The 3rd Global Forum on Pharmaceutical and Health Science (GFPHS)
Saturday, July 12th 2025

Rational Design of PPARγ Modulators: A Molecular Approach to Antidiabetic Drug Discovery

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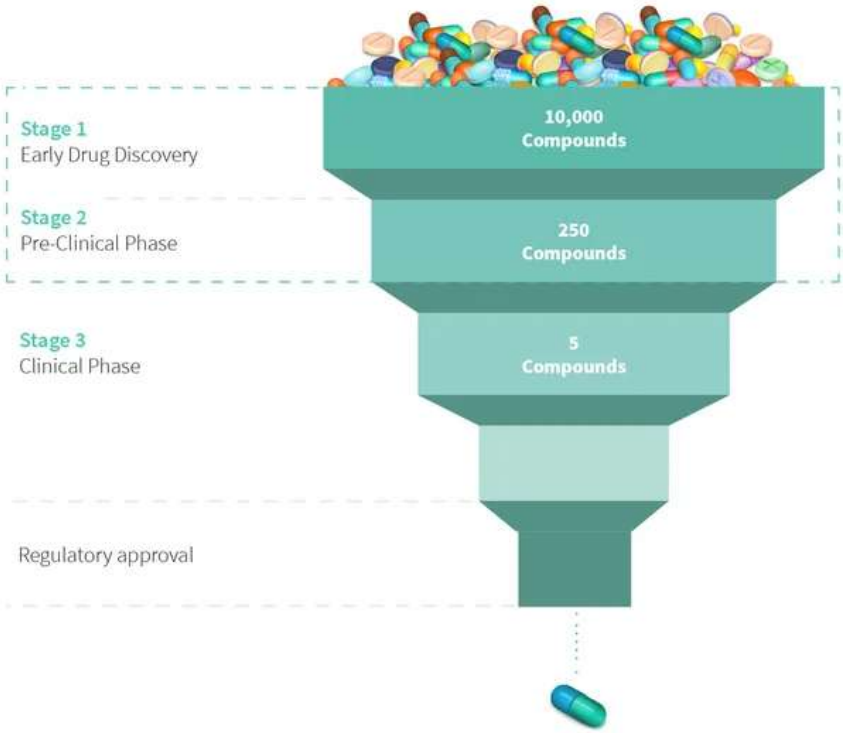
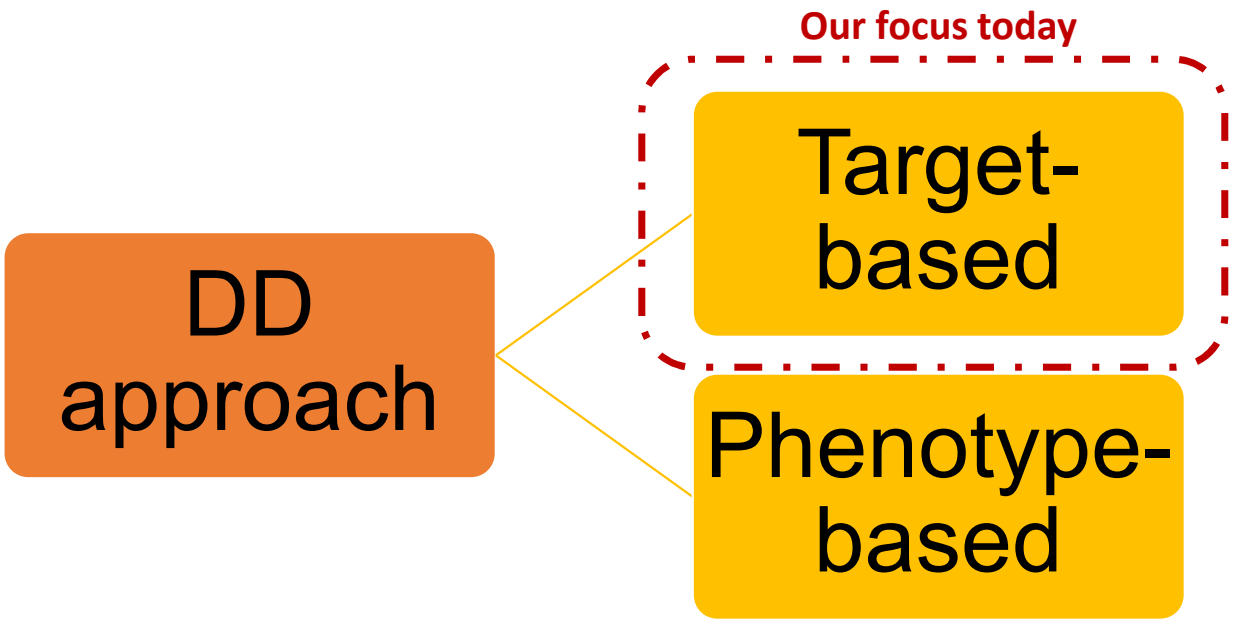
UAD Universitas Ahmad Dahlan

Drug discovery



Source picture: https://www.patheon.com/ Thermofisher Scientific

Drug discovery approach



Why?
Lower cost
Begins with known molecular target
Fast lead optimization process
Easier SAR (structure activity relationship)

Singh, V. K., et al. Expert Opinion on Drug Discovery, vol.14, 7, (2019).

Target-based Drug discovery

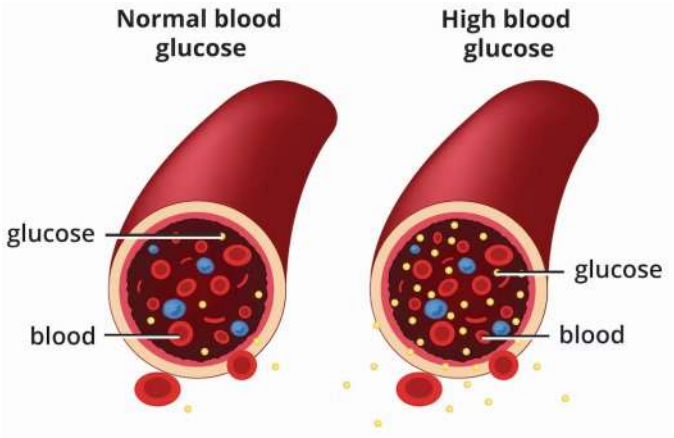
Outline

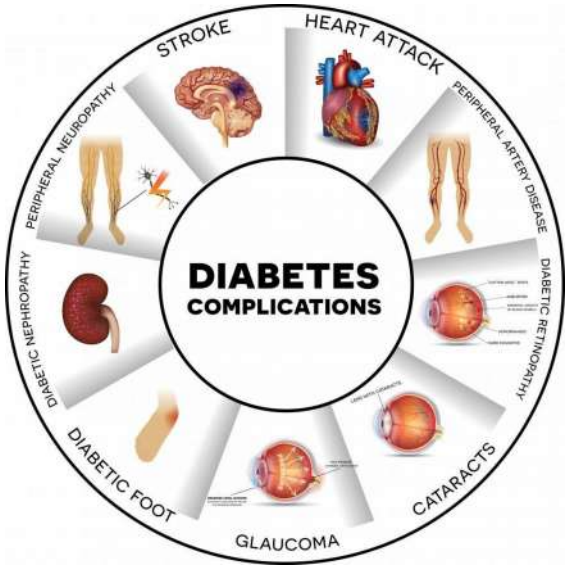
- **Diabetes Mellitus**
- PPARγ as Target Protein for T2DM
- Rational Design: Ligand Linking Strategy
- Screening System
- Synthesis of Agonist Candidates
- In vitro evaluation

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**





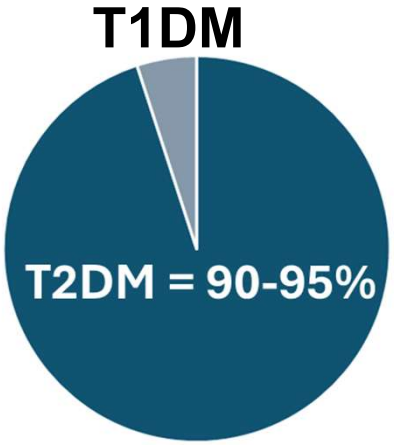
Sun, H. et al., *Diabetes Res. Clin. Pract.,*183, 109119 (2022); Antar, S.A. et al., *Biomed. Pharmacother;* 168, 115734 (*2023*)*;* Wu, Y. et al.*, Int. J. Med. Sci.* 11, 1185-1200 (2014).

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

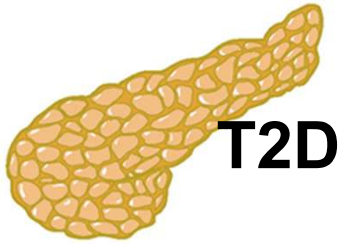


Optimal treatment of T2DM has been challenging to find.

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).

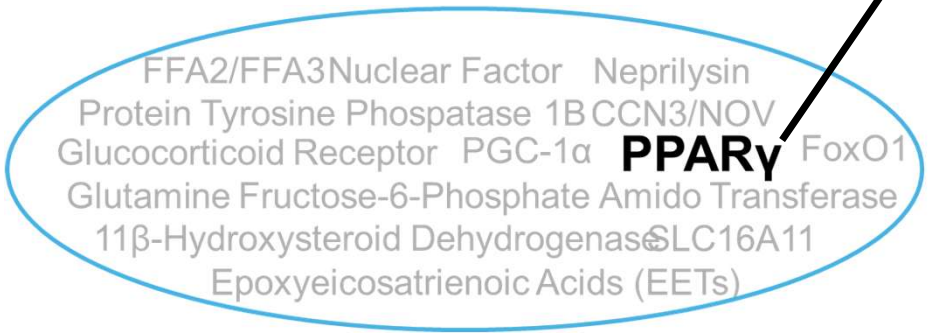
PPARγ is one of emerging target for T2DM



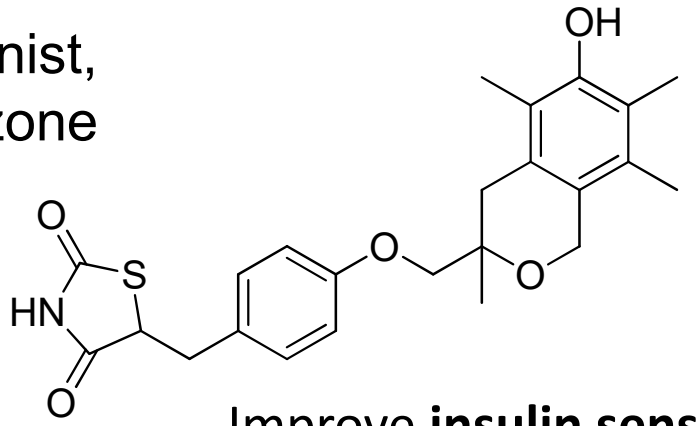
T2DM →

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.

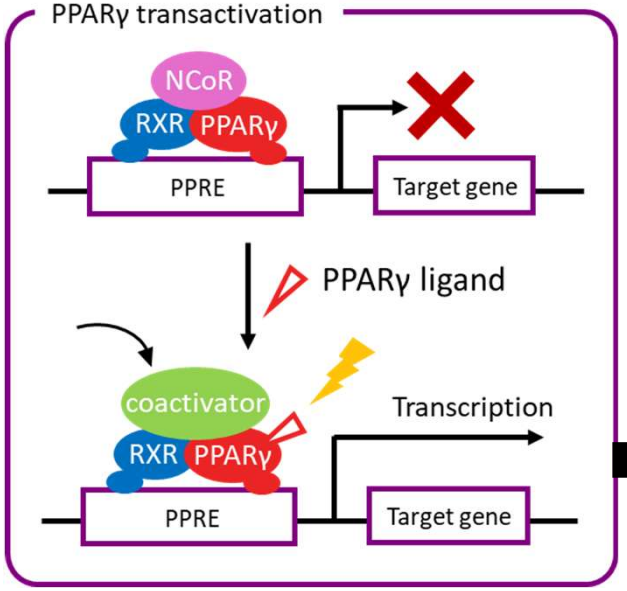
Unmet needs: design a new agonist of PPARγ with decreased side effect.

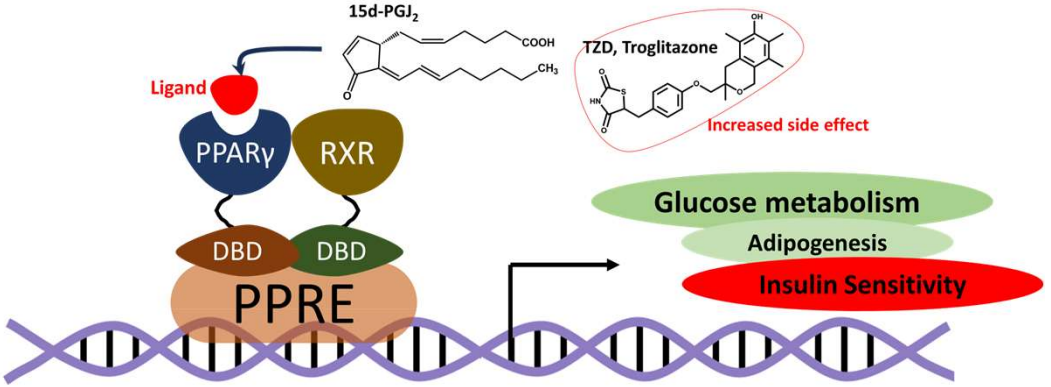
Kanwal. et al., *Biomedicines.* 10 (2), 331. (2022)

Outline

- Diabetes Mellitus
- **PPARγ as Target Protein for T2DM**
- Rational Design: Ligand Linking Strategy
- Screening System
- Synthesis of Agonist Candidates
- In vitro evaluation

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.



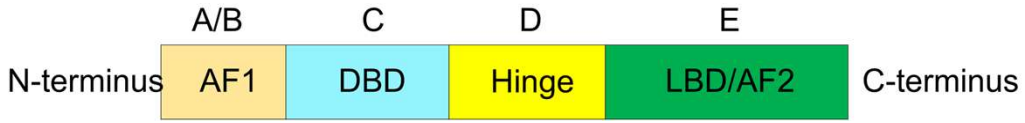


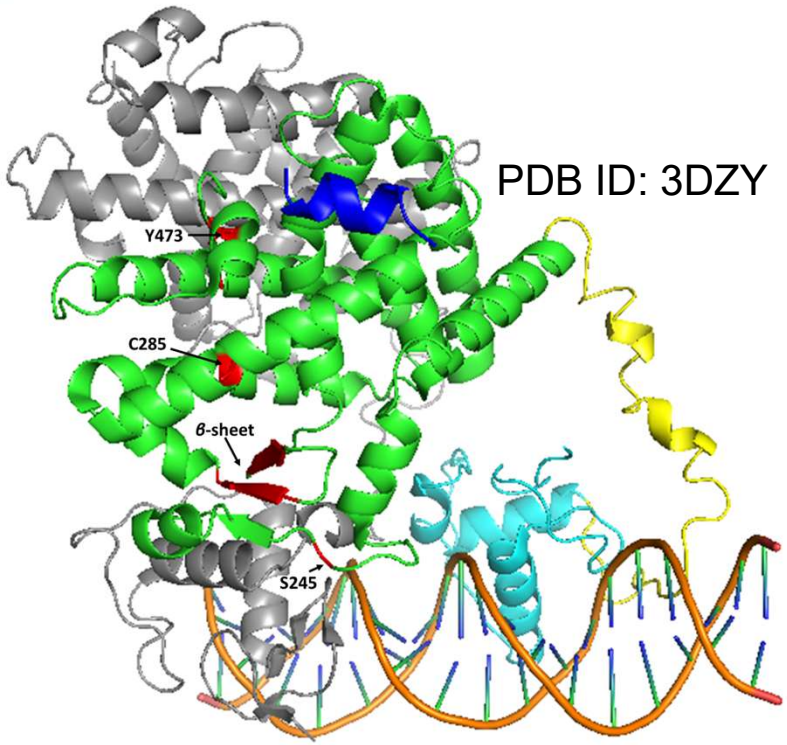
- Glucose homeostasis: ↑**GLUT4**, ↑CAP, etc
- Lipid metabolism: ↑LPL, ↑aP2, etc
- Adipokine regulation: ↑**adiponectin**, ↑FGF1, etc

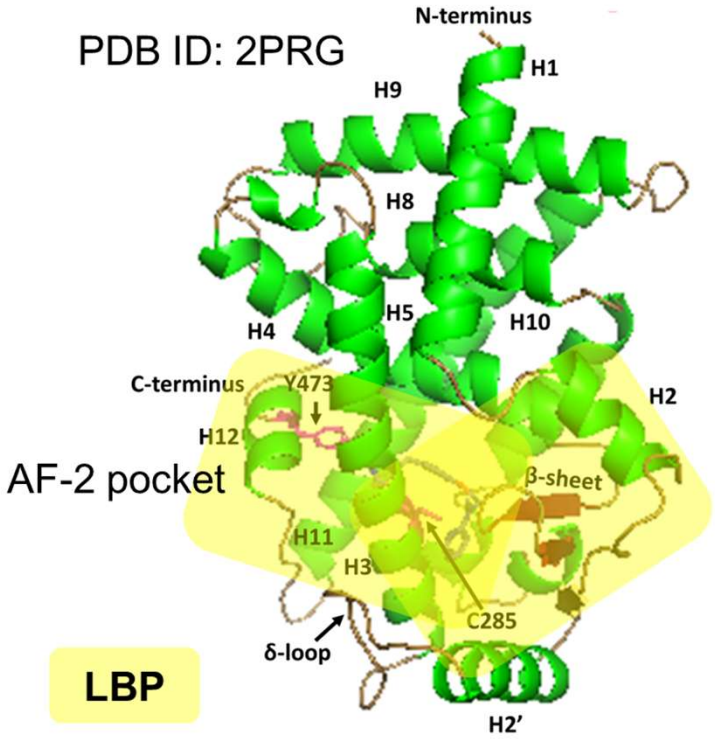
→ Improve insulin sensitivity

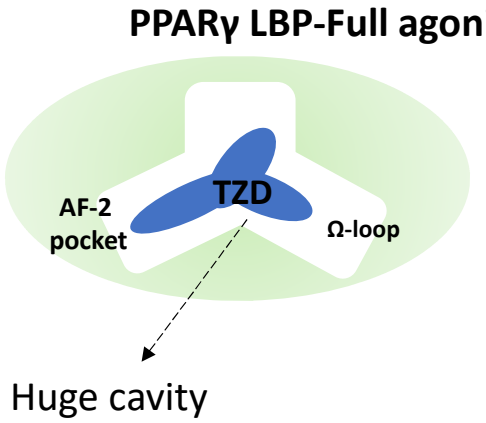
Chandra, V. et al., *Nature.* 456, 35-356, (2008); Ipjenberg, A. et al., *J. Biol. Chem.* 272, 20108-20117, (1997).

PPARγ structure and its LBP (ligand binding pocket)



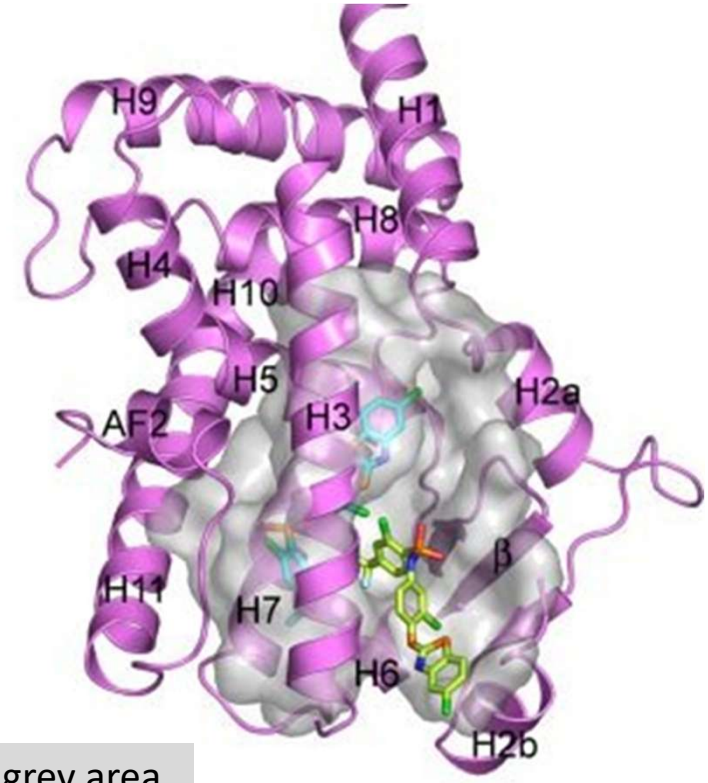




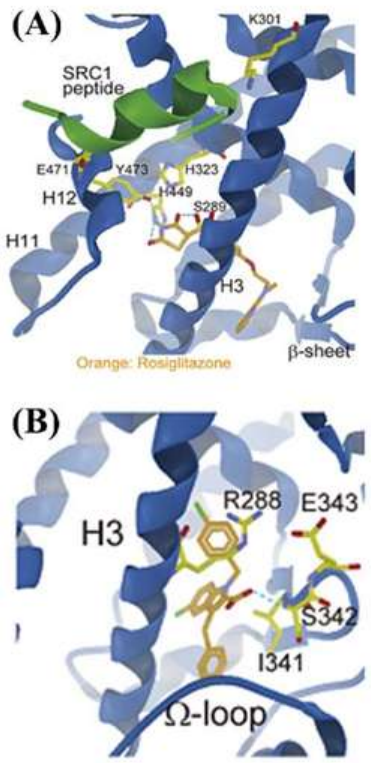


Chandra, V. et al., *Nature.* 456, 35-356, (2008); Ipjenberg, A. et al., *J. Biol. Chem.* 272, 20108-20117, (1997).

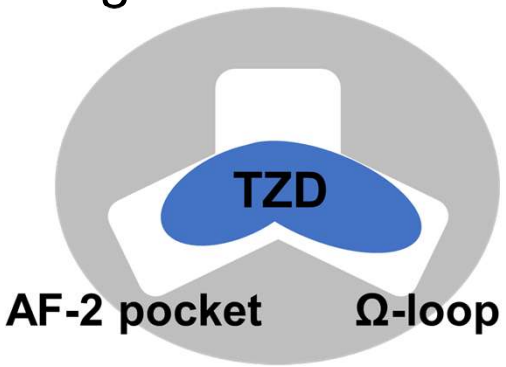
Understanding PPARγ LBP to design new agonist



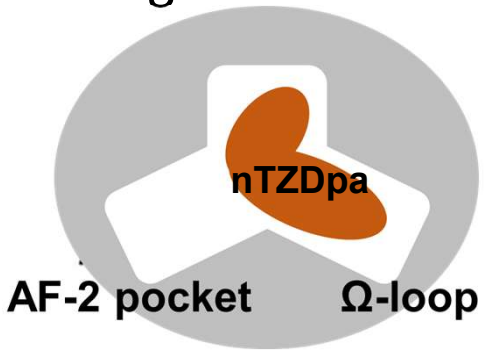
LBP: grey area



Full Agonist



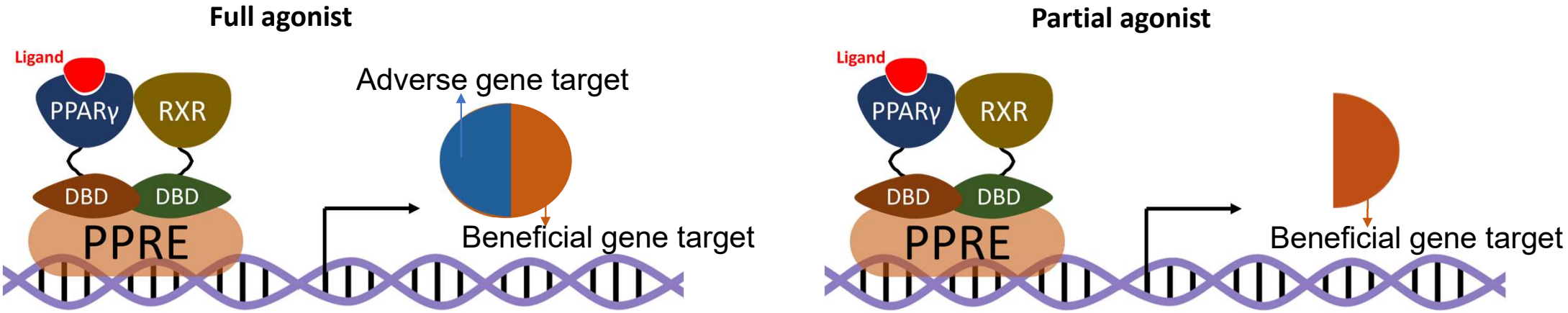
Partial Agonist



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)

Outline

- Diabetes Mellitus
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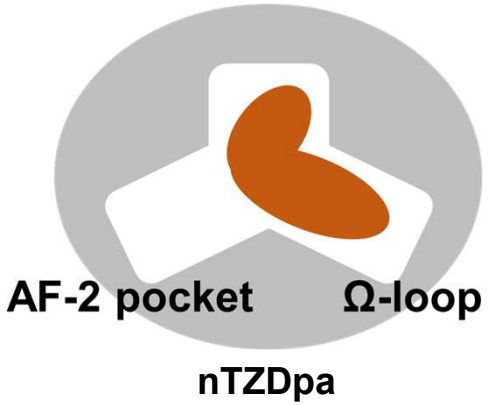
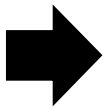
Establishment of a ligand-linking strategy

Partial Agonist

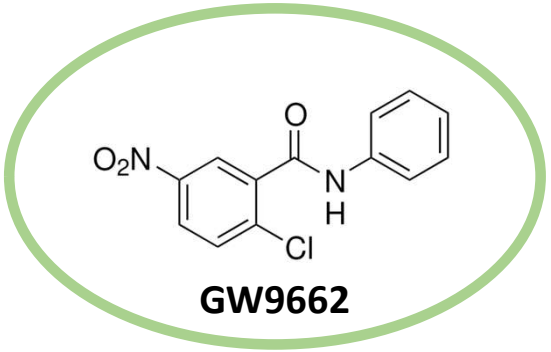


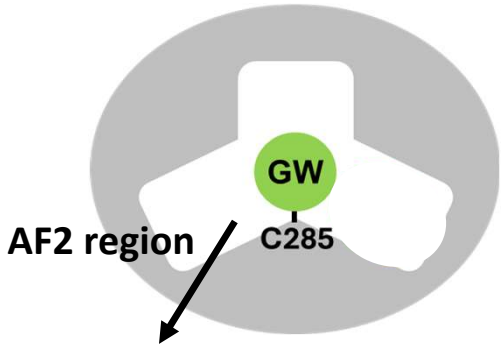


- Idea: Multiple ligands
- Omitting AF-2 region

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.

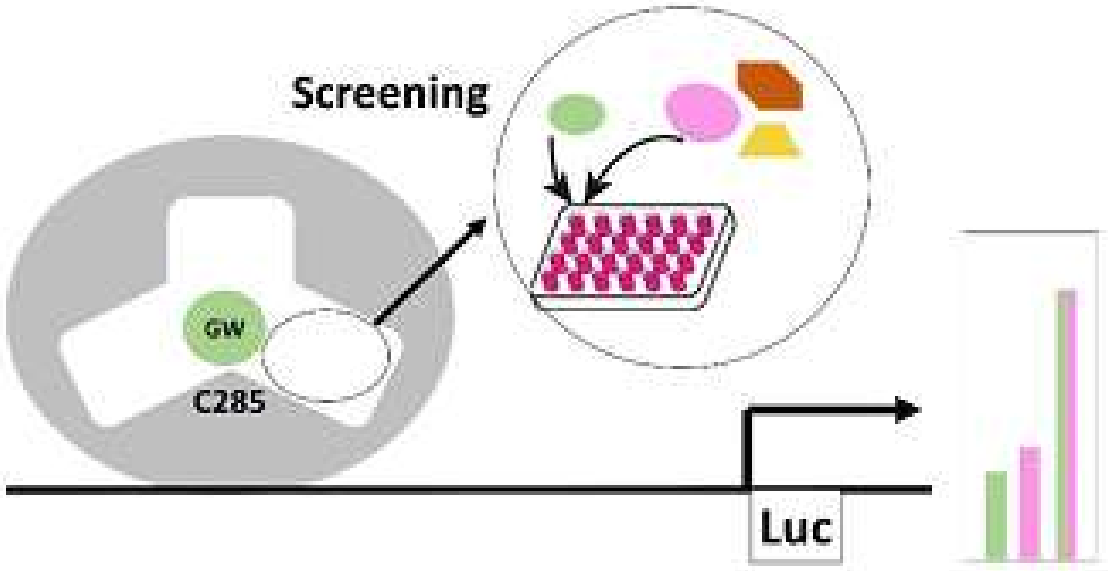
Blocking AF2 region

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)

Partner ligand of GW9662?

Partner ligand of GW9662 was decided on Cooperative activation on PPARγ



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

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Cell-based Assay Screening

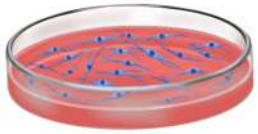
Conventional Assay

- Transfection cell was used. Limited number of compounds used.

High Throughput Screening

- Stable cell line. Thousand of compounds can be screened.

In vitro evaluation: conventional cell based assay

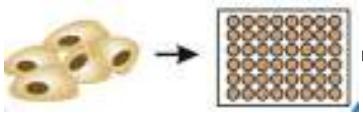


HepG2 cell line

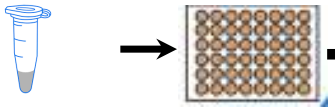
Transfection

DMEM (-),
pFN26A (BIND)-PPARγ2-LBD,
pGL4.35, pGL4.74, Hilymax

Incubate overnight



Seeding cells



Treatment of compounds

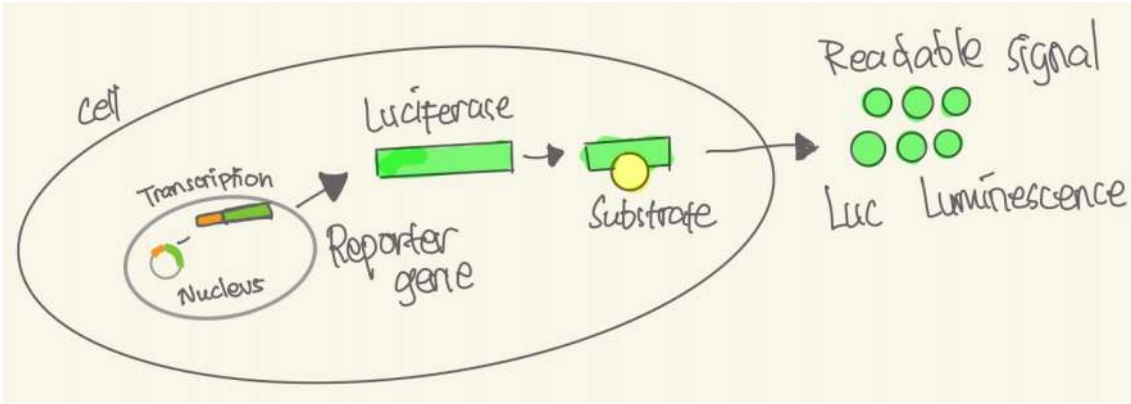
Incubate 6/12/24 h

Harvest - Cell lysis
Add luciferin reagent

Luciferase reporter assay principle

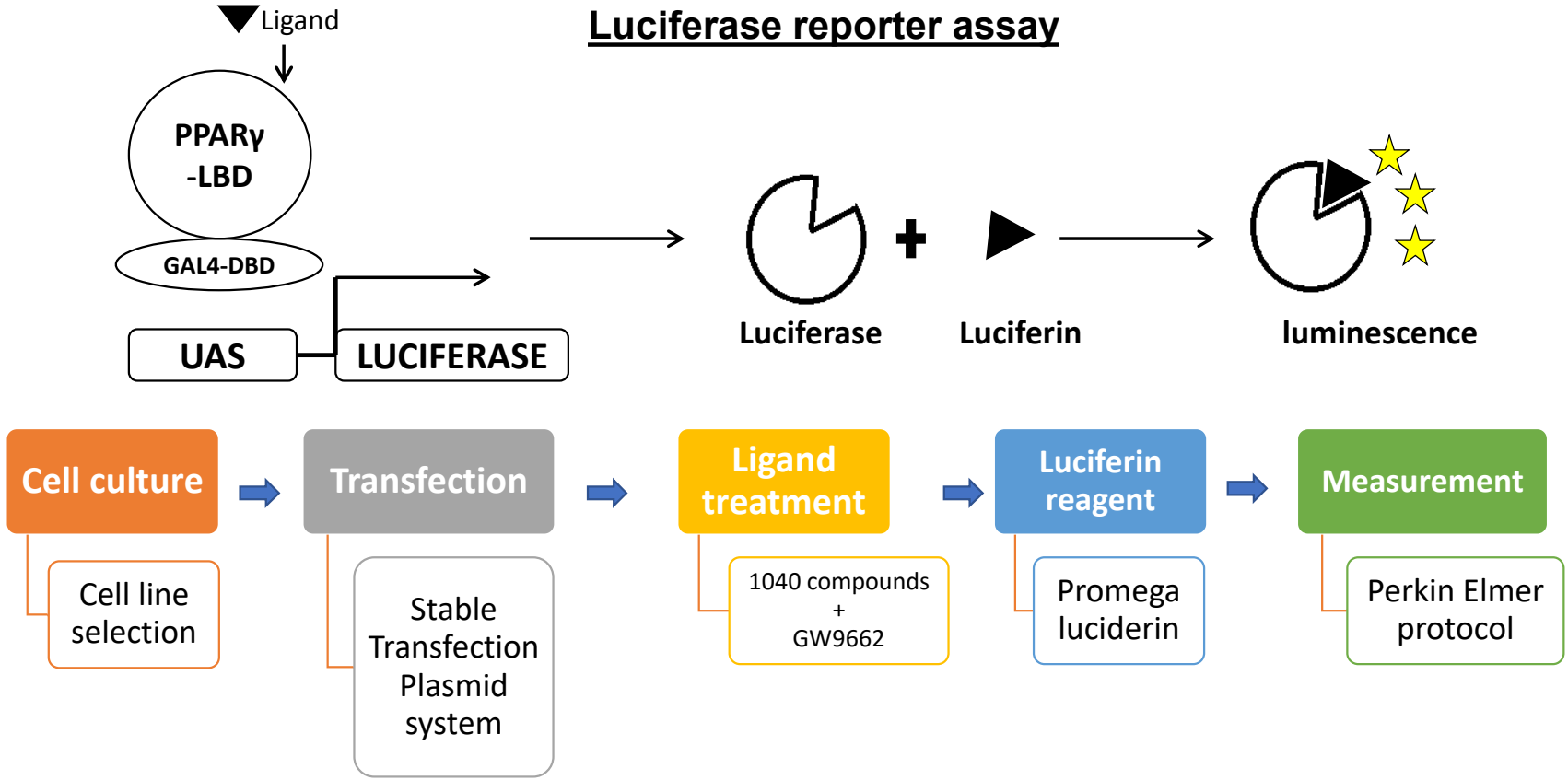
Reporter gene

Promoter | Luciferase



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

In vitro evaluation: stable cell line

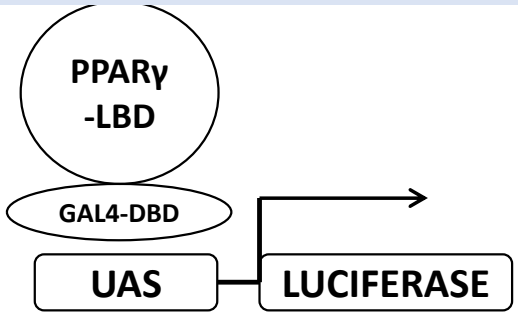


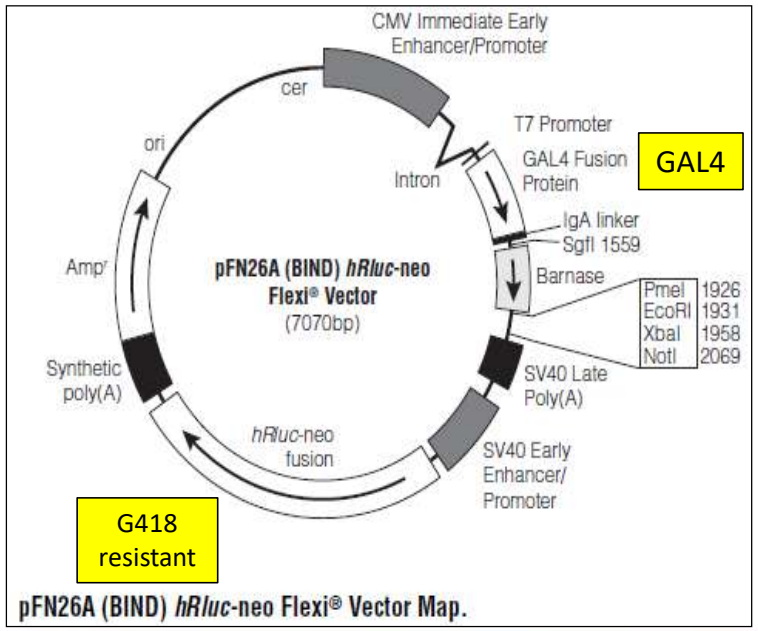
We want to simplify the invitro evaluation by establishment of stable cell ine

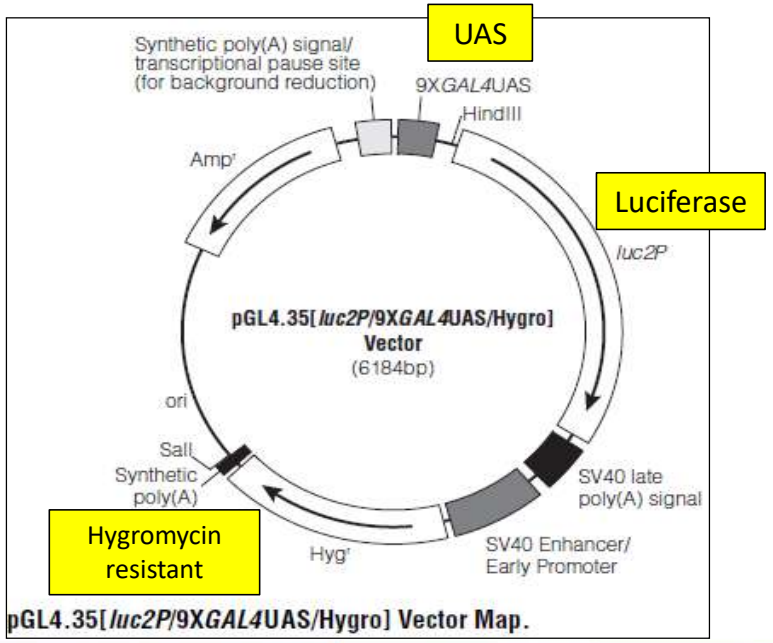
Mufidah, S., et al., Tetrahedron letters, Vol. 148, 15526-15530.

Plasmid preparation

PPARγ-Gal4 / UAS-luc system



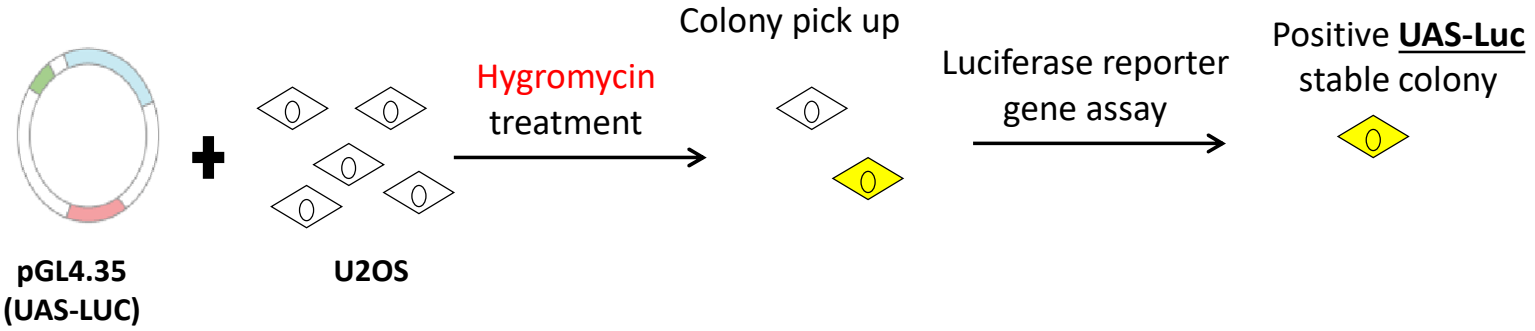




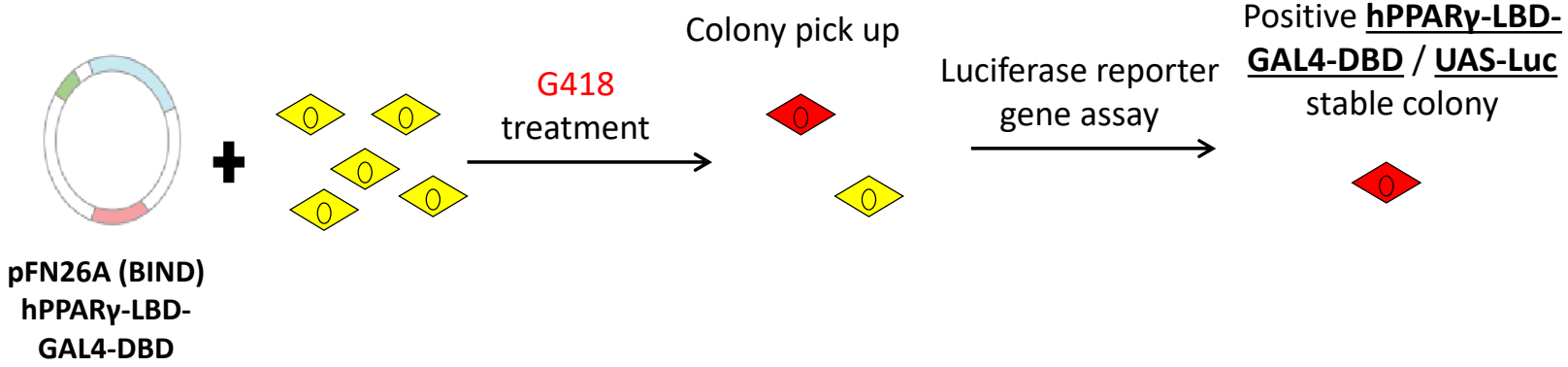
Protocol for stable cell line

Overview:

1- Establishment of UAS-Luciferase stable U2OS cell line:

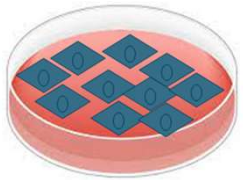


2- Establishment of hPPARγ-LBD-GAL4-DBD stable U2OS cell line:

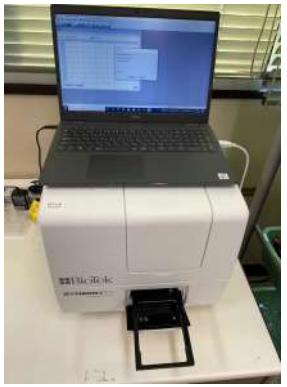


In vitro evaluation using stable cell line

Seeding cells and treatment



24 h

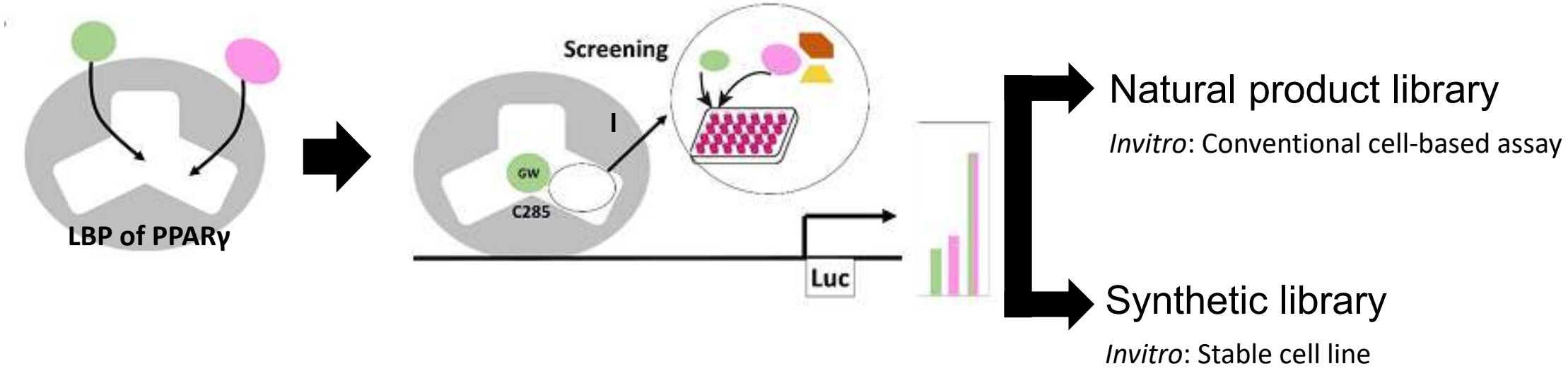


U2OS cells stably expressing **hPPARγ-LBD-GAL4-DBD** / **UAS-Luc**

Luminescence detection using microplate reader.

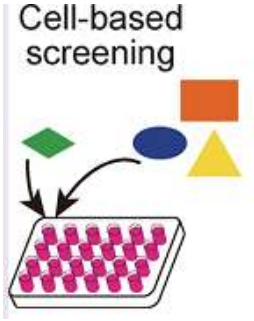
Screening of partner ligand

Screening partner ligand of GW9662 which cooperatively activate PPARγ transcription.



Ohtera, A., et. al. *ACS. Chem. Biol*., 10, 2794-2804 (2015); Utsugi, Y., et al., *Molecules,* 24, 2019 (2019); Mufidah, S., et al., Tetrahedron letters, Vol. 148, 15526-15530.

Screening partner ligand from natural product library



125 plant extracts

↓ luc assay

Kaempferia galanga L.

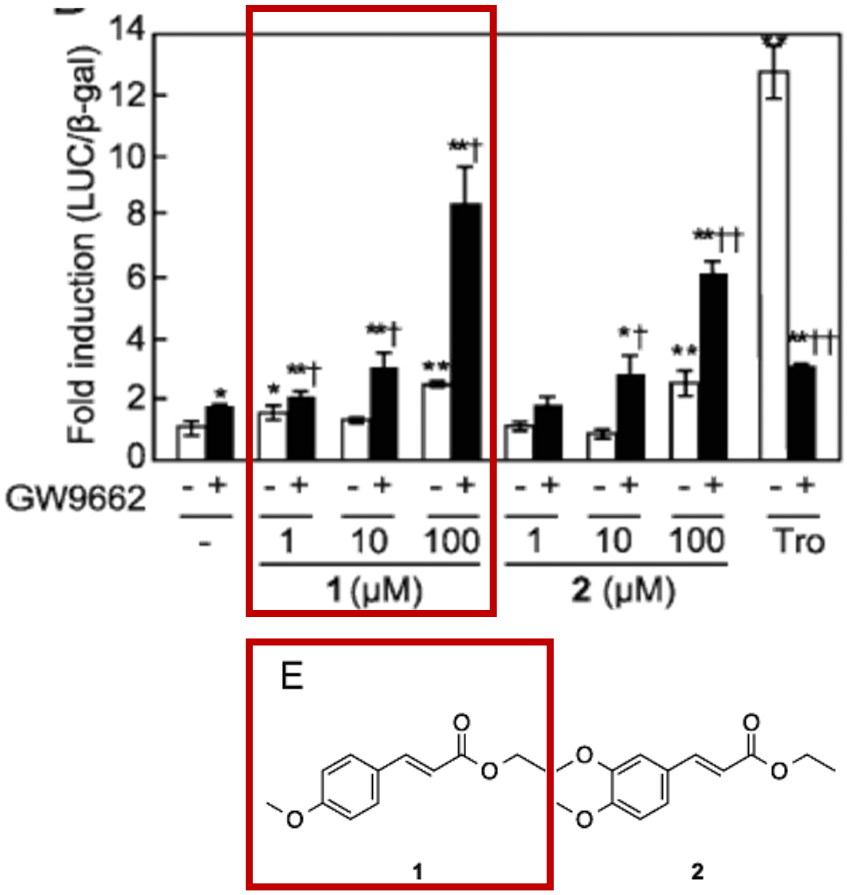
↓ bioassay-guided fractionation

1, **2**

CAEE (cinnamic acid ethyl ester)

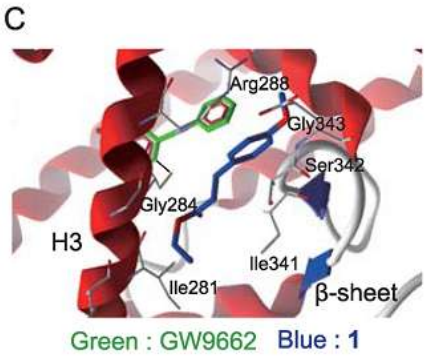
↓

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

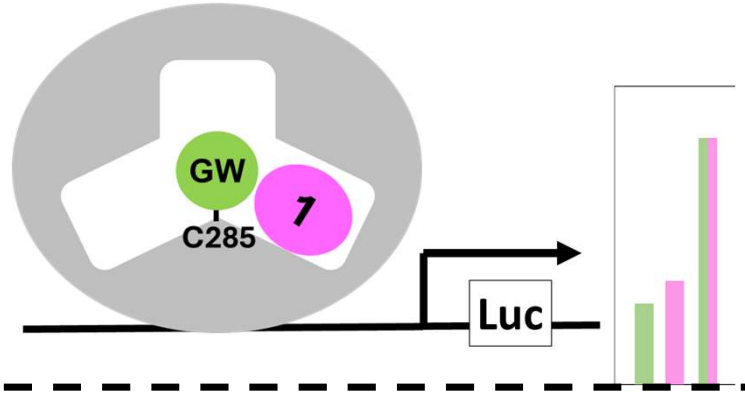


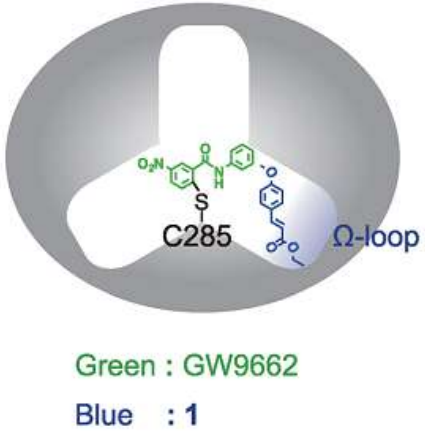
Ohtera, A., et. al. *ACS. Chem. Biol*., 10, 2794-2804 (2015)

Design new covalent agonist of PPARγ

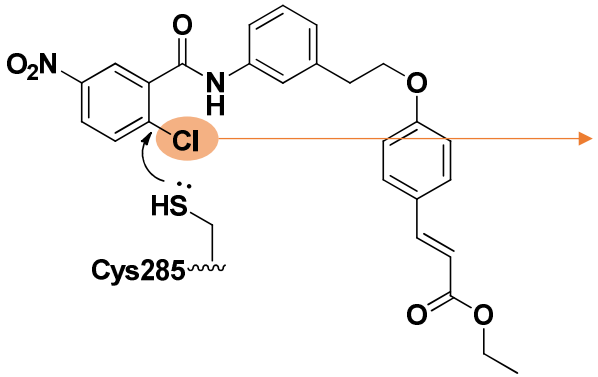


GW9662 and **1** showed cooperative activation





New Hybrid compounds



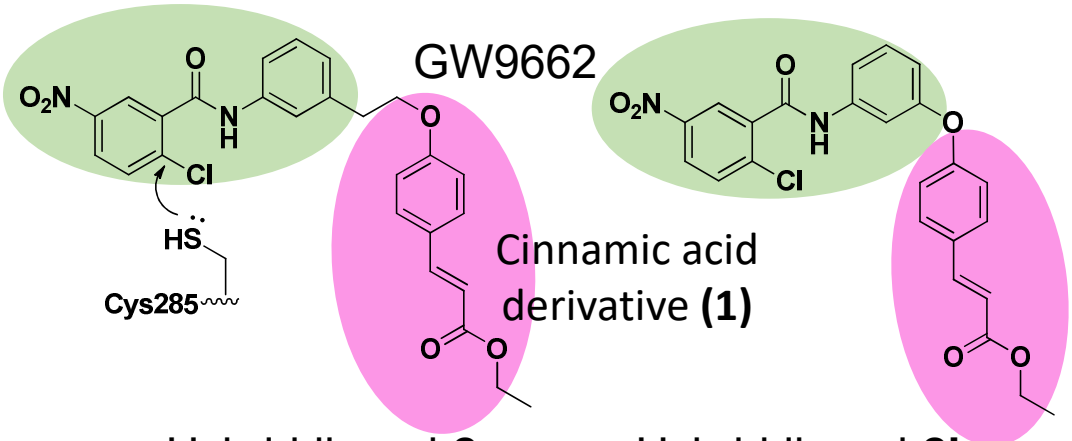
2A

Important for agonist activity.
Covalent binding with Cys285.

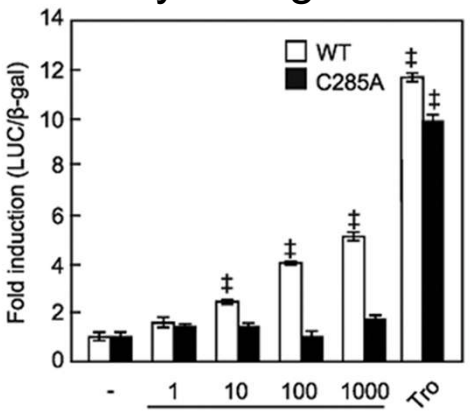
Changing from Cl to H, diminish the agonist activity.

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

New hybrid compounds based on a ligand-linking strategy

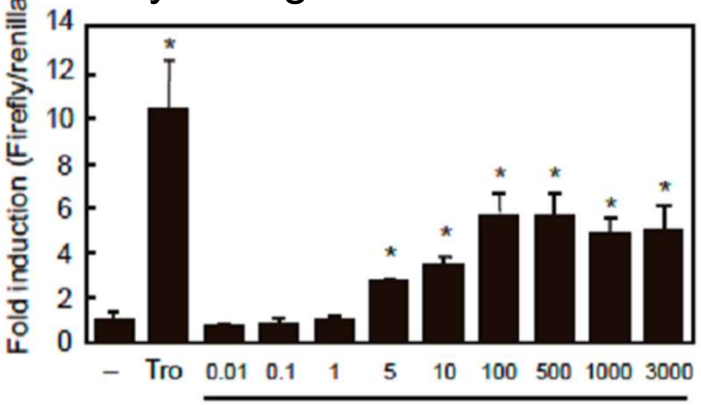


Hybrid ligand **2a**



2a (nM)

Hybrid ligand **2b**

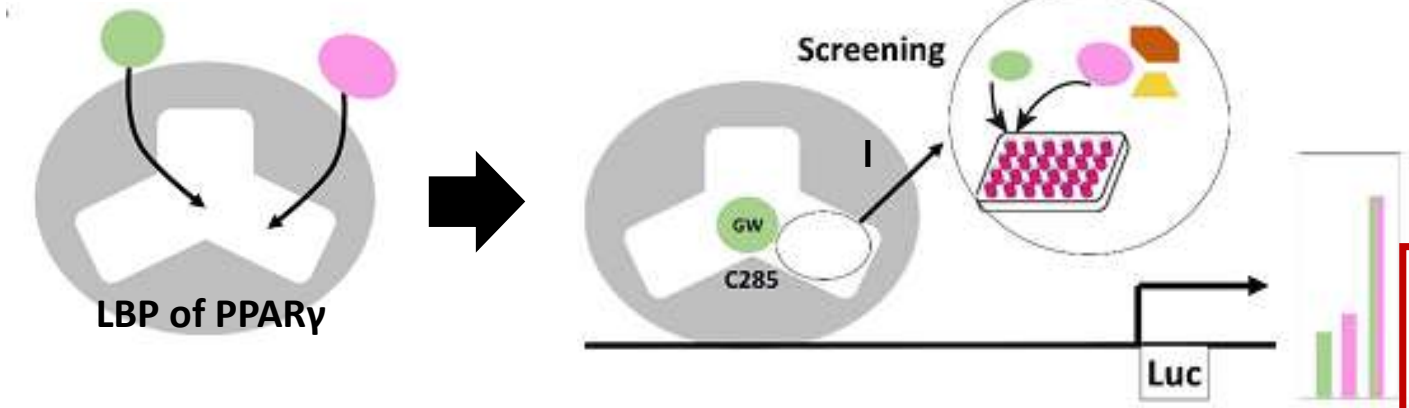


2b (nM)

Changing linker connecting GW9662 to compound 1 affected the agonist activity. Proximity between two ligands is important.

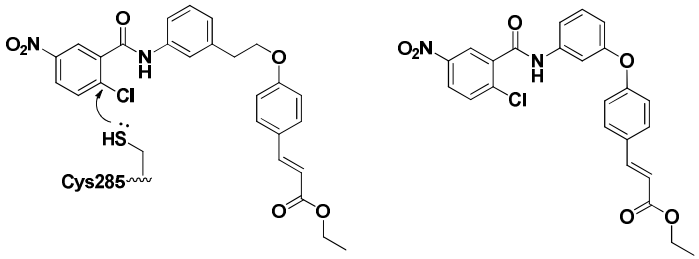
Utsugi, Y., et al., *Molecules,* 24, 2019 (2019)

Ligand linking strategy



Natural product library

Invitro: Conventional cell-based assay



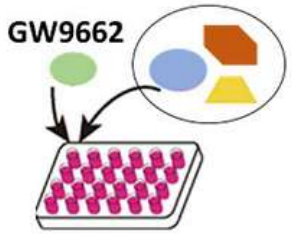
Synthetic library

Invitro: Stable cell line

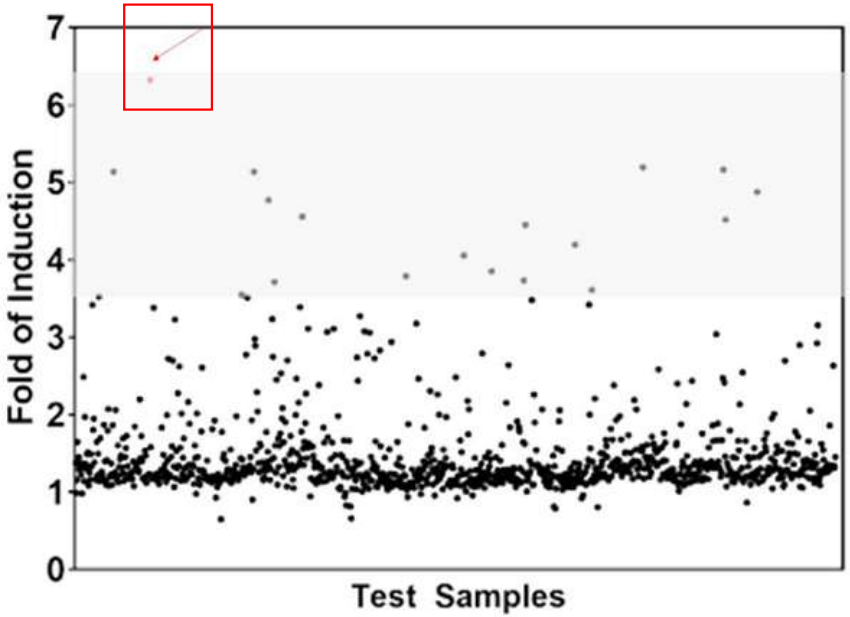
?

Ohtera, A., et. al. *ACS. Chem. Biol*., 10, 2794-2804 (2015); Utsugi, Y., et al., *Molecules,* 24, 2019 (2019); Mufidah, S., et al., Tetrahedron letters, Vol. 148, 15526-15530.

Screening partner ligand using synthetic library



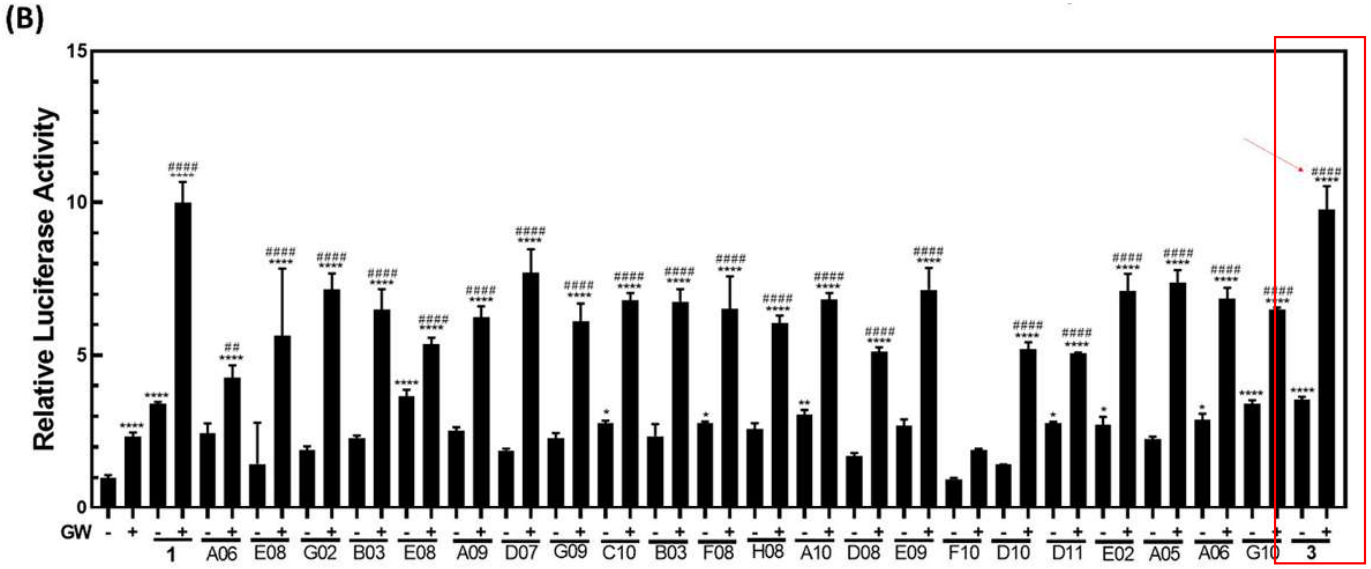
Screening partner ligand from fragment compound library (1040 samples)

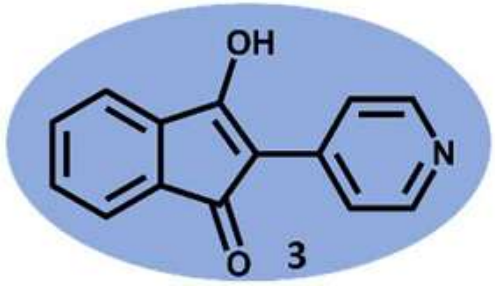


Mufidah, S., et al., Tetrahedron letters, Vol. 148, 15526-15530.

Collaboration with Drug Discovery Initiative, University of Tokyo

Screening partner ligand using synthetic library



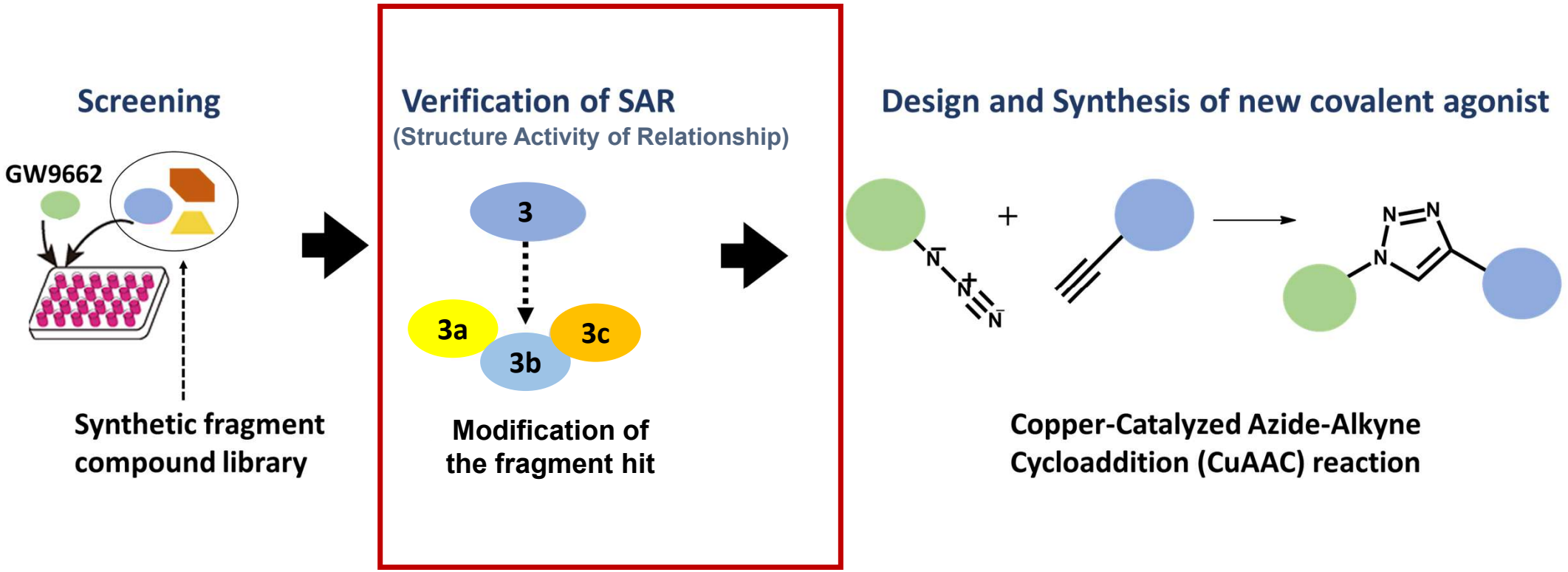


3 showed highest cooperative activation of PPARγ transcription with GW9662

Mufidah, S., et al., Tetrahedron letters, Vol. 148, 15526-15530.

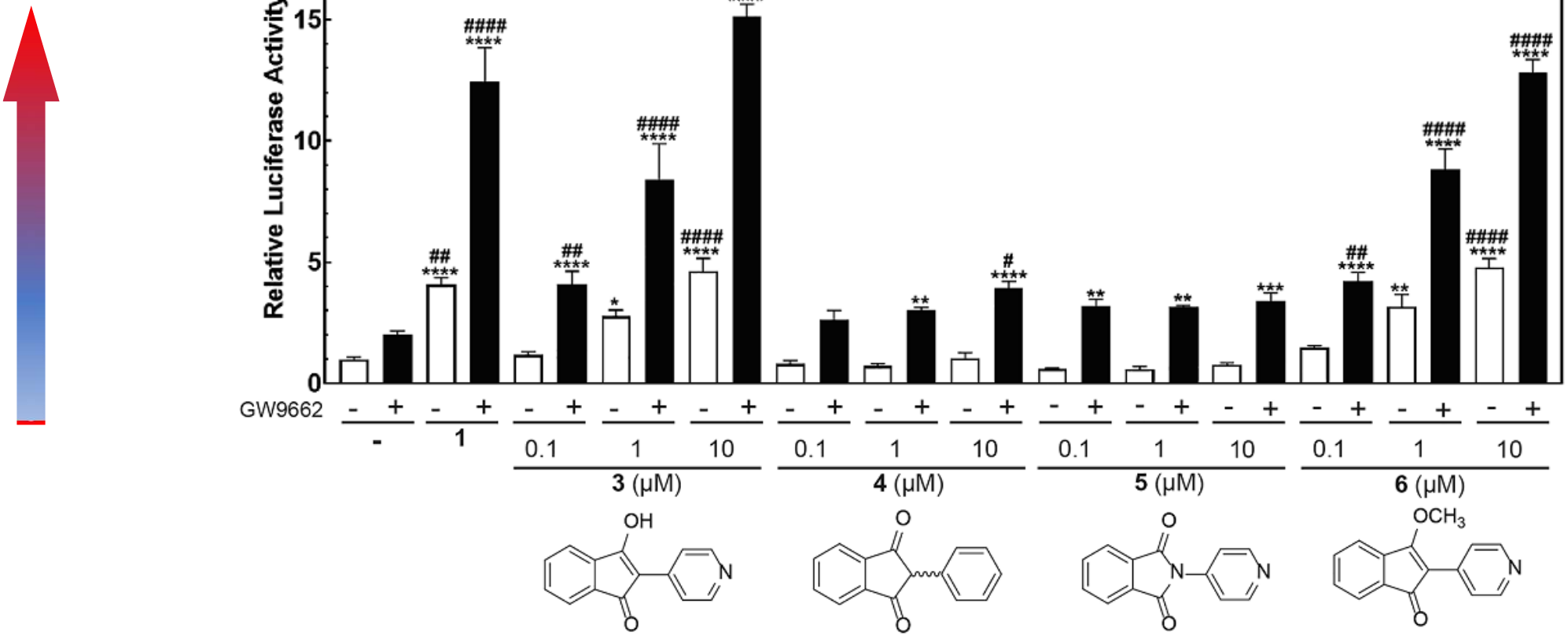
Collaboration with Drug Discovery Initiative, University of Tokyo

Design of hybrid ligands bearing triazole as linker



Luciferase assay for SAR study of 3

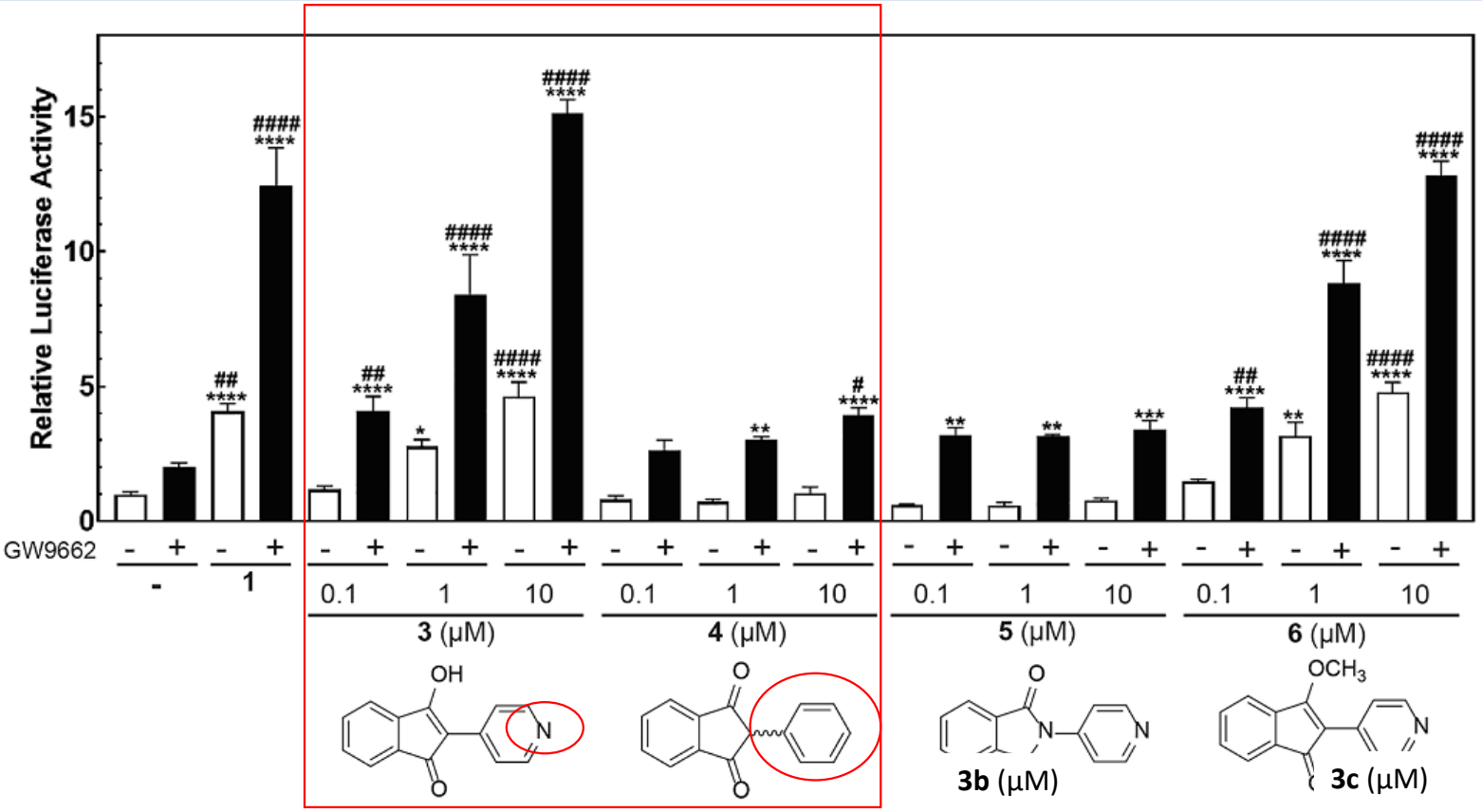
Strong Transcription activity



Mufidah, S., et al., Tetrahedron letters, Vol. 148, 15526-15530.

Luciferase assay for SAR study of 3

Strong Transcription activity

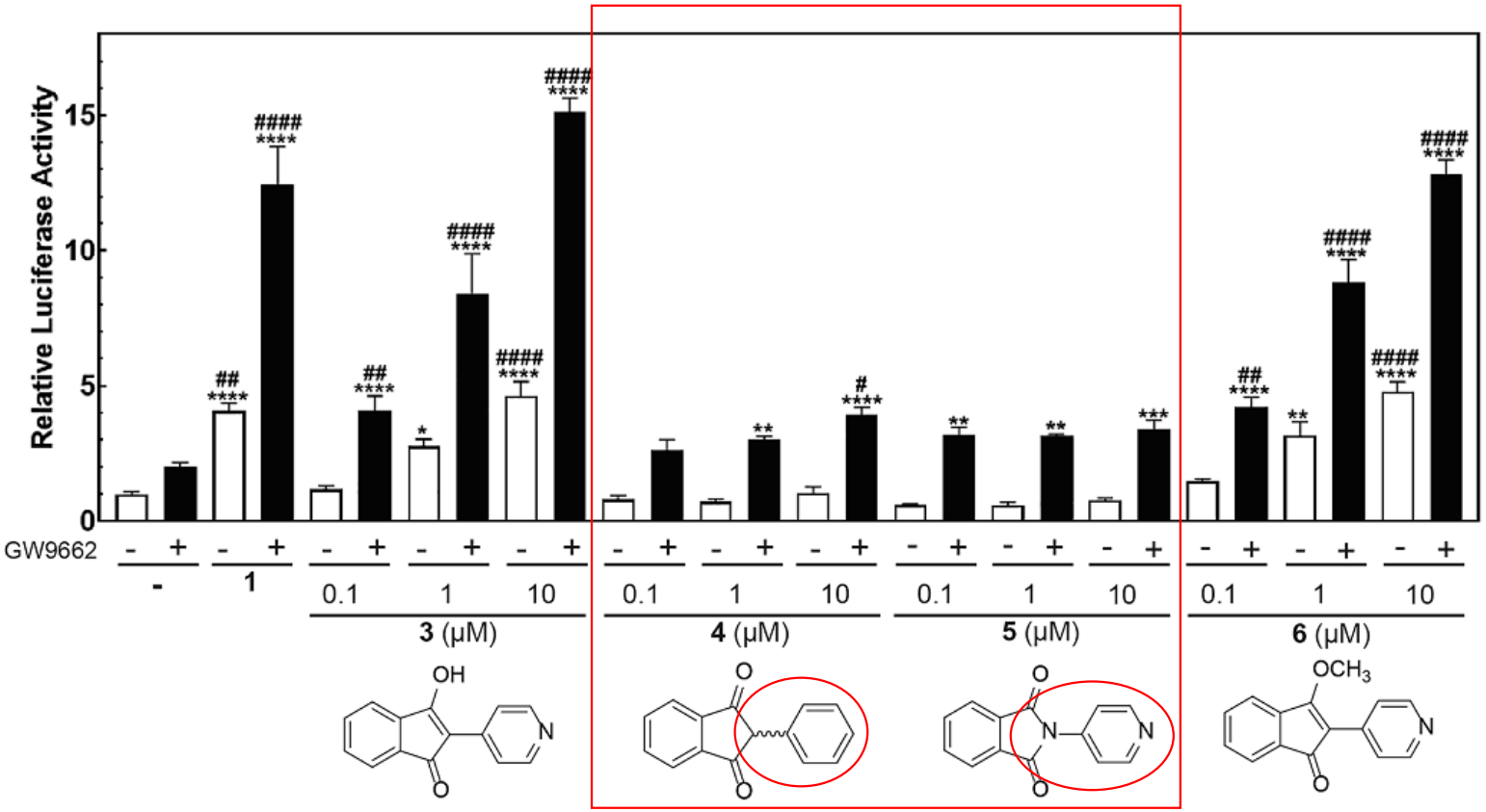


Pyridine or enol might be important

Mufidah, S., et al., Tetrahedron letters, Vol. 148, 15526-15530.

Luciferase assay for SAR study of 3

Strong Transcription activity

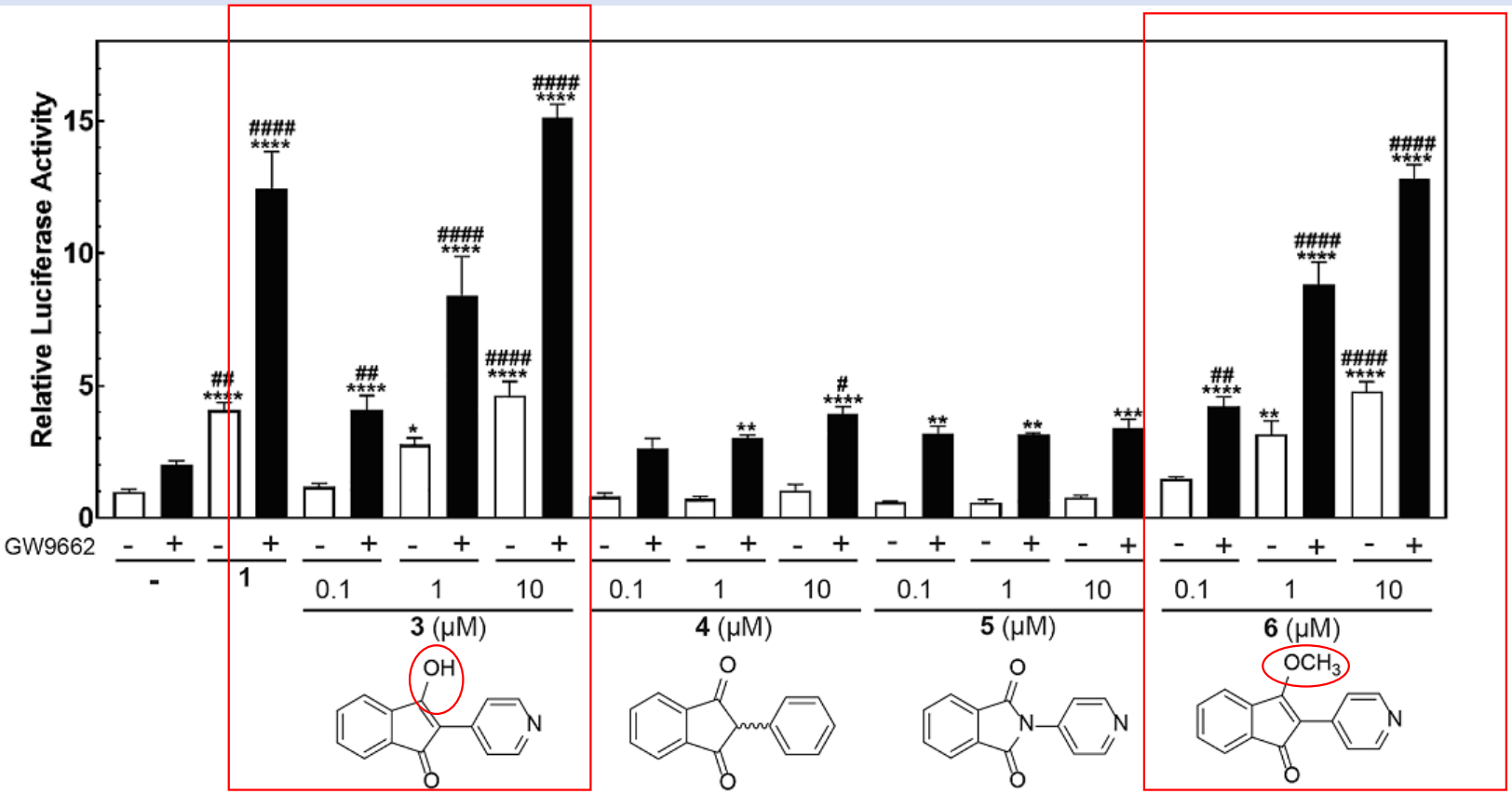


Pyridine itself did not have any important role for the activity.

Mufidah, S., et al., Tetrahedron letters, Vol. 148, 15526-15530.

Luciferase assay for SAR study of 3

Strong Transcription activity

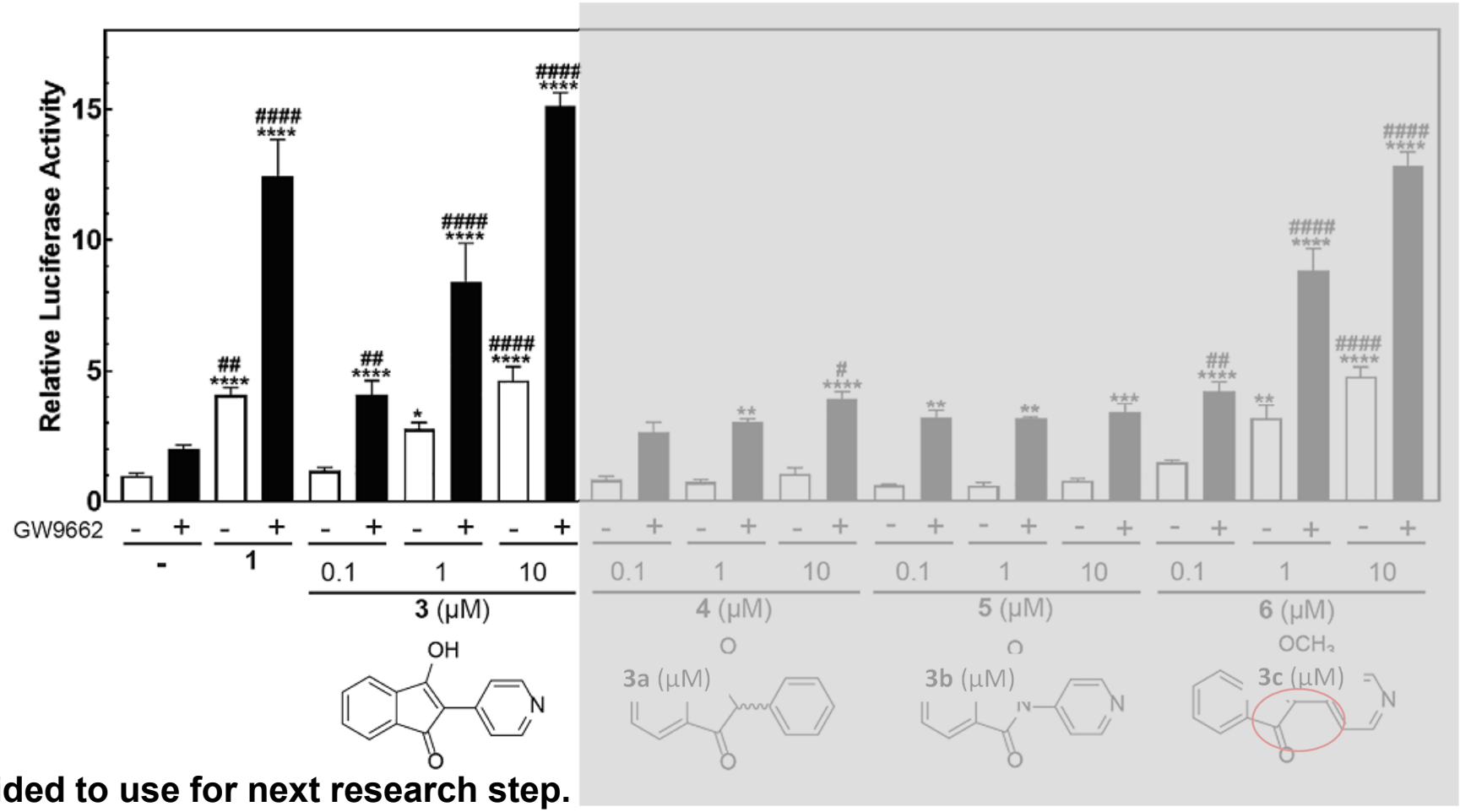


Enol might not have electrostatic interaction with PPARγ LBD, instead a gross structure of **3** was preferrable.

Mufidah, S., et al., Tetrahedron letters, Vol. 148, 15526-15530.

Luciferase assay for SAR study of 3

Strong Transcription activity



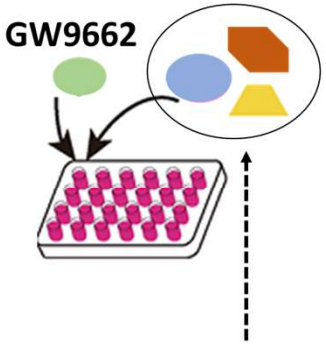
Compound 3 was decided to use for next research step.

Outline

- Diabetes Mellitus
- PPARγ as Target Protein for T2DM
- Rational Design: Ligand Linking Strategy
- Screening System
- **Synthesis of Agonist Candidates**
- In vitro evaluation

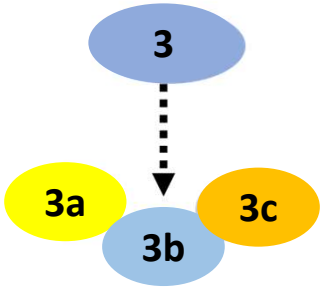
Design of hybrid ligands bearing triazole as linker

Screening



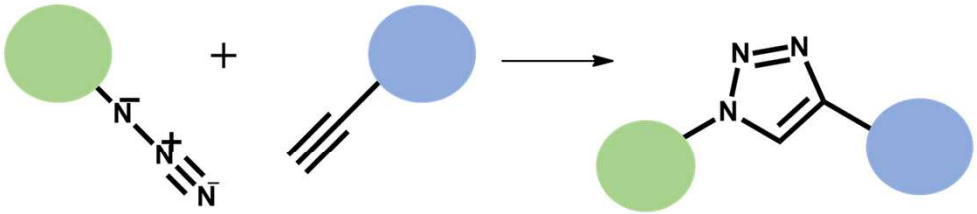
Synthetic fragment compound library

Verification of SAR
(Structure Activity of Relationship)



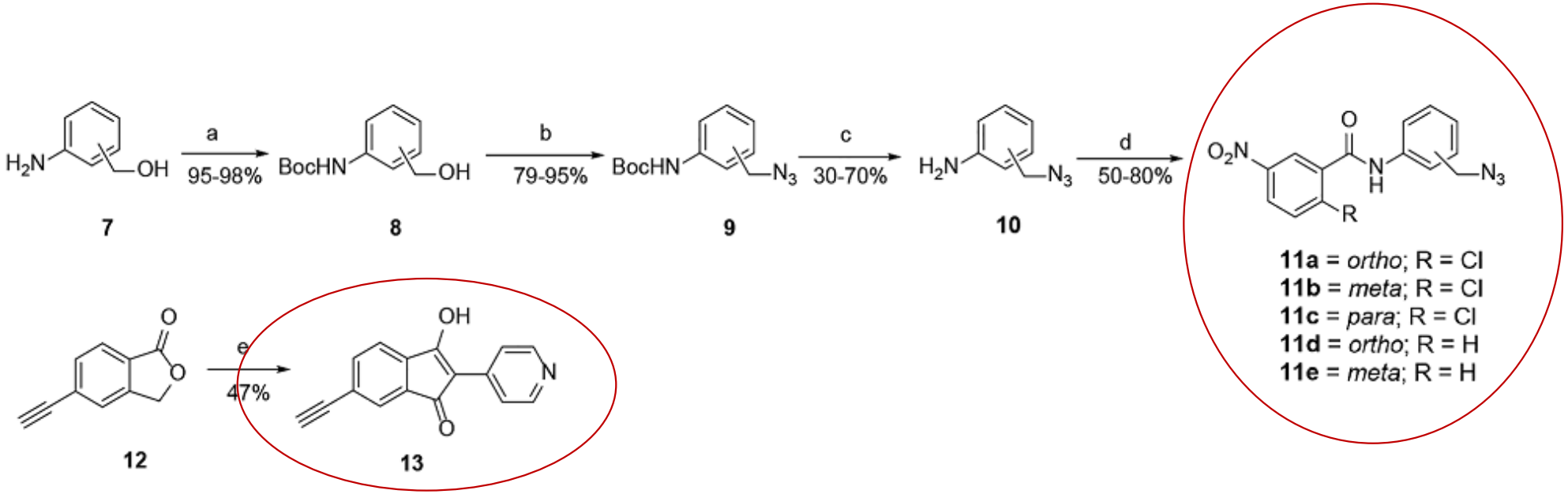
Modification of the fragment hit

Design and Synthesis of new covalent agonist



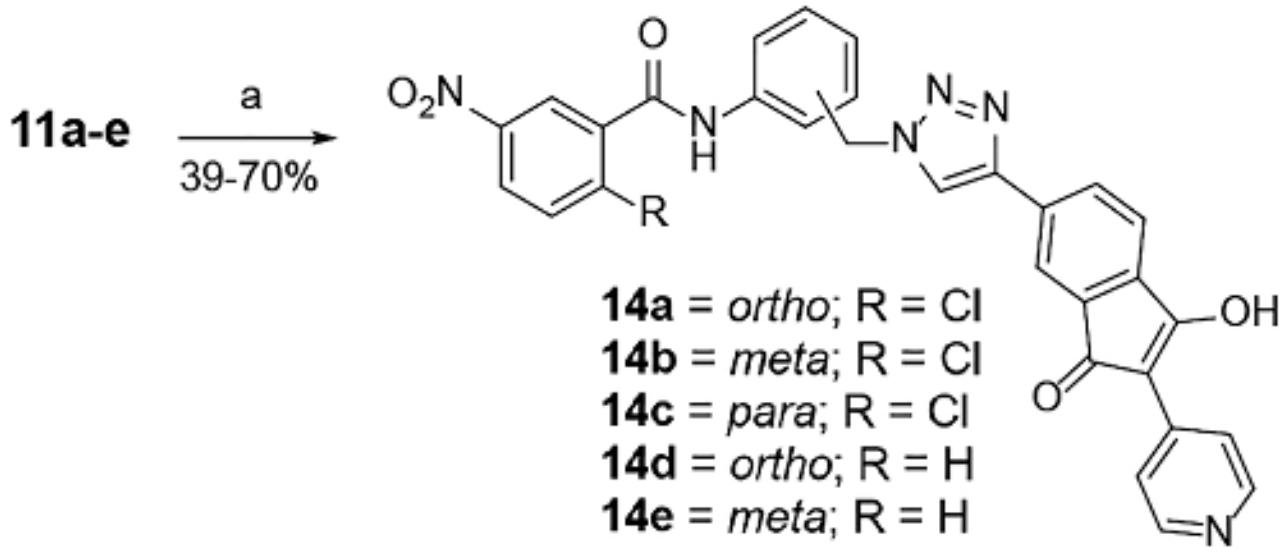
Copper-Catalyzed Azide-Alkyne Cycloaddition (CuAAC) reaction

Preparation building blocks



Scheme 1. Synthetic schemes of decorated structures of GW9662 and the identified fragment. Reagents and conditions: (a) $(Boc)_2O$, THF, rt, 18 h. (b) MsCl, TEA, CH_2Cl_2, 0 °C to rt, 3–5 h; NaN_3, DMF, 80 °C, 15 h. (c) TFA, CH_2Cl_2, 3–4 h. (d) Sodium acetate, MeOH, rt, 3–5 h. (e) Sodium methoxide, ethyl propionate, MeOH, rt for 1 h to reflux for 1 h.

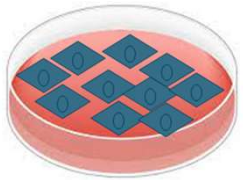
Synthesis of agonist candidates



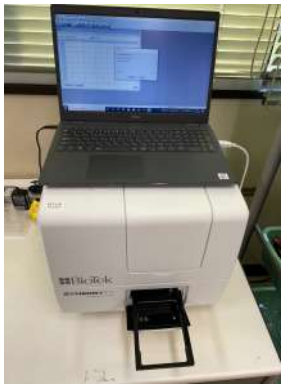
Scheme 2. Synthetic scheme of the coupled structure. Reagents and conditions: (a) **13**, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, TBTA, DMF/H_2O, rt to 60 °C, 24 h.

Biological evaluation by luciferase assay

Seeding cells and treatment



24 h

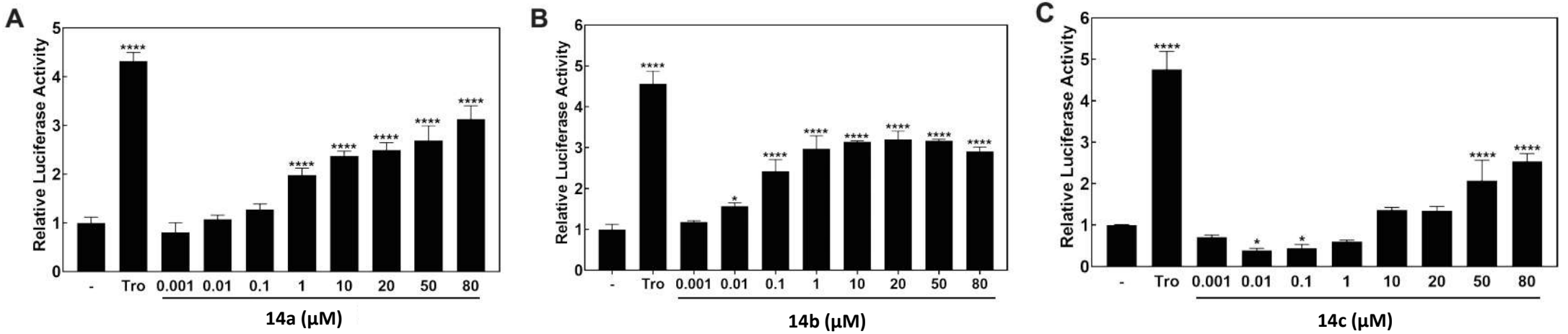


U2OS cells stably expressing **hPPARγ-LBD-GAL4-DBD** / **UAS-Luc**

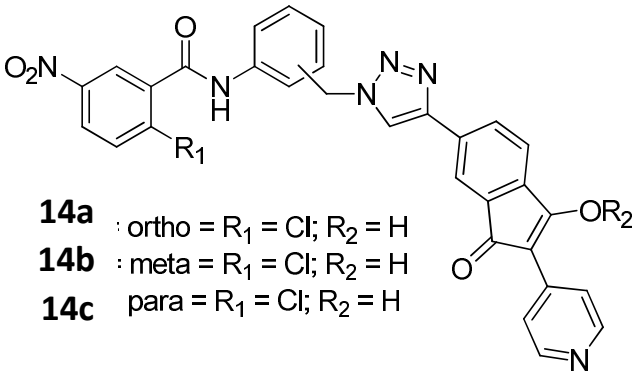
Seeding cell and treatment in the same day.
5 compounds, 8 different concentrations.

Luminescence detection using microplate reader.

14a-c showed agonist activity with different strength



Different tendency between ortho and meta



14a : ortho = R_1 = Cl; R_2 = H
14b : meta = R_1 = Cl; R_2 = H
14c para = R_1 = Cl; R_2 = H

Partial agonist
14c (para)
EC_{50} 39.85 µM

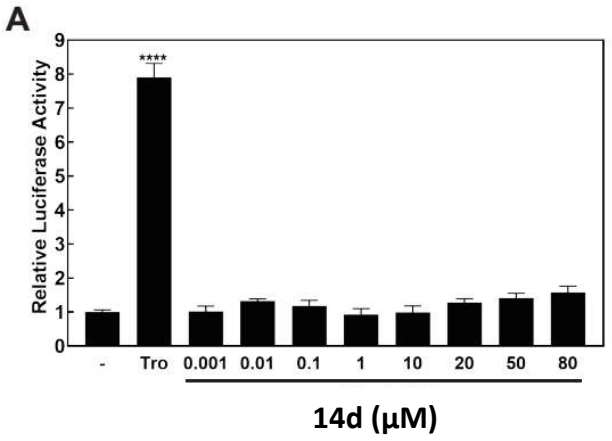
Partial agonist
14a (ortho)
EC_{50} 0.83 µM

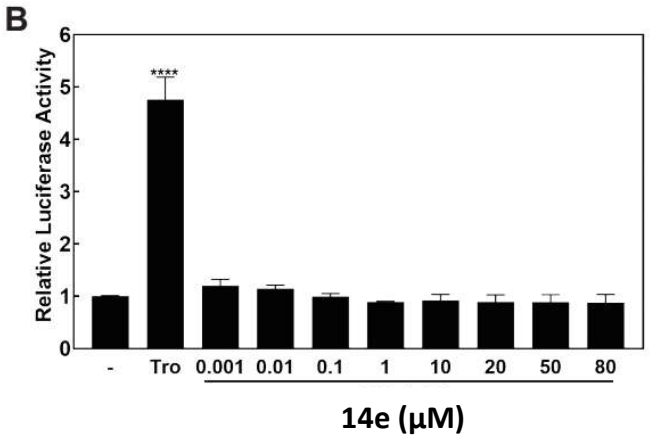
Partial agonist
14b (meta)
EC_{50} 0.05 µM

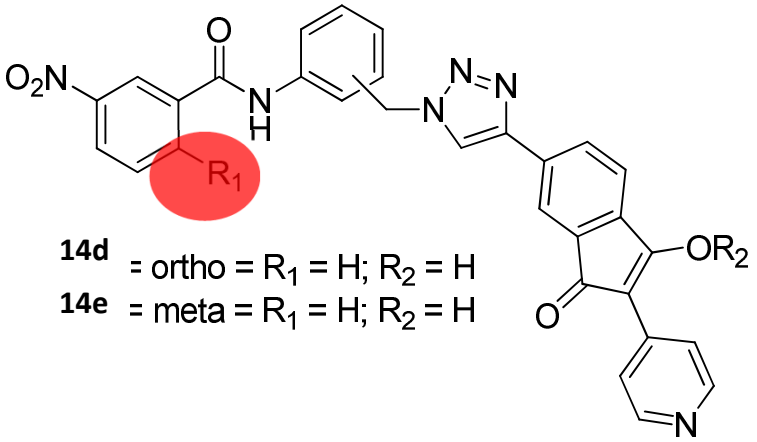
Weak → **Strong**

Linkage is important because it could give different strengths of agonist activity

Cl moiety is important for agonist activity.







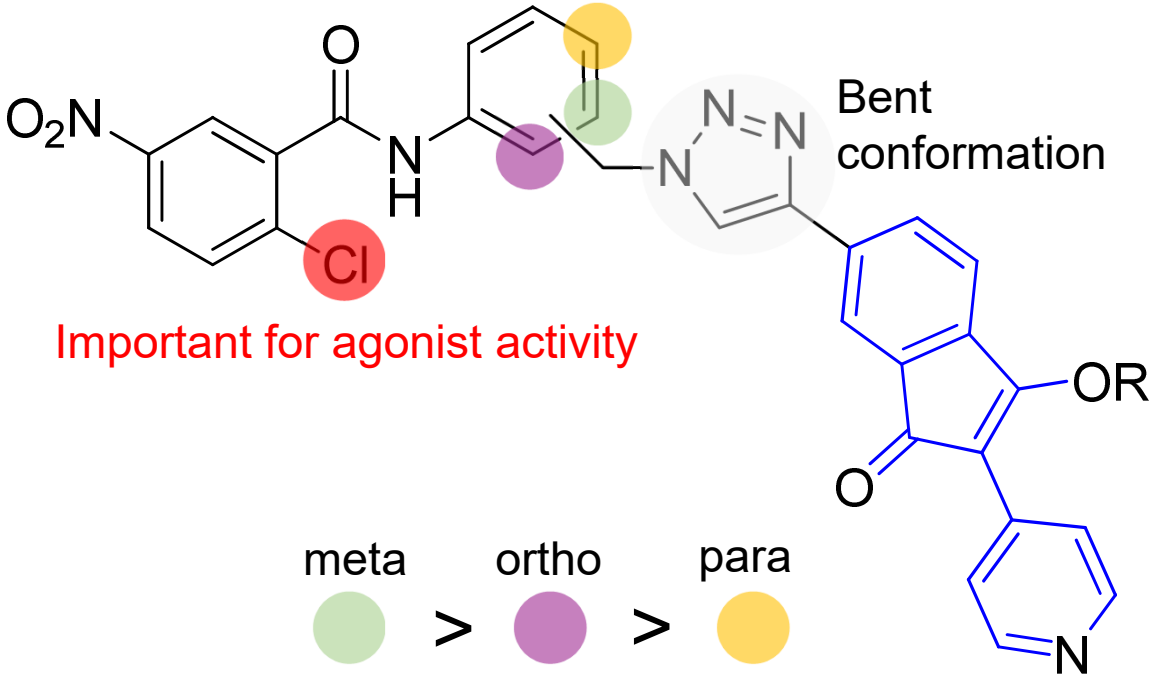
14d = ortho = R_1 = H; R_2 = H

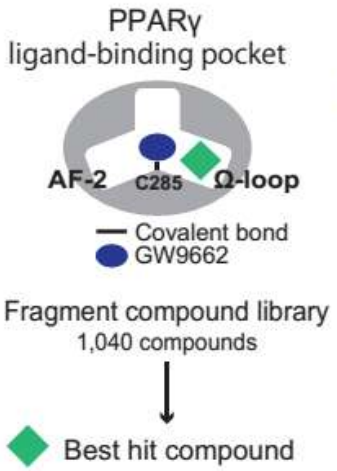
14e = meta = R_1 = H; R_2 = H

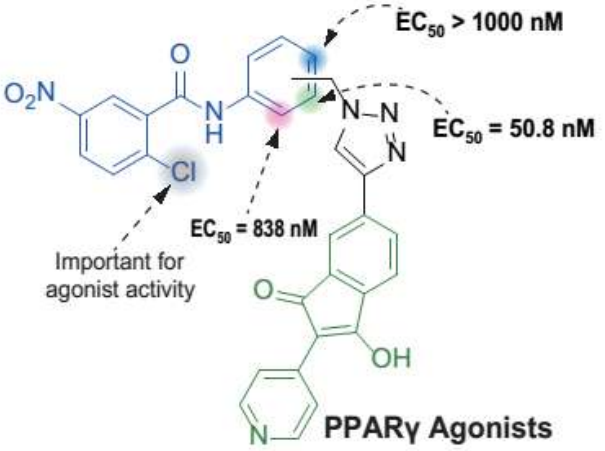
Changing Cl group into H, diminish the agonist activity.

Design of hybrid compounds from synthetic library screening

CuAAC reaction could be used to synthesize partial agonist of PPARγ by decorating GW9662 with the best fragment hit compound.







Mufidah, S., et al., Tetrahedron letters, Vol. 148, 15526-15530.

Conclusion

1. Drug design of antidiabetic agent targeting PPARγ could be done by ligand linking strategy, connecting GW9662 with partner ligand that cooperatively activate PPARγ transcription.
2. Partner ligand could be obtained from cell-based screening reporter gene assay of natural product library of synthetic library.
3. Linker of two could be obtained from click chemistry such as CuAAC reaction or ether linker which more preferable.
4. New hybrid compounds are interesting to be studied further about its molecular mechanism.

THANK YOU