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RESEARCH ARTICLE

Synthesis and theoretical calculations of carbazole substituted chalcone urea derivatives and studies their polyphenol oxidase enzyme activity

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Abstract

Synthesis of carbazole substituted chalcone urea derivatives and their polyphenol oxidase enzyme activity effects on the diphenolase activity of banana tyrosinase were evaluated. Tyrosinase has been purified from banana on an affinity gel comprised of Sepharose 4B-L-tyrosine-p-aminobenzoic acid. The results showed that most of the compounds (**3,4,5a,5d–h**) inhibited and some of them (**5c,5i–l**) activated the tyrosinase enzyme activity. The molecular calculations were performed using Gaussian software for the synthesized compounds to explain the experimental results.

Keywords: Carbazole containing chalcone, urea, thiourea, tyrosinase inhibitors

Introduction

Polyphenol oxidase (PPO), sometimes referred to as phenol oxidase, catecholase, phenolase, catechol oxidase, or even tyrosinase, is considered to be an o-dipenol[1]. PPO (EC 1.14.18.1), a multifunctional copper containing enzyme, is widely distributed in nature. It catalyzes two distinct reactions of melanin synthesis: a hydroxylation of monophenols to o-diphenols (monophenolase activity) and an oxidation of o-diphenols to o-quinones (diphenolase activity), both using molecular oxygen[2]. PPO is responsible, not only for melanisation in animals, but also for browning in plant. The unfavourable enzymatic browning of fruits and vegetables generally results in a loss of nutritional and commercial value[3]. An important group is constituted by compounds structurally analogous to phenolic substrate, which generally show competitive inhibition with respect to this substrate, although it can vary depending on enzyme source and substrate used[4].

Chalcones are one of the major classes of natural products with widespread distribution in fruits, vegetables, spices, tea and soy based foodstuff. They have recently received a great deal of attention due to their interesting pharmacological activities[5]. Some natural prenylated chalcones showed potent tyrosinase inhibitory activity. Three chalcones derivatives that are licuraside, isoisoliquritin, and Licochalcone A were isolated from the roots of the Glycyrrhiza species and competitively inhibited the monophenolase activity of mushroom tyrosinase[6]. Many biological activities have been attributed to this group, such as anticancer and antioxidant[7], antifungal[8], antimicrobial[9] and antibacterial[10] activities. A number of chalcone derivatives, have also been found to inhibit several important enzymes in cellular systems, including xanthine oxidase[11], heme oxygenase[12], protein tyrosine kinase[13], quinone reductase[14] and tyrosinase[15,16].

Nitrogen heterocycles have received a great deal of attention in the literature because of biological properties.

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Especially, among these heterocyclic systems, pyridine containing compounds have been the subject of expanding research efforts in heteroaromatic and biological chemistry[17]. The pyrido [2,3-d] pyrimidine heterocycles, which are those annelated to a pyrimidine ring, are important because of their wide range of biological[18] and pharmaceutical applications (i.e. bronchodilators, vasodilators) and their anti-allergic, cardiotonic, antihypertensive, and hepatoprotective activities[19].

In the present study, we have synthesized derivatives of carbazole substituted chalcone urea for evaluation as potential inhibitors of PPO that could be beneficial in the prevent enzymatic browning.

Materials and methods

General procedure of the carbazole substituted chalcone urea derivatives

Melting points were taken on a Yanagimoto micro-melting point apparatus and are uncorrected. IR spectra were measured on a SHIMADZU Prestige-21 (200 VCE) spectrometer. ^{1}H and ^{13}C NMR spectra were measured on spectrometer at VARIAN Infinity Plus 300 and at 75 Hz, respectively. ^{1}H and ^{13}C chemical shifts are referenced to the internal deuterated solvent. The elemental analysis was carried out with a Leco CHNS-932 instrument Flash column chromatography was performed using Merck silica gel 60 (230–400 mesh ASTM). All chemicals was purchased from Merck, Alfa Easer, Sigma-Aldrich and Fluka.

Synthesis of carbazole substituted chalcone urea derivatives was prepared according to Scheme 1.

*Acetylation of 9-ethylcarbazole (**2**)*

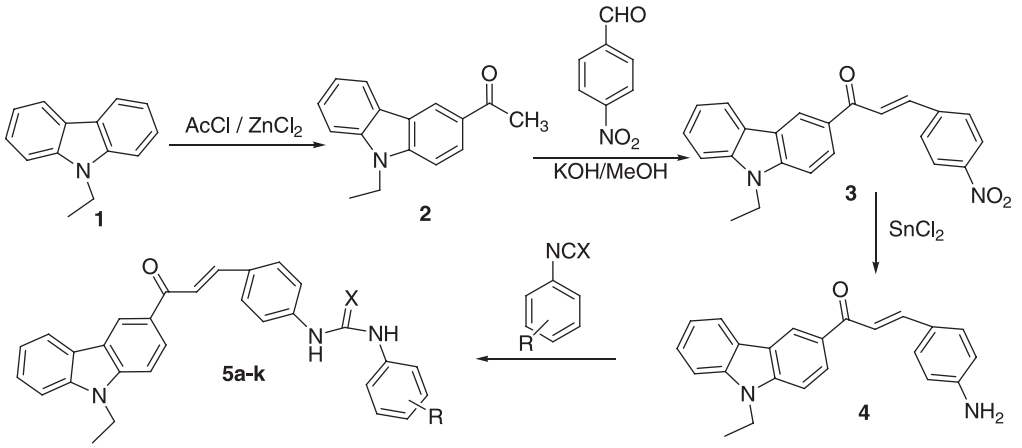
3-Acetyl-9-ethylcarbazole (**2**) necessary for the subsequent Claisen condensation was synthesized[20] in one step starting from 9-ethyl-carbazole (**1**) (7.80 g, 40 mmol) and acetyl chloride (7 mL, 100 mmol). The Friedel – Crafts acylation is commonly catalyzed by $BiCl_3$ (20.50 g, 65 mmol).

*General procedure for the synthesis of chalcones (**3**)*

The most useful method used to prepare chalcones is the condensation of acetophenones with benzaldehydes. Equimolar portions of 3-acetyl-9-ethylcarbazole (3.97 g, 16.77 mmol) and 4-nitrobenzaldehyde (2.48 g, 16.41 mmol) were dissolved in 30 mL of methanol. A 30 mL aliquot of 20% aqueous potassium hydroxide solution was then slowly added dropwise to the reaction mixture. The reaction solution was allowed to stir at room temperature for 24 h. The solution was extracted with AcOEt with an aqoues phase. Organic phase washed three times with water, dried with anhydrous magnesium sulphate and evaporated under reduced pressure.

*1-(9-Ethyl-9H-carbazol-3-yl)-3-(4-nitro-phenyl)-propenone (**3**)*

Recrystallized from ether to give as light yellow crystals. Yield 69.2%, m.p. 231°C; IR (KBr, υ, cm^{-1}): 3070 (C=C, aromatic), 1660 (C=O), 1589 (NO_2); $^{1}HNMR$ (300 MHz, $CDCl_3$,



Compound	R	X
5a	4-F	O
5b	4-NO_2	O
5c	4-NO_2	S
5d	2-F	S
5e	4-Cl	S
5f	H	S
5g	3-I	S
5h	3,5-dichlor	S
5i	3-F	S
5j	2,4-dichlor	S
5k	3-CF_3	S

Scheme 1. Synthesis of carbazole substituted chalcone urea derivatives.

δ, ppm): 8.85 (1H, s, = CH), 7.81-7.82 (1H, d, = CH), 7.33–8.30 (10H, m, - Ar-H), 7.27–7.31 (1H, t, = CH), 4.39–4.46 (2H, q, - CH_2), 1.46–1.61 (3H, t, - CH_3). Anal. Calcd. for $C_{23}H_{18}N_2O_3$: C, 74.58; H, 4.90; N, 7.56. Found: C, 74.40; H, 4.974; N, 7.492.

*General procedure for the synthesis of amino chalcones (**4**)*

A mixture of **3** (1.1 g, 3.24 mmol), $SnCl_2$ (3.66 g, 16.22 mmol) and tetrahydrofuran (30 mL) was stirred under reflux for 4 h. When the reaction completed, the solvent was evaporated under reduced pressure and the reaction mixture was diluted with water (15 mL), adjusted to pH = 10 with 1M sodium hydroxide solution, extracted with ethyl acetate (3 × 25 mL). The organic phase was dried over $MgSO_4$ and filtered.

*3-(4-Amino-phenyl)-1-(9-ethyl-9H-carbazol-3-yl)-propenone (**4**)*

Recrystallized from ether to give as dark red crystals. Yield 50%, m.p. 152°C; IR (KBr, υ, cm^{-1}): 1589 (- NH_2), 3052 (C=C, aromatic), 1624 (C=O); ^{1}HNMR (300 MHz, $CDCl_3$, δ, ppm): 9.17 (1H, s, = CH), 8.39-8.42 (1H, d, = CH), 8.30-8.33 (1H, d, = CH), 7.92–7.97 (1H, d, = CH), 7.32–7.80 (5H, m, - Ar-H), 7.51–7.56 (1H, t, = CH), 7.30–7.35 (1H, t, = CH), 6.71–6.74 (2H, d, = CH), 4.48–4.50 (2H, q, -CH_2), 4.47 (2H, s, -NH_2), 1.33–1.36 (3H, t, -CH_3). Anal. Calcd. for $C_{23}H_{20}N_2O$: C, 81.15; H, 5.92; N, 8.23. Found: C, 79.25; H, 6.03; N, 7.56.

General procedure for synthesis of ureas and thioureas

A solution of 3-(4-amino-phenyl)-1-(9-ethyl-9H-carbazol-3-yl)-propenone **(4)** (0.19 g, 0.559 mmol) and isocyanate or isothiocyanate derivatives (0.096 g, 0.704 mmol) in dry DMF (2 mL) was stirred at 40°C for 6 h, quenched with water and filtered.

*1-{4-[3-(9-Ethyl-9H-carbazol-3–yl)-3-oxo-propenyl]-phenyl}-3-(4-fluoro-phenyl)-urea (**5a**)*

Recrystallized from ethyl acetate/hexane to give as light brown powder. Yield 88.89%, m.p. 285.3°C; IR (KBr, υ, cm^{-1}): 3292 (-NH), 1707 (C=O), 1178 (C-F), 831 (C-H, aromatic); ^{1}HNMR (300 MHz, DMSO-d_6, δ, ppm): 8.67 (1H, s, -NH), 8.35 (1H, s, -NH), 8.14 (1H, s, = CH), 8.03-,Ar-H), 4.21–4.28 (2H, q, - CH_2), 1.26–1.31 (3H, t, - CH_3). Anal. Calcd. for $C_{30}H_{24}FN_3O_2$: C, 75.46; H, 5.07; N, 8.80. Found: C, 74.06; H, 4.67; N, 7.90.

*1-{4-[3-(9–Ethyl-9H-carbazol-3-yl)-3-oxo-propenyl]-phenyl}-3-(4-nitro-phenyl)-urea (**5b**)*

Recrystallized from eter/acetone/hexane to give as brown powder. Yield 85.9%, m.p. 172.4°C; IR (KBr, υ, cm^{-1}): 3252 (-NH), 3050 (C=C, aromatic), 1707 (C=O), 1501 (NO_2); ^{1}HNMR (300 MHz, DMSO-d_6, δ, ppm): 9.56 (1H, s, -NH), 9.26 (1H, s, -NH), 9.16 (1H, s = CH), 8.37–8.40 (1H, d, = CH), 8.30–8.31 (1H, d, = CH), 8.27–8.28 (1H, d, = CH), 8.21–8.24 (1H, d, = CH), 7.95–8.10 (1H, d, = CH), 7.57–7.91 (9H, m, - Ar-H), 7.30–7.33 (1H, t, = CH), 7.52–7.54 (1H, t, =CH), 4.52–4.54 (2H, q, - CH_2), 1.34–1.39 (3H, t, - CH_3). Anal. Calcd. for $C_{30}H_{24}N_4O_4$: C, 71.42; H, 4.79; N, 11.10. Found: C, 70.40; H, 4.19; N, 10.25.

*1-{4-[3-(9-Ethyl-9H-carbazol-3-yl)-3–oxo-propenyl]-phenyl}–3-(4-nitro-phenyl)-thiourea (**5c**)*

Recrystallized from acetone/hexane to give as light brown powder. Yield 85.3%, m.p. 215.5°C; IR (KBr, υ, cm^{-1},): 3225 (-NH), 3058 (C=C, aromatic), 1179 (C=S), 1591 (NO_2); ^{1}HNMR (300 MHz, DMSO-d_6, δ, ppm): 10.12 (1H, s, -NH), 10.01 (1H, s, -NH), 8.80 (1H, s, = CH), 8.07–8.16 (4H, m, - Ar-H), 7.81–7.84 (1H, d, = CH), 7.21–7.74 (11H, m, - Ar-H), 4.35–4.38 (2H, q, - CH_2), 1.35–1.40 (3H, t, - CH_3). Anal. Calcd. for $C_{30}H_{24}N_4O_3S$: C, 69.21; H, 4.65; N, 10.76; S, 6.16. Found : C, 68.18; H, 4.35; N, 10.75; S, 5.19.

*1-{4-[3-(9-Ethyl-9H-carbazol-3-yl)-3-oxo-propenyl]-phenyl}-3-(2-fluoro-phenyl)-thiourea (**5d**)*

Recrystallized from ethyl acetate/hexane to give as light brown powder. Yield 88.6%, m.p. 252°C; IR (KBr, υ, cm^{-1}): 3329 (-NH), 3019 (C=C, aromatic), 1194 (C=S), 1178 (C-F); ^{1}HNMR (300 MHz, DMSO-d_6, δ, ppm): 10.24 (1H, s, -NH), 9.69 (1H, s, -NH), 9.16 (1H, s, = CH), 8.14–8.18 (1H, d, = CH), 7.30–8.37 (15H, m, - Ar-H), 4.50–4.52 (2H, q, - CH_2), 1.33–1.36 (3H, t, - CH_3). Anal. Calcd. for $C_{30}H_{24}FN_3OS$: C, 73.00; H, 4.90; N, 8.51; S, 6.50. Found : C, 71.89; H, 4.45; N, 8.15; S, 5.29.

*1-(4-Chloro-phenyl)-3-{4-[3-(9-ethyl-9H-carbazol-3-yl)-3-oxo-propenyl]-phenyl}-thiourea (**5e**)*

Recrystallized from acetone/hexane to give as orange powder. Yield 77.6%, m.p. 163.9°C; IR (KBr, υ, cm^{-1}): 3262 (-NH), 2981 (C=C, aromatic), 2260 (-N=C=S), 748 (C-Cl); ^{1}HNMR (300 MHz, DMSO-d_6, δ, ppm): 10.16 (1H, s, -NH), 10.09 (1H, s, -NH), 9.16 (1H, s, = CH), 8.37–8.40 (1H, d, = CH), 8.31–8.32 (1H, d, = CH), 8.28–8.29 (1H, d, = CH), 7.92–7.95 (1H, d, =CH), 7.30–7.80 (12H, m, - Ar-H), 4.45–4.54 (2H, q, - CH_2), 1.31–1.38 (3H, t, - CH_3). Anal. Calcd. for $C_{30}H_{24}ClN_3OS$: C, 70.64; H, 4.74; N, 8.24; S, 6.29. Found : C, 70.04; H, 4.24; N, 7.24; S, 5.19.

*1-{4-[3-(9-Ethyl-9H-carbazol-3-yl)-3–oxo-propenyl]-phenyl}-3-phenyl-thiourea (**5f**)*

Recrystallized from eter/ethyl acetate/hexane to give as orange powder. Yield 91%, m.p. 171.8°C; IR (KBr, υ, cm^{-1}): 3327 (-NH), 3053 (C=C, aromatic), 2187 (-N=C=S); ^{1}HNMR (300 MHz, DMSO-d_6, δ, ppm): 10.07 (1H, s, -NH), 10.01 (1H, s, -NH), 9.15 (1H, s, = CH), 8.13–8.17 (1H, d, = CH), 7.16–8.38 (16H, m, - Ar-H), 4.50–4.52 (2H, q, - CH_2), 1.33–1.36 (3H, t, - CH_3). Anal. Calcd. for $C_{30}H_{25}N_3OS$: C, 75.76; H, 5.30; N, 8.84; S, 6.74. Found : C, 75.06; H, 4.50; N, 7.94; S, 5.74.

*1-{4-[3-(9-Ethyl-9H–carbazol-3-yl)-3-oxo-propenyl]-phenyl}-3-(3-iodo-phenyl)-thiourea (**5g**)*

Recrystallized from acetone/hexane to give as brown powder. Yield 76.9%, m.p. 207.8°C; IR (KBr, υ, cm^{-1}): 3307 (-NH), 3048 (C=C, aromatic), 1192 (C=S), 622 (C-I); ^{1}HNMR (300MHz, DMSO-d_6, δ, ppm): 10.22 (1H, s, -NH), 10.07 (1H, s, -NH), 9.16 (1H, s, = CH), 8.37–8.40 (1H, d, = CH), 8.29-8.31 (1H, d, = CH), 8.14–8.19 (1H, d, = CH), 7.50–7.99 (11H, m, - Ar-H), 7.30–7.35 (1H, t, = CH),

7.13–7.19 (1H, t, = CH), 4.51–4.53 (2H, q, - CH_2), 1.34–1.38 (3H, t, -CH_3). Anal. Calcd. for $C_{30}H_{24}IN_3OS$: C, 59.90; H, 4.02; N, 6.99; S, 5.33. Found : C, 58.73; H, 3.48; N, 6.598; S, 4.18.

1-(3,5-Dichloro-phenyl)-3-{4-[3-(9-ethyl-9H-carbazol-3-yl)-3-oxo-propenyl]-phenyl}-thiourea (5h)

Recrystallized from acetone/hexane to give as brown powder. Yield 64.1%, m.p. 201.4°C; IR (KBr, υ, cm^{-1}): 3294 (-NH), 3077 (C=C, aromatic), 2039 (-N=C=S), 750 (C-Cl); ^{1}HNMR (300 MHz, DMSO-d_6, δ, ppm): 9.47 (1H, s, -NH), 9.41 (1H, s, -NH), 8.71 (1H, s, =CH), 6.99–8.10 (15H, m, -Ar-H), 4.23–4.30 (2H, q, - CH_2), 1.28–1.34 (3H, t, - CH_3). Anal. Calcd. for $C_{30}H_{23}Cl_2N_3OS$: C, 66.18; H, 4.26; N, 7.72; S, 5.89. Found : C, 65.28; H, 3.56; N, 6.92; S, 4.89.

1-{4-[3-(9-Ethyl-9H-carbazol-3-yl)-3-oxo-propenyl]-phenyl}-3-(3–fluoro-phenyl)-thiourea (5i)

Recrystallized from ethyl acetate/hexane to give as dark orange powder. Yield 71.9%, m.p. 184.2°C; IR (KBr, υ, cm^{-1}): 3319 (-NH), 2037 (-N=C=S), 1192 (C-F), 748 (C-H, aromatic); ^{1}HNMR (300 MHz, DMSO-d_6, δ, ppm): 10.17 (1H, s, -NH), 10.12 (1H, s, -NH), 10.06 (1H, s, = CH), 9.13 (1H, s, = CH), 8.28–8.29 (1H, d, = CH), 8.25–8.26 (1H, d, = CH), 8.10–8.15 (1H, d, = CH), 7.89–7.93 (1H, d, = CH), 6.91–7.74 (11H, m, - Ar-H), 4.46–4.53 (2H, q, - CH_2), 1.31–1.36 (3H, t, - CH_3). Anal. Calcd. for $C_{30}H_{24}FN_3OS$: C, 73.00; H, 4.90; N, 8.51; S, 6.50. Found : C, 68.71; H, 4.07; N, 8.45; S, 6.79.

1-(2,4-Dichloro-phenyl)–3-{4-[3-(9-ethyl-9H-carbazol-3-yl)-3-oxo-propenyl]-phenyl}-thiourea (5j)

Recrystallized from ether/acetone/hexane to give as brown powder. Yield 83.6%, m.p. 193.8°C; IR (KBr, υ, cm^{-1}): 3329 (-NH), 3038 (C=C, aromatic), 1194 (C=S), 789 (C-Cl); ^{1}HNMR (300 MHz, DMSO-d_6, δ, ppm): 10.34 (1H, s, -NH), 9.69 (1H, s, -NH), 9.16 (1H, s, = CH), 8.37–8.40 (1H, d, = CH), 8.30–8.32 (1H, d, = CH), 8.14–8.19 (1H, d, = CH), 7.95–7.97 (1H, d, = CH), 7.30–7.89 (11H, m, - Ar-H), 4.51–4.53 (2H, q, - CH_2), 1.33–1.36 (3H, t, - CH_3). Anal. Calcd. for $C_{30}H_{23}Cl_2N_3OS$: C, 66.18; H, 4.26; N, 7.72; S, 5.89. Found : C, 64.80; H, 3.84; N, 7.15; S, 4.70.

1-{4-[3-(9-Ethyl-9H–carbazol–3- yl)–3-oxo-propenyl]-phenyl}-3-(3-trifluoromethyl-phenyl)-thiourea (5k)

Recrystallized from acetone/hexane to give as orange powder. Yield 73.7%, m.p.199.4°C; IR (KBr, υ, cm^{-1}): 3316 (-NH), 3058 (C=C, aromatic), 1175 (C=S), 1106 (C-CF_3); ^{1}HNMR (300 MHz, DMSO-d_6, δ, ppm): 10.30 (1H, s, -NH), 10.21 (1H, s, -NH), 9.15 (1H, s, = CH), 8.37–8.39 (1H, d, = CH), 8.31–8.32 (1H, d, = CH), 8.28–8.29 (1H, d, = CH), 7.49–8.19 (13H, m, -Ar-H), 7.30–7.35 (1H, t, = CH), 4.51–4.54 (2H, q, - CH_2), 1.34–1.39 (3H, t, - CH_3). Anal. Calcd. for $C_{31}H_{24}F_3N_3OS$: C, 68.49; H, 4.45; N, 7.73; S, 5.90. Found : C, 69.06; H, 4.37; N, 7.55; S, 5.12.

Purification of PPO

All purification steps were carried out at 25°C. The extraction procedure was adopted from Wesche-Ebeling & Montgomery[21]. The bananas were washed with distilled water three times to prepare the crude extract, 50 g of bananas were cut quickly into thin slices and homogenized in a Waring blender for 2 min using 100 ml of 0.1 M phosphate buffer, pH:7.3 containing 5% poly(ethylene glycol) and 10 mM ascorbic acid. After filtration of the homogenate through muslin, the filtrate was centrifuged at 15,000*g* for 30 min, and the supernatant was collected. A crude protein precipitate was made by adding $(NH4)_2SO_4$ to 80% saturation. The resulting precipitate was suspended in a minimum volume of 5 mM phosphate buffer and then dialyzed against 5 the same buffer overnight. The enzyme solution was then applied to the Sepharose 4B-tyrosine-p-amino benzoic acid affinity column[22], pre equilibrated with 5 mM phosphate buffer, pH 5.0. The affinity gel was extensively washed with the same buffer before the banana PPO (BPPO) was eluted with 1M NaCl, 5 mM phosphate, pH 7.0.

BPPO activity

Enzyme activity was determined; using catechol, by measuring the increase in absorbance at 420 nm[23], in a Biotek automated recording spectrophotometer. Enzyme activity was calculated from the linear portion of the curve. One unit of PPO activity was defined as the amount of enzyme that causes an increase in absorbance of 0.001 unit's min^{-1} for 1 ml of enzyme at 25°C.

Inhibition of BPPO activity

The inhibition type of diarylureas was determined by Lineweaver-Burk plots of 1/V vs. 1/S at two inhibitor concentrations. The inhibition constant, Ki, was deduced from the points of interception of the plots.

Results and discussion

Carbazole containing amino, nitro and urea chalcone derivatives were synthesized and examined effect on polyphenol oxidase enzyme. N-ethyl-3-acetylcarbazole (**2**) was prepared with N-ethylcarbazole and acetylchloride by $ZnCl_2$ ctalayst. Carbazole containing nitro (**3**) and amino (**4**) chalcones prepared by the condensing various acetophenones and 4-nitrobenzaldehyde with NaOH as base, were reducted with tin (II) chloride in ethanol.The 4-aminochalcones were reacted with isocyanates and isothiocyanates to get the products (**5a–l)** at high yields.

The prepared compounds were characterized by ^{1}H NMR, ^{13}C NMR, IR and elemental analysis. The hydrogens attached to the nitrogen rezonans synthesized urea and thiourea compounds between 8.50 and 10.40 ppm and was indicated from the ^{1}H NMR spectra. The signals for aromatic hydrogens and vinylic protons are between 6.55 and 8.15 ppm. From the ^{13}C NMR spectra,chalcone and urea carbonyl are seen at around 188 and 150 ppm respectively. In the infrared spectra of compounds **5a–k**, it was possible to observe the absorptions between 3250 and 3450 cm^{-1} relating to NH stretch, absorptions in 1600–1650 cm^{-1} from α, β-unsaturated carbonyl moiety stretch and absorptions in 1650–1750 cm^{-1} from urea carbonyl moiety stretching.

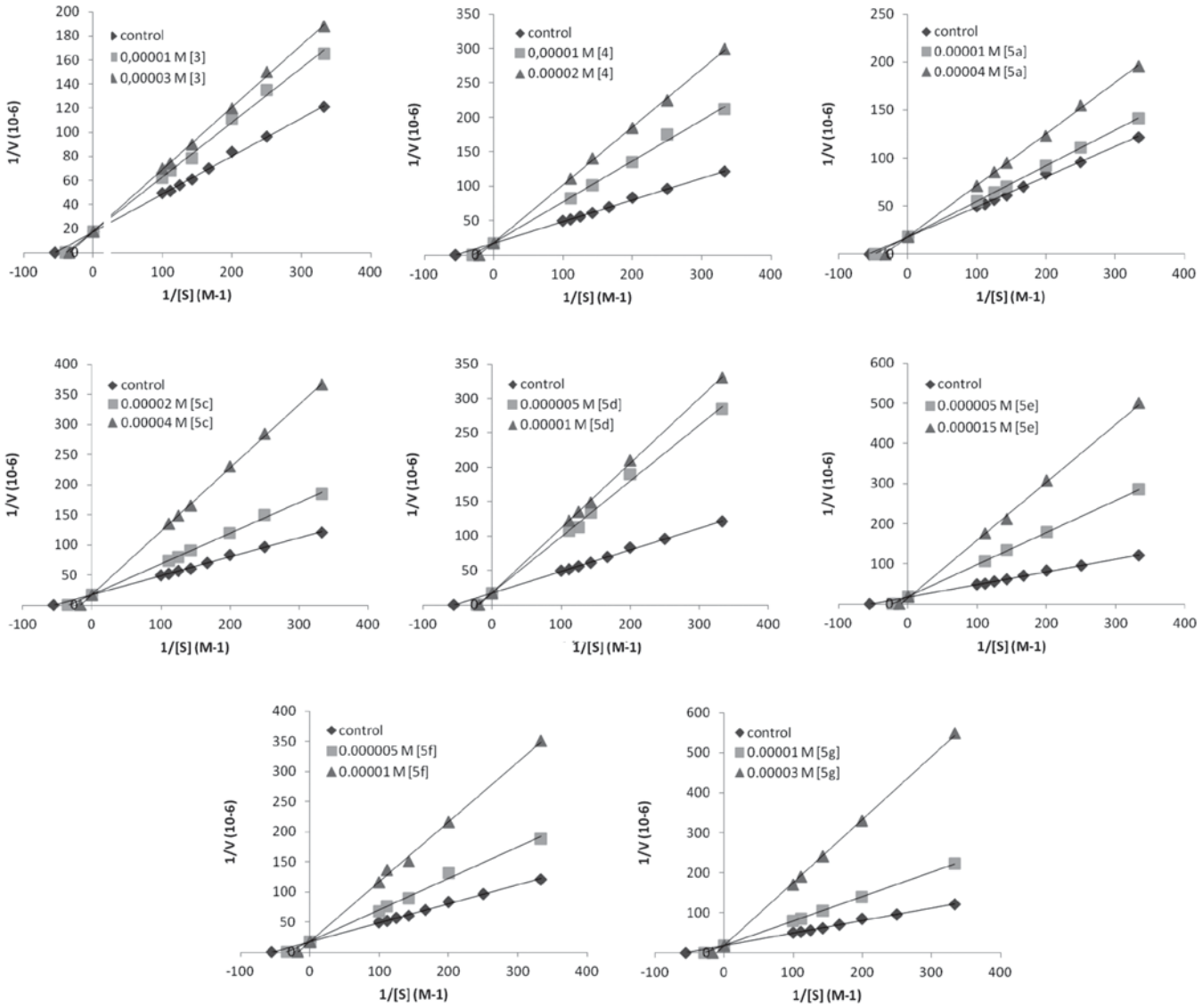


Figure 1. Ki graphics of chalcone urea derivatives on BPPO.

For evaluating the tyrosinase inhibitory activity, all the prepared compounds were subjected to tyrosinase inhibition assay with catechol as substrate. The results showed that most of the compounds (**3, 4, 5a, 5c–g**) inhibited and some of them (**5b, 5h–k**) activated the tyrosinase enzyme activity. Ki values were calculated from inhibition curves obtained with ureas (Figure 1). Ki values of 19.97, 14.96, 24.96, 29.91, 7.47, 9.96, 7.48, 19.95 μM were obtained for **((3), (4), (5a), (5c), (5d), (5e), (5f),** and **(5g)** respectively (Table 1). According to Ki values, **(5d)** was the most effective inhibitor for BPPO.

Table 1. Ki inhibition constants, dipol moment and $\Delta E_{HOMO\text{-}LUMO}$ (eV) values of the compounds.

Compound	Activity	Ki (μM)	Dipol moment (D)	$\Delta E_{HOMO\text{-}LUMO}$ (eV)
3	inhibitor	19.97	7.8467	0.24562
4	inhibitor	14.96	5.9614	0.25245
5a	inhibitor	24.96	4.4375	0.23121
5b	activator	—	3.4041	0.22925
5c	inhibitor	29.91	7.2517	0.23055
5d	inhibitor	7.47	7.1944	0,27405
5e	inhibitor	9.96	4.8791	0.24775
5f	inhibitor	7.48	4.3063	0.25965
5g	inhibitor	19.95	—*	—*
5h	activator	—	3.9318	0.22423
5i	activator	—	3.2735	0.22948
5j	activator	—	2.0184	0.22959
5k	activator	—	1.4482	0.22934

*Could not be calculated by the Gaussian.

Several compound reported as PPO inhibitors were also shown to have inhibitors effect on the BPPO. Among the tested anti-browning reagent, the most effective ones were dithiotreithol and sodium metabisulfite[24]. The action of sulfite in the prevention of enzymatic browning can usually be explained by several processes. One is the action on o-quinones. The formation of quinone-sulfite complexes prevents the quinone polymerization. The action of sulfite in the prevention of enzymatic browning can usually be explained by several processes. One is the action on o-quinones. The formation of quinone-sulfite complexes prevents the quinone polymerization[25]. The enzyme also seemed to be sensitive to thiourea since PPO contains copper as a co-factor, the irreversible

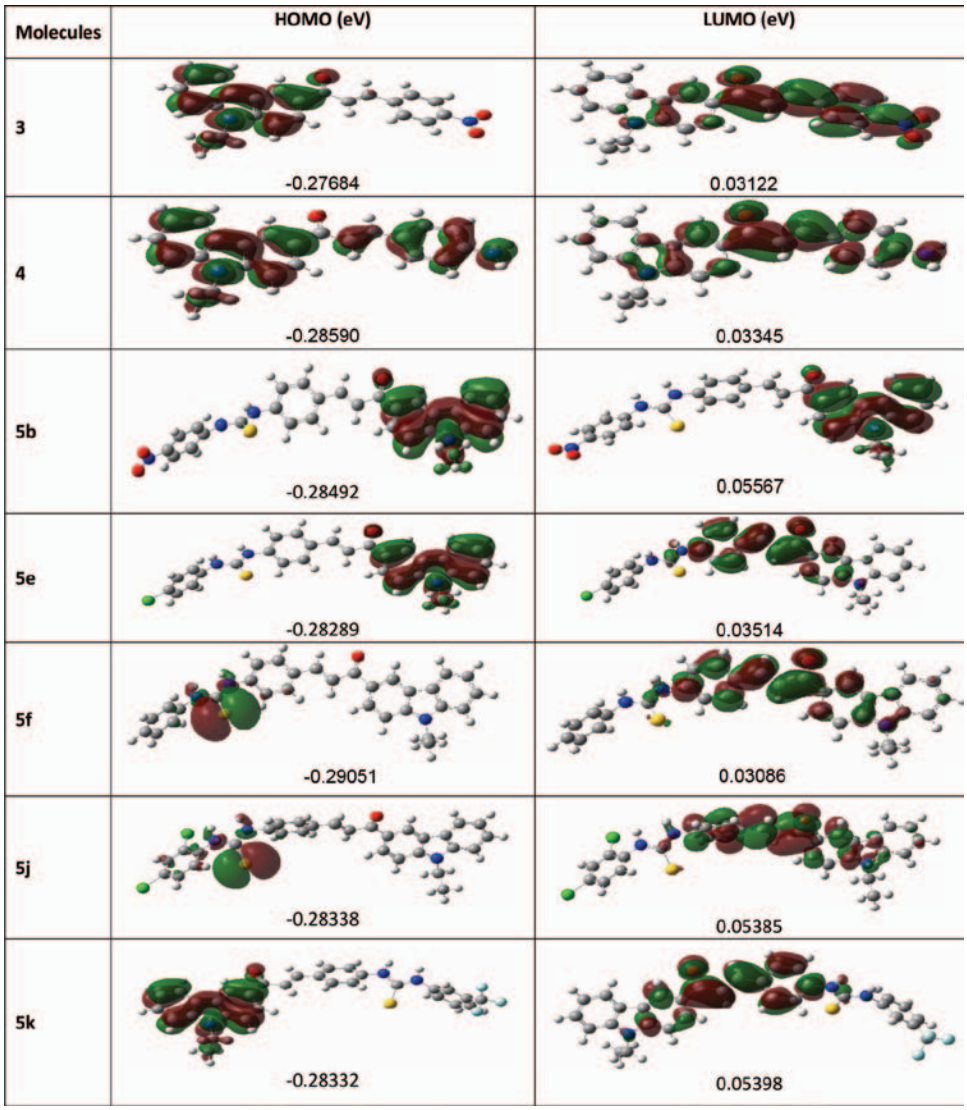


Figure 2. Calculated geometric structures of the some compounds using HF method with 6-31G basis set.

inactivation of this enzyme can be effected by substances (such as thiol compounds thiourea, -hydroxyquinoline, etc.), which remove copper from the active site of the enzyme[26]. Chilaka et al. reported that thiourea was a good inhibitor of PPO, with low Ki values of 0.15 mM and inhibition of PPO was uncompetitive[27]. We were determined that **(3), (4), (5a), (5c), (5d), (5e), (5f),** and **(5g)** was a better inhibitor of PPO according to thiourea. Gulcin et al. reported that sodium diethyl dithiocarbamate was the most effective inhibitor (Ki: 1.79 nM) on nettle PPO[28]. It was reported that the enzyme inhibited with diarylureas[15] and phenylthiourea[29], propanoic acid[30], alkyldithiocarbonates[31] and coumarin schiff-bases[32], n-alkyl dithiocarbamate compounds[33], sodium diethyl dithiocarbamate[28].

In order to understand and explain the experimental results obtained, molecular calculations were performed using Gaussian software[34a,b], for the synthesized compounds and some of the molecular structures are shown in Figure 2. Some of the prepared compounds **(5b, 5h–5k)** did not inhibit tyrosinase at 50 μM, as well as show activator effects on tyrosinase enzyme. The other compounds **(3,4, 5a, 5c–5g)** showed enzyme inhibitory effects. Carbazole substituted chalcones (**3** and **4**) are precursor for the final products which are carbazole substituted chalcone urea derivatives. The compounds **(3)** and **(4)** are good inhibitory effect on tyrosinase enzyme. That can be explained either HOMO-LUMO energy differences or electron releasing and electron withdrawing groups on the phenyl ring. The compound (**4**) is better inhibitory effect than (**3**) because HOMO-LUMO energy differences of **4** (0.25245 eV) higher than **3** (0.24562 eV). In addition to this, amine group on the phenyl ring donates electron to the carbonyl group of chalcone and higher electron density binds copper ions more effectively in the active cite of enzyme. Urea derivatives **(5a, 5c–5g)** have enzyme inhibitory effect due to HOMO-LUMO energy differences. Generally, differentiation between HOMO and LUMO reflects the intensity of electron affinity, and

lower differentiation suggests higher electron affinity[35]. The calculated HOMO-LUMO energy differences of the compounds(**(5a, 5c–5g)** showed a linear relationship for higher inhibitory effects with increasing $\Delta E_{HOMO\text{-}LUMO}$ values which are higher than 0.23eV and the compounds **(5b, 5h–5k)** which have $\Delta E_{HOMO\text{-}LUMO}$ values lower than 0.23eV showed enzyme activity effects. Among the urea compounds, **5d** (Ki, 7.47 μM and $\Delta E_{HOMO\text{-}LUMO}$, 0.27405 eV) is the most inhibition effect. **5c** (Ki, 29.91 μM and $\Delta E_{HOMO\text{-}LUMO}$, 0.23055 eV) is the least inhibition effect. This relation probably to be explained that the higher $\Delta E_{HOMO\text{-}LUMO}$ differences of the molecule increases enzyme inhibition activity for possibility of binding to copper ions in the active cite of enzyme. Moreover, the dipole moments of the molecules are higher than 4.0 shows enzyme inhibitory effect. The results are shown in Table 1.

Declaration of interest

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