Newly synthesized xanthonic derivatives as P-glycoprotein Modulators – in silico and in vitro studies

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INTRODUCTION

P-glycoprotein (P-gp) is an ATP-dependent efflux pump with a vital role in the defense mechanism against toxic substrates, by significantly decreasing their absorption and distribution, and reducing their intracellular accumulation and, subsequently, their toxicity. Given its broad substrate specificity, its cellular polarized expression in many excractory and barrier tissues, and its great efflux capacity, P-gp can be faced as a potential antitropical pathway, when activated and/or induced.
P-gp activators have the advantage of increasing P-gp activity without interfering with P-gp protein expression, conferring a higher speed of action when compared with P-gp inducers.

AIM

Xanthones are a group known to interact with P-gp as potential modulators. Therefore, this study aimed to evaluate the induction of and/or activation potential of 6 newly synthesized xanthonic derivatives (Xs), and their capacity to protect Caco-2 cells against the cytotoxicity induced by paraquat, a toxic P-gp substrate.

MATERIALS AND METHODS

Caco-2 cells were incubated for 24 h with the tested Xs (0.50 μM), to evaluate their cytotoxicity and select a non-cytotoxic working concentration. Neutral Red (NR) uptake assay was used to evaluate the cell viability.
The effect of the tested xanthones on P-gp expression was evaluated by flow cytometry, using a P-gp monoclonal antibody (UCHC2) conjugated with phycoerythrin (PE).
P-gp activity was measured through two different protocols, both using Rhodamine 123 (Rh123) as a fluorescent P-gp substrate. In the first protocol, the accumulation of Rh123 was evaluated in Caco-2 cells previously exposed to the tested xanthones for 24 h, assessing to eventual alterations in P-gp activity due to the possible effects on P-gp expression caused by the xanthones. Alternatively, in the second protocol, the accumulation of Rh123 was evaluated in the presence of the tested xanthonic derivatives, allowing a direct detection of alterations in P-gp activity without affecting protein expression.
To evaluate Xs potential protective effects against toxic P-gp substrates, Caco-2 cells were exposed, for 4 h, to increasing PQ concentrations (0-10000 μM), in the presence or absence of the tested Xs (20 μM, non-cytotoxic concentration); the incubations were also performed with or without simultaneous exposure to a potent P-gp inhibitor (Elacridar, 10 μM), to assess P-gp involvement in the possible cellular protection conferred by the Xs. P-gp cytotoxicity was evaluated by the NR uptake assay.

In silico, a P-gp model was constructed and validated to obtain a 3D structure of human P-gp that could be used for the structure-based virtual screening of an in-house library of xanthones, in search for new potential P-gp modulators. Docking simulations between the validated P-gp model and the tested compounds were undertaken. These compounds were also mapped onto previously described P-gp induction and activation pharmacophores.

RESULTS

In vitro results

Xanthones cytotoxicity assays

Figure 1. Xanthones (10 μM) cytotoxicity in Caco-2 cells evidenced by the Neutral Red uptake assay 24 h after exposure.

P-gp expression

Figure 2. Flow cytometry analysis of P-gp expression levels in Caco-2 cells exposed to the tested xanthones (3, 10, 100 μM) for 24 h, (Figures A to E). (Xs: X1, X2, X3, X4, X6, X12).

Figure 3. Flow cytometry analysis of P-gp expression levels in Caco-2 cells exposed to the tested xanthones (3, 10, 100 μM) for 24 h, (Figures A to E). (Xs: X1, X2, X3, X4, X6, X12).

Figure 4. P-gp activity (Fig. 4 A) and cytotoxicity (Fig. 4 B) of the tested xanthones (3, 10, 100 μM) for 24 h, in Caco-2 cells (Xs: X1, X2, X3, X4, X6, X12).

Parataque (PQ) cytotoxicity (Fig. 5 A) and P-gp activity (Fig. 5 B) of Caco-2 cells incubated with 0, 10 μM PQ, with or without Xs (X1, X2, X4, X6, X12) for 24 h.

Parataque (PQ) cytotoxicity (Fig. 6 A) and P-gp activity (Fig. 6 B) of Caco-2 cells incubated with 0, 10 μM PQ, with or without Xs (X1, X2, X4, X6, X12) for 24 h.

Docking studies

Figure 7. Docking of X1 and X2 in the paraquat P-gp binding site as predicted by the Glide program (XP, X-ray). (Xs: X1, X2, X6, X12).

Figure 8. Docking of X1 and X2 in the paraquat P-gp binding site as predicted by the Glide program (XP, X-ray). (Xs: X1, X2, X6, X12).

In silico results

- X1 bound with P-gp 10M with the highest affinity (highly negative docking scores), presenting values of free energy more negative and equal than known P-gp inhibitors (mean -16.06 kcal/mol).
- Figure 7B reveals that the xanthonic derivatives have a better docking scores (such as X1, X2, X16, and X6), with potential of being P-gp modulators (inhibition and/or activation), although further investigation is needed to better understand the biological mechanism of action.

Pharmacophore for P-gp activators/inducers

- X16 was the molecule with the best fitting to pharmacophore (Fig. 6A, Table 1).
- The best matches were obtained for compounds X12, X2 and on pharmacophores V and V, respectively. Accordingly, X12, X6, and X16 also have potential of being P-gp activators.
- Compound X2 is predicted as being the most active P-gp inductor and/or activator, as it has the highest potential with a score of 2.99.

CONCLUSIONS

- As previously reported for other xanthonic derivatives, the newly synthesized xanthonic derivatives demonstrated to interact with P-gp, both in silico and in vitro.
- The obtained results proved that the tested xanthonic derivatives did not reveal any significant toxicity (0-50 μM) in Caco-2 cells, 24 h after exposure.
- Dose-response analysis of P-gp expression demonstrated that none of the tested xanthonic derivatives had a significant increase in P-gp expression.
- P-gp activity measured 24 h after the exposure to the tested xanthonic derivatives demonstrated that, although no increase in P-gp expression was observed, X1, X2, X6, and X12 significantly increased P-gp activity without interfering with P-gp protein expression, given the short incubation with the xanthonic derivatives, demonstrating a direct and rapid process.
- Consequently, some of the tested xanthonic derivatives have shown potential in the protection of Caco-2 cells against PQ-induced toxicity, highlighting X1, X2, X12, and X16. Furthermore, these Xs revealed to protect Caco-2 cells through a mechanism mediated by P-gp.
- Given the demonstrated in vitro potential of these xanthonic derivatives as P-gp activators, they can be faced as potential therapeutic approach to achieve a new formulation of toxic substrates, such as PQ, and represent a promising source of new derivatives with P-gp modulation ability that worths to be further explored.