Synthesis and Evaluation of 3-Halobenzo[b]thiophenes as Potential Antibacterial and Antifungal Agents

Prerna J. Masih 1,*, Tanay Kesharwani 2, Elivet Rodriguez 1, Mia A. Vertudez 1, Mina L. Motakhaveri 1, Terelan K. Le 1, Minh Kieu T. Tran 1, Matthew R. Cloyd 2, Cory T. Kornman 2 and Aimee M. Phillips 1

1 Department of Biology, University of West Florida, 11000 University Pkwy, Pensacola, FL 32514, USA; elivet.rdz@gmail.com (E.R.); alaynsv1@gmail.com (M.A.V.); mmotakhaveri@umc.edu (M.L.M.); tkle@students.uwf.edu (T.K.L.); mtt15@students.uwf.edu (M.K.T.T.); legendaryraycharles@gmail.com (A.M.P.)
2 Department of Chemistry, University of West Florida, 11000 University Pkwy, Pensacola, FL 32514, USA; tkesharwani@uwf.edu (T.K.); mrc53@students.uwf.edu (M.R.C.); ctkornman@gmail.com (C.T.K.)
* Correspondence: pmasih@uwf.edu

Abstract: The global health concern of antimicrobial resistance has harnessed research interest to find new classes of antibiotics to combat disease-causing pathogens. In our studies, 3-halobenzo[b]thiophene derivatives were synthesized and tested for their antimicrobial activities using the broth microdilution susceptibility method. The 3-halo substituted benzo[b]thiophenes were synthesized starting from 2-alkynyl thioanisoles using a convenient electrophilic cyclization methodology that utilizes sodium halides as the source of electrophilic halogens when reacted along with copper(II) sulfate. This environmentally benign methodology is facile, uses ethanol as the solvent, and results in 3-halo substituted benzo[b]thiophene structures in very high yields. The cyclohexanol-substituted 3-chloro and 3-bromobenzo[b]thiophenes resulted in a low MIC of 16 µg/mL against Gram-positive bacteria and yeast. Additionally, in silico absorption, distribution, metabolism, and excretion (ADME) properties of the compounds were determined. The compounds with the lowest MIC values showed excellent drug-like properties with no violations to Lipinski, Veber, and Muegge filters. The time-kill curve was obtained for cyclohexanol-substituted 3-chlorobenzo[b]thiophenes against Staphylococcus aureus, which showed fast bactericidal activity at MIC.

Keywords: antimicrobial; antibacterial; antifungal; benzo[b]thiophene; ADME; time-kill

1. Introduction

There has been an increasing concern over the rapidly spreading resistance to existing antibiotics and antifungal drugs [1,2]. The antimicrobial resistance has been driven by antimicrobial exposure via underuse, overuse, and misuse in health care (human and veterinary medicine), agriculture, aquaculture, and the environment [3]. Antibiotic resistance threats report at least 18 antibiotic-resistant strains of bacteria and fungi. According to the Centers for Disease Control (CDC), 2.8 million people in the United States are infected with antibiotic-resistant bacteria or fungi each year, and over 35,000 of them die. The estimated national cost to treat infections in the USA alone is more than USD 4.6 billion annually [4]. The resistant microorganisms rapidly develop to evade antimicrobial effects by a wide range of complex biochemical and physiological mechanisms [5]. Unfortunately, there has not been a new class of antibiotics approved since the discovery of daptomycin in 1986 [6]. Therefore, we must continue to find new classes of antibiotics to keep up with the ever-changing evolution of pathogens.

Due to its diverse biological activities and drug-like properties, benzo[b]thiophene could be an interesting and potential pharmacophore to explore. These core structures are found in naturally occurring organic molecules and exist as a component of several...
Drug molecules, such as raloxifene, zileuton, and sertaconazole. Benzo[b]thiophenes exhibit a wide range of biological activities including antimicrobial [7], antifungal [8,9], anticancer [10], antidepressant [13,14], anti-inflammatory [15,16], antioxidant [17], antitubercular [18,19], and anticonvulsant [20]. In addition, benzo[b]thiophene derivatives act as histamine H3 antagonists [19], fatty acid amide hydrolase (FAAH) inhibitors [20], and rho kinase inhibitors [21]. Due to its aforementioned biological properties, benzo[b]thiophenes have not been explored. There is only one study by Liger and coworkers that showed an efflux pump resulting in resistance to fluoroquinolones (e.g., ciprofloxacin), quaternary ammonium compounds, biocides, and dyes [43,44]. However, more research is needed to realize the full potential of benzo[b]thiophene compounds, biocides, and dyes [43,44].

Some previously known benzo[b]thiophene derivatives, which more commonly contained other complex pharmacophores, such as quinazolines, coumarins, pyrimidines, carbamates, ureas, semicarbazides, and pyrazoles [7,9,27-32]. In the literature, the anti-microbial activity of benzo[b]thiophene derivatives appeared to be more dependent on substitution at the heterocyclic thiophene ring rather than at the aromatic moiety [18]. The correct placement of the substituents at the third position of the benzo[b]thiophene ring is the key to harnessing the desired antimicrobial activity (Figure 1). In the past, researchers have demonstrated that amine [8], amide [31,33], methyl [29,34], ether, and nitrile [35] functionalities enhance the desired antimicrobial activity of the benzo[b]thiophene rings. In addition, there have been several reports of the improved antimicrobial activity with the presence of chlorine in the third position [32,36,37]. However, there have been no systematic studies on the effect of other halogens at the third position. Halogen-containing carbo- and heterocycles comprise approximately 40% of drugs that are currently undergoing clinical trials or have been approved as drugs [38,39]. The halogen atoms, particularly chlorine and fluorine, could play important roles in significantly improving the drug-target binding affinity and absorption, distribution, metabolism, and excretion (ADME) properties of a molecule [40,41].

![Figure 1. Some previously known benzo[b]thiophene derivatives exhibiting antimicrobial activities.](image-url)

In the literature, the targets for benzo[b]thiophenes exhibiting antimicrobial properties have not been explored. There is only one study by Liger and coworkers that showed C2-arylated benzo[b]thiophene derivatives as a potent NorA pump inhibitor [42]. NorA is an efflux pump resulting in resistance to fluoroquinolones (e.g., ciprofloxacin), quaternary ammonium compounds, biocides, and dyes [43,44]. However, more research is needed to realize the full potential of benzo[b]thiophene and its derivatives and to better understand the unique biological properties of this interesting heterocycle.

Therefore, we decided to conduct a systematic structure–activity relationship (SAR) study of various 3-halo substituted (halo = Cl, Br and I) benzo[b]thiophene molecules for their antibacterial and antifungal activities to better understand the regiochemical effect of
the halogen moiety. Unfortunately, we did not include fluoro-substituted benzo[b]thiophenes because the synthesis of 3-fluoro analogues of benzo[b]thiophenes has proven to be challenging, and our electrophilic cyclization methodology did not result in the desired fluoro-substituted product. We hereby report the synthesis of novel and simpler halogenated benzo[b]thiophene derivatives and the evaluation of their antimicrobial activity and in silico ADME properties. The time-kill kinetics of the selected compound with the lowest minimum inhibitory concentration (MIC) was further investigated.

2. Results and Discussion

2.1. Synthesis of Benzo[b]thiophene Derivatives

The desired 3-halo substituted benzo[b]thiophene derivatives were synthesized using electrophilic cyclization reactions. Electrophilic cyclization of alkynes is a reaction that involves the activation of a C-C triple bond via halogen, boron, sulfur, and selenium electrophiles to undergo cyclization with an internally tethered C, O, N, P, S, or Se nucleophile. Electrophilic cyclization reactions have gained much attention in recent years due to their simplicity in generating various halogenated heterocycles with ease [45]. Larock and others have reported a simple two-step synthesis of 3-iodo- and 3-bromosubstituted benzo[b]thiophenes starting from readily available 2-iodothioanisole [46,47]. The first step is the synthesis of 2-alkynylthioanisole via a Sonogashira coupling reaction involving terminal alkyn and 2-iodothioanisoles. The second step consists of the cyclization reaction using I₂, ICl, Br₂, or N-bromo succinimide (NBS) electrophiles. Recently, Kesharwani and co-workers demonstrated that the seemingly difficult chlorocyclization of 2-alkynylthioanisole could easily be achieved using table salt as the source of electrophilic chlorine in the presence of copper sulfate in ethanol. This environmentally benign method has also been demonstrated to work for bromo- and iodocyclization by changing sodium chloride to sodium bromide and sodium iodide, respectively [48–50].

We employed the green halocyclization reaction above to synthesize a series of 3-halo substituted benzo[b]thiophenes as depicted in Scheme 1. The synthesis of benzo[b]thiophenes 10–27 has already been reported earlier [48–50]. However, the synthesis of 2-cyclohexyl, 2-(1,1-dimethylmethanol), and 2-cyclopentanol substituted 3-chloro and 3-bromobenzo[b]thiophene derivatives 28–32 have not been reported in the literature, and we report their first synthesis herein. The cyclization reaction of cyclohexyl substituted alkynyl thioanisole 7 with sodium chloride and sodium bromide worked with ease to give the corresponding 3-chloro and 3-bromo benzo[b]thiophene derivatives 28 and 29 in 90% and 92% yields, respectively. The cyclization reaction of substituted propargyl alcohol 8 resulted in the formation of 2-(3-chlorobenzo[b]thiophen-2-yl)propan-2-ol (30), and its bromo analogue 31 in excellent yields of 77% and 85%, respectively. The chlorocyclization of alkyn 9 resulted in a lower yield of 35% of the desired benzo[b]thiophene 32. However, our numerous synthetic efforts to cyclize 9 with Br electrophile failed, and the desired bromocyclized product 33 could not be obtained under various conditions. In addition to our green bromocyclization reaction condition, we employed other bromocyclization conditions involving electrophiles such as Br₂ and NBS. The final product, 33, seems to be very unstable; the alcohol moiety quickly dehydrates into the corresponding alkene. All of the synthesized compounds (10–33) were analyzed using ¹H NMR, ¹³C NMR, and high-resolution mass spectrometry (HRMS). The details of analysis of the newly reported molecules are provided in the Supplementary Materials.
positive bacteria, namely *S. aureus*, and *C. albicans*.

To evaluate the significance of the alcohol group, we chose to synthesize and evaluate cyclohexane substituted derivatives. The 2-halogeno cyclohexene substituted benzo[**b**]thiophene derivatives, with halogen moieties in various positions, were screened against all the Gram-positive bacteria and the fungus tested. It is known that alkene moieties can undergo hydration under biological conditions, thus further suggesting that the alcohol group could play a significant role in the inhibitory activity of these benzo[b]thiophene derivatives. Based on the encouraging activity of methyl alcohol and cyclohexene substituted benzo[b]thiophene derivatives, we decided to screen 2-(1-cyclohexanol)-3-halobenzo[b]thiophene derivatives 25–27. The chloro- and bromo-containing cyclohexanol compounds, 25 and 26, had a low MIC of 16 µg/mL against *B. cereus*, *S. aureus*, *E. faecalis*, and *C. albicans*. It was also concluded that out of the three halogens employed, only the bromo- and the chloro-substituted benzo[b]thiophene were active, whereas the iodo substitution did not demonstrate any inhibitory activity up to MIC > 512 µg/mL. We decided not to include the iodo-substituted derivatives further in compounds 28–33, as the previously iodo-substituted compound 27 did not show any significant antimicrobial activity compared to bromo-(26) and chloro-substituted (25) derivatives. To evaluate the significance of the alcohol group, we chose to synthesize and evaluate cyclohexane substituted benzo[b]thiophene derivatives 28–29, which showed insignificant or no activity against

![Scheme 1](image-url)
bacteria (MIC > 512 µg/mL) except for 2-cyclohexyl-3-chlorobenzo[b]thiophene (28), which resulted in a high MIC of 512 µg/mL against C. albicans. This result showed that alcohol contributed significantly to the inhibitory activity of 3-halo substituted benzo[b]thiophene compounds, 25 and 26, against B. cereus, S. aureus, E. faecalis, and C. albicans. Finally, we decided to change the cyclohexanol group to cyclopentanol and 2-hydroxypropan-2-yl groups to evaluate further whether the cyclohexanol group is required for high inhibition of bacteria and fungi. The 2-(hydroxypropan-2-yl)-3-chlorobenzo[b]thiophene 30 and its bromo analogue 31 resulted in a higher MIC value when compared with corresponding cyclohexanol derivatives 25 and 26. The MIC value for 2-hydroxypropan-2-yl was 64 µg/mL, whereas the MIC value for cyclopentanol was 128 µg/mL against S. aureus, E. faecalis, and C. albicans.

Table 1. MIC value against Gram-positive bacteria and fungi. The concentration is provided in µg/mL. The maximum concentration tested was 512 µg/mL. If the compound showed no/less inhibition until 512 µg/mL, the MIC is referred to as > 512 µg/mL.

| Compound | Bacteria | Fungi |
|----------|----------|-------|
|          | S. aureus | E. faecalis | B. cereus | C. albicans |
| 10       | >512      | >512    | >512      | >512        |
| 11       | >512      | >512    | >512      | >512        |
| 12       | >512      | >512    | >512      | >512        |
| 13       | >512      | >512    | >512      | >512        |
| 14       | >512      | >512    | >512      | >512        |
| 15       | >512      | >512    | >512      | >512        |
| 16       | >512      | >512    | >512      | >512        |
| 17       | >512      | >512    | >512      | >512        |
| 18       | >512      | >512    | >512      | >512        |
| 19       | 256       | 256     | 128       | 128         |
| 20       | >512      | >512    | >512      | >512        |
| 21       | >512      | >512    | >512      | >512        |
| 22       | 512       | 512     | 512       | 512         |
| 23       | 512       | 512     | 512       | 256         |
| 24       | >512      | >512    | >512      | >512        |
| 25       | 16        | 16      | 16        | 16          |
| 26       | 16        | 16      | 16        | 32          |
| 27       | >512      | >512    | >512      | >512        |
| 28       | >512      | >512    | >512      | >512        |
| 29       | >512      | >512    | >512      | >512        |
| 30       | 64        | 64      | 16        | 64          |
| 31       | 64        | 128     | 32        | 64          |
| 32       | 128       | 128     | 64        | 128         |
| Ampicillin | 8        | 8       | 32        | -           |
| Chloramphenicol | 8        | 4       | 2         | -           |
| Kanamycin  | 2        | 32      | 2         | -           |
| Fluconazole | -       | -       | 0.5       | -           |

With all of the above-mentioned inhibitory studies, we concluded that the most active compound had a chloro or bromo halogen group at the third position, and the MIC value was non-different between chloro 25 and bromo derivative 26. However, chloro-substituted methyl alcohol 19 was most active and 2-(hydroxypropan-2-yl)-3-chlorobenzo[b]thiophene 30 was only slightly more active than its bromo analogue 31 in E. faecalis and B. cereus. In contrast, the iodine-containing molecules did not show any antimicrobial activity until the highest concentration tested. It was also determined that the hydroxymethyl group at the second position of the benzo[b]thiophene seems to be important for the inhibitory activity against the Gram-positive bacteria and C. albicans. The cyclohexanol structure worked the best, but cyclopentanol and 2-hydroxypropan-2-yl groups were not as effective. It should also be noted that none of the tested compounds showed any activity against Gram-negative bacteria, even at the highest concentration. Gram-negative bacteria have an additional
outer membrane structure with the outer-lipid leaflet containing lipopolysaccharides [53]. This makes Gram-negative bacteria more resistant to antimicrobial agents that are unable to cross this additional layer [54] as shown by several studies [55,56].

2.3. Time-Kill Kinetics

The cyclohexanol- and halogen- (bromine and chlorine) containing benzo[b]thiophenes, 25 and 26, exhibited the lowest MIC value. However, the microdilution assay provides an end-point result that does not give enough information about the antimicrobial kinetics or whether the tested compound is bactericidal (killing an organism) or bacteriostatic (stalling the growth of an organism). Therefore, we decided to further evaluate the dose and time-dependent killing kinetics of the chloro-substituted cyclohexanol benzo[b]thiophene derivative 25 against S. aureus at 0.5× (8 μg/mL), 1× (16 μg/mL), and 2× (32 μg/mL) MIC using the time-kill assay [57]. The time-kill assay permits determining the rate of change in the number of viable bacteria compared to the initial starting inoculum at different concentrations of the compound of interest. A time-kill curve for compound 25 against S. aureus was plotted as log_{10} CFU/mL versus time (Figure 2). The cutoff point for determining the minimum bactericidal concentration (MBC) was ≥ 3 log_{10} CFU/mL reduction from the starting bacterial density [58].

![Figure 2. Time-kill curve for compound 25 against S. aureus. The data in the graph are depicted as mean ± S.D (n = 3, 4).](image)

The time-kill kinetics of compound 25 displayed rapid bactericidal activity towards S. aureus at both MIC and twice the MIC concentration within 1 hour of exposure, which resulted in >3 log_{10} reduction in viable cell count relative to the initial inoculum (Figure 2). However, 0.5× MIC concentration decreased the viable cells but did show ≤ 3 log_{10} reduction. The time-kill assay for compound 25 was consistent with bactericidal characteristics against S. aureus. Thus, compound 25 was determined to be a potent bactericidal agent against S. aureus.

2.4. In Silico ADME Properties

We analyzed the in silico physicochemical and pharmacokinetic properties of the benzo[b]thiophene derivatives with lower MIC values (25, 26, 30, 31, and 32) using the freely available Swiss ADME tool. This software gives access to a pool of fast yet modest predictive models that utilize simple molecular and physicochemical descriptors, such as molecular weight (MW), the count of specific types of bonds (the numbers of heavy atoms, aromatic heavy atoms, rotatable bonds, hydrogen-bond acceptors, hydrogen-bond donors), topological polar surface area (TPSA), and several others, which are vital determinants to predict good drug/lead-like molecules [59]. The key calculated/predicted values of
common physicochemical parameters of the benzo[\(b\)]thiophene derivatives are shown in Table 2.

Table 2. In silico calculated physicochemical parameter values for benzo[\(b\)]thiophene derivatives using Swiss ADME software.

| Compound | MW a | nHA b | nAHA c | nRotB d | nHBA e | nHBD f | MR g | TPSA h | MLOGP i | ESOL j |
|----------|------|-------|--------|---------|--------|--------|------|--------|---------|--------|
| 25       | 266.8| 17    | 9      | 1       | 1      | 1      | 74.77| 48.47  | 3.82    | MS     |
| 26       | 311.2| 17    | 9      | 1       | 1      | 1      | 77.46| 48.47  | 3.95    | MS     |
| 30       | 226.7| 14    | 9      | 1       | 1      | 1      | 62.46| 48.47  | 3.03    | S      |
| 31       | 271.2| 14    | 9      | 1       | 1      | 1      | 65.15| 48.47  | 3.17    | MS     |
| 32       | 252.8| 16    | 9      | 1       | 1      | 1      | 69.96| 48.47  | 3.57    | MS     |

a Molecular weight (MW), \(^b\) number of heavy atoms (nHA), \(^c\) number of aromatic heavy atoms (nAHA), \(^d\) number of rotatable bonds (nRotB), \(^e\) number of hydrogen bond acceptors (nHBA), \(^f\) number of hydrogen bond donors (nHBD), \(^g\) molecular refractivity (MR), \(^h\) topological polar surface area (TPSA), \(^i\) octanol/water partition coefficient (MLOGP), and \(^j\) ESOL (estimated SOLubility) with MS moderately soluble and S representing soluble.

The physicochemical parameters (shown in Table 2) were used to predict the drug-likeness of a molecule. The Lipinski rule-of-five is the most widely used rule-based filter of drug-likeness, which filters the molecules on a range of parameters, namely, molecular weight (MW) < 500 g/mol, hydrogen bond donors (HBDs) < 5, hydrogen bond acceptors (HBAs) < 10, and a logarithm of the octanol/water partition coefficient < 5 or (MlogP < 4.15) \[60\]. In addition, the number of rotatable bonds (nRotB) of \(\leq 10\) and a topological polar surface area (TPSA) of \(\leq 140 \text{Å}^2\) have been included in the Veber filter \[61\]. Swiss ADME software also includes Ghose (Amgen) \[62\], Egan (Pharmacia) \[63\], and Muegge (Bayer) \[64\] filters for drug-likeness predictions. Table 3 shows the results of the drug-likeness predictions based on all five filters. Interestingly, all analyzed compounds showed excellent drug-likeness with all five filters, with no violation of any of the physicochemical parameters. The molecules were also analyzed to identify potentially problematic or promiscuous fragments that could be putatively unstable, reactive, toxic, or prone to interfere with biological assays. Two complementary pattern recognition methods were implemented, namely PAINS (for pan assay interference structures) \[65\] and Brenk alerts \[66\]. None of the compounds analyzed showed any PAINS or Brenk alerts (Table 3).

Table 3. In silico, calculated drug-likeness values for benzo[\(b\)]thiophene derivatives using Swiss ADME software.

| Compound | Lipinski | Ghose | Veber | Egan | Muegge | PAINS \(^a\) | Brenk |
|----------|----------|-------|-------|------|--------|-------------|-------|
| 25       | Yes      | Yes   | Yes   | Yes  | Yes    | 0           | 0     |
| 26       | Yes      | Yes   | Yes   | Yes  | Yes    | 0           | 0     |
| 30       | Yes      | Yes   | Yes   | Yes  | Yes    | 0           | 0     |
| 31       | Yes      | Yes   | Yes   | Yes  | Yes    | 0           | 0     |
| 32       | Yes      | Yes   | Yes   | Yes  | Yes    | 0           | 0     |

\(^a\) PAINS (for pan assay interference structures).

In addition, the bioavailability radar plot of the compounds is shown in Figure 3. The pink area of the radar shows an optimum range of six physicochemical properties acceptable for drug-likeness. All of the analyzed compounds, 25, 26, 30, 31, and 32, were entirely within the pink area of the radar plot and thus considered to be drug-like.

Further, the pharmacokinetic properties predicted for the benzo[\(b\)]thiophene derivatives are included in Table 4, namely log of skin permeability (log Kp), blood–brain barrier (BBB) penetration, and gastrointestinal (GI) absorption. All of the compounds analyzed were predicted to have high GI absorption and BBB penetration; thus, these compounds could find applications in the treatment of brain-related infections.
Pharmaceuticals 2022, 15, x FOR PEER REVIEW...ives. In addition, all compounds except 30 and 31 were found to inhibit CYP2C9. Based on the protein interaction with P-gp and cytochrome P450 isoforms, hydroxypropan-2-yl derivatives 30 and 31 have the most favorable pharmacokinetic properties.

Table 4. In silico pharmacological properties of benzo[b]thiophene derivatives predicted using Swiss ADME software.

| Compound | GI a Absorption | BBB b Permeant | P-gp c Substrate | CYP1A2 Inhibitor | CYP2C19 Inhibitor | CYP2C9 Inhibitor | CYP2D6 Inhibitor | CYP3A4 Inhibitor | Log Kp (cm/s) |
|----------|-----------------|----------------|------------------|------------------|------------------|------------------|------------------|------------------|---------------|
| 25       | High            | Yes            | Yes              | Yes              | Yes              | Yes              | Yes              | No               | −4.86         |
| 26       | High            | Yes            | Yes              | Yes              | Yes              | Yes              | Yes              | No               | −5.09         |
| 30       | High            | Yes            | No               | Yes              | Yes              | No               | No               | No               | −5.33         |
| 31       | High            | Yes            | No               | Yes              | Yes              | No               | No               | No               | −5.56         |
| 32       | High            | Yes            | Yes              | Yes              | Yes              | Yes              | Yes              | No               | −5.16         |

a GI (gastrointestinal), b BBB (blood–brain barrier), c P-gp (permeability glycoprotein).

Additionally, the molecules were analyzed for their interactions with pharmaceutically important proteins, including permeability glycoprotein (P-gp) and Cytochrome P450 (CYP). P-gp primarily functions as an active efflux transporter and is widely distributed in the small intestine, blood–brain barrier capillaries, and several critical organs such as the kidney and liver [67]. It is associated with the efflux of xenobiotics from the brain and multi-drug resistance in cancer cells [68]. The compounds with the lowest MIC values, 25 and 26, were substrates for P-gp, whereas hydroxypropan-2-yl derivatives 30 and 31 were not the substrates for P-gp. Thus, a substrate of P-gp could be rendered less effective through efflux [69].

Cytochrome P450 (CYP450) is a superfamily of enzymes that plays a crucial role in the metabolism of drugs, steroids, fat-soluble vitamins, carcinogens, pesticides, and many other chemicals [70]. More than 50 isoforms of CYP enzymes exist, with 1A2, 2C9, 2C19, 2D6, and 3A4 isoforms accounting for over 90% of oxidative metabolic processes [71]. During drug development, studying the inhibitory activity of proposed derivatives against certain CYP isoforms is helpful to determine whether the molecules would be efficiently metabolized and cleared. Table 4 shows the results of the inhibitory prediction for five CYP isoforms. None of the proposed compounds inhibited CYP3A4, whereas just five derivatives were found to inhibit CYP2D6. Both CYP1A2 and CYP2C19 were found to be inhibited by all of the benzo[b]thiophenes derivatives. In addition, all compounds except 30 and 31 were found to inhibit CYP2C9. Based on the protein interaction with P-gp and cytochrome P450 isoforms, hydroxypropan-2-yl derivatives 30 and 31 have the most favorable pharmacokinetic properties.
In summary, in silico analysis predicted drug-like properties, high GI absorption, and BBB penetration for compounds 25, 26 (with the lowest MIC), 30, 31, and 32 (with intermediate MIC). In addition, compounds 30 and 31 showed excellent pharmacokinetic properties, including not being a P-gp substrate and not being CYP2C9, CYP2D6, or CYP3A4 inhibitors. These predictions would help us to design future benzo[b]thiophene derivatives.

3. Materials and Methods

A Bruker spectrometer operating at 400 and 100 MHz was used to record $^1$H and $^{13}$C NMR spectra, respectively. Electron ionization (EI) and direct probe sample introduction were used in a VG-70S magnetic sector mass spectrometer for recording high-resolution mass spectra (HRMS). Thin-layer chromatography was performed using glass plates coated with silica gel 60 F$_{254}$, and short-wave UV light was used to visualize the molecules to monitor the progress of reactions. ACS-grade hexanes and ethyl acetate were used as the eluent for flash chromatography, and silica gel (60–120 mesh) was used as the stationary phase. Benzo[b]thiophenes 10–27 were synthesized according to procedures in the literature, and the characterization data were in good agreement with previously reported data [48,49].

3.1. General Procedure for the Electrophilic Cyclization Reaction

In a 6-dram vial equipped with a magnetic stir bar, 2-alkynylthioanisole (0.3 mmol) and CuSO$_4$·5H$_2$O (1.5 mmol) were added, followed by 5 mL of EtOH. Finally, the desired sodium halide (1.5 mmol) was added to the reaction mixture in one portion, with continued stirring overnight. The reaction mixture was filtered using celite and concentrated under vacuum. The resulting concentrated reaction mixture was absorbed on silica gel, and the final product was purified by column chromatography using hexanes and ethyl acetate as the eluent.

3.1.1. 3-chloro-2-cyclohexylbenzo[b]thiophene (28)

Product was isolated as a pale yellow oil: $^1$H NMR (400 MHz, chloroform-d) $\delta$1.25–1.40 (m, 1H), 1.40–1.58 (m, 4H), 1.75–1.85 (m, 1H), 1.85–1.95 (m, 2H), 2.00–2.13 (m, 2H), 3.18–3.30 (m, 1H), 7.34 (td, $J =$ 1.2, 7.6 Hz, 1 H), 7.42 (td, $J =$ 0.8, 7.6 Hz, 1 H), 7.50–7.80 (m, 2H); $^{13}$C NMR (100 MHz, chloroform-d) $\delta$26.0, 26.7, 34.1, 38.5, 115.7, 121.4, 122.7, 124.7, 124.9, 136.3, 137.3, 145.3 HRMS (EI$^+$, $m/z$) calcd for (C$_{14}$H$_{15}$ClS)$^+$ 250.0583, found 250.0585.

3.1.2. 3-bromo-2-cyclohexylbenzo[b]thiophene (29)

Product was isolated as a pale yellow oil: $^1$H NMR (400 MHz, chloroform-d) $\delta$1.30–1.40 (m, 1H), 1.40–1.60 (m, 4H), 1.75–1.85 (m, 1H), 1.85–1.98 (m, 2H), 2.05–2.18 (m, 2H), 3.19–3.33 (m, 1H), 7.34 (td, $J =$ 1.2, 8.0 Hz, 1 H), 7.43 (td, $J =$ 0.8, 7.2 Hz, 1 H), 7.76–7.81 (m, 2H); $^{13}$C NMR (100 MHz, chloroform-d) $\delta$26.1, 26.8, 34.3, 40.2, 104.2, 122.7, 122.8, 124.7, 124.9, 136.3, 138.6, 147.2; HRMS (EI$^+$, $m/z$) calcd for (C$_{14}$H$_{15}$BrS)$^+$ 294.0078, found 294.0082.

3.1.3. 2-(3-chlorobenzo[b]thiophen-2-yl)propan-2-ol (30)

Product was isolated as an off-white solid: mp 99–101 °C; $^1$H NMR (400 MHz, chloroform-d) $\delta$1.81 (s, 6H), 2.36 (s, 1H), 7.36 (td, $J =$ 1.2, 8.0 Hz, 1H), 7.43 (t, $J =$ 8.0 Hz, 1H), 7.77 (d, $J =$ 8.0 Hz, 2H); $^{13}$C NMR (100 MHz, chloroform-d) $\delta$29.9, 72.9, 113.9, 121.7, 122.7, 125.1, 125.3, 135.7, 138.6, 147.1; HRMS (EI$^+$, $m/z$) calcd for (C$_{11}$H$_{11}$ClOS)$^+$ 226.0219, found 226.0214.

3.1.4. 2-(3-bromobenzo[b]thiophen-2-yl)propan-2-ol (31)

Product was isolated as an off-white solid: mp 92–94 °C; $^1$H NMR (400 MHz, chloroform-d) $\delta$1.83 (s, 6H), 2.22 (bs, 1H), 7.36 (td, $J =$ 1.2, 7.2 Hz, 1H), 7.43 (dt, $J =$ 0.8, 7.2 Hz, 1H), 7.78 (m, 2H); $^{13}$C NMR (100 MHz, chloroform-d) $\delta$29.9, 73.2, 101.5, 122.5, 123.0, 125.3, 125.4, 136.3, 140.1, 148.9; HRMS (EI$^+$, $m/z$) calcd for (C$_{11}$H$_{11}$BrOS)$^+$ 269.9714, found 269.9726.
3.1.5. 1-(3-chlorobenzothiophen-2-yl)cyclopentan-1-ol (32)

Product was isolated as a pale yellow oil; $^1$H NMR (400 MHz, chloroform-$d$) $\delta$ 1.85–2.08 (m, 4H), 2.08–2.19 (m, 2H), 2.31–2.50 (m, 3H), 7.36 (t, $J = 7.2$ Hz, 1H), 7.43 (t, $J = 7.6$ Hz, 1H), 7.77 (d, $J = 8.0$ Hz, 1H), 7.78 (d, $J = 8.0$ Hz, 1H); $^{13}$C NMR (100 MHz, chloroform-$d$) $\delta$ 24.3, 41.1, 114.9, 121.6, 122.6, 125.2, 125.3, 135.9, 138.5, 144.9.

3.2. Chemicals and Microbial Strains

The stock solutions of the compounds to be tested for antimicrobial activity were prepared in dimethyl sulfoxide (DMSO) as solvent and stored at −20 °C. The antimicrobial activity was tested against a total of six bacterial strains (three Gram-positive and three Gram-negative bacteria) and one fungal strain, namely *Bacillus cereus* (ATCC 10876), *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27853), and *Candida albicans* (ATCC 90028). All bacterial and fungal strains were purchased from American Type Culture Collection (ATCC). The cells were maintained according to the recommendation of CLSI in tryptic soy agar (TSA, BD Bacto™ DF0370173) for bacteria and potato dextrose agar (PDA, BD Difco DF0013176) plates for fungi [51,52].

3.3. Determination of Minimum Inhibitory Concentration Values

The broth micro-dilution method was used to determine the minimum inhibitory concentration (MIC) values in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines [51,52]. In summary, the test compounds were serially twofold diluted into 96-well microplates with cation-adjusted Muller Hinton Broth (CAMHB, BD BBL™ Cat number B12322) for bacteria and RPMI-1640 (Bioworld 306110981) for the fungus to achieve the final range of test concentrations of $512–8\ \mu g/mL$. The inoculum suspension was prepared by the colony-pick method, and the turbidity was adjusted to 0.5 McFarland standard. This suspension was further adjusted to obtain the final inoculum of $5 \times 10^5$ CFU/mL per well for bacteria and $2.5 \times 10^3$ CFU/mL per well for fungus. The 96-well plates were sealed and incubated for 16–18 h at 35 °C for bacteria and 24–48 h at 35 °C for fungus. Once the plates were retrieved from the incubator, the absorbance was read at 600 nm wavelength using the PerkinElmer instrument program. The MIC was determined as the lowest concentration of test compound able to inhibit the visible growth of bacteria. The concentration range tested for the 3-halobenzothiophenones was $512–8\ \mu g/mL$. All tests were performed in triplicate, and the highest MIC value obtained was reported. Four controls comprising medium with standard antibiotic (positive control), medium with DMSO (solvent control), medium with inoculum bacterial cells (negative control), and medium with broth only (negative growth control) were included in each test. Ampicillin, chloramphenicol, and kanamycin were the standard antibiotics used for antibacterial studies, while fluconazole was used as a standard for the antifungal studies.

3.4. Time-Kill Assay

Time-kill assays were performed using the broth macro-dilution method in accordance with the CLSI manual M-26A [57]. The experiments were performed in triplicate. Inoculum suspensions with approximately $5 \times 10^5$ CFU/mL of exponentially growing bacterial cells were used in this study. The test compound was two-fold serially diluted in borosilicate glass test tubes using CAMHB with final concentrations corresponding to $0.5 \times$ MIC, MIC, and $2 \times$ MIC value. A growth control comprising the bacterial strain without the test compound was included in each trial.

The inoculum cultures were incubated at 35 °C, and 100 µL aliquots were removed from the test tubes after timed intervals of incubation (i.e., 0, 1, 2, 4, 8, 12, and 24 h). The aliquots were serial tenfold diluted in saline as needed and plated on tryptic soy agar (TSA) plates. All plates were incubated at 35 °C for 24 h. The numbers of viable cells were determined by the plate count technique. Data were analyzed by plotting the $\log_{10}$ colony
forming unit per milliliter (CFU/mL) versus time (hours). In the time-kill curve, the change in bacterial concentration is analyzed over time. The bactericidal activity of a compound is defined as the reduction of viable bacterial cell count $\geq 3 \log_{10} \text{CFU/mL}$ as compared to the initial inoculum, while bacteriostatic activity corresponds to $<3 \log_{10} \text{CFU/mL}$ decrease in viable bacterial cell count relative to the initial inoculum.

3.5. Predicted In Silico ADME Properties

Swiss ADME software (https://www.swissadme.ch, accessed on 9 November 2021) was used to predict the physicochemical and pharmacokinetic properties of all 23 compounds in the study; these properties are vital determinants to predict a good drug/lead-like molecule [59].

4. Conclusions

In this study, we concluded that the bromo- and chloro-substituted cyclohexanol benzo[b]thiophene derivatives (25 and 26) showed the lowest MIC activity against Gram-positive bacteria ($S. aureus$, $E. faecalis$, and $B. cereus$) and $C. albicans$. In addition, compound 25 showed rapid bactericidal activity against $S. aureus$ at MIC. Using in silico methods, both 25 and 26 compounds were found to exhibit excellent drug-like properties, high GI absorption, and BBB penetration. Our data suggest that compound 25 could be a potent antibacterial and antifungal candidate, deserving of further investigation and mechanistic studies. In addition, the alcohol substitution seemed to enhance, whereas the iodo-substitution seemed to decrease, the antimicrobial activity of the benzo[b]thiophene derivatives of the compounds. The synthesized simple novel compounds possessed interesting attributes that could justify further confirmatory reactions to increase the number of new derivatives in future studies.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/ph15010039/s1. Figure S1. $^1$H NMR spectra of 25; Figure S2. $^{13}$C NMR spectra of 25; Figure S3. $^1$H NMR spectra of 26; Figure S4. $^{13}$C NMR spectra of 26; Figure S5. $^1$H NMR spectra of 28; Figure S6. $^{13}$C NMR spectra of 28; Figure S7. $^1$H NMR spectra of 29; Figure S8. $^{13}$C NMR spectra of 29; Figure S9. $^1$H NMR spectra of 30; Figure S10. $^{13}$C NMR spectra of 30; Figure S11. $^1$H NMR spectra of 31; Figure S12. $^{13}$C NMR spectra of 31; Figure S13. $^1$H NMR spectra of 32; Figure S14. $^{13}$C NMR spectra of 32.

Author Contributions: The authors’ contributions are as follows: Conceptualization, P.J.M. and T.K.; methodology, P.J.M. and T.K.; software, P.J.M.; validation, P.J.M., T.K., E.R., M.A.V., M.L.M., T.K.L., M.K.T.T., A.M.P., M.R.C. and C.T.K.; resources, P.J.M., T.K., E.R., M.A.V., M.L.M., T.K.L., M.K.T.T., A.M.P., M.R.C. and C.T.K.; writing—original draft preparation, P.J.M. and T.K.; writing—review and editing, P.J.M. and T.K.; visualization, P.J.M. and T.K.; supervision, P.J.M. and T.K.; project administration, P.J.M.; funding acquisition, P.J.M. and T.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article and Supplementary Materials.

Acknowledgments: We would like to acknowledge the financial support and resources provided by the Office of Undergraduate Research (OUR), Kugelman Honors Program, and the Department of Biology at the University of West Florida (UWF). We would also like to thank Pamela Benz and Peter Cavnar for providing lab space and resources during the research. The authors would also like to thank Jim Spain for insightful discussions and help with HPLC.

Conflicts of Interest: The authors declare no conflict of interest.
References

1. Guo, Y.; Song, G.; Sun, M.; Wang, J.; Wang, Y. Prevalence and Therapies of Antibiotic-Resistance in Staphylococcus aureus. Front. Cell. Infect. Microbiol. 2020, 10, 107. [CrossRef]

2. Joshi, S.; Shalal, A.; Zervos, M. Vancomycin-Resistant Enterococci: Epidemiology, Infection Prevention, and Control. Infect. Dis. Clin. N. Am. 2021, 35, 953–968. [CrossRef] [PubMed]

3. Holmes, A.H.; Moore, L.S.; Sundsfjord, A.; Steinbakk, M.; Regmi, S.; Karkey, A.; Guerin, P.J.; Piddock, L.J. Understanding the mechanisms and drivers of antimicrobial resistance. Lancet 2016, 387, 176–187. [CrossRef]

4. Centers for Disease Control and Prevention (CDC). Antibiotic Resistance Threats in the United States, 2019; U.S. Department of Health and Human Services, CDC: Atlanta, GA, USA, 2019.

5. Levy, S.B.; Marshall, B. Antibacterial resistance worldwide: Causes, challenges and responses. Nat. Med. 2004, 10, S122–S129. [CrossRef]

6. Durand, G.A.; Raoult, D.; Dubourg, G. Antibiotic discovery: History, methods and perspectives. Int. J. Antimicrob. Agents 2019, 53, 371–382. [CrossRef]

7. Naganagowda, G.; Padmashali, B. Utility of 3-Chlorobenzothiophene-2-carbonyliothiocyanate for the Synthesis of Some Novel Biheterocycles of Expected Biological Activity. Phosphorus Sulfur Silicon Relat. Elem. 2010, 185, 1369–1380. [CrossRef]

8. Pinto, E.; Queiroz, M.-J.R.P.; Vale-Silva, L.A.; Oliveira, J.F.; Begouin, A.; Begouin, J.-M.; Kirsch, G. Antifungal activity of synthetic di(hetero)arylamines based on the benzo[b]thiophene moiety. Bioorganic Med. Chem. 2008, 16, 8172–8177. [CrossRef]

9. Ryu, C.-K.; Lee, S.-K.; Han, J.-Y.; Jung, O.-J.; Lee, J.Y.; Jeong, S.H. Synthesis and antifungal activity of 5-arylamino-4,7-dioxobenzof[b]thiophenes. Bioorganic Med. Chem. Lett. 2005, 15, 2617–2620. [CrossRef]

10. Martorana, A.; Gentile, C.; Perricone, U.; Piccionello, A.P.; Bartolotta, R.; Terenzi, A.; Pace, A.; Mingoia, F.; Almerico, A.M.; Lauria, A. Synthesis, antiinflammatory activity, and in silico insights of new 3-benzoylaminobenzof[b]thiophene derivatives. Eur. J. Med. Chem. 2015, 90, 537–546. [CrossRef]

11. Jianqi, L.; Kai, G.; Na, L. Benzothiophene Alkanol Piperazine Derivatives and Their Use as Antidepressant. U.S. Patent No. 8,680,097, 25 March 2014.

12. Wardakhan, W.W.; Abdel-Salam, O.M.; Elmegeed, G.A. Screening for antidepressant, sedative and anaglesic activities of novel fused thiophene derivatives. Acta Pharm. 2008, 58, 1–14. [CrossRef]

13. Rask-Madsen, J.; Bukhave, K.; Laursen, L.S.; Lauritsen, K. 5-Lipoxygenase inhibitors for the treatment of inflammatory bowel disease. Agents Actions 1992, 36, C37–C46. [CrossRef]

14. Fakhr, I.M.I.; Radwan, M.A.A.; El-Batran, S.; Abd El-Salam, O.M.E.; El-Shenawy, S.M. Synthesis and pharmacological evaluation of 2-substituted benzo[b]thiophenes as anti-inflammatory and analgesic agents. Eur. J. Med. Chem. 2009, 44, 1718–1725. [CrossRef] [PubMed]

15. Ferreira, I.C.; Queiroz, M.J.; Vilas-Boas, M.; Estevinho, L.M.; Begouin, A.; Kirsch, G. Evaluation of the antioxidant properties of diarylamines in the benzo[b]thiophene series by free radical scavenging activity and reducing power. Bioorg. Med. Chem. Lett. 2006, 16, 1384–1387. [CrossRef]

16. Liu, F.; Hu, J.; Chang, L.; Jiang, Y. In vitro anti-mycobacterial activity of novel benzo(c)thiophene-1,3-dione: A novel scaffold against Mycobacterium tuberculosis. Microbiol. Pathog. 2020, 148, 104466. [CrossRef]

17. Chandrasheker, N.S.; Bailey, M.A.; Files, M.; Alling, T.; Florio, S.K.; Ollinger, J.; Odingo, J.O.; Parish, T. Synthesis and antibacterial activity of 3-substituted benzo[b]thiophene-1,1-dioxides. Peerel 2014, 2, e612. [CrossRef]

18. Keri, R.S.; Chand, K.; Budagumpi, S.; Balappa Somappa, S.; Patil, S.A.; Nagaraja, B.M. An overview of benzo[b]thiophene-based medicinal chemistry. Eur. J. Med. Chem. 2017, 138, 1002–1033. [CrossRef]

19. Santillan, A., Jr.; McClure, K.J.; Allison, B.D.; Morton, K.L.; Everson, A.M.; Nepomuceno, D.; Letavic, M.A.; Lee-Dutra, A.; et al. Indole- and benzothiophene-based histamine H3 antagonists. Bioorg. Med. Chem. Lett. 2010, 20, 6226–6230. [CrossRef] [PubMed]

20. Johnson, D.S.; Ahn, K.; Kesten, S.; Lazerverith, S.E.; Song, Y.; Morris, M.; Fay, L.; Gregory, T.; Stiff, C.; Dunbar, J.B.; et al. Benzothiophene piperazine and piperidine urea inhibitors of fatty acid amidase hydrolyase (FAAH). Bioorganic Med. Chem. Lett. 2009, 19, 2865–2869. [CrossRef] [PubMed]

21. Davis, R.L.; Kahraman, M.; Prins, T.J.; Beaver, Y.; Cook, T.G.; Cramp, J.; Cayanar, C.S.; Gardiner, E.M.M.; McLaughlin, M.A.; Clark, A.F.; et al. Benzothiophene containing Rho kinase inhibitors: Efficacy in an animal model of glaucoma. Bioorganic Med. Chem. Lett. 2010, 20, 3361–3366. [CrossRef] [PubMed]

22. Duc, X.D. Recent Progress in the Synthesis of Benzo[b]thiophene. Curr. Org. Chem. 2020, 24, 2256–2271. [CrossRef]

23. Matsuzawa, T.; Hosoya, T.; Yoshida, S. One-step synthesis of benzo[b]thiophenes by aryne reaction with alkynyl sulfides. Chem. Sci. 2020, 11, 9691–9696. [CrossRef]

24. Hari, D.P.; Hering, T.; König, B. Visible Light Photocatalytic Synthesis of Benzothiophenes. Org. Lett. 2012, 14, 5334–5337. [CrossRef] [PubMed]

25. Yan, K.; Yang, D.; Zhang, M.; Wei, W.; Liu, Y.; Tian, L.; Wang, H. Facile Access to Benzothiophenes through Metal-Free Iodine–Catalyzed Intermolecular Cyclization of Thiophenols and Alkynes. Synlett 2015, 26, 1890–1894. [CrossRef]

26. Ulyankin, E.B.; Kostyuchenko, A.S.; Chernenko, S.A.; Bystrushkin, M.O.; Samsonenko, A.L.; Shatsauskas, A.L.; Fisyuk, A.S. A Simple and Efficient Synthesis of Dibenzothiophene Derivatives. Synthesis 2021, 53, 2422–2434.
27. Sajal, S.; Barnali, D. Synthesis and evaluation of some novel thiophenes as potential antibacterial and mycolytic agents. *Der Pharma Chem* 2011, 3, 103–111.

28. Chawla, R.; Arora, A.; Parmeowar, M.K.; Sharma, P.C.; Michael, S.; Ravi, T.K. Synthesis of novel 1, 3, 4-oxadiazole derivatives as potential antimicrobial agents. *Synthesis* 2010, 181, 23.

29. Ferreira, I.C.; Calhelha, R.C.; Estevinho, L.M.; Queiroz, M.J.R. Screening of antimicrobial activity of diarylamines in the 2, 3, 5-trimethylbenzo[b]thiophene series: A structure–activity evaluation study. *Bioorganic Med. Chem. Lett.* 2004, 14, 5831–5833. [CrossRef]

30. Nagesh, H.; Padmeshali, B.; Sandeep, C.; Yuvaraj, T.; Siddesh, M.; Mallikarjuna, S. Synthesis and antimicrobial activity of benzothiophene substituted coumarins, pyrimidines and pyrazole as new scaffold. *Int. J. Pharm. Sci. Res.* 2014, 28, 6–10.

31. Kumara, T.; Mahadevan, K.; Harishkumar, H.; Padmeshali, B.; Naganagowda, G. Synthesis of Benzo[b]thiophene Substituted Carbamates, Ureas, Semicarbazides, and Pyrazoles and Their Antimicrobial and Analgesic Activity. *Phosphorus Sulfur Silicon Relat. Elem.* 2009, 184, 1866–1879. [CrossRef]

32. Naganagowda, G.; Petsom, A. Synthesis and antimicrobial activity of some new 2-(3-chloro-1-benzo[2-yl]-3-(substituted-phenyl)-4-(3H)-quinazolinones derivatives. [PubMed]

33. Gouda, M.A.; Berghot, M.A.; Abd El-Ghani, G.E.; Khalil, A.M. Synthesis and antimicrobial activities of some new thiazole and 5-trimethylbenzo[b]thiophene derivatives based on 4,5,6,7-tetrahydrobenzothiophene moiety. *Eur. J. Med. Chem.* 2010, 45, 1338–1345. [CrossRef] [PubMed]

34. Queiroz, M.J.R.P.; Ferreira, I.C.R.; Gaetano, Y.D.; Kirsch, G.; Calhelha, R.C.; Estevinho, L.M. Synthesis and antimicrobial activity studies of ortho-chlorodiarylamines and heteroaromatic tetracyclic systems in the benzo[b]thiophene series. *Bioorganic Med. Chem.* 2006, 14, 6827–6831. [CrossRef] [PubMed]

35. Fournier dit Chabert, J.; Marquez, B.; Neville, L.; Joucla, L.; Broussous, S.; Bouhours, P.; David, E.; Pellet-Rostaing, S.; Marquet, B.; Moreau, N.; et al. Synthesis and evaluation of new arylenzo[b]thiophene and diarylthiophene derivatives as inhibitors of the NorA multidrug transporter of *Staphylococcus aureus*. *Bioorganic Med. Chem. Med.* 2007, 15, 4482–4497. [CrossRef] [PubMed]

36. Naganagowda, G.; Thamyyongkit, P.; Klai-U-dom, R.; Ariyakriangkrai, W.; Luechai, A.; Petsom, A. Synthesis and biological activity of some more heterocyclic compounds containing benzothiophene moiety. *J. Sulfur Chem.* 2011, 32, 235–247. [CrossRef]

37. Isloor, A.M.; Kalluraya, B.; Sridhar Pai, K. Synthesis, characterization and biological activities of some new benzo[b]thiophene derivatives. *Eur. J. Med. Chem.* 2010, 45, 825–830. [CrossRef] [PubMed]

38. Hernandes, M.Z.; Cavalcanti, S.M.; Moreira, D.R.; de Azevedo Junior, W.F.; Leite, A.C. Halogen atoms in the modern medicinal chemistry: Hints for the drug design. *Curr. Drug Targets* 2010, 11, 303–314. [CrossRef]

39. Voth, A.R.; Khuu, P.; Oishi, K.; Ho, P.S. Halogen bonds as orthogonal molecular interactions to hydrogen bonds. *Nat. Chem.* 2009, 1, 74–79. [CrossRef] [PubMed]

40. Xu, Z.; Yang, Z.; Liu, Y.; Lu, Y.; Chen, K.; Zhu, W. Halogen Bond: Its Role beyond Drug–Target Binding Affinity for Drug Discovery and Development. *J. Chem. Inf. Modeling* 2014, 54, 69–78. [CrossRef] [PubMed]

41. Gillis, E.P.; Eastman, K.J.; Hill, M.D.; Donnelly, D.J.; Meanwell, N.A. Applications of Fluorine in Medicinal Chemistry. *J. Med. Chem.* 2015, 58, 8315–8359. [CrossRef] [PubMed]

42. Liger, F.; Bouhours, P.; Ganem-Elbaz, C.; Jolivalt, C.; Pellet-Rostaing, S.; Popowycz, F.; Paris, J.M.; Lemaire, M. C2 Arylated Benzo[b]thiophene Derivatives as Staphylococcus aureus NorA Efflux Pump Inhibitors. *ChemMedChem* 2016, 11, 320–330. [CrossRef] [PubMed]

43. Bhaskar, B.V.; Babu, T.M.C.; Reddy, N.V.; Rajendra, W. Homology modeling, molecular dynamics, and virtual screening of NorA efflux pump inhibitors of *Staphylococcus aureus*. *Drug Des. Dev. Ther.* 2016, 10, 3237. [CrossRef]

44. Fontaine, F.; Hequet, A.; Voinis-Chiret, A.; Bouillon, A.; Lesnard, A.; Cresteil, T.; Jolivalt, C.; Rault, S. First identification of boronic species as novel potential inhibitors of the *Staphylococcus aureus* NorA efflux pump. *J. Med. Chem.* 2014, 57, 2536–2548. [CrossRef]

45. Godoi, B.; Schumacher, R.F.; Zeni, G. Synthesis of Heterocycles via Electrophilic Cyclization of Alkynes Containing Heteroatom. *Chem. Rev.* 2011, 111, 2937–2980. [CrossRef] [PubMed]

46. Yue, D.; Larock, R.C. Synthesis of 2,3-Disubstituted Benzo[b]thiophenes via Palladium-Catalyzed Coupling and Electrophilic Cyclization of Terminal Acetylenes. *J. Org. Chem.* 2002, 67, 1905–1909. [CrossRef] [PubMed]

47. Larock, R.C.; Yue, D. Synthesis of benzo[b]thiophenes by electrophilic cyclization. *Tetrahedron Lett.* 2001, 42, 6011–6013. [CrossRef]

48. Kim, S.; Dahal, N.; Kesharwani, T. Environmentally benign process for the synthesis of 2, 3-disubstituted benzo[b]thiophenes using electrophilic cyclization. *Tetrahedron* 2013, 54, 4373–4376. [CrossRef]

49. Kesharwani, T.; Kornman, C.; Tonnaer, A.; Haynes, A.; Kim, S.; Dahal, N.; Romero, R.; Royappa, A. Sodium halides as the source of electrophilic halogens in green synthesis of 3-halo- and 3,4-dihalobenzo[b]thiophenes. *Tetrahedron* 2018, 74, 2973–2984. [CrossRef] [PubMed]

50. Kesharwani, T.; Kornman, C.T.; Tonnaer, A.L.; Royappa, A.D. Green synthesis of benzo[b]thiophenes via iron (III) mediated 5-endo-dig iodocyclization of 2-alkynylthioanisoles. *Tetrahedron Lett.* 2016, 57, 411–414. [CrossRef]

51. Clinical and Laboratory Standards Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeasts. In *CLSI Document M27*, 4th ed.; CLSI: Wayne, PA, USA, 2017; p. 46.

52. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. In *CLSI Standard M07*, 11th ed.; CLSI: Wayne, PA, USA, 2018; p. 112.
