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Original article

Identification of benzofuro[2,3-*b*]quinoline derivatives as a new class of antituberculosis agents

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ABSTRACT

A series of 11-alkoxylated and 11-aminated benzofuro[2,3-*b*]quinoline derivatives were designed, synthesized, and evaluated for their anti-TB and cytotoxic activities. The known 11-chlorobenzofuro[2,3-*b*]quinoline (**3**) was synthesized in a single step from anthranilic acid and 2-coumaranone in phosphorus oxychloride in 51% yield for the first time. Treatment of **3** with alcohols and amines gave 11-alkoxylated and 11-aminated benzofuro[2,3-*b*]quinoline derivatives respectively, which were evaluated for their anti-TB and cytotoxic activities. Our results indicated that 11-arylaminated derivatives were more active than their respective 11-aryloxylated isosteric isomers against *Mycobacterium tuberculosis*. Among the tested compounds, 11-methoxybenzofuro[2,3-*b*]quinoline (**4**), 11-methylamino- benzofuro[2,3-*b*]quinoline (**9**), and 11-dimethylaminobenzofuro[2,3-*b*]quinoline (**14**) exhibited significant activities against the growth of *M. tuberculosis* (MIC values of <0.20 μg/mL) and low cytotoxicities against VERO cell with IC_{50} values of 11.77, 5.55, and >30.00 μg/mL respectively. The selectivity index ($SI = IC_{50}/MIC$) for **4**, **9**, and **14** was greater than 58.85, 27.75, and 150 respectively.

1. Introduction

The first-line drugs currently used for the treatment of tuberculosis (TB) infection are streptomycin, isoniazid, ethambutol, pyrazinamide, and rifampicin [1]. However, the current TB treatment regimens, although highly effective, are far from ideal. Recently, the emergence of multi-drug resistant (MDR) strains and the global human immunodeficiency virus (HIV) pandemic have amplified the incidence of TB and have created an urgent need for alternative drug treatments for *M. tuberculosis* infection [2–7]. Over the past few years, we have been particularly interested in the synthesis of fluoroquinolones for anti-TB and anticancer evaluations [8–11]. We have also synthesized certain polycyclic heterocycles such as indolo[2,3-*b*]quinoline for anticancer evaluation on the premise that these tetracyclic heterocycles can bind strongly to double helical DNA by intercalation and cause inhibition of DNA replication and transcription [12,13]. The indoloquinoline skeleton is present in a number of natural alkaloids with broad biological effects including antibacterial, antihyperglycemic, antiinflammatory, and antimalarial activities [14–18]. Currently there is considerable interest in the synthesis of indoloquinoline derivatives, because of their potential biological activities [19–27]. However, the isomeric benzofuroquinoline derivatives have attracted only limited attention [28–32]. In continuation of our efforts to identify potential anti-TB agents with novel types of structures which are distinct from those of existing drugs, we herein describe the synthesis of certain alkoxy, aryloxy, alkylamino, or arylamino substituted benzofuro[2,3-*b*]quinoline derivatives for anti-TB evaluations. Recently, three quinoline alkaloids were isolated from the leaves of *Lunasia amara* Blanco (Rutaceae) which exhibited good inhibitory activities against *M. tuberculosis* H37Rv. One of the common structural features among these alkaloids is the presence of a 4-methoxy group [33]. We have also reported certain biologically active furo[2,3-*b*]quinoline and indolo[2,3-*b*]quinoline derivatives which possess alkoxy, aryloxy, alkylamino, or arylamino group [12,34].

Since the structures of these newly synthesized benzofuro[2,3-*b*]quinoline derivatives in which the 2-phenyl group and the bicyclic quinoline are locked by an oxygen bridge to form a coplanar tetracyclic structure [35], belong to potential DNA intercalators, their cytotoxicities against a 3-cell line panel consisting of MCF7 (Breast), NCI-H460 (Lung), and SF-268 (CNS) were also evaluated. The aim of our current study is to identify novel skeletons that confer anti-TB activities with low cytotoxicities.

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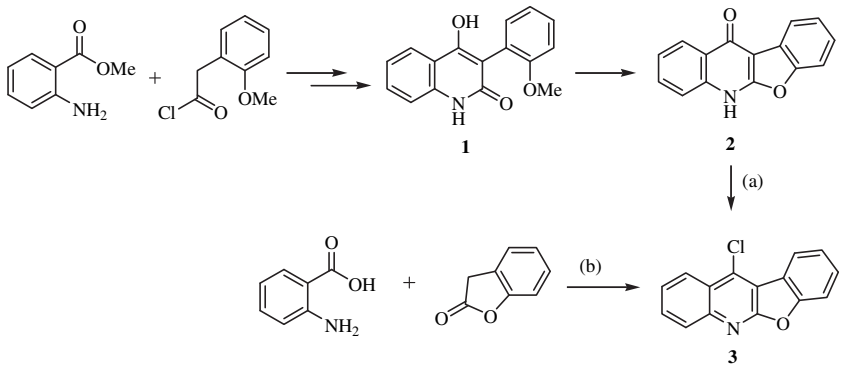


Scheme 1. Reactions and conditions (a) Procedures of Kawase, et. al. [29,30]; (b) $POCl_3$, reflux, 24 h.

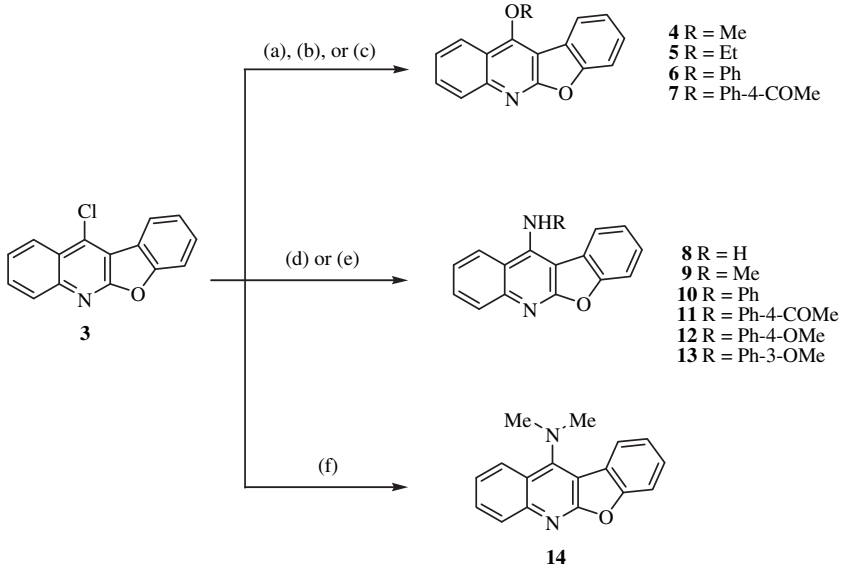
2. Chemistry

The known 11-chlorobenzofuro[2,3-*b*]quinoline (**3**) was selected as the starting material. According to the reported procedures of Kawase, et. al. [29,30], 4-hydroxy-3-(2-methoxyphenyl)-1,2-dihydroquinolin- 2-one (**1**), prepared from methyl anthranilate and 2-methoxyphenylacetyl chloride, was treated with pyridine hydrochloride to furnish a mixture of the linear benzofuro[2,3-*b*]quinolin-11-one (**2**) and its angular isomer. Purification of **2** followed by the chlorination with phosphorus pentachloride afforded desired **3** in an overall yield of 32%. Therefore, we decided to explore an alternative synthetic pathway for the preparation of **3** with a better overall yield. A survey of literature revealed that 10-chloro-11*H*-indeno[1,2-*b*]quinoline had been prepared from anthranilic acid and 1-indanone in phosphorous oxychloride in a single step [36]. Under similar reaction conditions, we were able to obtain **3** in a yield of 51% by refluxing anthranilic acid and 2-coumaranone in phosphorus oxychloride as outlined in Scheme 1.

By the treatment with various alcohols, compound **3** was converted into 11-alkoxylated benzofuro[2,3-*b*]quinoline derivatives **4**–**7** as described in Scheme 2. Although the preparation of 11-methoxybenzofuro[2,3-*b*]quinoline (**4**) and 11-ethoxybenzofuro [2,3-*b*]quinoline (**5**) had been previously described [30], biological activities especially anticancer and anti-infectious evaluations of these benzofuro[2,3-*b*]quinoline derivatives had not been explored. Conversion of **3** into 11-aminated benzofuro[2,3-*b*]quinoline derivatives **8–14** was carried out and their structures have been characterized by ESIMS, HRMSESI, elemental analysis, and NMR spectra.

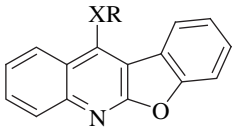
3. Results and discussion

The anti-TB activities of 11-substituted benzofuro[2,3-*b*]quinoline derivatives are summarized in Table 1. For comparison purposes, rifampin was used as the positive drug standard. 11-Methoxybenzofuro[2,3-*b*]quinoline (**4**) was found to be very potent in being able to inhibit 99% growth of *M. tuberculosis* at a concentration of <0.20 μg/mL. For the 11-alkoxy substituted derivatives, the less bulky methoxy derivative **4** was more active than its ethoxy counterpart **5** (MIC = 1.44 μg/mL) which in turn was more active than the most bulky phenoxy congener **6** (MIC > 100 μg/mL). The same order was observed for the 11-aminated derivatives in which the less bulky methylamino derivative **9** (MIC < 0.20 μg/mL) was a more potent anti-TB agent than its phenylamino counterpart **10** (MIC = 5.35 μg/mL). Introduction of a 4-methoxy substituent on the 11-phenylamino moiety was found to enhance anti-TB activity (**10**,



Scheme 2. Reactions and conditions (a) RONa, ROH, reflux for 3 h (for **4** and **5**); (b) PhOH, K_2CO_3, 100 °C, 2 h (for **6**); (c) 4-hydroxyacetophenone, K_2CO_3 in DMF, 100 °C, 6 h (for **7**); (d) RNH_2 (aq) in sealed vessel, 70 °C, 24 h (for **8** and **9**); (e) substituted aniline in ethoxyethanol, reflux, 24 h (for **10**–**13**); (f) 40% Me_2NH (aq) in sealed vessel, 70 °C, 24 h.

Table 1
Anti-*M. tuberculosis* H37Rv (ATCC 27294) activities of 11-substituted benzofuro[2,3-*b*]quinoline derivatives.



Compd	X	R	MIC (μg/mL)	IC_{50} (μg/mL)	SI (IC_{50}/MIC)
4	O	Me	<0.20	11.77	>58.85
5	O	Et	1.44	10.34	7.18
6	O	Ph	>100	ND[a]	ND
7	O	Ph-4-COMe	>100	ND	ND
8	NH	H	>100	ND	ND
9	NH	Me	<0.20	5.55	>27.75
10	NH	Ph	5.35	6.14	1.15
11	NH	Ph-4-COMe	14.57	ND	ND
12	NH	Ph-4-OMe	2.63	3.63	1.38
13	NH	Ph-3-OMe	17.83	ND	ND
14	N	$(Me)_2$	<0.20	>30.00	>150
Rifampin			0.125–0.25	100	400

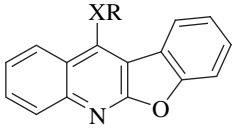
[a] Not determined.

5.35 *v.s.* **12**, 2.63) while introduction of a 4-methylcarbonyl or 3-methoxy substituent (**11**, 14.57 and **13**, 17.83 respectively) was found to decrease anti-TB activity. 11-Aminobenzofuro[2,3-*b*]quinoline (**8**), a primary amine derivative, was devoid of anti-TB activity while 11-dimethylaminobenzofuro[2,3-*b*]quinoline (**14**), a tertiary amine derivative, inhibited 99% growth of *M. tuberculosis* at a concentration of <0.20 μg/mL. In general, 1-arylaminated derivatives were more active than their respective 11-aryloxylated isosteric isomers (**6** *v.s.* **10**; **7** *v.s.* **11**) against *M. tuberculosis*. The anti-TB activities of compounds **4**, **9**, and **14** were comparable to the positive rifampin.

The cytotoxicity (IC_{50}) against mammalian VERO cells as well as the selectivity index (SI), defined as IC_{50}/MIC, for compounds **4**, **5**, **9**, **10**, **12**, and **14** were also determined. Compounds with an SI of >10 are of interest for further development as potential anti-TB agents. Results indicated that compounds **4**, **9**, and **14** exhibited significant activities against the growth of *M. tuberculosis* with MIC values of <0.20 μg/mL respectively and low cytotoxicities against VERO cell with IC_{50} values of 11.77, 5.55, and >30.00 μg/mL respectively. The selectivity index (SI) values for **4**, **9**, and **14** were greater than 58.85, 27.75, and 150 respectively.

All compounds were evaluated *in vitro* against a 3-cell line panel consisting of MCF7 (Breast), NCI-H460 (Lung), and SF-268 (CNS). In this protocol, each cell line was inoculated and preincubated on a microtiter plate. Test agents were then added at concentrations of 20.0 and 4.0 mg/mL respectively and the culture incubated for 48 h. End-point determinations were made with sulforhodamine B, a protein-binding dye. Results for each test agent are reported as the percent of growth of the treated cells against the untreated control cells. Results from Table 2 indicated that all the compounds are not cytotoxic at a concentration of 4.0 mg/mL.

Table 2
Cytotoxicity of 11-substituted benzofuro[2,3-*b*]quinoline derivatives.



Compd	X	R	MCF7 (Breast cancer)		NCI-H460 (Lung cancer)		SF-268 (CNS)	
			20 μg/mL	4 μg/mL	20 μg/mL	4 μg/mL	20 μg/mL	4 μg/mL
4	O	Me	92	102	68	90	98	98
5	O	Et	77	99	84	92	90	86
6	O	Ph	64	94	91	99	108	108
7	O	Ph-4-COMe	88	100	62	104	100	104
8	NH	H	113	114	107	110	72	84
9	NH	Me	99	100	99	102	86	98
10	NH	Ph	44	118	53	104	76	118
11	NH	Ph-4-COMe	49	106	20	64	63	109
12	NH	Ph-4-OMe	35	109	17	83	87	111
13	NH	Ph-3-OMe	84	107	46	99	60	97
14	N	$(Me)_2$	48	87	76	108	91	102

4. Conclusion

We have identified certain benzofuro[2,3-*b*]quinoline derivatives as potential anti-TB agents with low cytotoxicies. Among them, 11-dimethylaminobenzofuro[2,3-*b*]quinoline (**14**) exhibited the most potent anti-TB activity with MIC value of < 0.20 μg/mL and very low cytotoxicity against VERO cell with IC_{50} value of >30.00 μg/mL. The selectivity index (SI) for **14** was greater than 150. Further evaluation of **14** as an anti-TB drug candidate is on-going.

5. Experimental

5.1. General

Melting points were determined on a Electrothermal IA9100 melting point apparatus and are uncorrected. Nuclear magnetic resonance (^1H and ^{13}C) spectra were recorded on a Varian Gemini 200 spectrometer or Varian-Unity-400 spectrometer. Chemical shifts were expressed in parts per million (δ) with tetramethylsilane (TMS) as an internal standard. Thin-layer chromatography was performed on silica gel 60 F-254 plates purchased from E. Merck and Co. The elemental analyses were performed in the Instrument Center of National Science Council at National Cheng-Kung University and National Chung-Hsing University using Heraeus CHN-O Rapid EA, and all values are within ±0.4% of the theoretical compositions.

5.1.1. 11-Chlorobenzofuro[2,3-b]quinoline (*3*)

A mixture of anthranilic acid (1.37 g, 10.0 mmol) and 2-coumaranone (1.34 g, 10.0 mmol) in phosphorus oxychloride (60 mL) was refluxed for 24 h (TLC monitoring). After cooling, the mixture was poured into ice-water (50 mL) and neutralized with saturated $NaHCO_3$ until pH 7 resulted. The brown precipitate thus obtained was collected, purified by column chromatography (CH_2Cl_2), and crystallized from EtOH to give **3** (1.29 g, 51%). Mp: 208–210 (lit: 211–212 °C) [30]. ^1H NMR (400 MHz, DMSO-d_6): 7.53–7.57 (m, 1H, 9-H), 7.71–7.83 (m, 3H, 2-, 7-, 8-H), 7.90–7.94 (m, 1H, 3-H), 8.12 (d, 1H, $J = 8.4$ Hz, 10-H), 8.36–8.41 (m, 2H, 1-, 4-H). ^{13}C NMR (100 MHz, DMSO-d_6): 93.87, 111.88, 115.18, 120.68, 123.48, 123.50, 124.16, 126.47, 128.29, 130.45, 130.81, 145.34, 154.90, 159.11, 161.06. ESIMS (m/z): 254 $[M + H]^+$. HRMS (ESI): m/z calcd for $C_{15}H_9ClNO$ $[M + H]^+$ 254.0373, found 254.0372. Anal. calcd for $C_{15}H_8ClNO$: C 71.02, H 3.18, N 5.52; found: C 71.03, H 3.24, N 5.41.

5.1.2. 11-Methoxybenzofuro[2,3-b]quinoline (*4*)

A slurry of **3** (0.25 g, 1.0 mmol) in methanol (100 mL) was added to the stirred solution of sodium methoxide in methanol (prepared by dissolving 120 mg of freshly cut sodium in 12 mL of methanol under argon). The reaction mixture was refluxed for 3 h

(TLC monitoring). The solvent was removed *in vacuo*, and the residue was poured into H_2O (50 mL) and extracted with CH_2Cl_2 (50 mL × 3). The organic layer was washed with brine, dried over $MgSO_4$ and evaporated *in vacuo*. The resulting precipitate was purified by column chromatography (MeOH:CH_2Cl_2 1/50) and crystallized from EtOH to give **4** (0.15 g, 60%). Mp: 152–153 °C (lit: 165–165.5 °C) [30]. ^{1}H NMR (400 MHz, $CDCl_3$): 4.32 (s, 3H, OMe), 7.41–7.45 (m, 1H, 9-H), 7.53–7.64 (m, 3H, 2-, 7-, 8-H), 7.75–7.80 (m, 1H, 3-H), 8.10 (dd, 1H, J = 1.6, 8.0 Hz, 10-H), 8.14 (d, 1H, J = 8.4 Hz, 4-H), 8.32 (dd, 1H, J = 0.8, 8.4 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 61.96, 111.81, 113.87, 119.14, 120.95, 122.25, 123.57, 123.63, 124.66, 128.64, 129.20, 130.14, 147.34, 154.88, 159.54, 164.13. ESIMS (m/z): 250 $[M+H]^+$. HRMS (ESI): m/z calcd for $C_{16}H_{12}NO_2$ $[M+H]^+$ 250.0868, found 250.0869. Anal. calcd for $C_{16}H_{11}NO_2 \cdot 0.5H_2O$: C 74.40, H 4.69, N 5.42; found: C 74.41, H 4.83, N 5.06.

5.1.3. 11-Ethoxybenzofuro[2,3-b]quinoline (**5**)

Prepared from **3** and sodium ethoxide in ethanol by the same procedure as described for the preparation of **4**. Yield: 62%. Mp: 80–82 °C (lit: 72–73 °C) [30]. ^{1}H NMR (400 MHz, $CDCl_3$): 1.63 (t, 3H, J = 7.2 Hz, CH_3), 4.54 (q, 2H, J = 7.2 Hz, OCH_2), 7.39–7.43 (m, 1H, 9-H), 7.52–7.58 (m, 2H, 2-, 8-H), 7.61–7.63 (m, 1H, 7-H), 7.74–7.79 (m, 1H, 3-H), 8.06–8.08 (m, 1H, 10-H), 8.13 (d, 1H, J = 8.4 Hz, 4-H), 8.30–8.33 (m, 1H, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 16.02, 70.82, 108.39, 111.77, 121.19, 122.05, 122.56, 123.36, 123.54, 124.53, 128.40, 128.52, 130.07, 147.31, 154.83, 158.68, 164.14. ESIMS (m/z): 264 $[M+H]^+$. HRMS (ESI): m/z calcd for $C_{17}H_{14}NO_2$ $[M+H]^+$ 264.1024, found 264.1026. Anal. calcd for $C_{17}H_{13}NO_2 \cdot 0.5H_2O$: C 74.97, H 5.19, N 5.14; found: C 74.87, H 4.86, N 5.30.

5.1.4. 11-Phenoxybenzofuro[2,3-b]quinoline (**6**)

A mixture of **3** (0.25 g, 1.0 mmol), K_2CO_3 (0.28 g, 2.0 mmol) and phenol (15 mL) was stirred at 100 °C for 2 h (TLC monitoring). After cooling, the mixture was poured into 2 N NaOH solution (50 mL). The solution was extracted with CH_2Cl_2 (50 mL × 3), washed with brine, dried over $MgSO_4$ and evaporated *in vacuo*. Purification by column chromatography (MeOH:CH_2Cl_2 1/50) and crystallization from EtOH gave **6** (0.26 g, 84%). Mp: 120–122 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 7.10–7.28 (m, 5H, 8-, 9-, Ar–H), 7.36–7.42 (m, 2H, Ar–H), 7.55–7.66 (m, 2H, 2-, 7-H), 7.75–7.77 (m, 1H, 10-H), 7.87–7.91 (m, 1H, 3-H), 8.13–8.18 (m, 2H, 1-, 4-H). ^{13}C NMR (100 MHz, DMSO-d_6): 108.47, 111.66, 115.73 (2C), 119.90, 120.82, 122.16, 123.48, 123.63, 123.85, 125.51, 128.10, 129.48, 130.41 (2C), 130.74, 146.81, 152.43, 154.37, 156.87, 163.26. ESIMS (m/z): 312 $[M+H]^+$. HRMS (ESI): m/z calcd for $C_{21}H_{14}NO_2$ $[M+H]^+$ 312.1024, found 312.1025. Anal. calcd for $C_{21}H_{13}NO_2$: C 81.01, H 4.21, N 4.50; found: C 81.05, H 4.43, N 4.47.

5.1.5. 1-[4-(Benzofuro[2,3-b]quinolin-11-yloxy)phenyl]ethanone (**7**)

A mixture of **3** (0.25 g, 1.0 mmol), 4-hydroxyacetophenone (0.27 g, 2.0 mmol), and K_2CO_3 (0.28 g, 2.0 mmol) in DMF (50 mL) was stirred at 100 °C for 6 h (TLC monitoring). The solvent was removed *in vacuo*, and the residue was poured into H_2O (100 mL). The resulting precipitate that separated was collected, purified by column chromatography (MeOH:CH_2Cl_2 1/50), and crystallized from EtOH to give **7** (0.28 g, 80%). Mp: 162–163 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 2.17 (s, 3H, CH_3), 7.03–7.07 (m, 2H, Ar–H), 7.17–7.21 (m, 1H, 9-H), 7.37 (dd, 1H, J = 0.8, 8.0 Hz, 7-H), 7.47–7.56 (m, 2H, 3-, 8-H), 7.60 (d, 1H, J = 8.4 Hz, 10-H), 7.79–7.83 (m, 1H, 2-H), 7.94–7.98 (m, 2H, Ar–H), 8.13 (dd, 1H, J = 1.2, 8.4 Hz, 4-H), 8.21 (d, 1H, J = 8.4 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 26.42, 109.26, 111.71, 115.43 (2C), 120.13, 120.90, 122.06, 123.71, 123.85, 125.39, 128.57, 129.32, 130.53, 131.08 (2C), 132.42, 147.34, 151.88, 155.11, 160.64, 163.66, 196.47. ESIMS (m/z): 354 $[M+H]^+$. HRMS (ESI): m/z calcd for $C_{23}H_{16}NO_3$ $[M+H]^+$ 354.1130, found 354.1131. Anal. calcd for $C_{23}H_{15}NO_3$: C 78.17, H 4.28, N 3.96; found: C 78.05, H 4.46, N 3.81.

5.1.6. Benzofuro[2,3-b]quinolin-11-amine (**8**)

A mixture of **3** (0.25 g, 1.0 mmol) and ammonia water (20 mL) in a sealed vessel was heated at 70 °C for 24 h (TLC monitoring). The solvent was removed *in vacuo*, and the residue was poured into H_2O (100 mL). The resulting precipitate that separated was collected and crystallized from EtOH to give **8** (0.14 g, 60%). Mp: >300 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 7.43–7.56 (m, 3H, 3-, 8-, 9-H), 7.70 (dd, 1H, J = 0.8, 8.4 Hz, 7-H), 7.77–7.81 (m, 1H, 2-H), 7.88 (dd, 1H, J = 0.8, 8.4 Hz, 10-H), 8.08 (br s, 2H, NH_2), 8.54 (dd, 1H, J = 0.8, 7.2 Hz, 4-H), 8.03 (d, 1H, J = 8.0 Hz, 1-H). ^{13}C NMR (100 MHz, DMSO-d_6): 96.37, 110.91, 115.89, 121.67, 122.38, 123.35, 123.38, 123.69, 125.29, 126.37, 130.93, 143.18, 149.32, 152.39, 161.75. ESIMS (m/z): 235 $[M+H]^+$. HRMS (ESI): m/z calcd for $C_{15}H_{11}N_2O$ $[M+H]^+$ 235.0871, found 235.0873. Anal. calcd for $C_{15}H_{10}N_2O \cdot 1.0H_2O \cdot 1.0HCl$: C 62.38, H 4.55, N 9.70; found: C 62.77, H 4.92, N 9.87.

5.1.7. N-methylbenzofuro[2,3-b]quinolin-11-amine (**9**)

Compound **9** was prepared from **3** and 40% methylamine by the same procedure as described for the preparation of **8**. Yield: 64%. Mp: 214–216 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 3.50 (d, 3H, J = 5.2 Hz, CH_3), 7.35–7.48 (m, 4H, NH, 3-, 8-, 9-H), 7.62 (dd, 1H, J = 0.8, 8.0 Hz, 7-H), 7.67–7.72 (m, 1H, 2-H), 7.83 (dd, 1H, J = 0.8, 8.4 Hz, 10-H), 8.16 (dd, 1H, J = 0.8, 8.0 Hz, 4-H), 8.45 (dd, 1H, J = 0.8, 8.4 Hz, 1-H). ^{13}C NMR (100 MHz, DMSO-d_6): 34.85, 97.34, 110.75, 117.83, 121.67, 122.71, 122.97, 123.02, 123.04, 126.11, 127.89, 129.57, 146.17, 150.34, 152.81, 163.89. ESIMS (m/z): 249 $[M+H]^+$. HRMS (ESI): m/z calcd for $C_{16}H_{13}N_2O$ $[M+H]^+$ 249.1028, found 249.1029. Anal. calcd for $C_{16}H_{12}N_2O$: C 77.40, H 4.87, N 11.28; found: C 77.49, H 5.10, N 11.24.

5.1.8. N-phenylbenzofuro[2,3-b]quinolin-11-amine (**10**)

A mixture of **3** (0.25 g, 1.0 mmol) and aniline (0.28 g, 3.0 mmol) in ethoxyethanol (30 mL) was refluxed for 24 h (TLC monitoring). The solvent was removed *in vacuo*, and the residue was poured into H_2O (100 mL). The resulting precipitate that separated was collected and crystallized from EtOH to give **10** (0.12 g, 38%). Mp: 134–135 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 6.47 (d, 1H, J = 7.6 Hz, 7-H), 6.99–7.04 (m, 4H, 9-, Ar–H), 7.25–7.29 (m, 2H, Ar–H), 7.38–7.43 (m, 1H, 8-H), 7.57–7.61 (m, 1H, 3-H), 7.63 (d, 1H, J = 8.0 Hz, 10-H), 7.78–7.83 (m, 1H, 2-H), 8.00 (d, 1H, J = 8.4 Hz, 4-H), 8.54 (d, 1H, J = 8.4 Hz, 1-H), 9.62 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 104.76, 110.62, 118.34 (2C), 120.82, 121.60, 121.67, 122.60, 123.58, 123.94, 124.64, 127.50, 128.10, 129.32 (2C), 130.08, 142.29, 142.38, 146.41, 153.63, 163.43. ESIMS (m/z): 311 $[M+H]^+$. HRMS (ESI): m/z calcd for $C_{21}H_{15}N_2O$ $[M+H]^+$ 311.1184, found 311.1182. Anal. calcd for $C_{21}H_{14}N_2O \cdot 0.25H_2O$: C 80.10, H 4.65, N 8.90; found: C 80.16, H 4.65, N 8.78.

5.1.9. 1-[4-(Benzofuro[2,3-b]quinolin-11-ylamino)phenyl]ethanone (**11**)

Compound **11** was prepared from **3** and 4-aminoacetophenone by the same procedure as described for the preparation of **10**. Yield: 46%. Mp: 198–200 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 2.49 (s, 3H, CH_3), 6.86 (d, 1H, J = 7.6 Hz, 7-H), 6.95–7.98 (m, 2H, Ar–H), 7.17–7.21 (m, 1H, 9-H), 7.49–7.54 (m, 1H, 8-H), 7.64–7.68 (m, 1H, 3-H), 7.73 (d, 1H, J = 8.0 Hz, 10-H), 7.85–7.89 (m, 3H, 2-, Ar–H), 8.08 (dd, 1H, J = 0.4, 8.4 Hz, 4-H), 8.44 (dd, 1H, J = 0.8, 8.4 Hz, 1-H), 10.05 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 26.27, 108.40, 111.11, 115.52 (2C), 121.12, 121.81, 123.13, 123.66, 124.61 (2C), 128.24, 128.49, 129.17, 130.35 (2C), 131.024, 140.33, 146.41, 147.46, 154.18, 163.10, 195.97. ESIMS (m/z): 353 $[M+H]^+$. HRMS (ESI): m/z calcd for $C_{23}H_{17}N_2O_2$ $[M+H]^+$ 353.1290, found 353.1287. Anal. calcd for

$C_{23}H_{16}N_2O_2 \cdot 1.0H_2O$: C 74.57, H 4.91, N 7.56; found: C 74.37, H 4.91, N 7.56.

5.1.10. N-(4-methoxyphenyl)benzofuro[2,3-b]quinolin-11-amine (**12**)

Compound **12** was prepared from **3** and *p*-anisidine by the same procedure as described for the preparation of **10**. Yield: 35%. Mp: 166–167 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.80 (s, 3H, OCH_3), 6.60 (d, 1H, $J = 8.0$ Hz, 7-H), 6.83–6.87 (m, 2H, Ar–H), 6.97–7.04 (m, 4H, NH, 9-, Ar–H), 7.33–7.38 (m, 1H, 8-H), 7.44–7.48 (m, 1H, 3-H), 7.51 (d, 1H, $J = 8.0$ Hz, 10-H), 7.70–7.74 (m, 1H, 2-H), 8.05–8.12 (m, 2H, 1-, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): 55.60, 104.44, 110.82, 114.83 (2C), 119.84, 121.28 (2C), 121.31, 121.98, 122.68, 123.98, 124.53, 127.21, 129.01, 129.76, 135.00, 142.38, 146.81, 154.32, 155.88, 164.01. ESIMS (*m*/*z*): 341 $[M+H]^+$. HRMS (ESI): *m*/*z* calcd for $C_{22}H_{17}N_2O_2$ $[M+H]^+$ 341.1290, found 341.1292. Anal. calcd for $C_{22}H_{16}N_2O_2 \cdot 0.5H_2O$: C 75.62, H 4.91, N 8.02; found: C 75.95, H 5.03, N 7.83.

5.1.11. N-(3-methoxyphenyl)benzofuro[2,3-b]quinolin-11-amine (**13**)

Compound **13** was prepared from **3** and *m*-anisidine by the same procedure as described for the preparation of **10**. Yield: 32%. Mp: 134–135 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.65 (s, 3H, OCH_3), 6.49–6.55 (m, 2H, Ar–H), 6.60 (dd, 1H, $J = 2.0$, 8.4 Hz, Ar–H), 6.82 (d, 1H, $J = 7.6$ Hz, 7-H), 6.97 (br s, 1H, NH), 7.07–7.11 (m, 1H, 9-H), 7.15–7.20 (m, 1H, Ar–H), 7.38–7.42 (m, 1H, 8-H), 7.48–7.55 (m, 2H, 3-, 10-H), 7.72–7.76 (m, 1H, 2-H), 8.11–8.15 (m, 2H, 1-, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): 55.24, 104.04, 107.03, 108.08, 110.67, 110.95, 120.88, 121.62, 121.75, 122.82, 124.35, 124.85, 127.77, 129.00, 129.88, 130.40, 140.82, 143.07, 146.80, 154.60, 160.79, 163.79. ESIMS (*m*/*z*): 341 $[M+H]^+$. HRMS (ESI): *m*/*z* calcd for $C_{22}H_{17}N_2O_2$ $[M+H]^+$ 341.1290, found 341.1287. Anal. calcd for $C_{22}H_{16}N_2O_2 \cdot 1.25H_2O$: C 72.80, H 5.15, N 7.72; found: C 72.82, H 5.23, N 7.55.

5.1.12. N,N-dimethylbenzofuro[2,3-b]quinolin-11-amine (**14**)

Compound **14** was prepared from **3** and 40% dimethylamine by the same procedure as described for the preparation of **10**. Yield: 57%. Mp: 120–121 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.39 (s, 6H, $N(CH_3)_2$), 7.46–7.61 (m, 3H, 3-, 8-, 9-H), 7.71 (d, 1H, $J = 8.4$ Hz, 7-H), 7.74–7.78 (m, 1H, 2-H), 7.98 (d, 1H, $J = 8.4$ Hz, 10-H), 8.06 (d, 1H, $J = 8.0$ Hz, 4-H), 8.36 (dd, 1H, $J = 1.2$, 8.4 Hz, 1-H). ^{13}C NMR (100 MHz, $CDCl_3$): 43.87 (2C), 109.03, 111.13, 121.53, 123.32, 123.48, 123.62, 123.96, 125.53, 127.92, 128.45, 129.54, 147.13, 152.84, 153.84, 163.20. ESIMS (*m*/*z*): 263 $[M+H]^+$. HRMS (ESI): *m*/*z* calcd for $C_{17}H_{15}N_2O$ $[M+H]^+$ 263.1184, found 263.1185. Anal. calcd for $C_{17}H_{14}N_2O$: C 77.84, H 5.38, N 10.68; found: C 78.05, H 5.63, N 10.40.

5.2. Antimycobacterium activity

Primary screening is conducted against *M. tuberculosis* H37Rv (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay to determine the actual minimum inhibitory concentration (MIC) using the Microplate Alamar Blue Assay (MABA) [37]. The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls. Concurrent with the determination of MICs, compounds are screened by serial dilutions to assess cytotoxicity (IC_{50}) to a VERO cell. After 72 h exposure, viability is assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 Non-radioactive Cell Proliferation Assay.

5.3. Cell growth inhibitory assay

Human breast carcinoma MCF7 cells, non small cell lung carcinoma H460 cells, and human glioma (SF-268) cells were maintained in RPMI-1640 medium supplied with 5% fetal bovine serum. Cells in logarithmic phase were cultured at a density of 5000 cells/mL/well in a 24-well plate. The cells were exposed to various concentrations of the tested drugs for 72 h. The methylene blue dye assay was used to evaluate the effect of the tested compounds on cell growth as described previously [38].

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