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# Synthesis and Evaluation of Quinolino-Benzoxaboroles as Potential Antimicrobial Agents

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## Abstract

Several quinolino-benzoxaborole derivatives have been prepared to start from aminobenzoxaboroles. These derivatives have been evaluated for their anti-cancer activity on human and murine cancer cell lines and based on their relative non-toxicity, these compounds were further evaluated for their antibacterial activity against *E. coli*, *B. subtilis*, and *M. smegmatis*. The synthesized compounds were also evaluated for antifungal activity in *C. albicans* and *C. neoformans*.

## Keywords

Benzoxaboroles, Aminobenzoxaboroles, Quinolino-Benzoxaboroles, Anti-Microbial Agents

## 1. Introduction

Quinoline is a highly privileged nitrogen containing a bicyclic ring system in which a benzene ring is fused to a pyridine ring. The quinoline moiety is found in many natural products and many of these natural products have been traditionally used as medicines for treating a wide variety of diseases [1]. Quinoline-based synthetic molecules have been found to exhibit a diverse range of pharmacological properties and can be used as antimalarial, antibacterial, anti-convulsant, cardiotonic, anticancer, anthelmintic, antifungal, anti-inflammatory, and analgesic agents [2] [3] [4]. Quinoline-based clinical drugs also exhibit favorable pharmaceutical properties such as high metabolic stability, longer bio-

logical half-life, and high oral bioavailability [5]. For example, quinoline-based antimalarial drugs chloroquine and mefloquine have a $t_{1/2}$ of approximately one month and 70% to 100% oral bioavailability [6] [7].

Another structure with high potential as an antimicrobial agent is benzoxaborole. Benzoxaboroles contain a phenyl ring fused with a heterocyclic oxaborole ring moiety. Benzoxaboroles are considerably more stable and exhibit higher hydrolytic resistance in comparison to corresponding phenylboronic acids. The enhanced acidity of benzoxaboroles allows them to exist predominantly in anionic forms in aqueous solution at physiological pH, which results in benzoxaboroles exhibiting higher solubility and better pharmaceutical properties than phenylboronic acids [8] [9] [10]. Increasing interest in benzoxaborole compounds is mainly due to their broad-spectrum biological activity including antimicrobial, anti-inflammatory, anti-malarial and other medicinal properties [8] [9] [10].

Due to the significant antimicrobial activity of the benzoxaborole unit, we envisioned that the introduction of benzoxaboroles on quinolines would result in novel molecular entities with potent antimicrobial activities. In this regard, we developed a new synthetic methodology for the rapid creation of quinolino benzoxaboroles (Scheme 1).

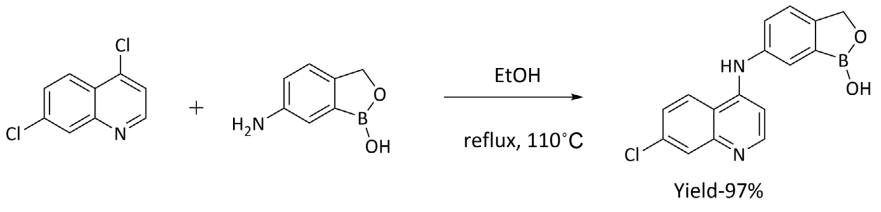
## 2. Experimental

4,7-dichloroquinoline, 4-chloroquinoline, 4-chloro-7-methoxyquinoline, 4-chloro-7-fluoroquinoline, 1,3-dihydroisobenzofuran-5-amine, 4-(4-aminophenoxy)-N-methylpicolinamide and (4-aminophenyl)boronic acid were purchased from Ambeed chemicals. 4-chloroaniline (Sigma-Aldrich), 4-fluoroaniline (Sigma-Aldrich), (2-formylphenyl) boronic acid (Oakwood Chemical) were purchased from their respective vendors. All other chemical reagents utilized for chemical synthesis were high grade quality and were purchased from Sigma-Aldrich. The $^{1}$H NMR and $^{13}$C NMR spectral analysis was carried out using a Bruker Ascend™ 400 MHz spectrometer. High resolution mass spectrometry (HRMS) was recorded using a Bruker micrOTOP-Q III ESI mass spectrometer.

### 2.1. Synthesis of 7-Substituted Quinoline-4-Amines

**General procedure:**

To the solution of 7-substituted 4-chloroquinoline (1.3 eq, 4 mmol) in absolute ethanol (7 mL/mmol), amine (1 eq, 3 mmol) was added and the mixture was




Scheme 1. Synthesis of 7-chloro substituted quinoline-aminobenzoxaborole 4.

refluxed (24 hours, heat source: sand bath). Reaction progress was monitored via thin layer chromatography (TLC) (30% of EtOAc & hexanes). Upon completion of the reaction the reaction mixture was brought to room temperature. The resulting precipitate was filtered using Buchner funnel and rinsed with acetone to remove impurity traces. The precipitate was dried under vacuum to yield the corresponding 7-substituted quinoline-4-amines with 90% yield.

#### 2.1.1. 6-((7-Chloroquinoline-4-Yl)Amino)Benzo[C] [1, 2]Oxaborol-1 (3 H)-Ol [Compound No. 4]

$^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.25 (s, 1 H), 9.43 (s, 1 H), 8.89 (d, $J$ = 9.2 Hz, 1 H), 8.51 (d, $J$ = 7.2 Hz, 1 H), 8.17(d, $J$ = 1.7 Hz, 1 H), 7.9 (m, 1 H), 7.82 (s, 1 H), 7.64 - 7.57 (m, 2 H), 6.73 (d, $J$ = 7.2 Hz, 1 H), 5.08 (s, 2 H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 155.69, 153.65, 143.73, 139.49, 138.89, 136.23, 132.96, 128.68, 127.94, 127.82, 126.67, 123.59, 119.64, 116.35, 100.57, 70.40; HRMS (ESI) m/z: calc'd for $C_{16}H_{12}BClN_2O_2$ $[M + H]^+$: 311.0753, found at 311.0680.

#### 2.1.2. 6-(Quinoline-4-Ylamino)Benzo[C] [1, 2] Oxaborol-1 (3 H)-Ol [Compound No. 8]

$^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.16 (s, 1 H), 9.47 (s, 1 H), 8.88 (d, $J$ = 7.8 Hz, 1 H), 8.51 (s, 1 H), 8.13 - 8.04 (m, 2 H), 7.86 - 7.83 (m, 2 H), 7.64 - 7.60 (m, 2 H), 6.74 (s, 1 H), 5.09 (s, 2 H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 155.77, 153.50, 143.00, 138.67, 136.46, 134.31, 132.90, 128.82, 128.11, 127.45, 124.30, 123.53, 120.63, 117.52, 100.03, 70.39; HRMS (ESI) m/z: calc'd for $C_{16}H_{13}BN_2O_2$ $[M + H]^+$: 277.1143, found at 277.1155.

#### 2.1.3. 6-(7-Methoxyquinoline-4-Ylamino)Benzo[C] [1, 2]Oxaborol-1 (3 H)-Ol [Compound No. 9]

$^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 10.95 (s, 1 H), 9.42 (s, 1 H), 8.75 (d, $J$ = 8.8 Hz, 1 H), 8.39 (d, $J$ = 6.8 Hz, 1 H), 7.81 (s, 1 H), 7.62 - 7.55 (m, 2 H), 7.45 - 7.43 (m, 2 H), 6.61 (d, $J$ = 7.2 Hz, 1 H), 5.07 (s, 2 H), 3.97 (s, 3 H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 163.47, 155.29, 153.33, 142.55, 141.05, 136.53, 132.81, 128.80, 128.04, 126.14, 123.46, 118.59, 111.82, 100.43, 99.39, 70.38, 56.50; HRMS (ESI) m/z: calc'd for $C_{17}H_{15}BN_2O_3$ $[M + H]^+$: 307.1245, found at 307.1176.

#### 2.1.4. 6-((7-Fluroquinoline-4-Yl)Amino)Benzo[C] [1, 2]Oxaborol-1 (3 H)-Ol [Compound No. 10]

$^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 9.24 (s, 1 H), 9.14 (s, 1 H), 8.52-8.43 (m, 2 H), 7.72 (s, 1 H), 7.57 (dd, $J$ = 2.4 Hz, 10.4 Hz, 1 H), 7.50 - 7.44 (m, 3 H) 6.79 (d, $J$ = 5.2 Hz, 1 H), 5.02 (s, 2 H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): 162.89 (d, $J$ = 245 Hz), 152.30 (d, $J$ = 14 Hz), 150.57, 150.43 (d, $J$ = 4.1 Hz), 149.20, 139.40, 132.22, 126.85, 125.57 (d, $J$ = 10 Hz), 125.27, 122.96, 117.09, 114.76 (d, $J$ = 24 Hz), 112.67 (d, $J$ = 20 Hz), 110.21, 70.32; HRMS (ESI) m/z: calc'd for $C_{16}H_{12}BFN_2O_2$ $[M + H]^+$: 295.1049, found at 295.1054.

#### 2.1.5. ((7-Chloroquinolin-4-Yl)Amino)Phenyl)Boronic Acid [Compound No. 11]

$^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.18 (s, 1 H), 8.87 (d, $J$ = 5.4 Hz, 1 H), 8.54

(d, $J$ = 11.1 Hz, 1 H), 8.19 (m, 2 H), 7.99 (d, $J$ = 1.8 Hz, 2 H), 7.89 (dd, $J$ = 1.9 Hz, 4.0 Hz, 1 H), 7.47 (d, $J$ = 1.9 Hz, 2 H), 6.88 (s, 1 H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 155.10, 143.80, 139.55, 139.04, 138.85, 136.15, 133.91, 127.81, 126.73, 124.47, 119.64, 116.52, 100.93; HRMS (ESI) m/z: calc'd for $C_{15}H_{12}BClN_2O_2$ [M + H]$^+$: 299.0753, found at 299.0752.

#### 2.1.6. 7-Chloro-N-(4-Chlorophenyl)Quinoline-4-Amine [Compound No. 12]

$^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.12 (s, 1 H), 8.85 (d, $J$ = 9.0 Hz, 1 H), 8.54 (d, $J$ = 6.9 Hz, 1 H), 8.17 (s, 1 H), 7.87 (d, $J$ = 9.0 Hz, 1 H), 7.64 (s, 1 H), 7.62 (d, $J$ = 8.7 Hz, 1 H), 7.53 (m, 2 H), 6.84 (d, $J$ = 6.9 Hz, 1 H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 155.22, 144.16, 139.65, 138.91, 136.53, 132.12, 130.43, 127.94, 127.68, 126.60, 119.86, 116.55, 101.00; HRMS (ESI) m/z: calc'd for $C_{15}H_{10}Cl_2N_2$ [M + H]$^+$: 289.0294, found at 289.0298.

#### 2.1.7. 7-Chloro-N-(4-Fluorophenyl)Quinoline-4-Amine [Compound No. 13]

$^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.19 (s, 1 H), 8.87 (d, $J$ = 9.1 Hz,1 H), 8.53 (d, $J$ = 7.0 Hz, 1 H), 8.18 (d, $J$ = 1.6 Hz, 1 H), 7.88 (m, 1 H), 7.55 (m, 2 H), 7.43 (m, 2 H), 6.73 (d, $J$ = 7.0 Hz, 1 H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): 161.36 (d, $J$ = 243 Hz), 155.65, 143.78, 139.49, 138.85, 133.75 (d, $J$ = 2.1 Hz), 128.41 (d, $J$ = 8.2 Hz), 127.8, 126.76, 119.62, 117.3 (d, $J$ = 22 Hz), 116.34, 100.66; HRMS (ESI) m/z: calc'd for $C_{15}H_{10}ClFN_2$ [M + H]$^+$: 273.0589, found at 273.0598.

#### 2.1.8. 7-Chloro-N-(1,3-Dihydroisobenzofuran-5-Yl)Quinoline-4-Amine [Compound No. 14]

$^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 11.26 (s, 1 H), 8.91 (d, $J$ = 9.2 Hz, 1 H), 8.51(d, $J$ = 7.0 Hz, 1 H), 8.19 (d, $J$ = 2.1 Hz, 1 H), 7.86 (dd, $J$ = 2.1 Hz, 9.2 Hz, 1 H), 7.50 (d, $J$ = 7.9 Hz, 1 H), 7.44 - 7.37 (m, 2 H), 6.77 (d, $J$ = 7.9 Hz, 1 H), 5.07 (s, 4 H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 155.57, 143.78, 141.59, 139.54, 138.99, 138.84, 136.61, 127.80, 126.67, 125.17, 123.07, 119.68, 119.00, 116.36, 100.78, 72.95, 72.90; HRMS (ESI) m/z: calc'd for $C_{17}H_{13}ClN_2O$ [M + H]$^+$: 297.0789, found at 297.0802.

### 2.2. Cell Culture and Cytotoxicity Assay

MDA-MB-231 cells (ATCC) were grown in DMEM media supplemented with 10% FBS (Fetal Bovine Serum) and 1% penicillin-streptomycin. MIA PaCa-2 cells (ATCC) were grown in DMEM media supplemented with 10% FBS, 2.5% Horse serum and 1% penicillin-streptomycin. WiDr cells (ATCC) were grown in MEM media supplemented with 10% FBS (Fetal Bovine Serum) and 1% penicillin-streptomycin. MCF-7 cells (ATCC) were grown in α-MEM supplemented with FBS (5%), insulin (10 μg/mL), non-essential amino acids (0.1 mM), sodium pyruvate (1 mM), EGF (100 μg/mL), hydrocortisone (10 μg/mL), HEPES (10 mM), and penicillin-streptomycin (1%). 4T1 cells (ATCC) and 67NR cells (ATCC) were cultured in RPMI-1640 supplemented with 10% FBS and penicillin-streptomycin (50 U/mL; 50 μg/mL).

Cells were seeded ($5 \times 10^3$ cells/well) in 96 well plates and incubated overnight

(37˚C, 5% $CO_2$). Test compounds were then added to 96-well plates in duplicate followed by serial dilution and were incubated (37˚C, 5% CO2). After incubation for 72 hours, 3-(4,5dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 0.5 mg/mL) was added and the plates were incubated for four hours. The formazan precipitate was then solubilized using sodium lauryl sulfate (SDS 10% w/v, 0.01 N HCl) and incubated for four hours. Later absorbance readings were recorded at 570 nm for each well and concentrations at which 50% of cell growth was inhibited ($IC_{50}$) were calculated, using control wells as 100% cell survival.

## 2.3. Cell Culture and Antimicrobial Assays

The compounds were screened against *Escherichia coli* (*E. coli*, gram-), *Bacillus subtilis* (*B. subtilis*, gram+), and *Mycobacterium smegmatis* (*M. smegmatis*, acid-fast). Although *E. coli* and *B. subtilis* are not highly pathogenic, initial screening of test compounds against these microbes aimed to verify potential activity towards highly pathogenic strains. Similarly, *M. smegmatis* is non-pathogenic in nature but shares a 90% genetic similarity to *M. tuberculosis*, which is highly pathogenic. We also screened the synthesized aminobenzoxaborole quinoline conjugates against two fungal species *Candida albicans* (*C. albicans*) and *Cryptococcus neoformans* (*C. neoformans*) to evaluate the broad-spectrum applications of these compounds. These two fungal species, *C. albicans* and *C. neoformans*, can be pathogenic for immunocompromised patients.

### 2.3.1. Zone of Inhibition

Zones of inhibition values were determined for the two fungal strains (*C. albicans* and *C. neoformans*) and three bacterial strains against *E. coli*, *B. subtilis*, and *M. smegmatis*. Luria-Bertani (LB) broth (DIFCO) and Sabouraud Dextrose Broth (SB, DIFCO) were made following previously reported procedure [11]. Agar plates were poured using the respective broth containing 5% agar (25 mL per culture dish). All media and their components were sterilized via autoclave at 250˚C for 15 minutes. Experiments were carried out using aseptic technique in a biohazard level 2 fume hood.

*M. smegmatis* cells were grown in 7H9 with Tween 80 (0.05% w/v) media maintained at pH 7, *E. coli* and *B. subtilis* cells were grown in LB broth with tryptone, yeast extract and NaCl maintained at pH 7.9, *C. neoformans* cultures were grown in SB growth media maintained at pH 6.9 and *C. albicans* cultures were grown in LB broth containing tryptone, yeast extract and NaCl maintained at pH 7. Bacterial and fungal strains of interest were cultured in their respective broths. Inoculated broth was spread evenly over the face of sterile agar plate. The white discs were placed on the sterile surface and 5 µL of the test compound was absorbed into disks. The disk was placed in specific coordinate on the petri dish using sterile tweezers. These dishes were incubated and allowed to grow for 48 hours until the culture was visible. The zone of inhibition values of the test compounds was recorded and compared with positive controls and negative control DMSO.

#### 2.3.2. Determination of Minimum Inhibitory Concentration (MIC) Values

The stock solution of each compound was prepared in DMSO to a concentration of 0.1 M (100 mM). 1 µL of the stock solution was added into 500 µL of the growth media in falcon tubes. This would make the final concentration 200 µM. All materials except for the culture were then placed under UV light for 1 - 2 minutes to ensure the solutions were sterile. In a 50 mL centrifuge tube, the media with the desired cell concentration was prepared with absorbance reading at 0.15 - 0.2 and 100 µL of the resulting solution was added to each well in the 96-well plate. The test compound in 500 µL of growth media was vortexed and 100 µL of this solution was added in row leaving the first and last column untreated (these columns were considered as blank). Serial dilutions were made by taking 100 µL from row 1 and adding to row 2 and subsequently 100 µL of the resulting solution into row 2 was removed and placed into row 3. This process was repeated all the way down to row 8. The absorbance values were recorded at 600 nm for each well after 24, 36, and 48 hours of treatment. The concentrations at which 50% of cell growth was inhibited ($MIC_{50}$) were tabulated, using control wells as 100% cell survival.

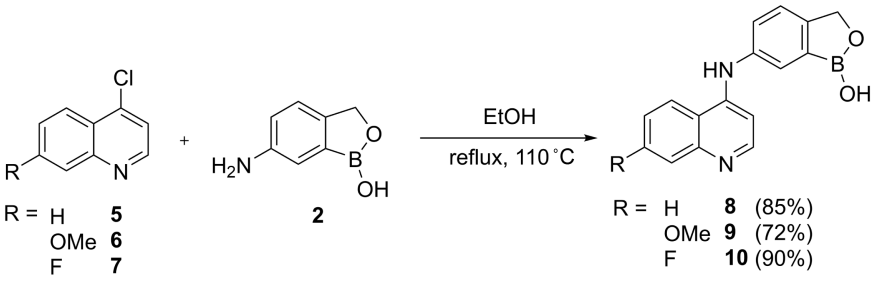
## 3. Results and Discussion

### 3.1. Synthesis Novel Compounds as Potential Antimicrobial Agents

Aryl amination is a key, one-step reaction that introduces a C-N bond between aryl halides and amines. 4-halo substituted quinolines are known for an efficient nucleophilic displacement with alkyl and aryl amines [12]. This reaction usually occurs under mild reaction conditions without the need for any organometallic catalysts or strong bases [12]. We envisioned that 4-halosubstitution on a simple quinoline **1** would serve as excellent starting material for nucleophilic aromatic substitution with aminobenzoxaborole **2**. If the nucleophilic displacement with **2** occurs, as is the case with normal aromatic amines, then this reaction will lead to an efficient synthetic protocol for creating novel benzoxaborole-containing aminoquinolines. First, we explored the reaction of 4,7-dichloroquinoline **3** with aminobenzoxaborole **2**. Initially, the reaction of **3** with **2** was carried out at room temperature in ethanol and no reaction occurred. Then the reaction was carried out under refluxing conditions at 100˚C for 10 hrs. With these conditions, the reaction took place smoothly as confirmed by TLC analysis of a more polar spot than 4,7-dichloroquinoline **3** and disappearance of both reactants **3** and **2**. After the workup, the crude solid product was washed with cold acetone to obtain the 7-chloroquinolino-4-aminobenzoxaborole **4** in pure form (Scheme 1).

Encouraged by the successful nucleophilic displacement reaction, we also carried out the reactions with other 7-halosubstituted quinolines. Examples of these included 4-chloroquinoline **5**, 4-chloro-7-methoxyquinoline **6** and 4-chloro-7-fluoroquinoline **7**. Again, refluxing in ethanol was required to obtain 4-aminobenzoxaborole substituted quinolines **8**, **9**, and **10** in good yields (Scheme 2).

To further understand the structure activity relationship (SAR) studies and




**Scheme 2.** Synthesis of 7-halosubstituted quinoline-aminobenzoxaboroles 8, 9, and 10.

evaluate the role of the benzoxaborole unit for pharmacological use, we have synthesized quinolino-aminophenylboronic acid derivative **11** (Scheme 3). Reaction of 4,7-dichloroquinoline **3** with 4-aminophenylboronic acid resulted in a 90% yield of compound **11**. In compound **11**, boron is attached to a carbocycle rather than being part of a heterocycle. Comparison of in-ring boron and outside-ring boron's activity provides key insights into how boron's position in benzoxaboroles contributes to its antimicrobial properties. To understand the role of the boron atom in providing biological activity to benzoxaboroles, we synthesized comparable non-boronyl derivatives **12** and **13** by the reaction of 4,7-dichloroquinoline **3** with 4-chloro and 4-fluoro anilines. To understand the role of the benzoxaborole unit in providing pharmacological activity, aminobenzofuran was used in place of aminobenzoxaborole **2** to result in the product quinolino-benzofuran **14**.

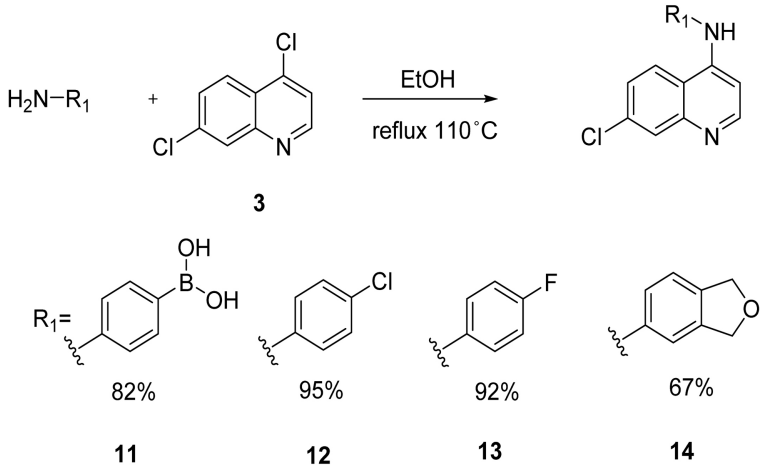
## 3.2. *In Vitro* Biological Evaluation of These Novel Compounds as Potential Antimicrobial Agents

### 3.2.1. Cell Proliferation Inhibition Studies in Cancer Cell Lines

To succeed as antimicrobial agents, the candidate compounds should exhibit low toxicities against proliferating human cells. In this regard, all the synthesized compounds were tested for their *in vitro* cell proliferation inhibition properties against rapidly proliferating human and murine cells. For this, we utilized human triple negative breast cancer (MDA-MBA-231) human pancreatic cancer (MIA PaCa-2), human colorectal adenocarcinoma (WiDr), human breast cancer (MCF-7), murine breast cancer (67NR), and murine metastatic breast cancer (4T1) cell lines. Cells were cultured in 96-well plates and were incubated with the test compound for 72 hours, at which time MTT was added. MTT is reduced to MTT formazan by mitochondrial succinate dehydrogenase with an absorbance at 570 nm [13]. The half maximal inhibitory concentration ($IC_{50}$) was calculated for each compound using GraphPad Prism 6 software. From this study, it was found that none of the tested compounds showed general cytotoxicity at 100 μM.

### 3.2.2. Antimicrobial Studies

Encouraged by their lack of cytotoxicity in proliferating cells even at high concentrations, we screened all the synthesized compounds for their biological




**Scheme 3.** Synthesis of quinolinoanilines 11-14 via aromatic amination.

activity against bacterial strains *E. coli*, *B. subtilis*, and *M. smegmatis*. We employed the Kirby-Bauer disk method for preliminary investigation of biological activity of test compounds. The candidate compounds were tested at 100 μM and were compared against three positive controls: 6-aminobenzoxaborole **2**, streptomycin, and ampicillin. DMSO solvent was used as a negative control. The results are summarized in Table 1. For gram-negative *E. coli*, only the H and methoxy substituted quinolino-aminobenzoxaborole **8** and **9** showed zones of inhibition slightly larger than the DMSO negative control (1 vs 1.4 and 1.3 cm, respectively, Table 1). Using phenylboronic acid in place of the benzoxaborole moiety showed no activity for the synthesized derivative **11**. The non-borono Cl and F substituted anilinoquinolones **12** and **13** in place of the aminobenzoxaborole template resulted in similar abolishment of activity comparative to the level of the DMSO negative control. Benzofuran derivative **14** was screened as a structural substitute to investigate the effect of boron in the 5-membered ring. The benzofuran derivative **14** also displayed a zone of inhibition identical to that of DMSO control.

Following these results, we then screened the test compounds against the gram-positive bacterial strain *B. subtilis* and found that overall inhibitory activity was equal to or slightly above DMSO control for all derivatives. For the benzoxaborole containing quinolines, only the chloro substituted derivative **4** gave a slightly larger zone of inhibition than the DMSO control (1 vs 1.4 cm, Table 1). No inhibitory activity was observed for other derivatives. Overall, the active derivatives **8**, **9** and **4** showed significantly lower of zone of inhibition values against *E. coli* and *B. subtilis* than the positive controls aminobenzoxaborole **2**, ampicillin, and streptomycin.

Additionally, we screened the test compounds on the acid-fast bacterial strain *M. smegmatis*, which is non-pathogenic in nature but shares ~90% genetic similarity to the disease-causing bacterial strain *M. tuberculosis* [14]. Initial drug screening on *M. smegmatis* can be carried out in biosafety level-2 hood and this

**Table 1.** Zone of inhibition values (cm) of the synthesized compounds against *E. coli*, *B. subtilis*, and *M. smegmatis*).

| Compound No. | Zone of inhibition (cm) | | |
|---|---|---|---|
| | *E. coli* | *B. subtilis* | *M. smegmatis* |
| **4** | 1 | 1.4 | 1.3 |
| **8** | 1.4 | 1.2 | 3.2 |
| **9** | 1.3 | 1 | 2.2 |
| **10** | 1 | 1 | 1 |
| **11** | 1 | 1 | 1.2 |
| **12** | 1 | 1 | 1 |
| **13** | 1 | 1 | 1 |
| **14** | 1 | 1 | 1 |
| **2** | 2.6 | 3.2 | 3.9 |
| **Streptomycin** | 2.6 | 2.7 | 2.4 |
| **Ampicillin** | 2.8 | 3.5 | 2.3 |
| **DMSO** | 1 | 1 | 1 |

bacterium acts as surrogate for *M. tuberculosis* [14]. In this study, we found our candidates gave modest to good zone of inhibition values in the range of 1.3 to 3.2 cm. However, the aminobenzoxaborole control **2** exhibited higher zone of inhibition than the test compounds (Table 1).

The synthesized derivatives were also tested against two fungal strains *C. neoformans* and *C. albicans* to explore their antifungal activity. The activity of the synthesized derivatives was compared against four controls for their efficacy. The controls used for this study were 6-aminobenzoxaborole **2**, fluconazole (antifungal drug), itraconazole (antifungal drug), and DMSO solvent. All the synthesized derivatives were tested at 100 μM in DMSO and were compared against controls for their antifungal efficacy. Unfortunately, none of the compounds including the control 6-aminobenzoxaborole 2 exhibited any zone of inhibition values against *C. neoformans* and *C. albicans*.

Since some of the quinolino-aminobenzoxaboroles showed good zone of inhibition values against *M. smegmatis*, we determined the MIC values of these candidates to explore their translational potential. This study indicated that boronated candidate compounds showed the $MIC_{50}$ values in the range of 27 to 110 μM concentration (Table 2). However, all these boronated quinolines showed lower activity than the parent aminobenzoxaborole **2**. It is interesting to note that none of the non-boron quinolines showed any significant activity even at 100 μM concentration.

**Table 2.** $MIC_{50}$ (μM) of the synthesized compounds against *Mycobacterium smegmatis*.

| Compound No. | *Mycobacterium smegmatis*-$MIC_{50}$ (μM) after 48 hrs (Mean + SEM) |
|---|---|
| **4** | 26.88 + 0.29 |
| **8** | 53.10 + 12.80 |
| **9** | 34.84 + 12.81 |
| **10** | 110.53 + 4.9 |
| **11** | 88.08 + 2.11 |
| **12** | NA |
| **13** | NA |
| **14** | NA |
| **2** | 11.37 + 3.51 |
| **Rifampin** | 7.33 + 2.34 |

## 4. Conclusion

In conclusion, we developed a novel synthetic methodology for preparing quinolino aminobenzoxaboroles. The synthesized compounds were evaluated for their cytotoxic properties against various human and murine cancer cells. All the compounds were found to be well tolerated as evidenced by their low toxicity even at 100 μM concentrations. Encouraged by the nontoxic nature, the test compounds were evaluated for their antibacterial activity against *E. coli*, *B. subtilis*, and *M. smegmatis*. Some of the synthesized borono-quinoline derivatives exhibited modest to good $MIC_{50}$ values against *M. smegmatis*. Further SAR and biological studies are required to identify the lead compound with the highest potency for clinical translation.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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