Synthesis, in Vitro and in Silico Studies of Some Novel 5-Nitrofuran-2-yl Hydrazones as Antimicrobial and Antitubercular Agents

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In this study, we synthesized two series of novel 5-nitrofuran-2-carbohydrazides 21a–h and 22a–e in addition to a third series of thiophene-2-carbohydrazides 23a–g to develop potent antimicrobial and/or antitubercular agents. The newly synthesized compounds were evaluated in vitro for their antimicrobial and antmycobacterial activities. Most of the 5-nitrofuran-2-carbohydrazides 21a–h and 22a–e displayed variable activity against Aspergillus fumigates, Staphylococcus aureus, Streptococcus pneumoniae, Bacillus subtilis, Salmonella typhimurium, Klebsiella pneumonia, Escherichia coli and Mycobacterium tuberculosis. The sulfonamide derivative 21f exhibited superior potency and broad-spectrum antimicrobial activity with minimum inhibitory concentration (MIC)=0.06–0.98 µg/mL and antitymocobacterial activity with MIC=3.9 µg/mL. The 5-nitrofuran-2-carbohydrazides 21a, b, g, h and 22a–c exhibited significant antibacterial activity with MIC values in the range of 0.12–7.81 µg/mL. The significances of the 5-nitrofuran moiety and sulfonamide function were explored via the structure–activity relationship (SAR) study. In addition, docking studies revealed that the p-amino benzoic acid (PABA) and binding pockets of the dihydropteroate synthase (DHPS) were successfully occupied by compound 21f. Furthermore, two quantitative structure–activity relationship (QSAR) models were built to explore the structural requirements which controlled the activity.

Key words synthesis; 5-nitrofuran; antimicrobial activity; antitubercular agent; quantitative structure–activity relationship (QSAR)

The Infectious Diseases Society of America, in 2010, underlined the urgent need of developing of new antibacterial drugs and it made a global commitment to develop ten novel antimicrobial agents before 2020.1) This urgent response was due to the unacceptable high rate of morbidity and mortality of infectious diseases around the world in addition to the growing threat of antibiotic resistant pathogens.2–4) Unfortunately, there is a drop in the number of authorization of new antimicrobial agents by the regulatory agencies and there is low probability to discover a novel lead compound in the preclinical studies.5) On the other hand, most of the international pharmaceutical companies have been moving away from the area of antimicrobial discovery and the number of scientists involved in the search for novel broad antimicrobial leads was reduced dramatically.6,7)

The discovery of the therapeutic potential of nitrofuran derivatives by Dodd and Stillman in 1944 during their research for antibacterial agents,8) paved the way to design and synthesis various 5-nitrofuran analogs with a broad-spectrum activity against Gram-negative, Gram-positive bacteria and even some protozoa.9) Although the mechanism of action of nitrofurans is not completely understood, previous studies accounted that under anaerobic conditions, the nitro group of the nitrofurans is reduced with formation of toxic free radical.10) So, the incorporation of a nitro group at position 5 of furan ring resulted in a marked increase in antibacterial activity.8) Nifuroxazide 1, Nitrofurantoin 2, Nitfuritoloin 3, Furazolidone 4, Nifuratel 5, Nitrofural (Nitrofurazone) 6 and Nifurzide 7 (Fig. 1) are synthetic members of 5-nitrofuran hydrazone agents that used broadly in Europe, Africa, Middle East and India in the treatment of bacterial infections.11–13) In addition, Nifurtimox 8 (Fig. 1) is often used to treat Chagas’ disease which caused by Trypanosoma cruzi.14) Although, some of the nitrofurans are not therapeutically employed due to their side effects,15) there is a resurgence of interest in the in evaluation of novel nitroheterocycles as antimicrobials.14,16–20)

Recently, several studies pointed out the importance of thiophene based derivatives as antibacterial agents.21–23) Besides, Epimerox is a thiophene containing compound that prevented the growth of several Gram-positive pathogens.24) Moreover, literature survey for functional groups which could be considered as pharmacophores for the antibacterial and antitubercular activities, revealed that the hydrazone moiety is common among many of the antibacterial drugs such as Nifuroxazide 125) (Fig. 1). Moreover, isonicotinoyl hydrazide (INH) and its isonicotinoyl hydrazone analogs are well established antitubercular agents.26–28)

Motivated by these findings, and in continuation of our ongoing interest in the synthesis and biological evaluation of different hydrazides and hydrazones as antimicrobial agents,29,30) it was contemplated to synthesize new series of N-arylpropanehydrazonoyl chlorides derivatives of 5-nitrofuran 21a–h, N’-(1-aryl/thiophenyl)-2-(phenylsulfonyl)ethylidene)-2-carbohydrazides of 5-nitrofuran 22a–e, and N-arylpropane-
hydrazonoyl chlorides derivatives of thiophene 23a–g with the prime aim of developing of potent antimicrobial and/or antitubercular agents. Furthermore, two-dimensional (2-D) quantitative structure–activity relationship (QSAR) models were generated to explore the structural requirements which controlled the different antimicrobial activities. Molecular docking studies were carried out to explain the significant results obtained by the most active compound.

RESULTS AND DISCUSSION

Discussion of Chemistry In continuation of our endeavor towards the development of potent antimicrobial agents,\textsuperscript{29–33} we synthesized a series of novel twenty 5-nitrofuran-2-carbohydrazide and thiophene-2-carbohydrazide derivatives bearing different aryl and heteroaryl rings. The synthetic route was initiated with the preparation of carbohydrazides 11 and 12\textsuperscript{34} (Chart 1). Diazotization of aromatic amines 13a–j with hydrochloric acid and sodium nitrite gave diazonium salts 14a–j which subsequently coupled with 3-chloropentane-2,4-dione (15) in ethanolic sodium acetate (Japp–Klingemann reaction) to afford oxo-N-arylpropanehydrazonoyl chlorides 16a–j, respectively\textsuperscript{35} (Chart 1). Moreover, bromination of ketones 17a–e was performed using cupric bromide to yield the corresponding α-bromo ketones 18a–e,\textsuperscript{36} respectively, which then reacted with sodium benzensulfinate (19a) or sodium toluenesulfinate (19b) to furnish the β-keto sulfones 20a–e, respectively\textsuperscript{37} (Chart 1).

Preparation of the target compounds 21a–h was achieved via the reaction of the appropriate 2-oxo-N’-(4-substitutedphenyl)propanehydrazonoyl chloride 16a–h with 5-nitrofuran-2-carbohydrazide 11 in refluxed tetrahydrofuran.
Next, we aimed to hybridize arylsulphone moiety, as promising class of antimicrobial agents, with 5-nitrofuran scaffold through hydrazone linker. Thus, arylsulphones were condensed with 5-nitrofuran-2-carbohydrazide in ethanol, in the presence of a catalytic amount of acetic acid, to give the corresponding arylsulphones (Chart 2). Finally, different 2-oxo-N"-(4-substitutedphenyl)propanehydrazonoyl chlorides were refluxed with thiophene-2-carboxyhydrazide in THF to furnish the target derivatives in THF (Chart 2). Reagents and conditions: i, EtOH/H₂SO₄/reflux 8 h; ii, NH₂NH₂·H₂O/EtOH/0–5°C/stirring 2 h; iii, NH₂NH₂·H₂O/EtOH/reflux 4 h; iv, HCl/NaNO₂/H₂O/0–5°C; v, CH₃COONa/EtOH/0–5°C; vi, CuBr₂/CHCl₃/EtOAc; vii, EtOH/reflux 3 h.

IR spectra of bis-hydrazones and revealed the presence of stretching vibrations of the carbonyl groups in the region 1636–1696 cm⁻¹, in addition to the absorption bands of two NH functions in the region 3246–3481 cm⁻¹. Chart 1. Synthesis of Compounds 11, 12, 16a–j and 20a–e

Chart 2. Synthesis of Compounds 21a–h and 22a–e
Also their 1H-NMR spectra showed two D2O exchangeable singlet signals in the regions δ 10.05–10.96 and 10.83–11.82 ppm attributable to two NH groups, in addition to the singlet signal of methyl group in the region δ 2.35–2.40 ppm. The sulfonamide 21f and substituted sulfonamide 21g and 21h analogs displayed another D2O exchangeable signal within 7.20–12.65 ppm. Compounds 21b–d, 21f–h, 23a–e and 23e–g were confirmed by their 13C-NMR and showed a characteristic signal resonating at 152.00–188.62 ppm that confirmed the presence of carbonyl group. The analyses of the latter products confirmed the assigned structure 21a–h and 23a–g (Charts 1, 2).

Recently, we reported the X-ray diffraction structure for an analogue of compounds 21, which confirmed the (1Z,2E)-configuration of these bis-hydrazone in solid state.30 On the other hand, structures of sulfones 22a–e were characterized using their IR, 1H-NMR, and MS spectra. The IR spectra of 22a–e exhibited characteristic absorption band at 1680–1700 cm⁻¹ due to C=O group, while that of the sulfonyl functionality was observed in the regions 1150–1158 cm⁻¹ and 1307–1412 cm⁻¹. Their 1H-NMR spectra exhibited the two

The synthesized compounds were also evaluated for their inhibitory activity against a variety of Gram-positive and Gram-negative bacteria and fungi. The table below summarizes the results obtained from the well-diffusion assay.

<table>
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<tr>
<th>Compd</th>
<th>Ar</th>
<th>R or R¹</th>
<th>Fungi</th>
<th>Gram-positive bacteria</th>
<th>Gram-negative bacteria</th>
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</tr>
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<td>20.4±0.58</td>
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<tr>
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</tr>
<tr>
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<td>OMe</td>
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<td>NA</td>
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</tr>
<tr>
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<td>OMe</td>
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<td>NA</td>
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<td>NA</td>
</tr>
<tr>
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<td>21.6±0.22</td>
<td>20.2±0.34</td>
<td>20.3±0.58</td>
<td>20.4±0.14</td>
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<td>CF</td>
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<td>NA</td>
<td>NA</td>
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</tbody>
</table>

NA: No activity. The screening organisms: Mould: Aspergillus fumigatus (RCMB 02568, An), An Yeasts: Candida albicans (RCMB 05036, Ca), Gram-positive bacteria: Staphylococcus aureus (RCMB 010028, Sa), Streptococcus pneumoniae (RCMB 010010, Sp), and Bacillus subtilis (RCMB 010069, Bs). Gram-negative bacteria: Pseudomonas aeruginosa (RCMB 010043, Pa), Salmonella typhimurium (RCMB 010315, St), Klebsiella pneumoniae (RCMB 0010093, Kp) and Escherichia coli (RCMB 010052, Ec), AB: Amphotericin B, CF: Ciprofloxacin.
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Table 2. Antimicrobial Activity as MICs (µg/mL) of Tested Standards and Synthesized Compounds against Tested Microorganisms

<table>
<thead>
<tr>
<th>Compd</th>
<th>Fungi</th>
<th>Gram-positive bacteria</th>
<th>Gram-negative bacteria</th>
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<tbody>
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<td></td>
<td>Af</td>
<td>Ca</td>
<td>Sa</td>
</tr>
<tr>
<td>21a</td>
<td>7.81</td>
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<td>1.95</td>
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<tr>
<td>21b</td>
<td>0.98</td>
<td>NA</td>
<td>1.95</td>
</tr>
<tr>
<td>21c</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>21d</td>
<td>62.5</td>
<td>NA</td>
<td>31.25</td>
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<tr>
<td>21e</td>
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<td>15.63</td>
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<tr>
<td>21f</td>
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<td>NA</td>
<td>0.12</td>
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<td>21g</td>
<td>1.95</td>
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<td>0.49</td>
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<tr>
<td>21h</td>
<td>7.81</td>
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<td>1.95</td>
</tr>
<tr>
<td>22a</td>
<td>31.25</td>
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<td>7.81</td>
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<tr>
<td>22b</td>
<td>3.90</td>
<td>NA</td>
<td>1.95</td>
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<tr>
<td>22c</td>
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<td>22d</td>
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<td>0.49</td>
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<tr>
<td>CF</td>
<td>1.95</td>
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</tr>
</tbody>
</table>

NA: No Activity. Results in bold indicate more or equal activity relative to standard drugs. The screening organisms, Mould: Aspergillus fumigatus (RCMB 02568, An), An Yeasts: Candida albicans (RCMB 05036, Ca), Gram-positive bacteria: Staphylococcus aureus (RCMB 010028, Sp), Streptococcus pneumoniae (RCMB 010010, Sp), and Bacillus subtilis (RCMB 010069, Bs). Gram-negative bacteria: Pseudomonas aeruginosa (RCMB 010043, Pa), Salmonella typhimurium (RCMB 010315, St), Klebsiella pneumoniae (RCMB 0010093, Kp) and Escherichia coli (RCMB 010052, Ei), AB: Amphotericin B, CF: Ciprofloxacin.

D$_2$O-exchangeable signal of hydrazone NH at δ 11.13–11.67 ppm. The compounds 22c–e showed characteristic 13C-NMR signal at δ 181.77–189.03 ppm attributed to the carbonyl group.

**Discussion of Anti-microbial Activity**

**Antibacterial,** antifungal and antimycobacterial activities were performed at The Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. The mean values of the inhibition zone diameter and the minimum inhibitory concentrations were listed in Table 3. Isoniazide and pyrazinamide were included in the experiments as reference drugs (MIC=0.40 and 3.21 µg/mL, respectively).

Analysis of the data in Table 3 illustrated that compounds of the first series 21a–h and second series 22a–e displayed good to fair antifungal activity, while the third series derivatives 23a–g displayed moderate antifungal and antibacterial activities, exploited the most potent antitubercular activity of the newly synthesized derivatives 21a–h, 22a–e and 23a–g against *M. tuberculosis* (RCMB 010126) was evaluated at The Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. The mean values of the inhibition zone diameter and the minimum inhibitory concentrations were listed in Table 3. Isoniazide and pyrazinamide were included in the experiments as reference drugs (MIC=0.40 and 3.21 µg/mL, respectively).

**Antifungal Activity** Target compounds 21a–h, 22a–e, 23a–g and amphotericin, a membrane-active polypeptide antibiotic and an antifungal reference drug, were evaluated *in vitro* for their antifungal activity by inhibition zone technique and minimum inhibitory concentration (MIC). Data in Tables 1 and 2 revealed that all the compounds showed no activity against *Candida albicans*, while compounds 21b, f, g and 22b showed a remarkable activity against *Aspergillus fumigatus*.

**Aspergillus fumigatus** is largely responsible for increasing the mortality rate in immunocompromised patients due to invasive aspergillosis (IA). The main reason for this unacceptable mortality rate is the limited number of antifungal agents. Whilst, the thiopeines containing counterparts 22e and 23a–g displayed no activity, the 5-nitrofuran derivatives 21a–h and 22a–d showed moderate to excellent activity toward the *Aspergillus fumigatus* with MIC values ranging from 0.49 to 125 µg/mL. In particular, the sulfonamide derivative 21f was found to be the most potent counterpart against *Aspergillus fumigatus* organism (MIC=0.49 µg/mL) as it was four times more active than the reference drug Amphotericin B (MIC=1.95 µg/mL).

**Antimycobacterial and in Vitro Cytotoxic Activities**

Antitubercular activity of the newly synthesized derivatives 21a–h, 22a–e and 23a–g against *M. tuberculosis* (RCMB 010126) was evaluated at The Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. The mean values of the inhibition zone diameter and the minimum inhibitory concentrations were listed in Table 3. Isoniazide and pyrazinamide were included in the experiments as reference drugs (MIC=0.40 and 3.21 µg/mL, respectively).

In *vitro* cytotoxicity of the most active antimicrobial and antitubercular compounds 21a, f–g and 22a–e was evaluated against human breast MDA-MB-231 cells using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. Doxorubicin was used as the positive control drug. The IC$_{50}$ values obtained for these compounds are listed in Table 3. None of the tested compounds displayed any significant cytotoxicity, thereby providing a high therapeutic index. Selectivity index (SI) is used to estimate the therapeutic effect of a drug and to identify drug candidates for further studies. SI
of each compound was determined as the ratio of IC<sub>50</sub> to MIC (Table 3). It was reported that, new drugs candidates must have SI equal or higher than 1.45). In this study, 21f could be considered as a promising new anti-tubercular drug candidate with SI >12.8.

**Antibacterial Activity** Initially, all the synthesized compounds (21a–h, 22a–e and 23a–g) were evaluated in vitro for their antibacterial activity, by inhibition zone technique, using three Gram-positive bacteria that are Staphylococcus aureus (RCMB 010028), Streptococcus pneumoniae (RCMB 010010), and Bacillus subtilis (RCMB 010069) and four Gram-negative bacteria that are Pseudomonas aeruginosa (RCMB 010043), Salmonella typhimurium (RCMB 010315), Klebsiella pneumoniae (RCMB 0010093) and Escherichia coli (RCMB 010052), as presented in Table 1. Subsequently, MIC of all the synthesized compounds was evaluated using the two-fold serial dilution technique. The lowest concentration showing no growth was taken as the MIC. The results of minimum inhibitory concentration were reported in Table 2. Ciprofloxacin, a broad-spectrum widely used antibacterial and the most potent marketed fluoroquinolone against Gram-negative bacteria, 21] was used as reference drug in this assay.

As shown in Tables 1 and 2, the tested compounds displayed different levels of antibacterial activity and possessed a distinctive pattern of selectivity against the tested strains. With the exception of compounds 21e and 22e, all compounds of the first and second series showed a remarkable broad-spectrum antibacterial activity against almost all tested strains used in the assay. On the other hand, the remaining compounds 23a–g had no significant activity against any of the tested strains at concentration up to 125 µg/mL. All the tested Gram-positive and Gram-negative strains were susceptible to the all active compounds influence, except P. aeruginosa affected only with compound 21b. Investigation of the activity against B. subtilis indicated that it was the most sensitive strain to the influence of the active members.

Observing the results, we could deduce valuable data about the structure activity correlation of the tested compounds. Firstly, we explored the impact of substitution of the 4-position of the pendant phenyl group in the first series compounds 21a–h. Incorporation of unsubstituted phenyl group led to compound 21a with broad and excellent activity against S. aureus, S. pneumonia, B. subtilis, S. typhimurium, K. pneumonia and E. coli (MIC=0.49–3.9 µg/mL). Since fluorine has a size and electronic properties similar to those of hydrogen, it is introduced as an isosteric to the hydrogen atom. Compound 21b bearing fluorine substituent at the 4-position, showed an increase in the activity against the tested Gram-negative bacteria, especially against P. aeruginosa (MIC=3.9 µg/mL). Also, it retained the activity of the Gram-positive one suggesting that the substitution in the 4-position may be tolerated also suggesting that the halogens incorporation may be advantageous. Conversely, substitution with more bulky chlorine atom, compound 21c, abolished the activity. Furthermore, grafting methyl group, compound 21d, and methoxy group, compound 21e, resulted in drop of the activity (MIC=3.9–31.25 µg/mL).

Concerning the effect of the substitution in the first series member’s 21a–e, the activities were decreased in the order of 4-F>unsubstituted>4-OME>4-Me>>,4-Cl, hinting that grafting a small electron-withdrawing group like fluorine is more beneficial than an electron-donating group like methyl or methoxy for the antibacterial activity.

Taking into consideration the importance of sulfonamido substituent as antimicrobial pharmacophore, 24) 4-sulfonamido functionalities were incorporated in 21f–g counterparts. Unsubstituted sulfonamide derivative 21f emerged as the most potent analog with outstanding antimicrobial activities (MIC=0.06–0.98 µg/mL), confirming the importance of sulfonamido substitution. Notably, 21f analog displayed more susceptibility towards Gram-positive bacteria (MIC=0.06–0.12 µg/mL) more than Gram-negative one (MIC=0.24–0.98 µg/mL), relative to the standard drug. Substitution with more bulky sulfonamide derivatives N-(thiazol-2-yl)sulfonamido and N-(pyrimidin-2-yl)sulfonamido produced compounds 21g and h with potent and broad-spectrum activity (MIC=0.12–3.9 µg/mL), respectively. Thence, the order of activities of the sulfonamides members in the first series, were decreased in the order of sulfanilamide>sulfathiazole>sulfadiazine for the Gram-positive strains, and in the order of sulfanilamide>sulfadiazine>sulfathiazole for the Gram-positive bacteria. Moreover, a molecular docking study was used to explain the obtained excellent biological data of 21f.

Considering the activities of 5-nitrofuran analogs containing sulfone group 22a–e, 4-F substituted analog 22b exhibited superior activity against Gram-positive bacteria (S. aureus, S. pneumonia and B. subtilis) with MIC values of 1.95, 0.49 and 0.49 µg/mL, respectively, confirming the particularity of the incorporation of small electron-withdrawing group. In addition, the unsubstituted 22a and the 4-Cl substituted 22c derivatives elicited good antibacterial activity against the
tested strains (MIC=0.98–7.81 µg/mL). On the other hand, compound 22d possessed modest activity against the tested bacteria (MIC=15.63–125 µg/mL). Also, it was found that the replacement of the phenyl group with 2-thiophenyl one, as compound 22e, led to complete loss of activity.

Interestingly, bioisosteric replacement of the 5-nitrofuran moiety with thiophene one in the third series led up to diminished activity, confirming the role of 5-nitrofuran group as a crucial element for the antibacterial activity.

**DISCUSSION OF IN SILICO STUDIES**

**Docking Study** It is well known that, the sulfonamides exert their antimicrobial activity through competition with p-amino benzoic acid (PABA) substrate for the DHPS enzyme active site and thus inhibiting the biosynthesis of dihydrofolic acid. (5) Crystallographic data of *Bacillus anthracis* dihydropterate synthase (BaDHPS) bound to sulfathiazole-6-hydroxy-methyl-7,8-dihydropterin-pyrophosphate (STZ-DHPP) adduct was obtained from Protein Data Bank (PDB code 3TYE). (56)

Similarly, data obtained from docking shows that 21f can interact with BaDHPS as STZ-DHPP with the same target. The benzene-sulfonamide moiety of 21f occupies virtually the same position observed in the STZ-DHPP-BaDHPS complex; thereby establishing the characteristic hydrogen bond between its sulfonamide moiety and the backbone NH group of Ser221, in addition to an extra hydrogen bond with Asn196 (Fig. 2). Concerning the PABA binding pocket, the 5-nitro furan moiety of compound 21f extends into the pteridine-binding pocket establishing hydrogen bond between the side chain of Lys220 and the oxygen of furan ring and another ionic bond between –NO₂ and the side chain of Lys220. Although, the pteridine and the oxygen of furan ring and another ionic bond between establishing hydrogen bond between the side chain of Lys220 extends into the pteridine-binding pocket.

**Table 4. Docking Scores of the Tested Compound and STZ**

<table>
<thead>
<tr>
<th>Compound</th>
<th>CDOCKER energy (kcal/mol)</th>
<th>CDOCKER interaction energy (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21f</td>
<td>−39.265</td>
<td>−52.744</td>
</tr>
<tr>
<td>STZ</td>
<td>−47.276</td>
<td>−63.925</td>
</tr>
</tbody>
</table>

**Table 5. Toxicity Risks Evaluation of Novel 5-Nitro Furan Hydrazones**

<table>
<thead>
<tr>
<th>Mutagenicity</th>
<th>Developmental toxicity potential</th>
<th>Skin irritation potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>21a</td>
<td>0.11</td>
<td>0</td>
</tr>
<tr>
<td>21b</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>21c</td>
<td>0.34</td>
<td>0</td>
</tr>
<tr>
<td>21d</td>
<td>0.17</td>
<td>0</td>
</tr>
<tr>
<td>21e</td>
<td>0.14</td>
<td>0</td>
</tr>
<tr>
<td>21f</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>21g</td>
<td>0</td>
<td>0.72</td>
</tr>
<tr>
<td>21h</td>
<td>0</td>
<td>0.61</td>
</tr>
<tr>
<td>22a</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>22b</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>22c</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Toxicity Risk Assessment Screening** As indicated in Table 5 toxicity risk assessment screening showed that only compounds 21a, c, 12d and 21e have low risk of mutagenicity on prolonged usage whereas compounds 21g and h exhibited risk of developmental toxicity. All the tested compounds have not showed any risk of irritation. It is noteworthy that the most active compound 21f showed no risk of tumorigenicity, developmental toxicity or reproductive toxicity.

**2D QSAR Study**

Development of QSAR Models

The establishment of quantitative relationships between structural features and measured bioactivities, QSAR, is one of the most useful strategies in the quest for new bioactive molecules. Thus, QSAR models were built with the objective of analyzing and correlating the physicochemical and biological behaviors of the newly synthesized compounds. The analysis was run by means of the DS 2.5 software (Discovery Studio 2.5, Accelrys, Co., Ltd.).

A set of the active members (21a–h and 22a–d) was used as a training set with their measured pMIC against *Bacillus subtilis* and *Mycobacterium tuberculosis* for QSAR modeling.

Various molecular properties of the training set compounds were calculated via the “Calculate Molecular Properties” module. 2D Descriptors involved: A logP, Finger prints, molecular properties, molecular property counts, surface area and volume and topological descriptors, while the 3D descriptors involved: Dipole, jurs descriptors, principle moments of inertia, shadow indices and surface area and volume. Genetic function approximation (GFA) was adopted to search for the best possible QSAR regression equation capable of correlating the variations in the biological activities of the training compounds with variations in the generated descriptors, i.e., multiple linear regression modeling (MLR). QSAR models were validated employing leave one-out cross-validation by setting the folds to a number much larger than the number of samples, \( r^2 \) (squared correlation coefficient value) and \( r^2 \) prediction (predictive squared correlation coefficient value), residuals between the predicted and experimental activity of the test set and training set.

**QSAR Study Results**

Equation 1 represents the best performing QSAR model for the activity against *Bacillus subtilis*:

\[
\begin{align*}
-\log\text{MIC} &= 4.0948 + 0.8749\text{CHI}_V - 3\_P \\
&+ 0.3273\text{AAC}\_\text{Total} \\
&- 0.1476\text{Molecular}\_\text{Volume}.
\end{align*}
\]

Equation 2 represents the best performing QSAR model for the activity against *Mycobacterium tuberculosis*:

\[
\begin{align*}
-\log\text{MIC} &= 5.2719 - 0.8597\text{\_Count}\_\text{asC} \\
&- 0.4935\text{Jurs}\_\text{RNCS}.
\end{align*}
\]

According to Eqs. 1 and 2, the QSAR models were represented graphically by scattering plots of the experimental versus the predicted bioactivity values—logMIC for the training set compounds as shown in Figs. 3 and 4. The method used to build the model was least-squares, \( r^2 = 0.871 \) and 0.772, respectively, \( r^2 \) (adj) = 0.822 and 0.722, respectively, \( r^2 \) (pred) = 0.754 and 0.570 respectively, least-squared error = 0.1026 and 0.0619,
respectively, where \( r^2 \) (adj) is \( r^2 \) adjusted for the number of terms in the model; \( r^2 \) (pred) is the prediction \( r^2 \), equivalent to \( q^2 \) from a leave-1-out cross-validation.

In conclusion, Eq. 1 suggested that antibacterial activity of the 5-nitrofuran derivatives 21a–h and 22a–d towards Bacillus subtilis is affected by the topological descriptors (CHI_V_3_P and IAC_Total) and the molecular volume. CHI_V (Kier & Hall valence-modified connectivity index), a 2-D topological descriptor, is a refinement of the molecular connectivity index where a vertex subgraph valence delta is enhanced to \( \delta_v \) to take into account electron configuration of the atom represented by the vertex: 
\[
\delta_v = (Z - h) / (Z - Z - 1)
\]
where \( v \) is the number of valence electrons in the atom, \( Z \) is its atomic number, and \( h \) is the number of hydrogens bound to it. This formula is designed to reproduce the unmodified molecular connectivity index for saturated hydrocarbons, for which \( \delta_v = \text{delta} \). However, \( \delta_v \) distinguishes between multiple and single bonds. The denominator introduces further distinction between element rows due to the presence of the atomic number.\(^{47,48}\) IAC_Total (Total Information of Atomic Composition) is a graph-theoretical infoContent topological descriptor that helps to differentiate molecules according to their size, degree of branching, flexibility, and overall shape using graph-theory concepts.\(^{49}\)

On the other hand Eq. 2 indicated that antimycobacterial activity of the 5-nitrofuran derivatives 21a–h and 22a–d is affected by the estate keys (ES_Count_aasC) and the Jurs (Jurs_RNCS) descriptors. Estate keys calculate the sums of the electrotopological state (E-state) values and/or the counts of each atom type.\(^{50,51}\) ES_Count_aasC calculates the E-state count for carbon with two aromatic bonds and one single bond. Moreover, Jurs descriptors are those ones that combine shape and electronic information to characterize molecules.\(^{52}\)

The descriptors are calculated by mapping atomic partial charges on solvent-accessible surface areas of individual atoms. Jurs_RNCS (relative negative charge surface area) is the solvent-accessible surface area of most negative atom divided by the relative negative charge.

### QSAR Validation

Robustness of the established QSAR models 1 and 2 was verified by using; Leave-one-out (LOO) internal validation (\( r^2 = 0.871 \) and 0.772, respectively). Cross-validation was also employed where \( q^2 \), which is equivalent to \( r^2 \) (pred), was 0.754 and 0.570, respectively. In addition, validation was employed by measuring the residuals between the experimental and the predicted activities of the training set (Tables 4, 5). Interestingly, the predicted activities by the QSAR models were very close to those experimentally observed, indicating that these models could be applied for prediction of more effective hits having the same skeletal framework.

### CONCLUSION

In the present investigation, initially, we synthesized the 5-nitrofuran hydrazides 21a–h and 22a–e and evaluated their antimicrobial and antimycobacterial activities. Amphotericin
of toxic free radical reported in 5-nitro furan derivatives. The
formation of microbial DHPS enzyme in addition to the formation
synthase (DHPS), suggesting that it could act by the inhibi-
PABA and pterin binding pockets of the dihydropteroate
ing studies indicated that compound 21f could occupy both
21f within the 5-nitrofuran derivatives. Finally, molecular dock-
in order to identify positive and negative structural features
the Discovery Studio 2.5 software
via
models were established
12f
safety and high therapeutic index of
counterpart. QSAR
screening and evaluation of selective index demonstrated the
the antibacterial activity. Moreover, toxicity risk assessment
firming the role of 5-nitrofuran group as a crucial element for
we synthesized and biologically evaluated
–
23a
we synthesized and biologically evaluated
on the activities of many thiophene containing antimicrobials,
–
the thiophene hydrazides
antimicrobial and antimycobacterial agents. Unfortunately, all
were devoid of activity, con-
g. Amongst the active
22e
22e were used as references for antimycobacterial activity. The
MIC (µg/mL) relative to the reference drugs. Based
upon cooling was filtered off and recrystallized from dioxan
was heated under reflux for 10 h. The solid product obtained
was added in THF (30 mL). The reaction mixture
of the 5-nitrofuran-2-carbohydrazide
MATERIALS AND METHODS

General Chemistry Melting points (mp) were measured
with a Stuart melting point apparatus and were uncorrected.
The NMR spectra were recorded by Varian Gemini-300BB
300 MHz FT-NMR spectrometers (Varian Inc., Palo Alto, CA,
U.S.A.). 1H and 13C spectra were run at 500 and 125 MHz,
respectively, in deuterated dimethyl sulfoxide (DMSO-d6). Chemical
shifts (δH) are reported relative to tetramethylsilane,
as internal standard. Electron impact mass spectra were measured
on a Varian MAT 311-A (70 eV). Reaction courses and
product mixtures were routinely monitored by thin layer chro-
matography (TLC) on silica gel precoated F254 Merck plates.
Unless otherwise noted, all solvents and reagents were
commercially available and used without further purification.

Synthesis of Hydrazones 21a–h To a stirred solution of
the 5-nitrofuran-2-carbohydrazide 11 (5 mmol), 2-oxo-
N’-(4-substitutedphenyl)propanehydrazonoyl chloride 16a–h
(5 mmol) was added in THF (30 mL). The reaction mixture
was heated under reflux for 10 h. The solid product obtained
upon cooling was filtered off and recrystallized from dioxan

Table 6. Experimental Activity of the Synthesized Derivatives against the Predicted Activity and Calculated Descriptors Governing Activity According
Eq. 1

<table>
<thead>
<tr>
<th>Compd</th>
<th>Experimental activity (−log MIC)</th>
<th>Predicted activity (−log MIC)</th>
<th>Residual</th>
<th>CHI_V_3_P</th>
<th>IAC_Total</th>
<th>Molecular Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>21a</td>
<td>0.3098</td>
<td>−0.4982</td>
<td>0.808</td>
<td>3.03</td>
<td>70.19</td>
<td>204.77</td>
</tr>
<tr>
<td>21b</td>
<td>0.3098</td>
<td>0.3802</td>
<td>−0.0704</td>
<td>3.1</td>
<td>75.15</td>
<td>210.25</td>
</tr>
<tr>
<td>21c</td>
<td>−0.1969</td>
<td>−1.9788</td>
<td>−0.1182</td>
<td>3.36</td>
<td>73.15</td>
<td>223.29</td>
</tr>
<tr>
<td>21d</td>
<td>−0.3927</td>
<td>−0.5763</td>
<td>−0.3164</td>
<td>3.31</td>
<td>74.61</td>
<td>216.77</td>
</tr>
<tr>
<td>21e</td>
<td>−0.5911</td>
<td>−0.3763</td>
<td>−0.2148</td>
<td>3.35</td>
<td>77.75</td>
<td>222.6</td>
</tr>
<tr>
<td>21f</td>
<td>1.2219</td>
<td>1.394</td>
<td>−0.1722</td>
<td>4.37</td>
<td>87.23</td>
<td>237.69</td>
</tr>
<tr>
<td>21g</td>
<td>0.9208</td>
<td>0.4953</td>
<td>0.4255</td>
<td>6.25</td>
<td>101.12</td>
<td>285.71</td>
</tr>
<tr>
<td>21h</td>
<td>0.3098</td>
<td>0.2642</td>
<td>0.0456</td>
<td>5.56</td>
<td>101.95</td>
<td>285.03</td>
</tr>
<tr>
<td>22a</td>
<td>0.0088</td>
<td>−0.0604</td>
<td>0.0691</td>
<td>5.47</td>
<td>80.64</td>
<td>239.41</td>
</tr>
<tr>
<td>22b</td>
<td>0.3098</td>
<td>0.6737</td>
<td>−0.3639</td>
<td>5.54</td>
<td>85.94</td>
<td>246.61</td>
</tr>
<tr>
<td>22c</td>
<td>0.0088</td>
<td>−0.1184</td>
<td>0.1271</td>
<td>5.79</td>
<td>85.94</td>
<td>253.47</td>
</tr>
<tr>
<td>22d</td>
<td>−1.194</td>
<td>−0.9744</td>
<td>−0.2196</td>
<td>6.05</td>
<td>92.68</td>
<td>275.77</td>
</tr>
</tbody>
</table>

Table 7. Experimental Activity of the Synthesized Derivatives against the Predicted Activity and Calculated Descriptors Governing Activity According
Eq. 2

<table>
<thead>
<tr>
<th>Compd</th>
<th>Experimental activity (−log MIC)</th>
<th>Predicted activity (−log MIC)</th>
<th>Residual</th>
<th>ES_Count_aasC</th>
<th>Jurs_RNCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>21a</td>
<td>−0.8927</td>
<td>−1.0877</td>
<td>0.1951</td>
<td>3</td>
<td>7.6602</td>
</tr>
<tr>
<td>21b</td>
<td>−1.4949</td>
<td>−1.7959</td>
<td>0.3011</td>
<td>4</td>
<td>7.3350</td>
</tr>
<tr>
<td>21c</td>
<td>−2.1614</td>
<td>−2.0299</td>
<td>−0.1315</td>
<td>4</td>
<td>7.8271</td>
</tr>
<tr>
<td>21d</td>
<td>−2.0969</td>
<td>−1.9143</td>
<td>−0.1827</td>
<td>4</td>
<td>7.5928</td>
</tr>
<tr>
<td>21e</td>
<td>−1.7959</td>
<td>−1.6574</td>
<td>−0.1385</td>
<td>4</td>
<td>7.0724</td>
</tr>
<tr>
<td>21f</td>
<td>−0.5911</td>
<td>−0.6608</td>
<td>0.0697</td>
<td>4</td>
<td>5.0529</td>
</tr>
<tr>
<td>21g</td>
<td>−0.8927</td>
<td>−1.0534</td>
<td>0.1607</td>
<td>5</td>
<td>4.1063</td>
</tr>
<tr>
<td>21h</td>
<td>−1.1940</td>
<td>−1.0878</td>
<td>−0.1061</td>
<td>5</td>
<td>4.1761</td>
</tr>
<tr>
<td>22a</td>
<td>−1.1940</td>
<td>−0.6694</td>
<td>−0.5246</td>
<td>4</td>
<td>5.0703</td>
</tr>
<tr>
<td>22b</td>
<td>−0.8927</td>
<td>−1.2696</td>
<td>0.3770</td>
<td>5</td>
<td>4.5445</td>
</tr>
<tr>
<td>22c</td>
<td>−1.1940</td>
<td>−1.3900</td>
<td>0.1960</td>
<td>5</td>
<td>4.7883</td>
</tr>
<tr>
<td>22d</td>
<td>−2.0969</td>
<td>−1.8807</td>
<td>−0.2162</td>
<td>6</td>
<td>4.0405</td>
</tr>
</tbody>
</table>

B and Ciprofloxacin were used as references for antifungal
and antibacterial screening, while, Isoniazid and Pyrazinamide
were used as references for antimycobacterial activity. The
results evidenced that all the compounds displayed moderate
to excellent activity except 21c and 22e. Amongst the active
compounds, the sulfonamide derivative 21f displayed the best
activity against A. fumigatus and panel of three Gram-positive
bacteria, four Gram-negative bacteria and M. tuberculosis
A. fumigatus activity and panel of three Gram-positive
compounds, the sulfonamide derivative 21f displayed the best
to excellent activity except 21c and 22e. Amongst the active
compounds, the sulfonamide derivative 21f displayed the best
activity against A. fumigatus and panel of three Gram-positive
bacteria, four Gram-negative bacteria and M. tuberculosis
A. fumigatus activity and panel of three Gram-positive

MATERIALS AND METHODS

Synthesis of Hydrazones 21a–h To a stirred solution of
the 5-nitrofuran-2-carbohydrazide 11 (5 mmol), 2-oxo-
N’-(4-substitutedphenyl)propanehydrazonoyl chloride 16a–h
(5 mmol) was added in THF (30 mL). The reaction mixture
was heated under reflux for 10 h. The solid product obtained
upon cooling was filtered off and recrystallized from dioxan
to afford the corresponding hydrazones 21a–h with 65–80% yield.

\((\text{1Z,2E})-2-(2-(\text{5-Nitrofuran-2-carbonyl})hydrazono)\text{-N'-phenylpropenehydrazonoyl Chloride (21a)}}

Orange powder (yield 75%), mp 230°C; IR (KBr, v cm⁻¹): 3414, 3315 (2NH), 1668 (C=O) and 1599 (C=N); 1H-NMR (DMSO-δ) δ ppm: 7.62 (d, J=6.7Hz, 2H, Ar-H), 7.36 (d, J=6.9Hz, 2H, Ar-H), 7.68–7.70 (m, 4H, ArH), 6.82 (d, J=7.5Hz, 2H, Ar-H), 7.70–7.75 (m, 2H, Ar-H and H² of furan), 10.13 (s, D₂O exch., 1H, =NH⁻⁻); 13C-NMR (DMSO-δ) δ ppm: 190.2, 162.5, 157.4, 146.6, 142.8, 138.8, 134.1, 131.8, 125.2, 125.1, 124.9, 124.6, 114.9, 113.4, 112.3, 106.1, 104.5, 102.9, 83.2, 83.3, 83.2, 28.0, 27.8, 106 [100].

\((\text{1Z,2E})-2-(2-(\text{5-Nitrofuran-2-carbonyl})hydrazono)\text{-N'-p-tolylpropenehydrazonoyl Chloride (21d)}}

Red powder (yield 75%), mp 239°C; IR (KBr, v cm⁻¹): 3414, 3315 (2NH), 1668 (C=O) and 1576 (C=N); 1H-NMR (DMSO-δ) δ ppm: 7.25 (s, 3H, CH₃), 6.82 (d, J=6.9Hz, 2H, Ar-H), 7.24 (d, J=6.7Hz, 2H, Ar-H), 7.68–7.70 (m, 4H, ArH), 6.82 (d, J=7.3Hz, 2H, Ar-H), 7.70–7.75 (m, 2H, Ar-H and H² of furan), 10.13 (s, D₂O exch., 1H, =NH⁻⁻); 13C-NMR (DMSO-δ) δ ppm: 190.2, 162.5, 157.4, 146.6, 142.8, 138.8, 134.1, 131.8, 125.2, 125.1, 124.9, 124.6, 114.9, 113.4, 112.3, 106.1, 104.5, 102.9, 83.2, 83.3, 83.2, 28.0, 27.8, 106 [100].

\((\text{1E,2Z})-2-(2-(\text{5-Nitrofuran-2-carbonyl})hydrazono)\text{-N'-(4-Methoxyphenyl)propenehydrazonoyl Chloride (21c)}}

Brown powder (yield 75%), mp 224°C; IR (KBr, v cm⁻¹): 3414, 3315 (2NH), 1668 (C=O) and 1576 (C=N); 1H-NMR (DMSO-δ) δ ppm: 7.25 (s, 3H, CH₃), 6.82 (d, J=6.9Hz, 2H, Ar-H), 7.24 (d, J=6.7Hz, 2H, Ar-H), 7.68–7.70 (m, 4H, ArH), 6.82 (d, J=7.3Hz, 2H, Ar-H), 7.70–7.75 (m, 2H, Ar-H and H² of furan), 10.13 (s, D₂O exch., 1H, =NH⁻⁻); 13C-NMR (DMSO-δ) δ ppm: 190.2, 162.5, 157.4, 146.6, 142.8, 138.8, 134.1, 131.8, 125.2, 125.1, 124.9, 124.6, 114.9, 113.4, 112.3, 106.1, 104.5, 102.9, 83.2, 83.3, 83.2, 28.0, 27.8, 106 [100].

\((\text{1Z,2E})-2-(2-(\text{5-Nitrofuran-2-carbonyl})hydrazono)\text{-N'-4-Sulfamoylphenylpropenehydrazonoyl Chloride (21f)}}

Yellow powder (yield 75%), mp 230°C; IR (KBr, v cm⁻¹): 3419, 3288 (2NH), 1700 (C=O), 1590 (C=N) and 1307, 1154 (SO₂); 1H-NMR (DMSO-δ) δ ppm: 5.36 and 5.44 (s, 2H, CH₂), 7.40–7.91 (m, 2H, Ar-H), 11.14 and 11.66 (s, D₂O exch., 1H, =CONH⁻⁻); MS m/z [%]: 449 [(M+2)⁺], 2.6, 447 [M⁺], 6.0, 139 [100].
ran-2-carboxyhydrazide (22d) Yellow powder (yield 75%), mp 203°C; IR (KBr, ν cm⁻¹): 3420, 3288 (2NH), 1680 (C=O), 1595 (C=N) and 1347, 1152 (SO₂); H-NMR (DMSO-d₆) δ ppm: 2.29 (s, 3H, CH₃), 5.41 (s, 2H, CH₂), 7.34 (d, J=8.0Hz, 1H, Hᵗ of furan), 7.43 (d, J=7.01Hz, 2H, Ar-H), 7.76–8.07 (m, 3H, ArH), 8.18 (d, J=7.75Hz, 2H, Ar-H), 8.32 (d, J=7.75Hz, 2H, Ar-H), 11.13 and 11.61 (s, D₂O exh., 1H, –CONH⁻); ¹³C-NMR (DMSO-d₆) δ ppm: 21.40, 63.30, 123.80, 124.19, 128.57, 128.75, 129.03, 130.18, 131.93, 136.10, 136.80, 140.62, 145.30, 150.80, 154.00, 189.03; MS m/z [%]: 472 [M⁺, 0.6], 140 [100].

(Z)-5-Nitro-N’-(2-(phenylsulfonyl)-1-(thiophen-2-yl)ethyldiene)uran-2-carboxyhydrazide (22e) Yellow powder (yield 75%), mp 140–150°C; IR (KBr, ν cm⁻¹): 3414, 3292 (2NH), 1700 (C=O), 1559 (C=N) and 1412, 1158 (SO₂); ¹³C-NMR (DMSO-d₆) δ ppm: 5.25 (s, 2H, CH₂), 7.25–8.10 (m, 10H, ArH), 11.13 and 11.61 (s, D₂O exh., 1H, –CONH⁻); ¹³C-NMR (DMSO-d₆) δ ppm: 63.04, 128.53, 128.73, 129.48, 129.70, 134.54, 137.11, 137.76, 139.73, 143.58, 181.77; MS m/z [%]: 420 [(M+1)⁺, 2.7], 419 [M⁺, 10.1], 109 [100].

Synthesis of Hydrazones 23a-g To a stirred solution of the appropriate 2-oxo-N’-(4-substitutedphenyl)propanehydrazonoyl chloride 16a–e, i, j (5mmol) in THF (20mL), thiophene-2-carboxyhydrazide 12 (5mmol) was added. The reaction mixture was heated under reflux for 10h. The solid product obtained upon cooling was filtered off and recrystallized from dioxan to afford the corresponding hydrazones 23a–g with 65–80% yield.

(1Z,2E)-N’-(4-Fluorophenyl)-2-(thiophene-2-carboxy)hydrazono)propanehydrazonoyl Chloride (23b) Yellow powder (yield 75%), mp 225°C; IR (KBr, ν cm⁻¹): 3420, 3312 (2NH), 1653 (C=O) and 1595 (C=N); H-NMR (DMSO-d₆) δ ppm: 2.38 (s, 3H, CH₃), 6.93–7.95 (m, 8H, ArH), 10.18 (s, D₂O exh., 1H, –CONH⁻), 11.00 (s, D₂O exh., 1H, –CONH⁻); ¹³C-NMR (DMSO-d₆) δ ppm: 13.95, 114.31 (2C), 121.65, 123.81, 127.63, 129.62, 134.56 (2C), 135.32, 143.96, 152.00; MS m/z [%]: 322 [(M+2)⁺, 3.7], 320 [M⁺, 9.5], 284 [100].

Antimicrobial Activity All strains were provided from culture collection of the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. Antibacterial and antifungal activities were expressed as the diameter of inhibition zones; agar well diffusion method was used. Holes (1 cm diameter) were digger in the agar using sterile cork borer in sterile melt agar plates for fungi and sterile nutrient agar plates for bacteria, which had previously been uniformly seeded with tested microorganisms. The holes were filled by fungal filtrates (100μL). Plates were left in a cooled incubator at 4°C for one hour for diffusion and then incubated at 37°C for tested bacteria and 28°C for tested fungi. Inhibition zones developed due to active antimicrobial metabolites were measured after 24h of incubation for bacteria and 48h of incubation for fungi. Ampthorici B and ciprofloxacin were used as antifungal and antibacterial positive control; respectively. The experiment was performed in triplicate and the average zone of inhibition was calculated.

Minimum Inhibitory Concentration MIC was performed by a serial dilution technique described by Irobi et al., starting with 100μmol concentration of all compounds dissolved in 1 mL DMSO and then reduced by successive two-fold dilutions of stock solution using a calibrated microspette. Ampthorici B and ciprofloxacin were used as the reference compounds for fungi and bacteria; respectively. The final solutions concentrations were 125, 62.50, 31.25, 15.63, 7.81, 3.90,
The microtiter plates were incubated at 37°C for tested bacteria and 28°C for tested fungi and were readed using microplate reader after 24 h for bacteria and after 48 h for fungi. In each case, triplicate tests were performed and the average was taken as final reading. MIC was expressed as the lowest concentration inhibiting test organism’s growth.54

Antimycobacterial Activity M. tuberculosis (RCMB 010126) strain was provided from culture collection of the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. The isolated M. tuberculosis (RCMB 010126) clone was cultivated under agitation on LB medium at 37°C for 24 h. The antitubercular activity was expressed as the diameter of inhibition zones using agar well diffusion method and as MIC using serial dilution technique. Isoniazide and pyrazinamide were used as the reference drugs. The final solutions concentrations were 125, 62.50, 31.25, 15.63, 7.81, 3.90, 1.95, 0.98, 0.49, 0.24 and 0.12 μmol/mL. The zones of inhibition were analyzed after 72 h of incubation at 37°C. Each test was repeated 3 times. MIC was expressed as the lowest concentration inhibiting test organism’s growth.

In Vitro Cytotoxicity MDA-MB231 cells were grown in Dulbecco’s modified Eagle’s medium (DMEM)/high glucose supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine and 1% penicillin/streptomycin. The new compounds were evaluated in a primary five cell line-one concentration (25 mg/mL) anticancer assay against MDA-MB231 cells cell lines. The cytotoxic effect of the newly synthesized compounds was evaluated by testing the capacity of the reducing enzymes present in viable cells to convert MTT to formazan crystals as previously described,55 with some modifications. Briefly, cells cultured in complete medium were seeded into 96-well microtiter plates (in quintuplicates) with 2×10^4 cells per well and incubated at 37°C under a humidified atmosphere of 5% CO₂ for 24 h. The cell medium in test wells were then changed to serum free medium (SFM) containing 25 mg/mL of the test compounds, while the cell medium in control wells were changed to SFM containing an equivalent volume of solvent (dimethyl sulfoxide “DMSO”). After incubation at 37°C for 24 h, SFM in control and test wells were replaced by 100 mL/well of MTT; 0.5 mg/mL) in phosphate-buffered saline (PBS) and incubated at 37°C for an additional 3 h. MTT solution were removed and the purple formazan crystals formed at the bottom of the wells were dissolved using 100 mL isopropyl alcohol/well with shaking for 1 h at room temperature. The absorbance at 549 nm was read on a microplate reader (ELX 800; Bio-Tek Instruments, Winooski, VT, U.S.A.). The dose response curves of the compounds effecting >50% inhibition in one-dose prescreening for each cell line were established with concentrations of 25, 12.5, 6.25, 3.125, 1.56 and 0.78 mg/mL, and the concentrations causing 50% cell growth inhibition (IC₅₀) were calculated.

Molecular Docking The molecular docking of the tested compounds was performed using Discovery Studio 4/CDOCKER protocol (Accelrys Software Inc.). The protein crystallographic structure of Bacillus anthracis dihydropyroloate synthase (PDB code 3TYE) was downloaded from the Protein Data Bank (PDB). The protein was prepared for docking process according to the standard protein preparation procedure integrated in Accelrys’s discovery studio 4 and prepared by prepare protein protocol. Sulfathiazole-6-hydroxy-methyl-7,8-dihydropyridin-pyrophosphate (STZ-DHPP) adduct and the 21f were drawn as a database and prepared by prepare ligand protocol to generate 3D structure and refine using CHARMM force field with full potential. Docking simulations were run using CDocker protocol where a maximum bad orientations was 800 and orientation van der Waals (vdW) energy threshold was 300. Simulated annealing simulation would be then carried out consisting of a heating phase 700 K with 2000 steps and a cooling phase back to 5000 steps. The binding energy was calculated as a score to rank the docking poses. The top 10 docking poses would be finally saved. Docking poses were ranked according to their –CDOCKER interaction energy, and the top poses were chosen for analysis of interactions for each compound.

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Conflict of Interest The authors declare no conflict of interest.

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