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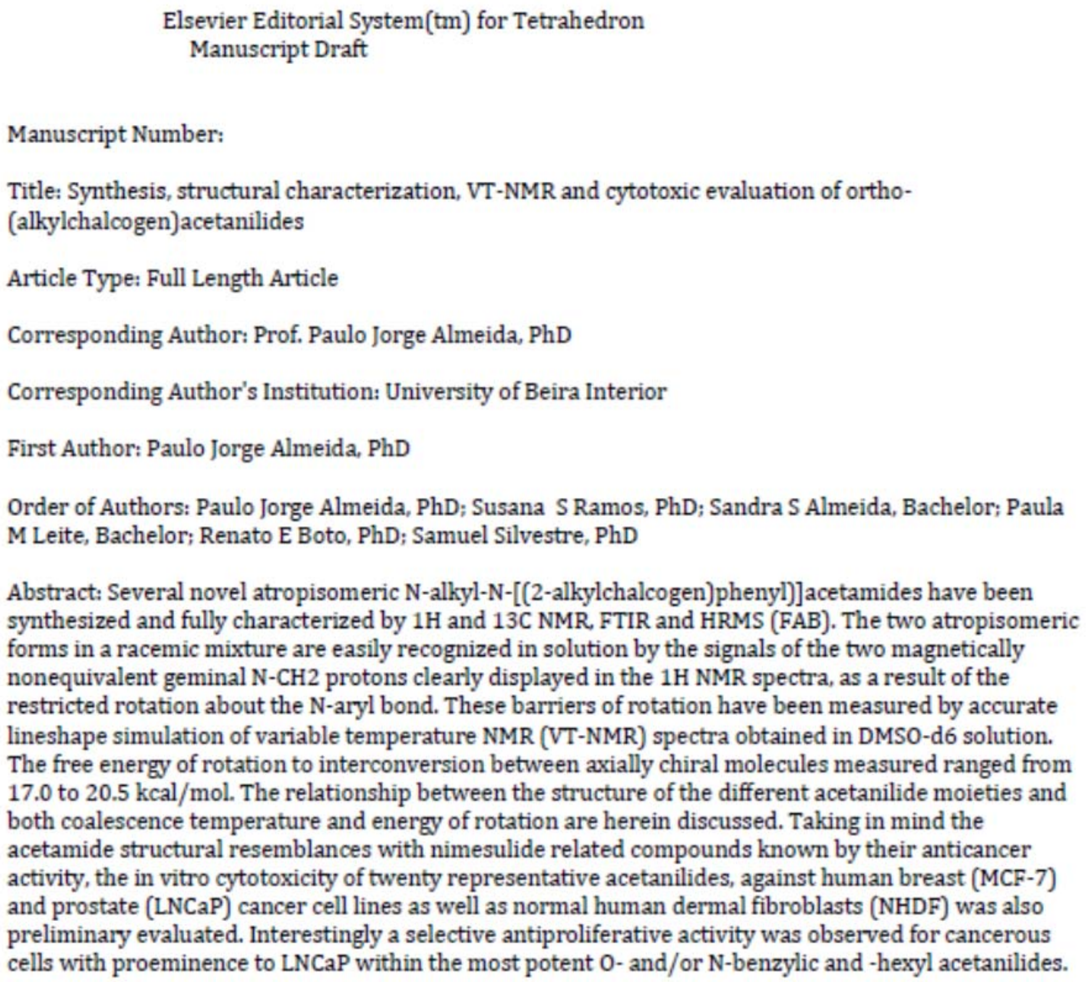

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Corresponding Author: Prof. Paulo Jorge Almeida, PhD

Corresponding Author's Institution: University of Beira Interior

First Author: Paulo Jorge Almeida, PhD

Order of Authors: Paulo Jorge Almeida, PhD; Susana S Ramos, PhD; Sandra S Almeida, Bachelor; Paula M Leite, Bachelor; Renato E Boto, PhD; Samuel Silvestre, PhD

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Graphical Abstract

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Synthesis, structural characterization, VT-NMR and cytotoxic evaluation of *ortho*-(alkylchalcogen)acetanilides

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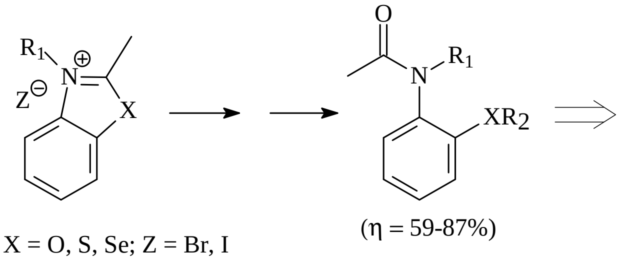
Susana S. Ramos[a,c] , Sandra S. Almeida[b] , Paula M. Leite[b] , Renato E. F. Boto[b,c], Samuel Silvestre[b] and Paulo Almeida[b,c]
[a]*UMTP-UBI, Unit of Textile and Paper Materials, University of Beira Interior, Rua Marquês d'Ávila e Bolama, 6201-001 Covilhã, Portugal*
[b]*CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal*
[c]*Department of Chemistry, University of Beira Interior, Rua Marquês d'Ávila e Bolama, 6201-001 Covilhã, Portugal*



- Structural characterization
- Atropisomeric *N*-aryl bond rotation by VT-RMN (17.0 to 20.5 kcal/mol)
- *In vitro* cytotoxicity against human breast (MCF-7) and prostate (LNCaP) cancer cell lines as well as normal human dermal fibroblasts (NHDF)

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Synthesis, structural characterization, VT-NMR and cytotoxic evaluation of *ortho*-(alkylchalcogen)acetanilides

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[b] *CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal*
[c] *Department of Chemistry, University of Beira Interior, Rua Marquês d'Ávila e Bolama, 6201-001 Covilhã, Portugal*

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ABSTRACT

Several novel atropisomeric *N*-alkyl-*N*-[(2-alkylchalcogen)phenyl)]acetamides have been synthesized and fully characterized by ^{1}H and ^{13}C NMR, FTIR and HRMS (FAB). The barriers of the restricted rotation about the N-aryl bond between the two atropisomeric forms were measured by accurate lineshape simulation of variable temperature NMR (VT-NMR) spectra obtained in DMSO-d_6 solution and ranged from 17.0 to 20.5 kcal/mol. The relationship between the structure of the different acetanilide moieties and both coalescence temperature and energy of rotation are herein discussed. Taking in mind the acetamide structural resemblances with nimesulide related compounds known by their anticancer activity, the *in vitro* cytotoxicity of twenty representative acetanilides, against human breast (MCF-7) and prostate (LNCaP) cancer cell lines as well as normal human dermal fibroblasts (NHDF) was also preliminary evaluated. Interestingly a selective antiproliferative activity was observed for cancerous cells with proeminence to LNCaP within the most potent *O*- and/or *N*-benzylic and -hexyl acetanilides.

1. Introduction

After the pioneering work by Curran *et al.* on atropisomeric anilides,[1] the potential use of non-biaryl atropisomers in medicinal chemistry has driven the interest and research around their synthesis and enantioselective synthesis, the stereoselectivity of their reactions as well as their use as chiral auxiliaries and chiral ligands.[2]

Recently, as part of our efforts to definitely clarify the identity of the products resulting from the hydroxylation of 2-methylbenzoazolium iodides, we described the synthesis of some representative *ortho*-(alkylchalcogen)-*N*-ethylacetanilides **3** (X=O, S or Se, scheme 1).[3] The acetanilides **3** were obtained as atropisomers, in moderate to good yields, after an alkylation step used to trap the unstable sulfur and selenium open (as well as the more stable oxygen analogues) *N*-ethyl-(2-thiol/selenol/hydroxyl)acetanilides **2b**, resulting from the cleavage of the 2-hydroxybenzoazole moiety of the initially formed intermediate **2a**.

N-alkyl-(2-alkylchalcogen)acetanilides **3** have restricted rotations around their N-aryl bond as well as amide N-CO bond, affording respectively enantiomeric and diasteroisomeric forms.[2q,3,4] This restricted rotation around the amide N-CO bond originates endo and exo isomers (benzene ring *cis* and *trans* to carbonyl oxygen), which are easily differentiated by ^{1}H NMR. In general, *N*-substituted anilides exist predominantly as the exo isomer.[2q,3-5] These atropisomeric forms resulting from the circumscribed rotation about the amide N-aryl bond are easily recognized in solution by the signals of the two magnetically nonequivalent geminal N-CH_2 protons clearly displayed in the ^{1}H NMR spectra. The dynamic process of the *E* diastereoisomer of five representative acetanilides **3** was thus investigated by variable temperature NMR (VT-NMR) spectroscopy and the corresponding rotation barriers at 298 K and the coalescence temperatures were determined, ever since is less than 413 K.[3]

Following our preliminary results,[3] herein we describe a full set of twenty four *ortho*-substituted acetanilides **3a-i** (X=O), **3j-v** (X=S), and **3w-x** (X=Se), nineteen of them are, to the best of our knowledge, novel (**3b**, **d-i**, **k**, **m-v**, **x**). In this serial, the nature of the chalcogen atom (X) and the nature of the alkyl group linked to both amide group (R$_1$) or chalcogen atom (R$_2$) were varied (scheme 1, table 1).

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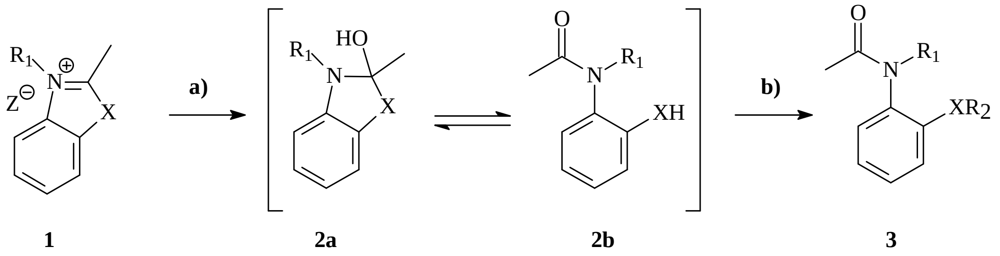
[*] *Corresponding author*. Tel.: +351 275329174; fax: +351 275319056; e-mail: paulo.almeida@ubi.pt (P. Almeida)



X = O, S, Se; Z = Br, I; R_1 and/or R_2 = Me, Et, Pr, Hex, Bz, 2-MeBz

Scheme 1. Reagents and conditions: **a**) NEt_3, 96% ethanol, reflux; **b**) ethanol, NaOH, RI or RBr, reflux.

Hence, the full characterization by ^{1}H and ^{13}C NMR, FTIR and HRMS (FAB) of these atropisomeric *N*-alkyl-*N*-[(2-alkylchalcogen)phenyl)]acetamides **3a-k**,**m-x** are here presented. The barriers of rotation between the two atropisomeric forms of acetanilides **3a-x**, with emphasis for alkyloxa- **3a-i** and alkylthioanilides **3j-v**, have been determined by accurate lineshape simulation of VT-NMR spectra obtained in DMSO-d_6 solution and ranged from 17.0 to 20.5 kcal/mol. The relationship between the structure of the different moieties of acetanilides and both coalescence temperature and energy of rotation ($\Delta G^{\ddagger}$) for all acetanilides is herein discussed. The cytotoxicity against normal human dermal fibroblasts (NHDF) and human breast (MCF-7) and prostate (LNCaP) cancer cell lines of several representative acetanilides **3a-d**, **h-n** and **r-x** and anilines **4a**,**b** were also evaluated and is here also presented and discussed.

2. Results and Discussion

In our previous described work,[3] the nature of both alkyl groups were restricted to *N*-ethylacetanilides (**3**, R_1=Et) and to *ortho*-(ethylchalcogen)- and *ortho*-(hexylchalcogen)acetanilides (**3**, X=O, S, Se; R_2=Et, Hex).

Thus, in order to determine a more accurate relationship between the structure of the different moieties of acetanilides and both coalescence temperature and energy of rotation, a set of nineteen new acetanilides was synthesized in addition to the five previously described. In this series, the nature of the chalcogen atom as well as the *N*-acetamide and chalcogen substituents were varied (X=O, S or Se; R_1 and/or R_2=methyl, ethyl, propyl, hexyl, benzyl and/or 2-methylbenzyl) (scheme 1).

All *N*-alkyl-(2-alkylchalcogen)acetanilides **3** were prepared from the appropriate 3-alkyl-2-methylbenzazol-3-ium iodides (or bromides **1)**, in moderate to good yields, following the same procedure already described[1] (scheme 1, table 1).

All the new acetanilides **3** were structurally characterized by ^{1}H and ^{13}C NMR, FTIR and HRMS (FAB).

As expected, the analysis by ^{1}H NMR reveals four esteroisomeric forms, with the exo diastereoisomers (S_a-endo and S_a-exo axially enantiomeric forms as example in figure 1) being predominant, as usual for most of *N*-substituted anilides,[4,5] with an *E/Z* ratio ≥ 95:5.

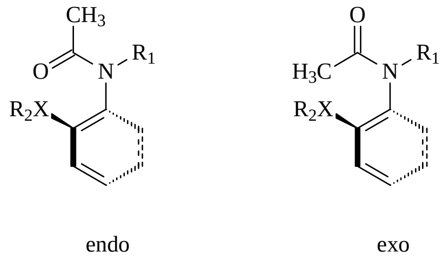


Figure 1. S_a-endo and S_a-exo axially enantiomeric forms of acetanilide.

Since the exo diastereoisomer is almost the exclusive form, the interconversion barriers of rotation ($\Delta G^{\ddagger}$) between the two axially enantiomeric forms (S_a and R_a) in solution have been determined by accurate lineshape simulation of VT-NMR spectra using gNMR modelling software (Cherwell Scientific). These lineshape simulations were based on the experimental spectra obtained for each compound, namely on the chemical shifts and coupling constants of both two geminal N-CH_2 protons magnetically nonequivalent and on the distance between both signals. ^{1}H NMR representative pattern signals of one of the *N*-CH_2 geminal proton of acetanilides are presented in figure 2.

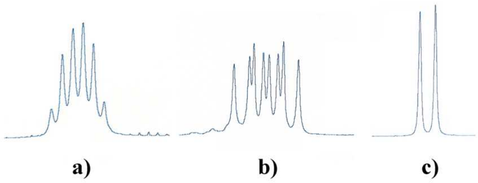


Figure 2. ^{1}H NMR representative pattern signals (400 MHz; DMSO-d_6) of one of the geminal *N*-CH_2 proton: a) R_1 = Et; b) R_1 = Pr or Hex; and c) R_1 = Bz.

By handing this information, the rate constants for interconversion (*K*) between the rotamers at a range of temperatures between 298 K and 413 K are obtained from each temperature once the simulated and experimental spectra present overlapped lineshape. From this data and using Eyring equation,[6] the barriers of rotation ($\Delta G^{\ddagger}$) at each temperature are then obtained.

$$\Delta G^{\ddagger} = 4.569 \text{ x } 10^{-3}\, T\, (10.319 + \log T / K)$$

Table 1. Reaction times, overall yields, free energies of rotation ($\Delta G^{\ddagger}$) and coalescence temperature (T_c) for *N*-alkyl-(2-alkylchalcogen)acetanilides **3**

				Reaction times				
Comp. 3	**X**	**R_1**	**R_2**	**Step a)** (min)	**Step b)** (h)	**Yield**[a] **(%)**	**$\Delta G^{\ddagger}$** (±0.2 kcal/mol)	**T_c** (K)
a[b]	O	Et	Et	60	3	79	18.1	388
b	O	Et	Pr	45	4	70	17.9	391
c[b]	O	Et	Hex	60	6	68	17.1	393
d	O	Et	Bz	45	6	66	17.2	393
e	O	Pr	Et	45	3	71	18.1	395
f	O	Pr	Hex	45	6	65	17.9	398
g	O	Hex	Et	60	3	75	17.8	398
h	O	Hex	Pr	60	5	71	17.4	401
i	O	Bz	Et	60	3	64	17.0	402
j[b]	S	Et	Et	30	3	87	18.3	[c]
k	S	Et	Pr	30	4	77	18.2	[c]
l[b]	S	Et	Hex	30	6	75	17.8	[c]
m	S	Et	Bz	30	6	78	18.0	[c]
n	S	Pr	Me	30	3	82	18.1	[c]
o	S	Pr	Et	30	3	81	18.2	[c]
p	S	Pr	Hex	30	6	69	18.1	[c]
q	S	Hex	Et	45	3	84	18.0	[c]
r	S	Hex	Pr	45	6	72	17.5	[c]
s	S	Bz	Et	30	3	83	17.5	[c]
t	S	Bz	Bz	45	5	65	17.9	[c]
u	S	MeBz	Et	45	5	62	18.1	[c]
v	S	MeBz	MeBz	60	6	59	18.2	[c]
w[b]	Se	Et	Et	30	4	85	20.5	[c]
x	Se	Et	Hex	45	5	74	17.8	[c]

[a] Isolated overall yield; [b] previously described;[3] [c] > 413 K

Further utilization of the Eyring equation furnishes the activation parameters by plotting ln (*K*/T) *vs.* 1/T which gives a straight line from which, using the equation y = mx + b, the slope (m) gives the enthalpy of rotation ($\Delta H^{\ddagger}$) and the y-intercept (b) gives the entropy of rotation ($\Delta S^{\ddagger}$). Inserting these values into Gibbs' equation for free energy ($\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$) gives a barrier of rotation ($\Delta G^{\ddagger}$) estimation at room temperature (taken to be 298 K). In our case, the linear regression analysis performed for each compound **3a-x** was obtained from a set of eight to twelve data points, with correlation coefficients between 0.9873 and 0.9947, showing consistently a very high degree of linearity for the data obtained. However, the coalescence temperature was not achieved for *ortho*-(alkylthio)- **3j-v** and *ortho*-(alkylseleno)acetanilides **3w-x** and therefore rate constants determined above this temperature could not be measured. The estimated free energies of rotation ranging from 17.0 to 20.5 kcal/mol (table 1, compounds **3a-x**) are in accordance with the values disclosed in the literature for anilides possessing a single bulky *ortho*-substituent.[2q,7] The coalescence temperatures from 388 to 402 K herein presented are restricted for the *ortho*-(alkyloxy)acetanilides **3a-i**, ever since the lowest temperatures at which the two rotamers of remaining acetanilides **3j-x** merge were determined to be above the maximum spectrometer's working temperature (413 K).

As a representative example, both experimental and simulated ^1H NMR N-CH_2 signals of the *N*-ethyl-*N*-(2-propoxyphenyl)acetamide **3b**, from 298 to 391 (*K* variation from 1 to 225 s^{-1}) are presented in figure 3.

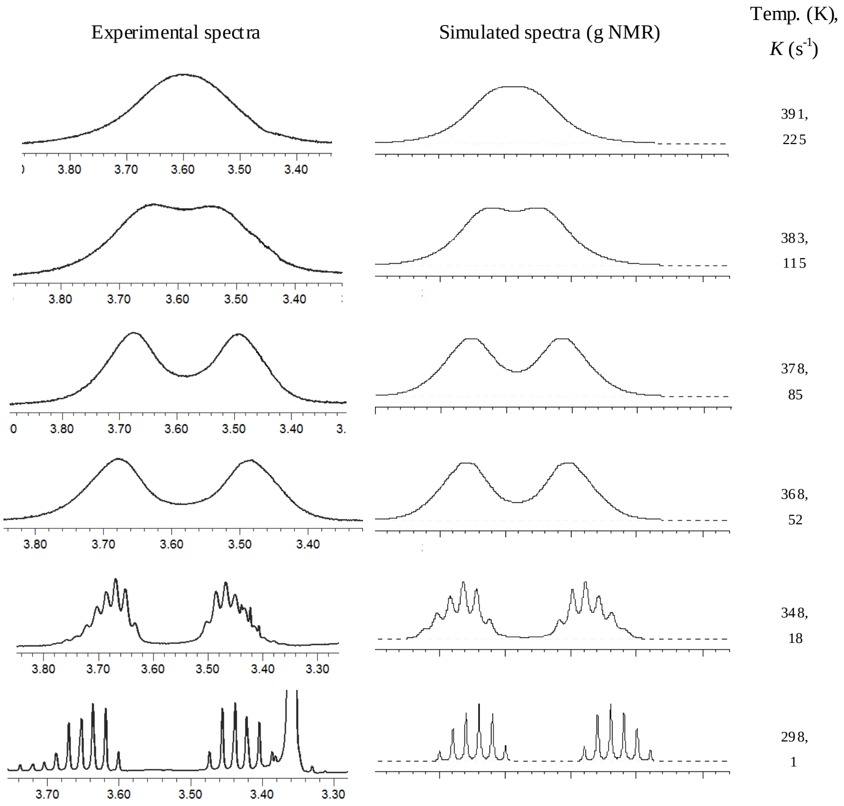


Figure 3. Experimental (left) ^{1}H NMR specta of N-CH_2 protons of **3b** in DMSO-d_6 as a function of temperature and lineshape simulation (right) for the same compound using gNMR software with the rate constants for the interconversion between the two rotamers.

Despite the fact that acetanilides **3** show the same N-CH_2 geminal protons splitting patterns within the same alkyl group linked to the chalcogen atom serial (figure 2), they differ in the region where these signals are displayed [δ 3.33-5.01 ppm (X=O); δ 2.97-5.42 ppm (X =S); δ 3.08-3.95 ppm (X =Se)]. Another significant difference lies on the chemical shift difference ($\Delta\delta_{AB}$) between the two geminal N-CH_2 protons, with the values being higher as the size of the chalcogen atom increases from oxygen to sulfur [($\Delta\delta_{AB}$ from 0.21 to 0.55 (X=O); $\Delta\delta_{AB}$ from 0.82 to 1.51 (X=S)]. This observation is in agreement with the $\Delta\delta_{AB}$ between nonequivalent methylene protons observed for other acetanilides already described, where $\Delta\delta_{AB}$ also depends on the size of the *ortho*-substituent of the aniline moiety.[4a,8] However, when the size of the chalcogen increases from sulfur to selenium atom, the shift differences remain almost the same within the two alkylselenoacetanilides **3w**,**x** compared to their thio- congeners **3j**,**l**.

Another direct consequence of the size of the chalcogen is the coalescence temperature of the acetanilides. As a matter of this fact, while the coalescence temperature of *ortho*-(alkyloxy)acetanilides **3a-i** was determined to be below 413 K, for *ortho*-(alkylthio) **3j-v** and (alkylseleno)acetanilides **3w-x** we are not able to reach the coalescence due to the limitation of the spectrometer's working temperature, as above mentioned. In addition, within the set of *ortho*-(alkyloxy)acetanilides **3a-i**, the coalescence temperature raises with the increase of the size of both R_1 and R_2 *n*-alkyl group (Hex>Prop>Et).

The previously described relationship between the rotation barrier values with both alkyl R_1 and R_2 size groups based just on five different acetanilides,[3] are herein reconfirmed. Specifically, $\Delta G^{\ddagger}$ values slightly increase with the size of the chalcogen (Se>S>O). Nevertheless, and in contrary to our expectations, the increase in the rotational barrier in function of the alkyl group size (Hex>Prop>Et) within each chalcogen serial renders lower $\Delta G^{\ddagger}$ values, in accordance with our previous report.[3] This statement is reinforced by the lower $\Delta G^{\ddagger}$ value observed when the hexyl or benzylic (benzyl and 2-methylbenzyl) group is introduced in R_1 or R_2 position.

Taking into account the structural similarly between the acetanilides herein described and nimesulide related compounds as anticancer agents,[9] we decided to evaluate the *in vitro* antiproliferative effect against normal human dermal fibroblasts (NHDF) and against the human hormone-dependent breast (MCF-7) and prostate (LNCaP) cancer cell lines, for several representative *ortho*-(alkyloxy)acetanilides **3a-d**,**h**,**i**, *ortho*-(alkylthio)acetanilides **3j-n**,**r-v** and *ortho*-(alkylseleno)acetanilides **3w-x**. In order to evaluate the carbonyl group role in the cytotoxicity of these acetanilides, two representative *ortho*-(alkylchalcogen)-*N*,*N*-dialkylanilines **4a**,**b** were additionally prepared as previously described[1] and their *in vitro* antiproliferative activity evaluated and compared to their *ortho*-(alkylthio)acetanilides **3j**,**n** congeners.

The evaluation of the relative cell viability of MCF-7, NHDF and LNCaP cells was performed through the MTT assay, following a previously described procedure.[10] Briefly, cells were seeded in 48-well plates and treated with a 30 µM concentration of the different compounds during approximately 48 h, with untreated cells serving as negative control and H_2O_2 (1 mM) as positive control and at the end of incubation the MTT test was effected. The results of these experiments are presented in table 2.

Table 2. Relative cell viability of MCF-7, NHDF and LNCaP cells incubated with compounds **3a-d**,**h-n**,**r-x** and **4a**,**b** at 30 µM, determined by the MTT assay after 48h of exposition. Mean values ± SD (% of the negative control) were obtained from five experimental determinations and data are expressed as a percentage of cell viability in comparison with the respective negative control.

Entry	**Compound** (30 µM)	**MCF-7**	**LNCaP**	**NHDF**	**LogP**[a]
1	**3a**	93.45 ± 3.38	68.32 ± 10.63	85.59 ±1 0.74	1.73
2	**3b**	92.54 ± 3.64	74.29 ± 10.14	83.97 ± 6.84	2.21
3	**3c**	55.53 ± 4.85	38.46 ± 2.62	78.34 ± 7.75	3.47
4	**3d**	88.84 ± 2.70	52.97 ± 4.41	75.77 ± 2.96	3.12
5	**3h**	51.52 ± 9.41	45.86 ± 2.01	84.34 ± 5.20	3.95
6	**3i**	87.59 ± 3.58	65.75 ± 10.13	91.07 ± 12.63	3.12
7	**3j**	99.50 ± 5.98	75.99 ± 9.89	82.65 ± 3.13	2.29
8	**3k**	89.14 ± 7.94	59.74 ± 4.03	76.19 ± 6.36	2.78
9	**3l**	67.65 ± 5.63	53.19 ± 6.17	83.48 ± 8.95	4.03
10	**3m**	95.33 ± 7.97	51.71 ± 5.02	79.17 ± 7.78	3.69
11	**3n**	95.34 ± 6.77	65.78 ± 2.66	81.49 ± 4.77	1.96
12	**3r**	47.58 ± 6.46	59.65 ± 9.99	82.99 ± 8.86	4.52
13	**3s**	89.37 ± 5.62	62.44 ± 3.19	80.05 ± 6.03	3.69
14	**3t**	79.81 ± 5.21	52.63 ± 6.08	96.68 ± 12.68	5.08
15	**3u**	77.00 ± 7.68	41.85 ± 1.75	75.21 ± 6.90	4.18
16	**3v**	13.71 ± 4.71	39.03 ± 2.59	79.02 ± 10.39	6.06
17	**3w**	98.23 ± 5.71	86.20 ± 8.98	95.45 ± 8.18	1.88
18	**3x**	82.63 ± 6.97	59.35 ± 1.60	98.90 ± 7.91	3.47
19	**4a**	99.72 ± 3.40	83.65 ± 6.86	93.00 ± 6.44	3.77
20	**4b**	97.19 ± 2.32	76.91 ± 16.91	88.27 ± 7.15	3.43
21	**H_2O_2 (1 mM)**	0.17 ± 0.32	11.22 ± 1.67	1.51 ± 2.56	--

[a] Calculated lipophilicity, using the software ChemDraw 9.0 (CambridgeSoft)

Considering the data presented in table 2 it is clear that, despite a low to moderate antiproliferative activity, generally these compounds seems to be more toxic to LNCaP cells than to MCF-7 and NHDF cells. Moreover, the benzylic **3d**,**i**,**s-v** and hexyl **3c**,**h**,**l**,**r**,**x** acetanilides (table 2, entries 3-6, 9, 12-16, 18) revealed to be more toxic and more selective (frequently up to 2-folder) to both cancer cell lines than the corresponding methyl, ethyl and propyl congeners.

These results prompt us to suspect that the lipophilicity of the compounds could be related to their antiproliferative activity. Thus, by using the software ChemDraw 9.0, the logP of all compounds considered in this assay were calculated (table 2) and the relationship between these two variables was evaluated. From this analysis, only a modest association between the lipophillicity and cytotoxic effects was found for LNCaP cells (r^2=0.5379) and for MCF-7 cells (r^2=0.3933) and no association was evidenced for NHDF cells (r^2=0.0032). Moreover, a carefully analysis within X2N2, X6N2 and X3N6 series [table 2, entries 1, 3, 5, 7, 9, 12, 17 and 18; Xn and Nn means the number of carbons (2=ethyl, 3=propyl and 6=hexyl) linked to the chalcogen (X=O, S or Se) or nitrogen respectively] reveals a generally higher cytotoxicity for the *ortho*-(alkyloxy)acetanilides (X=O) and a lower one for the *ortho*-(alkylseleno)acetanilides (X=Se).

The *ortho*-(alkylthio)anilines **4a**,**b** (Table 2, entries 19 and 20) revealed to be less active and less selective than their *ortho*-(alkylthio)acetanilides **3j**,**n** congeners (table 2, entries 7 and 11), pointing to the importance of the carbonyl amide group for the antiproliferative activity and to their's needless reduction into a methylene group.

The highest antiproliferative activity and superior selectivity against both cancerous cells (almost 6-fold and 2-fold higher for

MCF-7 and LNCaP, respectively, when compared to NHDF cells) observed for the 2-methylbenzyl disubstituted thioacetanilide **3v** (table 2, entry 16) should be emphasised. In fact, this compound revealed to have a higher selectivity to breast cancer cells (13.71% proliferation *vs* control) in comparison to both prostate (39.03%) and normal skin fibroblasts (79.02%). This important result is now being explored in order to clarify the potential interest of this compound as a future antitumor agent. Moreover, when comparing acetanilides **3v** and **3t** (table 2, entries 16 and 14) it is clear that the introduction of an *ortho*-methyl group increases the antiproliferative activity of the benzylic moiety as well as in their selectivity for MCF-7 cells in special. A similar effect can also be observed for compounds **3u** and **3s**, and again the *ortho*-methylated (**3u**) revealed a higher antiproliferative effect that the non-methylated derivative (**3s**) (table 2, entries 15 and 13).

Although more studies are needed to further explore the potential interest of this acetanilide family as antiproliferative agents, particularly those based in hexyl or substituted benzylic (as acetanilide **3v**) groups, the results herein presented, can be significantly important for the future development of more selective and powerful structures.

3. Conclusions

In summary, we prepared a series of atropisomeric *ortho*-(alkylchalcogen)-*N*-alkylacetanilides **3** from quaternary ammonium salts **1** in overall yields of 59-87%.

The dynamics of the N-aryl bond rotation of the major *E* diastereoisomers of these acetanilides have been evaluated by VT-NMR techniques and computed by gNMR calculations. While the magnitude of the energetic barrier to the interconversion between atropisomers is just slightly dependent on the chalcogen and on the nature of both alkyl groups linked to N-aryl or to the chalcogen atom, the coalescence temperature appears to be much more structurally dependent.

The evaluation of the *in vitro* antiproliferative effects of a range of representative acetanilides against normal human dermal fibroblasts (NHDF) and MCF-7 and LNCaP cancer cell lines was also performed. Curiously, this study revealed not only a general selective cytotoxicity against tumour cells *versus* normal cells, especially pronounced for prostate cancer cells, but also that benzylic and hexyl acetanilides were the most powerful compounds. The best result was observed for the methylphenyl disubstituted acetanilide (compound **3v**) which is now being studied as a potential future lead compound as anti-breast cancer agent. In order to enlarge structure-activity relationship data in this context, the development of new hexylic and/or benzylic related acetanilides is also now object of our interest.

Additionally, this new synthetic way to prepare *ortho*-(alkylchalcogen)-*N*-alkylacetanilides **3** appears to be a promising alternative to develop new compounds resembling nimesulide, especially in cases where the benzothiazole starting material possess a nitro group or other electrowithdrawing group derived from in the 6-position. Besides, other steps as acetanilide hydrolyze followed by mesylation could be considered.

Finally, it is our belief that our search for more interesting related compounds will forward us to acetanilides with a second bulky *ortho*-substituted group and therefore presenting a $\Delta G^{\square}$ sufficiently high to allow isolation of stereochemically stable atropisomers by this new simple, effective and regiospecific way herein depicted. Thus, an energy of rotation high enough to allow the separation and isolation of both atropisomers of each acetanilide will open to us the opportunity to study their biological interest, such as the cytotoxicity against various human cancer cell lines, both as racemic or as their isolated atropisomeric forms.

4. Experimental

4.1. General Considerations

Reagents and solvents were purchased from standard sources and purified and/or dried whenever necessary using standard procedures prior to use. TLC analysis was performed routinely using 0.20 mm Al-backed silica-gel plates (Macherey-Nagel 60 F_{254}). Compounds were visualized using UV light (254 nm). Attenued Total Reflectance (ATR) IR spectra were collected on a Thermoscientific Nicolet iS10: smart iTR, equipped with a diamond ATR crystal. For ATR data acquisition, a drop of the sample oil was placed onto the crystal and the spectrum was recorded. An air spectrum was used as a reference in absorbance calculations. The sample spectra were collected at room temperature in the 4000-400 cm^{-1} range by averaging 32 scans at a spectral resolution of 2 cm^{-1}. NMR spectra were acquired on a Bruker Avance 400 MHz spectrometer (^{1}H NMR at 400 MHz and ^{13}C NMR at 100 MHz) and were processed with the software TOPSPIN 2.0 (Bruker, Fitchburg, WI, USA). Deuterated dimethyl sulfoxide (DMSO-d_6) was used as solvent. Chemical shifts are reported in parts per million (δ) relative to TMS or deuterated solvent as an internal standard. Coupling constants (*J* values) are reported in Hertz (Hz) and splitting multiplicities are described as s = singlet; d = doublet; t = triplet; q = quartet; or combinations of the above; or m = multiplet. All new compounds were determined to be >95% pure by ^{1}H NMR. ESI-TOF mass spectrometry was performed on a microTOF (focus) mass spectrometer (Bruker Daltonics, Bremen, Germany). Ions were generated using an Apollo II (ESI) source. Ionization was achieved by electrospray, using a voltage of 4500 V applied to the needle, and a counter voltage between 100 and 150 V applied to the capillary. Samples were prepared by adding a spray solution of 70:30 (v/v) acetonitrile/water with 0.1% of formic acid into a solution of the sample in CH_2Cl_2 at a v/v ratio of 1 to 5‰ to give the best signal-to-noise ratio. Data acquisition was performed using the microTOFControl software version 2.1, and data processing was performed using the DataAnalysis software, version 3.4 both from Bruker Daltonics.

4.2. Synthesis and Structural Characterization

The acetanilides **3a-x** and anilines **4a**,**b** were prepared as already described.[1] The characterization of both novel or not yet fully characterized acetanilides **3a-h**,**l-x** and anilines **4a** described is herein present.

*4.2.1. N-(2-ethoxyphenyl)-N-ethylacetamide **3a***. IR (ν_{max}/cm^{-1}): 2980, 2931, 1682, 1651 (C=O), 1596, 1500, 1455, 1403, 1264, 1231, 1043, 924, 732, 702. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.34 (dt, 1H, *J* = 6.0 Hz, 1.7 Hz), 7.22 (dd, 1H, *J* = 7.5 Hz, 1.7 Hz), 7.13 (dd, 1H, *J* = 7.2 Hz, 1.1 Hz), 6.99 (dt, 1H, *J* = 6.2 Hz, 1.3 Hz), 4.02-4.15 (m, 2H), 3.65 (dq, 1H, *J* = 13.7 Hz, 7.0 Hz), 3.43 (dq, 1H, *J* = 13.7 Hz, 7.0 Hz), 1.64 (s, 3H), 1.30 (t, 3H, *J* = 7.0 Hz), 0.96 (t, 3H, *J* = 7.0 Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 169.6, 154.7, 131.4, 130.3, 129.8, 121.3, 113.7, 64.0, 42.6, 22.4, 15.1, 13.3 ppm. HRMS (ESI-TOF) *m/z* 208.13391 (208.13375 calcd. for $C_{12}H_{18}NO_2$, $[M+H^+]$).

4.2.2. N-ethyl-N-(2-propoxyphenyl)acetamide ***3b***. IR (ν_{max}/cm^{-1}): 2968, 2934, 2877, 1655 (C=O), 1596, 1499, 1454, 1395, 1286, 1265, 1231, 1043, 978, 750. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.34 (dt, 1H, J = 7.8 Hz, 1.7 Hz), 7.22 (dd, 1H, J = 7.6 Hz, 1.7 Hz), 7.13 (dd, 1H, J = 8.3 Hz, 1.0 Hz), 6.99 (dt, 1H, J = 7.5 Hz, 1.2 Hz), 3.98 (t, 2H, J = 5.9 Hz), 3.65 (dq, 1H, J = 13.9 Hz, 7.0 Hz), 3.43 (dq, 1H, J = 13.9 Hz, 7.0 Hz), 1.70 (sext, J = 6.9 Hz, 2H), 1.63 (s, 3H), 0.96 (dt, 3H, J = 7.4 Hz, 1.6 Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 169.7, 155.0, 131.4, 130.2, 129.8, 121.3, 113.6, 69.7, 42.8, 22.7, 22.4, 13.4, 11.0 ppm. HRMS (ESI-TOF) *m/z* 222.14940 (222.14940 calcd. for $C_{13}H_{20}NO_2$, [M+H$^+$]).

4.2.3. N-ethyl-N-[2-(hexyloxy)phenyl]acetamide ***3c***. IR (ν_{max}/cm^{-1}): 2960, 2931, 2871, 1657 (C=O), 1595, 1499, 1455, 1397, 1283, 1264, 1231, 1013, 935, 750. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.34 (dt, 1H, J = 7.8 Hz, 1.8 Hz), 7.21 (dd, 1H, J = 7.8 Hz, 1.8 Hz), 7.13 (dd, 1H, J = 8.3 Hz, 1.0 Hz), 6.99 (dt, 1H, J = 7.6 Hz, 1.3 Hz), 4.00 (t, 2H, J = 6.3 Hz), 3.64 (dq, 1H, J = 13.8 Hz, 6.9 Hz), 3.43 (dq, 1H, J = 13.8 Hz, 6.9 Hz), 1.64-1.72 (m, 2H), 1.62 (s, 3H), 1.36-1.43 (m, 2H), 1.26-1.32 (m, 4H), 0.95 (t, 3H, J = 7.1 Hz), 0.86 (t, 3H, J = 7.1 Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 169.5, 154.9, 131.3, 130.2, 129.8, 121.2, 113.5, 68.0, 42.6, 31.3, 29.0, 25.5, 22.5, 22.2, 14.3, 13.3 ppm. HRMS (ESI-TOF) *m/z* 264.19524 (264.19635 calcd. for $C_{16}H_{26}NO_2$, [M+H$^+$]).

4.2.4. N-[2-(benzyloxy)phenyl]-N-ethylacetamide ***3d***. IR (ν_{max}/cm^{-1}): 2972, 2932, 2872, 1652 (C=O), 1595, 1499, 1401, 1397, 1282, 1264, 1222, 1075, 950, 750, 735. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.41-7.44 (m, 3H), 7.35-7.40 (m, 2H), 7.30-7.34 (m, 1H), 7.24-7.27 (m, 2H), 7.03 (dt, 1H, J = 7.5 Hz, 1.5 Hz), 5.14-5.21 (m, 2H), 3.72 (dq, 1H, J = 13.4 Hz, 7.0 Hz), 3.41 (dq, 1H, J = 13.4 Hz, 7.0 Hz), 1.65 (s, 3H), 0.96 (t, 3H, J = 7.2 Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 169.7, 154.6, 137.4, 131.6, 130.5, 129.9, 129.0, 129.0, 128.4, 127.8, 127.8, 121.7, 114.2, 70.0, 42.7, 22.4, 13.4 ppm. HRMS (ESI-TOF) *m/z* 270.14819 (270.14940 calcd. for $C_{17}H_{20}NO_2$, [M+H$^+$]).

4.2.5. N-(2-ethoxyphenyl)-N-propylacetamide ***3e***. IR (ν_{max}/cm^{-1}): 2966, 2933, 2875, 1652 (C=O), 1596, 1499, 1455, 1398, 1281, 1249, 1221, 1122, 1043, 923, 751, 734. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.34 (dt, 1H, J = 7.8 Hz, 1.6 Hz), 7.22 (dd, 1H, J = 7.8 Hz, 1.6 Hz), 7.13 (dd, 1H, J = 8.0 Hz, 1.2 Hz), 6.99 (dt, 1H, J = 7.6 Hz, 1.2 Hz), 4.05-4.13 (m, 2H), 3.58 (ddt, 1H, J = 13.7 Hz, 7.0 Hz, 1.8 Hz), 3.36 (ddt, 1H, J = 13.7 Hz, 7.0 Hz, 1.8 Hz), 1.64 (s, 3H), 1.34-1.42 (m, 2H), 1.30 (t, 3H, J = 7.1 Hz), 0.81 (t, 3H, J = 7.6 Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 169.9, 154.7, 131.6, 130.2, 129.7, 121.2, 113.6, 64.2, 49.6, 22.2, 21.1, 15.1, 11.8 ppm. HRMS (ESI-TOF) *m/z* 222.14959 (222.14940 calcd. for $C_{13}H_{20}NO_2$, [M+H$^+$]).

4.2.6. N-[2-(hexyloxy)phenyl]-N-propylacetamide ***3f***. IR (ν_{max}/cm^{-1}): 2956, 2930, 2872, 1657 (C=O), 1595, 1499, 1455, 1395, 1280, 1248, 1221, 1123, 1044, 1014, 937, 750. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.33 (dt, 1H, J = 7.8 Hz, 1.8 Hz), 7.21 (dd, 1H, J = 7.6 Hz, 1.8 Hz), 7.12 (dd, 1H, J = 8.3 Hz, 1.2 Hz), 6.98 (dt, 1H, J = 7.5 Hz, 1.2 Hz), 4.00 (t, 2H, J = 6.1 Hz), 3.58 (ddt, 1H, J = 13.3 Hz, 7.0 Hz, 2.0 Hz), 3.33 (ddt, 1H, J = 13.3 Hz, 7.0 Hz, 2.0 Hz), 1.64-1.72 (m, 2H), 1.63 (s, 3H), 1.33-1.43 (m, 4H), 1.26-1.31 (m, 4H), 0.86 (t, 3H, J = 6.8 Hz), 0.80 (t, 3H, J = 7.5 Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 169.9, 154.9, 131.7, 130.1, 129.8, 121.2, 113.5, 68.0, 49.5, 31.2, 29.0, 25.5, 22.5, 22.2, 21.1, 14.3, 11.7 ppm. HRMS (ESI-TOF) *m/z* 278.21234 (278.21200 calcd. for $C_{17}H_{28}NO_2$, [M+H$^+$]).

4.2.7. N-(2-ethoxyphenyl)-N-hexylacetamide ***3g***. IR (ν_{max}/cm^{-1}): 2956, 2933, 2872, 1683, 1652 (C=O), 1595, 1501, 1402, 1265, 1232, 1015, 936, 780, 702. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.34 (t, 1H, J = 8.0 Hz), 7.22 (d, 1H, J = 7.8 Hz), 7.13 (d, 1H, J = 8.6 Hz), 6.99 (t, 1H, J = 8.4 Hz), 4.00 (t, 2H, J = 6.1 Hz), 3.64 (dq, 1H, J = 13.7 Hz, 7.0 Hz), 3.42 (dq, 1H, J = 13.7 Hz, 7.0 Hz), 1.66-1.72 (m, 2H), 1.65 (s, 3H), 1.38-1.62 (m, 2H), 1.28-1.36 (m, 4H), 0.95 (t, 3H, J = 7.2 Hz), 0.87 (t, 3H, J = 6.5 Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 169.6, 155.0, 131.4, 130.3, 129.8, 121.3, 113.6, 68.0, 42.6, 31.3, 29.1, 25.6, 22.6, 22.3, 14.3, 13.3 ppm. HRMS (ESI-TOF) *m/z* 264.19627 (264.19635 calcd. for $C_{16}H_{26}NO_2$, [M+H$^+$]).

4.2.8. N-hexyl-N-(2-propoxyphenyl)acetamide ***3h***. IR (ν_{max}/cm^{-1}): 2958, 2928, 2872, 2858, 1656 (C=O), 1596, 1499, 1454, 1395, 1274, 1257, 1235, 1122, 1044, 978, 750, 727. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.33 (dt, 1H, J = 7.9 Hz, 1.8 Hz), 7.21 (dd, 1H, J = 7.6 Hz, 1.8 Hz), 7.12 (dd, 1H, J = 8.2 Hz, 1.0 Hz), 6.99 (dt, 1H, J = 7.5 Hz, 1.0 Hz), 3.97 (t, 2H, J = 6.2 Hz), 3.60 (ddt, 1H, J = 13.4 Hz, 7.0 Hz, 1.4 Hz), 3.39 (ddt, 1H, J = 13.4 Hz, 7.0 Hz, 1.4 Hz), 1.70 (sext, 2H, J = 7.0 Hz), 1.63 (s, 3H), 1.29-1.39 (m, 2H), 1.15-1.25 (m, 6H), 0.96 (t, 3H, J = 7.6 Hz), 0.82 (t, 3H, J = 7.0 Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 169.8, 154.8, 131.6, 130.0, 129.7, 121.2, 113.5, 69.6, 47.8, 31.6, 27.8, 26.5, 22.5, 22.5, 22.2, 14.3, 10.9 ppm. HRMS (ESI-TOF) *m/z* 278.21166 (278.21200 calcd. for $C_{17}H_{28}NO_2$, [M+H$^+$]).

4.2.9. N-benzyl-N-(2-ethoxyphenyl)acetamide ***3i***. IR (ν_{max}/cm^{-1}): 2984, 2930, 1738, 1652 (C=O), 1500, 1396, 1264, 1217, 1044, 733, 701. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.20-7.35 (m, 4H), 7.17 (d, 2H, J = 7.4 Hz), 7.07 (d, 1H, J = 8.0 Hz), 7.02 (dd, 1H, J = 8.0 Hz, 1.4 Hz), 6.88 (t, 1H, J = 7.6 Hz), 5.01 (d, 1H, J = 15.7 Hz), 4.46 (d, 1H, J = 14.9 Hz), 3.88-4.10 (m, 2H), 1.74 (s, 3H), 1.27 (t, 3H, J = 7.4 Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 170.5, 154.4, 138.2, 131.3, 130.0, 129.8, 128.6, 128.6, 128.5, 128.5, 127.4, 121.0, 113.7, 64.0, 51.4, 22.2, 15.0 ppm. HRMS (ESI-TOF) *m/z* 270.14944 (270.14940 calcd. for $C_{17}H_{20}NO_2$, [M+H$^+$]).

4.2.10. N-ethyl-N-[2-(ethylthio)phenyl]acetamide ***3j***. IR (ν_{max}/cm^{-1}): 2972, 2930, 2872, 1655 (C=O), 1582, 1567, 1470, 1441, 1392, 1296, 1263, 1245, 1144, 1102, 1080, 1063, 1035, 994, 918, 759, 734. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.38-7.40 (m, 2H), 7.21-7.23 (m, 2H), 3.94 (dq, 1H, J = 14.0 Hz, 7.1 Hz), 3.08 (dq, 1H, J = 14.0 Hz, 7.1 Hz), 2.98 (q, 2H, J = 7.0 Hz), 1.62 (s, 3H), 1.25 (t, 3H, J = 6.9 Hz), 0.99 (t, 3H, J = 6.9 Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 169.3, 139.5, 137.3, 130.2, 129.3, 126.5, 125.9, 41.9, 24.7, 22.5, 14.0, 13.3 ppm. HRMS (ESI-TOF) *m/z* 224.11063 (224.11091 calcd. for $C_{12}H_{18}NOS$, [M+H$^+$]).

4.2.11. N-ethyl-N-[2-(propylthio)phenyl]acetamide ***3k***. IR (ν_{max}/cm^{-1}): 2965, 2932, 2873, 1660 (C=O), 1583, 1470, 1441, 1393, 1298, 1268, 1145, 1063, 1036, 761, 735. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.32-7.37 (m, 2H), 7.13-7.21 (m, 2H), 3.87 (dq, 1H, J = 13.6 Hz, 7.0 Hz), 3.05 (dq, 1H, J = 13.1 Hz, 6.9 Hz), 2.88 (t, 2H, J = 7.3 Hz), 1.59 (s, 3H), 1.50-1.58 (m, 2H), 0.95 (t, 3H, J = 7.6 Hz), 0.91 (t, 3H, J = 7.6 Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 170.4, 139.2, 137.1, 130.0, 129.5, 126.6, 126.0, 42.2, 32.4, 22.2, 21.9, 13.5, 13.1 ppm. HRMS (ESI-TOF) *m/z* 238.12530 (238.12656 calcd. for $C_{13}H_{20}NOS$, [M+H$^+$]).

4.2.12. N-ethyl-N-[2-(hexylthio)phenyl]acetamide ***3l***. Previously described.[1]

4.2.13. N-[2-(benzylthio)phenyl]-N-ethylacetamide ***3m***. IR (ν_{max}/cm^{-1}): 2974, 2922, 2849, 1663 (C=O), 1579, 1494, 1466, 1455, 1379, 1289, 1265, 1063, 1028, 994, 919, 762, 735. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.54-7.56 (m, 1H), 7.36-7.42 (m, 3H), 7.29-7.33 (m, 2H), 7.21-7.27 (m, 3H), 4.29 (s, 2H), 3.91 (dq, 1H, J = 13.4 Hz, 7.2 Hz), 3.04 (dq, 1H, J = 14.0 Hz, 7.2 Hz), 1.56 (s, 3H), 0.97 (t, 3H, J = 7.1 Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 169.2, 139.6, 137.3, 137.1, 130.2, 129.3, 129.3, 129.2, 128.9, 128.9, 127.7, 127.5, 126.4, 45.4, 35.2, 22.4, 13.4 ppm. HRMS (ESI-TOF) *m/z* 286.12683 (286.12656 calcd. for $C_{17}H_{20}NOS$, $[M+H^+]$).

4.2.22. N-[2-(methylthio)phenyl]-N-propylacetamide ***3n***. IR (ν_{max}/cm^{-1}): 2961, 2930, 2872, 1661 (C=O), 1579, 1470, 1374, 1265, 1124, 1068, 1042, 953, 731, 702. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.40-7.44 (m, 1H), 7.35 (d, 1H, *J* = 7.8 Hz), 7.23-7.24 (m, 2H), 3.88 (ddt, 1H, *J* = 13.1 Hz, 7.4 Hz, 2.5 Hz), 2.98 (ddt, 1H, *J* = 13.2 Hz, 7.4 Hz, 2.5 Hz), 2.47 (s, 3H), 1.64 (s, 3H), 1.34-1.51 (m, 2H), 0.82 (t, 3H, *J* = 7.4 Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 169.5, 139.3, 138.5, 129.9, 129.4, 125.6, 125.5, 48.5, 13.9, 11.7 ppm. HRMS (ESI-TOF) *m/z* 224.11042 (224.11091 calcd. for $C_{12}H_{18}NOS$, $[M+H^+]$).

4.2.14. N-[2-(ethylthio)phenyl]-N-propylacetamide ***3o***. IR (ν_{max}/cm^{-1}): 2965, 2930, 2873, 1656 (C=O), 1583, 1470, 1392, 1295, 1232, 1149, 1096, 1065, 1034, 973, 765, 733. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.37-7.42 (m, 2H), 7.20-7.26 (m, 2H), 3.88 (ddt, 1H, *J* = 14.1 Hz, 7.5 Hz, 2.5 Hz), 3.01 (q, 2H, *J* = 7.4 Hz), 2.97 (ddt, 1H, *J* = 14.1 Hz, 7.5 Hz, 2.5 Hz), 1.63 (s, 3H), 1.39-1.50 (m, 2H), 1.27 (t, 3H, *J* = 7.3 Hz), 0.82 (t, 3H, *J* = 7.7 Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 170.9, 139.4, 136.7, 130.0, 129.6, 126.6, 126.1, 49.0, 24.7, 22.3, 21.0, 14.0, 11.6 ppm. HRMS (ESI-TOF) *m/z* 238.12549 (238.12656 calcd. for $C_{13}H_{20}NOS$, $[M+H^+]$).

4.2.15. N-[2-(hexylthio)phenyl]-N-propylacetamide ***3p***. IR (ν_{max}/cm^{-1}): 2958, 2928, 2857, 1660 (C=O), 1583, 1469, 1437, 1392, 1295, 1255, 1232, 1149, 1096, 1064, 1036, 733. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.36-7.38 (m, 2H), 7.20-7.25 (m, 2H), 3.87 (ddt, 1H, *J* = 13.0 Hz, 7.3 Hz, 2.7 Hz), 2.99 (t, 2H, *J* = 7.5 Hz), 2.97 (ddt, 1H, *J* = 13.0 Hz, 7.3 Hz, 2.7 Hz), 1.63 (s, 3H), 1.56-1.62 (m, 2H), 1.37-1.50 (m, 4H), 1.24-1.28 (m, 4H), 0.86 (t, 3H, *J* = 7.0 Hz), 0.82 (t, 3H, *J* = 7.7 Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 169.4, 139.8, 137.3, 130.2, 129.3, 126.6, 125.9, 48.7, 31.2, 30.5, 28.5, 28.4, 22.5, 22.4, 21.2, 14.3, 11.7 ppm. HRMS (ESI-TOF) *m/z* 294.18792 (294.18916 calcd. for $C_{17}H_{28}NOS$, $[M+H^+]$).

4.2.16. N-[2-(ethylthio)phenyl]-N-hexylacetamide ***3q***. IR (ν_{max}/cm^{-1}): 2955, 2927, 2857, 1660 (C=O), 1583, 1528, 1469, 1456, 1392, 1374, 1309, 1241, 1171, 1156, 1126, 1096, 1065, 1034, 1015, 758, 733. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.37-7.42 (m, 2H), 7.21-7.26 (m, 2H), 3.91 (ddt, 1H, *J* = 14.1 Hz, 7.3 Hz, 3.0 Hz), 3.01 (q, 2H, *J* = 7.5 Hz), 2.94-3.02 (m, 1H), 1.63 (s, 3H), 1.36-1.47 (m, 2H), 1.27 (t, 3H, *J* = 7.2 Hz), 1.17-1.25 (m, 6H), 0.83 (t, 3H, *J* = 6.8 Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 170.8, 139.4, 136.8, 129.9, 129.5, 126.6, 126.1, 47.3, 31.3, 27.6, 26.3, 24.7, 22.3, 22.3, 14.2, 14.0 ppm. HRMS (ESI-TOF) *m/z* 280.17253 (280.17351 calcd. for $C_{16}H_{26}NOS$, $[M+H^+]$).

4.2.17. N-hexyl-N-[2-(propylthio)phenyl]acetamide ***3r***. IR (ν_{max}/cm^{-1}): 2959, 2927, 2857, 1660 (C=O), 1583, 1528, 1470, 1456, 1434, 1393, 1300, 1260, 1241, 1171, 1157, 1126, 1095, 1065, 1015, 795, 745, 729. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.35-7.42 (m, 2H), 7.18-7.26 (m, 2H), 3.91 (ddt, 1H, *J* = 13.5 Hz, 7.3 Hz, 2.9 Hz), 3.02 (ddt, 1H, *J* = 13.5 Hz, 7.3 Hz, 2.9 Hz), 2.98 (t, 2H, *J* = 7.1 Hz), 1.64 (s, 3H), 1.58-1.67 (m, 2H), 1.36-1.47 (m, 2H), 1.19-1.26 (m, 6H), 0.99 (t, 3H, *J* = 7.1 Hz), 0.83 (t, 3H, *J* = 6.8 Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 169.4, 139.9, 137.3, 130.3, 129.3, 126.6, 125.9, 47.0, 32.4, 31.5, 27.8, 26.6, 22.5, 22.4, 22.0, 14.4, 13.7 ppm. HRMS (ESI-TOF) *m/z* 294.18892 (294.18916 calcd. for $C_{17}H_{28}NOS$, $[M+H^+]$).

4.2.18. N-benzyl-N-[2-(ethylthio)phenyl]acetamide ***3s***. IR (ν_{max}/cm^{-1}): 2969, 2926, 2869, 1653 (C=O), 1582, 1467, 1434, 1388, 1280, 1249, 1209, 1064, 1030, 970, 774, 759, 700. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.41 (dd, 1H, *J* = 8.0 Hz, 1.4 Hz), 7.34 (dt, 1H, *J* = 7.6 Hz, 1.6 Hz), 7.22-7.30 (m, 3H), 7.17-7.20 (m, 2H), 7.06 (dt, 1H, *J* = 7.5 Hz, 1.6 Hz), 6.80 (dd, 1H, *J* = 7.9 Hz, 1.4 Hz), 5.42 (d, 1H, *J* = 14,2 Hz), 3,97 (d, 1H, *J* = 14,2 Hz), 3.02 (dq, 2H, *J* = 7.3 Hz, 3.0 Hz), 1.72 (s, 3H), 1.28 (t, 3H, *J* = 7.2 Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 170.0, 139.4, 137.9, 137.0, 130.2, 129.4, 128.9, 128.9, 128.7, 128.7, 127.7, 126.7, 125.7, 50.3, 24.7, 22.4, 14.1 ppm. HRMS (ESI-TOF) *m/z* 286.12667 (286.12656 calcd. for $C_{17}H_{20}NOS$, $[M+H^+]$).

4.2.19. N-benzyl-N-[2-(benzylthio)phenyl]acetamide ***3t***. IR (ν_{max}/cm^{-1}): 3028, 2926, 1655 (C=O), 1582, 1494, 1469, 1454, 1435, 1386, 1357, 1284, 1210, 1064, 1029, 971, 776, 755, 729, 698. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.56 (dd, 1H, *J* = 8.1 Hz, 1.6 Hz), 7.40-7.43 (m, 2H), 7.29-7.35 (m, 3H), 7.20-7.28 (m, 4H), 7.10-7.13 (m, 2H), 7.06 (dt, 1H, *J* = 7.5 Hz, 1.2 Hz), 6.77 (dd, 1H, *J* = 7.7 Hz, 1.2 Hz), 5.37 (d, 1H, *J* = 14.7 Hz), 4.30 (s, 2H), 3.86 (d, 1H, *J* = 13.9 Hz), 1.64 (s, 3H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 170.0, 139.7, 137.8, 137.7, 136.7, 130.2, 129.4, 129.3, 129.0, 128.9, 128.7, 128.0, 127.7, 127.6, 126.2, 50.5, 35.4, 22.4 ppm. HRMS (ESI-TOF) *m/z* 348.14149 (348.114221 calcd. for $C_{22}H_{22}NOS$, $[M+H^+]$).

4.2.20. N-[(2-ethylthio)phenyl]-N-(2-methylbenzyl)acetamide ***3u***. IR (ν_{max}/cm^{-1}): 2970, 2929, 2872, 1658 (C=O), 1584, 1471, 1440, 1386, 1379, 1359, 1301, 1271, 1248, 1049, 1040, 969, 764, 738. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.40 (dd, 1H, *J* = 8.0 Hz, 1.3 Hz), 7.32 (dt, 1H, *J* = 7.6 Hz, 1.4 Hz), 7.09-7.14 (m, 2H), 7.04-7.08 (m, 2H), 7.02 (dd, 1H, *J* = 7.5 Hz, 1.4 Hz), 6.74 (dd, 1H, *J* = 7.8 Hz, 1.3 Hz), 5.34 (d, 1H, *J* = 14,7 Hz), 4.18 (d, 1H, *J* = 14,7 Hz), 3.02 (q, 2H, *J* = 7.3 Hz), 2.06 (s, 3H), 1.71 (s, 3H), 1.27 (t, 3H, *J* = 7.3 Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 169.7, 139.1, 137.0, 136.8, 135.5, 130.4, 130.2, 129.8, 129.4, 127.8, 126.7, 126.2, 125.7, 47.2, 24.8, 22.4, 19.0, 14.2 ppm. HRMS (ESI-TOF) *m/z* 300.14192 (300.14221 calcd. for $C_{18}H_{22}NOS$, $[M+H^+]$).

4.2.21. N-(2-methylphenyl)-N-[2-(2-methylbenzylthio)phenyl]acetamide ***3v***. IR (ν_{max}/cm^{-1}): 3051, 3021, 2979, 2927, 1655 (C=O), 1584, 1493, 1471, 1391, 1265, 1050, 1037, 971, 730, 702. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.59 (dd, 1H, *J* = 8.0 Hz, 1.0 Hz), 7.34 (dt, 1H, *J* = 7.7 Hz, 1.4 Hz), 7.28 (d, 1H, *J* = 7.5 Hz), 7.17-7.22 (m, 2H), 7.01-7.14 (m, 5H), 6.94 (d, 1H, *J* = 7.3 Hz), 6.73 (dd, 1H, *J* = 7.8 Hz, 1.2 Hz), 5.30 (d, 1H, *J* = 14.7 Hz), 4.24-4.30 (m, 2H), 4.05 (d, 1H, *J* = 14.7 Hz), 2.40 (s, 3H), 2.03 (s, 3H), 1.64 (s, 3H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 169.6, 139.5, 137.2, 136.7, 135.4, 134.7, 130.9, 130.4, 130.2, 130.1, 129.8, 129.3, 128.3, 128.1, 127.8, 126.4, 126.3, 126.1, 55.4, 49.1, 34.2, 22.3, 19.0 ppm. HRMS (ESI-TOF) *m/z* 376.17314 (376.17351 calcd. for $C_{24}H_{26}NOS$, $[M+H^+]$).

4.2.23. N-ethyl-N-[2-(ethylseleno)phenyl]acetamide ***3w***. IR (ν_{max}/cm^{-1}): 2972, 2930, 2872, 1655 (C=O), 1582, 1470, 1441, 1392, 1376, 1297, 1263, 1144, 1161, 1102, 1063, 1035, 994, 760, 734. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.50 (dd, 1H, *J* = 7.9 Hz, 1.2 Hz),7.37 (dt, 1H, *J* = 7.3 Hz, 1.8 Hz), 7.30 (dt, 1H, *J* = 7.9 Hz, 1.2 Hz), 7.25 (dd, 1H, *J* = 7.6 Hz, 1.8 Hz), 3.94 (dq, 1H, *J* = 14.0 Hz, 7.0 Hz), 3.08 (dq, 1H, *J* = 14.0 Hz, 7.0 Hz), 3.00 (q, 2H, *J* = 7.5 Hz), 1.64 (s, 3H), 1.40 (t, 3H, *J* = 7.8 Hz), 1.02 (t, 3H, *J* = 7.8 Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 169.2, 141.4, 133.0, 130.4, 129.7, 129.5, 127.1, 42.1, 22.8, 18.7, 15.2, 13.5 ppm. HRMS (ESI-TOF) *m/z* 272.05426 (272.05536 calcd. for $C_{12}H_{18}NOSe$, $[M+H^+]$).

4.2.24. N-ethyl-N-[2-(hexylseleno)phenyl]acetamide ***3x***. IR (ν_{max}/cm^{-1}): 2957, 2928, 2855, 1656 (C=O), 1579, 1467, 1441, 1390, 1375, 1296, 1265, 1144, 1099, 1051, 1030, 993, 917, 759, 729. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.50 (dd, 1H, *J* = 7.8 Hz, 1.3 Hz), 7.36 (dt, 1H, *J* = 7.5 Hz, 1.6 Hz), 7.29 (dt, 1H, *J* = 7.2 Hz, 1.4 Hz), 7.24 (dd, 1H, *J* = 7.6 Hz, 1.6 Hz), 3.95 (dq, 1H, *J* = 13.7 Hz, 7.0 Hz), 3.08 (dq, 1H, *J* = 13.7 Hz, 7.0 Hz), 3.00 (t, 2H, *J* = 7.5 Hz), 1.65-1.70 (m, 2H), 1.64 (s, 3H), 1.29-1.41 (m, 2H), 1.24-1.28 (m, 4H), 1.02 (t, 3H, *J* = 7.2 Hz), 0.85 (t, 3H, *J* = 6.8 Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 169.1, 141.3, 133.2, 130.3, 129.7, 129.5, 127.0, 42.1, 31.1, 29.5, 29.4, 25.1, 22.7, 22.4, 14.3, 13.4 ppm. HRMS (ESI-TOF) *m/z* 328.11670 (328.11796 calcd. for $C_{16}H_{26}NOSe$, [M+H$^+$]).

4.2.25. N,N-diethyl-2-(ethylthio)aniline ***4a***. IR (ν_{max}/cm^{-1}): 2977, 2929, 1584, 1471, 1391, 1303, 1265, 1067, 1050, 730, 702. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.15-7.18 (m, 1H), 7.04-7.12 (m, 3H), 2.96 (q, 4H, *J* = 7.1 Hz), 2.85 (q, 2H, *J* = 7.3 Hz), 1.26 (t, 3H, *J* = 7.3 Hz), 0.92 (t, 6H, *J* = 7.1 Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 147.8, 136.5, 125.7, 124.9, 123.1, 47.1, 24.4, 14.1, 12.7 ppm. HRMS (ESI-TOF) *m/z* 210.13053 (210.13165 calcd. for $C_{12}H_{20}NS$, [M+H$^+$]).

4.2.26. N-ethyl-2-(methylthio)-N-propylaniline ***4b***. IR (ν_{max}/cm^{-1}): 2959, 2920, 2871, 2810, 1578, 1470, 1438, 1379, 1268, 1225, 1171, 1126, 1084, 1068, 1042, 752, 731. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.10-7.12 (m, 2H), 7.07-7.09 (m, 2H), 2.93 (q, 2H, *J* = 7.1 Hz), 2.87 (t, 2H, *J* = 7.2 Hz), 2.31 (s, 3H), 1.34 (sext, 2H, *J* = 7.3 Hz), 0.93 (t, 3H, *J* = 7.1 Hz), 0.82 (t, 3H, *J* = 7.4 Hz) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ = 147.7, 138.0, 125.1, 124.5, 124.2, 123.0, 54.5, 48.0, 20.6, 13.9, 12.5, 12.1 ppm. HRMS (ESI-TOF) *m/z* 210.13139 (210.13165 calcd. for $C_{12}H_{20}NS$, [M+H$^+$]).

4.3. VT-NMR spectroscopy

VT-NMR experiments were run on a Bruker Avance 400 MHz spectrometer equipped with a variable temperature probe. Temperature calibrations were performed before the experiments by means of a thermocouple with an uncertainly not exceeding ± 2 K. The conditions were kept as equal as possible with all subsequent work. To selectively irradiate the desired signal, a 50 Hz wide shaped pulse was calculated with a refocusing-SNOB shape[11] and a pulse width of 37 ms.

4.4. In vitro cell viability studies using MCF-7, NHDF and LNCaP cells

The cells used in this study were human breast (MCF-7) and prostate (LNCaP) cancer cells as well as normal human dermal fibroblasts (NHDF) (all acquired to ATCC – American Type Culture Collection).

Unless otherwise stated, chemicals (analytical grade), assay reagents, culture media and supplements are all from Sigma-Aldrich.

The studied compounds were dissolved in dimethylsulfoxide (DMSO) and the final solvent concentration in the MTT experiment was 0.3%. This concentration of DMSO has no significant effect on cell viability (data not shown).

4.4.1. Culture of cells

Cells were routinely maintained in 75 or 150 cm^2 *T-flasks* at 37°C in a humidified atmosphere containing 5% CO_2. Human dermal fibroblasts were cultured in RPMI medium supplemented with 10% fetal bovine serum (FBS), HEPES (0.01M), *L*-glutamine (0.02 M) and sodium pyruvate (0.001 M) and 1% antibiotic/antimycotic (10,000 units/ml penicillin, 10 mg/ml streptomycin and 25 µg/ml amphotericin B). Dubelco´s Modified Eagle´s Medium high glucose supplemented with 10% fetal bovine serum and 1% antibiotic/antimycotic was used to culture MCF-7 cells. LNCaP cells were cultured in RPMI medium supplemented with 10% FBS and 1% antibiotic (10,000 units/ml penicillin and 10 mg/ml streptomycin). The cells used in the experiments were in passages 5-6th (NHDF), 36-37th (MCF-7) and 23-24th (LNCaP).

4.4.2. MTT assay

Cell viability was evaluated by quantifying the extent of the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) according to a previously described procedure.[10] Briefly, cells were seeded in 48-well plates (0.5×10^4 cells/well) in the culture medium and after 48h they were treated with the different compounds at 30 µM for 48 h, with untreated cells serving as negative control and H_2O_2 (1mM) as positive control. At the end of incubation the media in wells were removed and replaced with fresh media and MTT solution (5mg/mL in phosphate buffer saline) and incubated at 37ºC for 4 h. Thereafter, media-containing MTT were removed and formazan crystals were dissolved with DMSO and absorbance was recorded in a Anthus 2020 microplate reader at 570 nm. The extent of cell death was expressed as the percentage of cell viability in comparison with control cells.

4.4.3. Statistics

The experiments were performed in quintuplicate and the results of the cell proliferation were expressed as average ± standard de**v**iation (SD). These calculations as well as the quantification of the potential association between the lipophilicity (logP) and cytotoxic effects were performed using the program Microsoft Excel 2010.

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