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RESEARCH ARTICLE

Synthesis, Antioxidant Activity and Molecular Docking Study of 1,2,4-Triazole and Their Corresponding Fused Rings Containing 2-Methylphenol

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ABSTRACT

Newly 4-amino-1,2,4-triazole-3-thione ring **2** was formed at position six of 2-methylphenol from the reaction of 6-(5-thio-1,3,4-oxadiazol-2-yl)-2-methylphenol **1** with hydrazine hydrochloride in the presence of anhydrase sodium acetate. Seven newly fused heterocyclic compounds were synthesized from compound **2**. First fused heterocyclic was 6-(6-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)-2-methylphenol **3** synthesized from reaction compound **2** with 3,5-di-*tert*-butyl-4-hydroxybenzoic acid in $POCl_3$. Reaction compound **2** with bromophencylbromide afford 6-(6-(4-bromophenyl)-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]-thiadiazin-3-yl)-2-methylphenol **4**. 6-(6-thio-1,7a-dihydro-[1,2,4]triazolo[3,4-*b*][1,3,4]-thiadiazol-3-yl)-2-methylphenol **5** was synthesized from reaction compound **2** with CS_2 in alkaline ethanolic solution. 6-(6-amino-1,7a-dihydro-[1,2,4]triazolo[3,4-*b*][1,3,4]-thiadiazol-3-yl)-2-methylphenol **6** was synthesized from cyclization of **2** with cyanogen bromide at room temperature. Compounds **7a-c** were synthesized from reaction compound **2** with arylisothiocyanate in dimethyl formamide. The antioxidant ability of these compounds was screened by 2,2-diphenyl-1-picrylhydrazyl radical assay (DPPH) and Ferric ion reducing antioxidant power assay (FRAP) assays. Compound **2** and **3** exhibited highest DPPH inhibition and FRAP value compared to rest synthesized compounds. The molecular docking studies of the newly synthesized compounds were screened for their A tubulin binding affinity with the aid of docking studies via MOE 2015. Compounds **4**, **7a**, **7b** and **7c** exhibited interaction compared with colchicine reference as potent tubulin inhibitor.

Keyword: Antioxidant, Docking, Fused Ring, Methylphenol, Triazole, Tubulin.

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INTRODUCTION

Recently, many researchers were emphasized that the free radical considered one of the major reasons that cause several diseases such as inflammation,[1] cancer,[2] degenerative diseases,[3] and chronic diseases.[4] Furthermore, the free radicals and their related species can cause serious damage in biomolecule cells, encompass proteins, lipids, Deoxyribonucleic Acid (DNA), and carbohydrates resulting in severe damage to the cell.[5] The antioxidant compounds are important materials due to their ability to prevent this damage.[6] The hindered phenolic derivatives are known for their significant antioxidant ability[7] and particularly those containing heterocyclic rings.[8-10] A large number of heterocyclic and fused heterocyclics displayed significant biological activities, including antioxidant ability,[11-14] antiplasmodial activity,[15] anti-inflammatory,[16] antibacterial activity,[17] antifungal[18] inhibitors of HIV.[19] Furthermore, fused heterocyclic, and especially the 1,2,4-triazole moiety, revealed various biological activity, for instance, antimicrobial activity,[20] antitumor,[21,22] herbicidal activity,[23] anti-inflammatory,[24] Jak1 kinase inhibitors,[25] antifungal activity,[26] anti-HIV-1 activity[27] and antioxidant activity.[28,29] It is too renowned in medicinal chemistry that merged two different pharmacophores in the same structure to promote the resulting compound's biological activities.[30-32] Recently, molecular docking studies are too beneficial to afford a prediction of the ligand-receptor complex structure using computation methods. Considering ligand-receptor interaction, one usually considers two points: strength of the interaction (what is known as 'affinity') and kind of an effect at the biochemical, electrophysiological, and behavioral level triggered by the ligand (what we refer to as intrinsic activity). To get an insight into the type of chemical forces involved in the

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interaction, one may build models of a specific pharmacophore or construct models of a receptor protein and examine the binding forces. Interaction of a ligand with a receptor may result in receptor activation (agonists).[33] The most popular approach to predict the correct binding pose and binding affinity (BA) is protein-based modeling (docking) in which physicochemical interactions between a ligand and receptor are deduced from the 3D structures of both molecules. In this work, docking method was performed to predict the antibacterial activity of the synthesized compounds.[34]

This work presented formation 4-amino 1,2,4-triazole at position six of 2-methylphenol and synthesized their corresponding fused ring to increases the sterical hindrances around the phenolic hydroxyl and investigate their antioxidant ability by DPPH and FRAP compared with 2-methylphenol, BHT, and ascorbic acid. The molecular docking study for the newly synthesized compounds was tested for their A tubulin binding affinity with the aid of docking studies via MOE 2015.

EXPERIMENTAL

General Chemistry

The melting point of synthesized compounds was established by open capillary tube using OMEGA MPS10 apparatus, and it is uncorrected. The end of the reactions was monitored by thin-layer chromatography (TLC) Plates (0.25 mm, 20 × 20 cm, 60F254, E. Merck). Silica gel 60 (230–400 mesh, E. Merck). They were utilized for column chromatography, brand Merck the spot located with UV lights at 254 nm. The FTIR spectrums were affirmed with Perkin Elmer 400 spectrometer. The NMR spectra were recorded on Bruker spectrometer 300 MHz (AL-Bayt University, Jordan) in DMSO-d_{6} with tetramethylsilane as internal standard. The Mass spectrum was recorded using Shimadzu GC MS-QP2010Ultra (Al-Mustansiriyah University, College of Science, Department of Chemistry). A Power Wave X340 (BIO-TEK Instruments, Inc., Winooski, VT, USA) UV spectrophotometer was used to record the DPPH and FRAP assays.

Synthesis of 6-(4-amino-1,2,4-triazol-3-yl-5-thione)-2-methylphenol 2

A mixture of 6-(5-thio-1,3,4-oxadiazol-2-yl)-2-methylphenol (3g, 14.4 mmol) which is synthesized according to previously reported method,[8] hydrazine hydrochloride (1.48 g, 21.6 mmol) and anhydrous sodium acetate (1.77 g, 21.6 mmol) dissolved 10 mL absolute ethanol then heated under reflux for 18 hours. After cooling, the solvent evaporated under reduced pressure. The residue washed with warm water (20 mL ×2). The crude precipitated recrystallized from methanol to afford white crystals. Yield 67%, m.p. (235-237) ˚C, IR (KBr, υ_{max}/ cm^{-1}); 3460 (OH_{phenol}), 3315, 3252 (NH_2), 3182 (NH), 3035 ($CH_{aromatic}$), 2939, 2785 ($CH_{aliphatic}$), 1617 (C=N), 15993-1435 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 2.23 (s, 3H, CH_3), 5.96 (bs, 2H, NH_2), 6.89 (t, *J* 7.6, 1H, H_4), 7.31 (d, *J* 7.2, 1H, H_3), 7.54 (d, *J* 7.2, 1H, H_5), 9.97 (bs, 1H, OH), 13.99 (bs, 1H, NH)); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 16.28 (1C, CH_3), 112.60 (1C, C_6), 119.21 (1C, C_4), 126.20 (1C, C_2), 127.84 (1C, C_5), 133.15 (1C, C_3), 149.17 (1C, C_1), 153.67 (1C, $C_{7(C=N)}$), 165.67 (1C, $C_{8(C=S)}$). EIMS m/z = 222 [$M^{\bullet+}$] (calc. for $C_9H_{10}N_4OS$, 222.06).

Synthesis of 6-(6-(3,5-di-tert-butyl-4-hydroxyphenyl)-[1,2,4] triazolo[3,4-b] [1,3,4]thiadiazol-3-yl)-2-methylphenol 3

To a mixture of 6-(4-amino-1,2,4-triazol-3-yl-5-thione)2-methylphenol (0.25 g, 1.12 mmol) and 3,5-di-tert-butyl-4-hydroxybenzoic acid (0.3 g, 1.12 mmol), in 50 mL round flask, 5 mL of phosphorusoxy chloride was added in a few portions at ambient temperature. The mixture stirred and refluxed for 3 hs in a water bath at 80-90°C. After cooling, the mixture was poured into 100 mL crushed ice and stirred for 15 minutes. Sodium bicarbonate was added in few portions until the pH adjusted to 7 to 8. The precipitate was filtered, washed with water and dried. The crude product was purified from column chromatography using hexane-ethyl acetate (3-1) as eluent to give pale pink precipitate. Yield 0.42 g, 77%, m.p. (240-243)˚C, IR (KBr, υ_{max}/ cm^{-1}); 3633 ($OH_{di\text{-}tert\text{-}butyl}$), 3602 ($OH_{phenol}$), 3042 ($CH_{aromatic}$), 2962-2869 ($CH_{aliphatic}$), 1606 (C=N), 1543-1421 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 1.45 (s, 18H, 2×C($\underline{CH_3}$)$_3$), 2.50 (s, 3H, CH_3), 5.62 (s, 1H, C_{13}-OH), 6.92 (t, *J* 7.9, 1H, H_4), 7.34 (d, *J* 7.5, 1H, H_3), 7.45 (d, *J* 7.9, 1H, H_5), 7.82 (s, 2H, H_{11}), 9.18 (bs, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 15.60 (1C, CH_3), 29.89 (6C, C_{15}, 2×C(CH_3)$_3$), 34.56 (2C, C_{14}, $\underline{C}(CH_3)_3$), 110.12 (1C, C_{10}), 114.86 (1C, C_6), 119.53 (1C, C_4), 123.16 (2C, C_{11}), 125.34 (1C, C_5), 132.87 (1C, C_2), 134.93 (1C, C_3), 139.64 (2C, C_{12}), 153.88 (1C, C_{13}), 157.30 (1C, C_1), 162.83 (1C, C_9), 163.94 (1C, $C_{8(C=N)}$), 164.88 (1C, $C_{7(C=N)}$); EIMs, m/z= 436 [$M^{\bullet+}$] (Calc. for $C_{24}H_{28}N_4O_2S$, 436.19).

Synthesis of 6-(6-(4-bromophenyl)-5H-[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazin-3-yl)-2-methylphenol 4

To a solution of 6-(4-amino-1,2,4-triazol-3-yl-5-thione)2-methyl-phenol (0.28 g, 1.25 mmol) in ethanol, 4-bromophencyl bromide (0.34 g, 1.25 mmol) was added with few portions at ambient temperature. The mixture was refluxed for 3 hs then sodium acetate (0.1 g, 1.25 mmol) was added and the mixture refluxed for another 3 hs. The crude material recrystallized from ethanol-water, to afford white crystal. Yield 0.32 g, 64%, m.p. (259-260)˚C, IR (KBr, υ_{max}/ cm^{-1}); 3450 (OH_{phenol}), 3086 ($CH_{aromatic}$), 2991-2852 ($CH_{aliphatic}$), 1603 (C=N), 1595-1477 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 2.27 (s, 3H, CH_3), 4.46 (s, 2H, CH_2, H_9), 6.96 (t, *J* 7.8, 1H, H_4), 7.32 (d, *J* 6.9, 1H, H_3), 7.84 (d, *J* 8.4, 2H, 2×H_{13}), 8.01 (m, 3H, H_5, 2×H_{12}), 11.46 (s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 16.05 (1C, CH_3), 22.45 (1C, C_9, CH_2), 112.63 (1C, C_6), 118.91 (1C, C_4), 125.34 (1C, C_{14}), 125.51 (1C, C_2), 127.85 (1C, C_5), 129.58 (2C, C_{12}), 132.25 (2C, C_{13}), 132.79 (1C, C_3), 133.14 (1C, C_{11}), 155.72 (1C, C_1), 163.82 (1C, C_{10}), 164.39 (1C, C_8), 166.01 (1C, C_7); EIMs, m/z= 400 [$M^{\bullet+}$] 100%, (Calc. for $C_{17}H_{13}BrN_4OS$, 400.00).

Synthesis of 6-(6-thio-1,7a-dihydro-[1,2,4]triazolo[3,4-b] [1,3,4]-thiadiazol-3-yl)-2-methylphenol 5

Excess of CS_2 (0.1 g, 1.25 mmol) was added to a mixture of

6-(4-amino-1,2,4-triazol-3-yl-5-thione)-2-methylphenol (0.28 g, 1.25 mmol) and KOH (0.07 g, 1.25 mmol). In ethanol The mixture was heated under refluxed 7 hours. The progress of the reaction was monitored by wet lead acetate paper. The solvent was reduced to half and the residue was poured in to 10 mL crashed ice. Hydrochloric acid (10%) was used to adjust the pH to 6-5 .The precipitate was filtrated, washed with water, dried and crystallized from methanol, to afford white crystal. Yield 0.28 g, 86%, m.p (230-232)˚C, IR (KBr, U_{max}/ cm^{-1}); 3309 (OH_{phenol}), 3101 (NH), 3033 ($CH_{aromatic}$), 2947-2781 ($CH_{aliphatic}$), 1610 (C=N), 1564-1477 (C=C);^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 2.22 (s, 3H, CH_3), 6.89 (t, *J* 6.87, 1H, H_4), 7.31 (d, *J* 7.56, 1H, H_3), 7.54 (d, *J* 8.01, 8.4, 1H, H_5), 9.98 (s, 1H, OH), 14.01 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 16.32 (1C, CH_3), 112.63 (1C, C_6), 119.23 (1C, C_4), 126.22 (1C, C_2), 127.88 (1C, C_5), 133.17 (1C, C_3), 149.21 (1C, C_1), 153.70 (1C, C_7, C=N), 165.69 (1C, C_8, C=N), 171.90 (1C, C_9, C=S); EIMs, m/z= 264 [$M^{\bullet+}$] (calc. for $C_{10}H_8N_4OS_2$, 264.01).

*Synthesis of 6-(6-amino-1,7a-dihydro-[1,2,4]triazolo[3,4-**b**][1,3,4]thiadiazol-3-yl)-2-methylphenol 6*

cyanogen bromide (0.13 g, 1.25 mmol) was added to a mixture of 6-(4-amino-1,2,4-triazol-3-yl-5-thione)2-methylphenol (0.28 g, 1.25 mmol) and sodium hydrogencarbonat (0.11 g, 1.25 mmol).in 10 mL methanol. The mixture was stirred at ambient temperature for 16 h. the precipitated was filtrated and washed with warm water. The crude material recrystallized from methanol, to afford white crystal. Yield 0.22 g, 70%, m.p (170-173)˚C, IR (KBr, U_{max}/ cm^{-1}); 3357 (OH_{phenol}) 3271-3097 (NH_2), 3026 ($CH_{aromatic}$), 2951-2785 ($CH_{aliphatic}$), 1610 (C=N), 1547-1481 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 2.25 (s, 3H, CH_3), 6.45 (s, 2H, NH_2), 6.86-6.95 (m, 1H, H_5), 7.32 (d, *J* 6.6, 1H, H_4), 8.02 (d, *J* 7.9, 1H, H_3), 10.92 (s, 1H, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 15.92 (1C, CH_3), 108.39 (1C, C_6), 119.85 (1C,C_4), 123.16 (1C, C_5), 125.66 (1C, C_2), 133.19 (1C, C_3), 154.21 (1C, C_9), 158.05 (1C, C_1), 163.15 (1C, C_7,C=N), 165.62 (1C, C_8,C=S); EIMs, m/z= 247 [$M^{\bullet+}$] (calc. for $C_{10}H_9N_5OS$, 247.05).

*General synthesis of 6-(6-((4-substituted-phenyl)amino)-1,7a-dihydro[1,2,4]-triazolo[3,4-**b**][1,3,4]thiadiazol-3-yl)-2-methylphenol 7a-c*

4-substituted-phenylisothiocyante (1.25 mmol) was added gradually to a solution of 6-(4-amino-1,2,4-triazol-3-yl-5-thione)2-methylphenol (0.28 g, 1.25 mmol) in 10 mL DMF. The mixture was heated under reflux for five hours. Upon cooling, the mixture was quenched with 15 mL distilled water and twice extracted from diethyl ether. The combined organic layer was washed with water several times, brine, and dried under sodium sulfate. The solvent was removed under reduced pressure and the crud solid crystallized from a suitable solvent.

6-(6-((4-chlorophenyl)amino)-1,7a-dihydro[1,2,4]-triazolo[3,4-b][1,3,4]thiadiazol-3-yl)-2-methylphenol 7a

The crud solid crystallized from chloroform: ethanol (1:6).to gives white Precipitate. Yield 0.3 g, 67%, m.p.(159-162) ˚C, IR (KBr, U_{max}/ cm^{-1}); 3425 (OH_{phenol}), 3180 (NH), 3093,3028 ($CH_{aromatic}$), 2958, 2860 ($CH_{aliphatic}$), 1647 (C=N), 1595, 1489 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 2.17 (s, 3H, CH_3), 5.91 (s, 1H, OH), 6.69-7.91 (m, 7H, H_3, H_4, H_5, $2H_{11}$, $2H_{12}$), 9.90 (bs, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 16.27 (1C, CH_3), 117.88 (1C, C_6), 119.21 (1C,C_4), 125.27 (2C, C_{11}), 127.85 (1C, C_2), 128.28 (1C, C_{13}), 128.97 (1C, C_5), 133.14 (1C, C_3), 135.05 (1C,C_{12}), 138.08 (1C,C_{10}), 153.68 (1C, C_9,C=N), 158.98 (1C, C_1), 159.08 (1C, C_7, C=N), 165.68 (1C, C_8,C=N); EIMs, m/z= 359 [$M^{\bullet+}$] (Calc. for $C_{16}H_{14}ClN_5OS$, 359.06).

6-{6-[(4-methylphenyl)amino][1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl}-2-methylphenol 7b

Recrystallized from acetonitrile to afford pale yellow precipitate ,Yield 57% M.P= 134-136 ºC, IR (KBr, U_{max}/ cm^{-1}); 3433 (OH_{phenol}), 3223 (NH), 3078 ($CH_{aromatic}$), 2967, 2883 ($CH_{aliphatic}$), 1638 (C=N), 1598,1492 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 2.10 (s, 3H, CH_3), 2.16 (s, 3H, CH_3) , 5.95 (s, 1H, OH), 6.81 (t, J 7.44, 1H, H4), 7.12 (d, 2H, J 7.8, H12), 7.42 -7.49 (m, 3H, H_3, H11), 7.52 (d, J 8.01, 1H, H_5),, 9.70 (bs, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 18.87 (1C, CH_3), 2.11 (1C, CH_3), 118.84 (1C, C_6), 120.09 (1C,C_4), 123.17 (2C, C_{11}), 128.05 (1C, C_2), 128.28 (1C, C_5), 129.33 (2C,C_{12}), 130.98 (1C, C_{13}), 131.24 (1C, C_3), 136.15 (1C,C_{13}), 136.08 (1C,C_{10}),152.83 (1C, $C_{9(C=N)}$), 157.66 (1C, C_1), 158.67 (1C, $C_{7(C=N)}$), 164.34 (1C, $C_{8(C=N)}$);); EIMs, m/z=337 [$M^{\bullet+}$] (Calc. for $C_{17}H_{15}N_5OS$, 337.1).

6-(6-((4-methoxyphenyl)amino)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl)-2-methylphenol 7c

Recrystallized from ethanol to gives off white precipitate; Yield 62%, M.P. 127-129 ºC, IR (KBr, U_{max}/ cm^{-1}); 3451 (NH), 3218 (OH_{phenol}), 3066 ($CH_{aromatic}$), 2972,2868 ($CH_{aliphatic}$), 1635 (C=N), 1596,1495 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ, ppm; 2.11 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3) , 5.92 (s, 1H, OH), 6.87 (t, J 8.04, 1H, H4), 7.17 (d, 2H, J 8.0, H11), 7.39 (d, 2H, J=8.22 Hz, H_3), 7.52 (d, J 8.01, 1H, H_5),7.92 (d.2H, J 8.0 H12), 9.74 (bs, 1H, NH).^{13}C NMR (75 MHz, DMSO-d_6) δ, ppm; 20.87 (1C, CH_3), 56.55 (1C, CH_3),114.35(2C, C11), 116.61(2C,C12) 119.06 (1C, C_6), 120.21 (1C,C_4), , 128.05 (1C, C_2), 128.28 (1C, C_5), 130.13 (1C, C_3), 136.08 (1C,C_{10}),152.83 (1C, $C_{9(C=N)}$), 157.66 (1C, C_1), 158.67 (1C, $C_{7(C=N)}$),160.52 (1C, C_{13}) 164.34 (1C, $C_{8(C=N)}$); EIMs, m/z=353 [$M^{\bullet+}$] (Calc. for $C_{17}H_{15}N_5O_2S$, 353.09).

DPPH Assay

The antioxidant capacity of the compounds was studied through their scavenging activity against DPPH radicals as reported by Gerhauser *et al.*[35] The percentage of DPPH and the IC_{50} (half maximal scavenging concentration) was calculated regarding the DPPH absorbance (0%) and the absorbance in the presence of ascorbic acid (100%) method.

FRAP Assay

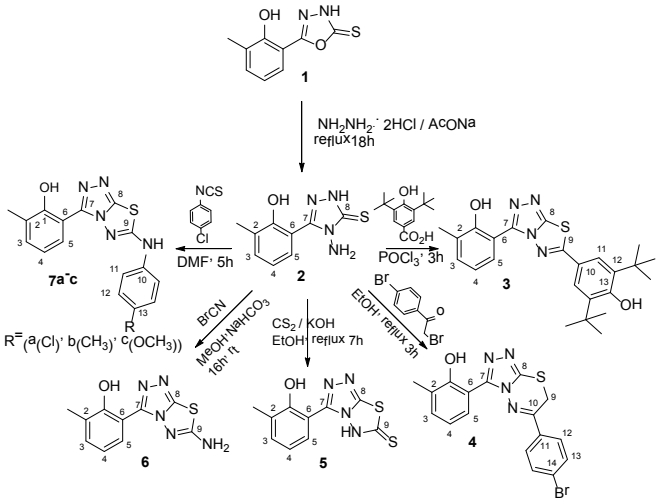
The FRAP assay was done according to Benzie et al., 1996.[36] This redox reaction occurs at a low pH. The ferric tri-pyridyltriazine (TPTZ- Fe3+) complex is reduced to the ferrous form (TPTZ- Fe2+) by electron-donating antioxidants;

the reduced complex is detected spectrophotometrically at 593 nm. Therefore, the reducing power of the sample may be referred as the total antioxidant power.

RESULTS AND DISCUSSION

Newly 6-(4-amino-1,2,4-triazole-3-yl-5-thione)-2-methylphenol (**2**) was synthesized from reaction 6-(5-thio-1,3,4-oxadiazol-2-yl)-2-methylphenol (**1**) with hydrazine hydrochloride in the presence of sodium acetate. Compound **2** was utilized as starting to synthesized seven newly fused heterocyclic. Reaction compound **2** with 3,5-di-tert-butyl-4-hydroxybenzoic acid in the presence of $POCl_3$ afforded compound **3**. Reaction compound **2** with bromophencyl bromide gave compound **4**, moreover reaction compound **2** with CS_2 in the presence of KOH afforded compound **5**. Compound **6** was synthesized from reaction compound **2** with cyanogen bromide. Three fused compounds, triazole-thiadiazole were synthesized by reacting compound **2** with three different 4-substituted phenylisothiocyante as depicted in Scheme 1.

The new compounds were characterized from their FTIR, ^{1}H NMR, ^{13}C NMR, and EIMs spectra. The FTIR for compound **2** exhibited three bands at 3460, 3315, and 3182 cm-1 attributed to OH, NH2, and NH. Besides the band of C=N of the 1,2,4-triazole ring assigned at 1617 cm^{-1}. The ^{1}H NMR spectrum showed the three aromatic protons of phenol ring as a triplet at 6.89 ppm for H_4 with J =7.6 Hz, doublet at 7.31 ppm for H_3 with J =7.2 Hz, and a doublet at 7.54 ppm for H_5 with J =7.2 Hz. The NMR spectrum exhibited the NH_2 as broadband at 5.96 ppm while the OH and NH appeared at 9.97 and 13.99 ppm, respectively. The ^{13}C NMR spectrum displayed nine peaks attributed to all carbons in compound **2**. The peak at 16.28 ppm assigned as a 2-methyl group and three phenolic carbon attached hydrogen atoms at (119.21, 127.84, and 133.15) ppm for C_4, C_5, and C_3, respectively. The two quaternary carbons are attached with 1,2,4-triazole ring and attached with methyl group located at (112.60 and 126.20) ppm, respectively. The last three carbons at low field at 149.17, 153.67, and 165.67 ppm assigned to C_1 (attached with OH), C=N, and C=S. The mass spectrum showed the molecular ion and their fragments, including the base peak, harmonized with the proposed structure.



Scheme 1: Synthetic route of compounds 2-7c

Besides to disappearing peaks of NH_2, NH, and C=S, the FTIR spectrum of compound **3** showed two peaks at 3633 cm^{-1} and 3602 cm^{-1} attributed to two phenolic hydroxyls; one for hindered phenol (incapable of forming hydrogen bonding) and the other one for capable formation hydrogen bonding. The strong absorption was observed at (2962–2869) cm^{-1}, which is compatible with the existence of two di-tert-butyl groups. The difference between C=N (1617 cm^{-1}) in the 1,2,4-triazole ring (compound **2**) and C=N (1606 cm^{-1}) in this fused heterocyclic compound enhances the evidence for the successful formation of this fused heterocyclic compound. The ^{1}HNMR spectrum of compound **3** exhibited the methyl group of *tert*-Butyl group as singlet peak at 1.45 ppm. The protons of the methyl group substituted at position two of phenol appeared at 2.19 ppm. The aromatic protons of 2-methyl-phenol appeared in their respective region. Moreover, the singlet peak at 7.82 ppm assigned for two protons of H_{11}. The NMR spectrum displayed two hydroxyls groups at 5.62 ppm, which were attributed to 4-OH (hindered phenol) and 9.18 ppm for 2-OH. The ^{13}C NMR spectrum exhibited six methyl groups for symmetrical two di-*tert*-butyl groups as singlet peaks at 29.89 ppm. The two quaternary carbons were located at 34.56 ppm. The aromatic carbons appeared for 2-methylphenol and 3,5-di-*tert*-butyl phenol. The interested three carbons of three C=N groups for the fused triazole-thiadiazole ring in this compound were located at 162.83, 163.94, and 164.88. The mass spectrum displayed the molecular ion as radical cation $M^{\bullet+}$ = 436, and their fragmentations corresponded to the structure's parts. The disappearance of essential peaks (NH_2, NH, C=S) from the IR spectrum of compound 4 were significant signs for success cyclization between compound **2** and 4-bromophencyl-bromide.The ^{1}H NMR of compound **4** showed characteristic peaks which indicated formation fused (1,2,4-triazolo and 1,3,4-thiadiazin) ring. Singlet peaked for two protons at 4.46 ppm attributed to CH2 at the fused ring, doublet at 7.84 for two protons assigned to H_{13}, and the multiple peaks at 8.01 ppm for three protons (one proton for H_5 and two protons for H_{12}). The existence of rest peaks such as three protons of 2-Me group at 2.27 ppm and the triplet peak at 6.94 ppm for H_4 and doublet at 7.32 ppm for H_3 confirmed the proposed structure of compound **4.** Furthermore, the NMR spectrum was compatible with the FTIR spectrum by no signal for NH_2 and NH protons. The protons of the hydroxyl group appeared at 11.46 ppm. The ^{13}C NMR spectrum showed four interested peaks at (22.45, 163.82, 164.39, and 166.01) ppm for CH_2 and three C=N at the fused ring (triazolo-thiadiazine). Furthermore, the new four peaks were located at 125.34, 129.58, 132.25, and 133.14 attributed to the 4-bromophenyl ring. The mass spectrum of compound **4** exhibited the molecular ion at $M^{\bullet+}$= 400, which is also the base peak. The peak at 402 with the intensity of 98% attributed to bromine 81 as isotope. The interested fragment

Table 1: Some physical properties of the synthesized compounds 2-7c

No.	*Yield %*	*M.p.℃*	*F.W*	*EIMs Calc.*	*EIMs Exp.*
2	83	235–237	$C_9H_{10}N_4OS$	222.06	222
3	77	240–243	$C_{24}H_{28}N_4O_2S$	436.19	436
4	64	259–260	$C_{17}H_{13}BrN_4OS$	401.28	401
5	86	230–232	$C_{10}H_8N_4OS_2$	264.32	264
6	70	170–173	$C_{10}H_{11}N_5OS$	247.05	247
7a	67	159–162	$C_{16}H_{14}ClN_5OS$	359.06	359
7b	57	134–136	$C_{17}H_{15}N_5OS$	337.1	337
7c	62	127–129	$C_{17}H_{15}N_5O_2S$	353.09	353

Table 2: DPPH inhibitions percentage and IC_{50} value of synthesized compounds 2-7c

No.	*DPPH Inhibition % ± SD a 100µg/mL*	*IC_{50} ±ESM b (100µg/ml)*
2	87.28 ± 0.01	42.36 ± 0.0141
3	89.05 ± 0.02	27.37 ± 0.022
4	73.57 ± 0.017	66.16 ± 0.035
5	78.96 ± 0.023	55.87 ± 0.023
6	76.72 ± 0.021	60.51 ± 0.0473
7a	80.82 ± 0.011	43.66 ± 0.024
7b	81.11 ± 0.031	41.25 ± 0.019
7c	80.54 ± 0.017	42.37 ± 0.022
2-Me phenol	20.15 ± 0.012	> 100
BHT	66.03 ± 0.022	79.835 ± 0.015
Ascorbic acid	90.65 ± 0.025	22.71 ± 0.020

m/z= 321 as carbocation represented the proposed structure losing Bromine radical. All the fragments have represented the part of the proposed structure of compound **4.** The FTIR spectrum of compound 5 showed the disappearance of NH_2 besides the shifting in value of C=S band. The present peak at 3309 cm^{-1}, attributed to NH enhances the formation of the thione tautomer, which is clearly known more stable than thiols tautomer.[37] The ^{1}H NMR spectrum of compound **5** exhibited no sign for the peak of NH_2 and exhibited all aromatic protons of phenol ring. The three protons of methyl group located as singlet peaks at 2.22 ppm. The phenolic hydroxyl group is located at 9.98 ppm. The peak assigned at 14.01 ppm compatible with the existence of thioamide proton. The ^{13}C NMR spectrum exhibited four peaks at low field (149.21, 153.70, 165.69, and 171.90 ppm) corresponding to C_1. Two carbons of C=N and C=S, respectively. As well, this spectrum was displayed all aromatic carbons and carbon of 2-methyl phenol. The mass spectrum showed the molecular ion as radical cation $M^{\bullet+}$= 264, and the other fragments included the base peak are harmonized with the proposed structure. The FTIR spectrum of compound **6** displayed the two bands for NH_2 at 3357 and 3271 as well the band of C=N was located at 1610 cm^{-1}. The ^{1}H NMR spectrum of this compound exhibited the two protons of NH_2 at 6.45 ppm as well it all rest peaks were exhibited at their expected region. The ^{13}C NMR confirms the proposed structure decisively. Existence three peaks at 154.21, 163.15, and 165.62 assigned for three imines group (C=N) at the structure of fused heterocyclic ring in compound **6**. The rest carbons were located in their expected region. The EIMs spectrum of this compound confirmed the molecular mass and exhibited all fragments, including the base peak. The base peak and these fragments correspond to the part of the proposed structure. The FTIR spectra of compounds **7a-c** showed disappearing of NH_2 Bands Furthermore, OH at 3425-3451 cm^{-1} and NH at 3180-3223 cm^{-1} besides to C=N band which located at 1635-1647 cm^{-1}. The ^{1}HNMR supports the IR spectrum, NH peak of aryl amine ring at 9.70–9.90 ppm. Furthermore, new aromatic protons of aryl amine attached with fused triazole-thiadiazole besides to the substituted group at aryl amine. The 4-methyl group for compound **7b** was located at 2.16 ppm, and the OCH_3 for compound **7c** was located at 3.86 ppm. The ^{13}C NMR confirms the structure of compounds **7a-c** by showing four peaks in the low field, three of them attributed to C=N of the fused heterocyclic and the forth for the C_1. Furthermore, the ^{13}C NMR spectrum showed all carbons of 2-methylphenol and for 4-chlorophenyl group. The mass spectra of these compounds confirmed the structure by exhibiting the molecular ion and the base peak and their fragments corresponding to the structure and their fragments. Some physical properties of synthesized compounds were tabulated in Table 1.

Antioxidant Ability

Compounds **2** and **3** exhibited significant DPPH inhibitions as well lowest IC_{50} this antioxidant ability is approaching antioxidant of ascorbic acid. This extraordinary antioxidant for compound **2** could be attributed to engaging the thiourea part and the NH_2 group, donating a hydrogen atom. The existence of 2,6-di-*tert*-butylphenol attached the1,3,4- thiadiazol ring at position five, which can stabilize the radical after donating proton atom by hindrance and by delocalization with C=N of the thiadiazol ring. Compounds **7a-c** as well showed good antioxidant with 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition. These compounds exhibited that the type of substituted group at position para does not enhance the antioxidant ability, as tabulated in Table 2.

The secondary amine as mentioned earlier well known as antioxidant which could attributed to enhancing the antioxidant ability. In general, all compounds in this group showed exceptional increases in antioxidant ability could be attributed to highly sterical hindrance in geometrical structure as the imaginary 3D structure depicted in Figure 1.

Although, FRAP value recorded antioxidant ability higher than DPPH when compared with the references. Compounds **2** and **3** displayed FRAP values slightly higher than ascorbic acid (Figure 2).

The FRAP value exhibited same sequence of antioxidant ability of these compounds. This observation could be attributed to two reasons. First, the sterical hindrance, which enhances ours belief that the geometrical structure is the predominated. It is well known that the DPPH stable free radical own steric hindrance which could cause antioxidant ability less than the real antioxidant ability with highly sterical hindrance substrate.[38,39] The second reason could these compounds prefers to undergo with Single electron transfer mechanism (SET) rather than a Hydrogen atom transfer mechanism (HAT). The antioxidant ability of compound **5** was higher than compound **6,** which reflected the important role of the thiourea group besides the steric hindrance. Compound **4** showed good antioxidants, and that could be due to participated the benzyl radical.[40]

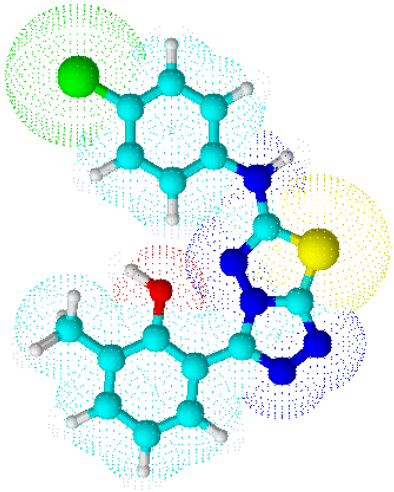


Figure 1: Stereogram of compound **7a**

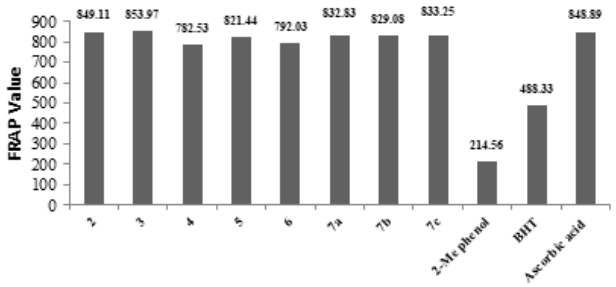


Figure 2

Table 3: The docking binding free energies of the synthesized compounds with tubulin pocket-forming hydrogen bonding and π interactions

Compound No.	*Binding free energies (DG) Kcal/mol.*	*No. of bonds*		*Bond. Length/ Å*
		H.b	*pi*	
2	-4.45	1	0	2.38 Å
3	-5.50	1	0	2.62 Å
4	-5.99	1	1	2.46 Å
5	-5.54	1	1	2.62 Å
6	-4.99	1	1	2.03 Å
7a	-4.94	0	2	-
7b	-6.89	1	3	2.22 Å
7c	-6.69	1	1	2.24 Å
Colchicine	-5.40	1	0	1.98 Å

Molecular Modeling

The synthesized compounds were tested for their A tubulin binding affinity with the aid of docking studies via MOE 2015. The 3D crystal structure of Tubulin was downloaded from the protein databank using PDB (ID 1SA0) at a resolution of *3.58 Å*. Firstly, water molecules were removed from the downloaded complex. The valence monitor option was used to correct crystallographic disorders and incorrect valence atoms. Then, the energy of the complex was minimized by applying CHARMM and MMFF94 force fields. The binding site of the Tubulin sequence (1SA0) was defined and prepared for docking. Our compounds and reference ligand Colchicine Structures were drawn using ChemBioDraw Ultra 14.0 and saved in MDL-SD file format. Next, the SD file was opened, 3D structures were protonated, and energy minimized by applying CHARMM and MMFF94 force fields, then prepared for docking. Docking studies were performed using CDOCKER protocol that employs CHARMM-based molecular dynamics (MD) scheme to dock ligands into a receptor binding site. In the docking analysis, a maximum of 7 conformers was considered for each molecule.

Finally, the ideal pose was selected according to the minimum free energy of the Tubulin ligand interactions. In this work, docking of the synthesized compounds and reference ligands with Tubulin crystal structure (ID: 1SA0) was carried out to predict the proposed binding mode, affinity, and preferred orientation of each docking pose. The results of the docking study were reported as Tubulin binding free energy (ΔG). The spontaneity of bindings was confirmed by the negative values of free energies (Table 3).

The works of literature have reported the key binding site of Tubulin, consisting of amino acid Cys241, Ala316, Val315, Ala317 and residues of Leu255

As reported on binding to Tubulin, Colchicine interacted with critical amino acid residues of the active site Cys241and Leu255. The proposed binding mode of compound **4** showed an affinity value of -4.45 kcal/mol. It demonstrated essential interactions with the Tubulin amino acids. The NH group at triazole moiety was involved in a hydrogen bonding interaction with ThrB353 with a distance equal 2.38Å. (Figure.3).

The proposed binding mode of compound **3** showed an affinity value of -5.50 kcal/mol. It showed one hydrogen bond interaction between the Nitrogen atom at triazole moiety and residues of Lys B254 amino acid with a distance equal 2.62 *Å*. (Figure.4).

The proposed binding mode of compound **4** showed an affinity value of -5.99 kcal/mol. It showed one hydrogen bond interaction between Nitrogen atom at triazole moiety and residues of Ala B250 amino acid with a distance equal 2.46 *Å*.

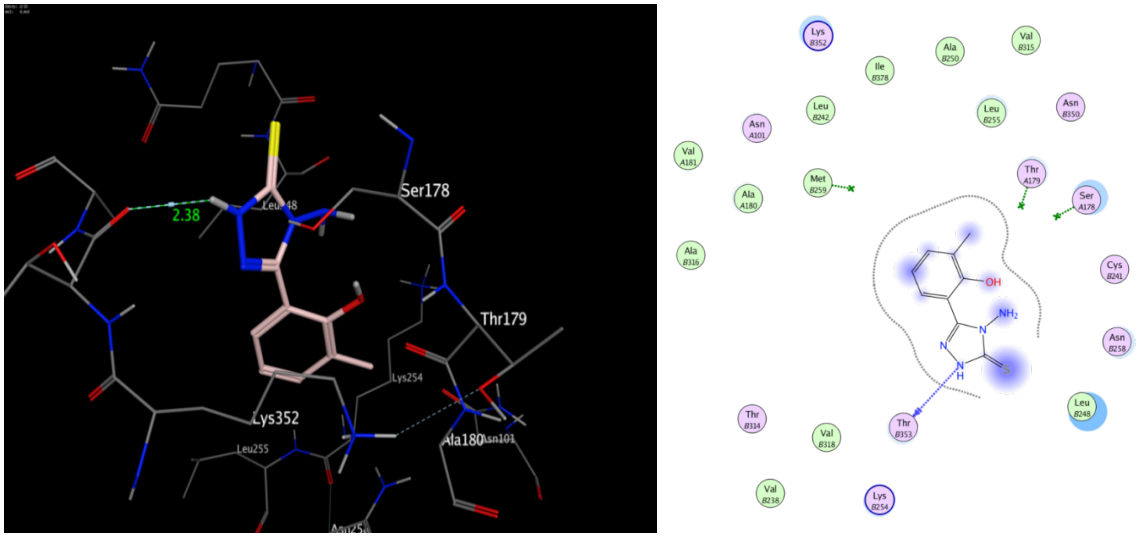


Figure 3: showing binding mode of compound **2** as potent tubulin inhibitor

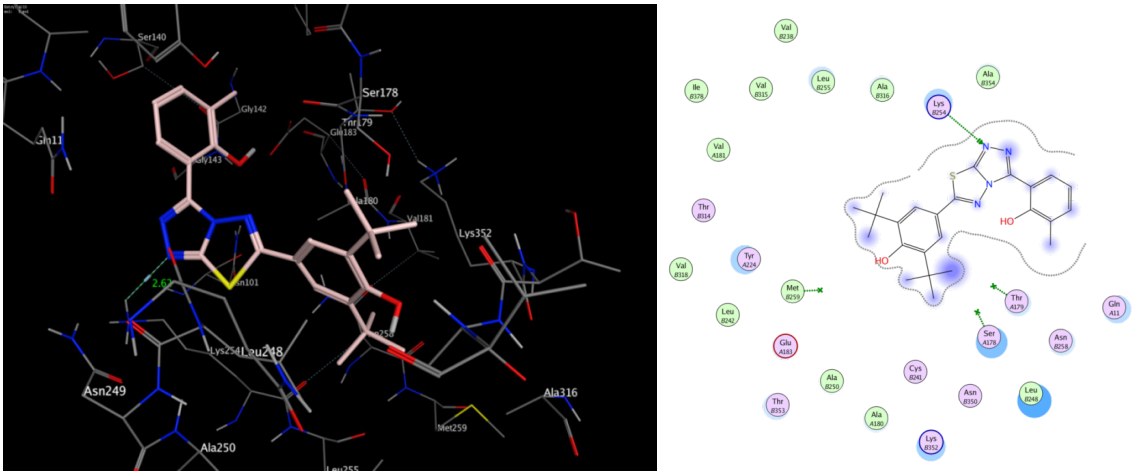


Figure 4: showing binding mode of compound **3** as potent tubulin inhibitor

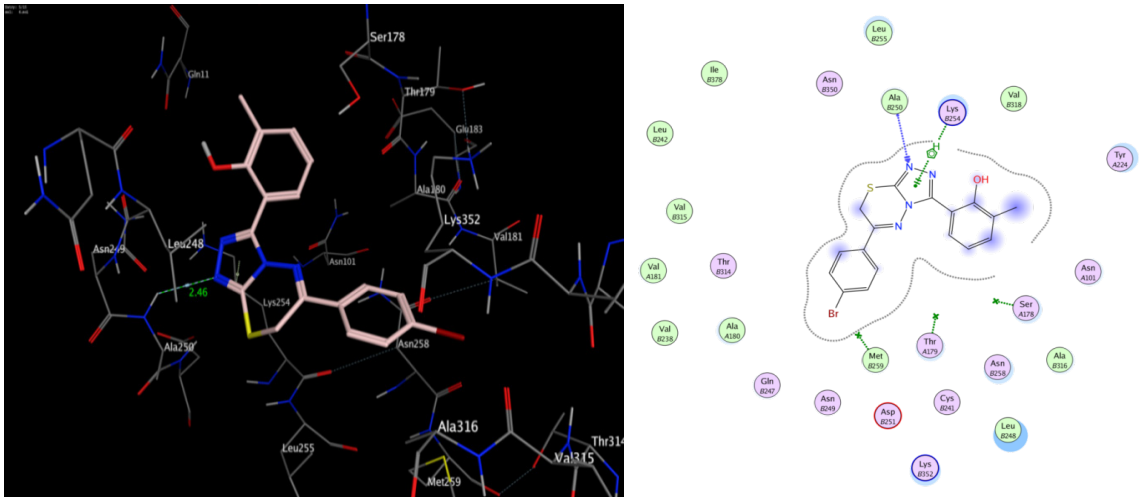


Figure 5: showing binding mode of compound **4** as potent tubulin inhibitor

In addition, the aromatics moieties demonstrated aromatic Stacking interactions with Lys B254. (Figure.5).

The proposed binding mode of compound **5** showed an affinity value of -5.54 kcal/mol. It showed one hydrogen bond between the Sulphur group at thiadiazole ring, and residues of Ala B317 with a distance equal 2.62 *Å* and then, the aromatics moieties demonstrated aromatic Stacking interactions with Ala B316. (Figure.6).

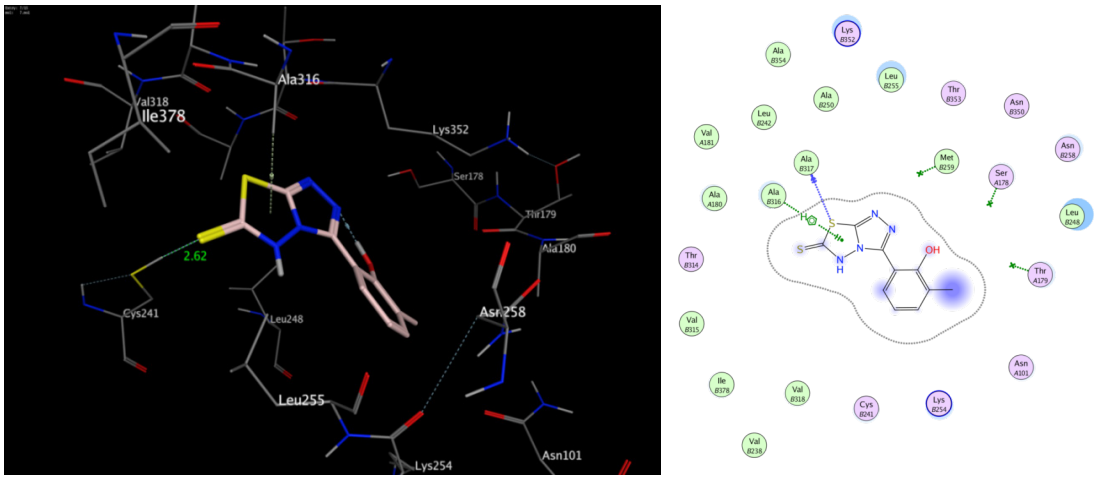


Figure 6: showing binding mode of compound 5 as potent tubulin inhibitor

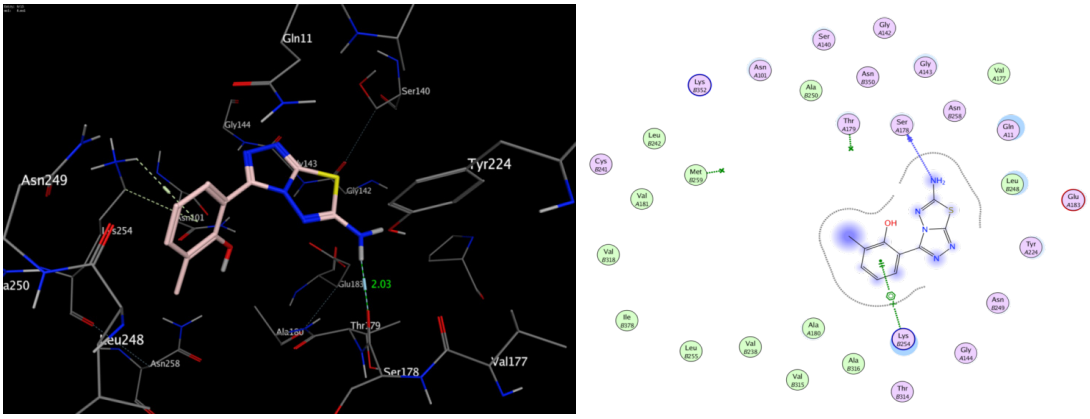


Figure 7: showing binding mode of compound **6** as potent tubulin inhibitor

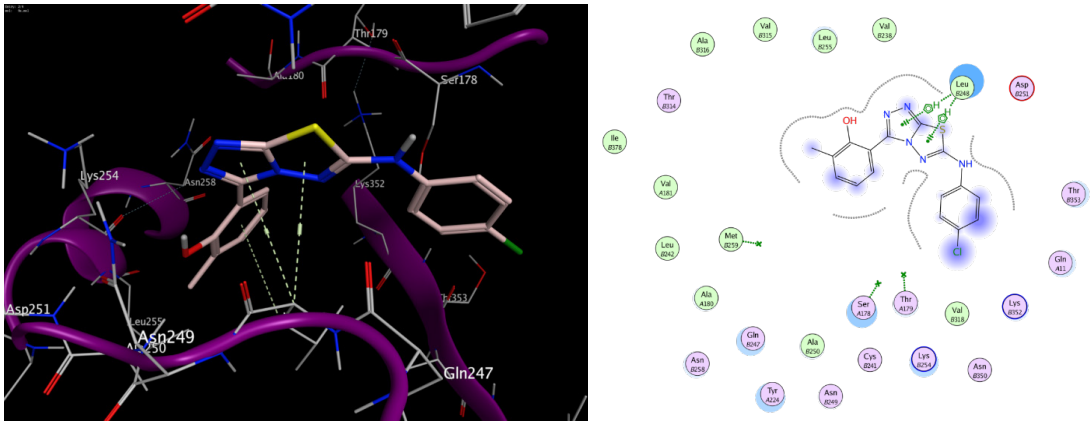


Figure 8: showing binding mode of compound **7a** as potent tubulin inhibitor

The proposed binding mode of compound **6** showed an affinity value of -4.99 kcal/mol. It showed one hydrogen bond interaction between NH_2 moiety and residues of SerA178 amino acid with a distance equal 2.03 *Å*. In addition, the aromatics moieties demonstrated aromatic Stacking interactions with Lys B254. (Figure.7).

The proposed binding mode of compound **7a** showed an affinity value of -5.94kcal/mol. It demonstrated important interactions with the Tubulin amino acids. The aromatics moieties demonstrated aromatic Stacking interactions with LeuB248 (Figure 8).

The proposed binding mode of compound **7b** showed an affinity value of -6.89kcal/mol. It showed one hydrogen bond interaction between Nitrogen atom at triazole moiety and residues of AlaB250 amino acid with a distance equal 2.22 Å. In addition, the aromatics moieties demonstrated aromatic Stacking interactions with LysB254 and LeuB248 (Figure 9)

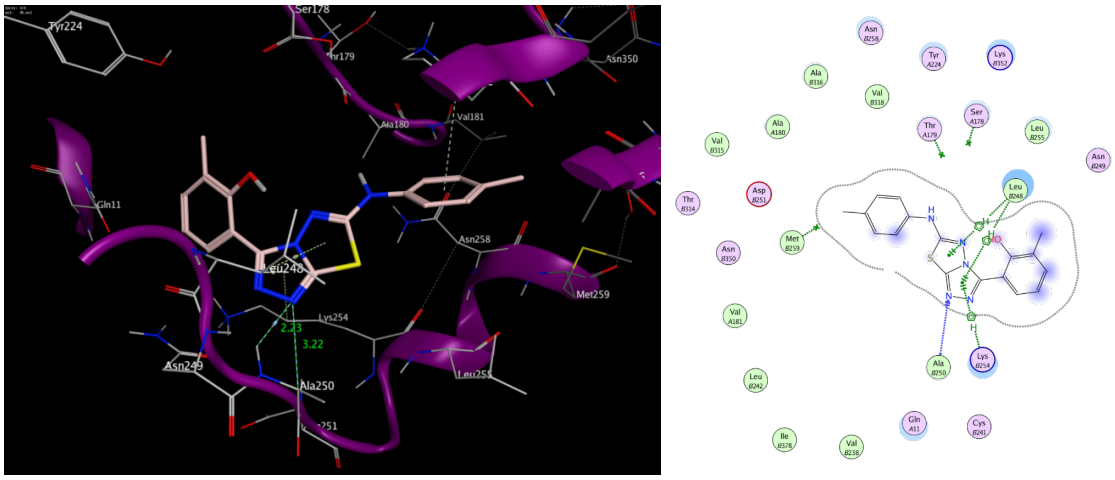


Figure 9: showing binding mode of compound **7b** as potent tubulin inhibitor

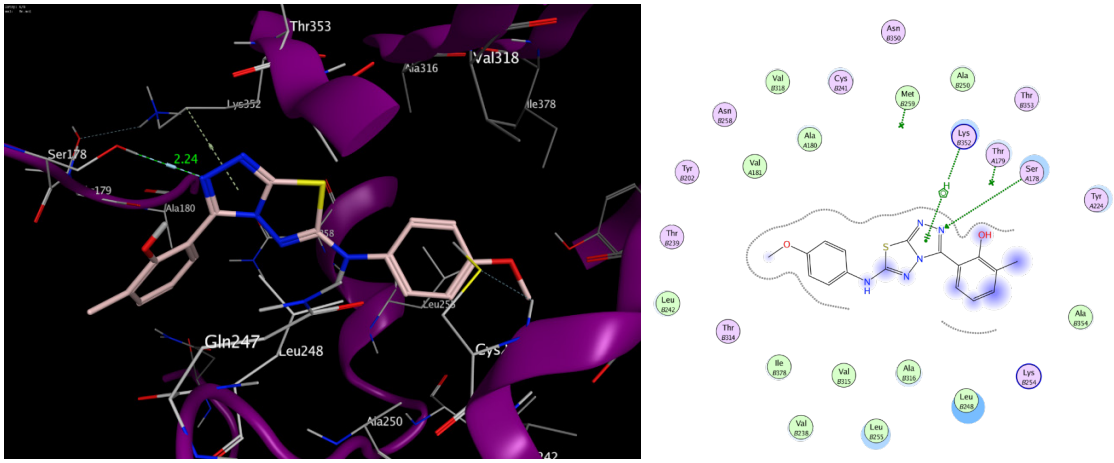


Figure 10: showing binding mode of compound **7c** as potent tubulin inhibitor

The proposed binding mode of compound **7c** showed an affinity value of -6.69 kcal/mol. It showed one bi-bi interaction, and the aromatics moieties demonstrated aromatic Stacking interactions with LysB352 and AsnA101. It showed one hydrogen bond interaction between Nitrogen atom at triazole moiety, and residues of SerA178 amino acid with a distance equal 2.24 Å. (Figure 10).

Compounds **7b** and **7c** exhibited significant potent tubulin inhibitor, while **7a** exhibited poor potent tubulin inhibitor. This result could be attributed to donating group (CH_3 and OCH_3) at position para for the phenyl in the molecular structure of compounds **7b** and **7c,** while compound **7a** consist of the drawing group (Cl). There is now a clear relationship between the antioxidant ability of these compounds and their potent tubulin inhibitor.

CONCLUSION

The 4-amine-1,2,4-triazole-5- thione **2** and their fused heterocyclic **3-7a-c** successfully synthesized. These compounds exhibit an extraordinary free radical scavenging in DPPH and FRAP assays, and that could be attributed to providing highly steric hindrance around the hydroxyl of 2-methylphenol. Furthermore, the significant antioxidant for compound **3,** which is slightly less than ascorbic acid, attributed to existents di-*tert*-butyl phenol besides the highly steric hindrance. The molecular docking simulation study for these compounds against tubulin as target exhibited that the tested compounds fit position with critical amino acid. Compounds **4**, **7a**, **7b,** and **7c** displayed good interaction compared to colchicine reference as potent tubulin inhibitor.

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