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Potential antitumor agents XVIII (1). Synthesis and cytotoxic activity of phenothiazine derivatives

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phenothiazine derivatives / cytotoxic activity

Even though the interest in phenothiazine and its derivatives within medicinal chemistry reached a peak in the 60's (the review by Schenker and Herbst [2] reports 6800 references) we can assume that it is still alive from the recent book edited by Gupta [3]. Apart from Dactinomycin, which is one of the most potent antitumor agents known at present and the well documented antitumor activity of phenothiazine itself [4–6], the study of antitumor agents among phenothiazine derivatives is mainly devoted to the search for: alkylating agents [7–11], compounds capable of enhancing the antitumor effect of well known drugs [12–18] and derivatives which can prove antitumor activity on their own [6, 19–34]. Within the latter group, the phenothiazine derivatives with psychotropic activity are the most widely studied compounds.

On the basis of these data we thought of manipulating the phenothiazine skeleton in order to produce a new molecule which, while maintaining the antitumor effect of the parent compound, could even show positive inotropic activity. Such a molecule could be useful, in combination therapy with doxorubicin, to potentiate its antitumor activity and to prevent its harmful effects on the heart.

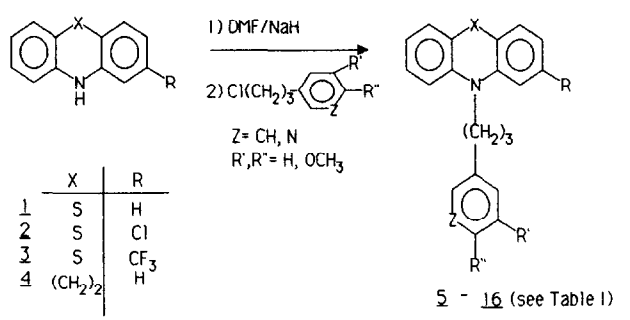
Chemistry

With this in mind, we decided to select 4 nuclei, to maintain the 3 carbon chain typical of psychotropic agents and to replace the dialkylamino group with a methoxyphenyl or a pyridyl group, because, in our experience, these fragments produced interesting cardiotonic agents when connected to an imidazothiazole moiety [35, 36].

The 12 compounds reported in scheme 1 and table I were prepared by treating the sodium salt of phenothiazine **1** (or 2-chlorophenothiazine **2**, 2-trifluoromethyl phenothiazine **3**, iminodibenzyl **4**) with the appropriate propyl chloride. The spectroscopic data of compounds **5–16** (table II) are in agreement with the assigned structures; an interesting review on the ^{1}H-NMR spectra of phenothiazine derivatives was published a few years ago [37].

Pharmacological results and discussion

The 12 compounds synthesized **5–16** were tested on guinea pig isolated atria [35, 36] but none of them proved positive inotropic activity. Nevertheless, they were submitted to a cytotoxicity test on P388 leukemia cells *in vitro*. The results obtained are reported in table III: for comparison purposes, we have also included the 4 unsubstituted molecules **1–4**.



Scheme 1.

Table I. Phenothiazine derivatives **5–16**.

Compound	X	R	Z	R'	R''	Formula (MW)	Anal.	Mp (°C)	Solvent
5	S	H	CH	H	OCH_3	$C_{22}H_{21}NOS$ (347.5)	C,H,N	70–73	Pet.ether
6	S	H	CH	OCH_3	OCH_3	$C_{23}H_{23}NO_2S$ (377.5)	C,H,N	66–70	Pet.ether
7	S	H	N	H	H	$C_{20}H_{18}N_2S$ (318.4)	C,H,N	85–88	Pet.ether
8	S	Cl	CH	H	OCH_3	$C_{22}H_{20}ClNOS$ (381.9)	C,H,N	57–60	Pet.ether
9	S	Cl	CH	OCH_3	OCH_3	$C_{23}H_{22}ClNO_2S$ (411.9)	C,H,N	98–100	Ethanol
10	S	Cl	N	H	H	$C_{20}H_{17}ClN_2S$ (352.9)	C,H,N	72–75	Pet.ether
11	S	CF_3	CH	H	OCH_3	$C_{23}H_{20}F_3NOS$ (415.5)	C,H,N	oil	
12	S	CF_3	CH	OCH_3	OCH_3	$C_{24}H_{22}F_3NO_2S$ (445.5)	C,H,N	oil	
13	S	CF_3	N	H	H	$C_{21}H_{17}F_3N_2S$ (386.4)	C,H,N	53–55	Pet.ether
14	CH_2CH_2	H	CH	H	OCH_3	$C_{24}H_{25}NO$ (343.4)	C,H,N	oil	
15	CH_2CH_2	H	CH	OCH_3	OCH_3	$C_{25}H_{27}NO_2$ (373.5)	C,H,N	108–110	Ethanol
16	CH_2CH_2	H	N	H	H	$C_{22}H_{22}N_2 \cdot HCl$ (350.9)	C,H,N	170–173	Ethanol

If we only consider compounds which gave $IC_{50} < 4$ μg/ml, we can confirm the cytotoxic activity of phenothiazine [4–6] and we can observe that the introduction of an arylpropyl chain at position 10 (**5–7**) leads to a drop in activity, especially when it is a 3,4-dimethoxyphenylpropyl chain; the deleterious effect of this group may even be seen in the iminodibenzyl derivative **15** whereas **14** and **16** were more active than the parent compound **4**. The contrary happens when the 3,4-dimethoxyphenylpropyl group is bound to the 2-substituted phenothiazines **2** and **3**: in particular, when the substituent at position 2 is a trifluoromethyl group, we obtained the most active compound (**12**). This becomes even more interesting if we consider that the 2-trifluoromethyl phenothiazine **3** was inactive.

In conclusion, we believe that these structures can be modulated by changing the substituent and the length of the chain in order to optimize the antitumor activity and to develop a concomitant cardiotonic potential. If antitumor activity of phenothiazine derivatives is related to their calmodulin antagonism [38], it is unlikely to reach this goal; but the interaction with calmodulin is not only mechanism of action suggested to explain the antitumor activity of this class of compounds [39–41] and forskolin, a well known positive inotropic agent [42], was also reported as an antimetastatic agent [43].

Experimental protocols

Chemistry

The melting points are uncorrected. Bakerflex plates (Silica gel IB2-F) were used for TLC and Kieselgel 60 (Merck 7734) for column chromatography: the eluent was a mixture of petroleum ether/acetone in various proportions. Analyses indicated by the symbols of the elements were within ± 0.4% of the theoretical values. The IR were recorded in Nujol on a Perkin–Elmer 298. The ^{1}H-NMR were recorded in DMSO–d_6 on a Varian EM390 (90 MHz) using TMS as internal standard. Phenothiazine and its derivatives are commercially available; 3-(4-methoxyphenyl)propyl chloride, 3-(3,4-dimethoxyphenyl)propyl chloride and 3-(3-pyridyl)propyl chloride were prepared from the corresponding hydroxypropyl derivatives [44, 45].

Table II. IR and ^{1}H-NMR of compounds **5–16**.

Compound	$\nu_{max.}$ (cm^{-1})	δ (ppm), J(Hz); hp = homopiperidine, py = pyridine
5	1325,1245, 1030,750	1.90(2H,qui,CH_2,J=7.5) 2.58(2H,t,CH_2,J=7.5) 3.67(3H,s, OCH_3) 3.80(2H,t,CH_2,J=7.5) 7.0(12H,m,ar)
6	1510,1305, 1235,740	2.0(2H,qui,CH_2,J=7) 2.60(2H,t,CH_2,J=7) 3.60(3H,s,OCH_3) 3.70(3H,s,OCH_3) 3.86(2H,t,CH_2,J=7) 6.20(1H,m,ar) 7.0 (10H,m,ar)
7	1570,1285, 1245,745	1.98(2H,qui,CH_2,J=7.5) 2.65(2H,t,CH_2,J=7.5) 3.84(2H,t, CH_2,J=7.5) 7.10(9H: 8H,m,ar + 1H,m,py) 7.50(1H,m,py) 8.40(2H,m,py)
8	1505,1295, 1235,740	1.90(2H,qui,CH_2,J=7.5) 2.60(2H,t,CH_2,J=7.5) 3.68(3H,s, OCH_3) 3.82(2H,t,CH_2,J=7.5) 7.0(11H,m,ar)
9	1515,1260, 1245,1155	1.98(2H,qui,CH_2,J=7)2.60(2H,t,CH_2,J=7) 3.60(3H,s,OCH_3) 3.70(3H,s,OCH_3) 3.86(2H,t,CH_2,J=7) 7.0(10H,m,ar)
10	1590,1565, 1250,745	1.95(2H,qui,CH_2,J=7.5) 2.65(2H,t,CH_2,J=7.5) 3.84(2H,t, CH_2,J=7.5) 7.10(8H: 7H,m,ar + 1H,m,py) 7.52(1H,m,py) 8.40(2H,m,py)
11	1510,1240, 1165,1120	1.94(2H,qui,CH_2,J=7.5) 2.64(2H,t,CH_2,J=7.5) 3.72(3H,s, OCH_3) 3.91(2H,t,CH_2,J-7.5) 7.15(11H,m,ar)
12	1510,1240, 1120,750	1.98(2H,qui,CH_2,J=7) 2.62(2H,t,CH_2,J=7) 3.60(3H,s,OCH_3) 3.70(3H,s,OCH_3) 3.90(2H,t,CH_2,J=7) 7.0(10H,m,ar)
13	1335,1240, 1155,1100	1.98(2H,qui,CH_2,J=7.5) 2.70(2H,t,CH_2,J=7.5) 3.95(2H,t, CH_2,J=7.5) 7.15(8H: 7H,m,ar + 1H,m,py) 7.54(1H,m,py) 8.42(2H,m,py)
14	1610,1510, 1245,745	1.70(2H,qui,CH_2,J=7.5) 2.50(2H,t,CH_2,J=7.5) 3.10(4H,s, hp) 3.65(3H,s,OCH_3) 3.68(2H,t,CH_2,J=7.5) 7.0(12H,m,ar)
15	1510,1480, 1255,1230	1.75(2H,qui,CH_2,J=7) 2.53(2H,t,CH_2,J=7) 3.13(4H,s,hp) 3.54(3H,s,OCH_3) 3.68(5H: 2H,t,CH_2,J=7 + 3H,s,OCH_3) 6.90 (11H,m,ar)
16	1600,1550, 745,685	1.80(2H,qui,CH_2,J=7.5) 2.80(2H,t,CH_2,J=7.5) 3.10(4H,s, hp) 3.72(2H,t,CH_2,J=7.5) 7.0(8H,m,ar) 7.95(1H,m,py) 8.35(1H,m,py) 8.80(2H,m,py)

General procedure for the synthesis of the 10-arylpropyl phenothiazines. 10 mmol of phenothiazine **1** (or **2–4**) were dissolved in 10 ml of anhydrous dimethylformamide and treated portionwise, under stirring at room temperature, with 12 mmol of sodium hydride. After 20 min the appropriate propyl chloride (12 mmol) was added dropwise: the mixture was maintained at room temperature for 30 min and refluxed for a time ranging from 15 to 90 min according to a TLC test. It was then poured onto ice and extracted with chloroform. A final purification by column chromatography gave **5–16** with a yield of 60–70%. From compound **16**, obtained as an oil, a crystalline hydrochloride was prepared.

Table III. Cytotoxic activity of compounds **1–16.**

Compound	IC_{50} *(μg/ml)* [a]
1	3
2	4
3	12
4	6
5	5
6	95
7	7
8	6
9	7
10	3
11	3
12	1
13	2
14	2
15	28
16	2
Doxorubicin	0.005
5-Fluorouracil	0.025
Lomustine (CCNU)	0.900
Chlorambucil	0.800

[a]Inhibitory concentration

Pharmacology

All the derivatives were tested against a culture of P388 leukemia cells derived from *in vivo* passaged tumor. Stock cultures of P388 cells were propagated *in vitro* in RPMI – 1640 medium (Whittaker MA Bioproducts, Walkersville, MD) supplemented with 10% foetal calf serum and 20 μM/l of 2-mercaptoethanol. The effect of the compounds on cell replication was determined in 2 ml cultures initially containing 100 000 cells. The compounds, dissolved in DMSO, were added to the culture medium at time 0. The cell cultures were incubated at 37°C in 5% CO2 for 48 h and the cells were then counted with a haemocytometer by counting the viable cells after staining with 0.5% trypan blue.

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