Contribució a l'Estudi dels Receptors de Serotonina. Molècules Basades en Indens i Indans

Sara López Pérez

UNIVERSITAT DE BARCELONA

FACULTAT DE FARMÀCIA

DEPARTAMENT DE FARMACOLOGIA I QUÍMICA TERAPÈUTICA

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7. Menció Europea al Títol de Doctor: Poly(ADP-ribose) polymerase (PARP) Inhibitors

As a part of my PhD program, I have undertook a placement of three months, in the laboratory in the "Dipartamento di Chimica e Tecnologia del Farmaco", at the "Università degli Studi di Perugia", Italy. During this period, the research project was aimed at the synthesis of new poly(ADP-ribose polymerase) inhibitors.

The poly(ADP-ribose) polymerase (PARP) is an abundant family of eukaryotic nuclear NAD-dependent enzymes that are able to catalyze the transfer of ADP-ribose units from NAD to substrate proteins, thereby contributing to the control of genomic integrity, cell cycle and gene expression.[1]

Characterized family members of the PARP family currently include different types of proteins: PARP-1, PARP-2, PARP-3, Tankyrase-1, Tankyrase-2 and vPARP. However, only two are activated in response to DNA damage: PARP-1 and PARP-2.[2]

PARP-1 was identified by Pierre Chambon et al[3], in 1963 as the enzyme responsible for the formation of the polymers of ADP-ribose or PAR. It is the most abundant and commonly studied member of this family, with a mass of 113 kDa constituted by three domains, the *N*-terminal that contains two zinc fingers which serve as DNA binding segments, an automodification domain or central regulatory and the C-terminal domain which contain the catalytic domain[4] or NAD^+ binding site. This catalytic domain is extremely conserved and contains the so-called PARP-1 signature, a stretch of 50 residues completely conserved between vertebrates (Figure 7.1).[5]

[1] Hassa P. O, Haenni S. S, Elser M and Hottiger M. O; *Microbiol Mol Biol Re,* **2006**, *70*, 789–829.
[2] Shieh, W. M., Ame, J. C., Wilson, M. V., Wang, Z. Q. and Koh, D. W.; *J. Biol. Chem*.,**1998**, *273*, 30069–30072; (b) Ame, J. C., Rolli, V., Schreiber, V., Niedergang, C., Apiou, F.,Decker, P., Muller, S., Hoger, T., Menissier-de Murcia, J. and De Murcia, G.; *J. Biol. Chem*., **1999**, *274***,** 17860–17868.
[3] Chambon P., Weill J. D., Mandel P.; *Biochem. Biophys. Res. Commun*., **1963**, *11*, 39–43.
[4] De Murcia, G. and Menissier-de Murcia, J.; *Trends Biochem. Sci*., **1994**, *19,* 172–176; (b) Lamarre, D., Talbot, B., de Murcia, G., Laplante, C., Leduc, Y., Mazen, A. and Poirier, G.G.; *Biochim. Biophys. Acta*, **1998**, *950*, 147–160; (c) Mazen, A., Menissier-de Murcia, J., Molinete, M., Simonin, F., Gradwohl, G., Poirier, G. and de Murcia, G.; *Nucleic Acids Res*., **1989**, *17*, 4689–4698.
[5] Uchida, K., Uchida, M., Hanai, S., Ozawa, Y., Ami, Y., Kushida, S. and Miwa, M.; *Gene*, **1993**, *137*, 293–297.

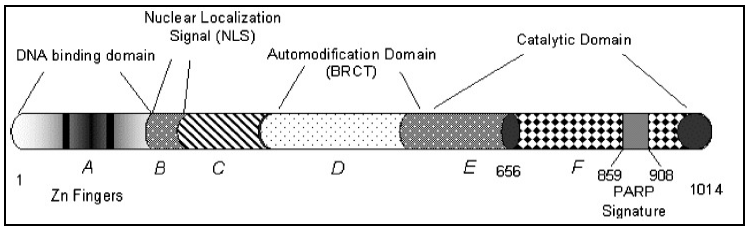


Figure 7.1. Modular architecture of PARP-1

In the presence of mild or moderate DNA damage, PARP-1 becomes activated and involves a variety of physiological functions related to genomic repair, including DNA replication and repair, cellular proliferation and differentiation, and apoptosis. PARP-1 functions as a DNA damage sensor and signalling molecule.

However, under massive genotoxic stimulus, like microbial aggression, trauma, hypoxia or ischemia it produces a variety of free radicals and reactive oxygen species that causes the double DNA strand break and results in an over-activation of PARP-1 with the rapid consume of NAD^+ as a substrate.[6] Attempts of the cell to resynthesize NAD^+ causes the depletion of the reservoir of ATP, leading to an energy crisis and subsequently cellular death and necrosis. In these cases, PARP-1 behaves as a death perpetrator (Figure 7.2).

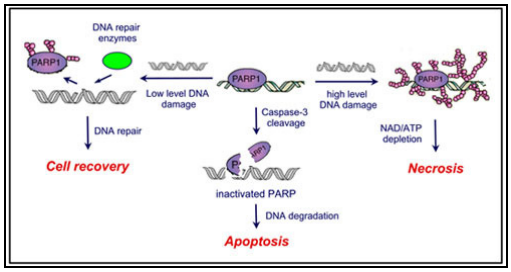


Figure 7.2. Upon mild, moderate or excessive DNA damage

Novel and promising therapeutic opportunities, in particular for the treatment of ischemia related CNS diseases, are becoming apparent for PARP modulators. The first PARP inhibitors were based on mimicking the structure of nicotinamide and benzamide analogs.[7] A clear consensus has been reached around the minimal pharmacophore needed for competitive inhibition of the nicotinamide binding site of PARP. This minimal pharmacophore includes a phenyl ring connected to an amide moiety in an anti disposition (Figure 7.3). Consistent with the X-ray structures of the catalytic fragment of PARP-1, this minimal pharmacophore is able to fill the nicotinamide binding pocket

[6] Soldani, C. and Scovassi, A.I.; *Apoptosis*, **2002**, *7*, 321–328; (b) Ha, H.C. and Snyder, S.H.; *Proc. Natl Acad. Sci*. USA, **1999**, *96*, 13978–13982.
[7] Halmosi, R.; Berente, Z.; Ősz, E.; Tóth, K.; Literati-Nagy, P.; Sümegi, B. *Mol. Pharmacol.* **2001**, *59*, 1497.

ant to interact with the key residues Gly-863 and Ser-904. Variations of this theme are sought to provide better pharmacokinetic profile and increase potency.

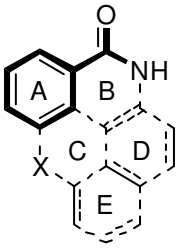
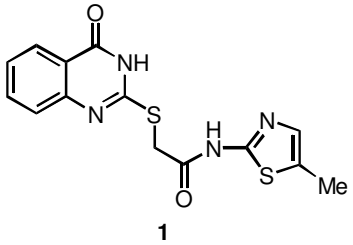


Figure 7.3. General pharmacophore for PARP-1 inhibitors

My project started as a result of a study by Virtual Screening focused on the discovery of new selective PARP-1 inhibitors carried out by Daniele Bellochi (research group by Prof. Pelliciari, Dip. Chimica e Tecnologia del farmaco, Perugia). Ten compounds were selected and acquired which were then tested in PARP-1 (bovin) and PARP-2 (mouse). The outcome of this screening showed compound **1** as a potent selective PARP-1 inhibitor at concentrations of 100 μM. With the purpose of increasing the activity and selectivity, my project has been devoted to the design and synthesis of new analogues of compound **1 (**Figure 7.4).

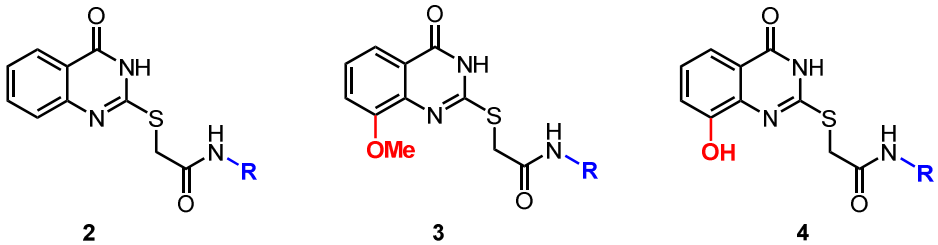


1

Figure 7.4

On the basis of this study, a library of substituted quinazolinone derivatives **2–4** was designed from a series of modifications around this scaffold **1**, by preserving the canonical PARP pharmacophore constituted by the constrained benzamide moiety.

- The linear amide group was substituted by differents heterocycles.
- The mercaptoquinazolinone core was decorated with methoxy and hydroxyl groups in the 8-position.

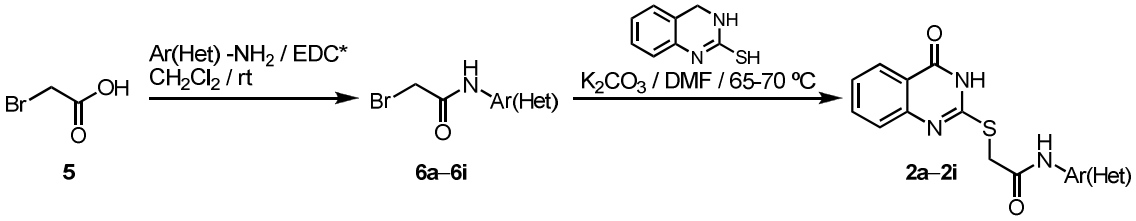


2 **3** **4**

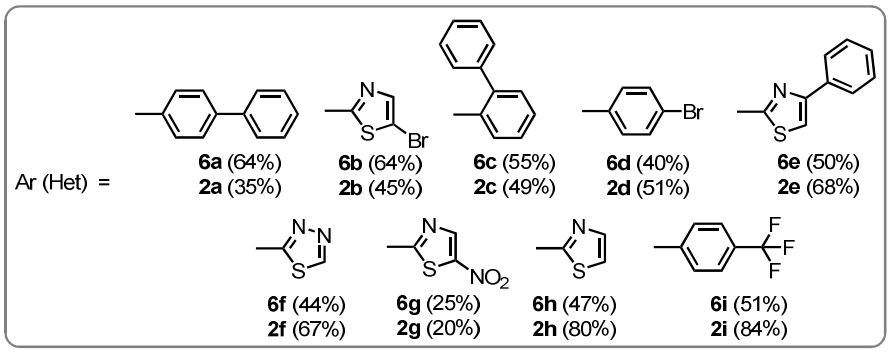
Figure 7.5

Chemistry

After analyzing different synthetic alternatives, compounds **2–4** were synthesized as outlined in Schemes 7.1 and 7.2. The 8-H derivatives **2a–2i** were prepared from commercial bromoacetic acid **5** by reaction with different amines to gave the corresponding bromoacetamides **6a–6i**, which by reaction with 2-mercaptoquinazolinone afforded the desired compounds **2a–2i** with variable yields (Scheme 7.1).

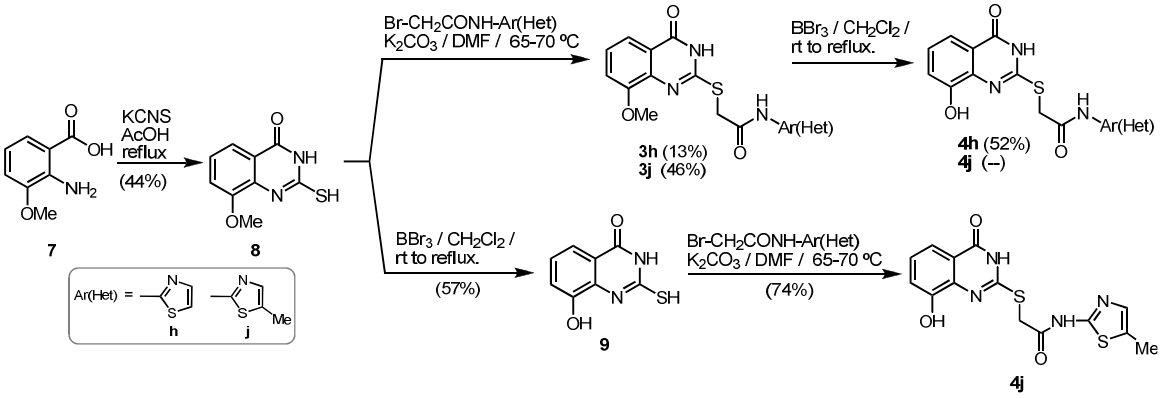


*EDC: *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide·HCl



Scheme 7.1

For the preparation of the compounds **3** and **4**-type was necessary, firstly the formation of the 2-mercapto-8-methoxyquinazolin-4(3*H*)-one **8** from the commercial 2-amino-3-methoxy-benzoic acid **7** with KCNS in acetic acid. This compound **8**, initially, was reacted with the corresponding amides **6h** and **6j**, following the same procedure previously explained, to obtain compounds **3h** and **3j**, in which hydrolysis to the respective alcohols **4h** with a 52% of yield but without success for **4j**. Consequently, the synthesis of **4j** was attempted by application of another procedure, where compound **8** was first hydrolyzed to the alcohol **9** followed by the nucleophilic substitution with the 2-bromo-*N*-(5-methylthiazol-2-yl)acetamide **6j** to give compound **4j** in goods yields (Scheme 7.2).



Scheme 7.2

Biological results

The newly synthesized compounds were screened at a concentration of 100 μM against recombinant bovine PARP-1 and mouse PARP-2 by monitoring the residual poly(ADP-ribosyl)transferase activity. The results are showed in the Figure 7.6 and their inspection allowed us to identify two possible selective PARP-1 inhibitors, **2c** (UPF-1504) and **2d** (UPF-1505) with a moderate activity, in comparison with the reference compound used UPF-1421, requiring further pharmacological studies that are in progress.

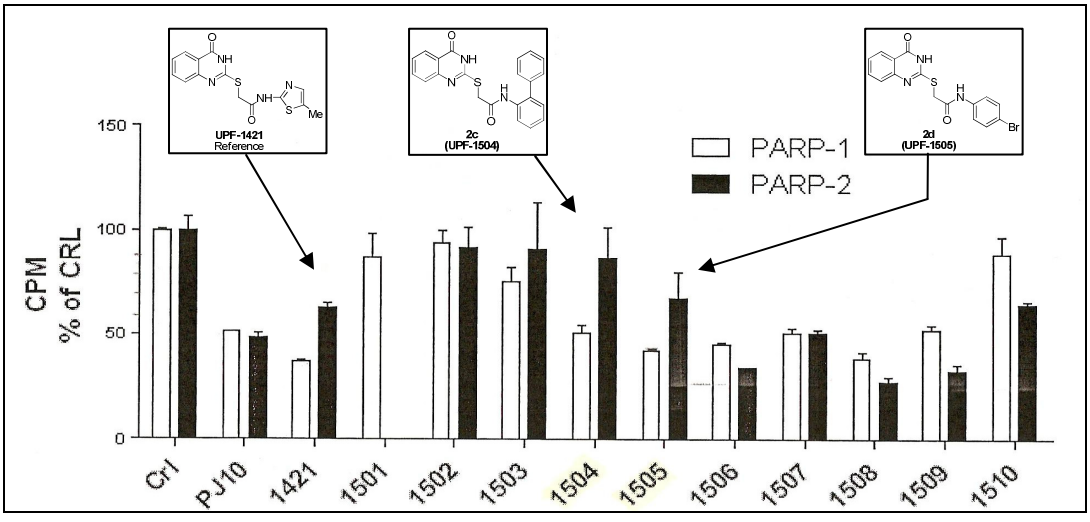
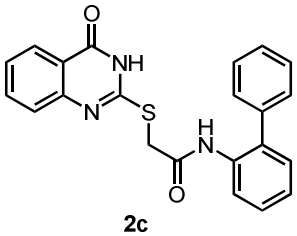


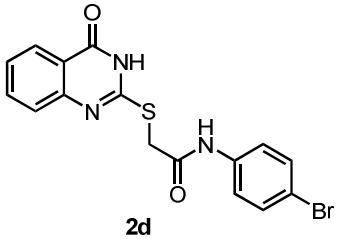
Figure 7.6. Structure-activity PARP-1 and PARP-2 of compounds

Conclusions

- We described the preparation and preliminary evaluation of a library of 4(*3H*)-quinazolinone derivatives for their study as a possible selective PARP-1 inhibitors, and the identification of compounds **2c** and **2d** with a good selectivity for PARP-1 over PARP-2.



2c



2d

- We thus demonstrated that selective inhibition of individual PARP isoforms can in fact be achieved through chemical manipulation of quinazolinone derivatives bearing the “canonical” pharmacophore.