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Evaluation of new chalcone derivatives as polyphenol oxidase inhibitors

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ABSTRACT

A newly series of 4-(phenylurenyl)chalcone (**4a–j**) and 4′-(phenylurenyl/thiourenyl)chalcone (**9a–l**) derivatives were synthesized and their inhibitory 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 result showed that **4a–j** inhibited the PPO enzyme activity. Conversely, **9a–h** and **9i–l** showed activator effect on tyrosinase enzyme activity.

Chalcones (1,3-diaryl-2-propen-1-ones) one of the major classes of natural products with widespread distribution in fruits, vegetables, spices, tea and soy based foodstuff have been recently the subjects of great interest for their interesting pharmacological activities.[1] They contain two aromatic rings with an unsaturated chain. Many biological activities have been attributed to this group, such as antitumoral,[2] anticancer and antioxidant,[3] antifungal,[4] antimitotic,[5] chemoprotective,[6] anti-inflammatory,[7,8] antimicrobial,[9] anti-nociceptive[10] and antibacterial[11] activities. A number of chalcone derivatives have also been found to inhibit several important enzymes in cellular systems, including xanthine oxidase,[12] aldose reductase,[13] heme oxygenase,[14] protein tyrosine kinase,[15,16] quinone reductase[17] and tyrosinase.[15] Additionally, the radioiodinated chalcones have been useful amyloid imaging agents for detecting b-amyloid plaques in the brain of Alzheimer's disease.[18] Reactions of 4-aminochalcones with isocyanates give unsymmetrically substituted urea derivatives which are linked to a series of biological activities including antiglycating,[19] MCH-R1 antagonists,[20] $P2Y_1$ receptor antagonists,[21] heparanase inhibitors,[22] anti-HIV,[23] cytostatic and antioxidant,[24] and proliferation inhibitors[25] properties.

Tyrosinase (monophenol or *o*-diphenol, oxygen oxidoreductase, EC 1.14.18.1), also known as polyphenol oxidase (PPO), is a copper-containing monooxygenase that is widely distributed in microorganisms, animals, and plants.[26] Tyrosinase could catalyze two distinct reactions involving molecular oxygen in the hydroxylation of monophenols to *o*-diphenols (monophenolase) and in the oxidation of *o*-diphenols to *o*-quinones (diphenolase).[27] Due to the high reactivity, quinines could polymerize spontaneously to form higher molecular weight brown pigments (melanins) or react with amino acids and proteins to enhance brown color of the pigment produced.[28,29] Previous reports confirmed that tyrosinase not only was involved in melanising in animals, but also was one of the main causes of most fruits and vegetables quality loss during post harvest handling and processing, leading to faster degradation and shorter shelf life.[30] Recently, investigation demonstrated that various dermatological disorders, such as age spots and freckle, were caused by the accumulation of an excessive level of epidermal pigmentation.[31,32] Tyrosinase has also been linked to Parkinson's and other neurodegenerative diseases.[33] In insects, tyrosinase is uniquely associated with three different biochemical processes, including sclerotization of cuticle, defensive encapsulation and melanisation of foreign organism, and wound healing.[34] These processes provide potential targets for developing safer and effective tyrosinase inhibitors as insecticides and ultimately for insect control. Thus, the development of safe and effective tyrosinase inhibitors is of great concern in the medical, agricultural, and cosmetic industries. However, only a few such as kojic acid, arbutin, tropolone, and 1-phenyl-2-thiourea are used as therapeutic agents and cosmetic products.[32,35]

In this study series of 10 new 4-(phenylurenyl)chalcone (**4a–j**) and 4 known[10] and 8 new 4′-(phenylurenyl/thiourenyl)chalcone (**9a–l**) derivatives were synthesized and the effect of them on tyrosinase have been evaluated. The 4-nitrochalcones **2a–d**, prepared by the condensing various acetophenones and 4-nitrobenzaldehyde with NaOH as base, were reducted with tin(II)

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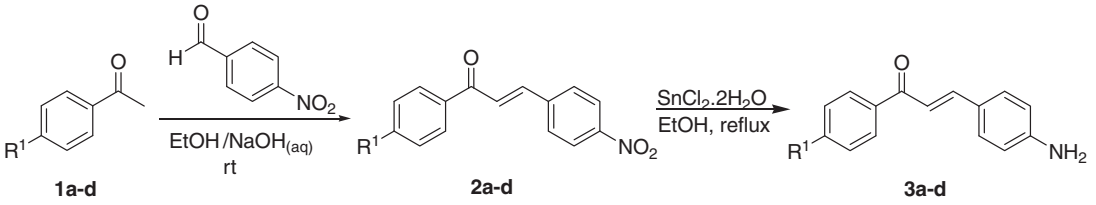
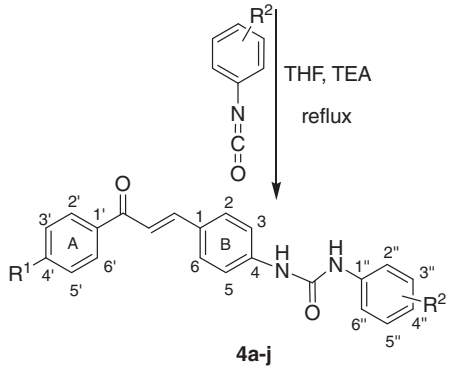
chloride in ethanol.[18] The 4-aminochalcones were reacted with isocyanates to get product 4-(phenylurenyl)chalcone (**4a–j)** (Scheme 1)[25] at high yields but synthesis of 4-(phenylthiourenyl)chalcones with 4-aminochalcone and isothiocyanate were unsuccessful. The syntheses of 4-(phenylurenyl/tiourenyl)acetophenones **7a–d** were obtained by reaction of *p*-aminoacetophenone with phenylisocyanate or phenylisothiocyanate derivatives. The subsequent treatment of these derivatives and substituted benzaldehydes with NaOH, in methanol/DMSO, at room temperature, (Scheme 2)[36] were given 4′-(phenylurenyl/thiourenyl)chalcone (**9a–l**).

All new compounds were characterized by ^{1}H NMR, ^{13}C NMR, IR and MS. In the infrared spectra of compounds **4a–j** and **9a–l**, it was possible to observe the absorptions between 3273 and 3410 cm^{-1} relating to NH stretch, absorptions in 1606–1650 cm^{-1} from α,β-unsaturated carbonyl moiety stretch and absorptions in 1654–1710 cm^{-1} from urea carbonyl moiety stretching. The ^{1}H NMR spectra for all the synthesized urea and thiourea compounds show signals between 8.65 and 10.12 ppm relating to hydrogens attached to the nitrogen. The signals for aromatic hydrogens are between 6.55 and 8.15 ppm. The vinylic protons are in this same regions. Through the ^{13}C NMR data, a sign can be seen about 187.53–189.72 ppm, relating to chalcone carbonyl. This is followed by the sign about 152.50–153.04 ppm for urea carbonyl and 179.67–179.79 ppm for thiourea carbonyl.

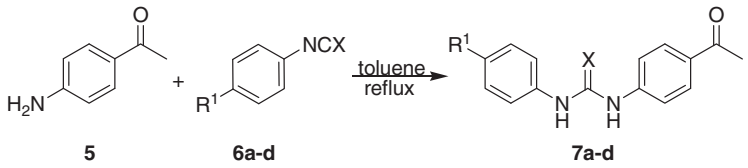
All purification steps were carried out at 25 °C. The extraction procedure was adopted from Wesche-Ebeling & Montgomery.[37] The enzyme was purified by Sepharose 4B-tyrosine-*p*-aminoben-



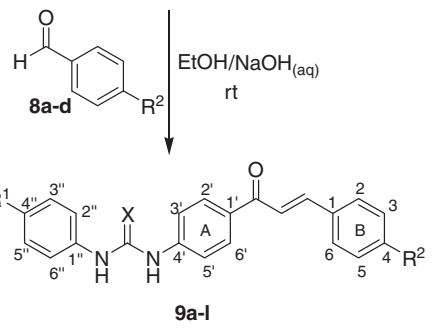
	4a	4b	4c	4d	4e
R^1	H	H	H	H	Cl
R^2	H	4"-F	4"-CH_3	3"-OCH_3	4"-F
	4f	**4g**	**4h**	**4i**	**4j**
R^1	Cl	Cl	CH_3	CH_3	OCH_3
R^2	4"-CH_3	3"-OCH_3	4"-F	4"-CH_3	4"-CH_3



Scheme 1. Synthesis of 4-(phenylurenyl)chalcone derivatives.



	9a	9b	9c	9d	9e	9f
X	O	O	O	O	O	O
R^1	H	H	H	H	CH_3	CH_3
R^2	Br	Cl	CH_3	OCH_3	Br	Cl
	9g	**9h**	**9i**	**9j**	**9k**	**9l**
X	O	O	S	S	S	S
R^1	CH_3	CH_3	H	H	CH_3	CH_3
R^2	CH_3	OCH_3	Cl	OCH_3	Cl	OCH_3



Scheme 2. Synthesis of 4′-(phenylurenyl)chalcone and 4′-(phenylthiourenyl)chalcone derivatives.

Table 1
Inhibitory effect of 4-(phenylurenyl) chalcone derivatives on banana tyrosinase activities

Entry	Catechol	
	IC_{50} (μM)	Type of inhibition (K_i, μM)
4a	0.172 (±0.005)	Competitive (0.037)
4b	0.177 (±0.003)	Competitive (0.125)
4c	0.241 (±0.007)	Competitive (0.057)
4d	0.244(±0.006)	Competitive (0.240)
4e	0.133 (±0.003)	Competitive (0.117)
4f	0.238 (±0.004)	Competitive (0.129)
4g	0.152 (±0.002)	Competitive (0.059)
4h	0.217 (±0.005)	Competitive (0.117)
4i	0.134 (±0.005)	Competitive (0.067)
4j	0.289 (±0.009)	Competitive (0.022)

zoic acid affinity column.[38] Enzyme activity was determined; using catechol, by measuring the increase in absorbance at 420 nm according to the method Espin et al.[39] For evaluating the tyrosinase inhibitory activity, all the synthesized compounds were subjected to tyrosinase inhibition assay with catechol as substrate. The result showed that 4-(phenylurenyl)chalcone derivatives (**4a–j**) inhibited the PPO enzyme activity. On the other hand, 4′-(phenylurenyl)chalcone (**9a–h**) and 4′-(phenylthiourenyl)chalcone derivatives (**9i–l**) showed activator effect on PPO enzyme activity.

The IC_{50} values and inhibition constants of **4a–j** analogues against PPO were summarized in Table 1. We have determined the IC_{50} values of 0.133–0.289 mM for the inhibition of banana PPO. It was determined that they are all competitive inhibitors. The result may be related to the structure of tyrosinase contained a type-3 copper center with a coupled dinuclear copper active site in the catalytic core.

As the concentration of inhibitors raised, the residual enzyme activity drastically decreased. All inhibitors manifested a similar relationship between the enzyme activity and enzyme concentration. From the progress curve obtained, compounds (**4a–j**) showing solid lines below the line of enzyme activity have indicated for enzyme inhibition. In each case, the type of inhibition was deduced from Lineweaver–Burk double reciprocal plots. The inhibitory constants were calculated from secondary plots of apparent K_m, against inhibitor concentration. Good straight lines were obtained, and a typical example is shown in Figure 1 for 4-(phenylurenyl) chalcone **4a**.

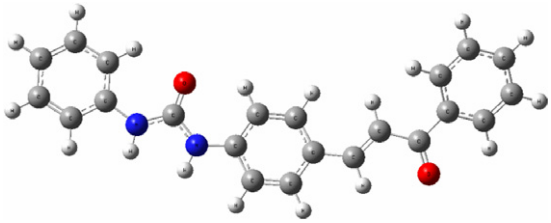
On the other hand, 4′-(phenylurenyl)-chalcone derivatives (**9a–l**) did not inhibit tyrosinase at 50 μM, as well as showed activator effect on tyrosinase enzyme. Generally, differentiation between HOMO and LUMO reflects the intensity of electron affinity, and lower differentiation suggests higher electron affinity.[40] Molecular calculations were performed using Gaussian software.[41] The explanation of the different effect of **4a–j** and **9a–l** on tyrosinase enzyme, we performed quantum chemical calculations of **4i** and **9g** structures to calculate HOMO and LUMO energy levels. HOMO–LUMO energy differences of **4i** (0.23709 eV) were lower than **9g** (0.25085 eV). These results were compatible with the inhibition effect of **4i** (IC_{50} = 0.134 μM) and **9g** (not active). We also calculated planarity of **4i** and **9g**. Our result showed a clear relation between the planar character of 4-(phenylurenyl) chalcones and the potency of these compounds as tyrosinase inhibitors. The E_{LUMO} and the delocation of these orbital, probably favored by the planarity of the molecule, were correlated with the inhibitory efficiency (Fig. 1).

The present investigation reported that 4-(phenylurenyl)chalcone derivatives (**4a–j**) had potent inhibitory effects on the diphenolase activity of banana tyrosinase. Interestingly, compound **4e** was found to be the most potent inhibitor with IC_{50} value of 0.133 μM. On the other hand, 4′-(phenylurenyl)chalcone derivatives (**9a–l**) were totally inactive. Different effects of these compounds were explained with quantum calculation method for structure of **4i** and **9g** which containing same group. Results showed good correlation intensity of electron affinity and inhibition of tyrosinase enzyme.

The enzyme PPO, a copper containing enzyme ubiquitously present in plants, has been studied as a model oxidizing enzyme since it contains metal ion (copper) and utilizes molecular oxygen.[26] Flavonoids, especially chalcones like butein, have been reported to possess a copper chelation activity.[42] The inhibition of PPO may be due to the chelation of copper, which is present in the active site of PPO.

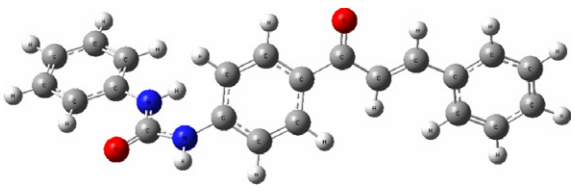
L-cycteine is an effective compound to prevent enzymatic browning. Direct inhibition of polyphenol oxidase by cystein through the formation of stable complexes with copper has also been proposed.[43] The enzyme also seemed to be sensitive to thiourea since PPO contains copper as a co-factor, the irreversible 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.[44]

Detailed information regarding the synthesis, spectroscopic characterization and biological evaluation of all compounds presented in this Letter can be found in the Supplementary data provided.



4i

Energy: -1101.81915184 a.u.
Dipole Moment: 4.1305 Debye
HOMO: -0.29432 eV
LUMO: -0.05569 eV
$\Delta E_{HOMO-LUMO}$= - 0.23863 eV



9g

Energy: -1101.81848948 a.u.
Dipole Moment: 6.0978 Debye
HOMO: -0.30556 eV
LUMO: -0.04168 eV
$\Delta E_{HOMO-LUMO}$= -0.26388 eV

Figure 1. Calculated geometric structures of **4i** and **9g** using HF method with 6-31G basis set.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.09.130.

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