

RESEARCH ARTICLE

Synthesis, Biological Profile, and Molecular Docking of Some New Bis-Imidazole Fused Templates and Investigation of Their Cytotoxic Potential as Anti-tubercular and/or Anticancer Prototypes

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Abstract: Background: There is a great need to discover more drugs with antimycobacterial activities to fight lung cancer and tuberculosis (two of the deadliest diseases worldwide). To our knowledge, the present study is the first to report the antimycobacterial activity of imidazole-fused heterocycles.

Objective: Construction of some bis-imidazole fused heterocycles with potential anti-tubercular and/or potent antitumor activities.

Methods: A series of bis-imidazole fused derivatives **6-8** and **13-16** was constructed using bis-phenacyl bromide derivative **2** as a synthetic platform. Compound **2** was also used to access bis-quinoxaline **20**, bis-benzothiazine derivatives **23**, and bis-thiazolopyrimidine derivatives **26**. The new bis-imidazole derivatives were evaluated for their anticancer activity against the lung carcinoma cell line (A-549) using Cisplatin as a reference drug. The new compounds were also screened for their anti-tubercular activity against *M. tuberculosis* (ATCC 25177) using Isoniazid as a reference drug.

Results: Among the new bis-imidazole derivatives, three examples showed remarkable antitumor activities while five other compounds showed high antimycobacterial activity.

Conclusion: A novel series of bis-imidazole fused heterocycles was developed. Multiple prototypes of this new series showed remarkable anti-tubercular and/or potent antitumor activities.

Keywords: Bis-heterocycles, imidazotriazoles, imidazothiazoles, thiazolopyrimidines, anti-tumor and anti-tubercular activities.

1. INTRODUCTION

In 2018, lung cancer (LC) was the leading cause of cancer death, with an estimate of 1.8 million deaths, 18.4% of the total cancer deaths globally [1]. Tuberculosis (TB), triggered by *Mycobacterium tuberculosis*, is one of the main lung infections causing pulmonary inflammation and tissue damage and the second most lethal infectious disease [2]. In 2017, TB infected 10 million people with 1.6 million fatalities [3]. Several reports have studied the correlation between TB and LC [4, 5]. As a result, TB was considered as one of the main suspects to potentially cause malignant alterations and LC [6–10]. Although Isoniazid and Rifampin are considered the two most powerful anti-TB drugs,

sometimes they face many therapeutic limitations, drug-drug interactions, and/or drug resistance. Therefore, there is a high demand to develop new drug targets with anti-TB and anti-tumor potentiality. Due to their broad spectrum of physiological activities (anticancer, anti-inflammatory, antioxidant, antiviral and antimicrobial), fused heterocycles constitute a robust cornerstone in today's medicinal chemistry [11–13]. Imidazo-thiazole derivatives with bridgehead nitrogen atom represent attractive heterocyclic scaffolds due to their remarkable biological activities [14–16]. Therefore, more attention has been paid to the chemistry and cytotoxicity of Imidazo-thiazoles. Some imidazo-thiazole derivatives exhibit good antiproliferative activities against a variety of human cancer cell lines [17–23]. Levamisole, the most popular commercial imidazo-thiazole derivative [24], is used as a biological response modifier in cancer adjuvant therapy [25]. The biological significance of levamisole triggered more scientific interest in structurally

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related imidazo-thiazoles. Many imidazo-triazole derivatives show antibacterial activity [26]. On the other hand, imidazo-pyrimidine and thiazolo-pyrimidine cores represent very versatile pharmacological building blocks [27-29], with some members known for their remarkable anticancer activities [30-34]. Thiazolo-pyrimidines possess the cytotoxic activity and have been used as CDC25BP phosphate inhibitors [35].

Encouraged by all these findings and in continuation of our previous work geared towards the synthesis of novel antitumor compounds [36-46], we designed a simple route to access several new bis-imidazo-azoles, bis-imidazo-azines, and bis-thiazolo-pyrimidine derivatives. Herein, we present the design, synthetic strategies, and molecular docking studies of some new bis-imidazo-fused templates. The study is also supported with a preliminary biological activity profile of all new compounds as potential tyrosine kinase (DYRK1A) inhibitors.

2. EXPERIMENTAL

2.1. Chemistry

Melting points were determined on a Gallenkamp apparatus and are uncorrected. IR spectra were recorded in potassium bromide using Pye-Unicam SP300 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded in deuterated DMSO- d_6 using a Varian Gemini 300 NMR spectrometer (300 MHz for ^1H NMR and 75 MHz for ^{13}C NMR) and the chemical shifts were related to that of the solvent DMSO- d_6 . Mass spectra were recorded on a GCMS-Q1000-EX Shimadzu and GCMS 5988-A HP spectrometers, the ionizing voltage was 70 eV. Elemental analyses of the products were carried out at the Microanalytical Centre of Cairo University, Giza, Egypt. The biological evaluation of the products was carried out at Regional Center for Mycology and Biotechnology at Al-Azhar University, Cairo, Egypt.

Synthesis of 1,2-Bis(4-bromoacetylphenoxy)methyl benzene (2)

To a stirred solution of 1,2-bis(4-acetylphenoxy-methyl)benzene **1** (10 mmol) and p-TsOH (5.6 g, 20mmol) in acetonitrile (50 mL), NBS (3.6 g, 20 mmol) was slowly added. The reaction mixture was heated under reflux with stirring for 1-2 h, then left to cool to room temperature. The solvent was evaporated in vacuo, and the residue was dissolved in chloroform (50mL), washed with water (2 × 20 mL), and dried over MgSO_4 . After evaporation of the solvent, the resulting solid was recrystallized from benzene to afford the corresponding bis(p-bromoketone) derivative **2**.

General procedure for the reaction of **2** with heterocyclic amines **3-5**, **9-12**, **17**, and **21**.

A solution of 1,1'-(ethane-1,2-diylbis(oxy))bis(4,1-phenylene)bis(2-bromoethan-1-one) derivative **2** (0.456 g, 1 mmol) and the appropriate heterocyclic amines **3-5**, **9-12**, **17**, and **21** (2 mmol) in ethanol (20 mL) was refluxed for 7-10 h (monitored by TLC). The reaction mixture was left to cool, and the formed solid product was collected by filtration,

washed with water, dried, and recrystallized from EtOH to give the corresponding products **6-8**, **13-16**, **20**, and **23**.

1,2-Bis(4-(1*H*-imidazo[1,2-*b*] [1,2,4]triazol-5-yl)phenoxy)ethane (6).

Yellow solid, 72% yield; mp. 140-142 °C; IR (KBr): ν 3420 (NH), 3108, 2933 (C-H), 1231, 1173 (CH_2) cm^{-1} ; ^1H NMR (DMSO- d_6): δ 4.50 (s, 4H, 2 CH_2), 7.13-8.32 (m, 8H, Ar-H), 8.48 (s, 2H, imidazole-H5), 8.71 (s, 2H, triazole-H5), 9.47 (br s, 2H, 2NH) ppm; ^{13}C NMR (DMSO- d_6): δ 66.0 (OCH_2), 115.1, 125.9, 127.4, 128.6, 130.6, 131.1, 132.5, 163.0 (Ar-H and C=N) ppm; MS m/z (%): 426 (M^+ , 39), 413 (60), 340 (62), 249 (26), 162 (82), 142 (61), 73 (80), 59 (100). Anal. Calcd for $\text{C}_{22}\text{H}_{18}\text{N}_8\text{O}_2$ (426.44): C, 61.96; H, 4.25; N, 26.28. Found: C, 62.23; H, 4.00; N, 26.00%

1,2-Bis(4-(1*H*-imidazo[1,2-*d*]tetrazol-5-yl)phenoxy)ethane (7).

Brown solid, 74% yield; mp. 209-210 °C; IR (KBr): ν 3429 (NH), 3073, 2926 (C-H), 1240, 1173 (CH_2) cm^{-1} ; ^1H NMR (DMSO- d_6): δ 4.36 (s, 4H, 2 CH_2), 7.01-7.95 (m, 8H, Ar-H), 8.22 (s, 2H, imidazole-H5), 10.55 (br s, 2H, 2NH) ppm; MS m/z (%): 428 (M^+ , 100), 395 (70), 309 (77), 277 (41), 238 (73), 177 (32), 128 (51), 64 (78). Anal. Calcd for $\text{C}_{20}\text{H}_{16}\text{N}_{10}\text{O}_2$ (428.42): C, 56.07; H, 3.76; N, 32.69. Found: C, 56.25; H, 3.45; N, 32.47%.

1,2-Bis(4-(9*H*-benzo[*d*]imidazo[1,2-*a*]imidazol-2-yl)phenoxy)ethane (8).

Green solid, 70% yield; mp. 289-291 °C; IR (KBr): ν 3425 (NH), 3062, 2926 (C-H), 1237, 1171 (CH_2) cm^{-1} ; ^1H NMR (DMSO- d_6): δ 4.38 (s, 4H, 2 CH_2), 7.21-7.50 (m, 16H, Ar-H), 8.40 (s, 2H, imidazol-H5), 11.67 (s, 2H, 2NH) ppm; ^{13}C NMR (DMSO- d_6): δ 65.3 (OCH_2), 112.9, 114.1, 116.7, 120.8, 128.6, 129.9, 131.5, 132.3, 139.3, 143.5, 157.0 (Ar-H and C=N); MS m/z (%): 524 (M^+ , 13), 412 (44), 382 (22), 250 (35), 131 (25), 91 (30), 63 (100). Anal. Calcd for $\text{C}_{32}\text{H}_{24}\text{N}_6\text{O}_2$ (524.58): C, 73.27; H, 4.61; N, 16.02. Found: C, 73.53; H, 4.47; N, 15.89%.

1,2-Bis(4-(imidazo[2,1-*b*]thiazol-6-yl)phenoxy)ethane (13).

Brown solid, 78% yield; mp. 225-226 °C; IR (KBr): ν 3061, 2927 (C-H) cm^{-1} ; ^1H NMR (DMSO- d_6): δ 4.44 (s, 4H, 2 CH_2), 7.20 (d, 1H, thiazole-H5), 7.45-8.10 (m, 16 H, Ar-H), 8.12 (d, 1H, thiazole-H4), 8.59 (s, 1H, imidazole-H5) ppm; MS m/z (%): 558 (M^+ , 12), 501 (12), 428 (100), 312 (32), 294 (29), 121 (30), 77 (45), 42 (57). Anal. Calcd for $\text{C}_{24}\text{H}_{18}\text{N}_4\text{O}_2\text{S}_2$ (458.55): C, 62.86; H, 3.96; N, 12.22. Found: C, 63.15; H, 3.70; N, 12.00%.

1,2-Bis(4-(benzo[*d*]imidazo[2,1-*b*]thiazol-2-yl)phenoxy)ethane (14).

Brown solid, 78% yield; mp. 130-132 °C; IR (KBr): ν 3061, 2927 (C-H) cm^{-1} ; ^1H NMR (DMSO- d_6): δ 4.44 (s, 4H, 2 CH_2), 6.72-7.99 (m, 16H, Ar-H), 8.14 (s, 2H, imidazole-H5) ppm; ^{13}C NMR (DMSO- d_6): δ 61.6 (OCH_2), 115.7, 120.8, 127.5, 129.3, 133.3, 137.9, 142.1, 143.1, 152.1, 156.8, 160.0 (Ar-H and C=N); MS m/z (%): 558 (M^+ , 12),

501 (12), 428 (100), 312 (32), 294 (29), 121 (30), 77 (45), 42 (57). Anal. Calcd for $C_{32}H_{22}N_4O_2S_2$ (558.67): C, 68.80; H, 3.97; N, 10.03. Found: C, 69.11; H, 3.71; N, 10.65%.

1,2-Bis(4-(imidazo[1,2-a]pyridin-2-yl)phenoxy)ethane (15).

Brown solid, 69% yield; mp. 138-140 °C; IR (KBr): ν 3061, 2929 (C-H) cm^{-1} ; 1H NMR (DMSO- d_6): δ 4.45 (s, 4H, 2CH₂), 6.73-7.97 (m, 16H, Ar-H), 8.24 (s, 2H, imidazole-H5) ppm; MS m/z (%): 446 (M⁺, 35), 415 (43), 358 (62), 316 (46), 269 (48), 208 (48), 120 (85), 94 (50), 76 (100), 57 (43). Anal. Calcd for $C_{28}H_{22}N_4O_2$ (446.51): C, 75.32; H, 4.97; N, 12.55. Found: C, 75.60; H, 4.65; N, 12.25%.

1,2-Bis(4-(imidazo[1,2-a]pyrimidin-2-yl)phenoxy)ethane (16).

Brown solid, 70% yield; mp. 179-180 °C; IR (KBr): ν 3072, 2957 (C-H) cm^{-1} ; 1H NMR (DMSO- d_6): δ 4.41 (s, 4H, 2CH₂), 7.09-8.34 (m, 14H, Ar-H), 8.56 (s, 2H, imidazole-H5) ppm; MS m/z (%): 448 (M⁺, 69), 412 (100), 372 (26), 285 (46), 192 (31), 133 (25), 83 (69), 73 (56). Anal. Calcd for $C_{26}H_{20}N_6O_2$ (448.49): C, 69.63; H, 4.50; N, 18.74. Found: C, 69.90; H, 4.21; N, 18.54%.

1,2-Bis(4-(quinoxalin-2-yl)phenoxy)ethane (20).

Pale yellow solid, 74% yield; mp. 249-251 °C; IR (KBr): ν 3054, 2932 (C-H), 1603 (C=N) cm^{-1} ; 1H NMR (DMSO- d_6): δ 4.48 (s, 4H, 2CH₂), 7.11-8.39 (m, 16H, Ar-H), 8.57 (s, 2H, pyrimidine-H3) ppm; ^{13}C NMR (DMSO- d_6): δ 66.3 (OCH₂), 114.9, 115.5, 129.4, 129.5, 129.9, 130.6, 131.0, 141.2, 141.9, 143.9, 151.0, 160.7 (Ar-C) ppm; MS m/z (%): 470 (M⁺, 100), 384 (5), 221 (6), 102 (10), 76 (27), 65 (5). Anal. Calcd for $C_{30}H_{22}N_4O_2$ (470.53): C, 76.58; H, 4.71; N, 11.91. Found: C, 76.86; H, 4.40; N, 11.60%.

1,2-Bis(4-(4H-benzo[b][1,4]thiazin-2-yl)phenoxy)ethane (23).

Green solid, 70% yield; mp. 160-161 °C; IR (KBr): ν 3423 (NH), 3048, 2928 (C-H) cm^{-1} ; 1H NMR (DMSO- d_6): δ 4.46 (s, 4H, 2CH₂), 6.63 (s, 2H, thiazine-H3), 7.03-8.06 (m, 16H, Ar-H), 9.68 (br s, 2H, NH) ppm; ^{13}C NMR (DMSO- d_6): δ 65.6 (OCH₂), 113.5, 116.6, 118.3, 120.9, 123.7, 125.5, 128.2, 129.9, 130.8, 134.4 (Ar-H and C=N); MS m/z (%): 508 (M⁺, 100), 494 (93), 480 (41), 384 (8), 210 (8), 153 (7). Anal. Calcd for $C_{30}H_{24}N_2O_2S_2$ (508.65): C, 70.84; H, 4.76; N, 5.51. Found: C, 71.11; H, 4.45; N, 5.29%.

Synthesis of bis-thiazolopyrimidines 26a,b

To a stirred hot solution of pyrimidinethione derivatives **24a** or **24b** (2 mmol) in dioxane (30 mL), 1,1'-((ethane-1,2-diylbis(oxy))bis(4,1-phenylene))bis(2-bromoethan-1-one) (**2**) (0.456 g, 1 mmol) and triethylamine (0.7 mL) were added, then the reaction mixture was refluxed for 6 h (monitored by TLC). After cooling, the formed solid product was collected by filtration and washed with aqueous ethanol to dissolve any triethylamine hydrochloride crystals, dried and crystallized from dioxane to give the respective thiazolopyrimidines **26a,b**.

2,2'-((Ethane-1,2-diylbis(oxy))bis(4,1-phenylene))bis(7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one) (26a).

Yellow solid, 76% yield; mp. 220-222 °C; IR (KBr): ν 3077, 2925 (C-H), 1669 (C=O) cm^{-1} ; 1H NMR (DMSO- d_6): δ 2.24 (s, 6H, 2CH₃), 4.38 (s, 4H, 2CH₂), 5.72 (s, 2H, pyrimidine-H5), 6.93-7.14 (m, 8H, Ar-H), 7.78 (s, 2H, thiazole-H5) ppm; ^{13}C NMR (DMSO- d_6): δ 18.5 (CH₃), 66.8 (OCH₂), 114.7, 121.4, 128.7, 131.1, 133.8, 144.8, 146.9, 148.5, 153.3 (Ar-C), 162.0 (C=O) ppm; MS m/z (%): 542 (M⁺, 40), 509 (42), 492 (38), 396 (34), 326 (49), 289 (44), 200 (100), 149 (67), 124 (26). Anal. Calcd for $C_{28}H_{22}N_4O_4S_2$ (542.63): C, 61.98; H, 4.09; N, 10.33. Found: C, 62.26; H, 3.85; N, 10.05%.

2,2'-((Ethane-1,2-diylbis(oxy))bis(4,1-phenylene))bis(7-phenyl-5H-thiazolo[3,2-a]pyrimidin-5-one) (26b).

Yellow solid, 73% yield; mp. 170-172 °C; IR (KBr): ν 3057, 2925 (C-H), 1671 (C=O) cm^{-1} ; 1H NMR (DMSO- d_6): δ 4.52 (s, 4H, 2CH₂), 6.64 (s, 2H, pyrimidine-H5), 7.18-7.97 (m, 18H, Ar-H), 8.14 (s, 2H, thiazole-H5) ppm; MS m/z (%): 666 (M⁺, 19), 632 (18), 506 (22), 413 (100), 341 (46), 285 (28), 183 (36), 116 (88), 58 (60). Anal. Calcd for $C_{38}H_{26}N_4O_4S_2$ (666.77): C, 68.45; H, 3.93; N, 8.40. Found: C, 68.74; H, 3.65; N, 8.11%.

3. BIOLOGICAL EVALUATION

3.2. Biological Activity

3.2.1. Anticancer Activity

The A-549 cells were obtained from "Holding company for biological products and vaccines (VACSERA)", Cairo, Egypt. The cells were spread in a Dulbecco's modified Eagle's medium (DMEM) supplemented with fetal bovine serum (10%), 50 μ g/mL gentamycin, 1% L-glutamine. All cells were kept at 37°C in a moistened atmosphere with CO₂ (5%) and were sub-cultured twice a week. Crystal violet and dimethyl sulfoxide were obtained from Sigma (St. Louis, Mo., USA) while DMEM, Fetal Bovine serum, L-glutamine, HEPES buffer solution, 0.25% Trypsin-EDTA, gentamycin, and RPMI-1640 were obtained from Lonza.

Evaluation of the Antitumor Activity Using Viability Assay

The antitumor evaluation of the new products was measured at Regional Center for Mycology and Biotechnology at Al-Azhar University, Cairo, Egypt.

The method of this MTT test was performed as previously described in detail [47, 48].

3.2.2. Anti-tubercular Activity

Antimycobacterial Activity

Mycobacterium tuberculosis (ATCC 25177) strain was obtained from American type culture collection (ATCC). Isoniazide was used as a reference drug. Antimycobacterial activity of the synthesized compounds was evaluated using the microplate Alamar blue assay (MABA) as described

previously [49] and modified [50], which was performed in black, clear-bottomed, 96-well microplates (in order to minimize background effect). Outer perimeter wells were filled with sterile water to prevent dehydration in experimental wells. Initial compound dilutions were prepared in dimethyl sulfoxide, and subsequent twofold dilutions were performed in the microplates. 0.1 ml of 10^5 CFU/ml *M. tuberculosis* inoculum was added to wells; additional control wells consisted of bacteria only (B). Plates were incubated at 37°C. Starting at day 4 of incubation, 20 μ L of alamarBlue solution (Alamar Biosciences/Accumed, Westlake, OH, USA) and 12.5 μ L of 20% Tween 80 were added to the entire plate. Plates were then incubated at 37°C, and results were recorded at 24 h post-reagent addition at 590 nm. MICs were defined as the lowest concentration of drug that prevented a color change.

Molecular Docking Study

All the molecular modeling studies were carried out using Molecular Operating Environment (MOE, 2015.10) software. All minimizations were performed with MOE until an RMSD gradient of $0.05 \text{ kcal}\cdot\text{mol}^{-1}\text{\AA}^{-1}$ with MMFF94x force field and the partial charges were automatically calculated.

The X-ray crystallographic structure of tyrosine kinase 1A (PDB ID: 2VX3) was downloaded from the protein data bank [51]. For the co-crystallized enzyme, water molecules and ligands which are not involved in the binding were removed, the protein was prepared for the docking study using *Protonate 3D* protocol in MOE with default options. Triangle Matcher placement method and London dG scoring function were used for docking.

4. RESULTS AND DISCUSSION

4.1. Chemistry

The starting bis-phenacyl bromide derivative **2** was readily made *via* direct NBS bromination in the presence of stoichiometric amounts of *p*-TsOH in acetonitrile [52]. Bis-phenacyl bromide derivative **2** reacted with a selected group of heterocyclic amines. Thus, reaction of compound **2** with two equivalents of 5-amino-1,2,4-triazole (**3**), 5-amino-

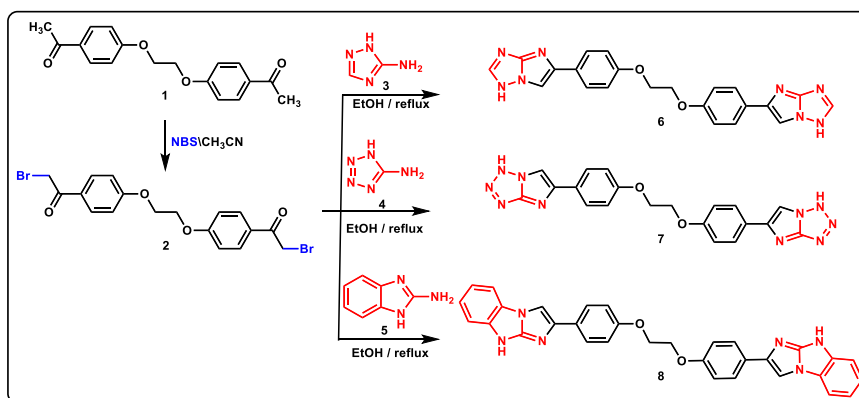
1,2,3,4-tetrazole (**4**), and 2-aminobenzimidazole (**5**) in refluxing ethanol gave the imidazo[1,2-*b*][1,2,4]triazole derivative **6**, imidazo[1,2-*d*]tetrazole derivatives **7**, and benzo[*d*]imidazo[1,2-*a*]imidazole derivative **8**, respectively (Scheme 1). All proposed structures of the isolated bis-compounds **6-8** were fully characterized by elemental as well as spectral analyses. Thus, the IR spectrum of **8**, as a representative example, showed absorption bands at 3425 cm^{-1} due to (NH) stretching frequency. Also, ^1H NMR spectra of **8** displayed the three characteristic singlets at $\delta = 4.38, 8.40,$ and 11.67 ppm assignable to the methylene ether linkage OCH_2 , imidazole-H5, and NH proton (D_2O exchangeable) respectively, in addition to the expected 16 aromatic protons. The mass spectra of compounds **6-8** showed typical fragmentation patterns.

To account for the formation of compounds **6-8**, it is suggested that the reaction proceeds through initial dehydrohalogenation to form intermediate A, followed intramolecular dehydrative cyclization to give the expected reaction products **6-8**, based on ^1H NMR, ^{13}C NMR, IR, Ms spectra and elemental analysis and in accordance with previous literature precedents [53, 54] (Scheme 2).

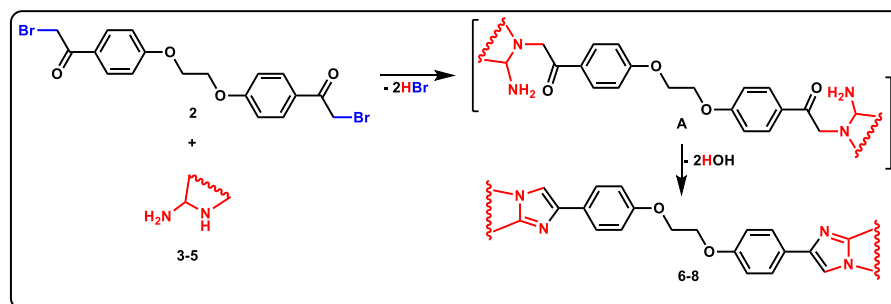
Similarly, the reaction of compound **2** with two equivalents of heterocyclic amines of the type $\text{NH}_2\text{-C=N}$ namely, 2-aminothiazole (**9**), 2-aminobenzo[*d*]thiazole (**10**), 2-aminopyrimidine (**11**) and 2-aminopyrimidine (**12**) in refluxing ethanol, afforded the respective fused imidazole derivatives, imidazo[2,1-*b*]thiazole **13**, benzo[*d*]imidazo[2,1-*b*]thiazole **14**, imidazopyridine **15**, and imidazopyrimidine **16**, respectively, as depicted in Scheme 2. The structures of compounds **13-16** were elucidated on the basis of elemental and spectral data (IR, ^1H NMR, and MS).

For example, the ^1H NMR spectrum of compound **13** revealed all characteristic signals at δ 4.44 (s, 4H, 2CH_2), 7.20 (d, 1H, thiazole-H5), 7.45–8.10 (m, 16 H, Ar-H), 8.12 (d, 1H, thiazole-H4), 8.59 (s, 1H, imidazole-H5) ppm. The mass spectra of the isolated products showed molecular ion peaks in accord with the assigned structures.

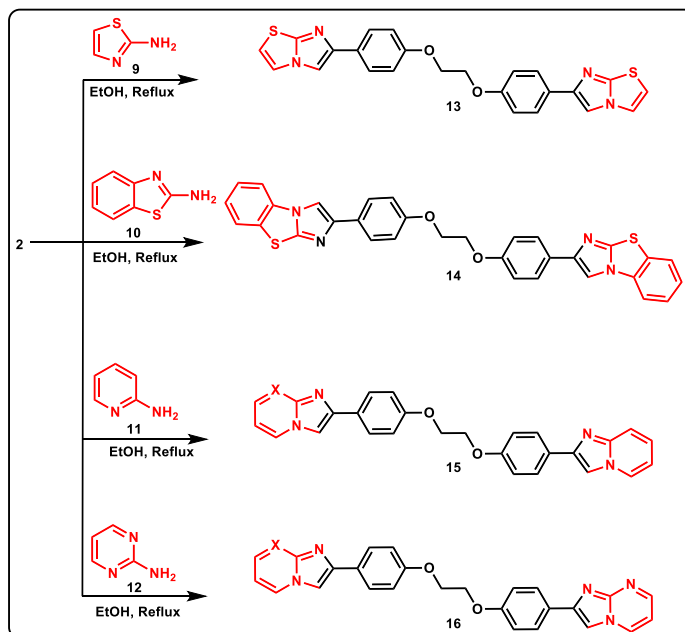
Mechanistically, we believe that the reaction starts with *N*-alkylation *via* simple nucleophilic substitution followed by intramolecular cyclization to give the isolated products **13-16**, as shown in Scheme 4.



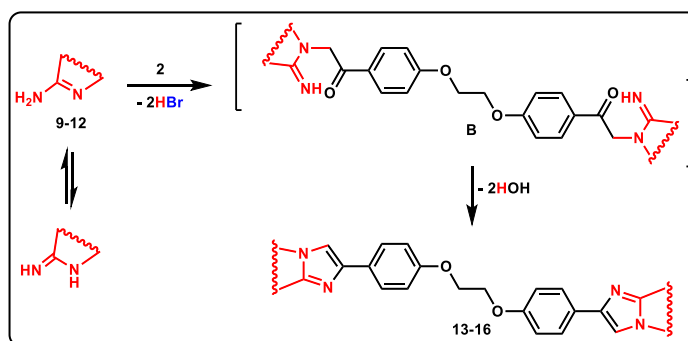
Scheme 1. Synthesis of bis-imidazo-azole derivatives **6-8**.



Scheme 2. Mechanism of formation of bis-imidazo-azole derivatives 6-8.



Scheme 3. Synthesis of bis-imidazo-thiazoles 13, 14 and bis-imidazo-azines 15, 16.

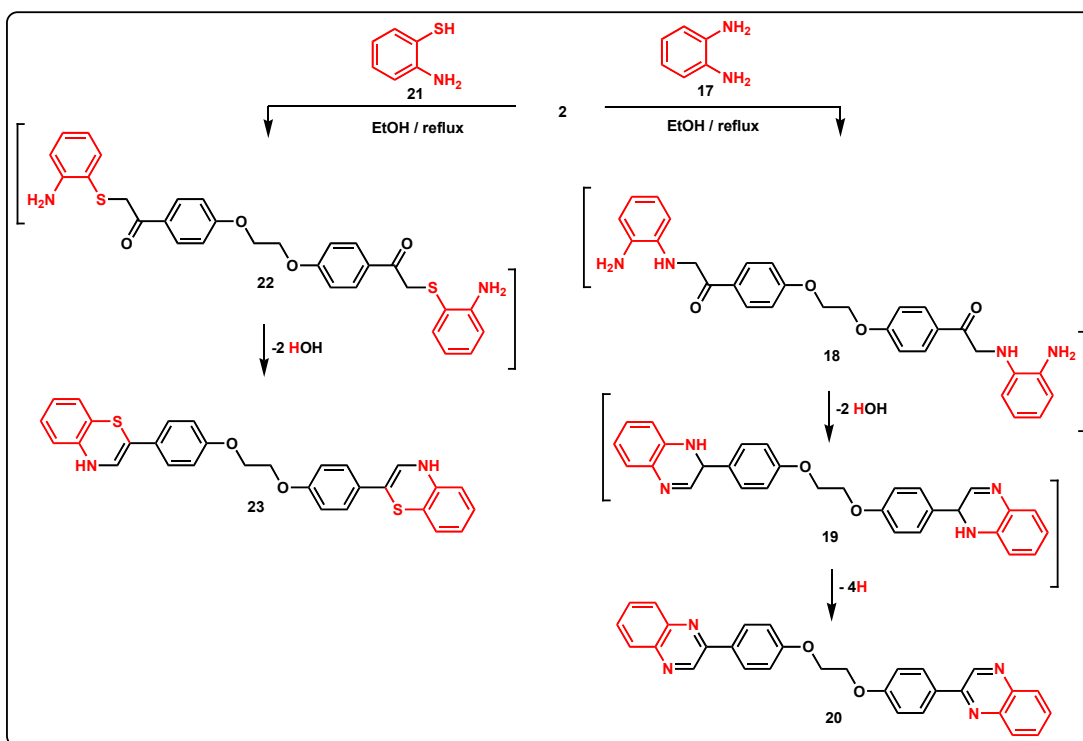


Scheme 4. Mechanism of formation of bis-imidazole-fused derivatives 13-16.

To showcase the diversity of our synthetic protocol, quinoxaline and benzothiazine were chosen as two synthetic targets. So, heating compound **2** with *o*-phenylene diamine (**17**) and 2-aminothiophenol (**21**) in boiling ethanol gave the desired quinoxaline (**20**) and benzothiazine (**23**) derivatives (Scheme 5). Structural confirmation of compounds **20** and **23** was based on their analytical and spectral data. For instance, the ^1H NMR spectrum of compound **23** displayed

two singlets at $\delta = 6.63$ and 9.68 ppm, assigned to 3-H and NH-thiazine protons, respectively. The mass spectra of compounds **20** and **23** showed characteristic molecular ion peaks in accordance with their proposed molecular formula, m/z (%): 470 and 508, respectively.

Finally, compound **2** reacted with pyrimidinethiones **24a,b** in refluxing dioxane containing TEA to give the



Scheme 5. Synthesis of bis-fused azine derivatives **20** and **23**.

corresponding 7-methyl-5*H*-thiazolo[3,2-*a*]pyrimidine **26a** and 7-phenyl-5*H*-thiazolo[3,2-*a*]pyrimidine **26b**, respectively, as depicted in Scheme 6. Structural elucidation of compounds **26a** and **26b** was based on elemental and spectral data. For example, the IR spectrum of thiazolopyrimidine **26a** showed the characteristic strong carbonyl absorption at $\nu = 1669 \text{ cm}^{-1}$. The ^1H NMR spectrum of **26a** showed the expected four singlets at $\delta = 2.24$ (s, 6H, 2CH₃), 4.38 (s, 4H, 2OCH₂), 5.72 (s, 2H, pyrimidine-H5) ppm, in addition to the aromatic multiplets at $\delta = 6.93$ -7.14 ppm (8H). Also, the ^{13}C NMR spectrum of **26a** showed 12 different carbons and its mass spectrum showed a molecular ion peak at m/z 542.

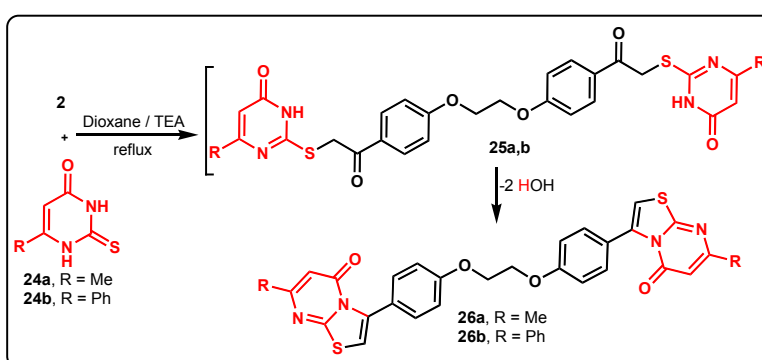
4.2. Biological Evaluation

4.2.1. *In vitro* Antitumor Activity

All the newly prepared compounds were screened for

their cytotoxic activity against the lung carcinoma cell line (A-549) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cisplatin was used as a reference drug. The data generated were used to plot a dose-response curve from which the concentration (μM) of test compounds required to kill 50% of the cell population (IC_{50}) was determined. Cytotoxic activity was expressed as the mean IC_{50} of three independent experiments. The difference between inhibitory activities of all compounds with different concentrations was statistically significant $P < 0.001$.

The results revealed that, the tested compounds showed high variation in the inhibitory growth rates and activities against the tested tumor cell lines in a concentration dependent manner compared to the reference drug as shown in Table 1 and Figure 1. The descending order of activity of the new compounds was as follows: **2** > **6** > **23** > **13** > **26a** > **14** > **26b** > **15** > **7** > **20** > **8** > **16**.



Scheme 6. Synthesis of bis-thiazolopyrimidines **26a,b**.

Structure Activity Relationship (SAR)

The activities of the synthesized compounds depend on the structural skeleton and electronic environment of the molecules. The lead compound, 1,2-bis(4-bromoacetyl-phenoxymethyl)benzene (**2**) ($IC_{50} = 5.5 \pm 1.8 \mu\text{g/mL}$) has promising *in vitro* inhibitory activity against the lung carcinoma cell line (A-549) greater than that of cisplatin ($IC_{50} = 5.8 \pm 1.1 \mu\text{g/mL}$). The bis-imidazotriazole derivative **6** ($IC_{50} = 23.8 \pm 1.8 \mu\text{g/mL}$) and the bis-benzopyridazine derivative **23** ($IC_{50} = 30.7 \pm 1.9 \mu\text{g/mL}$) have promising *in vitro* inhibitory activity but less than that of cisplatin ($IC_{50} = 5.8 \pm 1.1 \mu\text{g/mL}$). Compounds **13**, **26a**, **14**, **26b**, **15**, **7** and **20** have weak *in vitro* inhibitory activity ($IC_{50} = 75 - 230 \mu\text{g/mL}$). Compounds **8** and **16** have no noticeable *in vitro* inhibitory activity ($IC_{50} \sim 500 \mu\text{g/mL}$). Imidazotriazole **6** demonstrated more potent antitumor activity compared to imidazotetrazole **7** ($IC_{50} = 23.8 \pm 1.8 \mu\text{g/mL}$) and ($IC_{50} = 176 \pm 3.1 \mu\text{g/mL}$), respectively. This may be attributed to the extra nitrogen atom of the latter moiety (lower activity). Benzothiazine **23** demonstrated more potent antitumor activity compared to benzopyrimidine **20** ($IC_{50} = 30.7 \pm 1.9 \mu\text{g/mL}$) and ($IC_{50} = 230 \pm 3.1 \mu\text{g/mL}$), respectively. This may be due to the NH of thiazine moiety (higher activity). Imidazothiazole **13** demonstrated more potent antitumor activity compared to imidazobenzothiazole **14** ($IC_{50} = 75 \pm 1.8 \mu\text{g/mL}$) and ($IC_{50} = 118 \pm 10.2 \mu\text{g/mL}$), respectively. This may be attributed to the benzo group of the latter moiety (lower activity). 4-Methylthiazolopyrimidine **26a** demonstrated more potent antitumor activity compared to 4-phenyl thiazolopyrimidine **26b** ($IC_{50} = 114 \pm 2.9 \mu\text{g/mL}$) and ($IC_{50} = 154 \pm 0.5 \mu\text{g/mL}$), respectively. This may be due to the NH of thiazine moiety (higher activity).

4.2.2. Anti-tubercular study

The newly synthesized compounds were evaluated for their *in vitro* anti-tubercular activity against *M. tuberculosis* (ATCC 25177) using the microplate alamarBlue assay (MABA). Isoniazide was used as a reference drug. The result of antimycobacterial activity is presented in Table 2 as minimum inhibitory concentration (MIC). The analysis of antimycobacterial activity data revealed that, among all tested derivatives, compounds **2**, **13**, **16**, **26a** and **6** emerged as the most potent candidates with significant antimycobacterial activity (MIC: 0.12 - 3.9 $\mu\text{g/mL}$) compared to the

Table 1. The antitumor activities of the tested compounds against A-549 cell lines.

Tested compounds	IC ₅₀ ($\mu\text{g/mL}$).
2	5.5 ± 1.4
6	23.8 ± 1.8
7	176 ± 3.1
8	484 ± 9.7
13	75 ± 1.8
14	118 ± 10.2
15	170.3 ± 8.1
16	500 ± 8.2
20	230 ± 3.1
23	30.7 ± 1.9
26a	114 ± 2.9
26b	154 ± 0.5
Cisplatin	5.8 ± 1.1

reference drug, Isoniazide (MIC: 0.12 $\mu\text{g/mL}$). Meanwhile, compounds **15**, **20**, **23**, **8**, **14**, and **26b** were moderately active, against *M. tuberculosis* (ATCC 25177) (MIC: 7.81 - 15.63 $\mu\text{g/mL}$). Finally, compound **7** showed modest anti-tubercular activity (MIC: 62.5 $\mu\text{g/mL}$).

4.3. Molecular Docking Study

Molecular docking is carried out to investigate the structure-activity correlation between the potential drug prototypes and a specific target in order to predict both the strength and type of binding interactions of the possible ligand(s) with the target protein. In the current work, we tried to explore the mode of action of the synthesized derivatives as antitumor agents through searching for a suitable target and performing the molecular docking procedure. Validation of docking is performed by the self-redocking of the co-crystallized ligand, docking score and

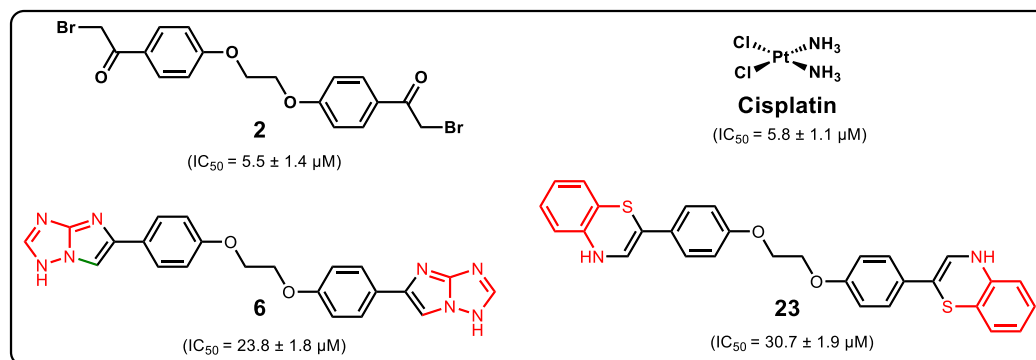


Fig. (1). Cytotoxic activities of the most active compounds against A-549 cell lines.

Table 2. Antitubercular activity as MIC of the synthesized derivatives.

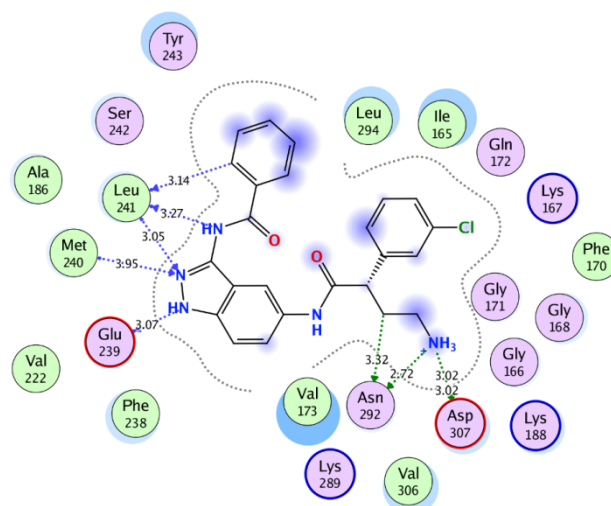
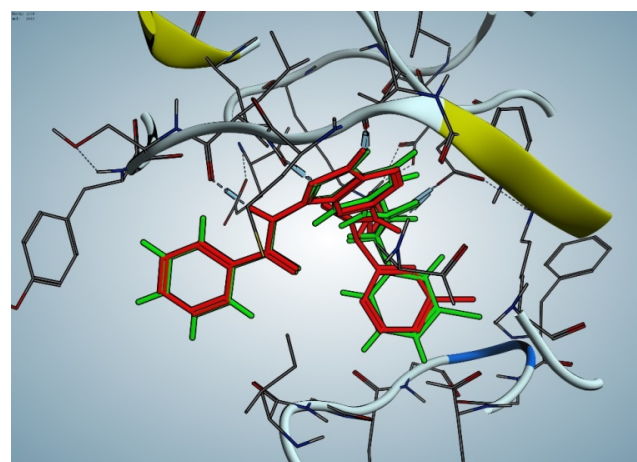
Compound	Minimum inhibitory concentration (MIC) (µg/ml)
2	0.12
6	3.9
7	62.5
8	15.63
13	0.12
14	15.63
15	7.81
16	0.49
20	7.81
23	7.81
26a	1.95
26b	15.63
Isoniazid	0.12

types of bonding interactions were determined. So, docking setup was first validated by self-docking of the co-crystallized ligand, N-(5-((2S)-4-amino-2-(3-chlorophenyl)butanoyl)amino)-1H-indazol-3-yl)benzamide, in the vicinity of the binding site of dual-specificity tyrosine phosphorylation regulated kinase (DYRK1A). The results are summarized in Table 3 and Figs. 2 and 3.

The protonation state of certain functional groups such as the triazole ring was adjusted to the most probable state in an aqueous environment by enabling the deprotonation of strong acids and protonation of strong bases. Docking procedure was first validated by self-docking of the co-crystallized ligand, N-(5-((2S)-4-amino-2-(3-chlorophenyl)butanoyl)amino)-1H-indazol-3-yl)benzamide, in the vicinity of the binding site of dual-specificity tyrosine phosphorylation regulated kinase (DYRK1A), docking score and types of bonding interactions were determined and are summarized in Table 3 and Figs. 2 and 3.

Table 3. Docking validation results.

Docking Score (S) (kcal/mol.)	RMSD (Å°)	Bounded Amino acids
-9.4123	0.7927	Glu239, Met240, Leu241, Asn292, Asp307

**Fig. (2).** 2D interaction diagram showing the co-crystallized ligand docking pose interactions with the key amino acids in tyrosine kinase 1A binding site. (A higher resolution / color version of this figure is available in the electronic copy of the article).**Fig. (3).** 3D representation of the superimposition of the co-crystallized (red) and the docking pose (green) of the ligand in tyrosine kinase 1A binding site with Root Mean Square Deviation (RMSD) of 0.7927 Å°. (A higher resolution / color version of this figure is available in the electronic copy of the article).

The validated setup was then used to predict the ligand-receptor interactions at the binding site for the series of bis-imidazole fused derivatives. All tested compounds showed good binding score and showed several binding interactions within the binding site. The results are summarized in Table 4 & Figs. 4-6. The validated procedure was used for docking representative bis-imidazotriazole **6**, bis-imidazothiazole **13**, and bis-benzothiazine derivative **23** in order to predict the ligand-receptor interactions at the binding site. All screened bis-heterocycles showed good binding scores indicating several binding interactions within the binding site. The results are summarized in Table 4 and Figs. 4-6.

Table 4. Docking results.

Compound	S (kcal/mol)	Amino acids	Interacting groups	Type of interaction	Length
6	-12.0004	Lys167	CH (phenyl)	Side chain donor	3.49
		Tyr243	=N (imidazole)	Side chain acceptor	3.38
		Asp287	NH (triazole)	Ionic	2.21
		Asp287	=N (triazole)	Ionic	3.55
		Asp307	CH (imidazole)	Backbone donor	1.82
		Asp307	CH (phenyl)	Backbone donor	2.62
		Asp307	NH (triazole)	Ionic	3.84
13	-10.45901	Ile165	N (imidazole)	Backbone acceptor	3.31
		Asp287	S	Backbone donor	3.36
		Lys289	N (imidazole)	Backbone acceptor	2.74
		Glu291	CH ₂	Side chain donor	2.31
23	-11.3463	Ile165	S	Side chain donor	4.14
		Gly166	CH ₂	Side chain donor	3.18
		Lys167	CH ₂	Side chain donor	3.38
		Leu241	NH	Side chain donor	2.13
		Asp287	S	Backbone donor	3.56
		Lys289	S	Backbone acceptor	3.51
		Asp307	CH (phenyl)	Backbone donor	2.73

Our molecular docking analysis showed that compounds **6**, **13**, and **23** can fit the LDH-5 active site with a high binding score (binding energy). The imidazo-triazole moiety in compound **6** was capable of binding strongly via three bonds to Asp307, an amino acid involved in binding with the co-crystallized drug, which explains the high binding score for this derivative (Fig. 4). However, the binding interaction between Asp307 and the thiazole moiety in compound **13** seems to be lost, which decreased the overall binding strength to the active binding site of the protein, as shown in Fig. 5 and Table 4. On the other hand, ring expansion from five- to six-membered benzothiazine derivative **23** seems to improve the binding interactions with Asp307 as well as Leu241 (Fig. 6). The latter interaction appeared to be missing in both imidazo-triazole and imidazothiazole derivatives.

This molecular docking analysis clearly suggests that our synthesized compounds, especially bis-imidazole derivatives, are promising lead molecules for further development of potent antitumor drugs.

CONCLUSION

A novel series of bis-fused imidazole derivatives **6-8** and **13-16** was constructed using bis-phenacyl bromide derivative **2** as a common structural motif. Compound **2** was also used to access bis-quinoxaline **20** and bis-benzothiazine **23**, and bis-thiazolopyrimidine derivatives **26a,b**. The new bis-fused imidazole heterocycles were screened for an antimycobacterial activity to investigate the case of TB and its most associative pathogen and its potential correlation

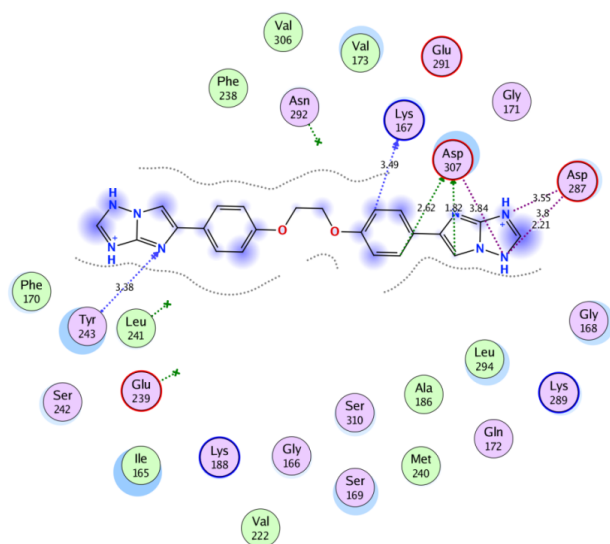


Fig. (4). 2D diagram of bis-imidazo-triazole derivative **6** showing its interaction with the tyrosine kinase 1A binding site. (A higher resolution / color version of this figure is available in the electronic copy of the article).

with LC. In vitro screening of the new bis-imidazole fused heterocycles for their cytotoxicity against lung cancer cell lines (A-549) using cisplatin as a reference drug revealed that compounds **6** and **23** have marked antitumor activity. The new compounds were also evaluated for their anti-tubercular activity against *M. tuberculosis* (ATCC 25177) using isoniazid as a

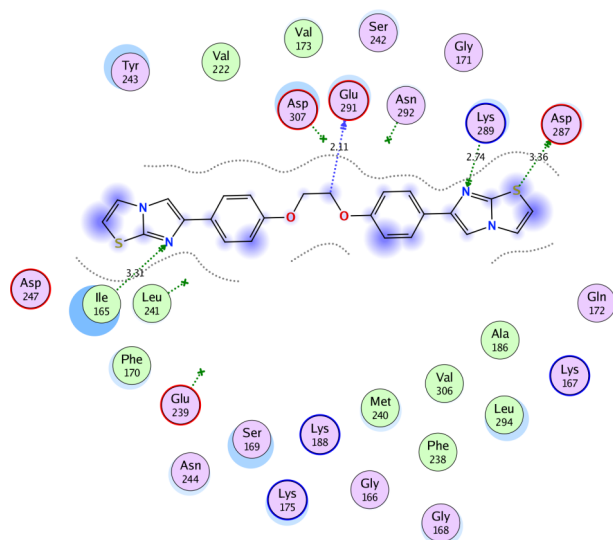


Fig. (5). 2D diagram of bis-imidazo-thiazole derivative **13** showing its interaction with the tyrosine kinase 1A binding site. (*A higher resolution / color version of this figure is available in the electronic copy of the article.*)

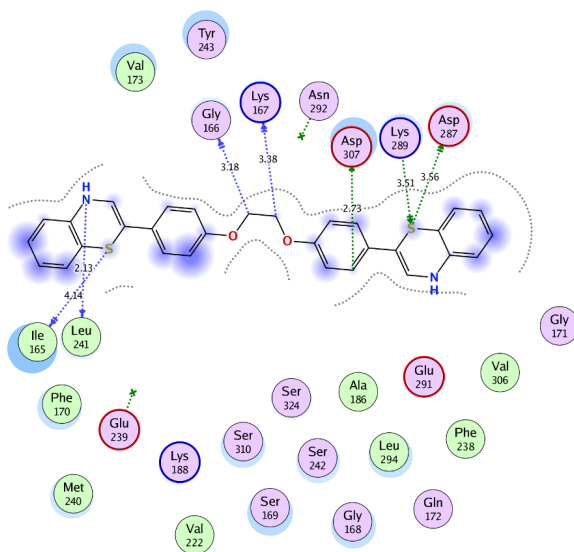


Fig. (6). 2D diagram of bis-thiazine derivative **23** showing its interaction with the tyrosine kinase 1A binding site. (*A higher resolution / color version of this figure is available in the electronic copy of the article.*)

reference drug and the results showed that compounds **2**, **13**, **16**, **26a** and **6** were the most potent compounds with significant antimycobacterial activity (MIC: 0.12-3.9 $\mu\text{g}/\text{mL}$) compared to the reference drug, Isoniazide (MIC = 0.12 $\mu\text{g}/\text{mL}$). A molecular docking study showed that compounds **6**, **13**, and **23** can fit the LDH-5 active site with high binding scores. These results suggest that these bis-imidazole derivatives are promising lead molecules for further development of potent anticancer prototypes.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Ethics Committee of Cairo University.

HUMAN AND ANIMAL RIGHTS

No animals were used for studies that are the base of this research. All human procedures were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT FOR PUBLICATION

Written informed consent has been obtained from all the participants of the study.

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available from the corresponding author, [Zeinab A. Muhammad], upon reasonable request.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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AUTHOR CONTRIBUTIONS

All the authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

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