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Antiproliferative activity of synthetic fatty acid amides from renewable resources

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ABSTRACT

In the work, the in vitro antiproliferative activity of a series of synthetic fatty acid amides were investigated in seven cancer cell lines. The study revealed that most of the compounds showed antiproliferative activity against tested tumor cell lines, mainly on human glioma cells (U251) and human ovarian cancer cells with a multiple drug-resistant phenotype (NCI-ADR/RES). In addition, the fatty methyl benzylamide derived from ricinoleic acid (with the fatty acid obtained from castor oil, a renewable resource) showed a high selectivity with potent growth inhibition and cell death for the glioma cell line—the most aggressive CNS cancer.

1. Introduction

In recent years, considerable efforts have been made to develop novel drugs for cancer treatment. Cancer is a major public health problem in both developed and developing countries, and it is the second leading cause of death in the United States. Annual estimates show an alarming increase of new cases worldwide.[1] The disease is characterized by the uncontrolled division and proliferation of cells, which can be caused, among other mechanisms, by DNA mutations, cell cycle defects, and deregulated apoptosis; compounds that induce apoptosis may be useful as targeted cancer therapies.[2,3] In addition, multidrug resistance (MDR) is the principal mechanism by which many cancers, such as breast, ovarian, lung among others, develop resistance to antitumor drugs,[4,5] and new drugs that can overcome this obstacle are important goals of anticancer studies.

Fatty acid amides (Fig. 1) are considered a new family of biologically important lipids as shown by different biochemical and pharmacological studies,[6,7] and they are part of the endocannabinoid family.[8] Studies with synthetic fatty acid amides showed antiproliferative activity against several tumor cells, suggesting that variation in the fatty acid moieties on groups attached to the nitrogen atom may be responsible for differences in antiproliferative profiles.[9–11] The effect of endocannabinoids and fatty acid amides in cancer cell proliferation was reported by the binding to cannabinoids and vanilloids receptors.[2,12,13]

Previously, we reported the synthesis and antituberculosis activity of new fatty acid amides and the fatty acid isoniazid, and we demonstrated that the fatty acid chain is of fundamental importance to biological activity, most likely by facilitating its permeability in bacterial cells.[14,15] To continue our efforts to discover fatty acid molecules with improved therapeutic potential, we evaluated the in vitro antiproliferative activity of a series of amide derivatives of long-chain fatty acids in different tumor cell lines.

2. Results and discussion

The fatty acid amides were readily obtained by the synthetic pathway shown in Figure 2[14–17] and were subjected to an in vitro antiproliferative study using a panel of seven human cancer cell lines—glioma (U251), breast epithelial carcinoma (MCF-7), ovarian with a phenotype of multiple drug resistance (NCI-ADR/RES), kidney (786-0), non-small cell lung cancer (NCI-H460), prostate (PC-3), ovarian (OVCAR-3)—as well as one non-tumor cell line, human keratinocyte (HaCaT), and kidney epithelial cells from the African green monkey (VERO). The chemotherapeutic agent doxorubicin was used as a standard positive control.

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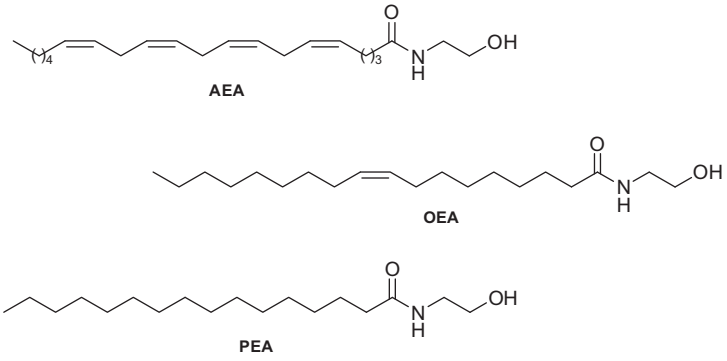


Figure 1. Fatty acid amides biologically actives.

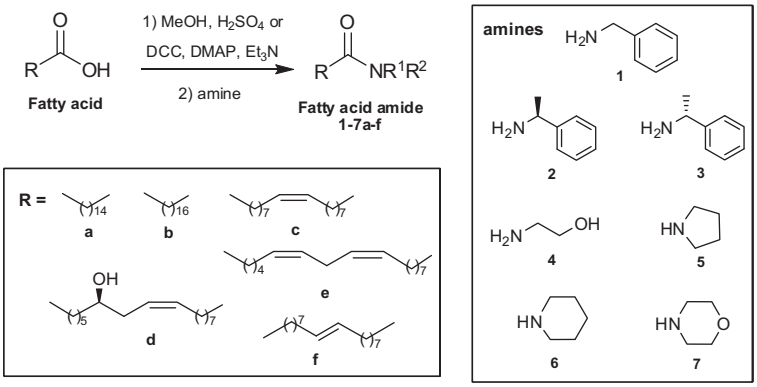


Figure 2. Fatty acid amides evaluated for antiproliferative activity on several cell lines.

Table 1
GI_{50} values of fatty acid amides against several cell lines

Amide	Tumor cell line							Mean GI_{50}	Non-tumor cell line[b]
	U251	MCF-7	NCI-ADR/RES	786-0	NCI-H460	PC-3	OVCAR-3		
	GI_{50}[a] ($\mu g\ mL^{-1}$)								
1a	–	–	–	–	–	–	–	–	–
1c	55.4	53.4	50.3	104.4	94.9	106.9	103.2		92.0
(*R*)-**1d**	8.6	37.8	8.6	32.3	32.3	122.6	31.6	39.1	30.8
(*R*)-**2b**	160.6	0.06	25.7	–	–	–	–	–	–
(*R*)-**2c**	6.1	6.1	3.0	26.8	36.6	46.9	56.9	26.5	10.3[c]
(*S*)-**3b**	–	–	–	–	–	–	–	–	69.5
(*S*)-**3c**	5.1	7.17	0.3	10.3	15.2	13.7	10.5	8.8	6.4
(*R,S*)-**3d**	3.6	5.1	1.9	6.7	8.3	11.7	11.1	6.9	9.7
(*S*)-**3e**	0.7	5.3	3.0	3.2	6.1	6.8	3.5	4.0	1.3
(*S*)-**3f**	–	110.2	–	–	–	–	21.7	–	24.6
4a	–	–	157.7	–	–	–	–	–	–
4b	116.9	129.5	157.7	–	–	75.6	157.7	–	117.7
4c	27.3	25.9	9.1	35.4	36.5	16.9	35.4	26.6	14.4
(*R*)-**4d**	3.6	6.5	2.8	6.6	4.1	6.3	4.1	4.8	3.4[c]
5a	4.6	4.6	4.6	5.5	5.9	4.7	6.3	5.2	4.8
5b	8.0	7.4	7.1	14.0	10.8	9.4	7.3	9.1	6.3
5c	3.8	5.2	0.2	5.2	45.6	13.0	11.5	12	3.9[c]
(*R*)-**5d**	1.5	4.4	4.0	4.1	6.9	6.2	4.3	4.4	4.0[c]
5e	4.2	4.2	4.7	4.7	7.0	4.7	5.7	5.0	13.7[c]
6c	6.7	7.6	7.7	10.6	23.0	8.7	9.3	10.5	7.7
(*R*)-**6d**	14.8	6.3	22.7	24.2	10.7	23.3	16.6	16.9	2.6
6e	44.2	22.1	63.2	58.4	29.1	53.6	48.9	45.6	4.2
7a	25	15.4	6.2	33.1	34.1	22.2	33.1	24.1	28.9
7b	39.9	42.8	33.1	41.1	53.1	41.1	43.3	42.0	41.5
7c	2.8	4.9	3.3	4.5	7.9	5.2	4.9	4.7	5.3
(*R*)-**7d**	33.1	41.8	29.4	36.0	49.1	38.5	31.0	37	41.3

[a] GI_{50} = 50% inhibition of cell growth.
[b] Non-tumor cell line = HaCat.
[c] Non-tumor cell line = VERO.

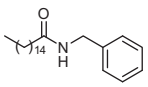
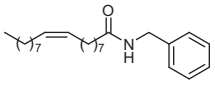
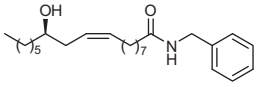
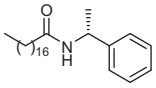
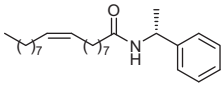
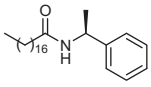
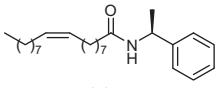
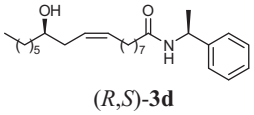
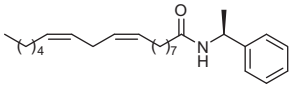
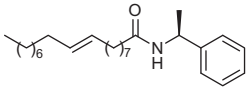
The antitumor activity ($\mu g\ mL^{-1}$) of tested fatty acid amides was given by three parameters for each cell line: GI_{50} (concentration which inhibits 50% cell growth), TGI (concentration for total inhibition of cell growth) and LC_{50} (concentration which leads to 50% cell death), calculated by origin 9.1 software. The dose–response curves for all synthesized compounds against the tested cell lines are given in the Supplementary material.

First, the data were analyzed in terms of the GI_{50} values (Table 1). For this parameter, compounds with GI_{50} values lower than 30 $\mu g\ mL^{-1}$ were considered to be active. Most of the compounds were active against most of the cell lines tested, in a dose-dependent manner. The compounds with the lowest values were **5c** (GI_{50} = 0.2 $\mu g\ mL^{-1}$) and (*S*)-**3c** (GI_{50} = 0.3 $\mu g\ mL^{-1}$), followed by compound (*R*,*S*)-**3d** (GI_{50} = 1.9 $\mu g\ mL^{-1}$).

To find a structure–activity relationship (SAR), we evaluated the fatty acid amides in terms of the TGI values. Here, the compounds were arranged into three sets according to the fragment attached to the nitrogen atom: (1) benzylamine derivatives (Table 2), (2) ethanolamine derivatives (Table 3) and (3) heterocyclic amine derivatives (Table 4).

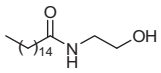
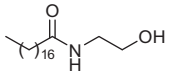
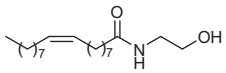
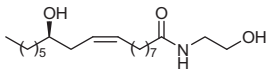
In Table 2, the antiproliferative activities of the benzylamine derivatives are shown. The derivatives encompass benzylamine

Table 2
Growth inhibition effect of the fatty acid benzylamide series **1–3** on several cell lines

Amide	Tumor cell line							Mean TGI	Non-tumor cell line[b]
	U251	MCF-7	NCI-ADR/RES	786-0	NCI-H460	PC-3	OVCAR-3		
	TGI[a] ($\mu g\ mL^{-1}$)								
 **1a**	>250	>250	>250	>250	>250	>250	>250	>250	>250
 **1c**	>250	>250	>250	>250	>250	>250	>250	>250	>250
 (*R*)-**1d**	47.5	104.5	46.6	71.5	178.8	122.6	86.0	95.5	66.5
 (*R*)-**2b**	>250	>250	>250	>250	>250	>250	>250	>250	>250
 (*R*)-**2c**	28.3	40.8	35.3	84.8	105.6	161.6	>250	>100.9	43.0[c]
 (*S*)-**3b**	>250	>250	>250	>250	>250	>250	>250	>250	>250
 (*S*)-**3c**	17.8	43.0	7.1	45.8	49.3	47.1	45.5	44.5	36.8
 (*R*,*S*)-**3d**	13.9	84.5	>250	60.1	132.1	141.9	>250	>133.2	59.1
 (*S*)-**3e**	5.1	38.6	16.4	7.7	38.9	30.0	20.0	22.4	77.2
 (*S*)-**3f**	>250	>250	>250	>250	>250	>250	>250	>250	>250
Doxorubicin	0.92	3.3	1.6	>25	4.9	11.7	7.6	7.9	4.1[c] 2.3

[a] TGI = total growth inhibition.
[b] Non-tumor cell line = HaCat.
[c] Non-tumor cell line = VERO.

Table 3
Growth inhibition effect of the fatty acid ethanolamides **4a–d** on several cell lines

Amide	Tumor cell line							Mean TGI	Non-tumor cell line[b]
	U251	MCF-7	NCI-ADR/RES	786-0	NCI-H460	PC-3	OVCAR-3		
	TGI[a] ($\mu g\ mL^{-1}$)								
 **4a**	>250	>250	>250	>250	>250	>250	>250	>250	>250
 **4b**	>250	>250	>250	>250	>250	>250	>250	>250	>250
 **4c**	51.3	66.7	39.5	64.6	81.0	44.2	71.7	59.9	53.5
 (*R*)-**4d**	6.5	3.7	5.4	20.4	9.8	23.5	8.8	11.2	7.6[c]
Doxorubicin	0.92	3.3	1.6	>25	4.9	11.7	7.6	7.9	4.1[c] 2.3

[a] TGI = total growth inhibition.
[b] Non-tumor cell line = HaCat.
[c] Non-tumor cell line = VERO.

(series **1**), (*R*)-methylbenzylamine (series **2**) and (*S*)-methylbenzylamine (series **3**). A clear structure-activity relationship was observed, as the various changes in the molecules influence its activity against the tested cell lines.

In this set, the structurally simplest compound, a benzylamine with a saturated fatty chain derivative (**1a**) was totally inactive (mean TGI >250 $\mu g\ mL^{-1}$). The introduction of an unsaturation in the fatty chain (*cis*-**1c**) or a methyl group in the benzylic position (*R*-**2a**, and *S*-**3b**) had no influence on activity (mean TGI >250 $\mu g\ mL^{-1}$), regardless of the configuration of the stereogenic center.

The derivative **1d**, with a hydroxyl functional group in the monounsaturated chain, showed a potential antiproliferative (mean TGI 95.5 $\mu g\ mL^{-1}$). It seems that functionalization of monounsaturated benzylamide derivatives is necessary for antiproliferative activity. Comparing with derivative **1d**, compounds (*R*)-**2c** and (*S*)-**3c**, with a methyl group in the benzylic position and a monounsaturated fatty chain, showed a lower to higher potential antiproliferative, respectively (mean TGI >100.9 $\mu g\ mL^{-1}$ and 44.5 $\mu g\ mL^{-1}$, respectively). In this instance, it was observed that the spatial orientation of the methyl group has a great influence on activity because, in terms of mean TGI, compound (*S*)-**3c** was twice more active than (*R*)-**2c**. These results suggest that the unsaturated chain was an important fragment only when coupled with a stereogenic center in the benzylic position or a hydroxyl group in the fatty chain.

Compound **3d** contains all the structural requirements for activity observed so far: an unsaturated chain coupled with a stereogenic center with *S* configuration in the benzylic position, and a hydroxyl group in the monounsaturated fatty chain. Comparing the derivative **3d** to its analogue **3c**, which lacks a hydroxyl group, reveals a mean TGI for **3c** that is three times lower than that of **3d** (Mean TGI >133.2 $\mu g\ mL^{-1}$ vs 44.5 $\mu g\ mL^{-1}$, respectively). However, comparing the derivative **3d** with the analogue **1d**, which lacks a methyl group, showed a smaller decrease in mean TGI (mean TGI >133.2 $\mu g\ mL^{-1}$ vs 95.5 $\mu g\ mL^{-1}$, respectively).

The compound **3e** contains one additional unsaturation in the fatty chain, substituting the hydroxyl group (**3d**), and this compound showed the best mean TGI value (22.4 $\mu g\ mL^{-1}$), which is six times more active than the hydroxyl analogue **3d** (Mean TGI >133.2 $\mu g\ mL^{-1}$) and twice as active as the monounsaturated analogue **3c** (Mean TGI = 44.5 $\mu g\ mL^{-1}$).

These data suggest that in the benzylamide series **1**, **2**, and **3**, a monounsaturated fatty acid along with a methyl group in the benzylic position and a hydroxyl group attached to the fatty chain is important for antiproliferative activity to a greater or lesser extent, and an additional unsaturation in the fatty chain caused a substantial drop in mean TGI.

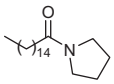
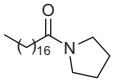
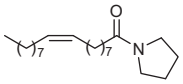
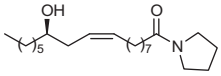
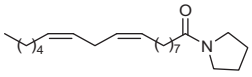
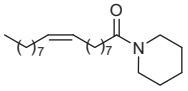
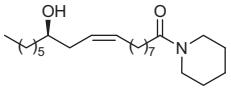
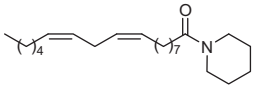
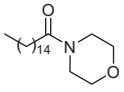
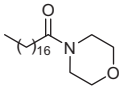
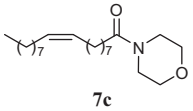
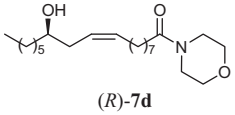
Finally, the double bound configuration was evaluated and was seen that the change of the configuration from *cis* (**3c**) to *trans* (**3f**) caused a total loss of antiproliferative activity.

Analysis of compounds derived from ethanolamine (Table 2, series **4**) showed that substitution of a saturated fatty chain (compounds **4a**, **b**) with a monounsaturated chain (compound **4c**) improved the antiproliferative activity from undetectable (mean TGI >250 $\mu g\ mL^{-1}$, **4a**) to some potential antiproliferative (mean TGI = 59.9 $\mu g\ mL^{-1}$, **4c**). This activity increased even further when a hydroxyl group was introduced to the monounsaturated fatty chain (mean TGI = 11.2 $\mu g\ mL^{-1}$, compound **4d**). Thus, in this series, it again appears that the presence of a hydroxyl group is important for antiproliferative activity when associated with an unsaturated chain.

For compounds in Table 3, it was observed that the presence of a five-member heterocyclic compound (series **5**) resulted in significantly greater antiproliferative activity compared with saturated chain analogues (series **4**). Moreover, changing the fatty chain from C16:0 (**5a**) to C18:0 (**5b**) resulted in a reduction in the mean antiproliferative activity (from 19.9 mg mL^{-1} for **5a**, to 46.5 $\mu g\ mL^{-1}$ for **5b**), whereas replacing the saturated chain with a monounsaturated chain (**5c**) further reduced the mean antiproliferative activity (from 46.5 $\mu g\ mL^{-1}$ for **5b**, to >67.7 $\mu g\ mL^{-1}$ for **5c**). The presence of a second double bond in the fatty acid chain (**5e**) resulted in increased potency (mean TGI = 24.6 $\mu g\ mL^{-1}$), and the presence of a hydroxyl group in the monounsaturated fatty chain resulted in the best observed mean antiproliferative activity (mean TGI = 4.5 $\mu g\ mL^{-1}$ for **5d**).

To evaluate the influence of heterocyclic ring size on antiproliferative activity, we changed the five-member ring (Table 3, series

Table 4
Growth inhibition effect of the fatty acid heterocyclic amide series **5**–**7** on several cell lines

Amide	Tumor cell line							Mean TGI	Non-tumor cell line[b]
	U251	MCF-7	NCI-ADR/RES	786-0	NCI-H460	PC-3	OVCAR-3		
	TGI[a] (μg mL^{-1})								
 **5a**	11.4	13.2	30.0	16.1	33.5	12.2	22.9	19.9	12.9
 **5b**	32.5	39.9	43.5	48.4	56.8	36.0	68.5	46.5	27.4
 **5c**	5.8	>250	5.6	23.3	153.1	21.9	14.0	>67.7	10.6[c]
 (*R*)-**5d**	4.4	1.5	4.0	6.9	6.2	4.3	4.1	4.5	6.8[c]
 **5e**	12.0	27.5	7.7	21.9	36.6	32.6	33.9	24.6	45.1[c]
 **6c**	25.0	33.3	47.4	40.7	40.7	31.8	35.4	36.3	47.9
 (*R*)-**6d**	6.3	14.8	22.7	10.7	23.3	16.6	24.2	16.9	12.8
 **6e**	22.1	44.2	63.2	29.1	53.6	48.9	58.4	45.6	30.5
 **7a**	55.9	54.7	70.3	69.9	91.0	36.7	72.7	64.5	73.7
 **7b**	82.6	81.7	86.9	85.6	160.8	102.4	111.1	101.6	94.2
 **7c**	6.6	13.1	10.6	11.2	34.7	14.8	13.9	15.0	23.2
 (*R*)-**7d**	54.5	115.6	142.8	75.7	152.4	72.6	62.2	96.5	97.4
Doxorubicin	0.92	3.3	1.6	>25	4.9	11.7	7.6	7.9	4.1[c] 2.3

[a] TGI = Total Growth Inhibition.
[b] Non-tumor cell line = HaCat.
[c] Non-tumor cell line = VERO.

5) to a six-member ring (Table 3, series **6**). However, a consequent improvement in mean antiproliferative activity was only observed with the monounsaturated compound (mean TGI >67.7 μg mL^{-1} for **5c** compared with 36.3 μg mL^{-1} for **6c**). Analysis of the compounds showed that the presence of one or two double bonds in the fatty acid chain resulted in a similar antiproliferative profile (mean TGI = 36.3 and 45.6 μg mL^{-1} for **6c** and **6e**, respectively). In addition, the presence of a hydroxyl group in the monounsatu-

rated fatty chain resulted in improved antiproliferative activity (mean TGI 16.9 μg mL^{-1}, **6d**).

The exchange of a carbon atom with an oxygen atom in the six-member ring (Table 3, series 7) caused a worsening in mean antiproliferative activity when compounds **7a**, **7b**, and **7d** were compared with compounds **5a**, **5b**, **5d**, and **6d**. However, when this oxygenated six-member ring was associated with a monounsaturated fatty chain, its mean antiproliferative activity improved (mean TGI = 15.0 μg mL^{-1} for **7c**) compared with that of **5c** (mean TGI >67.7 μg mL^{-1}) and **6c** (mean TGI = 36.3 μg mL^{-1}).

These data suggest that the antiproliferative activity is influenced by the structural variation in the fatty acid amides, including both the fatty acid derivative moiety and the moiety attached to the nitrogen atom.

The data in Tables 2–4 shows that most of the fatty acid amides studied showed activity against most of the cell lines, with a broad spectrum inhibition profile, and there were varied significant differences in cell selectivity and safety. Among the benzylamide derivatives (series **1**–**3**), based on the mean TGI, five compounds showed a potential antiproliferative: **1d**, (*R*)-**2c**, (*S*)-**3c**, (*R*,*S*)-**3d**, and (*S*)-**3e**. Among these, compounds (*S*)-**3c** and (*S*)-**3e** were noted for exhibiting the lowest mean TGI values. Furthermore, compound (*S*)-**3c** showed high activity and selectivity for ovarian cancer cells with a phenotype of multiple drug resistance (NCI-ADR/RES, TGI = 7.1 μg mL^{-1}) and glioma cell lines (U251, TGI = 17.8 μg mL^{-1}), as well as a favorable safety profile when compared with non-tumor cells (HaCat, TGI = 36.8 μg mL^{-1}). In contrast, compound (*S*)-**3e** showed high activity for glioma cells (U251, TGI = 5.1 μg mL^{-1}), prostate cancer cells (PC-3, TGI = 7.7 μg mL^{-1}) and the resistant ovarian cancer cells (NCI-ADR/RES, TGI = 16.4 μg mL^{-1}) cell lines but with relative selectivity and a favorable safety profile when compared with a non-tumor cell line (HaCat, TGI = 77.2 μg mL^{-1}). However, compound (*R*,*S*)-**3d**, derived from ricinoleic acid and methyl benzylamine, showed good growth inhibition and cell death for the glioma cell line (U251, TGI = 13.9 μg mL^{-1}, LC_{50} = 63.6), with a high degree of selectivity and a favorable safety profile when compared with a non-tumor cell line (VERO, TGI = 59.1 μg mL^{-1}). The dose–response analysis of cell growth inhibition by compound (*R*,*S*)-**3d** is displayed in Figure 3.

The ethanolamine derivative (*R*)-**4d** showed an excellent mean TGI, with growth inhibition and death for most of the cell lines, but with relative selectivity. Furthermore, no favorable safety profile

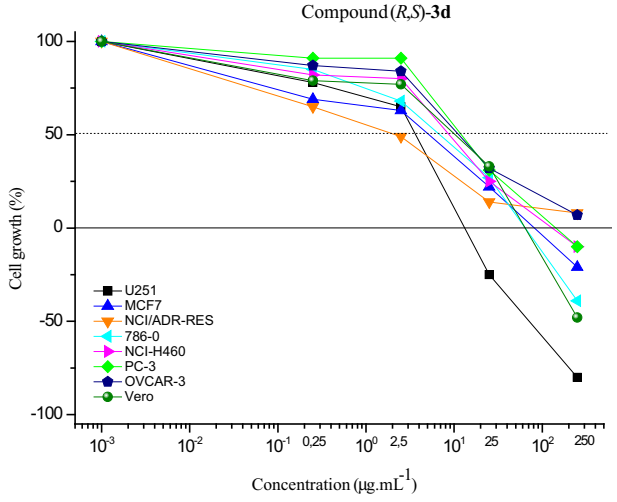


Figure 3. Dose–response analysis of cell growth inhibition by compound (*R*,*S*)-**3d** against several cell lines

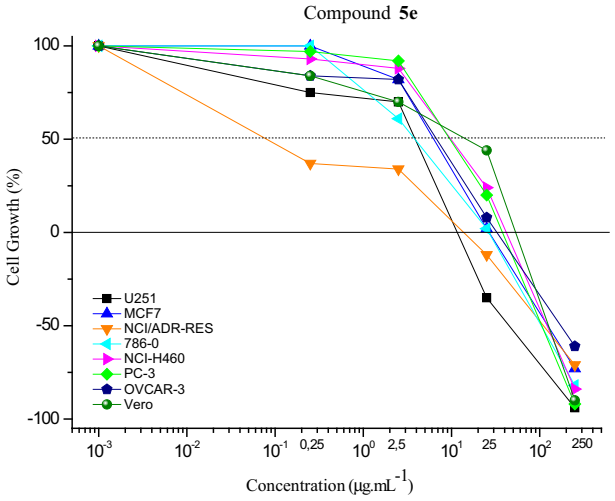


Figure 4. Dose–response analysis of cell growth inhibition by compound **5e** against several cell lines.

was observed when a comparison was made with a non-tumor cell line (VERO, TGI = 7.6 μg mL^{-1}).

Among the heterocyclic derivatives, two compounds were efficacious in inhibiting the proliferation of more than one cell line: compound **5c**, which demonstrated specificity of action in glioma (U251, TGI = 5.8 μg mL^{-1}), ovarian cancer cells with an MDR phenotype (NCI-ADR/RES, TGI = 5.6 μg mL^{-1}) and another ovarian cancer cell line (OVCAR-3, TGI = 14.0 μg mL^{-1}), as well as causing cell death to glioma cells (U251, LC_{50} = 14.2); and compound **5e**, which was specific for glioma (U251, TGI = 12.0 μg mL^{-1}), and ovarian cancer cells with an MDR phenotype (NCI-ADR/RES, TGI = 7.7 μg mL^{-1}) and caused cell death to glioma cells (U251, LC_{50} = 44.4). Although there was loss of both potency and specificity of action with an additional unsaturation in the fatty acid chain, compound **5e** appeared to have a more favorable safety profile than that of compound **5c**, as it yielded the greatest difference of activity between tumor and non-tumor cell lines (VERO, TGI = 45.1 and 10.6 μg mL^{-1}, respectively). The dose–response analysis of cell growth inhibition by compound **5e** is displayed in Figure 4. Ovarian cancer is difficult to diagnose, and recurrence may occur through multidrug resistance. Both compounds **5c** and **5e** showed a better potency for ovarian cancer cells with an MDR phenotype than for native ovarian cancer cells, a very relevant result given the importance of the search of new drugs for cancers with MDR.

Among all tested compounds, compound (*R*)-**5d** showed the best mean TGI value, exhibiting growth inhibition and cell death for most of the lines but without selectivity. This compound appears to have a general cytotoxic effect.

The morfolyl amine derivative **7c** showed a good mean TGI, with growth inhibition for the most of cell lines, but with relatively good selectivity and a moderately favorable safety profile when comparing tumor and non-tumor cell lines.

3. Conclusions

In conclusion, the work presented herein demonstrates that several fatty acid amides exhibited potent and selective antiproliferative activity in the cancer cell lines tested. In general, benzylamide derivatives showed a clear structure-activity relationship, with an interesting activity profile when there was a hydroxyl group in the fatty chain and/or a methyl group in the benzylamino moiety, as well as an unsaturated chain. For the other derivatives, the results of the growth inhibition assay demonstrate that TGI is

greatly influenced by structural modifications of the fatty acid amides, in particular, both the fatty acyl chain and the amino-containing moieties of the molecule. Compound (*R*,*S*)-**3d** showed a high selectivity with potent growth inhibition and cell death for the glioma cell line—the most aggressive CNS cancer. Also, compound **5e**, which was specific for glioma cells and ovarian cancer cells with an MDR phenotype, with good antiproliferative potential. Both compounds showed a favorable safety profile when analyzing their effects on a non-tumor cell line, and they can serve as templates for the development of candidate antitumor drugs, including those for cancers with MDR. Future studies with fatty acid amides are needed to confirm these important observations.

4. Experimental

4.1. Apparatus and chemistry

Reagents and fatty acids were purchased from Aldrich Chemical Co. and used without further purification. Ricinoleic acid (*cis*-C18:1,12-OH) was obtained from castor oil or castor oil biodiesel hydrolysis. Column chromatography was performed with Silica Gel 60 A (ACROS Organics 0.035–0.070 mesh). Analytical thin-layer chromatography was performed with plates containing silica gel (Merck 60GF245), and the spots were visualized using iodine. Yields refer to chromatographically and spectroscopically homogeneous materials. Melting points were obtained with a Fisatom 430D apparatus and are uncorrected. The NMR spectra were recorded on a Varian VNMRS 300 MHz spectrometer (^{1}H, 300 MHz and ^{13}C, 75.5 MHz—Universidade Federal do Rio Grande do Sul, UFRGS, Brazil) in deuterochloroform (CDCl3) solution. The chemical shift data are reported in units of δ (ppm) downfield from tetramethylsilane (TMS), which was used as an internal standard. Coupling constants (J) are reported in hertz (Hz) and refer to apparent peak multiplicities. GC/MS analysis was used to ascertain the purity of all compounds for which biological data was determined using a GCMS-QP2010Plus chromatographic system (Shimadzu) equipped with a split/splitless injector coupled with a mass detector.

4.2. Synthesis

All compounds used in this work were synthesized according to a published method. Amides **1**–**3** were synthesized by reacting the respective fatty acid (0.3 mmol) with amines (0.3 mmol), triethylamine (0.3 mmol), and a catalytic amount of (dimethylamino)pyridine (DMAP), and dicyclohexyl carbodiimide (DCC, 0.3 mmol) in CH_2Cl_2 was added dropwise to the above reaction mixture, which was then stirred at room temperature for 24 h. The solid dicyclohexylurea formed was removed by filtration and the solvent was evaporated under reduced pressure. Amides **4**–**7** were synthesized from fatty acid methyl esters (FAMEs) obtained via esterification of the respective fatty acid. The aminolysis reaction of FAMEs (0.3 mmol) was realized in the presence of the amines (1.8 mmol) and acetonitrile for 24 h. The progress of the reactions was monitored by silica gel TLC. The raw products were purified via column chromatography on silica gel (*n*-hexane/ethyl acetate, 7:3) and analyzed by proton and carbon NMR IR, and ESI–MS/MS. The presented spectroscopic data are in agreement with the literature.[14,15]

4.2.1. (*S*)-Elaidyl methylbenzylamide (3f)

$C_{26}H_{43}NO$. MW 385.33 g mol^{-1}. White solid. Yield 69%. mp 74 °C. R_f 0.5 (*n*-hexane/ethyl acetate, 7:3). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.3 (m, 5H), 6.0 (sl, 1H), 5.4 (m, 2H), 5.15 (m, 1H), 2.2 (t, 2H), 1.98 (m, 4H), 1.64 (m, 1H), 1.51 (d, 3H), 1.29 (m, 23H), 0.9 (t, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.5 (C=O), 143.2; 130.2; 128.6; 127.3; 76.7; 48.7; 36.7; 32.6; 31.9; 28.9; 25.8; 22.7; 21.7; 14.2. MS [*m*/*z*(%)] 385 (M^+, 5), 163 [–$CH_2C(OH)NHCH(CH_3)Ph$, 40], 120 [–$NHCH(CH_3)Ph$, 40], 105 [–$C(CH_3)Ph$, 100].

4.3. In vitro antiproliferative activity assay

The in vitro antiproliferative activity assay was performed as described by Monks et al. (1991).[18] Seven human tumor cell lines [U251 (glioma), MCF-7 (breast), NCI-ADR/RES (multiple drug-resistant ovarian cancer cells), 786-0 (renal), NCI-H460 (non-small cell lung cancer cells), PC-3 (prostate) and OVCAR-03 (ovarian)] were kindly provided by Frederick Ma (National Cancer Institute, Bethesda, MD, USA). Additionally, the HaCaT (human keratinocytes) cell line was used, a kind donation from Dr. Ricardo Della Coletta (FOP, UNICAMP). Stock and experimental cultures were grown in medium containing 5 mL RPMI 1640 (GIBCO BRL) supplemented with 5% fetal bovine serum (GIBCO BRL). Penicillin/streptomycin mixture (1000 U/mL:1000 μg/mL, 1 mL/L RPMI) was added to the experimental cultures. Cells in 96-well plates (100 μL cells well^{-1}) were exposed to sample concentrations in DMSO/RPMI (0.25, 2.5, 25, 250 μg mL^{-1}) at 37 °C and incubated in a 5% CO_2 atmosphere for 48 h. The final DMSO concentration did not affect cell viability. Before (T_0 plate) and after the sample addition (T_1 plates), cells were fixed with 50% trichloroacetic acid, and cell proliferation was determined by spectrophotometric quantification (540 nm) of cellular protein using the sulforhodamine B assay. Using the dose–response curve for each cell line, total growth inhibition (TGI, the concentration that produces total growth inhibition or cytostatic effects) was determined through non-linear regression analysis using ORIGIN software version 8.0 (OriginLab Corporation) (Shoemaker, 2006).[19]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.11.019.

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