Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 14 (2006) 7183–7186
Bioorganic & Medicinal Chemistry

Studies of interactions between uracil-based hybrid molecules and P-glycoprotein—Search for multidrug resistance modulators

Palwinder Singh* and Kamaldeep Paul

Department of Chemistry, Guru Nanak Dev University, Amritsar 143005, India

Received 4 June 2006; revised 22 June 2006; accepted 23 June 2006
Available online 14 July 2006

Abstract—The hybrid molecules having structural features of anticancer drug, 5-fluorouracil, and MDR modulator, propafenone, have been studied for their interactions with P-glycoprotein (P-gp). Some of the molecules (**5**, **8**, and **9**) show considerable interactions with P-gp and could be the potential candidates for their in vivo evaluation as MDR modulators. Further investigations show the dependence of P-gp interacting properties of these compounds on their physico-chemical parameters like log *P* and total polar surface area.

1. Introduction

Drug resistance continues to be a serious threat to the successful chemotherapy of a disease.[1] The establishment of the P-glycoprotein[2] (P-gp), responsible for transporting the drug out of the cell, as the causative agent for multidrug resistance (MDR), has started the search for suitable molecules (MDR modulators[3]) which could interact with P-gp and help in reducing its drug transporting potency. In continuation with our efforts[4] to develop hybrid molecules by combining the features of the drug and the modulator, in the present investigations we have studied the interactions of uracil-based hybrid molecules[5] (**C**, Fig. 1) with P-gp. These molecules (**C**, Fig. 1) carry the structural features of 5-fluorouracil[6] (anti-cancer drug, **A**, Fig. 1) and propafenone[7] (MDR modulator, **B**, Fig. 1). Since both the individual molecules (**A** and **B**, Fig. 1) interact with P-gp, the structural hybrids of the two (**C**, Fig. 1) might show better interactions with P-gp and when used in combination with a drug will avoid the contact of drug with P-gp and help to decrease the P-gp-mediated efflux of the drug.

Some of these hybrid molecules (**C**, Fig. 1) show considerable interactions with P-gp and a correlation with their physico-chemical properties indicates the dependence of these interactions on log *P* and total polar surface area (TPSA) of the molecules.

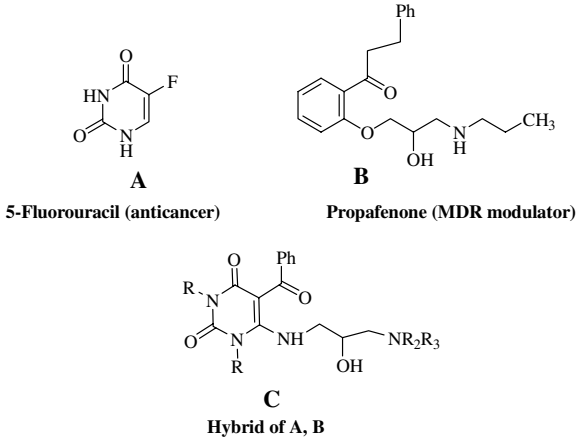


Figure 1.

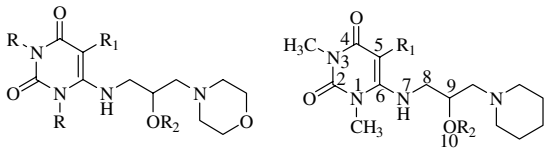
2. Results

2.1. Chemistry

The synthesis and characterization of the molecules (**C**, Fig. 1) under present investigation have been described in a recent report.[5] Compounds **1**–**9** (Fig. 2), with different substituents at N-1, N-3, C-5, and O-10, have been studied for their interactions with P-gp. The results of

Keywords: Multidrug resistance; P-Glycoprotein; Modulators; Hybrid molecules; Interactions; Physico-chemical properties.
* Corresponding author. Tel.: +91 183 2258802x3495; fax: +91 183 2258820; e-mail: palwinder_singh_2000@yahoo.com

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.06.060



1-6

1: R= CH_3, R_1 = R_2 = H;
2: R= CH_3, R_1= H, R_2 =COPh;
3: R= CH_3, R_1 = COPh, R_2 = H;
4: R= CH_3, R_1 = R_2 = COPh;
5: R= CH_2Ph, R_1 = R_2 = H;
6: R= CH_2Ph, R_1 = R_2 = COPh

7-9

7: R_1 = R_2 = H;
8: R_1 = COPh, R_2 = H;
9: R_1 = R_2 = COPh

Figure 2.

the interactions of these molecules with P-gp highlight the importance of the appropriate substituent at the suitable place in the molecule.

2.2. Biology

The nine molecules (Fig. 2), two MDR modulators (propafenone and verapamil), and two anti-cancer drugs (vinblastine and progesterone) were subjected to in vitro interactions with P-gp and evaluated in terms of the change in the basal activity of P-gp. These studies were carried out using 'Drug-P-glycoprotein' assay kit.[8] The kit contains the P-gp vesicles, prepared from highly resistant MDR cells, the DC-3F/ADX line, and permits to assess the interactions of the compounds with P-gp in terms of increase in the basal activity or decrease in the stimulated (verapamil, progesterone induced) activity of P-gp. In the present investigations, the modulation of basal ATPase activity of P-gp, measured by spectrophotometric method by continuous monitoring of ADP formation in the vesicle suspension medium, was studied. The basal ATPase activity of P-gp is its MgATP hydrolysis activity determined in the absence of any added drug. The interactions of the added compound (test compound) with P-gp result in the inhibition of ATPase activity of P-gp, which slows down the conversion of phosphoenolpyruvate (PEP) to pyruvate and further to lactate, and hence less conversion of NADH to NAD^+. Therefore, the wells (of the 96-well plate) with test compounds showing better interactions with P-gp have higher concentration of NADH in comparison to other wells in which compounds show less interactions with P-gp or wells without test compounds (basal activity of P-gp). As a result, the absorption of NADH at 340 nm, in the wells where compound-P-gp interactions are better, gets increased which is manifested as increase in the basal activity of P-gp. As per the manufacturer's specifications, a 30% increase (modulation) in the basal activity of P-gp, on the addition of a compound implies that the compound is interacting with P-gp.

3. Discussion

The results of the interactions of compounds **1**–**9** (Fig. 2) with P-gp in terms of the increase in basal activity of P-gp are given in Table 1. The MDR modulators, propafenone and verapamil, and the anticancer drugs, vinblastine and progesterone, have been taken for comparison.

The nine compounds evaluated for their interactions with P-gp differ from one another by the presence of CH_3/CH_2Ph groups at N-1, N-3; absence or presence of benzoyl group/s at C-5/O-10, and presence of either morpholine or piperidine moiety at the end of C-6 chain. Amongst compounds **1**, **5**, and **7** (without benzoyl group) only compound **5** with benzyl groups at N-1 and N-3 shows interactions with P-gp at 50 μM concentration. The results of interactions of compounds **2**, **3**, and **8** (with one benzoyl group either at C-5 or O-10) with P-gp indicate that along with the presence of benzoyl group, its position in the molecule and the nature of the cyclic tertiary nitrogen base at the end of C-6 chain are also important for exhibiting good interactions with P-gp. Compounds **2** and **3** differ from each other in the position of benzoyl group in the molecule and show considerably different interacting behavior toward P-gp. The presence of piperidine moiety in compound **8** in place of morpholine of compound **3** has remarkably increased the interactions of **8** with P-gp. The change of CH_3 groups present at N-1 and N-3 in compound **4** with benzyl groups in compound **6** has not made any improvement in the interactions of **6** with P-gp. However, replacement of morpholine moiety of **4** with piperidine in **9** has tremendously increased the interactions of compound **9** with P-gp. The studies of the interactions of these nine compounds with P-gp show that for a molecule to exhibit considerable interactions with P-gp, the correct combination of the substituents and their right placement in the molecule is very essential. Amongst the nine compounds studied here, compounds **8** and **9** exhibit appreciable interactions with P-gp at sub-micromolar concentration, while compound **5** shows interactions with P-gp at 50 μM concentration only. The compounds exhibiting better interactions at low concentrations do not show dose response as if the saturation of P-gp has taken place.

Therefore, the hybrid molecules obtained by mixing the structural features of 5-fluorouracil and propafenone exhibit interactions with P-gp somewhat better than those shown by MDR modulator propafenone and some of the anti-cancer drugs taken in the present studies (Table 1). Further tuning of the molecules could be made by modifying the substituents at the different positions of the central core of the molecule. Moreover, these studies point out that while mixing the structural features of two molecules in a single hybrid molecule, care must be taken for the correct placement of the substituents (present on the central core of individual molecules) onto the hybrid molecule. Compounds **8** and **9**, showing the best interactions with P-gp, when used in combination with a drug, will certainly avoid the contact of drug with P-gp because of their own preference for P-gp and could be the suitable candidates for MDR modulation.

The P-gp interacting behavior of these compounds was correlated with some of their physico-chemical properties, and it seems hydrophobicity/hydrophilicity of the

Table 1. Percentage increase of basal activity of P-gp by uracil derivatives at various concentrations

Compound	Percentage increase of basal activity of P-gp[a]		
	50 μM	5 μM	0.5 μM
1	−0.94	10	1
2	15	15	1.5
3	8	−0.29	9
4	19	−0.04	−0.31
5	31	6	3
6	22	4	6
7	6	−0.22	−0.01
8	32	29	30
9	36	33	31
Propafenone		31 (10^{-5} M)	
Verapamil		33 (6×10^{-5} M)	
Vinblastine		31 (10^{-5} M)	
Progesterone		34 (1.2×10^{-4} M)	

[a] A 30% increase in the basal activity of P-gp implies the interactions of the compound with P-gp.

Table 2. Calculated log *P* and TPSA of test compounds

Compound	log *P*	TPSA (Å^2)
1	−0.733	88.73
2	1.022	94.81
3	0.105	105.80
4	2.24	111.88
5	2.37	88.73
6	5.35	111.88
7	0.644	79.50
8	1.27	96.57
9	3.62	102.65

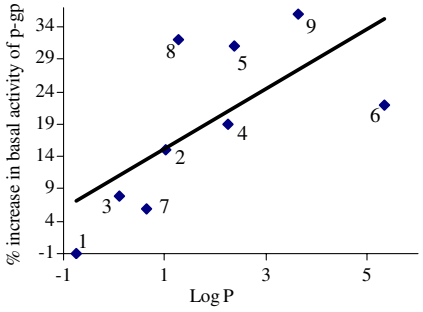


Figure 3. Graph between percentage increase in basal activity and log *P*.

molecules plays the key role for their interactions with P-gp. The calculated log *P* (partition coefficient, measure of hydrophobicity) and total polar surface area (TPSA)[9] (based on the H-donor/accepting sites in the molecule) values of these compounds are given in Table 2.

The relationship between the percentage increase of basal activity (at 50 μM) of P-gp by the compounds **1**–**9** and their log *P* values has been shown in Figure. 3. Compounds **1**, **3**, **7**, **2**, **4**, **5**, and **9** (in the increasing order of log *P*) exhibit a linear relationship between their P-gp interacting property and log *P* values. However, compounds **6** and **8** slightly deviate from the line. Taking log *P* as the only descriptor, a highly predictive equation for the activity of these compounds has been derived.

$$C = 8.61\log P + 4.89;\ r^2_{cv} = 0.84,\ r^2 = 0.92 \quad (1)$$

The graph between the percentage increase in basal activity of P-gp and TPSA of the molecules (Fig. 4) divides all the nine compounds into two groups. Compounds **7**, **5**, **8**, and **9** (with increasing order of TPSA) belong to one group and their interaction with P-gp increases linearly with increase in their TPSA. Compounds **1**, **2**, **3**, **4**, and **6** belong to the second group and they too exhibit a linear relationship between the two quantities and the deviations from linearity might be due to the more participation of hydrophobicity (log *P*) of the molecules in interacting with P-gp. The best interaction with P-gp has been observed for compound **9** with log *P* 3.62 and TPSA 102.65 Å^2.

Introducing the TPSA descriptor in Eq. 1, a slightly improved Eq. 2 with better predictive power has been obtained.

$$C = 8.7\log P - 0.05\mathrm{TPSA} + 9.6;\ r^2_{cv} = 0.86,\ r^2 = 0.93 \quad (2)$$

The activities of the compounds predicted from Eq. 2 show close resemblance with the experimental activities (Fig. 5).

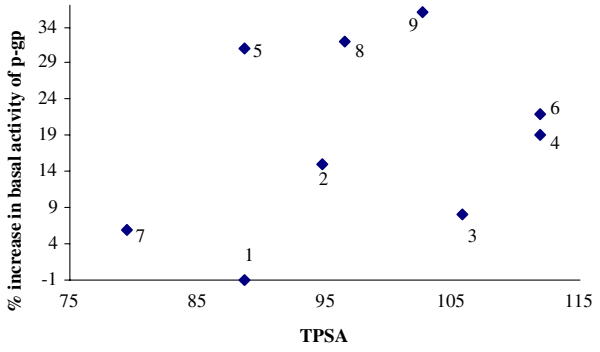


Figure 4. Graph between percentage increase in basal activity and TPSA. Compounds **5**, **7**, **8**, and **9** form one group, while compounds **1**, **2**, **3**, **4**, and **6** form the second group.

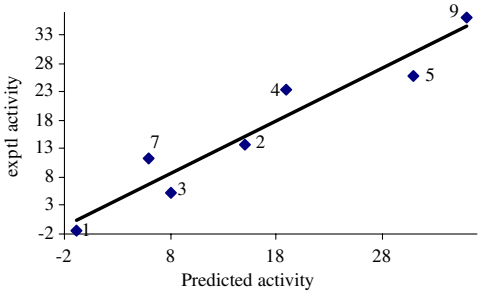


Figure 5. Graph between the activities (% increase in basal activity of P-gp on interaction with a compound) of compounds predicted from Eq. 2 and experimental activities.

Therefore, the two parameters viz. log *P* and TPSA seem to be collectively responsible for the interactions of compounds, studied here, with P-gp and a further refinement of the molecules, for developing more effective MDR modulators, could be made by making the suitable matching between these two physico-chemical properties of the molecules.

4. Conclusions

The hybrid molecules (**C**, Fig. 1) with structural features of anticancer drug, 5-fluorouracil, and MDR modulator, propafenone, have been evaluated for their interactions with P-gp. The identification of two molecules (**8** and **9**) with significant interactions with P-gp at submicromolar concentrations invites further exploration of the hybrid molecules as the potential candidates for the MDR modulation, an area which so far has received little attention. Moreover, the observations that P-gp interacting properties of hybrid molecules depend upon log *P* and TPSA of the molecules will be helpful in designing the new molecules with better MDR modulating properties.

5. Experimental

5.1. In vitro P-gp interaction studies

The bioassay for studying the interactions of the test compounds with P-gp was performed in duplicate. The 96-well plate and the reagents provided in the kit were stored at −20 °C and during the experiment, all of them were maintained at 4 °C in an ice bath. Besides the test compounds, the blank activity, non-specific activity, total activity, basal activity, and the activities of references (verapamil, propafenone, vinblastine, and progesterone) were also checked in duplicate. The blank wells contain 200 μl of enzymatic buffer; the non-specific activity wells have 120 μl of enzymatic buffer, 20 μl PK/LDH solution, 10 μl PEP solution, 10 μl NADH solution, 30 μl non-specific ATPase inhibitor solution, and 10 μl MgATP solution making the final volume 200 μl; the total activity wells contain 140 μl enzymatic buffer, 20 μl PK/LDH solution, 10 μl PEP solution, 10 μl NADH solution, and 10 μl each of membrane vesicles and MgATP solution making the final volume 200 μl; the basal activity wells have the same ingredients as in the non-specific activity wells but 10 μl of membrane vesicles and 110 μl enzymatic buffer; the filling of reference wells and the test wells is same as in basal activity wells except for the addition of 10 μl reference compounds in the reference wells and 20 μl of test compounds (of various concentrations) in the test well and accordingly the quantity of enzymatic buffer was decreased making the total volume 200 μl in each well. The plate was incubated for 20 min at 37 °C, shaken for 10 s, and read at 340 nm. The incubation and reading of the plate was repeated two times after an interval of 20 min. The average absorbance and the standard deviation were calculated. The activity was calculated from the mean value. The activity of the non-specific activity wells was subtracted from the activity of all other wells and the relative activity of each compound concentration was calculated in reference to the basal activity.

Acknowledgments

CSIR (01(1735)/02/EMR-II) and DST (SR/FTP/CS-20/2001), New Delhi, are gratefully acknowledged for financial assistance.

References and notes

1. (a) Teodori, E.; Dei, S.; Scapecchi, S.; Gualtieri, F. *Farmaco* **2002**, *57*, 385; (b) Krishna, R.; Mayer, L. D. *Eur. J. Pharmacol. Sci.* **2000**, *11*, 265.
2. Dantzig, A. H.; Law, K. L.; Cao, J.; Starling, J. J. *Curr. Med. Chem.* **2001**, *8*, 39.
3. (a) Kawasw, M.; Motohashi, N. *Curr. Drug Targets* **2003**, *4*, 31; (b) Hadjeri, M.; Barbier, M.; Ronot, X.; Mariotte, A.-M.; Boumendjel, A.; Boutonnat, J. *J. Med. Chem.* **2003**, *46*, 2125; (c) Langer, T.; Eder, M.; Hoffmann, R. D.; Chiba, P.; Ecker, G. F. *Arch. Pharm. Pharm. Med. Chem.* **2004**, *337*, 317; (d) Rosenbaum, C.; Rohrs, S.; Muller, O.; Waldmann, H. *J. Med. Chem.* **2005**, *48*, 1179; (e) Richter, M.; Molnar, J.; Hilgeroth, A. *J. Med. Chem.* **2006**, *49*, 2838.
4. Singh, P.; Paul, K.; Holzer, W. *Bioorg. Med. Chem.* **2006**, *14*, 5061.
5. Singh, P.; Paul, K.; Holzer, W. *Org. Biomol. Chem.* **2005**, *3*, 3958.
6. NIH database (NSC 19893).
7. Chiba, P.; Burghofer, S.; Richter, E.; Tell, B.; Moser, A.; Ecker, G. *J. Med. Chem.* **1995**, *38*, 2789.
8. The modulating activities of the compounds were evaluated using 'P-gp drug interaction' assay kit purchased from CEA, SPI-BIO mother company.
9. log *P* and TPSA of the molecules were calculated with software 'molinspiration,' www.molinspiration.com.