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Effect of Stilbene and Chalcone Scaffolds Incorporation in Clofibric Acid on PPARα Agonistic Activity

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Abstract: In an effort to develop safe and efficacious compounds for the treatment of metabolic disorders, new compounds based on a combination of clofibric acid, the active metabolite of clofibrate, and trans-stilbene, chalcone, and other lipophilic groups were synthesized. They were evaluated for PPARα transactivation activity; all branched derivatives showed an increase of the transcriptional activity of receptor compared to the linear ones. Noteworthy, stilbene and benzophenone branched derivatives activated the PPARα better than clofibric acid.

Keywords: PPARs, clofibrate, chalcone, stilbene, transactivation assay.

INTRODUCTION

The PPARs (peroxisome proliferator activating receptors) are ligand-activated transcription factors that are highly expressed in metabolically active tissues which regulate genes encoding lipid and glucose metabolism, and overall energy homeostasis. There are three PPAR isoforms, PPARα, β/δ, and γ, and these vary in tissue distributions, selectivity and responsiveness to ligands, thus leading to the regulation of different sets of genes [1]. PPARα is highly expressed in metabolically active tissues such as liver, muscle, intestine, and brown adipose tissue where it regulates gene expression involved in lipid metabolism. PPARα also controls inflammatory responses in the liver and other tissues [2]. PPARγ controls adipocyte differentiation in adipose tissue and its activation plays a major role in enhancing blood glucose uptake as well as in promoting the differentiation of adipocytes [3]. The activation of PPAR β/δ enhances fatty acids transport and oxidation, improves glucose homeostasis through improvement of insulin sensitivity and inhibition of glucose output, attenuates macrophage inflammatory responses, and increases plasma HDL concentrations [4]. PPARs subtypes share a similar structure: an amino terminal activation domain (AF-1), a DNA binding domain (DBD), a ligand binding domain (LBD) and a second carboxy terminal activation domain (AF-2). The specificity of the LBD ligand complex is largely based on hydrophobic interactions, hydrogen-bonding networks, and the steric size and shape of the binding pocket. PPAR ligands induce binding to PPAR responsive elements (PPREs) in the DNA after dimerization with another nuclear receptor, the retinoid-X receptor (RXR). The release of corepressors and recruitment of coactivators regulate the transcription of genes via direct activation of gene expression, ligand-dependent or -independent repression or transrepression [5].

At the time they were identified, in the early 1990s, no endogenous ligand was known to activate PPARs, but since then a number of lipid derivatives, mostly fatty acids and prostaglandins, have been proposed to be endogenous ligands [6].

In the last years, because of the broad roles of PPARs in regulating metabolism, inflammation, differentiation, and cellular growth, a number of approaches directed towards the development of new ligands for PPAR subtypes represented the logical evolution in the field of metabolic disorders treatment [7]. While PPARα is activated by the fibrate antilipidemic drugs [8], PPARγ is the receptor for the thiazolidinedione (TZD) class [9] of antidiabetic drugs.

In the past, we have synthesized a series of 2-heteroarylthioalkanoic acids derivatives of clofibric acid with the aim of obtaining new hypolipidemic compounds active as PPARα agonists [10]. The EC_{50} values of some new compounds within 3 μM demonstrated that the replacement of the phenyl ring of clofibric acid with other hindered groups leads up to PPARα agonists more potent than the reference compound clofibric acid. In an effort to develop safe and efficacious compounds for the treatment of metabolic disorders, natural products and their analogs are extensively studied as PPAR agonists. In this field, stilbenes and chalcones and some of their synthetic derivatives have shown to activate PPARα or to lower plasma lipid levels [11]. We have recently reported a series of molecules derived by the combination of antilipidemic drug gemfibrozil with natural α-asarone, stilbene, chalcone, and other bioisosteric modifications [12]. The highest agonistic activity was seen with the trans-stilbene derivative **1** (EC_{50} = 1.0 μM), showing that the wide electron delocalization could be an important factor to determine agonistic PPARα activity.

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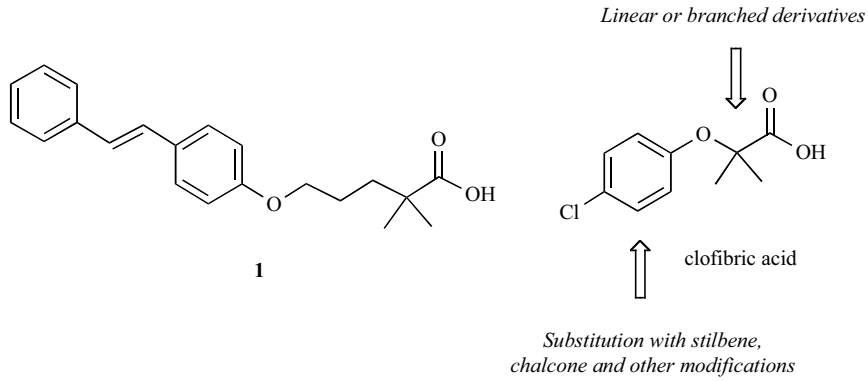


Fig. (1). Gemfibrozil derivative **1** and chemical modifications of clofibrate.

In this study, we describe the synthesis and structure-activity relationships (SARs) of newly designed compounds based on the combination of clofibric acid (the active metabolite of clofibrate) and lipophilic groups derived from natural products stilbene and chalcone (Fig. **1**).

MATERIALS AND METHODS

Chemistry

Melting points were determined on a Büchi B-540 apparatus and were uncorrected. Infrared spectra were recorded on a FT-IR 1600 Perkin–Elmer spectrometer. NMR spectra were run at 300 MHz on a Varian instrument; chemical shifts (δ) are reported in ppm. Microanalyses were carried out with an Eurovector Euro EA 3000 model analyzer and the analytical results were observed within 0.4% of the theoretical values. Commercial reagents were used as received from Aldrich or Fluka.

Procedure for the Preparation of Esters 7-16

The ethyl 2-bromo-2-methylpropanoate or ethyl bromoacetate (1.72 mmol) was added to a solution of (4-[(*E*)-2-phenylvinyl]phenol) (**2**), (2*E*)-1-(4-hydroxyphenyl) -3-phenylprop-2-en-1-one (**3**), (2*E*)-3-(4-hydroxyphenyl)-1-phenylprop-2-en-1-one (**4**), N-(4-hydroxyphenyl)-N-phenylurea (**5**) or 4-hydroxyphenyl)(phenyl)methanone (**6**) (1.72 mmol) and dry K_2CO_3 (6.19 mmol) dissolved in dry acetone (15 mL) under nitrogen atmosphere. After stirring for 18-20 h at reflux, the reaction was filtered and the precipitate was concentrated under reduced pressure. The residue was solubilized in chloroform (30 mL) and washed with NaOH 0.5 N (3 x 30 mL) and water (30 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (eluent cyclohexane/ethyl acetate 9:1).

Ethyl 2-methyl-2-{4-[(*E*)-2-phenylvinyl]phenoxy}propanoate (7). White solid, 54% yield, m.p. 62-64 °C. IR (KBr) 1722 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.26 (t, 3H, J = 7.2 Hz, CH_2CH_3), 1.61 (s, 6H, $C(CH_3)_2$), 4.25 (q, 2H, J = 7.2 Hz, CH_2CH_3), 6.83 (d, 2H, C*H* Ar), 6.94-7.08 (m, 2H, *H*C=C*H*), 7.23-7.41 (m, 5H, C*H* Ar), 7.48 (d, 2H, C*H* Ar); ^{13}C NMR ($CDCl_3$) δ 14.33 (CH_3CH_2), 25.60 ($C(CH_3)_2$), 61.74 (CH_2CH_3), 74.42 ($C(CH_3)_2$), 119.31, 126.54, 127.58, 128.22 and 128.88 (*C*H Ar), 127.45 and 128.25 (H*C*=*C*H), 131.56, 137.74 and 155.35 (*C* Ar), 174.51 (*C*=O).

Ethyl{4-[(*E*)-2-phenylvinyl]phenoxy}acetate (8). White solid, 81% yield, m.p. 107-108 °C. IR (KBr) 1761 cm^{-1}; ^{1}H NMR (DMSO) δ 1.19 (t, 3H, J = 7.2 Hz, CH_3CH_2), 4.15 (q, 2H, J = 7.2 Hz, CH_3CH_2), 4.78 (s, 2H, OCH_2CO), 6.91 (d, 2H, C*H* Ar), 7.05-7.19 (m, 2H, *H*C=C*H*), 7.20-7.39 (m, 3H, C*H* Ar), 7.47-7.52 (m, 4H, C*H* Ar); ^{13}C NMR (DMSO) δ 14.74 (CH_3CH_2), 61.33 (CH_2CH_3), 65.34 (OCH_2CO), 115.45 and 126.89 (*C*H Ar), 127.18 (=*C*H), 127.95 (=*C*H), 128.43, 128.54 and 129.36 (*C*H Ar), 131.03 and 137.97 (*C* Ar), 157.96 (*C* ArO), 169.38 (*C*=O).

Ethyl 2-methyl-2-{4-[(2*E*)-3-phenylprop-2-enoyl]phenoxy}propanoate (9). Yellow oil, 39% yield. IR (KBr) 1734, 1662 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.22 (t, 3H, J = 7.2 Hz, CH_3CH_2), 1.67 (s, 6H, $C(CH_3)_2$), 4.24 (q, 2H, J = 7.2 Hz, CH_2CH_3), 6.84 (d, 2H, J = 9.2 Hz, C*H* Ar), 7.40-7.42 (m, 3H, C*H* Ar), 7.50-7.55 (d, 1H, J = 15.7 Hz, HC=C*H*), 7.62-7.64 (m, 2H, C*H* Ar), 7.77-7.82 (d, 1H, J = 15.7 Hz, *H*C=CH), 7.98 (d, 2H, J = 9.2 Hz, C*H* Ar); ^{13}C NMR ($CDCl_3$) δ 14.28 (CH_3CH_2), 25.63 ($(CH_3)_2C$), 61.95 (CH_2CH_3), 79.58 ($C(CH_3)_2$), 117.69 (*C*H Ar), 122.O2 (=*C*H), 128.61, 129.17 and 130.62 (*C*H Ar), 131.86 (O=C*C* Ar), 135.25 (*C* ArC=), 144.37 (=*C*H), 159.91 (*C* ArO), 173.99 (O*C*=O), 189.01 (Ar*C*=O).

Ethyl {4-[(2*E*)-3-phenylprop-2-enoyl]phenoxy}acetate (10). Yellow solid, 72% yield, m.p. 62-63 °C. IR (KBr) 1763, 1656 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.31 (t, 3H, J = 6.9 Hz, CH_3CH_2), 4.29 (q, 2H, J = 6.9 Hz, CH_2CH_3), 4.71 (s, 2H, OCH_2CO), 6.99 (d, 2H, J = 9.0 Hz, C*H* Ar), 7.40-7.43 (m, 3H, C*H* Ar), 7.53 (d, 1H, J = 15.6 Hz, *H*C=C), 7.62-7.66 (m, 2H, C*H* Ar), 7.80 (d, 1H, J = 15.6 Hz, =C*H*), 8.04 (d, 2H, J = 9.0 Hz, C*H* Ar); ^{13}C NMR ($CDCl_3$) δ 14.40 (CH_3CH_2), 61.88 (CH_2CH_3), 65.45 (OCH_2CO), 114.67 (*C*H Ar), 121.O7 (=*C*HC=O), 128.63, 129.18, 130.67 and 131.07 (*C*H Ar), 132.23 (O=C*C*Ar), 135.2O (*C*ArCH=), 144.53 (Ar*C*H=), 161.67 (*C*ArO), 168.51 (EtO*C*=O), 188.77 (=CH*C*=O).

Ethyl 2-methyl-2-{4-[(1*E*)-3-oxo-3-phenylprop-1-enyl] phenoxy}propanoate (11). Yellow oil, 47% yield. IR (KBr) 1734 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.23 (t, 3H, J = 7.2 Hz, CH_3CH_2), 1.64 (s, 6H, $C(CH_3)_2$), 4.24 (q, 2H, J = 7.2 Hz, CH_2CH_3), 6.84 (d, 2H, J = 8.4 Hz, C*H* Ar), 7.41 (d, 1H, J = 15.6 Hz, =C*H*), 7.49-7.57 (m, 4H, C*H* Ar), 7.76 (d, 1H, J = 15.6 Hz, =C*H*), 8.03 (d, 2H, J = 8.4 Hz, C*H* Ar); ^{13}C NMR ($CDCl_3$) δ 14.30 (CH_3CH_2), 25.62 ($(CH_3)_2C$), 61.89 (CH_2CH_3), 79.52 ($C(CH_3)_2$), 118.71 (*C*H Ar), 120.49 (O=C*C*H=), 123.5 (*C*ArC=), 128.67, 128.82, 130.05 and

132.87 (*C*H Ar), 138.64 (O=C*C* Ar), 144.37 (=*C*H), 157.99 (*C*ArO), 166.10 (EtO*C*=O), 175.07 (=C*C*=O).

Ethyl{4-[(1*E*)-3-oxo-3-phenylprop-1-enyl]phenoxy} acetate (12). Yellow solid, 79% yield, m.p. 62-63 °C. IR (KBr) 1766 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.30 (t, 3H, J = 6.9 Hz, CH_3CH$_2$), 4.28 (q, 2H, J = 6.9 Hz, CH$_2$CH$_3$), 4.67 (s, 2H, OCH$_2$CO), 6.82 (d, 2H, J = 8.2 Hz, C*H* Ar), 7.43 (d, 1H, J = 15.3 Hz, =C*H*), 7.48-7.60 (m, 4H, C*H* Ar), 7.75 (d, 1H, J = 15.3 Hz, =C*H*), 7.99 (d, 2H, J = 8.2 Hz, C*H* Ar); ^{13}C NMR ($CDCl_3$) δ 14.40 (*C*H$_3$CH$_2$), 61.82 (*C*H$_2$CH$_3$), 65.49 (O*C*H$_2$CO), 115.27 (*C*H Ar), 120.57 (=*C*HC=O), 128.36 (*C* ArC=), 128.67, 128.83, 130.47 and 132.90 (*C*H Ar), 133.95 (O=C*C* Ar), 144.97 (=*C*HC Ar), 159.94 (*C* ArO), 168.57 (EtO*C*=O), 190.78 (*C*=OAr).

Ethyl 2-{4-[(anilinocarbonyl)amino]phenoxy}-2-methylpropanoate (13). White solid, 63% yield, m.p. 112-114 °C. IR (KBr) 3322, 1726, 1657 cm^{-1}, ^{1}H NMR ($CDCl_3$) δ 1.26 (t, 3H, J = 7.2 Hz, CH_3CH$_2$), 1.55 (s, 6H, C(CH_3)$_2$), 4.23 (q, 2H, J = 7.2 Hz, CH$_2$CH$_3$), 6.78 (d, 2H, J = 9.0 Hz, C*H* Ar), 7.01-7.06 (m, 2H, C*H* Ar), 7.14 (d, 2H, J = 9.0 Hz, C*H* Ar), 7.25-7.28 (m, 3H, C*H* Ar); ^{13}C NMR ($CDCl_3$) δ 14.33 (*C*H$_3$CH$_2$), 25.52 ((*C*H$_3$)$_2$C), 61.81 (*C*H$_2$CH$_3$), 79.74 (*C*(CH$_3$)$_2$), 120.6, 120.87, 122.92, 124.03 and 129.37 (*C*H Ar), 132.71 and 138.41 (*C* Ar), 152.12 (O=*C*NH), 154.04 (*C*ArO), 174.64 (O*C*=O).

Ethyl {4-[(anilinocarbonyl)amino]phenoxy}acetate (14). White solid, 74% yield, m.p. 162-164 °C. IR (KBr) 1735, 1642 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 1.25 (t, 3H, J = 7.2 Hz, CH_3CH$_2$), 4.20 (q, 2H, J = 7.2 Hz, CH$_2$CH$_3$), 4.67 (s, 2H, OCH$_2$CO), 6.88 (d, 2H, J = 9.3 Hz, C*H* Ar), 6.96-6.99 (m, 2H, C*H* Ar), 7.44 (d, 2H, J = 9.3 Hz, C*H* Ar), 7.51-7.54 (m, 2H, C*H* Ar), 7.95 (s, 1H, N*H*), 8.03 (m, 1H, N*H*); ^{13}C NMR (acetone-d_6) δ 14.03 (*C*H$_3$CH$_2$), 66.91 (O*C*H$_2$CO), 118.42, 119.53, 121.31 and 128.91 (*C*H Ar), 134.01 (NH*C* Ar), 139.72 (NH*C* Ar), 148.92 (C ArO), 152.73 (HN*C*=O), 173.10 (EtO*C*=O).

Ethyl 2-(4-benzoylphenoxy)-2-methylpropanoate (15). White solid, 29% yield, m.p. 85-87 °C. IR (KBr) 1725, 1654 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.23 (t, 3H, J = 6.9 Hz, CH_3CH$_2$), 1.67 (s, 6H, C(CH_3)$_2$), 4.23 (q, 2H, J = 6.9 Hz, CH$_2$CH$_3$), 6.86 (d, 2H, J = 9.0 Hz, C*H* Ar), 7.45-7.59 (m, 3H, C*H* Ar), 7.38 (d, 2H, J = 9.0 Hz, C*H* Ar), 7.61 (m, 2H, C*H* Ar); ^{13}C NMR ($CDCl_3$) δ 14.28 (*C*H$_3$CH$_2$), 24.90 ((*C*H$_3$)$_2$C), 60.71 (*C*H$_2$CH$_3$), 78.72 ((CH$_3$)$_2$*C*), 115.02, 128.63 and 128.70 (*C*H Ar), 129.54 (*C* Ar), 129.84 and 132.31 (*C*H Ar), 138.22 and 158.67 (*C* Ar), 170.12 (EtO*C*=O), 195.98 (Ar*C*=OAr).

Ethyl (4-benzoylphenoxy)acetate (16). White solid, 94% yield, m.p. 82-84 °C. IR (KBr) 1725, 1654 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.31 (t, 3H, J = 7.2Hz, CH_3CH$_2$), 4.29 (q, 2H, J = 7.2 Hz, CH$_2$CH$_3$), 4.70 (s, 2H, OCH$_2$CO), 6.97 (d, 2H , J = 9.0 Hz, C*H* Ar), 7.44-7.60 (m, 3H, C*H* Ar), 7.75 (m, 2H, C*H* Ar), 7.83 (d, 2H, J = 9.0 Hz, C*H* Ar); ^{13}C NMR ($CDCl_3$) δ 14.40 (*C*H$_3$CH$_2$), 61.87 (*C*H$_2$CH$_3$), 65.45 (O*C*H$_2$CO), 114.38, 128.46 and 130.00 (*C*H Ar), 131.34 and 132.29 (*C* Ar), 132.77 (*C*H Ar), 138.26, 161.47 (*C* Ar), 168.53 (EtO*C*=O), 195.71 (Ar*C*=OAr).

General procedure for the preparation of acids 17-26. A solution of NaOH 1N (3.9 mmol) was added to esters **7-16** (3.0 mmol) in EtOH (20 mL), and the mixture was stirred at r.t. for 10-15 h. The solvent was removed under reduced pressure and the residue was poured into water (20 mL) and acidified with conc HCl at 0 °C. The aqueous layer was extracted with dichloromethane (3 × 20 mL) and then the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by crystallization with cyclohexane or chloroform affording desired acids **17-26** with good yields.

2-Methyl-2-{4-[(*E*)-2-phenylvinyl]phenoxy}propanoic acid (17). White solid, 94% yield, m.p. 190-192 °C. IR (KBr) 3280, 1706 cm^{-1}; ^{1}H NMR (acetone-d_6) δ 1.59 (s, 6H, C(CH_3)$_2$), 6.90 (d, 2H, J = 8.7 Hz, C*H* Ar), 7.07-7.37 (m, 7H, C*H* Ar and *H*C=C*H*), 7.49-7.58 (m, 2H, C*H* Ar); ^{13}C NMR (acetone-d_6) δ 25.09 (C(*C*H$_3$)$_2$), 79.14 (*C*(CH$_3$)$_2$), 119.20, 126.47 (*C*H Ar), 127.07 (HC=*C*H), 127.43 and 127.56 (*C*H Ar), 128.24 (H*C*=*C*H), 128.84 (*C*H Ar), 131.33, 137.97 and 155.91 (*C* Ar), 175.17 (*C*=O).

Ethyl{4-[(*E*)-2-phenylvinyl]phenoxy}acetic acid (18). White solid, 51% yield, m.p. 206-207 °C. IR (KBr) 3210, 1706 cm^{-1}; ^{1}H NMR (DMSO) 4.78 (s, 2H, OCH_2CO), 6.90 (m, 2H, C*H* Ar), 7.05-7.19 (m, 3H, C*H* Ar and *H*C=C*H*), 7.34 (t, 2H, C*H* Ar), 7.50-7.56 (m, 4H, C*H* Ar) ^{13}C NMR (DMSO) δ 65.16 (O*C*H$_2$CO), 115.28, 126.87 (*C*H Ar), 127.05 (HC=*C*H), 127.92 and 128.41 (*C*H Ar), 128.59 (H*C*=CH), 129.35 (*C*H Ar), 130.83 and 137.99 (*C* Ar), 158.12 (*C* ArO), 170.84 (*C*=O).

2-Methyl-2-{4-[(2*E*)-3-phenylprop-2-enoyl]phenoxy} propanoic acid (19). Yellow solid, 91% yield, m.p. 149-151 °C. IR (KBr) 3300, 1733, 1635 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.66 (s, 6H, C(CH_3)$_2$), 6.97 (d, 2H, J = 9.0 Hz, C*H* Ar), 7.42-7.44 (m, 3H, C*H* Ar), 7.73-7.76 (m, 4H, C*H* Ar and HC=C*H*), 8.06 (d, 2H, J = 9.0 Hz, C*H* Ar); ^{13}C NMR (CD_3OD) δ 20.60 ((*C*H$_3$)$_2$C), 79.25 (*C*(CH$_3$)$_2$), 117.63 (*C*H Ar), 121.60 (HC=*C*H), 128.51, 128.88 and 130.45 (*C*H Ar), 131.34 (O=C*C* Ar), 135.19 (*C* ArC=), 144.39 (H*C*=CH), 160.43 (*C* ArO), 175.81 (HO*C*=O), 189.51 (Ar*C*=O).

{4-[(2*E*)-3-Phenylprop-2-enoyl]phenoxy}acetic acid (20). Yellow solid, 46% yield, m.p. 174-175 °C. IR (KBr) 3448, 1734, 1708 cm^{-1}; ^{1}H NMR (DMSO) δ 4.82 (s, 2H, OCH_2CO), 7.06 (d, 2H, J = 9.0 Hz, C*H* Ar), 7.43-7.45 (m, 3H, C*H* Ar), 7.70 (d, 1H, J = 15.6 Hz, *H*C=C), 7.85-7.89 (m, 2H, C*H* Ar), 8.12 (d, 1H, J = 15.6 Hz, =C*H*), 8.15 (d, 2H, J = 9.0 Hz, C*H* Ar); ^{13}C NMR (DMSO) δ 65.23 (O*C*H$_2$CO), 115.22 (*C*H Ar), 122.66 (=*C*HC=O), 129.52, 129.59 and 131.16 (*C*H Ar), 131.50 (O=C*C*Ar), 131.55 (*C*H Ar), 135.48 (*C*ArCH=), 143.95 (Ar*C*H=), 162.39 (*C*ArO), 170.49 (HO*C*=O), 188.07 (Ar*C*=O).

2-Methyl-2-{4-[(1*E*)-3-oxo-3-phenylprop-1-enyl] phenoxy}propanoic acid (21). Yellow solid, 66% yield, m.p. 200 °C (dec). IR (KBr) 3443, 1665, 1605 cm^{-1}; ^{1}H NMR (CD_3OD) δ 1.44 (s, 6H, C(CH_3)$_2$), 6.79 (d, 2H, J = 8.7 Hz, C*H* Ar), 7.13 (d, 1H, J = 15.2 Hz, =C*H*), 7.43-7.57 (m, 5H, C*H* Ar), 7.76 (d, 1H, J = 15.2 Hz, =C*H*), 7.93 (d, 2H, J = 8.7 Hz, C*H* Ar); ^{13}C NMR (CD_3OD) δ 26.03 ((*C*H$_3$)$_2$C), 78.85 (*C*(CH$_3$)$_2$), 119.62 (O=C*C*H=), 123.05 (*C*ArC=), 128.57(CAr*C*=), 129.11, 129.93 and 133.10 (*C*H Ar), 138.75 (O=C*C* Ar), 145.21 (=*C*H), 155.88 (*C*ArO), 175.89 (HO*C*=O), 191.31 (=C*C*=O).

{4-[(1*E*)-3-Oxo-3-phenylprop-1-enyl]phenoxy}acetic acid (22). Yellow solid, 80% yield, m.p. 200 °C (dec). IR

(KBr) 3440, 1732, 1707 cm^{-1}; ^1H NMR (CD_3OD) δ 4.59 (s, 2H, OCH_2CO), 7.01 (d, 2H, J = 8.6, C*H* Ar), 7.51-7.99 (m, 6H, C*H* Ar and *H*C=CH), 7.75 (d, 1H, =C*H*), 8.06 (d, 2H, J = 8.6 Hz, C*H* Ar); ^{13}C NMR (CD_3OD) δ 65.91 (O*C*H_2CO), 115.04 (*C*H Ar), 119.48 (=*C*HC=O), 128.05 (*C* ArC=), 128.39, 128.61, 130.38 and 132.84 (*C*H Ar), 138.41 (O=C*C* Ar), 145.16 (=*C*HC Ar), 160.99 (*C* ArO), 172.94 (HO*C*=O), 191.29 (*C*=OAr).

2-{4-[(Anilinocarbonyl)amino]phenoxy}-2-methylpropanoic acid (23). Beige solid, 80% yield, m.p. 195-196 °C. IR (KBr) 3310, 1707, 1635 cm^{-1}, ^1H NMR (CD_3OD) δ 1.53 (s, 6H, C(CH_3)$_2$), 6.59 (m, 2H, C*H* Ar), 6.97-7.03 (m, 2H, C*H* Ar), 7.24-7.31 (m, 3H, C*H* Ar), 7.39-7.41 (m, 2H, C*H* Ar); ^{13}C NMR (CD_3OD) δ 24.49 (C(*C*H$_3$)$_2$), 79.40 (*C*(CH$_3$)$_2$), 119.22, 120.62, 122.61, 128.65 and 129.32 (*C*H Ar), 134.11 (HN*C*Ar), 139.36 (*C*ArNH), 151.20 (*C* Ar), 154.56 (O=*C*NH), 174.70 (HO*C*=O).

{4-[(Anilinocarbonyl)amino]phenoxy}acetic acid (24). White solid, 73% yield, m.p. 210-212 °C. IR (KBr) 3318, 1723, 1658 cm^{-1}; ^1H NMR (CD_3OD) δ 4.87 (s, 2H, O-CH_2CO), 6.93 (m, 2H, C*H* Ar), 7.05-7.32 (m, 3H, C*H* Ar), 7.49 (m, 2H, C*H* Ar), 7.95-7.63 (m, 2H, C*H* Ar), 8.01-8.11 (s, 2H, N*H*); ^{13}C NMR (CD_3OD) δ 66.93 (O*C*H$_2$CO), 118.53, 119.43, 122.03 and 122.93 (*C*H Ar), 134.05 (NH*C* Ar), 140.07 (NH*C* Ar), 148.99 (C ArO), 152.75 (HN*C*=O), 173.05 (HO*C*=O).

2-(4-Benzoylphenoxy)-2-methylpropanoic acid (25). White solid, 87% yield, m.p. 123-125 °C. IR (KBr) 3460, 1742, 1706 cm^{-1}; ^1H NMR (CD_3OD) δ 1.65 (s, 6H, C(CH_3)$_2$), 6.95 (d, 2H, J = 9.0 Hz, C*H* Ar), 7.48-7.63 (m, 3H, C*H* Ar), 7.72 (d, 2H, C*H* Ar), 7.74 (d, 2H, J = 9.0 Hz, C*H* Ar); ^{13}C NMR (CD_3OD) δ 24.64 (C(*C*H$_3$)$_2$), 79.38 (*C*(CH$_3$)$_2$), 117.39, 128.26 and 129.54 (*C*H Ar), 130.40 (*C* Ar), 131.95 and 132.20 (*C*H Ar), 138.18 and 160.23 (*C* Ar), 176.02 (HO*C*=O), 196.42 (Ar*C*=OAr).

(4-Benzoylphenoxy)acetic acid (26). White solid, 74% yield, m.p.157-158 °C. IR (KBr) 3457, 1738, 1714 cm^{-1}; ^1H NMR (CD_3OD) δ 4.79 (s, 2H, OCH_2CO), 7.06 (d, 2H, J = 8.7 Hz, C*H* Ar), 7.49-7.65 (m, 3H, C*H* Ar), 7.72 (d, 2H, C*H* Ar), 7.79 (d, 2H, J = 8.7 Hz, C*H* Ar); ^{13}C NMR (CD_3OD) δ 60.58 (O*C*H$_2$CO), 114.24, 128.27 and 129.54 (*C*H Ar), 130.62 (*C* Ar), 132.20 and 132.38 (*C*H Ar), 138.20 and 162.17 (*C* Ar), 170.80 (HO*C*=O), 196.37 (Ar*C*=OAr).

Procedure for the preparation of (2*E*)-1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one (3). A mixture of *p*-hydroxyacetophenone (4.9 mmol) and benzaldehyde (4.9 mmol) in EtOH (7.5 mL) was stirred at 5 °C for 10 m. Then, a solution of KOH 40% (34.4 mmol) was added and the reaction mixture was stirred at room temperature for 4 h. The mixture was acidified with HCl 2N until pH 7 was reached. When the chalcone precipitated, it was filtered, washed with water and dried under reduced pressure. Yellow solid, 71% yield, m.p. 172-174 °C. IR (KBr) 3239, 1646 cm^{-1}; ^1H NMR (CD_3OD) δ 6.70 (d, 2H, J = 9.0 Hz, C*H* Ar), 7.41-7.43 (m, 3H, C*H* Ar and *H*C=CH), 7.71-7.75 (m, 4H, C*H* Ar and HC=C*H*), 8.02 (d, 2H, J = 9.0 Hz, C*H* Ar); ^{13}C NMR (CD_3OD) δ 115.29 (*C*H Ar), 121.71 (=*C*HC=O), 128.43 and 128.86 (*C*H Ar), 129.67 (O=C*C*Ar), 130.35, 131.25 and 135.27 (*C*H Ar), 143.90 (*C* Ar),143.95 (Ar*C*H=), 162.87 (*C*ArO), 189.49 (Ar*C*=O).

Procedure for the preparation of (2*E*)-3-(4-hydroxyphenyl)-1-phenylprop-2-en-1-one (4). A mixture of acetophenone (4.4 mmol) and *p*-hydroxybenzaldehyde (4.4 mmol) in EtOH (7.0 mL) was stirred at 5 °C for 10 m. Then, a solution of KOH 40% (30.8 mmol) was added and the reaction mixture was stirred at room temperature for 7 h. The mixture was acidified with HCl 2N until pH 7 was reached. The precipitate was filtered, washed with water and dried under reduced pressure to obtain compound 4. Brown solid, 34% yield, m.p. 181-183 °C. IR (KBr) 3231, 1650 cm^{-1}; ^1H NMR (acetone-d_6) δ 6.93 (d, 2H, J = 8.7 Hz, C*H* Ar), 7.51-7.79 (m, 7H, C*H* Ar and *H*C=C*H*), 8.12 (d, J = 8.7 Hz, 2H, C*H* Ar); ^{13}C NMR (acetone-d_6) δ 116.05 (*C*H Ar), 119.01 (=*C*HC=O), 126.99 (=C*C* Ar), 128.51, 128.82, 130.94 and 132.73 (*C*H Ar), 138.82 (O=C*C*Ar), 144.49 (Ar*C*H=), 160.14 (*C*ArO), 189.13 (Ar*C*=O).

Procedure for the preparation of 1-(4-Hydroxyphenyl)-3-phenylurea (5). To a solution of 4-amino-phenol (4.6 mmol) in acetonitrile (20 mL) was added the phenyl isocyanate (4.6 mmol). The reaction was refluxed under nitrogen atmosphere for 5 h. After removal of the solvent, the residue was subjected to crystallization from acetonitrile to afford compound **5**. White solid, 69% yield, m.p. 216-218 °C. IR (KBr) 3303, 1637 cm^{-1}; ^1H NMR (acetone-d_6) δ 6.67 (d, 2H, J = 9.0 Hz, CH Ar), 6.92-6.98 (m, 1H, CH Ar), 7.22-7.34 (m, 4H, CH Ar), 7.35 (d, 2H, J = 9.0 Hz, CH Ar); ^{13}C NMR (acetone-d_6) δ 115.37 and 118.61 (*C*H Ar), 121.00 (*C* Ar), 121.13 and 128.85 (*C*H Ar), 132.02 (HN*C*Ar), 140.50 (*C*ArNH), 153.13 (*C* Ar O), 205.60 (O=*C*NH).

Biology

Reporter Plasmids and Luciferase Assays

Human embryonic kidney cells (HEK293) were grown in Dulbecco's Modified Eagles's Medium (DMEM) containing 10% of FCS, penicillin/streptomycin, sodium pyruvate and nonessential amino acids. HEK293 cells were incubated at 37 °C in 5% CO_2 incubator until they were 80% confluent. One day before the experiment, the cells were plated in 96-well plates. The next day, the culture medium was replaced by a fresh one without FCS and the transient transfection was conducted using the calcium phosphate method. Cells were transfected with expression plasmids encoding the fusion protein GAL4-PPARαLBD (30 ng), reporter plasmid (50 ng), renilla luciferase normalization vector (20 ng), and pGEM carrier DNA (40 ng) to make a total of 140 ng of DNA per well. 8 h after transfection, cells were treated for 18 h with the indicated ligands. The two luciferase activities were measured using a dual luciferase assay kit (Promega) on a microplate luminometer (Labsystems Ascent LuminoskanReader). All transfection experiments were repeated at least twice.

RESULTS AND DISCUSSION

Compounds **17-26** (Fig. **2**) were easily obtained in good yields by standard esterification procedures followed by hydrolysis.

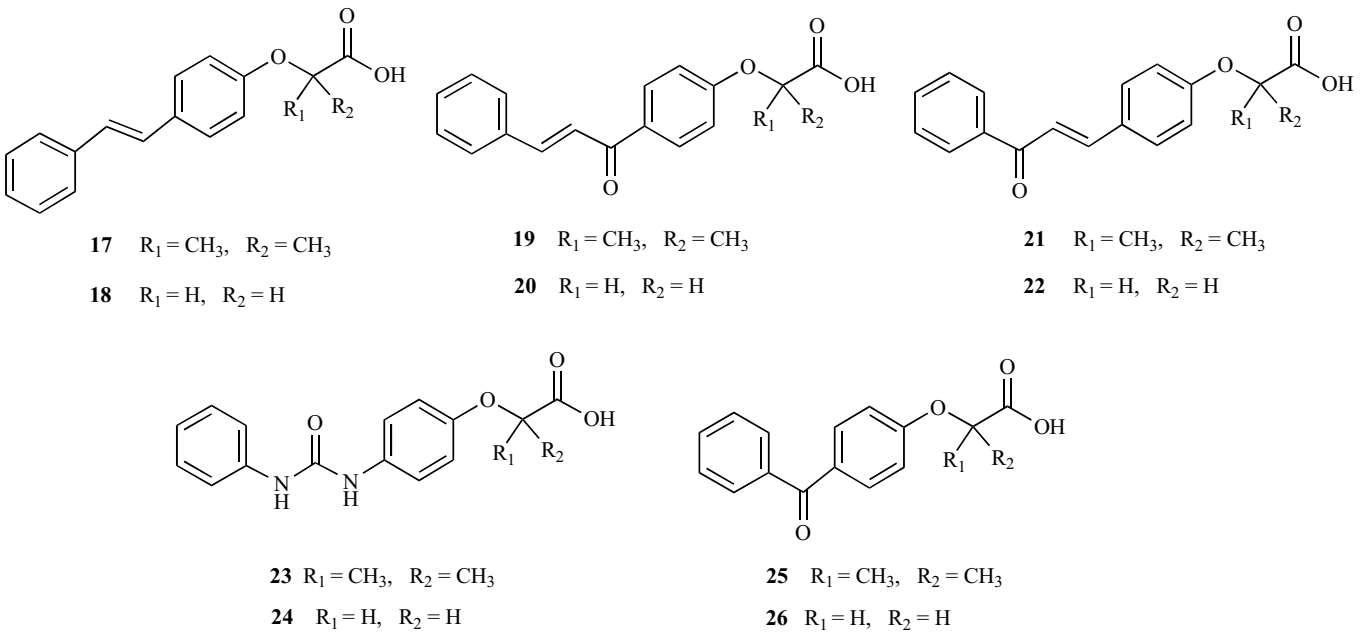
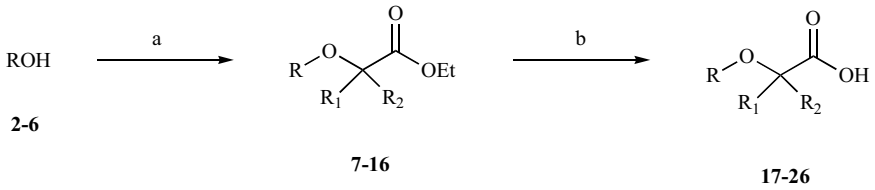


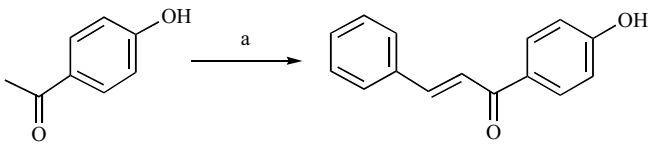
Fig. (2). New compounds.



Scheme 1. Reagents and conditions: (a) 2-bromo-2-methylpropanoate or ethyl bromoacetate, dry K_2CO_3, dry acetone, N_2, reflux; (b) 1N NaOH, EtOH, rt.

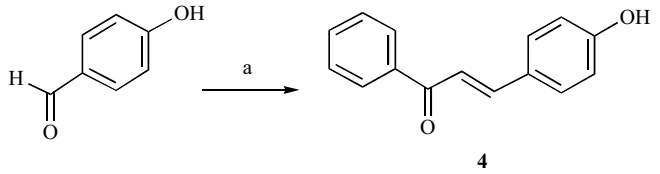
Esters **7**-**16** were obtained by S_N2 reaction of phenols **2**-**6** with ethyl 2-bromo-2-methylpropanoate or ethyl bromoacetate, in the presence of dry K_2CO_3 in dry acetone, under nitrogen atmosphere at reflux. The basic hydrolysis of **7**-**16** with 1N NaOH gave the acids **17**-**26** (Scheme **1**).

The phenols **2** and **6** are commercially available. The phenols (2*E*)-1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one (**3**) and (2*E*)-3-(4-hydroxyphenyl)-1-phenylprop-2-en-1-one (**4**) not commercially available, were synthesized by aldol condensations between *p*-hydroxyacetophenone and benzaldehyde (Scheme **2**) or between acetophenone and *p*-hydroxybenzaldehyde (Scheme **3**) in presence of KOH 40% in EtOH from 5 °C to room temperature.



Scheme 2. Reagents and conditions: C_6H_5CHO, KOH 40%, EtOH, 5 °C-rt.

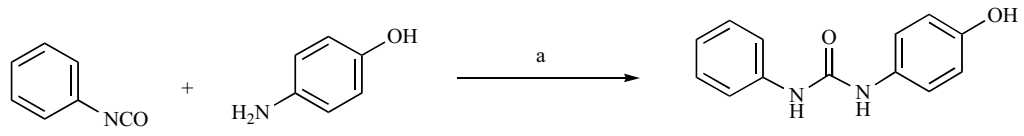
The 1-(4-hydroxyphenyl)-3-phenylurea (**5**) was obtained by a reaction between 4-amino-phenol and phenyl isocyanate in acetonitrile under nitrogen atmosphere at reflux (Scheme **4**).



Scheme 3. Reagents and conditions: $C_6H_5C(O)CH_3$, KOH 40%, EtOH, 5 °C-rt.

All new compounds were evaluated for human PPARα functional activity by a cell-based transactivation assay in eukaryotic cells [13]. In this method, we utilized firefly luciferase reporter gene that provided a good assay sensitivity, dynamic range when quantifying nuclear receptor activity and optimal correlation with *in vivo* activity. In this study, we used clofibric acid (150 μM, EC_{50} = 55.0 μM) as reference compound; results are expressed as efficacy (%) relative to positive control. The compounds with best efficacy were selected for the determination of their EC_{50} values. The results are shown in Fig. (**3**).

All linear compounds (**18**, **20**, **22**, **24**, and **26**) showed an activation of the PPARα lower than their branched analogs (**17**, **19**, **22**, **23**, and **25**) probably because the aromatic scaffold of these compounds can not fit the large hydrophobic pocket of the PPARα receptor, but can access in alternative hydrophobic site with displacement of carboxylic head. Between ramified compounds, only **17** and **25**, with an efficacy of 130% and 195% respectively, showed a better activation



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Scheme 4. Reagents and conditions: CH_3CN, N_2, reflux.

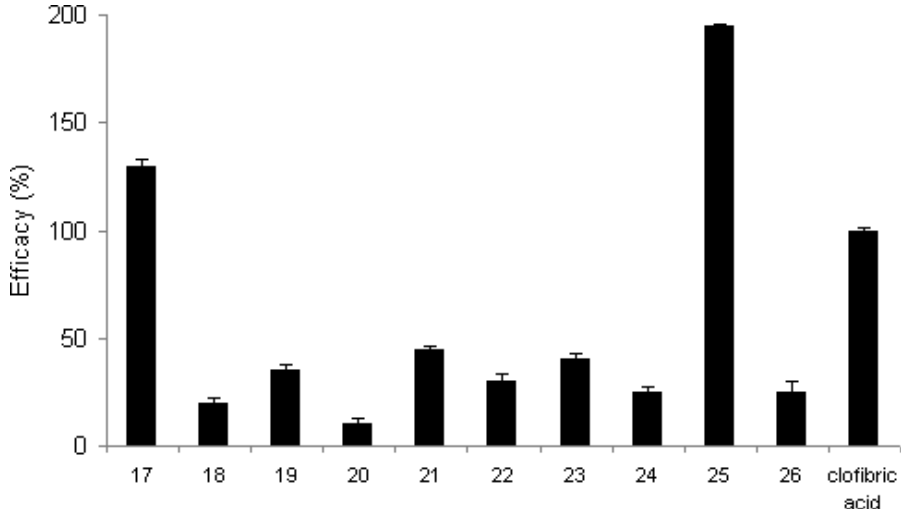

Fig. (3). PPARα functional activity by transactivation assay. Compounds were tested in at least three separate experiments at 150 μM. Efficacy values were calculated as percentage of the maximum obtained fold induction with the clofibric acid.

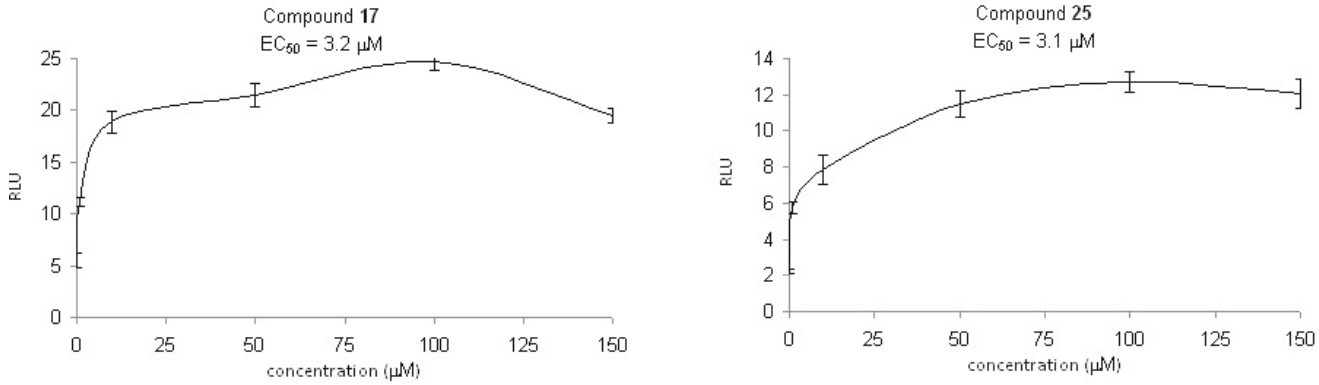

Fig. (4). Dose-responce curves for **17** and **25** for determination of EC_{50}. Compounds were tested in at least three separate experiments at five concentrations ranging from 1 to 150 μM.

of PPARα than clofibric acid. These compounds were selected for the determination of their EC_{50} values. The chalcone derivative **17** showed a good activation of the PPARα receptor with EC_{50}=3.2 μM; also the benzophenone analog **25** with EC_{50}=3.1 μM resulted in a significant activation of the receptor (Fig. **4**).

In conclusion, in this paper two series of clofibric acid analogs were synthesized and *in vitro* evaluated for human PPARα transactivation assay. Compounds with branched linker between carboxylic head and aromatic scaffold showed a better increase of the transcriptional activity of receptor than the linear derivatives. The best results were obtained with compound **17** and **25** that showed EC_{50} values of 3.1 μM and 3.2 μM respectively.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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