Journal of Advances in Medical and Pharmaceutical Sciences
6(4): 1-10, 2016, Article no.JAMPS.24351
ISSN: 2394-1111

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Four Compounds Suppressing Growth of *Mycobacterium tuberculosis*

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Authors' contributions

This work was carried out in collaboration among all authors. Author JDC designed the bacterial study. Authors PS and WK carried out the culture of bacteria and monitoring of growth inhibition. Author RB performed the statistical analysis, synthesis of compounds, molecular modeling and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMPS/2016/24351
Editor(s):
(1) Dongdong Wang, Department of Pharmacogonosy, West China College of Pharmacy, Sichuan University, China.
Reviewers:
(1) M. Luisa Jordao, National Institute of Health Dr. Ricardo Jorge, Portugal.
(2) Shweta Sharma, Dr. Ram Manohar Lohia Hospital, New Delhi, India.
(3) Charbell Miguel Haddad Kury, Medical School of the Municipality of Campos dos Goytcazes, Brazil.
Complete Peer review History: http://sciencedomain.org/review-history/13250

Original Research Article

Received 15th January 2016
Accepted 28th January 2016
Published 10th February 2016

ABSTRACT

Aims: To demonstrate the efficacy of several small molecular weight compounds having hydrazide groups, for inhibiting the growth of *Mycobacterium tuberculosis*. To show these same compounds have favorable drug-likeness properties.
Study Design: To synthesize tuberculostats and test their antibacterial activity *in-vitro*.
Place and Duration of Study: University of Nebraska, Durham Science Center, 6001 Dodge Street, Omaha NE 68182, and Texas A&M Health Science Center, Department of Microbial Pathogenesis and Immunology, 8447 State Hwy 47, Medical Research and Education Building, Room #3012, Bryan, TX 7780. From January 2015 to June 2015.
Methodology: Hydrazide groups were formed by covalently bonding hydrazine onto small molecules having a single aromatic ring by utilizing microwave excitation and evaluating for antibacterial activity. These compounds were placed into tissue culture media at various

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concentrations and then tuberculosis bacteria were added to determine the level of growth inhibition. Growth inhibition of the bacteria was measured as a function of compound concentration for assessment and comparison.
Results: Compounds A, B, C, and D carry hydrazide groups with various substituents that are bonded to a single aromatic ring. All four compounds show zero violations of Rule of 5, indicating favorable absorption and membrane permeation. All four compounds showed greater than 85% growth inhibition of bacteria at concentrations below 50 micrograms per milliliter, while assayed by colony forming units and luminescence. Values of Log BB suggests compounds A and C will have greater penetration into the central nervous system than isoniazid.
Conclusion: These four hydrazide compounds induced substantial inhibition of bacterial growth. Microwave excitation for the synthesis of hydrazide compounds is effective. These compounds have favorable drug-likeness properties and are highly effective inhibiting growth of *Mycobacterium tuberculosis*.

Keywords: Tuberculosis; TB; hydrazide; Mycobacterium tuberculosis; tuberculostatic.

ABBREVIATIONS

Term: TB, tuberculosis; A^2, Angstroms^2; A^3, Angstroms^3; SMILES, simplified molecular-input line-entry system; IUPAC, International Union of Pure and Applied Chemistry; MDR-TB, multi-drug resistant Mycobacterium tuberculosis; M/XDR-TB, extensively drug resistant Mycobacterium tuberculosis; TDR-TB, totally drug resistant tuberculosis; GPCR, G-protein-coupled receptors; CFU, colony forming units; CNS, central nervous system.

1. INTRODUCTION

Multi-drug resistant *Mycobacterium tuberculosis* (MDR-TB) and extensively drug resistant *Mycobacterium tuberculosis* (M/XDR-TB) is a substantial challenge for clinicians and public health workers worldwide [1]. The lack of new effective drugs, improper regimens, and unreliable drug susceptibility testing is believed to have added to the problem [1]. MDR-TB patients having localized disease, may benefit from utilization of surgery as an adjunct to chemotherapy [1]. Summation in 2005, indicated that an estimated 8.8 million new cases of active tuberculosis were reported and leading to an estimated 1.6 million deaths per year [2].

Tuberculosis (TB) infections of the central nervous system (CNS) accounts for about 1% of all cases of tuberculosis and carries a high mortality level as well as causing neurological morbidity [2,3]. Tuberculosis infection of the CNS is a devastating clinical manifestations of tuberculosis. Often described as meningitis, encephalitis is within the spectrum of clinical manifestations [4]. In a previously conducted epidemiological study of extrapulmonary tuberculosis within the United States, involvement of the CNS was noted in 5 to 10% of all extrapulmonary tuberculosis cases [2].

Treatment for *M. tuberculosis* meningitis includes isoniazid, rifampicin, pyrazinamide and ethambutol for a period of two months, which is then followed by isoniazid and rifampicin alone for a further ten months [5]. Interestingly, the administration of aspirin may actually reduce or even delay mortality [6]. TB meningitis is known to occur more frequently in children and especially children less than one year of age [7].

Totally drug-resistant tuberculosis (TDR-TB) has been reported in Iran, India and South Africa [8]. TDR-TB is most commonly described as an incidence that is resistance to all first- line drugs (isoniazid, rifampicin, pyrazinamide, ethambutol, and streptomycin) and second-line drugs administered to treat TB [8]. Adjunct TB therapies, including repurposing of drugs that target biologically molecular pathways, may achieve better clinical outcomes in combination with the standard chemotherapy [8]. Having general symptomatic signs of fever, chills, night sweats, appetite loss, and fatigue, cases of pulmonary tuberculosis may infect any part of the body where it is considered to be extrapulmonary TB which may coexist with pulmonary TB as well [9,10].

A latent tuberculosis infection is defined in a patient that is infected with *Mycobacterium tuberculosis* but has no active tuberculosis [11]. Although latent tuberculosis is not considered

contagious, approximately 10% of these patients will go on to develop active tuberculosis [11]. Identification and treatment of individuals having latent TB is an important aspect of controlling the disease, however treatment regimens generally need to be administered for several months [11].

Clearly, new drug designs continuing studies are well advised in the face of the threat of MDR-TB, M/XDR-TB, and TDR-TB. This study presents novel drug designs which apply the hydrazide group found in the first-line drug isoniazid, to an aromatic ring scaffolding that will modify molecular properties favorably for oral administration and penetration into the CNS. This approach, to beneficially modify molecular properties, has been shown to be highly efficacious for inhibiting *M. tuberculosis* [12-15], *Staphylococcus aureus* [16-18], and *Escherichia coli* [19-22]. The novel hydrazide compounds presented here are shown to significantly inhibit the growth of *M. tuberculosis* and favorable drug-likeness.

This study presents the efficacy of several small hydrazide compounds for inhibiting the growth of *Mycobacterium tuberculosis*. These same compounds have favorable drug-likeness properties.

2. METHODOLOGY

2.1 Reagents and Instrumentation

All reagents were obtained from Aldrich-Sigma Company (P.O. Box 2060, Milwaukee, WI 53201 USA). Infrared spectroscopy can be accomplished in a Mattson Galaxy FTIR in a dimethyl sulfoxide solvent that has been previously dried over molecular sieves to remove water prior to obtaining spectra.

2.2 Molecular Modeling and Numerical Analysis

Molecular modeling (2-D and 3-D) were accomplished by ChemSketch version 12.01 ACD/Labs Release: 12.00 (90 Adelaide Street West, Toronto, Ontario M5H 3V9, Canada) Molecular properties were determined by utilizing Molinspiration (Liscie Udolie 2, SK-841 04 Bratislava, Slovak Republic). Correlation statistic by Pearson r and other statistical analysis was accomplished by Microsoft Office Excel 2007 or PAST version 2.06 (copyright Hammer and Harper 1999-2011). Molecular dipole values were determined by package Gaussian-GaussView 5.0 (Gaussian Inc., 340 Quinnipiac St. Bldg. 40, Wallingford CT 06492 USA).

2.3 Bacterial Culture

2.3.1 Strain of bacteria

Mycobacterium tuberculosis strain CDC1551 wild type carrying vector plasmid pJDC174 and BCG carrying the same vector expressing a codon-optimized click beetle red gene (CBR) was used. The bacteria were grown to an optical density (OD) ~ 1.0 and then diluted to OD = 0.5 in media for following survival assays (read at 600 nm wavelength).

2.3.2 Media for *In vitro* evaluation of bacterial growth inhibition

M. tuberculosis were grown in Middlebrook 7H9 supplemented with albumin dextrose complex (M-ADC, per liter, 50 grams bovine serum albumen fraction V, 20 grams dextrose, 8.5 grams NaCl,) (Difco), 0.05% Tween 80 (M-ADC-Tw) and kanamycin at a final concentration of 10 µg/ml to select for plasmid maintenance in the strains.

2.3.3 *In vitro* evaluation of compounds

All compounds were dissolved to a final concentration of 5 mg/ml in aqueous solution. All compound solutions were sterilized by passage through 0.22 µm-syringe filters. Survival by Optical Density: Four clear 96-well flat bottom plates were filled with 108 µl per well of M-ADC-Tw media supplemented with 10 µg/ml kanamycin to maintain luminescence in the strain. Each tested compound, as well as isoniazid, was added in duplicate wells at 72 µl per well at a final concentration of 2 mg/ml. Two-fold serial dilutions were carried out six times for all compounds and isoniazid. The last row of wells was maintained without any antibiotic. A 10 µl per well of *M. tuberculosis* (bacteria at equal concentration in 10 µL for all wells) was added to 96-well plates to give a final volume of 100 µl per well. Plates were incubated at 37℃ for 3 days, 7 days and 14 days.

2.3.4 Measurement of survival by luminescence

Four solid white 96-well flat bottom plates were prepared as before. Plates were incubated at 37℃ for 3, 7, and 14 days. Luminescence

measurements were taken for day 0, 3, 7, and 14 in the presence of tested compounds [23]. Bacterial luminescence was measured measured immediately after addition of 20 µl of 5 mM D-luciferin in 0.45 M sodium citrate buffer pH 5.0 (Gold Biotechnology) using an EnVision (Perkin Elmer) plate reader. Photon collection time was one second per well.

2.4 Synthesis of Compounds

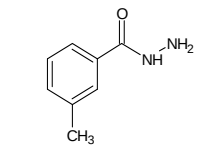
Prior to use, the hydrazine (NH_2NH_2) must be distilled over CaO and NaOH. The anhydrous NH_2NH_2 was collected at 113℃ and was stored sealed at -20ºC. Hydrazide derivatization: All compounds were treated similarly, place 120 mg of compound into pyrex open test tube with 64 microliter of $SOCl_2$. Microwave 3 to 5 minutes (avoid over-heating as that will cause degradation of the agent), allowed to cool to room temperature. Vacuum pump removal of any unreacted thionyl chloride is recommended. Add 400 microliter of anhydrous NH_2NH_2 and microwave at 45 second intervals up to five minutes (avoid excess heating during process). Excess hydrazine was removed by pump vacuum at room temperature. The derivatives were not heated or allowed to become wet. After thorough drying they are stored dried in air tight containers at -20℃. The presence of the hydrazine group can be confirmed by use of FTIR, observing for peaks at 944 cm^{-1} for hydrazine and 1000 cm^{-1} to 1200 cm^{-1} for C-N stretch, and around 3000 cm^{-1} to 3500 cm^{-1} for N-H stretch. Furthermore, the presence of the hydrazide group on all final products can be confirmed by the colorimetric protocol and UV-Visible spectrophotometric analysis utilizing Gibb's reagent as described previously [24]. Assignments of C-13 peaks for compounds A, B, C, and D (ppm): (A) Aromatic carbons C-1 133.4, C-2 128.0, C-3 137.8, C-4 132.6, C-5 128.5, C-6 124.3, 3-methyl carbon 20.9, carbonyl carbon 167.3; (B) Aromatic carbons C-1 133.9, C-2 120.2, C-3 153.2, C-4 124.8, C-5 129.0, C-6 124.1; carbonyl carbon 167.3; carbonyl carbon 168.0 and methyl carbon 16.9; (C) Aromatic carbons C-1 133.5, C-2 127.5, C-3 128.9, C-4 141.2, C-5 128.9, C-6 127.5; carbonyl carbon 167.3; methylene bridge carbon 50.5; (D) Aromatic carbons C-1 139.6, C-2 128.2, C-3 123.7, C-4 151.8, C-5 123.7, C-6 128.2; carbonyl carbon 167.3.

3. RESULTS AND DISCUSSION

The investigation and development of novel drugs for the treatment of *Mycobacterium tuberculosis* infection is of pressing importance. The appearance of multi-drug resistant and extensively drug-resistant types of *Mycobacterium tuberculosis* will be a substantial problem for clinical intervention. Four hydrazide compounds have been synthesized utilizing microwave excitation, followed by property characterization, then in-vitro testing for bacterial growth inhibition.

These four compounds, A, B, C, and D are presented for comparison in Fig. 1, with molecular structure, IUPAC name, SMILES (simplified molecular-input line-entry system notation), and formula weight. Notably, each compound has a single aromatic ring with a hydrazide substituent ($-C(O)NHNH_2$). The range of formula weights runs from 150.18 (compound A) to 194.19 (compound B). The hydrazide group is common to all these compounds, as well as to isoniazid (isonicotinylhydrazide), which is an antibacterial used as a first-line agent in the prevention and treatment of both latent and active *Mycobacterium tuberculosis* infections [1,8,9]. For compound A there is a methyl group ($-CH_3$) in meta position to the hydrazide group. For compound B there is a $–OC(O)CH_3$ group in meta position to the hydrazide group. For compound C there is a $–CH_2Cl$ group in para position to the hydrazide group. Lastly, compound D has a nitro group $–NO_2$ in para position to the hydrazide functional group. The substituents of the aromatic ring plays an important role in defining molecular properties of each compound, which in turn defines the efficacy of the compound for clinical application.

Various important properties such as Log P, polar surface area, number of oxygen atoms, number of nitrogen atoms, number of amine groups ($-NH_n$), and molecular volume have been determined for each compound, and presented in Table 1. One-way ANOVA test of all properties for all compounds shown in Table 1 indicate that the means of summed numerical values are essentially equal (P = .99) [24]. Also, summed properties of all compounds have equal medians by Kruskal-Wallis test (P = .93) [24]. In addition, the correlations of each compound's properties to another are extremely high, with Pearson r greater than 0.9700 for all compounds and with all properties inclusive (see Table 1).

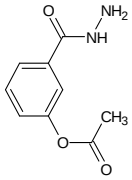


Compound A

3-methylbenzohydrazide

Cc1cccc(c1)C(=O)NN

Formula Weight = 150.18

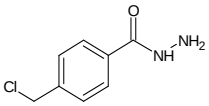


Compound B

3-(hydrazinylcarbonyl)phenyl acetate

O=C(C)Oc1cc(ccc1)C(=O)NN

Formula Weight = 194.19

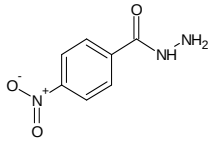


Compound C

4-(chloromethyl)benzohydrazide

ClCc1ccc(cc1)C(=O)NN

Formula Weight = 184.62



Compound D

4-nitrobenzohydrazide

O=[N+]([O-])c1ccc(cc1)C(=O)NN

Formula Weight = 181.15

Fig. 1. Molecular structures, IUPAC names of compounds, SMILES notation, and formula weight of compounds A, B, C, and D. Note that each compound has one aromatic ring one hydrazide group (-C(=O)NHNH$_2$). Compound A has a methyl group (-CH$_3$) in the meta position to the hydrazide group. For compound B the substituent (-O-C(=O)CH$_3$) is in the meta position to the hydrazide group. However, for compound C the substituent (-CH$_2$Cl) is in the para position to the hydrazide group; as is (-NO$_2$) for compound D

Molecular properties of compounds A, B, C, D, and isoniazid are presented in Table 1. Searching for any outliers in numerical values utilizing extreme studentized deviate (Grubbs' test) [25], results showed no outliers in values of Log P, polar surface area, molecular weight, molecular volume, number of oxygen atoms, number of nitrogen atoms, and number of rotatable bonds. Ranges in numerical values for important descriptors such as Log P, polar surface area, molecular weight, and molecular volume, inclusive of isoniazid, are as follows: -0.969, to 0.896; 55.12 Angstroms^2 to 100.95 Angstroms^2, 137.43 to 194.19, 122.56 Angstroms^3 to 171.25 Angstroms^3, respectively.

Notably, all compounds shown in Table 1 show zero violations of the Rule of 5, which indicates favorable drug absorption and permeation [26]. Previous studies have determined that poor absorption of drug can occur when there are one or more violations of the following parameters, known as Rule of 5 [26]: 1) Molecular weight less than 500; 2) Log P is less than 5; 3) There is no more than 10 hydrogen bond acceptors (nitrogen and oxygen atoms); 4) There is no more than 5 hydrogen bond donors (-OH & -NH$_n$). Outcomes for these compounds indicate an opportune absorption into the body and efficient membrane permeation predicted for compounds A, B, C, and D. Oxygen and Nitrogen centered polar fragments are considered in determination of polar surface area and have been shown to be good descriptors for characterizing drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability and blood-brain barrier penetration [26].

Number of rotatable bonds is a simple topological parameter considered to be a measure of molecular flexibility [26]. Previous studies have shown the descriptor of BB (Concentration in the brain*/*Concentration in blood) can be accurately estimated and utilized to determine if any perspective drug can be expected to penetrate into the central nervous system (CNS) [27,28]. Studies of drugs known to efficiently pierce into the CNS have revealed an equation for effectively predicting extent of drug penetration, with that equation (1) presented below [29]:

$$\text{Log BB} = -0.0148(\text{PSA}) + 0.152(\text{Log P}) + 0.139 \quad (1)$$

Applying equation (1) to PSA and Log P values for compounds A, B, C, D, and isoniazid resulted in the Log BB (from which BB is determined) values for each, are presented in Table 2. Interestingly, the BB value (Concentration in brain/Concentration in blood) is 0.0966 for first-line tuberculostat isoniazid, whereas for compounds A, B, and C are higher at 0.273, 0.0977, and 0.288, respectively. Essentially, compounds A and C are predicted to have significantly greater penetration into the central nervous system for treatment of bacterial infection due to *Mycobacterium tuberculosis*. This is a very efficacious result, supporting the positive potential of these compounds, as well as

the study of structure modification that enhances the absorption/permeation of tuberculostats. Note that compound A has a methyl group substituent, compound C has a $–CH_2Cl$ substituent on the aromatic ring (see Fig. 1). Small molecules have a greater chance to penetrate into the central nervous system [27], and molecules having a molecular weight less than 400 have greater penetration [28].

Tuberculous meningitis is associated with high morbidity and mortality with incidence of CNS tuberculosis directly proportional to the prevalence of tuberculous infection in general [30].

Bioactivity scores for compounds A, B, C, and D also indicate that these molecular scaffolds have structures similar to optimal drug-likeness in six major categories defined in Table 3. This strategy leads to drug-likeness score, with focus on particular drug classes and development of specific activity score for each of these classes. Drug-likeness scores assist in determining which drug should be synthesized and screened [31]. Drug-likeness is often considered a qualitative property of chemicals which is widely integrated into the early stages of lead and drug discovery [32]. Methods that estimate drug-likeness are valuable in the early stages of lead discovery, and can be used to filter out compounds with undesirable properties from screened libraries of candidates and to prioritize candidates from primary screens [32]. The goal is the development of orally available drugs but these approaches are also useful to optimize drug-like pharmacokinetic properties [32]. Optimal ranges for each drug category are indicated in the first column labeled Category.

Note that compounds A, B, C, and D all show bioactivity scores falling within the optimal ranges for drug categories GPCR ligand, ion channel modulator, kinase inhibitor, protease inhibitor, and enzyme inhibitor. Only in the category of nuclear receptor ligand that compounds A and C fall slightly outside the optimal range, while compounds B and D fall within the optimal range. This approach is known to provide useful evaluation of the drug structure as to whether there is consistency in scaffolding and similarity to related clinical drugs.

Table 1. Comparison of properties (Isoniazid and compounds A, B, C, and D)

Property	Isoniazid	Compound A	Compound B	Compound C	Compound D
Log P	-0.969	0.745	0.372	0.896	0.279
Polar Surface Area (Angstroms^2)	68.01	55.12	81.43	55.12	100.95
Number of Atoms	10	11	14	12	13
Molecular Weight	137.43	150.18	194.19	184.63	181.15
Number of Oxygen Atoms	1	1	3	1	3
Number of Nitrogen Atoms	3	2	2	2	3
Number of -OH & -NH_n	2	2	2	2	2
Number Rotatable Bonds	1	1	3	2	2
Molecular Volume (Angstroms^3)	122.56	143.28	171.25	157.06	150.05
Violations Rule of 5	0	0	0	0	0
Dipole (Debye)	4.835	4.947	2.522	5.614	3.481

Table 2. Penetration into central nervous system based on Log BB

Compound	Log BB	BB = Cbrain/Cblood
A	-0.563	0.273
B	-1.01	0.0977
C	-0.541	0.288
D	-1.31	0.0487
Isoniazid	-1.02	0.0966

The scores for compounds A, B, C, and D indicate favorable drug-likeness within the six categories examined. These favorable outcomes show these four compounds to have potential for successful drug candidates.

Compounds A, B, C, and D were placed into tissue culture at various concentrations, then subjected to bacterial presence to determine their effects on bacterial growth. Similarly, this was accomplished with first-line tuberculostat isoniazid, for comparison to novel compounds.

Results that are presented in Fig. 2, show clearly that all four novel compounds A, B, C, D, as well as isoniazid very strongly inhibit the growth of bacteria (more than 85%) at concentrations less than 50 micrograms per milliliter. Near complete bacterial inhibition is accomplished at all concentrations greater than 100 micrograms per milliliter. These results were observed at day 3 and day 14 (see Fig. 2).

Table 3. Bioactivity scores of compounds

Category (Range)	Compound A	Compound B	Compound C	Compound D
GPCR ligand (-1.50 to 0.50)	-1.40	-1.04	-1.41	-1.31
Ion channel modulator (-2.00 to 0.50)	-1.59	-1.13	-1.43	-1.22
Kinase inhibitor (-2.00 to 0.5)	-1.29	-1.02	-1.49	-1.16
Nuclear receptor ligand (-2.00 to 0.50)	-2.04	-1.24	-2.23	-1.76
Protease inhibitor (-2.00 to 0.50)	-1.25	-0.83	-0.84	-1.15
Enzyme inhibitor (-1.50 to 0.50)	-0.84	-0.48	-0.66	-0.73

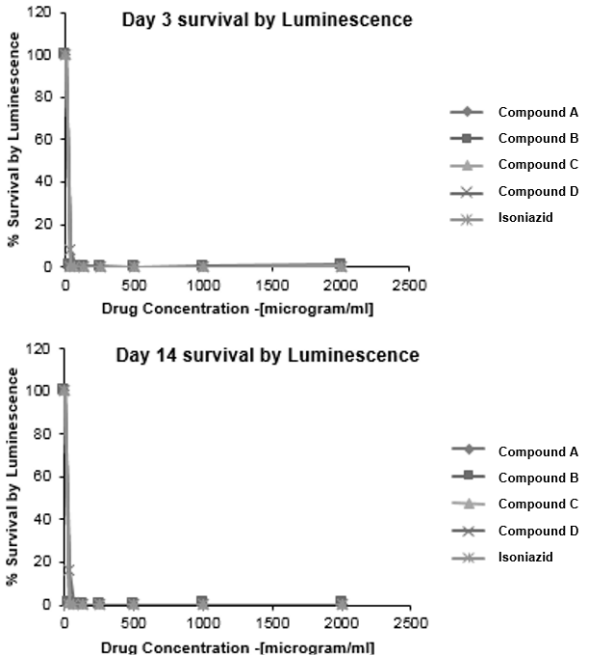


Fig. 2. Inhibition of bacterial growth induced by compounds A, B, C, and D; compared to isoniazid at day 3 and day 14. Bacterial presence measured by luminescence. There is very substantial inhibition of growth at concentrations less than 100 micrograms per milliliter. Essentially complete inhibition occurs at higher concentrations

Therefore, it can be concluded that the suppression of bacteria occurs soon after administration and follows strongly and effectively for time period reaching from day 3 of administration to day 14 (two weeks after). Following bacterial inhibition by luminescence provides accurate information to the extent of microbial suppression and conveys a measure of the bacterial metabolic activity, that in turn, suggests that compounds A, B, C, and D rapidly shuts down metabolism preceding bacterial death.

Similarly, when measured by colony-forming unit (unit to estimate the number of viable bacteria) all four compounds A, B, C, and D substantially reduces bacteria survival (see Fig. 3). The level of bacterial inhibition is similar to that induced by first-line tuberculostat isoniazid. Bacterial inhibition greater than 85% is accomplished at concentrations less than 50 micrograms per milliliter. This very strong growth suppression is sustained at concentrations greater than 100 micrograms per milliliter. Inhibition of growth as measured by colony-forming units is very strong and apparent soon after exposure at day 3 and extends to day 14 (two week time period). These results show clearly the very strong inhibitory effect of compounds A, B, C, and D on the growth of this deadly bacteria.

The design of new drugs for use in the ongoing battle against the deadly infections of *Mycobacterium tuberculosis* is a very necessary endeavor. The appearance of multiple drug resistant, extensively drug resistant, and totally drug resistant bacterial types is a very strong concern. Numerous nations are particularly prone to have problems of containment due to poor communication, poor transportation, poor economy, and lower quality of medical support.

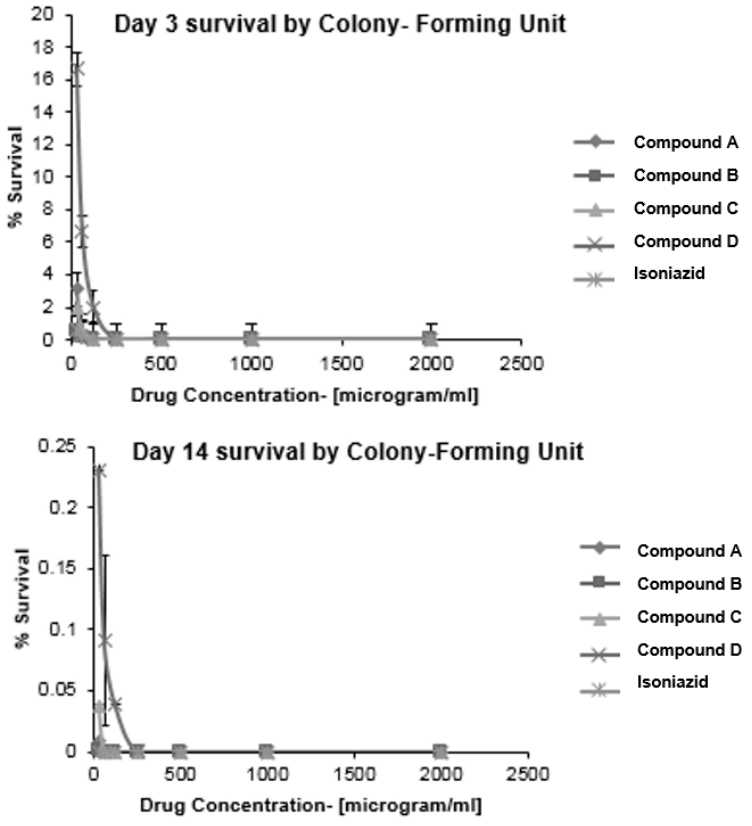


Fig. 3. Inhibition of bacterial growth induced by compounds A, B, C, and D; compared to isoniazid. Bacterial inhibition measured by colony-forming units (CFU). There is substantial inhibition of growth at concentrations less than 100 micrograms per milliliter. Essentially complete inhibition occurs at higher concentrations

The development of new drugs will assist in containment of bacterial outbreaks and proliferation. The compounds presented in this study are shown to substantially suppress bacterial proliferation and possess properties highly favorable in drug-likeness, absorption, and bacterial inhibition.

4. CONCLUSION

Four novel compounds that are presented here have a single hydrazide group covalently bonded to a single aromatic ring. In addition, there various substituents in the meta or para position to the hydrazide group. Molecular properties were calculated and showed values statistically comparable to first-line tuberculostat isoniazid. All four compounds A, B, C, and D showed zero violations of the Rule of 5, indicating these compounds have properties enabling favorable absorption into the body and membrane penetration. Compounds A and C show potential for substantially greater penetration into the central nervous system than that for isoniazid, on order to treat bacterial infection by *Mycobacterium tuberculosis*. All four compounds showed bioactivity scores within the optimal ranges for five major categories of clinical drugs. Substantial bacterial growth inhibition was accomplished by all four novel compounds and to a level comparable to isoniazid. Measured by luminescence and colony forming units, compounds A, B, C, and D initiated greater than 85% growth inhibition of bacteria at concentrations less than 50 micrograms per milliliter. Compounds A, B, C, and D showed very strong bacterial growth inhibition and molecular properties favorable for drug absorption, drug-likeness, and penetration into the central nervous system. The investigation and design of novel drugs to treat infections of *Mycobacterium tuberculosis* is a highly desirable endeavor to assure continued effective clinical treatment of this deadly microbe.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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