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Towards new neuroprotective agents: design and synthesis of 4*H*-thieno[2,3-*c*] isoquinolin-5-one derivatives as potent PARP-1 inhibitors

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This paper is dedicated to the memory of Prof. Piero Pratesi.

Abstract

An excessive activation of poly(ADP-ribose) polymerase-1 (PARP-1), a nuclear enzyme able to catalyze the transfer of ADP-ribose from NAD to acceptor proteins, is involved in the progression of neuronal damage after brain insult. Potent and selective PARP-1 inhibitors have neuroprotective properties in experimental models of brain ischemia. As a follow up of our previous structure–activity relationship study and in search for novel potent PARP-1 inhibitors, a series of 4*H*-thieno[2,3-*c*]-isoquinolin-5-one derivatives was designed and synthesized. Tested for their ability to inhibit PARP-1, these novel derivatives showed high inhibitory potency. The unsubstituted derivative TIQ was selected for further characterization and found to be endowed with strong neuroprotective properties in models of cerebral ischemia.

Keywords: Neuroprotection; PARP; Inhibitors; Ischemia

1. Introduction

The poly(AD-Pribosyl)polimerase (PARP) family of enzymes catalyzes the post-translational modification of several nuclear proteins in response to DNA damages [1]. The family of PARP enzymes contains at least five members, termed PARP-1–PARP3, tankyrase, and VPARP. These five proteins share moderate to low sequence homology and their physiological functions are largely undetermined [2]. The by far best characterized member is PARP-1, a 113 kDa protein constituted by several domains, including a Zn-finger domain, a nuclear localization signal peptide, an automodification domain and a C-terminal catalytic domain, which contains the NAD^+ binding site.

Activated by DNA strand breaks, PARP-1 uses NAD^+ as substrate and promotes the elongation and branching of poly(ADP-ribose) covalently linked to the acceptor protein, including histones, p53, caspases and PARP itself, an event which alters the function of the target protein and which has functional relevance in processes related to DNA repair, genomic integrity and modulation of chromatin structure. Massive DNA damage, however, causes a hyperactivation of PARP-1 which results in a rapid depletion of the NAD^+ stores and, in succession, of the ATP reservoirs, leading ultimately to cell death (Fig. 1) [3].

Since the variety of the cellular events, known as excitotoxicity, induced by the interruption of local blood flow and mainly triggered by overactivation of glutamate receptors, results in the production of a variety of DNA-damaging species, and peroxynitrite in particular, it is conceivable that PARP-1 activation may play a key role in some form of neuronal loss following cerebral

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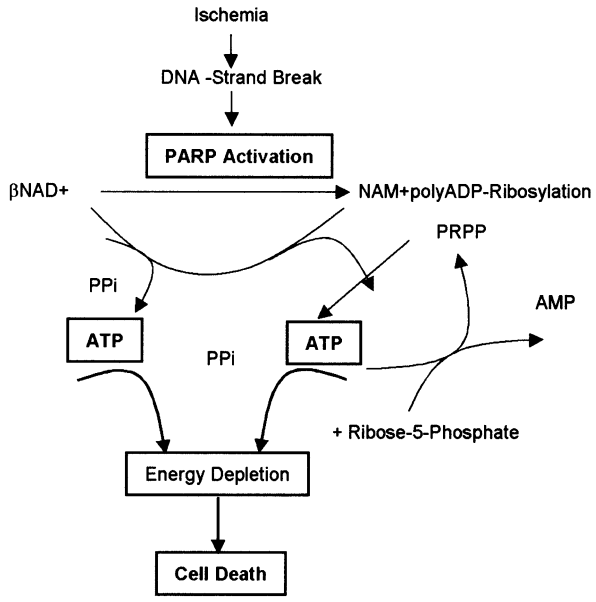


Fig. 1. Pathway of PARP-1 mediated excitotoxic death.

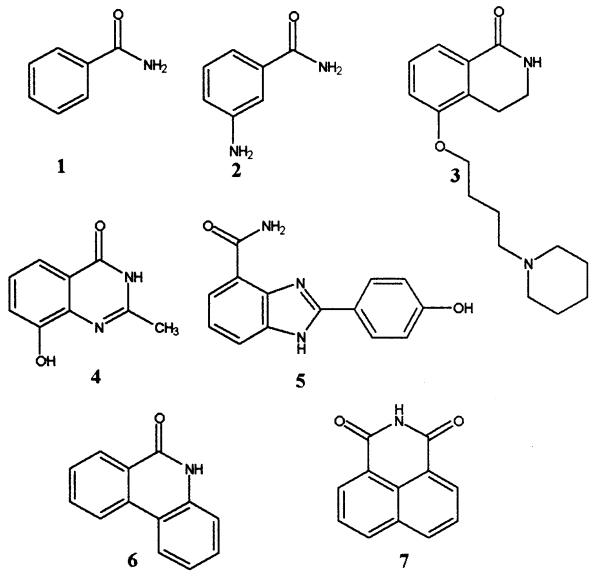


Plate 1.

ischemia [4]. Indeed, several studies have provided indications that PARP-1 inhibition is able to preserve neurons from the ischemia induced death, block the propagation of the penumbra area and result in significant neuroprotection [5]. Experiments with PARP-knocked out animals, furthermore, did not evidentiate the somehow expectable strong increment in tumor insurgence while confirming the decreased susceptibility to NMDA-induced excitotoxicity [6]. Taken together, these observations make the PARP family as a most promising target for the development of novel antiexcitotoxic agents with sustained therapeutic opportunity in ischemic insults.

A variety of NAD^+-competitive PARP-1 inhibitors have been reported and evaluated for their antitumoral or, more recently, antiexcitotoxic activities, (Plate 1); they include benzamides, such as benzamide itself (**1**) and 3-aminobenzamide (**2**) [7], 5-substituted dihydroisoquinolin-1-ones, among which 5-(4-piperidin-1-yl-butoxy)-3,4-dihydro-*2H*-isoquinolin-1-one (DPQ, **3**) has been used to characterize the role of PARP in excitotoxic insults [6a] 2,8-substituted quinazolin-4-ones (**4**) [7,8], benzimidazoles (**5**) [9], lactames such as 5*H*-phenanthridin-6-one (PND, **6**) and 1,8-naphtalamides (**7**) [7].

Furthermore, several high resolution crystallographic structures of the PARP-1 catalytic site complexed with inhibitors have been elucidated [10]. Inspection of these complexes reveals a conserved pattern of interactions, shared by all the available competitive inhibitors, which include: (i) a hydrogen bonding interaction between the required amido group of inhibitors and Gly863; (ii) a π–π interaction between an aromatic ring of inhibitors and Tyr907–Try896. Occasional SAR and QSAR studies have been carried out in the past aimed at understanding the structural basis of action of individual classes of inhibitors. More recently, we have reported a more thorough QSAR/docking study based on the analysis of as many as 46 competitive PARP-1 inhibitors. A relevant outcome of that work was the suggestion that high PARP-1 inhibitory potency could be achieved by increasing the total hydrophobic contacts between inhibitors and enzyme [11]. As a follow up of that previous study and in the frame of our continous activity in the field of medicinal chemistry of neuroprotection, we report here the synthesis and the preliminary evaluation of a series of 4H-thieno[2,3-*c*]isoquinolinon-5-ones, **8**–**10** (Plate 2) as novel PARP-1 inhibitors.

2. Chemistry

The preparation of this heterocyclic system has been not reported so far and naturally occurring products containing this heterocyclic system are unknown. In

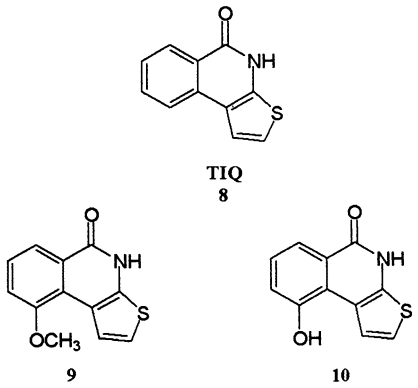
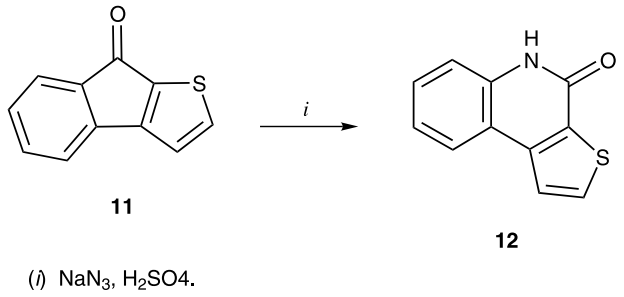


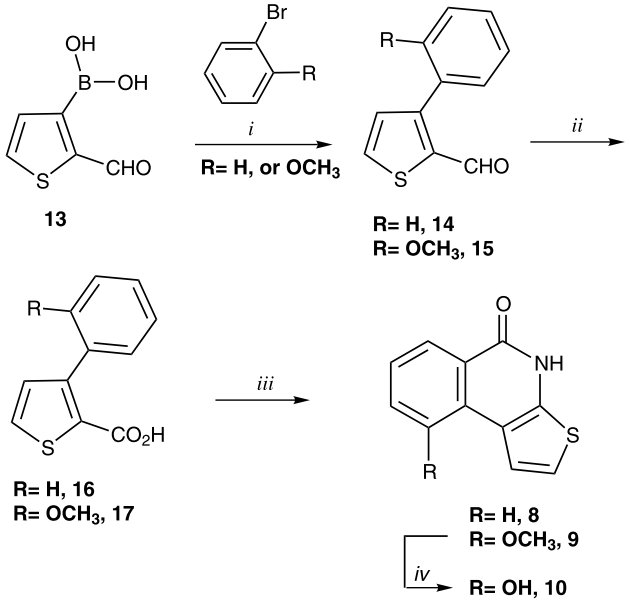
Plate 2.



Scheme 1.

1960, Arcus and Barrett [18], reported the Schmidt reaction of the 8*H*-indeno[2,1-*b*]thiophen-8-one (**11**) to give a single product derived from the ring-expansion. The next year was assigned to the compound the structure of the thieno[2,3-*c*]quinolin-4-one (**12**) deriving from a preferential migration of the phenyl ring with respect to the thienyl ring (Scheme 1 and Scheme 2) [19].

Our synthetic route was planned according to the sequence reported by Ito et al. [14] for the preparation of furo[2,3-*c*]isoquinolin-5-ones. Thus, reaction of 2-formylthiophene-3-boronic acid (**13**) with bromobenzene or 1-bromo-2-methoxybenzene in ethylenglicole dimethyl ether and aqueous sodium bicarbonate gave the corresponding 3-phenyl-2-thiophenecarbaldehyde (**14**) [12] and 3-(2-methoxyphenyl)-2-thiophenecarbaldehyde (**15**) in 68 and 75% yield, respectively. Jones oxidation of aldehydes **14** and **15** afforded the corresponding 3-phenyl-2-thiophenecarboxylic acid (**16**) [13] and 3-(2-methoxyphenyl)-2-thiophenecarboxylic acid (**17**) in 46 and 51% yield, respectively. Chlorination of **16** and **17** with thionyl chloride followed by treatment with sodium azide yielded the corresponding acylazide intermediate. The subsequent thermolysis via Curtius rearrangement in boiling *o*-dichlorobenzene [14], gave the desired derivatives 4*H*-thieno[2,3-*c*]isoquinolin-5-one (TIQ, **8**) and 9-methoxy-4*H*-thieno[2,3-*c*]-isoquinolin-5-one (MTIQ, **9**) in 44 and 79% yield, respectively. Finally, deprotection of the methoxy group of derivative **9** with boron tribromide yielded the corresponding compound 9-hydroxy-4*H*-thieno[2,3-*c*]-isoquinolin-5-one (HTIQ, **10**) in 81% yield.



Scheme 2.

3. Biology

The new derivatives **8**–**10** were evaluated as PARP-1 inhibitors by using bovine recombinant PARP-1. The results are reported in Table 1, along with those obtained with standard inhibitors such as DPQ (**3**) and PND (**6**) used as reference compounds. All new derivatives inhibited PARP-1; indeed, **8**–**10** have submicromolar IC_{50}s, and, in our testing condition, appear to be more potent than DPQ (**3**) or PND (**6**) used as reference compounds. Among the newly synthesized compounds, the more available TIQ (**8**) was then selected for further characterization and was tested in the middle cerebral artery occlusion (MCAO) model of transient focal ischemia in rats. Briefly, TIQ (**8**) was administered (3 mg/kg i.p.) immediately before and 2 h after the transient MCAO. When compared with control, animals treated with TIQ (**8**) at the above dosage showed a sustained reduction (ca. 40%) of the necrotic brain volume after ischemic insult (Fig. 2), thus confirming its potential as neuroprotective agent.

4. Molecular modeling

The analysis of the results of the enzyme inhibition assays (Table 1) revealed interesting features also from a chemical point of view. Indeed, derivatives **8**–**10** are potent PARP-1 inhibitors, with potencies up to 20-fold higher than DPQ (**3**) and PND (**6**) used as reference compounds. It is interesting to note that the replacement of the phenyl ring C of PND (**6**) by the thienyl ring as in **8** resulted in more than one order of magnitude gain in potency. In contrast, the introduction of a methoxy (**9**) or hydroxy (**10**) group, able in principle to form

Table 1
IC_{50} values obtained by enzymatic inhibition of bovine recombinant PARP-1

Comp.	IC50 (μM)
8	0.45±0.5
9	0.3±0.2
10	0.1±0.05
3 (DPQ)	2.2±0.1
6 (PND)	5.7±0.2

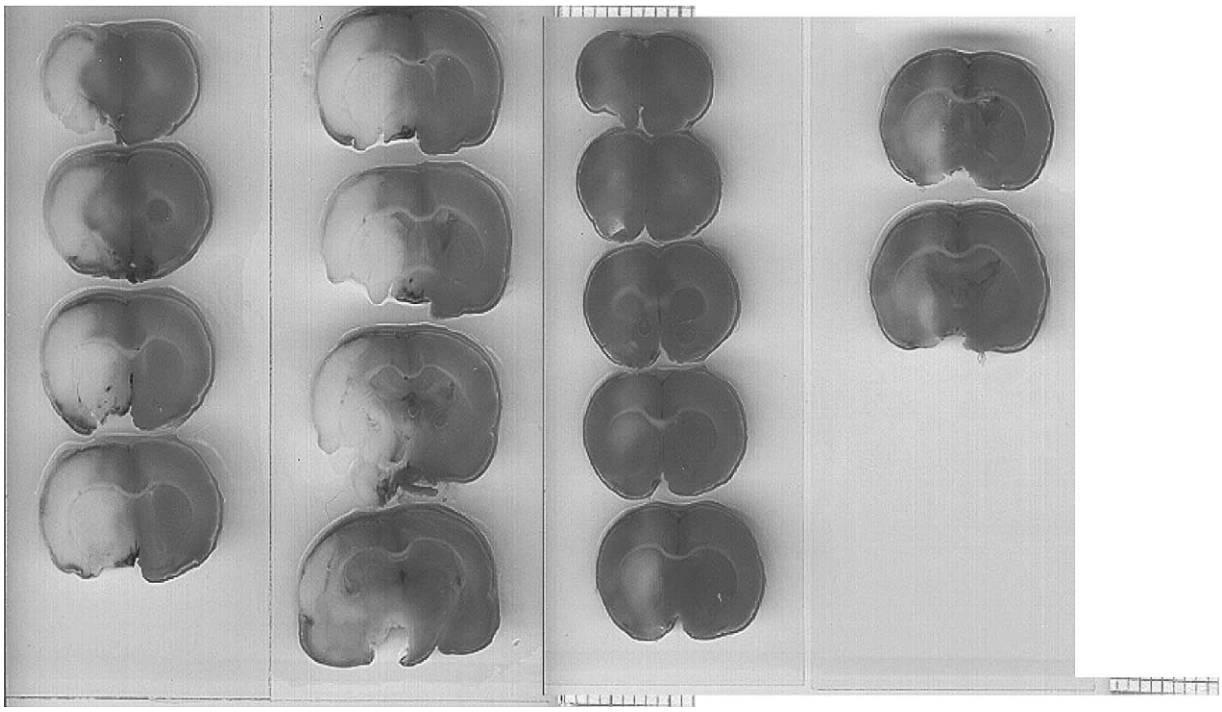


Fig. 2. Effect of TIQ-A (**8**) on the MCAO model of transient focal ischemia in rats. Left, vehicle; right, TIQ-A (**8**) (3 mg/kg i.p.).

hydrogen bonding, increased the potency with respect of the parent derivative **8** only of 1.5 or 3.5 times, respectively. In order to gain insights into the binding mode of the novel inhibitors, we have analyzed the docking of derivative **8** into the catalytic site of PARP-1. The results of the docking experiments, carried out by using standard setting of Autodock 3.0 and reported in Fig. 3, did not evidentiate any specific interaction between active site residues and the sulfur atom which can eventually account for the much higher potency of **8** versus **6**. A possible interaction is however noticed between the sulfur atom of **8** and a crystallized water (HOH77 in *2pax*). Obviously, this interaction cannot be present in **6**. Furthermore, in the best docking solution **8**, as well as **9** and **10**, had their planar, conjugated moiety sandwiched between two tyrosine residues. Since we have previously shown that this π–π interaction between the aromatic ring of the inhibitors and the two tyrosine Tyr907–Tyr863 can be a major driving force for binding [11], we have investigated, at the AM1 level of theory, the frontier orbitals and the molecular electrostatic potential in the case of PND (**6**) and **8**. The results of the computations are shown in Fig. 4, where it can be seen that **8** and **6** have a similar frontier orbital profile, although there is an inversion of symmetry in the case of the HOMO orbitals. It can be speculated that one of the effects of the thiophen ring is that of increasing the magnitude of the π-stacking interactions with Tyr863 and Tyr907.

5. Discussion

We have reported the novel of thieno[2,3-*c*]isoquinolinon-5-one derivatives **8**–**10** as PARP-1 inhibitors. Tested in vitro, all the new derivatives potently inhibited PARP-1, with IC50 values, in our conditions, lower than reference compounds such as **6** or **7**. The unsubstituted derivative **8** was chosen for a more detailed characterization, including evaluation in in vivo models of cerebral ischemia. In these experiments, **8** demonstrated to be endowed with marked neuroprotective properties, thus bringing additional support to the idea that silencing PARP-1 activity after ischemic insult may provide protection from excitotoxic neuronal death, at least when the necrotic component is predominant over the apoptotic one. The availability of X-ray structure of the catalytic domain of PARP-1 in its apo form and complexed with diverse inhibitors, coupled with our previous results in the field of molecular modeling of PARP-1 inhibitors [11], has allowed us to investigate the binding mode of the novel derivatives. The modest increase in potency observed in going from compound **8** to **10** indicated that the substituents in the 5-position is likely to not be involved in any specific interaction with the enzyme counterpart and this was indeed confirmed by the docking calculations. More challenging is the explanation for the higher activity of compound **8** versus its parent derivative **6**. Indeed, the substitution of the benzene ring by a thiophene moiety increased the

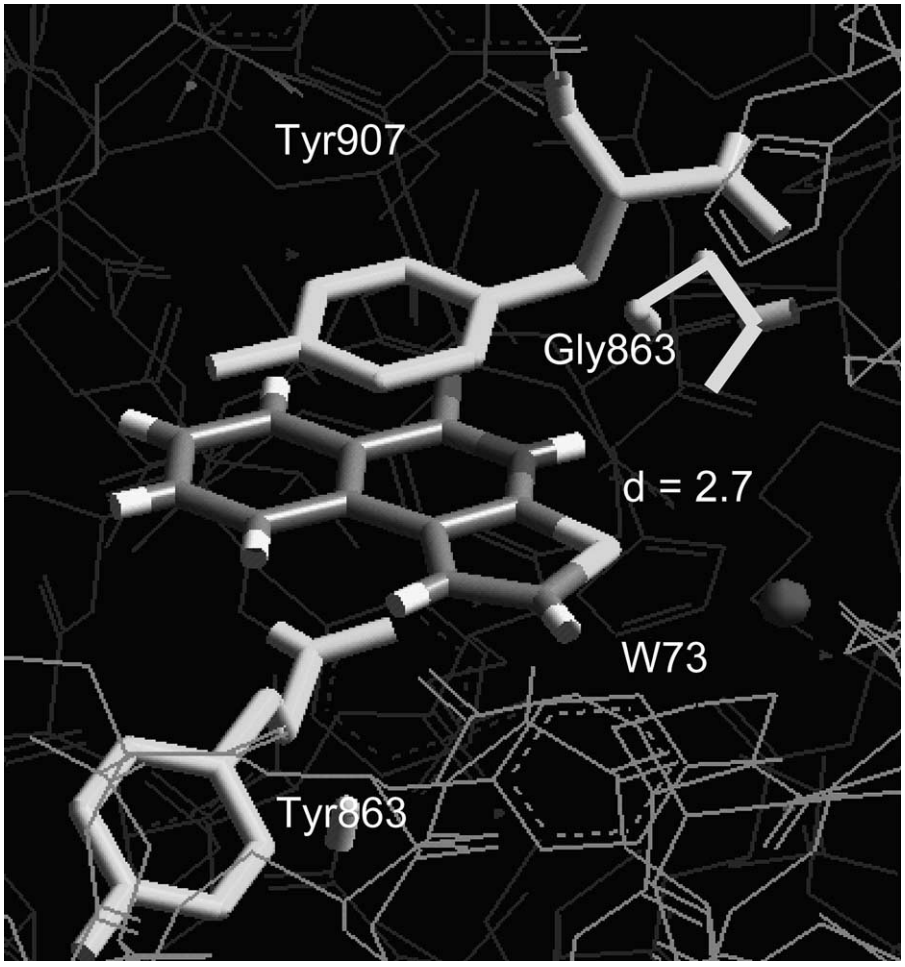


Fig. 3. Docking of **8** (left) into the active site of PARP-1 (PDB code: *2pax*). Relevant active site residues are colored in yellow, and water 73 which may interact with **8** is red colored.

activity of more than an order of magnitude. Again, the docking experiments did not evidentiate any particular interaction between the sulfur-containing ring and the enzyme which could eventually justify the increase in potency of **8** versus **6**. A possible explanation is that the thiophene ring interacts with the stacked tyrosine residues more favourably than the benzene ring. Whether short range interactions, such as charge transfer, or long range interactions, such as dispersion or electrostatic forces, are involved in such a π–π stabilization is presently unknown, although recent reports [15,16] indicate a crucial role for dispersion forces.

In conclusion, we have reported a series of novel PARP-1 inhibitors endowed with good potency and neuroprotective properties in model system of brain ischemia. In addition, our data indicate a peculiar behaviour of the thiophene ring compared with the benzene one, in a way that may be instrumental to extend the scope of this bioisosteric replacement.

6. Experimental

6.1. Chemistry

Melting points were determined with a Buchi 535 electrothermal apparatus and are uncorrected. NMR spectra were obtained with a Bruker AC 200 MHz spectrometer, while for compounds **8**–**10** a Bruker AC 400 MHz spectrometer was used. The abbreviations used are as follows: s, singlet; d, doublet; dd, double doublet; m, multiplet. TLC were carried out on pre-coated TLC plates with silica gel 60 F-254 (Merck). Flash column chromatography was performed on Merck silica gel 60 (0.040–0.063 mm). Elemental analyses were carried out on a Carlo Erba 1106 elemental analyzer.

6.2. 3-Phenyl-2-thiophenecarbaldehyde (*14*)

2-Formyl-3-thiopheneboronic acid (**13**) (0.89 g, 5.7 mmol) was added to a mixture of 2-bromobenzene (0.66

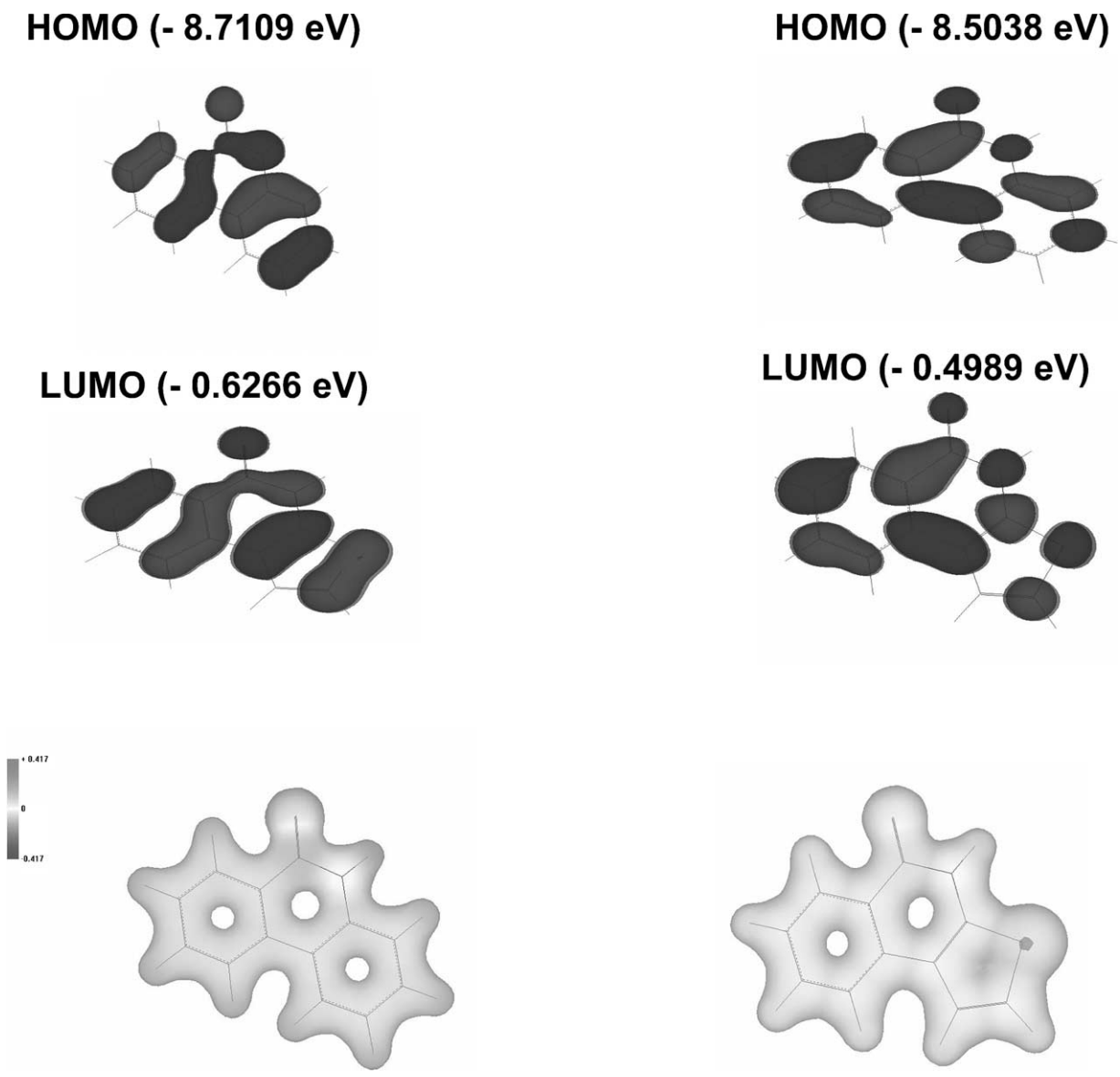


Fig. 4. Comparison between the frontier orbitals and the electrostatic potential of **6** (left) and **8** (right). HOMO and LUMO (with the respective energies) are plotted in the first two rows. Electrostatic potential is reported in the bottom row.

ml, 6.2 mmol), $(Ph_3P)_4Pd$ (197 mg, 0.15 mmol) in ethylenglicoledimethylether (20 ml). An acqueous solution of 2 M $NaHCO_3$ (6.3 ml) was successively added and the reaction mixture and was refluxed for 6 h. The mixture was cooled to room temperature (r.t.), the solvent was partially removed under reduced pressure and the resulted mixture was extracted with ethyl acetate (4×100 ml). The collected organic layer was washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure. The mixture was submitted to the flash chromatography, elution with light petroleum–ethyl acetate (95:5) afforded the compound **14** (0.74 g, 68% yield) as amorphous solid.

^{1}H NMR ($CDCl_3$): δ 7.19 (d, $J = 5.0$ Hz, 1H), 7.40–7.43 (m, 5H), 7.70 (d, $J = 5.2$ Hz, 1H), 9.83 (s, 1H).

*6.3. 3-(2-Methoxyphenyl)-2-thiophenecarbaldehyde (**15**)*

As described for **14**, 2-formyl-3-thiopheneboronic acid (**13**) (1.05 g, 6.7 mmol) was reacted with 1-bromo-2-methoxybenzene (0.9 ml, 7.35 mmol) thus obtaining derivative **15** (1.09 g, 75% yield).

^{1}H NMR ($CDCl_3$): δ 3.75 (s, 3H), 6.98 (m, 3H), 7.15 (d, $J = 5.0$ Hz, 1H), 7.21–7.35 (m, 3H), 7.65 (d, $J = 5.2$ Hz, 1H), 9.66 (s, 1H) as amorphous solid.

*6.4. 3-Phenyl-2-thiophenecarboxylic acid (**16**)*

Eight normality of Jones reagent (about 10 ml) was added dropwise to a solution of 3-phenyl-2-thiophe-

nealdehyde (**14**) (0.58 g, 3.1 mmol) in acetone (32 ml), until persistent orange colour. The mixture was stirred at r.t. for 18 h. Isopropyl alcohol (5 ml) was added to remove Jones reagent excess and then the mixture was filtered, the organic solvents were removed under vacuum, taken up with water (100 ml) and extracted with ethyl acetate (4 × 100 ml). The collected organic layer was washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure. The mixture was submitted to the flash chromatography, elution with chloroform–methanol (95:5) gave the compound **16** (0.44 g, 46% yield) as pure solid (m.p.: 200–202 °C).

^{1}H NMR ($CDCl_3$): δ 6.87–7.37 (m, 5H), 7.05 (d, J = 5.0 Hz, 1H), 7.53 (d, J = 5.0 Hz, 1H).

6.5. 3-(2-Methoxyphenyl)-2-thiophenecarboxylic acid (*17*)

As described for **16**, starting from 3-(2-methoxyphenyl)-2-thiophenecarbaldehyde (**15**) (0.6 g, 2.75 mmol) derivative **17** (0.32 g, 51% yield) was obtained as pure solid (m.p.: 141–143 °C).

^{1}H NMR ($CDCl_3$): δ 3.65 (s, 3H), 5.19 (s, 1H), 6.81 (m, 3H), 6.97 (d, J = 5.0 Hz, 1H), 7.11–7.24 (m, 3H), 7.45 (d, J = 5.2, 1H).

6.6. 4H-Thieno[2,3-c]isoquinolin-5-one (*8*)

Thionyl chloride (0.08 ml, mmol) was added to a solution of 3-phenyl-2-thiophenecarboxylic acid (**16**) (0.22 g, 0.68 mmol) in dry benzene (5 ml) and the mixture was refluxed for 2 h. The solvent and the excess of thionyl chloride were removed under vacuum. The residue was taken up using THF (3 ml), and NaN_3 (0.066 g, 1.02 mmol) in water (1 ml) was added quickly to this solution stirred at 0 °C. The mixture was stirred for 1 h at r.t. and poured into cracked ice and water (10 ml) and extracted with ethyl ether (4 × 10 ml), the collected organic layer was washed, dried (Na_2SO_4) and evaporated under vacuum. The residue was dissolved, in 2 ml of *o*-dichlorobenzene and then added dropwise to 8 ml of boiling *o*-dichlorobenzene under stirring. The mixture was further refluxed for 10 h, then was cooled, and evaporated under reduced pressure. The mixture was submitted to the flash chromatography, elution with chloroform afforded the derivative **8** (0.061 g, 0.3 mmol, 44% yield) as pure solid (m.p.: 266–268 °C).

^{1}H NMR (DMSO): δ 7.23 (d, J = 5.6 Hz, 1H), 7.51 (m, 1H), 7.70 (d, J = 5.3 Hz, 1H), 7.78 (m, 1H), 8.10 (dd, J = 7.4, 1.1 Hz, 1H), 8.25 (dd, J = 8.0, 0.9 Hz, 1H), 12.3 (s, 1H).

^{13}C NMR ($CDCl_3$): δ 116.4, 119.2, 120.6, 122.6, 123.3, 126.2, 128.4, 133.1, 134.1, 139.9, 163.2.

Elemental *Anal*. Calc. (%) for $C_{11}H_7NOS$: C, 65.65; H, 3.51; N, 6.96. Found: C, 66.03; H, 3.44; N, 6.81%.

6.7. 9-Methoxy-4H-thieno[2,3-c]isoquinolin-5-one (*9*)

As described for **8**, starting from 3-(2-methoxyphenyl)-2-thiophenecarboxylic acid (**17**) (0.6 g, 2.75 mmol) derivative **9** (0.78 g, 79% yield) was obtained as pure solid (m.p.: 198–201 °C).

^{1}H NMR ($CDCl_3$): δ 4.00 (s, 3H), 6.95 (d, J = 5.7 Hz, 1H), 7.20 (m, 1H), 7.44 (t, J = 8.0 Hz, 1H), 7.98 (d, J = 5.7 Hz, 1H), 8.13 (dd, J = 8.0 and 1.1 Hz, 1H).

^{13}C NMR (DMSO): δ 56.8, 114.7, 115.7, 116.1, 120.4, 124.3, 125.9, 126.3, 127.5, 155.7, 161.7.

Elemental *Anal*. Calc. (%) for $C_{12}H_9NO_2S$: C, 62.32; H, 3.92; N, 6.06. Found: C, 62.51; H, 3.99; N, 6.00%.

6.8. 9-Hydroxy-4H-thieno[2,3-c]isoquinolin-5-one (*10*)

BBr_3 1 M dichloromethane solution (7.2 ml, 7.2 mmol) was added to a solution of 9-methoxy-4*H*-thieno[2,3-*c*]isoquinolin-5-one (**9**) (0.74 g, 3.45 mmol) in dichloromethane (20 ml) and the reaction mixture was refluxed for 16 h. The mixture was cooled to r.t. and pored into ice and the resulted mixture was extracted with dichloromethane (4 × 50 ml). The collected organic layer was washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure. The mixture was submitted to the flash chromatography, elution with chloroform–methanol (90:10) gave the compound **10** (0.56 g, 81% yield) as pure solid (m.p.: 298–301 °C).

^{1}H NMR (CD_3OD): δ 7.01 (d, J = 5.7 Hz, 1H), 7.15 (dd, J = 7.8 and 1.1 Hz, 1H), 7.28 (t, J = 7.9 Hz, 1H), 7.82 (dd, J = 7.8 and 1.1 Hz, 1H), 8.05 (d, J = 5.7 Hz, 1H).

^{13}C NMR (CD_3OD): δ 116.4, 117.2, 117.5, 118.3, 123.3, 124.8, 125.6, 126.2, 153.4, 162.8, 172.3.

Elemental *Anal*. Calc. (%) for $C_{11}H_7NO_2S$: C, 60.82; H, 3.25; N, 6.45. Found: C, 61.03; H, 3.55; N, 6.13%.

7. Biology

7.1. PARP inhibitors enzymatic activity assay

PARP Inhibitors, at different concentrations, were incubated at 37 °C for 50 min, in a mixture reaction containing ~1.5 μg ml^{-1} PARP (Alexix Biochemicals, Cat. 202 042 C010, Specific Activity: ~1400 U mg^{-1}), 0.2 mg ml^{-1} DNA (Sodium Salt from Calf Thymus SIGMA, D-3664) and 0.002 mCi ml^{-1} ^{3}H-NAD (NEN, NET 443). PARP, DNA and ^{3}H-NAD were diluted in Working Buffer (20 mM $MgCl_2$, 100 mM Trizma-Base, 5 mM DTT). One hundred millimolar PARP Inhibitors stocks were in *N*,*N*-dimethyl-formamide and the following dilutions in H_2O/DMF (1:1). The reaction was performed in a final volume of 100 μl. After the incubation time, 50 μl of 50% ice cold TCA was added to each sample and left at 4 °C for 1 h, to precipitate

proteins. Samples were centrifuged at 14,000 rpm for 50 min. Surnatantes were removed and after washing two times with 1 ml of ddH_2O, pellets were resuspended in 500 μl of toluene and incubated at 37 °C for 1 h. CPMs were counted (3 min) after adding 4 ml of Liquid Scintillation Cocktail to each sample.

7.2. Middle cerebral artery occlusion (MCAO)

The infarction volume was measured 72 h after occlusion according to Cozzi et al. [17].

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