Res Chem Intermed
DOI 10.1007/s11164-014-1542-8

Access to antimycobacterial and anticancer potential of some fused quinazolines

Amit B. Patel · Kishor H. Chikhalia · Premlata Kumari

Received: 12 December 2013 / Accepted: 12 January 2014
© Springer Science+Business Media Dordrecht 2014

Abstract Two series of some various *N*-phenyl/benzothiazolyl acetamide-fused quinazoline derivatives were synthesized and tested for their antimycobacterial activity against *M. tuberculosis* H37Rv. Moreover, the synthesized analogs were also screened against human PC3 cells in order to explore their anticancer activity. The in vitro antimycobacterial screening revealed that, among the synthesized analogs, *N*-benzothiazolyl acetamide derivatives showed remarkable antimycobacterial activity. However, the best anticancer results were observed amongst the *N*-phenyl acetamide-substituted quinazoline derivatives. The newly synthesized compounds were characterized through IR, ^{1}H NMR, ^{13}C NMR, MS, and elemental analysis.

Keywords Quinazoline · Benzothiazole · Suzuki coupling · Anticancer Antimycobacterial activity

Introduction

The new therapeutic modulations for the chemotherapy of some life-threatening diseases is a subject of immense interest owing to the fact that many present treatments have failed or fall short in terms of multi-drug resistance or toxicity problems associated with them [1]. According to the recent WHO global TB report, 1.4 million people died from TB in 2011, including almost 1 million deaths among

A. B. Patel (✉) · P. Kumari (✉)
Department of Applied Chemistry, S. V. National Institute of Technology, Surat 395007, India
e-mail: amit.svnit10@gmail.com

P. Kumari
e-mail: premlatakumari1@gmail.com

K. H. Chikhalia
Department of Chemistry, School of Science, Gujarat University, Ahmedabad 380 009, India

Published online: 01 February 2014

HIV-negative individuals and 0.43 million HIV-positive people [2, 3]. Cancer is a disease of striking significance in the world today. It represents the second leading cause of human mortality after cardiovascular diseases [4, 5]. The current scenario highlights the need for the discovery and development of new lead compounds of simple structure, exhibiting optimal biological potency with novel mode of actions. Quinazoline derivatives are also an important class of heterocycles, which can serve as unique and versatile scaffolds for experimental drug design. The quinazoline derivatives have received much attention owning to their diverse biological activity such as antifungal [6], antibacterial [7], analgesic [8], anticancer [9, 10], anti-HIV [11], antimicrobial [12, 13], anti-inflammatory [14], and anticonvulsant [15], etc. 2-Aminobenzothiazoles have attracted considerable attention as they are also endowed with a wide range of pharmaceutical activities including anticonvulsant [16], analgesic [17], anticancer [18–20], antituberculosis [21], antibacterial [22], antifungal [23], anti-HIV [24], and cytotoxic [25], etc. The development of new synthetic methods leading to structures, which incorporate various biologically active moieties in a single molecule, has attracted much attention in organic chemistry. In an attempt to find new promising pharmacologically active molecules, we report here the antimycobacterial and anticancer activities of various *N*-phenyl acetamides and *N*-benzothiazolyl acetamide-fused quinazoline derivatives.

Experimental

General

All solvents and chemicals were purchased from Sigma Aldrich Chemicals (Mumbai, India) and Rankem (Surat, India). The TLC plates (silica gel 60 F254) were obtained from Merck, with visualization under UV light. The uncorrected melting points were determined in open capillaries on a Veego electronic apparatus VMP-D. FT-IR spectra (4,000–400 cm^{-1}) of the synthesized analogs were recorded on a Shimadzu 8400-S FT-IR spectrophotometer by preparing KBr pellets. The elemental analysis was performed by Perkin Elmer 2400 CHN Elemental Analyser. ^{1}H and ^{13}C NMR spectra were recorded on an Avance-II-400 MHz (Bruker) spectrometer using DMSO as a solvent and TMS as internal standard. Chemical shifts were reported as parts per million (ppm) downfield from TMS (Me_4Si). The mass spectra were measured with Waters Micromass Q-Tof Micro instrument (time of flight mass spectrometer). Column chromatography was performed on 45-cm (2.5 cm diameter) glass column using silica gel LC 60A (70–200 μ).

Synthesis of 2-chloro-4-(m-tolyloxy)quinazoline (**5**)

An oven-dried schlenk tube was charged with a magnetic stir bar, 2,4-dichloroquinazoline **4** (2 mmol), *m*-cresol (2 mmol), potassium carbonate (10 mmol), and anhydrous ethanol (20 mL). The tube was then evacuated and back-filled with nitrogen. The protocol was repeated two times. The tube was placed in a preheated oil bath at refluxed temperature and the reaction slurry was stirred vigorously for

12 h. After cooling to the room temperature, the reaction mixture was treated with 200 mL ice-cold water, and the resulting precipitate was filtered off. The crude product **5** was recrystallized from DMF [32]. White solid, Yield: 81 %, m.p. 133–135 °C; White solid, Yield: 81 %, m.p. 133–135 °C; IR (KBr, cm^{-1}): 2,989 (Ar–CH), 1,578(CN), 1,245 (C–O–C), 645(C–Cl). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.29 (dd, $J = 6.8$, 1.4 Hz, 1H, quinazoline), 7.98 (dd, $J = 6.8$, 1.4 Hz, 1H, quinazoline), 7.66–7.17 (m, 3H, Ar–H), 6.58–6.48 (m, 3H, Ar–H, *m*-tolyloxy ring), 2.15 (s, 3H, $–CH_3$).

Synthesis of 4-(4-(m-tolyloxy)quinazolin-2-yl)aniline (**6**)

An oven-dried 100-mL flat-bottomed flask was charged with a magnetic stir bar, intermediate **5** (2 mmol), tetrakis-(triphenylphosphine)palladium (0) (0.025 mmol, 1.3 mol %), 2 M sodium carbonate solution (2 mL), and 4-aminophneylboronic acid pinacol ester (3 mmol) in dimethoxyethane (DME) solvent (100 mL). The flask was then evacuated and back-filled with nitrogen. The protocol was repeated two times. The flask was placed in a preheated oil bath at refluxed temperature and the reaction slurry was stirred vigorously for 24 h. The solvent was recovered at reduced pressure. Then, the reaction mixture was cooled to room temperature. The residue was dissolved in methylene dichloride and filtered through a pad of Celite. The filtrate was washed with water (2× 100 mL), and the organic layer was dried with sodium sulphate. The filtrate was concentrated and the resulting residue was purified by column chromatography (20 % EA in Hex) to yield the desired analog **6** [33]. White solid, Yield: 68 %, m.p. 201–204 °C; White solid, Yield: 68 %, m.p. 201–204 °C; IR (KBr, cm^{-1}): 3,360 (–NH), 1,245 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.42 (dd, $J = 6.8$, 1.4 Hz, 1H, quinazoline), 8.13 (dd, $J = 6.6$, 1.5 Hz, 1H, quinazoline), 7.62–7.21 (m, 7H, Ar–H), 6.74–6.62 (m, 3H, Ar–H, *m*-tolyloxy ring), 4.67 (br s, 2H, –NH), 2.32 (s, 3H, $–CH_3$).

General synthetic procedure for analogs (**7a–g/8a–g**)

An oven-dried 250-mL flat-bottomed flask was charged with a magnetic stir bar, intermediate **6** (50 mmol), 2-chloro-*N*-(6-substituted-phenyl/benzothiazolyl)-acetamides **2a–j/3a–j** (52 mmol), potassium carbonate (54 mmol), and acetone (50 mL). The tube was then evacuated and back-filled with nitrogen. The protocol was repeated two times. The tube was placed in a preheated oil bath at refluxed temperature and the reaction slurry was stirred vigorously for 6–12 h. After the completion of the reaction, the reaction mass was poured onto crushed ice and the resulting precipitate was filtered, washed with water, dried, and crystallized with a suitable solvent to obtain **7a–j**. The progress of the reaction was monitored by Thin Layer Chromatography (20 % EA in Hex) [34, 35].

*N-phenyl-2-(4-(4-(m-tolyloxy)quinazolin-2-yl)phenylamino)acetamide (**7a**)*

Pale white solid, Yield: 72 %, m.p. 232–234 °C; IR (KBr, cm^{-1}): 3,385 (–NH), 3,034 (–CH), 1,665 (C=O), 1260 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6): δ

10.32 (s, 1H, –CONH), 10.14 (s, 1H, –NH), 8.31(dd, $J = 7.2$, 1.6 Hz, 1H, quinazoline), 8.06 (dd, $J = 7.8$, 1.5 Hz, 1H, quinazoline), 8.27–7.55 (m, 8H, Ar–H), 7.43–7.01 (m, 6H, Ar–H, *m*-tolyloxy ring), 4.15 (s, 2H, $–CH_2$), 2.05 (s, 3H, $–CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6): 178.1 (1C, C=O), 164.4 (1C, C–O–C, quinazoline to *m*-tolyloxy linkage), 162.4 (1C, C–C, quinazoline to phenyl linkage), 158.5, 155.7, 155.1, 146.0, 145.9, 145.5, 140.6, 138.2, 135.8, 134.0, 128.7, 128.4, 128.3, 127.5, 127.1, 125.8, 125.7, 124.9, 123.6, 120.8, 119.3, 118.7, 113.3, 113.1 (24C, Ar–C), 43.3 (1C, CH_2), 29.0 (1C, CH_3). MS, *m/z* 460.2 $[M+1]^+$. Anal calcd for $C_{29}H_{24}N_4O_2$: C, 75.63; H, 5.25; N, 12.17 Found C, 75.42; H, 5.56; N, 11.89.

N-(4-chlorophenyl)-2-(4-(4-(m-tolyloxy)quinazolin-2-yl)phenylamino)acetamide ***(7b)***

Light greenish solid, Yield: 76 %, m.p. 192–193 °C; IR (KBr, cm^{-1}): 3,369 (–NH), 2,987 (–CH), 1,674 (C=O), 1241 (C–O–C), 740 (C–Cl). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.89 (s, 1H, –CONH), 9.81 (s, 1H, –NH), 8.25 (dd, $J = 7.6$, 1.8 Hz, 1H, quinazoline), 7.92 (dd, $J = 6.8$, 2.0 Hz, 1H, quinazoline), 7.81 (td, $J = 6.8$, 1.2 Hz, 1H), 7.58–7.46 (m, 8H, Ar–H), 7.37–7.04 (m, 5H, Ar–H, *m*-tolyloxy ring), 3.61 (s, 2H, $–CH_2$), 2.33 (s, 3H, $–CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6): 180.2 (1C, C=O), 164.9 (1C, C–O–C, quinazoline to *m*-tolyloxy linkage), 161.7 (1C, C–C, quinazoline to phenyl linkage), 159.3, 156.2, 156.1, 147.2, 145.0, 144.9, 140.1, 138.7, 136.3, 134.7, 129.8, 129.1, 127.9, 127.2, 126.8, 125.6, 125.0, 123.8, 122.7, 119.7, 118.9, 118.1, 114.5, 114.2 (24C, Ar–C), 46.7 (1C, CH_2), 21.3 (1C, CH_3). MS, *m/z* 495.6 $[M+1]^+$. Anal calcd for $C_{29}H_{23}ClN_4O_2$: C, 70.37; H, 4.68; N, 11.32 Found C, 70.25; H, 4.76; N, 11.28.

N-(4-bromophenyl)-2-(4-(4-(m-tolyloxy)quinazolin-2-yl)phenylamino)acetamide ***(7c)***

Pale yellow solid, Yield: 70 %, m.p. 178–182 °C; IR (KBr, cm^{-1}): 3,319 (–NH), 2,928 (–CH), 1,680 (C=O), 1,240 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.21 (s, 1H, –CONH), 9.98 (s, 1H, –NH), 8.38 (dd, $J = 7.8$, 1.6 Hz, 1H, quinazoline), 8.09 (dd, $J = 6.4$, 1.8 Hz, 1H, quinazoline), 7.76 (td, $J = 6.6$, 1.4 Hz, 1H), 7.62–7.51 (m, 10H, Ar–H), 7.39–6.98 (m, 3H, Ar–H, *m*-tolyloxy ring), 3.71 (s, 2H, $–CH_2$), 2.38 (s, 3H, $–CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6): 179.8 (1C, C=O), 164.3 (1C, C–O–C, quinazoline to *m*-tolyloxy linkage), 162.1 (1C, C–C, quinazoline to phenyl linkage), 158.2, 156.7, 155.9, 146.8, 145.4, 145.1, 142.3, 138.9, 136.1, 134.2, 130.1, 129.7, 128.6, 127.7, 127.3, 125.8, 124.3, 122.7, 121.4, 120.1, 118.5, 118.0, 115.3, 113.9 (24C, Ar–C), 48.3 (1C, CH_2), 27.4 (1C, CH_3). MS, *m/z* 540 $[M+1]^+$. Anal calcd for $C_{29}H_{23}BrN_4O_2$: C, 64.57; H, 4.30; N, 10.39 Found C, 64.46; H, 4.28; N, 10.31.

N-(4-fluorophenyl)-2-(4-(4-(m-tolyloxy)quinazolin-2-yl)phenylamino)acetamide ***(7d)***

Brown solid, Yield: 78 %, m.p. 225–227 °C; IR (KBr, cm^{-1}): 3,326 (–NH), 2,937 (–CH), 1,678 (C=O), 1,255 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.03 (s,

1H, –CONH), 9.85 (s, 1H, –NH), 8.17 (dd, $J = 8.2$, 1.4 Hz, 1H, quinazoline), 7.83 (dd, $J = 6.6$, 1.5 Hz, 1H, quinazoline), 7.69 (td, $J = 6.8$, 1.5 Hz, 1H), 7.54–7.43 m(m, 8H, Ar–H), 7.36–7.02 (m, 5H, Ar–H, *m*-tolyloxy ring), 3.68 (s, 2H, $–CH_2$), 2.25 (s, 3H, $–CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6): 180.6 (1C, C=O), 165.1 (1C, C–O–C, quinazoline to *m*-tolyloxy linkage), 162.8 (1C, C–C, quinazoline to phenyl linkage), 158.7, 156.1, 155.5, 147.4, 146.3, 145.8, 144.0, 139.2, 135.5, 133.8, 128.6, 128.2, 127.4, 126.8, 126.2, 125.5, 124.8, 123.4, 121.9, 120.3, 119.0, 118.3, 114.8, 114.1 (24C, Ar–C), 47.7 (1C, CH_2), 25.2 (1C, CH_3). MS, *m/z* 480.2 $[M+1]^+$. Anal calcd for $C_{29}H_{23}FN_4O_2$: C, 72.79; H, 4.84; N, 11.71 Found C, 72.53; H, 4.76; N, 11.78.

*N-(4-nitrophenyl)-2-(4-(4-(m-tolyloxy)quinazolin-2-yl)phenylamino)acetamide (**7e**)*

Yellow solid, Yield: 65 %, m.p. 249–251 °C; IR (KBr, cm^{-1}): 3,326 (–NH), 2,942 (–CH), 1,687 (C=O), 1,215 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.92 (s, 1H, –CONH), 9.76 (s, 1H, –NH), 8.22(dd, $J = 7.6$, 1.5 Hz, 1H, quinazoline), 8.01 (dd, $J = 7.0$, 1.9 Hz, 1H, quinazoline), 7.83 (td, $J = 7.2$, 1.2 Hz, 1H), 7.62–7.49 (m, 9H, Ar–H), 7.40–7.03 (m, 4H, Ar–H, *m*-tolyloxy ring), 3.78 (s, 2H, $–CH_2$), 2.31 (s, 3H, $–CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6): 181.1 (1C, C=O), 164.7 (1C, C–O–C, quinazoline to *m*-tolyloxy linkage), 162.2 (1C, C–C, quinazoline to phenyl linkage), 158.4, 156.6, 155.1, 147.8, 146.0, 145.6, 143.8, 139.3, 135.9, 133.4, 130.7, 129.9, 128.5, 127.4, 126.5, 125.2, 124.3, 123.7, 122.2, 119.9, 119.3, 118.6, 115.9, 114.5 (24C, Ar–C), 46.8 (1C, CH_2), 23.9 (1C, CH_3). MS, *m/z* 506.2 $[M+1]^+$. Anal calcd for $C_{29}H_{23}N_5O_4$:C, 68.90; H, 4.59; N, 13.85 Found C, 68.97; H, 4.67; N, 13.69.

*N-(4-cyanophenyl)-2-(4-(4-(m-tolyloxy)quinazolin-2-yl)phenylamino)acetamide (**7f**)*

White solid, Yield: 72 %, m.p. 216–218 °C; IR (KBr, cm^{-1}): 3,287 (–NH), 2,892 (–CH), 1,670 (C=O), 1,233 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.09 (s, 1H, –CONH), 9.93 (s, 1H, –NH), 8.41(dd, $J = 7.2$, 1.8 Hz, 1H, quinazoline), 8.12 (dd, $J = 7.2$, 1.6 Hz, 1H, quinazoline), 7.91 (td, $J = 7.8$, 2.4 Hz, 1H), 7.71–7.56 (m, 8H, Ar–H), 7.45–7.28 (m, 5H, Ar–H, *m*-tolyloxy ring), 4.01 (s, 2H, $–CH_2$), 2.44 (s, 3H, $–CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6): 178.4 (1C, C=O), 165.4 (1C, C–O–C, quinazoline to *m*-tolyloxy linkage), 163.0 (1C, C–C, quinazoline to phenyl linkage), 159.1, 157.3, 156.2, 146.5, 145.2, 144.8, 141.4, 138.7, 134.9, 133.3, 130.2, 129.7, 129.1, 128.6, 127.3, 126.7, 124.9, 124.3, 122.7, 120.4, 118.4, 117.8, 114.3, 113.9 (24C, Ar–C), 106.3 (1C, $–C\equiv N$), 48.1 (1C, CH_2), 23.3 (1C, CH_3). MS, *m/z* 486.3 $[M+1]^+$. Anal calcd for $C_{30}H_{23}N_5O_2$: C, 74.21; H, 4.77; N, 14.42 Found C, 74.26; H, 4.83; N, 14.57.

*N-p-tolyl-2-(4-(4-(m-tolyloxy)quinazolin-2-yl)phenylamino)acetamide (**7g**)*

Brown solid, Yield: 69 %, m.p. 259–262 °C; IR (KBr, cm^{-1}): 3,318 (–NH), 3,004 (–CH), 1,668 (C=O), 1,227 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.15 (s,

1H, –CONH), 10.01 (s, 1H, –NH), 8.29(dd, $J = 7.8$, 1.6 Hz, 1H, quinazoline), 8.02 (dd, $J = 6.5$, 1.4 Hz, 1H, quinazoline), 7.87 (td, $J = 7.6$, 2.2 Hz, 1H), 7.63–7.48 (m, 9H, Ar–H), 7.33–6.98 (m, 4H, Ar–H, *m*-tolyloxy ring), 3.81 (s, 2H, $–CH_2$), 2.39 (s, 6H, $–CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6): 178.9 (1C, C=O), 164.6 (1C, C–O–C, quinazoline to *m*-tolyloxy linkage), 162.3 (1C, C–C, quinazoline to phenyl linkage), 159.4, 155.8, 155.0, 146.7, 145.8, 145.0, 141.7, 139.2, 135.8, 134.5, 129.8, 129.4, 128.7, 127.5, 127.1, 126.4, 126.0, 125.7, 123.2, 119.3, 117.9, 117.1, 115.2, 113.8 (24C, Ar–C), 46.3 (1C, CH_2), 24.1 (2C, CH_3). MS, *m/z* 475 $[M+1]^+$. Anal calcd for $C_{30}H_{26}N_4O_2$: C, 75.93; H, 5.52; N, 11.81 Found C, 76.01; H, 5.37; N, 11.96.

N-(4-methoxyphenyl)-2-(4-(4-(m-tolyloxy)quinazolin-2-yl)phenylamino)acetamide ***(7h)***

Light brown solid, Yield: 78 %, m.p. 195–196 °C; IR (KBr, cm^{-1}): 3,302 (–NH), 2,948 (–CH), 1,680 (C=O), 1,253 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.96 (s, 1H, –CONH), 9.80 (s, 1H, –NH), 8.12(dd, $J = 8.0$, 1.9 Hz, 1H, quinazoline), 7.90 (dd, $J = 6.6$, 1.7 Hz, 1H, quinazoline), 7.69 (td, $J = 7.4$, 1.8 Hz, 1H), 7.57–7.40 (m, 10H, Ar–H), 7.31–7.12 (m, 3H, Ar–H, *m*-tolyloxy ring), 4.09 (s, 3H, $–OCH_3$), 3.65 (s, 2H, $–CH_2$), 2.40 (s, 3H, $–CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6): 180.9 (1C, C=O), 164.1 (1C, C–O–C, quinazoline to *m*-tolyloxy linkage), 161.8 (1C, C–C, quinazoline to phenyl linkage), 159.0, 155.5, 154.9, 147.2, 145.1, 144.3, 140.8, 138.4, 135.6, 133.7, 129.4, 128.7, 127.9, 127.3, 126.7, 126.0, 125.1, 124.5, 122.8, 120.2, 118.1, 117.5, 114.8, 114.0 (24C, Ar–C), 56.3 (1C, $–O\underline{C}H_3$), 43.7 (1C, CH_2), 22.8 (1C, CH_3). MS, *m/z* 491 $[M+1]^+$. Anal calcd for $C_{30}H_{26}N_4O_3$: C, 73.45; H, 5.34; N, 11.42 Found C, 73.39; H, 5.28; N, 11.35.

N-(4-ethoxyphenyl)-2-(4-(4-(m-tolyloxy)quinazolin-2-yl)phenylamino)acetamide ***(7i)***

Brown solid, Yield: 77 %, m.p. 223–225 °C; IR (KBr, cm^{-1}): 3,267 (–NH), 2,892 (–CH), 1,672 (C=O), 1,245 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.25 (s, 1H, –CONH), 10.08 (s, 1H, –NH), 8.33(dd, $J = 8.2$, 1.2 Hz, 1H, quinazoline), 7.98 (dd, $J = 7.2$, 1.5 Hz, 1H, quinazoline), 7.72 (td, $J = 6.8$, 1.5 Hz, 1H), 7.61–7.45 (m, 8H, Ar–H), 7.39–7.24 (m, 5H, Ar–H, *m*-tolyloxy ring), 4.13 (q, $J = 6.2$ Hz, 2H, $–OC\underline{H}_2CH_3$), 3.52 (s, 2H, $–CH_2$), 2.27 (s, 3H, $–CH_3$), 1.92 (t, $J = 6.8$ Hz, 3H, $–OCH_2C\underline{H}_3$). ^{13}C NMR (100 MHz, DMSO-d_6): 178.6 (1C, C=O), 165.0 (1C, C–O–C, quinazoline to *m*-tolyloxy linkage), 163.2 (1C, C–C, quinazoline to phenyl linkage), 158.7, 155.9, 154.8, 147.0, 145.4, 145.1, 140.3, 138.7, 135.9, 133.5, 129.7, 129.1, 128.6, 127.4, 126.5, 125.8, 124.7, 124.3, 121.6, 119.0, 118.5, 118.1, 114.9, 113.7 (24C, Ar–C), 58.3 (1C, $–O\underline{C}H_2CH_3$), 43.9 (1C, CH_2), 24.6 (1C, CH_3), 13.8 (1C, $–OCH_2\underline{C}H_3$). MS, *m/z* 504.7 $[M+1]^+$. Anal calcd for $C_{31}H_{28}N_4O_3$: C, 73.79; H, 5.59; N, 11.10 Found C, 73.84; H, 5.64; N, 11.19.

*N-(4-acetamidophenyl)-2-(4-(4-(m-tolyloxy)quinazolin-2-yl)phenylamino)acetamide (**7j**)*

White solid, Yield: 69 %, m.p. 239–241 °C; IR (KBr, cm^{-1}): 3,240 (–NH), 2,967 (–CH), 1,678 (C=O), 1,238 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.37 (s, 1H, –CONH), 10.11 (s, 1H, –NHCOCH$_3$), 9.72 (s, 1H, –NH), 8.21(dd, J = 7.6, 1.4 Hz, 1H, quinazoline), 8.03 (dd, J = 6.8, 1.2 Hz, 1H, quinazoline), 7.81 (td, J = 7.2, 2.0 Hz, 1H), 7.59–7.42 (m, 8H, Ar–H), 7.28–7.02 (m, 5H, Ar–H, *m*-tolyloxy ring), 3.69 (s, 2H, –CH$_2$), 2.41 (s, 3H, –CH$_3$), 2.04 (s, 3H, –NHCOCH$_3$). ^{13}C NMR (100 MHz, DMSO-d_6): 179.3 (1C, C=O), 171.6 (1C, –NH–CO–CH$_3$), 164.8(1C, C–O–C, quinazoline to *m*-tolyloxy linkage), 162.7 (1C, C–C, quinazoline to phenyl linkage), 158.2, 156.0, 155.3, 146.3, 145.2, 144.8, 141.2, 139.2, 136.4, 134.8, 130.2, 129.5, 128.4, 127.1, 126.6, 126.2, 124.9, 122.8, 121.3, 120.7, 119.5, 118.3, 115.6, 114.8 (24C, Ar–C), 46.1 (1C, CH$_2$), 25.3 (2C, CH$_3$). MS, *m/z* 519.3 $[M+1]^+$. Anal calcd for $C_{31}H_{27}N_5O_3$: C, 71.94; H, 5.26; N, 13.53 Found C, 72.05; H, 5.33; N, 13.41.

*N-(benzo[d]thiazol-2-yl)-2-((4-(4-(m-tolyloxy)quinazolin-2-yl)phenyl)amino)acetamide (**8a**)*

Light brown solid, Yield 75 %, m.p. 233–236 °C. IR (KBr, cm^{-1}): 3,303 (–NH), 2,958 (–CH), 1,678 (C=O), 1,236 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.22 (s, 1H, –CONH), 8.67 (s, 1H, –NH), 8.46 (dd, J = 7.4, 1.6 Hz, 1H, quinazoline), 8.04 (dd, J = 6.6, 1.5 Hz, 1H, quinazoline), 7.92–7.21 (m, 11H, Ar–H), 6.89–6.58 (m, 3H, Ar–H, *m*-tolyloxy ring), 3.32 (s, 2H, –CH$_2$), 2.22 (s, 3H, –CH$_3$). ^{13}C NMR (100 MHz, DMSO-d_6): δ170.4 (1C, C=O), 168.5 (1C, C–C, quinazoline to phenyl linkage), 165.3 (1C, C=N, benzothiazole), 154.5, 153.3 (2C, C–O–C, quinazoline to *m*-tolyloxy linkage), 150.7, 150.9, 149.4, 148.9, 147.1, 146.6, 144.4, 138.8, 133.2, 131.3, 130.7, 129.5, 129.3, 128.4, 127.6, 126.8, 125.8, 125.5, 122.3, 121.8, 121.1, 119.8, 119.7 (23C, Ar–C), 46.3 (1C, CH$_2$), 21.5 (1C, CH$_3$). MS, *m/z* 517.3 $[M+1]^+$. Anal calcd for $C_{30}H_{23}N_5O_2S$: C, 69.61; H, 4.48; N, 13.53 Found C, 69.45; H, 4.33; N, 13.69.

*N-(6-chlorobenzo[d]thiazol-2-yl)-2-((4-(4-(m-tolyloxy)quinazolin-2-yl)phenyl)amino)acetamide (**8b**)*

Light green solid, Yield 73 %, m.p. 271–272 °C. IR (KBr, cm^{-1}): 3,320 (–NH), 2,938 (–CH), 1,679 (C=O), 1,201 (C–O–C), 753 (–Cl). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.51 (s, 1H, –CONH), 9.02 (s, 1H, –NH), 8.21 (dd, J = 7.2, 1.5 Hz, 1H, quinazoline), 8.09 (dd, J = 6.8, 1.1 Hz, 1H, quinazoline), 7.68–7.24 (m, 10H, Ar–H), 6.71–6.65 (m, 3H, Ar–H, *m*-tolyloxy ring), 3.20 (s, 2H, –CH$_2$), 2.31 (s, 3H, –CH$_3$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.5 (1C, C=O), 164.8 (1C, C–C, quinazoline to phenyl linkage), 159.9 (1C, C=N, benzothiazole), 153.2, 151.9 (2C, C–O–C, quinazoline to *m*-tolyloxy linkage), 148.0, 147.1, 146.3, 145.9, 144.6, 143.2, 142.7, 141.3, 140.9, 139.2, 138.2, 134.5, 132.8, 131.3, 129.5, 128.3, 127.4, 126.0, 125.8, 125.3, 124.7, 122.0, 118.8 (23C, Ar–C), 38.6 (1C, CH$_2$), 22.9 (1C,

CH_3). MS, *m/z* 551.1 $[M+1]^+$. Anal calcd for $C_{30}H_{22}ClN_5O_2S$: C, 65.27; H, 4.02; N, 12.69 Found C, 65.42; H, 3.78; N, 12.80.

*N-(6-bromobenzo[d]thiazol-2-yl)-2-((4-(4-(m-tolyloxy)quinazolin-2-yl)phenyl)amino)acetamide (**8c**)*

Light yellow solid, Yield 78 %, m.p. 264–265 °C. IR (KBr, cm^{-1}): 3,288 (–NH), 2,890 (–CH), 1,680 (C=O), 1,245 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.38 (s, 1H, –CONH), 8.72(s, 1H, –NH), 8.56 (dd, $J = 7.7$, 1.3 Hz, 1H, quinazoline), 8.01 (dd, $J = 7.2$, 1.4 Hz, 1H, quinazoline), 7.71–7.35 (m, 10H, Ar–H), 6.83–6.62 (m, 3H, Ar–H, *m*-tolyloxy ring), 3.30 (s, 2H, $–CH_2$), 2.25 (s, 3H, $–CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6): δ172.1 (1C, C=O), 164.5 (1C, C–C, quinazoline to phenyl linkage), 162.5 (1C, C=N, benzothiazole), 154.4, 152.0 (2C, C–O–C, quinazoline to *m*-tolyloxy linkage), 150.2, 149.7, 149.1, 148.8, 146.7, 145.6, 144.4, 142.1, 141.8, 139.1, 138.7, 137.8, 136.3, 133.0, 132.3, 129.7, 128.5, 128.1, 127.6, 126.6, 125.5, 121.8, 118.9 (23C, Ar–C), 46.8 (1C, CH_2), 21.5 (1C, CH_3). MS, *m/z* 595.4 $[M+1]^+$. Anal calcd for $C_{30}H_{22}BrN_5O_2S$: C, 60.41; H, 3.72; N, 11.74 Found C, 60.26; H, 3.68; N, 11.87.

*N-(6-fluorobenzo[d]thiazol-2-yl)-2-((4-(4-(m-tolyloxy)quinazolin-2-yl)phenyl)amino)acetamide (**8d**)*

Light yellow solid, Yield 72 %, m.p. 271–273 °C. IR (KBr, cm^{-1}): 3,328 (–NHs), 2,995 (–CH), 1,668 (C=O), 1,180 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6): ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.29 (s, 1H, –CONH), 8.82 (s, 1H, –NH), 8.57 (dd, $J = 7.4$, 1.6 Hz, 1H, quinazoline), 8.12 (dd, $J = 6.8$, 1.5 Hz, 1H, quinazoline), 7.65–7.29 (m, 10H, Ar–H), 6.78–6.55 (m, 3H, Ar–H, *m*-tolyloxy ring), 3.21 (s, 2H, $–CH_2$), 2.19 (s, 3H, $–CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6): δ171.0 (1C, C=O), 164.8 (1C, C–C, quinazoline to phenyl linkage), 162.2 (1C, C=N, benzothiazole), 155.2, 152.8 (2C, C–O–C, quinazoline to *m*-tolyloxy linkage), 151.6, 149.6, 149.2, 148.8, 147.9, 146.1, 145.5, 145.0, 144.7, 142.2, 141.8, 141.3, 140.7, 140.0, 139.2, 138.4, 133.1, 131.3, 128.4, 127.6, 126.5, 125.2, 121.3 (23C, Ar–C), 46.2 (1C, CH_2), 21.4 (1C, CH_3). MS, *m/z* 535.0 $[M+1]^+$. Anal calcd for $C_{30}H_{22}FN_5O_2S$: C, 67.28; H, 4.14; N, 13.08 Found C, 67.39; H, 4.04; N, 13.21.

*N-(6-nitrobenzo[d]thiazol-2-yl)-2-((4-(4-(m-tolyloxy)quinazolin-2-yl)phenyl)amino)acetamide (**8e**)*

Light yellow solid, Yield 69 %, m.p. 279–280 °C. IR (KBr, cm^{-1}): 3,311 (–NH), 3,010 (–CH), 1,675 (C=O), 1,192 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.25 (s, 1H, –CONH), 8.68 (s, 1H, –NH), 8.45 (dd, $J = 7.2$, 1.3 Hz, 1H, quinazoline), 8.05 (dd, $J = 7.2$, 1.3 Hz, 1H, quinazoline), 7.70–7.21 (m, 10H, Ar–H), 6.72–6.51 (m, 3H, Ar–H, *m*-tolyloxy ring), 3.35 (s, 2H, $–CH_2$), 2.23 (s, 3H, $–CH_3$). ^{13}C NMR (100 MHz, DMSO-d_6): δ171.3 (1C, C=O), 165.5 (1C, C–C, quinazoline to phenyl linkage), 161.9 (1C, C=N, benzothiazole), 156.0, 152.7 (2C, C–O–C, quinazoline to *m*-tolyloxy linkage), 150.4, 149.6, 148.9, 146.6, 145.2, 144.7, 144.0, 142.5, 140.2,

138.7, 133.6, 131.8, 129.5, 128.4, 127.6, 126.8, 125.3, 124.2, 123.1, 121.8, 121.0, 120.3, 119.2 (23C, Ar–C), 47.0 (1C, CH_2), 21.9 (1C, CH_3). MS, *m/z* 561.9 $[M+1]^+$. Anal calcd for $C_{30}H_{22}N_6O_4S$: C, 64.05; H, 3.94; N, 14.94 Found C, 64.13; H, 4.15; N, 15.08.

*N-(6-cyanobenzo[d]thiazol-2-yl)-2-((4-(4-(m-tolyloxy)quinazolin-2-yl)phenyl)amino)acetamide (**8f**)*

Light yellow solid, Yield 71 %, m.p. 277–278 °C. IR (KBr, cm^{-1}): 3,286 (–NH), 2,980 (–CH), 2,218 (-CN), 1,670 (C=O), 1,160 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.42 (s, 1H, –CONH), 8.91 (s, 1H, –NH), 8.48 (dd, $J = 7.5$, 1.2 Hz, 1H, quinazoline), 8.11 (dd, $J = 6.9$, 1.4 Hz, 1H, quinazoline), 7.63–7.27 (m, 10H, Ar–H), 6.82-6.57 (m, 3H, Ar–H, *m*-tolyloxy ring), 3.39 (s, 2H, –CH_2), 2.35 (s, 3H, –CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ172.1 (1C, C=O), 166.4 (1C, C–C, quinazoline to phenyl linkage), 162.0 (1C, C=N, benzothiazole), 155.3, 151.7 (2C, C–O–C, quinazoline to *m*-tolyloxy linkage), 149.7, 148.9, 148.1, 147.2, 146.7, 144.9, 142.2, 141.3, 140.8, 140.06, 137.7, 132.1, 131.4, 130.0, 129.4, 128.3, 127.6, 126.2, 125.3, 124.1, 122.6, 121.8, 120.4 (23C, Ar–C), 107.6 (1C, –C≡N) 46.3 (1C, CH_2), 21.5 (1C, CH_3). MS, *m/z* 541.7 $[M+1]^+$. Anal calcd for $C_{31}H_{22}N_6O_2S$: C, 68.62; H, 4.09; N, 15.49 Found C, 68.73; H, 3.88; N, 15.30.

*N-(6-methylbenzo[d]thiazol-2-yl)-2-((4-(4-(m-tolyloxy)quinazolin-2-yl)phenyl)amino)acetamide (**8g**)*

Light yellow solid, Yield 75 %, m.p. 272–273 °C. IR (KBr, cm^{-1}): 3,292 (–NH), 3,012 (–CH), 1,665 (C=O), 1,220 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.23 (s, 1H, –CONH), 8.59 (s, 1H, –NH), 8.44 (dd, $J = 7.6$, 1.3 Hz, 1H, quinazoline), 8.03 (dd, $J = 7.1$, 1.1 Hz, 1H, quinazoline), 7.68–7.32 (m, 10H, Ar–H), 6.77–6.49 (m, 3H, Ar–H, *m*-tolyloxy ring), 3.25 (s, 2H, –CH_2), 2.32 (s, 6H, –CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ171.5 (1C, C=O), 164.2 (1C, C–C, quinazoline to phenyl linkage), 159.8 (1C, C=N, benzothiazole), 155.6, 152.2 (2C, C–O–C, quinazoline to *m*-tolyloxy linkage), 150.5, 148.8, 147.5, 146.2, 146.3, 144.7, 144.1, 143.4, 142.6, 141.2, 140.8, 139.0, 134.6, 132.7, 131.1, 129.2, 127.0, 127.5, 126.2, 125.4, 123.6, 121.0, 118.8 (23C, Ar–C), 46.7 (1C, CH_2), 21.1 (2C, CH_3). MS, *m/z* 531.3 $[M+1]^+$. Anal calcd for $C_{31}H_{25}N_5O_2S$: C, 70.04; H, 4.74; N, 13.17 Found C, 69.81; H, 4.58; N, 13.24.

*N-(6-methoxybenzo[d]thiazol-2-yl)-2-((4-(4-(m-tolyloxy)quinazolin-2-yl)phenyl)amino)acetamide (**8h**)*

Light yellow solid, Yield 70 %, m.p. 265–267 °C. IR (KBr, cm^{-1}): 3,315 (–NH), 3,015 (–CH), 1,680 (C=O), 1,205 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.35 (s, 1H, –CONH), 9.02 (s, 1H, –NH), 8.51 (dd, $J = 7.2$, 1.3 Hz, 1H, quinazoline), 7.98 (dd, $J = 6.8$, 1.6 Hz, 1H, quinazoline), 7.64–7.25 (m, 10H, Ar–H), 6.73–6.52 (m, 3H, Ar–H, *m*-tolyloxy ring), 3.84 (s, 3H, –OCH_3), 3.22 (s, 2H, –CH_2), 2.25 (s, 3H, –CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ170.4 (1C, C=O), 164.2 (1C, C–C,

quinazoline to phenyl linkage), 162.7 (1C, C=N, benzothiazole), 156.0, 153.9 (2C, C–O–C, quinazoline to *m*-tolyloxy linkage), 150.3, 150.1, 149.6, 148.9, 147.6, 146.7, 144.1, 143.5, 142.8, 142.0, 140.1, 138.7, 137.9, 137.1, 136.3, 134.7, 133.1, 132.4, 129.0, 128.4, 122.8, 121.2, 120.3 (23C, Ar–C), 55.2 (1C, –O$\underline{C}$H$_3$), 46.0 (1C, –CH$_2$), 21.4 (1C, –CH$_3$). MS, *m/z* 547.4 $[M+1]^+$. Anal calcd for $C_{31}H_{25}N_5O_3S$: C, 67.99; H, 4.60; N, 12.79 Found C, 68.12; H, 4.49; N, 12.63.

*N-(6-ethoxybenzo[d]thiazol-2-yl)-2-((4-(4-(m-tolyloxy)quinazolin-2-yl)phenyl)amino)acetamide (**8i**)*

Light yellow solid, Yield 80 %, m.p. 282–283 °C. IR (KBr, cm^{-1}): 3,282 (–NH), 2,980 (–CH), 1,670 (C=O), 1,195 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.26 (s, 1H, –CON$\underline{H}$), 8.85 (s, 1H, –NH), 8.67 (dd, J = 7.2, 1.8 Hz, 1H, quinazoline), 8.09 (dd, J = 6.6, 1.2 Hz, 1H, quinazoline), 7.74–7.27 (m, 10H, Ar–H), 6.81–6.63 (m, 3H, Ar–H, *m*-tolyloxy ring), 4.02 (q, J = 5.9 Hz, 2H, –OC$\underline{H}_2$CH$_3$) 3.29 (s, 2H, –CH$_2$), 2.21 (s, 3H, –CH$_3$), 1.99 (t, J = 6.4 Hz, 3H, –OCH$_2$C$\underline{H}_3$). ^{13}C NMR (100 MHz, DMSO-d_6): δ171.6 (1C, C=O), 164.9 (1C, C–C, quinazoline to phenyl linkage), 161.4 (1C, C=N, benzothiazole), 155.7, 153.0 (2C, C–O–C, quinazoline to *m*-tolyloxy linkage), 151.1, 150.7, 149.5, 148.3, 147.5, 146.9, 145.7, 144.1, 143.8, 142.2, 141.6, 140.3, 139.6, 138.3, 133.1, 132.2, 128.4, 127.3, 126.7, 124.5, 121.3, 120.1, 119.5 (23C, Ar–C), 64.3 (1C, –O$\underline{C}$H$_2$CH$_3$), 46.9 (1C, –CH$_2$), 21.6 (1C, –CH$_3$), 14.4 (1C, –OCH$_2$C$\underline{H}_3$). MS, *m/z* 560.6 $[M+1]^+$. Anal calcd for $C_{32}H_{27}N_5O_3S$: C, 68.43; H, 4.85; N, 12.47 Found 68.52; H, 4.89; N, 12.38.

*N-(6-acetamidobenzo[d]thiazol-2-yl)-2-((4-(4-(m-tolyloxy)quinazolin-2-yl)phenyl)amino)acetamide (**8j**)*

Light yellow solid, Yield 72 %, m.p. 289–291 °C. IR (KBr, cm^{-1}): 3,320 (–NH), 2,990 (–CH), 1,675 (C=O), 1,225 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.46 (s, 1H, –CON$\underline{H}$), 9.10 (s, 1H, –N$\underline{H}$COCH$_3$), 8.63 (s, 1H, –NH), 8.42 (dd, J = 7.7, 1.4 Hz, 1H, quinazoline), 8.07 (dd, J = 6.4, 1.3 Hz, 1H, quinazoline), 7.69–7.33 (m, 10H, Ar–H), 6.85–6.61 (m, 3H, Ar–H, *m*-tolyloxy ring), 3.31 (s, 2H, –CH$_2$), 2.32 (s, 3H, –CH$_3$), 2.06 (s, 3H, –NHCOC$\underline{H}_3$). ^{13}C NMR (100 MHz, DMSO-d_6): δ171.3 (1C, –C=O), δ168.5 (1C, –NH–$\underline{C}$O–CH$_3$), 165.0 (1C, C–C, quinazoline to phenyl linkage), 162.8 (1C, C=N, benzothiazole), 154.4, 152.5 (2C, C–O-C, quinazoline to *m*-tolyloxy linkage), 150.9, 149.7, 148.1, 147.9, 146.3, 145.1, 144.6, 143.2, 141.5, 140.3, 139.1, 138.6, 137.4, 135.5, 132.8, 131.2, 129.1, 128.6, 127.2, 126.1, 125.5, 121.8, 119.7 (23C, Ar–C), 46.2 (1C, -CH$_2$), 21.5 (2C, –CH$_3$). MS, *m/z* 574 $[M+1]^+$. Anal calcd for $C_{32}H_{26}N_6O_3S$: C, 66.88; H, 4.56; N, 14.62 Found 66.73; H, 4.42; N, 14.87.

Methods for in vitro evaluation of antimycobacterial activity

BACTEC MGIT method

The mycobacteria growth indicator tubes (MGIT) containing 4 mL of modified Middle brook 7H9 Broth Base were numbered as per the title compounds to be

tested for antituberculosis efficacy by means of various prepared concentrations. The suspension was allowed to sit for 20 min and the tubes were centrifuged at 3,000 rpm for 15 min. After that, 10^4–10^7 CFU/mL of prepared *M. tuberculosis* H37RV strain suspension was added in the medium to be incubated and 0.1 mL of egg-based medium was also added. The MGIT tubes were then tightly recapped, mixed well, and incubated into a BACTEC MGIT instrument at 37 °C until positivity was observed. The readings were measured daily starting from the second day of incubation. Positive cultures were usually detected within 10 days. For reading the actual results, the MGIT tubes were removed from the incubator and placed on the UV light next to a positive control tube and an uninoculated tube. Bright fluorescence detected by the corresponding MGIT tube was noticed in the form of bright orange color in the bottom of the tube and also an orange reflection on the meniscus [36]. The primary screening was conducted at concentration of 6.25 μg/mL against *M. tuberculosis* H37Rv in a BACTEC MGIT system. Compounds demonstrating 99 % inhibition in the primary screen were described as the most potent. All the other compounds to be tested were re-examined for their actual MIC by adopting conventional the L.J. agar dilution method. The MIC was defined as the lowest concentration inhibiting 99 % of the inoculum.

Lowenstein and Jensen method

The secondary antimycobacterial screening for test compounds was obtained for *M. tuberculosis* H37Rv, by adopting the L.J. (Lowenstein and Jensen) agar dilution method [37] for the measurement of MIC, and is defined as the lowest concentration of drug, which inhibits ≥99 % of bacterial population present at the beginning of the assay. Stock solutions of 250, 200, 100, 62.5, 50, 25, 12.5, 6.25, 3.12, and 1.56 μg/mL dilutions of each test compound in DMSO (dimethylsulfoxide) were added in the liquid L.J. Medium and then the media were sterilized by the inspissation method. A culture of *M. tuberculosis* H37Rv growing on L. J. Medium was harvested in 0.85 % saline in bijou bottles. These tubes were then incubated at 37 °C for 24 h followed by streaking of *M. tuberculosis* H37Rv (5 × 104 bacilli per tube). These tubes were then incubated at 37 °C. Growth of bacilli was seen after 12, 22, and finally 28 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with *M. tuberculosis* H37Rv. The concentration at which no development of colonies occurred or <20 colonies was taken as the MIC concentration of the test compound. The standard strain *M. tuberculosis* H37Rv was tested with the known drugs, Isoniazid, Rifampicin, Ethambutol, and Pyrazinamide.

Methods for in vitro evaluation of anticancer activity

SRB assay

The cell lines were grown in RPMI 1640 medium containing 10 % fetal bovine serum and 2 mM L-glutamine. For the present screening experiment, cells were inoculated into 96-well microtiter plates, in 90 μL at plating densities, depending on

the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at (37 ± 1) °C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 h prior to the addition of the experimental drugs. After 24 h, one plate of each cell line was fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs were solubilized in an appropriate solvent at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to 10 times the desired final maximum test concentration, with the complete medium containing test article at a concentration of 10^{-3}. An additional three 10-fold serial dilutions were made to provide a total of four drug concentrations plus control. Aliquots of 10 μL of these different drug dilutions were added to the appropriate microtiter wells already containing 90 μL of medium, resulting in the required final drug concentrations [38].

Endpoint measurement

After compound addition, plates were incubated at standard conditions for 48 h and assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 μL of cold 30 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 min at 4 °C. The supernatant was discarded and the plates were washed five times with tap water and air-dried. Sulforhodamine B (SRB) solution (50 μL) at 0.4 % (w/v) in 1 % acetic acid was added to each of the wells, and plates were incubated for 20 min at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1 % acetic acid. The plates were air-dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on an Elisa plate reader at a wavelength of 540 nm with 690 nm as the reference wavelength. Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells ×100. Using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as: $[(Ti - Tz)/(C - Tz)] \times 100$ for concentrations for which $Ti \geq Tz$ and $[(Ti - Tz)/Tz] \times 100$ for concentrations for which $Ti < Tz$.

The dose response parameters were calculated for each test article. Growth inhibition of 50 % (GI_{50}) was calculated from $[(Ti - Tz)/(C - Tz)] \times 100 = 50$, which is the drug concentration resulting in a 50 % reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) was calculated from $Ti = Tz$. The LC_{50} (concentration of drug resulting in a 50 % reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from $[(Ti - Tz)/Tz] \times 100 = -50$. Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not

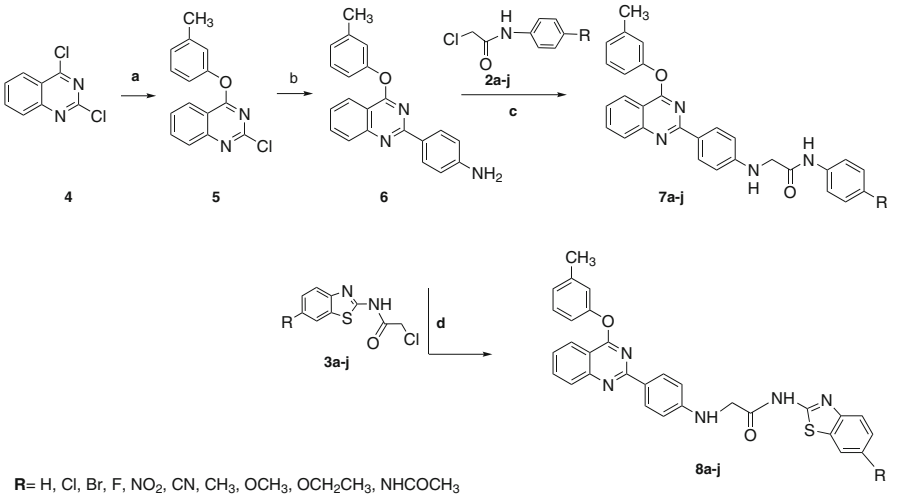
reached or was exceeded, the values for that parameter were expressed as greater or less than the maximum or minimum concentration tested [39].

Results and discussion

Chemistry

The synthetic route for the target analogs **7a–j/8a–j** is outlined in Scheme 1. The initial compound 2,4-dichloroquinazoline (**4**) was synthesized according to the reported literature [26–31]. Treatment of **4** with *m*-cresol in the presence of anhydrous K_2CO_3 at 80 °C in ethanol provided the ether intermediate **5**, which was further reacted with 4-aminophenylboronic acid pinacol ester in the presence of a catalytic (1.25 mol%) amount of tetrakis-(triphenylphosphine)palladium in basic media to obtain the intermediate **6**. Synthesis of analog **6** involved the Suzuki cross-coupling reaction to facilitate the C–C bond formation between the quinazoline ring and phenyl ring with aniline functionality. Finally, the intermediate **6** was reacted with different *N*-phenyl acetamides (**2a–j**) and *N*-benzothiazolyl acetamides (**3a–j**) at reflux temperature to furnish the final analogs **7a–j** and **8a–j**, respectively.

All the analogs (**7a–j/8a–j**) were characterized through IR, ^{1}H NMR, ^{13}C NMR, MS, and elemental analysis. The spectral data for the first analog **7a** is discussed below. Other derivatives showed similar spectral results indicating no apparent changes in their structural composition. The FT-IR spectrum of analog **7a** displayed absorption bands at 3,267 cm^{-1} for NH stretching, 2,919 cm^{-1} for aromatic CH



Scheme 1 Synthetic protocol for the analogs **7a–j/8a–j.** Reagents and conditions : *a* K_2CO_3, *m*–Cresol, EtOH, 80 °C; *b* 4–aminophenylboronic acid pinacol ester, $Pd(PPh_3)_4$, Na_2CO_3, DME, 90 °C; *c*, *d* K_2CO_3, acetone, reflux

Table 1 Results of in vitro antimycobacterial screening of the analogs **7a–j/8a–j**

Entry	R	BACTEC MGIT method[a]		L.J. MIC method[a]	
		MIC (μg/mL)	% Inhibition	MIC (μg/mL)	% Inhibition
7a	–H	>6.25	ND	250	95
7b	–Cl	>6.25	ND	100	98
7c	–Br	>6.25	ND	25	99
7d	–F	>6.25	ND	62.5	97
7e	$-NO_2$	>6.25	ND	200	95
7f	–CN	>6.25	ND	62.5	94
7g	$-CH_3$	>6.25	ND	100	96
7h	$-OCH_3$	>6.25	ND	62.5	99
7i	$-OCH_2CH_3$	>6.25	ND	100	97
7j	$-NHCOCH_3$	>6.25	ND	200	98
8a	–H	>6.25	ND	100	96
8b	–Cl	6.25	99	6.25	99
8c	–Br	6.25	99	3.12	99
8d	–F	>6.25	ND	12.5	98
8e	$-NO_2$	>6.25	ND	100	92
8f	–CN	>6.25	ND	62.5	96
8g	$-CH_3$	>6.25	ND	100	95
8h	$-OCH_3$	6.25	99	6.25	99
8i	$-OCH_2CH_3$	6.25	99	12.5	98
8j	$-NHCOCH_3$	>6.25	ND	100	94
Ethambutol		3.12	99		
Pyrazinamide		6.25	99		
Rifampicin		0.25	99		
Isoniazid		0.20	99		
DMSO		–	–		

Highest inhibition values in bold

ND not determined

[a] Each value is the mean of three independent experiments

stretching, 1,673 cm^{-1} for C=O stretching, and 1,252 cm^{-1} for the ether group. In the ^{1}H NMR spectrum, the peaks due to methyl protons and methylene protons displayed signals at 2.05 and 4.15 ppm, respectively. The protons corresponding to the quinazoline nucleus moiety resonated at the 8.06–8.31 ppm region, whereas the NH proton adjacent to the carbonyl carbon at 10.32 ppm. From the ^{13}C NMR spectrum of **7a**, a signal at 178.1 ppm indicates the presence of a carbonyl group adjacent to the NH group. The methylene carbon showed a signal at 43.3 ppm, while the methyl carbon displayed a signal at 29.0 ppm.

Table 2 Results of in vitro anticancer screening of analogs **7a–j/8a–j**

Entry	R	Anti PC3 cell line activity[a] (μg/mL)		
		LC_{50}^{b}	TGI[b]	GI_{50}^{b}
7a	–H	>80	>80	>80
7b	–Cl	>80	>80	55.6
7c	–Br	>80	>80	>80
7d	–F	>80	>80	>80
7e	$-NO_2$	>80	>80	55.6
7f	–CN	>80	>80	>80
7g	$-CH_3$	>80	>80	>80
7h	$-OCH_3$	>80	>80	45.6
7i	$-OCH_2CH_3$	>80	>80	>80
7j	$-NHCOCH_3$	>80	>80	>80
8a	–H	>80	>80	>80
8b	–Cl	>80	>80	>80
8c	–Br	>80	>80	78.4
8d	–F	>80	>80	>80
8e	$-NO_2$	>80	>80	53.9
8f	–CN	>80	>80	>80
8g	$-CH_3$	>80	>80	>80
8h	$-OCH_3$	>80	>80	>80
8i	$-OCH_2CH_3$	>80	>80	>80
8j	$-NHCOCH_3$	>80	>80	>80
ADR[c]		58.8	18.6	<10

[a] Each value is the mean of three independent experiments

[b] LC_{50} concentration of drug causing 50 % cell kill; *TGI* concentration of drug causing total inhibition of cell growth; GI_{50} concentration of drug causing 50 % inhibition of cell growth, with active group values in bold

[c] ADR = Adriamycin, positive control compound

Pharmacology

Antimycobacterial activity

The investigation of in vitro antimycobacterial screening (Table 1) revealed that only *N*-benzothiazolyl acetamide-fused quinazoline analogs showed moderate to good inhibition against *Mycobacterium tuberculosis* H37Rv in the range of 3.12–25 μg/mL. The results observed from BACTEC MGIT method indicated that analog **8b**, **8c**, **8h**, and **8i** exhibited highest inhibition (99 %) at a constant concentration level (6.25 μg/mL). These compounds were considered as the most potent analogs against mycobacteria and were found to indicate equivalent antituberculosis potency as that of the standard drug pyrazinamide. However, the results of secondary biological screening using the Lowenstein-Jensen MIC method

revealed that the bromo group-endowed analog **8c** showed the highest inhibition against *M. tuberculosis* H37Rv at 3.12 μg/mL MIC, equipotent to ethambutol. In addition, two analogs with the substitution of chloro (**8b**) and methoxy (**8h**) displayed 6.25 μg/mL of inhibition, which is equipotent to pyrazinamide. However, *N*-phenyl acetamide-fused quinazoline derivatives were found to exhibit moderate to poor activity at MIC ranging from 25 to 100 μg/mL.

Anticancer activity

The activity of new compounds against prostate cancer (PC3) cell proliferation reported in Table 2 suggest that analogs **7b**, **7e**, **7h**, **8c**, and **8e** were active in terms of GI_{50}. Interestingly, *N*-phenyl acetamide-fused quinazolines with the substitution of an electron-donating methoxy (**7h**) group exhibited highest activity at 45.6 μg/mL inhibition of cell growth. However, electron-withdrawing chloro (**7b**) and nitro (**7e**) groups-endowed similar derivatives displayed 55.6 μg/mL of GI_{50}. Moreover, *N*-benzothiazolyl acetamide-fused quinazolines with the substitution of electron-withdrawing bromo (**8c**) and nitro (**8e**) groups exhibited moderate activity at 78.4 and 53.9 μg/mL inhibition of cell growth, respectively. In addition, all the synthesized analogs were found to be non-toxic as they possessed LC_{50} values >80 μg/mL.

Conclusion

In summary, we developed an efficient synthetic route for the synthesis of *N*-phenyl/benzothiazolyl acetamide-fused quinazolines using the Suzuki coupling reaction. The bioassay results revealed that most of the benzothiazolyl-fused quinazoline analogs displayed an exceptional in vitro antimycobacterial activity (MIC, 3.12–25 μg/mL) against *M. tuberculosis* H37Rv. However, the *N*-phenyl acetamide derivatives exhibited noticeable cell growth inhibition against prostate cancer PC3 cells. Finally, these compounds represent new scaffolds that could be further optimized for future development of more potent and selective antimycobacterial/anticancer agents.

Acknowledgments The authors are thankful to Director, S.V. National Institute of Technology, Surat for the scholarship, encouragement and facilities. We wish to offer our deepest gratitude to Microcare Laboratory, Surat, India, and Tata Memorial Advanced Centre for Treatment, Research and Education in Cancer, Mumbai, India, for carrying out the biological screenings. We are also grateful to SAIF Panjab University and Centre of Excellence, Vapi, India, for spectral analysis.

References

1. H. Nikaido, Annu. Rev. Biochem. **78**, 119 (2009)
2. R.V. Patel, P. Kumari, D.P. Rajani, K.H. Chikhalia, Acta Pol. Pharm. **69**, 423 (2012)
3. WHO global tuberculosis report; 2012. http://apps.who.int/iris/bitstream/10665/75938/1/9789241564502_eng.pdf. Accessed 31 Nov 2013
4. J.B. Gibbs, Science **278**, 1969 (2000)

5. C. Unger, Drug Future **22**, 1337 (1997)
6. C. Ryu, Y.H. Kim, H.A. Im, J.Y. Kim, J.H. Yoon, A. Kim, Bioorg. Med. Chem. Lett. **22**, 500 (2012)
7. A. Kumar, P. Sharma, P. Kumari, B.L. Kalal, Bioorg. Med. Chem. Lett. **21**, 4353 (2011)
8. A.M. Alafeefy, A.A. Kadi, O.A. Al-Deeb, K.E.H. El-Tahir, N.A. Al-Jabber, Eur. J. Med. Chem. **45**, 4947 (2010)
9. S. Yan, Y. Dong, Q. Peng, Y. Fan, J. Zhang, J. Lin, RSC Adv. **3**, 5563 (2013)
10. N.M. Raghavendra, P. Thampi, P.M. Gurubasavarajaswamy, D. Sriram, Arch. Pharm. **340**, 635 (2007)
11. D. Li, P. Zhan, H. Liu, C. Pannecouque, J. Balzarini, E. De Clercq, X. Liu, Bioorg. Med. Chem. **21**, 2128 (2013)
12. B.S. Kuarm, Y.T. Reddy, J.V. Madhav, P.A. Crooks, B. Rajitha, Bioorg. Med. Chem. Lett. **21**, 524 (2011)
13. S.S. Ibrahim, A.M. Abdel–Halim, Y. Gabr, S. El–Edfawy, R.M. Abdel–Rahman, J. Chem. Res. (S). 154 (1997). doi:10.1039/A600184J
14. V. Alagarsamy, D. Shankar, M. Murugan, A.A. Siddiqui, R. Rajesh, Arch. Pharm. **340**, 41 (2007)
15. U. Kabra, C. Chopde, S. Wadodkar, J. Heterocycl. Chem. **48**, 1351 (2011)
16. N. Siddiqui, A. Rana, S.A. Khan, M.A. Bhat, S.E. Haque, Bioorg. Med. Chem. Lett. **17**, 4178 (2007)
17. S. Shafi, M.M. Alam, N. Mulakayala, C. Mulakayala, G. Vanaja, A.M. Kalle, R. Pallu, M.S. Alam, Eur. J. Med. Chem. **49**, 324 (2012)
18. S.E. O'Brien, H.L. Browne, T.D. Bradshaw, A.D. Westwell, M.F.G. Stevens, C.A. Laughton, Org. Biomol. Chem. **1**, 493 (2003)
19. Hutchinson, S.A. Jennings, B.R. Vishnuvajjala, A.D. Westwell, M.F.G. Stevens, J. Med. Chem. **45**, 744 (2002)
20. G. Turan-Zitouni, Y. Ozkay, Z.A. Kaplancikli, M.D. Altintop, Lett. Drug. Des. Discov. **8**, 830 (2011)
21. V.N. Telvekar, V.K. Bairwa, K. Satardekar, A. Bellubi, Bioorg. Med. Chem. Lett. **22**, 649 (2012)
22. P. Bandyopadhyay, M. Sathe, S. Ponmariappan, A. Sharma, P. Sharma, A.K. Srivastava, M.P. Kaushik, Bioorg. Med. Chem. Lett. **21**, 7306 (2011)
23. H. Bujdakova, M. Muckova, Int. J. Antimicrob. Ag. **4**, 303 (1994)
24. D. Bhavsar, J. Trivedi, S. Parekh, M. Savant, S. Thakrar, A. Bavishi, A. Radadiya, H. Vala, J. Lunagariya, M. Parmar, L. Paresh, R. Loddo, A. Shah, Bioorg. Med. Chem. Lett. **21**, 3443 (2011)
25. K. Kanuma, K. Omodera, M. Nishiguchi, T. Funakoshi, S. Chaki, Y. Nagase, I. Iida, J. Yamaguchi, G. Semple, T. Tran, Y. Sekiguchi, Bioorg. Med. Chem. **14**, 3307 (2006)
26. R.M. Kumbhare, T. Dadmal, U. Kosurkar, V. Sridhar, J.V. Rao, Bioorg. Med. Chem. Lett. **22**, 453 (2012)
27. S. Kumar, N. Shakya, S. Gupta, J. Sarkar, D.P. Sahu, Bioorg. Med. Chem. Lett. **19**, 2542 (2009)
28. V.J.R. Farhanullah, B.K. Tripathi, A.K. Srivastava, Bioorg. Med. Chem. **11**, 2439 (2003)
29. Z. Zeng, Q. He, Y. Liang, X. Feng, F. Chen, E. De Clercq, J. Balzarini, C. Pannecouque, Bioorg. Med. Chem. **18**, 5039 (2010)
30. I. Pogorelic, M. Filipan-Litvic, S. Merkas, G. Ljubic, I. Cepanec, M.J. Litvic, Mol. Catal. A Chem. **274**, 202 (2007)
31. L. Zhu, J. Jin, C. Liu, C. Zhang, Y. Sun, Y. Guo, D. Fu, X. Chen, B. Xu, Bioorg. Med. Chem. **19**, 2797 (2011)
32. H. Wang, C. Wei, X. Deng, F. Li, Z. Quan, Arch. Pharm. **342**, 671 (2009)
33. A.B. Patel, R.V. Patel, P. Kumari, D.P. Rajani, K.H. Chikhalia, Med. Chem. Res. **22**, 367 (2013)
34. T. Akhtar, S. Hameed, N.A. Al-Masoudi, R. Loddo, P.L. Colla, Acta Pharm. **58**, 135 (2008)
35. A.B. Patel, K.H. Chikhalia, P. Kumari, Res. Chem. Intermed. **21**, 3119 (2013). doi:10.1007/s11164-013-1377-8
36. H.D. Isenberg, *Clinical Microbiology Procedures Handbook* (American Society for Microbiology, Washington DC, 1992)
37. P. Anargyros, D.S. Astill, I.S. Lim, J. Clin. Microbiol. **28**, 1288 (1990)
38. V. Vanicha, K. Kanyawim, Nat. Protoc. **1**, 1112 (2006)
39. R.V. Patel, P. Kumari, D.P. Rajani, K.H. Chikhalia, Eur. J. Med. Chem. **46**, 4354 (2011)