2014

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British Journal of Pharmaceutical Research
4(12): 1535-1551, 2014

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Novel Tuberculostatic Agents Suitable for Treatment of *Mycobacterium tuberculosis* Infections of the Central Nervous System

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Authors' contributions

This work was carried out in collaboration between all authors. Author RB synthesized the compounds of this study, wrote the first draft of the manuscript, and completed all molecular modeling. Author PS completed the bacterial culture study and analyzed growth data. Author SLGC designed, analyzed, and managed the culture study. Author JDC contributed to analysis and design of experiments. All authors read and approved the final manuscript.

Original Research Article

Received 25th March 2014
Accepted 5th May 2014
Published 18th June 2014

ABSTRACT

Aims: To demonstrate the efficacy of five small molecule compounds for inhibiting the growth of *Mycobacterium tuberculosis.* To present evidence that these compounds will penetrate into the central nervous system.
Study Design: Five small molecule compounds bearing a hydrazide group were synthesized utilizing microwave excitation. These compounds were then placed into tissue culture with *Mycobacterium tuberculosis* at various concentrations for evaluation of bacterial growth inhibition.
Place and Duration of Study: The compounds to be tested were prepared at the University of Nebraska Chemistry Department August 2013. The evaluation of antibacterial activity was determined at the Texas A&M Health Science Center during October to December of 2013.
Methodology: Applying microwave excitation for generation of hydrazide groups within the structure of small molecule carboxylic acids, five agents were prepared for evaluation

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of bacterial growth inhibition. These agents were dissolved into tissue culture media at various concentrations. Having various levels of tuberculostatic agents, then tuberculosis bacteria were added to determine level of growth inhibition. Growth inhibition of the bacteria was achieved and measured by compound concentration for comparison and evaluation.

Results: Five compounds having a hydrazide functional group greatly inhibited the growth of *Mycobacterium tuberculosis*. All five agents had molecular weight less than 215 grams/mole and polar surface area of less than 70 Angstroms^2. Values of Log P ranged from -0.226 to 0.998. Values of Log BB (Log [C_{brain}/C_{blood}]) ranged from -0.711 to - 0.525, with a range in central nervous system penetration C_{brain}/C_{blood} of 0.195 to 0.299. All compounds showed zero violations of the Rule of 5. Substantial inhibition of bacterial growth was observed at concentrations as low as 30 micrograms/mL, as measured by optical density and colony forming units.

Conclusion: These five hydrazide compounds substantially decreased the proliferation of tuberculosis bacteria at concentrations as low as 30 micrograms/mL. In addition, their physicochemical properties are shown to allow high levels of penetration into the central nervous system.

Keywords: Tuberculosis; Mycobacterium tuberculosis; tuberculostatic; isoniazid.

ABBREVIATIONS

Term: SMILES, Simplified Molecular-Input Line-Entry System; TB, tuberculosis; CNS, central nervous system; mcg, microgram; PSA, polar surface area.

1. INTRODUCTION

The causative agent for the disease referred to as tuberculosis (TB) is *Mycobacterium tuberculosis*. Infections due to *M. tuberculosis* are still regarded as a major world-wide health problem. Some estimates place the number of infections at approximately one-third of the entire world's population [1]. The World Health Organization estimates that by the year 2015 there will be as many as one billion newly infected people and as many as 200 million will develop the active disease [1]. Approximately 2 million deaths per year are attributed to TB and each year there can be as many as 9 million new cases [1]. As of the year 2013, it is believed that as much as 95% of new cases and 98% of deaths attributed to TB occur in low-income and middle-income countries [1]. The decay of health services, social-economic decline, and emergence of drug-resistant forms of TB contribute the most to these alarming trends [1].

An involvement of the central nervous system (CNS) will occur in up to 10% of all individuals having TB infection [2,3]. This CNS involvement can develop tuberculoma (non-neoplastic mass usually appearing in the lungs or brain), meningitis (inflammation of the protective membranes covering the brain and spinal cord), abscess (area of pus that has accumulated due to inflammation from an infection), or other form of manifestation [2]. The meningitis form of TB is devastating and accounts for up to 5% of all extrapulmonary cases of TB [4]. Central nervous system tuberculosis infection can produce the debilitating form referred to as tuberculosis meningitis which has a high rate of morbidity and mortality [5], this with the infection originating in the lungs [6].

A hematogenous (carried by the blood) proliferation of *Mycobacterium tuberculosis* from the primary pulmonary infection to incur an infection of the CNS can form small subpial (beneath the pia mater) and subependymal foci (located under the ependymal) inside the brain and spinal cord resulting in meningitis and/or tuberculoma [7]. A TB infection of the CNS carries a high rate of mortality and neurological morbidity [8]. In children the manifestation is usually tubercular meningitis, post-tubercular meningitis hydrocephalus, and tuberculomas [8]. Symptoms in young children include weight loss, sleep disturbance, vomiting, and seizures; whereas in older children it many manifest as headache, fever, vomiting, weight loss, and photophobia [7].

Risk factors for CNS involved TB infection has been identified to include malnutrition and recent measles infection [9]. Previous studies have shown that nearly all tuberculomas of the brain, even irrespective of their size, can be cured by medical treatment that includes steroids to control brain edema (accumulation of fluid) [10]. This being fortuitous due to the fact that 40% to 66% of adults presenting TB meningitis will have extra meningeal TB infection at the time of diagnosis [11]. Treatment of CNS tuberculosis general includes the use of four drugs; isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and ethambutol (EMB) [12]. Previous studies have shown that children having tuberculomas of the brain will respond well to this four drug regimen [13]. The functional group within the molecular structure for the first-line drug isoniazid is a hydrazide group ($-C(O)NHNH_2$). The hydrazide group is the bases for the antibacterial activity expressed by the compounds synthesized and tested in this study. Side effects to the drug isoniazid includes weakness, nausea, and seizures [12]. One of the difficulties of diagnosis of TB meningitis is the slow develop of symptoms that include fatigue, malaise, and fever with only the following symptoms appearing after significant progression [14]: lethargy, unconsciousness, alterations in mental status, vomiting/nausea, and fever.

The first-line drug isoniazid (isonicotinohydrazide) has been shown to be effective in the treatment of TB [15]. Isoniazid is a prodrug that is activated by bacterial catalase-peroxidase enzyme named KatG. The enzyme KatG couples the isonicotinic acyl with NADH to form isonicotinic acyl-NADH complex that binds tightly to the enoyl-acyl carrier protein reductase InhA, blocking the action of fatty acid synthase. This process inhibits the synthesis of mycolic acid, required for the mycobacterial cell wall [15].

Findings of previous studies and the spread of TB world-wide clearly bespeaks for the investigation and development of additional drugs for the treatment of CNS associated tuberculosis. This work presents five compounds shown to be suitable for clinical application in the treatment of CNS associated TB infections.

2. METHODOLOGY

2.1 Reagents and Instrumentation

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

2.2 Molecular Modeling and Numerical Analysis

Molecular modeling and molecular properties were determined by utilizing ChemSketch v. 5 (90 Adelaide Street West, Toronto, Ontario M5H 3V9, Canada) and 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. Determination for hierarchical cluster analysis was accomplished by by PAST v. 1.97(Copyright Oyvind Hammer and Harper 1999-2010) and KyPlot version 2.0 beta 15 (copyright Koichi Yoshioka 1997 – 2001). Toxicity assessment was accomplished by OSIRIS Property Explorer version 2 (Actelion, Gewerbestrasse 16, Ch-4123 Allschwil, Switzerland).

2.3 Bacterial Culture

2.3.1 Strain

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 survival assays.

2.3.2 Media

M. tuberculosis were grown in Middlebrook 7H9 supplemented with albumin dextrose complex (M-ADC) (Difco), 0.05% Tween 80 (M-ADC-Tw) and kanamycin at a final concentration of 10µg/ml to select for plasmid maintenance in the strains.

2.3.3 Compounds

All compounds were dissolved to a final concentration of 5 mg/ml. Both isoniazid and compounds were sterilized by passage through 0.22 µm-syringe filters.

Survival by Optical Density: Four clear 96-well flat bottom plates were filled with 108µl per well of M-ADC-Tw media supplemented with 10 µg/ml kanamycin to maintain luminescence in the strain. Each tested compound was added in duplicate wells at 72µl per well at a final concentration of 2 mg/ml. Similar amounts of isoniazid were added to the first two wells at a concentration of 5 microgram/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* was added to 96-well plates to give a final volume of 100µl per well. Plates were incubated at 37ºC for 7 days and 14 days. Bacterial survival was measured at 600 nm using an EnVision (PerkinElmer) plate reader at day 0, 2, 7 and 14 in the presence of compounds.

2.3.4 Survival by luminescence

Four solid white 96-well flat bottom plates were prepared as before. Plates were incubated at 37ºC for 2, 7 and 14 days. Luminescence measurements were taken for day 0, 2, 7, and 14 in the presence of tested compounds. Bacterial luminescence was measured 5 minutes after addition of 10µl of 5mM D-luciferin in 0.45M sodium citrate buffer pH 5.0 (Gold Biotechnology) using a 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 113ºC and was stored sealed at -20ºC. Hydrazide derivatization: All compounds were treated similarly, place 120mg 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 -20ºC. The presence of the hydrazine group can be confirmed by use of FTIR, observing for peaks at 944 cm^{-1} for hydrazine and 1000cm^{-1} to 1200cm^{-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 [16]. Assignments of C-13 peaks for compounds A, B, C, D, and E (ppm): (A) Aromatic carbons C-1 134.2, C-2 136.5, C-3 129.3, C-4 131.8, C-5 125.6, C-6 127.2, 2-methyl 18.6, carbonyl carbon 163.3; (B) Aromatic carbons C-1 130.5, C-2 127.2, C-3 129.3, C-4 141.1, C-5 129.3, C-6 127.2; 4-methyl 20.9; carbonyl carbon 167.3; (C) Aromatic carbons C-1 135.9, C-2 129.8, C-3 129.0, C-4 127.4, C-5 129.0, C-6 129.8; α-carbon 41.5; carbonyl carbon 174.8; (D) Aromatic carbons C-1 131.6, C-2 128.7, C-3 137.2, C-4 129.0, C-5 128.7, C-6 128.7; carbonyl carbon 167.3; (E) Carbonyl carbon 174.1, α-carbon 36.0, β-carbon 25.3, γ-carbon 28.6, δ-carbon 33.8, ε-carbon 32.9.

3. RESULTS AND DISCUSSION

The threat to personal health that tuberculosis presents as well as its proliferation world-wide necessitates the study and development of additional clinical therapeutics. The morbidity and mortality of tuberculosis infection of the CNS presents unique problems even in diagnosis, in addition to clinical treatment due to the difficulty of drugs to penetrate through the blood-brain barrier. Here in this study are presented five small molecule compounds showing physicochemical properties suitable for penetration into the central nervous system. This capability requires unique and rigorous parameters for their chemical properties in addition for the required bacterial inhibition that is specifically targeting *Mycobacterium tuberculosis*.

Five small molecule compounds were synthesized applying microwave excitation to emplace a hydrazide ($-C(O)NHNH_2$) group to replace the initial carboxyl group (-C(O)OH). This functional group as well as other structural features lend unique physicochemical properties suitable for passive absorption into the CNS. Structures of compounds A, B, C, D, and E are presented in Fig. 1. These small molecule compounds have some properties shared and important to their efficacy to target TB infections of the CNS. Straight forward inspection reveals the hydrazide group ($-C(O)NHNH_2$) in position without steric hindrance inhibiting activation by the necessary reaction to bacterial catalase-peroxidase enzyme named KatG [15]. The hydrazide group is covalently bonded onto an aromatic ring in the case of compounds A, B, C, and D. However, for compound E the hydrazide group is attached to an aliphatic chain having a terminal end bromine atom.

Compound A has a methyl group (-CH_3) in ortho position (the C-2 position) relative to the hydrazide group. For compound B there is a methyl group in para position (the C-4 position) relative to the hydrazide group. Compound C (2-phenylacetohydrazide) has an aromatic ring with a (-CH_2-) group bridging the hydrazide functional group. The compound D has a chlorine atom in para position (the C-4 position) relative to the hydrazide group. Compound E contains an aliphatic chain in place of the aromatic ring (compared to compounds A, B, C, and D). These structural features will influence the physicochemical parameters of these compounds. In addition the molecular formula, elemental composition, and SMILES notation for these five drugs are shown in Fig. 1.

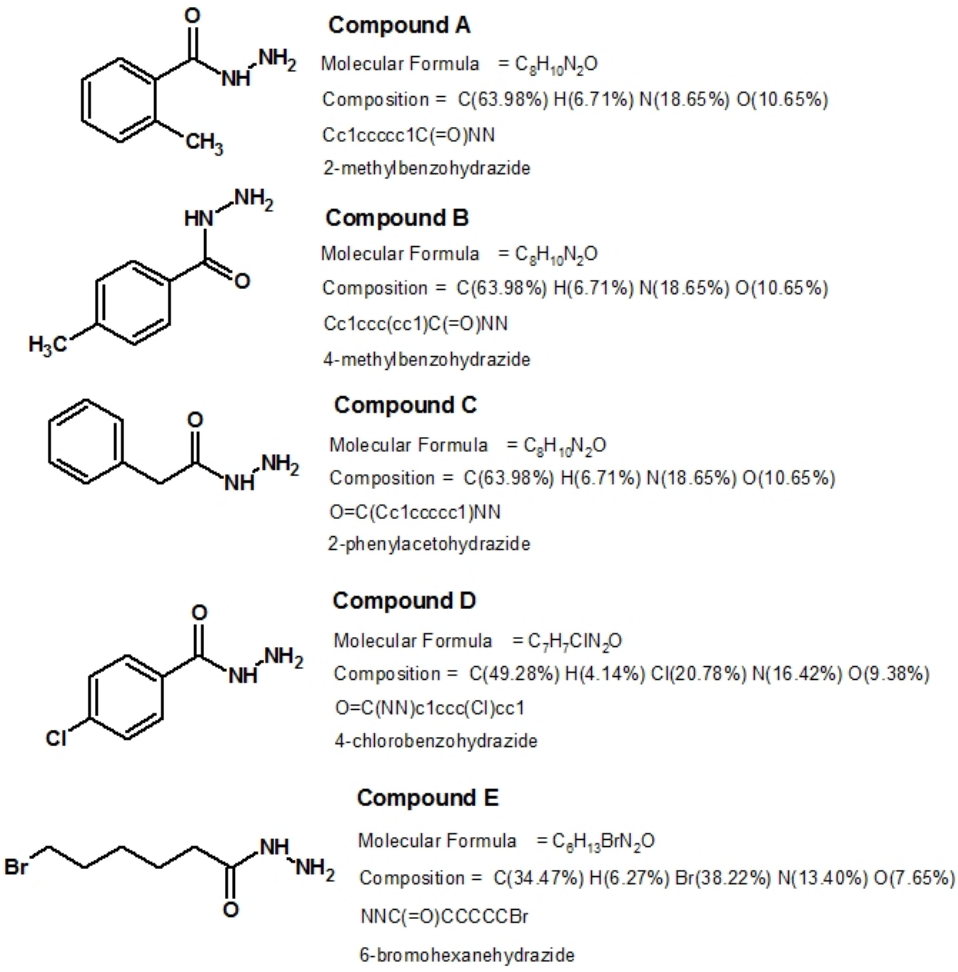

Fig. 1. Molecular structures of compounds A, B, C, D, and E. The structures show the position of the hydrazide functional group (-C(O)NHNH$_2$), along with molecular formula, percent elemental composition, SMILES nomenclature, and IUPAC name. Note that compounds A, B, C, and D possess an aromatic ring, while compound E contains an aliphatic carbon group with a terminal bromine atom. The aromatic ring of compound B contains a methyl group (-CH_3), while compound D contains a chlorine atom in the para- position to the hydrazide group. These structural features significantly affect the physicochemical properties of the compounds

Pharmacological properties of compounds A to E are presented in Table 1. The ranges in numerical values for Log P and molecular weight are -0.226 to 0.998 and 150.2g/mole to 209.1g/mole, respectively. The average molecular weight is 161.2g/mole with only a small standard deviation of 25.8 g/mole (16% of the average). The polar surface area (PSA) is constant for A through E at 55.121 A^2, as is the number of oxygen atoms, nitrogen atoms, and NH_n groups. For compounds, A to E the number of oxygen and nitrogen atoms (hydrogen bond acceptors) is constant at 3. For all compounds, A to E the number of hydroxyl groups (-OH) and number of amine groups (-NH_n) (hydrogen bond donors) remains constant at 3. Based on the Log P values, it is observed that compounds A to E are more lipophilic than isoniazid that has Log P equal to -0.969 (more hydrophilic), as well as having smaller PSA values at 55.121 A^2. The Pearson r correlation coefficient for Log P to the number of atoms (r=0.6398) and molecular volume (r=0.5586) is considered strong. The correlation for molecular volume to molecular weight is very strong at r=0.7926 and very strong for the number of rotatable bonds to molecular weight (r=0.8733). Toxicity risk assessment analysis showed no sizeable consideration for these five compounds having a hydrazide functional group that is similar to the first-line drug isoniazid. Evaluation of risk assessment for compound A showed no irritant, reproductive, or mutagenic risk. Similarly, compound B showed no irritant, reproductive, or mutagenic risk. For compound C, there is no irritant or reproductive risk, with only a slight mutagenic risk. Likewise, compound D shows no irritant, reproductive, or mutagenic risk. The outcome for compound E showed a slight risk of irritant, reproductive, and mutagenic risk. Overall this is a supporting risk assessment determination for all compounds A, B, C, D, and E which supports their overall potential as tuberculostats.

As values of Log P become more positive then the level of lipophilic tendency becomes greater. The other way around the more negative the Log P numerical value the greater the hydrophilic tendency of the drug. By Log P values (-0.969 for isoniazid) the first-line tuberculostat isoniazid is the most hydrophilic of the drugs shown in Table 1.

Drug discovery is an important process of identifying potential drug candidates. In drug development lipophilicity and molecular weight are often increased in order to improve the affinity and selectivity of any particular drug candidate. But this step makes it more difficult to maintain drug-likeness optimization. Drug-likeness is a qualitative concept applied in drug design for how drug-like a potential compound is with respect to factors like bioavailability. Furthermore, bioavailability is a principal pharmacokinetic property of drug design involving the absorption of the drug or the fraction of an administered dose of unchanged drug that reaches the systemic circulation.

The Rule of five defines parameters to evaluate drug-likeness or determine if a chemical compound having pharmacological or biological activity has properties that would make it a likely orally active drug in humans. This set of rules states that an orally active drug has no more than one violation of the following criteria [17]: 1) No more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms); 2) Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms); 3) A molecular mass less than 500 daltons; and 4) An octanol-water partition coefficient (Log P) not greater than 5. All five compounds A, B, C, D, E, and isoniazid are shown to have zero violations of the Rule of 5, which indicates favorable drug-likeness and bioavailability.

All compounds A, B, C, D, and E were placed in bacterial culture to evaluate efficacy of growth inhibition that is targeting *Mycobacterium tuberculosis*. The results show clearly that the growth and proliferation of *Mycobacterium tuberculosis* are very strongly inhibited. Results presented in Fig. 2, shows clearly the very strong inhibition of bacterial growth.

Looking at Fig. 2, it is clearly seen that significant inhibition of bacteria occurs as early as day 2 at all concentrations of drugs (measured by OD 600nm). By day 7 the bacterial inhibition is greater than 60% (e.g. the percent survival is at or less than 40%) for all compounds A, B, C, D, and E at concentration of 120 microgram/mL. There is growth inhibition greater than 60% for compounds D, E, and C at a level of 60 microgram/mL at time day 7. By day 14 the percent bacterial growth inhibition for all drugs is greater than 80% (e.g. percent survivial is $\leq$ 20%) for all compounds A, B, C, D, and E at 60 microgram/mL concentration and greater. This is clearly a substantial bacterial inhibition induced by these small-molecule compounds and at very low concentration and within a time period of seven days.

To determine the colony-forming unit (CFU) is to obtain an estimation of viable bacterial numbers. Unlike direct microscopic counting where all cells, whether they are dead or living are counted; utilizing CFU counting estimates viable cells. Evaluation of bacterial growth inhibition for compounds A, B, C, D, and E by CFU are determined and presented in Fig. 3.

The measurement of viable bacteria (CFU) after treatment with all five compounds shows clearly that there is a complete collapse of bacterial proliferation as early as day 2 (see Fig. 3). Furthermore, complete bacterial cell death is achieved by all five compounds by day 7 (see Fig. 3). Likewise, by day 14 there is total bacterial cell death for all compounds A, B, C, D, and E. This is a strong and substantial antibacterial activity that contributes to the clinical potential of these five compounds. A very strong antibacterial action would be highly advantageous for drugs targeting TB infections of the central nervous system. Similarly, the survival of bacteria was monitored by luminescence (see METHODOLOGY) and is shown in Fig. 4. Cell number correlates with luminescent output by this assay. Note that essentially a complete collapse of bacterial growth is accomplished by day 2. On day 2, there was greater than 50% cell death achieved at concentration of 30 microgram/mL and greater (i.e. survival less than 50% by Total Flux luminescence). This is also seen at day 7 and day 14 after determination by luminescence.

By day 7 all compounds, A to E have induced greater than 50% cell death (bacteria cell survival less than 50%). By day 14, the substantial level of growth inhibition is sustained and cell death is greater than 60% at all concentration levels for all compounds A, B, C, D, and E.

Various studies investigating the physicochemical properties of drugs known to penetrate into the CNS, allow for determination of relevant properties that enable that action. Previous studies have shown conclusively that small drugs penetrate through the blood-brain barrier and into the CNS with much better efficiency [18]. Furthermore, it has been shown clearly that drugs which strongly penetrate into the CNS will have a molecular weight of less than 400 Daltons and have PSA of less than 90 Angstroms^2 [19]. Looking at Table 1 then it is clearly seen that all drugs A, B, C, D, and E fulfil these criteria. This outcome strongly suggests that all five of these compounds will effectively and substantially pass into the CNS from bloodstream delivery.

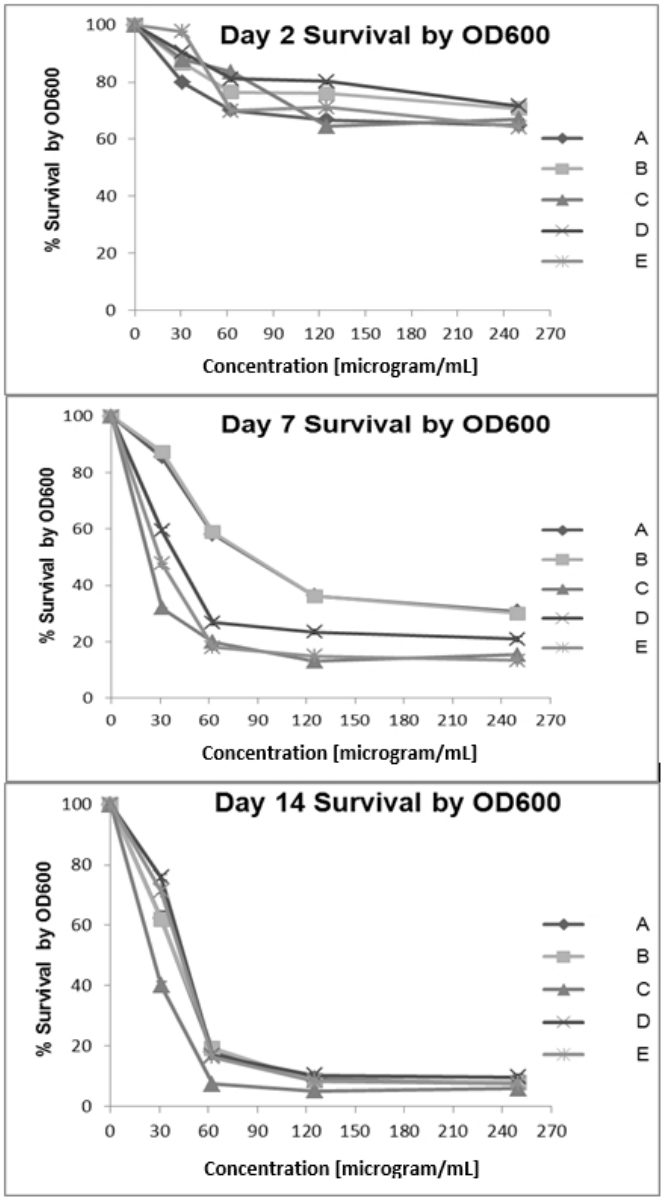

Fig. 2. Survival rate of bacteria at day 2, day 7, and day 14 when compounds A, B, C, D, and E are dosed at different concentrations (see X-axis, concentration of drugs at microgram/mL). Note that by day 7, the bacterial inhibition is greater than 50% at concentrations as low as 60 micrograms/mL for compounds C, D, and E

Other studies have found that orally active drugs targeting the CNS can be transported into the CNS passively if they have PSA of less than 120 Angstroms^2 and these drugs can be highly effective for CNS penetration if the PSA is less than 60 to 70 Angstroms^2 [20]. Again, physicochemical properties shown in Table 1 indicate that all drugs A, B, C, D, and E having a PSA of 55.121 A^2 will strongly penetrate into the CNS to target TB bacteria. Other studies

have applied these molecular property parameters to design antibacterial agents for targeting infections in the CNS: *Staphylococcus aureus* [21], *Mycobacterium tuberculosis* [22], methicillin-resistant *Staphylococcus aureus* [23], and ampicillin-resistant *Escherichia coli* [24].

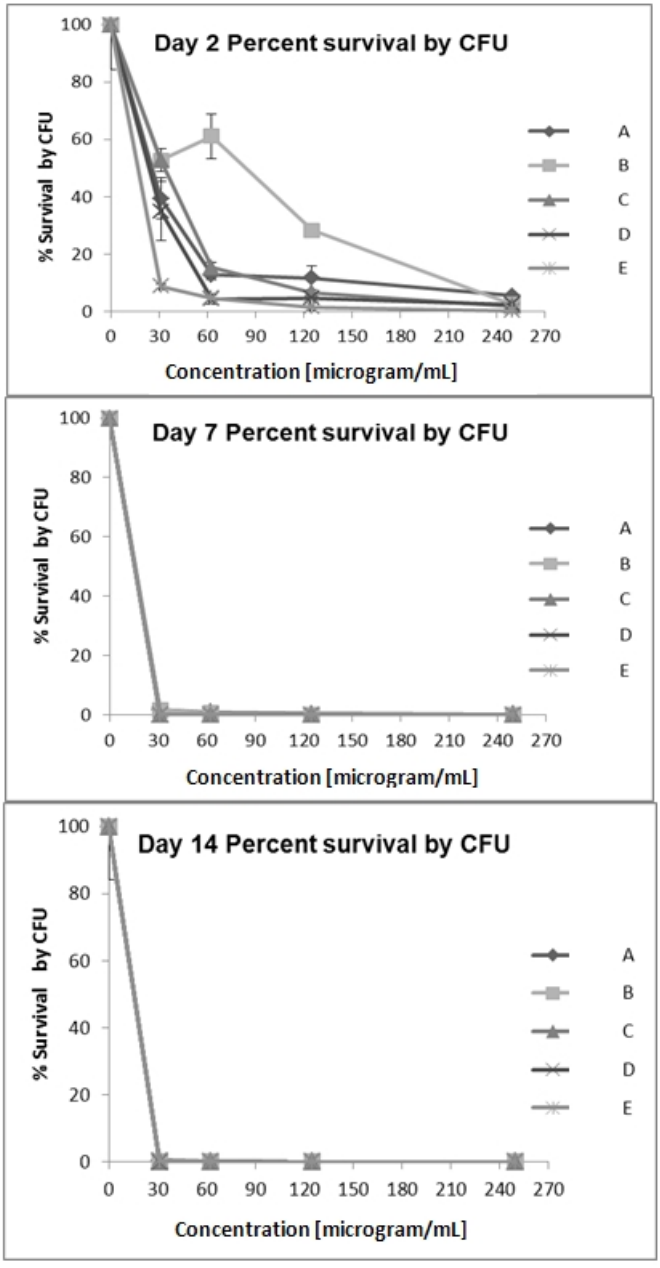

Fig. 3. Results for bacterial inhibition by compounds A, B, C, D, and E by CFU are shown. Clearly there is essentially complete bacterial cell death by day 2 at all concentrations. At day 7 and day 24 there is essentially complete bacterial cell death at concentrations as low as 30 micrograms/mL for all compounds A, B, C, D, and E

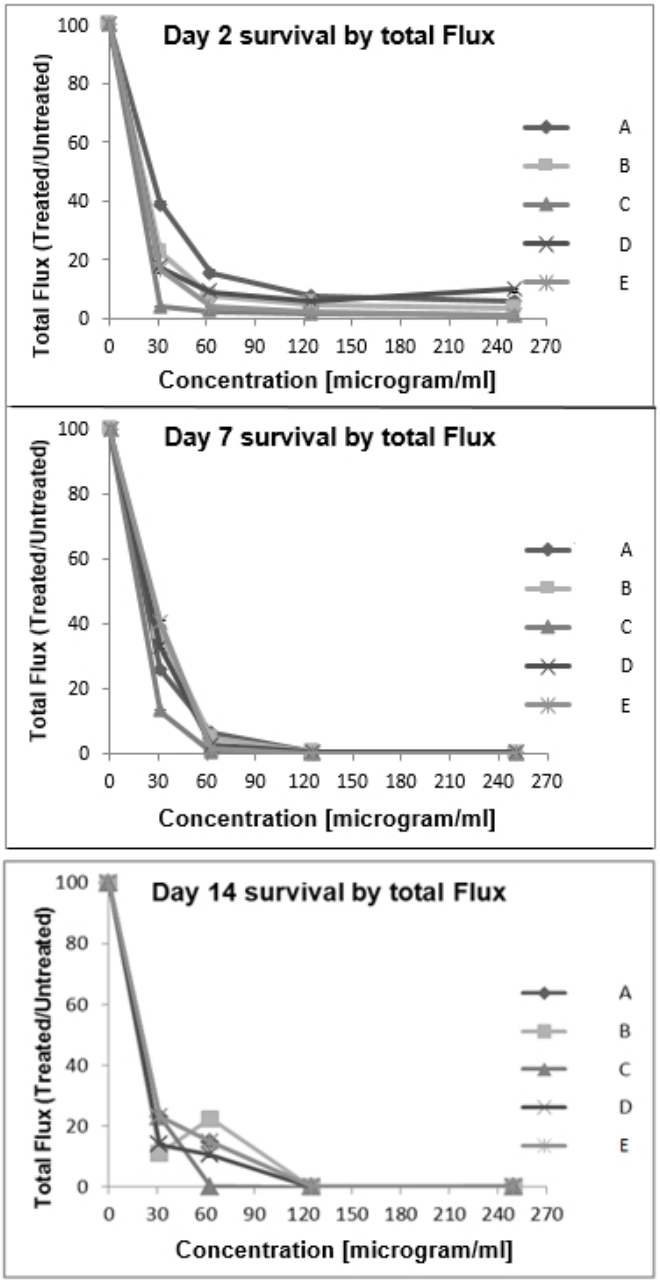

Fig. 4. Reading of luminescence of bacteria also shows drastic and effective action on bacterial growth for each compound A, B, C, D, and E at concentrations as low as 30 microgram/mL. Further increased inhibition of bacteria growth was achieved at greater concentrations. The cell number correlates with luminescent output, as shown in the figure

Table 1. Physicochemical properties of drugs

Compound	Log P	Polar surface area (Angstroms^2)	Number of atoms	Molecular weight (g/mole)	Number of Oxygens & Nitrogens	Number of –OH & -NH_n	Violations of rule of five	Number of rotatable bonds	Molecular volume (Angstroms^3)
A	0.721	55.121	11	150.2	3	3	0	1	143.28
B	0.769	55.121	11	150.2	3	3	0	1	143.27
C	-0.226	55.121	11	150.2	3	3	0	2	143.52
D	0.998	55.121	11	170.6	3	3	0	1	140.25
E	0.258	55.121	10	209.1	3	3	0	5	157.20
isoniazid	-0.969	68.013	10	137.1	4	3	0	1	122.56

Earlier studies have demonstrated that the parameter 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 CNS [25]. Study of drugs known to effectively pierce into the CNS reveal an equation for activity prediction, that equation shown below [25]:

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

Applying equation (1) to values of PSA and Log P for drugs A, B, C, D, and E resulted in the values for each, that are presented in Table 2.

Table 2. Penetration into CNS based on log bb

Compound	Log BB	BB = C brain/C blood
A	-0.567	0.271
B	-0.560	0.275
C	-0.711	0.195
D	-0.525	0.299
E	-0.638	0.230
Isoniazid	-1.02	0.0966

The average value of Log BB for all drugs in Table 2 is - 0.670 (standard deviation = 0.18) with the minimum of - 1.02 to a maximum of - 0.525. The average value of BB for all compounds is 0.228 (standard deviation = 0.074) with the minimum of 0.0966 and maximum of 0.299. The Pearson r correlation of Log BB to BB is extremely strong at 0.9882. Note that based on BB numerical values, the effective concentration of drugs A, B, C, D, and E into the CNS are all substantially greater than that for the first-line tuberculostat isoniazid (BB = 0.0966). The level of CNS penetration increases as Log P becomes more lipophilic. This is a powerful support for these five drugs as efficacious drugs for treatment of TB infections of the CNS. The relationship of Log P values to numerical values of BB is shown to be linear and presented in Fig. 5. The linear relationship of Log P to BB has a correlation of 0.9936 and coefficient of determination R^2 of 0.9872. The coefficient of determination of 0.9872 indicates the relationship accounts for 98.72 % of variance in the model. This is an extremely linear relationship with the positive correlation coefficient of 0.9936.

The purpose of cluster analysis is to discover a system of organizing observations or objects, into groups in which members of these groups share properties in common. It is a data reduction tool that creates subgroups that have increased manageability than the individual datum [26,27]. The actual task of clustering has the objective of grouping a set of objects in such a way that objects in the same group (called a cluster) are more similar (in some sense or another) to each other than to those in other groups (clusters) [26,27]. In the method of hierarchical cluster analysis as applied here, there is no prior knowledge as to the number of clusters that will be produced.

Results of hierarchical clustering utilizing Euclidean distance and single linkage clustering are shown in Fig. 6. Note that when using the physicochemical properties presented in Table 1, inclusive of isoniazid, there is a high resolution of the drugs as to the most similar.

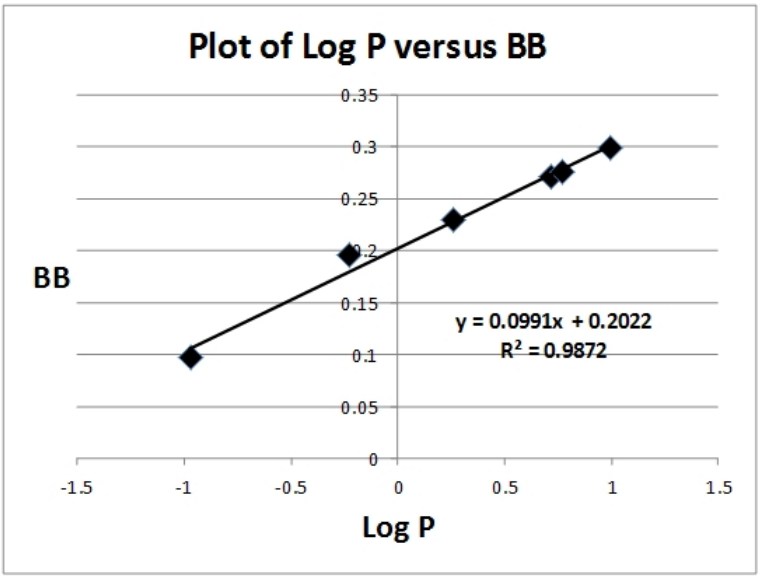

Fig. 5. The relationship of Log P to values of BB for these compounds is highly linear. Note that the coefficient of determination of 0.9872 indicates that the relationship accounts for 98.72 % of variance in the model. Equation of the line: y = 0.0991x + 0.2022 has a correlation of 0.9936.

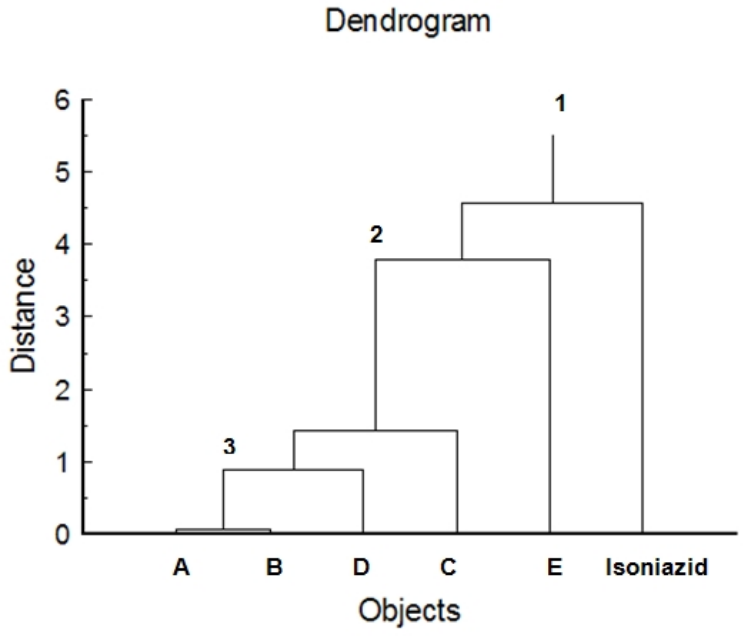

Fig. 6. Hierarchical cluster analysis of compounds A, B, C, D, E, and isoniazid (see Table 1) utilizing Euclidean distance measurement and single linkage clustering conditions

Compounds A and B are determined to be of highest similarity and are joined at node 3. However, the cluster of A and B are joined to compound D and C (next level of similarity) at node 2. Further resolution appears to indicate that compound E and isoniazid are less similar to A, B, and D; with compound C closer but distinct from those drugs. Isoniazid having a more negative Log P, greater PSA, and additional nitrogen atom accounts for some difference.

The socio-economic damage evoked by tuberculosis infection across the populations are substantial and requires alertness and methodical application of appropriate public health measures to counter measure. The continued study and evaluation of new tuberculostat agents are vital due to the apparent facile nature of neurons to act as host cells for M. tuberculosis [28] and the need for treatment improvements over mere empirical and conventional regimens applied for pulmonary tuberculosis [29]. This study has shown that the physicochemical properties of potential drugs can be altered to improve penetration into the central nervous system and target very debilitating *Mycobacterium tuberculosis* infections.

4. CONCLUSION

In summary, this study presents five small hydrazide-type compounds that have physicochemical properties suitable for penetrating into the CNS. Specifically, this includes polar surface area values of less than 60 Angstroms^2. Furthermore, all five compounds show zero violations of the Rule of 5, which indicates favorable drug-likeness and bioavailability. Importantly, all five compounds A, B, C, D, and E inhibit growth of *M. tuberculosis* at concentrations as small as 30 microgram/mL. For all five compounds and by all methods of measurement, very substantial to complete inhibition of bacteria growth was achieved at concentrations greater than 30 micrograms/mL. This outcome is shown by optical density measurement at 600 nm and measurement of colony-forming units (CFU), as well as following viable bacteria by luminescence. These trends of bacterial growth inhibition and cell death were highly consistent when comparing OD assay, CFU assay, and cell viability measured by luminescence. The relationship of Log P to BB is shown to be highly linear, an outcome suggesting a linear relationship between lipophilic tendency measured by Log P and ability to cross into the CNS from the blood stream. Calculation of Log BB and BB indicates that compounds A, B, C, D, and E will have significant and even greater penetration into the CNS than the first-line tuberculostat isoniazid. Pattern recognition analysis indicated that compounds A and B had highest similarity, followed by D, C, and C in order. While isoniazid was determined to be distinct from compounds A, B, C, D, and E. That capability and the very high inhibition of *Mycobacterium tuberculosis* support the potential of these agents to be effective in targeting CNS infections. A significant number of pulmonary TB infections becomes infections of the CNS, which itself has a high morbidity and mortality. The further investigation of novel drugs and their development will highly benefit the clinical treatment of TB infections of the central nervous system.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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The peer review history for this paper can be accessed here:
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