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Original article

Effects of a series of dihydroanthracene derivatives on drug efflux in multidrug resistant cancer cells

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Abstract

A set of 9,10-dihydro-9,10-ethano and ethenoanthracene derivatives was tested with the aim to quantify the effect observed on drug efflux. Structure activity relationships and molecular modeling studies allowed to define topological display of pharmacophoric groups for these reversal agents.

Keywords: Drug efflux; P-glycoprotein; Multidrug resistance; Cancer cells; 9,10-Dihydro-9,10-ethano and ethenoanthracene derivatives; Chemosensitizers; Structure activity relationships

1. Introduction

Clinical resistance to chemotherapeutic drugs is a major problem, in particular in the treatment of cancer. Multidrug resistance (MDR) is defined as the ability of cells exposed to a single drug to develop resistance to a broad range of structurally and functionally unrelated drugs. MDR is associated with a reduced intracellular drug accumulation and an increased cellular drug efflux [1]. This phenomenon has been shown to be caused by overexpression of the energy dependent efflux pump P-glycoprotein (P-gp) 170 [2,3]. One way to overcome MDR is to design inhibitors able to block the P-gp mediated efflux. The diversity of chemical structures encountered in MDR reversal compounds [4,5], i.e. calcium channel blockers like verapamil and analogs, calmodulin antagonists, anthracycline and vinca alkaloid analogs, steroids and hormonal analogs, miscellaneous hydrophobic cationic compounds and cyclosporins, has led to define limited common features for chemosensitizers. They are: protonatable nitrogen; aromatic ring and high lipophilicity [4–8]. However, compounds without any protonatable nitrogen such as steroids and colchicine are also active [4,5].

Due to the fact that 9-γ-methylaminopropyl-9,10-dihydro-9,10-ethanoanthracene, known as maprotiline (Fig. 1), exhibits anti-MDR activity on cancer cells [9], 37 dihydro-ethano and ethenoanthracene (DEEA) derivatives (Fig. 2) were tested as chemosensitizers against L5178 MDR cancer cells. A structure activity relationships (SAR) study was conceivable owing to the semi-rigid structure provided by the bridged dihydroanthracene nucleus on which various substituents can be branched. Finally, a molecular modeling study allowed to suggest a putative topology of pharmacophoric groups for drugs capable to reverse MDR.

Abbreviations: MDR, multidrug resistance; SAR, structure activity relationships; P-gp, P-glycoprotein; HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; DEEA, dihydro-ethano and ethenoanthracene.

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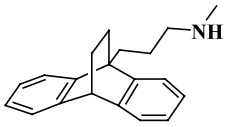
2. Chemistry

Compounds **18** [10], **27**, **31**, **32**, **33**, **35**, **36** and **37** (Fig. 2) were prepared by the general routes given in Fig. 3.



Other DEEA derivatives (Fig. 2) used in this study are described in Refs. [11–14].

Product **18** was obtained by a Diels–Alder reaction between anthracene and methyl 4-bromocrotonate in xylene under reflux.

Amide **27** was prepared by condensation of commercially available methylamine dissolved in THF, with acid chloride intermediate of the corresponding carboxylic acid [15], then reduced to the secondary amine **39** which is the precursor of phosphinamide **33**. Secondary amine **13** [14] yielded the corresponding urea derivative **36** by action of carbamoyl chloride.

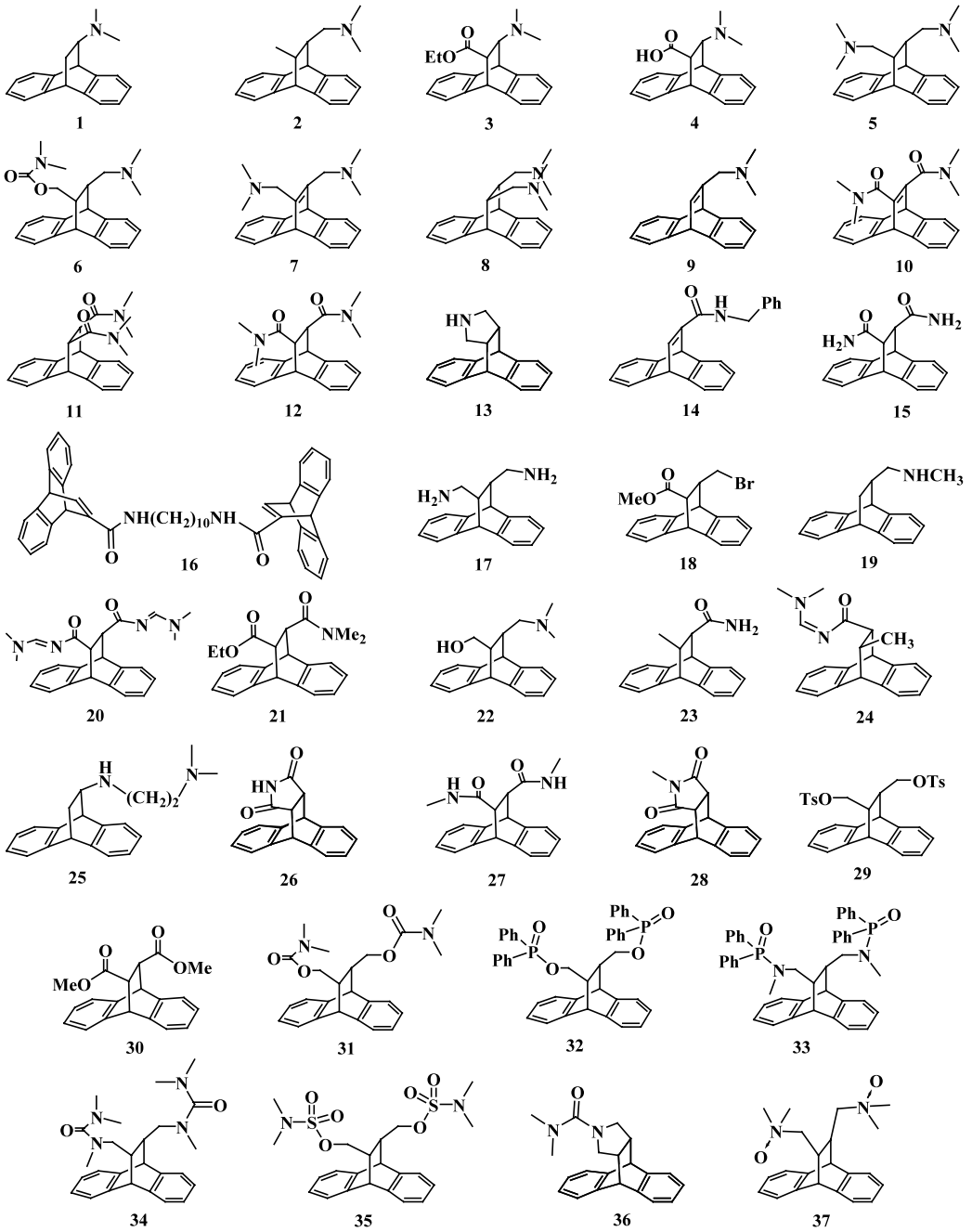


Fig. 2. Studied molecules.

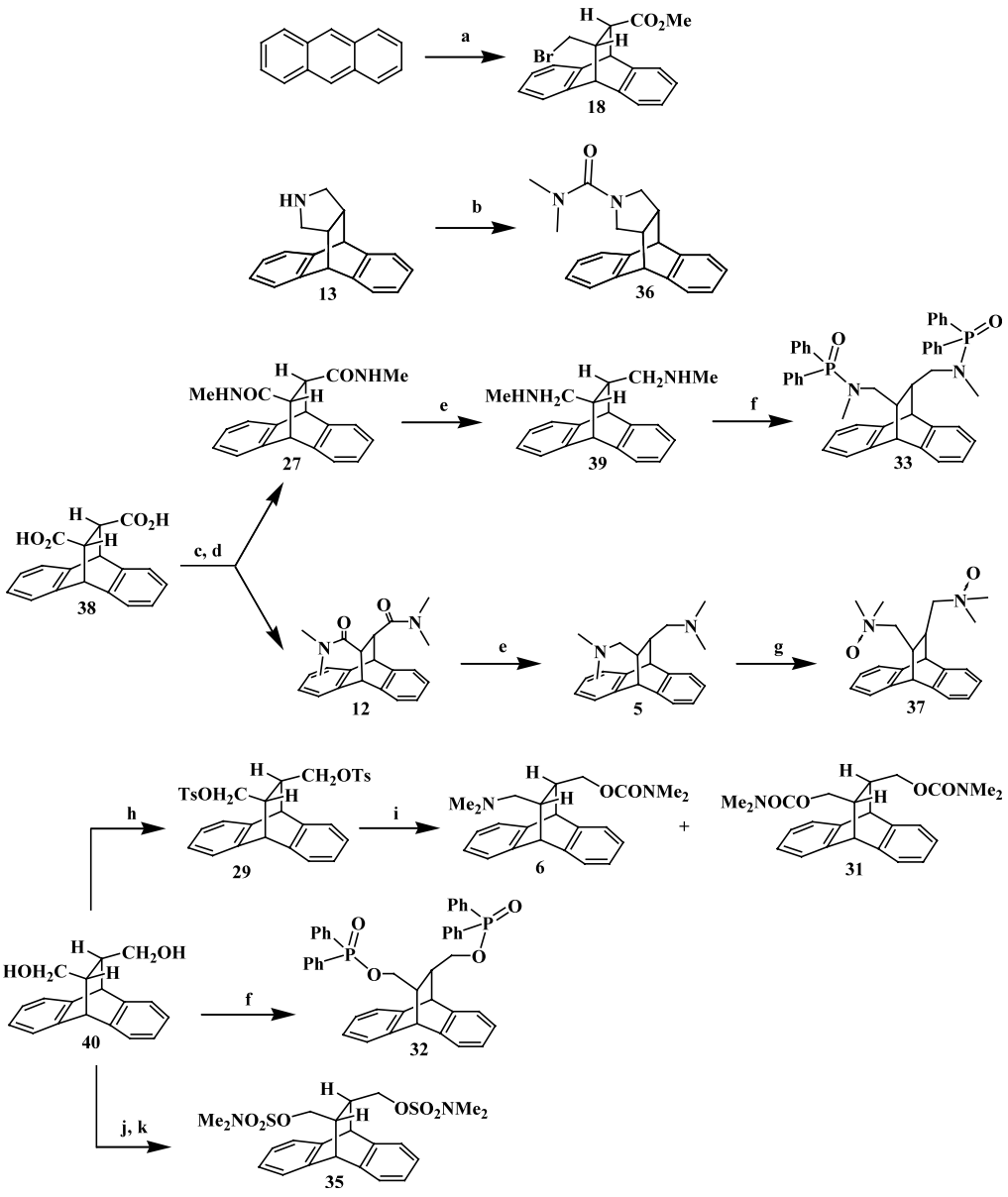


Fig. 3. Synthetic pathways. Reagents: (a) $BrCH_2CH{=}CHCO_2Me$/xylene; (b) Me_2NCOCl/THF; (c) $SOCl_2$; (d) $MeNH_2$ or Me_2NH/THF; (e) $LiAlH_4$; (f) Ph_2POCl/TEA/CH_2Cl_2; (g) H_2O_2/MeOH; (h) TsCl/pyridine; (i) Me_2NH, HCl/$CsCO_3$/DMF; (j) NaH/DMF; (k) Me_2NSO_2Cl.

In previous investigations, in which tosylate **29** and dimethylamine hydrochloride were heated in DMF in the presence of cesium carbonate, the major product was not the diamine derivative **5** but amino carbamate **6** and dicarbamate **31**. A direct way to **31** would consist in the addition of *N*,*N*-dimethyl carbamoyl chloride to sodium alkoxide of 9,10-dihydro-9,10-ethanoanthracene-11,12-dimethanol. This procedure was successfully applied to the synthesis of amino carbamate **6** [14] from the corresponding amino alcohol. From diol **40** [16], the expected dicarbamate is formed besides products of close polarity render uneasy the purification. This diol also led to phosphinate **32** and sulfamate **35**.

Diamine oxide **37** results from the oxidation of amine **5** by H_2O_2 in methanol.

3. Pharmacology

As the efflux of drugs is the major character of P-gp mediated MDR and as rhodamine efflux is more sensitive than that of anticancer drugs [17], to quantify the anti-MDR activity, we have chosen to measure the efflux inhibition by chemosensitizers, with rhodamine 123 as substrate. In each assay, the calcium channel blocker verapamil was used as reference drug since it has

Table 1
Biological data

Compounds	$C_{max} \leqslant 5$ (μM)	Compounds	$C_{max} \leqslant 125$ (μM)	Compounds	$C_{max} > 125$ (μM)
32	100% at 0.6	Verapamil	100% at 50	**14**	26% at 235
33	100% at 2	**35**	100% at 80	**17**	19% at 300
31	100% at 5	**6**	100% at 125	**19**	7% at 320
29	100% at 5	**2**	75% at 125	**24**	6% at 200
–	–	**34**	60% at 125	**25**	4% at 220
–	–	**5**	54% at 125	**3**	2% at 250
–	–	**20**	48% at 125	**22**	2% at 275
–	–	**8**	30% at 125	**23**	1% at 300
–	–	**9**	30% at 125	**1**	1% at 320
–	–	**16**	28% at 125	**4**	ND
–	–	**13**	28% at 125	**15**	ND
–	–	**12**	20% at 125	**18**	ND
–	–	**21**	20% at 125	**26**	ND
–	–	**7**	5% at 125	**27**	ND
–	–	**11**	3% at 125	**28**	ND
–	–	**10**	3% at 125	**30**	ND
–	–	–	–	**36**	ND
–	–	–	–	**37**	ND

been widely studied as effective antagonist of resistance to a number of drugs in most MDR cell lines in vitro. Concentration of chemosensitizers which gives the highest response (C_{max}), was evaluated. Results are summarized in Table 1.

4. Results and discussion

Products **29**, **31**, **32**, and **33** are 10–80 times more potent than verapamil.

Except the presence or not of the double bond in the bridge, the DEEA moiety was kept structurally identical to keep constant influence of this part of the molecule on biological activity. In doing that, only the side chain interactions with a putative receptor are put into evidence. Minor variations on the side chain allowed optimization of the reversal potency as well as identification of important structural moiety responsible for the biological activity.

The inhibiting activity is under the influence of several features of synthesized compounds: (i) the presence or not of the double bond in the bridge and, in the latter case; (ii) the diastereochemistry of the bridge; (iii) the nature and the number of substitutions (R^1, R^2).

The double bond in ethenoanthracene derivatives **9** and **7**, decreases the reversal activity when compared to ethanoanthracene derivatives **2** and **5**. The same must be noted with diastereoisomers RS **8** versus RR-SS **5** (Fig. 4). Therefore, the pharmacophoric group(s) must be borne on a 9,10-dihydro-9,10-ethanoanthracenic structure (RR-SS).

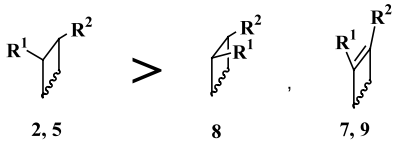


Fig. 4. Nature of the bridge and effect on the anti-MDR activity.

Moreover, if structure of 11,12-disubstituted dihydroethanoanthracenes (**20**, **34**) is more favorable for reversal activity than that of the monosubstituted derivatives (**24**, **36**), we noted that monoamines (**2** and **9**) display better activity than the corresponding disubstituted compounds (**5** and **7**).

The anti-MDR activity of DEEA derivatives seems to be dependent on the presence of strong hydrogen bond acceptor (HBA) group(s) instead of that of protonatable nitrogen. Indeed, strength of the different HBA groups can be ranked according to the pK_{HB} (converted to $\Sigma pK_x(HB)$ for polyfunctional solutes) scale [18] which measures hydrogen bond basicity as shown in Table 2. Some substructures have been found to be predominantly present in inactive molecules [7]: carboxylic acid, primary amine or hydroxyl groups (hydrogen bond donor, HBD groups) branched on aromatic ring and quaternary ammonium. Whatever be their location in the molecule, they cause log P lessening (smaller than 3 [14]), while log P value ranges between 3 and 4 in active molecules. The presence of both HBA and HBD sites (**14**, **15**, **22**, **23**, **25**, **26**, **27**) impairs the activity. In addition, in active compounds, replacement of HBA group by the HBD one is detrimental. This is clearly demonstrated when comparing the amine alcohol **22** to the amine carbamate **6** but also tertiary amine **2** to the secondary ones **13** or **19**. Concerning diamides, a tertiary group in **10** is preferable to a secondary one in **27** or a primary one in **15**.

Furthermore, the distance of HBA groups from the bridge is also a determining factor (Fig. 5). Although

Table 2
$\Sigma pK_x(HB)$ of DEEA derivatives

Compounds	Model derivatives		$\Sigma pK_x(HB)$
1	$MeNMe_2$	–	3.14 [41]
2	$EtNMe_2$	–	3.18 [41]
3	MeCOOEt	$MeNMe_2$	5.22 [18,41]
4	MeCOO[Me] [a]	$MeNMe_2$	5.16 [18,41]
5	$EtNMe_2$	$EtNMe_2$	6.36 [41]
6	$EtOCONMe_2$	$EtNMe_2$	6.02 [20,41]
7	$CH_2{=}CHCH_2NMe_2$	$CH_2{=}CHCH_2NMe_2$	5.86 [41]
8	$EtNMe_2$	$EtNMe_2$	6.36 [41]
9	$CH_2{=}CHCH_2NMe_2$	–	2.93 [41]
11	$MeCONMe_2$	$MeCONMe_2$	6.90 [21]
12	$MeCONMe_2$	$MeCONMe_2$	6.90 [21]
13	EtNHEt	–	3.26 [20]
15	$MeCONH_2$	$MeCONH_2$	3.06 [b]
17	$EtNH_2$	$EtNH_2$	4.36 [22]
19	EtNHMe	–	3.25 [20]
20	[Ph]CON=$CHNMe_2$ [a]	[Ph]CON=$CHNMe_2$ [a]	6.66 [23]
21	MeCOOEt	$MeCONMe_2$	5.53 [19,21]
22	EtOH	$EtNMe_2$	5.21 [21,24]
23	$MeCONH_2$	–	3.06 [b]
24	$PhCON{=}CHNMe_2$	–	3.33 [23]
25	$MeNH(CH_2)_2NHMe$	–	6.60 [20]
27	MeCONHMe	MeCONHMe	6.62 [21]
28	*N*-methylsuccinimide	–	4.14 [b]
29	$EtOSO_2$[Me] [a]	$EtOSO_2$[Me] [a]	6.92 [25]
30	MeCOOMe	MeCOOMe	4.02 [19]
31	$EtOCONMe_2$	$EtOCONMe_2$	5.68 [19]
32	Ph_2PO[Ph] [a]	Ph_2PO[Ph] [a]	8.02 [b]
33	Ph_2PO[Ph] [a]	Ph_2PO[Ph] [a]	8.62 [b]
34	[Me]$NMeCONMe_2$ [a]	[Me]$NMeCONMe_2$ [a]	6.90 [21]
35	[Ph]SO_2NMe_2 [a]	[Ph]SO_2NMe_2 [a]	6.96 [b]
36	[Me]$NMeCONMe_2$	–	3.45 [21]
37	$MeN(O)Me_2$	$MeN(O)Me_2$	14.58 [35]
Verapamil	$PhCH_2CN$	$EtNMe_2$	5.00 [41,43]
o-propafenone analogs	4-MeOPhCOMe	*N*-methylpiperidine	5.43 [41,42]
m-propafenone analogs	4-MeOPhCOMe	*N*-methylpiperidine	5.43 [41,42]
p-propafenone analogs	4-MeOPhCOMe	*N*-methylpiperidine	5.43 [41,42]

[a] A small correction has been applied when the substituent between brackets does not exactly match the fragment of the molecule under study.
[b] Unpublished results.

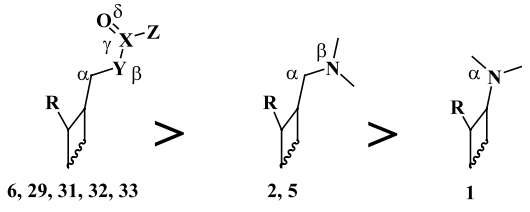


Fig. 5. Position of the HBA group on the bridge.

tertiary amido group is known to be good HBA group [26–28] and carbamidine derivatives [23] as well, we observed low reversal activity for **8**, **9**, **11**, **12**, **20**, and **24**. This can be explained by wrong position of HBA groups.

Results indicate that HBA group in the δ position confers better activity than those in the β or the α one. In a rough evaluation X, Y, and Z atoms can be ranked in the following decreasing order of activity:

$$X = P > C > S$$

$$Y = O > N$$

$$Z = C > N$$

These observations demonstrate that products which cannot be protonated, are yet capable to inhibit efflux as recently emphasized [29]. Thus, side chain interactions of the DEEA derivatives with the putative receptor would be governed by hydrogen bonds. It is the reason

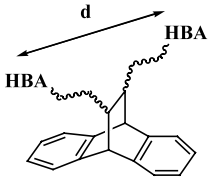


Fig. 6. Distance between two HBA groups.

why strong hydrogen bonding acceptors are necessary and conversely, why donor effects are antagonistic for the reversal activity.

SAR suggest that anti-MDR activity of DEEA compounds is mediated by the *d* distance between two HBA (Fig. 6) since the δ position is better than the β and α ones. The distance variation and DEEA activity can roughly be depicted using molecular modeling of **5**, **6**, **8**, **20**, **29**, **31**, **32**, **33**, **34**, and **35**; IC_{50} values have only been evaluated (Table 3) for these ten molecules in order to be compared.

The flexibility of side chains of these DEEA compounds is different. Indeed, these chains can be ranked in a decreasing order of degree of freedom as it is shown in Fig. 7.

Thus, distances which are spaced from 3 to 8 Å in compounds investigated are dependent on the molecular strain energy. Models of these ten molecules were built from the experimental crystallographic structure of ethano derivative **5** [30]. The side chain conformation was considered as the sole geometrical variable. For each compound, conformational analysis was performed and strain energy of the most stable conformer (E_{min}, Table 3) was calculated as detailed in Section 6. Then, energy strain E_s can be deduced and $\Delta E = E_s - E_{min}$ (Table 4) was calculated from each distance variation, in order to correlate activity with this value which expresses the distance allowed. These results show that significant correlations are only obtained for distance values around 7 Å. As the correlation coefficient is very low at a distance of 6 Å, the study was refined by varying distance between 6.5 and 7 Å (Table 5). The best correlation was obtained when distance *d* is contained between 6.6 and 6.7 Å. Nearly 65% of the activity of the compounds studied could be explained in terms of the energy variation ΔE.

The multilinear regression which involves both the energy variation ΔE and the sum of the HBA strengths

Table 3
Molecular modeling data

Compounds	IC_{50} (μM)	E_{min} (kcal mol^{-1})
5	97.5	29.6
6	16.0	27.3
8	970	31.9
20	139	28.1
29	1.8	97.1
31	4.0	25.8
32	0.25	88.8
33	0.62	97.5
34	63.9	44.4
35	8.8	106.1
Verapamil	30	35.4
o-propafenone analogs	0.6	16.7
m-propafenone analogs	0.71	16.2
p-propafenone analogs	2.7	26.0

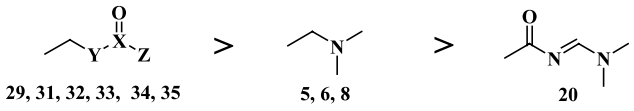


Fig. 7. Degree of freedom of DEEA side chains.

of the different sites, $\Sigma pK_x(HB)$, gave the following results:

For $d = 6.6$Å

$$\log(1/IC_{50}) = -0.1584(\pm 0.042)\Delta E + 0.4942(\pm 0.233) \times \sum pK_x(HB) - 3.6945(\pm 1.675)$$

$n = 10$; $s = 0.5887$; $r = 0.889$ $F_{2,7} = 13.18$

For $d = 6.7$Å

$$\log(1/IC_{50}) = -0.1181(\pm 0.031)\Delta E + 0.5016(\pm 0.229) \times \sum pK_x(HB) - 3.8417(\pm 1.646)$$

$n = 10$; $s = 0.5841$; $r = 0.891$ $F_{2,7} = 13.44$

Parameters defined in this study only explain around 79% of the anti-MDR activity. These results suggest that the remaining 21% could be clarified by the conformational study of the two HBA groups related to each other and to the aromatic rings. Molecule with strong HBA groups at (i) convenient distance and (ii) included in a ring, could give conformational information.

From several data, Klopman et al. [7] have proposed that P-gp modulators display basic nitrogen and aromatic ring in relative mutual disposition, and that their volume and shape affect the activity [31,32]. From a computer search of the NCI database concerning 40 000 compounds, a requirement for P-gp modulator was a HBA group near an aromatic ring [8,29]. In a study comparing structural data elements of hundred compounds tested as P-gp substrates [33], recognition elements are formed by two electron donor groups (type I) or three electron donor groups (type II) with a fixed spatial separation: a 2.5 Å distance for type I, and 4.6 Å for the outer groups of type II. Finally, study of propafenone-type modulators [34] leads to the conclusion that nitrogen atom present in the modulator gives non ionic contribution. For the drug-Pgp interaction, the only requirement is the presence of HBA groups. For three of the propafenone analogs studied, the distance between two HBA groups (carbonyl oxygen and nitrogen) was calculated: 5.757, 8.830 and 10.904 Å and corresponding IC_{50} values are 0.60, 0.70 and 2.70 μM. The *d* value determined above—6.6–6.7 Å—would be in agreement with the latter figures if one assumes a graphic distance-activity as depicted in Fig. 8.

As the correlation study is only based on the distance between HBA groups which influence energy strain of the molecule, we extended this study to verapamil and three propafenone analogs (Table 6) which have been

Table 4
Regression coefficient from correlation study between anti-MDR activity of DEEA compounds and energy variation ΔE calculated for several d values.

Compounds	d (Å)						
		3	4	5	6	7	8
5	ΔE (kcal mol^{-1})	58.1	27.8	11.5	3.2	19.7	103.2
6	–	12.2	1.4	1.0	5.6	0.7	4.8
8	–	51.0	10.0	7.6	0.6	30.0	122.7
20	–	1.5	3.0	0.8	0.7	39.0	154.7
29	–	13.7	11.3	9.1	6.7	4.5	2.5
31	–	1.3	0.1	0.3	0.4	0.9	1.2
32	–	3.3	4.8	3.0	1.8	1.1	0.3
33	–	9.2	5.5	8.3	2.2	0	1.7
34	–	3.8	1.1	0.7	3.8	6.6	0.8
35	–	11.6	8.8	4.4	2.4	0.6	0
	r	0.571	0.247	0.024	0.208	0.774	0.755

tested in the same conditions to that of DEEA derivatives (Tables 2 and 3). Results confirm that parameters defined in this study can be applied to structurally unrelated drugs.

Finally, amine oxide group is far the best HBA group ($\Sigma pK_x(HB) = 14.58$) [35], moreover, when we compare the γ position of this group with the β one of the corresponding amine **5** (Fig. 5), a higher activity would be expected for the *N*-oxide derivative. Surprisingly **37** shows no activity at all. Considering amine oxide behavior, it was noted for example that *N*-methylmorpholine oxide monohydrate is an industrial solvent for cellulose but when the H_2O content is greater than 2 moles H_2O per mole of oxide, dissolution is no longer possible [36]. This was attributed to the fact that H_2O forms stronger hydrogen bonds in the 2.5 mole hydrate than in the monohydrate and this prevents competition of cellulose with H_2O for amine oxide acceptor sites. In our case, the lack of biological activity of **37** could be similarly related to the fact that H_2O molecules (5 per mole) are too tightly bonded to the *N*-oxide groups preventing the necessary interaction with the protein, involving a lower lipophilicity and a steric hindrance.

5. Conclusion

Some modulators with DEEA structure show high ability to reverse MDR in cancer cells. This could be of high interest the more so as these compounds are not cytotoxic [14]. Numerous compounds have been listed as P-gp modulators [7,33]. Taking that into account all functional groups capable to play a role in the inhibitory effects, basic structural features were deduced from these tests. DEEA compounds are simple molecules. Therefore, SAR and molecular modeling studies allow to show that reversal activity depends on the presence of two HBA (not particularly a protonatable nitrogen) separated by a distance of 6.6–6.7 Å. For the compounds studied here, only the variations of side chains were taken into account, the cyclic structure being kept unchanged. Hence, no information is available for

Table 5
Refinement of correlation study

Compounds		d (Å)					
		6.5	6.6	6.7	6.8	6.9	7.0
5	ΔE (kcal mol^{-1})	4.9	6.6	8.8	11.6	15.0	19.7
6	–	2.7	2.2	1.6	1.0	0.4	0.7
8	–	10.2	13.3	16.9	20.8	25.3	30.0
20	–	10.0	13.7	18.0	28.9	29.0	39.0
29	–	5.4	5.2	5.0	4.9	4.6	4.5
31	–	0.9	0.9	0.9	0.9	0.9	0.9
32	–	1.7	1.5	1.4	1.3	1.2	1.1
33	–	0.5	0.4	0.2	0.1	0	0
34	–	5.4	5.7	6.0	6.3	6.7	6.6
35	–	1.4	1.2	1.0	0.8	0.7	0.6
	r	0.796	0.809	0.808	0.754	0.796	0.774

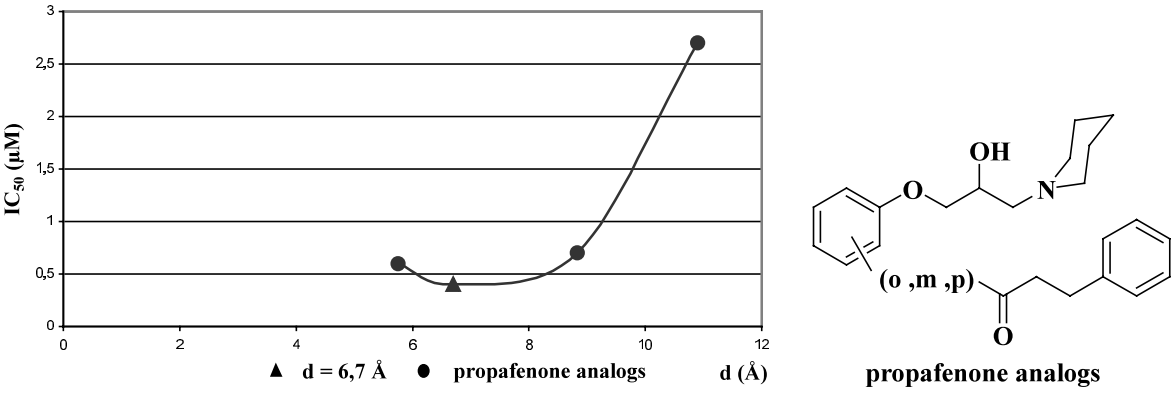


Fig. 8. Distance-activity graph for propafenone analogs.

Table 6
Correlation study with structurally unrelated drugs

Compounds		ΔE (kcal mol^{-1})
5	$d = 6.6$ Å	6.6
6	–	2.2
8	–	13.3
20	–	13.7
29	–	5.2
31	–	0.9
32	–	1.5
33	–	0.4
34	–	5.7
35	–	1.2
Verapamil	–	6.6
o-propafenone analogs	–	2.0
m-propafenone analogs	–	0.5
p-propafenone analogs	–	0.7
	r	0.829

$\log(1/IC_{50}) = -0.1956$ (± 0.039) $\Delta E + 0.1616$ (± 0.168) $\Sigma pK_x(HB) - 1.0956$ (± 1.110) $n = 14$; $s = 0.6261$; $r = 0.843$; $F_{2,11} = 13.53$.

aromatic rings, as well as for their optimum number and position. In order to get information on aromatic rings and to ascertain the minimal structure providing active compounds, investigations similar to those of the present study are currently carried out, using benzobarrelene analog derivatives.

6. Experimental protocols

6.1. Chemistry

Liquid chromatography was performed on silica gel 60 (230-400 Mesh) and TLC on silica gel 60 F_{254}. Melting points were determined on Büchi apparatus and are given uncorrected. ^{1}H and ^{13}C-NMR spectra were performed on Brüker spectrometer (300 MHz) with TMS as internal reference; chemical shifts δ are given in the parts per million scale with J values in Hertz. Elemental analysis are within $\pm 0.4\%$ of the theoretical values.

6.1.1. Methyl-12-bromomethyl-trans-9,10-dihydro-9,10-ethanoanthracene-11-carboxylate (**18**)

A mixture of anthracene (6.2 g, 35 mmol) and methyl 4-bromocrotonate (5 mL, 42 mmol) in xylene (20 mL) was heated under reflux during 4 days. Xylene was then eliminated under vacuum and the residue obtained was recrystallized in CH_3CN and purified by liquid chromatography (ether/pentane, 20/80).

Yield: 10%. Mp: 134–135 °C. ^{1}H-NMR ($CDCl_3$): 7.37 (m, 4H); 7.18 (m, 4H); 4.71 (d, $J = 2.4$ Hz, 1H); 4.60 (s, 1H); 3.65 (s, 3H); 3.29 (m, 1H); 2.90 (m, 2H); 2.40 (m, 1H). ^{13}C-NMR ($CDCl_3$): 172.3 (s); 142.7 (s); 141.7 (s); 139.7 (s); 139.6 (s); 126.3 (d); 126.2 (d); 126.1 (d); 126.0 (d); 125.5 (d); 124.7 (d); 123.6 (d); 123.5 (d); 52.0 (d); 51.1 (d); 46.7 (d); 46.6 (d); 44.7 (t); Anal. ($C_{19}H_{17}BrO_2$) C, H.

6.1.2. N,N′-Dimethyl-trans-9,10-dihydro-9,10-ethanoanthracene-11,12-dicarboxamide (**27**)

A mixture of corresponding acid derivative **38** [13] (2.9 g, 10 mmol) and $SOCl_2$ (20 mL) was heated 3 h under reflux. After elimination of $SOCl_2$ in excess under vacuum, the acid chloride obtained was used for the next step without further purification. Acid chloride (10 mmol) and a solution of 2N methylamine in THF (40 mL) were stirred 1 day at room temperature. The solvent was eliminated under vacuum. The residue obtained was extracted with CH_2Cl_2 and washed with NaOH 2N solution (pH 10) before the organic phase was dried under drierite. Pure amide derivative was obtained using column chromatography (ether/methanol, 80/20).

Yield: 60%. Mp: >300 °C. ^{1}H-NMR (DMSO): 8.05(d, $J = 4.4$ Hz, 2H); 7.26 (m, 2H); 7.11 (m, 2H); 7.01 (m, 4H); 4.54 (s, 2H); 3.12 (s, 2H); 2.51 (d, $J = 4.4$ Hz, 6H). ^{13}C-NMR (DMSO): 171.9 (s); 143.4 (s); 140.8 (s); 125.5 (d); 125.4 (d); 124.5 (d); 123.1 (d); 47.4 (d); 46.9 (d); 25.9 (q). Anal. ($C_{20}H_{20}N_2O_2$) C, H, N.

*6.1.3. N-Methyl-9,10-dihydro-9,10-ethanoanthracene-11,12-dicarboximide (**28**) [11,12]*

A mixture of anthracene (10 g, 56 mmol) and methyl *N*-methyl maleimide (5 g, 45 mmol) in xylene (100 mL) was heated under reflux during 7 h. The compound obtained was filtrated when hot and then recrystallized in chloroform/acetone.

Yield: 88%. Mp: 268–269 °C. ^{1}H-NMR ($CDCl_3$): 7.37 (m, 2H); 7.25 (m, 2H); 7.14 (m, 4H); 4.78 (s, 2H); 3.20 (s, 2H); 2.50 (s, 3H). ^{13}C-NMR ($CDCl_3$): 176.9 (s); 141.4 (s); 138.4 (s); 126.9 (d); 126.7 (d); 124.8 (d); 124.2 (d); 46.9 (d); 45.4 (d); 24.2 (q).

*6.1.4. trans-9,10-Dihydro-9,10-ethanoanthracene-11,12-dimethyl-bis-p-toluenesulfonate (**29**) [13]*

Diol **40** [16] (3.5 g, 13 mmol) was added to a mixture of *p*-TsCl (5.3 g, 28 mmol) in pyridine (35 mL) at O °C, then left 2 days in a refrigerator. The mixture was poured onto ice water, a brown precipitate was separated and recrystallized in acetonitrile.

Yield: 66%. Mp: 108–110 °C. ^{1}H-NMR ($CDCl_3$): 7.73 (d, $J = 8.3$ Hz, 4H); 7.34 (d, $J = 8.3$ Hz, 4H); 7.08 (m, 8H); 4.20 (d, $J = 1.2$ Hz, 2H); 3.68 (m, 2H); 3.32 (t, $J = 9.6$ Hz, 2H); 2.46 (s, 6H); 1.56 (m, 2H). ^{13}C-NMR ($CDCl_3$): 145.0 (s); 142.0 (s); 139.1 (s); 132.5 (s); 129.9 (d); 127.9 (d); 126.5 (d); 126.1 (d); 125.5 (d); 123.6 (d); 71.2 (t); 44.6 (d); 41.9 (d); 30.9 (q).

*6.1.5. trans-9,10-Dihydro-9,10-ethanoanthracene-11,12-dimethyl-bis-N,N-dimethylcarbamate (**31**)*

Method A: Ditosylate derivative **29** (2.5 g, 4.4 mmol) and dimethylamine hydrochloride (2.7 g, 33 mmol) in presence of cesium carbonate (13 g, 40 mmol) in DMF (50 mL) were heated (100 °C) during 3 days. DMF was then eliminated under vacuum and the residue obtained was extracted in $CHCl_3$ and washed with NaOH solution. The organic phase was dried under drierite, filtered and concentrated under vacuum. The residue obtained was purified by liquid chromatography (CH_2Cl_2/MeOH). Compounds **31** (19% yield) and **6** (58% yield) were obtained.

Method B: Alcohol derivative **40** (0.9 g, 3.4 mmol), NaH 50% (0.4 g, 8.3 mmol) and carbamoyl chloride (0.8 g, 7.4 mmol) in THF were stirred, under nitrogen, at room temperature during 1 day. The mixture was hydrolyzed with water and extracted with CH_2Cl_2. The organic phase was dried under drierite, filtered and concentrated under vacuum. By-products obtained were not separated by liquid chromatography because of their close related polarity (whereas this method gives good yield (90%) in preparing **6**).

Yield: 22% (oil). ^{1}H-NMR ($CDCl_3$): 7.41 (m, 4H); 7.25 (m, 4H); 4.39 (s, 2H); 4.02 (d, d, $J = 10.7$ Hz, $J = 5.5$ Hz, 2H); 3.73 (d, d, $J = 10.7$ Hz, $J = 8.1$ Hz, 2H); 3.05 (s, 12H); 1.97 (m, 2H). ^{13}C-NMR ($CDCl_3$): 156.7 (s); 143.5 (s); 140.8 (s); 126.5 (d); 126.3 (d); 125.8 (d); 123.8 (d); 68.1 (t); 46.5 (d); 43.0 (d); 36.8 (q); 36.3 (q). $C_{24}H_{28}N_2O_4$ (C, H, N).

*6.1.6. trans-9,10-Dihydro-9,10-ethanoanthracene-11,12-dimethyl-bis-diphenylphosphinate (**32**)*

Diol **40** (0.53 g, 2 mmol) and triethylamine (5 mmol) in CH_2Cl_2 (25 mL) were added at 0 °C to Ph_2POCl (0.99 g, 4.2 mmol) in CH_2Cl_2 (5 mL). The mixture was stirred 1 day at room temperature and then washed with a solution of NaOH 5%. The organic phase was dried over drierite, filtered and concentrated under vacuum. The residue obtained was purified by liquid chromatography (ether/methanol, 95/5).

Yield: 70%. Mp: 92–95 °C. ^{1}H-NMR ($CDCl_3$): 7.83–7.63 (m, 8H); 7.45–7.28 (m, 12H); 7.21–7.14 (m, 4H); 7.05–6.92 (m, 4H); 4.32 (s, 2H); 3.72 (m, 2H); 3.43 (m, 2H); 1.77 (m, 2H). ^{13}C-NMR ($CDCl_3$): 142.6 (s); 139.8 (s); 131.5 (s); 131.4 (d); 131.2 (d); 130.2 (d); 128.4 (d); 126.1 (d); 125.8 (d); 125.5 (d); 123.3 (d); 66.6 (t) ($J_{PC} = 5.7$ Hz); 45.2 (d); 43.5 (d) ($J_{PC} = 6.1$ Hz). ^{31}P NMR ($CDCl_3$): 31.8. $C_{42}H_{36}O_4P_2$ (C, H).

*6.1.7. N,N′-Dimethyl-trans-9,10-dihydro-9,10-ethanoanthracene-11,12-dimethanamine (**39**)*

Amide **27** (8 g, 25 mmol) was added to a slurry of $LiAlH_4$ (6.6 g, 173 mmol) in anhydrous THF (170 mL) heated at 60 °C during 1 day. After cooling, H_2O (6.6 mL), NaOH 1.25N (6.6 mL) and H_2O (13.2 mL) were successively added. The suspension was filtered over celite. Filtrates were concentrated and acidic water (pH 1) was added and then extracted with ethylacetate. The aqueous phase was basified to pH 10 with NaOH and extracted with CH_2Cl_2. The organic phase was dried over drierite, filtered and concentrated under vacuum. Amine was isolated after chromatography on silicagel (methanol/ammonia, 95/5). Yield 27%. F°C (hydrochloride): 187–188 °C. ^{1}H-NMR (CD_3OD): 7.27–7.26 (m, 4H), 7.04 (m, 4H), 4.21 (s, 2H), 2.27 (s, 6H), 2.16 (m, 4H), 1.42 (br. s, 2H). ^{13}C-NMR (CD_3OD): 145.53 (s), 142.28 (s), 127.58 (d), 127.19 (d), 126.75 (d), 124.72 (d), 57.27 (t), 48.35 (d), 46.64 (d), 36.49 (q). $C_{20}H_{24}N_2$ (C, H, N).

*6.1.8. N,N′-Dimethyl-trans-9,10-dihydro-9,10-ethanoanthracene-11,12-dimethyl-bis-diphenylphosphinamide (**33**)*

Diamine **39** (0.44 g, 1.5 mmol) and triethylamine (0.70 g, 6.9 mmol) in CH_2Cl_2 (30 mL) were added at 0 °C to Ph_2POCl (0.78 g, 3.3 mmol) in CH_2Cl_2 (5 mL). The mixture was stirred 1 day at room temperature and then washed with a solution of NaOH 2N. The organic phase was dried over $CaSO_4$ filtered and concentrated under vacuum. The solid is purified by liquid chromatography (methanol/ammonia, 98/2).

Yield: 44%. Mp: 250–250.5 °C. ^{1}H-NMR ($CDCl_3$): 7.90–7.67 (m, 8H); 7.53–7.32 (m, 12H); 7.16 (d, 4H);

6.99–6.86 (m, 4H); 4.26 (br. s, 2H); 2.62 (d, $J = 10.6$ Hz, 6H); 2.47 (m, 4H); 1.31 (m, 2H). ^{13}C-NMR ($CDCl_3$): 144.06 (s); 139.86 (s); 132.56 (s) ($J_{PC} = 17.8$ Hz); 132.31 (d); 132.25 (d); 132.19 (d); 132.13 (d); 131.66 (d); 130.87 (s) ($J_{PC} = 17.8$ Hz); 128.55 (d); 128.48 (d); 128.39 (d); 128.31 (d); 126.19 (d); 125.72 (d); 125.33 (d); 122.72 (d); 52.61 (t) ($J_{PC} = 2.3$ Hz); 45.69 (d); 43.68 (d) ($J_{PC} = 3.4$ Hz); 35.40 (q) ($J_{PC} = 3.4$ Hz). $C_{44}H_{42}N_2O_2P_2$ (C, H, N).

6.1.9. *trans-9,10-Dihydro-9,10-ethanoanthracene-11,12-dimethyl-bis-N,N-dimethylsulfamate (**35**)*

Diol **40** (0.50 g, 1.87 mmol) in DMF (5 mL) was added to NaH 50% (5.6 mmol) in suspension in DMF (20 mL) at 0 °C under nitrogen. The mixture was stirred 3 h at room temperature. Dimethyl sulfamoyl chloride (4.5 mmol) was then added at 0 °C, stirred 12 h at room temperature. Solvent was evaporated under vacuum. The residue obtained was extracted with $CHCl_3$, washed to neutrality. The organic phase was dried over drierite and concentrated under vacuum. The residue obtained was purified by chromatography (eluent: chloroform/methanol, 99/1).

Yield: 35%. Mp: 78.5–80.5 °C. ^{1}H-NMR ($CDCl_3$): 7.28–7.22 (m, 4H); 7.12–7.08 (m, 4H); 4.27 (s, 2H); 3.80 (dd, $J = 9.8$ Hz, $J = 5.7$ Hz, 2H); 2.76 (s, 6H); 3.62 (dd, $J = 9.7$ Hz, $J = 8.8$ Hz, 2H); 2.82 (s, 12H); 1.80 (m, 2H). ^{13}C-NMR ($CDCl_3$): 142.39 (s); 139.53 (s); 126.66 (d); 126.33 (d); 125.61 (d); 123.75 (d); 71.90 (t); 45.15 (d); 42.48 (d); 38.46 (q). $C_{22}H_{28}N_2O_6S_2$ (C, H, N, S).

6.1.10. *1′-(N,N-Dimethylcarbamoyl)-9,10-(3′,4′-pyrrolidino)-9,10-dihydroanthracene (**36**)*

Amine **13** [14] (0.25 g, 1 mmol), triethylamine (0.2 g, 2 mmol) and carbamoyl chloride (0.16 g, 1.5 mmol) in toluene (15 mL) were stirred under reflux during 19 h and then washed with water. The organic phase was dried over drierite, filtered and concentrated under vacuum. The solid was recrystallized in methanol/water.

Yield: 82%. Mp: 171–171.5 °C. ^{1}H-NMR ($CDCl_3$): 7.23–7.17 (m, 4H); 7.06–7.02 (m, 4H); 4.13(d, $J = 2.0$ Hz, 2H); 3.41 (m, 2H); 2.81 (dd, $J = 110.9$, $J = 3.9$ Hz, 2H); 2.68 (m, 2H); 2.49 (s, 6H). ^{13}C-NMR ($CDCl_3$): 163.00 (s); 143.49 (s); 140.73 (s); 125.92 (d); 125.79 (d); 125.38 (d); 123.55 (d); 50.74 (t); 48.20 (d); 43.90 (d); 38.11 (q). $C_{21}H_{22}N_2O$ (C, H, N).

6.1.11. *N,N,N′,N′-Tetramethyl-trans-9,10-dihydro-9,10-ethanoanthracene-11,12-dimethanamine-N,N′-dioxide (**37**)*

Diamine **5** [14] (0.5 g, 1.56 mmol) in methanol (10 mL) was added to H_2O_2 30% (8.8 mmol). The mixture was stirred 4 h at room temperature and then concentrated under vacuum. The residue obtained was crystallized in acetone–ether.

Yield: 100%. Mp: 94–96 °C. ^{1}H-NMR (D_2O): 7.45 (m, 4H); 7.25 (m, 4H); 4.69 (s, 2H); 3.22 (m, 6H); 3.17 (s, 6H); 3.11 (m, 4H); 2.14 (br. s, 2H). ^{13}C-NMR (D_2O): 143.74 (s); 140.25 (s); 127.55 (d); 127.19 (d); 126.72 (d); 124.71 (d); 73.84 (t); 59.28 (q); 58.53 (q); 47.87 (d); 42.59 (d). $C_{22}H_{28}N_2O_2$, $5H_2O$ (C, H, N). Mp: (hydrochloride) 168 °C, $C_{22}H_{28}N_2O_2$, 2HCl (C, H, N).

6.2. Computational chemistry

6.2.1. Molecular modeling

Molecular modeling was performed using PcModel version 6.0 Molecular modeling Software (SERENA Software). Models were built from the experimental structure of **5** [30]. Only side chains were then modified keeping the bridged tricycle constant. For each compound, we took into consideration arbitrarily the SS and SR diastereoisomers. All calculations were made 'in vacuo' with RHF calculation option modulated by the 'non-planar' option for the total Pi system calculation. Geometry optimizations were carried out using minimizer based on the MMX force field. Conformational analysis of each compound was made using the Dihedral Driver option with a 10° stepwise increment for dihedral angles. No conformations were eliminated from the search based on energy. Angle files were thus produced, and the MMX energy corresponding to each conformation evaluated. We thus optimized the geometry in order to obtain for each molecule estimation of strain energy of the most stable conformers (E_{min}). For each molecule investigated, the d distance between the two HBA atoms varying from 3 to 8 Å, was fixed. Then each molecule was minimized to provide strain energies (E_s) which thus depend on the d distance imposed.

6.2.2. Multilinear regression

Multilinear regression calculation was performed with QSAR-PC: PAR program [37,38].

6.2.3. Evaluation of the HBA strengths ΣpK_x(HB)

All the hydrogen bond basicities have been estimated from the pK_{HB} values of model compounds representing the side chain functional group [26]. The simplified molecular fragments used in the calculation are presented in Table 2 together with the estimated overall basicity ΣpK_x(HB) of the molecule after a necessary conversion of the equilibrium constants in mole fraction units [18]. In compounds **29** and **35**, the presence of two SO accepting groups by sulfonyl function has been taken into account. In propafenone analogs, the ether and alcohol oxygen HB basicities are neglected. The HB accepting points of verapamil are considered to be the cyano and the amine functions.

6.3. Pharmacology

6.3.1. Cells

The 5178Y mouse T-lymphoma parent cell line was infected with the pHa MDR1/A retrovirus as previously described by Pastan et al. [39]. The L 5178 MDR cell line and the L5178Y parent cell line were grown in McCoy's 5A medium supplemented with 10% heat-inactivated horse serum, L-glutamine and antibiotics (and 60 ng mL^{-1} colchicine for MDR cell line).

6.3.2. Rhodamine 123 (R123) uptake assay

L5178 cells (2×10^6 mL^{-1}) were resuspended in serum-free medium and distributed (0.5 mL aliquot) to Eppendorf tubes. Compounds to be tested were added at different concentrations and samples were incubated for 10 min at room temperature. Then, R123 indicator was added to the samples at a final concentration of 5.2 μM and cells were incubated for 20 min at 37 °C, washed twice and resuspended in 0.5 mL phosphate buffer saline for analysis. The fluorescence of the cell population was measured by flow cytometry using a Beckton Dickinson FACScan instrument. Since R123 is a substrate of P-gp, there was a significant fluorescence between MDR and parental cells. Untreated MDR cells accumulate R123 only at low level. Verapamil was used as reference drug [40].

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