European Journal of Medicinal Chemistry 101 (2015) 126–132

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

# The interaction of 4-thiazolidinone derivatives containing indolin-2-one moiety with P-glycoprotein studied using K562 cell lines

Feng Wang [a], Zijian Liu [b], Jian Wang [b], Jun Tao [c], Ping Gong [b], Xue Bao [d], Yanfang Zhao [b, **, 1], Yulin Wang [d, *, 1]

[a] Department of Interventional Radiology, The First Affiliated Hospital of Dalian Medical University, Dalian, 116021, China
[b] Key Laboratory of Structure-Based Drug Design and Discovery (Shenyang Pharmaceutical University), Ministry of Education, School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, China
[c] Department of Radiology, Hubei Cancer Hospital, 116 Zhuodaoquan Southern Road, Hongshan District, Wuhan, 430079, China
[d] Department of Parasitology, College of Basic Medical Sciences, Dalian Medical University, 9 South Lvshun Road Western Section, Dalian 116044, Liaoning, China

ARTICLE INFO

*Article history:*
Received 18 February 2015
Received in revised form
31 May 2015
Accepted 1 June 2015
Available online 5 June 2015

*Keywords:*
P-glycoprotein
4-Thiazolidinone derivatives
Cytotoxicity assay
Molecular docking

ABSTRACT

P-glycoprotein (P-gp) is an active drug efflux pump, which exists widely in various MDR tumor cells, conferring drug resistance to tumor cells during chemotherapy. Some 4-thiazolidinone derivatives containing indolin-2-one moiety are novel anti-tumor compounds. The aim of this study was to evaluate the transport activity of P-gp towards 4-thiazolidinone derivatives containing indolin-2-one moiety (as mixtures of 2Z, 5Z and 2E, 5Z isomers) and the transport inhibition activities of the derivatives to P-gp, the results of which could provide crucial information for the further separation of and development on the derivatives with excellent anti-tumor activities. The results indicate that the further separation and development should be focused on compounds 7, 10, 12 and 13 (tumor cell cytotoxic P-gp modulators) and compounds 8, 9, 17 and 18 (non-substrates of P-gp), which exhibit anti-tumor activities and could overcome P-gp mediated MDR. Furthermore, the results of molecular docking indicate that Ser222, a residue in TM4 domain of P-gp, exhibits an intriguing feature in that it interacts with all of the derivatives related with P-gp transport in a significant way, including both typical substrates and modulators of P-gp. Meanwhile, the compounds showing no interaction with Ser222 are mainly from the category of non-substrates of P-gp. Therefore, the interaction between Ser222 and the tested derivative would provide useful information for the further development on 4-thiazolidinone derivatives containing indolin-2-one moiety.



## 1. Introduction

Multidrug resistance (MDR) is defined as the simultaneous resistance to many structurally and functionally unrelated drugs, even to those to which the tumor cells have not been exposed previously [1]. As reported, most tumors developing MDR are often associated with the over-expression of permeability-glycoprotein (P-gp), a 170-kDa transmembrane protein discovered in 1976 [2,3]. It is firmly established that P-gp-mediated multi-drug resistance is a major obstacle to successful cancer chemotherapy [4]. P-gp acts as an active drug efflux pump at the membrane surface of tumor cells and confers drug-resistance to tumor cells by reducing the intracellular concentration of anti-tumor drugs [5]. Therefore, it is necessary to evaluate the transport activity of P-gp towards novel anti-tumor compounds during anti-tumor drug development. Furthermore, the data on the transport inhibition activity of a novel anti-tumor compound to P-gp could provide very important information for future rational combination therapy against tumors in clinical works as P-gp inhibitor anti-tumor drugs increase the efficacy of P-gp substrate anti-tumor drugs.

4-Thiazolidinone derivatives are known for their broad spectrum of biological activities, including anticancer activity [6–10]. Among them, MMPT and DBPT have been reported to have remarkable cytotoxicities against lung cancer cell line H460, and

* Corresponding author.
** Corresponding author.
*E-mail addresses:* yanfangzhao@126.com (Y. Zhao), wangyulin1971@126.com (Y. Wang).
[1] These two authors contribute equally to this work.

http://dx.doi.org/10.1016/j.ejmech.2015.06.002
0223-5234/

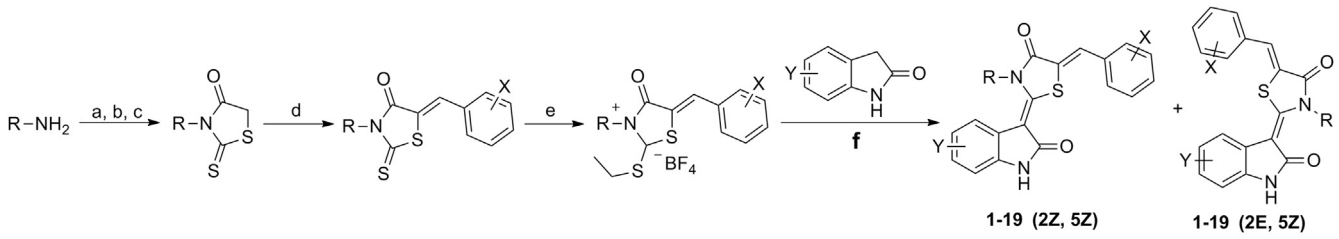
even some MDR cell lines, such as paclitaxel-resistantH460$_{taxR}$ and navelbine-resistant H460$_{VinR}$ [11–13]. Our group have synthesized a series of 4-thiazolidinone derivatives containing indolin-2-one moiety, which are designed and synthesized based on the idea that the combination of two privileged structures in one molecule leads to drug-like molecules [14]. Most of the prepared compounds exhibited significant antitumor activities against different human cancer cell lines including HT-29, H460, MDA-MB-231 and SMMC-7721. However, the interaction between P-gp and these derivatives still remains . The aim of this study was to evaluate the transport activity of P-gp to 4-thiazolidinone derivatives containing indolin-2-one moiety (as mixtures of 2Z, 5Z and 2E, 5Z isomers) and the transport inhibition activities of the derivatives to P-gp by cell (K562/S and K562/ADM) based cytotoxicity assay and P-gp mediated rhodamine 123 efflux inhibition assay, respectively. The K562/ADM subline expresses P-gp at the membrane surface at a high level, whereas the parent line (K562/S) does not [15]. 4-thiazolidinone derivatives would kill K562/S and K562/ADM by the same mechanism, while the $IC_{50}$ value of the tested derivative against K562/ADM would be higher significantly than that of against K562/S when it could be transported by P-gp. Therefore, the ratio between the $IC_{50}$ values of the tested derivative against K562/ADM and K562/S is an excellent standard for the evaluation of the transport activity of P-gp towards this derivative. In this study, the transport inhibition ratios of 4-thiazolidinone derivatives at different concentrations to P-gp were measured by P-gp mediated rhodamine 123 efflux inhibition assay. The $IC_{50}$ values were calculated by using sigmoidal plot. Our previous work shows that the $K_m$ value of P-gp transporting towards rhodamine 123 is 7.2 μM [16]. The $K_i$ values were then calculated using $IC_{50}$ values, $K_m$ value of P-gp transporting towards rhodamine 123 and rhodamine 123 concentration with the Cheng–Prusoff equation [17].

The results of cytotoxicity assay and P-gp mediated rhodamine 123 efflux inhibition assay indicate that 4-thiazolidinone derivatives containing indolin-2-one moiety show different types of interaction with P-gp, even with very similar molecular structures. Therefore, those derivatives could be classified into different categories, including P-gp substrates, non-P-gp substrates and P-gp modulators. AutoDock 4 was then used to identify the binding residues in P-gp with the derivatives from different categories. The results indicate that there is no significant difference amongst the binding energies between P-gp and derivatives from each category. However, Ser222, a residue in TM4 domain of P-gp, exhibits an intriguing feature in that it interacts with all of the derivatives related with P-gp transport in a significant way, including both typical substrates and modulators of P-gp. Meanwhile, the compounds showing no interaction with Ser222 are mainly from the category of non-substrates of P-gp. The homology modeled human P-gp structure used for docking represents the initial stage of the transport cycle of P-gp [3,18]. Combined with the research of the others [19,20], we proposed that Ser222 should act as one of the triggers for the initiation of the transport cycle of P-gp.

## 2. Chemistry

The preparation of target compounds **1**–**19** was described in Scheme 1 as reported in our previous article [14]. The indolin-2-ones were synthesized from appropriate anilines according to the reported procedures [21,22].

The 5-benzylidene-3-substitutedrhodanine intermediates **Ib** and **IIb** were synthesized by reaction of the primary amine *N,*$^{1}$*N*$^{1}$-dimethylethane-1,2-diamine or *N*$^{1}$,*N*$^{1}$-diethylpropane- 1,3-diamine and carbon disulfide under basic conditions followed by ring closure with chloroacetic acid and subsequent the intermediate **Ia** or **IIa** was subjected to Knoevenagel condensation with suitable benzaldehydes in refluxing ethanol. The *S*-ethylation of **Ib** and **IIb** with boron trifluoride diethyl etherate and triethyl orthoformate produced thiazolinium salts **Ic** and **IIc**, which were condensed with




| | | | |
|---|---|---|---|
| **1** | **R= diethylaminopropyl** | **X= 2-hydroxy** | **Y= 5-methyl** |
| **2** | **R= dimethylaminoethyl** | **X= 4-hydroxy** | **Y= H** |
| **3** | **R= diethylaminopropyl** | **X= 3, 4-dioxymethylene** | **Y= H** |
| **4** | **R= diethylaminopropyl** | **X= 3, 4-dioxymethylene** | **Y= 5-methyl** |
| **5** | **R= diethylaminopropyl** | **X= 3, 4-dioxymethylene** | **Y= 5-fluoro** |
| **6** | **R= diethylaminopropyl** | **X= 3, 4-dioxymethylene** | **Y= 6-fluoro** |
| **7** | **R= diethylaminopropyl** | **X= 3, 4-dioxymethylene** | **Y= 5-chloro** |
| **8** | **R= diethylaminopropyl** | **X= 3, 4-difluoro** | **Y= 5-fluoro** |
| **9** | **R= dimethylaminoethyl** | **X= 4-hydroxy** | **Y= 5-methyl** |
| **10** | **R= diethylaminopropyl** | **X= 2, 4-difluoro** | **Y= 6-fluoro** |
| **11** | **R= diethylaminopropyl** | **X= 3,4,5-trimethoxy** | **Y= H** |
| **12** | **R= diethylaminopropyl** | **X= 3,4,5-trimethoxy** | **Y= 5-methyl** |
| **13** | **R= diethylaminopropyl** | **X= 3,4,5-trimethoxy** | **Y= 5-fluoro** |
| **14** | **R= diethylaminopropyl** | **X= 3,4,5-trimethoxy** | **Y= 6-fluoro** |
| **15** | **R= diethylaminopropyl** | **X= 3,4,5-trimethoxy** | **Y= 5-chloro** |
| **16** | **R= dimethylaminoethyl** | **X= 4-hydroxy** | **Y= 5-fluoro** |
| **17** | **R= dimethylaminoethyl** | **X= 4-hydroxy** | **Y= 6-fluoro** |
| **18** | **R= dimethylaminoethyl** | **X= 3, 4-dioxymethylene** | **Y= H** |
| **19** | **R= diethylaminopropyl** | **X= 4-hydroxy** | **Y= 5-chloro** |

**Scheme 1.** Reagents and conditions:(a) $CS_2$, $Et_3N$, $Et_2O$, 25 °C, 0.5 h; (b) $ClCH_2COOH$, $K_2CO_3$, MeOH, $H_2O$, 25 °C, 7–8 h; (c) concd. $H_2SO_4$, pH = 4.0, 35–40 °C, 12 h; (d) Benzaldehyde, piperidine, EtOH, reflux 3–4 h; (e) $BF_3{\cdot}ET_2O$, $HC(OEt)_3$, 1,4-dioxane, 80 °C; (f) $Et_3N$, $CH_3CN$, 25 °C, 4h.

substituted indolin-2-ones in the presence of triethylamine to form target compounds **1**–**19**.

## 3. Results and discussion

### *3.1. Biological evaluation and molecular docking study on the interaction of 4-thiazolidinone derivatives containing indolin-2-one moiety (as mixtures of both isomers) with P-glycoprotein*

The transport activity of P-gp to 4-thiazolidinone derivatives containing indolin-2-one moiety and the transport inhibition activities of the derivatives to P-gp were evaluated by cell (K562/S and K562/ADM) based cytotoxicity assay and P-gp mediated rhodamine 123 efflux inhibition assay, respectively. $IC_{50}$ values of the derivative against K562, the $IC_{50}$ ratio between the $IC_{50}$ values of the tested derivative against K562/ADM and K562/S and the $K_i$ values of the derivatives against P-gp mediated rhodamine 123 efflux are shown in Table 1. The values of $IC_{50}$ are the mean of at least three independently performed experiments. Generally, inter-experimental variation was below 20%. The control of adriamycin, a representative substrate of P-gp, shows an $IC_{50}$ ratio of 6.75 (data not shown). AutoDock 4 and homology modeled human P-gp structure [18] were then used to study the interaction between P-gp and the derivatives. Meanwhile, rhodamine 123 and verapamil, the representative substrate and modulator of P-gp, are used as control compounds for the study on the interaction between P-gp and its substrates and modulators. The interaction between the derivatives and Ser222 within P-gp is shown in Table 1.

As Table 1 shows, 4-thiazolidinone derivatives containing indolin-2-one moiety exhibit multi-interaction types with P-gp, even with similar molecular structures. Based on the data of $IC_{50}$ ratio and $K_i$, the tested derivatives could be classified as typical substrates of P-gp (The compounds show $IC_{50}$ ratio values more than 2 and $K_i$ less than 2.23 μM. The compounds were labelled with the head note of "a"), typical modulators of P-gp (The compounds show $IC_{50}$ ratio values less than 1.3 and $K_i$ less than 2.23 μM. The compounds were labelled with the head note of "b") and typical non-substrates of P-gp (The compounds show $IC_{50}$ ratio values less than 1.3 and $K_i$ more than 2.23 μM. The compounds were labelled with the head note of "c") [23]. Meanwhile, compounds 2, 5, 6, 14 and 19 show $IC_{50}$ ratio values ranging from 1.32 to 1.98 and $K_i$ more than 2.23 μM. Therefore, those compounds were classified as weak substrates of P-gp. Compounds 1, 2, 3, 4, 5, 6, 11, 14, 15, 16 and 19 are of substrates of P-gp, which means those compounds could not overcome the MDR mediated by P-gp. Therefore, those compounds show no value for further development. Compounds 7, 10, 12 and 13 are of tumor cell cytotoxic P-gp modulators, which show $IC_{50}$ ratios ranging from 0.48 to 1.20 and $K_i$ ranging from 0.12 μM to 1.78 μM. Many anti-tumor drugs in clinical use are being challenged by MDR caused by multi-mechanisms [24]. P-gp mediated MDR is one of the major obstacles for tumor clinical chemotherapy. Therefore, a P-gp modulator anti-tumor drug will increase the efficacy of another anti-tumor drug with the property of P-gp substrate when they are administrated together to tumor patients. Thus, the four derivatives show a more promising future than the others at this point. Furthermore, there are still a few of the compounds which can not be transported by P-gp, nor can they inhibit P-gp mediated rhodamine 123 transport, such as compounds 8, 9, 17 and 18. Those derivatives that are of non-substrates of P-gp, also demonstrate a promising future for further separation and development.

Understanding the interaction mechanism between anti-tumor compounds and P-gp is crucial for the further optimization of these compounds. The molecular mechanism of drug transport by P-gp is still not well understood at present, due to the lack of a high resolution crystal structure for human P-gp. Molecular docking was performed to understand the interaction between 4-thiazolidinone derivatives containing indolin-2-one moiety and P-gp, which would provide useful information for the further optimization of those derivatives. The results indicate that there is no significant difference amongst the binding energies between P-gp and derivatives from each category (data not shown). However, Ser222, a residue in TM4 domain of P-gp [19], exhibits an intriguing feature in that it interacts with all of the derivatives related with P-gp transport in a significant way, including both typical substrates and modulators of P-gp. Furthermore, Ser222 even interacts with all of the weak substrates of P-gp, except for the 2Z, 5Z isomer of compound 19. Meanwhile, the compounds showing no interaction with Ser222 are mainly from the category of non-substrates of P-gp (only the 2Z, 5Z isomer of compound 19 is from the category of weak substrates of P-gp). Ser222 also could interact with rhodamine 123 and verapamil, the control compounds for substrate and modulator of P-gp, respectively, at preferred ligand docking clusters. Furthermore, the results of molecular docking also showed that most of the P-gp substrate and modulator derivatives could interact with Pro223, the neighbor residue of Ser222 within P-gp (data not show). This could mean that Pro223 may act as an assistant for Ser222 and could increase the interaction efficacy between P-gp and the derivatives, regardless of substrates or modulators. The representative results could be seen in Fig. 1 and Fig. 2.

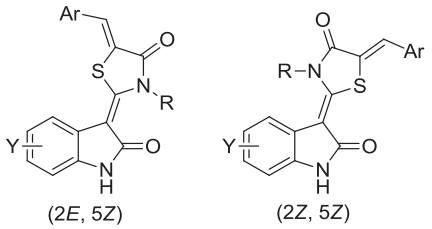
The homology modeled human P-gp structure represents the initial stage of the transport cycle of P-gp [3]. Furthermore, Ser222 locates at the entrance gate to the drug binding cavity of P-gp and is involved in the interaction between P-gp and its substrates [19]. The research of Loo and Clark also has confirmed that Ser222 could bind with verapamil, a representative modulators of P-gp [20]. Therefore, we proposed that Ser222 should act as one of the triggers for the initiation of the transport cycle of P-gp, which means that the binding of Ser222 with substrate will activate ATPase activity of P-gp and cause P-gp to undergo large structural changes to transport substrate outside of the cell. The initiation of P-gp transport should be independent with the following substrate translocation during the whole transport cycle. The modulators inhibit the transport activity of P-gp towards substrate by blocking the binding between the trigger residues within P-gp and substrate. However, they could not be transported by P-gp as they could not bind with the residues responsible for substrates translocation. Therefore, the molecular docking study based on the initial stage structure of P-gp only could be used to distinguish non-substrates of P-gp from those that could interact with P-gp, including both substrates and modulators of P-gp.

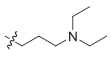
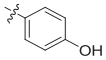
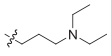
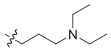
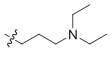
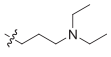
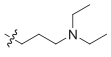
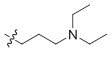
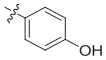
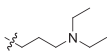
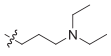
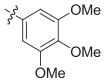
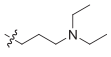
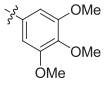
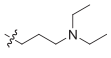
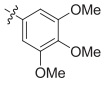
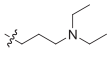
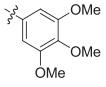
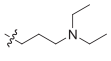
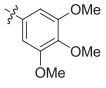
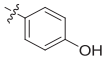
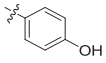
Accurate computational prediction of P-gp protein structure represents a good starting point for molecular docking study. The annotation of the function of Ser222 in P-gp has proven a successful homology model constructed by G.E. Jara. The issue of substrate translocation within P-gp should be addressed by the combination of biochemical experiment, molecular docking and molecular dynamics simulations.

## 4. Conclusions

In summary, 4-thiazolidinone derivatives containing indolin-2-one moiety exhibit multi-interaction types with P-gp, which would provide crucial information for the further separation and development on those derivatives. The further separation and development should be focused on compounds 7, 10, 12 and 13 (tumor cell cytotoxic P-gp modulators) and compounds 8, 9, 17 and 18 (non-substrates of P-gp). Ser222 may be acting as one of the triggers

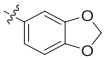
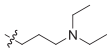
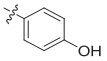
**Table 1**
Structures, interaction with Ser222, $IC_{50}$ values against K562/A, K562/S and $K_i$ values towards P-gp mediated Rho123 transport of compounds 1–19.




| # | R | Ar | Y | $IC_{50}$/μM | | $IC_{50}$ ratio | $K_i$/μM | Interaction with Ser222 | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | K562/A | K562/S | | | 2E, 5Z | 2Z, 5Z |
| 1[a] |  |  | 5-Me | 30.58 | 14.76 | 2.07 | 1.71 | + | + |
| 2 |  |  | H | 6.16 | 4.28 | 1.44 | >2.23 | + | + |
| 3[a] |  |  | H | 7.95 | 1.44 | 5.53 | 0.48 | + | + |
| 4[a] |  |  | 5- Me | 5.03 | 2.04 | 2.46 | 0.94 | + | + |
| 5 |  |  | 5-F | 12.52 | 7.38 | 1.70 | >2.23 | + | + |
| 6 |  |  | 6-F | 2.98 | 2.26 | 1.32 | >2.23 | + | + |
| 7[b] |  |  | 5-Cl | 5.05 | 4.86 | 1.04 | 1.78 | + | + |
| 8[c] |  |  | 5-F | 6.48 | 13.39 | 0.48 | >2.23 | – | – |
| 9[c] |  |  | 5-Me | 6.8 | 6.74 | 1.01 | >2.23 | – | – |
| 10[b] |  |  | 6-F | 4.12 | 3.69 | 1.12 | 1.01 | + | + |
| 11[a] |  |  | H | 6.65 | 2.58 | 2.58 | 0.73 | + | + |
| 12[b] |  |  | 5- Me | 2.02 | 4.17 | 0.48 | 0.32 | + | + |
| 13[b] |  |  | 5-F | 2.86 | 2.39 | 1.20 | 0.12 | + | + |
| 14 |  |  | 6-F | 1.64 | 1.18 | 1.39 | 0.47 | + | + |
| 15[a] |  |  | 5-Cl | 1.65 | 0.82 | 2.02 | 0.22 | + | + |
| 16[a] |  |  | 5-F | 6.14 | 1.30 | 4.73 | 1.34 | + | + |
| 17[c] |  |  | 6-F | 7.16 | 5.59 | 1.28 | >2.23 | + | – |

(*continued on next page*)

**Table 1** (*continued*)

| # | R | Ar | Y | $IC_{50}$/μM | | $IC_{50}$ ratio | $K_i$/μM | Interaction with Ser222 | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | K562/A | K562/S | | | 2E, 5Z | 2Z, 5Z |
| 18[c] |  |  | H | 0.78 | 1.32 | 0.59 | >2.23 | + | – |
| 19 |  |  | 5-Cl | 48.81 | 24.63 | 1.98 | >2.23 | + | – |

*Note*: Based on the data of $IC_{50}$ ratio and $K_i$, compounds with the head note of "a" were classified as typical substrates of P-gp, compounds with the head note of "b" were classified as typical modulators of P-gp, compounds with the head note of "c" were classified as typical non substrates of P-gp, and compounds without head note were classified as weak substrates of P-gp,.

within P-gp, which is responsible for the initiation of P-gp transport cycle towards 4-thiazolidinone derivatives containing indolin-2-one moiety. The interaction between the tested compound and Ser222 would provide important information for the further optimization of 4-thiazolidinone derivatives containing indolin-2-one moiety.

## 5. Experiment

### 5.1. Chemistry

The preparation of the target compounds **1**–**19** was achieved in 6 steps from commercially available $N^1$,$N^1$-dimethylethane-1,2-diamine or $N^1$,$N^1$-diethylpropane-1,3-diamine as shown in Scheme 1, which has been illustrated in detail in our previous study [14] (see also Supplementary information).

### 5.2. Cytotoxicity assay of 4-thiazolidinone derivatives containing indolin-2-one moiety against K562/S and K562/ADM

SRB assay was performed based on the method of Vichai and Kirtikara with minor modifications [25]. In brief, 100 μl of serially diluted tested compound was loaded into 96-well plates. Cells were seeded into 96-well plates at 6000 viable cells per well. Blank wells (no cells and no compounds) and control (no compounds) wells were set up. After 48 h incubation, the plates were centrifuged at the speed of 3000 rpm for 10 min. Afterwards, the medium was removed. The cells were fixed by addition of 100 μl of cold 16% trichloroacetic acid (TCA, 4 °C) in each well. The plate was incubated at 4 °C for 1 h before being gently washed for five times with tap water to remove TCA. The plates were air dried. Then, 50 μl of 0.4% (w:v) SRB dissolved in 1% acetic acid (v:v) was added to each well for 45 min. At the end of the staining period, unbound SRB was removed by washing it four times with 1% acetic acid. The plate was air dried again, and 100 μl of 10 mM Tris base solution (pH = 10.5) was added into each well to make the cell-bound dye soluble. The plate was shaken for 15 min on a gyratory shaker followed by reading the optical density (OD) at 570 nm in a microplate spectrophotometer. Survival rates were calculated as follows: Cell survival rate (%)=(T-B)/(C−B) *100%, T is the mean absorbance in the presence of a defined drug concentration, C is the mean absorbance of controls, and B is the mean absorbance of blanks. The $IC_{50}$ values were calculated by using sigmoidal plot.

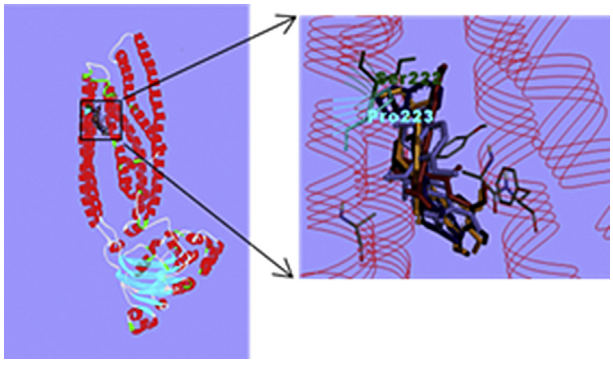



**Fig. 1.** Superimposition of the docking of compound 11 (substrate of P-gp, black), compound 12 (modulator of P-gp, gold), compound 18 (non-substrate of P-gp, blue), Rhodamine 123 (representative substrate of P-gp, dark red) and S-(-) Verapamil (representative modulator of P-gp, grey) in P-gp. Ser222 and Pro223 are labeled as green and cyan, respectively. All the three 4-thiazolidinone derivatives containing indolin-2-one moiety above are in the isomeric form of 2Z, 5Z. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

### 5.3. P-gp mediated rhodamine 123 efflux inhibition assay

250 μl of serially diluted tested compound in PRMI 1640 containing 17.84 μM of rhodamine 123 were loaded into 1.5 ml Eppendorf tubes. Then they were loaded with 250 μl of K562/A cells at a density of $1.2 \times 10^6$ cells/ml. At the same time, AR tubes (no tested compounds) and AVR tubes (AR tube containing 100 μM verapamil) were set up. All the tubes are duplicated for one test. Influx was stopped by centrifugation at 30 min, and the cells were then washed with ice-cold PBS containing verapamil 100 μM. Thereafter the cells were resuspended by 100 μl of ice-cold PBS containing verapamil 100 μM. The fluorescence was measured by a spectrofluorometer (Molecular Devices:GEMINI XPS) with an excitation wave length of 488 nm and an emission wavelength of 585 nm. Efflux inhibition rate was calculated as follows: Efflux inhibition rate (%)=($F_T$-$F_{AR}$)/($F_{AVR}$-$F_{AR}$) *100%, $F_T$ is the mean fluorescence with the presence of a defined tested compound concentration, $F_{AR}$ is the mean fluorescence of AR, and $F_{AVR}$ is the mean fluorescence of AVR. The $IC_{50}$ values were calculated by using the sigmoidal plot. Our previous work shows that the $K_m$ value of P-gp transporting towards rhodamine 123 is 7.2 μM. Then, the $K_i$ values were calculated using $IC_{50}$ values, $K_m$ value of P-gp transporting towards rhodamine 123 and rhodamine 123 concentration (8.92 μM) with the Cheng–Prusoff equation [17].

### 5.4. Molecular docking

AutoDock 4 was used to perform docking calculations. The homology modeled human P-gp structure by Jara et al. [18] was used in the docking calculations. Polar hydrogens were added and energy minimization was made employing both steepest descent and conjugate gradients protocols. Atomic solvation parameters and fragmental volumes for the proteins were assigned using the addsol utility in the AutoDock 4 program. Resides around rhodamine 123

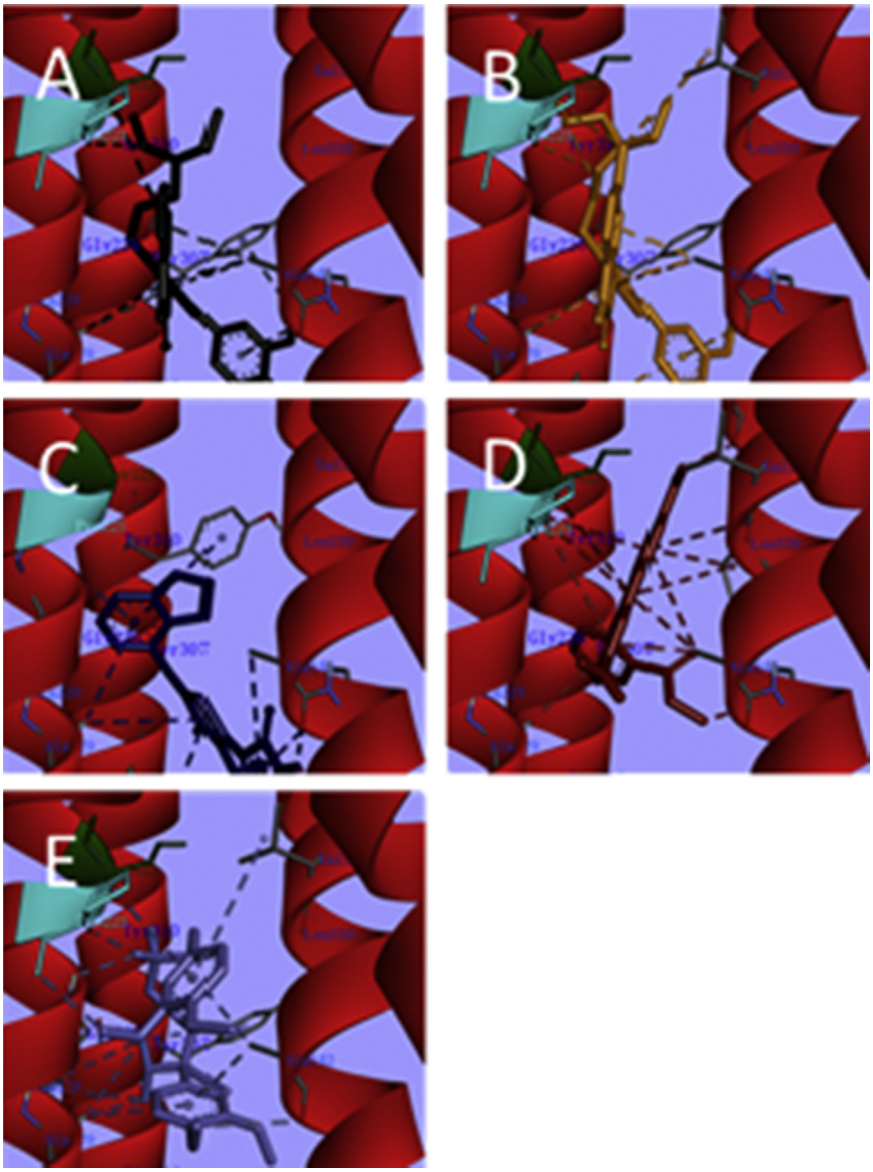



**Fig. 2.** Binding geometry of compound 11 (A), compound 12 (B), compound 18 (C), Rhodamine 123 (D) and S-(-) Verapamil (E) into the P-gp binding pocket and the interaction of the compounds with P-gp predicted by the Autodock docking algorithms. Ser222 and Pro223 are labeled as green and cyan, respectively. All the three 4-thiazolidinone derivatives containing indolin-2-one moiety above are in the isomeric form of 2Z, 5Z. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

binding site (R-site), including S222, F303, V338, L339, F343, were used to define binding pocket for docking. A 60 × 60 × 60 Å grid box with a grid spacing of 0.375 Å was generated for the receptor. Affinity grid fields were generated using the auxiliary program AutoGrid 4.

Ligand structures were built with Tripos Sybyl 6.9.1 software package, assigned charges using the Gasteiger–Hückel method and minimized with the Powell method (Tripos force field) to an energy gradient of 0.05 kcal/(mol•Å). Flexible torsions in the ligands were assigned and all dihedral angles were allowed to rotate freely.

The Lamarckian genetic algorithm (LGA) was used to find the appropriate binding positions, orientations, and conformations of the ligands. The optimized AutoDocking parameters are as follows: the maximum number of energy evaluations was increased to 25,000,000 per run; the iterations of Solis & Wets local search was 3000; the number of individuals in population was 300 and the number of generations was 100. Results differing by less than 2 Å in a positional root mean square deviation (RMSD) were clustered together. In each group, the lowest binding energy configuration with the highest % frequency was selected as the group representative. All other parameters were maintained as default. Accelrys Discovery Studio Visualizer 4.0 was used for graphic display.

## Acknowledge

This work was financially supported by the National Natural Science Foundation of China (No. 81202405), Program for Innovative Research Team of the Ministry of Education and program for Liaoning Innovative Research Team in University (No. IRT1073).

## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.06.002.

## References


[1] S.B. Kaye, The multidrug resistance phenotype, Br. J. Cancer 58 (1988) 691–694.
[2] R.L. Juliano, V. Ling, A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants, Biochim. Biophys. Acta 455 (1976) 152–162.
[3] S.G. Aller, J. Yu, A. Ward, Y. Weng, S. Chittaboina, R. Zhuo, P.M. Harrell, Y.T. Trinh, Q. Zhang, I.L. Urbatsch, G. Chang, Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding, Science 323 (2009) 1718–1722.
[4] F.J. Sharom, ABC multidrug transporters: structure, function and role in chemoresistance, Pharmacogenomics 9 (2008) 105–127.
[5] J.M. Ford, Experimental reversal of P-glycoprotein-mediated multidrug resistance by pharmacological chemosensitisers, Eur. J. Cancer 32A (1996) 991–1001.
[6] T. Tomasic, L.P. Masic, Rhodanine as a privileged scaffold in drug discovery, Curr. Med. Chem. 16 (2009) 1596–1629.
[7] A. Verma, S.K. Saraf, 4-thiazolidinone–a biologically active scaffold, Eur. J. Med. Chem. 43 (2008) 897–905.
[8] M.E. Welsch, S.A. Snyder, B.R. Stockwell, Privileged scaffolds for library design and drug discovery, Curr. Opin. Chem. Biol. 14 (2010) 347–361.
[9] I. Kucukguzel, G. Satilmis, K.R. Gurukumar, A. Basu, E. Tatar, D.B. Nichols, T.T. Talele, N. Kaushik-Basu, 2-Heteroarylimino-5-arylidene-4-thiazolidinones as a new class of non-nucleoside inhibitors of HCV NS5B polymerase, Eur. J. Med. Chem. 69 (2013) 931–941.
[10] M.O. Taha, M.A. Al-Sha'er, M.A. Khanfar, A.H. Al-Nadaf, Discovery of nanomolar phosphoinositide 3-kinase gamma (PI3Kgamma) inhibitors using ligand-based modeling and virtual screening followed by in vitro analysis, Eur. J. Med. Chem. 84 (2014) 454–465.
[11] F. Teraishi, S. Wu, J. Sasaki, L. Zhang, J.J. Davis, W. Guo, F. Dong, B. Fang, JNK1-dependent antimitotic activity of thiazolidin compounds in human non-small-cell lung and colon cancer cells, Cell. Mol. Life Sci. 62 (2005) 2382–2389.
[12] F. Teraishi, S. Wu, J. Sasaki, L. Zhang, H.B. Zhu, J.J. Davis, B. Fang, P-glycoprotein-independent apoptosis induction by a novel synthetic compound, MMPT [5-[(4-methylphenyl)methylene]-2-(phenylamino)-4(5H)-thiazolone], J. Pharmacol. Exp. Ther. 314 (2005) 355–362.
[13] S. Wu, W. Guo, F. Teraishi, J. Pang, K. Kaluarachchi, L. Zhang, J. Davis, F. Dong, B. Yan, B. Fang, Anticancer activity of 5-benzylidene-2-phenylimino-1, 3-thiazolidin-4-one (BPT) analogs, Med. Chem. 2 (2006) 597–605.
[14] S. Wang, Y. Zhao, G. Zhang, Y. Lv, N. Zhang, P. Gong, Design, synthesis and biological evaluation of novel 4-thiazolidinones containing indolin-2-one moiety as potential antitumor agent, Eur. J. Med. Chem. 46 (2011) 3509–3518.
[15] C. Loetchutinat, C. Saengkhae, C. Marbeuf-Gueye, A. Garnier-Suillerot, New insights into the P-glycoprotein-mediated effluxes of rhodamines, Eur. J. Biochem. 270 (2003) 476–485.
[16] Y. Wang, D. Hao, W.D. Stein, L. Yang, A kinetic study of Rhodamine123 pumping by P-glycoprotein, Biochim. Biophys. Acta 1758 (2006) 1671–1676.
[17] Y. Cheng, W.H. Prusoff, Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction, Biochem. Pharmacol. 22 (1973) 3099–3108.
[18] G.E. Jara, D.M. Vera, A.B. Pierini, Binding of modulators to mouse and human multidrug resistance P-glycoprotein. A computational study, J. Mol. Graph Model 46 (2013) 10–21.
[19] I.K. Pajeva, C. Globisch, M. Wiese, Comparison of the inward- and outward-open homology models and ligand binding of human P-glycoprotein, FEBS J. 276 (2009) 7016–7026.
[20] T.W. Loo, D.M. Clarke, Defining the drug-binding site in the human multidrug resistance P-glycoprotein using a methanethiosulfonate analog of verapamil, MTS-verapamil, J. Biol. Chem. 276 (2001) 14972–14979.
[21] A. Beauchard, H. Laborie, H. Rouillard, O. Lozach, Y. Ferandin, R. Le Guevel, C. Guguen-Guillouzo, L. Meijer, T. Besson, V. Thiery, Synthesis and kinase inhibitory activity of novel substituted indigoids, Bioorg. Med. Chem. 17 (2009) 6257–6263.
[22] L. Mologni, R. Rostagno, S. Brussolo, P.P. Knowles, S. Kjaer, J. Murray-Rust, E. Rosso, A. Zambon, L. Scapozza, N.Q. McDonald, V. Lucchini, C. Gambacorti-Passerini, Synthesis, structure-activity relationship and crystallographic studies of 3-substituted indolin-2-one RET inhibitors, Bioorg. Med. Chem. 18 (2010) 1482–1496.
[23] L. Chen, Y. Li, H. Yu, L. Zhang, T. Hou, Computational models for predicting substrates or inhibitors of P-glycoprotein, Drug Discov. Today 17 (2012) 343–351.
[24] G. Housman, S. Byler, S. Heerboth, K. Lapinska, M. Longacre, N. Snyder, S. Sarkar, Drug resistance in cancer: an overview, Cancers (Basel) 6 (2014) 1769–1792.
[25] V. Vichai, K. Kirtikara, Sulforhodamine B colorimetric assay for cytotoxicity screening, Nat. Protoc. 1 (2006) 1112–1116.