ACS Medicinal Chemistry Letters

Letter

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Syntheses and Antituberculosis Activity of 1,3-Benzothiazinone Sulfoxide and Sulfone Derived from BTZ043

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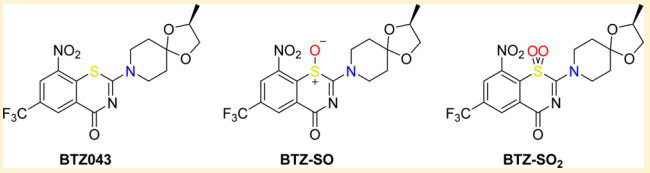
Supporting Information

ABSTRACT: The discovery of 1,3-benzothiazin-4-ones (BTZs), especially BTZ043 and PBTZ-169 as potent agents for the treatment of tuberculosis, prompted intensive research related to development of potential antituberculosis agents based on electron deficient nitroaromatic scaffolds. Herein we report the syntheses, computational and NMR studies and anti-TB activity of oxidation products, 1,3-benzothiazinone sulfoxide (BTZ-SO) and 1,3-benzothiazinone sulfone (BTZ-SO_2) derived from BTZ043. The combined computational and NMR work revealed differences in the total charge densities and molecular shapes of the oxidation products. While docking studies still suggested similar interactions and binding patterns for both products with the target DprE1 enzyme, antituberculosis assays indicated remarkable differences in their activity. Interestingly, BTZ-SO possesses potent activity against nonpathogenic and pathogenic mycobacterial strains, but BTZ-SO_2 is only weakly active.



KEYWORDS: *BTZ043, oxidation, sulfone, sulfoxide, DprE1, tuberculosis*

Tuberculosis (TB) remains a serious threat to global health. The discovery of 1,3-benzothiazin-4-ones (BTZs), especially BTZ043 and PBTZ-169 (Figure 1) as impressively

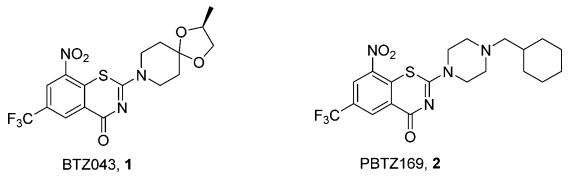
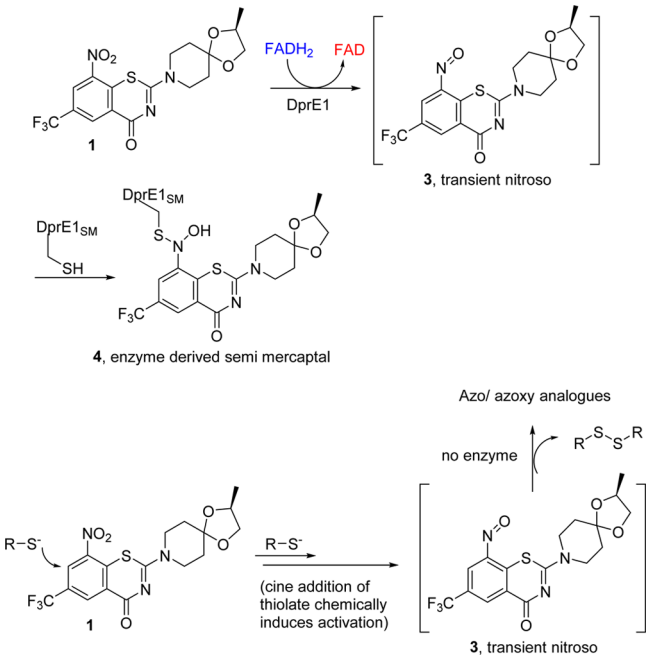


Figure 1. Structures of 1,3-benzothiazinone anti-TB agents, BTZ043 and PBTZ169.

potent and selective agents for the treatment of tuberculosis (TB), prompted highly intensive research in the area of electron deficient nitroaromatic warheads as anti-TB agents.[1,2]

Prior to the discovery of BTZs, several nitroaromatic compounds were found to have interesting anti-TB activity, especially PA-824,[3,4] OPC67683,[5] nitrofuranylamides, and nitrofuran isooxazolines.[6−11] The mode of activation of BTZ and related compounds has been shown to involve reduction of the essential nitro group to a reactive nitroso moiety **3** apparently mediated by cofactor $FADH_2$, which then reacts with cysteine 387 of DprE1 to form a covalent, semimercaptal adduct, **4** (Scheme 1). Subsequently, several other related electron deficient aromatic compounds have been shown to cause similar covalent inhibition of DprE1, including dinitrobenzamides (e.g., DNB1),[12,13] benzoquinoxalines (e.g.VI-9376),[14] and nitrosubstituted triazoles (e.g., 377790).[15]As DprE1 is an essential enzyme involved in the

Scheme 1. Enzymatic and Chemical Activation of BTZ



Received: August 25, 2014
Accepted: November 29, 2014
Published: November 29, 2014

arabinogalactan synthesis, an integral component of the *M.tb* cell wall, its covalent inhibition compromises the mycobacterial cell wall assembly. Recently, our work related to the metabolic activation of BTZ043 and other nitroaromatic agents revealed that the key nitroso intermediate (i.e., **3**) can also be generated by nucleophilic cine addition of thiolates or possibly hydrides.[16] While such a reaction can be envisioned to take place in the enzyme active site, in the absence of the enzyme, azoxy and related nitroso derived dimers were generated and characterized. We also reported that further reduction produces the corresponding inactive hydroxylamine and amine.[16]

Additionally, Makarov et al. recently also demonstrated that both compounds **1** and **2** undergo reduction in the presence of purified nitro reductase to the corresponding hydroxylamine and azoxy analogues.[1] Thus, BTZ and other electrophilic nitroaromatic anti-TB compounds are susceptible to redox activation both *in vitro* and *in vivo*.[16]

Discussions in the literature on nitroaromatic anti-TB warheads related to BTZ043 have mostly been focused on the reductive metabolism catalyzed either by DprE1 or nitroreductases. A potential alternative metabolic fate involves oxidative processes. S-Oxidation of sulfur containing therapeutics is a well-known metabolic transformation catalyzed by cytochrome P450s or by flavin monoxygenases.[17] Since oxidation of the sulfur was anticipated to also affect the electrophilicity of the active aromatic ring of BTZ and possibly alter the planar conformation of its fused ring system that might influence enzyme active site recognition, it was of interest to evaluate the anti-TB activity of the sulfone and sulfoxide analogues of BTZ043. Herein, we report preparation and studies of these oxidized forms of BTZ043 and their interesting anti-TB activity.

The Mulliken charges calculated by the semiempirical AM1 method indicate that the sulfur atom of the benzothiazinone scaffold has slightly positive character. This is possibly because of the delocalization of its lone pair of electrons within the ring (see Table 1). Such delocalization affords negative character to the carbons that are adjacent to sulfur, whereas the unsubstituted carbons on the central aromatic rings remain highly electron deficient. This is reflected in the dramatic downfield shift of the aromatic protons in the nuclear magnetic resonance (NMR) of BTZ043 (Figure 2) and is consistent with its planarity. Consequently, it was also anticipated that the sulfur in question is not very nucleophilic and thus not prone to oxidation leading to the formation of either the sulfoxide (BTZ-SO, compound **5**) or sulfone (BTZ-SO_2, compound **6**). It should be noted that our preliminary *in vitro* experiment to explore the oxidation of BTZ043 in the presence of mouse liver microsomes to either BTZ-SO or BTZ-SO_2 revealed no oxidation products within limits of detection (<0.15%) by liquid chromatography/mass spectrometry (LC/MS) experiments (data not shown). More studies are needed in the *M.tb* infected models to unequivocally rule out the oxidative metabolism of BTZs.

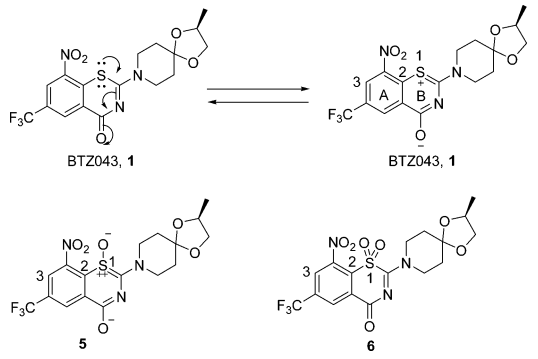
Our initial synthetic attempts to oxidize BTZ043 to either **5** or **6** by several reaction conditions including use of 30% H_2O_2 in glacial acetic acid at room temperature or under reflux conditions resulted in either no reaction or decomposition. Similarly treatment of BTZ043 with oxone in DCM/H_2O gave no reaction even after 24 h.

Finally, reaction of BTZ043 with 2 equiv of *m*-chloroperbenzoic acid (mCPBA) with BTZ043 generated small but usable quantities of both the sulfoxide (BTZ-SO, compound **5**) in 2% yield and sulfone (BTZ-SO_2, compound **6**) in 5% yield after 5 days (Scheme 2).

Notably, BTZ043 was recovered, substantiating the diminished nucleophilicity of the BTZ sulfur as emphasized by its ionic resonance form in which the lone pair on sulfur is highly delocalized into the benzothiazinone scaffold making the sulfur resistant to oxidation (see Table 1).

While characterizing the products of this oxidation reaction by ^{1}H NMR, we found that the experimentally observed chemical shifts of **1**, **5**, and **6** are unique in their own right. Classically, oxidation of phenyl thio ethers shifts the resonance of the aromatic protons downfield with the sulfone having a larger effect than the sulfoxide as would be expected from inductive effects. However, the actual ^{1}H NMR spectra of **1**, **5**, and **6** reflect an opposite trend, which is consistent with the influence of sulfur lone pair delocalization as represented by the resonance structure of **1**, and to a lesser degree, sulfoxide **5**. For sulfone **6** such lone pair delocalization is not possible, and the oxidized moiety can only exert an inductive effect (see Table 1). The near coincidence of the aromatic proton chemical shifts of the sulfoxide (**5**) and sulfone (**6**) illustrate the contribution of the ionic resonance form of **1** (Figure 2 and Table 1). Less dramatic, but notable differences in the aliphatic regions of the proton NMR spectra are consistent with conformational changes due to sulfur oxidation state. Computational studies also correlate with this observation and indicate that, while the bicyclic aromatic core of BTZ043 is planar, ring B of sulfoxide **5** and sulfone **6** are puckered and are nonaromatic. This loss of aromaticity of the 1,4-thiazinone ring (ring B, see Table 1 footnote) carried out by the oxidation of **1** into **5** (1,4-thiazinone-1-oxide) and **6** (1,4-thiazinone-1,1-dioxide) may explain the poor yields of the oxidation reactions. Comparison of Mulliken charge calculations (Table 1) indicate now expected differences in electrophilicity of the unsubstituted aromatic carbons (e.g., C3, Table 1) and hence their potential susceptibility to nucleophilic attack in a related cine reaction as observed for BTZ043.

Table 1. Resonance Structures and Calculated Mulliken Charges for Compounds 1, 5, and 6[a]



Mulliken charges	1	5	6
S1	0.51	1.55	2.80
C2	−0.18	−0.44	−0.67
C3	0.21	0.18	0.17

[a]The Mulliken charges are shown only for the numbered atoms for comparison purposes. See Supporting Information for the complete list of charges. The benzothiazinone ring of **1** is divided into ring A as the nitroaromatic and ring B, which is a 1,4-thiazinone.

dx.doi.org/10.1021/ml5003458 | ACS Med. Chem. Lett. 2015, 6, 128−133

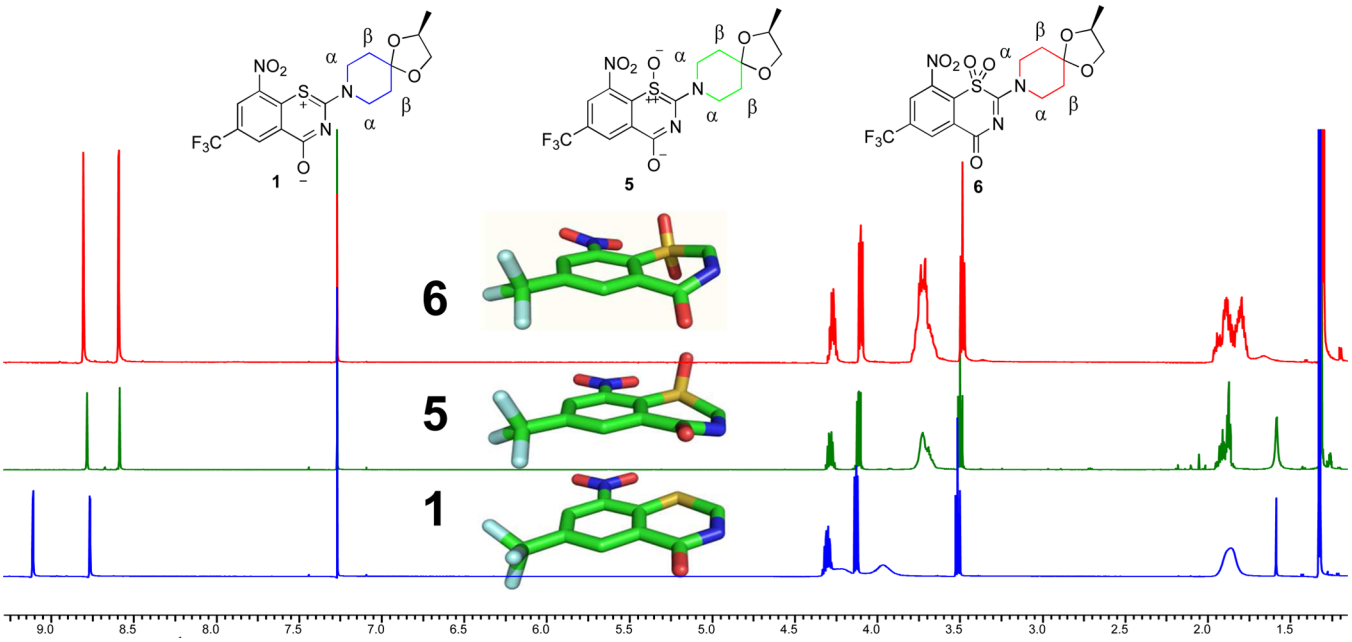
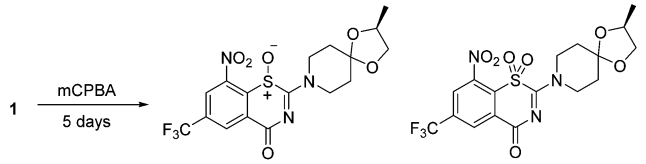


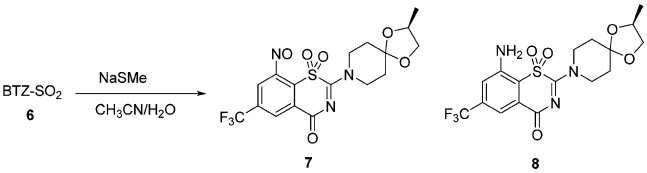
Figure 2. ^{1}H NMR spectra of **1**, **5**, and **6**. The signals of the piperidine protons β to nitrogen atom for each of **1**, **5**, and **6** appear in the region of 1.8−1.95 ppm, whereas the signals for the protons α to the nitrogen atom appear in the 3.7−4.25 ppm region. The 3-dimensional pictures of benzothiazinone rings of **1**, **5**, and **6** are shown to illustrate the effect of oxidation on the thiazinone ring.

Scheme 2. Synthesis of BTZ-SO and BTZ-SO$_2$



On the basis of these NMR and computational analyses, we anticipated that, like BTZ043 itself, sulfoxide **5** and sulfone **6** should be prone to cine reactions with thiolates to initiate redox chemistry similar to that seen in our earlier studies with BTZ043 and related electron deficient aromatic compounds. Indeed, treatment of **6** with methanethiolate in acetonitrile/water resulted in immediate color formation reminiscent of our earlier studies with BTZ043 and other electron deficient aromatic compounds.[16] Analysis of the reaction mixture by LC/MS revealed generation of the corresponding nitroso derivative **7** and amine **8** (see Supporting Information) (Scheme 3).

Scheme 3. Reaction of 6 with Sodium Methane Thiolate



Extended reaction resulted in further conversion of the transient nitroso agent to the amine, as expected. To confirm generation of the nitroso intermediate, the reaction was repeated in the presence of dienes, 1,3-cyclohexadiene and α-terpinene, and as expected, the corresponding nitroso cycloaddition products were detected by HRMS (see Supporting Information), again, just as with our earlier studies of BTZ043 and other model compounds. However, treatment of **5** with methanethiolate in acetonitrile/water in the presence of α-terpinene only indicated the formation of the corresponding hydroxylamine product (see Supporting Information). While in this case the formation of the nitroso cycloaddition product was not observed unlike for the reaction of **6**, the formation of the hydroxylamine form was still consistent with the nucleophilic addition of the methane thiolate at the cine position of **5** leading to subsequent generation of the hydroxylamine form.

The combined NMR, computational, and chemical reactivity studies suggested that sulfoxide **5** and sulfone **6** should be active anti-TB agents if they would still be recognized by the DprE1 target. Additionally, to explore the interactions of the slightly puckered **5** and puckered **6**, both **5** and **6** were docked, using the protocol published previously,[18] into the crystal structure of DprE1 (PDB code 4F4Q)[19] with Glide.[20,21] The docking study revealed that both **5** and **6** mimicked the binding pattern of **4** in the active site of DprE1 (Figure 3).

We were anxious to learn if the results of the chemical, NMR, and computational studies would correlate with antimycobacterial activity. Both compounds **5** and **6**, along with **1**, were evaluated against our in-house nonpathogenic mycobacterial strains, namely, *M. smegmatis*, *M. vaccae*, and *M. aurum*. Interestingly, the sulfoxide analogue **5** showed potent activity against these strains, which was comparable to that of **1**; however, except against *M. vaccae*, sulfone analogue **6** turned out to be less active, consistent with the studies described (Table 2). Additionally we evaluated compounds **1**, **5**, and **6** against representative Gram-positive (*S. aureus* and *M. luteus*) and Gram-negative (*P. aeruginosa* and *A. baumannii*) strains. Consistent with the remarkable anti-TB selectivity of BTZ043, none of the compounds were effective inhibitors of any of these strains (Table 2). Therefore, both compounds **5** and **6** selectively inhibit growth of mycobacteria (potentially targeting DprE1) and not the other bacteria tested.

Next, these compounds were evaluated against pathogenic mycobacterial strains, namely, *M. tuberculosis* ($H_{37}Rv$) and *M.*

dx.doi.org/10.1021/ml5003458 | *ACS Med. Chem. Lett.* 2015, 6, 128−133

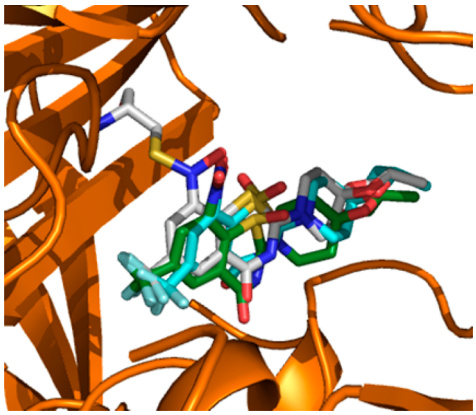


Figure 3. Overlay of the docked poses of **5** and **6** on the semimercaptal adduct, **4** (carbons in off white). The carbons of **5** are colored in green, whereas carbons of **6** are in cyan.

bovis (Table 3). While both of these strains cause TB, the former is a causative agent in humans, whereas the latter causes TB in cattle.[22] As observed with our in-house testing with nonpathogenic mycobacteria, only BTZ043 itself and sulfoxide analogue **5** showed impressive activity against both *M. tuberculosis* and *M. bovis*, whereas the sulfone analogue **6** was only weakly active. Thus, as now anticipated, the sulfoxide analogue **5** has activity against pathogenic and nonpathogenic strains of mycobacteria, while the sulfone analogue has much weaker activity.

Pleased by the potent activity of the BTZ-SO (**5**) and weaker activity of BTZ-SO_2 (**6**), we were interested to see if this activity trend would be mimicked across other pathogenic mycobacterial strains such as *M. marinum* and *M. kansasii*, which cause TB in immunocompromised individuals.[23,24] Overall, the sulfoxide analogue **5** demonstrated activity against all mycobacterial strains tested, whereas the sulfone analogue **6** was found to be inactive against the evaluated mycobacterial strains (Table 3).

Divalent sulfur in many therapeutic agents has been known to undergo metabolic oxidation to sulfoxides and sulfones. The subtle differences in the activity of the synthesized analogues **5** and **6** will not only help predict the metabolic fate of the BTZ class of compounds but also help evaluate such compounds against various pathogenic mycobacterial strains.

We also evaluated the cytotoxicity of **1**, **5**, and **6** against some representative cell lines including PC3 (prostate cancer),[25] MCF-7 (breast cancer),[26] and HeLa (ovarian cancer).[27] In general, none of these compounds showed any significant cytotoxicity issues (Table 4).

In conclusion, the studies described herein indicate that the oxidation products of benzothiazinone derived nitroaromatic warheads have variable anti-TB activity of their own. While it appears that these oxidized products are unlikely to be in vivo metabolites of BTZ043, the NMR, computational, and reactivity studies suggest that they merited investigation as potential anti-TB agents. Our study related to changes in the conformation brought about by the oxidations of **1** and the impact on the NMR chemical shifts of **5** and **6** will be additionally helpful to understand the chemical reactivity of 1,3-benzothiazine-4-ones.

Table 3. *In Vitro* Activity of the BTZ043 and Related Sulfur Oxidation Products against H37Rv and Other Pathogenic Mycobacterial Strains[a]

	M. tb ($H_{37}Rv$)				
	MABA:MIC 7H12 (μM)	MABA:MIC GAS (μM)	*M. marinum*	*M. bovis*	*M. kansasii*
1	<0.02	<0.02	0.037	<0.02	<0.02
5	0.06	<0.02	0.19	0.21	0.30
6	0.48	0.51	>1	>1	>1
INH	0.1	0.03	>8	0.50	3.86
rifampin	0.05	0.04	0.23	0.02	0.10

[a]GAS, glycerol-alanine-salts medium; 7H12, 7H9 medium plus casitone, palmitic acid, albumin, and catalase; MABA, Microplate Alamar Blue Assay; *M. marinum*, *Mycobacterium marinum*; *M. bovis*, *Mycobacterium bovis*; *M. kansasii*, *Mycobacterium kansasii*.

Table 4. Cytotoxicity of 1, 5, and 6 (μM) against Representative Cell Lines[a]

	PC3	MCF-7	HeLa
1	>20	>20	>20
5	15	>20	>20
6	>20	>20	>20

[a]PC3, prostate cancer cell line; MCF-7, breast cancer cell line; HeLa, ovarian cancer cell line.

■ ASSOCIATED CONTENT

Supporting Information

Complete experimental details along with the characterizations of the synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Funding

This research was supported in part by grant 2R01AI054193 from the National Institutes of Health (NIH). We thank the University of Notre Dame, especially the Mass Spectrometry

Table 2. *In Vitro* Activity (MIC in μM) of BTZ043 and Related Sulfur Oxidation Products against Representative Gram-Positive, Gram-Negative and Nonpathogenic Mycobacterial Strains[a]

	M. smegmatis MC^{2}155	*M. vaccae* IMET10670	*M. aurum* SB66	*S. aureus* SG511	*M. luteus* ATCC10240	*P. aeruginosa* KW 799/61	*A. baumannii* ATCC17961
1	0.002	0.002	>200	>0.2	>200	>200	>200
5	<0.013	<0.013	3.13−12.5	12.5	50	>200	>200
6	>0.5	<0.013	>200	0.2	>200	>200	>200
ciprofloxacin	0.32	0.32	0.020	0.32	1.25	0.156	0.156

[a]*M. smegmatis*, *Mycobacterium smegamatis*; *M. vaccae*, *Mycobacterium vaccae*; *M. aurum*, *Mycobacterium aurum*; *S. aureus*, *Staphylococcus aureus*; *M. luteus*, *Micrococcus luteus*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *A. baumannii*, *Acinetobacter baumannii*.

and Proteomics Facility (Bill Boggess, Michelle Joyce, and Nonka Sevova), which is supported by Grant CHE-0741793 from the National Science Foundation (NSF).

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

BTZ, 1,3-benzothiazin-4-one; DprE1, decaprenylphosphoryl-β-D-ribose 2′oxidase; MDR, multidrug resistant; *M tuberculosis*, *Mycobacterium tuberculosis*; XDR, extensively drug resistant; TB, tuberculosis; PC3, prostate cancer cell line; MCF-7, breast cancer cell line; HeLa, ovarian cancer cell line

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