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Antiobesity, antioxidant and cytotoxicity activities of newly synthesized chalcone derivatives and their metal complexes

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Dedicated to Professor R. R. Schmidt on the occasion of his 79th birthday.

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ABSTRACT

Four sets of rationally designed chalcones were prepared for evaluation of their antiobesity, antioxidant and cytotoxicity activities. These sets include nine oleoyl chalcones as mimics of oleoyl estrone, three monohydroxy chalcones (chalcone ligands), Schiff base-derived chalcones and four copper as well as zinc complexes. Oleoyl chalcones **4d**, **4e** and particularly **6a** as an isosteric isomer of oleoyl estrone, were as active as Orlistat on weight loss and related metabolic parameters using male SD rats *in vivo*. Chalcone ligands **10a**–**c** and Schiff base-derived chalcones **11** and **14a**,**b** were weakly antioxidants, while, the copper and zinc complexes **15a**–**d** were good antioxidants with zinc chelates **15b**,**d** being more active than their copper analogues **15a**,**c** *in vitro*. Compounds **10c** and **14a** showed good cytotoxicity activities as Doxorubicin against PC3 cancer cell line *in vitro*, while, the copper complex **15c** showed promising activity with IC_{50} value of 5.95 μM. The estimated IC_{50} value for Doxorubicin was 8.7 μM. Chalcones **14a,b** are bifunctional probes for potential investigations in cancer diagnosis and radiotherapy by complexation with Gd^{3+} or metal radioisotopes followed by posttranslation of Shiga toxin B-subunits that target globotriosyl ceramide expressing cancer cells.

1. Introduction

Chalcones **I** (1,3-diaryl-2-peopen-1-ones) (Scheme 1) are structurally divergent natural products in edible and medicinal plants having benefits for human health from life style to protection from life threatening diseases [1,2]. Besides being natural products, they are precursors for flavones, isoflavones, aurones and anthocyanins which are regarded as cyclic chalcones and have closely related physiological and medical relevancies. Of the biological significant aspects of natural as well as synthetic chalcones are their antiobesity [3,4], antioxidant [5,6] and anticancer activities [7,8].

Oleoyl estrone (OE) **II**, is an estrone hormone synthesized in the white adipose tissue then released into the blood stream in animal and human as well [9–11]. It is able to produce rapid and sustained weight loss through induction of body fat loss while preserving protein stores, a common side effect of antiobesity formulations. Besides being able to reduce circulating plasma lipids, OE is able to reduce plasma insulin and improve insulin resistance [12,13]. It functions through hampering the uptake of substrates essential for lipogenesis and thus favoring lipolysis indirectly, *i.e.* imbalance the lipogenesis–lipolysis equilibrium. Its sustained action arises from its tendency to decrease expression of lipogenic enzyme genes without affecting expression of lipolytic enzyme genes [14]. Obesity accounts for approximately 20% of all cancer cases; weight loss was reported to reduce risk for, at least, breast cancer [15]. The

Abbreviations: OE, oleoyl estrone; ROS, reactive oxygen species; SD, sprague–dawley; Pgp, P-glycoprotein; BCRP, breast cancer resistant protein; MRP1, multi-drug resistance-associated protein 1; TGA, thermal gravimetric analysis; DTG, differential thermogravimetry; TNF-α, tumor necrosis factor-α; FFAs, free fatty acids; PTPs, protein tyrosine phosphatase; IRS-1, insulin receptor substrate-1; LDL, low density lipoprotein; HDL, high density lipoprotein; LFD, low fat diet; HFD, high fat diet; FI, free insulin; ISI, insulin sensitivity index; TG, triglycerides; FBs, fasting blood sugar; DPPH, 1,1-diphenyl-2-picryl-hydrazyl; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; AsAc, ascorbic acid; ELISA, enzyme-linked immunosorbent assay; ANOVA, analysis of variance.

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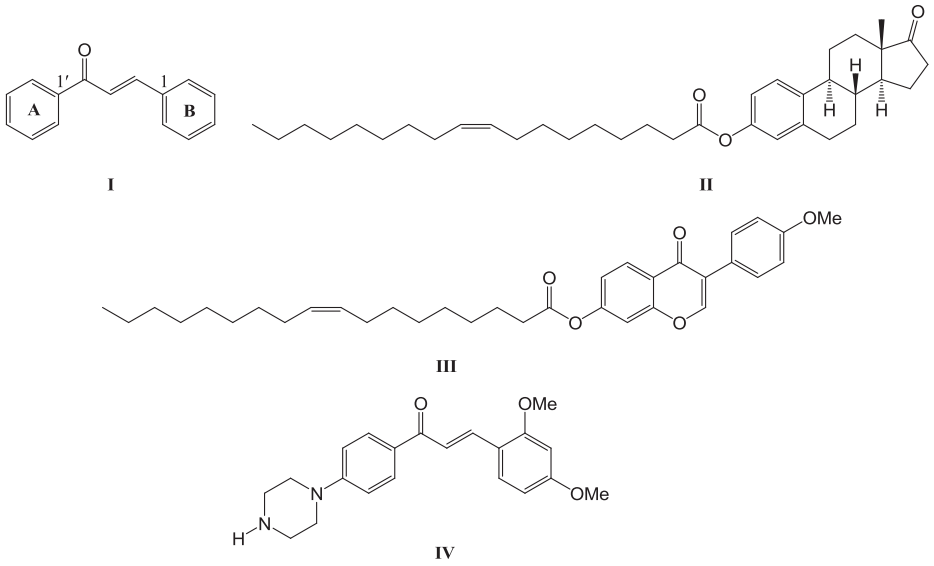


Scheme 1. Structure of model compounds.

physiologic disorders accompanying obesity include hyperglycaemia, insulin resistance and growth factors excretion. All these factors are involved in a complex physiologic pathways lead to hormone receptor-positive tumors or the so-called obesity-related cancer [16].

Based on the known benefits of natural isofavones on human obesity and plasma cholesterol levels, besides, their close similarity to estrogen ring structure, Xiang et al. [17], reported the synthesis of fatty acid substituted isoflavone derivatives, *e.g.* **III**, as potential antiobesity candidates that might retain the antiobesity effect of OE and bypass the estrogenic effect of OE if applied as antiobesity agent. **III** was able to lower the body weight gain, adiposity, improved plasma lipid profile and reduced plasma insulin with low toxicity LD_{50} 2.1638 g/kg. Isoflavone derivatives with other lipid chains were not as active as **III**, thus, reflecting the known importance of the oleoyl moiety for the antiobesity effect [18].

As antioxidants, hydroxylated, methoxylated and prenylated chalcones are known for their powerful antioxidant activities. They are able to scavenge reactive oxygen species (ROS), free radicals and inhibit their implication in damage of cell membranes, DNA, proteins and consequent potential prognosis of ageing, cancer and atherosclerosis [5].

Chalcone derivatives of diverse chemical architectures are quite significant in anticancer drug discovery. They are known to induce apoptosis [19], DNA [20] and mitochondrial damage [21], inhibit angiogenesis [22], tubulin [23], Kinases [24] and drug efflux protein activities [25–27]. They are implemented in cancer diagnosis too [28].

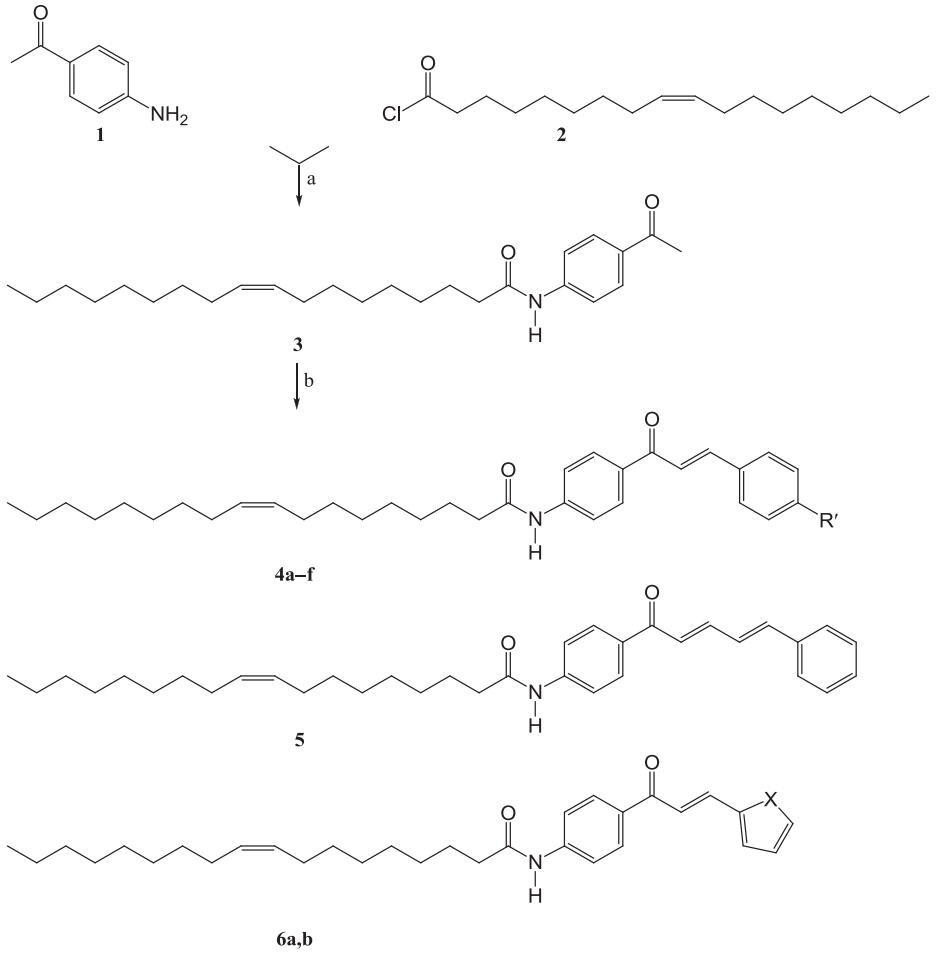
In this paper, a series of oleoylamidochalcones were prepared as easily accessible isosteric isomers of OE and oleoylisoflavones and screened as antiobesity agents on SD rat model. Another set of monohydroxy chalcones (chalcone ligands), Schiff base-derived chalcones and their copper and zinc complexes were also prepared and investigated as antioxidants and anticancer agents. While zinc and chalcones are individually antioxidants we welled that merging should enhance the overall antioxidant power. On the other hand, cancer cells are known for their high affinity to copper ions [29], thus, upon chelation of chalcones with copper they will be more liable to target cancer cells in micro Dendron architecture. Copper (II) complexes of Schiff base-derived curcumin ligands are highly cytotoxic [30].

Putting in mind the affinity of the oleoyl chain to cross cellular membranes [31], it might be concluded that the oleoyl moiety as well as the copper ions might act as penetration enhancers of chalcones into cancer cells. Even if they are non cytotoxic to cancer cells, they might act as inhibitors of drug efflux proteins characteristic to cancer cells and responsible for drug resistance of cancer cells, for instance, P-glycoproteins (Pgp), breast cancer resistance protein (BCRP) [25,26] and multidrug resistance-associated protein 1 (MRP1) [26]. Chalcones with basic functionalities on Ring A, for instance **IV** [25], were proven to act as Pgp-inhibitors, thus, exerted synergistic effect with doxorubicin [25,27].

2. Results and discussion

2.1. Chemistry

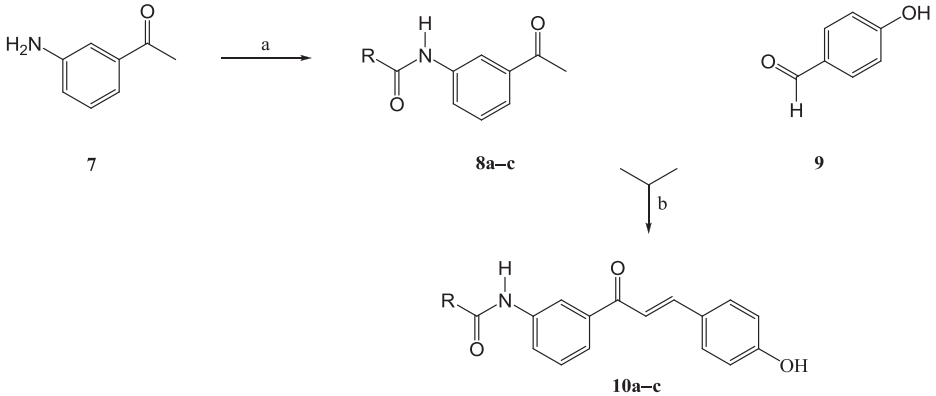
To obtain 4′-(*N*-oleoylamido)chalcones **4**–**6** as oleoyl estrone analogues for screening mainly as potential antiobesity agents, *p*-aminoacetophenone **1** was treated with oleoyl chloride **2** in Et_3N to afford substrate **3** in nearly quantitative yield (Scheme 2). Claisen–Schmidt condensation of **3** with a set of aromatic aldehydes, including basically 2-furancarboxaldehyde and 2-thiophenecarboxaldehyde, afforded the desired chalcones in very good yields (63%–qu) except in the case of aldehydes having *p*-electron withdrawing groups, chalcones **4e**,**f**. Molecular ion peaks were recognizable with good intensities and were in accordance with the calculated empirical formulas and the same was for elemental analyses except for **5** where it was not possible to obtain complete right analysis for it. In the IR spectra, diagnostic bands for NH stretching, enone C=O stretching overlapped with *Amide I* and finally *Amide II* bands were all clearly visual near to 3275, 1655 and 1610 cm^{-1}, respectively. In some cases, for instance **4f**, the enone C=O stretching band was shifted a little to a higher frequency 1675 cm^{-1} while the NO_2 symmetric and asymmetric stretching vibrations were strongly visible at 1515 and 1340 cm^{-1}, respectively. ^1H NMR spectra of compounds **4**–**6** showed the oleoyl olefinic protons as multiplet at δ 5.43 ppm and well separated from the aromatic protons. The H_β of the enone system was more deshielded than the H_α due to π-bond delocalization and both appeared in the aromatic region with large coupling constant *J* 15.6 Hz characteristic for *S-trans* configuration of the enone system [32].



Scheme 2. *Reagents and conditions*: (*a*) DCM, Et_3N, 0 °C–rt (99%); (*b*) Aldehyde, Et_2O/EtOH, NaOH, rt (**4a**, R′ = H, 82%), (**4b**, R′ = OCH_3, qu), (**4c**, R′ = CH_3, 73%), (**4d**, R′ = NMe_2, 63%), (**4e**, R′ = Cl, 31%), (**4f**, R′ = NO_2, 78%), (**5**, 78%), (**6a**, X = O, 90%), (**6b**, X = S, 89%).

In ^{13}C NMR, the carbonyl carbon of the enone system was more deshielded than the amide C=O carbon, presumably, the tautomerism in the amide shields the C=O carbon more effectively than the delocalization of the π-orbitals in the enone moiety.

Tracing the fact that hydroxylated chalcones are potential antioxidant and cytotoxic, besides, the high affinity of cancer cells to intake copper ions, a series of chalcone ligands having an OH group as donor group, compounds **10a**–**c** as well as Schiff base-derived chalcones **14a**,**b** were prepared (Scheme 3). Thus, Claisen–Schmidt condensation of **8a**–**c** with *p*-hydroxybenzaldehyde **9** afforded chalcone ligands **10a**–**c** in low yields. These chalcones showed overlapped NH/OH stretching vibration bands within the range 3200–3300 cm^{-1}. Enone C=O stretching as well as *Amide I* and *Amide II* bands appeared quite similar to those in chalcones **4**–**6**. In the ^1H NMR spectra, the deuterium exchangeable phenolic OH signal appeared downfield at δ 10.0–10.5 ppm. The amide proton in the aromatic amide **10c**, δ 10 ppm, was more deshielded compared with the aliphatic analogues **10a**,**b**, δ 1.6–1.2 ppm.

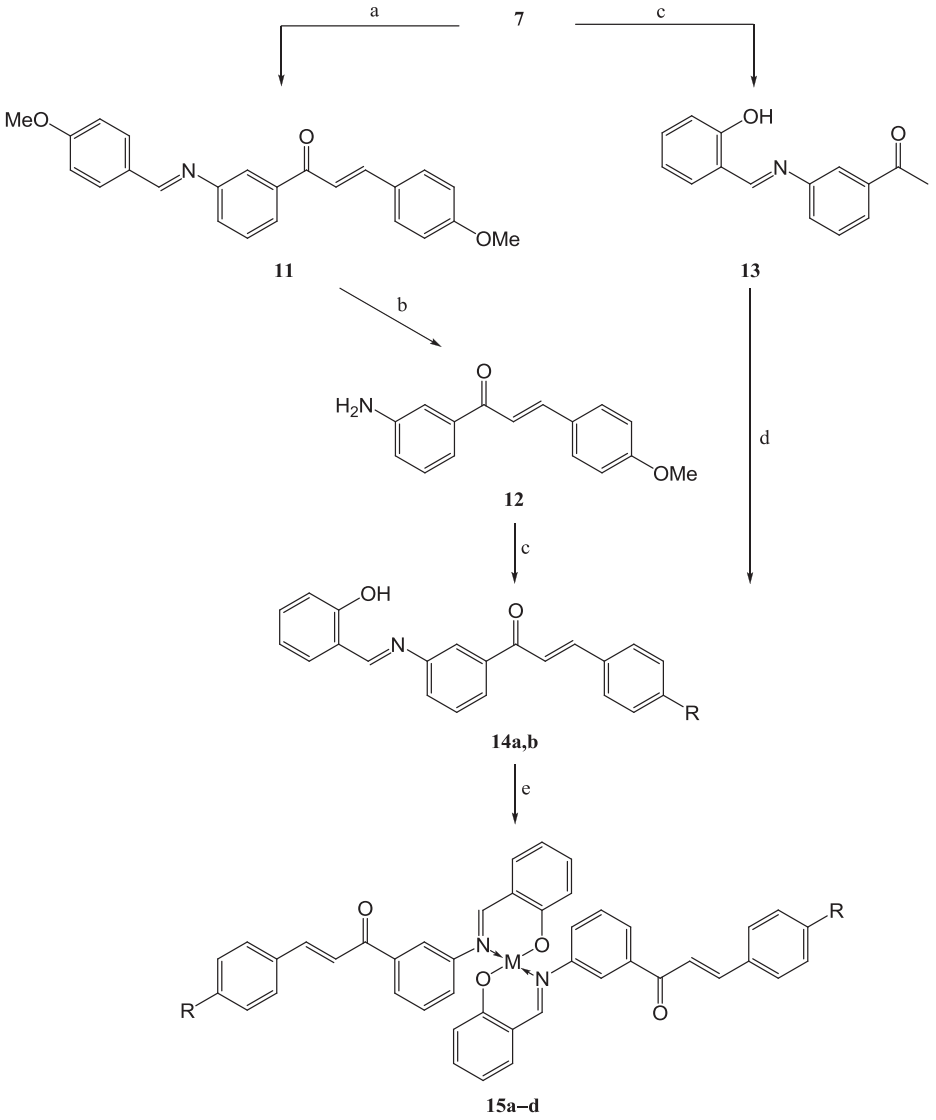


Scheme 3. *Reagents and conditions*: (*a*) $C_{15}H_{31}COCl$ or CH_3COCl or PhCOCl, Et_2O/DCM, Et_3N (**8a**, R = $C_{15}H_{31}$, 43%), (**8b**, R = CH_3, 91%), (**8c**, R = Ph, 96%); (*b*) EtOH, NaOH (**10a**, R = $C_{15}H_{31}$, 66%), (**10b**, R = CH_3, 21%), (**10c**, R = Ph, 43%).

Chalcones **14a**,**b** were prepared from Schiff's base **13** by Claisen–Schmidt condensation with anisaldehyde and *p*-tolualdehyde affording **14a** in weak yield (27%) as single medium sized prisms and **14b** (57%) as sun-shaped crystals (Scheme 4). Besides, the desire to check chalcones having non-chelating Schiff's base tags for SAR analysis, compound **11** was prepared in 76% yield in one step by treatment of **7** with anisaldehyde in the presence of NaOH. Upon acid hydrolysis of **11** (86%), intermediate amine **12** was treated with salicylaldehyde affording **14a** in much better yield (75%) but most interestingly is the crystal color and morphology which was better and enabled obtaining of X-ray ORTEP structure shown in (Fig. 1). The OH IR-stretching band of **14a**,**b** could not be seen due to strong intramolecular H-bonding with the azomethine nitrogen which appears, instead, at about 2970 cm^{-1}. For the same reason it was highly deshielded $\delta \approx 13$ ppm and D_2O exchangeable. The imine hydrogen appeared as singlet at δ 8.7 ppm. Attempts to prepare more derivatives of the type **14** having different substituents at Ring B were unsuccessful. Despite, products were visible on TLC, all trials to purify them even by flash chromatography were unsuccessful. It was anticipated that it decomposes by silica due to acidity, however, elution in the presence of 1% Et_3N or chromatography on alumina as stationary phase failed to purify the products.

The ORTEP diagram (Fig. 1) of **14a** crystallized in a monoclinic $P2_1/c$ system illustrates the *E*-geometry around the double bond of the enone moiety. This is in good agreement with the observed high coupling constant, ^1H NMR, of H_α and H_β. The O–H and the azomethine nitrogen are well oriented for intramolecular hydrogen bonding and supports planarity of the whole molecule. Bond lengths are given in (Table 1).

To investigate the effect of merging chalcones with metal ions on the antioxidant and cytotoxicity of chalcones, chalcones **14a**,**b** were treated with copper and zinc acetates in refluxing EtOH to afford **15a**–**d** as ML_2 chelate complexes, despite, the reaction proportions were 1:1 (Scheme 4). A set of diagnostic variations in absorption bands could be recognized by comparing the IR spectra of **15a**–**d** and their parent chelators **14a**,**b**. These variations include, disappearance of the phenolic OH-azomethine intramolecular H-bond (OH–N) at ≈2970 cm^{-1}, shift of the azomethine (C=N) absorption to lower frequency (1618 → 1605 cm^{-1}), and the phenolic



Scheme 4. *Reagents and conditions*: (*a*) Anisaldehyde, NaOH, EtOH (76%); (*b*) HCl, Acetone (86%); (*c*) Salicylaldehyde, EtOH, rfx. [Only **14a** from **12**, 75% (*Method A*); 68% for **13**]; (*d*) Anisaldehyde or tolualdehyde, NaOH, EtOH [27% for **14a,** R = OMe (*Method B*) and 57% for **14b**, R = Me]; (*e*) $Cu(OAc)_2$. $5H_2O$ or $Zn(OAc)_2$. $2H_2O$, EtOH, rfx. (62% for **15a**, R = OMe, M = Cu^{2+}; 61% for **15b**, R = OMe, M = Zn^{2+}; 66% for **15c**, R = Me, M = Cu^{2+}; 46% for **15d**, R = Me, M = Zn^{2+}).

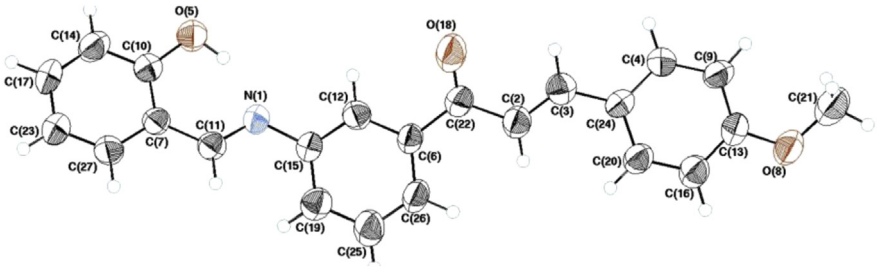


Fig. 1. Ortep diagram of **14a.**

C–O absorption ≈ 1290 cm^{-1} to higher frequency 1330 cm^{-1} due to enhancement of the mesomeric effect upon complexation. The formation of complexes **15a**–**d** gives rise to extra bands at the low frequency region, definitively, around 530 and 450 cm^{-1} which point to formation of M–O and M–N bonds, respectively.

The UV-spectra (Figs. 2 and 3) supports the IR data, thus, the absorption intensity of **14a** at 346 and **14b** at 333 nm decreased from about 3.1 to 2.2 in case of zinc chelates **15b**,**d** and more pronounced to about 0.5 in case of copper complexes **15a**,**c**. These variations denote to incorporation of the lone-pair of electrons of the azomethine nitrogen atom in chelation and it is no more available for efficient transition motions.

In the ^1H NMR spectra of the diamagnetic zinc complexes **15b**,**d**, the phenolic proton disappeared clearly as a good evidence for chelation, however, the shift in the imine proton was minute and negligible. The absence of acetate CH_3 signal in DMSO at δ 1.91 ppm [33] supports the proposed structure of ML2 and the spontaneous deprotonation of phenolic protons under the neutral reaction conditions. Thus, the complexes are non-electrolytes and no acetates are available outside the coordination sphere, therefore, they showed very low electrical conductivity, Λ_M values of 1–2 Ω^{-1} cm^2 mol^{-1} in DMF, as further confirmation of their neutral and non-electrolytic nature.

Thermal analysis (TGA–DTG) of complexes **15a**–**d** up to 800 °C **(**Figs. 4–7**)** showed the thermal stability of zinc complexes **15b**,**d** compared with the copper chelates **15a**,**c**. Complexes **15b**,**c** are half hydrated due to weight loss of 1.2% (Calcd. 1.14%) at 547.15 K in the case of **15b** and 1.24% (Calcd. 1.19%) at 537.15 K in the case of **15c**. This water of crystallization was neither visible in the IR spectra nor effective on the elemental analysis. Copper complexes **15a,c** showed main endothermic decomposition at 618.87 and 604.19 K due to loss of one chalcone moiety 35.75–39.96% (Calcd. 36.23–41.46%), while the zinc analogues **15b,d** showed their main decomposition event at 625.38 and 618.97 K due to loss of only cinnamoyl fragment 19.96–20.92% (Calcd. 20.47–19.45%).

2.2. Biology

2.2.1. Antiobesity screening

Adipocytes in obese individuals function as endocrine gland releases a variety of molecules including protein hormones such as leptin, cytokines like TNF-α, free fatty acids (FFAs) and enzymes like protein tyrosine phosphatases (PTPs). PTPs dephosphorylate multiple tyrosine residues of the insulin receptor tyrosine kinase, thus, retarding insulin-stimulated glucose uptake. The pancreas responds by pumping more insulin to push glucose uptake with concurrent elevation of both insulin and glucose levels, prognosis of insulin resistance and *Type II* diabetes [34,35]. Increased resistance to insulin mediated inhibition of lipolysis lead to elevation of plasma free fatty acid levels, blood pressure, impair large artery endothelial functions and cardiovascular diseases. Theses elevated plasma fatty acid levels assist also in insulin receptor substrate-1 (IRS-1) dephosphorylation and insulin resistance [36]. Proinflammatory cytokines released from excess adipose tissues are responsible for elevation of blood pressure and elevation in both insulin resistance and glucose. They are major risk factors of cardiovascular diseases [37]. Elevation of plasma triglycerides (hypertriglyceridemia), and total cholesterol (hypercholesterolemia), *i.e.* low density lipoprotein-cholesterol (LDL-cholesterol) and high density lipoprotein-cholesterol (HDL-cholesterol), are related physiologic disorders to obesity [38].

Nine oleoylamidochalcones **4a**–**f**, **5** and **6a,b** were prepared as isosteric isomers of oleoyl estrone for screening their antiobesity and related metabolic disorders. Choose of the chalcone moiety as alternative for the estrone ring was based on successful results exhibited by oleoylisoflavones, while, the oleoyl group was retained as necessary component for antiobesity stimulation where other saturated and unsaturated alternatives were ineffective. Besides being easily accessible in more diverse chemical architectures than isoflavones, chalcones have closely related physiological profiles as isoflavones which gives better opportunity for potential physiologic effects. Thus, eight of these chalcones **4a**–**f** and **6a,b** were screened as weight loss stimulants and some related metabolic disorders including levels of triglycerides, free fatty acids, total cholesterol, fast blood sugar, free insulin and insulin sensitivity index. Experiments were done *in vivo* using male SD rats. Animals were divided into eleven groups, one low-fat-diet (LFD) and another high fat diet (HFD) as negative control. A third group was treated with Orlistat as positive control while the remaining eight groups were treated with compounds **4a**–**f** and **6a,b** individually. Vehicle doses (30 mg/day/kg of rat weight) were given by intragastric administration once daily for 30 days.

In body weight experiments (Fig. 8A) feeding with HFD significantly increases body weight to 49.5% of the normal group, while, it was attenuated significantly by 23.5% upon treatment of HFD individuals with Orlistat. Variation between Orlistat treated obese rats and normal one was insignificant. Chalcones **4d** with *p*-dimethylaminophenyl, **4e** with *p*-chlorophenyl and **6a** with 2-furanyl moieties, as Ring B terminals were able to reduce the body weight significantly by 28.0, 22.0 and 36.6%, respectively, compared with HFD rats without having significant variations with normal rats. Other chalcones varied insignificantly with HFD rats reflecting their negative effects on body weight loss. Chalcone **4a** was regarded as pseudo active chalcone, where it has insignificant variation with both Orlistat and HFD rats.

In case of free fatty acids analysis (Fig. 8B), HFD rats showed a significant increment in FFAs level by 63% compared with normal rats, while, Orlistat treatment could attenuate significantly its level again by 38.9% and return it to its normal levels. Chalcones **4d**, **4e** and **6a** varied significantly with HFD rats without having significant variations with Orlistat treated and normal rats.

Free insulin augmentation by HFD feeding (95.5%) was attenuated significantly by Orlistat treatment (51.4%) without significant variation with normal rats (Fig. 8C). In this case, only **4d** and **6a**

Table 1
Bond lengths [Å] for compound **14a**.

N1–C11	1.282 (2)	C9–C13	1.371 (3)	O5–H5	0.921 (14)
N1–C15	1.422 (3)	C10–C14	1.392 (3)	C9–H9	0.960 (2)
C2–C3	1.319 (3)	C12–C15	1.388 (3)	C11–H11	0.960 (2)
C2–C22	1.477 (3)	C13–C15	1.394 (3)	C12–H12	0.960 (2)
C3–C24	1.459 (3)	C14–C17	1.367 (3)	C14–H14	0.960 (2)
C4–C9	1.391 (3)	C15–C19	1.390 (3)	C16–H16	0.960 (2)
C4–C24	1.396 (3)	C16–C20	1.368 (3)	C17–H17	0.960 (2)
O5–C10	1.359 (2)	C17–C23	1.392 (3)	C19–H19	0.960 (2)
C6–C12	1.390 (3)	O18–C22	1.222 (2)	C20–H20	0.960 (2)
C6–C22	1.493 (3)	C19–C25	1.383 (3)	C21–H21A	0.960 (3)
C6–C26	1.393 (3)	C20–C24	1.389 (3)	C21–H21B	0.960 (3)
C7–C10	1.396 (3)	C23–C27	1.395 (3)	C21–H21C	0.960 (3)
C7–C11	1.449 (3)	C25–C26	1.384 (3)	C23–H23	0.960 (2)
C7–C27	1.399 (3)	C2–H2	0.960 (2)	C25–H25	0.960 (2)
O8–C13	1.365 (2)	C3–H3	0.960 (2)	C26–H26	0.960 (2)
O8–C21	1.421 (3)	C4–H4	0.960 (2)	C27–H27	0.960 (2)

reduced significantly this metabolic parameter by 49.2 and 61.6%, respectively, while, **4e** varied insignificantly with HFD rats.

Regarding insulin sensitivity (Fig. 8D), HFD feeded rats acquired significant depression in insulin sensitivity than normal LFD rats by 21.5%, while, Orlistat treatment augmented this parameter significantly by 48.4% compared with HFD group. Chalcones **4d**, **4e** and **6a** augmented free insulin significantly quite as Orlistat.

HFD individuals exhibited significant triglyceride and fast blood sugar gain, while, total cholesterol varied insignificantly (Fig. 8E). Orlistat treated group got significant drop in triglyceride level by 42.7% without significant variation with normal rats. The drug has no statistical significant effect on total cholesterol or FBs on comparison with HFD rats. As observed in last parameters, only compounds **4d**, **4e** and **6a** could attenuate significantly TG levels compared with HFD rats by 39.7, 34.1 and 49.7%, respectively, but they varies insignificantly with Orlistat and normal rats.

Despite, Orlistat, **4d** and **4e** could not depress significantly FBs levels, **6a** could do by 41.3% compared with HFD and return it to its normal levels. Neither Orlistat nor test compounds could affect total cholesterol significantly.

In terms of these results, the compounds that showed significant activities are arranged in comparison to Orlistat in the following order **6a** > Orlistat, **4d** > **4e** > **4a**. Thus, the *p*-dimethylaminophenyl and *p*-chlorophenyl moieties are valuable as Ring B terminals, while, the 2-furanyl is much better for relieving weight gain and related metabolic disorders. Comparing the inertness of **6b** with the activity of **6a** reflects the preference of the furanyl oxygen rather than the thiophenyl sulfur, the unique difference, for the antiobesity effect of **6a**. On the other hand, comparing the

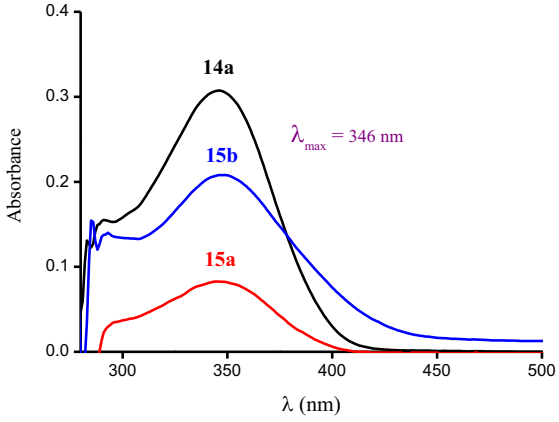


Fig. 2. UV spectra of **14a**, **15a**,**b**.

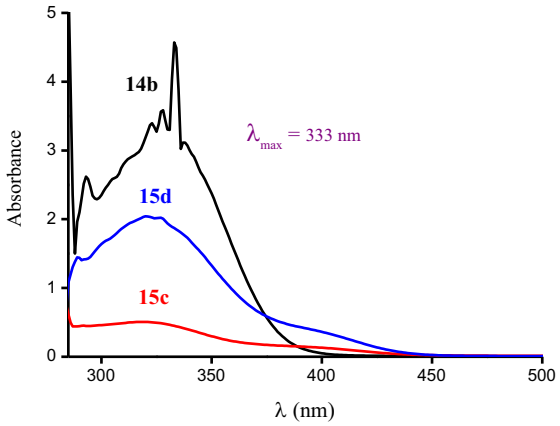


Fig. 3. UV spectrum of **14b**, **15c**,**d**.

structure of **6a** with oleoyl estrone gives insight to the importance of the oxygenated terminal five membered ring in both compounds besides the oleoyl moiety for the antiobesity activity, whatever, the oxygen is exocyclic as in OE or endocyclic as in **6a** which are the most similar structural features between both compounds.

2.2.2. Cytotoxicity assay

Many natural as well as synthetic chalcones are continually reported for their anticancer activity against various cancer cell lines with hydroxylated and methoxylated species being in front of anticancer known chalcones. Nineteen compounds and Doxorubicin as positive control were evaluated for their cytotoxicity against prostate cancer cell line PC3 *in vitro*. In terms of these results (Fig. 9) the cytotoxic activity of compounds with fatty acylamido groups as Ring A **4a**–**f, 5, 6a,b** and **10a** regardless their Ring B structure were weakly significantly compared with the control. Other small sized chalcones **10b,c**, **11** and **14a,b** showed good cytotoxic significant activities with IC_{50} values within the range 15.74–10.91 μM, while, it was 8.91 μM for the control. Compound **10c** was significantly more cytotoxic, IC_{50} 10.92 μM, than **10b**, IC_{50} 15.74 μM, thus, the phenyl group has positive impact on the cytotoxicity compared with the aliphatic one, particularly, if it is lipidic as in **10a**. All Schiff-base derived chalcones either nonchelating **11** or chelating **14a,b** were significantly active within the range 12.52–10.90 μM. Compounds **10c** and **14a** were significantly as active as Doxorubicin without any significant variations between them.

As it was planned, copper and zinc complexes **15a**–**d** of chelating chalcones **14a**,**b** were screened as well. The cytotoxicity of the parent chalcones **14a**,**b** was reduced upon complexation

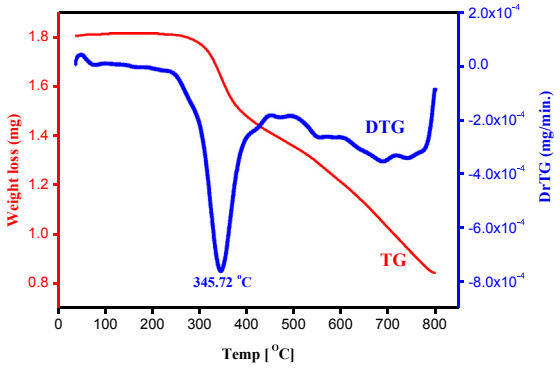


Fig. 4. TGA–DTG thermogram of **15a**.

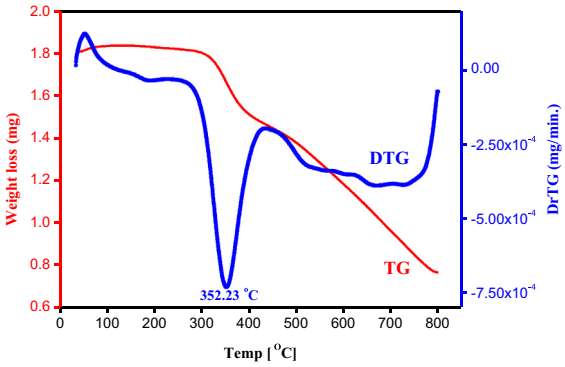


Fig. 5. TGA–DTG thermogram of **15b**.

(**15a**,**b**,**d**) with the exception of the copper chelate **15c** whose activity was increased two-fold significantly compared with **14b** and showed significant cytotoxicity than doxorubicin (31.6% more active), the unique in this investigation. Probably, these complexes due to their expected high stability did not liberate their parent ligands whose chelating centres are necessary for cytotoxicity and that the OH group is necessary for the activity. However, this is opposed by the high activity of **15c**. Eventually, the activity is attributed to the presence of the *p*-methylphenyl moiety of Ring B and the copper ion.

2.2.3. Antioxidant activity

Compounds showed potential cytotoxic activity including chalcone ligands **10a**,**b**, precursor **11**, as well as Schiff-base derived chalcones **14a**,**b** and metal complexes **15a**–**d** of the later species were selected for antioxidant activity estimation using both the DPPH-radical scavenging and the hydroxyl-radical scavenging assays at 30 nM. The DPPH-scavenging assay of test compounds was recorded spectrophotometrically at 517 nm taking 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) as positive control [39,40]. As shown in (Fig. 10), Precursor **11**, chalcone ligands **10a**,**b** and chelating chalcones **14a**,**b** showed low significant scavenging activities within the range 19–25% of Trolox reflecting their weak antioxidant power.

However, upon metal complexation of Schiff base-derived chalcone ligands **14a**,**b** with copper and zinc acetates, significant increment in the antioxidant power was observed. Zinc complexes **15b**,**c** were a little more powerful than the copper analogues **15a**,**c**. Thus, in comparison with the significant differences with Trolox, the zinc complex **15b** which showed the highest activity was less active by 10.7%. Its analogue **15d** was less active by 20.9%, while the copper complexes **15a** and **15c** were less active by 27–28% without significant variations between both of them.

In the second approach, Hydroxyl radical scavenging activity was measured according to Fenton method [41] (Fig. 11). Results showed that chalcones **10a**,**b, 11** and **14a**,**b** were significantly weakly active exactly as in the DPPH• Assay and increased significantly upon metal complexation. Metal complexes of **14b** either copper **15c** or zinc **15d** were significantly more active than those of **14a. 15d** was 6.7% significantly more active than **15c** and variation between **15a** and **15b** was insignificant.

The antioxidant activity of compounds is related with their electron or hydrogen radical releasing ability to DPPH• and •OH so that they become stable diamagnetic molecules. Once, phenolic derivatives **10a,b** and **14a,b** showed low antioxidant activities, these derivatives were regarded as weak hydrogen radical releasing species and attention was turned to estimate the electron releasing affinity which is well related to the antioxidant power on quantum molecular basis from the E_{HOMO} and E_{LUMO} values [42].

Thus, the energy of the highest occupied molecular orbital (E_{HOMO}) measures the electron-donating character of a compound (ionization potential), while (E_{LUMO}) measures its electron-acceptor character (electron affinity). Compounds of high E_{HOMO} and low E_{LUMO} values and, consequently, low energy gab (Δ_E) are classified as good electron-releasing species. As shown in (Table 2), the powerful antioxidants in this study **15a**–**d** exhibited the lowest Δ_E ($E_{LUMO} - E_{HOMO}$) values (2.75–7.79) compared with (11.61–11.99) for the rest of compounds under consideration reflecting their high electron-releasing affinities [43].

Medically, antioxidants are auxiliary drugs co-administered with various formulations, particularly anticancer drugs, to scavenge free radicals and reactive oxygen species stemming from their oxidative stress. Therefore, compounds combining an antioxidant and anticancer activities, compound **15c** in this consideration, are desirable in cancer chemotherapy [44].

3. Conclusion

In summary, a series of oleoyl chalcones, hydroxy chalcones, Schiff base-derived chalcones as well as their copper and zinc complexes were prepared for screening their antiobesity, cytotoxicity and antioxidant activities. Oleoyl chalcone **6a** graphted with furanyl moiety exhibited interesting antiobesity activity. The structure similarity between **6a** and **OE** suggest it to mimic **OE**, therefore, its least effective dose and mechanism of action, either by pancreatic lipase inhibition or interference with gene expression of lipolytic and lepogenic enzymes have to be considered. This work also showed the importance of hydroxy chalcones modified with simple amide and Schiff base moieties as anticancer agents. The copper complex **15c** of a Schiff' base-derived chalcone **14b** showed preference to Doxorubicin against PC3 cell line. As antioxidants, complexation of Schiff base-derived ligands **14a,b** with copper and zinc, led to potent antioxidant complexes **15a**–**d**. Thus, compound **15c** as powerful cytotoxic complex and good antioxidant is another fruit of this work for further investigations. The toxicity of **6a** and **15c** will be considered in due course.

4. Experimental section

4.1. Chemistry

Melting points were determined on Gallenkamp apparatus and are uncorrected. Flash chromatography was carried out on silica gel (Baker, 30–60 μm), *Type I*, or [Lichroprep Si 60 (E. Merck; ϕ 15–25 μm)], *Type II*. TLC Monitoring tests were carried out using plastic sheets precoated with silica gel 60 F_{245} (layer thickness 0.2 mm) purchased from Merck. Spots were visualized by their fluorescence under UV-lamp (λ 245 and 366 nm) or staining with iodine vapor, 15% H_2SO_4, $KMnO_4$, or Ce(IV)SO_4 in H_2SO_4. NMR spectra were recorded on Bruker 600 MHz spectrometer, central laboratory, King Abd El Aziz, Jeddah, Saudi Arabia. IR-spectra were recorded on ATR-Alpha FT–IR Spectrophotometer from 400 to 4000 cm^{-1}. Mass spectra were recorded on GCMS-QP 1000Ex Shimadzu spectrometers in the microanalysis unit at Cairo University. UV–vis. Measurements were recorded by Perkin-Elmer Lambda 25 UV/vis double-beam Spectrophotometer. Electrical conductivity measurements were carried out using Equiptronics digital conductivity meter model JENWAY 4070 at room temperature for 1×10^{-3} mol L^{-1} solutions. Thermal analysis measurements were recorded on a Schimadzu model 50 instrument using 20 mg samples, micro analytical unit of Cairo University. The nitrogen flow rate and heating were 20 cm^3/min and 10 °C/min, respectively.

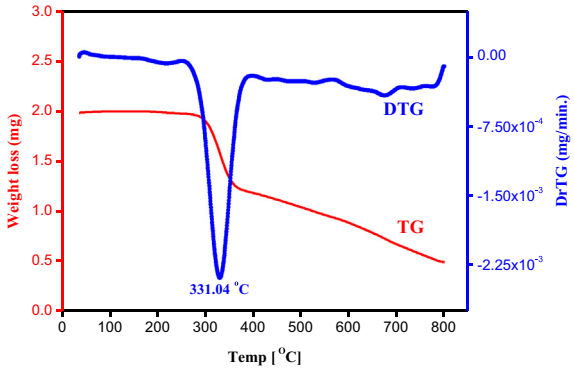


Fig. 6. TGA–DTG thermogram of **15c**.

Elemental analyses were carried out on PerkinElmer 2400 Series II CHNS/O System, Chemistry Department, Faculty of Science at Taif University, Saudi Arabia.

*4.1.1. N-(4-Acetylphenyl)oleamide (**3**)*

A mixture of **1** (2.0 g, 14.8 mmol) and Et_3N (7.5 ml, 54.0 mmol) in dry DCM (25 ml) was stirred in ice-salt bath, while, oleoyl chloride **2** (8.0 ml, 20.4 mmol) in dry DCM (10 ml) was added dropwise. The mixture was allowed to warm up gradually to ambient temperature with continuous stirring over 1 h then evaporated *in vacuo*. The residue was taken in Et_2O (50 ml), washed with H_2O (20 ml), dried over Na_2SO_4, evaporated *in vacuo* and purified by flash chromatography (toluene:ethyl acetate, 7:1) to afford **3** (5.86 g, 99%) as colorless tiny star-like crystals. R_f 0.35 (toluene:ethyl acetate, 7:1); Mp. 62–64 °C. IR (ν, cm^{-1}): 3332 (NH_{str}), 1678 (C=O_{str}), 1656 (*Amide I*), 1604 (*Amide II*); ^1H NMR (600 MHz, $CDCl_3$): δ 7.93 (d, 2H, *J* 8.4 Hz, H-3_{Ar}, H-5_{Ar}), 7.64 (d, 2H, *J* 8.4 Hz, H-2_{Ar}, H-6_{Ar}), 7.57 (brs., 1H, NH), 5.37–5.32 (m, 2H, CH=CH), 2.57 (s, 3H, $COCH_3$), 2.39 (t, 2H, *J* 7.2, 7.8 Hz, $COCH_2$), 2.04–1.99 (m, 4H CH_2CH=CHCH_2), 1.73 (quin, 2H, *J* 7.2, 7.8 Hz, $COCH_2CH_2$), 1.37–1.26 (m, 20H, 10 CH_2), 0.88 (t, 3H, *J* 6.6, 7.2 Hz, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ 197.03 (CH_3CO), 173.73 (C=O_{Amide}), 142.40 (C-1_{Ar}), 132.74 (C-4_{Ar}), 130.06, 129.77 (CH=CH, C-3_{Ar}, C-5_{Ar}), 118.79 (C-2_{Ar}, C-6_{Ar}), 37.89 ($COCH_3$), 31.91, 29.77, 29.70, 29.53, 29.33, 29.32, 29.28, 29.22, 29.11, 27.23, 27.16, 26.45, 25.44, 22.70 (13 CH_2, $COCH_2$), 14.27 (CH_3). Exact mass calculated for $C_{26}H_{41}NO_2$ (399.31), EI MS, *m/z* (%), Found: 399.30 (M^+, 79), 368 (33), 337 (100). Anal. Calcd. for $C_{26}H_{41}NO_2$ (399.61): C, 78.15; H, 10.34; N, 3.51. Found. C, 77.68; H, 10.52; N, 3.62.

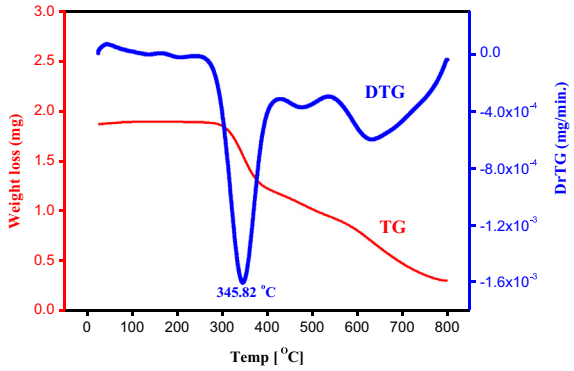


Fig. 7. TGA–DTG thermogram of **15d**.

*4.1.2. General procedure for the synthesis of 4-(arylacryloylphenyl) oleamides (**4**–**6**)*

Ethanolic NaOH (0.7 M, 0.5 ml) was added to a solution of **3** (0.5 g, 1.2 mmol) and the appropriate aldehyde (1.8 mmol) in Et_2O and the resulting suspension was stirred at ambient temperature for 24 h. The mixture was neutralized with AcOH (0.5 ml), evaporated *in vacuo* and the residue was purified by flash chromatography.

*4.1.2.1. N-(4-Cinnamoylphenyl)oleamide (**4a**).* Yield: 82% as fine colorless crystals upon flash chromatography with gradient of toluene then (toluene:ethyl acetate, 14:1) and crystallization from Et_2O; R_f 0.41 (toluene:ethyl acetate, 9:1); Mp. 104 °C; IR (ν, cm^{-1}): 3290 (NH_{str}), 1650 (C=O_{str}, *Amide I*), 1598 (*Amide II*); ^1H NMR (600 MHz, $CDCl_3$): δ 8.04–8.02 (m, 1H, Ar), 8.03 (d, 1H, *J* 6.6 Hz, Ar), 7.81 (d, 1H, *J* 15.6 Hz, COCH=*CH*), 7.68 (d, 2H, *J* 9.0 Hz, Ar), 7.65 (d, 1H, *J* 7.8 Hz, Ar), 7.65–7.64 (m, 1H, Ar), 7.54 (d, 1H, *J* 15.6 Hz, CO*CH*=CH), 7.43–7.41 (m, 2H, Ar), 7.42 (dd, 1H, *J* 1.8, 4.8 Hz, Ar), 7.36 (brs, 1H, NH), 5.35–5.34 (m, 2H, CH=CH), 2.40 (t, 2H, *J* 7.2, 7.8 Hz, $COCH_2$), 2.02–1.99 (m, 4H, CH_2CH=CHCH_2), 1.74 (quin, 2H, *J* 7.8 Hz, $COCH_2CH_2$), 1.40–1.25 (m, 20H, 10 CH_2), 0.88 (t, 3 H, *J* 6.6 Hz, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ 188.94 (*COCH*=CH), 171.59 (CONH), 144.54, 142.13, 134.95, 133.71, 130.50, 130.06, 130.00, 129.70, 128.96, 128.44, 121.77, 118.89 (12 C_{Ar}, 2 *CH*=*CH*), 37.94, 31.91, 29.77, 29.70, 29.53, 29.33, 29.32, 29.28, 29.22, 29.11, 27.23, 27.16, 25.43, 22.69 (14 CH_2), 14.13 (CH_3). Exact mass calculated for $C_{33}H_{45}NO_2$ (487.3450), EI MS, *m/z* (%), Found: 487.25 (M^+, 4), 474.25 (10), 433.15 (31), 397.25 (98), 379.20 (31.5). Anal. Calcd. for $C_{33}H_{45}NO_2$ (487.72): C, 81.27; H, 9.30; N, 2.87. Found. C, 80.90; H, 9.07; N, 2.57.

*4.1.2.2. N-(4-((E)-3-(4-methoxyphenyl)acryloyl)phenyl)oleamide (**4b**).* Yield: (qu.) as brilliant yellow solid upon flash chromatography using solvent gradient of toluene then (toluene:ethyl acetate, 15:1); R_f 0.13 (toluene:ethyl acetate, 15:1); Mp. 64 °C; IR (ν, cm^{-1}): 3307 (NH), 1671 (C=O_{str}), 1658 (*Amide I*), 1598 (*Amide II*); ^1H NMR (600 MHz, $CDCl_3$): δ 8.05, 6.94 (2 d, 4H, *J* 8.4 Hz, Ar), 7.79 (d, 1H, *J* 15.6 Hz, COCH=*CH*), 7.60, 7.22 (2 d, 4H, *J* 9.0 Hz, Ar), 7.32 (s, 1H, NH), 7.39 (d, 1H, *J* 15.6 Hz, CO*CH*=CH), 5.36–5.35 (m, 2H, CH=CH), 3.86 (s, 3H, OCH_3), 2.59 (t, 2H, *J* 7.2, 7.8 Hz, $COCH_2$), 2.04–2.00 (m, 4H, CH_2CH=CHCH_2), 1.76 (quin, 2H, *J* 7.8 Hz, $COCH_2CH_2$), 1.44–1.27 (m, 20H, 10 CH_2), 0.88 (t, 3H, *J* 7.2 Hz, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ 189.36 (*COCH*=CH), 171.82 (CONH), 161.75, 154.10, 144.91, 135.97, 130.28, 130.08, 130.00, 129.70, 127.55, 121.76, 119.49, 114.45 (12 C_{Ar}, 2 CH=CH), 55.44 (OCH_3), 34.42, 31.91, 29.77, 29.69, 29.53, 29.34, 29.33, 29.16, 29.09, 29.08, 27.24, 27.16, 24.85, 22.69 (14 CH_2), 14.13 (CH_3); Exact mass calculated for $C_{34}H_{47}NO_3$ (517.3555), EI MS, *m/z* (%), Found: 517.00 (M^+, 20), 505.00 (31), 423.00 (44), 340.00 (100). Anal. Calcd. for $C_{34}H_{47}NO_3$ (517.74): C, 78.87; H, 9.15; N, 2.71. Found. C, 79.19; H, 8.74; N, 2.75.

*4.1.2.3. N-(4-((E)-3-p-Tolylacryloyl)phenyl)oleamide (**4c**).* Yield: 73% As yellowish fine crystals upon flash chromatography using solvent gradient of toluene then (toluene:ethyl acetate, 11:1) followed by recrystallization from Et_2O; R_f 0.44 (toluene:ethyl acetate, 9:1); Mp. 116 °C; IR (ν, cm^{-1}): 3274 (NH), 1655 (C=O_{str}, *Amide I*), 1601 (*Amide II*); ^1H NMR (600 MHz, $CDCl_3$): δ 8.03, 7.67 (2d, 4H, *J* 8.4 Hz, Ar), 7.79 (d, 1H, *J* 15.6 Hz, COCH=*CH*), 7.67 (d, 2H, *J* 8.4 Hz, Ar), 7.55, 7.23 (2d, 4H, *J* 7.8 Hz, Ar), 7.50 (d, 1H, *J* 15.6 Hz, CO*CH*=CH), 7.31 (brs, 1H, NH), 5.35–5.34 (m, 2H, CH_2CH=*CH*CH_2), 2.40 (t, 2H, *J* 7.2, 7.8 Hz, $COCH_2$), 2.39 (s, 3H, CH_{3Tol}), 2.02–2.00 (m, 4H, CH_2CH=CHCH_2), 1.74 (quin, 2H, *J* 7.2 Hz, $COCH_2CH_2$), 1.35–1.25 (m, 20H, 10 CH_2), 0.88 (t, 3H, *J* 6.6, 7.2 Hz, CH_3); ^{13}C NMR (150 MHz, DMSO): δ 189.02 (*COCH*=CH), 171.55 (CONH), 144.63, 142.00, 141.03, 132.21, 130.52, 129.95, 129.70, 129.49, 128.98, 128.48, 120.76, 118.86 (12 C_{Ar}, 2 CH=

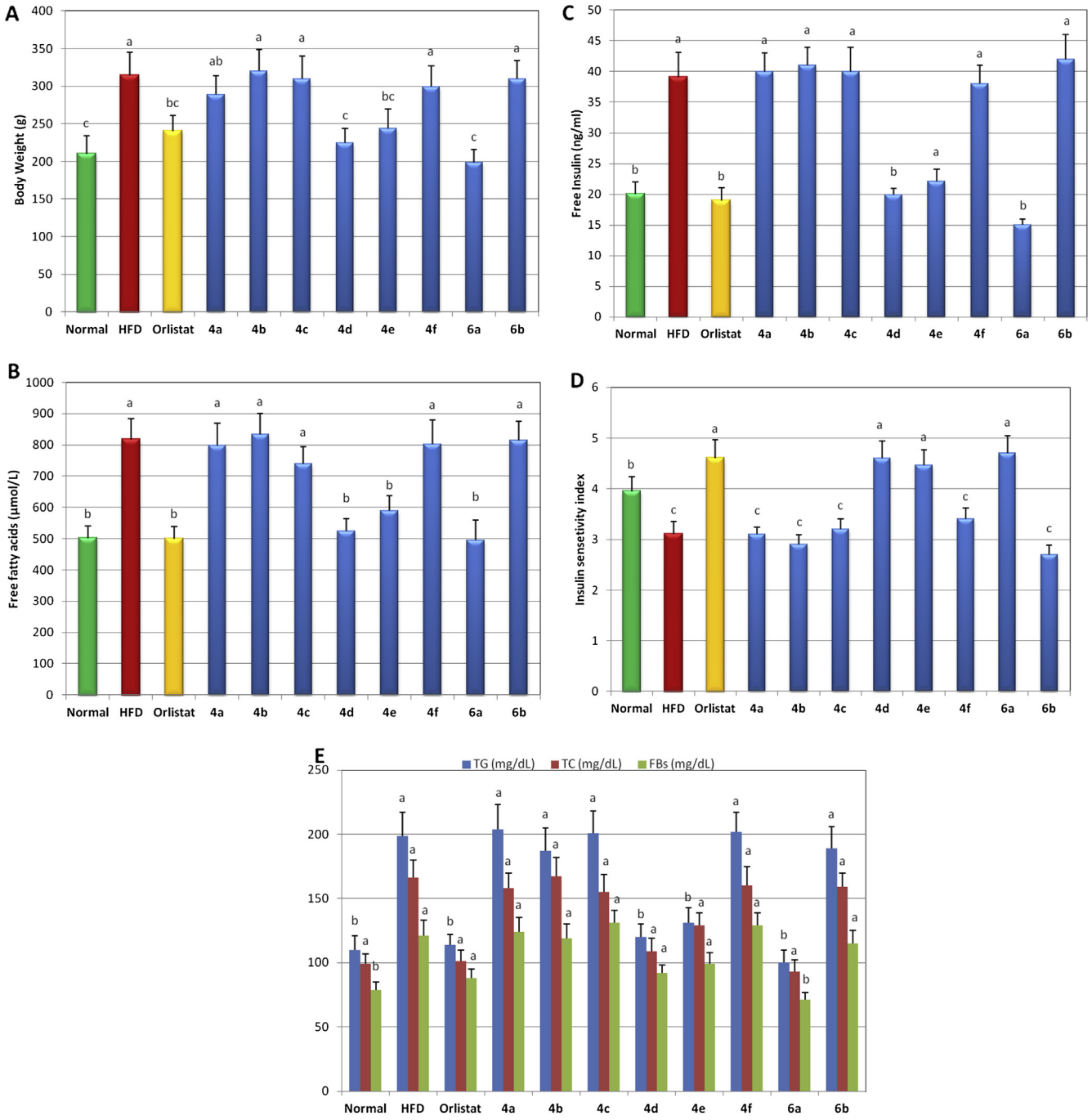


Fig. 8. Effects of treatment of HFD fed male SD rats with compounds **4a**–**f** and **6a,b** compared with Orlistat treated rats and normal control. (**A**) for body weight in grams; (**B**) for FFAs in µmol/L; (**C**) for FI in ng/ml; (**D**) for ISI and (**E**) for TG, TC and FBs expressed as mg/dL. Different letters on the column for each parameter varied significantly at $p \leq 0.05$.

CH), 37.95, 29.77, 29.70, 29.53, 29.33, 29.32, 29.28, 29.22, 29.11, 27.23, 27.16, 25.43, 22.69, 21.55, (14 $-CH_2-$, CH_{3Tol}), 14.13 (CH_3); Exact mass calculated for $C_{34}H_{47}NO_2$ (501.3606), EI MS, *m/z* (%), Found: 501.00 (M^+, 14), 484.00 (10), 399.00 (26), 120.00 (100). Anal. Calcd. for $C_{34}H_{47}NO_2$ (501.74): C, 81.39; H, 9.44; N, 2.79. Found. C, 80.99; H, 9.15; N, 2.92.

*4.1.2.4. N-(4-((E)-3-(4-Dimethylaminophenyl)acryloyl)phenyl)oleamide (**4d**).* Yield: 63% As orange fine solid upon flash chromatography using solvent gradient of toluene then (toluene:ethyl acetate, 9:1); R_f 0.24 (toluene:ethyl acetate, 9:1); Mp. 54–6 °C. IR (*v*, cm^{-1}): 3331 (NH_{str}), 1678 ($C{=}O_{str}$), 1656 (*Amide I*), 1591 (*Amide II*); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.00 (d, 1H, *J* 8.4 Hz, Ar), 7.73 (d, 2H, *J* 7.8 Hz, Ar), 7.79 (d, 1H, *J* 15.0 Hz, COCH=*CH*), 7.66–7.60 (m, 3H, *J* 8.4 Hz, Ar), 7.54 (d, 1H, *J* 7.8 Hz, Ar), 7.50 (brs, 1H, NH), 7.34 (d, 1H, *J* 15.0 Hz, COCH=CH), 6.69 (d, 1H, *J* 9.0 Hz, Ar), 5.35–5.33 (m, 2H, $CH_2CH{=}CHCH_2$), 3.05 (s, 6H, NMe_2), 2.39 (t, 2H, *J* 7.8 Hz, $COCH_2$), 2.02 (m, 4H, $CH_2CH{=}CHCH_2$), 175–1.71 (m, 2H, *J* 7.2 Hz, $COCH_2CH_2$), 1.31–1.26 (m, 20H, 10 CH_2), 0.88 (t, 3H, *J* 6.6, 7.2 Hz, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ 196.99 (COCH=CH), 171.68 (CONH), 152.04, 142.39, 130.43, 130.05, 129.76, 129.71, 129.70, 118.78, 111.83 (12 C_{Ar}, 2 CH=CH), 40.14 (NMe_2), 37.89 ($COCH_2$), 31.91, 29.77, 29.70, 29.53, 29.33, 29.27, 29.22, 29.23, 27.11, 27.16, 26.45, 25.43, 22.70 (13 CH_2), 14.13 (CH_3); Exact mass calculated for $C_{35}H_{50}N_2O_2$ (530.3872), EI

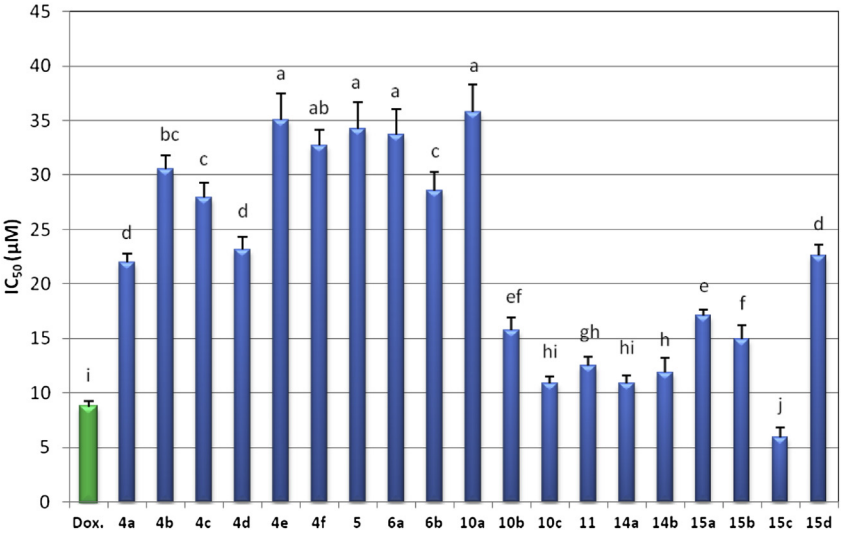


Fig. 9. Cytotoxicity results. Different letters on the column varied significantly at $p \leq 0.05$.

MS, *m/z* (%), Found: 530.50 (M^+, 12), 526.00 (42), 514.00 (38), 502 (56), 444 (60), 313 (100). Anal. Calcd. for $C_{35}H_{50}N_2O_2$ (530.78): C, 79.18; H, 9.49; N, 5.28. Found. C, 78.75; H, 9.96; N, 4.81.

4.1.2.5. N-4-((E)-3(4-Chlorophenylacryloyl)phenyl)oleamide (***4e***). Yield: 31% As faint creamy powder upon twice flash chromatography on *Type I* silica followed by *Type II* using solvent gradient of toluene then (toluene:ethyl acetate, 9:1) and finally recrystallization from toluene; R_f 0.28 (toluene:ethyl acetate, 9:1); Mp. 134–135 °C; IR (*v*, cm^{-1}): 3276 (NH_{str}), 1655 ($C{=}O_{str}$, *Amide I*), 1609 (*Amide II*); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.01, 7.68, 7.57, 7.39 (4d, 8H, *J* 8.4 Hz, Ar), 7.74 (d, 1H, *J* 15.6 Hz, COCH=C*H*), 7.51 (d, 1H, *J* 15.0 Hz, COC*H*=CH), 7.49 (brs, 1H, NH), 5.36–5.33 (m, 2H, $CH_2CH{=}CHCH_2$), 2.40 (t, 2H, *J* 7.2, 7.8 Hz, $COCH_2$), 2.04–1.99 (m, 4H, $CH_2CH{=}CHCH_2$), 1.74 (quin, 2H, *J* 7.2, 7.8 Hz, $COCH_2CH_2$), 1.38–1.25 (m, 20H, 10 CH_2), 0.88 (t, 3H, *J* 7.2 Hz, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ 188.63 (*C*OCH=CH), 171.70 (CONH), 143.02, 142.32, 136.38, 133.42, 130.06, 130.00, 129.69, 129.59, 129.24, 122.14, 118.94 (12 C_{Ar}, 2 CH=CH), 37.92 ($COCH_2$), 31.91, 29.77, 29.70, 29.53, 29.33, 29.32, 29.28, 29.23, 27.11, 27.23, 26.16, 25.43, 22.69 (13 CH_2), 14.13 (CH_3); Exact mass calculated for $C_{33}H_{44}ClNO_2$ (521.3060), EI MS, *m/z* (%), Found: 521.45 (M^+, 12), 518.30 (18), 368.30 (28), 368.30 (100). Anal. Calcd. for $C_{33}H_{44}ClNO_2$ (522.16): C, 75.91; H, 8.49; N, 2.68. Found. C, 75.66; H, 8.43; N, 2.97.

4.1.2.6. N-(4-((E)-3-(4-Nitrophenyl)acryloyl)phenyl)oleamide (***4f***). Yield: 52% As faint yellow powder upon flash chromatography on *Type I* silica followed by *Type II* using solvent gradient of toluene then (toluene:ethyl acetate 8:1); R_f 0.3 (toluene:ethyl acetate, 8:1); Mp. 128–130 °C; IR (*v*, cm^{-1}): 3326 (NH_{str}), 1675 ($C{=}O_{str}$), 1660 (*Amide I*), 1594 (*Amide II*), 1515 ($NO_{2asy.str.}$), 1340 ($NO_{2sym.str}$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.28, 7.79 (2d, 4H, *J* 8.4 Hz, Ar), 8.04, 7.70 (2d, 4H, *J* 9.0 Hz, Ar), 7.82 (d, 1H, *J* 15.6 Hz, COCH=C*H*), 7.64 (d, 1H, *J* 15.6 Hz, COC*H*=CH), 7.40 (brs, 1H, NH), 5.35–5.34 (m, 2H, $CH_2CH{=}CHCH_2$), 2.41 (t, 2H, *J* 7.8 Hz, $COCH_2$), 2.02–2.00 (m, 4H, $CH_2CH{=}CHCH_2$), 1.78–1.73 (quin, 2H, *J* 7.2, 7.8 Hz, $COCH_2CH_2$), 1.38–1.25 (m, 20H, 10 CH_2), 0.88 (t, 3H, *J* 6.6, 7.2 Hz, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ 187.97 (*C*OCH=CH), 171.66 (CONH), 148.52, 142.64, 141.21, 141.14, 136.25, 133.01, 130.54, 130.15, 130.08, 129.87, 129.68, 128.93, 125.47, 124.23, 123.53, 118.99 (12 C_{Ar}, 2 CH=CH), 37.93 ($COCH_2$), 29.76, 29.70, 29.53, 29.32, 29.27, 29.21, 29.11, 27.23, 27.16, 25.40, 22.69 (13 CH_2), 14.13 (CH_3); Exact mass calculated for $C_{33}H_{44}N_2O_4$ (532.3301), EI MS, *m/z* (%), Found: 532.50 (M^+, 52),

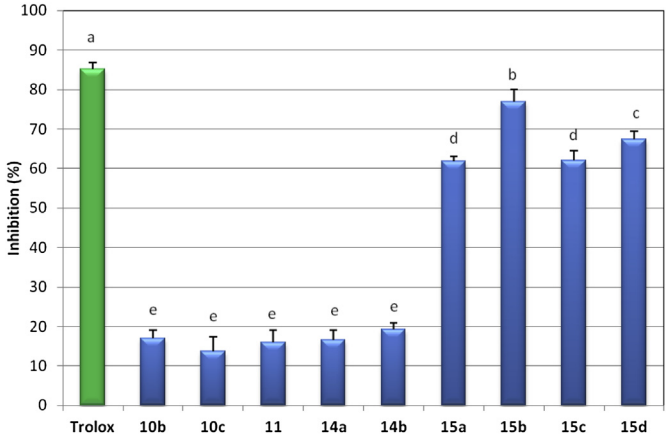


Fig. 10. DPPH Radical scavenging assay. Different letters on the column varied significantly at $p \leq 0.05$.

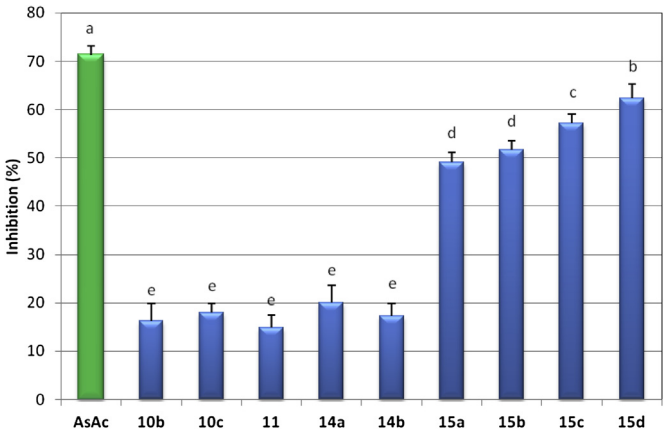


Fig. 11. OH• Scavenging assay. Different letters on the column varied significantly at $p \leq 0.05$.

Table 2
Energy calculations using ZNDO1 semi empirical method built in HyperChem Molecular package.

	E_{TOTAL} (kcal/mol)	$H_{FORMATION}$ (kcal/mol)	E_{HOMO} (eV)	E_{LUMO} (eV)	Δ_E (eV)
10b	−114130	−7748.8	−6.970	4.977	11.947
10c	−135289	−9681.0	−6.960	4.980	11.940
11	−145161	−10732.0	−6.749	4.860	11.609
14a	−140241	−10222.0	−6.850	4.890	11.740
14b	−129582	−10093.0	−6.860	5.132	11.992
15a	−312831	−20379.0	−1.275	1.478	2.753
15b	−281254	−21270.9	−3.285	4.507	7.792
15c	−291443	−20050.7	−2.352	0.7083	3.06
15d	−259938	−21014.3	−3.273	4.448	7.721

522.50 (43), 509.0 (36), 495.0 (21). Anal. Calcd. for $C_{33}H_{44}N_2O_4$ (532.71): C, 74.40; H, 8.33; N, 5.26. Found. C, 73.97; H, 8.28; N, 5.15.

*4.1.2.7. N-(4-((2E,4E)-5-phenylpenta-2,4-dienoyl)phenyl)oleamide (**5**).* Yield: 78% As brilliant yellow amorphous mass upon flash chromatography using solvent gradient of toluene then (toluene:ethyl acetate, 14:1); R_f 0.20 (toluene:ethyl acetate, 14:1); IR (*v*, cm^{-1}): 3339 (NH_{str}), 1672 (C=O_{str}), 1650 (*Amide I*), 1605 (*Amide II*); ^1H NMR (600 MHz, $CDCl_3$): δ 7.98, 7.65, 7.50, 7.03 (4d, 8H, *J* 8.4 Hz, Ar), 7.60 (dddd, 1H, *J* 1.2, 1.8, 2.4, 8.4 Hz, $H_{Olefinic}$), 7.39–7.32 (m, 4H, 1 Ar, 2 $H_{Olefinic}$, NH), 7.10 (d, 1H, *J* 15.0 Hz, $H_{Olefinic}$), 5.35–5.34 (m, 2H, CH_2CH=CH=$CHCH_2$), 2.40 (t, 2H, *J* 7.2, 7.8 Hz, $COCH_2$), 2.02–1.99 (m, 4H, CH_2CH=$CHCH_2CH_2$), 1.74 (quin, 2H, *J* 7.2, 7.8 Hz, $COCH_2CH_2$), 1.37–1.25 (m, 20H, 10 CH_2), 0.88 (t, 3H, *J* 7.2 Hz, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ 188.90 (*C*OCH=CH), 171.62 (CONH), 144.55, 141.99, 141.80, 136.15, 130.06, 129.87, 129.70, 129.20, 128.86, 128.57, 127.29, 126.99, 124.14, 118.86 (12 C_{Ar}, 3 CH=CH), 37.94 ($COCH_2$), 31.91, 29.77, 29.70, 29.53, 29.33, 29.28, 29.22, 29.11, 27.23, 27.16, 25.44, 22.69 (13 CH_2), 14.13 (CH_3); Exact mass calculated for $C_{35}H_{47}NO_2$ (513.3606), EI MS, *m/z* (%), Found: 513.40 (M^+, 60), 495.40 (1), 422.32 (9), 249.10 (100). Anal. Calcd. for $C_{35}H_{47}NO_2$ (513.75): N, 2.73. Found. N, 2.26.

*4.1.2.8. N-(4-((E)-3-(Furan-2-yl)acryloyl)phenyl)oleamide (**6a**).* Yield: 90% As tiny star-like yellow crystals upon flash chromatography using solvent gradient of toluene then (toluene:ethyl acetate 8:1); R_f 0.28 (toluene:ethyl acetate, 8:1); Mp. 67 °C; IR (*v*, cm^{-1}): 3353 (NH_{str}), 1677 (C=O_{str}), 1647 (*Amide I*), 1595 (*Amide II*); ^1H NMR (600 MHz, $CDCl_3$): δ 8.03, 7.67 (2d, 4H, *J* 8.4 Hz, Ar), 7.59 (d, 1H, *J* 15.6 Hz, COCH=*C*H), 7.53 (d, 1H, *J* 1.2 Hz, H-5$_{Fur}$), 7.46 (d, 1H, *J* 15.6 Hz, COC*H*=CH), 7.45 (brs, 1H, NH), 6.71 (d, 1H, *J* 3.6 Hz, H-3$_{Fur}$), 6.51 (d, 1H, *J* 1.2 Hz, H-4$_{Fur}$), 5.37–5.31 (m, 2H, CH=CH), 2.39 (t, 2H, *J* 7.2, 7.8 Hz, $COCH_2$), 2.04–1.99 (m, 4H, CH_2CH=$CHCH_2$), 1.74 (quin, 2H, *J* 7.2 Hz, $COCH_2CH_2$), 1.38–1.17 (m, 20H, 10 CH_2), 0.88 (t, 3H, *J* 6.6, 7.6 Hz, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ 188.35 (COCH=CH), 171.80 (CONH), 151.73, 144.88, 130.45, 130.06, 129.92, 129.71, 119.06, 118.89, 116.16, 112.67 (10 C_{Ar}, 2 CH=CH), 37.93 ($COCH_2$), 31.91, 29.77, 29.70, 29.53, 29.33, 29.32, 29.28, 29.22, 29.11, 27.23, 27.16, 25.44, 22.69 (13 CH_2), 14.13 (CH_3); Exact mass calculated for $C_{31}H_{43}NO_3$ (477.3242), EI MS, *m/z* (%), Found: 477.40 (M^+, 1), 466.45 (7), 452.0 (14), 434.0 (38), 399.0 (36), 368 (100). Anal. Calcd. for $C_{31}H_{43}NO_3$ (477.68): C, 77.95; H, 9.07; N, 2.93. Found. C, 77.79; H, 8.88; N, 2.77.

*4.1.2.9. N-(4-((E)-3-(Thiophen-2-yl)acryloyl)phenyl)oleamide (**6b**).* Yield: 89% As tiny yellow crystals upon flash chromatography using solvent gradient of toluene then (toluene:ethyl acetate, 9:1) followed by recrystallization from toluene or Et_2O; R_f 0.32 (toluene:ethyl acetate, 8:1); Mp. 62 °C; IR (*v*, cm^{-1}): 3342 (NH_{str}), 1671 (C=O_{str}), 1652 (*Amide I*), 1589 (*Amide II*); ^1H NMR (600 MHz, $CDCl_3$): δ 8.02, 7.67 (2d, 4H, *J* 8.4 Hz, Ar), 7.94 (d, 1H, *J* 15.6 Hz, COCH=*C*H), 7.42 (d, 1H, *J* 4.8 Hz, H-5$_{Thiop}$), 7.36 (d, 1H, *J* 3.6 Hz, H-3$_{Thiop}$), 7.33 (d, 1H, *J* 15.6 Hz, COC*H*=CH), 7.32 (brs, 1H, NH), 7.09 (dd, 1H, *J* 3.6, 4.8 Hz, H-4$_{Thiop}$), 5.37–5.33 (m, 2H, CH_2CH=$CHCH_2$), 2.40 (t, 2H, *J* 7.2, 7.8 Hz, $COCH_2$), 2.01–1.99 (m, 4H, CH_2CH=$CHCH_2$), 1.75 (quin, 2H, *J* 7.2, 6.6 Hz, $COCH_2CH_2$), 1.39–1.26 (m, 20H, 10 CH_2), 0.88 (t, 3H, *J* 6.6, 7.2 Hz, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ 188.30 (COCH=CH), 171.55 (CONH), 1420.10, 140.47, 136.94, 133.63, 132.00, 131.06, 19.90, 129.70, 128.72, 128.35, 120.51, 118.88 (10 C_{Ar}, 2 CH=CH), 37.94 ($COCH_2$), 31.91, 29.76, 29.70, 29.53, 29.33, 29.32, 29.27, 29.22, 29.11, 27.23, 27.16, 25.43, 22.69 (13 CH_2), 14.13 (CH_3); Exact mass calculated for $C_{31}H_{43}NO_2S$ (493.3014), EI MS, *m/z* (%), Found: 493.50 (M^+, 4.7), 483.30 (8), 463.30 (13), 437.40 (15). Anal. Calcd. for $C_{31}H_{43}NO_2S$ (493.74): C, 75.41; H, 8.78; N, 2.84. Found. C, 74.93; H, 8.34; N, 3.35.

*4.1.3. N-(3-Acetylphenyl)palmitamide (**8a**)*

Compound **7** (1.0 g, 7.4 mmol) was treated with palmitoyl chloride (2.5 g, 9.1 mmol) as described for **3**. Recrystallization of the residue from EtOH affords **8a** as fine creamy crystals (1.5 g, 54%); Mp. 95–96 °C; R_f 0.25 (toluene:ethyl acetate, 6:1); IR (*v*, cm^{-1}): 3288 (NH_{str}), 1672 (C=O_{str}), 1657 (*Amide I*), 1590 (*Amide II*); ^1H NMR (600 MHz, DMSO-d_6): δ 8.04 (s, 1H, H-2$_{Ar}$), 7.91 (d, 1H, *J* 7.8 Hz, H-6$_{Ar}$), 7.68 (d, 1H, *J* 7.8 Hz, H-4$_{Ar}$), 7.43 (t, 1H, $J_{4,5} = J_{5,6}$ 7.8 Hz, H-5$_{Ar}$), 2.61 (s, 3H, $COCH_3$), 2.39 (t, 2H, *J* 7.8 Hz, $COCH_2$), 1.74 (m, 2H, $COCH_2CH_2$), 1.63 (br.s, 1H, N–H), 1.38–1.25 [m, 24H, $(CH_2)_{12}$], 0.88 (dd, 3H, *J* 6.6, 7.2 Hz, CH_2CH_3); Anal. Calcd for $C_{24}H_{39}NO_2$. 0.5H_2O (382.59) C: 75.34, H: 10.56, N: 3.66. Found: C: 75.95, H: 10.56, N: 4.04.

*4.1.4. General procedure for the synthesis of (**10a–c**)*

A mixture of **8a–c** (0.4 g, 1.1 mmol), **9** (0.6 g, 5.0 mmol) and NaOH (0.5 g, 12.5 mmol) in EtOH (12 ml) was stirred at room temperature for 48 h then neutralized with AcOH and evaporated *in vacuo*.

*4.1.4.1. (E)-N-(3-(3-(4-hydroxyphenyl)acryloyl)phenyl)palmitamide (**10a**).* The residue was purified by flash chromatography using solvent gradient of (toluene:ethyl acetate, 10:1 → 8:1) to afford **10a** (0.33 g, 66%) as orange sun-like crystals; Mp. 106 °C; R_f 0.34 (toluene:ethyl acetate, 4:1); IR (*v*, cm^{-1}): 3197–3171 (O–H_{str}, N–H_{str}), 1671 (C=O_{str}, *Amide I*), 1601 (*Amide II*); ^1H NMR (600 MHz, $CDCl_3$): δ 9.86 (s, 1H, OH), 7.83–7.81 (m, 5H, Ar, COCH=*CH*), 6.99–6.98 (m, 5H, Ar, COC*H*=CH), 2.37 (t, 2H, *J* 7.8 Hz, $COCH_2$), 1.83 (br.s, 1H, NH), 1.64 (m, 2H, $COCH_2CH_2$), 1.35–1.25 [m, 24H, $(CH_2)_{12}$], 0.88 (t, 3H, *J* 7.2 Hz, CH_2CH_3); Exact mass calculated for $C_{31}H_{43}NO_2$ (477.32), EI MS, *m/z* (%), Found: 479.00 (17, M+2), 423 (17), 383 (46), 381 (18), 369 (8). Anal. Calcd. for $C_{31}H_{43}NO_2$ (477.69): N: 2.95. Found: N 2.48.

*4.1.4.2. (E)-N-(3-(3-(4-hydroxyphenyl)acryloyl)phenyl)acetamide (**10b**).* The residue was purified by flash chromatography (toluene:ethyl acetate, 1:1) to afford **10b** (50 mg, 21%) as colorless powder; Mp. 228–30 °C; R_f 0.2 (toluene:ethyl acetate, 1:1); IR (*v*, cm^{-1}): 3339 (O–H_{str}, N–H_{str}), 1670 (C=$O_{str.}$), 1640 (*Amide I*), 1601 (*Amide II*); ^1H NMR (600 MHz, DMSO-d_6): δ 10.16 (br.s, 1H, OH), 8.17 (s, 1H, H-2$_{Anilide}$), 7.87 (d, 1H, $J_{5,6}$ 7.8 Hz, H-6$_{Anilide}$), 7.82 (d, 1H, $J_{7,8}$ Hz, H-4$_{Anilide}$), 7.71 (d, 2H, *J* 8.4 Hz, H-2$_{Phenol}$, H-6$_{Phenol}$), 7.67 (d, 1H, *J* 15.6 Hz, COCH=*CH*), 7.61 (d, 1H, *J* 15.6 Hz, COC*H*=CH), 7.47 (t, 1H, $J_{4,5} = J_{5,6}$ 7.8 Hz, H-5$_{Anilide}$), 6.82 (d, 2H, *J* 8.4 Hz, H-3$_{Phenol}$, H-5$_{Phenol}$), 2.54 (s, 1H, NH), 2.06 (s, 3H, $COCH_3$); Exact mass calculated for $C_{17}H_{15}NO_3$ (281.11), EI MS, *m/z* (%), Found: 281 (100, M^+), 264 (6), 238 (45), 222 (16), 210 (24). Anal. Calcd. for $C_{17}H_{15}NO_3$. 0.25H_2O (285.83) C: 71.42, H: 5.24. Found: C: 71.42, H: 5.47.

*4.1.4.3. (E)-N-(3-(3-(4-hydroxyphenyl)acryloyl)phenyl)benzamide (**10c**).* The residue was purified by flash chromatography with solvent gradient (toluene then toluene:ethyl acetate, 8:1 → 5:1) to

afford **10c** (0.37 g, 44%) as brilliant yellow crystals; Mp. 154–156 °C; R_f 0.15 (toluene:ethyl acetate, 6.5:1); IR (ν, cm^{-1}): 3298 (O–H_{str}, N–H_{str}), 1661 (C=$O_{str.}$, *Amide I*), 1611 (*Amide II*); ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.47, 10.15 (2s, 2H, $NH_{D_2O\ Exchangeable}$, $OH_{D_2O\ Exchangeable}$), 8.41 (s, 1H, H-$2_{Anilide}$), 8.10, 7.89 (2d, 2H, *J* 7.8 Hz, Ar), 7.99 (d, 2H, *J* 8.4 Hz, H-2_{Phenol}, H-6_{Phenol}), 7.72 (d, 2H, *J* 9.0 Hz, Ar), 7.68 (d, 1H, *J* 14.4 Hz, COCH=C*H*), 7.60 (d, 1H, *J* 7.2 Hz, Ar), 7.54 (m, 3H, COC*H*=CH, Ar), 7.16 (d, 1H, *J* 7.2 Hz, Ar), 6.83 (d, 2H, *J* 8.4 Hz, H-3_{Phenol}, H-5_{Phenol}); ^{13}C NMR (150 MHz, DMSO-d_6): δ 188.90 (*C*OCH=CH), 165.76 (CONH), 160.27, 144.75, 139.66, 138.47, 137.41, 134.66, 131.86, 131.09, 129.16, 128.97, 128.52, 128.28, 127.77, 125.77, 125.39, 124.60, 123.88, 119.99, 118.49, 115.91 (18C_{Ar}, COCH=*C*H); Exact mass calculated for $C_{22}H_{17}NO_3$ (343.12), EI MS, *m/z* (%), Found: 343.00 (28, M^+), 326 (0.7), 315 (0.6), 284 (0.3), 254 (0.3), 238 (7). Anal. Calcd. for $C_{22}H_{17}NO_3$ (343.38) C: 76.95, H: 4.99. Found: C: 76.80, H: 5.08.

4.1.5. (E)-1-(3-((E)-4-methoxybenzylideneamino)phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**11**)

A mixture of **7** (1.0 g, 7.4 mmol), anizaldehyde (2.2 ml, 18.1 mmol) and NaOH (0.2 g, 5.0 mmol) in EtOH–DMF (2:5, 7 ml) was stirred overnight, filtered at the pump, washed with little H_2O then EtOH and dried well. Recrystallization from EtOH affords **11**(2.1 g, 76%) as fine yellow crystals; Mp. 132 °C; R_f 0.5 (toluene:acetone:Et_3N, 9 : 0.2 : 0.1); IR (ν, cm^{-1}): 1661 (C=$O_{str.}$), 1621 (C=$N_{str.}$); ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.46 (s, 1H, N=CH), 7.86–7.89 (m, 2H, Ar), 7.88 (d, 1H, *J* 9.0 Hz, Ar), 7.86 (t, 1H, *J* 1.8, 3.6 Hz, Ar), 7.64–7.61 (m, 1H, Ar), 7.62 (d, 1H, *J* 8.4 Hz, Ar), 7.52 (t, 1H, *J* 7.8 Hz, Ar), 7.46, 7.83 (2 d, 2H, J_{vic} 15.6 Hz, COC*H*=C*H*), 7.41, 7.42 (2 dd, 1H, *J* 1.2, 1.8, Ar), 7.02–7.00 (m, 1 H, Ar), 7.02 (d, 1H, *J* 9.0 Hz, Ar), 6.96–6.93 (m, 1H, Ar), 6.95 (d, 1H, *J* 8.4 Hz, Ar), 3.87, 3.89 (2s, 6H, 2 OCH_3); Exact mass calculated for $C_{24}H_{21}NO_3$ (371.15), EI MS, *m/z* (%), Found: 371 (100, M^+), 340 (22), 262 (23), 328 (13), 237 (36), 297 (10). Anal. Calcd. for $C_{24}H_{21}NO_3$ (371.43) C: 77.61, H: 5.70, N: 3.77. Found: C: 77.89, H: 5.72, N: 4.18.

4.1.6. (E)-1-(3-((E)-2-hydroxybenzylideneamino)phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**14a**)

4.1.6.1. Method A. A mixture of **11** (0.2 g, 0.5 mmol) and conc. HCl (0.3 ml) in dry acetone (10 ml) was heated under reflux for 2 h then coevaporated with toluene *in vacuo*. The residue was taken in ethyl acetate (30 ml), washed with saturated K_2CO_3 solution (10 ml), dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by flash chromatography (toluene:ethyl acetate, 5:1) to afford **12** (0.11 g, 86%). R_f 0.3 (toluene:ethyl acetate, 5:1). Compound **12** (0.11 g, 0.4 mmol) was taken in EtOH (4 ml) treated with salicylaldehyde (0.2 ml, 1.9 mmol) and heated under reflux for 1 h and left to be crystallized overnight to afford **14a** (0.12 g, 75%) as single yellow crystals.

4.1.6.2. Method B. Compound **7** was treated with salicylaldehyde as described for the synthesis of **14a** in *Method A* to afford **13** (68%) upon washing with Et_2O as yellow crystals and was pure enough to be used in the next step without further purification; R_f 0.3 (toluene:ethyl acetate, 5:1); Mp. 90–92 °C; IR (ν, cm^{-1}): 3329 (O–H_{str}, very weak), 3060 (C–$H_{Ar\ str}$), 2993 (C–$H_{Alip.\ str}$), 1671 (C=$O_{str.}$), 1614 (C=$N_{str.}$).

A mixture of **13** (0.45 g, 1.9 mmol), anisaldehyde (0.3 ml, 2.5 mmol) and NaOH (0.2 g, 5.0 mmol) in EtOH (5 ml) was stirred at ambient temperature for 48 h then acidified with AcOH. The precipitate formed was filtered at the pump, washed with MeOH–Et_2O and dried well. The residue was recrystallized from EtOH to afford **14a** (0.18 g, 27%); R_f 0.3 (toluene); Mp. 129–30 °C. IR (ν, cm^{-1}): 2947 (OH–N), 1668 (C=O_{str}), 1616 (C=N_{str}), 1294 (C–O_{str}). ^{1}H NMR (600 MHz, $CDCl_3$): δ 13.10 (s, 1H, OH), 8.70 (s, 1H, CH=N), 7.92 (d, 1H, *J* 7.8 Hz, Ar), 7.90 (t, 1H, *J* 1.8 Hz, Ar), 7.83 (d, 1H, *J* 15.6 Hz, COCH=C*H*), 7.63 (d, 2H, *J* 8.4 Hz, Ar), 7.55 (t, 1H, *J* 7.2, 7.8 Hz, Ar), 7.48 (m, 1H, Ar), 7.44 (d, 1H, *J* 15.6 Hz, COC*H*=CH), 7.44–7.42 (m, 1H, Ar), 7.41–7.39 (m, 1H, Ar), 7.04 (d, 1H, *J* 7.8 Hz, Ar), 6.97 (dd, 1H, *J* 1.2, 7.2 Hz, Ar), 6.95 (d, 1H, *J* 7.2 Hz, Ar), 6.94 (m, 1H, Ar), 3.86 (s, 3H, OCH_3); ^{13}C NMR (150 MHz, DMSO-d_6): δ 189.85 (C=O), 163.69 (C=N), 161.80, 161.11, 148.91, 145.26, 139.73, 133.53, 132.56, 130.40, 129.64, 127.39, 126.69, 125.66, 120.64, 119.27, 119.24, 119.00, 117.29, 114.42 (18 C_{Ar}, COCH=*C*H), 55.42 (OCH_3); Exact mass calculated for $C_{23}H_{19}NO_3$ (357.14), EI MS, *m/z* (%), Found: 357.00 (100, M^+), 355 (0.8), 342 (9), 314 (4), 298 (1.5), 284 (0.9). Anal. Calcd. for $C_{23}H_{19}NO_3$ (357.40) C: 77.29, H: 5.36. Found: C: 77.22, H: 5.40.

4.1.7. (E)-1-(3-((E)-2-hydroxybenzylideneamino)phenyl)-3-p-tolylprop-2-en-1-one (**14b**)

Compound **13** (0.8 g, 3.3 mmol) was treated with *p*-tolualdehyde (0.5 ml, 4.2 mmol) and NaOH (0.2 g, 5.0 mmol) in EtOH (10 ml) then worked up as described for **14a** in *Method B*. The crude precipitate formed was filtered at the pump then recrystallized from MeOH to afford **14b** (0.62 g, 54%) as buff colored crystals; Mp. 118 °C; R_f 0.25 (Toluene); IR (ν, cm^{-1}): 2947 (OH–N), 1662 (C=$O_{str.}$), 1618 (C=$N_{str.}$), 1283 (C–$O_{str.}$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 13.09 (s, 1H, OH), 8.71 (s, 1H, N=CH), 9.43 (d, 1H, *J* 7.8 Hz, Ar), 7.92 (t, 1H, *J* 1.8 Hz, Ar), 7.84 (d, 1H, *J* 15.6 Hz, COCH=C*H*), 7.597.56 (m, 3H, Ar), 7.52 (d, 1H, *J* 15.6 Hz, COC*H*=CH), 7.51–7.50 (m, 1H, Ar), 7.44–7.40 (m, 2H, Ar), 7.25–7.24 (m, 2H, Ar), 7.05 (d, 1H, *J* 8.4 Hz, Ar), 6.98 (t, 1H, *J* 7.8, 7.2 Hz, Ar), 1.62 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ 189.99 (C=O), 163.76 (C=N), 161.12, 148.99, 145.55, 141.39, 139.60, 133.57, 132.57, 131.93, 129.75, 129.69, 128.62, 126.75, 125.80, 120.68, 120.64, 119.26, 119.00, 117.31 (18 C_{Ar}, COCH=*C*H), 21.60 (CH_3); Exact mass calculated for $C_{23}H_{19}NO_2$ (341.14), EI MS, *m/z* (%), Found: 343 (M+2, 9), 342 (M+1, 7), 341 (M, 43), 328 (11), 327 (12), 293 (17), 149 (100). Anal. Calcd. for $C_{23}H_{19}NO_2$ (341.40) C: 80.92, H: 5.61, N: 4.10. Found: C: 80.51, H: 5.37, N: 3.61.

4.1.8. General procedure for the synthesis of metal complexes (**15a–d**)

Metal acetate (0.42 mmol) in EtOH (15 ml) was added dropwise to a refluxing solution of the ligand **14a** or **14b** (0.42 mmol) in EtOH (15 ml). Reflux was continued for further 10 min after complete addition of the salt. The precipitate formed was filtered, washed with EtOH (3 ml) and dried well.

*4.1.8.1. Bis(2-((E)-(3-((E)-3-(4-methoxyphenyl)acryloyl)phenylimino)methyl)phenoxy)copper (**15a**).* Brown precipitate formed immediately during addition of the salt (0.1 g, 62%); IR (ν, cm^{-1}): 1650 (C=O_{str}), 1604 (C=N_{str}). Anal. Calcd. For $C_{46}H_{36}CuN_2O_6$ (776.33): C, 71.17; H, 4.67; N, 3.61. Found: C, 70.67; H, 4.46; N, 3.51.

*4.1.8.2. Bis(2-((E)-(3-((E)-3-(4-methoxyphenyl)acryloyl)phenylimino)methyl)phenoxy)zinc (**15b**).* Yellow precipitate formed upon concentration of the reaction mixture to half of its volume and cooling to ambient temperature (0.1 g, 61%); IR (ν, cm^{-1}): 1651 (C=O_{str}), 1603 (C=N_{str}); ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.71 (s, 2H, 2 CH=N), 8.02 (s, 2H, Ar), 7.94 (d, 2H, *J* 7.2 Hz, Ar), 7.72 (d, 4H, *J* 9.0 Hz, Ar), 7.66 (d, 2H, *J* 15.6 Hz, 2 C*H*=CHCO), 7.61 (d, 2H, *J* 15.6 Hz, 2 CH=*CH*CO), 7.55–7.54 (m, 2H, Ar), 7.51 (t, 2H, *J* 7.2, 7.8 Hz, Ar), 7.45 (dd, 2H, *J* 1.8, 7.8 Hz, Ar), 7.27 (m, 2H, *J* 1.2, 1.8 Hz, Ar), 6.97 (d, 4H, *J* 9.0 Hz, Ar), 6.73 (d, 2H, *J* 8.4 Hz, Ar), 6.60 (t, 2H, *J* 7.2 Hz, Ar), 3.80 (s, 6H, 2 OCH_3). Anal. Calcd. For $C_{46}H_{36}N_2O_6Zn$. $0.5H_2O$ (778.21): C, 70.18; H, 4.74. Found: C, 70.76; H, 4.46.

*4.1.8.3. Bis(2-((E)-(3-((E)-3-p-tolylacryloyl)phenylimino)methyl)phenoxy)copper (**15c**).* Brown precipitate formed upon concentration of the reaction mixture to half of its volume and cooling to

ambient temperature (0.11 g, 66%); IR (ν, cm^{-1}): 1655 (C=O_{str}), 1605 (C=N_{str}). Anal. Calcd. For $C_{46}H_{36}CuN_2O_4$. 0.5H_2O (753.35): C, 73.33; H, 4.96; N, 3.71. Found: C, 72.68; H, 4.68; N, 3.41.

*4.1.8.4. Bis(2-((E)-(3-((E)-3-p-tolylacryloyl)phenylimino)methyl) phenoxy)zinc (**15d**).* Yellow precipitate formed upon concentration of the reaction mixture to half of its volume and cooling to ambient temperature (0.075 g, 46%); IR (ν, cm^{-1}): 1655 (C=$O_{str.}$), 1607 (C=$N_{str.}$); ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.70 (s, 2 H, 2 CH=N), 8.01 (s, 2 H, Ar), 7.95 (d, 2 H, *J* 7.8 Hz, Ar), 7.71 (d, 2 H, *J* 15.6 Hz, 2 *CH*=CHCO), 7.66 (d, 2 H, *J* 15.6 Hz, 2 CH=*CH*CO), 7.65 (d, 4 H, *J* 7.8 Hz, Ar), 7.55–7.54 (m, 2 H, Ar), 7.51 (t, 2 H, *J* 7.2, 7.8 Hz, Ar), 7.45 (dd, 2 H, *J* 1.8, 7.8 Hz, Ar), 7.26 (m, 2 H, *J* 1.8, 8.4 Hz, Ar), 7.23 (d, 4 H, *J* 7.8 Hz, Ar), 6.72 (d, 2 H, *J* 8.4 Hz, Ar), 6.59 (t, 2 H, *J* 7.2 Hz, Ar), 2.33 (s, 6 H, 2 CH_3). Anal. Calcd. For $C_{46}H_{36}N_2O_4Zn$ (746.20): C, 74.04; H, 4.86; N, 3.75. Found: C, 74.18; H, 4.72; N, 3.73.

4.2. Crystallographic structure determination

Single crystal X-ray diffraction data were collected using the Mo $K\alpha$ radiation (λ = 0.71073 Å) on maXus (Bruker Nonius, Delft & MacScience, Japan) at 298 K. Structure was solved by direct methods using SIR97 [45] and refined by full-matrix least-squares on all F^2 using maXus [46]. Cell refinement according to HKL Scalepack and data reduction using Denzo and Scalepak [47]. Molecular graphics according to Johnson et al. [48]. Crystallographic data of **14a** was deposited with the Cambridge Crystallographic Data Centre (http://www.ccdc.cam.ac.uk/services/structure_deposit), deposition number: CCDC 960986 (E-mail: deposit@ccdc.cam.ac.uk).

4.3. Biology

4.3.1. Antiobesity

4.3.1.1. Animal experiments. Body weight loss and hypolipidemic activities in SD Male rats (Purchased from Experimental Animal Center of Suez Canal University, Ismailia, Egypt) were divided into eleven groups each group contain 10 rats: rats fed with a low fat diet (normal), rats treated with high-fat diet-fed (obese), high-fat diet fed rats treated with Tetrahydrolipstatin (Orlistat) (30 mg/day/kg of rat weight) as positive control, high-fat diet-fed rats treated with compound (**4a**–**f** and **6a,b**) at a dose of 30 mg/kg. Low fat diet was composed of protein 20%, carbohydrate 70%, and fat 10%, whereas high fat diet was composed of protein 20%, carbohydrate 35%, and fat 45%. They were allowed free access to tap water and food. Every group of rats, the normal control rats were exception, were fed with HFD for two months. Then every group of rats were given vehicle or compounds by intragastric administration respectively, once daily for one month. After this feeding period, the rats were sacrificed.

4.3.1.2. Sample collection. Blood was taken in the morning, after 12–14 h fasting. Sera were obtained after at least 30 min clotting by centrifugation at 2000 rpm for 15 min. Sera were removed and frozen at −20 °C.

4.3.1.3. Measurements of metabolic parameters. Serum levels of glucose, TC, TG and FFAs were determined by enzymatic tests from Roche Diagnostic using the cobase 311 autoanalyser. Insulin levels were determined by an enzyme-immunoassay which does not cross-react with proinsulin. These hormone measurements were done in duplicate and results were accepted if the coefficients of variation were less than 10%. Inter assay coefficients of variation were approximately 10%. Insulin sensitivity index was calculated as FBs/Free insulin level.

4.3.2. Antioxidant activity

4.3.2.1. 1,1-Diphenyl-2-picryl-hydrazyl (DPPH•) radical scavenging activity assay. Each test compound (10 μL, 0.03 mM in DMSO) was added to DPPH (990 μL, 0.1 mM in MeOH) and shacked thoroughly reaching a final concentration of (30 nM). Absorbance at 517 nm was determined spectrophotometrically over a period of 2 h. Changes in absorbance were minimal within 30 min. 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was used as positive antioxidant control under the same conditions and all measurements were undertaken on three replicates. The activity of the test compounds to scavenge DPPH was calculated as percentage inhibition from the relation:

$$\text{Inhibition } \% = [(A_B - A_A)/A_B]\ 100$$

where A_A is the absorbance of the test compound or control at t = 30 min, while, A_B is that for the blank at t = 0 min.

4.3.2.2. Hydroxyl radical (HO•) scavenging assay. Samples (10 μl) of selected tested compounds and standard compound (Ascorbic acid) at 30 nM were dissolved in DMSO, then incubated with 9.0 mM $FeSO_4$ (1.0 ml), 0.3% H_2O_2 (1.0 ml) in 0.5 ml salicylic acid–EtOH solution (9.0 mM) for 30 min at 37 °C. Hydroxyl radicals were detected by monitoring absorbance at 510 nm. The total volume of the mixture in each tube was made up to 3 ml by adding the required amount of distilled water. Inhibition (I) percentage of deoxyribose degradation in percent was calculated according following equation: $I\ (\%) = [(A_0 - A_1/A_0)]\ 100$. A_0 is the absorbance of the control reaction and A_1 is the absorbance of the test compound. Measurements were done in the Chemistry Department, Faculty of science at Taif University.

4.4. Cytotoxicity screening

In vitro anticancer-drug discovery screening of newly synthesized compounds was performed using the colorimetric cytotoxicity assay for anticancer-drug screening using prostate cancer cell line PC3 at the National cancer Institute, Cairo University. Cells were plated in 96-multiwell plates (10^4 cells per well) for 24 h to allow attachment of cells to the walls. Monolayer cells were incubated with test compounds individually at concentrations of 5.0, 10.0, 20.0, 40.0 and 80.0 μM at 37 °C under 5% CO_2 atmosphere. After 48 h, cells were fixed, washed then stained with sulfo-rhodamine-B stain. Excess stain was washed with acetic acid and attached stain was recovered with *tris*–EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration was plotted to get the survival curve for each compound [49].

4.5. Statistical analysis

All results were subjected to one-way ANOVA and the means were compared according to the Student–Newman–Keuls (SNK) multiple range test ($p \leq 0.05$).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.02.021.

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