Bioorganic & Medicinal Chemistry 17 (2009) 2423–2427

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Search for MDR modulators: Design, syntheses and evaluations of N-substituted acridones for interactions with *p*-glycoprotein and Mg^{2+}

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ARTICLE INFO

Article history:
Received 9 December 2008
Revised 3 February 2009
Accepted 4 February 2009
Available online 8 February 2009

Keywords:
Multi drug resistance
p-Glycoprotein
Hybrid molecules
Acridone derivatives
Modulators

ABSTRACT

By combining the structural features of acridone based anti-cancer drugs (like amsacrine) and MDR modulator propafenone, acridones with hydroxyl amine chain at *N*-10 have been designed and synthesized. These molecules exhibit appreciable interactions with *p*-gp and Mg^{2+} indicating their suitability to modulate *p*-gp mediated multi drug resistance.

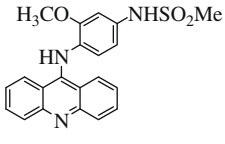
1. Introduction

Today, most drug therapies involve multiple agents or multiple target agents, as it is almost universally the case that single drugs or single-target drugs encounter resistance. Drug resistance (Multiple Drug Resistance, MDR)[1] which emanates due to the decrease in the intracellular drug concentration is a great hurdle in the successful practice of chemotherapy of various diseases like cancer, AIDS and even malaria. It is becoming a matter of great concern to develop such chemical entities (MDR reversers) which could maintain the chemotherapeutic level of the drug inside the cell by blocking *p*-glycoprotein (*p*-gp, transporter protein of the ABC family of drug transporters)[2–7] mediated efflux of the drug.

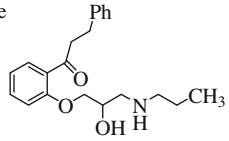
The planar, heterocyclic and considerably hydrophobic nature of acridone, making it to interact with several biomolecular targets, led to the investigations of a number of acridone derivatives for their anti-tumor,[8–10] anti-protozoan[11–13] and anti-viral[14] properties. Some of the acridone derivatives have also been studied for multi drug resistance (MDR) modulating[8,15,16] properties among which GF 120918 was chosen for phase I clinical trials.

For energy requirement, *p*-gp mediated drug efflux is linked with ATP hydrolysis for which Mg^{2+} plays the key role.[17–19] It was envisaged that the molecules interacting with *p*-gp, if also bind Mg^{2+}, could provide an extra advantage for modulation of *p*-gp mediated MDR via blockage of ATP hydrolysis and hence the energy supply to *p*-gp. Here, taking acridone as the heterocyclic moiety (present as the central core of a number of anti-tumor agents;[8] A, Fig. 1) and introducing hydroxylamine fragment (active part of MDR modulators;[20] B, Fig. 1) at its *N*-10 position, molecules C (Fig. 1) have been designed, synthesized and investigated for their interactions with *p*-gp and Mg^{2+} and therefore a multiple target approach has been adopted for modulating the functioning of *p*-gp.

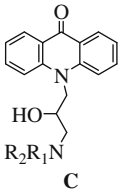
A parallelism has been observed between the modulation of basal activity of *p*-gp by these molecules and the extent of their inter-



A
Amsacrine - Anticancer



B
Propafenone - MDR modulator



C

Figure 1.

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doi:10.1016/j.bmc.2009.02.002

actions with Mg^{2+}. Further insight into the nature of interactions between the acridones **C** and *p*-gp was explored by the dockings of these molecules in the ATP binding site of *p*-gp.

2. Results

2.1. Chemistry

The synthesis of the target molecules have been achieved from the commercially available materials. The Ullmann condensation of *o*-chlorobenzoic acid and aniline provided the acridone skeleton of the molecule. Treatment of acridone **1** with NaH in DMSO followed by stirring with epichlorohydrin gave N-substituted acridone **2**. NMR and mass spectral data confirmed the formation of this compound. Irradiating an equimolar mixture of acridone **2** and pyrrolidine (solventless conditions) in microwave oven for 5 min resulted in the formation of compound **3** (86%) and likewise the reactions of acridone **2** with other amines provided compounds **4**–**8** (74–84%) in 5–7 min (Scheme 1). Therefore, epoxy ring opening with secondary amines under microwave irradiations partially provides a green approach to the synthesis of target compounds.

2.2. Biology

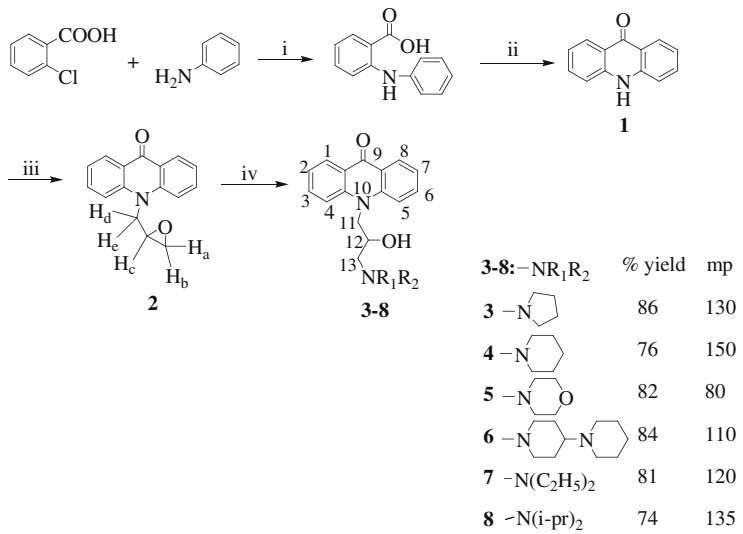
The interactions of compounds **3–8** with *p*-gp were studied using 'Drug–*p*-glycoprotein Interaction' assay kit which contains the *p*-gp vesicles prepared from highly resistant MDR cells, the DC-3F/ADX line. The interactions of compounds with *p*-gp are assessed in terms of modulation of basal activity (MgATP hydrolysis activity in the absence of drug) of *p*-gp measured by spectrophotometric method by continuous monitoring of ADP formation in the vesicle suspension medium. The interactions of added compound (test compound) with *p*-gp result in the inhibition of ATPase activity of *p*-gp—slowing down of conversion of phosphoenolpyruvate to pyruvate and slow formation of lactate. This will decrease the conversion of NADH to NAD^+ and hence higher absorption at 340 nm (due to NADH). Therefore, the absorption of NADH at 340 nm, in the wells (96 well plate) where compound–*p*-gp interactions are better is higher which is manifested as increase in the basal activity of *p*-gp. Compounds are tested for their interactions with *p*-gp at 0.5 μM, 5 μM and 50 μM concentrations making final concentrations as 0.05 μM, 0.5 μM and 5 μM (after dilutions in the wells). Two MDR modulators propafenone, verapamil and two anti-cancer drugs vinblastine, progesterone are taken for comparison.

3. Discussion

As per the manufacturer's specifications for the 'drug–*p*-gp interactions' assay kit, a 30% increase in the basal activity of *p*-gp, on the addition of a compound implies that the compound is interacting with *p*-gp. It is evident from the data given in Table 1 (Fig. 2), compounds **3–7** exhibit appreciable interactions with *p*-gp. Six compounds evaluated in the present investigations for their interactions with *p*-gp differ from one another by the nature of amine group present at the end of *N*-10 substituent. Compounds **3**, **4** and **7** with respectively pyrrolidine, piperidine and diethyl moiety at the end of *N*-10 chain show better interactions with *p*-gp in comparison to compounds **5**, **6** and **8**. Compound **4** with 44% increase in the basal activity of *p*-gp shows the best interactions with *p*-gp followed by compounds **3** and **7**. Compounds **3**, **4** and **7** exhibit significant interactions with *p*-gp even at 0.05 μM concentration. It seems as if an optimum value of log*P* (~2) for compounds **3**, **4** and **7** (1.73, 2.14 and 1.98, respectively) contributes towards the better interactions of these compounds with *p*-gp. Compounds **5**, **6** and **8** with respective log*P* values 1.08, 2.56 and 2.58 exhibit less interactions with *p*-gp. Moreover, the interactions of compounds **3** and **4** with *p*-gp are similar as exhibited by the known MDR modulator propafenone and better than some of the anti-cancer drugs taken in the present investigations. Therefore, these results support the design of acridones **3–8** and also identify compounds **3** and **4** as suitable leads for their development into MDR modulators.

Since sequestering of Mg^{2+} could result in slowing down of ATP hydrolysis and hence the supply of energy to *p*-gp during drug effluxing, the new designed acridones were investigated for their interactions with Mg^{2+} with the help of UV spectral studies. Compounds **3–8** at 10^{-4} M concentrations (prepared in HEPES buffer (10^{-2} M) at pH 7.2) were titrated with Mg^{2+} solutions (0–0.5×10^{-4} M). All these compounds exhibit a hyperchromicity in the region 395–405 nm on addition of Mg^{2+} solution (Fig. 3) with a concomitant hypochromicity in the region 320–330 nm.

The association constants of compounds **3–8** with Mg^{2+} (Table 2) indicate the extent of their bindings. Compound **3**, **4**, **6**, **7** and **8** show appreciable interactions with Mg^{2+}. Compound **4** (which also shows best interaction with *p*-gp) exhibits strongest binding



3-8: $-NR_1R_2$	% yield	mp
3 –N⟨ ⟩	86	130
4 –N⟨ ⟩	76	150
5 –N⟨ O ⟩	82	80
6 –N⟨ ⟩N⟨ ⟩	84	110
7 $-N(C_2H_5)_2$	81	120
8 $-N(i\text{-}pr)_2$	74	135

Scheme 1. Reagents and reaction conditions: (i) K_2CO_3, CuO, reflux; (ii) concd H_2SO_4, heat; (iii) NaH, DMSO, epichlorohydrin, 60–70 °C; (iv) NHR_1R_2, MWI, 5–7 min.

Table 1
Percentage increase of basal activity of *p*-gp by compounds **3–8**

Compound	Percentage increase of basal activity of *p*-gp		
	5 (μM)	0.5 (μM)	0.05 (μM)
3	38	34	31
4	44	40	33
5	30	27	24
6	31	32	25
7	36	32	29
8	28	21	18
Propafenone	40	31	—
Verapamil	33	30	—
Vinblastine	35	31	—
Progesterone	34	30	—

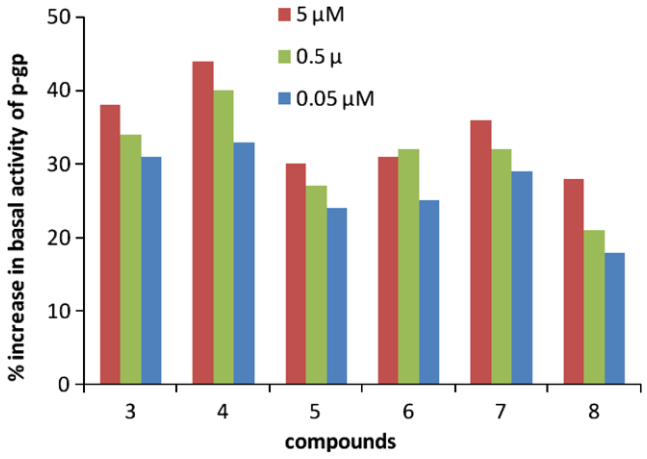


Figure 2. Percentage increase in basal activity of *p*-gp on interaction with compounds **3–8**.

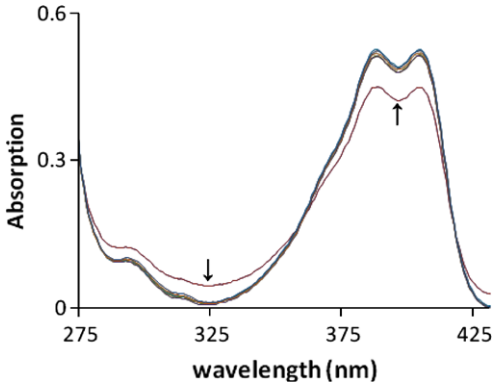


Figure 3. Absorption spectra of compound **3** in the presence of increasing concentration of Mg^{2+} (0–0.5 × 10^{-4} M). Arrows denote the change in absorption with increasing concentration of Mg^{2+}.

Table 2
Association constants for Mg^{2+} binding in HEPES buffer (M^{-1})

Compound	K_a
3	8.3×10^4
4	1.08×10^5
5	6.8×10^3
6	8.3×10^4
7	2.2×10^4
8	2.22×10^4

with Mg^{2+} (K_a 1.08 × 10^5 M^{-1}). Small differences in the bindings of these compounds with Mg^{2+} are almost in the same trend as observed in their interactions with *p*-gp except in compound **6** which interacts with *p*-gp weakly irrespective of its appreciable binding with Mg^{2+}.

Therefore, these investigations viz. *p*-gp interaction studies and Mg^{2+} binding studies indicate the potential of the acridones **3–8**, especially compounds **3** and **4**, to act as MDR modulators. Parallel trends of the results of both these investigations indicate the possibilities of modulations of *p*-gp activities by these molecules through Mg^{2+} binding along with their interactions with *p*-gp.

To get further insight into the nature of interactions between the acridones and *p*-gp and to supplement the experimental results, dockings[21] of acridones **3–8** in the ATP binding site of *p*-gp were performed. The crystal structure of *p*-gp in complexation with ATP and ADP was taken from protein data bank (pdb ID 1MV5) and refined for docking studies. ATP molecule is bound to *p*-gp through H-bonds between its phosphate residue and S383, L382, G381 and G380 amino acids of *p*-gp. The adenine moiety of ATP is present in a parallel position to the phenyl ring of Y352, at a distance of 4.12 Å, sufficiently close to exhibit π–π interactions. Docking programme was validated by docking ATP in the binding site of *p*-gp (Fig. 4) where a close overlapping between the docked ATP (ATP1) and one present with the crystal of *p*-gp (ATP) was observed.

Since the drug binding site of *p*-gp is near to the ATP binding site (cavity between the intracellular binding domain and nucleoside binding domain),[22] we have taken 5 Å around ATP as the binding pocket of *p*-gp for the docking purpose. Dockings of compounds **3–8** in the binding site pocket of *p*-gp indicate that all these molecules are held in the binding site through H-bond and π–π interactions between the acridones and amino acid residues (Fig. 5). Compounds **3–5** show H-bonds with Y393 through their carbonyl group. The acridone moiety of compounds **3–8** exhibit π–π interactions with Y352. However, compounds **6** and **8**, after docking in *p*-gp are placed in a position parallel to ATP. Therefore, the docking studies also support the better interactions of compounds **3** and **4** with *p*-gp due to their H-bondings with active site amino acid residues.

4. Conclusions

In conclusion, we have synthesized the rationally designed acridone derivatives following a convenient synthetic methodology. The investigations of these molecules for their interactions with *p*-gp and Mg^{2+} have identified compounds **3** and **4** as suitable candidates for *p*-gp mediated MDR modulation. Moreover, these stud-

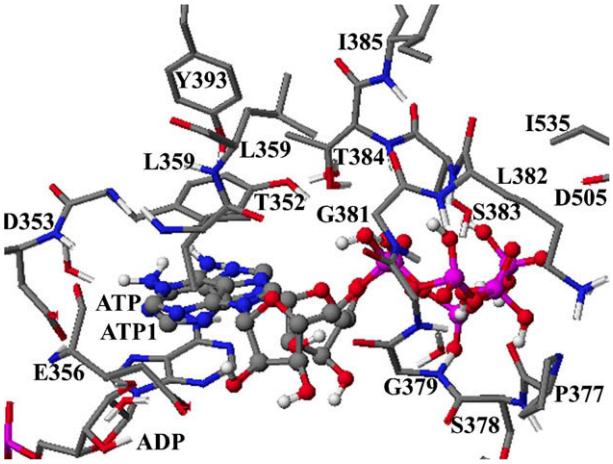


Figure 4. Validation of docking programme. ATP1 (ATP docked in the ATP binding site of *p*-gp) closely overlaps with the ATP molecule present in the crystal structure of the protein. Hs' are suppressed for clarity.

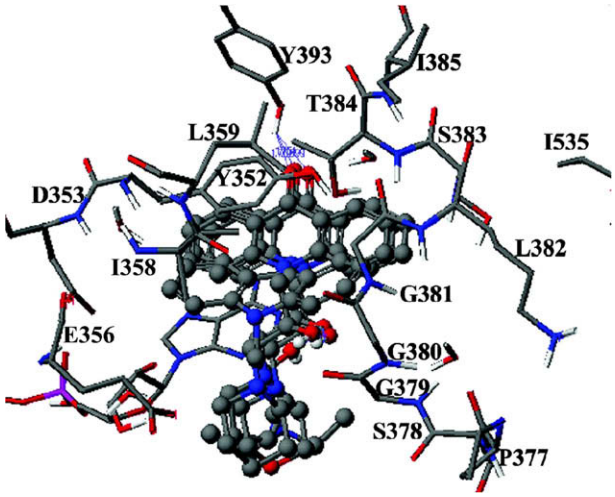


Figure 5. Compounds **3**, **4**, **5** and **7** docked in the binding site pocket of *p*-gp. H-bonds between the carbonyl oxygens of **3**, **4**, **5** and O**H** of Y393 are visible. Hs' are suppressed for clarity.

ies show that Mg^{2+} sequestering behavior of these compounds along with their interactions with *p*-gp could prove as an appropriate approach for developing multiple target agents as MDR modulators.

5. Experimental

Melting points were determined in capillaries and uncorrected. ^1H and ^{13}C NMR spectra were run on JEOL 300 MHz and 75 MHz NMR spectrometer respectively using $CDCl_3$ as solvent. Chemical shifts are given in ppm with TMS as an internal reference. *J* values are given in hertz. Chromatography was performed with silica 100–200 mesh and reactions were monitored by thin layer chromatography (TLC) with silica plates coated with silica gel HF-254. In ^{13}C NMR spectral data, +ve, −ve terms correspond to CH_3, CH, CH_2 signals in DEPT-135 NMR spectra.

5.1. 10-Oxiranylmethyl-10*H*-acridin-9-one (2)

Acridone **1** (1 mmol) was treated with NaH (1.2 mol) in DMSO followed by the addition of epichlorohydrin (1.2 mmol) and stirred at 60–70 °C until the completion of reaction (TLC). The reaction mass was treated with water and extracted with ethyl acetate (4 × 25 ml). Organic layer was dried over Na_2SO_4 and concentrated under vacuum. Column chromatography of the crude residue provided brownish solid, mp 180 °C, yield 47%, ^1H NMR (300 MHz, $CDCl_3$): δ *2.67–2.70* (dd, 1H, J^2 = 4.5 Hz, J^3 = 2.7 Hz, H_b), 2.92–2.95 (dd, 1H, J^2 = 4.5 Hz, J^3 = 4.5 Hz, H_a), 3.48–3.52 (m, 1H, (8 lines are visible), H_c), 4.37–4.44 (dd, 1H, J^2 = 13.2 Hz, J^3 = 4.8, H_e), 4.83–4.89 (dd, 1H, J^2 = 17.2 Hz, J^3 = 2.1 Hz, H_d), 7.26–7.33 (m, 2H, ArH), 7.55–7.60 (m, 2H, ArH), 7.68–7.75 (m, 2H, ArH), 8.51–8.54 (dd, 2H, *J* = 8.4 Hz, *J* = 1.8 Hz, ArH); ^{13}C (normal/DEPT-135): δ 44.98 (−ve, CH_2), 47.55 (−ve, CH_2), 50.17 (+ve, CH), 115.06 (+ve, ArC), 121.70 (+ve, ArC), 127.73 (+ve, ArC), 133.98 (+ve, ArC), 178.15 (C=O), MS (FAB): *m/z* 252 (M^++1). Anal. Calcd for $C_{16}H_{13}NO_2$: C, 76.48; H, 5.21; N, 5.57. Found: C, 75.04; H, 5.60; N, 5.79. IR (KBr, cm^{-1}): 1604 (C=O).

5.2. General procedure for synthesis of compounds 3–8

An equimolar mixture of compound **2** and appropriate amine was irradiated in a domestic oven for 5 min and the completion of the reaction monitored by TLC. The reaction mixture was washed with diethyl ether to get pure compounds **3**–**8**.

5.2.1. 10-(2-Hydroxy-3-pyrrolidin-1-yl-propyl)-10*H*-acridin-9-one (3)

Yellowish Solid, mp 130 °C, yield 86%; ^1H NMR (300 MHz, $CDCl_3$): δ *1.78–1.84 (br m,* 4H, $C_{16}H_2/C_{17}H_2$), 2.59–2.91 (m, 6H, $C_{15}H_2/C_{18}H_2$, $C_{13}H_2$), 4.31–4.36 (m, 1H, $C_{12}H$), 4.40–4.46 (dd, J^2 = 15.75 Hz, J^3 = 3.45 Hz, 1H, $C_{11}H$), 4.50–4.58 (dd, J^2 = 16.05 Hz, J^3 = 7.35 Hz, 1H, $C_{11}H$), 7.17–7.26 (m, 2H, ArH), 7.63–7.72 (m, 2H, ArH), 8.40–8.43 (m, 2H, ArH), 8.52–8.56 (dd, 2H, *J* = 8.4 Hz, *J* = 1.8 Hz, ArH); ^{13}C NMR (normal/DEPT-135): δ 23.56 (−ve CH_2), 50.33 (−ve, CH_2), 54.22 (−ve, CH_2), 59.80 (−ve, CH_2), 67.81 (+ve, CH), 115.46 (+ve, ArC), 121.26 (+ve, ArC), 127.52 (+ve, ArC), 133.57 (+ve, ArC), 142.53 (C=O); FAB-MS *m/z* 323 (M^++1). Anal. Calcd for $C_{20}H_{22}N_2O_2$: C, 74.51; H, 6.88; N, 8.69. Found: C, 74.86; H, 7.03; N, 8.68. IR (KBr cm^{-1}): 1593 (C=O), 3301 (OH).

5.2.2. 10-(2-Hydroxy-3-piperidin-1-yl-propyl)-10*H*-acridin-9-one (4)

Yellow crystalline solid, mp 150 °C, yield 76%; ^1H NMR (300 MHz, $CDCl_3$): δ *1.25–1.60* (m, 6H, $C_{16}H_2/C_{17}H_2/C_{18}H_2$), 2.45–2.60 (m, 6H, $C_{15}H_2/C_{19}H_2$,$C_{13}H_2$), 4.30 (m, 1H, $C_{12}H$), 4.39–4.46 (dd, J^2 = 16.05 Hz, J^3 = 3.75 Hz, 1H, $C_{11}H$), 4.51–4.58 (dd, J^2 = 16.05 Hz, J^3 = 7.05 Hz, 1H, $C_{11}H$), 7.23–7.28 (m, 2H, ArH), 7.67–7.72 (m, 2H, ArH), 8.49–8.52 (d, *J* = 8.1, 2H, ArH), 8.56–8.60 (dd, 2H, *J* = 8.4 Hz, *J* = 1.8 Hz, ArH); ^{13}C NMR (normal/DEPT-135): δ 25.86 (−ve, CH_2), 50.24 (−ve, CH_2), 54.74 (−ve, CH_2), 62.36 (−ve, CH_2), 65.98 (+ve, CH), 115.41 (+ve, ArC), 121.34 (+ve, ArC), 127.66 (+ve, ArC), 133.66 (+ve, ArC), 142.53 (C=O); FAB- MS *m/z* 337 (M^++1). Anal. Calcd for $C_{21}H_{24}N_2O_2$: C, 74.97; H, 7.19; N, 8.33. Found: C, 74.66; H, 7.27; N, 8.47. IR (KBr cm^{-1}): 1693 (C=O), 3334 (OH).

5.2.3. 10-(2-Hydroxy-3-morpholin-4-yl-propyl)-10*H*-acridin-9-one (5)

Light yellow solid, mp 80 °C; yield 82%; ^1H NMR (300 MHz $CDCl_3$): δ *2.59–2.71* (m, 6H, $C_{15}H_2/C_{18}H_2$,$C_{13}H_2$), 3.65–3.77 (m, 4H, $C_{16}H_2/C_{17}H_2$), 4.55 (m, 3H, $C_{12}H/C_{11}H_2$), 7.05–7.52 (m, 2H, ArH), 7.56–7.59 (m, 2H, ArH), 7.61–7.70 (m, 2H, ArH) 8.16–8.26 (dd, *J* = 8.6 Hz, *J* = 1.5 Hz, 2H, ArH); ^{13}C NMR (normal/DEPT-135): δ 50.5 (−ve, CH_2), 53.98 (−ve, CH_2), 62.36 (−ve, CH_2), 66.41 (−ve, CH_2), 66.89 (+ve, CH), 115.44 (+ve, ArC), 121.24 (+ve, ArC), 127.20 (+ve, ArC), 133.56 (+ve, ArC), 177.70 (C=O); FAB-MS *m/z* 339 (M^++1). Anal. Calcd for $C_{20}H_{22}N_2O_3$: C, 70.09; H, 6.55; N, 8.28. Found: C, 70.12; H, 6.10; N, 8.64. IR (KBr cm^{-1}): 1593 (C=O), 3323 (OH).

5.2.4. 10-(3-[1,4′]Bipiperidinyl-1′-yl-2-hydroxy-propyl)-10*H*-acridin-9-one (6)

Yellowish solid, mp 110 °C, yield 84%; ^1H NMR (300 MHz, $CDCl_3$): δ *1.43–1.67* (m, 8H, $C_{22}H_2/C_{24}H_2/C_{16}H_2/C_{18}H_2$), 1.79–1.90 (m, 2H, $C_{23}H_2$), 2.22–2.34 (m, 2H, $C_{21}H_2$), 2.48–2.59 (br m, 6H, $C_{25}H_2/C_{19}H_2/C_{15}H_2$), 2.00–2.07 (m, 1H, $C_{17}H$), 2.97–3.72 (m, 2H, $C_{13}H_2$), 4.27–4.30 (m, 1H, $C_{12}H$), 4.40–4.46 (dd, J^2 = 15.9 Hz, J^3 = 3.3 Hz, 1H, $C_{11}H$), 4.50–4.58 (dd, J^2 = 15.9 Hz, J^3 = 7.2 Hz, 1H, $C_{11}H$), 7.21–7.26 (m, 2H, ArH), 7.66–7.70 (m, 4H, ArH), 8.46–8.49 (d, *J* = 8.1 Hz, 2H, ArH); ^{13}C NMR (normal/DEPT-135): δ 25.88 (−ve, CH_2), 28.07 (−ve, CH_2), 50.14 (−ve, CH_2), 50.33 (−ve, CH_2), 52.48 (−ve, CH_2), 54.78 (−ve, CH_2), 61.69 (+ve, CH), 66.42 (+ve, CH), 121.29 (+ve, ArC), 127.56 (+ve, ArC), 133.62 (+ve, ArC), 178.00 (C=O), FAB-MS *m/z* 420 (M^++1). Anal. Calcd for $C_{26}H_{33}N_3O_2$: C, 74.43; H, 7.93; N, 10.82. Found: C, 74.03; H, 8.01; N, 10.52.

5.2.5. 10-(3-(Diethylamino)-2-hydroxypropyl)acridin-9(10*H*)-one (7)

Yellowish solid, mp 120 °C, yield 81%; ^1H NMR (300 MHz, $CDCl_3$): δ *1.04–1.27* (m, 6H, $C_{16}H_3/C_{18}H_3$), 2.53–2.74 (m, 6H,

$C_{15}H_2/C_{17}H_2/C_{13}H_2$), 4.19–4.27 (m, 1H, $C_{12}H$), 4.38–4.45 (dd, J^2 = 16.2 Hz, J^3 = 3.45 Hz, 1H, $C_{11}H$), 4.49–4.57 (dd, J^2 = 16.05 Hz, J^3 = 7.35 Hz, 1H, $C_{11}H$), 7.21–7.26 (m, 2H, ArH), 7.65–7.74 (m, 4H, ArH), 8.46–8.49 (d, J = 7.5 Hz, 2H, ArH); ^{13}C NMR (normal/DEPT-135): δ 11.94 (+ve, CH_3), 47.27 (−ve, CH_2), 50.44 (−ve, CH_2), 57.31 (−ve, CH_2), 66.74 (+ve, CH), 115.4 4 (+ve, ArC), 121.30 (+ve, ArC), 127.57 (+ve, ArC), 133.63 (+ve, ArC), 142.52 (C=O); FAB-MS m/z 325 (M^++1). Anal. Calcd for $C_{20}H_{24}N_2O_2$: C, 74.04; H, 7.46; N, 8.64. Found: C, 74.14; H, 7.89; N, 8.93. IR (KBr): 1593 (C=O), 3342 (OH).

5.2.6. 10-(3-(Diisopropylamino)-2-hydroxypropyl)acridin-9(10*H*)-one (8)

Creamish solid, mp 135 °C, yield 74%; ^{1}H NMR (300 MHz, $CDCl_3$): δ *1.04–1.10* (m, 12H, $C_{16}H_3/C_{17}H_3/C_{19}H_3/C_{20}H_3$), 2.67–2.69 (m, 1H, $C_{13}H$), 2.80–2.93 (m, 1H, $C_{13}H$), 2.95–3.10 (m, 1H, $C_{15}H$), 3.46–3.50 (m, 1H, $C_{18}H$), 4.37–4.89 (m, 3H, $C_{12}H/C_{11}H_2$), 7.23–7.32 (m, 2H, ArH),7.56–7.59 (m, 2H, ArH), 7.69–7.75 (m, 2H, ArH), 8.51–8.59 (m, 2H, ArH); ^{13}C NMR (normal/DEPT-135): δ 19.88 (+ve CH_3), 22.06 (+ve, CH_3), 45.05 (−ve, CH_2), 47.59 (−ve, CH_2), 50.23 (+ve, CH), 50.89 (+ve, CH), 66.61 (+ve, CH), 115.10 (+ve, ArC), 115.49 (+ve, ArC), 121.31 (+ve, ArC), 122.39 (+ve, ArC), 178.08 (C=O); FAB-MS m/z 353 (M^++1). Anal. Calcd for $C_{22}H_{28}N_2O_2$: C, 74.97; H, 8.01; N, 7.95. Found: C, 74.64; H, 8.17; N, 8.26.

5.3. Biological studies

The modulating activities of compounds **3**–**8** were studied using 'drug–*p*-gp interaction' assay kit purchased from CEA, SPI-BIO mother company. The bioassay for studying the interactions of the test compounds with *p*-gp was performed in triplicate in accordance with the previously reported procedure.[23]

5.4. Mg^{2+} ion binding studies

Stock solutions (10^{-3} M concentrations) of compounds **3**–**8** were prepared by dissolving in two drops of ethanol and diluting with HEPES buffer (10^{-2} M) at pH 7.2. The complex formation was studied by continuous addition of increasing mole fraction of metal ion to 100 μL of ligand solution, making final volume 1 ml (final concn of solution was 10^{-4} M). After plotting the Job plot, binding constants of compounds **3**–**8** with Mg^{2+} were calculated using following equation.

$$K_d = [C_0 - (\Delta A/\Delta A_{max})C_0][C_m - (\Delta A/\Delta A_{max})C_0]/[\Delta A/\Delta A_{max})C_0]$$

$K_a = 1/K_d$; C_0 is the initial concentration of ligand, C_m is the concentration of Mg^{2+}, ΔA is the increase in absorbance at the wavelength of maximum absorption upon addition of each mole fraction of Mg^{2+}, ΔA_{max} is the increase in absorbance when the ligand is totally bound to Mg^{2+}.

Acknowledgements

DST, UGC, CSIR New Delhi are gratefully acknowledged for financial assistance and CDRI Lucknow for recording mass spectra and CHN analysis. JK thanks UGC for fellowship.

References and notes

1. Teodori, E.; Dei, S.; Scapecchi, S.; Gualtieri, F. *Il Farmaco* **2002**, *57*, 385.
2. Szakacs, G.; Paterson, J. K.; Ludwig, J. A.; Booth-Genthe, C.; Gottesman, M. M. *Nat. Rev. Drug Discov.* **2006**, *1*, 585.
3. Raub, T. J. *Mol. Pharm.* **2006**, *3*, 3. and references cited therein.
4. Kawase, M.; Motohashi, N. *Curr. Drug Target* **2003**, *4*, 31.
5. Collnot, E.-M.; Baldes, C.; Wempe, M. F.; Kappl, R.; Huttermann, J.; Hyatt, J. A.; Edger, K. J.; Schaefer, U. F.; Lehr, C.-M. *Mol. Pharm.* **2007**, *4*, 465.
6. Liu, X.-L.; Tee, H.-W.; Go, M.-L. *Bioorg. Med. Chem.* **2008**, *16*, 171.
7. Colabufo, N. A.; Berardi, F.; Cantore, M.; Perrone, M. G.; Contino, M.; Inglese, C.; Niso, M.; Perrone, R.; Azzariti, A.; Simone, G. M.; Porcelli, L.; Paradiso, A. *Bioorg. Med. Chem.* **2008**, *16*, 362.
8. Belmont, P.; Bosson, J.; Godet, T.; Tiano, M. *Anti-Cancer Agents Med. Chem.* **2007**, *7*, 139.
9. Goodell, J. R.; Ougolkov, A. R.; Hiasa, H.; Kaur, H.; Remmel, R.; Billadeau, D. D.; Ferguson, D. M. *J. Med. Chem.* **2008**, *51*, 179.
10. Kawaii, S.; Tomono, Y.; Katase, E.; Ogawa, K.; Yano, M.; Takemura, Y.; Ju-ichi, M.; Ito, C.; Furukawa, H. *J. Nat. Prod.* **1999**, *62*, 587.
11. Winter, R. W.; Kelly, J. X.; Smilkstein, M. J.; Dodean, R.; Bagby, G. C.; Rathbun, R. K.; Levin, J. I.; Hinrichs, D.; Riscoe, M. K. *Exp. Parasitol.* **2006**, *114*, 47.
12. Dheyongera, J. P.; Geldenhuya, W. J.; Dekker, T. G.; Matsabisa, M. G.; Van der Schyf, C. J. *Bioorg. Med. Chem.* **2005**, *13*, 1653.
13. Di Giorgio, C.; Shimi, K.; Boyer, G.; Delmas, F.; Galy, J.-P. *Eur. J. Med. Chem.* **2007**, *42*, 1277.
14. Goodell, J. R.; Madhok, A. A.; Hiasa, H.; Ferguson, D. M. *Bioorg. Med. Chem.* **2006**, *14*, 5467.
15. Gopinath, V. S.; Thimmaiah, P.; Thimmaiah, K. N. *Bioorg. Med. Chem.* **2008**, *16*, 474.
16. Boumendjel, A.; Macalou, S.; Ahmed-Belkacem, A.; Blanc, M.; Di Pietro, A. *Bioorg. Med. Chem.* **2007**, *15*, 2892.
17. Lehninger, A. L.; Nelson, D.L.; Cox, M. M. In: Principles of Biochemistry, 2nd ed., CBS ISBN:81-239-0295-6, 1992, p 374.
18. Gomez-Puyou, A.; Ayala, G.; Muller, U.; Gomez-Puyou, T. de. *J. Biol. Chem.* **1983**, *258*, 13673.
19. Phillips, R. C.; George, S. J. P.; Rutman, R. J. *J. Am. Chem. Soc.* **1966**, *88*, 2631.
20. Chiba, P.; Burghofer, S.; Richter, E.; Tell, B.; Moser, A.; Ecker, G. *J. Med. Chem.* **1995**, *38*, 2789. and references cited therein.
21. The dockings were carried out using 'Dock in active site' module of BioMed CaChe 7.0.5.85.
22. Battisti, R. F.; Zhong, Y.; Fang, L.; Gibbs, S.; Shen, J.; Nadas, J.; Zhang, G.; Sun, D. *Mol. Pharm.* **2007**, *4*, 140.
23. Singh, P.; Paul, K. *Bioorg. Med. Chem.* **2006**, *14*, 7183.