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Environmental pollutants directly affect the liver X receptor alpha activity: Kinetic and thermodynamic characterization of binding

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ABSTRACT

Liver X receptor is a ligand-activated transcription factor, which is mainly involved in cholesterol homeostasis, bile acid and triglycerides metabolism, and, as recently discovered, in the glucose metabolism by direct regulation of liver glucokinase. Its modulation by exogenous factors, such as drugs, industrial by-products, and chemicals is documented. Owing to the abundance of these synthetic molecules in the environment, and to the established target role of this receptor, a number of representative compounds of phthalate, organophosphate and fibrate classes were tested as ligands/modulators of human liver X receptor, using an integrated approach, combining an *in silico* molecular docking technique with an optical SPR biosensor binding study. The compounds of interest were predicted and proved to target the oxysterols-binding site of human LXRα with measurable binding kinetic constants and with affinities ranging between 4.3×10^{-7} and 4.3×10^{-8} M. Additionally, non-cytotoxic concentration of these chemicals induced relevant changes in the *LXRα* gene expression levels and other target genes (*SREBP-1c* and *LGK*) in human liver hepatocellular carcinoma cell line (HepG2), as demonstrated by q-RT-PCR.

1. Introduction

Liver X receptors (LXRs) are nuclear receptors (NRs) mainly involved in the gene transcription process of constituents of lipid and carbohydrate metabolism, including reverse cholesterol transport (RCT) [1–4]. Furthermore, they regulate inflammatory response in macrophages [5]. Two isoforms of this receptor are well characterized: LXRα (or Nr1h3), principally expressed in liver and, to a lesser extent, in adipose tissue, adrenal glands, kidneys, macrophages, lungs and intestine, and LXRβ (or Nr1h2), expressed ubiquitously [6]. LXR, like other members of NR1 family, works together with co-activators and co-repressors, forming for example heterodimers with the retinoid X receptor (RXR) [7]. Upon interaction with specific endogenous ligands (oxysterols like 22(*R*)-hydroxycholesterol, 24(*S*)-hydroxycholesterol and 24(*S*), 25-epoxycholesterol [8,9], oxysterol metabolites [10], and some bile acids [10]), it activates the transcription by binding LXR responsive elements (LXREs), a series of direct repeats (DRs) of the core sequence AGGTCA separated by four nucleotides (DR-4) [11]. In particular, LXRs have been characterized as main transcriptional regulators of lipid and sugar metabolism, acting on sterol regulatory element-binding protein-1c (*SREBP-1c*) gene and liver glucokinase (*LGK*) gene, respectively [12,13].

Other recent studies documented the modulation of NRs by a series of small molecules, such as common pharmaceutical drugs [14], and environmental pollutants, such as industrial by-products and widely used chemicals, which can eventually act also as endocrine disruptors [15–17]. Among these compounds, phthalates, organophosphate and fibrates are extensively studied because of their significant reactivity against NRs.

Phthalates or phthalic acid esters (PAEs) are synthetic organic compounds used as additives or plasticizers of polyvinyl chloride (PVC) in several consumer products [18,19]. They can hence be found in numerous products such as toys, childcare articles, food packaging materials, vinyl gloves, floor and wall coverings, and medical devices [20]. Since these molecules are not covalently bound to PVC, they could be easily released into the environment, and come into contact with humans via intravenous, oral, inhalation and dermal routes [21]. Among these, di-2-ethyl hexyl phthalate (DEHP) and mono-2-ethyl hexyl phthalate (MEHP) were demonstrated to interact with constitutive androstane receptor

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(CAR) [22] and LXRα [23], respectively. These results were also confirmed by an *in silico* analysis of some phthalates with the three subtype of human peroxisome proliferator activated receptor (PPAR) and retinoid X receptor (RXR) [18].

Organophosphates (OPs), derivatives of phosphoric, phosphonic and phosphinic acids, are commonly used as some ophthalmic agents, antihelmintics, herbicides, insecticides, nerve chemical weapons, in addition to plasticizers, solvents, and extreme pressure additives for lubricants, having a high probability to interact with humans. In this respect, recently, a large number of studies have reported dangerous relationships between the extensive use of OPs and the increase of some diseases like Parkinson, Alzheimer, diabetes, obesity, and chronic obstructive respiratory syndrome [24]. In particular, farnesyl pyrophosphate (FPP) was reported to bind some NRs, thus activating the transcription [25].

Finally, fibrates are amphipathic carboxylic acids used for some time as lipid regulators, reducing plasma triglyceride and modulating the apolipoprotein content in low-density lipoproteins (LDL), acting on PPARs [26]. Specifically, a number of studies conducted in United States, United Kingdom and Germany reported the presence of bezafibrate mainly in aquatic environments, like streams, river and effluents, acting as endocrine disruptor by affecting the gonadal steroidogenesis and spermatogenesis and regulating the PPAR activity [16,27].

To obtain further information on the molecular basis of the observed effects caused by these chemicals, a representative selection of the phthalate, organophosphate and fibrate class of compounds was taken into consideration in this work; the kinetic and thermodynamic characterization of the binding to LXRα and the evaluation of the modulatory capability toward the receptor have been the main focus of the present paper. Starting from a preliminary *in silico* screening data on the interaction with LXRα, five out of seventeen potential pollutant molecules, namely bisphenol A (BPA), diisodecyl phthalate (DiDP), diisononyl phthalate (DiNP), bezafibrate (BZF) and tri-m-tolyl phosphate (TMTP) were selected on the basis of their predicted affinity for this receptor and their chemical class. These compounds were used for a molecular docking analysis, for the biosensor binding *in vitro* assay with LXRα, and tested as modulators of transcription of specific genes regulated by this receptor in a polarized human liver hepatocellular carcinoma cell line (HepG2).

2. Materials and methods

2.1. Molecular docking

A preliminary screening of a capability of some pollutants to bind the liver X receptor was performed using molecular docking, a structural bioinformatics tool that provides geometric and affinity information about the complex. In particular, Autodock Vina [28] (version 1.1.2), the automated molecular docking software, was used on an Intel Core i7/Mac OS X 10.9 – based platform. All pollutant molecules were retrieved from Pubchem database [29], minimized (with a universal force field, UFF, and a conjugate gradient algorithm until a ΔE lower than 0.001 kJ/mol) using Avogadro software (Version 1.1.0. http://avogadro.openmolecules.net/) [30] and saved as pdbqt files. The three-dimensional structure of LXRα (PDB ID:3IPQ [31]) was obtained from Protein Data Bank [32] and its pdbqt file was prepared removing water molecules, all ligands included in the crystal and considering polar hydrogen atoms. Then, LXRα molecule was set as receptor, pollutant molecules as ligands and docking grid box was created around the entire receptor and the oxysterols-binding site with a size of $60 \times 65 \times 55$ Å and $26 \times 27 \times 25$ Å, respectively.

Predicted binding affinities were expressed throughout as equilibrium dissociation constants, resulting from the equation:

$$K_d = e^{\Delta G}\text{bind}\frac{1000}{RT} \quad (1)$$

and obtained using both default settings and *ad-hoc* optimized scoring function weights. The latter were derived carrying on a multivariate liner regression of the predicted energy contributions (Supplementary Table 1) versus the experimental free energy of binding (ΔG) of 12 available LXRα/ligand complexes (see Fig. 3 legend), using the Matlab R2014b estimation algorithms (the ordinary multivariate normal maximum likelihood estimation, the maximum likelihood estimation via the expectation conditional maximization algorithm, and covariance-weighted least squares estimation) [33].

All models and images were rendered using Mac PyMOL software (Python Molecular Graphics – version 1.3), whereas the intermolecular interaction features of each complex were obtained using LigandScout software (Inte:Ligand version 3.12) [34].

2.2. Biosensor studies

The liver X receptor, BPA, DiDP, DiNP, BZF and TMTP, Na_2HPO_4, CH_3COONa, KCl, NaCl, Tween-20, were all purchased from Sigma–Aldrich (Milan, Italy). The carboxylate cuvette used for the interaction studies and the immobilization kit (NHS, *N*-hydroxysuccinimide; EDC, 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide; ethanolamine) were obtained from Farfield Group (Cheshire, UK). All chemicals were of the highest grade available.

After PBS-T wash (Na_2HPO_4 10 mM, KCl 2.7 mM, NaCl 138 mM, Tween-20 0.05% $_{(v/v)}$, pH 7.4) and equilibration with Tween-20 free PBS for approximately 15 min, the surface of a IAsys biosensor carboxylate cuvette was covalently charged with a given concentration of liver X receptor (100 μg/mL) by a standard EDC/NHS coupling procedure [35]. The non-coupled receptor was removed by washing with PBS buffer and the remaining reactive groups of the surface were deactivated by a treatment with ethanolamine (1 M). Then, each pollutant was added to the LXR-functionalized surface at increasing concentrations in the range 1.6×10^{-6} -1.6×10^{-7} M, and association kinetics were routinely followed up to the equilibrium. Dissociation and surface regeneration steps were obtained by a PBS buffer addition.

The measured LOD values for the pollutants under study were in the range 7.02×10^{-9}–6.41×10^{-10} M. Kinetic raw data were analysed with Fast Fit software (Fison Applied Sensor Technology; Affinity Sensors) and globally fitted [36] with a standard monophasic time course equation:

$$R_t = R_{eq,[L]}\left(1 - e^{-(K_{ass[L]} + K_{diss})t}\right) \quad (2)$$

where the response at equilibrium (extent) is:

$$R_t = R_{eq,[L]} = \frac{R_{max}K_{ass[L]}}{K_{ass[L]} + K_{diss}} \quad (3)$$

and R_{max} is the extent at asymptotically high concentrations of a ligand *[L]*. Time courses measured at several ligand concentrations are simultaneously analysed using Eqs. (2) and (3), sharing k_{ass},k_{diss}, and R_{max} parameters.

2.3. Cell culture and viability assays

Cells were grown in a 5% CO_2 atmosphere at 37 °C in 100 mm tissue culture dishes. Growth media were MEM supplemented with 10% FBS, 1% sodium pyruvate, antibiotic, and antimycotic for HepG2. Media and reagents for cell culture were purchased from EuroClone S.p.A. (Milan, Italy).

The effect of each pollutant molecule on HepG2 cells viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [37]. After individual treatments with each compound, MTT was added to the culture medium at a final concentration of 0.5 mg/mL and incubated for 2 h at 37 °C. The medium was replaced with 100 μL of DMSO, and the optical density was measured at 550 nm after 10 min on a microplate reader. At least six cultures were used for each time point.

2.4. Cell treatment, preparation of mRNA and Real-time quantitative PCR

Cells cultures were treated for 24 h at 37 °C with each pollutant compound (1 μM) in growing medium (control cells were treated with the same concentration of DMSO). Upon treatment, cells were harvested in PBS and centrifuged, and RNA was extracted using TRI reagent kit (Sigma–Aldrich, Milan, Italy). Cells were incubated with 1 mL TRI reagent (10^7 cells/mL) for 5 min at 25 °C, and supplemented with 200 μL of chloroform. After vigorous mixing and centrifugation at 12,000 × *g* for 15 min at 4 °C, the upper layer was transferred to a new tube. An aliquot of 500 μL isopropanol was added and the resulting mixture incubated for 10 min at 25 °C, followed by centrifugation at 12,000 × *g* for 15 min at 4 °C to pellet RNA. The pellet was washed with 1 mL 75% ethanol and air-dried for 5 min. RNA was dissolved in 20 μL RNase-free water. Quality controls on isolated RNA were performed through Nanodrop (Thermo Scientific, Wilmington, USA). First strand cDNA was synthesized from 1 μg of total RNA in a 20 μL volume using random hexamer primer and RevertAid H Minus M-MuLV Reverse transcriptase (Thermo Scientific S.p.A., Milan, Italy) and RNA retrotranscription was carried out at 25 °C for 5 min followed by 60 min at 42 °C and terminate by heating at 70 °C for 5 min. The amount of cDNA was determined through Nanodrop (Thermo Scientific, Wilmington, USA). 500 ng of template cDNA was amplified with specific primers (300 nM each) using Maxima SYBR Green/ROX qPCR Master Mix (Fermentas, Burlington, Canada) following the manufacturer's instructions. Target sequence of the genes *SREBP-1c*, *LXRα* and *LGK* were amplified using specific primers: SREBP-1c-f, 5′-AACGCTTCCATGCTGATCCT-3′; SREBP-1c-r, 5′-TGGCATGGTAGTGCTGGAAG-3′; LXRα-f, 5′-CCCTCAGCCTTTCCCCAAAT-3′; LXRα-r, 5′-ACATCTCTTCCTGGAGCCCT-3′; LGK-f, 5′-ACCCAGTCCTGGCCATTTTC-3′; LGK-r, 5′-TCTGTCTCTTGCACCTGCTG-3′. Each reaction was normalized to β-actin mRNA level using primers ACTβ-f, 5′-GCCGCCAGCTCACCAT-3′ and ACTβ-r, 5′-TCGATGGGGTACTTCAGGGT-3′. The expected sizes of the amplicons were 189, 296, 178, and 229 bp for *SREBP-1c*, *LXRα*, *LGK* and *ACTβ*, respectively. The thermal cycle was carried out at the following conditions: 10 min at 95 °C per one cycle; 30 s at 95 °C, 1 min at 60 °C and 30 s at 72 °C per 40 cycles. Real time quantitative PCRs were performed using Stratagene Mx3000P q-RT-PCR System (M-Medical S.r.l., Milan, Italy) and analysed by MxPro software for comparative quantitation.

3. Results and discussion

3.1. Molecular docking

Complexes between LXRα and seventeen pollutant compounds (listed in Table 1) were evaluated according to an *in silico* molecular docking analysis. Those chemicals that yielded to the most energetically favorable complexes (namely, BPA, DiDP, BZF, DiNP and TMTP) showed a predictive range of free energies of binding between −8.84 and −11.03 kcal/mol, with the corresponding equilibrium dissociation constants ranging between 3.24×10^{-7} and 8.06×10^{-9} M (Table 2). All binding models obtained from molecular docking analysis (considering the entire receptor as potential binding site) reported the ligand docked into the oxysterol binding site, with a minor electrostatic contribution

Table 1
Predicted free energy of binding (ΔG) of seventeen pollutants complexed with LXRα obtained by molecular docking.

Name	*ΔG* (kcal/mol)	Name	*ΔG* (kcal/mol)
Acetyl triethyl citrate (ATEC) - 6504	−6.3	**Diisononyl phthalate (DiNP) - 34,277**	**−9.6**
Bisphenol A (BPA) - 6623	**−8.8**	Gemfibrozil - 3463	−8.3
Bezafibrate (BZF) - 39,042	**−9.6**	Mono-2-ethylhexyl phthalate (MEHP) - 20,393	−8.5
di-2-Ethylhexyl phthalate (DEHP) - 8343	−8.8	Nafenopin - 19,592	−10.1
di-2-Ethylhexyl terephthalate (DEHT) - 22,932	−8.5	Tributyltin chloride (TBT) - 15,096	−8.2
Diisobutyl phthalate (DiBP) - 6782	−8.6	**tri-*m*-tolyl Phosphate (TMTP) - 11,232**	**−11.3**
Diisodecyl phthalate (DiDP) - 33,599	**−8.9**	**tri-*o*-tolyl Phosphate (TOTP) - 6527**	**−10.9**
Diethylene glycol dibenzoate - 8437	−8.8		
Diisononyladipate (DiNA) - 6,427,097	−7.9	tri-*p*-tolyl Phosphate (TPTP) - 6529	−10.4

In bold fonts are indicated the five pollutants selected for the experimental analysis. Pubchem ID is indicated after the compound name and abbreviation.

Table 2
Predicted ($K_{d,pred}$) and experimental (K_d) equilibrium dissociation constants obtained by molecular docking and biosensor analysis, respectively.

name	$K_{d,pred}$(M)	K_d (M)	k_{ass} ($M^{-1}s^{-1}$) and k_{diss} (s^{-1})	K_d* (M)
BPA	3.24×10^{-7}	$(2.55 \pm 0.44) \times 10^{-7}$	44105.8 ± 2669.25 $(11.23 \pm 1.81) \times 10^{-3}$	$(2.81 \pm 0.14) \times 10^{-7}$
DiDP	3.15×10^{-7}	$(4.32 \pm 1.09) \times 10^{-7}$	23228.8 ± 4117.25 $(10.03 \pm 1.82) \times 10^{-3}$	$(4.70 \pm 0.16) \times 10^{-7}$
BZF	9.18×10^{-8}	$(5.43 \pm 0.70) \times 10^{-8}$	99963.9 ± 1116.92 $(5.43 \pm 0.73) \times 10^{-3}$	$(6.25 \pm 0.26) \times 10^{-8}$
DiNP	8.44×10^{-8}	$(1.20 \pm 0.15) \times 10^{-7}$	44445.4 ± 1058.22 $(5.34 \pm 0.67) \times 10^{-3}$	$(1.23 \pm 0.15) \times 10^{-7}$
TMTP	8.06×10^{-9}	$(4.38 \pm 2.07) \times 10^{-8}$	23986.8 ± 1986.01 $(1.05 \pm 0.49) \times 10^{-3}$	$(5.49 \pm 2.01) \times 10^{-8}$
24(S)-HC	1.39×10^{-7}	$(1.10 \pm 0.10) \times 10^{-7}$ [2]	/	/
7-KC	1.3×10^{-5}	$>5 \times 10^{-6}$ [2]	/	/

Kinetic constants of the compound/receptor complexes and equilibrium dissociation constants (K_d*), derived by the extent of binding analysis (using Eq. (3) as fitting function and Levenberg–Marquardt algorithm), are also added. 24(*S*)-hydroxycolesterol (24(S)-HC) and 7-ketocholesterol (7-KC) were included in the table as a positive control and as a poorly active agonist, respectively.

(and a low number of theoretical H-bonds), and a predominant hydrophobic influence on the free energy of binding (Supplementary Table 1 and Fig. 1 right panels), in agreement with the non polar nature of this binding site. All the energy contributions are reported in Supplementary Table 1. The general preference of ligands for this site with sub-micromolar affinity indicates the presence of specific binding site for these species on the receptor. Moreover, TMTP and DiNP (the second compound in terms of theoretical affinity) were predicted to establish a π–π interaction with Phe^{326} and Phe^{315}, respectively. These amino acids, together with some other non-polar ones in the binding site, namely Ile-Leu and Thr (see Fig. 1), are crucial for the hydrophobic stabilization of the complexes, in agreement with the experimental evidences by Qian et al. [27]. Additionally, BZF and DiNP are extra-stabilized through the formation of one theoretical h-bond with Thr^{302}, whereas BPA can establish two theoretical h-bonds with Phe^{257} and Ile^{336} using its hydroxyl groups (Fig. 1 and Supplementary Table 1).

3.2. Biosensor studies

A SPR biosensor-based strategy was applied to confirm the results of the *in silico* studies and to characterize the kinetics of binding in the LXRα/compound complexes formation. The association and dissociation kinetics of each LXRα/compound complex showed monophasic behaviors, confirming a single binding site on the receptor (Fig. 2a). To assess the validity of the monophasic model in fitting each time course, a standard *F*-test procedure was used: the biphasic model was statistically non-significant at 95% confidence (data not shown). Equilibrium dissociation constants (K_d), derived by both kinetic (k_{ass} and k_{diss}) and extent (R_{max}) analysis, were in strong agreement with those predicted by molecular docking (Table 2 and Fig. 3) confirming the high-affinity interaction of this class of molecules for the LXRα and the reliability of the molecular docking method used. A reliability also confirmed by the best-predicted affinity values obtained using the default scoring function weights with respect to those obtained using the *ad-hoc* optimized weights (data not shown). All compounds tested targeted the receptor with (moderately) high affinity, showing equilibrium dissociation constants in the range 4.3×10^{-7}–4.3×10^{-8} M. These affinities were similar to, or even higher than, those reported for oxysterol/receptor complexes (ranging from 1×10^{-7} to 4×10^{-7} M [38]), and are summarized in Table 2. In particular, BZF and TMTP showed K_d values comparable to those reported for most potent drugs (T0901317 [39], GW3965 [40], LXR623 [41]).

The binding responses at equilibrium (extent of binding) were calculated for each time course with fully comparable equilibrium dissociation constants. The hyperbolic nature of the saturation plot (Fig. 2b) demonstrated a non-cooperative binding of these compounds to the receptor. Further analysis of association and dissociation kinetic parameters revealed a significant difference in the recognition processes of these two chemicals with LXRα: specifically, the stabilization of BZF/LXRα complex was mainly dependent on faster association phase, conversely a slower dissociation phase was critical in the stability of TMTP/LXRα complex. Nevertheless, the kinetic analysis generally revealed favored recognition events (most likely due to hydrophobic interactions (Supplementary Table 1 and Fig. 1)) with dissociation phases playing an important role in the stabilization of the ligand/receptor complexes.

3.3. Quantitative mRNA expression analyses

The analysis of mRNA levels after cell treatments with non-cytotoxic level of these pollutant compounds (as resulted by MTT

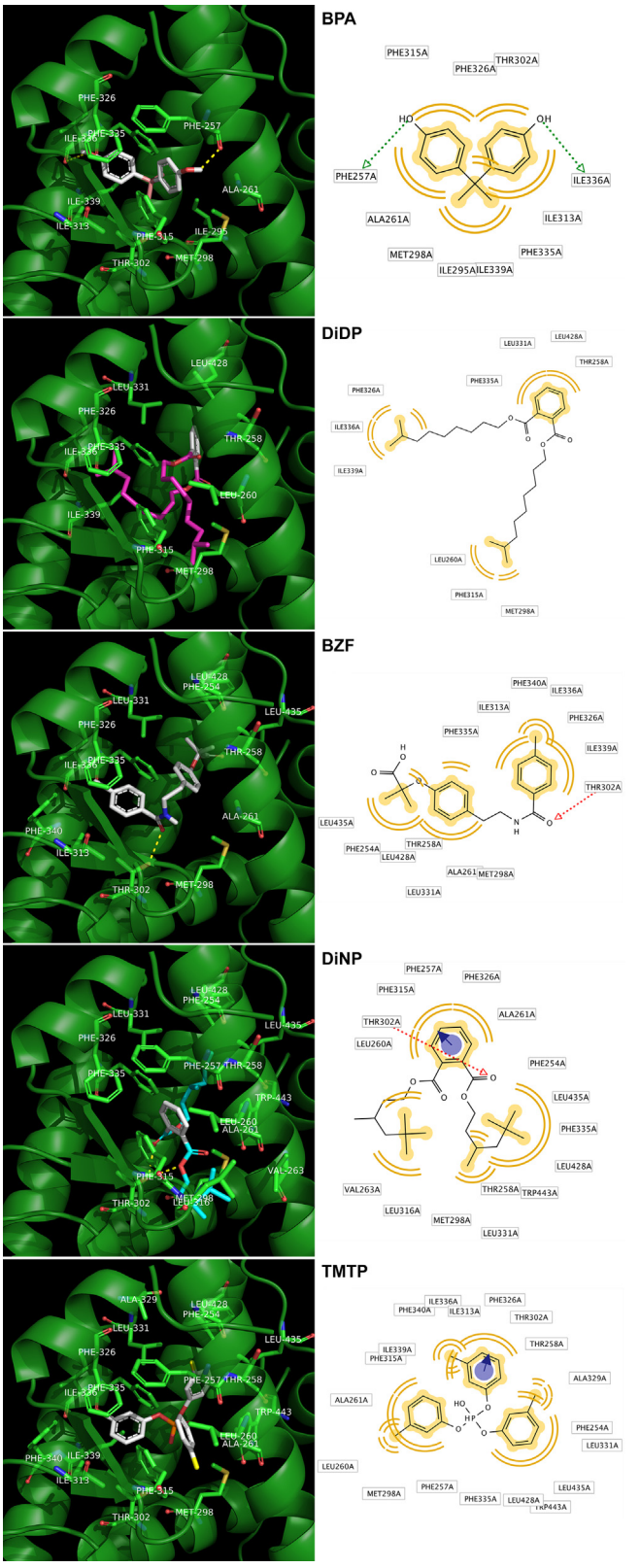

Fig. 1. Three-dimensional representation of the five pollutant compounds docked into the LXRα. The LXRα is represented in cartoon style in the left panels and all amino acids of the receptor, involved into the interaction with the ligand, are labelled and represented as stick. 2D visualizations, obtained by LigandScout, of the theoretical H-bonds (red and green dotted lines), the π–π or aromatic–aromatic ring interactions (blue circle and bold arrows) and the hydrophobic interactions (yellow areas) are shown in the right panels.

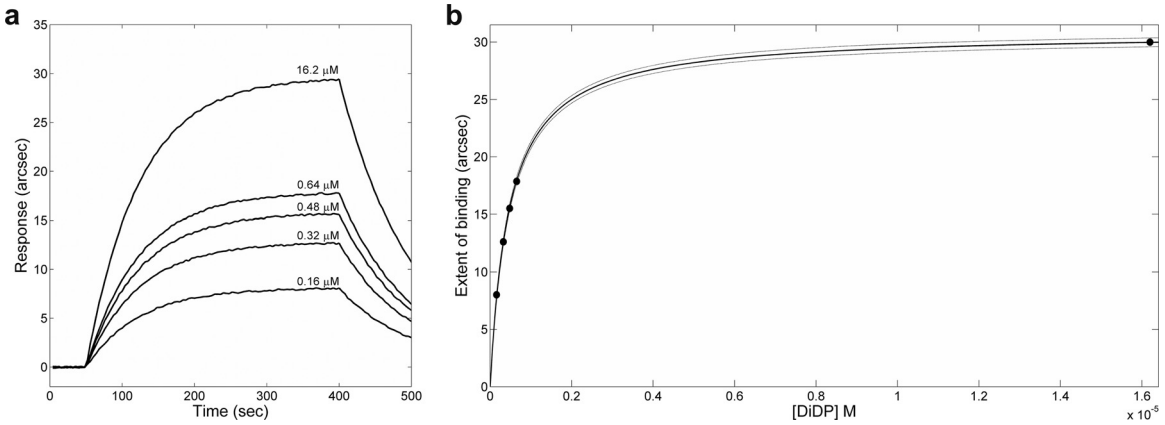

Fig. 2. Kinetics of the interaction between DiDP and LXRα. Overlay of association and dissociation kinetics of soluble DiDP to surface-blocked LXRα (a) and dependence of the extent of binding on DiDP concentration (b), obtained by SPR biosensor analysis. Nonlinear fit (solid line) and 95% confidence-bound (dashed lines) are shown.

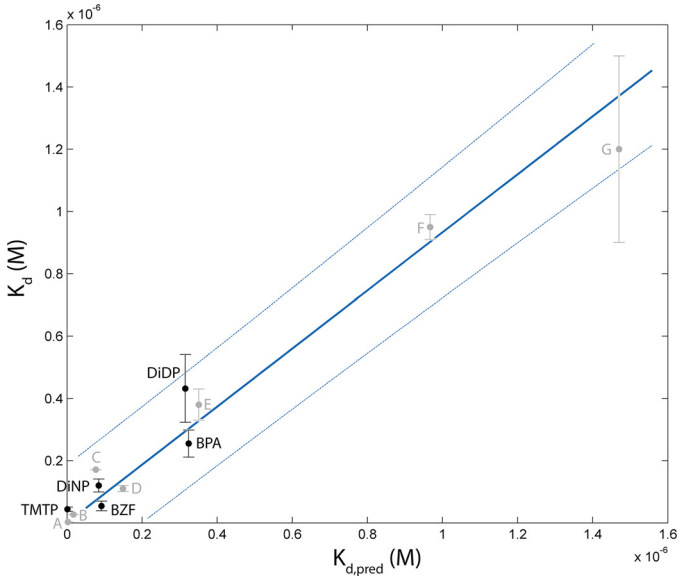

Fig. 3. Linear relationship between equilibrium dissociation constants obtained in silico ($K_{d,pred}$) and by SPR biosensor assay (K_d). Experimental K_d values of T0901317 (A), GW3965 (B), LXR623 (C), 24(S)-hydroxycolesterol (D), 22(R)-hydroxycolesterol (E), 22(R), 24(*S*)-hydroxycolesterol (F) and 24(R), 25-epoxycholesterol (G) were derived from available literature [2,39–41], whereas in silico values were predicted using the molecular docking procedure descripted in the methods section. Linear fit (solid line) and 95% confidence-bound (dashed lines) are shown.

assay, data not shown) was carried out to further and decisively confirm their effect on the LXR activity. Among the main genes regulated by LXRα, we selected *SREBP-1c*, *LGK* and *LXRα* genes, and we investigated their regulation by q-RT-PCR. As shown in Fig. 4, consistently with available evidences reporting the effect of different treatments with BPA on the expression of some genes under the transcriptional control of LXR [42,43], each compound significantly affected the expression of all the studied genes. Specifically, the extent of the modulation displayed a trend in line with the calculated equilibrium dissociation constants (within the experimental errors) (Table 2). Compared to control, the *SREBP-1c* gene expression was increased by at least 4-fold, whereas the *LGK* and *LXRα* genes expression was decreased by about 10-folds.

The observed effects in the expression of *SREBP-1c* and *LGK* can be attributed to both the direct and the indirect interaction between these molecules and the receptor. In particular, the increase in *SREBP-1c* expression, described also by Kim et al. [13], can be considered as an LXRα-mediated response, whereas the decrease in *LGK* expression as a SREBP-mediated response. In fact, being *LGK* a direct target gene of SREBP [44] (and consequently an indirect target gene of LXR), whose transcription activity is inhibited by this class of molecules [45], their interaction produces a decrease in *LGK* expression. Accordingly, being T0901317 a specific LXR binder, it causes the increase in *SREBP* expression and, subsequently/indirectly, the increase in *LGK* expression (Fig. 4, in agreement with Kim et al. [13]). In addition, it has been

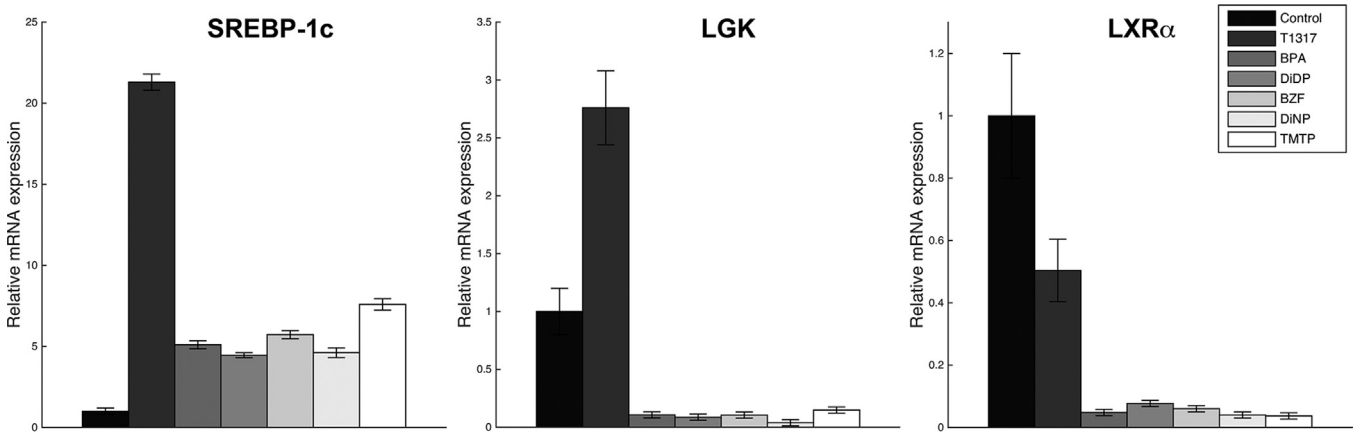

Fig. 4. Effect of pollutant compounds on SREBP-1c, LGK and LXRα mRNA levels in HepG2 cells. The quantity of mRNAs, showed as the mean ± S.D. of three independent experiments performed in triplicate and expressed as -fold increase relative to the basal transcription level in the absence of ligands, was normalized with respect to β-actin mRNA. Data were analysed by the comparative C_T method. Control cells were treated with the same DMSO concentration used to solubilize each compound. T0901317 is included as the positive control.

demonstrated that LXRα-mediated activation of *LGK* can be inhibited via a negative mechanism involving a small heterodimer partner (SHP) [13]. SHP was found to repress the transcriptional activity of LXRα by interacting with its common heterodimer partner RXRα. The inhibition of LXRα transcriptional activity results in down-regulation of *LGK* gene expression. Interestingly, one recent study has found that fenofibrate increased SHP mRNA levels in cultured liver cells [46]. Conversely, the decrease in *LXRα* expression may be due either to an auto-regulation through a negative feedback process [47,48] or to a regulation exerted by other transcription factors, which are in turn activated by TNFα in response to the exposure to some environmental endocrine-disrupting chemicals [49,50].

In conclusion, these evidences substantiated the reliability of the predictive computational procedure proposed in terms of both binding affinities, as confirmed by SPR studies, and drug-binding domain, as phenomenologically demonstrated by cell-based assay and q-RT-PCR. In particular, our study demonstrated the existence of a specific interaction between LXR and an heterogeneous set of synthetic environmental pollutants, which can affect the expression of LXR target genes, with consequent potential alteration in lipid and glucose cellular homeostasis. Future studies will be focused on the interplay of other glucose/lipid transcription factors.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jsbmb.2015.04.011.

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