

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



# Benzo-Fused Seven- and Six-Membered Derivatives Linked to Pyrimidines or Purines Induce Apoptosis of Human Metastatic Breast Cancer MCF-7 Cells *In Vitro*

Joaquín M. Campos, M. Carmen Núñez,  
Ana Conejo-García and Olga Cruz-López

*Departamento de Química Farmacéutica y Orgánica, Facultad de Farmacia,  
Universidad de Granada, c/ Campus de Cartuja s/n, Granada,  
Spain*

## 1. Introduction

In recent years, strategy in cancer therapy has been the use of high doses of toxic non-specific agents and to investigate a range of new agents that specifically target tumour-related molecules, in a variety of biological pathways. A basic knowledge of these pathways in the cancer cell is becoming fundamental for clinical practice since it can provide prognostic as well as predictive information for established therapies, and lead to the discovery of potential new targets. Two main therapeutic strategies may be followed to optimize cancer treatment: a better selection of patients who will benefit the most from a given hormonal or cytotoxic therapy, through the use of predictive markers determined by genomics and/or proteomics techniques and the development of new agents with innovative and tumour-specific mechanism of action. We have reviewed the consideration of Choline Kinase as a novel target for the development of new anticancer drugs (Campos et al., 2003).

Breast cancer is a common and often fatal disease. Excluding cancers of the skin, that of the breast is the most common cancer among women, accounting for nearly one out of every three cancers diagnosed in American women. Each year over 186,000 new cases and 46,000 deaths are reported in the United States alone (Harris et al., 1996). Five main molecular pathways are of particular interest in terms of new drug development in breast cancer: the estrogen receptor pathway, the tyrosine signal transduction pathway, the angiogenesis pathway, the cell cycle regulation pathway and the apoptosis (programmed cell death) pathway. We will focus in this review on new cytotoxic, apoptotic and cell-cycle-regulator agents, designed by our Group.

As part of their action on neoplastic cells, many anticancer drugs activate apoptosis that may be a primary mechanism of antineoplastic agents (Hickman, 1992). Although breast cancer is most often treated with conventional cytotoxic agents it has proved difficult to induce apoptosis in breast cancer cells using these drugs (Rasbridge et al., 1994). Improved clinical response may be obtained by identifying therapies that are particularly effective in

activating apoptosis and determining how those therapies may be modified to effect maximum apoptosis induction. The cell cycle apparatus and apoptosis have recently attracted the attention of researchers intent on developing new types of anticancer therapy (Lundberg & Weinberg, 1999; Qin & Ng, 2002). On the other hand, the MCF-7 human breast cancer cell line has been used as an excellent experimental model to improve the efficacy of different therapies before its use in patients (Matsuo et al., 2000; Trouet et al., 2001). We will concentrate in this review on the evolution of the chemical structures and the biological properties whilst, in general, the chemical syntheses will be referred to through the corresponding original references.

## 2. Benzo-fused seven-membered derivatives linked to pyrimidines

Having previously reported the synthesis and anticancer activities of acyclic 5-fluorouracil (5-FU) *O,N*-acetalic compounds **1-2** (Campos et al., 1996), cyclic *O,N*-acetalic compounds was synthesized with the objective of increasing the lipophilicity of the target molecules. In this way we have reported the synthesis and anticancer activities of compounds **3** (Campos et al., 1997), **4-6** (Saniger et al., 2003a), **7** (Marchal et al., 2007), **8-9** (Saniger et al., 2003a) and *trans*-**10** (Saniger et al., 2003b) (Figure 1). In all cases, the linkage between the 5-FU moiety and the seven-membered ring was carried out through its *N*-1 atom.

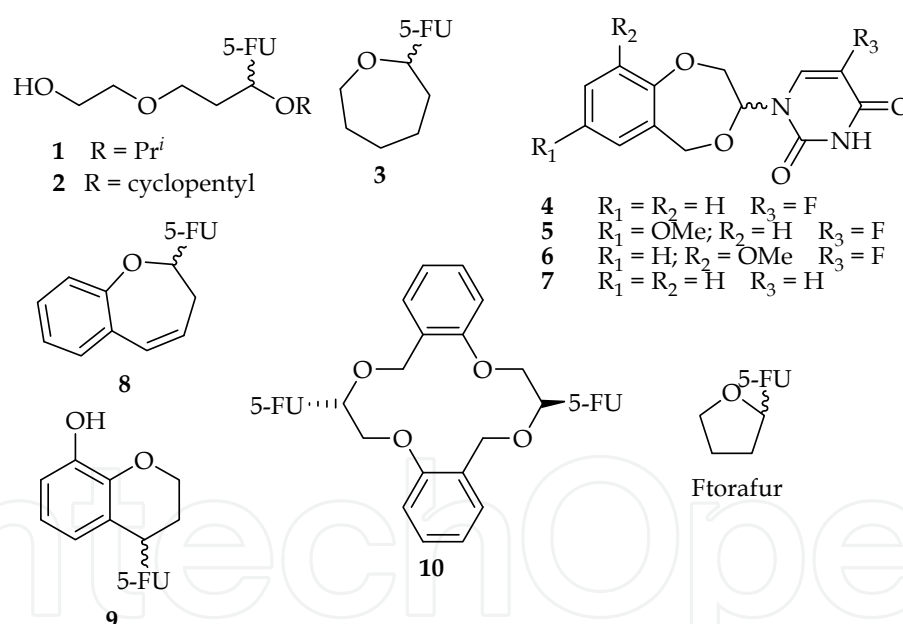


Fig. 1. Several 5-FU derivatives showing interesting antitumour activities.

### 2.1 Antiproliferative activities of cyclic *O,N*-acetals

The IC<sub>50</sub> values of the 5-FU cyclic *O,N*-acetals are shown in Table 1 (entries 4-6, 8-10).

The most active 5-FU-derived compounds are **4**, **5** and **10** (entries 5, 6 and 11). Compound **3** (entry 4) shows the least antiproliferative activity (IC<sub>50</sub> = 23 ± 0.88 μM). The lipophilicity in this structure has been increased by means of a fused benzene ring, and an unsaturation has been introduced to give **8**. An increase has been obtained in its antiproliferative activity (IC<sub>50</sub> = 14 ± 1.02 μM, entry 9). On comparing structures **8** and **4**, it is worth emphasizing that the

Entry	Compound	clog <i>P</i> <sup>a</sup>	IC <sub>50</sub> (μM) <sup>b</sup>	Cell Cycle (48 h) <sup>c</sup>			Apoptosis <sup>d</sup>	
				G <sub>0</sub> /G <sub>1</sub>	G <sub>2</sub> /M	S	24 h	48 h
1	Control			68.39	12.04	19.57	1.24	1.24
2	5-FU	-0.89 <sup>e</sup>	2.75	58.07	2.10	39.38	56.75	52.81
3	Ftorafur	-0.18 <sup>f</sup>	3 ± 0.11	45.62	0.00	54.38	52.20	58.06
4	3	0.86	23 ± 0.88	50.99	18.51	30.49	46.63	53.92
5	4	1.09	7 ± 0.61	74.41	15.77	9.82	8.45	12.17
6	5	1.16	4.5 ± 0.33	73.41	13.15	13.44	1.50	3.50
7	6	1.16	22 ± 0.93	71.76	10.08	18.16	40.08	46.73
8	7	0.87	5 ± 0.25	69.01	11.38	19.61	5.30	5.22
9	8	2.54	14 ± 1.02	86.14	1.60	12.26	57.33	51.37
10	9	0.70	69 ± 2.31	68.61	9.60	21.79	54.33	35.49
11	10	2.18	5.5 ± 0.58	82.48	5.13	12.40	14.37	19.05

<sup>a</sup> Calculated by a fragmental system based on Rekker’s method (Rekker & de Kort,1979), the CDR option of the PALLAS 2.0 programme (PALLAS FRAME MODULE, a prediction tool of physicochemical parameters, is supplied by CompuDrug Chemistry Ltd, PO Box 23196, Rochester, NY 14696, USA). <sup>b</sup>See ref. (Campos et al., 2000). <sup>c</sup>Determined by flow cytometry: see ref. (Saniger et al., 2003a). <sup>d</sup>Apoptosis was determined using an annexin V-based assay (Chadderton et al., 2000). The data indicate the percentage of cells undergoing apoptosis in each sample. All experiments were conducted in duplicate and gave similar results. The data are means ± SEM of three independent determinations. <sup>e</sup>The experimental log *P* value is -0.83 (Buur et al., 1985). <sup>f</sup>The experimental log *P* value, measured by the octanol-buffer partitioning technique at pH 7.4 is -0.36 ± 0.5 (Gulyaeva et al., 2003).

Table 1. Antiproliferative activities, cell cycle dysregulation, and apoptosis induction in the MCF-7 human breast cancer cell line after treatment for 24 and 48 h for the compounds.

bioisosteric change of carbon for oxygen and the saturation of the double bond in compound **4** increases the antiproliferative activity twice (IC<sub>50</sub> = 7 ± 0.61 μM, entry 5). The introduction of a methoxy group into the benzene ring of **4** provokes different influences on the antiproliferative activities. Thus, the C-7 substitution produces an increase of the antiproliferative activity (**5**, IC<sub>50</sub> = 4.5 ± 0.33 μM, entry 6), whilst if C-9 is the substituted position it gives rise to a decrease in the antiproliferative activity (**6**, IC<sub>50</sub> = 22 ± 0.93 μM, entry 7). The structural nature of **9** (entry 10, Table 1) implies that this compound cannot be considered as a 5-FU prodrug and we suspected that the remaining compounds (entries 4-7, 9-11, Table 1) would not be 5-FU prodrugs. To start with and to confirm it we decided to change the 5-FU moiety of **4** for the naturally occurring pyrimidine base uracil to produce **7**, with the prospect of finding an antiproliferative agent endowed with a new mechanism of action (Marchal et al., 2007).

2.2 Apoptosis induction of cyclic O,N-acetals

Apoptosis has been studied in terms of cancer development and treatment with attempts made to identify its role in chemotherapeutic agent-induced cytotoxicity. Cytotoxic agents often induce only a fraction of the cells to become apoptotic. To fully exploit apoptosis as a mechanism of antineoplastic agent response, a larger proportion of cells need to be

recruited into apoptosis. Paclitaxel (Taxol®), cyclophosphamide and cytosine arabinoside are the only commonly used cytotoxic agents shown to elicit apoptosis in breast cancer cells (Meyn et al., 1995; Milas et al., 1995). Quantitation of apoptotic cells was done by monitoring the binding of fluorescein isothiocyanate (FITC)-labelled annexin V (a phosphatidylserine-binding protein) to cells in response to our title compounds as described (Chadderton et al., 2000). The apoptosis study shows that compounds **3**, **6**, **8** and **9**, at their IC<sub>50</sub> concentrations, provoke early apoptosis in the cells treated for 24 and 48 h. It is worth pointing out that compound **6** (entry 7) induces greater apoptosis at 48 h (46.73%) than at 24 h (40.08%) and so does compound **3** [48 h (53.92%) and 24 h (46.63%), entry 4]. The compounds that show the most important apoptotic indexes at 24 h are **8** (57.33%, entry 9) and **9** (54.33%, entry 10), whereas at 48 h are **3** (53.92%, entry 4) and **8** (51.37%, entry 9). These compounds are more potent as apoptosis inducers against the MCF-7 human breast cancer cells than paclitaxel (Taxol®), which induced programmed cell death of up to 43% of the cell population (Saunders et al., 1997). Accordingly, the early apoptotic inductions and the low IC<sub>50</sub> values give rise to a significant antitumor activity.

### 2.3 Cell cycle distribution of cyclic 5-FU *O,N*-acetals

Cell cycle regulation has attracted a great deal of attention as a promising target for cancer research and treatment (Sampath & Plunkett, 2001; Gali-Muhtasib & Bakkar, 2002). The use of cell-cycle-specific treatments in cancer therapy has greatly benefited from the major advances that have been recently made in the identification of the molecular actors regulating the cell cycle and from the better understanding of the connections between cell cycle and apoptosis. As more and more “cell cycle drugs” are being discovered, their use as anticancer drugs is being extensively investigated (Gali-Muhtasib & Bakkar, 2002). To study the mechanisms of the antitumour and antiproliferative activities of the compounds, the effects on the cell cycle distribution were analyzed by flow cytometry. Control DMSO-treated cell cultures contained 68.39% G<sub>0</sub>/G<sub>1</sub>-phase cells, 12.04% G<sub>2</sub>/M-phase cells and 19.57% S-phase cells. Cyclic *O,N*-acetals **4-10** (entries 5-11) provoke a G<sub>0</sub>/G<sub>1</sub>-phase cell cycle arrest whereas ftorafur [1-(2-tetrahydrofuran-5-yl)-5-fluorouracil], a known prodrug of 5-FU, induces a S-phase cell cycle arrest.

In fact, a correlation between treated cells with compounds **4-10** recruited in the G<sub>0</sub>/G<sub>1</sub> phase when treated at their IC<sub>50</sub> concentrations, and their calculated lipophilicities by the CDR option of the PALLAS 2.0 programme:

$$\log (\% G_0/G_1) = 1.801 (\pm 0.009) + 0.055 (\pm 0.003) \log P \quad (1)$$

$$n = 7, r^2 = 0.976, s = 0.006, F_{1,5} = 206.10, \alpha < 0.001$$

where  $n$  is the number of compounds,  $r^2$  is correlation coefficient,  $s$  is the standard deviation,  $F$  is the  $F$  ratio between the variances of observed and calculated activities, and data within parentheses are standard errors of estimate. This equation means that the more lipophilic the compound is the more cells are recruited in the G<sub>0</sub>/G<sub>1</sub> phase.

### 2.4 Modification of the molecular markers caused by the cyclic 5-FU *O,N*-acetals

Due to the fact that the cyclic *O,N*-acetals accumulate the cells in the G<sub>1</sub>-phase the expression pattern of cyclin D1 was studied. This cyclin is one of the cyclin-dependent

kinase (CDK) activator subunits, specifically to CDK4, being responsible of the progression of the cell through the G<sub>1</sub>-phase. Compounds **4** and **10** gave rise to a spectacular inhibition of cyclin D1 up to its total disappearance. This fact did not take place with 5-FU because the cyclin D1 level increased in relation to those of the parental MCF-7 cells. On one hand, this would explain why these compounds accumulate the cells in the G<sub>1</sub>-phase (on inhibiting cyclin D1 the cell cannot progress to the S phase) and on the other, they show a different mechanism of action from the one shown by 5-FU: in short, they are not prodrugs. 5-FU Increases the cyclin D1 production so that cells pass in most cases toward the S phase where they are held back. It has been reported (Stacey, 2003) that cyclin D1 works as an active “switch” in the progression of the cellular cycle and that elevated levels of cyclin D1 promote the entry of the cell into the S phase. Moreover, compound **10** increases the expression of proteins p21 or p27 even up to double of the control. These proteins belong to the family INK2 of the CDK inhibiting proteins that work by hindering the association and activation of cyclins with their complexes (Sherr, 2000) and hence they halt cells in the G<sub>1</sub> and G<sub>2</sub>/M phases. Compounds also affect the cdc2 activity that, regulated by their corresponding cyclins A or B, is essential for the entrance into mitosis during the cellular cycle (Sherr, 1993). Compounds **4**, **10** and **5**, with the exception of **8** and 5-FU, significantly decrease the cdc2 activity. Cdc2 is needed during the cellular cycle in the final phase of G<sub>1</sub>, in the control point named “start” to be committed to the mitotic cycle. This is because at the end of G<sub>2</sub> (at the beginning of the mitosis) (Lees & Harlow, 1993), the inhibition of cdc2 by the *O,N*-acetals implies the halting of the cycle in G<sub>1</sub> and the non-entrance of the tumor cells in mitosis. The increase of the cdc2 expression caused by 5-FU is due to the fact that this higher activity is necessary for the cells to pass rapidly to the S phase, where cells are stopped by this fluoropyrimidine. Finally, compound **8** also significantly increases the cdc2 levels, which may be because its premature activation is one of the requirements for apoptosis (Shi et al., 1994); in fact this compound is the one that induces a higher proportion of programmed cellular death in the MCF-7 treated cells.

## 2.5 Apoptosis markers

Since the synthesized compounds induce very important apoptosis, we have carried out studies of the expression of some of the genes that intervene in this phenomenon, among which p53 and the family bcl-2 are outstanding. The tumour suppressor gene p53 protects the integrity of the genome so that if the DNA of the cell is damaged by an agent, an overexpression of it is produced inducing the stopping in G<sub>1</sub> for the repair of the damage, or if this is not possible, enter in apoptosis (Agarwal et al., 1995). On the other hand, the members of the family of proteins Bcl-2 work as regulators of apoptosis, Bcl-2 and Bcl-XL protecting against apoptosis. Bax, Bak and Bad induce such a phenomenon (Reed, 1997).

The treatment of the MCF-7 cells (wild-type p53) with these compounds provoked in general an increase in the protein expression of p53, mainly for 5-FU and **8**, and a marked decrease of the levels of bcl-2 for all of them. These data show that p53 activity is restored with the compounds, allowing the entrance of the tumour cells in apoptosis, which permits their elimination by this mechanism. In the same way bcl-2 inhibition facilitates the entrance of cells into the programmed cell death.

### 3. Benzo-fused seven-membered derivatives linked to purines

Later on, we substituted the pyrimidine bases for the purine one (with several substituents at its position 6), with the objective of increasing both the lipophilicity and the structural diversity of the target molecules (Figure 2). Their syntheses (using conventional heating and microwave irradiation) and biological activities have been recently published (Conejo-García et al., 2008).

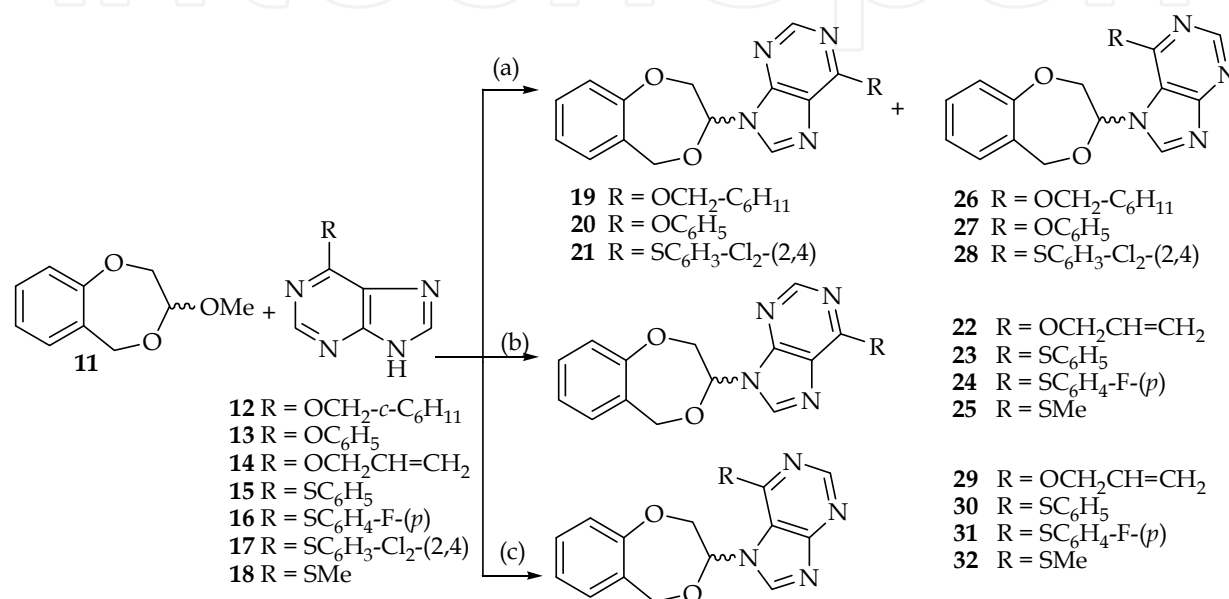


Fig. 2. *Reagents and conditions*: Purine bases (12-18), TCS, HMDS,  $\text{SnCl}_4$ , anhydrous MeCN. Method (a), (b) or (c): 45 °C, 24-72 °C; (b) microwave, 130 °C, 5 min; (c) microwave, 100 °C, 5 min.

#### 3.1 Biological activities

The antitumour potential of the target molecules is reported against the MCF-7 human breast cancer cell line including 5-FU as reference drug (Table 2) (Conejo-García et al., 2008). The purine *O,N*-acetals 19-32 are more active than their corresponding purine bases 12-18. The differences in the antiproliferative effect of the *N*-7' and *N*-9' regioisomers are not significant with the exception of the allyloxy derivatives 22 and 29. The biological effect is dependent on the substituent present on position 6 of the purine ring although a clear structure-activity relationship between the size of this moiety and the antiproliferative effect of the MCF-7 human breast cancer cell line is not observed. The most active compound (22), that presents an allyloxy group as substituent at position 6 of the purine ring, shows an  $\text{IC}_{50} = 5.04 \pm 1.68 \mu\text{M}$  nearly equipotent as 5-FU. The following two more active compounds, 23 and 28, present bulky substituents as the phenylthio and 2,4-dichlorophenylthio ones, respectively.

Compound	IC <sub>50</sub> (μM)	Compound	IC <sub>50</sub> (μM)	Compound	IC <sub>50</sub> (μM)
5-FU	4.32 ± 0.02	19	11.6 ± 0.93	27	21.1 ± 2.93
12	32.3 ± 1.42	20	24.7 ± 3.82	28	8.4 ± 0.91
13	28.4 ± 0.45	21	12.0 ± 0.59	29	20.9 ± 1.24
14	44.7 ± 0.74	22	5.04 ± 1.68	30	15.6 ± 3.74
15	31.0 ± 0.16	23	7.12 ± 0.46	31	14.1 ± 0.67
16	31.6 ± 0.54	24	11.2 ± 1.32	32	31.8 ± 5.46
17	21.4 ± 0.42	25	24.0 ± 1.95		
18	23.6 ± 4.11	26	13.4 ± 1.94		

Table 2. Antiproliferative activities against the MCF-7 cell line for 5-FU (Núñez et al., 2007), for the purine bases (12-18), and for the seven-membered alkylated purine derivatives (*N*-9' isomers: 19-25; *N*-7' isomers: 26-32).

To study the mechanisms of the antitumour and antiproliferative activities of the most active compounds (22, 23 and 28), the effects on the cell cycle distribution were analyzed by flow cytometry (Table 3). DMSO-treated cell cultures contained a 58.62 ± 0.74 of the G<sub>0</sub>/G<sub>1</sub>-phase cells, a 33.82 ± 0.72 of the S-phase cells and a 7.55 ± 1.34 of the G<sub>2</sub>/M-phase cells. In contrast, MCF-7 cells treated during 48 h with the IC<sub>50</sub> concentrations of 22, 23 and 28 showed important differences in cell cycle progression compared with DMSO-treated control cells. The cell cycle regulatory activities can be divided into the following two groups: (a) the breast cancer cells showed an accumulation in the S-phase, up to 37.00 ± 2.00 of the cells, mainly at the expense of the G<sub>0</sub>/G<sub>1</sub>-phase population that decreased to a percentage of 55.63 ± 1.57 of the cells; (b) compounds 23 and 28 accumulated the cancerous cells in the G<sub>2</sub>/M-phase (11.08 ± 1.01 and 19.16 ± 0.56, respectively) at the expense of the S-phase cells (26.82 ± 1.26 and 22.73 ± 0.37, respectively). In response to 23 (and 28), the percentage of apoptotic cells increased, from 0.22 ± 0.31 in control cells to a maximum of 73.37 ± 0.12 (and 65.28 ± 1.92) apoptotic cells at a concentration equal to their IC<sub>50</sub> against the MCF-7 cell line. This is a remarkable property because the demonstration of apoptosis in MCF-7 breast cancer cells by known apoptosis-inducing agents has proved to be difficult.

Compound	Cell cycle <sup>a</sup>			Apoptosis <sup>b</sup>
	G <sub>0</sub> /G <sub>1</sub>	S	G <sub>2</sub> /M	
Control	58.62 ± 0.74	33.82 ± 0.72	7.55 ± 1.34	0.22 ± 0.16
22	55.63 ± 1.57	37.00 ± 2.00	7.37 ± 0.43	44.47 ± 2.98
23	59.10 ± 1.28	26.82 ± 1.26	11.08 ± 0.01	73.37 ± 0.12
28	58.10 ± 0.19	22.73 ± 0.37	19.16 ± 0.56	65.28 ± 1.92

<sup>a</sup> Determined by flow cytometry (Boulaiz et al., 2003). <sup>b</sup> Apoptosis (48 h) was determined using an Annexin V-based assay (Boulaiz et al., 2003). The data indicate the percentage of cells undergoing apoptosis in each sample. All experiments were conducted in duplicate and gave similar results. The data are means ± SEM of three independent determinations.

Table 3. Cell cycle distribution and apoptosis induction in the MCF-7 human breast cancer cell line after treatment for 48 h for the three most active compounds.

#### 4. Anticancer and SAR studies on (1,2,3,5-tetrahydro-4,1-benzoxazepine-3-yl)-pyrimidine and -purine derivatives

The synthesis and the mechanistic aspects of bioisosteres containing a 4,1-benzoxazepine *N*-alkylated pyrimidine (**33–45**) or purine (**46–58**) (Figure 3) have been thoroughly discussed (Díaz-Gavilán et al., 2006; Díaz-Gavilán et al., 2007).

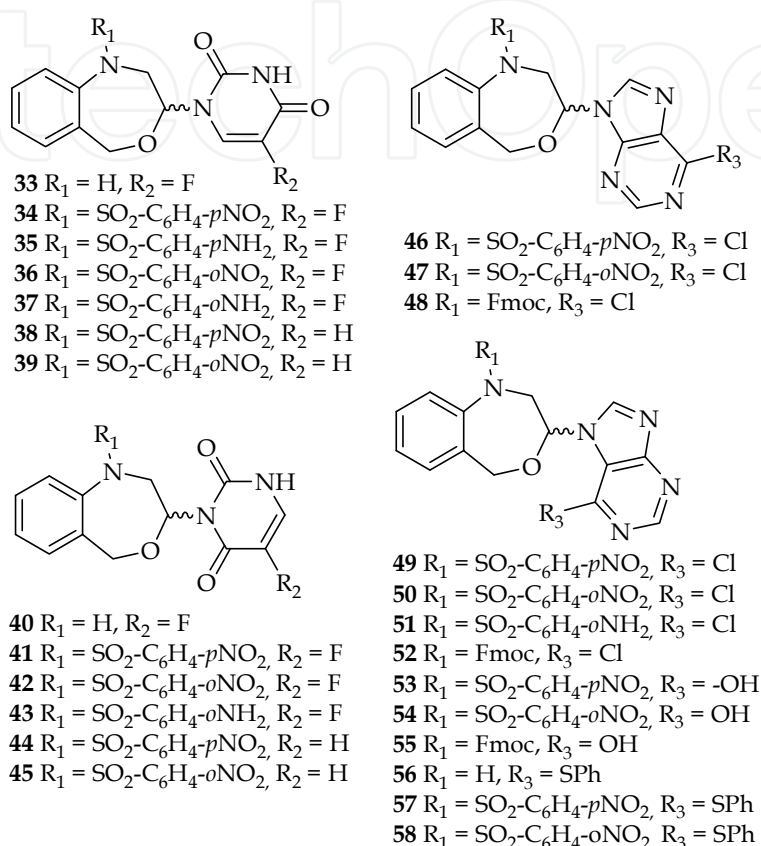


Fig. 3. New benzoxazepine *O,N*-acetals containing pyrimidine and purine rings.

##### 4.1 Antiproliferative activities

Table 4 show the antiproliferative activity ( $\text{IC}_{50}$  values) on MCF-7 human breast cancer cells found for the pyrimidine and purine derivatives **33–58** (Díaz-Gavilán et al., 2008a). The most potent molecules were the purine derivatives. Compounds **48**, **50**, **52** and **57** presented  $\text{IC}_{50}$  values below 1  $\mu\text{M}$ . Between the pyrimidine derivatives **33–45**, those containing 5-fluorouracil ( $R_2 = \text{F}$ ) showed improved activities than those derived of uracil ( $R_2 = \text{H}$ ). Bonding of the pyrimidine ring through *N*-1'' or *N*-3'' affected the activity though only slightly, when  $R_1 = \text{H}$ , **33** versus **40**. The substitution on C-6'' of the purine ring is essential for the activity<sup>1</sup>. Bulky, lipophilic groups afforded the best values of  $\text{IC}_{50}$  [ $R_3 = \text{Cl}$  (**46–52**), -SPh (**57**, **58**)] while purinone compounds [ $R_3 = \text{OH}$  (**53–55**)] were comparable to the pyrimidine analogues in terms of activity. Bonding of the purine ring through *N*-7'' or *N*-9''

<sup>1</sup> For the numbering of the compounds, the atoms of the benzoxazepine are tagged with numbers without primes, the atoms of the  $R_1$  group are numbered with primes ('), while the pyrimidine and purine bases are numbered with double primes (').

affected the activity to a lesser extent, and the positive or negative character of this effect depended on the nature of  $R_1$  (see **46/49**  $R_1 = pNs$ , **47/50**  $R_1 = oNs$ , **48/52**  $R_1 = Fmoc$ ). Both, pyrimidine and purine derivatives, were more potent when  $R_1$  is not hydrogen. The lipophilic character of  $R_1$  increased the activity and no limit of volume had been observed for the studied groups. The electron-withdrawing character of  $R_1$  could help to increase the activity (Díaz-Gavilán et al., 2004). Carbonyl derivatives were more potent than the sulfonyl ones.

Compds	IC <sub>50</sub> (μM)	Compds	IC <sub>50</sub> (μM)	Compds	IC <sub>50</sub> (μM)	Compds	IC <sub>50</sub> (μM)
33	>100	40	72.40 ± 11.3	47	2.10 ± 0.69	54	19.66 ± 5.27
34	19.33 ± 1.04	41	19.81 ± 0.08	48	0.67 ± 0.18	55	53.57 ± 13.1
35	14.37 ± 0.69	42	22.63 ± 0.11	49	1.22 ± 0.12	56	48.92 ± 9.89
36	19.70 ± 0.15	43	43.70 ± 0.09	50	0.92 ± 0.01	57	0.86 ± 0.12
37	54.82 ± 1.04	44	44.28 ± 4.65	51	9.14 ± 1.24	58	2.59 ± 0.57
38	39.78 ± 2.60	45	50.90 ± 3.87	52	0.84 ± 0.09		
39	45.17 ± 0.48	46	2.73 ± 0.17	53	>100		

Table 4. Antiproliferative activities against the MCF-7 cells for  $N$ -1''- (**33-39**) and  $N$ -3''- (**40-45**) pyrimidines and for  $N$ -9''- (**46-48**) and  $N$ -7''-purines (**49-58**).

When  $R_1 =$  benzensulfonamido, it can be observed for pyrimidine and purine derivatives that the *ortho*-substitution on  $R_1$  is preferred to *para*, in terms of potency, and the nitro group renders better results than the amino one. As an exception, compound **57** ( $R_1 = pNs$ ,  $R_3 = SPh$ ), is more potent than **58** ( $R_1 = oNs$ ,  $R_3 = SPh$ ). The new related acyclic  $O,N$ -acetals **59-69** (Figure 4) were obtained as minor products in the condensation reaction between the  $O,O$ -acetals and pyrimidine (Díaz-Gavilán et al., 2006) or purine (Díaz-Gavilán et al., 2007) bases. Their antiproliferative activities have also been studied on MCF-7 human breast cancer cells and the  $IC_{50}$  values obtained are shown in Table 5. Acyclic purine  $O,N$ -acetals (**67-69**) show higher potency than the pyrimidine acyclic derivatives (**59-66**). The  $N$ -7''-alkylated purine **68** presented an excellent value of  $IC_{50}$ . In contrast to the cyclic analogues, the presence of an  $o$ - $NO_2$  or  $p$ - $NO_2$  group does not modify the activity of the  $N$ -9''-isomers (**67** and **69**).

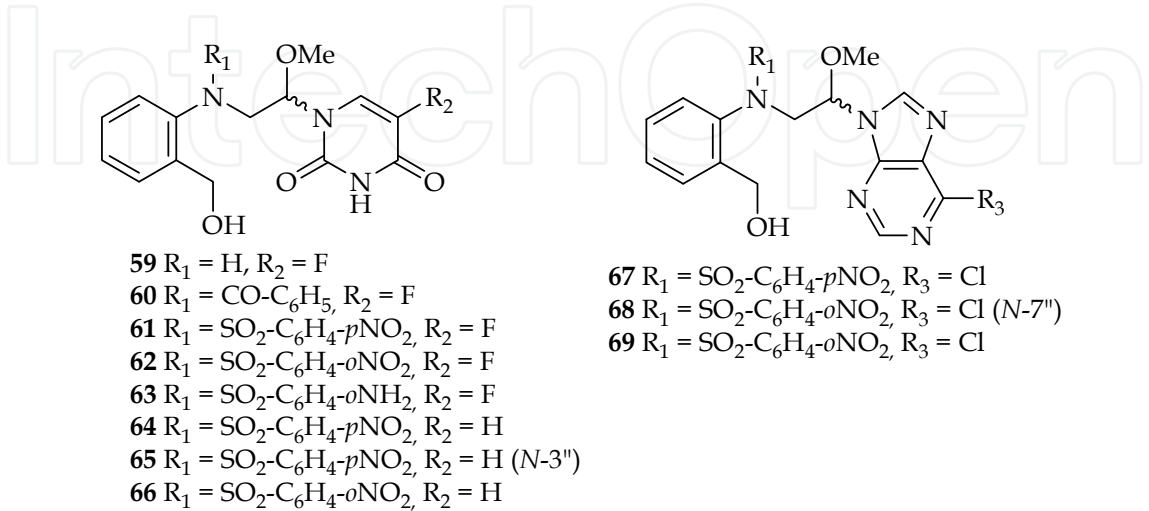


Fig. 4. New acyclic  $O,N$ -acetals containing pyrimidine and purine moieties.

Compds	IC <sub>50</sub> (μM)	Compds	IC <sub>50</sub> (μM)
59	35.97 ± 0.40	65	55.22 ± 12.14
60	16.14 ± 0.77	66	64.81 ± 0.05
61	55.22 ± 12.14	67	18.70 ± 0.08
62	90.99 ± 6.06	68	3.25 ± 0.23
63	>100	69	11.30 ± 1.27
64	45.76 ± 2.45		

Table 5. Antiproliferative activities against the MCF-7 cell line for acyclic *N*-1''- and *N*-3''-pyrimidines (59-66) and *N*-9''- and *N*-7'' purines (67-69).

Compounds **48** and **57** were selected to identify the molecular key targets of its anti-cancer activity (Díaz-Gavilán et al., 2008a). Completion of the human genome sequence and the advent of DNA microarrays using cDNAs enhanced the detection and identification of hundreds of differentially expressed genes in response to anticancer drugs. In this way gene-expression patterns of treated human breast cancer cells in comparison with parental MCF-7 cells were obtained. For this purpose, the expression of about 22,000 different human genes was analyzed using the Agilent 60-mer oligo microarray platform and the Human 1A Oligo Microarray Kit (V2) (Agilent Technologies, CA, USA).

The up-regulated and the down-regulated genes include genes that encode for different metabolic pathways, cellular development process, signal molecules, response to stress, regulation of the cell cycle and apoptosis, etc. Analysis of the mRNAs, which are deregulated (up-regulated or down-regulated) at least 2-fold in treated cells, revealed the following results: 26 genes up-regulated and 59 genes down-regulated in **48**-MCF-7 treated cells; and 26 genes up-regulated and 17 genes down-regulated in **57**-treated human breast cancer cells. Each compound revealed a somewhat unique expression pattern together with the up-regulation of significant genes involved in different cellular functions and a significant down-regulation of genes for **48**. One of the more important results in the current study was the ability of **48** to modulate the expression of genes involved in apoptosis or its delay of mitosis. This effect can be explained by the accumulation of cells in the G<sub>2</sub>/M checkpoint of cell cycle, particularly GP132, the receptor for an unknown ligand, which activates a G2 alpha protein (Díaz-Gavilán et al., 2008a). This is transcriptionally up-regulated by stress-inducing and cell-damaging agents and that is involved in caspase-mediated apoptosis (Lin & Ye, 2003). Similarly, the ERN1 gene that belongs to the Ser/Thr protein kinase family is a potent unfolded-protein response transcriptional activator and acts by triggering growth arrest and apoptosis (Yoneda et al., 2001). However, **57** induced the down-regulation of a gene involved in the metastatic progression of cancer such as RAC1, a Ras-like protein member of the Rho family of the GTPase key downstream target in Ras signalling (Baugher et al., 2005).

The studies by microarray technology showed that the main molecular targets of some of these compounds (**48** and **57**) are pro-apoptotic genes with protein kinase activity such as GP132, ERN1 or RAC1, which prevent the metastatic progression (Díaz-Gavilán et al., 2008a).

**5. Synthesis and anticancer activity of (RS)-9-(2,3-dihydro-1,4-benzoxathiin-3-ylmethyl)-9H-purines**

The 2,3-dihydro-1,4-benzodioxin ring system is present in a large number of structures of therapeutic agents possessing important biological activities (Guillaumet, 1996). Some of them

are antagonists of  $\alpha$ -adrenergic receptors, giving them antihypertensive properties (Quaglia et al., 1999; Pallavicini et al., 2006). Others have affinities with serotonin receptors which are involved in nervous breakdown and schizophrenia (Birch et al., 1999). Sixteen years ago, 2,3-dihydro-1,4-benzodioxins were developed as inhibitors of 5-lipoxygenase, an enzyme involved in the oxygenation of arachidonic acid to the leukotriens; they are also useful for the treatment of inflammatory diseases such as asthma and arthritis (Satoh et al., 1995). The occurrence of the 2,3-dihydro-1,4-benzodioxin structure in various naturally abundant compounds has been also reported (Fukuyama et al., 1992). Paradoxically, despite the considerable development of biologically active compounds with the 2,3-dihydro-1,4-benzodioxin moiety, the 2,3-dihydro-1,4-benzoxathiin skeleton has still remained inaccessible. The importance of 5-FU as the first-choice drug in carcinomas of the gastrointestinal tract is well known despite its side-effects. With the aim of diminishing the toxicity and obtaining biologically active derivatives of 5-FU suitable for oral administration great effort has been made in the preparation of 5-FU prodrug derivatives. A review of the literature on the various prodrugs of 5-FU has been published (Malet-Martino et al., 2002). Various 5-FU prodrugs are active against certain malignant cell lines due to an inhibition of thymidilate synthase by the formation of 5-fluorodeoxyuridine monophosphate or by the incorporation of 5-fluorouridine triphosphate into RNA. During various synthetic studies on masked 5-FU derivatives, it has been found that the bond strength between the N-1 atom in the 5-FU ring and its N-1 substituent is an important factor influencing the antitumour activity and the toxicity of the compounds. The previous results indicated that the weaker the bond strength, the stronger are the antitumour activity and the toxicity of the masked compounds (Ozaki, 1996). In the case of *N*-alkyl-5-FU derivatives, the strong *N*-1<sub>5-FU</sub>-C<sub>exocyclic</sub> bond conversely prevented these derivatives from being easily hydrolyzed *in vivo* and showed no antitumour activity against L1210 leukaemia (Ozaki, 1996). When oxygen was introduced at the  $\alpha$ -position to the alkyl group, the N-C bond became labile under hydrolytic conditions and the resulting derivatives showed antitumour activity.

As it has been demonstrated before, compounds **4-6**, **8-10** may be considered as drugs with their own entity and antitumour activity independent of that of 5-FU. If the previously described compounds are not prodrugs, it is not necessary to maintain the *O,N*-acetalic characteristic with the corresponding weakness of the *O,N*-acetalic bond. Therefore, molecules are being designed in which both structural entities (such as the benzoheterocyclic ring and the purine base) are linked by a heteroatom-C-C-base-N-atom bond. Very recently the design, synthesis and biological evaluation of a series of 2- and 6-disubstituted (*RS*)-9-(2,3-dihydro-1,4-benzoxathiin-3-ylmethyl)-9*H*-purine derivatives **70-80** were described [Figure 5, Table 6] (Díaz-Gavilán et al., 2008b).

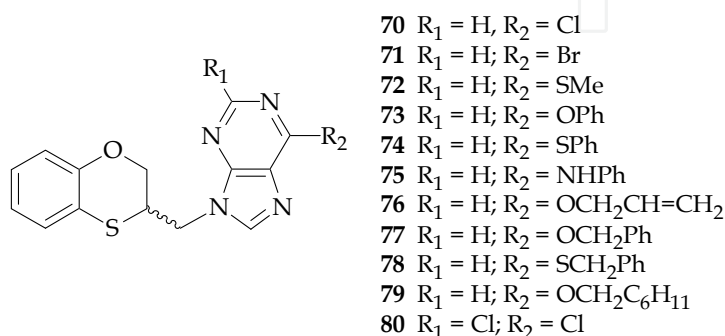


Fig. 5. The 1,4-benzoxathiin system linked to several purines.

Compound	IC <sub>50</sub> (μM)	Compound	IC <sub>50</sub> (μM)	Compound	IC <sub>50</sub> (μM)
5-FU	4.32 ± 0.02	73	20.5 ± 1.11	77	23.2 ± 1.26
70	10.6 ± 0.66	74	10.5 ± 1.06	78	16.7 ± 3.03
71	6.18 ± 1.70	75	11.2 ± 2.73	79	17.4 ± 1.60
72	20.5 ± 1.81	76	17.5 ± 0.25	80	8.97 ± 0.83

Table 6. Antiproliferative activities against the MCF-7 cell line for 5-FU (Villalobos et al., 1995), and the six-membered alkylated purine derivatives.

The three most potent compounds (**70**, **71** and **80**) were subjected to cell cycle and apoptosis studies on the MCF-7 human breast cancer cell line (Table 7). The following two consequences can be stated: (a) in contrast to 5-FU, the six-membered compounds **70**, **71** and **80**, provoked a G<sub>0</sub>/G<sub>1</sub>-phase cell cycle arrest when the MCF-7 cells were treated during 48 h with the IC<sub>50</sub> of the compounds, mainly at the expense of the S-phase populations. The fact that at similar doses the novel derivatives exhibit different sequences of cell cycle perturbations in comparison with 5-FU indicates that these compounds act by different pathways (Marchal et al., 2004). In the case of **71** it is worth pointing out that, moreover, there is an increase in the G<sub>2</sub>/M-phase of the cancerous cells; and (b) the apoptotic indices of the target compounds are very important, especially for **80** (58.29% for **70**, 63.05% for **71**, and 76.22% for **80**). Up to now and according to our knowledge, compound **80** is the most important apoptotic inducer against the MCF-7 human breast cancer cell line so far reported.

Compound	Cell cycle <sup>a</sup>			Apoptosis <sup>b</sup>
	G <sub>0</sub> /G <sub>1</sub>	S	G <sub>2</sub> /M	
Control	58.62 ± 0.74	33.82 ± 0.72	7.55 ± 1.34	0.22 ± 0.16
5-FU <sup>c</sup>	58.07 ± 0.11	39.38 ± 0.98	2.10 ± 0.12	52.81 ± 1.05
70	69.71 ± 1.50	23.73 ± 1.65	6.56 ± 0.17	58.29 ± 0.75
71	62.85 ± 0.87	26.71 ± 1.25	10.43 ± 0.38	63.05 ± 0.26
80	70.30 ± 0.32	23.67 ± 2.40	6.06 ± 2.72	76.22 ± 2.02

<sup>a</sup> Determined by flow cytometry (Marchal et al., 2004).  
<sup>b</sup> Apoptosis was determined using an Annexin V-based assay (Marchal et al., 2004). The data indicate the percentage of cells undergoing apoptosis in each sample.  
<sup>c</sup> Data were taken from ref. (Campos et al., 2005). All experiments were conducted in duplicate and gave similar results. The data are means ± SEM of three independent determinations.

Table 7. Cell cycle distribution and apoptosis induction in the MCF-7 human breast cancer cell line after treatment for 48 h for the three most active compounds as antiproliferative agents.

6. Conclusion

Breast cancer is the commonest malignancy in women and comprises 18% of all cancers in women. Normal breast development is controlled by a balance between cell proliferation and apoptosis, and there is strong evidence that tumour growth is not just a result of uncontrolled proliferation but also of reduced apoptosis. The balance between proliferation

and apoptosis is crucial in determining the overall growth or regression of the tumour in response to chemotherapy, radiotherapy and more recently, hormonal treatments. All of these approaches act in part by inducing apoptosis. Understanding these relationships could allow individually tailored treatments to maximize tumour regression and efficacy of treatment. It could also help to answer why some tumours fail to respond and thereby indicate new routes of drug development.

Starting from Ftorafur, a known 5-FU prodrug, which shows an 58% apoptosis induction in the MCF-7 human breast cancer cell line after treatment for 48 h, the seven-membered cyclohomologue 5-FU *O,N*-acetal **3** and the benzo-fused dihydro oxepine *O,N*-acetal **8** present apoptosis inductions higher than 50%. By using molecular modification strategies widely used in medicinal chemistry, lately compounds **23** and **28**, having in common the benzo-fused 2,3-dihydro-5*H*-1,4-dioxepin and a 6-substituted purine moieties, show 73% and 65% apoptosis inductions. Finally and following our Drug Anticancer Programme, the benzo-fused 1,4-oxathiane moiety linked to the *N*-9 atom of a 2,6-dichloropurine **80** was designed and synthesized. According to our knowledge this is the most important apoptotic inducer against the MCF-7 human breast cancer cell line so far reported. This compound is a more potent apoptosis inducer than the clinically-used drug paclitaxel (Taxol®), which induced programmed cell death up to 43% of cell population. Their mechanisms of action at molecular level are being studied at present.

## 7. Acknowledgements

This study was supported by the Instituto de Salud Carlos III (Fondo de Investigación Sanitaria) through project no. PI10/00592.

## 8. References

- Agarwal, M. L.; Agarwal, A.; Taylor, W. R. & Stark, G. R. (1995). p53 controls both the G2/M and the G1 cell cycle checkpoints and mediates reversible growth arrest in human fibroblasts. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.92, No.18, (August 1995), pp. 8493-8497, ISSN 0027-8424.
- Baugher, P. J.; Krishnamoorthy, L.; Price, J. E. & Dharmawardhane, S. F. (2005). Rac1 and Rac3 isoform activation is involved in the invasive and metastatic phenotype of human breast cancer cells. *Breast Cancer Research*, Vol.7, No.6, (September 2005), pp. R965-R974, ISSN 1465-542X.
- Birch, A. M.; Bradley, P. A.; Gill, J. C.; Kerrigan, F. & Needham, P. L. (1999). N-Substituted (2,3-dihydro-1,4-benzodioxin-2-yl)methylamine derivatives as D(2) antagonists/5-HT(1A) partial agonists with potential as atypical antipsychotic agents. *Journal of Medicinal Chemistry*, Vol.42, No.17, (January 1999), pp. 3342-3355, ISSN 0022-2623.
- Boulaiz, H.; Prados, J.; Melguizo, C.; García, A. M.; Marchal, J. A.; Ramos, J. L.; Carrillo, E. & Aránega, A. (2003). Inhibition of growth and induction of apoptosis in human breast cancer by transfection of gef gene. *British Journal of Cancer*, Vol.89, (July 2003), pp. 192-198, ISSN 0007 - 0920/03.
- Buur, A.; Bundgaard, H. & Falch, E. (1985). Prodrugs as drug delivery systems. Part 37. Prodrugs of 5-fluorouracil. IV. Hydrolysis kinetics, bioactivation and physicochemical properties of various *N*-acyloxymethyl derivatives of 5-

- fluorouracil. *International Journal of Pharmaceutics*, Vol.24, No.1, (April 1985), pp. 43-60, ISSN 0378-5173.
- Campos, J.; Pineda, M. J.; Gómez, J. A.; Entrena, A.; Trujillo, M. A.; Gallo, M. A. & Espinosa, A. (1996). 5-Fluorouracil Derivatives. 1. Acyclonucleosides through a Tin (IV) Chloride-Mediated Regiospecific Ring Opening of Alkoxy-1,4-Diheteroepanes. *Tetrahedron*, Vol.52, No.26, (June 1996), pp. 8907-8924, ISSN 0040-4020.
- Campos, J.; Gómez, J. A.; Trujillo, M. A.; Gallo, M. A. & Espinosa, A. (1997). Diheterocyclanes as Synthons for the Preparation of Novel Series of Nucleoside and Acyclonucleoside Analogues. *Il Farmaco*, Vol.52, No.5, (May 1997), pp. 263-269, ISSN 0014-827X.
- Campos, J.; Núñez, M. C.; Rodríguez, V.; Gallo, M. A. & Espinosa, A. (2000). QSAR of 1,1'-(1,2-Ethylenebisbenzyl)bis(4-substitutedpyridinium) Dibromides as Choline Kinase Inhibitors: a Different Approach for Antiproliferative Drug Design. *Bioorganic & Medicinal Chemistry Letters*, Vol.10, No.8, (April 2000), pp. 767-770, ISSN 0960-894X.
- Campos, J.; Núñez, M. C.; Conejo-García, A.; Sánchez-Martín, R. M.; Hernández-Alcoceba, R.; Rodríguez-González, A.; Lacal, J. C.; Gallo, M. A. & Espinosa, A. (2003). QSAR-derived choline kinase inhibitors: How rational can antiproliferative drug design be? *Current Medicinal Chemistry*, Vol.10, No.13, (July 2003), pp. 1095-1112, ISSN 0929-8673.
- Campos, J.; Saniger, E.; Marchal, J. A.; Aiello, S.; Suárez, I.; Boulaiz, H.; Aránega, A.; Gallo, M. A. & Espinosa, A. (2005). New Medium Oxacyclic O,N-Acetals and Related Open Analogues: Biological Activities. *Current Medicinal Chemistry*, Vol.12, No.12, (June 2005), pp. 1423-1438, ISSN 0929-8673.
- Chadderton, A.; Villeneuve, D. J.; Gluck, S.; Kirwan-Rhude, A. F.; Gannon, B. R.; Blais, D. E. & Parissenti, A. M. (2000). Role of specific apoptotic pathways in the restoration of paclitaxel-induced apoptosis by valspodar in doxorubicin-resistant MCF-7 breast cancer cells. *Breast Cancer Research and Treatment*, Vol.59, No.3, (February 2000), pp. 231-244, ISSN 0167-6806.
- Conejo-García, A.; Núñez, M. C.; Marchal, J. A.; Rodríguez-Serrano, F.; Aránega, A.; Gallo, M. A.; Espinosa, A. & Campos, J. M. (2008). Regioespecific Microwave-Assisted Synthesis and Cytotoxic Activity against Human Breast Cancer Cells of (RS)-6-Substituted-7- or 9-(2,3-Dihydro-5H-1,4-Benzodioxepin-3-yl)-7H- or -9H-Purines. *European Journal of Medicinal Chemistry*, Vol.43, No.8, (October 2007), pp. 1742-1748, ISSN 0223-5234.
- Díaz-Gavilán, M.; Rodríguez-Serrano, F.; Gómez-Vidal, J. A.; Marchal, J. A.; Aránega, A.; Gallo, M. A.; Espinosa, A. & Campos, J. M. (2004). Synthesis of Tetrahydrobenzoxazepine Acetals with Electron-Withdrawing Groups on the Nitrogen Atom. Novel Scaffolds Endowed with Anticancer Activity against Breast Cancer Cells. *Tetrahedron*, Vol.60, No.50, (December 2004), pp. 11547-11557, ISSN 0040-4020.
- Díaz-Gavilán, M.; Gómez-Vidal, J. A.; Entrena, A.; Gallo, M. A.; Espinosa, A. & Campos, J. M. (2006). Study of the factors that control the ratio of the products between 5-fluorouracil, uracil, and tetrahydrobenzoxazepine O,O-acetals bearing electron-

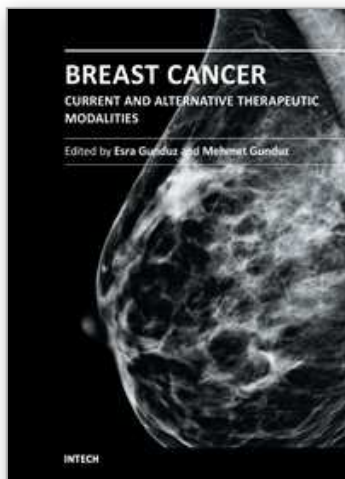
- withdrawing groups on the nitrogen atom. *Journal of Organic Chemistry*, Vol.71, No.3, (October 2005), pp. 1043-1054, ISSN 0022-3263.
- Díaz-Gavilán, M.; Choquesillo-Lazarte, D.; González-Pérez, J. M.; Gallo, M. A.; Espinosa, A. & Campos, J. M. (2007). Synthesis and Reactivity of (RS)-6-Chloro-7- or 9-(1,2,3,5-Tetrahydro-4,1-Benzoxazepin-3-yl)-7H- or 9H-Purines Bearing a Nitrobenzene-sulfonyl Group on the Nitrogen Atom. *Tetrahedron*, vol.63, No.24, (June 2007), pp. 5274-5286, ISSN 0040-4020.
- Díaz-Gavilán, M.; Gómez-Vidal, J. A.; Rodríguez-Serrano, F.; Marchal, J. A.; Caba, O.; Aránega, A.; Gallo, M. A.; Espinosa, A. & Campos, J. M. (2008a). Anticancer Activity of (1,2,3,5-Tetrahydro-4,1-Benzoxazepine-3-yl)-Pyrimidines and -Purines against the MCF-7 Cell Line: Preliminary cDNA Microarray Studies. *Bioorganic & Medicinal Chemistry Letters*, Vol.18, No.4, (February 2008), pp. 1457-1460, ISSN 0960-894X.
- Díaz-Gavilán, M.; Conejo-García, A.; Cruz-López, O.; Núñez, M. C.; Choquesillo-Lazarte, D.; González-Pérez, J. M.; Rodríguez-Serrano, F.; Marchal, J. A.; Aránega, A.; Gallo, M. A.; Espinosa, A. & Campos, J. M. (2008b). Synthesis and Anticancer Activity of (RS)-9-(2,3-Dihydro-1,4-Benzoxathiin-3-ylmethyl)-9H-Purines. *ChemMedChem*, Vol.3, No.1, (January 2008), pp. 127-135, ISSN 1860-7179.
- Fukuyama, Y.; Hasegawa, T.; Toda, M.; Kodama, M. & Okazaki, H. (1992). Structures of americanol A and isoamericanol A having a neurotrophic property from the seeds of *Phytolacca americana*. *Chemical & Pharmaceutical Bulletin*, Vol.40, No.1, (January 1992), pp. 252-254, ISSN 0009-2363.
- Gali-Muhtasib, H. & Bakkar, N. (2002). Modulating cell cycle: current applications and prospects for future drug development. *Current Cancer Drug Targets*, Vol.2, No.4, (December 2002), pp. 309-336, ISSN 1568-0096.
- Guillaumet, G. (1996). 1,4-Dioxins, oxathiins, dithiins and their benzo derivatives. In : *Comprehensive Heterocyclic Chemistry II*, (first edition) Katritzky, A.R. ; Rees, C. W. & Scriven, E. F. V., Vol.6, pp. 447-481, Elsevier, ISBN: 978-0-08-096518-5, Oxford, UK.
- Gulyaeva, N.; Zaslavsky, A.; Lechner, P.; Chlenov, M.; McConnell, O.; Chait, A.; Kipnis, V. & Zaslavsky, B. (2003). Relative hydrophobicity and lipophilicity of drugs measured by aqueous two-phase partitioning, octanol-buffer partitioning and HPLC. A simple model for predicting blood-brain distribution. *European Journal of Medicinal Chemistry*, Vol.38, No.4, (April 2003), pp. 391-396, ISSN 0223-5234.
- Harris, J. R.; Lippman, M. E.; Morrow, M. & Hellman, S. (1996). *Diseases of the Breast* (first edition), Lippincott-Raven, ISBN 0-397-51470-0, New York.
- Hickman, J. A. (1992). Apoptosis induced by anticancer drugs. *Cancer and Metastasis Reviews*, Vol.11, No.2, (April 1992), pp. 121-139, ISSN 0167-7659.
- Lees, E. M. & Harlow, E. (1993). Sequences within the conserved cyclin box of human cyclin A are sufficient for binding to and activation of cdc2 kinase. *Molecular and Cellular Biology*, Vol.13, No.2, (January 1993), pp. 1194-1201, ISSN:0270-7306.
- Lin, P. & Ye, R.D. (2003). The Lysophospholipid Receptor G2A Activates a Specific Combination of G Proteins and Promotes Apoptosis. *Journal of Biological Chemistry*, Vol.278, No.16, (February 2003), pp. 14379-14386, ISSN 0021-9258.

- Lundberg, A. S. & Weinberg, R. A. (1999). Control of the cell cycle and apoptosis. *European Journal of Cancer*, Vol.35, No.4, (December 1998), pp. 531, ISSN 0959-8049.
- Malet-Martino, M.; Jolimaitre, P. & Martino, R. (2002). The prodrugs of 5-fluorouracil. *Current Medicinal Chemistry: Anti-Cancer Agents*, Vol.2, No2, (March 2002), pp. 267-310, ISSN 1568-0118.
- Marchal, J. A.; Boulaiz, H.; Suárez, I.; Saniger, E.; Campos, J.; Carrillo, E.; Prados, J.; Gallo, M. A.; Espinosa, A. & Aránega, A. (2004). Growth inhibition, G1-arrest, and apoptosis in MCF-7 human breast cancer cells by novel highly lipophilic 5-fluorouracil derivatives. *Investigational New Drugs*, Vol.22, No.4, (November 2004), pp. 379-389, ISSN 0167-6997.
- Marchal, J. A.; Nuñez, M. C.; Suarez, I.; Diaz-Gavilan, M.; Gomez-Vidal, J. A.; Boulaiz, H.; Rodriguez-Serrano, F.; Gallo, M. A.; Espinosa, A.; Aranega, A. & Campos, J. M. (2007). A synthetic uracil derivative with antitumor activity through decreasing cyclin D1 and Cdk1, and increasing p21 and p27 in MCF-7 cells. *Breast Cancer Research and Treatment*, Vol.105, No.3, (November 2006), pp. 237-246, ISSN:0167-6806.
- Matsuo, S.; Tanako, S.; Yamashita, J. & Ogawa, M. (2000). Synergistic cytotoxic effects of tumor necrosis factor, interferon- $\gamma$  and tamoxifen on breast cancer cell lines. *Anticancer Research*, Vol.12, No.5, (September 2000), pp. 1575-1579, ISSN 0250-7005.
- Meyn, R. E.; Stephens, L. C.; Hunter, N. R. & Milas, L. (1995). Kinetics of cisplatin-induced apoptosis in murine mammary and ovarian adenocarcinomas. *International Journal of Cancer*, Vol.60, No.5, (July 2006), pp. 725-729, ISSN 0020-7136.
- Milas, L.; Hunter, N. R.; Kurdoglu, B.; Mason, K. A.; Meyn, R. E.; Stephens, L. C. & Peters, L. (1995). Kinetics of mitotic arrest and apoptosis in murine mammary and ovarian tumors treated with taxol. *Cancer Chemotherapy and Pharmacology*, Vol.35, No.4, (April 1995), pp. 297-303, ISSN 0344-5704.
- Núñez, M. C.; Rodríguez-Serrano, F.; Marchal, J. A.; Caba, O.; Aránega, A.; Gallo, M. A.; Espinosa, A. & Campos, J. M. (2007). 6'-Chloro-7- or 9-(2,3-dihydro-5H-4,1-benzoxathiepin-3-yl)-7H- or 9H-purines and their corresponding sulfones as a new family of cytotoxic drugs. *Tetrahedron*, Vol.63, No.1, (January 2007), pp. 183-190, ISSN 0040-4020.
- Ozaki, S. (1996). Synthesis and antitumor activity of 5-fluorouracil derivatives. *Medicinal Research Reviews*, Vol.16, No.1, (January 1996), pp. 51-86, ISSN 0198-6325.
- Pallavicini, M.; Fumagalli, L.; Gobbi, M.; Bolchi, C.; Colleoni, S.; Moroni, B.; Pedretti, A.; Rusconi, C.; Vistoli, G. & Valotti, E. (2006). QSAR study for a novel series of ortho disubstituted phenoxy analogues of  $\alpha$ 1-adrenoceptor antagonist WB4101. *European Journal of Medicinal Chemistry*, Vol.41, No.9, (September 2006), pp. 1025-1040, ISSN 0223-5234.
- Qin, L. F. & Ng, I. O. (2002). Induction of apoptosis by cisplatin and its effect on cell cycle-related proteins and cell cycle changes in hepatoma cells. *Cancer Letters*, Vol.175, No.1, (January 2002), pp. 27-38, ISSN 0304-3835.
- Quaglia, W.; Pigini, M.; Piergentili, A.; Giannella, M.; Marucci, G.; Poggesi, E.; Leonardi, A. & Melchiorre, C. (1999). Structure-Activity Relationships in 1,4-Benzodioxan-Related Compounds. 6. Role of the Dioxane Unit on Selectivity for  $\square$ 1-

- Adrenoreceptor Subtypes. *Journal of Medicinal Chemistry*, Vol.42, No.15, (March 1999), pp. 6359-6370, ISSN 0022-2623.
- Rasbridge, S. A.; Gillet, C. E.; Seymour, A. M.; Patel, K.; Richards, M. A.; Rubens, R. D. & Millis, R. R. R. (1994). The effects of chemotherapy on morphology, cellular proliferation, apoptosis and oncoprotein expression in primary breast carcinoma. *British journal of cancer*, Vol.70, No.2, (August 1994), pp. 335-341, ISSN 0007-0920.
- Reed, J. C. Double identity for proteins of the Bcl-2 family. (1997). *Nature*, Vol.387, No.6635, (June 1997), pp. 773-776, ISSN 0028-0836.
- Rekker, R. F. & de Kort, H. M.(1979). The hydrophobic fragmental constant; an extension to a 1000 data point set. *European Journal of Medicinal Chemistry*, Vol.14, No.6, (July 1979), pp. 479-488, ISSN 0009-4374.
- Sampath, D. & Plunkett, W. (2001). Design of new anticancer therapies targeting cell cycle checkpoint pathways. *Current Opinion in Oncology*, Vol.13, No.6, (November 2001), pp. 484-490, ISSN 1040-8746.
- Saniger, E.; Campos, J. M.; Entrena, A.; Marchal, J. A.; Boulaiz, H.; Aranega, A.; Gallo, M. A. & Espinosa, A. (2003a). Medium Benzene-fused Oxacycles with the 5-Fluorouracil Moiety: Synthesis, Antiproliferative Activities and Apoptosis Induction in Breast Cancer Cells. *Tetrahedron*. Vol.59, No.29, (July 2003), pp. 5457-5467, ISSN 0040-4020.
- Saniger, E.; Campos, J. M.; Entrena, A.; Marchal, J. A.; Boulaiz, H.; Aránega, A.; Gallo, M. A. & Espinosa, A. (2003b). Neighbouring Group Participation as the Key Step in the Reactivity of Acyclic and Cyclic Salicyl-Derived *O,O*-Acetals with 5-Fluorouracil. Antiproliferative Activity, Cell Cycle Dysregulation and Apoptotic Induction of New *O,N*-Acetals against Breast Cancer Cells. *Tetrahedron*. Vol.59, No.40, (September 2003), pp. 8017-8026, ISSN 0040-4020.
- Satoh, Y.; Powers, C.; Toledo, L. M.; Kowalski, T. J.; Peters, P. A. & Kimble, E. F.(1995). Derivatives of 2-[[N-(Aminocarbonyl)-N-hydroxyamino]methyl]-1,4-benzodioxan as Orally Active 5-Lipoxygenase Inhibitors *Journal of Medicinal Chemistry*, Vol.38, No.1, (August 1994), pp. 68-75, ISSN 0022-262.
- Saunders, D. E.; Lawrence, W. D.; Christensen, C.; Wappler, N. L.; Ruan, H. & Deppe, G. (1997). Paclitaxel-induced apoptosis in MCF-7 breast-cancer cells. *International Journal of Cancer*, Vol.70, No.2, (January 1997), pp. 214-220, ISSN 0020-7136.
- Sherr, C. J. (1993). Mammalian G1 cyclins. *Cell*, Vol.73, No.6, (June 1993), pp. 1059-1065, ISSN 0092-8674.
- Sherr, C. J. (2000). The Pezcoller lecture: cancer cell cycles revisited. *Cancer Research*. Vol.60, No.14, (July 2000), pp. 3689-3695, ISSN 0008-5472.
- Shi, L; Nishioka, W. K.; Th'ng, J.; Bradbury, E. M.; Litchfield, D. W. & Greenberg, A. H. (1994). Premature p34cdc2 activation required for apoptosis. *Science*, Vol.263, No.5150, (February 1994), pp. 1143-1145, ISSN 0036-8075.
- Stacey, D. W. (2003). Cyclin D1 serves as a cell cycle regulatory switch in actively proliferating cells. *Current Opinion in Cell Biology*, Vol.15, No.2, (April 2003), pp. 158-163, ISSN 0955-0674.
- Trouet, A.; Passioukov, A.; Derpooten, K. V.; Fernández, A. M.; Abarca-Quñones, J.; Baurain, R.; Lobl, T. J.; Olilla, C. & Dubois, V. (2001). Extracellular tumour-activated prodrugs for the selective chemotherapy of cancer: Application to

- doxorubicin and preliminary in vitro and in vivo studies. *Cancer Research*, Vol.61, No.7, (April 2001), pp. 2843-2846, ISSN 0008-5472.
- Villalobos, M.; Olea, N.; Brotons, J. A.; Olea-Serrano, M. F.; Ruiz de Almodovar, J. M. & Pedraza, V. (1995). The E-screen assay: a comparison of different MCF-7 cell stocks. *Environmental Health Perspectives*, Vol.103, No.9, (September 1995), pp. 844-850, ISSN 0091-6765.
- Yoneda T.; Imaizumi, K.; Oono, K.; Yui, D.; Gomi, F.; Katayama, T. & Tohyama, M. (2001). Activation of caspase-12, an endoplasmic reticulum (ER) resident caspase, through tumor necrosis factor receptor-associated factor 2-dependent mechanism in response to the ER stress. *Journal of Biological Chemistry*, Vol.276, No.17, (January 2001), pp. 13935-13940, ISSN 0021-9258.

IntechOpen



## **Breast Cancer - Current and Alternative Therapeutic Modalities**

Edited by Prof. Esra Gunduz

ISBN 978-953-307-776-5

Hard cover, 540 pages

**Publisher** InTech

**Published online** 09, November, 2011

**Published in print edition** November, 2011

Cancer is the leading cause of death in most countries and its consequences result in huge economic, social and psychological burden. Breast cancer is the most frequently diagnosed cancer type and the leading cause of cancer death among females. In this book, we discussed various therapeutic modalities from signaling pathways through various anti-tumor compounds as well as herbal medicine for this deadly cancer. We hope that this book will contribute to the development of novel diagnostic as well as therapeutic approaches.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Joaquín M. Campos, M. Carmen Núñez, Ana Conejo-García and Olga Cruz-López (2011). Benzo-Fused Seven- and Six-Membered Derivatives Linked to Pyrimidines or Purines Induce Apoptosis of Human Metastatic Breast Cancer MCF-7 Cells In Vitro, Breast Cancer - Current and Alternative Therapeutic Modalities, Prof. Esra Gunduz (Ed.), ISBN: 978-953-307-776-5, InTech, Available from:  
<http://www.intechopen.com/books/breast-cancer-current-and-alternative-therapeutic-modalities/benzo-fused-seven-and-six-membered-derivatives-linked-to-pyrimidines-or-purines-induce-apoptosis-of->

**INTECH**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen