Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXIX

Synthesis of a Click Linker for the release of a synthetic antitumor small molecule from nanocarrier in redox environment

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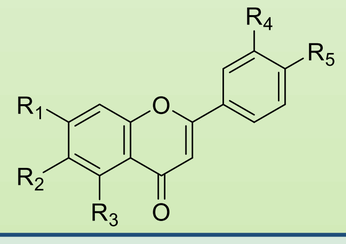
Background and purpose



A **structure-activity relationship** study **(SAR)** on a **flavone scaffold** identified APF-1 as a compound capable of performing cytotoxic activity in different cancer cells with a good selectivity index value compared to non-cancer cells (HDF).

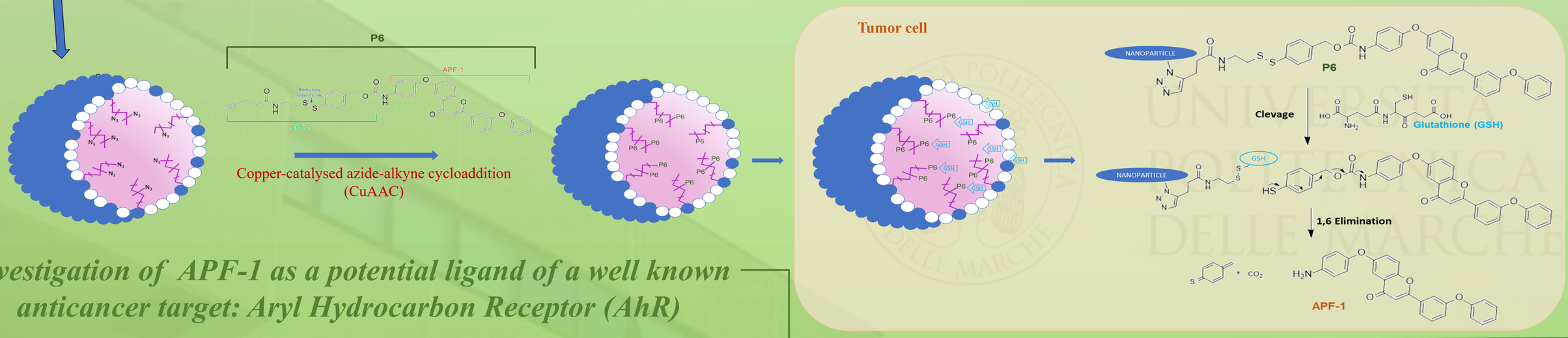
Discovery of APF-1: flavone based compound with high cytotoxic activity in cancer cell

Cmpd	R_1	R_2	R_3	R_4	R_5	MCF7	HepG	A549	H1975	HDF
APF1	H_2N-Ph-O	H	H	Ph-O	H	2.1 ± 3	4.6 ± 1	4.2 ± 0.4	2.3 ± 0.2	62 ± 6
APF2	H_2N-Ph-O	H	H	H	H	42 ± 5	15.8 ± 1	22 ± 3	50 ± 5	>160
APF3	H	H_2N-Ph-O	-	Ph-O	H	61 ± 3	10 ± 2	33 ± 2	40 ± 4	80 ± 3
APF4	H	H	H_2N-Ph-O	Ph-O	H	>160	44 ± 6	32 ± 5	>160	>160
APF5	H_2N-Ph-O	H	H	H	Ph-O	21 ± 3	>160	7 ± 3	10 ± 5	55 ± 2

Table: Cytotoxicity of APF compounds in cancer cells (MCF7, HepG, A549, H1975) and non tumor cells (HDF); IC_{50} values (μM).

Nanocarriers loaded with drugs can provide significant advantages, such as prolonged circulation time of the drug, cellular internalization, protection of the drug from premature degradation, controlled drug release, and, most importantly, selective targeting of tissues, thereby reducing side effects caused by non-specific drug targeting.

We want to develop a delivery system consisting of a **versatile click linker** capable of releasing **APF-1** from the nanocarriers following **the cleavage of a reduction-sensitive trigger by glutathione** within the cancer cells and tumor microenvironment.



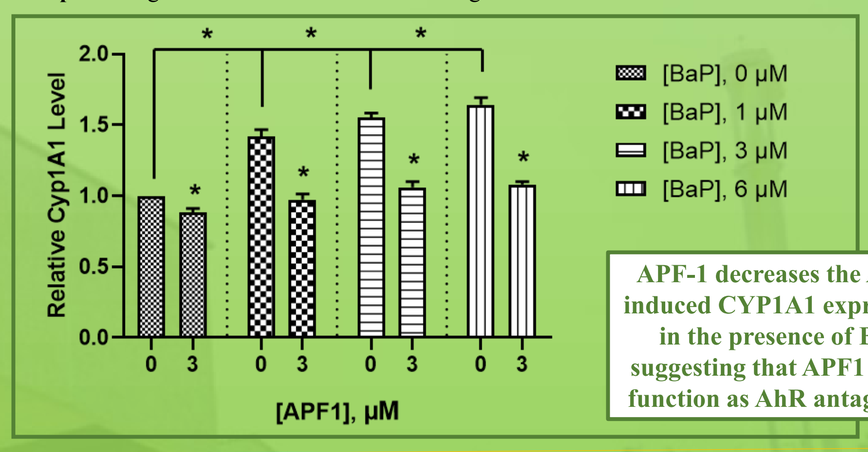
Investigation of APF-1 as a potential ligand of a well known anticancer target: Aryl Hydrocarbon Receptor (AhR)

- ❖ AhR is a key transcription factor involved in detecting environmental chemicals and regulating gene expression, notably CYP1A1, impacting xenobiotic metabolism.
- ❖ In cancer, AhR exhibits a dual role: its activation can promote tumor progression by influencing cell growth, migration, and immune responses, while its inhibition can suppress tumor development.
- ❖ Natural ligands like Benzo[a]pyrene (BaP) activate AhR and may contribute to carcinogenesis through sustained receptor activation.
- ❖ Synthetic compounds, such as aminoflavones, can act as either agonists or antagonists of AhR.

Starting from these considerations, we investigated whether APF-1 could modulate AhR activity by examining the CYP1A1 expression induced by BaP.

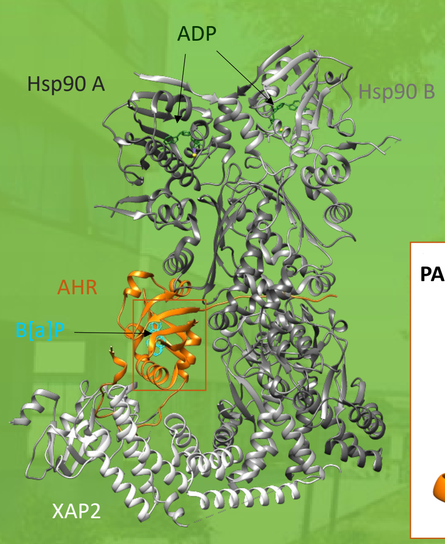
Figure. Western Blotting analysis in A549 cancer cell line. The GADPH was used as a control.

Graph. Histogram related to the western blotting.



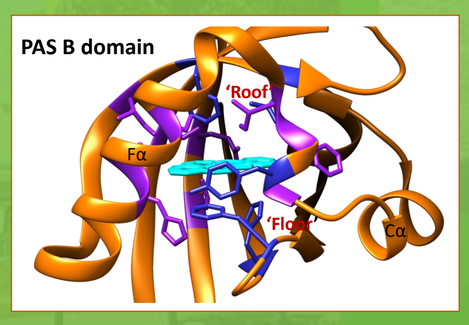
APF-1 decreases the AhR-induced CYP1A1 expression in the presence of BaP suggesting that APF1 could function as AhR antagonist.

Binding mode of B[a]P in PAS B domain of AhR



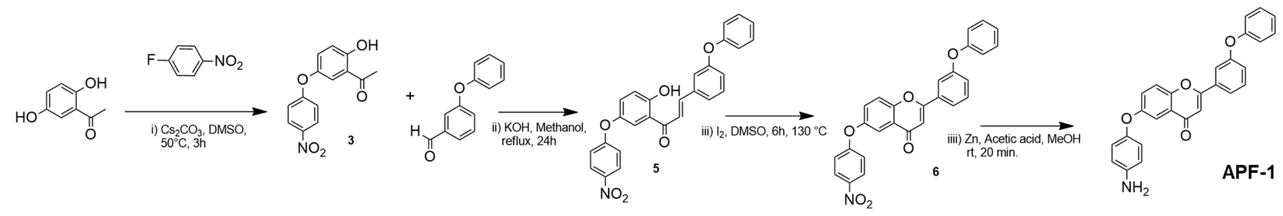
PDB: 8QMO

- Residues engaged **in van der Waals interaction** with B[a]P: G321, F324, I325, C333 (Fα), S336, H337, I349, S365, V381, Q383.
- Residues involved in **Π-Π interaction**: F351 and L353 on the Roof, F295 and H291 on the Floor, Y322 at the edge.

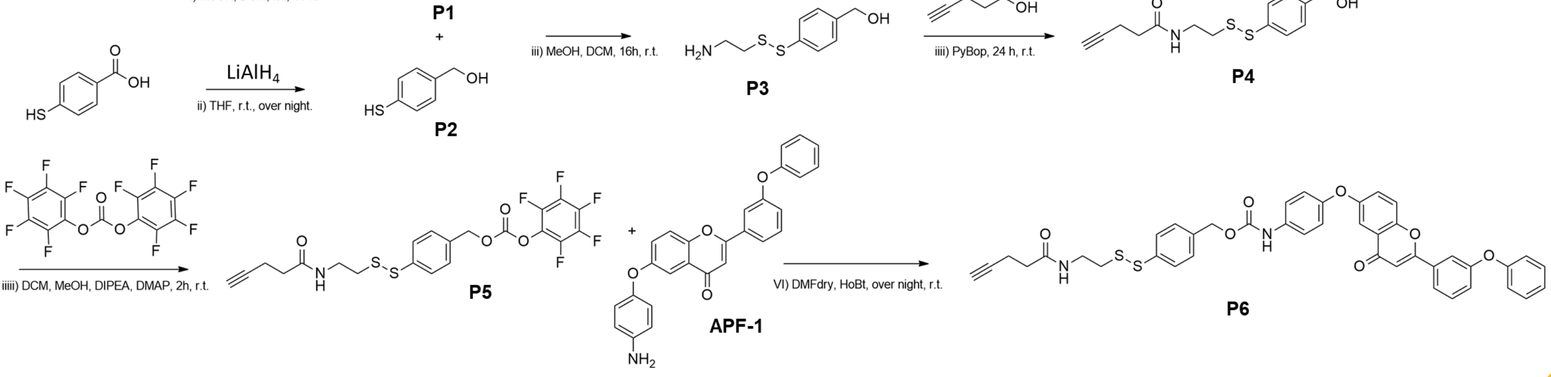


Chemical synthesis

APF-1 synthesis



Linker - APF-1 (P6) synthesis



Next steps...in the Netherlands

- ➢ Polymer Derivatization and characterization;
- ➢ Core-crosslinked polymeric micelle (CCPM) formation and drug loading;
- ➢ Investigation of APF-1 release and evaluation of cellular internalization and cytotoxicity.

References:

- G. Mobbili et Al, Molecules **2023**, 28, 3239
- Hebels E.R. et Al, Bioconjugate Chem. **2023**, 34, 2375−2386
- Kennedy L. et Al, Biomolecules **2020**, 10, 1429.
- Goya-Jorge E. et Al, Molecules **2021**, 26, 2315.
- Kwong H.S. et Al, Journal of Molecular Biology, **2024**, 436, 168411.