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Synthesis and Biological Evaluation of Ethacrynic Acid Derivatives Bearing Sulfonamides as Potent Anti-Cancer Agents

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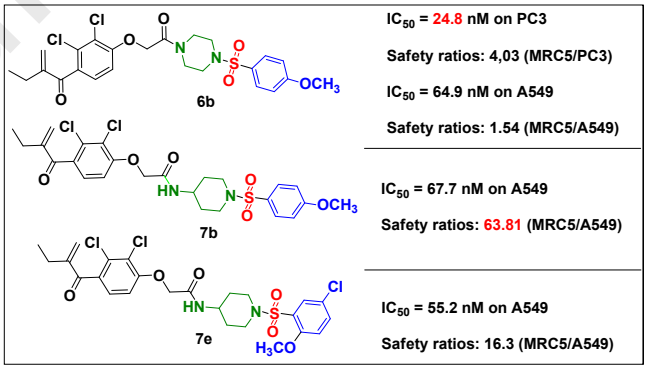
Abstract

A series of ethacrynic acid (2-[2,3-dichloro-4-(2-methylidenebutanoyl)phenoxy]acetic acid) (**EA**, Edecrin) containing sulfonamides linked *via* three types of linkers namely 1,2-ethylenediamine, piperazine and 4-aminopiperidine was synthesized and subsequently evaluated *in vitro* against HL60 and HCT116 cancer cell lines. All the **EA** analogs, excluding **6a** and **6c**, showed anti-proliferative activity with IC_{50s} in the micromolar range (less than 4 uM). Three derivatives **6b**, **7b** and **7e** were selected for their interesting dual activity on HL60 cell line in order to be further evaluated against a panel of cancer cell lines (HCT116, A549, MCF7, PC3, U87-MG and SKOV3) as well as on MRC5 as a normal cell line. These compounds displayed IC_{50} values in nanomolar range against A549, MCF7, PC3 and HCT116 cell lines, deducing the discovery that piperazine or 4-aminopiperidine is the linker's best choice to develop **EA** analogs with highly potent anti-proliferative activities own up to 24 nM. Besides, in terms of selectivity, those linkers are more suitable offering safety ratios of up to 63.8.



Chemotherapeutic agents are the primary way to brawl against cancer and represent the first level of research in cancer therapeutics. Therefore, the development of new anticancer agents represents an important and challenging field in medicinal chemistry.[1–3] Generally, the main sources of compounds for drug development are natural compounds,[4–9] analogues of existing drugs[10–15] and new synthetic products.[16–24]

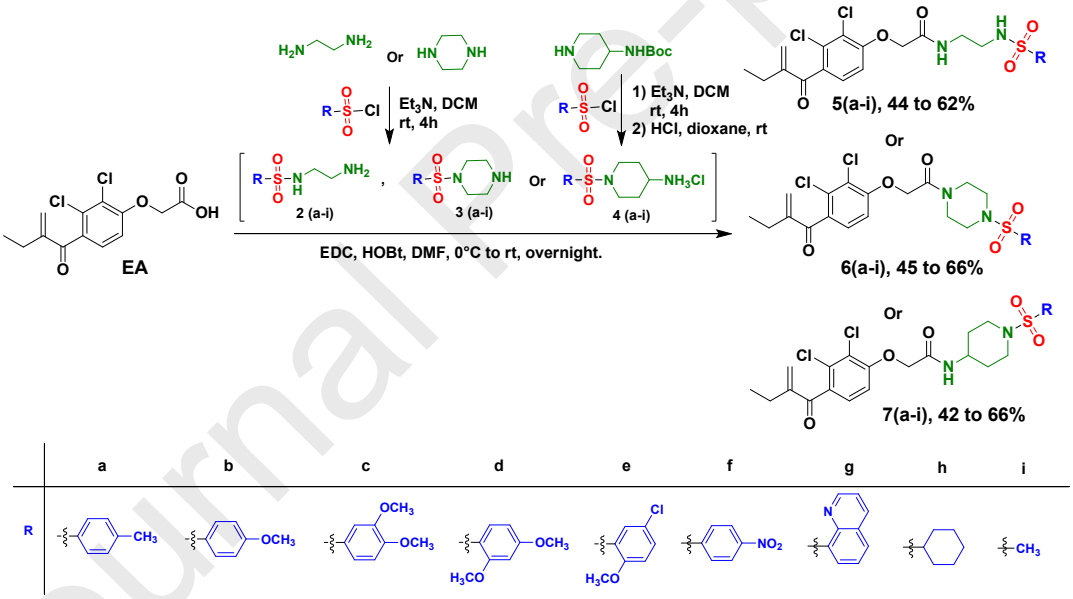
A myriad of natural and synthetic α,β-unsaturated carbonyl based compounds including chalcones, curcumin and their synthetic analogs are identified to exhibit antitumor activities.[25,26] α, β-unsaturated carbonyl unit has an extraordinary advantage in the development of biologically active and therapeutically relevant compounds in medicinal chemistry. Recently, a number of derivatives containing α, β-unsaturated carbonyl group has been reported to display versatile biological activities such as antitumor activity, antimalarial effects, antiviral activity, and reduce intraocular pressure. Besides, the **EA** is a well-known drug used not only as a diuretic agent [27,28] but also as a treatment of high blood pressure and edema.[29,30] The ethacrynic acid's α,β-unsaturated carbonyl unit acts as a Michael acceptor involved in the inhibition of the major classes of glutathione S-transferases (GSTs) (α, μ, and π) that plays a key role in regulating multidrug resistance.[31,32] In addition to its modest anti-proliferative effect against tumor cells,[33] the **EA** was therefore used as an adjuvant to improve the therapeutic efficacy of several anticancer agents.[34–37] Several analogues were synthetized in order to enhance the biological activities.[38–43] All the modification of the α,β-unsaturated unit by reduction of the double bound and/or the carbonyl have resulted in a total loss of the anti-proliferative activities.[40,41] Similar results are found when one or the two chlorines of the **EA** are removed or replaced by other groups. The major improvement relied on the modification of the carboxylic acid part into ester, amide or heterocycle derivatives.[41–43] Recently, the carboxylic acid in the **EA** was replaced by either aromatic esters or aromatic amides and the isolated new compounds exhibited promising anti-proliferative activities especially when the aromatic amides were used.[15,43]

Sulfonamide has been confirmed to be an important functional group in drug discovery.[44] Drugs containing sulfonamides were the first chemical substances used to cure and prevent bacterial infections in humans.[45] Similarly, they are known as key parts of many chemotherapeutic agents. Regarding this, an extensive work has been performed over the last few years the synthesis, structure-activity relationships (SAR), and the anticancer activities evaluation of compounds bearing sulfonamides.[46–50]

In continuation of our recent report on **EA** derivatives exhibiting anti-proliferative activities by the activation of caspase cascade which induce the apoptosis,[15] we wish to report here the synthesis and anti-proliferative activities of sulfonamide containing analogues of **EA**. This original series of **EA**-modified compounds was first evaluated *in vitro* against HL60

(promyelocytic leukemia) and HCT116 (human colon carcinoma) cancer cells. Then, the most promising analogues were tested on a panel of cancer and non-cancer cells.

The synthesis of compounds **5(a-i)**, **6(a-i)** and **7(a-i)** is illustrated in scheme 1. The sulfonamide moieties were introduced on **EA** *via* three types of linkers namely 1,2-ethylenediamine, piperazine and 4-aminopiperidine. The series of **EA** sulfonamide derivatives **5(a-i)**, **6(a-i)** and **7(a-i)** were synthesized through a two-step sequence starting from commercially available **EA** according to scheme 1. First, the key intermediates **2(a-i)**, **3(a-i)** and **4(a-i)** were prepared by reacting a variety of sulfonyl chlorides **1(a-i)** with either 1,2-ethylenediamine, piperazine or 4-(*N*-Boc-amino) piperidine *via* an *N*-sulfonylation reaction in the presence of triethylamine at room temperature in DCM.[51] These intermediates were dried and used directly in the next step without further purification. Afterwards, a conventional condition of peptide synthesis was employed using the intermediates **2(a-i)**, **3(a-i)** or **4(a-i)** and **EA** in the presence of EDC/HOBt as activators in DMF to afford **EA** derivatives **5(a-i)**, **6(a-i)** and **7(a-i)** in moderate to acceptable yields.



Scheme 1: Synthesis of compounds **5(a-i)**, **6(a-i)** and **7(a-i)**.

All the synthesized compounds, which were purified by column chromatography, were characterized by ^{1}H and ^{13}C NMR and high-resolution mass spectrometry and were in full accordance with their depicted structures.

Twenty seven **EA** derivatives described in this work were tested *in vitro* to evaluate their anti-proliferative effects on two different cancer cell lines namely, HL60 (promyelocytic leukemia) and HCT116 (human colon carcinoma). The most promising compounds were then tested against a panel of cancer and normal cells.

The anti-proliferative activities are based on the evaluation of the percentage of viability using the sensitive CellTiter-Glo® luminescent assay, which is a homogeneous method to determine the number of viable cells in culture and is based on the quantitation of the ATP (an indicator of metabolically active cells).[52] The amount of ATP is directly proportional to the number of viable cells present in the culture (supporting info). The viability of both HL60 and HCT116 were measured for all compounds at the 10μM concentration (Table 1). The results show that most of the **EA** analogues, except **6c,** inhibited the cell proliferative growth of HL60 (11 to 64% of cell viability). In the case of HCT116, only compound **6b** is very potent with 21% cell viability. Using the Graph Prism software from polynomial curves (four or five-parameter logistic equations), the IC_{50} values were determined for all the compounds induced a reduction of the viability over 50%. All the **EA** analogs, excluding **6a** and **6c,** showed anti-proliferative activity against HL60 with IC_{50} ranging between 0.58 and 3.39 μM. SAR discussion based on our previously reported **EA** analogues[15] and the current compounds bearing sulfonamide moiety SO_2NRR' (see lead compound **III**, figure 1) showed that the introduction of the sulfonamide group on the previously used linkers maintained the anti-proliferative activity against HL60. For example, compound **5b** with *N*-(2-aminoethyl)-4-methoxybenzenesulfonamide linker and compound **6b** with 1-((4-methoxyphenyl)sulfonyl)piperazine linker, and containing both of them a methoxy group on the aromatic ring, display almost the same inhibitory activity in comparison with their previously reported analogues **I** and **II** bearing 2-(4-methoxyphenyl)ethan-1-amine and 2-(4-methoxyphenyl)ethan-1-amine linker, respectively.[15] IC_{50} values of **5b** and **6b** are 0.86, 0.59 μM, respectively, while, their corresponding compounds **I** and **II** displayed the IC_{50} values of 1.3 and 0.8 μM, respectively.[15] In contrast, compound **5c** bearing *N*-(2-aminoethyl)-3,4-dimethoxybenzenesulfonamide revealed a sudden drop of potency with IC_{50} value of 3.38 μM compared to its previously reported analogue **IV** containing 2-(3,4-dimethoxyphenyl)ethan-1-amine moiety which displayed an IC_{50} of 0.8 μM.[15]

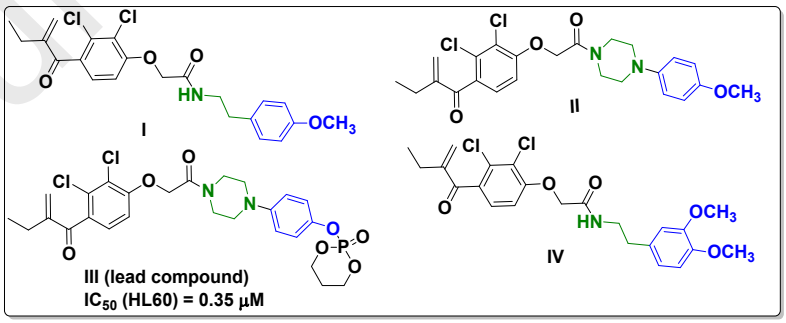
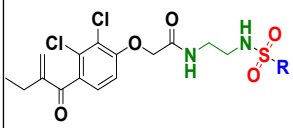
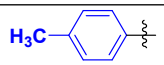
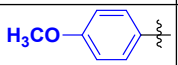
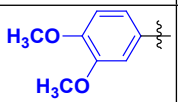
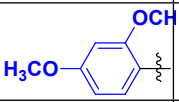
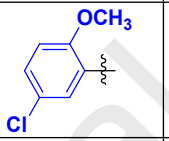
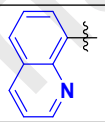
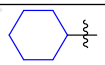
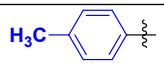
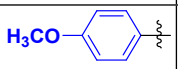
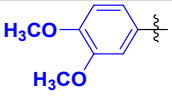
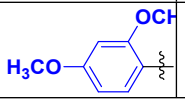


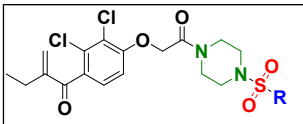
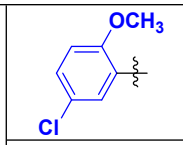
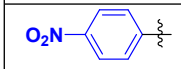
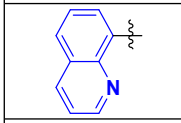
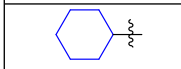
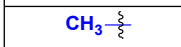
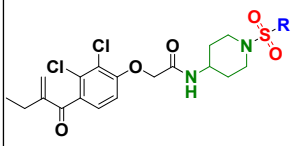
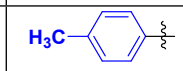
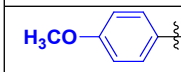
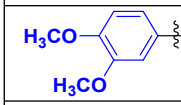
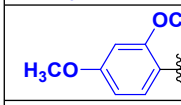
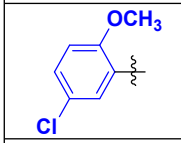
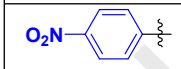
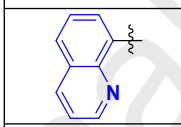
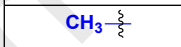
Figure 1. Chemical structures of previously reported **EA** analogues.[15]

Subsequently, compounds **6b**, **7b** and **7e** (Figure 2) were selected for their interesting dual activity on both cancer cell lines. The IC_{50} against HCT116 for these three compounds display good to very good activity (IC_{50} values of 0.05, 1.15 and 0.79 μM, respectively). Interestingly,

compound **6b** exhibited good selectivity towards HCT116 (almost 12 folds more active on HCT116 cell line than on HL60 cell line). SAR discussion based on the IC_{50} values of compounds **6b**, **7b** and **7e** and the previously reported compounds[15] upon cancer cell HCT116, revealed that the piperazine linker along with the presence of the sulfonamide moiety (compound **6b**) are crucial for the achievement of selective and very potent anti-proliferative **EA** analogues.

Table 1. Percentage of viability at 10 μM of **EA** derivatives on HL60 and HCT116 and IC_{50} of selected **EA** analogues

Structure	R-	Compounds	HL60 (%)	HCT116 (%)	IC_{50} (HL60) (μM)	IC_{50} (HCT116) (μM)
		5a	17±0.8	100±6.0	2.37±1.3	
		5b	24±3.0	97±5.0	0.86±0.1	
		5c	19±0.5	97±3.0	3.36±1.0	
		5d	35±0.2	77±2.0	2.79±0.3	
		5e	18±0.7	78±6.0	1.60±0.4	
		5f	27±3.0	59±0.4	0.58±0.1	
		5g	20±0.9	100±2.0	1.33±0.4	
		5h	28±2.0	91±3.0	1.34±0.4	
		5i	21±1.0	62±0.4	0.94±0.4	
		6a	64±2.0	100±5.0		
		6b	17±0.3	21±2.0	0.59±0.1	0.05±0.001
		6c	100±2.0	89±1.0		
		6d	15±0.2	40±2.0	3.19±0.7	

		**6e**	**24±1.0**	**75±2.0**	**1.41±0.3**	
		**6f**	**24±0.4**	**100±3.0**	**3.26±0.9**	
		**6g**	**28±0.2**	**100±1.0**	**2.98±0.9**	
		**6h**	**32±2.0**	**100±6.0**	**2.32±1.3**	
		**6i**	**24±0.3**	**68±0.3**	**3.39±1.1**	
		**7a**	**25±2.0**	**100±0.2**	**0.98±0.02**	
		**7b**	**25±2.0**	**45±1.0**	**0.63±0.004**	**1.15±0.11**
		**7c**	**25±0.8**	**99±1.0**	**1.31±0.3**	
		**7d**	**11±0.8**	**90±3.0**	**0.86±0.03**	
		**7e**	**16±0.8**	**nc**	**0.70±0.01**	**0.79±0.14**
		**7f**	**23±2.0**	**100±2.0**	**2.47±0.8**	
		**7g**	**34±0.5**	**84±1.0**	**0.97±0.02**	
		**7h**	**41±2.0**	**61 ± 3.0**	**2.94±0.8**	
		**7i**	**26±0.3**	**73±1.0**	**3.33±0.7**	

nc : not calculated

To go further in our study, these compounds were engaged in additional investigations regarding their anti-proliferative activities on a panel of cancer cell lines. These cancer cell lines are representative of diverse tissues/organ tumors: human breast cancer (MCF7), human glioblastoma cancer (U87-MG), human prostate cancer (PC3), ovarian carcinoma (SK-OV3), human lung epithelial carcinoma (A549), human colon cancer (HCT116) and proliferative human lung fibroblasts as non-cancer cell line (MCR5) (Table 2).

The data in table 2 show that all compounds display a potent anti-proliferative activity with IC_{50s} in the nanomolar range (less than 100 nM) on at least one type of cancer cell line. Interestingly, **6b**, **7b** and **7e** inhibited the proliferation of A549 cells with IC_{50} values of 0.064, 0.067 and 0.055 µM, respectively. These potencies are similar to doxorubicin, which displays an IC_{50} of

0.056 µM against A549. On the MCF7 cell line, the most potent compounds are **6b** with IC_{50} of 0.056 µM and **7e** with IC_{50} of 0.097 µM. In the case of PC3 and HCT116 cell lines, **6b** showed a very high potency with IC_{50} of 0.024 µM and 0.050 µM, respectively. Compound **6b** is even more active than doxorubicin on both cell lines. Concerning U87-MG and SKOV3 cell lines, **6b, 7b** and **7e** exhibited the same range of activities with more moderate IC_{50} values between 0.150 and 0.309 µM.

Encouraged by these very interesting results, we calculated the selectivity index of **6b** against HCT116 compared to compounds **7b** and **7e**. For compound **6b** and **7b** having the same sulfonamide moiety and differentiated only by the type of linker, the selectivity index which is IC_{50}(**7b**)/IC_{50} (**6b**) show 23 fold. While the selectivity index of **6b** versus **7e** by calculating IC_{50}(**7e**)/ IC_{50}(**6b**) is 15.8 fold.

It is well known that lipophilicity, which associates both biological and physicochemical properties, is a crucial property for drug absorption, distribution, potency and elimination. Thus, using Marvin application ChemAxon, we calculated the membrane-water partition coefficient (LogP) for the three compounds **6b**, **7b** and **7e.** All the three compounds displayed similar LogP (between 3.78 and 4.38) suitable for the future development of drug-like compounds.[53]

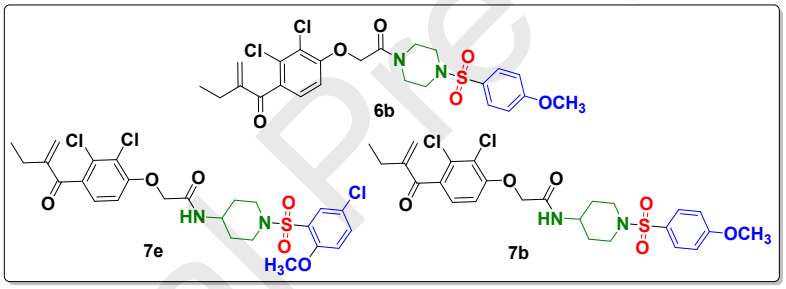


Figure 2. The structures of sulfonamide-**EA** derivatives **6b, 7b** and **7e**

Table 2. IC_{50} Values (µM) determined for **6b**, **7b, 7e** and **doxorubicin** upon various cancer human cells.

	Compounds IC_{50} (µM)			
	6b	**7b**	**7e**	**Doxorubicin**
HL60	**0.590 ± 0.100**	**0.630 ± 0.040**	**0.700 ± 0.100**	**0.010 ± 0.001**
A549	**0.064 ± 0.003**	**0.067 ± 0.004**	**0.055 ± 0.001**	**0.056 ± 0.0008**
MCF7	**0.561 ± 0.0009**	**0.128 ± 0.056**	**0.097 ± 0.009**	**0.120 ± 0.009**
PC3	**0.024 ± 0.007**	**0.112 ± 0.011**	**0.076 ± 0.003**	**0.002 ± 0.0001**
U87-MG	**0.207 ± 0.012**	**0.238 ± 0.047**	**0.307 ± 0.089**	**0.09 ± 0.0023**
SKOV3	**0.219 ± 0.033**	**0.309 ± 0.004**	**0.150 ± 0.060**	**nd±**

HCT116	**0.050 ± 0.001**	**1.150 ± 0.110**	**0.790 ± 0.140**	**0.090 ± 0.003**
MRC5	**0.100 ± 0.050**	**4.320 ± 0.380**	**0.900 ± 0.010**	**0.039 ± 0.001**

LogP[a]	**3.82**	**3.78**	**4.38**	**1.27**

[a]Partition coefficient, a measure of lipophilicity.

To select the compounds with the highest selectivity for cancer cells, the safety ratios for MRC5/cancer cells were calculated for compounds **6b**, **7b** and **7e**. The results summarized in table 3 show that compound **7b** has the highest selectivity for cancer cells A549, PC3 and MCF7 with safety ratios of 63.8, 38.57 and 33.75, respectively. This compound is also selective for U87-MG and SKOV3 cell lines with safety ratios of 18.15 and 13.98, respectively. Interestingly, compound **7b** is more selective for all cancer cells than doxorubicin, which exhibited very low safety ratios (between 19.08 to 0.33 fold). The compound **7e** is also more selective than doxorubicin, except for PC3, but its safety ratios (16.3 to 1.13) are lower than those obtained with **7b**. The compound **6b** is the less selective **EA** analogues for all cancer cells with low safety ratios. Finally, considering that the structure of compounds **7b** and **6b** which only differs by their linkers, those results suggest that 4-aminopiperidine is the best choice of linker to develop **EA** analogs with very potent anti-proliferative activities. However, in terms of selectivity, the 4-aminopiperidine linker is more suitable regarding the fact that **EA** analogue **7b** offers safety ratios between 3.75 and 63.8.

Table 3. Safety ratios of compounds **6b**, **7b**, **7e** and doxorubicin

Compounds / **SI**	**6b**	**7b**	**7e**	**Doxorubicin**
MRC5/HL60	0.16	6.85	1.28	3.98
MRC5/A549	1.54	**63.81**	16.3	0.70
MRC5/MCF7	1.78	33.75	9.27	0.33
MRC5/PC3	4.03	38.57	11.78	19.08
MRC5/ U87-MG	0.48	18.15	2.93	0.40
MRC5/SKOV3	0.45	13.98	6.00	nd±
MRC5/HCT116	2.00	3.75	1.13	0.44

SI: selectivity index, the ratio of IC_{50} MRC5/IC_{50} cancer cell.

In this study, twenty-seven **EA-**sulfonamide analogues were synthetized, fully characterized and biologically evaluated. The percentages of viability of HL60 and HCT116 were measured and IC_{50} on HL60 were calculated except for two inactive compounds (**6a** and **6c**).

Satisfactorily, the original **EA**-sulfonamides analogues inhibited HL60 cell line with IC_{50} values in the low to sub-micromolar range. From this first screening, three compounds were selected (**6b**, **7b** and **7e**) for further tests on a panel of cancer cell lines as well as on MRC5 as a normal cell line. In the cases of cancer cell lines A549 and MCF7, compounds **6b**, **7b** and **7e** display IC_{50}, in the nanomolar range, similar to those obtained with doxorubicin. The IC_{50} of **6b**, **7b** and **7e** are higher than doxorubicin in the case and HCT116 cell. In contrast, for PC3 cell line, even if the antiproliferative activities of compounds **6b**, **7b** and **7e** are very interesting 0.0248, 0.112 and 0.0764 µM, respectively, these potencies are lower than doxorubicin (IC_{50}=0.0021 µM).

The second important discovery in this work is distinct abilities of the selected compounds **6b, 7b** and **7e** to significantly discriminate between cancer cells (e.g. A549, MCF7, PC3 and HCT116) and normal MRC5 cells. The safety ratios (IC_{50} MRC5/IC_{50} cancer cell) calculated for the selected compounds, are much better than doxorubicin for A549, MCF7, U87-MG and HCT116 cell lines. The most selective compound for all cancer cells is **7b**, with the best safety ratio of 63.8 on A549 cell line over MRC5 cell line.

Finally, our finding bode well for the future development of drug-like based on **EA** analogues capable of differentiating between normal and cancer cells (good safety ratios, especially for compound **7b** and good LogP values).The development of new **EA** analogues that will show high antiproliferative activities and selectivities by expanding the chemical domain is undergoing in our laboratory.

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