Asian Journal of Chemistry; Vol. 36, No. 9 (2024), 2197-2202

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2024.32150

Synthesis and Antitubercular Activity of Novel 1,5-Naphthyridin-2(1*H*)-one based Carbohydrazide Derivatives

NAGENDRA BABU CHILAKALA[1], VISHNU THUMMA[2], B. RAJU[1], SHARADA ETNOORI[1], LAKSHMI SATYA BODDU[3] and K. PREMALATHA[1,4,*]

[1]Department of Chemistry, Osmania University, Hyderabad-500007, India
[2]Department of Sciences and Humanities, Matrusri Engineering College, Hyderabad-500059, India
[3]Department of Pharmaceutics, Vishnu Institute of Pharmaceutical Education and Research, Narsapur-502313, India
[4]Department of Chemistry, Telangana Mahila Viswavidyalayam, Hyderabad-500095, India

*Corresponding author: E-mail: premalathasheshu@gmail.com

Received: 15 June 2024; *Accepted*: 24 August 2024; *Published online*: 30 August 2024; AJC-21748

A library of novel 1,5-naphthyridin-2(1*H*)-one based carbohydrazide derivatives (**6a-j**) were synthesized involving functional group interconversion, esterification and coupling reactions. All the newly synthesized carbohydrazide derivatives (**6a-j**) were evaluated for their ability to inhibit the growth of *M. tuberculosis* mc^{2}6230 by determining their minimum inhibitory concentration (MIC), with rifampicin used as the standard reference. Compound **6f** (3-cyano) displayed potent activity with a MIC value of 4 µg/mL, compound **6g** (4-cyano) displayed activity with an MIC value of 8 µg/mL, whereas the *ortho*-substituted cyano compound **6e** showed activity a little lower with MIC of 16 µg/mL. Whereas all other compounds displayed good to moderate activity. The molecular docking study of potent compound **6f** against crystal structure of dihydrofolate demonstrated a significant docking score and binding interactions in support of experimental investigations.

Keywords: Antitubercular activity, Carbohydrazide, 1,5-Naphthyridin-2(1*H*)-one, Molecular docking.

INTRODUCTION

Tuberculosis (TB) remains a substantial threat to global public health, particularly in instances where individuals are affected by multidrug-resistant tuberculosis (MDR-TB) [1]. Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, has been present for a considerable period of time [2]. The treatment period for this condition is longer and requires the daily use of medications that are more toxic, expensive and less effective than those used for treating tuberculosis that is susceptible to drugs [3]. The international community has made a resolute pledge to eliminate tuberculosis by the year 2030 [4]. While there exists an international standard for "free TB care" programs, several countries only offer coverage for select diagnostic tests and initial treatment choices [5]. Managing MDR-TB presents more challenges for patients, healthcare professionals and the healthcare systems [6].

1,5-Naphthyridin-2(1*H*)-one is a structural constituent of natural compounds amarastelline A and nigakinone which had luminescent properties [7]. Some of the drugs containing 1,5-naphthyridin-2(1*H*)-one such as ACEA-0762 is a N-methyl-D-aspartate (NMDA) receptor antagonist [8], napthyridinone is a inhibitor of immunodeficiency virus type 1 integrase in HIV treatment [9], 4-methoxy-5-hydroxycanthin-6-one (CAN) is an anti-inflammatory agent [10] and BMS-502 is a dual inhibitor of diacylglycerol kinase alpha (DGK) [11] (Fig. 1). Certain compounds of 1,5-napthyridine have been recognized as promising inhibitors of *c*-Met kinase and the transforming growth factor (TGF-β), as well as effective treatments against leishmaniasis [12-14]. The metal complexes of these naphthyridines are potential antimicrobial agents [15].

Carbohydrazides and their Schiff bases are significant types of heterocycles which are not only useful in organic chemistry but also have extensive applications in physical and inorganic chemistry [16]. A set of potentially bioactive compounds containing carbohydrazide functionality and its hydrazone derivatives have been synthesized and evaluated for their anticancer, antibacterial, antifungal and anti-inflammatory activities [17-20].

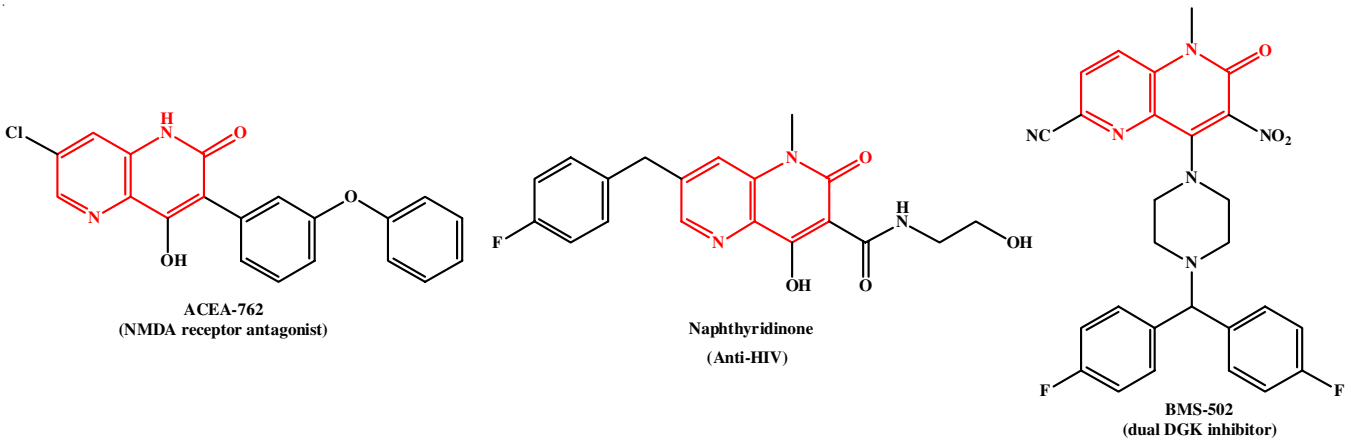


Fig. 1. Structures of drugs containing 1,5-naphthyridin-2(1*H*)-one moiety

Combining of carbohydrazide with 1,5-naphthyridin-2(1*H*)-one is completely novel and there is no literature available. This inspired us to design and synthesize novel carbohydrazide derivatives based on 1,5-naphthyridin-2(1*H*)-one for their antitubercular efficacy against *M. tuberculosis*, a drug-resistant strain.

EXPERIMENTAL

The synthetic reagents, catalysts and solvents were procured from commercial industrial suppliers Merck, Sigma-Aldrich, TCI, Spectrochem and Avra chemicals and used without undergoing any additional purification processes. The progress of the reaction was monitored using thin-layer chromatography (TLC) with comprehending silica gel Merk $60F_{254}$ plates. The melting points of the synthesized compounds were measured using the Stuart SMP3 melting point apparatus and are uncorrected. The mixtures were purified using the recrystallization process and the column chromatography procedure using silica gel 60-120 mesh. The characterization of compounds was determined by analyzing ^{1}H and ^{13}C NMR with Varian 400 spectrometer (400 and 101 MHz) using $CDCl_3$ and DMSO-d_6 as solvents and TMS as an internal standard. The mass spectra were obtained using a Shimadzu GC-MS QP 1000 spectrometer.

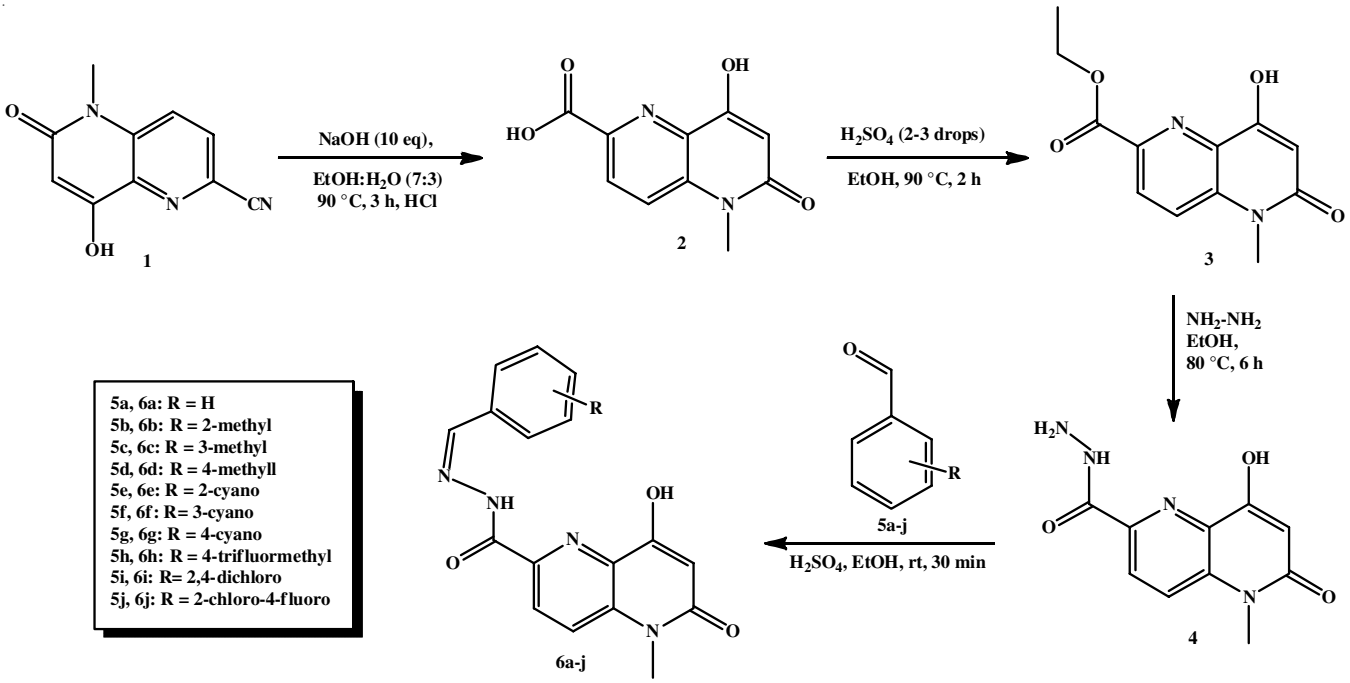
Synthesis of 8-hydroxy-5-methyl-6-oxo-5,6-dihydro-1,5-naphthyridine-2-carboxylic acid (2): To a solution of 8-hydroxy-5-methyl-6-oxo-5,6-dihydro-1,5-naphthyridine-2-carbonitrile (**1**) (2.1 g, 0.01 mol) in aqueous ethanol (50 mL), added powdered NaOH and refluxed the mixture at 90 ºC for 3 h. The crude product was neutralized using 10% HCl (5 mL) and then the obtained solid was filtered off and finally dried under vacuum. White solid, yield: 80%. m.p.: 127-129 ºC. IR (KBr, ν_{max}, cm^{-1}): 3332, 2971, 1721; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 12.65 (s, 1H), 10.17 (s, 1H), 8.38 (d, *J* = 8.4 Hz, 1H), 8.24 (d, *J* = 8.4 Hz, 1H), 7.68 (s, 1H), 2.89 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 172.5, 150.4, 141.4, 140.7, 136.0, 128.7, 124.8, 99.7, 31.2. ESI-MS: *m/z* 221.12 $[M+H]^+$.

Synthesis of ethyl 8-hydroxy-5-methyl-6-oxo-5,6-dihydro-1,5-naphthyridine-2-carboxylate (3): About 2.2 g of 8-hydroxy-5-methyl-6-oxo-5,6-dihydro-1,5-naphthyridine-2-carboxylic acid (**2**) (0.01 mol) was dissolved in ethanol (50 mL) and stirred the reaction mixture at 90 ºC for 2 h by adding catalytic amount of sulfuric acid. After completion, the reaction mixture was poured on ice cold water, the obtained solid was filtered off and dried under vacuum. White solid, yield: 82%. m.p.: 145-147 ºC. IR (KBr, ν_{max}, cm^{-1}): 3332, 2995, 1727; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 12.52 (s, 1H), 8.36 (d, *J* = 8.4 Hz, 1H), 8.27 (d, *J* = 8.4 Hz, 1H), 7.77 (s, 1H), 4.55 (q, 2H), 2.85 (s, 3H), 1.30 (t, *J* = 4.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 175.8, 166.3, 150.4, 142.4, 138.9, 132.0, 129.0, 119.1, 99.7, 61.3, 31.3, 14.7. ESI-MS: *m/z* 249.15 $[M+H]^+$.

Synthesis of 8-hydroxy-5-methyl-6-oxo-5,6-dihydro-1,5-naphthyridine-2-carbohydrazide (4): Compound **3** (2.4 g, 0.01 mol) was dissolved in ethanol (50 mL) and added hydrazine (0.01 mol, 1 equiv.), the reaction mass was stirred at 80 ºC for 6 h. The obtained solid was filtered off and dried under vacuum. White solid, yield: 70%. m.p.: 141-143 ºC. IR (KBr, ν_{max}, cm^{-1}): 3332, 2995, 2442, 1735; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 12.50 (s, 1H), 9.55 (s, 1H), 8.37-8.35 (m, 1H), 8.22-8.20 (m, 1H), 7.65 (s, 1H), 4.82 (bs, 2H), 2.87 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ ppm: 163.5, 162.5, 150.4, 140.9, 139.0, 134.3, 127.5, 121.7, 99.7, 31.3. ESI-MS: *m/z* 235.17 $[M+H]^+$.

General procedure for the synthesis of (*Z*)-8-hydroxy-5-methyl-N′-(substituted benzylidene)-6-oxo-5,6-dihydro-1,5-naphthyridine-2-carbohydrazide derivatives (6a-j): To a individual mixture of compound **4** (0.023 g. 0.001 mol) and various substituted benzaldehydes (0.001 mol, 1 equiv.) added sulfuric acid in catalytic amount and stirred reaction mixture for 30 min at room temperature. After completion, the reaction mass was poured on to ice-cold water, obtained solid was collected and purified employing column chromatography using ethyl acetate:*n*-hexane (3:7) as eluent (**Scheme-I**).

(*Z*)-N′-Benzylidene-8-hydroxy-5-methyl-6-oxo-5,6-dihydro-1,5-naphthyridine-2-carbohydrazide (6a): White solid, yield: 78%. m.p.: 165-167 ºC. IR (KBr, ν_{max}, cm^{-1}): 3300, 2225, 1735; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.53 (s, 1H), 9.25 (s, 1H), 8.37-8.32 (m, 1H), 7.58-7.54 (m, 1H), 7.35-7.30 (m, 5H), 7.02 (s, 1H), 6.15 (s, 1H), 2.94 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 172.3, 162.5, 159.0, 150.4, 141.0, 138.0, 135.2, 135.0, 129.0, 128.8, 128.7, 127.0, 125.7, 99.7, 31.2. ESI-MS: *m/z* 323.12 $[M+H]^+$. Elemental analysis calcd. (found) % for $C_{18}H_{16}N_4O_3$: C, 63.35 (63.31); H, 4.38 (4.34); N, 17.38 (17.34).



Scheme-I: Synthesis of 1,5-naphthyridin-2(1*H*)-one based carbohydrazide derivatives (**6a-j**)

(*Z*)-8-Hydroxy-5-methyl-N′-(2-methylbenzylidene)-6-oxo-5,6-dihydro-1,5-naphthyridine-2-carbohydrazide (6b): White solid, yield: 76%. m.p.: 176-178 ºC. IR (KBr, ν_{max}, cm^{-1}): 3332, 2225, 1735; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.37 (s, 1H), 8.82 (s, 1H), 8.34 (d, *J* = 8.8 Hz, 1H), 8.16 (d, *J* = 7.2 Hz, 1H), 7.91-7.89 (m, 1H), 7.37-7.21 (m, 4H), 6.21 (s, 1H), 3.58 (s, 3H), 2.53 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 162.3, 160.0, 159.3, 147.0, 141.4, 137.7, 137.0, 132.1, 130.9, 130.5, 129.9, 126.2, 126.1, 124.2, 124.0, 101.8, 28.6, 19.3. ESI-MS: *m/z* 337.11 $[M+H]^+$. Elemental analysis calcd. (found) % $C_{18}H_{16}N_4O_3$: C, 64.28 (64.21); H, 4.79 (4.76); N, 16.66 (16.61).

(*Z*)-8-Hydroxy-5-methyl-N′-(3-methylbenzylidene)-6-oxo-5,6-dihydro-1,5-naphthyridine-2-carbohydrazide (6c): White solid, yield: 76%. m.p.: 176-178 ºC. IR (KBr, ν_{max}, cm^{-1}): 3310, 2227, 1721; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.37 (s, 1H), 9.00 (s, 1H), 8.35 (d, *J* = 8.8 Hz, 1H), 8.27 (d, *J* = 7.6 Hz, 1H), 7.50-7.49 (m, 1H), 7.39-7.37 (m, 1H), 7.24-7.20 (m, 1H), 7.17-7.15 (m, 2H), 6.03 (s, 1H), 2.93 (s, 3H), 2.31 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 172.6, 162.5, 159.0, 150.4, 141.0, 138.0, 137.8, 136.1, 135.2, 129.7, 128.7, 128.5, 128.3, 125.7, 123.5, 99.7, 31.2, 21.2. ESI-MS: *m/z* 337.18 $[M+H]^+$. Elemental analysis calcd. (found) % for $C_{18}H_{16}N_4O_3$: C, 64.28 (64.22); H, 4.79 (4.75); N, 16.66 (16.62).

(*Z*)-8-Hydroxy-5-methyl-N′-(4-methylbenzylidene)-6-oxo-5,6-dihydro-1,5-naphthyridine-2-carbohydrazide (6d): White solid, yield: 74%. m.p.: 174-176 ºC. IR (KBr, ν_{max}, cm^{-1}): 3332, 2222, 1721; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.37 (s, 1H), 9.26 (s, 1H), 8.39 (d, *J* = 7.2 Hz, 1H), 8.32 (d, *J* = 7.2 Hz, 1H), 7.49 (d, *J* = 7.6 Hz, 2H), 7.19 (d, *J* = 7.6 Hz, 2H), 6.81 (s, 1H), 6.09 (s, 1H), 2.95 (s, 3H), 2.32 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 172.3, 162.5, 159.0, 150.6, 141.3, 138.5, 138.0, 135.2, 131.9, 129.1, 128.7, 127.3, 125.7, 99.7, 31.2, 21.1. ESI-MS: *m/z* 337.12 $[M+H]^+$. Elemental analysis calcd. (found) % for $C_{18}H_{16}N_4O_3$: C, 64.28 (64.23); H, 4.79 (4.74); N, 16.66 (16.61).

(*Z*)-N′-(2-Cyanobenzylidene)-8-hydroxy-5-methyl-6-oxo-5,6-dihydro-1,5-naphthyridine-2-carbohydrazide (6e): White solid, yield: 77%. m.p.: 178-180 ºC. IR (KBr, ν_{max}, cm^{-1}): 3332, 3102, 2257, 1721; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.35 (s, 1H), 9.36 (s, 1H), 8.37-8.32 (m, 2H), 7.78-7.76 (m, 1H), 7.61-7.57 (m, 2H), 7.52-7.48 (m, 1H), 6.99 (s, 1H), 6.16 (s, 1H), 2.94 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 167.1, 162.5, 159.0, 150.4, 141.0, 138.0, 135.2, 132.5, 130.9, 129.8, 129.3, 128.7, 127.5, 125.7, 119.4, 113.2, 99.7, 31.2. ESI-MS: *m/z* 348.10 $[M+H]^+$. Elemental analysis calcd. (found) % for $C_{18}H_{13}N_5O_3$: C, 62.24 (62.17); H, 3.77 (3.71); N, 20.16 (20.10).

(*Z*)-N′-(3-Cyanobenzylidene)-8-hydroxy-5-methyl-6-oxo-5,6-dihydro-1,5-naphthyridine-2-carbohydrazide (6f): White solid, yield: 75%, m.p.: 182-184 ºC. IR (KBr, ν_{max}, cm^{-1}): 3330, 3112, 2242, 1727; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.35 (s, 1H), 9.39 (s, 1H), 8.35-8.32 (m, 2H), 7.84-7.82 (m, 2H), 7.79-7.77 (m, 1H), 7.56-7.53 (m, 1H), 7.50-7.46 (m, 1H), 6.06 (s, 1H), 2.93 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 172.6, 162.5, 159.0, 150.4, 141.0, 138.0, 136.6, 135.2, 133.4, 131.7, 129.8, 128.7, 128.7, 125.7, 119.3, 116.3, 99.7, 31.2. ESI-MS: *m/z* 348.15 $[M+H]^+$. Elemental analysis calcd. (found) % for $C_{18}H_{13}N_5O_3$: C, 62.24 (62.16); H, 3.77 (3.72); N, 20.16 (20.11).

(*Z*)-N′-(4-Cyanobenzylidene)-8-hydroxy-5-methyl-6-oxo-5,6-dihydro-1,5-naphthyridine-2-carbohydrazide (6g): White solid, yield: 78%. m.p.: 181-183 ºC. IR (KBr, ν_{max}, cm^{-1}): 3312, 3100, 2247, 1722; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.65 (s, 1H), 8.79 (s, 1H), 8.67 (s, 1H), 8.32 (d, *J* = 8.8 Hz, 1H), 8.13 (d, *J* = 8.8 Hz, 1H), 7.94 (s, 4H), 6.12 (s, 1H), 3.57 (s, 3H);

^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 172.3, 162.56 159.0, 150.4, 141.0, 138.6, 138.0, 135.2, 132.9, 129.0, 128.7, 125.7, 119.1, 116.2, 99.7, 31.2. ESI-MS: *m/z* 348.14 $[M+H]^+$. Elemental analysis calcd. (found) % for $C_{18}H_{13}N_5O_3$: C, 62.24 (62.20); H, 3.77 (3.71); N, 20.16 (20.12).

(*Z*)-8-Hydroxy-5-methyl-6-oxo-N′-(4-(trifluoromethyl)-benzylidene)-5,6-dihydro-1,5-naphthyridine-2-carbohydrazide (6h): White solid, yield: 75%. m.p.: 192-194 ºC. IR (KBr, ν_{max}, cm^{-1}): 3300, 2247, 1762; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.65 (s, 1H), 9.40 (s, 1H), 8.36-8.26 (m, 2H), 7.55-7.50 (m, 5H), 6.03 (s, 1H), 2.93 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 172.3, 162.5, 159.0, 150.4, 141.0, 138.0, 135.9, 135.2, 130.2, 130.2, 130.2, 130.1, 129.9, 129.6, 129.4, 129.1, 128.7, 128.4, 127.3, 127.2, 127.1, 127.0, 125.7, 125.7, 123.1, 120.5, 99.7, 31.2. ESI-MS: *m/z* 391.09 $[M+H]^+$. Elemental analysis calcd. (found) % for $C_{18}H_{13}F_3N_4O_3$: C, 55.39 (55.35); H, 3.36 (3.30); N, 14.35 (14.31).

(*Z*)-N′-(2,4-Dichlorobenzylidene)-8-hydroxy-5-methyl-6-oxo-5,6-dihydro-1,5-naphthyridine-2-carbohydrazide (6i): White solid, yield: 72%. m.p.: 190-192 ºC. IR (KBr, ν_{max}, cm^{-1}): 3312, 2227, 1747; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.65 (s, 1H), 9.49 (s, 1H), 8.38-8.30 (m, 2H), 7.45-7.42 (m, 2H), 7.19-7.17 (m, 2H), 5.96 (s, 1H), 2.93 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 166.5, 162.5, 159.0, 150.4, 141.0, 138.0, 135.2, 134.6, 134.6, 131.0, 129.6, 129.0, 128.7, 128.4, 125.7, 99.7, 31.2. ESI-MS: *m/z* 391.02 $[M+H]^+$. Elemental analysis calcd. (found) % for $C_{17}H_{12}Cl_2N_4O_3$: C, 52.19 (52.15); H, 3.09 (3.05); N, 14.32 (14.27).

(*Z*)-N′-(2-Chloro-4-fluorobenzylidene)-8-hydroxy-5-methyl-6-oxo-5,6-dihydro-1,5-naphthyridine-2-carbohydrazide (6j): White solid, yield: 79%. m.p.: 188-190 ºC. IR (KBr, ν_{max}, cm^{-1}): 3330, 2227, 1721; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.62 (s, 1H), 9.41 (s, 1H), 8.37-8.31 (m, 2H), 7.57-7.54 (m, 2H), 7.15-7.13 (m, 1H), 6.98-6.94 (m, 1H), 6.11 (s, 1H), 2.94 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm): 166.5, 162.5, 160.7, 159.0, 158.1, 150.4, 141.0, 138.0, 135.2, 134.7, 134.6, 130.4, 130.3, 128.7, 126.4, 126.4, 125.7, 118.4, 118.1, 113.2, 113.0, 99.7, 31.2. ESI-MS: *m/z* 375.05 $[M+H]^+$. Elemental analysis calcd. (found) % for $C_{17}H_{12}ClFN_4O_3$: C, 54.49 (54.4); H, 3.23 (3.18); N, 14.95 (14.90).

Bacterial strain and growth conditions: *M. tuberculosis* mc^26230 strain used in this study was grown in Middlebrook 7H9 broth (BD Difco) supplemented with 0.05% (v/v) Tween 80, 0.2% (v/v) glycerol and 10% albumin-dextrose-catalase (ADC) and kept in incubator at 37 ºC.

Determination of minimum inhibitory concentration (MIC): Novel 5-naphthyridin-2(1*H*)-one based carbohydrazide derivatives (**6a-j**) were screened against *M. tuberculosis* mc^26230 to determine the minimum inhibitory concentration (MIC) using 96-well liquid broth micro dilution assay. The minimum inhibitory concentration (MIC) is the lowest concentration of a drug that can inhibit bacterial growth. Compound concentration was started from 128 µg/mL and then serially diluted with media.

Molecular docking procedure: The crystal structure of *Mycobacterium tuberculosis* dihydrofolate reductase (PDB ID: 2CIG) was retrieved from Protein Data Bank (www.rcsb.org). The protein was prepared by removing water molecules and heteroatoms, added polar hydrogens. The ligands were sketched in ChemDraw Professional tool in MDL file format. Employed Autodock Vina fit in PyRx tool was used for molecular docking screening. The grid box was assigned with dimensions of center_x = 8.84451319628, center_y = -17.9510531418, center_z = -8.35695796159, size_x = 20.4476976671, size_y = 27.2567592612 and size_z = 26.1726685581 to cover active site pocket. The results were visualized using Pymol and Biovia Discovery Studio softwares.

RESULTS AND DISCUSSION

The synthetic route for the synthesis of 1,5-naphthyridin-2(1*H*)-one based carbohydrazide derivatives (**6a-j**) is outlined in **Scheme-I**. Initially, 8-hydroxy-5-methyl-6-oxo-5,6-dihydro-1,5-naphthyridine-2-carbonitrile (**1**) precursor was treated with NaOH to obtain 8-hydroxy-5-methyl-6-oxo-5,6-dihydro-1,5-naphthyridine-2-carboxylic acid (**2**). It was verified by carboxylic acid and phenolic protons as two singlets at δ 10.17 ppm and δ 12.65 ppm and the N-methyl protons appeared as a singlet at δ 2.89 ppm in ^{1}H NMR spectrum. The ^{13}C NMR spectrum of compound **2** also confirmed the carboxylic acid carbon signal at δ 180.9 ppm and naphthyridine carbonyl carbon signal at δ 172.5 ppm. The IR spectrum of compound **2** also verified C=O and OH stretching frequency peaks near 1721 cm^{-1} and 2971 cm^{-1} corresponding to carboxylic group. Compound **2** was subjected to esterification by ethanol in presence of a catalytic amount of sulfuric acid to obtain ethyl 8-hydroxy-5-methyl-6-oxo-5,6-dihydro-1,5-naphthyridine-2-carboxylate (**3**). It was evidenced by disappearance of singlet at δ 10.17 ppm and appearance of a quartet at δ 4.25 ppm and a triplet at δ 1.30 ppm corresponding to ethyl carboxylate group in ^{1}H NMR spectrum. The ^{13}C NMR confirmed two carbon signals of ethyl function at δ 61.3 ppm and δ 14.7 ppm. Compound **3** was treated with hydrazine by refluxing in ethanol to obtain 8-hydroxy-5-methyl-6-oxo-5,6-dihydro-1,5-naphthyridine-2-carbohydrazide (**4**). It was characterized by the appearance of singlet at δ 9.55 ppm and a broad singlet at δ 4.82 ppm corresponding to NH and NH_2 protons of carbohydrazide group. In ^{13}C NMR, there was disappearance of two carbon signals of ethyl function at δ 61.30 ppm and δ 14.70 ppm. Finally, the carbohydrazide function of intermediate scaffold **4** was allowed to react with various substituted benzaldehydes **5a-j** individually in presence of sulfuric acid to obtain corresponding (*Z*)-8-hydroxy-5-methyl-N′-(substituted benzylidene)-6-oxo-5,6-dihydro-1,5-naphthyridine-2-carbohydrazide derivatives (**6a-j**).

Antitubercular activity: Novel 5-naphthyridin-2(1*H*)-one based carbohydrazide derivatives (**6a-j**) were screened against *M. tuberculosis* mc^26230 to determine the minimum inhibitory concentration (MIC) using 96-well liquid broth micro dilution assay. Compound concentration was started from 128 µg/mL and then serially diluted with DMSO and rifampicin was employed as standard drug, the results are tabulated in Table-1. According to the MIC values obtained the activity of the synthesized compounds was reported to be good to moderate. Interestingly, compound **6f** (3-cyano) displayed potent activity with a MIC value of 4 µg/mL. When cyano

TABLE-1
MIC OF COMPOUNDS **6a-j** AGAINST *M. tuberculosis* mc^{2}6230

Compd.	**6a**	**6b**	**6c**	**6d**	**6e**	**6f**	**6g**	**6h**	**6i**	**6j**	Rifampicin
MIC (µg/mL)	8	8	>128	32	16	4	8	16	32	16	0.03

group was placed at the *para*-position in compound **6g** the activity was slightly diminished to MIC value of 8 µg/mL, whereas the *ortho*-substituted cyano compound showed activity a little lower with MIC of 16 the 3-cyano g/mL. Compound **6a**, absence of substituents and 2-methyl substituted compound **6b** showed good activity with MIC value of 8 µg/mL. The other compounds with 4-methyl (**6d**), trifluoromethyl (**6h**), dichloro (**6i**) and 2-chloro-4-fluoro (**6j**) displayed moderate activity. Only one compound with 3-methyl group (**6c**) presented very poor inhibition.

Molecular docking studies: To get an insight into the binding mode of potent molecule **6f**, the molecular docking simulations were performed against crystal structure of *M. tuberculosis* dihydrofolate reductase (PDB ID: 2CIG), it is an essential enzyme that facilitates the synthesis of nucleic acids [21] and employed Autdock Vina integrated PyRx tool for present study [22]. Validated the results by re-docking the co-crystalized ligand (4*R*)-isonicotinic-acetyl-nicotinamide-adenine dinucleotide (1DG) which has presented an RMSD of 1.08 Å with a docking score of -8.2 kcal/mol. Compound **6f** scored binding affinity value of -9.9 kcal/mol and displayed two key interactions with amino acid sites Ala7 and Ile94 of dihydrofolate with a bond distance of 2.20 Å and 3.02 Å, two π-π stacked interactions with Phe31 and other hydrophobic interactions with Gly15, Ile20, Val46, Leu50 and Leu57 of dihydrofolate (Fig. 2). The co-crystalized ligand 1DG presented four H-bond interactions with Ala7, Ile14 and Ser49(2) and hydrophobic interactions with Ile14 and Phe31 (π-π stacked) of dihydrofolate (Fig. 3). The super imposed images of ligands **6f** and 1DG presented in Fig. 4, which clearly indicates that ligand **6f** was best fit into cavity same as reference ligand 1DG.

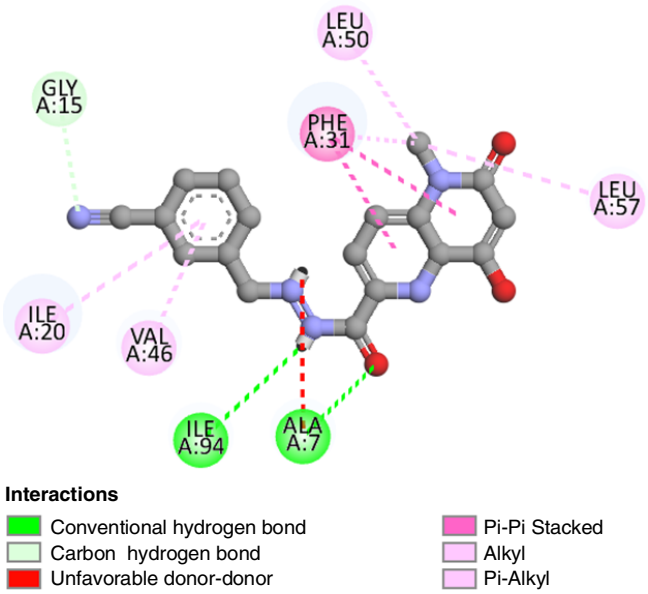


Fig. 2. Binding interactions of compound **6f** against dihydrofolate reductase

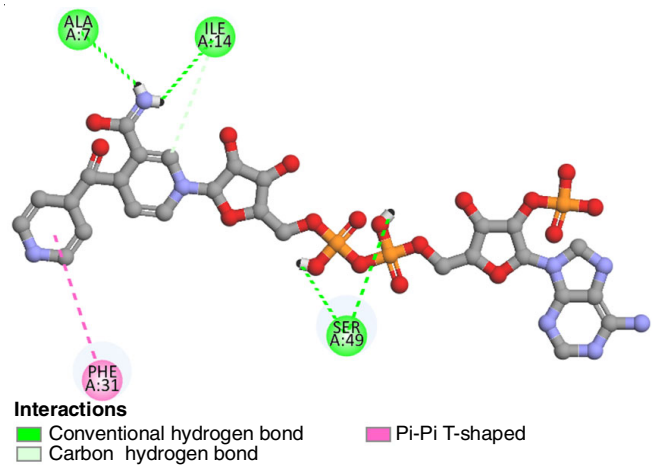


Fig. 3. Binding interactions of 1DG against dihydrofolate reductase

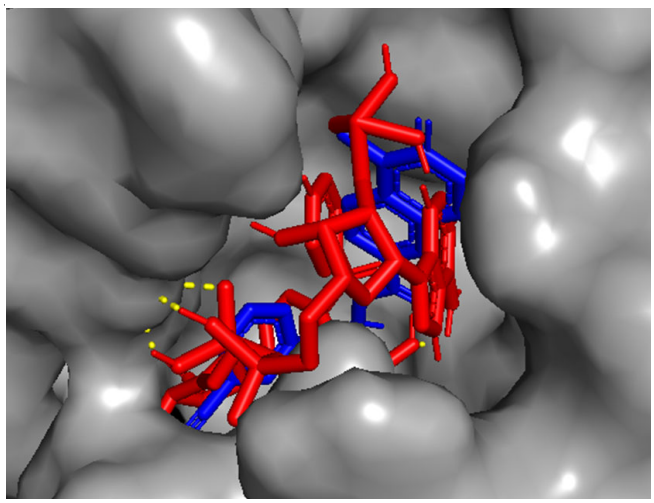


Fig. 4. Docking pose of ligand **6f** (blue) and 1DG (red) in cavity of dihydrofolate reductase

The binding interactions such as H-bond and π-π stacked were coinciding with interactions of 1DG. In turn it proved that these molecules could present promising inhibition.

Conclusion

In conclusion, a series of 1,5-naphthyridin-2(1*H*)-one based carbohydrazide derivatives (**6a-j**) was synthesized and characterized by IR, ^{1}H, ^{13}C NMR and mass spectral data. The antitubercular efficacy against *M. tuberculosis* mc^{2}6230 was evaluated using rifampicin as standard drug. Compound **6f** (3-cyano) displayed the potent activity with a MIC value of 4 µg/mL. Compound **6g** (4-cyano) displayed moderate activity with an MIC value of 8 µg/mL, whereas the *ortho*-substituted cyano compound **6e** showed activity a little lower with MIC of 16 µg/mL. Whereas all other compounds displayed good to moderate activity. The molecular docking analysis of compound **6f** against the crystal structure of dihydrofolate revealed

a significant docking score and binding interacations, providing support for the experimental investigations. Therefore, these compounds have the potential to be highly effective agents against tuberculosis and can be further developed in the drug research and design process.

ACKNOWLEDGEMENTS

The authors thank the Head, Department of Chemistry, Osmania University, Hyderabad, India for providing research facilities.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. N.B. Chilakala, A. Roy, N.P. Kalia, V. Thumma, R. B, S. Etnoori and P. K, *Chem. Biodivers.*, e202401491 (2024); https://doi.org/10.1002/cbdv.202401491
2. K. Sakamoto, *Vet. Pathol.*, **49**, 423 (2012); https://doi.org/10.1177/0300985811429313
3. C.A. Peloquin and G.R. Davies, *Clin. Pharmacol. Ther.*, **110**, 1455 (2021); https://doi.org/10.1002/cpt.2261
4. C. Lange, R.E. Aarnoutse, J.W.C. Alffenaar, G. Bothamley, J. Costa, F. Brinkmann, D. Chesov, R. van Crevel, M. Dedicoat, J. Dominguez, R. Duarte, H.P. Grobbel, G. Günther, L. Guglielmetti, J. Heyckendorf, A.W. Kay, O. Kirakosyan, O. Kirk, R.A. Koczulla, G.G. Kudriashov, L. Kuksa, F. van Leth, C. Magis-Escurra, A.M. Mandalakas, B. Molina-Moya, C.A. Peloquin, M. Reimann, R. Rumetshofer, H.S. Schaaf, T. Schön, S. Tiberi, J. Valda, P.K. Yablonskii and K. Dheda, *Int. J. Tuberc. Lung Dis.*, **23**, 645 (2019); https://doi.org/10.5588/ijtld.18.0622
5. K. Lönnroth, P. Glaziou, D. Weil, K. Floyd, M. Uplekar and M. Raviglione, *PLoS Med.*, **11**, e1001693 (2014); https://doi.org/10.1371/journal.pmed.1001693
6. Y. Zhang, X. Liu, L. Yang, G. Zhang, Z. Gu, Z. Chen and J. Sun, *Infect. Drug Resist.*, **13**, 3679 (2020); https://doi.org/10.2147/IDR.S256128
7. H. Yokoo, A. Ohsaki, H. Kagechika and T. Hirano, *Eur. J. Org. Chem.*, **2018**, 679 (2018); https://doi.org/10.1002/ejoc.201701609
8. I.G. Tikhonova, I.I. Baskin, V.A. Palyulin and N.S. Zefirov, *J. Med. Chem.*, **46**, 1609 (2003); https://doi.org/10.1021/jm0210156
9. B.A. Johns, T. Kawasuji, J.G. Weatherhead, E.E. Boros, J.B. Thompson, C.S. Koble, E.P. Garvey, S.A. Foster, J.L. Jeffrey and T. Fujiwara, *Bioorg. Med. Chem. Lett.*, **24**, 3104 (2014); https://doi.org/10.1016/j.bmcl.2014.05.011
10. J.-F. Liu, M. Shao, D.-W. Zhai, K. Liu and L.-J. Wu, *Planta Med.*, **75**, 142 (2009); https://doi.org/10.1055/s-0028-1088390
11. L. Chupak, M. Wichroski, X. Zheng, M. Ding, S. Martin, C. Allard, J. Shi, R. Gentles, N.A. Meanwell, J. Fang, D. Tenney, J. Tokarski, C. Cao and S. Wee, *ACS Med. Chem. Lett.*, **14**, 929 (2023); https://doi.org/10.1021/acsmedchemlett.3c00063
12. J.-F. Wu, M.-M. Liu, S.-X. Huang and Y. Wang, *Bioorg. Med. Chem. Lett.*, **25**, 3251 (2015); https://doi.org/10.1016/j.bmcl.2015.05.082
13. F. Gellibert, J. Woolven, M.-H. Fouchet, N. Mathews, H. Goodland, V. Lovegrove, A. Laroze, V.-L. Nguyen, S. Sautet, R. Wang, C. Janson, W. Smith, G. Krysa, V. Boullay, A.-C. de Gouville, S. Huet and D. Hartley, *J. Med. Chem.*, **47**, 4494 (2004); https://doi.org/10.1021/jm0400247
14. A. Tejería, Y. Pérez-Pertejo, R.M. Reguera, R. Balaña-Fouce, C. Alonso, M. González, G. Rubiales and F. Palacios, *Eur. J. Med. Chem.*, **152**, 137 (2018); https://doi.org/10.1016/j.ejmech.2018.04.033
15. S. Đuric, S. Vojnovic, A. Pavic, M. Mojicevic, H. Wadepohl, N.D. Savic, M. Popsavin, J. Nikodinovic-Runic, M.I. Djuran and B.Đ. Glišic, *J. Inorg. Biochem.*, **203**, 110872 (2020); https://doi.org/10.1016/j.jinorgbio.2019.110872
16. E.L. Onyeyilim, M.A. Ezeokonkwo, D.I. Ugwu, C.P. Uzoewulu, F.U. Eze, V.I. Okonkwo, C.C. Eze and J.A. Ezugwu, *Mini Rev. Med. Chem.*, **22**, 661 (2022); https://doi.org/10.2174/1389557521666210831154935
17. H.M. Abd El-Lateef, A.A. Elmaaty, L.M.A. Abdel Ghany, M.S. Abdel-Aziz, I. Zaki and N. Ryad, *ACS Omega*, **8**, 17948 (2023); https://doi.org/10.1021/acsomega.3c01156
18. Y. Shang, Q. Hao, K. Jiang, M. He and J. Wang, *Bioorg. Med. Chem. Lett.*, **30**, 127118 (2020); https://doi.org/10.1016/j.bmcl.2020.127118
19. I. Benjamin, C.U. Benson, S.A. Adalikwu, F.A. Nduoma, F.O. Akor, M.O. Odey, E.C. Ezeani, I.A. Anyambula, M.A. Odume and H. Louis, *Chem. Phys. Impact*, **7**, 100275 (2023); https://doi.org/10.1016/j.chphi.2023.100275
20. R. Saruengkhanphasit, C. Butkinaree, N. Ornnork, K. Lirdprapamongkol, W. Niwetmarin, J. Svasti, S. Ruchirawat and C. Eurtivong, *Bioorg. Chem.*, **110**, 104795 (2021); https://doi.org/10.1016/j.bioorg.2021.104795
21. A. Argyrou, M.W. Vetting, B. Aladegbami and J.S. Blanchard, *Nat. Struct. Mol. Biol.*, **13**, 408 (2006); https://doi.org/10.1038/nsmb1089
22. V. Thumma, V. Mallikanti, R. Matta, R. Dharavath and P. Jalapathi, *RSC Med. Chem.*, **15**, 1283 (2024); https://doi.org/10.1039/D3MD00479A