

Microwave-Assisted Synthesis, Characterization, Molecular Docking and In Vitro Anti-Tubercular Evaluation of Novel Schiff Bases of 2-Aminobenzothiazole Targeting Glutamine Synthetase 1

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Abstract: Tuberculosis (TB) remains a leading infectious cause of death, and drug-resistant strains make new antitubercular scaffolds and targets necessary. Glutamine synthetase 1 (GS1, GlnA1) of *Mycobacterium tuberculosis* forms glutamine from glutamate and ammonia and is linked to nitrogen metabolism and to the poly-L-glutamate/glutamine component of the cell wall. Five Schiff bases of 2-aminobenzothiazole (C1–C5) were chosen from a designed library on the basis of docking against GS1 (PDB ID: 4ACF) and were synthesized by microwave-assisted condensation of 2-aminobenzothiazole with cinnamaldehyde, 4-nitrobenzaldehyde, 3-methyl-4-nitrobenzaldehyde, 5-chlorosalicylaldehyde and 5-fluoro-4-nitrobenzaldehyde (350 W, 75 °C, 3–5 min; yields 81–88%). The products were characterized by melting point, TLC, IR, ¹H NMR and mass spectrometry. All five compounds satisfied Lipinski's rule of five, and OSIRIS Property Explorer flagged no mutagenic, tumorigenic, irritant or reproductive-effect risk. Docking scores ranged from –7.6 to –8.5 kcal/mol (highest for C3, –8.5; C5, –8.4). In the Microplate Alamar Blue Assay (MABA) against *M. tuberculosis* H37Rv the MIC values were 100 (C1), 6.25 (C2), 3.12 (C3), 25 (C4) and 1.6 µg/mL (C5). C5 was the most active compound; its MIC equals the values reported for isoniazid and ethambutol (1.6 µg/mL) and is twofold higher than those for rifampicin and streptomycin (0.8 µg/mL). Docking scores ranked the compounds only weakly in agreement with MIC (Spearman $\rho = 0.6$, $n = 5$), and no enzyme-inhibition assay was performed, so GS1 inhibition remains a hypothesis. C5 is regarded as a candidate for further optimization, enzyme assays and cytotoxicity testing.

Keywords: 2-aminobenzothiazole; Schiff base; glutamine synthetase 1; anti-tubercular; microwave-assisted synthesis; molecular docking; PyRx; MABA; in silico drug design.

INTRODUCTION

Tuberculosis (TB) remains one of the leading infectious causes of death worldwide. The World Health Organization estimated that 10.7 million people fell ill with TB and 1.23 million died of it in 2024 [1]. Resistance to first-line drugs and the long duration of therapy keep the search for new antitubercular

scaffolds and new molecular targets active. Glutamine synthetase (GS; EC 6.3.1.2) catalysis the ATP-dependent condensation of glutamate and ammonia to give glutamine. In pathogenic mycobacteria the enzyme is exported, and an inhibitor of the exported enzyme blocked bacterial growth in broth and in human monocytes, with a fall in the poly-L-

glutamate/glutamine content of the cell wall [2]. The GS inhibitor methionine sulfoximine reduced bacterial counts in a guinea pig model and acted with isoniazid [3], and glnA1 was shown to be essential for growth of *M. tuberculosis* in human THP-1 macrophages and in guinea pigs [4]. GS1 has therefore been proposed as a drug target [5]. Crystal structures of the enzyme, including complexes with a transition-state mimic [6], ATP-competitive inhibitors [7] and imidazopyridine inhibitors (PDB 4ACF) [8], provide a basis for structure-based design. A note of caution comes from a screening programmed in which nanomolar GlnA1 inhibitors showed no whole-cell activity [9]. Benzothiazole is a privileged bicyclic heterocycle found in compounds with antimicrobial, anticancer, anticonvulsant and other activities [10]. Schiff bases (azomethines, $-\text{CH}=\text{N}-$) are readily made from primary amines and aldehydes and have been widely studied as antimicrobial agents [11], and microwave irradiation shortens such condensations from hours to minutes [12]. In this work, Schiff bases of 2-aminobenzothiazole were designed against GS1, screened in silico for drug-likeness and toxicity risk, synthesized by a microwave-assisted method, characterized spectroscopically and tested against *M. tuberculosis* H37Rv by the Microplate Alamar Blue Assay (MABA).

2. Materials and Methods

2.1 Target selection and molecular design

The crystal structure of *M. tuberculosis* GS1 (PDB ID: 4ACF; X-ray diffraction, 2.00 Å; six chains A–F; in complex with an imidazopyridine inhibitor and L-methionine-S-sulfoximine phosphate) was taken from the RCSB Protein Data Bank [8,18]. 2-Aminobenzothiazole was chosen as the lead nucleus on the basis of the literature on its biological activity. A library of Schiff bases was drawn in ACD/ChemSketch, and the five compounds with the best docking scores (C1–C5) were selected for synthesis. Molecular formula, formula weight and composition were calculated in ChemSketch.

2.2 Drug-likeness, ADME and toxicity prediction

Physicochemical and drug-likeness parameters were predicted with Molinspiration [16] and SwissADME [15]. Drug-likeness was judged against Lipinski's rule of five [13]: molecular weight below 500, no more than 10 hydrogen-bond acceptors, no more than 5 hydrogen-bond donors, log P not above 5 and

molar refractivity of 40–150. Rotatable-bond (10 or fewer) and polar-surface-area (140 Å² or less) limits belong to Veber's rules [14]. Mutagenic, tumorigenic, irritant and reproductive-effect risks were predicted with OSIRIS Property Explorer [17], a rule-based tool that matches fragments of the drawn structure to a precompiled toxicity-fragment list.

2.3 Molecular docking

Docking was performed in PyRx [19] with the AutoDock Vina wizard [20]. The protein was converted to a macromolecule (pdbqt) and each ligand was energy-minimized in the Open Babel module and converted to pdbqt. The grid box was maximized to cover the protein (blind docking). Binding affinity is reported in kcal/mol, with more negative values read as more favorable predicted binding. The docked poses were saved as .pdb files and the interactions were visualized in BIOVIA Discovery Studio [21].

2.4 Chemistry

General procedure. 2-Aminobenzothiazole (0.01 mol) and the appropriate aldehyde (0.01 mol) were placed in a microwave reaction vessel with ethanol (10 mL) and 2–3 drops of glacial acetic acid. The mixture was irradiated at 350 W (75 °C) for 3–5 min, and the reaction was followed by thin-layer chromatography (TLC). After completion the mixture was cooled to room temperature and poured onto crushed ice; the precipitate was filtered, washed with cold ethanol, dried and recrystallized from ethanol. The aldehydes were cinnamaldehyde (C1), 4-nitrobenzaldehyde (C2), 3-methyl-4-nitrobenzaldehyde (C3), 5-chlorosalicylaldehyde (C4) and 5-fluoro-4-nitrobenzaldehyde (C5). The compounds were named (E)-N-[(E)-3-phenylprop-2-en-1-ylidene]-1,3-benzothiazol-2-amine (C1), (E)-N-(4-nitrobenzylidene)-1,3-benzothiazol-2-amine (C2), (E)-N-(3-methyl-4-nitrobenzylidene)-1,3-benzothiazol-2-amine (C3), (E)-N-[(5-chloro-2-hydroxyphenyl)methylidene]-1,3-benzothiazol-2-amine (C4) and (E)-N-(5-fluoro-4-nitrobenzylidene)-1,3-benzothiazol-2-amine (C5).

2.5 Characterization

Melting points were determined by the open capillary method. Purity and reaction progress were checked by TLC on pre-coated silica gel plates with ethyl acetate–hexane (4:6) as mobile phase and visualization in an iodine chamber. IR

spectra were recorded on an ABB IR spectrophotometer (Model 3000) as KBr pellets. ¹H NMR spectra were recorded on a 400 MHz Bruker Avance III spectrometer in CDCl₃. Mass spectra were recorded on a Shimadzu mass spectrometer with electrospray ionization, with the samples dissolved in ethanol.

2.6 In vitro anti-tubercular activity (MABA)

Anti-tubercular activity was determined by the Microplate Alamar Blue Assay [22,23] against *M. tuberculosis* H37Rv (ATCC 27294). The compounds were tested at 100, 50, 25, 12.5, 6.25, 3.12, 1.6 and 0.8 µg/mL. A pink color of the Alamar Blue indicator was read as bacterial growth and a blue color as inhibition, and the MIC was taken as the lowest concentration that kept the well blue. The standard-drug values reported with the assay were isoniazid 1.6, ethambutol 1.6, pyrazinamide 3.125, rifampicin 0.8 and streptomycin 0.8 µg/mL.

3. Results

3.1 Drug-likeness and predicted toxicity

All five compounds met the Lipinski criteria (Table 1). Molecular weights were 264.34–301.30 g/mol, hydrogen-bond donors 0–1, acceptors 3–5, log P 3.95–4.71 and molar refractivity 79.92–84.70 cm³/mol. OSIRIS Property Explorer showed no mutagenic, tumorigenic, irritant or reproductive-effect risk for any compound (Table 2). The OSIRIS drug-likeness values, however, were positive only for C1 (0.18) and C4 (2.19) and were negative for the three nitro-substituted compounds C2, C3 and C5 (–9.00, –7.21 and –6.53); drug scores were 0.44–0.78. These are rule-based predictions and do not replace experimental ADME and toxicity testing.

Table 1. Lipinski parameters of C1–C5 (CMP-1 to CMP-5 in the source data). MW, molecular weight; HBA, hydrogen-bond acceptors; HBD, hydrogen-bond donors; MR, molar refractivity.

Code	MW (g/mol)	HBA	HBD	Log P	MR (cm ³ /mol)	Rule violations
C1	264.34	3	0	4.71	83.25	0
C2	283.31	5	0	3.95	79.97	0
C3	297.33	5	0	4.26	84.70	0
C4	288.75	4	1	4.40	79.99	0
C5	301.30	5	0	4.09	79.92	0

Table 2. OSIRIS Property Explorer predictions for C1–C5. TPSA, topological polar surface area.

Code	cLogP	TPSA (Å ²)	Drug-likeness	Drug score	Toxicity risks
C1	3.85	53.49	0.18	0.66	None
C2	2.91	99.31	–9.00	0.46	None
C3	3.26	99.31	–7.21	0.44	None
C4	4.10	73.72	2.19	0.78	None
C5	2.22	99.31	–6.53	0.47	None

3.2 Molecular docking against GS1

The docking scores against GS1 (4ACF) were –8.1 (C1), –7.8 (C2), –8.5 (C3), –7.6 (C4) and –8.4 kcal/mol (C5) (Table 3). C3 had the most negative score and C5 the second most negative. The pose of C5 (Figure 1) shows hydrogen bonds with Phe232, His278 and Glu133, π – π stacking of the benzothiazole ring with Phe232 and of the aryl ring with His278, a π –cation contact with Arg364, a π –anion contact with Glu214, a carbon–hydrogen bond with Gly131 and a halogen (fluorine) contact with Tyr230. Because blind docking was used, the poses were not restricted to the site of the co-crystallized ligand.

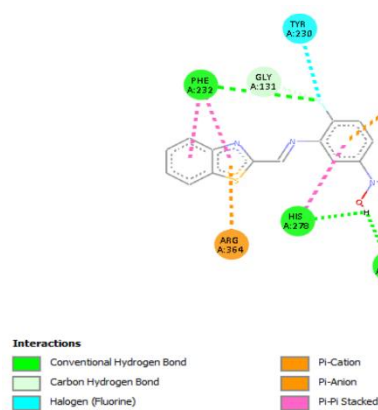


Figure 1. Two-dimensional interaction diagram of C5 docked into GS1 (PDB ID: 4ACF); docking score –8.4 kcal/mol.

3.3 Synthesis and physical characterization

The five Schiff bases were obtained in 81–88% yield as off-white to bright-yellow solids that dissolved in methanol and acetone (Table 3). Each gave a single spot on TLC with an R_f different from that of the reactants, and the melting ranges were 211–255 °C.

Table 3. Docking scores, yields and physical data of C1–C5.

Code	Formula (MW)	Docking (kcal/mol)	Yield (%)	M.p. (°C)	R _f
C1	C ₁₆ H ₁₃ N ₃ S (264.34)	–8.1	85	211–215	0.7
C2	C ₁₄ H ₉ N ₃ O ₂ S (283.31)	–7.8	82	243–248	0.5
C3	C ₁₅ H ₁₁ N ₃ O ₂ S (297.33)	–8.5	81	231–234	0.5
C4	C ₁₄ H ₉ ClN ₃ OS (288.75)	–7.6	88	219–223	0.6
C5	C ₁₄ H ₉ FN ₃ O ₂ S (301.30)	–8.4	81	248–255	0.3

Appearance: C1 off-white; C2 light yellow powder; C3 yellow crystalline; C4 pale yellow; C5 bright yellow powder. TLC: ethyl acetate–hexane (4:6).

3.4 Spectral characterization

The IR spectra of C1, C2, C4 and C5 showed the azomethine C=N stretch at 1612–1621 cm^{−1} (Table 4); no C=N band is listed for C3. The nitro compounds C2, C3 and C5 showed N–O stretching bands (1513, 1321 and 1520 cm^{−1}, respectively), C4 showed a phenolic O–H band at 3425 cm^{−1} and a C–Cl band at 689 cm^{−1}, and C5 showed a C–F band at 1164 cm^{−1}. In the ¹H NMR spectra the azomethine proton appeared at δ 8.35–8.65 ppm, with the benzothiazole and aryl protons between δ 7.0 and 8.5 ppm, the methyl group of C3 at δ 3.45 and the phenolic proton of C4 at δ 11.05. The mass spectra of C3 and C5 showed molecular-ion peaks at *m/z* 297 and 301, matching the calculated masses; the base peak was *m/z* 135 for C2, C3 and C5.

Table 4. Key spectral data of C1–C5. Highlighted entries disagree with the calculated mass or structure and must be checked against the original spectra.

Code	IR (cm ^{−1})	¹ H NMR δ (ppm)	MS <i>m/z</i> (calc.)
C1	3050 (ar C–H), 2924 (C–H), 1621 (C=N), 1598 (C=C)	7.20–8.05 (ar), 7.78 (cinnamylidene), 8.35 (CH=N)	272 (calc. 264.34)
C2	3092, 2918, 1621 (C=N), 1513 (NO ₂), 835 (p-subst.)	7.35–7.95 (BT), 8.15, 8.30 (p-nitrophenyl), 8.55 (CH=N)	301 (calc. 283.31)
C3	no C=N band listed, 1471 (C=C), 1321 (NO ₂), 1215 (C–N)	3.45 (CH ₃), 7.05–7.55 (BT), 8.10, 8.30 (ar), 8.55 (CH=N)	297 (calc. 297.33); base peak 135
C4	3425 (O–H), 1612 (C=N), 1582 (C=C), 1235 (C–O), 689 (C–Cl)	1.35, 2.45, 5.10, 6.15, 8.35 (CH=N), 11.05 (OH)	297 (calc. 288.75)
C5	3062, 1615 (C=N), 1586 (C=C), 1520 (NO ₂), 1164 (C–F)	7.30, 7.60 (BT), 7.95, 8.20, 8.45 (ar), 8.65 (CH=N)	301 (calc. 301.30); base peak 135

3.5 Anti-tubercular activity

The MIC values against *M. tuberculosis* H37Rv were 100 μ g/mL (C1), 6.25 μ g/mL (C2), 3.12 μ g/mL (C3), 25 μ g/mL (C4) and 1.6 μ g/mL (C5) (Table 5, Figure 2). At 50 μ g/mL, C2–C5 kept the wells blue (inhibition), whereas C1 did not; at 0.8 μ g/mL no compound inhibited growth. C5 was therefore the most active compound. Its MIC equals the values given for isoniazid and ethambutol (1.6 μ g/mL) and is twofold higher than those for rifampicin and streptomycin (0.8 μ g/mL).

Table 5. MABA results (S, sensitive/inhibited; R, resistant/growth) and MIC of C1–C5 (RX1–RX5 in the assay).

Code	100	50	25	12.5	6.25	3.12	1.6	0.8
C1	S	R	R	R	R	R	R	R
C2	S	S	S	S	S	R	R	R
C3	S	S	S	S	S	S	R	R
C4	S	S	S	R	R	R	R	R
C5	S	S	S	S	S	S	S	R

Concentrations in μ g/mL. MIC: C1 100; C2 6.25; C3 3.12; C4 25; C5 1.6 μ g/mL.

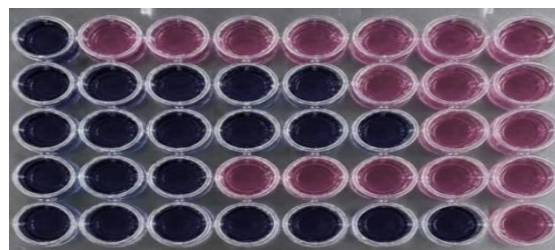


Figure 2. MABA plate for C1–C5 (rows 1–5) at 100, 50, 25, 12.5, 6.25, 3.12, 1.6 and 0.8 μ g/mL (columns 1–8). Blue: inhibition; pink: growth.

4. Discussion

All five Schiff bases were made in good yield by a short, solvent-light microwave procedure and were active against *M. tuberculosis* H37Rv in the MABA. The most active compound, C5 (MIC 1.6 μ g/mL), carries a fluoro-nitro-substituted aryl ring. The three nitro-substituted compounds C2, C3 and C5 (MIC 6.25, 3.12 and 1.6 μ g/mL) were more active than the cinnamylidene derivative C1 (100 μ g/mL) and the chloro-hydroxy derivative C4 (25 μ g/mL). With only five compounds this is an observation, not a structure–activity relationship. The relation between docking and activity is weaker than a simple reading suggests. C3 had the best docking score (−8.5 kcal/mol) but not the lowest MIC, and C1 docked better (−8.1) than C2 and C4 yet was the least active. Ranking the five compounds by docking score and by MIC gives a Spearman coefficient of 0.6, which for *n* = 5 is not statistically significant. Docking therefore helped to choose compounds that were synthetically accessible, but it did not rank their potency reliably. The results do not show that the compounds act on GS1. No enzyme-inhibition assay was done, a whole-cell MIC can arise from any mechanism, and the docking was blind and was not validated by re-docking of the co-crystallized ligand.

The screening study cited above also found that potent GlnA1 inhibitors can lack whole-cell activity [9], which makes enzyme-level confirmation especially important here. The OSIRIS output also needs careful reading: no toxicity alert was raised, but three of the five compounds have negative drug-likeness values, and nitroaromatic groups are a recognized liability that needs experimental checking. Further limitations are that the MABA gives MIC only at two-fold dilution steps, replicate data are not reported, no cytotoxicity or selectivity index against mammalian cells was determined, and the spectral data for C1, C2 and C4 need to be reconciled with the calculated masses and structures (Table 4). Enzyme-inhibition assays against GS1, cytotoxicity testing, confirmatory MIC determination in replicate and docking restricted to the ATP-binding site with re-docking validation would be the next steps.

5. Conclusions

Five 2-aminobenzothiazole Schiff bases were designed against GS1, synthesized by a microwave-assisted method in 81–88% yield and characterized. All satisfied Lipinski's rule of five and raised no OSIRIS toxicity alert. In the MABA the MIC values ranged from 100 to 1.6 µg/mL, and C5, the 5-fluoro-4-nitrobenzylidene derivative (docking score –8.4 kcal/mol), was the most active (MIC 1.6 µg/mL). Docking ranked the compounds only weakly in agreement with activity, and GS1 inhibition has not been demonstrated. C5 is a candidate for further optimization, enzyme-level studies and cytotoxicity testing.

Acknowledgments: We sincerely acknowledge the support and facilities provided by the Department of Pharmaceutical Chemistry, College of Pharmacy, Madurai Medical College, for carrying out this research work. We are grateful to the laboratory and technical staff for their assistance with the synthesis, spectral analysis and biological evaluation of the compounds.

Author Contributions: Aravindh Rao S: conceptualization, in silico design and docking, synthesis, characterization, data analysis and writing—original draft. Ramesh Kumar S: supervision, project administration, validation and critical review. All authors reviewed the manuscript and approved the final version.

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

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