Asian Journal of Chemistry; Vol. 33, No. 8 (2021), 1923-1928

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2021.23364

Design, Synthesis, Molecular Docking Studies and Antitubercular Evaluation of Hexahydroquinolin-2-yl Benzamide Derivatives

N. RAGHAVENDRA BABU[1,2,*], G.S.N. KOTESWARA RAO[2], RAJASEKHAR REDDY ALAVALA[2] and P.S. LAKSHMI SOUKYA[3]

[1]Department of Pharmaceutical Chemistry, G. Pulla Reddy College of Pharmacy, Mehdipatnam, Hyderabad-500028, India
[2]Department of Pharmaceutical Chemistry, K.L. College of Pharmacy, K.L. Deemed to be University, Vaddeswaram-522502, India
[3]Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research, Hyderabad-500037, India

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

Received: 1 June 2021; *Accepted*: 6 July 2021; *Published online*: 26 July 2021; AJC-20447

A new series of hexahydroquinolin-2-yl benzamide derivatives (**BZ_{1-10}**) were designed and synthesized. The synthesized compounds were characterized by ^{1}H NMR, IR and ESI-MS spectra and also subjected for molecular docking studies with the target DNA gyrase enzyme (PBD ID: 4B6C). The molecular docking results of synthesized derivatives indicated the best docking score of -5.105 and -5.02 for **BZ_9** and **BZ_4**, respectively. All the synthesized compounds were screened for antitubercular activity against H37RV strain, among all, two compounds exhibited significant activity at 12.5 µg/mL and 25 µg/mL concentrations. Thus, the MIC values are in between range of 12.5 and 6.25 µg/mL concentrations. The teratogenicity assay of synthesized compounds was performed in zebrafish larvae, out of the ten compounds, **BZ_4**, **BZ_6** and **BZ_8** compounds were found to safer at 0.5 µM concentration without any abnormalities.

Keywords: Docking, Hexahydroquinolin, Antitubercular, Teratogenicity.

INTRODUCTION

Tuberculosis (TB) is a communicable disease which is a major cause of ill health, one of the top 10 causes of death worldwide and the leading cause of death from a single infectious agent (ranking above HIV/AIDS). In 2019, approximately 10 million people developed TB and 1.4 million died [1]. Coughing is one mode of spreading tuberculosis bacteria. The disease usually affects the lungs (pulmonary tuberculosis), but it can also affect other parts of the body (extrapulmonary TB). Every year, nearly 2 million individuals die of tuberculosis and up to 2 billion people have the bacteria in their bodies. Tuberculosis can be treated through antibiotic regimes and frontline antimycobacterial drugs isonicotinylhydrazine and rifampicin are being used to treat tuberculosis for at least 50 years [2]. Longer treatment periods result in lower patient adherence to treatment. Moreover, failure to discover new classes of antimycobacterial medications has led to the evolution of drug-resistant *Mycobacterium tuberculosis* strains. These consist of multidrug-resistant TB, extensively drug-resistant TB and more recently, totally drug-resistant TB, which are resistant to all currently available antibiotics [3]. Therefore, an urgent need has risen to develop new classes of drug molecules with newer targets and with an alternative action mechanism [4].

Among heterocyclic compounds, quinoline [5] is the most ubiquitous heterocyclic aromatic compound and can be applied in industrial and medicinal processes. Quinolone is alternatively called 1-azanapthalene and benzo[*b*]pyridine. Quinoline derivatives can be found in a wide range of natural compounds, particularly alkaloids. It exhibits similar reactions to pyridine and benzene and can also participate in both electrophilic and nucleophilic substitution reactions [6]. This moiety is primarily utilized as a core template to produce a variety of medications. In recent years, quinolines and their derivatives have been found greater consideration because of their varied biological activities like anti-cancer [7], anti-bacterial [8], anti-inflammatory [9], antitubercular [10,11], anti-viral properties [12]. Quinoline is a basic pharmacophore used for the design of antitubercular agents, like mefloquine, ciprofloxacin, moxifloxacin and bedaquiline which are already available in the market [13,14]. The literature survey revealed most of them have hexahydroquinoline intermediates [15-26] and we tried to synthesize new deri-

vatives by reacting hexahydroquinoline intermediates with benzoyl chloride to form the target compounds hexahydroquinolin-2-yl benzamide derivatives. Molecular docking study was performed for synthesized compounds against target DNA gyrase enzyme (PBD ID: 4B6C) to explore their binding interactions at the active site. The synthesized compounds were screened for antitubercular activity against H37RV strain by using microplate alamar blue assay method. In addition, teratogenic assay studies were also performed for the synthesized compounds.

EXPERIMENTAL

All chemicals and solvents used were of synthetic grade and obtained from S.D. fine chemicals Ltd. (Mumbai, India) and Avra chemicals Pvt. Ltd. (Hyderabad, India). Reaction completion was monitored through thin layer chromatography (TLC) by using E. Merck 0.25-mm silica gel plates and observed under UV light (256 nm) and iodine chamber. Synthesized compounds were purified through the recrystallization of methanol and acetone mixture, and compound purity was checked using a single spot in TLC. The mobile phase for TLC was determined through the trial-and-error method. Melting points were determined in open capillary tubes by using ANALAB melting apparatus and are uncorrected. All the ^{1}H NMR spectra were recorded on Variant 400 MHz spectrometer by using DMSO-d_6 and $CDCl_3$ as solvent and tetramethyl silane (TMS) as the internal standard. FTIR spectra were recorded through Shimadzu FT-IR spectrophotometry by using 1% KBr discs. The mass spectra of the compounds were recorded on Agilent 1100 series.

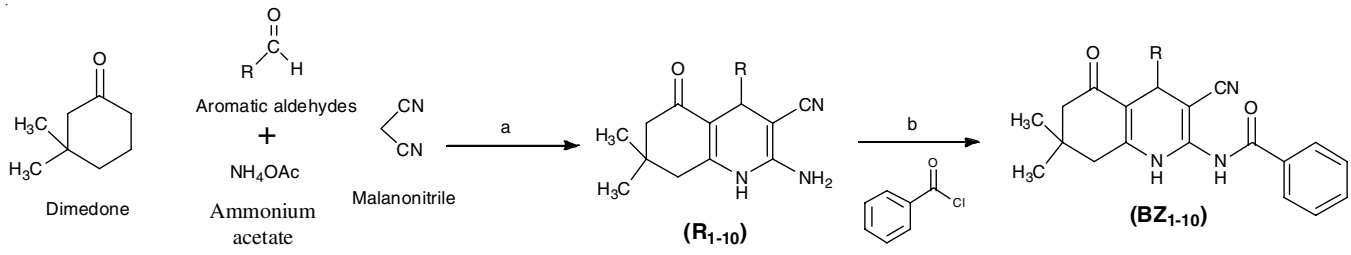
Synthesis of hexahydroquinoline-3-carbonitrile intermediates (R_{1-10}): In a 50 mL round bottom flask introduced 5,5-dimethyl cyclohexane-1,3-dione (1 mmol), malanonitrile (1 mmol), appropriate aromatic aldehydes (1 mmol) and excess of ammonium acetate (3.3 mmol) were dissolved in ethanol and 2-3 drops of piperidine was added. The reaction mixture was refluxed for 2-3 h. After completion of reaction [monitored by TLC ethyl acetate:*n*-hexane (1:1)], the seperated solid was filtered off and recrystallized with methanol and acetone mixture to afford the product (**Scheme-I**).

2-Amino-7,7-dimethyl-5-oxo-4-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (R_1): White solid; yield: 82%; m.p.: 280-281 ºC. IR (KBr, ν_{max}, cm^{-1}): 3396, 3323, 3213, 2960, 2198, 1658, 1604, 1487, 1368; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.10-7.40 (m, 5H, ArH), 6.97-7.10 (s, 2H, NH_2), 4.10 (s, 1H, CH), 2.30-2.40 (m, 2H, CH_2), 2.08-2.20 (m, 2H, CH_2), 0.95-1.04 (d, 6H, $(CH_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 193.9, 162.5, 151.5, 146.1, 129.2, 128.7, 127.6, 127.4, 125.7, 117.3, 111.9, 58.6, 52.3, 51.2, 41.0, 33.1, 28.2, 27.3.

2-Amino-4-(4-methoxyphenyl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (R_2): Yellow solid; yield: 89%; m.p.: 289-291 ºC. IR (KBr, ν_{max}, cm^{-1}): 3377, 3321, 3186, 2962, 2196, 1656,1603, 1485, 1369; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 6.90-7.10 (m, 4H, ArH), 6.82-6.90 (s, 2H, NH_2), 4.1 (s, 1H, CH), 3.7 (s, 3H, OCH_3), 2.22-2.45 (s, 2H, CH_2), 2.07-2.11 (m, 2H, CH_2), 0.94-1.03 (d, 6H, $(CH_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 194.2, 162.3, 157.6, 149.6, 138.4, 132.2, 130.3, 117.3, 116.2, 114.2, 111.8, 58.6, 55.8, 52.3, 42.0, 38.4, 32.7, 28.3, 27.6.

2-Amino-4-(4-bromophenyl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (R_3): White solid; yield: 79%; m.p.: 295-296 ºC. IR (KBr, ν_{max}, cm^{-1}): 3392, 3319, 3187, 2962, 2192, 1683, 1656, 1604, 1485, 1365; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.40-7.50 and 7.10 -7.20 (m, 4H, ArH), 7.04-7.10 (s, 2H, NH_2), 4.1 (s, 1H, CH), 2.40-2.50 (s, 2H, CH_2), 2.00-2.30 (m, 2H, CH_2), 0.94-1.03 (s, 6H, $(CH_3)_2$). ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 194.6, 150.8, 150.4, 150.0, 133.2, 130.5, 127.2, 126.2, 126.0, 120.8, 108.9, 58.7, 51.4, 37.5, 32.4, 29.2, 28.3.

Synthesis of hexahydroquinolin-2-yl benzamide derivatives (BZ_{1-10}): To a clean dry 50 mL round bottom flask, an appropriate compound (R_{1-10}) (0.001mol) dissolved in ethanol and benzoyl chloride (0.001 mol) was added dropwise for the period of 5 min. The reaction mixture was refluxed under stirring for 8 h. After completion of reaction [monitored by TLC ethyl acetate:*n*-hexane (2:1)], separated solid was filtered and then



	R
BZ_1	C_6H_5
BZ_2	4-OCH_3 C_6H_4
BZ_3	4-Br C_6H_4
BZ_4	4-Cl C_6H_4
BZ_5	2,4$(OCH_3)_2C_6H_3$

	R
BZ_6	2,4$(Cl)_2C_6H_3$
BZ_7	4$(C_2H_5)_2C_6H_4$
BZ_8	4$(CH=CH)C_6H_4$
BZ_9	4-F C_6H_4
BZ_{10}	2-thienyl

Scheme-I: Preparation of *N*-(3-cyano-4-(4-substitutedphenyl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinolin-2-yl) benzamide derivatives [Reagents and conditions, (a) Ethanol, piperidine, reflux for 2-3 h, (b) Ethanol, potassium hydroxide, potassium iodide, reflux for 8-9 h]

kept at room temperature for 24 h. Finally, the solid was recrystallized with methanol (**Scheme-I**).

N-(3-Cyano-7,7-dimethyl-5-oxo-4-phenyl-1,4,5,6,7,8-hexahydroquinolin-2-yl) benzamide (BZ_1): White solid; yield: 70%; m.p.: 208-209 ºC. IR (KBr, ν_{max}, cm^{-1}): 3395, 3324, 3085, 3028, 2960, 2922, 2199, 1738, 1680, 1604, 1454 , 1426, 1330, 1214, 1159, 738; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 11.80 (s, 1H, NH), 7.30-7.70 (m, 10H, ArH), 5.50-5.60 (s, 1H CH), 2.62-2.76 (m, 2H, CH_2), 2.23-2.35 (m, 2H, CH_2), 1.0-1.1 (d, 6H, $(CH_3)_2$). ^{13}C NMR (100MHz, DMSO-d_6) δ ppm: 195.6, 167.5, 158.4, 144.7, 132.7, 129.6, 128.5, 128.3, 127.1, 126.5, 119.7, 112.7, 58.2, 49.9, 35.5, 31.8, 28.4, 26.8; ESI-MS (*m/z*): $[M+H]^+$; calcd.for $C_{25}H_{23}N_3O_2$: 397.4, found: 397.18.

N-(3-Cyano-4-(4-methoxyphenyl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8 hexahydroquinolin-2-yl)benzamide (BZ_2): Light yellow solid; yield: 68%; m.p.: 148-149 ºC. IR (KBr, ν_{max}, cm^{-1}): 3377, 3321, 3184, 3080, 2931, 2833, 2198, 1789, 1683, 1606, 1454, 1370, 1323, 1212, 1160, 776; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.80 (bs, 1H, NH), 6.80-8.10 (m, 9H, ArH), 4.10-4.20 (s, 1H, CH), 3.70 (s, 3H, OCH_3) 2.22-2.26 (m, 2H, CH_2), 2.06-2.10 (m, 2H, CH_2), 0.94-1.03 (d, 6H, $(CH_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 195.6, 167.2, 162.1, 158.4, 157.9, 136.8, 135.1, 132.8, 13.3, 129.3, 129.2, 128.5, 128.2, 119.8, 113.6, 112.9, 58.5, 54.9, 50.0, 34.7, 31.7, 28.3, 26.7; ESI-MS (*m/z*): $[M+H]^+$; calcd.for $C_{26}H_{25}N_3O_3$: 427.4, found: 429.2. [M+2]

N-(-4-(4-Bromophenyl)-3-cyano-7,7-dimethyl-5-oxo-1,4,5,6,7,8 hexahydroquinolin-2-yl)benzamide (BZ_3): White solid; yield: 61%; m.p.: 170-172 ºC. IR (KBr, ν_{max}, cm^{-1}): 3394, 3319, 3210, 3188, 2962, 2888, 2192, 1773, 1682, 1604, 1450, 1366, 1315, 1213, 1159, 774; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.1 (s, 1H, NH), 7.1-8.0 (m, 9H, ArH), 4.17 (s, 1H, CH), 2.26-2.50 (m, 2H, CH_2), 2.08-2.22 (m, 2H, CH_2), 0.94-1.03 (s,6H, $(CH_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 195.6, 167.3, 162.6, 158.4, 144.1, 132.8, 131.2, 13.7, 129.5, 129.2, 128.5, 119.6, 119.5, 112.2, 57.6, 49.9, 35.2, 31.8, 30.7, 28.3, 26.8; ESI-MS (*m/z*): $[M+H]^+$; calcd. for $C_{25}H_{22}N_3O_2Br$: 476.3, found: 491.1. [M+CH_3]

N-(-4-(4-Chlorophenyl)-3-cyano-7,7-dimethyl-5-oxo-1,4,5,6,7,8 hexahydroquinolin-2-yl)benzamide (BZ_4): White solid; yield: 67%; m.p.: 182-184 ºC. IR (KBr, ν_{max}, cm^{-1}): 3392, 3321, 3212, 3079, 2942, 2834, 2196, 1786, 1682, 1608, 1453, 1372, 1326, 1213, 1159, 772; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 11.7 (s, 1H, NH), 7.2-7.8 (m, 9H, ArH), 4.7 (s, 1H, CH), 2.22-2.28 (m, 2H, CH_2), 2.08-2.21 (m, 2H, CH_2), 0.96-1.04 (s, 6H, $(CH_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 194.1, 168.1, 163.8, 156.4, 148.5, 133.2, 132.1, 128.8, 127.5, 118.6, 117.5, 112.2, 59.8, 51.2, 40.0, 38.4, 32.8, 27.2, 26.8; ESI-MS (*m/z*): $[M+H]^+$; calcd.for $C_{25}H_{22}N_3O_2Cl$: 431.9, found: 431.2.

***N*-(3-Cyano-4-(2,4-dimethoxyphenyl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinolin-2-yl)benzamide (BZ_5):** White solid; yield: 70%; m.p.: 151-152 ºC. IR (KBr, ν_{max}, cm^{-1}): 3386, 3334, 3204, 3086, 2962, 2818, 2193, 1787, 1684, 1609, 1456, 1364, 1316, 1211, 1112, 763; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 12.1 (s, 1H, NH), 7.1-7.6 (m, 8H, ArH), 4.1 (s, 1H, CH), 3.83 (s, 6H, $(OCH_3)_2$), 2.61-2.65 (m, 2H, CH_2), 2.01-2.26 (m, 2H, CH_2), 0.99-1.00 (s, 6H, $(CH_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) d ppm: 198.9, 166.0, 159.6, 158.6, 147.5, 133.2, 132.1, 128.8, 127.6, 120.1, 113.3, 106.5, 56.1, 51.5, 45.0, 39.6, 33.1, 27.5, 27.2; ESI-MS (*m/z*): $[M+H]^+$; calcd. for $C_{27}H_{27}N_3O_4$: 457.5, found: 457.9.

***N*-(3-Cyano-4-(2,4-dichlorophenyl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinolin-2-yl)benzamide (BZ_6):** White solid; yield: 69%; m.p.: 157-158 ºC. IR (KBr, ν_{max}, cm^{-1}): 3393, 3318, 3165, 3064, 2945, 2841, 2196, 1790, 1672, 1605, 1456, 1382, 1313, 1261, 1184, 748; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 11.1 (s,1H, NH), 7.1-8.0 (m, 8H, ArH), 4.31 (s,1H, CH), 2.81-2.86 (m, 2H, CH_2), 2.01-2.29 (m, 2H, CH_2), 0.97-1.01 (s, 6H, $(CH_3)_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 197.8, 165.7, 158.6, 154.3, 148.5, 134.2, 132.8, 127.4, 127.1, 120.8, 114.1, 107.1, 58.1, 52.6, 46.1, 38.5, 31.1, 26.8, 26.4; ESI-MS (*m/z*): $[M+H]^+$; calcd.for $C_{25}H_{21}N_3O_2Cl_2$: 466.3, found: 467.8 [M+1].

Antitubercular activity: The anti-mycobacterial activity of the synthesized compounds (**BZ_{1-10}**) were assessed against *M. tuberculosis* using microplate Alamar blue assay (MABA) [27]. This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. The sterile deionized water (200 µL) was added to all outer perimeter wells of sterile 96 wells plate to minimize the evaporation of medium in the test wells during incubation. The 96-wells plate received 100 µL of Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/mL. Plates were covered and sealed with parafilm and incu-bated at 37 ºC for five days. After this time, 25 µL of freshly prepared 1:1 mixture of Alamar blue reagent and 10% Tween 80 was added to the plate and incubated for 24 h. A blue colour in the well was interpreted as no bacterial growth and pink colour was scored as growth. The MIC was defined as lowest drug concentration which prevented the colour change from blue to pink.

Molecular docking: Molecular docking study of the synthesized compounds (**BZ_{1-10}**)) was performed using DNA gyrase protein the crystal structure of DNA (PBD ID: 4B6C) was downloaded from the protein data bank and used for the docking studies by using Schrödinger maestro software in order identify the binding interactions with the targeted protein.

Teratogenicity assay: The teratogenicity assay for these compounds was performed in zebrafish larvae.

Animals: Adult zebrafish were housed in a rectangular aquatic housing tank and fed normal fish food [28]. The water in the tank was maintained at room temperature and the fish were kept on 14 h dark/14 h light cycle.

Embryo collection: Male and female zebrafishes in a 2:3 ratio was fed and kept in breeding tank at 6:00 p.m. The embryos were collected on the next morning, washed thrice with E3 medium and incubated in E3 medium at 25-28 ºC. At 6 h post fertilization, the embryos were transferred into well plates containing 1 mL of vehicle for control group and 10 test compounds in each well. For each drug, the following concentrations (n = 10 for each concentration) were tested: 50, 10, 5, 3, 1, 0.5, 0.25, 0.01 µM until day 5. On 5-day post fertilization the larvae were anaesthetized with tricaine methasulfonate and observed under microscope, therefore images were obtained and morpho-

TABLE-1
ANTITUBERCULAR ACTIVITY OF STANDARD AND TEST COMPOUNDS

Compound	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.12 µg/mL	1.6 µg/mL	0.8 µg/mL
BZ_1	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
BZ_2	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
BZ_3	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
BZ_4	Sensitive	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant
BZ_5	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
BZ_6	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
BZ_7	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
BZ_8	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
BZ_9	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant
BZ_{10}	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
INH	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant
Ciprofloxacin	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant

logical abnormalities were observed and compared with control group.

RESULTS AND DISCUSSION

A series of *N*-(3-cyano-4-(4-substituted phenyl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinolin-2-yl)benzamide derivatives (**BZ_{1-10}**) were synthesized in two steps. In the first step, 2-amino-4-(4-substituted phenyl)-7,7-dimethyl-5-oxo-1,4,5,6,7,8-hexahydro quinoline-3-carbonitriles (**R_{1-10}**) were synthesized by the condensation of dimedone, appropriate substituted aryl aldehydes, malanonitrile and ammonium acetate. In the second step, compounds (**R_{1-10}**) were reacted with the benzoyl chloride in the presence of KOH and KI to yield the final compounds (**BZ_{1-10}**). All the synthesized derivatives were characterized by ^{1}H & ^{13}C NMR, IR and ESI-MS spectra. In ^{1}H NMR of compounds aromatic protons appeared as a multiplet in the region δ 6.8-8.1 ppm, six protons of $(CH_3)_2$ appeared as singlets/doublet in between δ 0.9-1.1 ppm, dione ring CH_2 protons appeared as multiplet at δ 2.08 and 2.2 ppm, while quinoline CH proton appeared in between δ 4.1-5.5 ppm.

Antitubercular activity: Synthesized compounds (**BZ_{1-10}**) were evaluated for antitubercular activity against *Mycobacterium tuberculosis* H37RV strain by using microplate Alamar blue assay method. The results of the antitubercular activity of synthesized compounds are presented in Table-1, which revealed that compounds **BZ_9** and **BZ_4** exhibited good activity against *M. tuberculosis* strain to the level of 12.5 and 25 µg/mL, respectively. Compounds **BZ_9** and **BZ_4** were found to contain *p*-flourophenyl and *p*-chlorophenyl groups as substituents at 4th position of quinoline nucleus. Thus, the MIC value may be in between the range of 6.25 and 12.5 µg/mL and the other synthesized compounds showed the moderate activity. Results were also compared with the standard drugs isoniazid and ciprofloxacin.

Docking studies: *in-silico* docking studies gives insight into the binding ability in the form of docking/glide score and the orientation of the molecule in different poses based on the conformers. Amino acid interactions and epic state penalties were predicted with the help of XP docking module. Initial docking studies of GSP co-crystal gave a docking score of -8.07 (Fig. 1) with hydrogen bond interactions with ASP 79, GLY 83 and hydrophobic interaction with ARG 82. Table-2 shows the docking scores and interaction with amino acids of the synthesized compounds. The docking results of the synthesized derivatives indicated the best docking scores of -5.015 and -5.02 for **BZ_9** and **BZ_4**, respectively as shown in Fig. 2. The two derivatives successfully retained the co-crystal binding interactions.

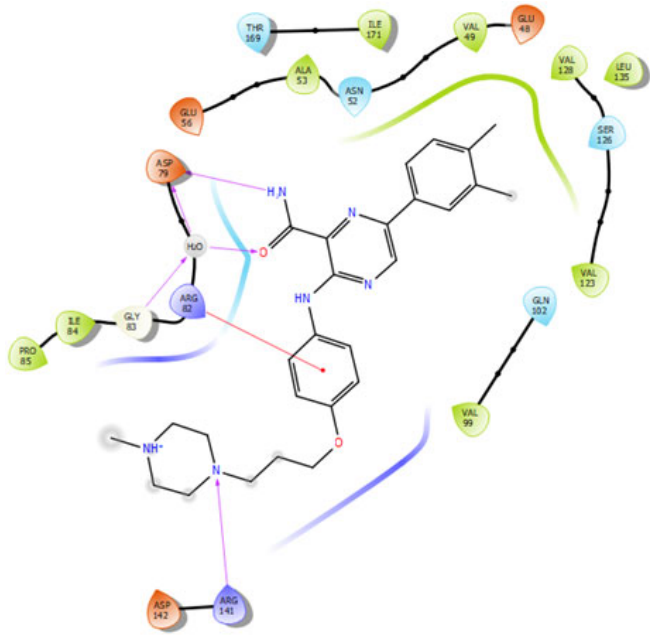


Fig. 1. GSP co-crystal gave a docking score of -8.0

TABLE-2
CALCULATED DOCKING SCORES WITH AMINO ACID INTERACTIONS OF THE SYNTHESIZED COMPOUNDS

Compound	Docking score	Interaction amino acids
BZ_1	-3.997	ARG82, GLY83, ASP79
BZ_2	-4.009	VAL127
BZ_3	-4.591	ARG82, GLY83, ASP79
BZ_4	-5.02	ARG82, GLY83, ASP79
BZ_5	-4.743	ASP55
BZ_6	-4.169	ARG82
BZ_7	-4.215	VAL127
BZ_8	-2.759	VAL127
BZ_9	-5.015	ARG82, GLY83, ASP79
BZ_{10}	-4.67	ASN52, ARG82

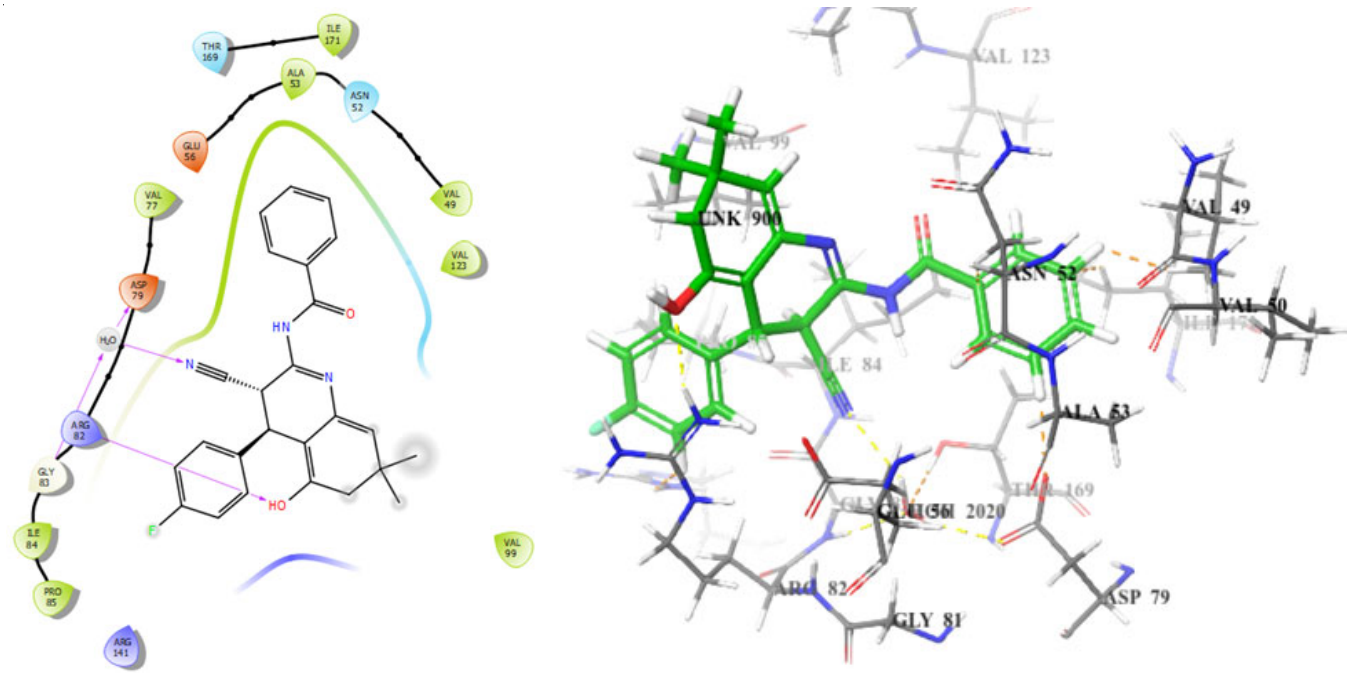


Fig. 2. **BZ$_9$** showing a docking score of -5.015 and 3D interaction image of **BZ$_9$**

Teratogenicity assay: The teratogenicity assay of the synthesized compounds was performed in zebrafish larvae. Out of the ten compounds, seven were highly teratogenic for zebrafish larvae. Three compounds **BZ$_4$**, **BZ$_6$** and **BZ$_8$**, were found to be safer at 0.5 µM without any abnormalities. The result of the three compounds is shown in Fig. 3.

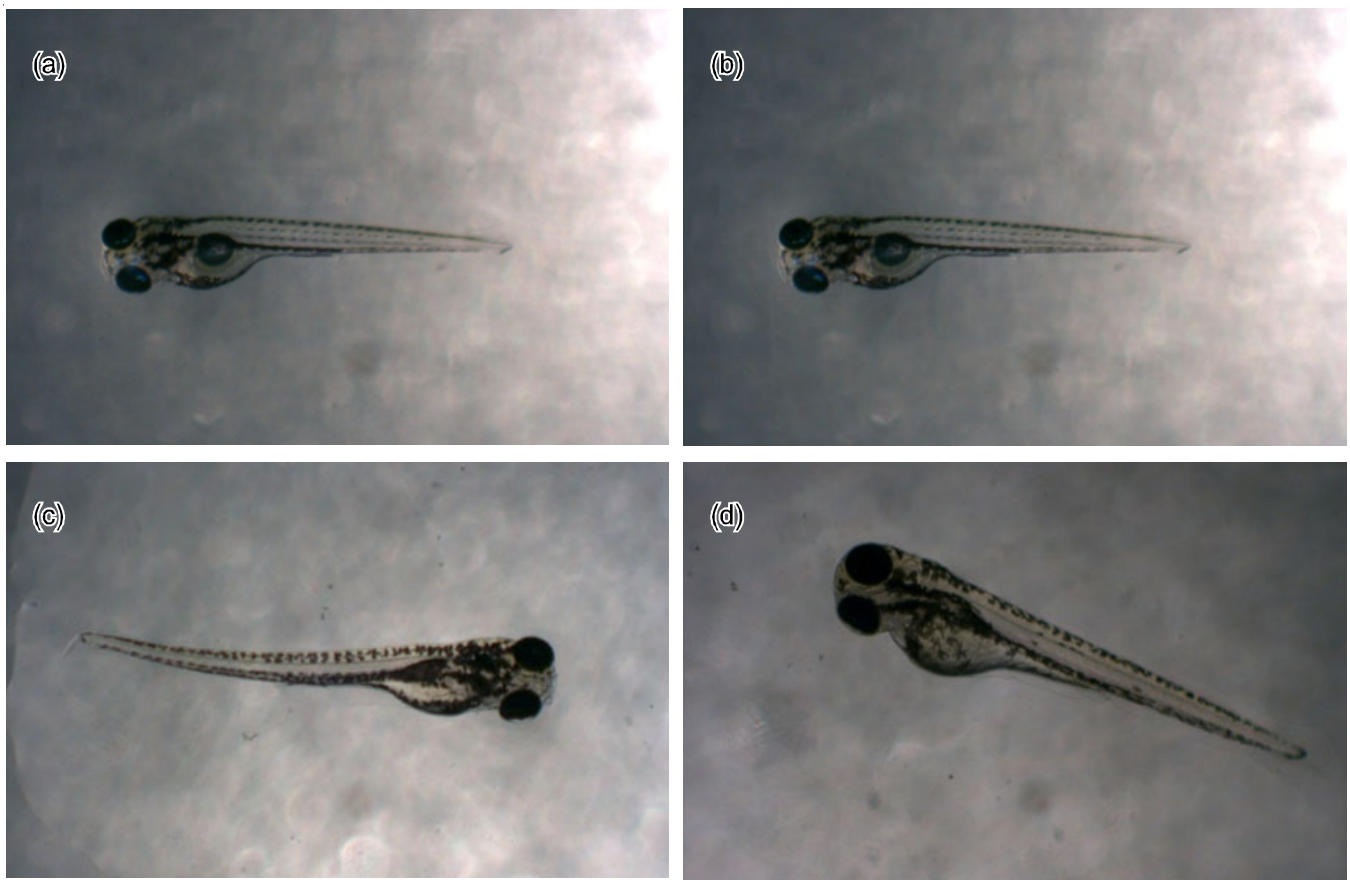


Fig. 3. (a) Control (b) **BZ$_4$** (0.5 µM) (c) **BZ$_6$** (0.5 µM) (d) **BZ$_8$** (0.5 µM) – all the larvae had no abnormalities when compared to the control group

Conclusion

The present study reports the synthesis of hexahydroquinolin-2-yl-benzamide derivatives and *in silico* evaluation for their efficacy as antitubercular compounds through docking against target DNA gyrase (PBD ID: 4B6C). Compounds **BZ$_9$** and **BZ$_4$** are recognized as the most hopeful antitubercular compounds among all the synthesized derivatives based on their docking scores. Compounds **BZ$_9$** and **BZ$_4$** exhibited good activity against *Mycobacterium tuberculosis* strain to the level of 12.5 and 25 µg/mL, respectively. The teratogenicity assay for the synthesized compounds was also performed. Three compounds **BZ$_4$**, **BZ$_6$** and **BZ$_8$**, were found to be safer at 0.5 µM without any abnormalities. Therefore, the synthesized benzamide derivatives would represent a fruitful matrix for the development of potent antitubercular agents. Hence, the synthesized derivatives would deserve further investigation and derivatization involving the molecular modifications and further work on this moiety may be quite rewarding.

CONFLICT OF INTEREST

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

REFERENCES

1. World Health Organization, Multidrug and Extensive Drug Resistant Tuberculosis: 2020 Global Report on Surveillance and Response (2020).
2. T.A. Cinu, S.K. Sidhartha, B. Indira, B.G. Varadaraj, P.S. Vishnu and G.G. Shenoy, *Arab J. Chem*., **12**, 3316 (2019); https://doi.org/10.1016/j.arabjc.2015.09.003
3. K.A. El Sayed, P. Bartyzel, X. Shen, T.L. Perry, J.K. Zjawiony and M.T. Hamann, *Tetrahedron*, **56**, 949 (2000); https://doi.org/10.1016/S0040-4020(99)01093-5
4. K. Dheda, T. Gumbo, N.R. Gandhi, M. Murray, G. Theron, Z. Udwadia, G.B. Migliori and R. Warren, *Lancet Respir. Med*., **2**, 321 (2014); https://doi.org/10.1016/s2213-2600(14)70031-1
5. A. Marella, O.P. Tanwar, R. Saha, M.R. Ali, S. Srivastava, M. Akhter, M. Shaquiquzzaman and M.M. Alam, *Saudi Pharm. J*., **21**, 1 (2013); https://doi.org/10.1016/j.jsps.2012.03.002
6. S.M. Prajapati, K.D. Patel, R.H. Vekariya, S.N. Panchal and H.D. Patel, *RSC Adv*., **4**, 24463 (2014); https://doi.org/10.1039/C4RA01814A
7. M.J. Ahsan, S. Shastri, R. Yadav, M. Hassan, M.A. Bakht, S.S. Jadav and S. Yasmin., *Org. Chem. Int*., **2016**, 9589517 (2016); https://doi.org/10.1155/2016/9589517
8. D. Zeleke, R. Eswaramoorthy, Z. Belay and Y. Melaku, *J. Chem.*, **2020**, 1324096 (2020); https://doi.org/10.1155/2020/1324096
9. X. Wen, S.B. Wang, D.C. Liu, G.H. Gong and Z.S. Quan, *Med. Chem. Res*., **24**, 2591 (2015); https://doi.org/10.1007/s00044-015-1323-y
10. S. Alegaon, K. Kashniyal, S. Kuncolienkar, R. Kavalapure, P. Salve, M. Palled, S. Suryawanshi and S. Jalalpure, *Future J. Pharm. Sci*., **6**, 1 (2020); https://doi.org/10.1186/s43094-020-0019-4
11. R. Yang, W. Du, H. Yuan, T. Qin, R. He, Y. Ma and H. Du, *Mol. Divers*., **24**, 1065 (2020); https://doi.org/10.1007/s11030-019-10012-1
12. R.S. Keri and S.A. Patil, *Biomed. Pharmacother*., **68**, 1161 (2014); https://doi.org/10.1016/j.biopha.2014.10.007
13. A. Jallapally, D. Addla, P. Yogeeswari, D. Sriram and S. Kantevari, *Bioorg. Med. Chem. Lett*., **24**, 5520 (2014); https://doi.org/10.1016/j.bmcl.2014.09.084
14. S. Alegaon, K. Kashniyal, S. Kuncolienkar, R. Kavalapure, P. Salve, M. Palled, S. Suryawanshi and S. Jalalpure, *Future J. Pharm. Sci*., **6**, 1 (2020); https://doi.org/10.1186/s43094-020-0019-4
15. D. Patil, D. Chandam, A. Mulik, S. Jagdale, P. Patil and M. Deshmukh, *J. Saudi Chem. Soc*., **21**, S329 (2017); https://doi.org/10.1016/j.jscs.2014.04.001
16. M. Maheswara, V. Siddaiah, G.L. Damu and C.V. Rao, *Arkivoc*, 201 (2006); https://doi.org/10.3998/ark.5550190.0007.223
17. B. Das, B. Ravikanth, R. Ramu and B.V. Rao, *Chem. Pharm. Bull*., **54**, 1044 (2006); https://doi.org/10.1002/chin.200650158
18. M.S. Al-Said, M.S. Bashandy and M.M. Ghorab, *Arzneimittelforschung*, **61**, 527 (2011); https://doi.org/10.1055/s-0031-1296239
19. F.S. Hosseini and M. Bayat M, *J. Iran Chem. Soc*., **17**, 2267 (2020); https://doi.org/10.1007/s13738-020-01920-3
20. H. Jiang, X.S. Wang, M.M. Zhang, Y.L. Li and D.Q. Shi, *Acta Cryst. E*, **62**, o1184 (2006); https://doi.org/10.1107/s1600536806006465
21. S. Das, S. Santra, A. Roy, S. Urinda, A. Majee and A. Hajra, *Green Chem. Lett. Rev*., **5**, 97 (2012); https://doi.org/10.1080/17518253.2011.584073
22. K.A. Undale, Y. Park, K. Park, D.H. Dagade and D.M. Pore, *Synlett*., 791 (2011); https://doi.org/10.1055/s-0030-1259924
23. C.S. Reddy and M. Raghu, *Chem. Lett*., **19**, 775 (2008); https://doi.org/10.1016/j.cclet.2008.04.040
24. B.V. Lichitsky, A.A. Dudinov and M.M. Krayushkin, *Arkivoc*, 73 (2001); https://doi.org/10.3998/ark.5550190.0002.911
25. K.A. Undale, T.S. Shaikh, D.S. Gaikwad and D.M. Pore, *C.R. Chim*., **14**, 511 (2011); https://doi.org/10.1016/j.crci.2010.09.009
26. H.M. Faidallah, A.A. Saqer, K.A. Alamry, K.A. Khan and A.M. Asiri, *J. Enzyme Inhib. Med. Chem.,* **29**, 367 (2014); https://doi.org/10.3109/14756366.2013.787421
27. M.C.S. Lourenco, M.V.N. DeSouza, A.C. Pinheiro, M. de L. Ferreira, R.S.B. Goncalves, T.C.M.T Nogueira and M.A. Peralta, *Arkivoc*, 181 (2007); https://doi.org/10.3998/ark.5550190.0008.f18
28. J.M. Panzica-Kelly, C.X. Zhang, T.L. Danberry, A. Flood, J.W. DeLan, K.C. Brannen and K.A. Augustine-Rauch, *Birth Defects Res. B Dev. Reprod. Toxicol*., **89**, 382 (2010); https://doi.org/10.1002/bdrb.20260