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1. Introduction

Peroxisome proliferator-activated receptor gamma (PPAR_{γ}), a ligand-activated transcription factor of the nuclear receptor (NR) superfamily, directly binds to and regulates the target genes involved in lipid and carbohydrate metabolism, cell proliferation, apoptosis, and angiogenesis. Recent preclinical study and epidemiological analysis postulate an important role for PPAR_{γ} in carcinogenesis,^{1,2} and PPAR_{γ} agonists

Computational identification of potential chemoprophylactic agents according to dynamic behavior of peroxisome proliferator-activated receptor gamma[†]

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Peroxisome proliferator-activated receptor gamma (PPAR_Y) is an attractive target for chemoprevention of lung carcinoma, however its highly dynamic nature has plagued drug development for decades, with difficulties in receptor modeling for structure-based design. In this work, an integrated receptor-based virtual screening (VS) strategy was applied to identify PPAR_Y agonists as chemoprophylactic agents by using extensive docking and conformational sampling methods. Our results showed that the conformational plasticity of PPAR_Y, especially the H2 & S245 loop, H2' & Ω loop and AF-2 surface, is markedly affected by binding of full/partial agonists. To fully take the dynamic behavior of PPAR_Y into account, the VS approach effectively sorts out five commercial agents with reported antineoplastic properties. Among them, ZINC03775146 (gusperimus) and ZINC14087743 (miltefosine) might be novel PPAR_Y agonists with the potential for chemoprophylaxis, that simultaneously take part in a flexible switch of the AF-2 surface and state change of the Ω loop. Furthermore, the dynamic structural coupling between the H2 & S245 and H2' & Ω loops offers enticing hope for PPAR_Y-targeted therapeutics, by blocking kinase accessibility to PPAR_Y. These results might aid the development of chemopreventive drugs, and the integrated VS strategy could be conducive to drug design for highly flexible biomacromolecules.

thiazolidinediones (TZDs, *e.g.* rosiglitazone, RSG) possess antiproliferative effects and stimulate apoptosis in lung carcinoma cell lines, especially non-small-cell lung cancer (NSCLC).^{3,4} More interestingly, the cancer-preventing effects of several natural products are invariably associated with activation of PPAR_γ.⁵⁻⁷ Existing evidence intensively indicates the latency of PPAR_γ agonists in lung cancer chemoprevention, and stimulates a viable pursuit in chemoprophylactic agents.^{8,9}

PPAR_γ ligand-binding domain (PPAR_γ-LBD), which is necessary and sufficient for the transcriptional activation, is composed of 13 α-helices (H1–H12 and H2'), and includes a Yshaped internal ligand-binding pocket (LBP) and an activation function-2 surface (AF-2, formed by H3-5 and H12, see Fig. 1).^{10,11} The binding of endogenous and synthetic agonists to LBP causes conformational alterations in the AF-2 region to promote the displacement of a corepressor and the recruitment of a transcriptional coactivator, resulting in the activation and differentiation of gene expression.¹⁰ Meanwhile, the β-sheet (surrounded by H2, H3 and H2') and Ω loop can be mediators in the cyclin-dependent kinase 5 (Cdk5)-mediated PPAR_γ phosphorylation at Ser245, which leads to the dysregulated expression of target genes.¹¹ Aiming at these conformational alterations, numerous efforts have been made to explore

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selective full/partial agonists with distinct binding profiles.^{11,12} Full agonists (*e.g.* RSG) in general favor direct contacts with AF-2,¹³ while partial agonists (*e.g.* (2*S*)-2-(biphenyl-4-yloxy)-3phenylpropanoic acid, LRG) occasionally stabilize the β -sheet/ Ω loop (see Fig. 1).^{13,14} Unfortunately, PPAR_Y-LBD is a highly dynamic entity with multiple low-energy conformations and ligand-dependent conformational dynamics, especially the AF-2, β -sheet and Ω loop regions (Fig. 1).^{11,15,16} The highly structurally dynamic nature adds to not only the difficulties in advanced understanding of PPAR_Y function, but also hinders the rational discovery of PPAR_Y agonists.^{15,16}

Nowadays, receptor-based virtual screening (RBVS) is routinely implemented in drug discovery to increase the efficiency of the development pipeline.¹⁷ It not only allows successful predictions (*e.g.* antivirals for influenza and COVID-19) at the leading edge of a battle against a pandemic, but also provides a better understanding about the complexity of living systems.¹⁸⁻²² Among the sequential stages of RBVS, the initial receptor structures have become a prerequisite for success, with a significant practical problem to determine the major physiological states or important transition states, especially for receptors with highly dynamic behavior.²³⁻²⁵ To address this issue, a variety of algorithms and strategies have been developed for modeling receptor dynamics and minimizing disturbance of false positives, such as Markov state models (MSMs) and ensemble docking.²⁵⁻²⁸

In our previous works,^{9,29} we applied an ensemble-based docking method to determine the binding profiles between ligands and PPAR, with the ideal computational costs. The outcomes aroused our interest to integrate and extend existing flexible docking and conformational sampling methods to identify novel agents based on the dynamic behavior of PPARy. In this work, an integrated RBVS strategy (Scheme 1) was proposed to identify PPAR, agonists as cancer chemopreventive agents and their binding profiles, with the necessary flexibility to PPARy-LBD, desirable chemical space of drug-like compound, and more cost-effective. The dynamic behavior of PPAR, -LBD (especially the AF-2, β -sheet and Ω loop regions) was firstly studied on a set of five representative structures by explicitly solvated molecular dynamics (MD) simulations, consisting of four crystal structures and a homology model (Fig. 1). Then, VS experiments were carried out against the ZINC library,³⁰ utilizing the multiple conformations exacted from the MD simulations. Ultimately, top ten compounds were selected for further MD simulations to clarify their binding profiles and interaction mechanisms. The data presented here showed that



Scheme 1 Flowchart of virtual screening based on the dynamic behavior of PPAR_{γ}-LBD. The compounds were firstly filtered by rules formulated by Lipinski and Veber, then evaluated (LibDock) against a representative conformation of **Full**, with a 15.0 Å binding sites sphere. In terms of cDocker algorithm, representative conformations of **Full** and **Partial-1** were used with a 10.0 Å binding site sphere (conformation selection, see Fig. 2–4 and main text).

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the remarkable flexibility of AF-2, β -sheet and Ω loop regions results in correspondingly different conformations of PPAR_{γ}, and five of selected compounds have been reported to the antitumor effects. Our results could be of value in the development of PPAR_{γ} agonists with the chemopreventive effects, and the integrated VS strategy is expected to contribute to rational drug discovery of biomacromolecules with high dynamics.

2. Materials and methods

2.1. Representative structure setup

The coordinates of apo-active form (**Apo-active**, 1PRG³¹), active form with full agonist rosiglitazone (RSG) and coactivator

peptide (Full, 1FM6 (ref. ³²)), active form with partial agonist *S*enantiomer of LRG (Partial-1, 3B3K³³), and active form with partial agonist *R*-enantiomer of LRG (Partial-2, 3D6D³³) were retrieved from RCSB Protein Data Bank. The apo-inhibition form with corepressor peptide (Apo-inhibited) was modelled using the MODELER module,^{34,35} with the templates of PPAR_{α}-LBD (1KKQ)³⁶ and 1PRG,³¹ and then evaluated by Profile-3D module³⁵ and Procheck program.³⁷ Note that the differences between the five structures are shown in Fig. 1 and ESI Fig. 1.† All the hetero-atoms of non-protein parts were removed, and missing hydrogen atoms were added using Discovery Studio,³⁵ based on the expected charge distributions of amino acids at neutral pH.



Full (PDB ID: 1FM6)

Partial-1 (PDB ID: 3B3K)

Partial-2 (PDB ID: 3D6D)

Fig. 1 Ribbon diagrams of PPAR_Y-LBD and five structures used in the simulations: Apo-active, apo-active form of PPAR_Y-LBD, Apo-inhibited, apo-inhibition form of PPAR_Y-LBD with corepressor peptide (in yellow), Full, active form of PPAR_Y-LBD with full agonist rosiglitazone (RSG) and coactivator peptide (in yellow), Partial-1, active form of PPAR_Y-LBD with partial agonist LRG^S (S-enantiomer of LRG), Partial-2, active form of PPAR_Y-LBD with partial agonist LRG^R (*R*-enantiomer of LRG).



Fig. 2 Superimposition of various representative PPAR_γ-LBD conformations which were derived from simulation trajectories by using root mean-square difference (RMSD) clustering. The volumes and pocket sizes were separately determined by Discovery Studio client³⁵ and Fpocket program.⁵⁰

The energy minimizations were performed with the Charmm27 force field,³⁸ until converged to 0.01 kcal mol⁻¹ Å⁻¹. The explicit solvent MD simulations were conducted using GROMACS5.1.4 (ref. ³⁹) and the Charmm27 force field.³⁸ All investigated systems are summarized in Table 1, and the details of simulation setup are in agreement with our previous work.^{9,20,29} In brief, each system was solvated in a cubic box of SPC/E (simple-point-charge) water molecules extending at least 9.0 Å from any solute atom. Na⁺ counter-anions were placed to neutralize the system. To mimic physiological conditions, the NPT ensemble was applied at constant pressure (1 atm) and 300 K.⁴⁰ Particle-Mesh Ewald (PME) method⁴¹ and LINCS algorithm⁴² were applied to handle long-range electrostatics and

constrain all covalent bonds. The cutoff radii for coulomb and van der Waals interactions were set to 8.0 Å. Free dynamics were performed using a 2.0 fs time step, and coordinates were collected every 10.0 ps. The representative conformation of each system was chosen using the g_cluster tool implemented in GROMACS.⁴³

2.2. Virtual screening with representative structure

The 'Drugs-Now' and 'TCM Database (a) Taiwan' subsets of ZINC database (downloaded on 28 August 2018)³⁰ were used in virtual screening, and firstly filtered by rules formulated by Lipinski (rule of five)⁴⁴ and Veber.⁴⁵ The selected compounds

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Fig. 3 The root-mean-square fluctuations (RMSF) per residue of various PPAR_{γ}-LBD structures over (A) 100 ns and (B) 600 ns MD trajectories. The values of Full^R were averaged based on three independent 600 ns MD trajectories. H2' & Ω loop (residues 250–266 and residues 263–278), S245 loop (residues 237–253) and H12 (residues 465–477).

were then treated with 'Prepare Ligands' and 'Minimize Ligands' tools³⁵ to handle the geometries and partial atomic charges, especially correct ionization and low-energy conformers (converged to 0.001 kcal mol^{-1} Å⁻¹). Virtual screening was performed via two programs, LibDock46 and cDocker.47 The former is used for shape-based fast filtering, and the latter for semi-flexible fine screening. In our virtual screening protocol (see the flowchart of Scheme 1), the compounds were firstly evaluated (LibDock) against the MDgenerated representative conformation of Full, with a 15.0 Å binding site sphere. The compounds with LibDock scores larger than those of full agonist RSG and partial agonist LRG^S (\geq 118), were selected for the second filter. In terms of cDocker algorithm, MD-generated representative conformations of Full and Partial-1 were used, and the binding site was assigned with a sphere of 10.0 Å. The binding poses of best ten compounds were selected and further refined by 100.0 ns MD simulations. The details of screening parameters can be found in the ESI.†

2.3. Free energy calculation and geometric analysis

Binding free energies (ΔG_{bind}) were evaluated by the molecular mechanics generalized Born surface area method (g_mmpbsa),⁴⁸ which has been extensively used to expound the interactions between ligands and protein binding pockets.^{9,29} Details of parameters are similar to those performed the previous works,^{9,29} and in the ESI.† All values were calculated in averages over 200 snapshots evenly extracted from the 60–100 ns MD trajectories.

Apart from standard methods, secondary structures, volumes and binding pocket size were separately determined by the defined secondary structure of proteins (DSSP) method (do_dssp),⁴⁹ Discovery Studio client³⁵ and Fpocket program.⁵⁰ Principal component analysis (PCA) and dynamics cross-correlation matrices (DCCM) were calculated using Bio3D

package⁵¹ and GROMACS implemented tools.³⁹ Structural plotting and visualization were accomplished by Discovery Studio client.³⁵ Details of these analytical approaches can be found in the ESI.[†]

3. Results and discussion

3.1. Representative conformations and essential dynamics of PPAR_{γ}

First of all, dynamic behavior of PPAR_y-LBD (especially the AF-2, β -sheet and Ω loop regions) was studied with the four crystal structures (Apo-active, Full, Partial-1 and Partial-2) and one homology model structure (Apo-inhibited). Their structural differences have been shown in Fig. 1 and ESI Fig. 1,† and system setup of each MD simulation can refer to Table 1. The equilibration reliabilities were verified by monitoring the time evolution of structural parameters,52 and each PPARy-LBD structure (except Full) was well-behaved during the 100 ns MD simulations (ESI Fig. 2[†]), consistent with previous atomistic MD simulations of PPAR_y-LBD with agonists.^{9,29} As shown in Fig. 2, the representative conformations, extracted by RMSD clustering method, represent obvious structural differences. The representative conformation of Full has the maximal volume and pocket size with the values of \sim 33 937 and \sim 1200 Å³, while those of Partial-1 are moderate (~ 25712 and ~ 909 Å³) with the predictably different ligand-binding manner. While the volumes (pocket sizes) of Apo-active, Apo-inhibited and Partial-2 are $\sim 28\ 094\ (\sim 993)$, $\sim 23\ 530\ (\sim 832)$ and $\sim 24\ 895\ (\sim 880)\ \text{Å}^3$. To further explore the structural adjustments, the root mean square fluctuations (RMSF) and principal component analysis (PCA) analyses were separately performed with the 60-100 ns MD trajectories using the GROMACS implemented tools39 and Bio3D package.⁵¹ According to the analysis results (Fig. 3 and 4), it was not difficult to observe that Full, Partial-1 and Partial-2



Fig. 4 Analysis of various PPAR_Y-LBD structures from MD simulations. Projection of snapshots onto the principal planes defined by the three most significant PCs (PC1, PC2 and PC3) *via* Bio3D package.⁵¹ The magnitude of each eigenvalue is expressed as the percentage of the total variance (mean-square fluctuation) captured by the corresponding eigenvector. Eigenvalue rank was obtained from diagonalization of the atomic displacement correlation matrix of Ca atom coordinates. The analyses of **Apo-active**^R, **Full**^R and **Full**^{NP} were from 600 ns MD simulations, while others from 100 ns MD simulations (see ESI Fig. 2 and 3,† and main text).

exhibit dramatic fluctuations of S245 loop (residues 237–253), H2' & Ω loop (residues 250–266 and residues 263–278) and H12 (residues 465–477). In addition, the overall correlated motions

of Full, Partial-1 and Partial-2 are substantially dramatic, and their structures require a relatively long period to adjust and equilibrate, in which the AF-2, β -sheet and Ω loop regions

Simulation	System setup	Time (ns)
Apo-active	Apo-active PPAR _y -LBD (PDB source: 1PRG chain A)	100
Apo-inhibited	Apo-inhibited $PPAR_{\gamma}$ -LBD with corepressor (homology model)	100
Full	Active PPAR _y -LBD with rosiglitazone and coactivator (PDB source: 1FM6)	100
Partial-1	Active PPAR _{γ} -LBD with partial agonist LRG ^{s} (PDB source: 3B3K)	100
Partial-2	Active PPAR _y -LBD with partial agonist LRG^{R} (PDB source: 3D6D)	100
Apo-active ^R	Apo-active PPARy-LBD (PDB source: 1PRG chain A)	600
Full ^R (triplicate)	Active PPAR,-LBD with rosiglitazone and coactivator (PDB source: 1FM6, repeated for three times)	600
Full ^{NP}	Active PPAR, LBD with rosiglitazone (PDB source: 1FM6)	600

^a Fig. 1 shows the corresponding structures. ^b All simulations include SPC/E water box, which extends at least 9.0 Å from any solute atom.

occupy the accessible conformations for the recruitment of coregulator and agonist (Fig. 2–4), in accord with previous NMR and MD results.^{10,15}

The dynamic features of apo-active $PPAR_{\gamma}$ -LBD (Apo-active^R), active PPAR_y-LBD with RSG and coactivator (Full^R), as well as active PPAR_y-LBD with RSG (Full^{NP}) were further validated throughout the extra 600 ns MD simulations (Table 1 and ESI Fig. 3[†]). Note that the analyses of **Full^R** were performed over three replicate simulations to ensure the consistency of the individual simulations. The above-described motion patterns all reappeared, with more speculative locations of coactivator peptide within Full^R, viewed by RMSF and PCA results (Figure 3, 4, and ESI Fig. 4[†]). Structural fluctuates from our atomistic MD simulations reveal that the bound full/partial agonist (e.g. RSG/ LRG) could consolidate the PPAR_y-LBD conformations into active state, which favors coactivator binding (activation). While the H2' & Ω loop, S245 loop and AF-2 surface (especially H12) are much perturbed during the agonist-interaction process (Fig. 2-4), in agreement with above descriptions.^{10,15} Though the dynamic nature of PPAR_v-LBD is restricted to favor coactivator binding with the present of RSG (Full^{NP} vs. Apo-active^R), the binding stability of coactivator peptide with PPAR_v-LBD is less than previous affirmative expectations (Full^R vs. Full^{NP}).^{13,53} It means that current full/partial agonists cannot fully facilitate the PPAR_y activation in modulating target gene expression, and may be attributed to the insufficient consideration of PPAR,-LBD dynamic behavior in rational design.^{16,54} In fact, most of agonists were explored based on the steady-states of Apo-active, Full and Partial-2, with rarely considering the structural fluctuation of β -sheet and Ω loop regions (e.g. motion patterns of Full and Partial-1).55-57 Thereby the representative MD-derived conformations of Full and Partial-1 were adopted in the subsequent VS process to fully take the structural dynamics of AF-2, β -sheet and Ω loop regions, because of the dramatic motions (e.g. pocket size, structural fluctuation and ligandbinding manner, see Fig. 2-4).

3.2. Docking hits with representative conformations of PPAR_{γ}

Highly efficient and robust RBVS is a kind of art which aims at the achievement of balance between accurate optimization of ligand and receptor geometries, ligand-receptor complex representation and computational costs.58 To take distinct ligand geometries, dynamic ensembles of PPAR, -LBD and effective efficiency into account in the docking procedures, an integrated VS platform (see Scheme 1) was applied to accurately predict the binding poses of candidates. The Lipinski (rule of five)⁴⁴ and Veber⁴⁵ rules quickly narrow the candidates ($\sim 10^8$) down to 22 840 (TCM) and 7 377 031 (ZINC) compounds, with reasonable drug-like physicochemical properties. Site feature docking (LibDock46) ulteriorly picks out 1599 (TCM) and 1271 (ZINC) compounds to form favorable binding interactions, using the protein site feature of MD-generated conformation of Full which has the maximal internal pocket size. Note that this motion not only ensures the chemical space of filtered compounds, but also successfully sorts out positive controls RSG and LRG^S, with the LibDock score of \sim 118. In terms of cDocker algorithm,47 MD-generated conformations of Full and Partial-1 present the representative dynamic features, and the predicted binding poses of RSG and LRG^S with Full and Partial-1 are in a manner consistent with the crystallographic poses (1FM6 and 3B3K), with the cDocker interaction energies (E_{int}) being -61.29 and -66.62 kcal mol⁻¹, respectively (ESI Table 1[†]). As is evident, 1260 (TCM) and 991 (ZINC) compounds selectively bind with Full and Partial-1 (Scheme 1), and the binding patterns of evaluated top ten compounds seem separately to be comparable to those of RSG and LRG^S (ESI Fig. 5[†]). Among the top ten compounds, compound ZINC15120682 and ZINC85568445 are the natural products (TCM), and other eight compounds are commercial agents, with one of them (ZINC12381030) being the proved PPAR agonist (ESI Scheme 1[†]).³⁰ More important, several experimental studies have shown the five commercial agents ZINC03775146 (gusperimus),59 ZINC03831462 (sofalcone),⁶⁰ ZINC14087743 (miltefosine),⁶¹ ZINC17719775 (ascorbyl palmitate)62 and ZINC58581064 (dolutegravir)63 to have the miscellaneous antineoplastic activities, especially hindering the signal transduction of cellular proliferation. The binding properties will be further discussed in the following section.

3.3. Binding features of compounds predicted by MM-PBSA

As shown in ESI Fig. 6 and 7,† each docked complex reached the fundamental convergence after \sim 40 ns, indicated by the time evolutions of backbone-atom and ligand positional RMSDs, and

interaction energy profiles (short-range energy components).64,65 It reveals that a 100 ns simulation is sufficient for the structural relax, and relatively stable MD trajectories (60-100 ns) are subsequently selected for further analyses of energy and geometry.9,29 In accord with our simulations, ZINC03775146 (gusperimus), ZINC03831462 (sofalcone), ZINC14087743 (miltefosine) and ZINC17719775 (ascorbyl palmitate) are preferred agonists, with the average binding free energies (average between values of Full and Partial-1, ΔG_{bind} being -109.04, -8.12, -22.94 and -34.91 kcal mol⁻¹, respectively (Table 2). Note that the ΔG_{bind} values of RSG and LRG^S with PPAR_v-LBD are -40.04 and -43.23 kcal mol⁻¹, respectively. van der Waals components ($\Delta E_{vdw} + \Delta G_{sur}$) primarily drive the binding processes of the four compounds, with the contributions over -48 kcal mol⁻¹, which is consistent with previous MD simulations of PPAR_y-LBD with agonists (ESI Tables 2 and 3⁺).^{9,29} In contrast to current agonist (e.g. RSG/LRG), the four compounds seem to induce more favorable coactivator binding, with the possibly preferable PPAR_{γ} activation (Fig. 5 and ESI Fig. 5–7^{\dagger}).

ZINC03775146 (gusperimus) is a conventional immunosuppressor drug with the antitumor activities.59 It can enter and fill the ligand-binding pocket (LBP) of PPAR_y-LBD (Full), with the binding pose resembling that of full agonist RSG (Fig. 5A). Guanidino group of ZINC03775146 has electrostatic (Hbonding) interactions with residue Glu259 of H2', residues Leu270 and Ser274 of Ω loop. N-(Hydroxymethyl)acetamide group of ZINC03775146 occupies the portion of the large twolobe entry of the pocket, 13,66 where residue Arg288 of H3, residues Leu340 and Ser342 of β-sheet region are in electrostatic contacts. ZINC03775146 also has H-bonding interactions with residue Ser289 of H3 and residue Tyr473 of H12 (Fig. 5A). In addition, the alkyl portion of ZINC03775146 has hydrophobic stacking with residues Cys285 (H3) and Tyr327 (H4/5). ZINC03831462 (sofalcone)60 normally treats gastric ulcer and chronic gastritis, and upregulates the nuclear factor (erythroidderived 2)-like 2/heme oxygenase-1 pathway. Its binding with **Partial-1** is close to the case of partial agonist LRG^{S} (Fig. 5B). ZINC03831462 demonstrates hydrophobic interactions with

residues Cys285 and Arg288 of H3, residues Leu330 and Leu333 of H4/H5, residues Met364 and Lys367 of H7, residues His449, Leu453, Ile456 and Lys457 of H11, as well as residue Leu469 of H12. The result agrees with a previous proposal that $PPAR_{\gamma}$ partial agonists interact with residues inside the region between H3 and β -sheet (Fig. 1), thereby promoting the stabilization of β sheet surface.67,68 ZINC14087743 (miltefosine)61 is an oral drug used for the treatment of visceral leishmaniasis, can inhibit phosphatidylinositol-3-kinases (PI3K)/protein-serine-threonine kinase (Akt) activity in the cancer cell lines A431 and HeLa. It occupies the canonical binding pocket of PPAR, -LBD, with the binding pose resembling those of RSG and ZINC03775146 (Fig. 5C and ESI Fig. 5[†]). Residues Arg280, Phe282 and Gly284 of H3, residue Phe363 of H7, residues His449 and Leu453 of H11, residue Glu259 of H2', residues Leu270 and Ser274 of Ω loop, as well as residue Ser342 of β-sheet region are in hydrophobic contacts within the ZINC14087743-Full complex. The binding of ZINC17719775 (ascorbyl palmitate, an antioxidant)62 with Partial-1 is close to the cases of LRG^S and ZINC03831462 (Fig. 5D). However, only residues Glu460, Met463 and Leu465 of AF-2 (portion of H11 and H12) are in hydrophobic interactions with ZINC17719775.

3.4. Insights about structural movements of $PPAR_{\gamma}$ and chemoprophylactic agents

In general, full agonists (*e.g.* RSG) adopt a common horseshoe conformation centered about H3 to stabilize the AF2 surface (Fig. 1), and have the extensive hydrogen bond network with residues Ser289, His323, His449 and Tyr473.¹³ Instead, partial agonists (*e.g.* LRG) bind in the part of LBP with a hydrophobic manner (*e.g.* residue Cys285) and stabilize the β-sheet (*e.g.* residue Ser342)/Ω loop.^{13,14} Our MD results indicated that the top four compounds are located near the canonical LBP of PPAR_γ-LBD, associated with the stabilization of AF-2 (*e.g.* residue Leu453 of H11, and residues Leu465, Leu469 and Tyr473 of H12, Fig. 5 and ESI Fig. 5[†]). This motion is similar as the cases of current full/partial agonists, while shows relatively few H-bonding interactions with H12 (Fig. 5).^{10,15} The four

Table 2	Docking results	of top ten	compounds	(binding f	ree energies,	$\Delta G_{\text{bind}})^{\prime}$

#	ZINC no.	Vendors	Catalog number	Full	Partial-1	Average
	Zirte no.	Venuorb	Guturog Humber	i un	Turtiur 1	meruge
1	ZINC03775146	AK Scientific	K529	-124.17	-93.90	-109.04
2	ZINC03831462	3B Scientific Corporation	3B2-0795	-4.16	-12.09	-8.12
3	ZINC15120682			-0.92	15.31	7.20
4	ZINC12381030	Vitas-M	STK170412	14.60	20.74	17.67
5	ZINC14087743	BioSynth	M-7200	-24.58	-21.29	-22.94
6	ZINC03874917	3B Scientific Corporation	3B3-013268	18.02	29.13	23.58
7	ZINC17719775	Acros Organics	44 948	-29.52	-40.29	-34.91
8	ZINC03874915	3B Scientific Corporation	3B3-013268	35.25	36.13	35.69
9	ZINC58581064	AK Scientific	X7595	6.69	9.12	7.91
10	ZINC85569445	_	_	24.85	29.74	27.30
	RSG^b	Vitas-M	STL350047	-40.04	_	-40.04
	LRG ^{Sc}			_	-43 23	-43 23

^{*a*} Energy units in kcal mol⁻¹, obtained from the equilibrium structures. ^{*b*} Rosiglitazone, control for full agonist. ^{*c*} S-(2S)-2-(Biphenyl-4-yloxy)-3-phenylpropanoic acid, control for partial agonist.



Fig. 5 Views of the preferred binding modes of selected compounds with the active-site residues of PPAR_Y-LBD: (A) ZINC03775146–**Full**, (B) ZINC03831462–**Partial-1**, (C) ZINC14087743–**Full** and (D) ZINC17719775–**Partial-1**. Key residues are represented by stick models, with C atoms in pink. Compounds are represented by ball and stick models. The O, N, C, S atoms are colored in red, blue, green and dark yellow, respectively. The important H-bonds (or electrostatic interactions) are labeled in the dashed black lines. For comparison, cocrystallized orientation of rosi-glitazone (RSG) and *S*-(*2S*)-2-(biphenyl-4-yloxy)-3-phenylpropanoic acid (LRG^S) within **Full** and **Partial-1** are shown in ball and stick models, with C atoms in purple.

compounds take a middle-of-the-road approach, and supplement the hydrophobic (H-bonding) interactions with few important residues of H3 (*e.g.* residue Cys285 and Arg288), β sheet (residue Ser342) and Ω loop (*e.g.* residues Leu270 and Ser274) regions, especially ZINC03775146 (gusperimus) and ZINC14087743 (miltefosine). For all this, the residue fluctuations of H12 remain relatively stable, however those in the ZINC03775146/ZINC14087743 bound complexes have much larger changes (Fig. 5, ESI Fig. 5 and 6†). It indicates that H12 of the four bound complexes occupies more stability (less subensembles), seemed to correlate positively with agonist activity. For instance, RSG holds the H12 in narrow energy well with rare structural exchange, and has the substantial IC₅₀ value.^{15,33}

To further explore the influence of the four compounds on the structural movements of PPAR_{γ}-LBD, the large amplitude conformational changes and secondary structure alterations were monitored during the 100.0 ns MD simulations (Fig. 6 and 7). H2 & S245 loop and H2' & Ω loop in ZINC03775146–**Full** and ZINC14087743–**Full** exhibit a much larger fluctuation than those in the other two bound complexes, especially residues Thr238 and Gly239 of H2, residues Ser245 and Pro246 of S245 loop, and residues Leu270 and Ser274 of Ω loop (Fig. 6 and 7). These results intensely indicate that H2 and H2' of **Full** with the present of ZINC03775146 (gusperimus)/ZINC14087743 (miltefosine) turn the conformational alteration process over to the configuration adjustment of Ω loop (adjacent to H2'), to achieve an optimal fit of compound with the PPAR_y-LBD (Fig. 5 and ESI Fig. 5[†]). This observation agrees with the previous points that some partial agonists likely induce a closed state of the Ω loop, to inhibit the PPAR, phosphorylation induced by Cdk5.57,68 The differences in dynamic cross-correlation maps (DCCM) between the four docked complexes further support the altered motions of the S245 and Ω loops upon binding of the corresponding compound (ESI Fig. 8[†]). So in conclusion, the binding of selective compound (at least ZINC03775146, gusperimus) induces the conformational adjustments of the PPARy-LBD (e.g. H12 and Ω loop regions), associated with the correlated motions (shared community) between H2 & S245 loop and H2' & Ω loop regions, that analogously viewed by the conformational dynamics of allosteric proteins.54 Consequently, the four compounds could be looked as a new class of agonist, in view of AF-2 surface's flexible switch (full agonist-like binding model) and Ω loop's state change (partial agonist-like binding model) simultaneously (Fig. 1 and 5). More importantly, the middle-ofthe-road approach of ZINC03775146 (gusperimus) and ZINC14087743 (miltefosine) with PPAR_{γ} gives conceivable clues to explain the relevance of them in inhibiting and blocking the signal transduction of cellular proliferation, as well as the inhibitions of cancer cells.59,61 Thereby ZINC03775146 (gusperimus) and ZINC14087743 (miltefosine) may be preferred PPAR_{γ}



Fig. 6 Analysis of various docked complexes from MD simulations. Projection of snapshots onto the principal planes defined by the three most significant PCs (PC1, PC2 and PC3) via Bio3D package.⁵¹ The magnitude of each eigenvalue is expressed as the percentage of the total variance (mean-square fluctuation) captured by the corresponding eigenvector. Eigenvalue rank was obtained from diagonalization of the atomic displacement correlation matrix of C α atom coordinates. The analyses were from 100 ns MD simulations (see ESI Fig. 6 and 7,† and main text).

agonists, and lead candidate agents for the lung cancer chemoprevention.

In this study, the acquired binding profiles of top four compounds satisfy some general features, including proper orientation of the protein–substrate interface (AF-2 surface stabilization) and compatibility with the representative of heterodimer PPAR_{γ}-RXR α bound to a DNA stretch.^{10,15,31} The predicted binding poses of ZINC03775146 (gusperimus) and

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Fig. 7 Secondary structures of H2 \oplus S245 loop (resides 228–240 and residues 237–253) and H2' \oplus Ω loop (residues 250–266 and residues 263–278) in the (A) ZINC03775146–Full, (B) ZINC03831462–Partial-1, (C) ZINC14087743–Full and (D) ZINC17719775–Partial-1 complexes. Content rate of turn, α -helix, bend and coil conformations of the individual residues was calculated through the DSSP tool implemented in GROMACS.⁴⁹

ZINC14087743 (miltefosine) also reproduce the adequate structural arrangement for the phosphorylation reaction at the catalytic site (S245 loop), with the necessary flexibility to the β sheet and Ω loop regions.¹¹ In addition to the conventional structural adjustments (e.g. AF-2), the collaborative structural alterations of H2 & S245 loop and H2' & Ω loop regions should play an important role in the interaction of Cdk5 with the nuclear receptor, and then affect the phosphorylation of residue Ser245.68 It means that a structural coupling between H2 & S245 loop and H2' & Ω loop regions controls PPAR_y/Cdk5 axis, and PPAR_y ligands break the collaborative structural alterations may also hold promise for the drug design, not just on the stabilizations of β -sheets and Ω -loop regions.¹¹ It is consistent with recent NMR and hydrogen-deuterium exchange (HDX) results that the salt-bridge disruption involving Ω loop and H3 switch are expected to destabilize the AF2 surface.¹⁶ From there, the hydrophobic groups (e.g., biphenyl group) might contribute

a lot to the ligand binding with the region and benefit a more effective regulator, on basis of the steric and hydrophobicity/ hydrophilicity characteristics of the region between H3, Ω loop and β -sheet of PPAR_{\gamma}-LBD (Fig. 1 and 5).^{15,57}

4. Conclusion

PPAR_{γ} plays a key role in the control of several cellular signaling and gene expression, and is an attractive target for the lung cancer chemoprevention.^{3,4} Unfortunately the highly structural dynamic nature of PPAR_{γ}-LBD adds to not only the difficulties in basic understanding of PPAR_{γ} function, but also hinders the rational drug design.¹⁵ To solve the problem, we presented an integrated VS platform (details in Scheme 1) to identify the PPAR_{γ} agonists and the binding profiles, with the dynamic behavior of PPAR_{γ}.

Through the analyses of ligand-binding pocket (LBP) volume and correlated motions of AF-2, β -sheet and Ω loop regions, it

was found that the conformational plasticity of PPAR_{γ} is markedly affected by binding of selective agonist (*e.g.* RSG/ LRG), and the intrinsically dynamic behavior of PPAR_{γ} should be carefully considered in the virtual screening, with multiple acceptable conformations (*e.g.* MD-generated conformations of **Full** and **Partial-1**). The integrated VS platform could quickly sort out favorable compounds (*e.g.* ZINC03775146 (gusperimus) and ZINC14087743 (miltefosine)) and determine their binding profiles, with the considerable balance between accuracies and computational costs. In addition, the intrinsically dynamic behavior of PPAR_{γ} aroused a therapeutic window for the regulation of target genes *via* PPAR_{γ}, using a ligand that blocks kinase accessibility to PPAR_{γ} Ser245, and offered hope for resurrecting PPAR_{γ}-targeted therapeutics to lung cancer chemoprevention.

The recent unified view of drug screening emphasizes that the appropriate selection of the initial structure will determine the success of rational drug design.^{69,70} In effect, a considerable proportion of receptors are discovered without the presence of endogenous substrates or synthetic ligands. The allosterically perturbed signals transmit from apo state to ligand bound state (or transition state) can affect the functional activities of receptors. As a result, the presented integrated VS strategy should be of value in the rational drug screening, especially aiming at biomacromolecules with considerable flexibilities or protein–protein association.

Conflicts of interest

The authors have declared that no competing interests exist.

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