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Synthesis and structure–activity relationships of fibrate-based analogues inside PPARs

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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 lipophilic groups derived from natural products chalcone and stilbene were synthesised. Some of them were found to be active at micromolar concentrations only on PPARα or PPARγ, while others were identified as dual agonists PPARα/γ.

The PPARs (peroxisome proliferator activating receptors) are a subfamily of ligand-activated nuclear hormone receptors that are highly expressed in metabolically active tissues which regulate genes encoding lipid and glucose metabolism, and overall energy homeostasis.[1] PPARs are activated by fatty acids and cyclooxygenase-derived eicosanoids;[2] similar to other nuclear hormone receptors, they bind their ligand in the cytoplasm and translocate to the cell nucleus. Once within the nucleus, PPARs form a heterodimer with RXR (retinoid X receptor) and bind to specific DNA-response elements in the promoter of target genes. Binding of ligands to PPARs leads to a conformational change in the receptor, resulting in the recruitment of coactivators and chromatin remodelling, which allows the initiation of DNA transcription.[3]

Three PPAR subtypes, commonly designated as PPARα, PPARγ, and PPARβ/δ, have been identified; they have different tissue distribution, binding of ligands, and recruitment of co-activators or co-repressors. PPARα plays a pivotal role in lipid metabolism by decreasing both serum triglycerides and free fatty acid levels, and increasing HDL (high-density lipoproteins) level.[4] The PPARγ has a critical role in fatty acid storage and glucose metabolism by coordinating the expression of genes involved in lipid metabolism, adipogenesis and inflammation.[5] PPARβ/δ functional roles are still the least defined of all the PPARs; however, several studies have suggested that PPARδ regulates fatty acid catabolism, insulin sensitivity, and energy homeostasis in muscle and adipose tissue.[6]

Two classes of compounds, namely fibrates,[7] as antihyperlipedimic agents, and thiazolidinediones,[8] as antidiabetic agents, are currently marketed and are PPARα and γ agonists, respectively. There are, however, factors that limit the use of these drugs: fibrates are poor activators of PPARα and their subtype selectivity is not high. Probably due to the high doses requested to exert therapeutic effect, therapy with fibrates is associated with increased risk for myopathy and hepatotoxicity.[9] Frequent side effects of thiazolidinediones are weight gain, edema, and heart failure.[10] In the last years, a number of approaches directed towards the development of new potent agonists specific for PPAR subtypes represented the logical evolution in the field of metabolic disorders treatment.[11] In addition, also PPARα/γ dual agonists, termed as '*glitazars*', have been identified as very attractive candidates in the treatment of metabolic disorders, by combining the beneficial effects of two different subtype receptors (Fig. 1).[12] Currently, none of new PPAR agonists has been approved by FDA due to the toxic side effects, although some of them have reached different stages of clinical trials.[13] So, the safety concerns seem to discourage research in this field; however, the importance of metabolic diseases and the fact that the reasons of failure of various PPAR agonists are quite different from each other require the development of newer and safer drugs, by evaluating the adverse effects case by case.

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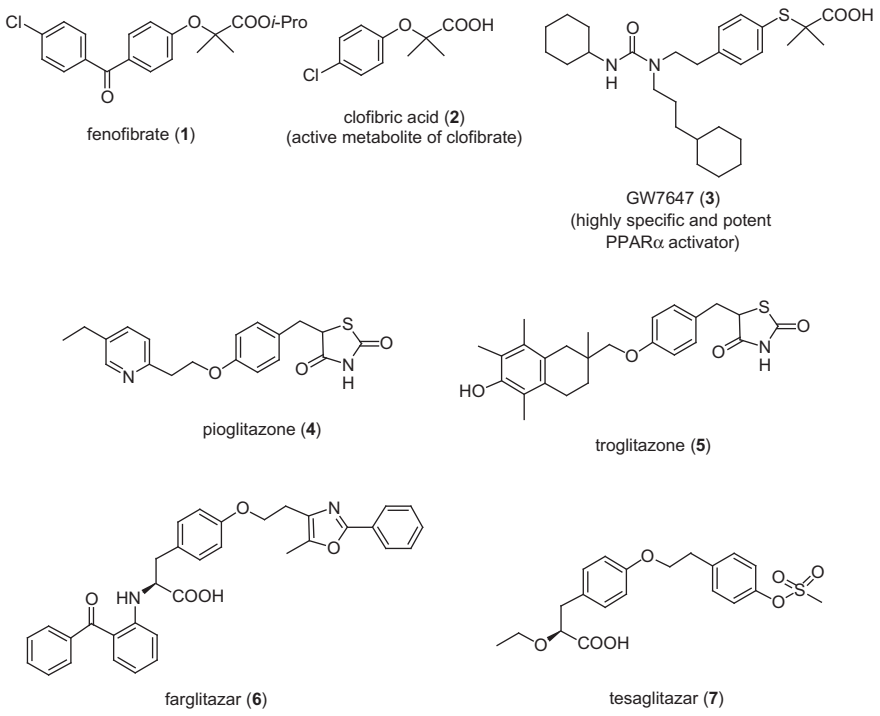


Figure 1. Chemical structures of representatives fibrates, specific and potent PPARα activators, thiazolidinones and '*glitazars*'.

The chemical structures of synthetic PPAR agonists are referable to a general pharmacophore, including a carboxylic acid head, an aromatic ring, and a lipophilic cyclic tail, connected by linkers; the nature of these fragments affects the potency and subtype selectivity. In the search for new PPAR ligands that fit these common structural features, 'nutraceuticals' and natural products derived from plants, like stilbenes[14] and chalcones[15] have attracted attention as novel scaffolds because of their health benefits including antioxidant, antilipidemic and antiplatelet properties. It was envisaged that structures containing these moieties may show PPAR agonist activity with enhanced pharmacological effects. In this field, we have recently reported the synthesis and PPARα activation of a series of molecules derived by the combination of antilipidemic drug gemfibrozil with natural α-asarone, stilbene, chalcone, and other bioisosteric modifications.[16] In continuation of these studies, and in order to gain more insight on the structure–activity relationship, we herein describe the synthesis and in vitro evaluation of new compounds based on a combination of two key pharmacophores: the classical clofibric acid, the active metabolite of clofibrate, and lipophilic groups derived from natural products chalcone and stilbene. We kept unalterate the clofibrate scaffold and systematically varied the cyclic tail and the length of linker between the aromatic centre and the lipophilic tail (Fig. 2). The new compounds were in vitro bioevaluated for their PPARα and PPARγ agonistic activity using the reporter gene assay and a preliminary screening of gene activation by RTqPCR (real-time quantitative PCR analysis) was performed.

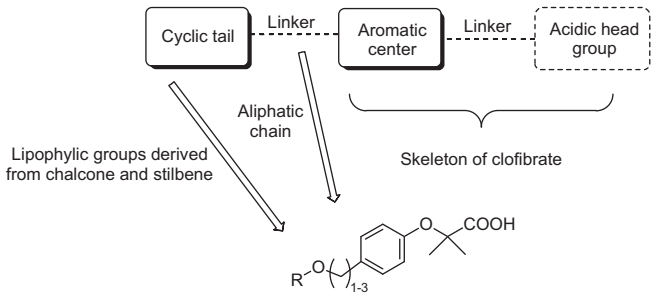
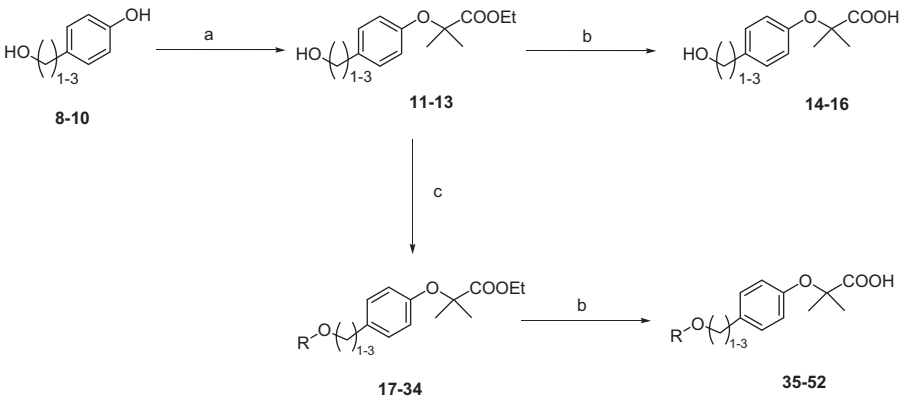


Figure 2. General features of synthetic PPAR agonists and structural development of presented compounds.

All compounds were easily obtained in good yields by standard esterification procedures followed by hydrolysis.[17] Esters **11**–**13** were obtained by S_N2 reaction of phenols **8**–**10** with ethyl 2-bromo-2-methylpropanoate, in the presence of dry K_2CO_3 in DMF at reflux. The basic hydrolysis of **11**–**13** with 1 N NaOH gave the acids **14**–**16**. Esters **17**–**34** were synthesized by Mitzunobu reaction between **11**–**13** and the appropriate commercial phenols. Only the phenol (2*E*)-1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one, not commercially available, was synthesized according to well established procedures.[18] Hydrolysis of **17**–**34** with 1 N NaOH gave desired acids **35**–**52** (Scheme 1).

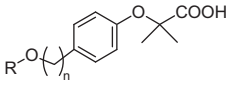
All new compounds were assessed for their PPARα and PPARγ functional activity by a transactivation assay using the GAL4–PPARγ and GAL4-responsive luciferase reporter in HEK293 (human embryonic kidney 293 cells).[19] As control for PPARα, clofibric acid (**2**), the active metabolite of clofibrate, and GW7647 (**3**), a highly specific and potent PPARα activator,[20] were used; the PPARγ activity was examined in comparison with pioglitazone (**4**). The results are shown in Table 1.

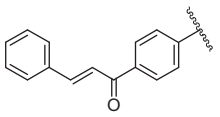
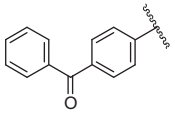
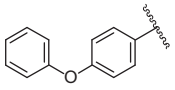
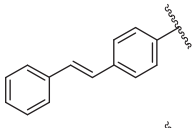
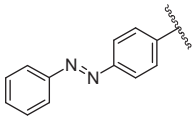
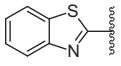
As depicted in Figure 2, our strategy was based on the combination of clofibric acid with lipophilic groups derived from natural products and their modifications. Also the length of the linker between the aromatic centre and the lipophilic tail (one, two or three carbons) was investigated. In general, the tested compounds displayed a very different profile of efficacy from PPARα to PPARγ. Most of the PPARα agonists had an efficacy superior to clofibrate; in contrast, none of the compounds active on PPARγ was able to exhibit full agonist activity and all appear partial agonists. Initial SAR was focused on the introduction of chalcone and its analogues obtained by removing the double bond (benzoylphenoxy) and replacing the carbonyl function with the oxygen (phenoxyphenoxy). Almost all compounds of this series showed a selective activation of



Scheme 1. (a) Ethyl 2-bromo-2-methylpropanoate, dry K_2CO_3, DMF, reflux; (b) 1 N NaOH, EtOH, rt; (c) appropriate phenol (ROH), DIAD, Ph_3P, dry THF, N_2, rt.

Table 1
In vitro transactivation activity of compounds with various lipophilic tails and linkers



Compound	R	*n*	hPPARα EC_{50} (μM)[a] (% max)[b,c]	hPPARγ EC_{50} (μM) (% max)
35		1	na[d]	na
36		2	64.0 ± 0.9 (118)	5.9 ± 0.8 (21)
37		3	na	0.8 ± 0.04 (21)
38		1	na	5.4 ± 0.2 (19)
39		2	na	54.6 ± 1.0 (12)
40		3	na	5.8 ± 0.3 (33)
41		1	na	0.8 ± 0.04 (13)
42		2	na	6.9 ± 0.3 (13)
43		3	na	75.2 ± 1.2 (5)
44		1	40.5 ± 3.1 (156)	6.0 ± 0.2 (36)
45		2	11.6 ± 0.4 (81)	12.6 ± 0.5 (18)
46		3	9.9 ± 0.2 (125)	0.8 ± 0.1 (32)
47		1	5.6 ± 0.3 (350)	21.0 ± 0.9 (19)
48		2	0.6 ± 0.01 (158)	1.4 ± 0.02 (21)
49		3	42.8 ± 0.4 (81)	10.0 ± 0.7 (36)
50		1	44.6 ± 0.6 (125)	32.0 ± 0.7 (35)
51		2	18.3 ± 0.7 (169)	39.3 ± 0.3 (8)
52		3	45.8 ± 0.8 (100)	7.9 ± 0.1 (23)
14	H	1	34.5 ± 1.0 (119)	30.0 ± 0.3 (6)
15	H	2	75.1 ± 1.3 (94)	na
16	H	3	30.6 ± 0.8 (181)	na
2			55.0 ± 3.9 (100)	na
3			0.2 ± 0.02 (164)	na
4			na	0.8 ± 0.01 (100)

[a] Compounds were tested in at least three separate experiments at five concentrations ranging from 1 to 150 μM. The results are expressed with ± SEM.
[b] Compounds were tested in at least two separate experiments at concentration of 150 μM. Only GW7647 was tested at 1 μM. The results are expressed with ± SEM.
[c] Efficacy values were calculated as percentage of the maximum obtained fold induction with the clofibric acid (**2**) and pioglitazone (**4**).
[d] Not active.

PPARγ receptor, while they are inactive on PPARα. The linker length influences the activity in a different way depending on the lipophilic group in the molecule. For chalcone derivatives, a spacer of one carbon leads to a molecule completely inactive (**35**), while the PPARγ activity gradually reaches a value approximately equal to that of pioglitazone; compound **37**, containing a linker of three methylenic groups, showed an EC_{50} = 0.8 μM. Among the compounds containing the benzoylphenoxy group those with a spacer of one (**38**) or three carbon atoms (**40**) were active, while the intermediate chain leads to an almost inactive compound (**39**). The molecules with a phenoxyphenoxy lipophilic tail (**41**–**43**) show values of PPARγ activity decreasing with the increasing of chain spacer. Among them,

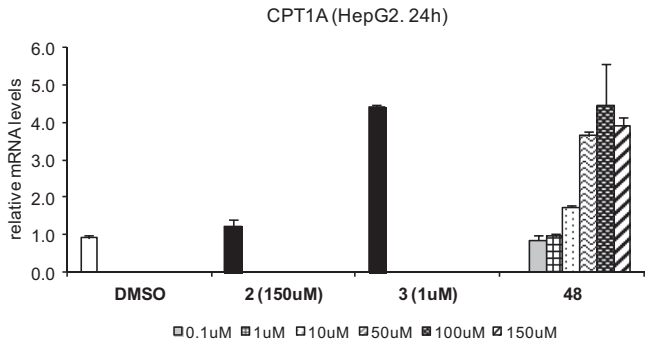


Figure 3. CPT1A expression in HepG2 following treatment. HepG2 cells were treated with vehicle (DMSO), **2** (150 μM), **3** (1 μM), **48** (0.1–150 μM) for 48 h. RTqPCR was performed to measure CPT1A mRNA levels.Values shown represent mean ± SEM for four independent determinations performed in duplicate. Cyclophilin was used as reference gene and values were normalised to data obtained from vehicle treated cells.

compound **41** showed equipotent PPARγ activity (EC_{50} = 0.8 μM) compared to pioglitazone. The introduction of stilbene scaffold and the related phenyldiazenyl derivative completely changes the profile of activity. All the analogues are active on PPARα and PPARγ. Analogues with stilbene were found to have an increasing potency for both receptors with the length of the spacer chain. Compound **44** activated the PPARγ receptor better than PPARα, while **45** was a weak dual PPARα/γ activator. The analogue with the three-carbon linker (**46**) showed an EC_{50} = 0.8 μM; it was also a dual PPARα activator (EC_{50} = 9.9 μM). The replacement of double bond of stilbene with a diazenyl function substantially does not change the trend of activity. However, a considerable increase of activity against PPARα was observed for compound **48** (linker of two carbon atoms), which was also a good activator of PPARγ (PPARα EC_{50} = 0.6 μM, PPARγ EC_{50} = 1.4 μM); this molecule probably fits better into the receptor pockets than the other two analogues (**47** and **49**). Further modifications involved the introduction of a benzothiazole and the total removal of the lipophilic tail. In the series of benzothiazole derivatives, all compounds (**50**–**52**) did not show improvements in activity even if the EC_{50} values on both PPARα and PPARγ were good. Also in the series of alcohols, modifications of linker length did not influence the PPARα activation very much. The acid **14** containing the methanol group in *para* at the aromatic ring and the ethanol analogue **15** activated the PPARα slightly better than clofibric acid; than, the introduction of propanol group (**16**) caused a little worsening of activity. Only **14** was a weak activator of PPARγ. The transactivation studies reported in Table 1 show that the lipophilic tail and the length of linker seem to be correlated to activity; indeed, there were remarkable differences when varying these two fragments regarding the potency and the isoform selectivity. Among all the compounds tested, stand out as PPARγ agonists molecules **37** and **41**,[21] while dual agonists may be represented by **46** and **48**.[22]

In the liver, the import of fatty acids into hepatocyte mitochondria is regulated by the mitochondrial key enzyme CPT1A (carnitine palmitoyl acyl-CoA transferase 1).[23] The expression pattern of this gene is a well established in vitro model to study PPARα activation.[24] On the basis of the transactivation assay results, we selected compound **48** for the preliminary in vitro analysis of CPT1α expression in HepG2 (human hepatocellular liver carcinoma cell line) by using RTqPCR. The expression level of CPT1A was measured in the presence of various concentrations of compound **48**. Cells were stimulated with increasing amounts of selected compound **48** (from 0.1 to 100 μM) and compared with **2** and **3**; control cells were treated with DMSO alone. As shown in Figure 3, our molecule led to a significant and concentration-dependent increase of mRNA levels; its activity was comparable with that of superagonist **3**, acting as a PPARα agonist for regulating the expression of PPARα target gene CPT1A involved in lipid metabolism in hepatocytes.

In summary, we have investigated new molecules designed from the general pharmacophore for PPAR agonists, based on clofibric acid (carboxylic acid head and aromatic ring) and a lipophilic tail derived from natural products chalcone and stilbene and their modifications, connected by a linker of one, two or three methylenic groups. Compounds **37** and **41** were found to be PPARγ agonists with the same potency of pioglitazone, and compounds **46** and **48** were identified as dual PPARα/γ agonists, although the values of efficacy seem to indicate a behaviour as partial agonists. All new molecules were tested in vitro with the transactivation assay and a preliminary test of gene expression was performed on compound **48**. This molecule is candidate as new lead compound for the design of more potent and selective PPARαγ agonists analogues of fibrates.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.09.111.

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21. *Spectral data for **37** and **41**. 2-Methyl-2-[4-(3-{4-[(2E)-3-phenylprop-2-enoyl] phenoxy}propyl)phenoxy] propanoic acid (**37**)*: yellow solid, 53% yield, mp 121–123 °C. IR (KBr) 3420, 1735, 1662 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.58 (s, 6H, $C(CH_3)_2$), 2.11 (m, 2H, J_1 = 6.3 Hz, J_2 = 7.8 Hz, $OCH_2CH_2CH_2Ph$), 2.78 (t, 2H, J = 7.8 Hz, $OCH_2CH_2CH_2Ph$), 4.04 (t, 2H, J = 6.3 Hz, $OCH_2CH_2CH_2Ph$), 6.87 (d, 2H, J = 8.5 Hz, CH Ar), 6.95 (d, 2H, J = 9.0 Hz, CH Ar), 7.11 (d, 2H, J = 8.5 Hz, CH Ar), 7.40–7.42 (m, 3H, CH Ar), 7.54 (d, 2H, J = 15.6 Hz, CH Vin), 7.63–7.66 (m, 2H, CH Ar), 7.81 (d, 2H, J = 15.6 Hz, CH Vin), 8.02 (d, 2H, J = 9.0 Hz, CH Ar); ^{13}C NMR ($CDCl_3$) δ 25.17 ($C(CH_3)_2$), 30.83 ($OCH_2CH_2CH_2$), 31.50 ($OCH_2CH_2CH_2$), 67.26 ($OCH_2CH_2CH_2$), 78.18 ($C(CH_3)_2$), 114.55 and 121.27 (CH Ar), 122.09 (C=OCH Vin), 128.61, 129.16, 129.58, 130.60 and 131.10 (CH Ar), 131.26, 135.29 and 136.68 (C Ar), 144.32 (CH Vin), 158.03 and 163.14 (C Ar), 189.13 (COOH), 201.01 (ArC=O). Anal. Calcd for $C_{28}H_{28}O_5$: C, 75.65; H, 6.35. Found: C, 75.58; H, 6.37. *2-Methyl-2-{4-[(4-phenoxyphenoxy)methyl]phenoxy}propanoic acid (**41**)*: white solid, 78% yield, mp 115–116 °C. IR (KBr) 3224, 1703 cm^{-1}; ^1H NMR (CD_3OD) δ 1.56 (s, 6H, $C(CH_3)_2$), 4.98 (s, 2H, OCH_2), 6.87–7.04 (m, 9H, CH Ar), 7.26–7.37 (m, 4H, CH Ar); ^{13}C NMR (CD_3OD) δ 24.57 ($C(CH_3)_2$), 69.95 (OCH_2Ph), 79.05 ($C(CH_3)_2$), 115.90, 117.42, 119.15, 120.49, 122.37, 128.69, 129.51 (CH Ar), 131.08, 150.60, 155.38, 155.52 and 158.74 (C Ar), 161.03 (COOH). Anal. Calcd for $C_{23}H_{22}O_5$: C, 73.00; H, 5.86. Found: C, 73.15; H, 5.83.
22. *Spectral data for **46** and **48**. 2-Methyl-2-[4-(3-{4-[(E)-2- phenylvinyl]phenoxy} propyl)phenoxy]propanoic acid (**46**)*: white solid, 86% yield, mp 258 °C dec, IR (KBr) 3423, 1703 cm^{-1}; ^1H NMR (CD_3OD) δ 1.49 (s, 6H, $C(CH_3)_2$), 2.02 (m, 2H, J_1 = 6.3 Hz, J_2 = 7.2 Hz, $OCH_2CH_2CH_2Ph$), 2.71 (t, 2H, J = 7.2 Hz, $OCH_2CH_2CH_2Ph$), 3.95 (t, 2H, J = 6.3 Hz, $OCH_2CH_2CH_2Ph$), 6.82–6.89 (m, 4H, CH Ar), 6.97–7.08 (m, 4H, CH Ar), 7.17–7.22 (m, 1H, CH Ar), 7.29–7.34 (m, 2H, CH Ar), 7.44–7.52 (m, 4H, CH Ar); ^{13}C NMR (CD_3OD) δ 25.31 ($C(CH_3)_2$), 31.04 ($OCH_2CH_2CH_2Ph$), 31.19 ($OCH_2CH_2CH_2Ph$), 66.77 ($OCH_2CH_2CH_2Ph$), 80.37 ($C(CH_3)_2$), 114.51, 118.76, 126.04 (CH Ar), 126.18 (CH Vin), 126.91 (CH Ar), 127.56 (CH Ar), 128.12 (CH Vin), 128.44 and 128.60 (CH Ar), 130.25, 133.71, 138.04, 154.91 and 159.10 (C Ar), 180.97 (COOH). Anal. Calcd for $C_{27}H_{28}O_4$: C, 77.86; H, 6.78. Found: C, 77.75; H, 6.80. *2-Methyl-2-[4-(2-{4-[(E)-phenyldiazenyl]phenoxy}ethyl)phenoxy]propanoic acid (**48**)*: orange solid, 91% yield, mp 239–240 °C. IR (KBr) 3423, 1705 cm^{-1}; ^1H NMR(CD_3OD) δ 1.50 (s, 6H, $C(CH_3)_2$), 3.02 (t, 2H, J = 6.9 Hz, OCH_2CH_2), 4.22 (t, 2H, J = 6.9 Hz OCH_2CH_2), 6.87 (d, 2H, J = 8.7 Hz CH Ar), 7.04 (d, 2H, J = 9.0 Hz CH Ar), 7.15 (d, 2H, J = 8.7 Hz CH Ar), 7.44–7.54 (m, 3H, CH Ar), 7.83–7.87 (m, 2H, CH Ar), 7.88 (d, 2H, J = 9.0 Hz CH Ar); ^{13}C NMR (CD_3OD) δ 25.31 ($C(CH_3)_2$), 34.67 (OCH_2CH_2), 69.33 (OCH_2CH_2), 80.41 ($C(CH_3)_2$), 114.71, 118.70, 122.29, 124.55, 128.97, 129.17 and 130.34 (CH Ar), 131.85, 147.02, 154.75, 155.86 and 163.75 (C Ar), 175.21 (COOH). Anal. Calcd for $C_{24}H_{24}N_2O_4$: C, 71.27; H, 5.98; N, 6.93. Found: C, 71.33; H, 5.96; N, 6.97.
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