120.1, 126.3, 128.1, 129.6, 131.1, 132.7, 138.5, 146.5, 147.6, 189.3.

Synthesis of 6-substituted carbaldehyde hydrazide analog (3a, 3d)[40]

A mixture containing 1-mmol of 6-substituted 2-chloroquinoline-3-carbaldehyde (2a and 2d) and 1.1 mmol of 4-hydroxybenzoic acid hydrazide was prepared in 20 mL of absolute ethanol and stirred at ambient temperature for 1 to 5 hours. Two drops of hydrochloric acid are added to catalyze the process. TLC is used to track the reaction's progress. After the reaction was completed, the mixture is neutralized using a solution of sodium bicarbonate in water (10%). After filtering and washing with 20 mL of water, the resulting precipitate was recrystallized using ethanol.

Characterization data of 6-substituted-2-chloroquinoline-3-carbaldehydehydrazidederivatives (3a & 3d)

- *N'- [(2-chloro-6-ethoxyquinoline -3-yl) methylidene]-4-hydroxybenzohydrazide (3a)*

IR υ (cm^{-1}): 3400 (O-H), 1610 (C=O), 1458–1500 (C-C Aromatic), 1550 (C=N), 780 (C-Cl)
^{1}H-NMR (500 MHz, $CDCl_3$: δ 11.87 (s, 1 H), 9.68(s, 1 H), 8.98 (s, 1 H), 6.88-7.85 (Aromatic, 8 H), 4.06 (m, 2H), 1.34(t, 3H)
^{13}C-NMR: δ 14.2, 64.3, 106.4, 115.7, 121.2, 124.5, 127.6, 128.1, 129.6, 130.9, 131.7, 146.5, 146.5, 150.6, 156.2, 157.4, 164.7.

- *N'-[(2-chloro-6-Bromoquinolin-3-yl) methylidene]-4-hydroxybenzohydrazide (3d)*

IR υ (cm^{-1}): 3470 (O-H), 1690 (C=O), 1458–1515 (C-C Aromatic), 1510 (C=N), 780 (C-Cl), 700 (C-Br)
^{1}H-NMR (500MHz, $CDCl_3$: δ 12.04 (s, 1H), 9.68 (s, 1H), 9.04 (s, 1H), 8.14–8.39 (Aromatic, 8H)
^{13}C-NMR: δ 115.7, 120.1, 124.5, 128.0, 128.1, 129.6, 129.6, 131.1, 132.7, 146.5, 146.5, 150.6, 157.4, 164. 7.

- *Synthesis of ester derivatives of 6-substituted-2-chloroquinoline-3-carbaldehyde hydrazide (4a-f)[41]*

At room temperature, acetyl chloride (12 mmol) was added to a blend of compound 3 (10 mmol) and ZnO (10 mol%), while stirring. The advancement of the reaction was tracked *via* TLC. Following its completion, ethyl acetate (EtOAc) (2×5 mL) was used to extract the reaction mixture. ZnO was removed by filtering. The organic aliquot is subsequently washed with a 10% $NaHCO_3$ solution and water, then dried over Na_2SO_4 to further retrieve the product. An alternative method of isolating the product would be to mix water and 10% $NaHCO_3$ solution, separate the organic layer, and then dry it on Na_2SO_4. The physicochemical properties of these molecules, including percent yield, melting point, and retention factor (Rf) of thin layer chromatography (TLC) analysis, are presented in Table 1.

Characterization data of ester derivatives of 6-substituted-2-chloroquinoline-3-carbaldehyde hydrazine (4a-f)

- *4-{2-[(2-chloro-6-ethoxyquinolin-3-yl) methylidene] hydrazine carbonyl} phenyl acetate (4a)*

IR υ (cm^{-1}): 3500 (N-H), 1620 (C=O), 1550 (C=N)
^{1}H-NMR (500 MHz, $CDCl_3$: δ 11.87 (s, 1H), 8.98 (s, 1H), 7.16–8.51 (Aromatic, 8H), 4.06 (m, 2H), 2.31 (s, 3H), 1.34 (s, 3H)
ESI-MS (m/z): 411.26 (M^+, $C_{21}H_{18}ClN_3O_4$), 412.48 ($M+H^+$, $C_{21}H_{19}Cl\ N_3O_4$), 413.10($M+2H^+$, $C_{21}H_{20}Cl\ N_3O_4$)
^{13}C-NMR: δ 9.1, 14.2, 27.8, 64.3, 106.4, 114.3, 121.2, 127.1, 128.9, 129.6, 130.9, 131.7, 146.5, 146.6, 150.5, 150.6, 156.1. 164.6, 172.8.

- *4-{2-[(2-chloro-6-ethoxyquinolin-3-yl) methylidene] hydrazine carbonyl} phenyl propanoate (4b)*

IR υ (cm^{-1}): 3480 (N-H), 1610 (C=O), 1520 (C=N)
^{1}HNMR (500MHz, $CDCl_3$: δ 12.06 (s,1H), 8.98 (s,1H), 7.16-8.51 (Aromatic,8H), 4.07 (q,2H), 2.61 (q,2H), 1.34 (s,3H),1.08 (s,3H).
ESI-MS (m/z):425.67 (M^+, $C_{22}H_{20}ClN_3O_4$), 426.34 ($M+H^+$, $C_{22}H_{21}ClN_3O_4$), 427.78($M+2H^+$, $C_{22}H_{22}ClN_3O_4$)

Table 1: Physicochemical data of ester derivatives of 6-substituted-2-chloroquinoline-3-carbaldehyde hydrazide

Synthesized compound	*R*	*R^1*	*Molecular formula*	*MW*	*%yield*	*M.P.*	*Rf**
4a	OCH_2CH_3	$COCH_3$	$C_{21}H_{18}ClN_3O_4$	411.84	61	273–274	0.66
4b	OCH_2CH_3	$COCH_2CH_3$	$C_{22}H_{20}ClN_3O_4$	425.67	75	287–289	0.71
4c	OCH_2CH_3	$COCH_2CH_2CH_3$	$C_{23}H_{22}ClN_3O_4$	439.16	78	301–303	0.57
4d	Br	$COCH_3$	$C_{19}H_{13}BrClN_3O_3$	446.26	63	308–310	0.71
4e	Br	$COCH_2CH_3$	$C_{20}H_{15}BrClN_3O_3$	460.38	79	322–324	0.63
4f	Br	$COCH_2CH_2CH_3$	$C_{21}H_{17}BrClN_3O_3$	474.03	69	336–337	0.73

*TLC analysis using mobile phase: n-Hexane: Ethyl Acetate (2:8)

^{13}C NMR: δ 9.3, 14.2, 27.8, 64.3, 106.8, 114.9, 121.1, 127.3, 128.8, 129.2, 130.5, 131.9, 146.5 ,146.6, 150.6,150.8, 156.3, 164.1 ,172.6.

- *4-{2-[(2-chloro-6-ethoxyquinolin-3-yl) methylidene] hydrazine carbonyl} phenyl butanoate (4c)*

IRυ (cm^{-1}): 3350 (N-H), 1590 (C=O), 1520 (C=N)
^{1}H-NMR (500MHz, $CDCl_3$: δ 12.17 (s,1H), 8.98 (s,1H), 7.16–8.50 (Aromatic, 8H), 4.06 (q, 2H), 2.59 (t, 3H) 1.51 (m, 2H), 1.35 (t, 2H), 1.08 (t, 3H)
ESI-MS (m/z): 439.16 (M^+, $C_{23}H_{22}ClN_3O_4$), 440.65 ($M+H^+$, $C_{23}H_{23}ClN_3O_4$), 441.63($M+2H^+$, $C_{23}H_{24}ClN_3O_4$)
^{13}C-NMR: δ 9.1, 14.2, 27.8, 64.3, 106.4, 114.3, 121.2, 124.5, 127.1, 128.9, 129.6, 130.9, 131.7, 146.5, 146.5, 150.6, 150.6, 156.1, 164.6, 172.8.

- *4-{2-[(6-bromo-2-chloroquinolin-3-yl) methylidene] hydrazine carbonyl} phenyl acetate (4d)*

IR υ (cm^{-1}): 3490 (N-H), 1620 (C=O), 1570 (C=N)
^{1}H-NMR (500MHz, $CDCl_3$: δ 12.04 (s, 1H), 9.04 (s, 1H), 7.51–8.39 (Aromatic, 8H), 2.59 (s, 3H)
ESI-MS (m/z): 446.26 (M^+, $C_{19}H_{13}Br\,Cl\,N_3O_3$), 447.64 ($M+H^+$, $C_{19}H_{14}Br\,Cl\,N_3O_3$), 448.37($M+2H^+$, $C_{19}H_{15}Br\,Cl\,N_3O_3$)
^{13}C-NMR: δ 21.0, 114.3, 120.7, 124.1, 128.0-128.1, 129.5-129.7, 131.1, 132.7, 146.4-146.6, 150.5-150.7, 164.7 , 169.1.

- *4-{2-[(6-bromo-2-chloroquinolin-3-yl) methylidene] hydrazine carbonyl} phenyl propanoate (4e)*

IR υ (cm^{-1}): 3500 (N-H), 1690 (C=O), 1540 (C=N)
^{1}H-NMR (500MHz, $CDCl_3$: δ 12.28 (s, 1H), 9.04 (s, 1H), 7.51-8.39 (Aromatic, 8H), 2.61 (q, 2H), 1.28 (t, 3H)
ESI-MS (m/z): 460.38 (M^+, $C_{20}H_{15}Br\,Cl\,N_3O_3$), 461.48 ($M+H^+$, $C_{20}H_{16}Br\,Cl\,N_3O_{33}$), 462.54($M+2H^+$, $C_{20}H_{17}Br\,Cl\,N_3O_3$)
^{13}C-NMR: δ 27.8, 114.3, 120.1, 124.5, 128.0-128.2 ,129.5-129.7, 131.1, 132.7, 146.4-146.6, 150.5-150.7, 164.7, 172.8.

- *4-{2-[(6-bromo-2-chloroquinolin-3-yl) methylidene] hydrazine carbonyl} phenyl butanoate (4f)*

IR (KBR) υ (cm^{-1}): 3350 (N-H), 1650 (C=O), 1490 (C=N)
^{1}H-NMR (500 MHz, Chloroform-d): δ12.22 (s, 1 H), 9.04 (s, 1H), 7.51–8.39 (Aromatic, 8H), 2.59 (t, 3H) 1.68 (m, 2H), 0.99 (t, 2H)
ESI-MS (m/z): 474.03 (M^+, $C_{21}H_{17}BrClN_3O_3$), 475.84 ($M+H^+$, $C_{21}H_{19}BrClN_3O_3$), 476.32($M+2H^+$, $C_{21}H_{20}BrClN_3O_3$)
^{13}C-NMR: δ 20.2, 114.3, 120.1, 124.5, 128.0-128.2, 129.5-129.7,131.1, 132.7, 146.4-146.6, 150.5-150.7, 164.7, 169.4

Antitubercular Activity Screening

Antitubercular action of the compounds towards *M. tuberculosis* was estimated by the microplate Alamar blue assay (MABA)[42] an innocuous method that makes use of a reagent that is stable to heat. This method has demonstrated good correlation with BACTEC radiometric techniques.

Media and Microorganism

The standard strain of *M. tuberculosis* vaccine, ATCC No. 27294 (H37 RV), was employed in the investigation. For the sake of comparison, benchmarks including streptomycin, pyrazinamide, ethambutol, isoniazid, and rifampicin were used.
The Alamar blue solution was made by mixing a 10% solution of Tween 80 in water to enhance dye solubility with the Alamar blue reagent. Additionally, Middlebrook 7H9 broth was supplemented with appropriate additives to support the growth of *M. tuberculosis.*

Assessment of anti-TB activity

To curtail the evaporation loss of the medium from the test wells during the course of incubation, 200 µL of sterile deionized water was initially dispensed into each of the outermost wells of a sterile 96-well plate. Subsequently, Middlebrook 7H9 broth (100 µL) was instilled into each well of the 96-well plate, which was then inoculated with a standardized suspension of *M. tuberculosis* to achieve the desired final inoculum size.
Final concentrations of 100 to 0.2 µg/mL were achieved by serially diluting the compounds directly on the plate. A positive control containing known anti-TB drugs was included for comparison. The plates were covered and concealed by parafilm and then subjected to an incubation for five days at 37°C. 25 µL of freshly prepared blend (1:1, Alamar blue reagent and 10% Tween 80) was added to each well after the incubation period and another 24 hours were spent incubating the plates. In the experimental well, pink and blue coloration is a marker of the presence and absence of bacterial growth, respectively. The minimum inhibitory concentration (MIC) was defined as the minimal drug content required to prevent the conversion from blue to pink coloration in the test well.

Data Analysis

Linear comparison of the MIC values of the experimentally synthesized anti-TB derivatives with reference to the control was employed to confirm the bioefficacies. All procedures involving *M. tuberculosis* were conducted in a biosafety level 3 (BSL-3) laboratory, employing suitable containment equipment and safety protocols to uphold the highest level of safety. Stringent biosafety guidelines were followed to guarantee proper disposal of all infectious materials and mitigate any risk of exposure to hazardous pathogens. These precautions were implemented to maintain a secure laboratory environment and ensure the safety of personnel.

Prediction of ADME characteristics

SwissADME, an online dedicated software program, was employed to forecast the pharmacokinetic aspects of the developed anti-TB derivatives. The drug-likeness of chemical compounds was determined by employing the "Lipinski's Rule of Five for oral bioavailability".

The synthesized compounds (labeled 4a–4f) were forwarded to SwissADME following the conversion of their structure to simplified molecular input line-entry system (SMILES).[43]

Through this process, *in-silico* prediction of pharmacokinetic parameters was conducted, providing valuable insights into the compounds' potential as drug candidates.

Results And Discussion

New ester derivatives of 6-substituted-2-chloroquinoline-3-carbaldehyde hydrazide analogues were synthesized, and their antitubercular action was then assessed.

In the study, our purpose was to explore a better antitubercular treatment strategy by synthesizing hybrid molecules composed with hydrazide and chloroquinoline backbones.

Chemistry

The conversion of substituted acetanilide to 6-substituted-2-chloroquinoline-3-carbaldehyde derivatives (2a, 2d) using phosphoryl chloride ($POCl_3$) in dimethylformamide (DMF) involves a series of chemical reactions involving Vilsmeier-Haack reaction, a form of electrophilic aromatic substitution, and cyclization reaction. The initial reaction involves activation of DMF, the formylating agent by $POCl_3$ to form Vilsmeier-Haack complex (an iminium chloride or chloroiminium ion). The activated complex then interacts with the substituted acetanilide, which acts as the nucleophile. The nucleophilic aromatic ring of the acetanilide attacks the carbon atom of the iminium ion, leading to the introduction of a formyl group (-CHO) into the acetanilide structure. The critical step aromatic rig is formylated to give an intermediate.

Subsequent intramolecular cyclization is facilitated in the presence of $POCl_3$, aiding in the dehydration process. This cyclization results in the closure of the nitrogen-containing ring, ultimately forming the quinoline skeleton. The reaction mixture is then worked up, usually involving hydrolysis to neutralize any remaining reagents and to fully form the derivative.[39]

The presence of aldehyde proton at δ 9.73 (2a) and 9.86 (2d), and aromatic protons in the range of 7.24 to 7.90 (2a) and 8.14 to 9.04 (2d) in proton NMR confirm formation of chloroquinoline carbaldehyde nucleus. FTIR and ^{13}C-NMR further confirm the presence of functional groups and carbon skeleton, respectively, thereby elucidating the structures.

A condensation reaction occurs involving 4-hydroxybenzoic acid hydrazide and 6-substituted -2-chloroquine-3-carbaldehyde in absolute ethanol and hydrochloric acid to yield hydrazide derivatives.[40] The reaction commences with an attack of a hydrazide nitrogen atom on the carbonyl carbon of the 2-chloroquinoline-3-carbaldehyde as a nucleophile. This step is facilitated by the acidic environment, which protonates the carbonyl oxygen, rendering the carbonyl carbon more vulnerable to nucleophilic attack. The nucleophilic attack results in the formation of a tetrahedral intermediate, a common intermediate in reactions involving carbonyl groups. Subsequently, the elimination of water from the tetrahedral intermediate forms a carbon nitrogen double bond to give hydrazide linkage.

The stretching vibration around 3400 cm^{-1} confirms the presence of O-H in FTIR. The ^{1}H-NMR shows characteristic peaks at δ 8.98 (3a) and 9.04 (3d) for methylene protons, hydrazide N-H protons at 9.68, and hydroxy protons at 11.87 (3a) and 12.04 (3d). ^{13}C-NMR helped to confirm the carbon skeleton of the hydrazide compounds.

The hydrazide group is conjugated to the quinoline ring system through the former aldehyde carbon. This introduces a significant degree of structural complexity and potential for biological activity, as both the quinoline and hydrazide moieties are known for their pharmacological properties.

Esterification of quinoline hydrazide derivatives involves the process of acylation followed by esterification as shown in the scheme (Fig. 1). The reaction proceeds with attack of hydrazide nitrogen on the carbonyl carbon of acetyl chloride to give a tetrahedral intermediate. After the nucleophilic attack, the tetrahedral intermediate releases a chloride ion, thus forming the acylated hydrazide.[41]

It undergoes esterification, potentially involving an intramolecular reaction. The introduction of an ester group can affect the solubility, absorption, and distribution of the compound within biological systems, making it a valuable step in drug design and development for the treatment of tuberculosis.

Mass spectrometry with the electrospray ionization method confirmed the molecular weights of synthesized hybrid compounds. ^{13}C-NMR and FTIR verified the presence of carbon backbone and functional groups, respectively. The absence of signal for O-H and the presence of characteristic peaks at δ 8.98 to 9.04 for methylene protons and around 12 for hydrazide protons helped elucidate the structures of compounds 4a-4f.

Antitubercular activity using MABA

The MABA methodology was employed for evaluating the anti-TB action of the prepared analogues. MABA provides a unique combination of sensitivity, specificity, and operational simplicity, making it an efficient framework for rapidly screening potential anti-TB agents.[42] As a significant outcome of this study, the compounds 4a and 4f demonstrated 6.25 µg/mL as MIC, indicating a potent inhibitory effect against *M. tuberculosis*.

In comparison, as exhibited in Table 2 and Fig. 2, MIC values of 12.5 µg/mL for compounds 4b, 4c, 4d, and 4e demonstrate they are moderately active, in contrast to the minimum threshold concentrations of reference anti-TB drugs, e.g., isoniazid and ethambutol at

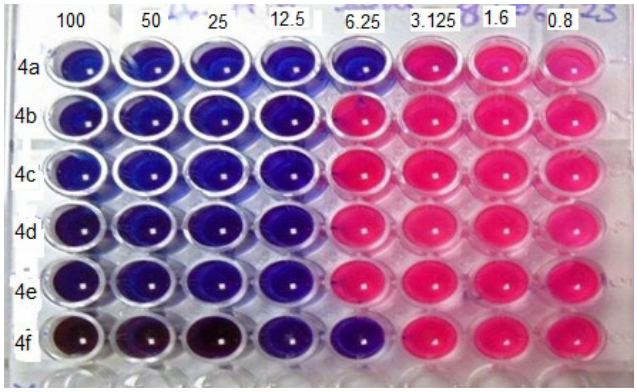

Fig. 2: Micro titer plate showing color change in Alamar blue assay method for synthesized ester derivatives of 6-substituted-2-chloroquinoline-3-carbaldehyde hydrazide 4a-4f depicting MIC values in µg/mL

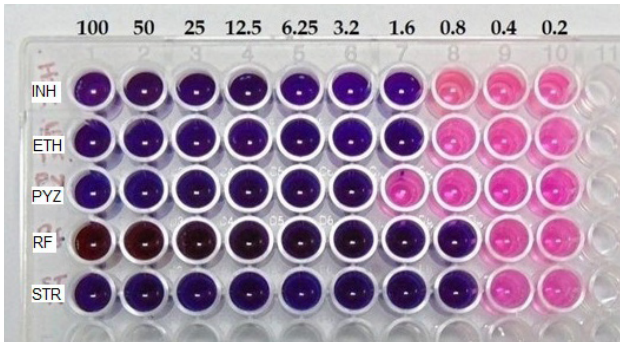

Fig. 3: Micro titer plate showing color change in Alamar blue assay method for standard anti-TB drugs depicting MIC values; INH: Isoniazide (1.6 µg/mL), ETH: Ethambutol (1.6 µg/mL), PYZ: Pyrazinamide (3.125 µg/mL), RF: Rifampicin (0.8 µg/mL), STR: Streptomycin (0.8 µg/mL)

1.6 µg/mL, pyrazinamide at 3.125 µg/mL, rifampicin and streptomycin at 0.8 µg/mL each (Fig. 3).

The MABA method's significance extends beyond its application in this study. Its non-toxic, thermally stable and colorimetric-based approach enables the high-throughput screening of compounds without relying on radioactive markers or biohazardous materials, presenting a safer and more environmentally friendly alternative to traditional methods. Furthermore, the good correlation of MABA with proportional and BACTEC radiometric methods affirms its reliability and reproducibility in assessing the antimicrobial activity of novel compounds.

Prediction of ADME properties

The utilization of SwissADME for prediction provides valuable theoretical attributes related to pharmacokinetic parameters such as ADME of potent drug candidates. This approach is essential for gaining an understanding of how the synthesized compounds may interact within biological systems, thereby offering guidance for subsequent research and development endeavors.[43]

ADME properties of synthesized hybrid molecules were comprehensively predicted using SwissADME and the representative results are presented in Table 3. All six molecules, 4a to 4f adhered to Lipinski's Rule of Five, indicating no violations. This rule serves as a fundamental guideline to evaluate drug likeliness, suggesting that these compounds may have favorable pharmacokinetic profiles. The adherence of molecules to this rule highlights their potential suitability for oral administration, as it indicates predicted favorable absorption and distribution characteristics. The presence of a moderate number of rotatable bonds (6-8) in these molecules suggests a level of molecular flexibility that can potentially improve binding interactions with biological targets while still maintaining structural integrity and avoiding undesirable metabolic instability.

The prediction of high gastrointestinal (GI) absorption for all molecules suggests a promising bioavailability profile, which is critical for oral drug efficacy. Conversely, the absence of blood-brain barrier (BBB) permeation for these compounds indicates specificity in targeting peripheral sites without central nervous system effects. This attribute is particularly advantageous for drugs intended for systemic but not central actions, reducing the risk of central side effects. The analysis revealed that none of the molecules are substrates for P-glycoprotein (Pgp), a transporter protein that can lead to drug resistance by effluxing drugs out of cells. This characteristic is

Table 2: Alamar blue assay results showing MIC values for synthesized derivatives

S. No.	*Synthesized derivatives*	*Concentration of synthesized derivatives (µg/mL) **							
		100	*50*	*25*	*12.5*	*6.25*	*3.125*	*1.6*	*0.8*
01	4a	S	S	S	S	**S**	R	R	R
02	4b	S	S	S	S	R	R	R	R
03	4c	S	S	S	S	R	R	R	R
04	4d	S	S	S	S	R	R	R	R
05	4e	S	S	S	S	R	R	R	R
06	4f	S	S	S	S	**S**	R	R	R

*R- Resistant, S- Sensitive

Table 3: Physicochemical properties predicted for synthesized compounds 4a – 4f using SwissADME

*Compounds properties**	*4a*	*4b*	*4c*	*4d*	*4e*	*4f*
Rotatable bonds no.	7	7	8	6	7	8
H-bond acceptors no	5	5	5	5	5	5
H-bond donors no	1	1	1	1	1	1
Consensus Log Po/w	4.25	3.73	4.04	3.91	4.25	4.54
Molar refractivity	109.24	104.23	109.03	104.43	109.24	114.04
Drug likeliness	Yes	Yes	Yes	Yes	Yes	Yes
Lipinski (violation)	0	0	0	0	0	0
Log S (ESOL) (Solubility)	-5.47	-4.88	-5.11	-5.17	-5.47	-5.71
Synthetic accessibility	2.79	2.78	2.9	2.69	2.79	2.91

*Few representative properties are listed in the table.

beneficial for maintaining therapeutic concentrations at target sites. The consensus log *p-values*, ranging from 3.7 to 4.5, fall within an optimal range that balances solubility and permeability, further affirming the compounds' drug likeliness. Molar refractivity values between 104 and 114, along with 6 to 8 rotatable bonds, suggest an appropriate balance of molecular flexibility and spatial occupancy, enhancing the likelihood of effective receptor interaction. The synthetic accessibility scores fell within the range of 2.7 to 2.9, suggesting that these molecules are relatively straightforward to synthesize. This ease of synthesis is advantageous for drug development processes. The uniform bioavailability score of 0.55 across all compounds, coupled with their ADME characteristics, predicts a moderate likelihood of success in preclinical development stages.

In summary, the ADME predictions for the hybrid molecules 4a to 4f using SwissADME offer promising prospects for their pharmacokinetic profiles. These predictions suggest favorable attributes, such as congruence to the "Lipinski's rule of five", optimal physicochemical properties and high gastrointestinal absorption which is conducive to drug development. Consequently, these findings justify the need for further experimental validation and exploration of these compounds as potential therapeutic agents. This lays the foundation for subsequent pharmacological studies (*in-vitro* and *in-vivo*)

Overall, the results of our investigation represent a significant contribution to the field of antitubercular drug discovery, highlighting the promising antitubercular activity of the synthesized chloroquinoline-hydrazide ester derivatives. It opens new avenues for further research into the optimization and development of these compounds as potential antitubercular therapies. A feasible route for the creation of novel antitubercular drugs that can tackle the problem of drug-resistant TB strains has been suggested by the investigation of chloroquinoline and hydrazide derivatives as the bases for hybrid compounds. Promising preliminary findings have been obtained in this regard.

Conclusion

Pursuing the goal of finding effective antitubercular medicines, this work synthesized and assessed new ester derivatives of 6-substituted-2-chloroquinoline-3-carbaldehyde hydrazide analogs. We succeeded in synthesizing and characterizing the chemicals by means of rigorous purification and precise multi-step synthesis procedures. The derivatives 4a and 4f indicated marked *in-vitro* activity against *M. tuberculosis in-vitro* exhibiting MIC value - 6.25 µg/mL, according to the Alamar blue technique assessment of their antitubercular activity. This finding underscores the therapeutic potential of the chloroquinoline and hydrazide combination, considering their documented antitubercular properties to enhance the efficacy of the novel hybrid molecules. In conclusion, the innovative approach of combining chloroquinoline and hydrazide to create novel antitubercular agents has yielded compounds with significant *in-vitro* efficacy against *M. tuberculosis*. These findings provide a way for future investigations to further understand the mechanisms of action, optimize the lead compounds, and evaluate their *in-vivo* efficacy and safety profiles.

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