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MW Assisted Synthesis of New 4,6-diaryl-3,4-Didhydropyrimidines-2(1*H*)-thione Derivatives: Tyrosinase Inhibition, Antioxidant, and Molecular Docking Studies

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ABSTRACT

A number of new methoxy-substituted 4,6-diaryl-3,4-dihydropyrimidine-2(1*H*)-thiones (DH-Pyr-S, 17-28) were designed and synthesized by the reaction of methoxy-substituted chalcones (1–14) with thiourea using solid-phase microwave method (MW) in view of the structural requirements as suggested in the pharmacophore model for tyrosinase inhibition (TI). Synthesized compounds were assessed for their *in vitro* TI potential and compounds 16, 17, and 21 exhibited notable tyrosinase inhibitory properties at the concentrations of 31.86 ± 2.45 µM, 44.58 ± 0.46 µM, and 48.47 ± 0.66 µM, respectively. Compounds (16, 17, and 21) were exhibited experimentally more potent TI than the standard used in terms of the IC_{50} value (Kojic acid, 55.38 ± 2.30 µM; $p<0.0001$). Additionally, DPPH activity of 15-28 were evaluated and compound 17 showed the moderate DPPH activity (45.64 ± 0.34%). Binding affinities of synthesized molecules to the tyrosinase catalytic core were further investigated through *in silico* molecular docking studies using AutoDock Vina (version 1.2.5), discovery studio accelyrs (BIOVIA, Dassault Systèmes) and predicting small-molecule pharmacokinetic properties using graph-based signatures (pkCSM) programs were used for ADMET calculations. Among synthesized compounds 15, 21, and 24 revealed high binding affinity to tyrosinase active site with lowest binding free energy (ΔG) values of -7.9 kcal/mol, thereby outperformed kojic acid affinity. In conclusion most modeling results were in agreement with their experimental data, suggesting the TI potential of lead compounds.

Keywords: Dihydropyrimidine-2(1*H*)-thione, Tyrosinase inhibition, DPPH, ADMET, Molecular docking.

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Introduction

Heterocycles play a role in different fields, inclusive of medicinal chemistry, biochemistry, and others. Pharmaceuticals, agrochemicals, and veterinary items are the main applications of heterocyclic compounds [1]. Pyrimidine is the one of the general names of nitrogenous heterocyclic aromatic organic compounds in the structure of nucleic acids, some coenzymes, and vitamins. Pyrimidine and its derivatives, found in many natural products, are essential for the synthesis of pharmaceutical compounds [2], antitumor, antitubercular, antileukemia, antileishmanial, analgesic, anticonvulsant, anticancer, antifungal, and anti-inflammatory [3]. Pyrimidine derivatives known to be effectively used in the cosmetic sector for wrinkle treatment, strengthening hair roots, and strengthening the epidermis layer to prevent gray hair formation. Many methods have been employed to synthesize 3,4-dihydropyrimidine-2-one/thione compounds, e.g., the condensation reaction of malonate derivatives and urea in a basic medium, the cyclocondensation reaction of aldehyde, dicarbonyl compound, and urea/thiourea in one step with the Biginelli reaction [4], *α,β*-unsaturated esters by the Michael addition reaction [5], condensation of carbonyls with diamines, and the condensation reaction of chalcones with urea, thiourea, and guanidine derivatives [6]. Chalcones are the most used and attractive bioactive starting materials to synthesize various heterocyclic compounds, such as pyrimidine, pyrazoline [7]. Substituted chalcones and thiourea can easily react under basic conditions according to the 1,4-Michael addition reaction to yield DH-Pyr-S compounds [8].

Some of the drugs used as azathioprine (immunosuppressive), zidovudine, erlotinib, and 5-fluorouracil (anticancer), sulfadiazine and minoxidil (antibacterial), lamivudine (anti-HIV), etravirine (antiviral), and moxonidine (antihypertensive) have pyrimidine structure [9]. In this context, to treat over pigmentation in human bodies, there is a strong urge to find out new and efficient tyrosinase inhibition (TI). Several TI have been reported, but a few of these inhibitors have been commercially in use [10]. There are a few sulfurs containing tyrosinase inhibitors in the literature. It is well known that oxidation resulted by reactive oxygen species is a major cause of melanogenesis

[11]. Sulfur containing compounds possess good antimelanogenic and antioxidant activities, which shows their capacity to affect key redox enzymes. Literature work have shown that small molecules which has sulfur resulted a key role in a wide range of various biological activities [12]. Thus, these finding attracted many scientists to find out new S analogs for the prevention and therapy. Some substances such as kojic acid and arbutin have been well described as TI. However, some of these compounds possess undesirable side effects like carcinogenicity and cytotoxicity [13].

Due to the above problems, we tried to develop new safer and more effective tyrosinase inhibitors. Literature revealed that some thioxo pyrimidine compounds, including compound 15, showed good antiparasitic activity but low cytotoxicity against L1210 and B16 cells [14]. The insecticidal activity of a series of methoxy and various halo-substituted thiopyrimidine compounds, including compound 16, has been studied in the literature [14, 15]. Moreover, the binding affinities of 17 and 21 to the tyrosinase catalytic core (2Y9X) were studied by molecular docking studies [16-18]. The current research focuses on synthesizing methoxy DH-Pyr-S derivatives from the reaction of methoxy chalcones with thiourea and tested their TI, DPPH activity, and molecular docking studies.

Materials and Methods

Materials and Equipment

2-/4-methoxyacetophenone, 2-/3-/4-methoxybenzaldehyde, 2,3-/2,5-dimethoxybenzaldehyde, 2,3,4-trimethoxybenzaldehyde, benzaldehyde and thiourea were of Merck, Fluka, Lancaster, and Sigma-Aldrich brands and they are in analytical purity. Solvents, including ethanol, chloroform, ethyl acetate, and *n*-hexane used in synthesis, purification, and analysis in instrumental devices were purified by fractional distillation. The NMR solvent was $CDCl_3$ (Merck, 99.8%). The NMR spectra were recorded on a Varian Mercury 200 MHz and Bruker 400 MHz NMR instruments, UV spectra were taken on a Unicam UV2-100 spectrophotometer, FT-IR spectra were acquired on a Perkin-Elmer 1600 (ATR, 4000-400 cm^{-1}) spectrophotometer, mass spectra were recorded using an Agilent LC-QTOF-MS spectrophotometer. Melting points were found with a Thermovar device attached to the microscope. The reactions of the synthesized compounds were performed on a laboratory microwave device (Milestone).

Synthesis

Synthesis of 1–14

The preparation of known 1,3-diaryl-2-propene-1-one derivatives (1–14) was carried out in line with the procedure described in the literature [19-20]. The stirring of the equimolar quantities of substituted acetophenone (10 mmol), benzaldehyde (10 mmol), and NaOH (40%, 4 mL) in ethanol (10 mL) was carried out at a temperature of 0-5 ^{0}C for half an hour. Reaction mixture was stirred for a period of 12 h at room temperature and the reactions were terminated after TLC monitoring. The reaction mixture was acidified with 6 N HCl and poured into crushed ice. The precipitated solid filtrated and washed with cold water and crystallized from ethanol to provide white or yellow products or they were purified by column chromatography when required. Structure of synthesized chalcones (1-14) were confirmed by their NMR data (^{1}H and ^{13}C/APT) and by comparing them with data reported in the literature [19-20].

Synthesis of 15-28

Basic alumina (5 g) was added in a successive way to a solution of 1,3-diaryl-2-propen-1-one (1-14) (4 mmol) and thiourea (4 mmol), and chloroform (15 mL) in a round bottom flask (100 mL), and the reaction mixture was carefully mixed and dried on the rotary evaporator. The material adsorbed on basic silica was transferred to a Pyrex tube (2 cm diameter, 30 mL) and inserted inside the MW oven (Milestone) and heated using a fixed power of 600 W for 5-6 min at 85 $^{\circ}$C. The reaction mixture was solved in methanol and neutralized with 2N HCl solution. The solvent was evaporated, and the residue was dissolved in water (50 mL) then extracted with chloroform (20 mL x 3). Then they were purified by column chromatography (Silica gel) using *n*-hexane (50 mL) and a *n*-hexane-ethyl acetate solvent mixture (4:1, 4:2, and 1:1; 100 mL each). After the TLC control, corresponding pure products (DH-Pyr-S, 15-28) were obtained in the range of 60-80% yields, respectively. The structures of 15-28 were identified by their spectral properties (^{1}H and ^{13}C/APT-NMR, FT-IR, LC-QTOF-MS) and comparison of the data reported in the literature [14-15].

4-(2-Methoxyphenyl)-6-phenyl-3,4-dihydropyrimidine-2(1H)-thione (17)

Yield: 75%, mp = 221-223 ^{o}C, Rf: 0.67 (*n*-hexane–ethyl acetate, 1:1); FTIR (ATR, cm^{-1}): 3149 (-NH), 3026(=CH), 2900, 2974 (-CH), 1573 (C=C, aromatic ring), 1245 (-OCH_3), 1270 (C=S); UV-vis λ nm (log): 284 (0.229); ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm), 3.92 (s, 3H, -OCH_3,), 5.15-5.16 (m, 1H, H_6), 5.29-5.30 (m, 1H, H_5), 6.94-6.96 (m, 1H, $H_{3'}$), 7.28-7.32 (m, 2H, $H_{4'}$/$H_{4''}$), 7.32-7.42 (m, 6H, $H_{5'}$/$H_{6'}$/$H_{2''}$/$H_{3''}$/$H_{5''}$/$H_{6''}$), 6.84, 8.20 (s, 2H, -NH). ^{13}C/APT-NMR (100 MHz, $CDCl_3$, δ, ppm): 55.77 (-OCH_3), 58.41 (C_6), 102.37 (C_5), 111.32 ($C_{3'}$), 121.14 ($C_{5'}$), 121.22 ($C_{1'}$), 126.99 ($C_{2''}$/$C_{6''}$), 129.14 ($C_{3''}$/ $C_{5''}$), 128.55 ($C_{6'}$), 129.36 ($C_{4'}$), 130.91 ($C_{4''}$), 133.17 (C_4), 142.55 ($C_{2'}$), 156.60 ($C_{1''}$), 174.56 (C_2)]. LC-QTOF-MS: (*m/z*) (%) $[M+Na]^+$: 319.0877 (100), calc. 319.0879.

4,6-Bis(2-methoxyphenyl)-3,4-dihydropyrimidine-2(1H)-thione (18)

Yield: 78%, mp = 194-195 ^{o}C, Rf: 0.68 (*n*-hexane–ethyl acetate, 1:1); FTIR (ATR, cm^{-1}): 3168 (-NH), 3005,3073(=CH), 2835, 2965 (-CH), 1562 (C=C, aromatic ring), 1242 (–OCH_3), 1261 (C=S); UV-vis λ nm (log): 212 (3.754), 276 (0.624); ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm), 3.90, 3.92 (s, 6H, -OCH_3), 5.23-5.24 (m, 1H, H_6), 5.65-5.66 (m, 1H, H_5), 7.28-7.41 (m, 4H, $H_{3'}$/$H_{4'}$/$H_{3''}$/$H_{5''}$), 7.42-7.44 (m, 4H, $H_{5'}$/$H_{6'}$/$H_{4''}$/$H_{6''}$), 6.98, 8.06 (s, 2H, -NH). APT-NMR (100 MHz, $CDCl_3$ δ, ppm): 50.86, 55.44 (-OCH_3), 55.57 (C_6), 100.59 (C_5), 110.45 ($C_{3''}$), 111.33 ($C_{3'}$), 121.03 ($C_{5'}$), 121.15 ($C_{6'}$), 127.72 ($C_{4'}$), 122.04 ($C_{1'}$), 129.52 ($C_{1''}$), 129.53 ($C_{4''}$),

129.70 ($C_{6''}$), 130.84 ($C_{5''}$), 134.27 (C_4), 156.21 ($C_{2'}$), 156.56 ($C_{2''}$), 174.70 (C_2) , LC-QTOF-MS: (*m/z*) (%) $[M+Na]^+$: 349.0992 (60), calc. 349.0993.

4-(2-Methoxyphenyl)-6-(3-methoxyphenyl)-3,4-dihydropyrimidine-2(1H)-thione (19)

Yield: 73%, mp = 170-171 °C, Rf: 0.69 (*n*-hexane–ethyl acetate, 1:1); FTIR (ATR, cm^{-1}): 3149 (-NH), 3102(=CH), 2835, 2937 (-CH), 1577, 1602 (C=C, aromatic ring), 1241 (-OCH_3), 1271 (C=S); UV-vis λ nm (log): 243 (0.433), 282 (0.425); ^{1}H-NMR (400 MHz, $CDCl_3$, *δ*, ppm), 3.84, 3.91 (s, 6H, -OCH_3), 5.14-5.15 (m, 1H, H_6), 5.26-5.27 (m, 1H, H_5), 6.94-6.98 (m, 4H, $H_{3'}$/$H_{4'}$/$H_{5'}$/$H_{4''}$), 7.32-7.35 (m, 4H, $H_{6'}$/$H_{2''}$/$H_{5''}$/$H_{6''}$), 6,84, 8.19 (s, 2H, -NH). ^{13}C/APT-NMR (100 MHz, $CDCl_3$, *δ*, ppm): 55.36, 55.77 (-OCH_3), 57.17 (C_6), 102.26 (C_5), 111.31 ($C_{4''}$), 112.59 ($C_{2''}$), 113.94 ($C_{3'}$), 119.20 ($C_{5'}$), 121.15 ($C_{6''}$), 121.70 ($C_{1'}$), 129.37 ($C_{6''}$), 130.21 ($C_{4'}$), 130.93 ($C_{5''}$), 133.26 (C_4), 144.05 ($C_{1''}$), 156.59 ($C_{2'}$), 160.20 ($C_{3''}$), 174.55 (C_2). LC/ MS-TOF: (*m/z*) (%) $[M+23]^+$: 349.0995 (100), calc. 349.0992. LC-QTOF-MS: (*m/z*) (%) $[M+Na]^+$: 349.0972 (60), calc. 349.0975.

4-(2-Methoxyphenyl)-6-(4-methoxyphenyl)-3,4-dihydropyrimidine-2(1H)-thione (20)

Yield: 79%, mp = 161-162 °C, Rf: 0.71 (*n*-hexane–ethyl acetate, 1:1); FTIR (ATR, cm^{-1}): 3175 (-NH), 2835, 2971 (-CH), 1575, 1601 (C=C, aromatic ring), 1241 (-OCH_3), 1265 (C=S); UV-vis λ nm (log): 223 (3.620), 276 (0.510); ^{1}H-NMR (400 MHz, $CDCl_3$, *δ*, ppm), 3.83, 3.92 (s, 6H, -OCH_3), 5.13 (s, 1H, H_6), 5.26 (s, 1H, H_5), 6.95-6.96 (m, 1H, $H_{3'}$), 7.28-7.33 (m, 4H, $H_{4'}$/$H_{5'}$/$H_{3''}$/$H_{5''}$), 7.33-7.35 (m, 3H, $H_{2''}$/$H_{6''}$/$H_{6'}$), 6.60, 8.14 (s, 2H, -NH). ^{13}C/APT-NMR (100 MHz, $CDCl_3$, *δ*, ppm): 55.39, 55.78 (-OCH_3), 56.76 (C_6), 102.59 (C_5), 111.31 ($C_{3'}$), 114.44 ($C_{3''}$/$C_{5''}$), 121.16 ($C_{5'}$), 121.74 ($C_{1'}$), 128.34 ($C_{2''}$/ $C_{6''}$), 129.35 ($C_{6'}$), 130.90 ($C_{4'}$), 134.81 (C_4), 133.08 ($C_{1''}$), 156.58 ($C_{2'}$), 159.80 ($C_{4''}$), 174.37 (C_2). LC-QTOF-MS: (*m/z*) (%) $[M+Na]^+$: 349.0982 (100), calc. 349.0980.

4-(2-Methoxyphenyl)-6-(2,3-dimethoxyphenyl)-3,4-dihydropyrimidine-2(1H)-thione (21)

Yield: 70%, mp = 175-176 °C, Rf: 0.65 (*n*-hexane–ethyl acetate, 1:1); FTIR (ATR, cm^{-1}): 3177 (-NH), 2835, 2979 (-CH), 1570 (C=C, aromatic ring), 1241 (-OCH_3), 1271 (C=S); UV-vis λ nm (log): 224 (3.723), 275 (0.798); ^{1}H-NMR (400 MHz, $CDCl_3$, *δ*, ppm), 3.89, 3.92 (s, 9H,-OCH_3), 5.19-5.21 (m, 1H, H_6), 5.63-5.64 (m, 1H, H_5), 6.93-6.95 (m, 3H, $H_{3'}$/$H_{4''}$/$H_{5''}$), 7.07-7.08 (m, 2H, $H_{4'}$/$H_{6''}$), 7.35-7.36 (m, 2H, $H_{5'}$/$H_{6'}$), 6.80, 8.12 (s, 2H, -NH). ^{13}C/APT-NMR (100 MHz, $CDCl_3$, *δ*, ppm): 51.24, 55.76, 55.90 (-OCH_3), 61.07 (C_6), 101.37 (C_5), 111.32 ($C_{4''}$), 112.54 ($C_{3'}$), 119.61 ($C_{6''}$), 121.13 ($C_{5'}$), 121.90 ($C_{1'}$), 124.56 ($C_{6'}$), 129.34 ($C_{4'}$), 130.82 ($C_{5''}$), 133.70 ($C^{1''}$), 135.54 (C_4), 145.76 ($C_{2'}$), 152.56 ($C_{2''}$), 156.55 ($C_{3''}$), 174 (C_2). LC-QTOF-MS: (*m/z*) (%) $[M+Na]^+$: 379.1076 (100), calc. 379.1077.

4-(2-Methoxyphenyl)-6-(2,5-dimethoxyphenyl)-3,4-dihydropyrimidine-2(1H)-thione (22)

Yield: 72%, mp = 148-151 °C, Rf: 0.72 (*n*-hexane–ethyl acetate, 1:1); FTIR (ATR, cm^{-1}): 3262 (-NH), 3000,3070(=CH), 2835, 2963 (-CH), 1561,1600 (C=C, aromatic ring), 1242 (-OCH_3), 1259, 1273 (C=S); UV-vis λ nm (log): 244 (0.425), 283 (0.412); ^{1}H-NMR (400 MHz, $CDCl_3$, *δ*, ppm), 3.79, 3.91 (s, 9H, -OCH3), 5.19 (s,1H, H_6), 5.62 (s, 1H, H_5), 6.84-7.03 (m, 5H, $H_{3'}$/$H_{5'}$/$H_{3''}$/$H_{4''}$/$H_{6''}$), 7.36-7.41 (m, 2H, $H_{4'}$/$H_{6'}$), 8.08 (s, 2H, -NH). ^{13}C/APT-NMR (100 MHz, $CDCl_3$, *δ*, ppm): 50.81, 55.75 (-OCH_3), 55.82 (C_6), 100.28 (C_5), 111.35 ($C_{4''}$), 111.38 ($C_{6''}$), 113.33 ($C_{3'}$), 114.48 ($C_{3''}$), 120.58 ($C_{1'}$), 121.13 ($C_{5'}$), 129.30 ($C_{6'}$), 130.66 ($C_{1''}$), 130.84 ($C_{4'}$), 134.47 (C_4), 150.41 ($C_{2''}$), 153.91 ($C_{5''}$), 156.67 ($C_{2'}$), 174.77 (C_2). LC-QTOF-MS: (*m/z*) (%) $[M+Na]^+$: 379.1077 (100), calc. 379.1077.

4-(2-Methoxyphenyl)-6-(2,3,4-trimethoxyphenyl)-3,4-dihydropyrimidine-2(1H)-thione (23)

Yield: 80%, mp = 210-211 °C, Rf: 0.66 (*n*-hexane–ethyl acetate, 1:1); FTIR (ATR, cm^{-1}): 3263 (-NH), 3098 (=CH), 2827, 2951 (-CH), 1599,1569 (C=C, aromatic ring), 1238 (-OCH_3), 1263 (C=S); UV-vis λ nm (log): 231 (3.623), 274 (1.205), 283 (1.041); ^{1}H-NMR (400 MHz, $CDCl_3$, *δ*, ppm), 3.87, 3.88 (s, 12H, -OCH_3), 5.52 (s, 1H, H_5), 5.17 (s, 1H, H_6), 6.68-6.78 (m, 2H, $H_{5'}$/$H_{5''}$), 6.95-6.98 (m, 2H, $H_{3'}$/$H_{6''}$), 7.36-7.39 (m, 2H, $H_{4'}$/$H_{6'}$), 7.10, 8.09 (s, 2H, -NH). ^{13}C/APT-NMR (100 MHz, $CDCl_3$, *δ*, ppm): 51.12, 55.76, 56.06, 60.84 (-OCH_3), 61.22 (C_6), 101.25 (H_5), 107.23 ($C_{5''}$), 111.33 ($C_{3'}$), 121.15 ($C_{6''}$), 121.15 ($C_{1''}$), 122.42 ($C_{5'}$), 127.67 ($C_{1'}$), 129.29 ($C_{6'}$), 130.82 ($C_{4'}$), 133.86 (C_4), 141.86 ($C_{3''}$), 150.68 ($C_{2''}$), 153.93 ($C_{4''}$), 156.55 ($C_{2'}$), 174.48 (C_2). LC-QTOF-MS: (*m/z*) (%) $[M+Na]^+$: 409.1206 (100), calc., 409.1205.

4-(4-Methoxyphenyl)-6-(2-methoxyphenyl)-3,4-dihydropyrimidine-2(1H)-thione (24)

Yield: 69%, mp = 233-234 °C, Rf: 0.65 (*n*-hexane–ethyl acetate, 1:1); FTIR (ATR, cm^{-1}): 3159 (-NH), 3010(=CH), 2841, 2961 (-CH), 1553,1611 (C=C, aromatic ring), 1240 (-OCH_3), 1273 (C=S); UV-vis λ nm (log): 241 (0.620), 276 (0.615), 283 (0.524); ^{1}H-NMR (400 MHz, $CDCl_3$, *δ*, ppm), 3.85, 3.90 (s, 6H, -OCH_3), 5.19-5.20 (s, 1H, H_6), 5.63-5.65 (m, 1H, H_5), 6.92-7.01 (m, 2H, $H_{3''}$/$H_{5''}$), 6.94 (d, 2H, *J*=8 Hz, H3'/H5'), 7.01-7.04 (m, 2H, H4''/H6''), 7.42 (d, 2H, *J*=8 Hz, $H_{2'}$/$H_{6'}$), 7.28, 7.63 (s, 2H, -NH). ^{13}C/APT-NMR (100 MHz, $CDCl_3$, *δ*, ppm): 55.42, 55.87 (-OCH_3), 55.46 (C_6), 97.46 (C_5), 110.52 ($C_{3''}$), 114.36 (C3'/$C_{5'}$), 121.04 ($C_{2'}$/$C_{6'}$), 126.03 ($C_{1''}$), 126.52 ($C_{4''}$), 127.54 ($C_{6''}$), 129.67 ($C_{1'}$), 129.67 ($C_{5''}$), 134.76 (C_4), 156.20 ($C_{2''}$), 160.53 ($C_{4'}$), 175.40 (C_2). LC-QTOF-MS: (*m/z*) (%) $[M+Na]^+$: 349.0972 (100), calc. 349.0975.

4-(4-Methoxyphenyl)-6-(3-methoxyphenyl)-3,4-dihydropyrimidine-2(1H)-thione (25)

Yield: 60%, mp = 155-156 °C, Rf: 0.70 (*n*-hexane–ethyl acetate, 1:1); FTIR (ATR, cm^{-1}): 3200 (-NH), 3102 (=CH), 2838, 2971 (-CH), 1566,1606 (C=C, aromatic ring), 1247 (-OCH_3), 1268 (C=S); UV-vis λ nm (log): 226 (4.345), 268 (2.212); ^{1}H-NMR (400 MHz, $CDCl_3$, *δ*, ppm), 3.74, 3.75 (s, 6H, -OCH_3), 5.06-5.07 (m, 1H, H_6), 5.13-5.14 (m, 1H, H_5), 6.77-6.90 (m, 5H, $H_{3'}$/$H_{5'}$/$H_{2''}$/$H_{4''}$/ $H_{6''}$), 7.22-7.26 (m, 1H, $H_{5''}$), 7.39-7.41 (m, 2H, $H_{2'}$/$H_{6'}$), 8.89, 9.36 (s, 2H, -NH). ^{13}C/APT-NMR (100 MHz, $CDCl_3$, *δ*, ppm) : 55.30, 55.41, 55.45 (-OCH_3), 64.17 (C_6), 99.48 (C_5), 112.64 ($C_{2''}$), 112.94 ($C_{4''}$), 113.97 ($C_{3'}$/$C_{5'}$), 118.90 ($C_{6''}$), 126.10 ($C_{1'}$), 127.37 ($C_{2'}$/$C_{6'}$), 129.95 ($C_{5''}$), 134.40 (C_4), 145.85 ($C_{1''}$), 155.58 ($C_{4'}$), 156.78 ($C_{3''}$), 175.40 (C_2). LC-QTOF-MS: (*m/z*) (%) $[M+Na]^+$: 349.0995 (100), calc. 349.0993.

4-(4-Methoxyphenyl)-6-(2,3-dimethoxyphenyl)-3,4-dihydropyrimidine-2(1H)-thione (26)

Yield: 73%, mp = 102-103 °C, Rf: 0.69 (*n*-hexane–ethyl acetate, 1:1); FTIR (ATR, cm^{-1}): 3197 (-NH), 3002, 3100 (=CH), 2833, 2963 (-CH), 1599, 1607 (C=C, aromatic ring), 1252 (-OCH_3), 1282 (C=S); UV-vis λ nm (log): 227 (4.182), 264 (3.542); ^{1}H-NMR (400 MHz, $CDCl_3$, *δ*, ppm), 3.70-3.91 (m, 9H, -OCH_3), 5.15 (s, 1H, H_6), 5.60 (s, 1H, H_5), 6.90-7.10 (m, 5H, $H_{4''}$/$H_{5''}$/ $H_{6''}$/$H_{3'}$/ H5'), 7.28-7.38 (m, 2H, $H_{2'}$/$H_{6'}$), 6.90, 7.84 (s, 2H, -NH). ^{13}C/APT-NMR (100 MHz, $CDCl_3$, *δ*, ppm): 51.31, 55.45, 55.93 (-OCH_3), 61.13 (C_6), 98.40 (C5), 112.58 (C4''), 114.34 (C3'/C5'), 119.47 (C6''), 124.62 (C5''), 126.59 (C2'/C6'), 125.95 (C1''), 134.14 (C1'), 135.60 (C_4), 145.78 ($C_{2''}$), 152.63 ($C_{3''}$), 160.51 ($C_{4'}$), 175.48 (C_2). LC/ MS-TOF: (*m/z*) (%) $[M+Na]^+$: 379.1085 (100), calc. 379.1087.

4-(4-Methoxyphenyl)-6-(2,5-dimethoxyphenyl)-3,4-dihydropyrimidine-2(1H)-thione (27)

Yield: 74%, mp = 241-242 °C, Rf: 0.68 (*n*-hexane–ethyl acetate, 1:1); FTIR (ATR, cm^{-1}): 3127 (-NH), 2835, 2959 (-CH), 1599, 1608 (C=C, aromatic ring), 1244 (-OCH_3), 1271 (C=S); UV-vis λ nm (log): 242 (0.425), 275 (0.410); ^{1}H-NMR (400 MHz, $CDCl_3$, *δ*, ppm), 3.78, 3.86 (s, 9H, -OCH_3), 5.17 (s, 1H, pyrimidine-H_5), 5.61 (s, 1H, H_6), 6.84-6.99 (m, 5H, $H_{3''}$/$H_{4''}$/ $H_{6''}$/$H_{3'}$/ $H_{5'}$), 7.41-7.43 (m, 2H, $H_{2'}$/$H_{6'}$), 7.62, 8.12 (s, 2H, -NH), ^{13}C/APT-NMR (100 MHz, $CDCl_3$, δ, ppm): 50.87, 55.41 (-OCH3), 55.84 (C6), 97.19 (C5), 111.34 ($C_{4''}$), 113.27 ($C_{6''}$), 114.33 ($C_{3''}$), 114.37 ($H_{3'}$/$H_{5'}$), 126.55 ($H_{2'}$/$H_{6'}$), 126.55 ($C_{1''}$), 130.85 ($C_{1'}$), 135.11 (C_4), 150.38 ($C_{2''}$), 154.04 ($C_{5''}$), 160.55 ($C_{4'}$), 175.58 (C_2). LC-QTOF-MS: (*m/z*) (%) $[M+Na]^+$: 379.1063 (40), calc.379.1067.

4-(4-Methoxyphenyl)-6-(2,3,4-trimethoxyphenyl)-3,4-dihydropyrimidine-2(1H)-thione (28)

Yield: 61%, mp = 124-125 °C, Rf: 0.70 (*n*-hexane–ethyl acetate, 1:1); FTIR (ATR, cm^{-1}): 3192 (-NH), 2850, 2920 (-CH), 1557,1599 (C=C, aromatic ring), 1247 (-OCH_3), 1283 (C=S); UV-vis λ nm (log): 309 (0.426), 327 (0.315); ^{1}H-NMR (400 MHz, $CDCl_3$, *δ*, ppm), 3.85-3.99 (m, 12H, -OCH_3), 5.11-5.14 (m, 1H, H_6), 5.51-5.52 (m, 1H, H_5), 6.69 (m, 1H, $H_{4''}$), 6.68 (d, 1H, *J*=8 Hz, $H_{6''}$), 6.94 (d, 2H, *J*=8 Hz, $H_{3'}$/$H_{5'}$), 7.45 (d, 2H, *J*=8 Hz, $H_{2'}$/$H_{6'}$), 6.86, 7.59 (s, 2H, -NH). ^{13}C/APT-NMR (100 MHz, $CDCl_3$, *δ*, ppm): 51.20, 55.42, 56.06, 60.85 (-OCH_3), 61.23 (C_6), 98.05 (C_5), 107.19 ($C_{5''}$), 114.37 ($C_{3'}$/$C_{5'}$), 122.23 ($C_{6''}$), 125.96 ($C_{1''}$), 126.47 ($C_{2'}$/$C_{6'}$), 127.53 ($C_{1'}$), 134.34 (C_4), 141.90 ($C_{3''}$), 150.71 ($C_{2''}$), 154.01 ($C_{4''}$), 160.53 ($C_{4'}$), 175.22 (C_2). LC-QTOF-MS: (*m/z*) (%) $[M+Na]^+$: 409.1192 (100), calc. 409.1199.

Tyrosinase Inhibition Assay of 15-28

The tyrosinase inhibitory impacts of the compounds were assayed in line with our previous research with slight modifications [21]. Kojic acid was utilized as a standard compound, whereas DMSO (1%) was used as a negative control. The solutions were newly prepared in phosphate buffer (100 mM, pH 6.8). The compounds (12.5-250 µM) in wells were treated with tyrosinase from mushroom (Sigma-Aldrich, T3824) (250 U/mL, 20 µL). The incubation of the mixtures was done for a period of 15 min at room temperature. After incubation, 3,4-dihydroxy-*L*-phenylalanine (*L*-DOPA) (Sigma-Aldrich, D9628) was added to the wells with the objective of starting the enzymatic reaction, and the incubation of the mixture was performed for a 15 min period at room temperature. Optical density was found to be 475 nm with a microplate reader. The IC_{50} values were computed with the formula below; % inhibition: (A-B) × 100 / A, where A refers to the activity of tyrosinase without the compounds, and B denotes tyrosinase activity with the compounds [21].

DPPH Radical Scavenging Assay of 15-28

The DPPH radical scavenging properties of the compounds were assayed following our previous research [21]. Ascorbic acid was used as a standard compound, whereas DMSO (1%) was utilized as a negative control. DPPH solution (0.2 mM) (Sigma-Aldrich, D9132) was prepared in methanol. DPPH solution, and the compounds (100 µM) were added to each other in wells. The incubation of the mixtures was carried out for 30 min at room temperature in the dark. Optical density (OD) was found to be 517 nm with a microplate reader. The IC_{50} values were computed with the formula above [22].

Molecular Docking

Protein Preparation

The 3D structure of the *Agaricus bisporus* tyrosinase enzyme (PDB ID: 2Y9X) was retrieved from the Protein Data Bank (https://www.rcsb.org/). Water molecules, bound ligand compounds and metal ion groups were simply excluded from the structure. The protonation states of 2Y9X were calculated by Propka 3.1 software [16-17] at pH=7.0. For the receptor protein, all basic amino acids were positively charged, while acidic amino acids were negatively charged by employing the Kollman charges embedded within AutoDock software. The 3D structures of ligand compounds were also prepared before molecular docking studies. OpenBabel software was utilized to build specific pdbqt files for each compound [18]. Each compound was also charged by executing the Gasteiger charges in AutoDock Vina. The prepared ligands and target protein structures were eventually used as input files during the execution of AutoDock Vina-based molecular docking.

Statistical analysis

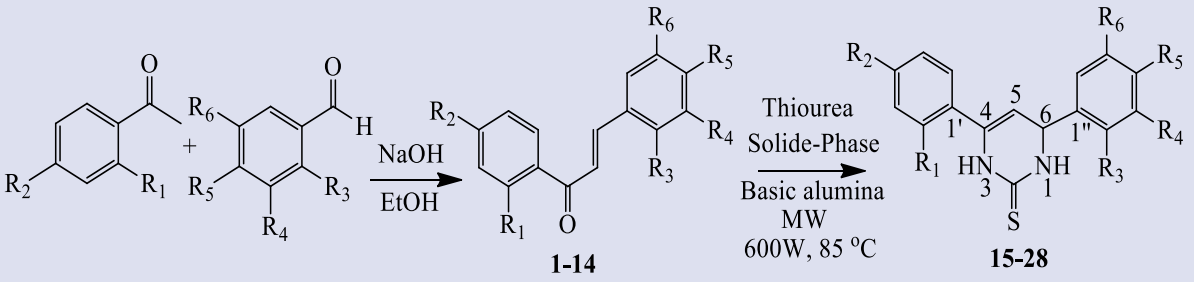
GraphPad Prism 5.0 was utilized to analyze the data. The results are presented as mean ± standard deviation (n = 3). The differences between the compounds and standard compound were studied by conducting a one-way analysis of variance (ANOVA), followed by Tukey's tests.

Results and Discussion

Chemistry

In order to find a new potential TI, a diverse range of methoxy DH-Pyr-S (15-28) were designed and synthesized in two step. The complete synthetic scheme of target DH-Pyr-S (15-28) is shown in Scheme 1. The first step was the

base-catalyzed condensation of the methoxy acetophenone with methoxy benzaldehydes in ethanol solvent to yield intermediate methoxy chalcones (1-14). The second step was the condensation of methoxy chalcones with thiourea by the solid-phase MW method to give methoxy DH-Pyr-S (15-28) analogous [14-15]. Structure of target DH-Pyr-S (15-28) were identified by their spectroscopic data (^{1}H NMR, ^{13}C/APT NMR, FT-IR, UV-vis, and LC-QTOF-MS). The spectral data of already known methoxy chalcones (1-14) [19-20] and compounds 15-16 [14-15] have been reported. However, the spectral data of the all new methoxy DH-Pyr-S (17-28) are given in the experimental part. In the literature, DH-Pyr-S compounds have been synthesized by reflux, and MW methods [7-8, 10-11, 14-15].



No	R_1	R_2	R_3	R_4	R_5	R_6
15	-H	$-OCH_3$	-H	-H	-H	-H
16	-H	$-OCH_3$	-H	-H	$-OCH_3$	-H
17	$-OCH_3$	-H	-H	-H	-H	-H
18	$-OCH_3$	-H	$-OCH_3$	-H	-H	-H
19	$-OCH_3$	-H	-H	$-OCH_3$	-H	-H
20	$-OCH_3$	-H	-H	-H	$-OCH_3$	-H
21	$-OCH_3$	-H	$-OCH_3$	$-OCH_3$	-H	-H
22	$-OCH_3$	-H	$-OCH_3$	-H	-H	$-OCH_3$
23	$-OCH_3$	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	-H
24	-H	$-OCH_3$	$-OCH_3$	-H	-H	-H
25	-H	$-OCH_3$	-H	$-OCH_3$	-H	-H
26	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	-H	-H
27	-H	$-OCH_3$	$-OCH_3$	-H	-H	$-OCH_3$
28	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	$-OCH_3$	-H

Figure 2. N_2 adsorption isotherm of the Ni/GCE and Ni@s-rGO/GCE

Biological Activities of Compounds (15-28)

Tyrosinase Inhibition of 15-28

In a continuation of our prior investigations to find out a new TI [22], methoxy DH-Pyr-S were studied for their antityrosinase activity. All the synthetic methoxy DH-Pyr-S (15-28) showed moderate to good inhibition against tyrosinase enzyme (Table 1) [21]. IC_{50} values of 15-28 and the reference kojic acid (IC_{50} = 55.38 ± 2.30 µM) are in the table 1. The IC_{50} values of 15-28 ranged from 31.86 ± 2.45 to 229.77 ± 8.033 µM. Among all the synthesized methoxy DH-Pyr-S, the compound 17 exhibited excellent tyrosinase inhibitory activity with the lowest IC_{50} = 31.86 ± 2.45µM. Therefore, compounds 21 (44.58 ± 0.46 µM) and 16 (IC_{50} = 48.47 ± 0.66 µM) may be useful as a potential lead candidate to cure tyrosinase-mediated hyperpigmentation in the future. In the literature, cytotoxicity of eleven synthesized 1,2,3,4-tetrahydro-2-thioxopyrimidine analogs were reported against the growth of two cancer cell lines (B16 melanoma and L1210 leukemia). It was mentioned that two of those analogs exhibited significant activity (IC_{50} < 1 µM for L1210 and <10 µM for B16 cells) [23]. Biological activity of 2-thiopyrimidine derivatives had shown that some of the compound gave good antiinflammatory (37.4% at 100 mg/kg) and analgesic activity (75% at 100 mg/kg). Some of the compounds also had shown moderate analgesic (25–75%), protein kinase (CDK-5, GSK-3), and antiinflammatory (5–20%) inhibitory activities (IC_{50} > 10 µM) [24]. Antiproliferative activities of a fused thiazolo[2,3-b]pyrimidinones were reported against K562 (chronic myelogenous leukemia), HepG2 (hepatocellular carcinoma), MDA-MB-231 (breast cancer), MCF-7 (breast cancer), COLO 205 (colorectal adenocarcinoma) cell lines. Thus, 3,4-dihydropyrimidine-2(1*H*)-one/thione analogs are highly valuable bioactive compounds [9].

Table 1. Tyrosinase inhibition and DPPH of 15-28

Compounds	Tyrosinase (IC_{50} values µM)	DPPH (%) (at 100 µM)
15	128.64 ± 6.70	34.90 ± 0.26
16	48.47 ± 0.66*	42.30 ± 2.50
17	31.86 ± 2.45*	45.64 ± 0.34
18	182.64 ± 4.36	31.87 ± 1.48
19	229.77 ± 8.03	34.35 ± 0.27
20	223.56 ± 4.06	37.95 ± 1.68
21	44.58 ± 0.46*	30.30 ± 0.63
22	> 250	40.39 ± 1.93
23	> 250	36.06 ± 0.34
24	191.22 ± 3.55	34.22 ± 0.35
25	125.70 ± 3.04	33.39 ± 0.77
26	100.59 ± 2.49	39.18 ± 1.36
27	159.35 ± 2.05	36.68 ± 0.53
28	> 250	40.27 ± 0.39
Kojic acid	55.38 ± 2.30	-
Ascorbic acid	-	86.03 ± 0.09

*p<0.0001

DPPH Activity of 15-28

In this study, the DPPH radical scavenging impacts of the compounds were investigated, and Table 1 shows the inhibition ratios (%) of the compounds at 100 µM. Ascorbic acid was utilized as a standard compound. Compound 17 exhibited the best DPPH radical scavenging effect, with 45.64 ± 0.34%, among the compounds tested [22]. According to the results, these compounds had lower radical scavenging effects than ascorbic acid. Literature revealed that DH-Pyr-S gave significant biological activities such as good response to antimicrobial activity and possessed good antioxidant activities [25]. DPPH of various synthesized pyrimidinones and pyrimidinethiones were mentioned and they showed significant antioxidant activity. Significant anticancer activity of pyrimidines against HeLa and HepG2 cell lines were also reported [26]. Synthetic dihydropyrimidines had shown better activity than other compounds due to presence of the electron withdrawing groups [27]. In another work, 2,3-dihydropyrimidin-2(1H)-one derivatives were clearly indicated that the dihydropyrimidines gave both antioxidant and anti-inflammatory activities. Due to the pharmacologic and antimicrobial activities of synthesized tetrahydropyrimidine derivatives have been evaluated as drug candidates [28]. In a study, synthesized pyrimidine-thiones analogs gave good acetylcholinesterase, and human carbonic anhydrase isoforms I and II activities. The antioxidant activity of the DH-Pyr-S was also reported by DPPH and Fe^{+2} chelating activities. The antioxidant, antiinflammatory and antituberculosis screenings of pyrimidine-2-one was reported. The result of all heterocyclic analogs indicated that they all have significant antioxidant and anti-inflammatory activities when compared to standard drugs used. Moderate antituberculosis activity for the synthesized pyrimidine-2-one compounds were also reported. Thus, 3,4-dihydropyrimidine-2(1*H*)-one/thione analogs are highly valuable bioactive compounds. These studies showed that 3,4-dihydropyrimidine-2(1*H*)-one/thione compounds resulted very different biological activities.

ADMET calculations

Pharmacokinetic and toxicity properties of synthesized DH-Pyr-S molecules including adsorption, distribution, metabolism, excretion, and toxicity predictions (ADMET) were predicted accordingly and (pkCSM) program were used for ADMET (Table 2).

Molecular Docking Data

Binding affinities and TI potentials of newly synthesized DH-Pyr-S molecules were examined by employing AutoDock Vina (version 1.2.5) program and the obtained results were summarized in Table 3 [16-18]. Molecular docking results showed that compounds 15, 21 and 24 share the lowest but identical binding free energies (ΔG: -7.9 kcal/mol) for the 2Y9X tyrosinase catalytic core, whereas compounds 17, 18, 21 and 25 have the potential to form 2 hydrogen bonds with the 2Y9X catalytic residues.

Among newly synthesized DH-Pyr-S molecules, tyrosinase binding affinities of two compounds with the lowest experimental half-maximal inhibitory concentration (IC_{50}) values were investigated in detail to decipher their interaction modes to 2Y9X. For this, 2Y9X-17 and 2Y9X-21 complexes from molecular docking studies were comprehensively studied using the Discovery studio Accelyrs program (BIOVIA, Dassault Systèmes). Both compound 17 and compound 21 showed higher binding affinity to the tyrosinase active site than kojic acid, with low binding free energies of −7.6 kcal/mol and −7.9 kcal/mol, respectively (Table 3). Furthermore, the presence of numerous alkyl and pi-alkyl interactions between tyrosinase and compound 21 (2Y9X-21 complex), as well as Pi-sigma and carbon-hydrogen bond interactions, appeared to contribute to the strong TI activity. This suggests that the multiple hydrophobic interactions as well as robust H bonds identified from the 2Y9X-21 complex are able to promote the tyrosinase inhibitory activity of compound 21.

Considering its low IC_{50} value, the biochemical interaction pattern of compound 17 was also investigated through 2D plot analysis. The descriptive biochemical interaction analysis revealed that compound 17 also forms two close hydrogen bonds with 2Y9X catalytic residues ASN260 and ARG268 (Figure 1A and 1B). This explains the experimentally proved stronger tyrosinase inhibition potential of 17 compared to both 15 and 16 (Figures 1A-B). Furthermore, in-depth intermolecular interaction analysis revealed that the catalytic site of 2Y9X is more accessible for compounds 15, 21, and 24 than compound 17, suggesting that the basic scaffold of these compounds is well suited for catalytic core of 2Y9X (Table 3). On the other hand, molecular docking data also suggested compounds 15 and 24 as promising tyrosinase inhibitors with high binding affinity to 2Y9X with a low

binding free energy of -7.9 kcal/mol. However, *in silico* predictions for these compounds did not correlate well with the experimental IC_{50} values (Table 3). Taking together, both *in vitro* experimental outputs and *in silico* molecular docking findings elucidated that among synthesized molecules, 21 is the leading compound for tyrosinase inhibition.

Table 2. ADMET analysis of synthesized DH-Pyr-S molecules

Property	ADMET Properties	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Absorption	Water solubility (log mol/L)	-3.874	-3.991	-3.961	-4.084	-4.084	-4.084	-4.236	-4.236	-4.404	-3.991	-3.991	-4.144	-4.143	-432
	Caco2 permeability (log Papp in 10-6cm/s)	1.797	1.916	1.685	1.803	1.803	1.803	1.144	1.144	1.136	1.916	1.916	1.144	1.144	1.136
	Intestinal absorption (human) (% Absorbed)	90.327	90.435	90.786	90.894	90.894	90.894	91.002	90.787	91.007	90.435	90.435	90.543	90.328	90.548
	Skin Permeability (log Kp)	-2.798	-2.864	-2.804	-2.875	-2.875	-2.875	-2.921	-2.928	-2.943	-2.864	-2.864	-2.905	-2.911	-2.923
	P-glycoprotein substrate	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	P-glycoprotein I inhibitor	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	P-glycoprotein II inhibitor	No	No	No	No	No	No	No	No	Yes	No	No	No	No	Yes
Distribution	VDss (human) (log L/kg)	0.245	0.105	0.305	0.166	0.166	0.166	0.0243	0.0039	0.108	0.105	0.105	0.004	-0,025	-0,172
	Fraction unbound (human) (Fu)	0.091	0.09	0.094	0.092	0.092	0.092	0.089	0.089	0.085	0.09	0.09	0.088	0.088	0.084
	BBB permeability (log BB)	0.297	0.049	0.3	0.053	0.053	0.053	-0,195	-0,195	-0,563	0,049	0,049	-0,199	-0,199	-0,566
	CNS permeability (log PS)	-1.215	-1.277	-1.159	-1.221	-1.221	-1.221	-2.205	-2.194	-2.358	-1.277	-1.277	-2.205	-2.199	-2.363
Metabolism	CYP2D6 substrate	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	CYP3A4 substrate	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	CYP1A2 inhibitior	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	CYP2C19 inhibitior	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	CYP2C9 inhibitior	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	CYP2D6 inhibitior	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	CYP3A4 inhibitior	No	No	No	No	No	No	Yes	No	Yes	No	No	No	No	Yes
Excretion	Total Clearance (log ml/min/kg)	-0.222	-0.193	-0.037	0.116	-0.222	0,009	-0,243	0.207	0.325	-0,069	-0,186	0.059	0.023	0.141
	Renal OCT2 substrate	Yes	Yes	Yes	No	No	No	No	No	No	Yes	Yes	Yes	No	No
Toxicity	AMES toxicity	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Max. tolerated dose (log mg/kg/day)	-0.046	-0.136	0.009	-0,082	-0,082	-0,082	-0,142	-0,142	-0,159	-0,136	-0,136	-0,195	-0,195	-0,211
	hERG I inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	hERG II inhibitor	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Oral Rat Acute Toxicity (LD50) (mol/kg)	2.691	2.747	2.741	2.797	2.797	2.797	2.855	2.843	2.908	2.747	2.747	2.803	2.791	2.855
	Oral Rat Chronic Toxicity (LOAEL) (log mg/kg_bw/day)	1.693	1.422	1.713	1.432	1.432	1.432	1.085	1.085	1.055	1.422	1.422	1.138	1.138	1.108
	Hepatotoxicity	Yes	No	No	No	Yes	Yes	Yes	No	No	Yes	No	Yes	No	No
	Skin Sensitisation	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	T.pyriformis toxicity (log ug/L)	1.849	1.884	1.782	1.827	1.827	1.827	1.718	1.714	1.451	1.884	1.884	1.762	1.757	1.479
	Minnow toxicity (log mM)	0.298	0.349	0.137	0.188	0.188	0.188	0.337	0.337	0.585	0.349	0.349	0.498	0.498	0.745

Table 3. Estimated binding affinity of docked compounds (16, 17, and 21) against *Agaricus bisporus* tyrosinase and the profile of the interacting residues in the binding site

Ligand ID	2Y9X Binding energy (kcal/mol)	H bond number
15	-7.9	1
16	-7.4	NA
17	-7.6	2
18	-7.7	2
19	-7.3	NA
20	-7.4	1
21	-7.9	2
22	-7.4	2
23	-7.2	NA
24	-7.9	1
25	-7.6	2
26	-7.1	NA
27	-7.3	NA
28	-6.9	NA
Kojic acid	-5.7	2

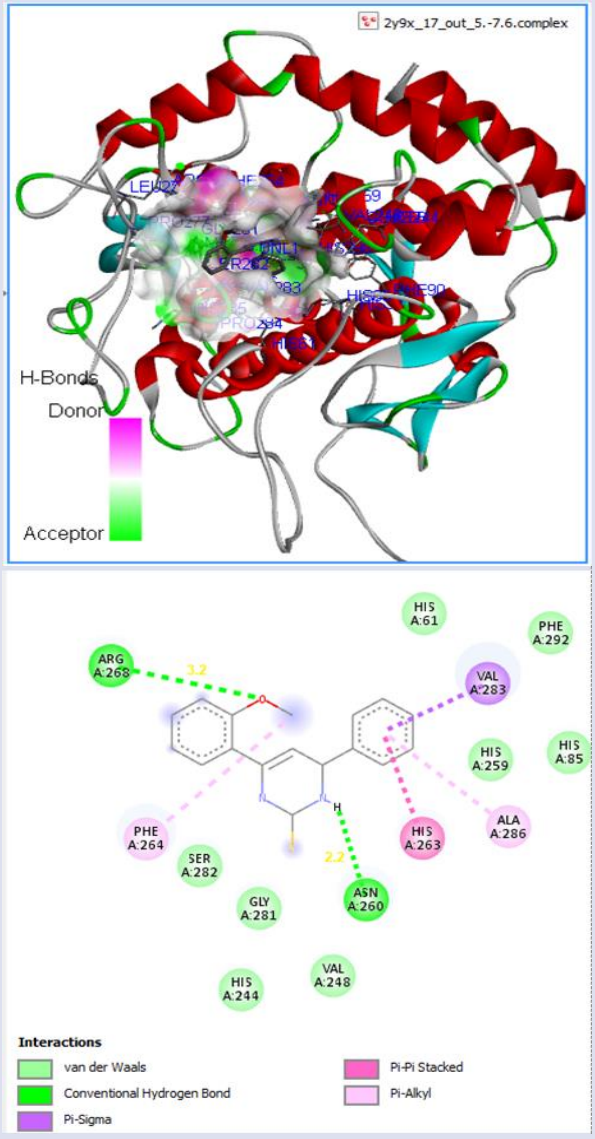


Figure 1. A. Binding pose profile of 2Y9X-17 complex. B. 2D plot analysis of interacting residues with compound 17.

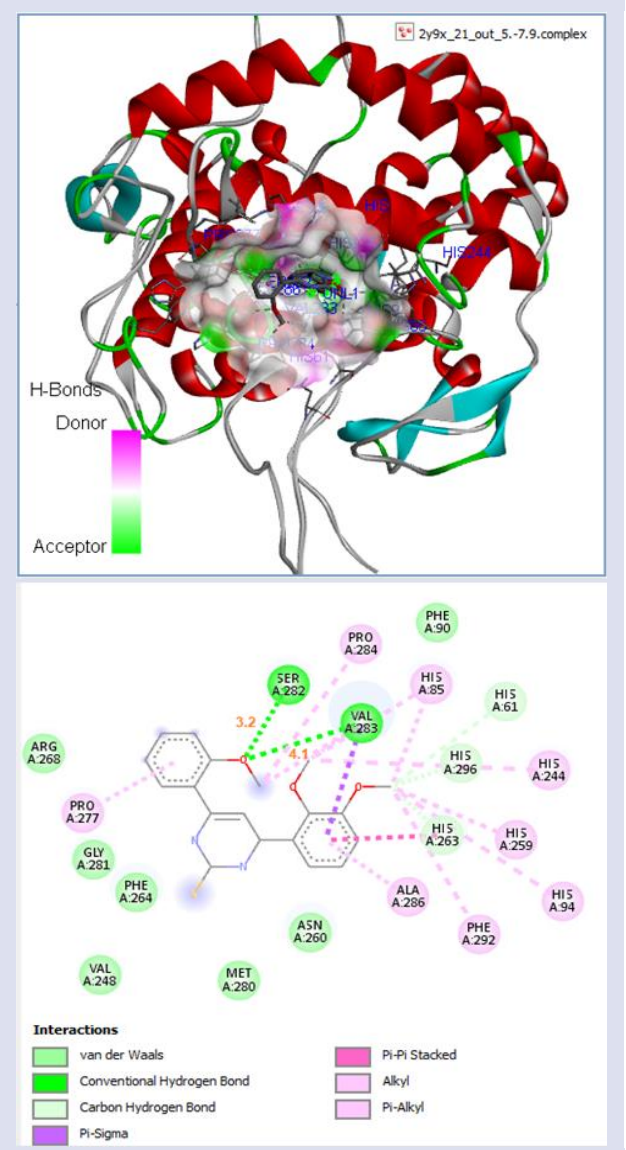


Figure 2. A. Binding pose profile of 2Y9X-21 complex. B. 2D plot analysis of interacting residues with compound 21

In the literature, docking study for the dihydropyrimidine compounds as a potential inhibitors of gyrase enzyme were reported and one of the dihydropyrimidine compound had shown significant inhibition with binding free energies higher than novobiocin that was used as standard [29].

Conclusion

In summary, a series of new methoxy DH-Pyr-S (17-28) were synthesized using known methodologies starting from methoxy acetophenone and methoxy benzaldehydes over two steps reaction. Structure of them were identified by various spectral techniques (NMR, FT-IR, UV, and LC-QTOF-MS). All the target methoxy DH-Pyr-S compounds (17-28) were tested against the tyrosinase enzyme. In order to find activities of methoxy DH-Pyr-S in terms of potent TI, we herein, report that some of the synthetic methoxy DH-Pyr-S analogs are potential new TI.

Among the synthesized methoxy DH-Pyr-S, the new compound 17 exhibited excellent TI with the lowest IC_{50} value of 31.86 ± 2.45 µM. Furthermore, compounds 21 (IC_{50} = 44.58 ± 0.46 µM) and 16 (IC_{50} = 48.47 ± 0.66 µM) may prove to be effective inhibitors against the tyrosinase enzyme. All the synthesized target methoxy DH-Pyr-S (15-28) exhibited weak DPPH activities. A molecular modeling studies of methoxy DH-Pyr-S (16, 17, and 21) were performed against 2Y9X enzyme to find the binding interactions against the target protein. The best binding affinities of these molecules (15, 21, and 24) to the tyrosinase catalytic core were found to be -7.9 kcal/mol. All three compounds showed higher binding affinity to the tyrosinase active site compared to the standard molecule kojic acid. Calculated molecular docking studies of the most active compounds 16, 17, and 21 revealed that they could be considered as TI candidates. Moreover, *in silico* binding analyzes specific to the synthesized molecules revealed that the catalytic core of 2Y9X was more accessible to compounds 15, 21, and 24; however, this suggests that functional groups added to compound 17 can be used to modify these three lead compounds towards the development of more selective tyrosinase inhibitors.

Ultimately, experimental and theoretical analysis results confirmed that DH-Pyr-S molecules could be a good starting point for the development of more selective tyrosinase inhibitors.

Acknowledgment

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Conflicts of interest

There are no conflicts of interest in this work.

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