The 7th International Seminar on Smart Molecule of Natural Resources (ISSMART)
Thursday, August 7th 2025

7th ISSMART 2025
International Seminar on Smart Molecule of Natural Resource

In Silico Screening of Flavonoid Glycosides and Their Aglycones as Potential PPARγ Modulators

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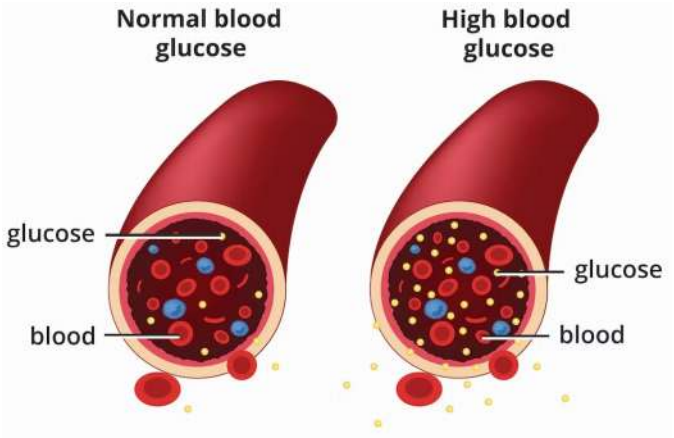
Outline

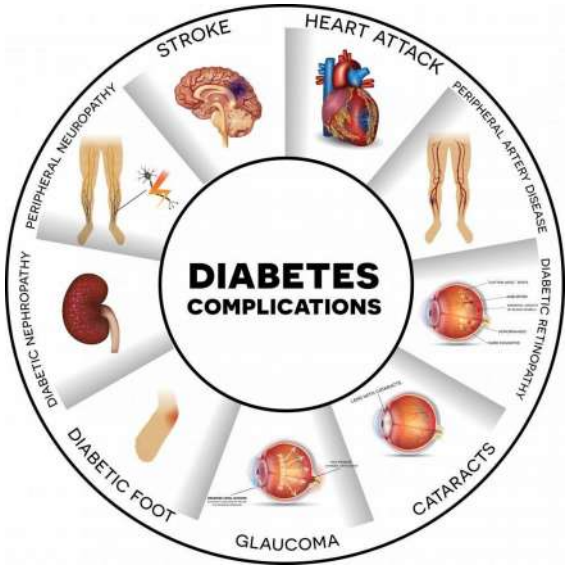
- **Diabetes Mellitus**
- PPARγ as Target Protein for T2DM
- Flavonoid as Source for Antidiabetic Agent
- Screening partner ligand of GW9662
- Conclusion

Diabetes Mellitus

Diabetes mellitus (DM) is a chronic progressive metabolic disorder in which the body is unable to utilize glucose.

High blood glucose → **Long term complication**





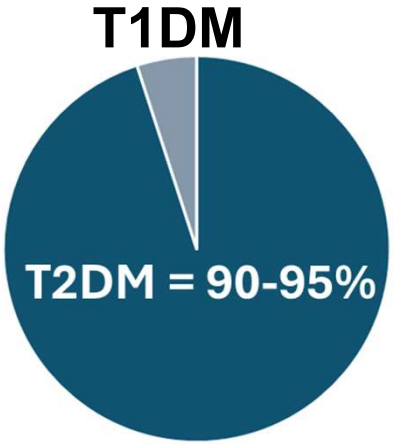
Sun, H. et al., *Diabetes Res. Clin. Pract.,*183, 109119 (2022); Antar, S.A. et al., *Biomed. Pharmacother;* 168, 115734 (*2023*)*;* Wu, Y. et al.*, Int. J. Med. Sci.* 11, 1185-1200 (2014).

High numbers of DM cases require expansion of therapeutic options

Diabetes around the world in 2021

537 million adults (20-79 years) are living with diabetes

Projected to rise to 783 million by 2045

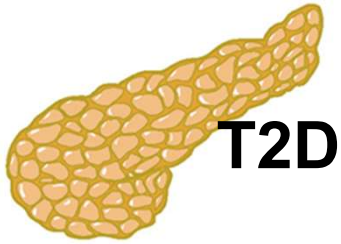


Optimal treatment of T2DM has been challenging to find.

Expansion of theraupetic options

Sun, H. et al., *Diabetes Res. Clin. Pract.,*183, 109119 (2022); Antar, S.A. et al., *Biomed. Pharmacother;* 168, 115734 (*2023*)*;* Wu, Y. et al.*, Int. J. Med. Sci.* 11, 1185-1200 (2014).

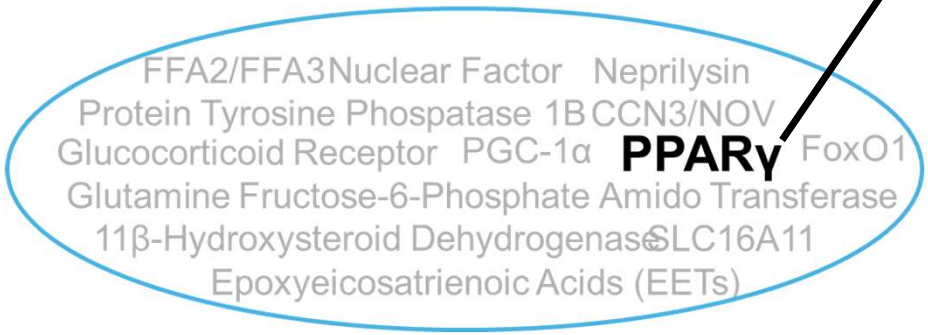
PPARγ is one of emerging target for T2DM



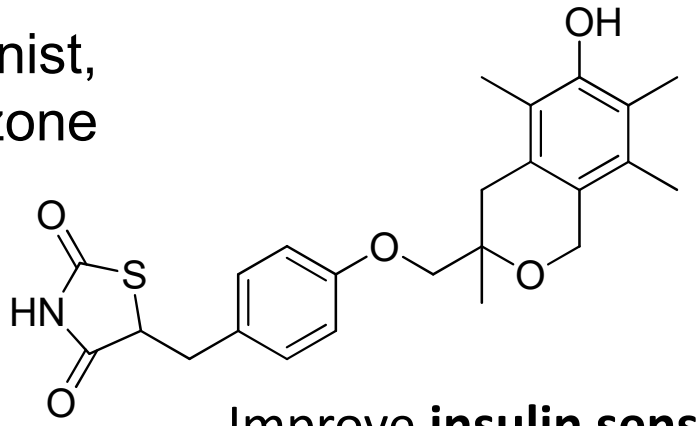
T2DM →

Associated with insulin deficiency/resistant.
Significant emerging targets have been reported to improve insulin's action on target tissues and help restore β-cell functions.

Emerging target for T2DM



Full Agonist,
Troglitazone



Improve **insulin sensitivity**. However, it increased the side effects; such as cardiovascular disease.

Unmet needs: design a new agonist of PPARγ with decreased side effect.

Kanwal. et al., *Biomedicines.* 10 (2), 331. (2022)

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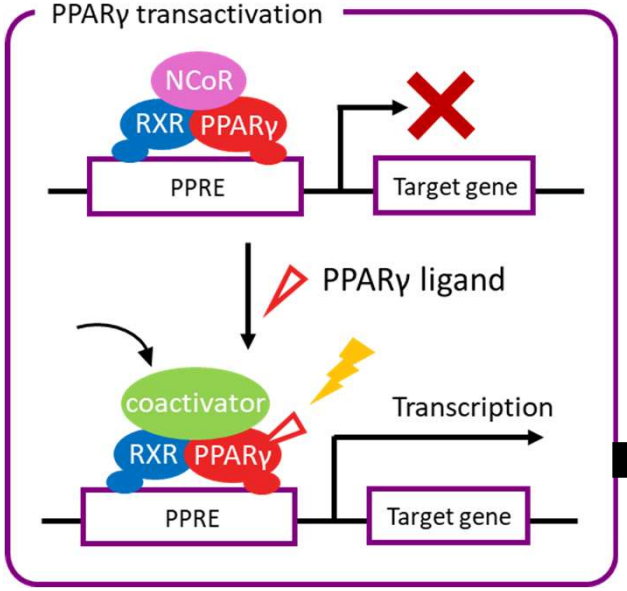
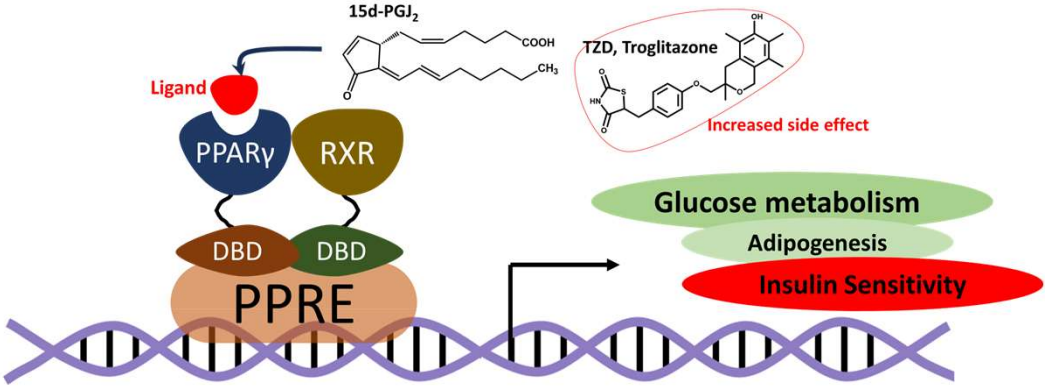
PPARγ is a ligand-activated transcription factor and belongs to the nuclear receptor superfamily. PPARγ controls the transcription of target genes by forming the heterodimer with the retinoid X receptor (RXR) and binding to specific PPAR response elements (PPREs) in the promoter region of target genes.



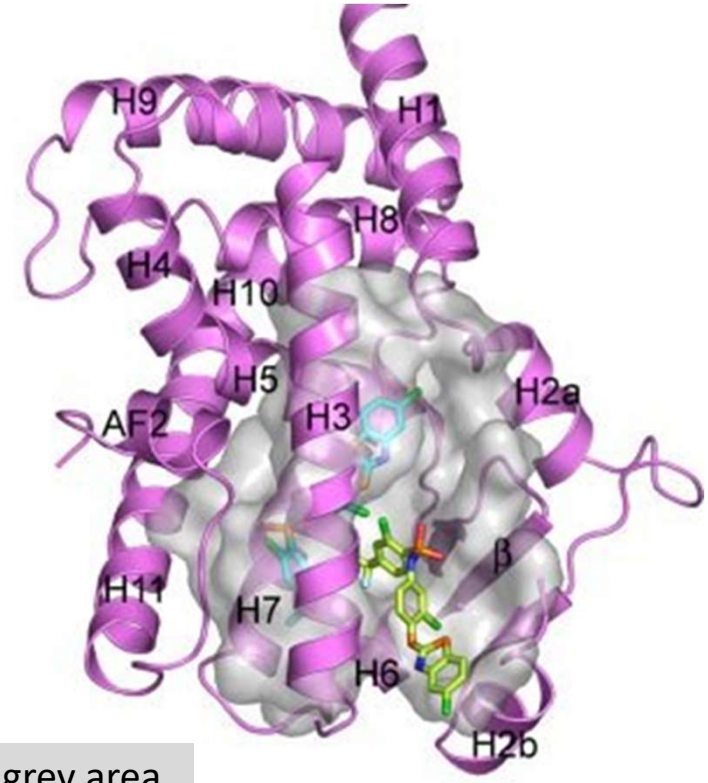


- Glucose homeostasis: ↑**GLUT4**, ↑CAP, etc
- Lipid metabolism: ↑LPL, ↑aP2, etc
- Adipokine regulation: ↑**adiponectin**, ↑FGF1, etc

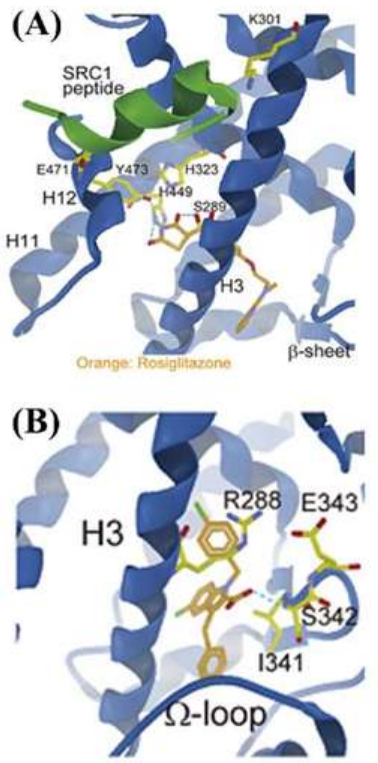
→ Improve insulin sensitivity

Chandra, V. et al., *Nature.* 456, 35-356, (2008); Ipjenberg, A. et al., *J. Biol. Chem.* 272, 20108-20117, (1997).

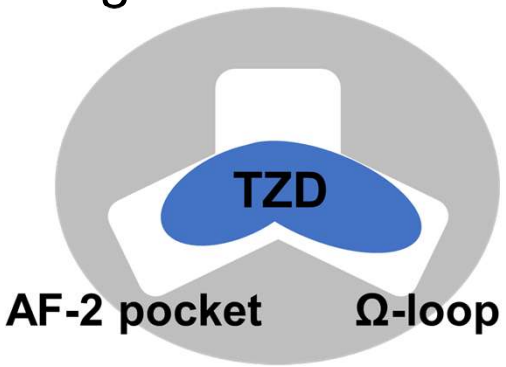
Understanding PPARγ LBP to design new agonist



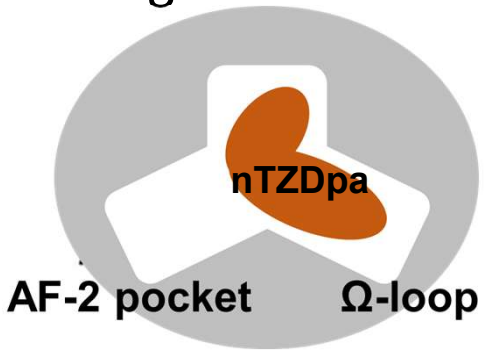
LBP: grey area



Full Agonist



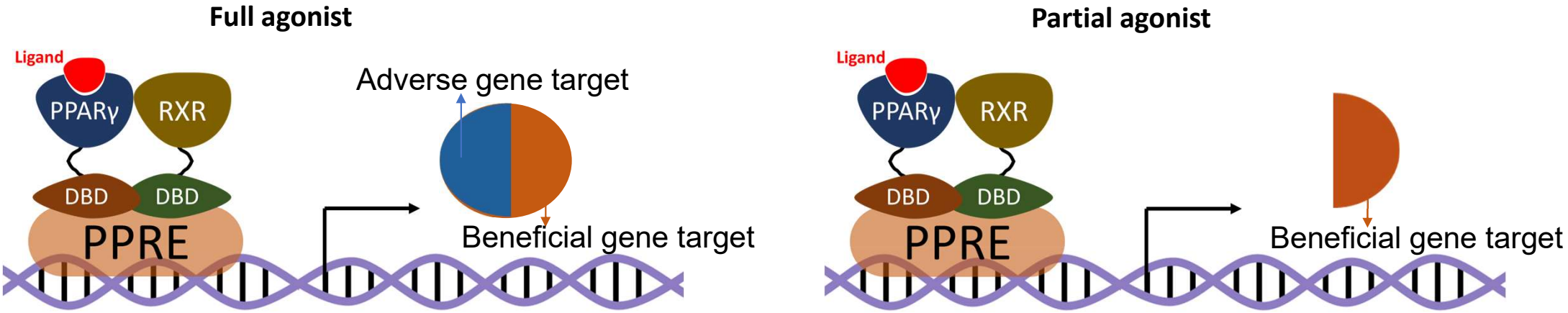
Partial Agonist



Omitting the interaction of AF-2 pocket could design partial agonist.

Li, Y., et al. J. Biol. Chem., 283,14, 9168 – 9176 (2008); Miyamae, Y., *Biol. Pharm. Bull.* 44, 1185–1195 (2021)

Partial agonist of PPARγ



Selective PPARγ Modulator

- **Binding in distinct manner to PPARγ LBP**
- **Resulting in SELECTIVE gene expression**

Discovery of new PPARγ partial agonist is a great of concern

Berger, J.P. et al., *Mol. Endocrinol.,* 17, 662–676 (2003); Miyamae, Y., *Biol. Pharm. Bull.* 44, 1185–1195 (2021)

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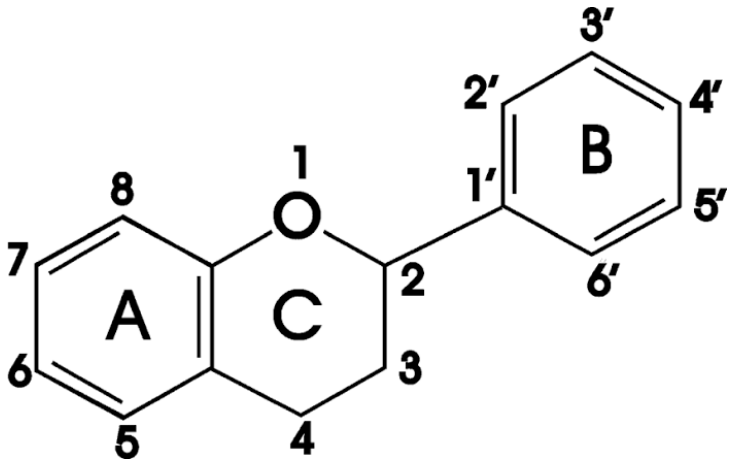
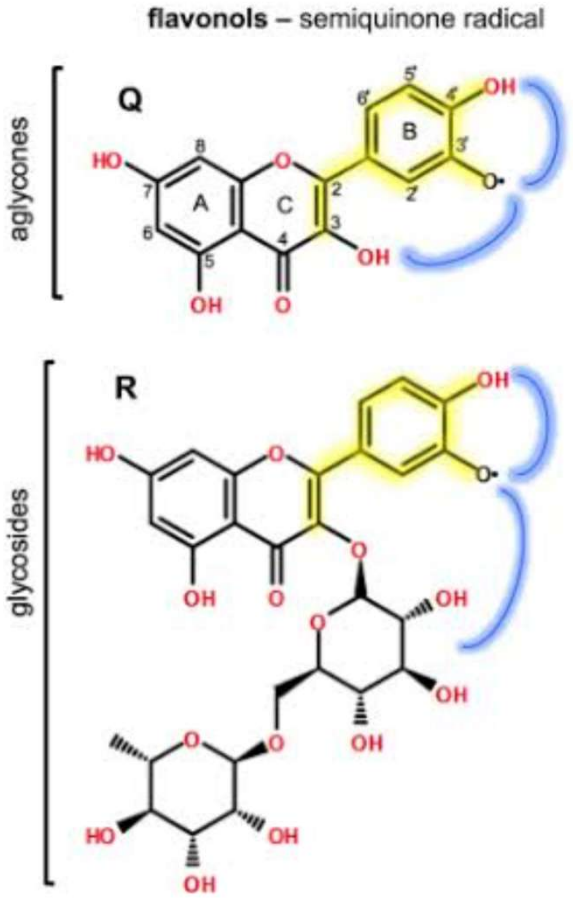
Flavonoid, natural compounds isolated from medicinal plants

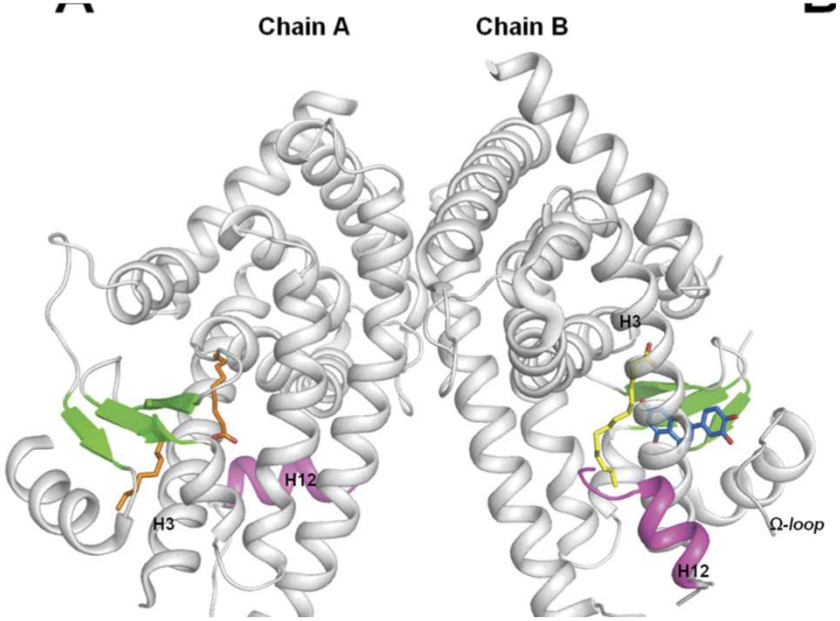




Flavonoid is commonly isolated from plants with the core structure attached.
Based on the specific structure, it can be categorised into many groups.
One of the group is flavonoid glycoside with a glucose structure and it can be hydrolysed into aglycone structure.
It has many biological activities, such as anti-oxidant, antiinflammation and antidiabetic.

Baranowska et al., Scientific reports, 12282 (2021)

Luteolin bind to PPARγ and acted like partial agonist.



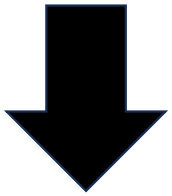
Luteolin can bind to PPARγ without stabilisation of H-12 which related to full agonist and side effect.

It can be categorized as **partial agonist.**

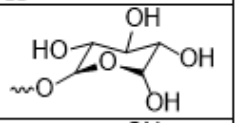
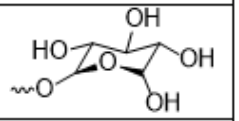
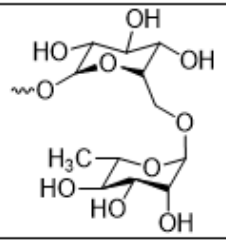
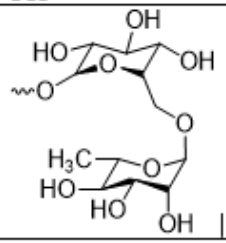
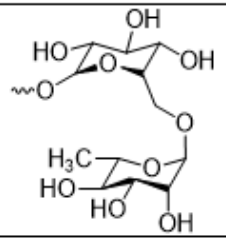
Ana et al., Mol. Pharmacol., 81:788-799 (2012)

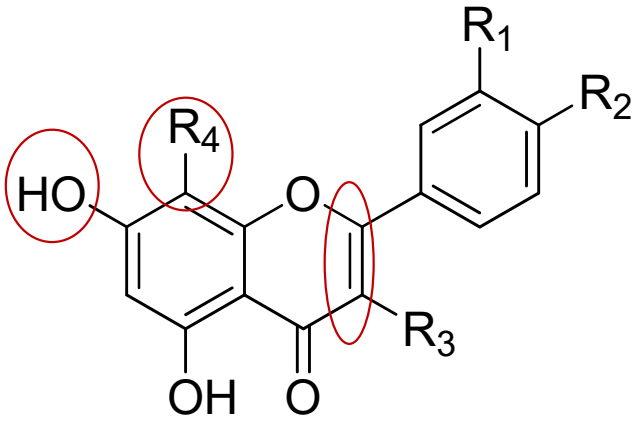
Research aim

To screen flavonoid glycoside and its aglycone as PPARγ modulator by molecular docking

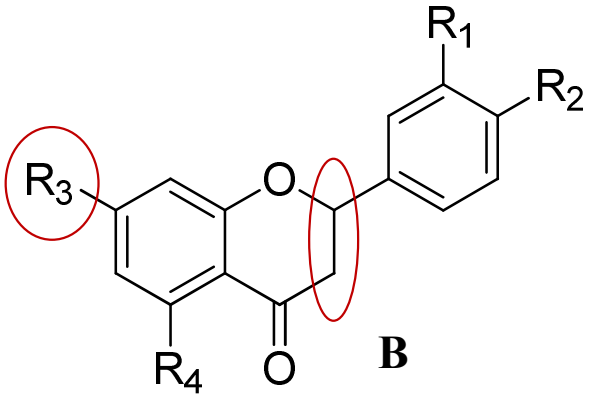


Pyrx Software was used for molecular docking using PPARγ protein and flavonoid compounds as ligand.

Structure **A**					
No	Compounds name	R_1	R_2	R_3	R_4
1	Luteolin	OH	OH	H	H
2	Apigenin	H	OH	H	H
3	Quersetin	OH	OH	OH	H
4	Orientin	OH	OH	H	
5	Vitexin	H	OH	H	
6	Rutin	OH	OH		OH
Structure **B**					
7	Naringenin	H	OH	OH	OH
8	Naringin	H	OH		OH
9	Hesperetin	OH	OCH_3	OH	OH
10	Hesperidin	OH	OCH_3		OH



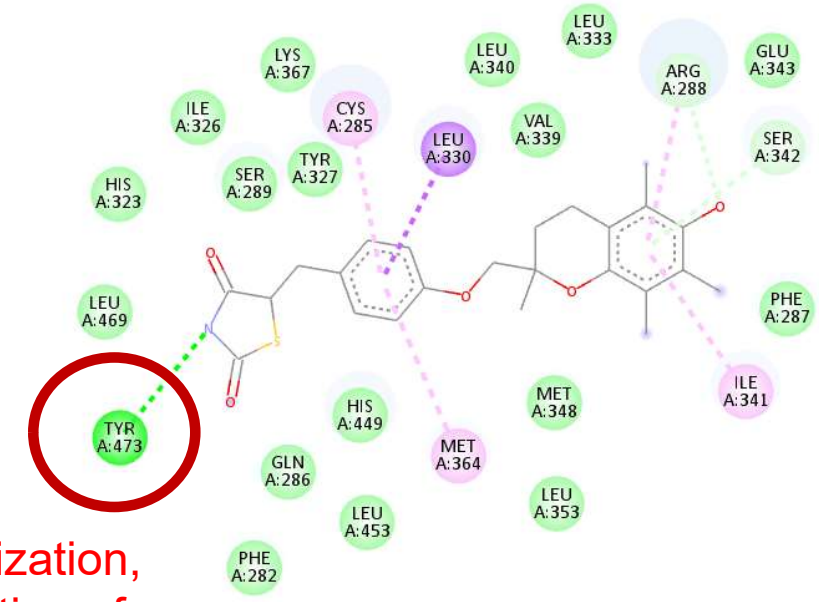
A



B

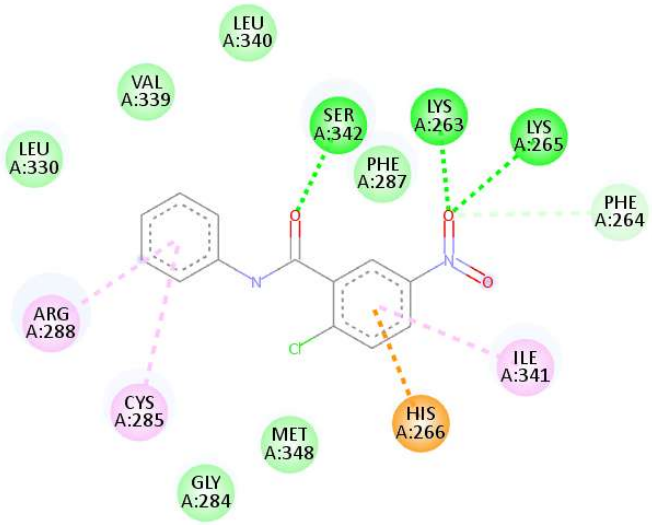
Troglitazone and GW9662 compounds as control

Full agonist



H12 stabilization, full activation of PPAR transcription

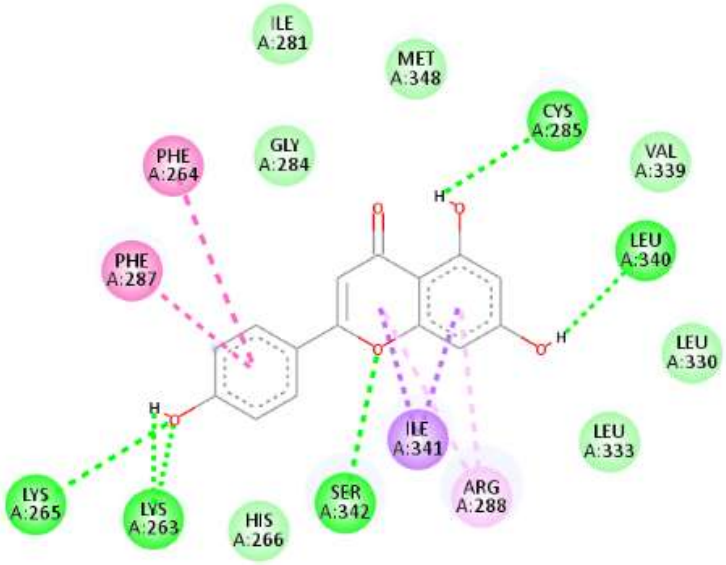
GW9662, covalent antagonist



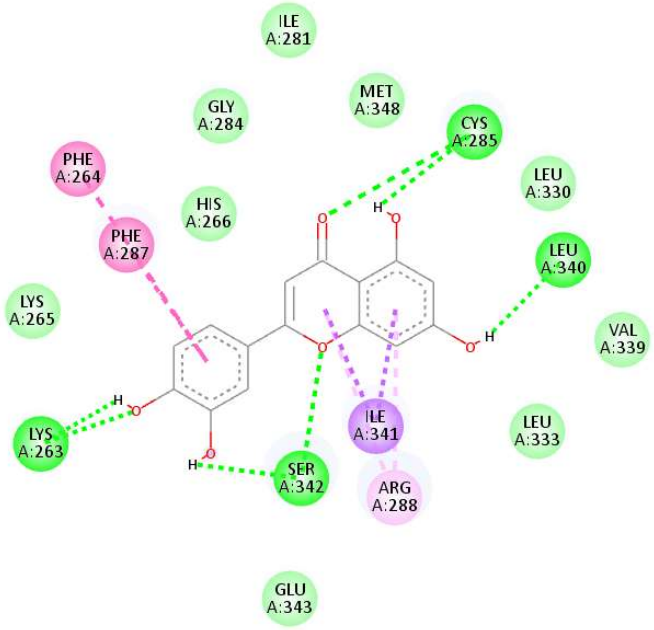
Covalent binding with Cys285

Aglycone compounds showed promising interaction

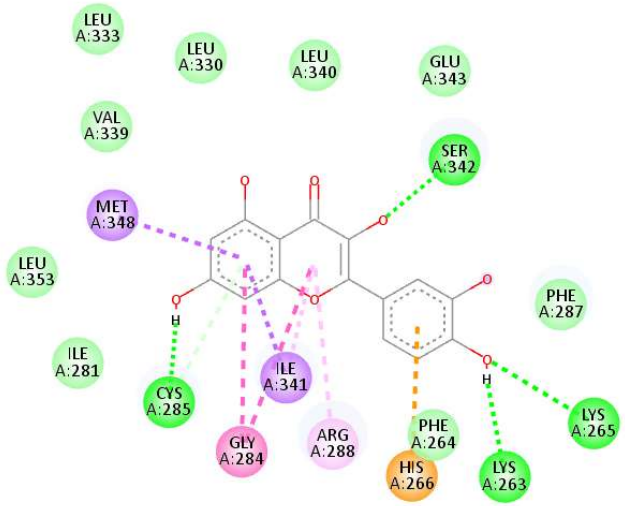
Luteolin



Apigenin

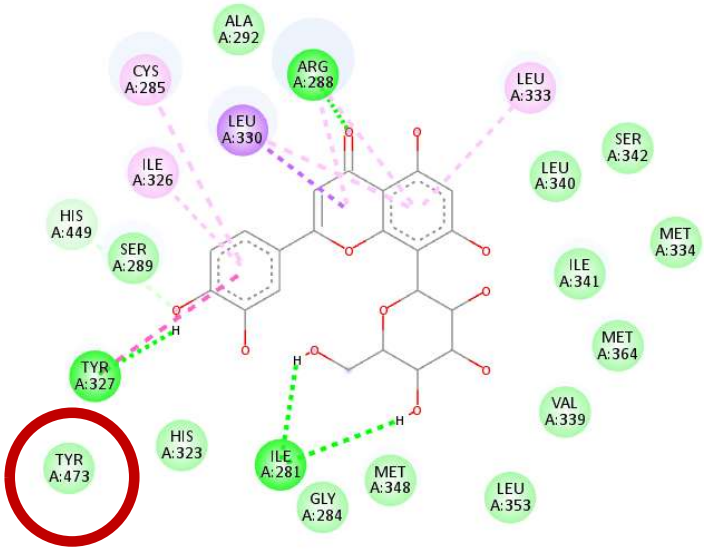


Quercetin



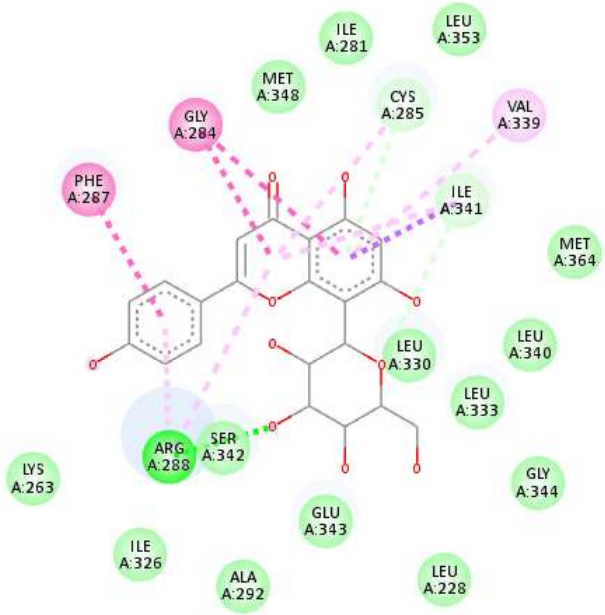
Flavonoid glycoside compounds showed less potent

Orientin

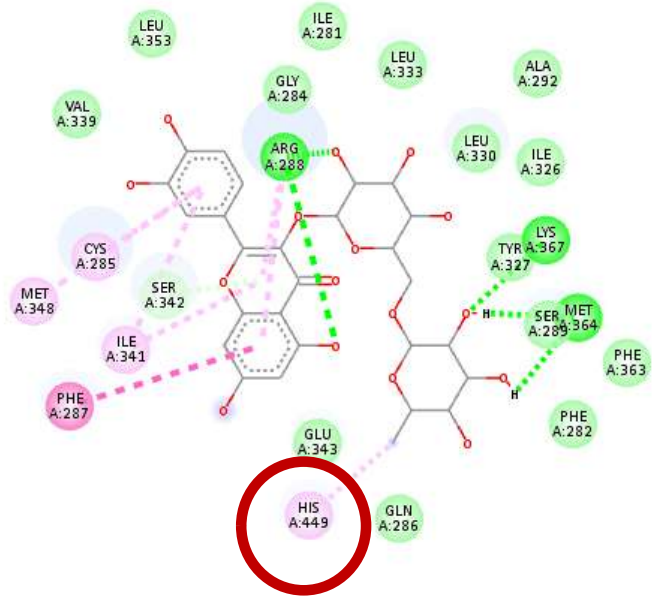


H12 stabilization, full activation of PPAR transcription

Vitexin

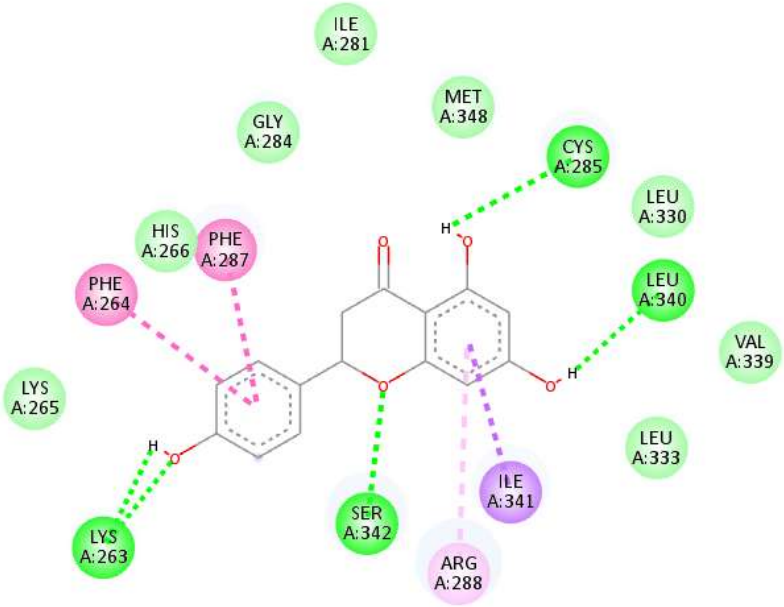


Rutin



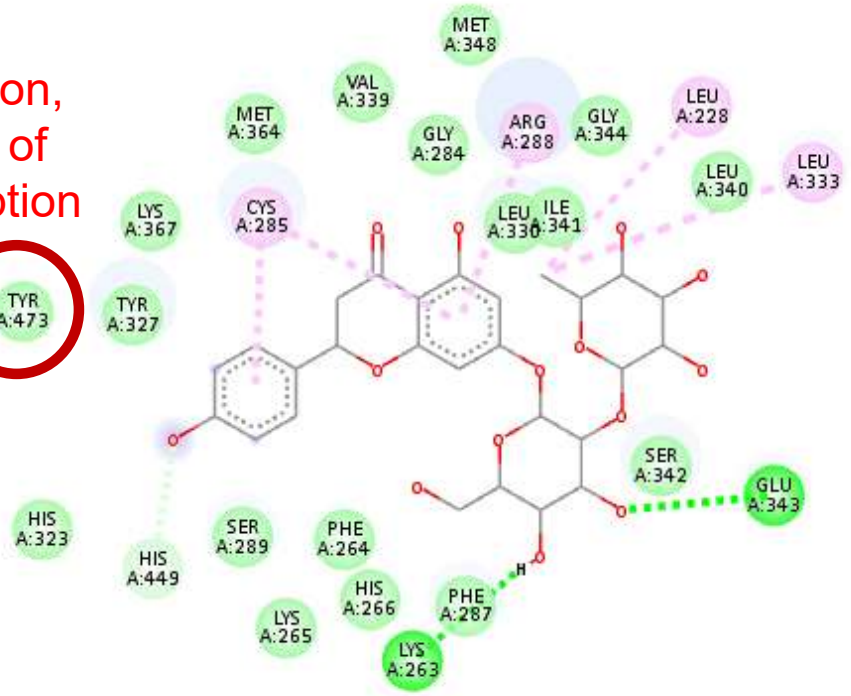
Naringenin and Naringin showed different binding mode

Naringenin



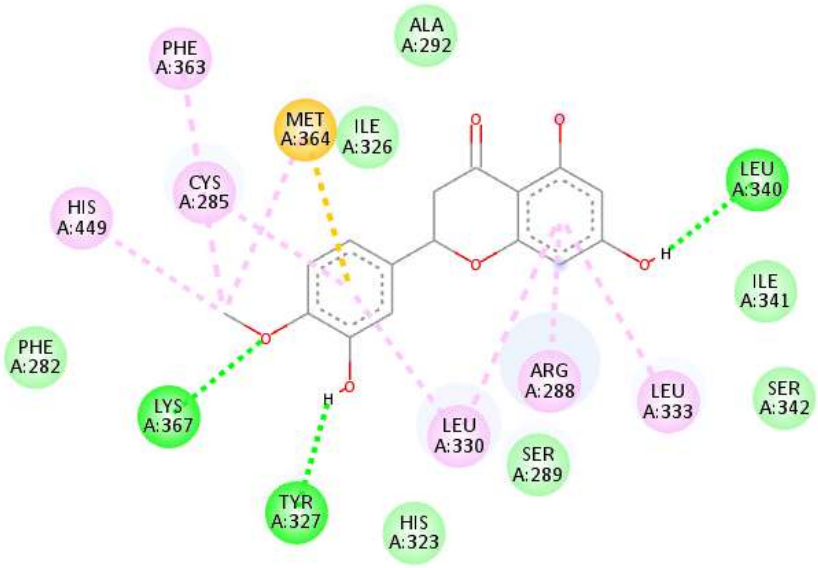
H12 stabilization, full activation of PPAR transcription

Naringin

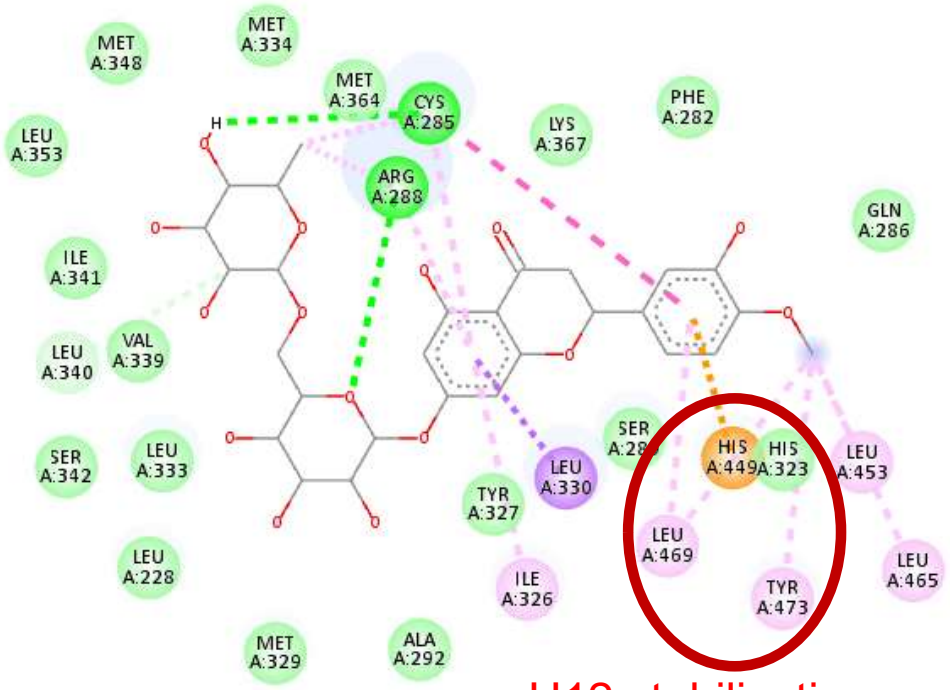


Hesperetin and Hesperidin showed different binding mode

Hesperetin

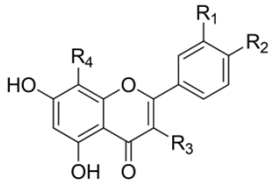


Hesperidin



H12 stabilization, full activation of PPAR transcription

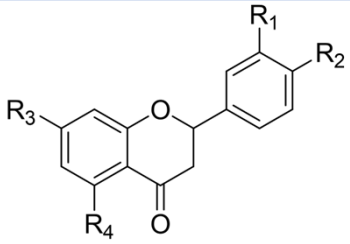
Aglycone compounds showed more potent activity.



A

No	Binding energy (kcal/mol)	Hydrogen bonds	π interactions				Van der waals/carbon hydrogen bond
			π- π	π-σ	π-alkyl	π-cation	
Tro	-9.68	Tyr473		Leu330	Cys285, Arg288, Met364, Ile341,		Leu469, His323, Ile326, Ser289, Tyr327, Lys367, Leu340, Val339, Leu333, Glu343, Phe287, Met348, Leu353, His449, Gln286, Leu453, Phe282
GW	-7.74	Cys285 (halogen)	Gly284		Leu255, Arg288, Ile341,	His266	Arg280. Glu259, Phe264, Phe287, Leu340, Ser342, Glu343
1	-7.55	Lys263, Cys285, Leu340, Ser342	Phe264, Phe287	Ile341,	Arg288		His266, Ile281, Gly284, Leu330, Leu333, Met348, Val339, Glu343
2	-7.58	Lys265, Lys263, Cys285, Leu340, Ser342	Phe264, Phe287	Ile341,	Arg288		His266, Ile281, Gly284, Leu330, Leu333, Met348, Val339
3	-6.18	Lys263, Lys265, Cys285	Gly284	Ile341, Met348	Arg288	His266	Val339, Leu333, Leu330, Leu340, Glu343, Phe287, Phe264, Ile281, Leu353
4	-6.13	Tyr327, Ala292, Arg288, Ile281		Leu330	Cys285, Ile326, Leu333		Tyr473, His323, His449, Ala292, Leu340, Ser342, Met334, Ile341, Met364, Val339, Leu353, Met348, Gly284
5	-6.23	Arg288, Cys285	Phe287, Gly284	Ile341	Val339		Met348, Ile281, Leu353, Met364, Leu340, Leu333, Leu330, Gly344, Leu228, Glu343, Ala292, Ser342, Ile326, Lys263
6	-1.33	Arg288, Lys367, Met364	Phe287	Cys285	Met348, Ile341, His449		Val339, Leu353, Gly284, Ile281, Leu333, Leu330, Ala292, Tyr327, Ser289, Phe363, Phe282, Gln 286, Glu343

Aglycone compounds showed more potent activity.



B

No	Binding energy (kcal/mol)	Hydrogen bonds	π interactions				Van der waals/carbon hydrogen bond
			π- π	π-σ	π-alkyl	π-cation	
Tro	-9.68	Tyr473		Leu330	Cys285, Arg288, Met364, Ile341,		Leu469, His323, Ile326, Ser289, Tyr327, Lys367, Leu340, Val339, Leu333, Glu343, Phe287, Met348, Leu353, His449, Gln286, Leu453, Phe282
GW	-7.74	Cys285 (halogen)	Gly284		Leu255, Arg288, Ile341,	His266	Arg280, Glu259, Phe264, Phe287, Leu340, Ser342, Glu343
7	-7.55	Cys285, Leu340, Ser342, Lys263	Phe287, Phe264	Ile341	Arg288		Lys265, His266, Gly284, Ile281, Met348, Leu330, Val349, Leu333
8	-1.63	Glu343, Lys263			Cys285, Arg288, Leu228, Leu333		Tyr473, Tyr327, Lys367, Met364, Va;339, Gly284, Met348, Leu330, Ile341, Gly344, Leu340, Ser342, Phe287, His266, Phe264, Lys265, Ser289, His449, His323
9	-6.92	Lys367, Leu340, Tyr327			Phe363, Cys285, His449, Leu330, Arg288, Leu330	Met364	Ile326, Ala292, Ile341, Ser342, Ser289, His323, Phe282
10	-3,28	Arg288	Cys285	Leu330	Leu453, Leu465, Tyr473, Leu469, Ile326	His449	Ile341, Leu353, Met348, Met334, Met364, Lys367, Phe282, His323, Ser289, Tyr327, Ala292, Met329, Leu333, Leu228, Ser342, Val339, Leu340

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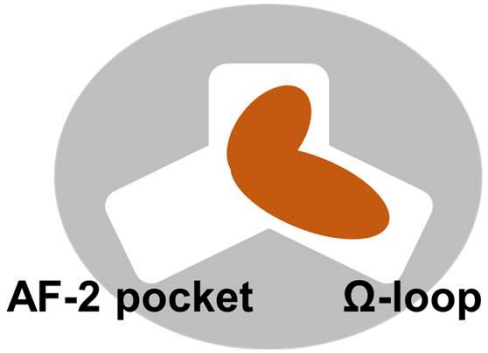
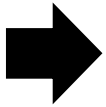
Establishment of a ligand-linking strategy

Partial Agonist

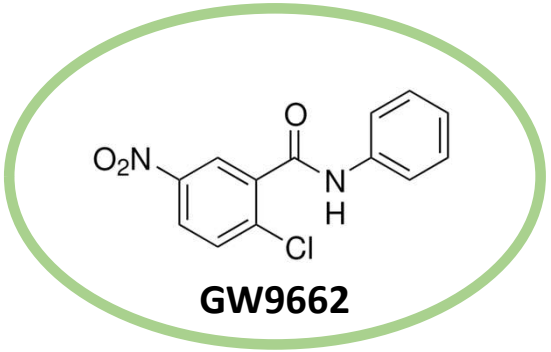


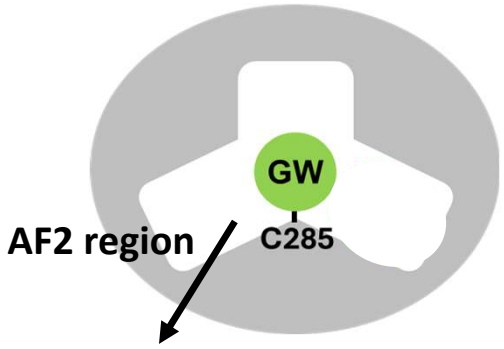


- Idea: Multiple ligands
- Omitting AF-2 region

Ohtera, A., et. al. *ACS. Chem. Biol*., 10, 2794-2804 (2015); Utsugi, Y., et al., *Molecules,* 24, 2019 (2019)

GW9662 as Anchor Structure





- Idea: Multiple ligands
- Omitting AF-2 region

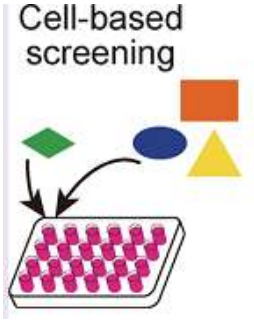
GW9662 is PPARγ antagonist, irreversibly binds to Cys285. The irreversible binding able to block AF-2 region occupation.

Blocking AF2 region

Purpose: design covalent partial agonist, effective with less dose.

Ohtera, A., et. al. *ACS. Chem. Biol*., 10, 2794-2804 (2015); Utsugi, Y., et al., *Molecules,* 24, 2019 (2019)

Screening partner ligand from natural product library



125 plant extracts

↓ luc assay

Kaempferia galanga L.

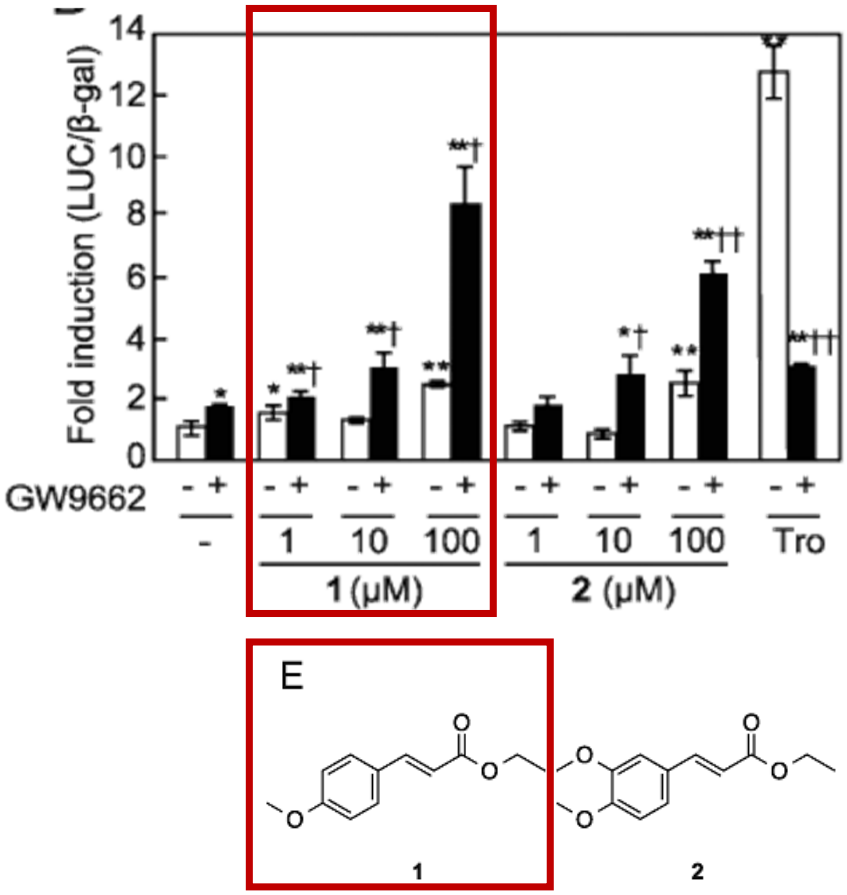
↓ bioassay-guided fractionation

1, **2**

CAEE (cinnamic acid ethyl ester)

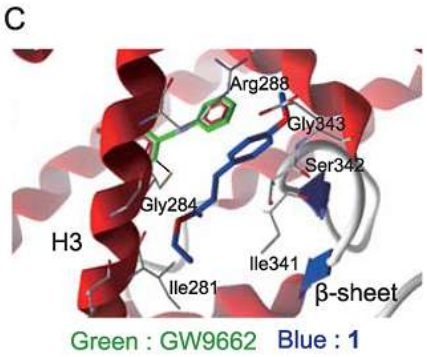
↓

Isolated compound 1 and 2 co-treated with GW9662 to see cooperative activation of PPARγ transcription

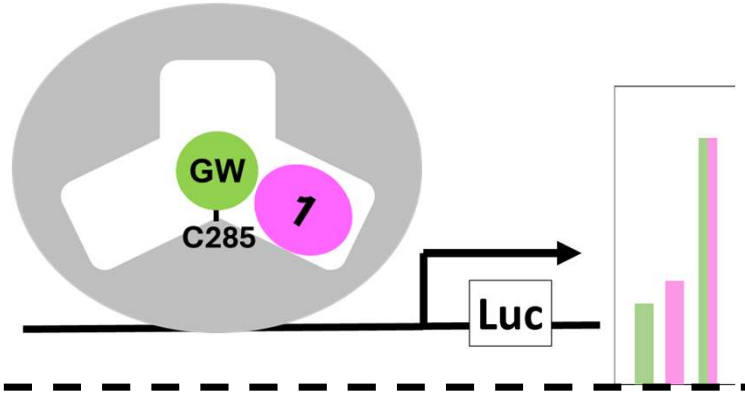


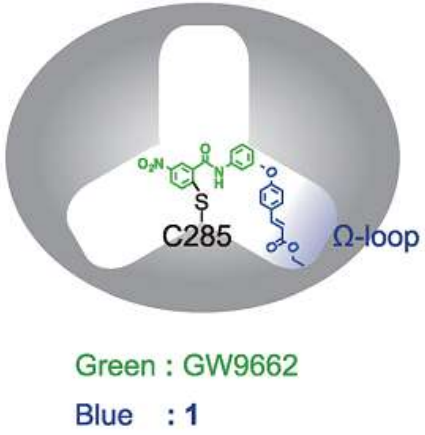
Ohtera, A., et. al. *ACS. Chem. Biol*., 10, 2794-2804 (2015)

Design new covalent agonist of PPARγ

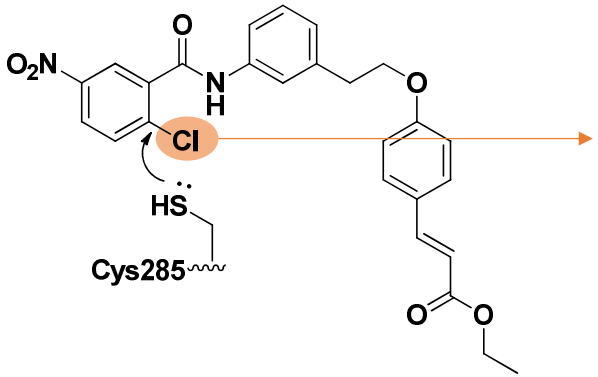


GW9662 and **1** showed cooperative activation





New Hybrid compounds



2A

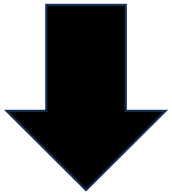
Important for agonist activity.
Covalent binding with Cys285.

Changing from Cl to H, diminish the agonist activity.

Ohtera, A., et. al. *ACS. Chem. Biol.*, 10, 2794-2804 (2015)

Research aim

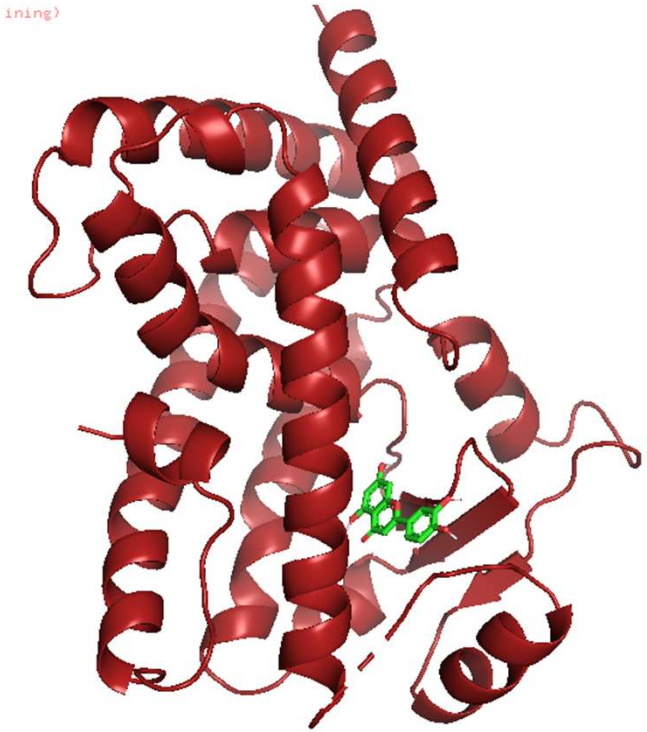
To screen aglycone compound which can paired with GW9662 for design PPARγ modulator



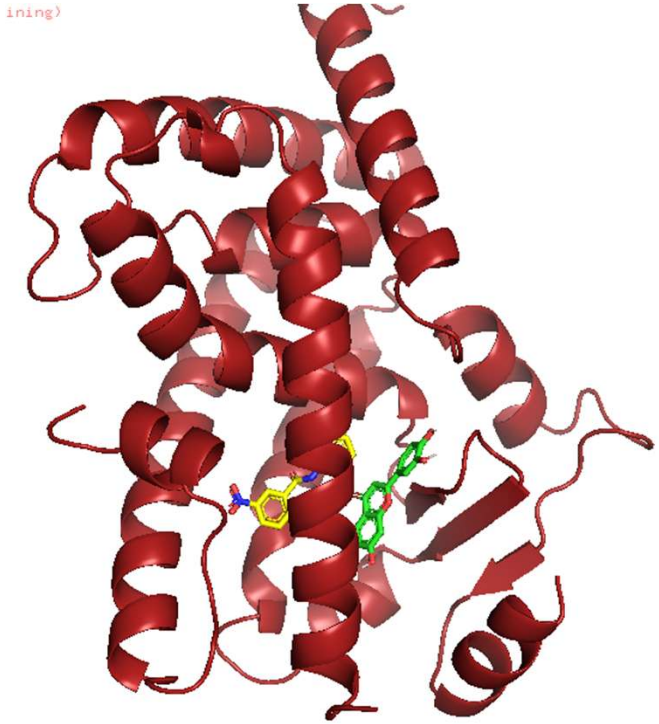
Pyrx Software was used for molecular docking using PPARγ protein with GW9662 and flavonoid compounds as ligand.

GW9662 and Luteolin bind with different manner

Luteolin

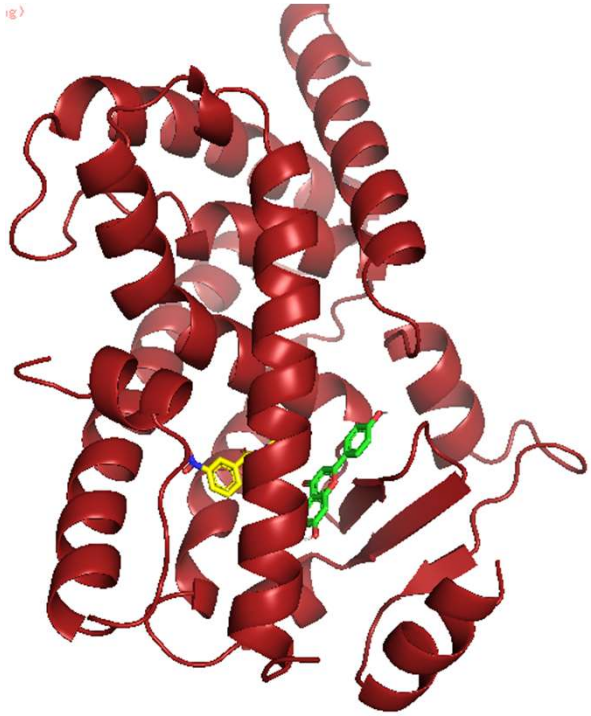


GW9662 and Luteolin



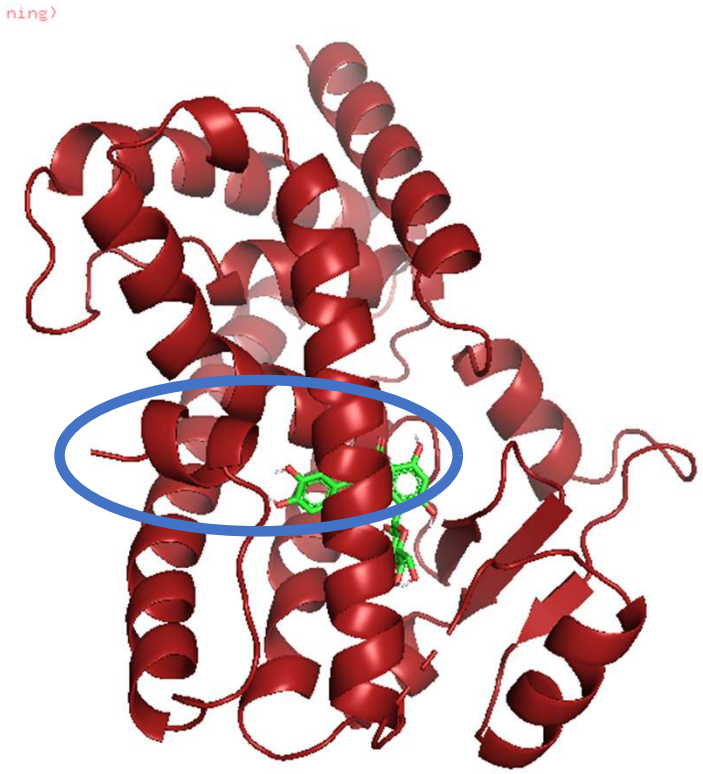
Yellow: GW9662
Green: flavonoid

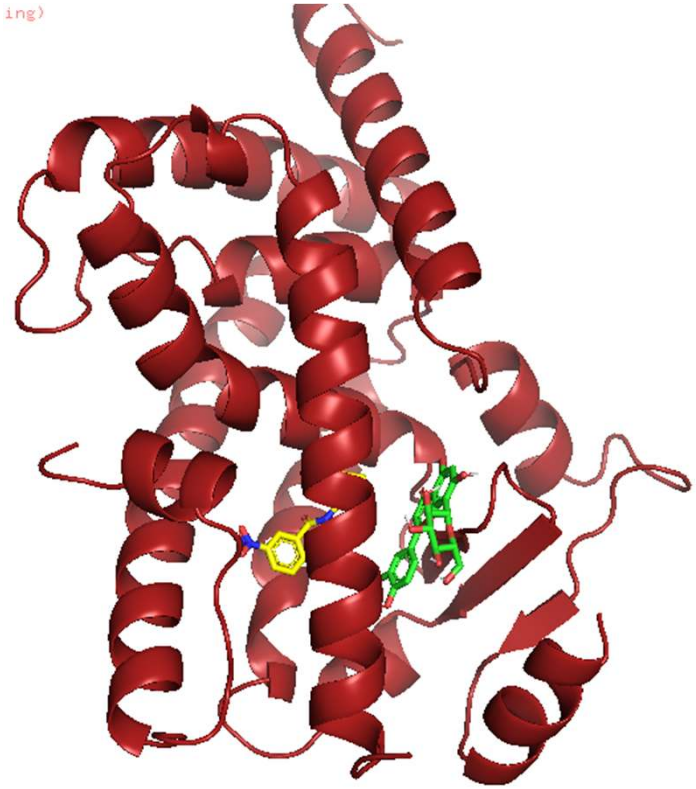
GW9662 and Apigenin



GW9662 and Orientin bind with different manner but Orientin closely interact with H-3

Yellow: GW9662
Green: flavonoid

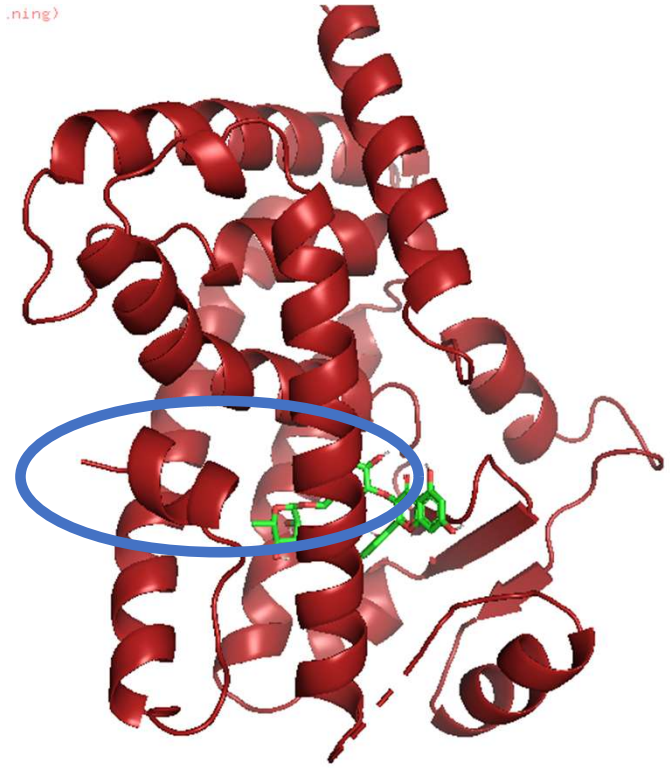


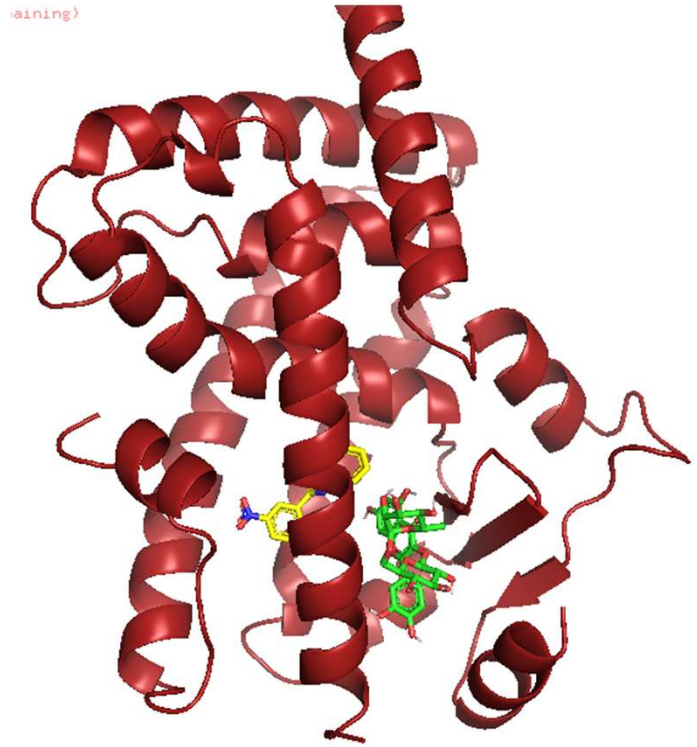


Orientin: One unit of glucose

GW9662 and Rutin bind with different manner but Rutin closely interact with H-3

Yellow: GW9662
Green: flavonoid





Rutin: Two unit of glucose

Conclusion

1. Aglycone compounds showed potent activity compared with flavonoid glycoside, with Luteolin and Apigenin the most potent.
2. The hydroxyl group of aglycone compounds is important for hydrogen bond interaction with PPARγ.
3. Tyr473 correlated with stabilization of Helix 12 and most related to the side effect of full agonist of PPARγ, Flavonoid glycoside showed interaction with Tyr473 and Aglycone compounds were binding to PPARγ with no occupation at AF-2 region.
4. More glucose unit attached to the flavonoid resulted in a similar manner of binding with full agonist of PPARγ.
5. Aglycone compounds might be used for partner ligand with GW9662 to discover new PPARγ modulators.

THANK YOU