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Design and Synthesis of New Thienopyrimidine Derivatives Along With Their Antioxidant Activity.

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Abstract

Antioxidants are one of major defenses against toxicity caused by free radicals which makes them effective in the prevention and treatment of diseases, like atherosclerosis, stroke, diabetes, Alzheimer's disease, and cancer. The present study discusses the design and synthesis of a new series of cycloheptathiophene/ thienopyrimidine derivatives along with their antioxidant biological evaluation. Virtual screening was performed to build a pharmacophore model based on reported natural active antioxidant compound. All resulted outputs were carefully analyzed for the best matched hits. Moreover, molecular docking was done at the active site of tyrosinase as a predictive tool for exploring the biological activity of the targeted compounds. Free radical scavenging activity was measured using DPPH method in reference to Ascorbic acid. Six out of fifteen synthesized compounds showed more significant antioxidant activity than that recorded by the reference standard.

Keywords: Antioxidant; Cycloheptathiophene; DPPH; Molecular docking; Thienopyrimidine; Virtual screening.

1. Introduction

Oxidative processes are fundamental metabolic processes for all living organisms [1]. However, reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, hydroxyl and nitric oxide radicals produced during chain reactions in the oxidation process play an important role in oxidative stress related to the pathogenesis of various important diseases [1]. Antioxidants act as a major defense against radical mediated toxicity by protecting the damages caused by free radicals. Antioxidant agents are effective in the prevention and treatment of complex diseases, like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer [1, 2]. Moreover, skin is quite suspectable to oxidative stress that is enhanced by direct sunlight/ ultraviolet (UV) exposure that can cause many manifestations from which is pre-matured aging [3]. Melanogenesis is a process for skin protection from damages caused by UV exposure. However, melanin accumulation or excessive production might cause critical skin disorders as hyperpigmentation. In such cases, tyrosinase inhibitors are crucial to adjust the level of secreted melanin and accordingly protect skin from pre-matured aging [4].

Ring systems containing the thiopyrimidine moiety are of interest because of their diverse pharmacological and biological activities.[5-7]. They are bio isosteres to purine having thiophene ring intertwined with pyrimidine. Many active antioxidant compounds were bearing thiophene or thienopyrimidine ring structure[8,9](figure 1). Based on this, we considered synthesis and biological evaluation of compounds 6 a, 6 b, 8, 10a-c, 11, 15 having piperazine or thiourea substituents.

Surveying the literature disclosed many active antioxidant compounds were bearing arylideneaminoquinazolin-4(3H)-ones [10]. On this basis, modifications to synthesize thienopyrimidine analogues were performed by replacing the phenyl ring in the quinazolinone core by its isostere thiophene ring, in addition to the hybridization with a lipophilic moiety represented by cycloheptyl ring. The arylidine moieties were chosen to be of varying lipophilicity, electron (donating/withdrawing) properties and H-bonding ability. Further

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modification was designed by substituting the 2-methyl group with a phenylamino moiety in compounds 13a-c to explore the effect of the aromatic substitution on the antioxidant activity.

Moreover, the literature [2] revealed that the CH=CH–C=O chain allows for electronic delocalization of a free-radical position via extensive mesomeric effects and free-radical stabilization through the so-called capto-dative effect , thus, the incorporation of the benzal-acetophenone moiety declared to promote the antioxidant performance [2]. Accordingly, compounds 7 and 14 were designed and synthesized by incorporating the biologically active pharmacophoric moiety benzalacetophenone into the (3-amino-2-methyl and 3-amino-2-phenylamino) thienopyrimidine precursors 3 and 12 respectively in order to evaluate their antioxidant behavior.

Based on the above-mentioned facts, we found thienopyrimidine a promising core for synthesis of new antioxidant compounds. Virtual screening was performed to build a pharmacophore model using a reported naturally occurring antioxidant agent"ethyl-2-(2-(benzylthio)-4(4-(dihydroxyamino)phenyl)-2H-imidazol-1(5H)-yl)acetate" [11] whose activity was related to the presence of the sulphur atom linked to the imidazole ring found as a core nucleus in its structure[11]. Furthermore, Molecular docking was performed at the active site of tyrosinase as a predictive tool for exploring the biological activity of the targeted compounds.

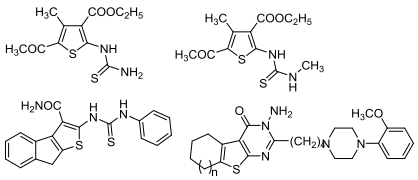


Figure 1: examples for thienopyrimidines with antioxidant activity

2. Experimental:

2.1….. molecular docking

Docking poses were analyzed and compared to the standard antioxidant ascorbic acid, Docking was performed on crystal structure of tyrosinase (PDB entry:1ZDW). 3D protonation was added and Water molecules were ignored from the proteins. The ligands were protonated, prepared and docked after energy minimization. Settings were applied by default as London dG and GBVI/WSA dG functions.

2.2. Chemistry:

2.2.1. General procedure for the synthesis of compounds: 4 a-e

A reaction mixture of the 3-amino thienopyrimidine derivative **3** (2.49g, 0.01 mol) and the appropriate aldehyde (0.01 mol) in ethanol (10 mL) and in presence of glacial acetic acid (1mL) was heated under reflux for 6 h. The reaction mixture was then left to cool to room temperature, poured onto ice/ water, the formed solid was filtered, dried and crystallized from absolute ethanol.

2.2.1.1. 2-Methyl-3-(p-hydroxybenzylidenamino)-5,6,7,8,9-pentahydro cyclohepta [4,5] thieno [2,3-*d*] pyrimidin-4(3*H*)-one. 4 a

Yield (86%), m.p. 298-301 ^{o}C ; IR (KBr disc) (cm^{-1}): 3430 (OH), 3192 (CH-aromatic), 1673 (C=O), 1516 (C=N). ^{1}H-NMR (DMSO, δ ppm):1.58-3.22 (m, C<u>H</u>$_3$, 10H cycloheptane), 7.14-7.57 (m, 4<u>H</u> aromatic), 8.90 (s, C<u>H</u> azomethene), 11.00 (O<u>H</u>, D_2O exchangeable), MS: m/z (%): M$^{\cdot+}$; 353 (34.56); Anal. Calcd. for $C_{19}H_{19}N_3O_2S$ (353.44): C, 64.57; H, 5.42; N, 11.89; Found: C, 64.75; H, 5.60; N, 12.01.

2.2.1.2. 2-Methyl-3-(p-chlorobenzylidenamino)-5,6,7,8,9-pentahydrocyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4(3*H*)-one. 4 b

Yield (84%), m.p. 170-172 ^{o}C; IR (KBr disc) (cm^{-1}): 3100 (CH-aromatic), 1669 (C=O), 1506 (C=N). MS: m/z (%):M$^{\cdot+}$: 371 (17.76) M+2: 373 (8.41); Anal.Calcd. for $C_{19}H_{18}ClN_3OS$ (371.884): C, 61.36; H, 4.88; N, 11.30; Found: C, 61.41; H, 4.94; N, 11.52.

2.2.1.3. 2-Methyl-3-(3,4,5-trimethoxybenzylideneamino)-5,6,7,8,9-pentahydrocyclohepta [4,5]thieno[2,3-*d*] pyrimidin-4(3*H*)-one. 4 c

Yield (85%), m.p. 173-176 ^{o}C; IR (KBr disc) (cm^{-1}): 3069 (CH-aromatic), 2859 (CH$_3$), 1667 (C=O), 1520 (C=C), 1509 (C=N); MS: m/z (%): M$^{\cdot+}$: 427 (23.01); Anal. Calcd. for $C_{22}H_{25}N_3O_4S$ (427.517): C, 61.81; H, 5.89; N, 9.83; Found: C, 61.98; H, 5.64; N, 9.71.

2.2.1.4. 2-Methyl-3-(2,5-dimethoxybenzylidenamino)-5,6,7,8,9-pentahydrocyclohepta[4,5]thieno [2,3-*d*]pyrimidin-4(3*H*)-one. 4 d

Yield (85 %), m.p. **173-176** ^{o}C; IR (KBr disc) (cm^{-1}): 3091 (CH-aromatic), 2865 (CH aliphatic), 1671 (C=O); ^{1}H-NMR (DMSO, δ ppm): 1.58-3.22 (m, C<u>H</u>$_3$ and 10<u>H</u> cycloheptane), 3.85 (m, 6H, OC<u>H</u>$_3$), 7.14-7.57 (m, 3<u>H</u> aromatic), 9.01 (s, C<u>H</u> azomethene); MS: m/z (%): M$^{\cdot+}$: 397 (29.56); Anal. Calcd. for $C_{21}H_{23}N_3O_3S$ (397.491): C, 62.64; H, 5.52; N, 10.96; Found: C, 62.51; H, 5.33; N, 10.79.

2.2.1.5. 2-Methyl-3-((3-methoxy-4-hydroxy)benzylidenamino)-5,6,7,8,9pentahydroyclohepta[4,5] thieno[2,3-*d*]pyrimidin-4(3*H*)-one. 4 e

Yield (82 %), m.p. 236-238 ^{o}C; IR (KBr disc) (cm^{-1}): 3372 (OH), 3103 (CH-aromatic), 2882 (CH aliphatic), 1668 (C=O).); ^{1}H-NMR (DMSO, δ ppm): 1.49-3.98 (m, C<u>H</u>$_3$, 10<u>H</u> cycloheptane and OC<u>H</u>$_3$), 7.22-7.63 (m, C<u>H</u> aromatic), 6.5(s, N=C<u>H</u>), 8.11(s, O<u>H</u>). Anal. Calcd. for $C_{20}H_{21}N_3O_3S$ (383.464): C, 62.64; H, 5.52; N, 10.96; Found: C, 62.51; H, 5.33; N, 10.79.

2.2.2. General procedure for the synthesis of compounds: 6 a, b.

The choroacetamido derivative **5** (2.4g, 10 mmol) and appropriate secondary amine (10 mmol) were refluxed in dry benzene (15 mL) in presence of 3-7 drops TEA for 6 h. The solvent was removed under reduced pressure, the obtained solid was filtered, dried and crystallized from ethanol.

2.2.2.1. 2-Morpholino-N-(2-methyl-4-oxo-5,6,7,8,9-pentahydrocyclohepta[4,5]thieno[2,3-*d*] pyrimidine-3(4*H*)-yl)acetamide: 6 a

Yield (72 %), m.p. 169-172 °C; IR (KBr disc) (cm^{-1}): 3322 (NH), 2899 (CH_2), 2852 (CH_3), 1667 (C=O). Mass spectrum: m/z (%): $M^{\cdot+}$; 376 (1.01); Anal. Calcd. (%) for $C_{18}H_{24}N_4O_3S$ (376.473): C, 57.43; H, 6.43; N, 14.88; Found (%): C, 57.57; H, 6.51; N, 14.92.

2.2.2.2. 2-(2,6-Dimethoxyphenylamino)-N-(2-methyl-4-oxo-5,6,7,8,9-pentahydro cyclohepta[4,5]thieno [2,3-*d*] pyrimidine-3(4*H*)-yl) acetamide:6 b

Yield (67%), m.p. 150-152 °C; IR (KBr disc) (cm^{-1}): 3389 (NH), 1664 (C=O); ^{1}H-NMR (DMSO, δ ppm):1.26-2.98 (CH_3, 10H cycloheptane), 4.35 (s, CH_2), 5.36 (s,6H, 2 CH_3), 7.41-7.79 (m, 3H aromatic), 11.10 (NH, D_2O exchangeable) ; Anal. Calcd.(%) for $C_{22}H_{26}N_4O_4S$ (442.531): C, 59.71; H, 5.89; N, 12.66; Found (%): C, 59.88; H, 5.96; N,12.91.

2.2.3. 3-(3-Oxo-1,3-diphenylpropylamino)-2-methyl-5,6,7,8,9-pentahydrocyclohepta[4,5]thieno [2,3-*d*] pyrmidin-4(3*H*)-one: 7

An equimolar amount of the 3-aminothienopyrmidine **3** (2.49g, 10 mmol) and benzalacetophenone (2.10g, 10 mmol) was refluxed in DMF (15 mL) for 8 h. The reaction mixture was left to cool then poured onto ice/cold water, filtered, dried and crystallized from absolute ethanol. Yield 67%, m.p. 147-149 °C. IR (KBr disc) (cm^{-1}): 3350 (NH), 3058 (CH aromatic), 1660 (C=O); MS m/z(%): $M^{+\cdot}$457(1.10), 57(100); Anal. Calcd. (%) for $C_{27}H_{27}N_3O_2S$ (457.587): C, 70.87; H, 5.95; N, 9.18; Found (%): C, 70.84; H, 6.01; N, 9.21.

2.2.4. 1-(2-Methyl-4-oxo-5,6,7,8,9-pentahydrocyclohepta[4,5]thieno[2,3-*d*]pyrimidin-3(4*H*)-yl)-3-phenylthiourea: 8

Phenyl isothiocyanate (5.6mL, 40 mmol) was added to the 3-aminothienopyrimidine **3** (2.49g, 10 mmol) in dichloromethane (30 mL), the mixture was refluxed for 12 h. Excess solvent was removed under vacuum, the remained solid was collected, dried and crystallized from absolute ethanol. Yield 73%, m.p :138-140°C; IR (KBr disc) (cm^{-1}): 3340 (NH broad), 3090 (CH aromatic), 1656 (C=O), 2922 (CH-aliphatic), 2200 (-N=C=S); ^{1}H-NMR (DMSO, δppm):1.22 (s, C<u>H</u>$_3$), 1.26-3.31 (m, 10<u>H</u> cycloheptane), 7.12-7.45 (m, C<u>H</u> aromatic), 10.83 (s, N<u>H</u> disappeared on deutration), 11.50 (s, N<u>H</u> replaceable in D_2O; ^{13}C-NMR (DMSO, δppm): 14.20(CH_3), 26.52-31.76 (5C-cycloheptane), 120.8-155.36 (4C-thiophene), 124.23-139.40 (6C-aromatic), 161.13 (C=O), 165.37 (C-2 pyrimidine), 175.64 (C=S); MS: m/z (%): $M^{+\cdot}$384 (62.76), 77(100%); Anal. Calcd. (%) for $C_{19}H_{20}N_4OS_2$ (384.52): C, 59.35; H, 5.24; N, 14.57; Found: C, 59.48; H, 5.46; N, 14.88.

2.2.5. General procedure for synthesis of compounds 10 a-c.

A mixture of 2-thioxothienopyrimidine derivative **9** (6.4 g, 20 mmol) and the appropriate secondary amines namely morpholine or piperazine derivative (40 mmol) was refluxed in DMF (15mL) for 15h. The reaction mixture was then left to cool, the separated solid was filtered and crystalized from ethanol / DMF.

2.2.5.1. 3-Phenyl-2-morpholino-5,6,7,8,9-pentahydrocyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-one: 10 a.

Yield (62%), m.p. 230-233°C; IR (KBr disc)(cm^{-1}): 3104 (CH-aromatic), 1660(C=O), 1514 (C=N); MS: m/z (%): $M^{\cdot+}$:381 (14.56) Anal. Calcd. (%) for $C_{21}H_{23}N_3O_2S$ (381.491): C, 66.12; H, 6.08; N, 11.01; Found: C, 66.40; H, 6.25; N, 11.25.

2.2.5.2. 3-Phenyl-2-(4-methylpiperazin-1-yl)-5,6,7,8,9-pentahydrocyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-one: 10 b.

Yield (55%), m.p. 120-122 °C; IR (KBr disc) (cm^{-1}): 3115 (CH-aromatic), 2859 (CH_3), 1664 (C=O). MS: m/z (%): $M^{\cdot+}$; 394 (48.58); Anal. Calcd. (%) for $C_{22}H_{26}N_4OS$ (394.533): C, 66.97; H, 6.64; N, 14.20; Found: C, 67.21; H, 6.81; N, 14.38.

2.2.5.3. 3-Phenyl-2-(4-phenylpiperazin-1-yl)-5,6,7,8,9-pentahydrocyclohepta[4,5]thieno[2,3-*d*]pyrimidin-4-one: 10 c.

Yield (52%), m.p. 180-183°C; IR (KBr disc) (cm^{-1}): 3101 (CH-aromatic), 1667 (C=O), 1517 (C=N); ^{1}H-NMR (DMSO, δ ppm): 1.29-3.51 (m, 10H cycloheptane), 3.51-4.01 (m, piperazine-8H), 7.11-7.56 (m, 5H aromatic); Anal.Calcd.(%) for $C_{27}H_{28}N_4OS$ (456.602): C, 71.02; H, 6.18; N, 12.27; Found (%): C, 71.27; H, 6.36; N, 12.40.

2.2.6. Ethyl-2-(3-phenylthioureido)-4,5,6,7,8-pentahydrocyclohepta[*b*]thiophene-3-carboxylate: 11

The 2-aminothiophene derivative **1** (2.4g, 10 mmol) was dissolved in hot ethanol (10 mL), then phenyl isothiocyanate (~2mL, 15 mmol) was added drop wise while stirring. The reaction mixture was heated under reflux on a water bath for 2 h, then left over night at room temperature. The separated solid was filtered, washed with ethanol and crystalized from ethanol. Yield 96%; m.p:150-153 °C. IR (KBr disc) (cm^{-1}):3330 (NH), 2223 (-S=C=N-), 1702(C=O).^{1}H-NMR (DMSO, δppm): 1.22 (t, C<u>H</u>$_3$), 1.34-2.97 (m, 10<u>H</u> cycloheptane), 4.22 (q, C<u>H</u>$_2$-CH$_3$), 7.12-7.49 (m, aromatic 5<u>H</u>) ,10.83 (s, N<u>H</u>, D_2O exchangeable), 11.50 (s, N<u>H</u>, disappeared on deuteration); ^{13}C-NMR (DMSO, δppm): 14.29(CH_3), 26.53-31.50 (5C-cycloheptane), 60.45(CH_2), 113.87-155.21 (4C-thiophene), 123.53-136.80 (6C-aromatic), 161.13

(C=O), 175.64(C=S). MS: m/z (%): $M^{+\cdot}$ 374(62.76), 193(100); Anal. Calcd. (%): for $C_{19}H_{22}N_2O_2S_2$ (374.52): C, 60.93; H, 5.92; N, 7.48. Found %: C, 60.87; H, 5.89; N, 7.24.

2.2.7. General method for synthesis of compounds: 13 a-d

Equimolar amounts of the substituted 3-aminothienopyrimidine derivative (3.2g, 10 mmol) **12** and the appropriate aldehyde (10 mmol) were refluxed in ethanol (10 mL) in presence of glacialacetic acid (1mL) for 4-6 h, then left to cool. The reaction mixture was poured onto ice/H_2O, the precipitated solid was filtered, washed with water, dried and crystalized from absolute ethanol.

2.2.7.1. 3-[N\-(3,4,5Trimethoxybenzylidenamino)]-2-phenylamino–5,6,7,8,9-pentahydrocyclohepta [4,5]thieno[2,3-*d*]pyrimidine-4(3*H*)-one: 13 a.

Yield (79%), m.p. 240-243 ºC; IR (KBr disc) (cm^{-1}): 3380 (NH), 3093 (CH-aromatic), 2880 (CH-aliphatic), 1665 (C=O), 1511 (C=N). MS: m/z (%): $M^{\cdot+}$: 504(100); Anal. Calcd. (%) for $C_{27}H_{28}N_4O_4S$ (504.601): C, 64.27; H, 5.59; N, 11.10; Found: C, 64.25; H, 5.55; N, 11.13.

2.2.7.2. 3-[N\-(2,5-Dimethoxybenzylidenamino)]-2-phenylamino–5,6,7,8,9-pentahydrocyclohepta [4,5]thieno[2,3-*d*]pyrimidine-4(3*H*)-one: 13 b.

Yield (84%), m.p. 190-192 ºC; IR (KBr disc) (cm^{-1}): 3396 (NH), 3104 (CH-aromatic), 2852 (CH aliphatic), 1666 (C=O), 1509(C=N). MS: m/z (%): $M^{\cdot+}$: 474 (100); Anal. Calcd. (%) for $C_{26}H_{26}N_4O_3S$ (474.575): C, 66.80; H, 5.52; N, 11.81; Found: C, 66.82; H, 5.50; N, 11.79.

2.2.7.3. 3-[N\-(p-Hydroxybenzylidenamino)]-2-phenylamino-5,6,7,8,9-pentahydrocyclohepta[4,5]thieno[2,3-*d*]pyrimidine-4(3*H*)-one: 13 c.

Yield (81%), m.p.210-212 ºC; IR (KBr disc) (cm^{-1}): 3410-3360 (OH), 3384 (NH masked), 31 (CH-aromatic), 2859 (CH_3), 1671 (C=O), 1517 (C=N); ^{1}H-NMR (DMSO, δppm): 1.23-2.99 (m,10H cycloheptane), 7.14-7.57 (m, 4H aromatic), 9.11 (s, CH azomethene), 13.59 (s, NH, D_2O exchangeable), 14.00 (s, OH , D_2O exchangeable); Anal. Calcd. (%) for $C_{24}H_{22}N_4O_2S$ (430.522): C, 66.96; H, 51.15; N, 13.01; Found (%): C, 66.68; H, 5.11; N, 13.19.

2.2.7.4. 3-[N\-(p-Chlorobenzylidenamino)]-2-phenylamino-5,6,7,8,9-pentahydrocyclohepta[4,5] thieno [2,3-*d*]pyrimdine-4(3*H*)-one: 13 d.

Yield (74%), m.p.200-202 ºC; IR (KBr disc) (cm^{-1}): 3410 (NH), 3098 (CH-aromatic), 1669 (C=C), 1506 (C=N); ^{1}H-NMR (DMSO, δppm): 1.26-3.23 (m,10H, cycloheptane), 7.11-7.90 (m, 4H aromatic), 9.01(s, CH azomethene), 12.94(s, NH, D_2O exchangeable); Anal. Calcd. (%) for $C_{24}H_{21}ClN_4OS$ (448.968): C, 64.20; H, 4.71; N, 12.48; Found (%): C, 64.42; H, 4.76; N, 12.52.

2.2.8. 3-(3-Oxo-1,3-diphenylpropylamino)-2-phenylamino-5,6,7,8,9pentahydrocyclohepta[4,5] thieno [2,3-*d*]pyrimidin-4(3*H*)-one: 14

A mixture of the 3-amino-2-phenylamino thienopyrimidine derivative **12** (3.2 g, 10 mmol) and benzalacetophenone (2.0 g, 10 mmol) were refluxed in DMF (15 mL) for 4 h. The reaction mixture was then left to cool, poured onto ice/water, the formed precipitate was then filtered, washed with water, dried, and crystallized from ethanol/DMF. Yield 57%, m.p > 250ºC; IR (KBr disc) (cm^{-1}): 3337 (NH), 3100 (CH-aromatic), 2982 (CH-aliphatic), 1661 (C=O); ^{1}H-NMR (DMSO, δppm): 1.27-2.51 (m, 10H cycloheptane), 2.24 (s, NH), 3.92 (s, CH_2), 4.44 (m, CH), 6.85 7.19– 7.52 (m, CH aromatic), 13.59 (s, NH); MS: m/z (%): $M^{+\cdot}$: 534 (20.4), 57 (100); Anal. Calcd. (%) for $C_{32}H_{30}N_4O_2S$ (534.67), C, 71.88; H, 5.66; N, 10.48; Found %: C, 71.68; H, 5.42; N, 10.21.

2.2.9. Ethyl-2-thioureido-4,5,6,7,8-pentahydrocyclohepta[*b*]thiophene-3-carboxylate: 15

A mixture of the 2-aminothiophene-3- carboxylate **1** (2.4g, 10 mmol) and potassium thiocyanate (~0.15g,15 mmol) was refluxed in 10% HCl (10 mL) for 12 h. The reaction mixture was allowed to stand overnight at room temperature. The formed solid was filtered, washed with ethanol, dried and crystallized from ethanol. Yield 59%, m.p:180-182)ºC; IR (KBr disc) (cm^{-1}):3430 (NH_2), 2982(CH-aliphatic), 2259 (-N=C=S), 1661(C=O); ^{1}H-NMR spectrum (DMSO, δ ppm):1.22-3.40 (m,CH_3 and 10 H of cycloheptane), 4.26 (q, CH_2-CH_3), 8.30 (s, NH_2, D_2O exchangeable), 11.00 (s, NH, D_2O exchangeable); MS: m/z (%): $M^{+\cdot}$:298 (11.19), 60 (100); Anal. Calcd. (%) for $C_{13}H_{18}N_2O_2S_2$ (298.42): C, 52.32; H, 6.08; N, 9.39; Found %: C, 52.43; H, 6.14; N, 9.52.

2.3. Free radical scavenging activity:

The *in vitro* antioxidant activity method was performed in the Bioassay-Cell Culture Laboratory, National Research Centre, Dokki, Giza .

2.3.1. Materials and method:

The free radical scavenging activity of the compounds was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH•) [15]. Briefly, 0.1mM solution of DPPH• in methanol was prepared. Then, 1 ml of this solution was added to 3 ml of the solution of the tested compounds at different concentrations (25-100 µM/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at λ_{max} 517 nm in Asys microplate reader. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.

DPPH scavenging effect (%) = $100 - [((A_0-A_1)/A_0)\times 100]$

A_0 = the absorbance of the control reaction.

A_1 = the absorbance in the presence of the sample [16]

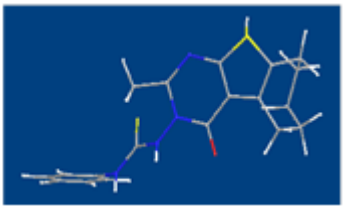
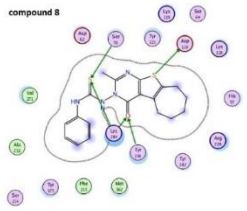
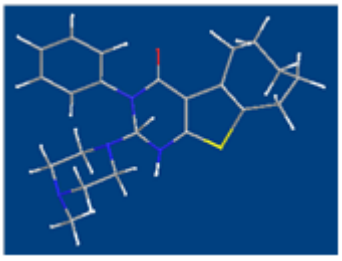
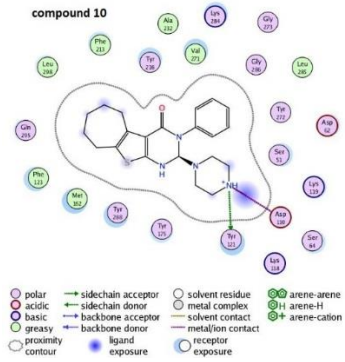
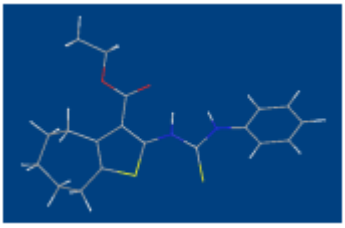
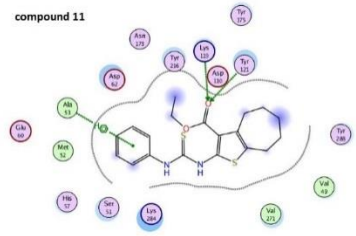
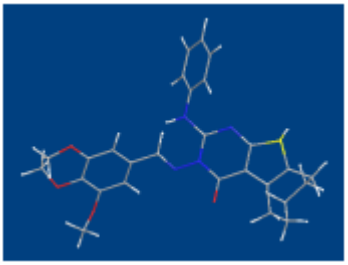
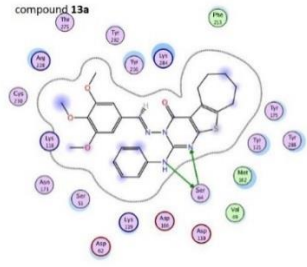
The IC_{50} value for each sample, defined as the concentration of the test sample leading to 50%

reduction of the initial DPPH concentration, was calculated from the nonlinear regression curve of Log concentration of the test compound (µM/ml) against the mean percentage of the radical scavenging activity The *in vitro* antioxidant activity of the selected fifteen compounds was performed using the DPPH free radical scavenging method using Ascorbic acid as a reference standard.

All compounds were screened at 100 µM/mL and the % scavenging activity was illustrated in **table 1** as well as the IC_{50} and the IC_{90} of the most active compounds.

Additionally, further screening was performed on the most potent compounds that gave more than 70% free radical scavenging activity by assaying them at lower gradual concentration ranging from ~1-100 µM/ mL. The observed responses were recorded in **table 2.**

Table1: Docking scores for the synthesized compounds.

Compound	energy	RMSD	2D-interactions
8 	-6.05045938	1.51750565	
10b 	-7.42152166	1.34332049	
11 	-6.92367887	1.72667885	
13a 	-8.31740093	1.64837205	

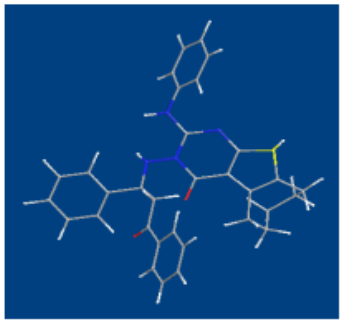
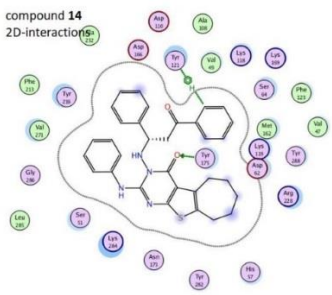
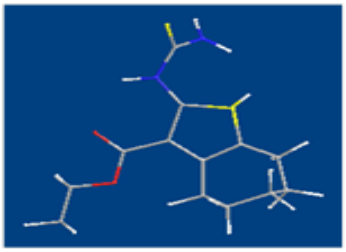
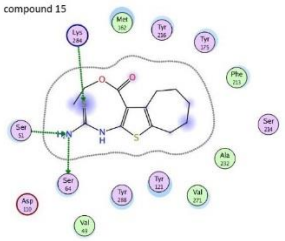
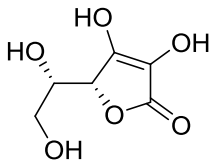
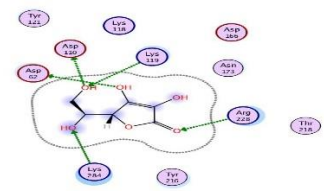
14 	-8.79497623	1.2849406	
15 	-5.85524845	2.32229424	
 Ascorbic acid	-4.6659317	1.36003506	

Table 2: IC_{50}, IC_{90} and %free radical scavenging at 100 µM

Compound No.	**IC_{50}** (µM/ml)	**IC_{90}** (µM/ml)	%free radical scavenging at 100 µM
4a	----------	----------	3.4
4b	----------	----------	8.2
4c	----------	----------	0
4d	----------	----------	23.7
4e	----------	----------	42.7
6a	----------	----------	18.3
7	----------	----------	0
8	34.1	58.8	100
10a	----------	----------	0
10b	56.3	102.2	84.9
11	63.4	110.2	76.7
13a	14.1	37.2	100
13b	----------	----------	52.6
14	17.8	42.4	100
15	23.5	45.1	100
Ascorbic acid	58.7	113.5	73.5

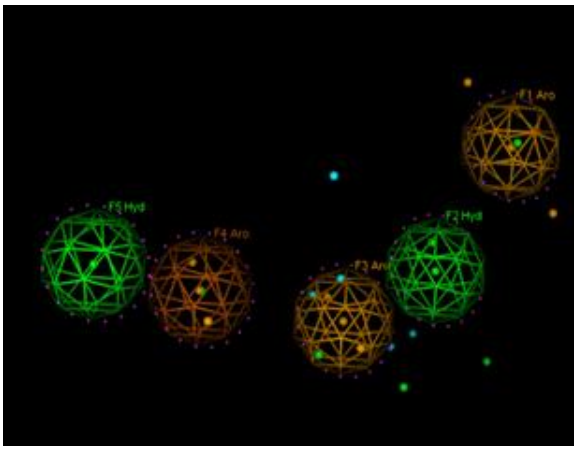


Figure 2:pharmacophore model built using MOE

3. Results and Discussion

3.1. Virtual screening:

Molecular modeling study in addition to the literature survey was a predictive tool for exploring the biological activity of the targeted compounds.

Building of the Pharmacophore Model

Using the chemical structure of a reported natural product ethyl-2-(2-(benzylthio)-4(4 (dihydroxy amino)phenyl)-2H-imidazol-1(5H)-yl)acetate that exerted antioxidant performance [11], a pharmacophore model composed of five features was built using MOE 2012.10 [12]. All features of the compound were taken into account; Aromatic feature and hydrophobic feature (**figure 2**).

Searching of Databases:

The model was used in searching for drug-like databases downloaded from Zinc databases. According to the search results, no hits were resulted from all the used databases. The pharmacophore model was then modified to allow only four features to be introduced and the search was repeated.

Modification of the Structures and Synthesis Decision:

The first group of compounds was designed to be prepared by modification of a selected hit zinc 16808 with good RMSD value (**figure 3a**).

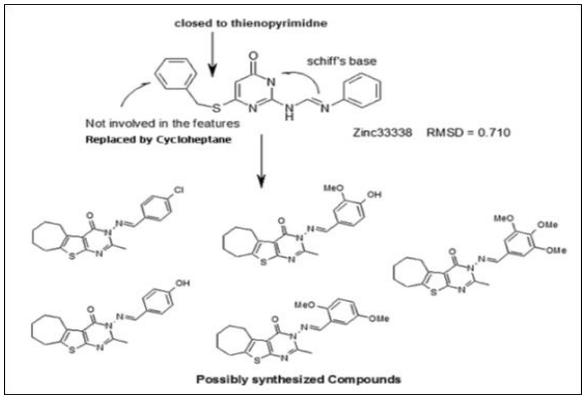


Figure 3a: First group design

The Second group was the Schiff's base derivatives. They were derived from selected hit zinc 33338 of RMSD 0.710 and modified to the most similar, easily synthesized form (**figure 3b**)

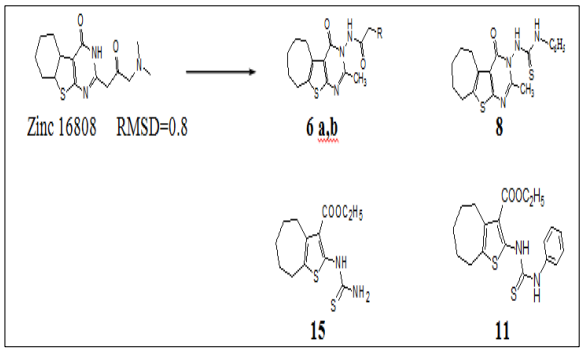


Figure 3b: Design of Sciff's base derivatives

There were different chemical scaffolds found in the resulted hits. The above mentioned two hits were the only hits that are related to or could be modified to thienopyrimidines

Analysis of the Output:

All resulted outputs were carefully analyzed for the best matched hits. The top ranked hits that could be easily synthesized were selected. Some chemical modifications were introduced to the hit structure by removing the part of the structure that was not involved in the built features or by adding new pharmacophoric moieties.

3.2. Molecular Docking

Molecular docking was performed using MOE software. The 3D structure of tyrosinase was downloaded from the PDB site (PDB entry1ZDW; www.rcsb.org). Scoring and re-scoring of the docked poses were followed and achieved. Best poses were saved and reported in **figures 4a-c**. Best energy scores and root mean square deviation values(RMSD) were selected and tabulated along with their 2D-interactions **Table 1**.

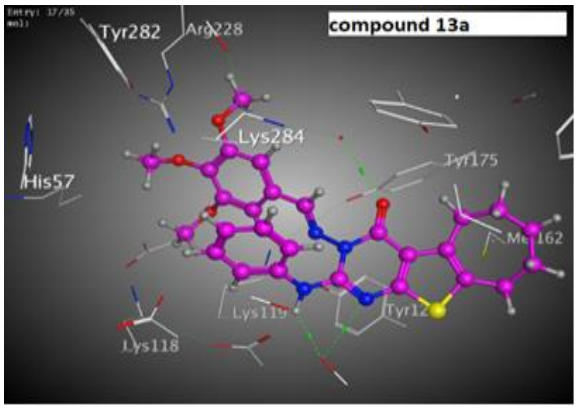


Figure 4a: compound 13a in pink ball and stick structure showing H-bonds in green inside tyrosinase pocket.

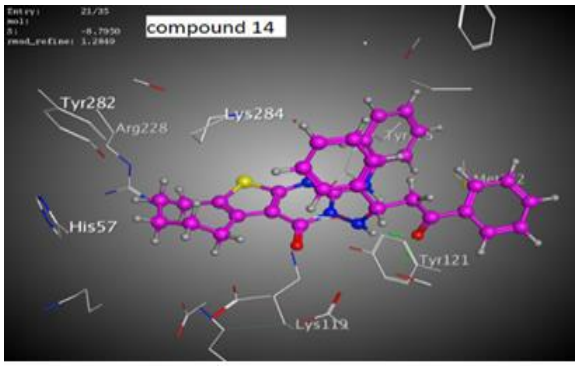


Figure 4b compound 14 in pink ball and stick structure showing H-bonds in green inside tyrosinase pocket.

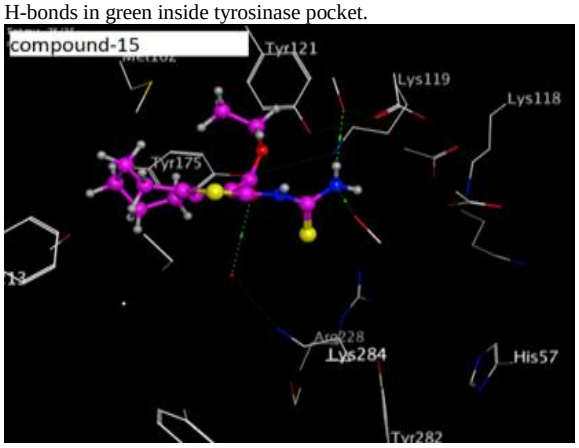
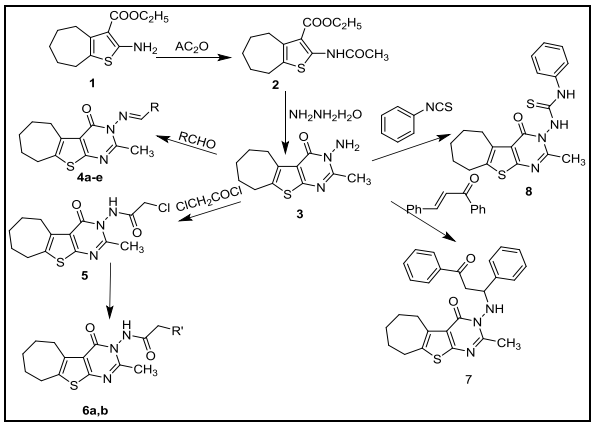


Figure 4c: compound 15 in pink ball and stick structure showing H-bonds in green inside tyrosinase pocket.

3.3. Chemistry

Infrared analysis was performed on Bruker FT-IR spectrophotometer using KBr discs, at the Micro-analytical center, Faculty of Science, Cairo University, Cairo, Egypt and were expressed as wave number (cm^{-1}). The ^{1}H-NMR analysis was performed at the Faculty of Science Micro-analytical cetnter, Cairo University, Cairo, Egypt on Varian Mercury VX-300 NMR spectrophotometer at 300MHZ. The ^{13}C nuclear magnetic resonance was done on apparatus Varian Mercury VX-300 NMR spectrophotometer at 75.446 MHZ using (DMSO-d6), at main defense chemical laboratories, Cairo, Egypt. Elemental analyses were detected at Al-Azhar University Laboratory of the Regional Center for Mycology and Biotechnology. Melting points (M.p.) were recorded on electrothermal IA9100 apparatus (Shimadzu, Japan).

The synthetic strategy to synthesize the target thienopyrimidines **1-15** is depicted in **Schemes 1,2**.The aminothiophene ester [13] **1** was readily available through Gewald reaction [14] using cycloheptanone, ethyl cyanoacetate, sulfur, and a secondary amine. Reaction of the aminothiophene ester **1** with acetic anhydride afforded the corresponding acetylated derivative **2** [9]**.** Refluxing 2 with hydrazine hydrate in ethanol gave the 3-amino-2-methyl thienopyrimidine derivative **3** [9] which acted as a key intermediate in (**Scheme 1**).



Scheme 1

In the present work, Schiff's bases **4a-e** were obtained by reacting the 3-amino carboxylate derivative **3** with the appropriate aldehyde namely p-hydroxybenzaldehyde, p-chlorobenzaldehyde, 3,4,5,trimethoxybenzaldehyde, 2,5-dimethoxybenzaldehyde and 3-methoxy-4-hydroxybenzaldehyde in absolute ethanol and in the presence of a catalytic amount of glacial acetic acid. IR spectrum for **4 a** and **e:** revealed the disappearance of NH_2 band, and the appearance of a broad band at 3430 and 3372 cm^{-1} equivalent to OH group, in addition to the presence of a band at 1673 cm^{-1} representing C=O. IR spectrum for **4 b-d:** confirmed the absence of the NH_2 band at the range of 3400-3250 cm^{-1}, and showed a band at about 1669 cm^{-1} corresponding to C=O. Moreover, ^{1}H-NMR spectrum for **4 a**, **d** : lacked the signal at δ 5.78 ppm equivalent to the NH_2 protons, and, indicated a multiplet signal assigned for the aromatic protons at δ 7.14-7.57 ppm, and a singlet signal at δ 8.90 - 9.01 ppm attributed to the benzylidene proton , in addition to the multiplet pattern representing the cycloheptane protons and the CH_3 protons at C-2-of pyrimidine at δ 1.58-3.22 ppm. Furthermore**,** Mass spectrum for **4 a-d:** clarified the molecular ion peaks at their expected values. Chloroacetylation took place in dry benzene with stirring at room temperature to afford **5**. Structure of compound **5** was confirmed by its IR spectrum which confirmed the disappearance of NH_2 band and presence of bands at 1705, 1655 cm^{-1} equivalent to C=O groups in addition to its ^{1}H-NMR spectrum that lacked the singlet signal attributed to NH_2 protons at δ 5.78 ppm and indicated a singlet signal at δ 4.30 ppm corresponding to CH_2 protons of the chloroacetamido, a singlet signal at δ 11.32 ppm equivalent to NH protons that disappeared upon deuteration , also the multiplet signals assigned for the cycloheptane protons and the CH_3 protons at C-2 of pyrimidine were observed at their predicted values. ^{13}C-NMR spectrum, illustrated a signal at δ 60.67 ppm corresponding to the methylene carbon of the acetamido group, two signals at δ 158.90 and δ 166.33 ppm attributed to C=O of the pyrimidinone and that of acetyl group respectively, and a signal at δ 164.60

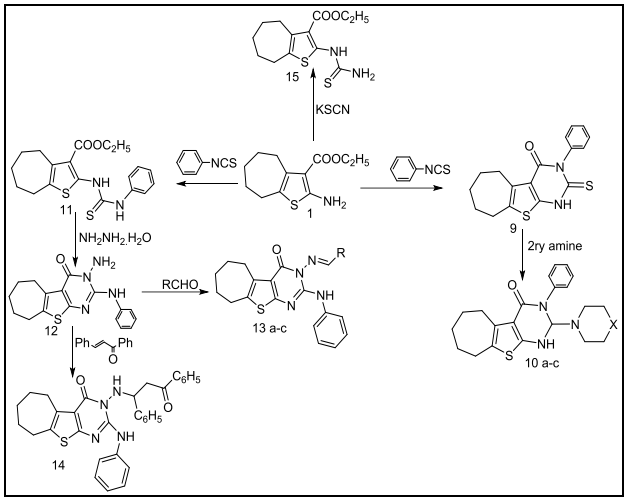
ppm equivalent to C-2 of pyrimidine ring, in addition to the signals assigned for the cycloheptane carbons and those representing the thiophene ring carbons at their expected values . Furthermore, Mass spectrum disclosed $M^{+\cdot}$, at m/z 325 (10.85 %) , $[M+2]^+$ at m/z 327, and m/z77 (100 %)

Compounds **6 a** and **b** were achieved by reaction of the chloroacetamido derivative **5** with the appropriate amine namely morpholine and 2,6-dimethoxy phenylamine in dry benzene using few drops of TEA. IR spectrum showed, a band at 3389-3322cm^{-1} corresponding to NH group ,in addition to band at 1667 cm^{-1} attributed to C=O groups. Also, ^{1}H-NMR spectrum of **6 b,** a singlet

at δ 4.35 ppm equivalent to the CH_2 protons of the acetamide , and a singlet at δ 11.10 ppm representing the NH protons, a singlet at δ 3.56 ppm assigned for the 2-OCH_3 protons ,a multiplet at δ 7.41-7.79 ppm equivalent to the aromatic protons, in addition to the muliplet pattern of cycloheptane protons and CH_3 protons that appeared at their expected region of the spectra, Mass spectrum of **6 a:** revealed $M^{+\cdot}$ at m/z 376 (1.01 %) and a base peak at m/z 100 (100%) equivalent to the fragment

In the present investigation, reaction of the 3- amino derivative **3** and benzalacetophenone was performed in DMF to give 3-oxo-1,3-diphenyl propylamino derivative **7**. IR spectrum confirmed the disappearance of the forked band equivalent to NH_2 group with concomitant appearance of a band at 3350 cm^{-1} attributed to NH group and a band at 1660 cm^{-1} assigned to C=O groups. Also, Mass spectrum revealed $M^{+\cdot}$, at m/z 457 (1.10 %) and a base peak at m/z 57**.** Synthesis of the thiourea derivative **8** was achieved via refluxing the amino derivative **3** with phenyl isothiocyanate in ethanol in presence of few drops of TEA. IR spectrum showed the disappearance of the forked NH_2 band and the appearance of a band at 3340 cm^{-1} equivalent to NH groups of thiourea , in addition to a band at 2200 cm^{-1} corresponding to(-N=C=S) group. ^{1}H-NMR spectrum indicated the presence of a multiplet signal representing the aromatic protons at δ 7.12-7.45 ppm and two singlet signals at δ10.83 and δ 11.50 ppm assigned for the two exchangeable NH protons, in addition to the multiplet pattern of the cycloheptane ring and the CH_3 protons at C-2 of pyrimidine that appeared at their expected region of the spectra. Moreover, ^{13}C-NMR spectrum disclosed a signal at δ 175.64 ppm attributed to the C=S carbon and signals equivalent to the aromatic ring carbons at the range δ 124.23- δ 139.40 ppm, in addition to the previously mentioned pattern of cycloheptane, thiophene and pyrimidine rings carbons. Also, Mass spectrum revealed $M^{+\cdot}$ at m/z 384 (62.76 %) and m/z 77 (100%).

Regarding **scheme-2** compounds **9, 11, 12** and **15** were readily prepared as reported [8].



Scheme 2

The titled compound **10 a-c** was prepared by reaction of the thioxopyrimidine derivative **9** with the appropriate secondary amine namely morpholine, N-methylpiperazine and N-phenylpiperazine in pyridine. IR spectrum lacked any bands at 3400-3300 cm^{-1} corresponding to the NH group ,and showed a band at ~1660 cm^{-1} equivalent to C=O group. Furthermore ,the ^{1}H-NMR spectrum of **10 c** revealed a multiplet at δ 3.51- 4.01 ppm corresponding to the piperazine protons, in addition to increase in the integeration of the multiplet attributed to the aromatic protons, and the multiplet pattern equivalent to the cycloheptane protons. Moreover, Mass spectrum of **10 a** and **b** clarified their molecular ion peaks.

Compounds **13 a-d** were synthesized by reacting the 3-amino-2-phenylamino derivative **12** with the appropriate aldehyde in absolute ethanol catalyzed by few drops of glacial acetic acid. IR spectrum of **13 a-d**: disclosed the absence of forked NH_2 band at the range of 3400- 3300 cm^{-1}. Moreover, ^{1}H-NMR spectrum of **13 c** illustrated an increase in the integeration of the multiplet signal assigned for the aromatic protons, a singlet signal at δ 9.11 ppm assigned for the benzylidene proton, and singlet signals at δ13.59 and δ 14.00 ppm attributed to the NH and OH protons respectively that disappeared upon deuteration, in addition to the multiplet indicating the cycloheptane protons . Also**,** Mass spectrum of **13 a** and **b**: showed $M^{+\cdot}$, at m/z 504 (100 %) and at m/z 474 (100 %) respectively.

Preparation of compound **14** was accomplished by reaction between the 3-amino derivative **12** and benzalacetophenone in DMF. IR spectrum confirmed the disappearance of the forked band equivalent to NH_2 group with concomitant appearance of new band at 3337 cm^{-1} attributed to NH group and the appearance of a new band at 1661 cm^{-1} assigned for the introduced C=O group. ^{1}H-NMR spectrum clarified mulitplet signals corresponding to the aromatic protons at δ 7.19-7.52 ppm , singlet signals equivalent to NH protons at δ 2.24 and 13.59 ppm ,

singlet signal assigned for the methylene protons at δ 3.92 ppm and a singlet signal at δ 4.44 ppm due to the CH proton, in addition to the muliplet pattern representing the cycloheptane protons at their predicted positions. Furthermore, Mass spectrum showed $M^{+\cdot}$, at m/z 534 (20.90 %) and base peak at m/z 57 (100 %).

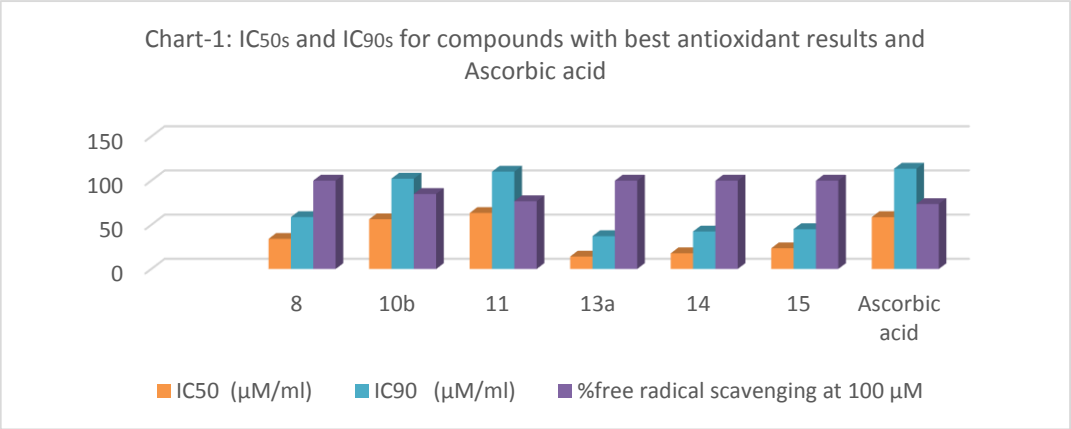
The *in-vitro* antioxidant activity of the selected fifteen compounds was performed using the DPPH free radical scavenging method using Ascorbic acid as a reference standard. All compounds were screened at 100 µM/mL and the % scavenging activity was illustrated in **Table 2** as well as the IC_{50} and the IC_{90} of the most active compounds.

3.4. Biology:

Additionally, screening was performed on the most potent compounds that gave more than 70% free radical scavenging activity by assaying them at lower gradual concentration ranging from ~1-100 µM/ mL. The observed responses were recorded in **Table 3.**

Table 3: The % free radical scavenging activity of the most potent compounds at gradual concentrations.

	Compound No. and its % inhibition					
concentration µM/ml	**(%) 8**	**(%) 10b**	**(%) 11**	**(%) 13a**	**(%) 14**	**(%) 15**
100	100	84.9	76.7	100	100	100
50	73.4	45.2	48.2	90.4	89.4	88.8
25	40.6	29.9	22.2	82.3	71.2	60.3
12.5	19.7	16.7	9.8	68.7	57.7	39.3
6.25	11.3	10.2	5.3	42.1	32.8	19.7
3.13	3.4	3.7	2.4	29.1	19.7	9.8
1.56	0	1.2	1	11.8	12.4	4.2
0.78	0	0	0	5.7	7.5	0



<u>According to the antioxidant results, the following could be noticed:</u>

- Concerning the benzylideneamino derivatives: that are introduced as two groups **4 a-e** in scheme-1 and **13 a**, **b** in scheme-2 with varied antioxidant activity (inactive to moderate). The nature of the substituents on the benzylidene ring greatly affected their antioxidant activity:
  - The presence of an electron donating group (4-OH) in **4 a** didn't show any promising antioxidant activity as illustrated from the % free radical scavenging value (3.4%).
  - The electron withdrawing group (4-Cl) in **4 b** slightly enhanced the activity (8.2%) relative to **4 a**.
  - The 3,4,5-trimethoxy substituents in **4 c** yielded a completely inactive compound, whereas, the 2,5-dimethoxy derivative **4 d** exhibited a relatively increased antioxidant activity (23.7%) than the trimethoxy analogue.
  - 4-Hydroxy-3-methoxy benzylidene derivative **4 e** disclosed a moderate free radical inhibition (42.7%).
  - 3,4,5-Trimethoxybenzylideneamino of the 2-phenyl aminothienopyrimidinone derivative **13 a** revealed a prominent antioxidant activity (100%) among the tested compounds and even more scavenging activity than the positive control Ascorbic acid (73.5%) , whereas, the 2,5-dimethoxy one **13 b** showed a decrease in activity (52.6%) up to half the value expressed by the trimethoxy analogue **13 a** .
- 2-Morpholino-N-acetamido derivative **6 a** exhibited a low antioxidant activity (18.3%).
- Regarding the 3-oxo-1,3-diphenylpropylamino derivatives: the reaction of benzalacetophenone with the 3-amino-2-methyl cycloheptathienopyrimidin-4-one **3** afforded the 3-oxo-1,3-diphenylpropylamino-2-methyl derivative **7** which didn't show any antioxidant

activity (0%) , whereas, its reaction with the 3-amino-2-phenylaminocycloheptathienopyrimidin-4-one **12** gave the 3-oxo-1,3-diphenylpropylamino-2-phenylamino analogue **14** that displayed a marked free radical scavenging activity (100%).

- Reaction of phenyl isothiocyanate with the 3-aminothienopyrimidin-4-one derivative **3** afforded the 3-phenylthioureido derivative **8** which exhibited a distinguished antioxidant activity (100%), while its reaction with the 2-aminocycloheptathiophene derivative **1** gave the 2-phenyl thioureido derivative **11** which displayed a marked activity (76.7%). both compounds **8** and **11** exhibited a free radical scavenging performance higher than that of the reference drug Ascorbic acid (73.5%) .
- Treatment of the 3-phenyl-2-thioxocycloheptathieno pyrimidin-4-one **9** with morpholine produced 3-phenyl-2-morpholino derivative **10 a** with no antioxidant activity, while the activity was greatly increased in the N-methyl piperazine analogue **10 b** (84.9%).
- Finally, reaction of 2-aminocyclohepta[b]thiophene carboxylate **1** with potassium thiocyanate afforded the corresponding 2-thioureido derivative **15** with a prominent antioxidant activity (100%).

4. Conclusion

Antioxidants act as one of the main defense mechanisms against radical mediated toxicity. They are effective in prevention and treatment of complex diseases. Many active antioxidant compounds were having thiophene or thienopyrimidine ring structure which prompted our interest to synthesize fifteen new compounds bearing thienopyrimidine core and analyze them for their antioxidant activity. Six compounds showed significant free radical scavenging activity at 100µM ranging from (7.67%-100%) compared to reference standard (73.5%) with IC50s values ranging from (14.1 µM/ml- 63.4 µM/ml) in relation to reference standard that recorded (58.7 µM/ml). Molecular docking was performed and best docking scores were for compounds 13a and 14. Interestingly, the docking scores were consistent with the compounds' antioxidant profile that expressed the lowest IC50 values (14.1 µM/ml -17.8 µM/ml) respectively.

Conflicts of interest

"There are no conflicts to declare".

5. References

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