Supporting Information

Structure-Dependent Activity of Phthalate Esters and Phthalate Monoesters Binding to Human Constitutive Androstane Receptor

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Contents:

Chemicals and Reagents.

6-(4-Chlorophenyl)imidazo[2,1-*b*][1,3]thiazole-5-carbaldehyde *O*-(3,4-dichlorobenzyl)oxime (CITCO, >99.0%) and Dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dimethyl phthalate (DMP, >99.7%), diethyl phthalate (DEP, >99.5%), di-n-propyl phthalate (DPRP, >99.7%), diisopropyl phthalate (DIPRP, >98%), diallyl phthalate (DAP, AR), di-n-butyl phtalate (DnBP, >99.5%), diisobutyl phthalate (DIBP, >99%), di-n-pentyl phthalate (DnPP, >98%), diheptyl phthalate (DHP, AR), di-n-octyl phthalate (DnOP, >99%), diisononyl phthalate (DINP, >99%), diisodecyl phthalate (DIDP, >99.5%), dicyclohexyl phthalate (DCHP, >99%), benzyl butyl phthalate (BBP, >98%), and di(2-ethylhexyl) phthalate (DEHP, >99.4%) were purchased from Aladdin Reagent Co. Ltd. (Shanghai, China). Di-n-hexyl phthalate (DHXP, >99%), diisopentyl phthalate (DIPP, >99%), di-n-nonyl phthalate (DnNP, >99%), di-iso-octyl phthalate (DIOP, >99.5%), phthalic acid

23 bis-4-methyl-2-pentyl ester (BMPP, >99.0%), mono-methyl phthalate (MMP, >99%),
24 mono-hexyl phthalate (MHXP, >99%), mono-isobutyl phthalate (MIBP, >99%), mono-n-butyl
25 phthalate (MnBP, >99%), mono-cyclohexyl phthalate (MCHP, >99%), mono-ethyl phthalate
26 (MEP, >99%), mono-2-ethylhexyl phthalate (MEHP), monooctyl phthalate (MOP), and
27 mono-benzyl phthalate (MBZP) were purchased from J&K Scientific Ltd (Beijing, China).
28 Diphenyl 1,2-phthalate (DPHP, >98.0%) and dibenzyl phthalate (DBzP, >98.0%) were
29 purchased from the Hengyezhongyuan Company (Beijing, China). Bis-(2-ethylhexyl)
30 tetrabromophthalate (TBPH) and mono-(2-ethyhexyl) tetrabromophthalate (TBMEHP) were
31 purchased from Wellington Laboratories (Guelph, Ontario, Canada). Zymolyase (20T) was
32 purchased from Seikagaku Kogyo Co. Ltd. (Tokyo, Japan), and 2-nitrophenyl-β-D-galactoside
33 (ONPG) was purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan). All reagents used
34 in this study were of molecular biology grade unless otherwise described. All the target
35 chemicals were prepared in DMSO solution with the concentration of 100 μM and stored at
36 -20°C.

37 **Measurement of Atom-Atom Distance.**

38 The atom-atom distances between activated phthalates (DMP, DEP, DPRP, DAP, DIPRP,
39 DnBP, DIBP, DIPP, DnPP, DHXP, DPHP, DBzP, BBP, DCHP, and BMPP) and the neighbored
40 aromatic amino acid residues (Phe132, Phe161, Phe217, Tyr224, Phe234, Phe238, Phe243,
41 and Tyr326) were measure by means of Scigress Explorer user interface and adjust tools. The
42 distances are shown in red dashed lines (Figure S3).

43

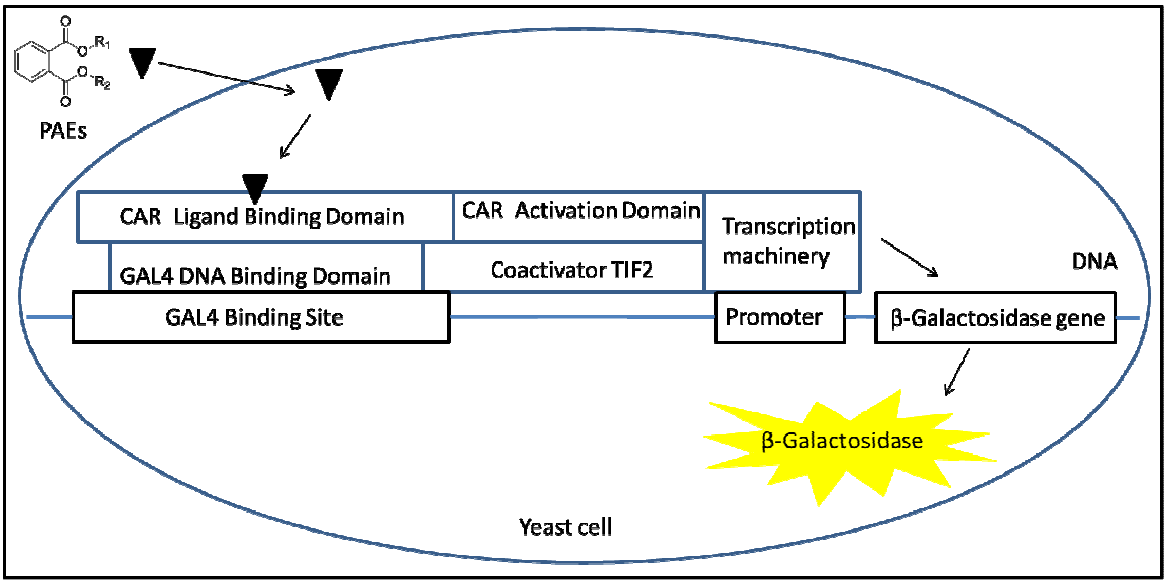
Supplementary Figure Legends

Figure S1: Mechanism of the yeast two-hybrid assay to determine the human CAR binding activities of phthalate esters.

Figure S2 : Molecular docking conformation of DINP in human CAR pocket. DINP represented as ball and cylinder, and the amino acid residues in the active site are represented as sticks. They are all colored according to element type: carbon atom (grey), oxygen atom (red), and nitrogen atom (blue).

Figure S3 : Distances between activated phthalates (DMP, DEP, DPRP, DAP, DIPRP, DnBP, DIBP, DIPP, DnPP, DHXP, DPHP, DBzP, BBP, DCHP, and BMPP) and the neighbored aromatic amino acid residues (red dashed line). Activated phthalates are represented as ball and cylinder, and the amino acid residues in the active site are represented as sticks. They are all colored according to element type: carbon atom (grey), oxygen atom (red), and nitrogen atom (blue). Numbers in the picture are the measured distances.

Figure S4: Molecular docking conformation of MHXP in human CAR pocket. MHXP represented as ball and cylinder, and the amino acid residues in the active site are represented as sticks. They are all colored according to element type: carbon atom (grey), oxygen atom (red), and nitrogen atom (blue).



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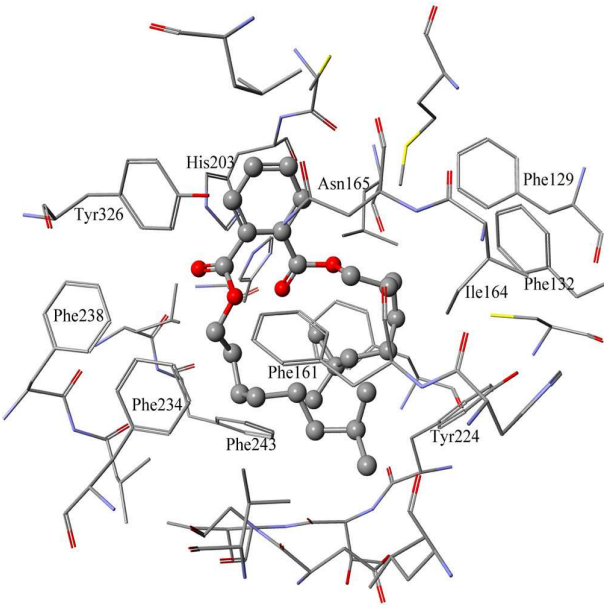
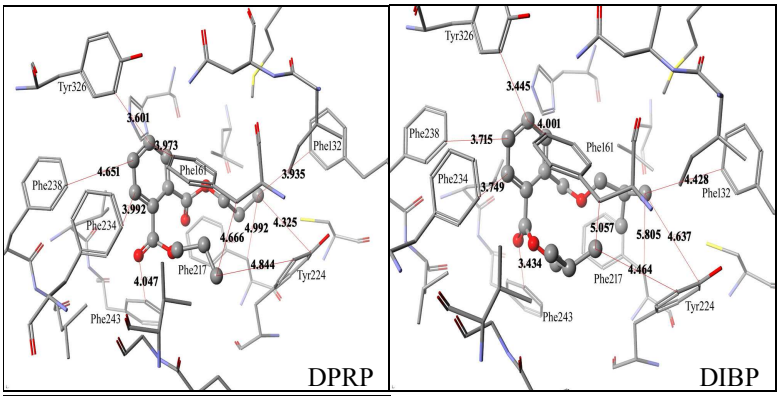


Figure S2. Molecular docking conformation of DINP in human CAR pocket. DINP represented as ball and cylinder, and the amino acid residues in the active site are represented as sticks. They are all colored according to element type: carbon atom (grey), oxygen atom (red), and nitrogen atom (blue).

75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Phe132
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Phe238
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Phe234
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4.666
Phe217
4.844
Tyr224
4.047
Phe243
DPRP
Tyr326
3.445
4.001
Phe238
3.715
Phe161
Phe234
3.749
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Tyr224
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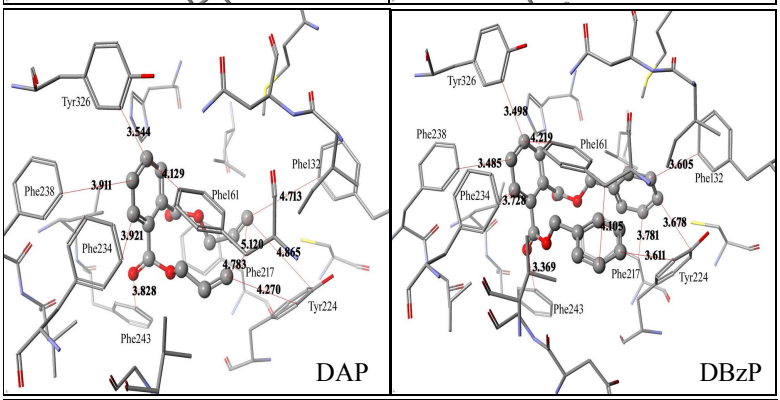
DIBP

76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Phe217
3.828
4.270
Tyr224
Phe243
DAP
Tyr326
3.498
4.219
Phe238
Phe161
3.485
3.605
Phe132
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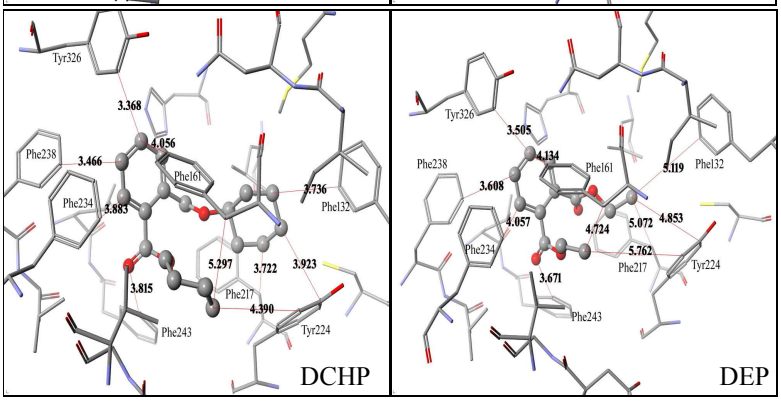
Tyr224
Phe243
DBzP

77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3.815
Phe217
4.390
Phe243
Tyr224
DCHP
Tyr326
3.505
4.134
Phe238
Phe161
5.119
Phe132
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DEP

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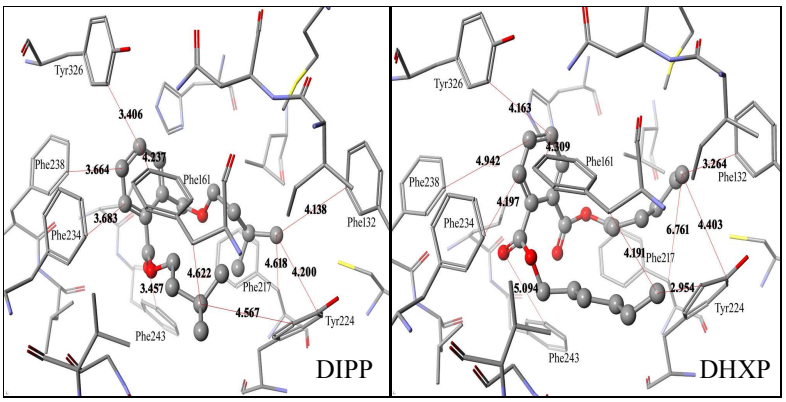
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4.622
3.457
Phe217
4.567
Tyr224
Phe243
DIPP
Tyr326
4.163
4.309
4.942
Phe161
3.264
Phe238
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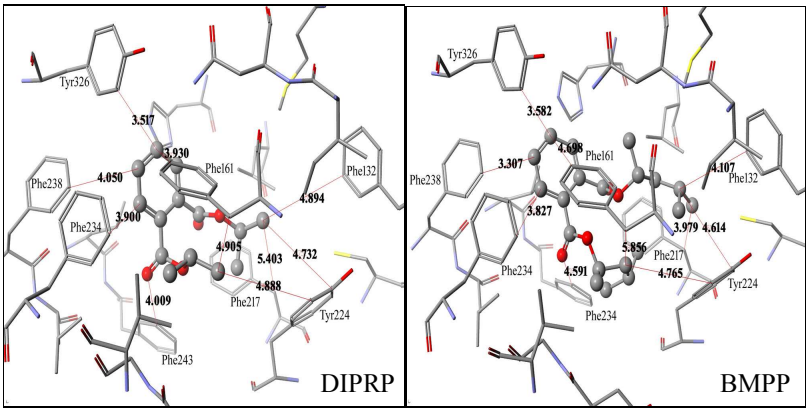
Tyr224
Phe243
DHXP

79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Phe234
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4.905
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4.732
4.888
4.009
Phe217
Tyr224
Phe243
DIPRP
Tyr326
3.582
4.698
Phe161
Phe238
3.307
4.107
Phe132
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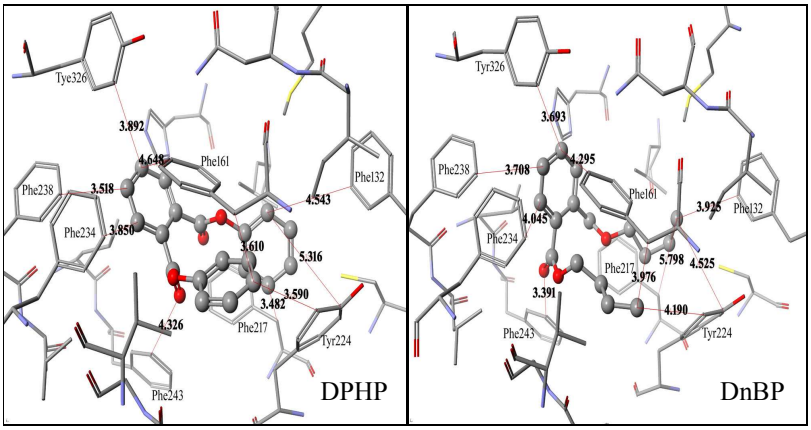
BMPP

80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Phe234
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3.610
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3.590
3.482
4.326
Phe217
Tyr224
Phe243
DPHP
Tyr326
3.693
Phe238
3.708
4.295
Phe161
3.925
Phe132
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4.190
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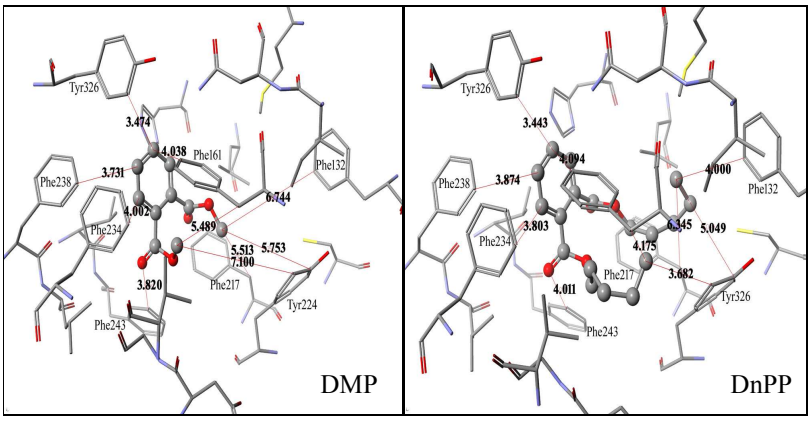
DnBP

81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Phe238
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7.100
3.820
Phe217
Tyr224
Phe243
DMP
Tyr326
3.443
4.094
4.000
Phe238
3.874
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4.175
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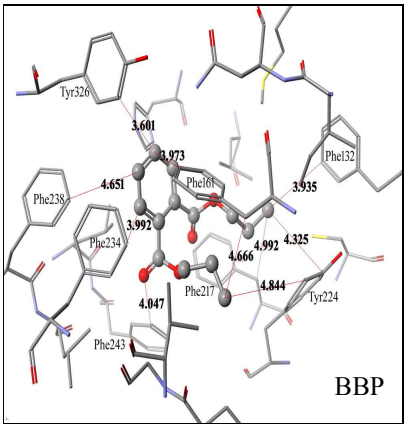
4.011
Tyr326
Phe243
DnPP

82


Tyr326
3.601
3.973
Phe132
Phe238
4.651
Phe161
3.935
3.992
Phe234
4.992
4.325
4.666
Phe217
4.844
Tyr224
4.047
Phe243
BBP

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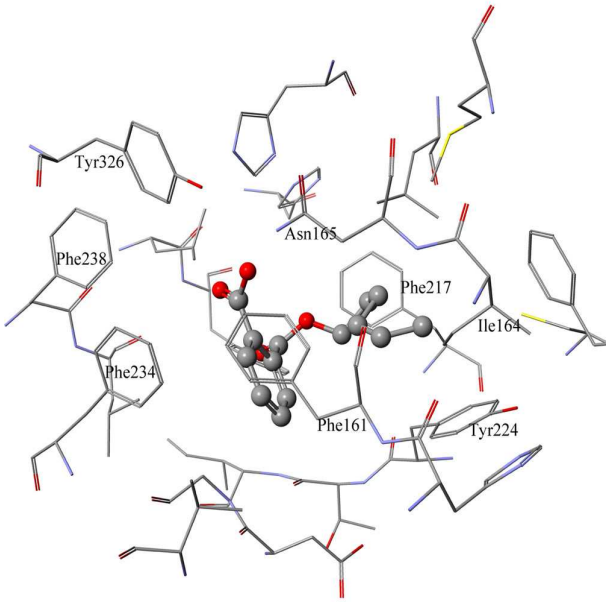

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