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STRUCTURE–ACTIVITY RELATIONSHIPS FOR A SERIES OF PHENYL (MORPHOLINO) METHANETHIONE DERIVATIVES: CYTOTOXICITY AGAINST ARTEMIA SALINA AND CANCER CELL LINES

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ABSTRACT

A series of phenyl (morpholino) methanethione derivatives (a-h) has been evaluated for antiproliferative activity *vitro* against MCF-7 and HCT-116 cancer cells by the MTT assay and toxicity on the larvae of *Artemia salina* Leach. From the toxicity studies and structural activity relationship of compounds (a-h), it is clear that mesomeric donor effect groups on benzene ring have increased actively this toxicity. Moreover, for cytotoxicity studies on MCF-7 and HCT-116 cancer cells in breast and colon cancer respectively, the compounds (a) and (f) have showed an interesting activity with 100 µM of (f) on MCF-7 cells (35.7 ± 3.1%) and 100 µM of the compound (a) on the HCT-116 cells (58.1 ± 1.5%). A cytotoxicity on MCF-7 of these compounds has expressed a toxicity on the larvae of *Artemia salina.* This does phenyl (morpholino) methanethione derivatives a potential source of anti-cancer molecules.

KEYWORD: *Artemia salina* Leach, MCF-7, HTC-116, phenyl (morpholino) methanethione derivatives.

INTRODUCTION

Cancer is one of the leading global health problems and the second major cause of death in the developing countries [1,2] with increasing incidences every year. The advanced treatment opportunities of these cancers haven't overcame the major problems of chemotherapy such as drug resistance and severe side effects due to the lack of specificity and high toxicity. Therefore identification of novel molecules with potent anti-proliferative activity, low toxicity and with minimum side effects is necessary.

Sulphur Compounds continues to generate intense research interests through interesting biological, pharmaceutical, industrial and/or materials properties.[3,4,5]

Thioamide and derivatives, sulphur compounds, have interesting anticancer activity, in particulary, benzhydrylpiperazine thioamide derivatives that were recently screened against breast (MCF-7) and colorectal (HCT-116) cancer cell lines.[6]

In 2008, Kapanda *et al*., showed that other thioamides, in particular phenyl (morpholino) methanethione derivatives, inhibit the activity of the enzyme MGL (Monoacylglycerol Lipase) and FAAH (Fatty Acid Amide Hydrolase).[7]

Some phenyl (morpholino) methanethione derivatives synthesed as that phenyl (morpholino) methanethione and dimethylaminophenyl (morpholino) methanethione were discussed for their toxicity on larvae of *Artemia salina* Leach and trypanocide activity[8] thougth our study and reported.

In a continuation of our work, a series phenyl (morpholino) methanethione derivatives was synthesed, characterized and reported.[9]

The paper presents cytotoxicity of these phenyl (morpholino) methanethione derivatives is reported. These compounds were evaluated for their antiproliferative potency against cancer cell lines (MCF-7) and (HCT-116) respectively of the breast and colon which has never been studied, to our knowledge.

Structure-activity relationship of phenyl (morpholino) methanethione derivatives based on their results of cytotoxicity and toxicity on *Artemia salina* Leach was determined.

MATERIAL AND METHODS

Cytotoxicity on MCF-7 and HCT-116

Cytotoxicity of samples were estimated respectively on the MCF-7 and HCT-116 cancer cell lines of the breast and colon respectively as described by Bekir *et al*[10] with some modifications.

Cells were incubated for 24 h at 37 ° C and distributed in 96-well plates at 12000 cells/well in 100 µL before the treatment with the sample solution.

The sample solution of 100 µM concentration is obtained by dissociating an amount of the sample (pure molecule) in 5% DMSO. After 48 h at 37 ° C ; cell growth was estimated by the test of 3- (4-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) bromide. MTT is a yellow water soluble tetrazolium salt. Optical density was measured at 540 nm.

The results are expressed as a percentage of survival relative to untreated cells according according to the following formula:

$$\%\text{Cell viability} = \frac{\text{OD mean of treated cells}}{\text{OD mean of controlled cells}} \times 100$$

Toxicity against *Artemia salina* Leach

This test was performed on larvae of brine shrimp (*Artemia salina* Leach) by method of Michael *et al* [11] resumed by Vanhaecke *et al* [12] and by Sleet Sleet and Brendel.[13] Thus, encysted *Artemia salina* eggs are incubated in seawater at pH 7-8 (48h). Then, series of solutions of test substances at varying concentrations and progressive were prepared in DMSO (dimethyl sufoxide)/seawater. A defined number of larvae introduced into each solution. All solution sand control solutions containing no active substance were left stirring for 24hours.Counting under a microscope the number of death larvae in each solution used to evaluate the toxicity of the solution. In the case where there was death in the control medium, the data was corrected by Abbott's formula.[14]

$$\%\ \text{décès} = \frac{\text{test} - \text{contol}}{\text{control}} \times 100$$

Data (dose-response) are transformed by logarithm and the half-lethal concentration LC_{50} is determined by linear regression.[15]

Statistical analysis

All data were expressed as means ± standard deviations of triplicate measurements. The confidence limits were set at $p<.05$. Correlations and regressions were carried using Excel program. Data analysis procedure (ANOVA) was performed in to assess the data.

RESULT AND DISCUSSION

The series of thiobenzamides[9] that constitute this study has a general chemical structure illustrated in "fig. 1".

Theoretical study based on the pharmacokinetic properties rules[16,17] and drug availability of compounds of the series of thiobezamides are summarized in the table 1. The scaffold has advantageous properties as low molecular weight, reasonable *C*log *P*, good hydrogen bond donating and accepting capabilities (Table 1), easy and economical synthetic routes.

It showed that they constitute good candidates drugs if they have interesting biological activities.[17]

*C*log *P* estimated Lipophilicity which is a main physico-chemical determinant influencing the bioavailability, permeability and frequently the toxicity of drugs.[17]

A substance is the more lipophilic as log P is positive. The log P values were calculated using Chem Draw Ultra 8.0.[18]

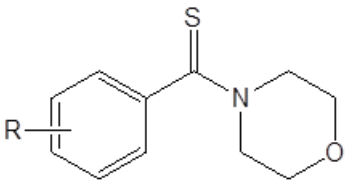
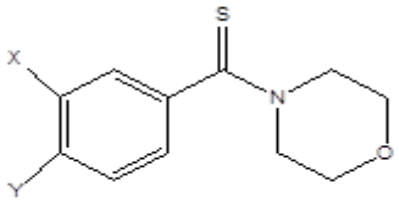


Figure 1: General chemical structure of thiobenzamides synthesed (scaffold).

Table 1: Properties compatible with reasonable pharmacokinetics and drug availability, Rules of Lipinski applied to thiobenzamides.



Compounds			Molecular weight ($g.mol^{-1}$)	Clog P	No of H bond donors	No of H bond donor acceptors	No of criteria met
	X	Y					
a	H	H	207.07	1.581	0	3	all
b	NO_2	H	252.06	1.519	0	4	all
c	H	NO_2	252.06	1.519	0	4	all
d	OH	H	223.07	0.749	1	3	all
e	H	OH	223.07	0.749	1	3	all
f	OH	OH	39.06	0.152	2	4	all
g	H	$N(CH_3)_2$	250.11	1.416	0	2	all
h	H	COOH	251.06	1.351	1	4	all
Rules			< 500	< 5	≤ 5	< 10	At least 3

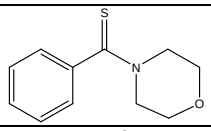
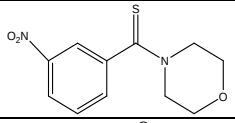
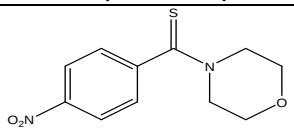
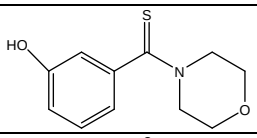
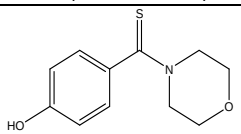
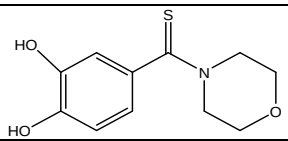
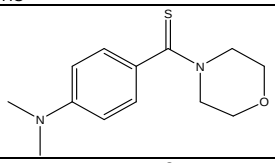
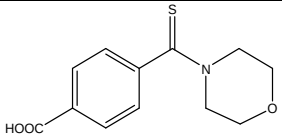
log P values were calculated using ChemDrawUltra 8.0

Cytotoxicity

The compounds synthesed are good drug candidate through their bioavailability; they have been submitted to cytotoxicity and toxicity evaluation.

The LC_{50} values on larvae of *Artemia salina* and the 100 µM inhibitory concentration (IC_{50}) values of the compound on cells of the cancer cell lines MCF-7 and HCT-116, respectively cancer cell lines of breast and human colon cancer, are summarized in Table 2.

Table 2: Cytotoxic activities and toxicity on *Artemia Salina* Leach of thiobenzamides.

Compounds		Inhibition (IC_{50}) of 100µM Compounds		Activity on larvae of *Artemia salina*	
		MCF-7	HCT-116	LC_{50} en µM	Toxicity
a		14.80 ± 0.30	58.10 ± 1.50	1463.06 ± 7.00	no toxic
b		na	na	309.95 ± 9,20	no toxic
c		na	na	309.95 ± 0.70	no toxic
d		na	na	248.50 ± 3.30	actif
e		na	na	246.03± 0.60	actif
f		35.70 ± 3.10	1.20 ± 0.10	242.07 ± 0.30	actif
g		na	na	214.00 ± 9.00	actif
h		na	na	294.51 ± 5.00	no toxic

Toxicity by using Lapachol value (IC_{50} = 281mM) as reference; na : no actif.

Cytotoxicity on MCF-7 and HCT-116

Inhibition potential study with 100 µM of each compound on the cells of the MCF-7 and HCT-116 cancer cell lines, respectively cancer cell lines of breast and human colon cancer showed that the compounds (f) and (a) have an activity on MCF-7 and HCT-116 cells.

Thus, in our series of thiobenzamide molecules, (a) is more active on HCT-116 cells (58.1 ± 1.5%) and (f) more active on MCF-7 cells (35.7 ± 3, 1%).

Moreover, a structure-activity relationship shows that the substitution of the groups: NO_2; $N(CH_3)_2$, OH, -COOH on the phenyl group of (a) attenuated the cytotoxicity of the molecules on HCT-116 cells.

Although the substitution of two hydroxyl groups at positions (meta) and (para) on the aromatic ring decreased the inhibitory activity on HCT-116 cells (58.1 to 1.2%), it significantly increased inhibitory activity on MCF-7 cells (14.8 to 35.7%).

The presence of phenolic hydroxyl groups in the structure of molecule is responsible for its antioxidant activity[19] allow so to cytotoxic effects interesting in particular on the breast human cancer.[20] So, the presence of phenolic hydroxyl groups in the structure of compound (f) could explicate the increase cytotoxic activity observed on MCF-7 cells.

Toxicity to *Artemia salina* Leach

Lapachol [2-hydroxy-3-(3-methylbut-2-enyl) naphthalene-1,4- dione] (LC_{50} = 281 µM) a reference compound was used as positive control. According to Santos-Pimenta *et al* [21] and Angelia *et al* [22] a compound having an LC_{50} less than 281 µM, was active on larvae of *Artemia salina.*

The toxicity test on larvae of *Artemia salina* shows that compounds (d), (f) and (g) are active, so toxic on the larvae.

A structure - toxicity relationship of these thioamides shows that bioactivity is enhanced by the attachment of a substituent to the phenyl group.

Taking as reference the thiobenzamide not substituted namely phenyl (morpholin-4-yl) methanethione (a), the attachment of a nitro group in the ortho position to the aromatic ring (b) (309.95 ± 9.20 µM) induced the toxicity of thiobenzamide (a) (1463.06 ± 7 µM).

This induced toxicity remains the same if this substituent was in the para (c) position (309.95 ± 0.70µM). However, a changement of this nitro group with dimethylamino increased this toxicity (g) (214 ± 90 µM); thus making this compound most toxic on *Artemia salina*.

Furthermore, binding of a hydroxyl group in the meta position to the aromatic ring (d) (248.50 ± 3.30 µM) of (a) also increased the toxicity considerably compared to its nitro homolog (b). Toxicity is increased when two hydroxyl groups are attached to meta and para

(f) (242 ± 0.30 µM), respectively. We can deduce that the hydroxyl substituents favour the toxicity of thiobenzamide relative to the nitro homolog.

Compound (h) (294 ± 50 µM) where a carboxylic group is in the para position is not active on the larvae. The introduction of this substituent has nevertheless increased the toxicity with respect to the molecule (a) (1463 ± 70 µM).

Regarding the mesomeric effect of each group, it is clear that mesomeric donor effect groups on benzene ring have increased actively this toxicity. The position of this donor mesomere groups and degree of mesomere effect influence this toxicity.

The changement a hydroxyl group (donor group) the meta position (d) to para position is increased the toxicity (248.50 - 246.03 µM). This increase is observed too with dimethylamino group (g; 214.00 µM) that are a superior effect than hydroxyle group (d; 248.50 µM).

Correlation; toxicity of the shrimp larvae and anticancer agents

Study of the toxicity on larvae of *Artemia salina* is interesting. Many studies have shown the existence of a good correlation between the test of toxicity on Artemia salina larvae and many other biological activities.[21,23,24]

The toxicity of the shrimp larvae and cytotoxicity on MCF-7 relationship of these thioamides shows that cytotoxicity on MCF-7 induced the toxicity on shrimp larvae.

The high degree of correlation between the cytotoxicity of the shrimp larvae and MCF-7 has already been observed by Ramesh *et al* (2009) during his work on piperidinyl-diethylstilbestrol (DES) and pyrrolidinyl-diethylstilbestrol (DES)[25] and others studies.[26,27]
The present work also revealed that there is a correlation between cytotoxicity on MCF- 7 and toxicity of the shrimp larvae for phenyl (morpholino) methanethione derivatives.

(3,4-dihydroxyphenyl) (morpholin-4-yl) methanethione (f) owned an activity interesting on MCF-7 cell is active on shrimp larvae.

A correlation exists too between this toxicity on the larvae of *Artemia salina* and cytotoxicity on cells 9PS and 9KB of carcinoma human, on the one hand[28] A - 549 cells of pulmonary carcinoma and HT-29 colon carcinoma cells, on the other hand.[29] Therefore phenyl(

morpholino)methanethione derivatives of less LC_{50} than 281 µM could be used in the treatment of these cancers.

CONCLUSION

In this study, eight (08) phenyl (morpholino) methanethione derivatives were evaluated for their biological potential activity. It is revealed that a larger series of phenyl (morpholino)methanethione derivatives would be potential sources of anticancer molecules especially on the cancer cells MCF-7 and HCT-116. To our knowledge, this is the first time that the anti-cancer activities of these molecules are evaluated on the cancer cells MCF-7 and HCT-116. The toxicity on the larvae of *Artemia salina* showed a structure - toxicity relationship and a correlation between cytotoxicity on MCF-7 and toxicity.

These compounds with their good pharmacokinetic properties open an interesting opportunity for exploration of other biological study.

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CONFLICT OF INTEREST STATEMENT

The authors declared no conflict of interest.

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