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Medium benzene-fused oxacycles with the 5-fluorouracil moiety: synthesis, antiproliferative activities and apoptosis induction in breast cancer cells

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Abstract—On the basis of the increase in lipophilicity, novel benzannelated six- and seven-membered derivatives have been synthesized, starting from 2-hydroxybenzyl alcohols, 2-hydroxybenzaldehydes, and catechol. The X-ray structure of (*RS*)-1-(2,3-dihydro-5*H*-1,4-benzodioxepin-3-yl)-5-fluorouracil (**5**) is presented and compared with its conformational analysis at the semiempirical (AM1) and ab initio (6-31G*) levels and NOE effects. A good agreement between both experimental and theoretical data was found showing a chair conformation for the 2,3-dihydro-5*H*-1,4-dioxepin ring and an axial orientation of the 5-FU moiety on the C3 position. Compounds **5** and (*RS*)-1-(7-methoxy-2,3-dihydro-5*H*-1,4-benzodioxepin-3-yl)-5-fluorouracil (**6**) were found to be the most potent inhibitor of MCF-7 cells growth. (*RS*)-1-(2,3-Dihydrobenzoxepin-2-yl)-5-fluorouracil (**8**) induced apoptosis up to 57.33% of cell population after 24 h.

1. Introduction

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.[1]

As part of their action on neoplastic cells, many anticancer drugs activate apoptosis (programmed cell death). Apoptosis may be a primary mechanism of antineoplastic agents.[2] Although breast cancer is most often treated with conventional cytotoxic agents it has proven difficult to induce apoptosis in breast cancer cells using these drugs.[3] 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.

Novel derivatives of 5-fluorouracil (5-FU, **1**) possessing a broader spectrum of antitumor activity and fewer side effects than 5-FU, have been diligently sought in a number of laboratories. *seco*-Nucleosides are non-classical nucleosides in which the 'sugar' is linear instead of cyclic as is usual. Among them, the isopropoxy **2** and cyclopentoxy **3** analogues were reported to exhibit antitumor activity against the HEp human cells.[4] The most lipophilic structure proved to be the most active compound of all those tested, being 4-fold more active than 5-FU. Afterwards, we embarked on a program to synthesize a wide range of 5-FU derivatives linked to saturated annelated heptatomic moieties.[5,6] Nevertheless, since systematic study of structure–activity relationship in seven-membered 5-FU *O,N*-acetals has not been realized so far, it is thought that much effort should be made in this class of derivatives to search for new anticancer agents. Compound **4** has been previously described by us.[7] On one hand, with the idea of increasing the lipophilicity of **4**, in this paper, we propose a series of bioisosteric benzannelated seven-membered *O,N*-acetals such as **5**–**8**. On the other, the preparation of a six-membered benzannelated derivative such as **9** could emphasize the significance of the biological activity of the seven-membered 5-FU *O,N*-acetals. In all the cases, the attachment of the 5-FU moiety occurs at the N^1-position of the pyrimidine ring. To our knowledge, up to now, no attempt has been made to synthesize benzannelated 5-FU *O,N*-acetals. Our aim was to fill this gap and in this paper we report the synthesis and antitumor activity of the title

Keywords: acetals; benzodioxepins; X-ray crystal structure; antitumor compounds.
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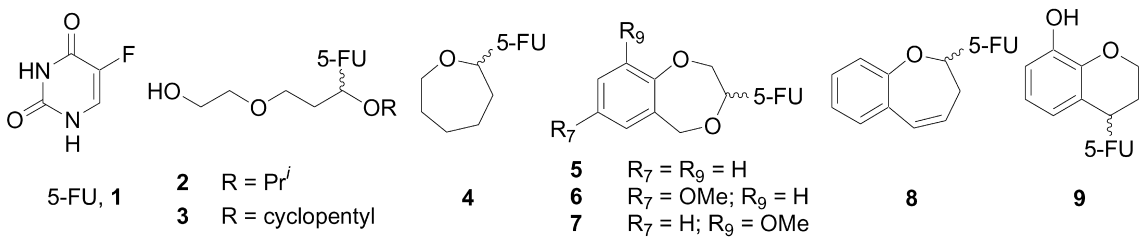


Chart 1.

compounds, starting from salicyl alcohol or salicyl aldehyde (Chart 1).

2. Results and discussion

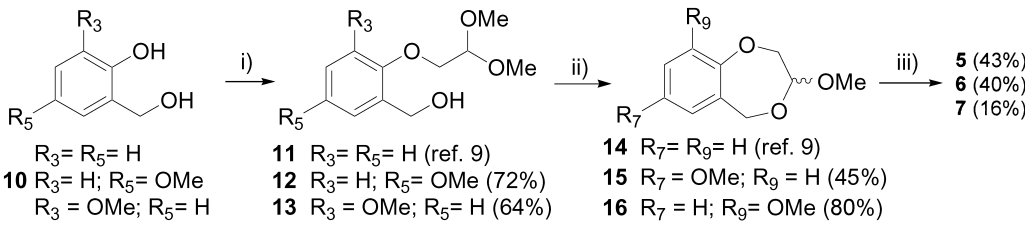
2.1. Chemistry

At this stage of our research we have carried out the work with racemic mixtures. Our synthetic strategy was to synthesize the seven-membered cycloacetal and then condense it with the 5-FU base. The synthesis of compounds **5**–**7** is depicted in Scheme 1. Reduction of 2-hydroxy-5-methoxybenzaldehyde with sodium borohydride gave the 2-hydroxy-5-methoxybenzyl alcohol[8] **10** whilst 2-hydroxybenzyl and 2-hydroxy-3-methoxybenzyl alcohols were commercially available. Alkylation of the phenolic group with bromoacetaldehyde dimethyl acetal using sodium hydride as a base in dimethylformamide (DMF) gave the hydroxyacetals **11**–**13**. Compound **11** has been previously reported[9] and we have used the same protocol to synthesize **12** and **13**. The cyclization reaction under conditions previously reported by us[6] produced the 3-methoxy-2,3-dihydro-5*H*-1,4-benzodioxepins **14**–**16**. Subsequent substitution of the acetalic methoxy groups by the 5-FU moiety in the presence of the silylating agents, 1,1,1,3,3,3-hexamethyldisilazane (HMDS) and trimethylchlorosilane (TCS), under acid catalysis ($SnCl_4$) in acetonitrile during 24 h yielded **5** (45%), **7** (20%) with a reaction time of 24 h, and **6** (40%) when the reaction time was increased up to 144 h (6 days). All condensations were carried out in a one-pot reaction under our standard conditions.[4–7]

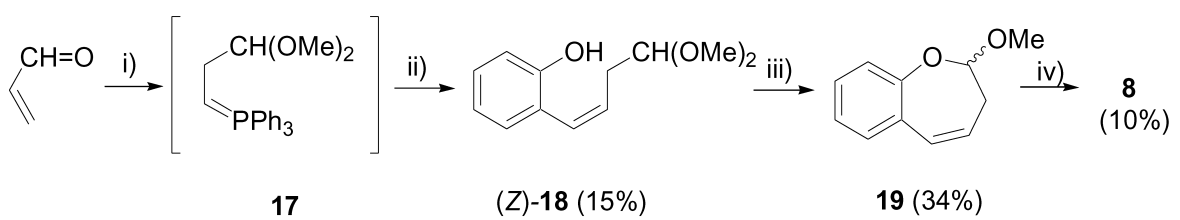
The preparation of **8** is shown in Scheme 2. Starting from acrolein the corresponding non-isolated phosphonium salt **17**[10] reacted via a Wittig reaction with 2-hydroxybenzaldehyde and formed (*Z*)-**18** (15%). The cyclization reaction using boron trifluoride diethyl etherate in diethyl ether as a solvent during six days gave 2-methoxy-2,3-dihydrobenzoxepin **19** (34%). Formation of (*Z*)-**18** must be justified by means of two factors that have an influence on the stereoselectivity of the Wittig reaction: the ylide structure and the reaction conditions. As a rule of thumb the non-stabilized ylides predominantly give (*Z*) alquenes.[11] In our case, the reaction conditions have been very mild and **17** is a non-stabilized ylide that gives only isomer (*Z*) in a very fast process. The experimental evidence that **18** is a *Z* isomer is the following: for this compound $J_{3',4'}$=11.3 Hz and this value is very close to $J_{2,3}$ in *cis*-2-butene ($J_{2,3}$=10.9 Hz),[12] and very far from the corresponding $J_{2,3}$ value in *trans*-2-butene ($J_{2,3}$=15.1 Hz).[12] Besides this, in compound **19** the double bond configuration has to be *Z* and in this case, $J_{4,5}$=11.9 Hz. The substitution of the methoxy group was carried out as described previously for 24 h to yield **8** (10%).

The synthesis of **9** followed a rather unexpected route in which the key step was the formation of 4-methoxy-8-chromanol **20**.

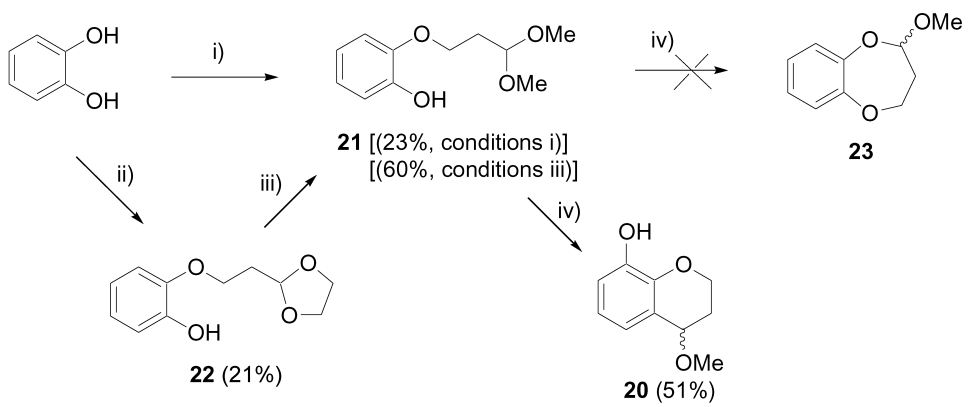
The alkylation of catechol to give the *o*-hydroxyphenoxypropanal dimethyl acetal **21** was carried out in two different



Scheme 1. *Reagents*: (i) $BrCH_2CH(OMe)_2$, HNa, anhydrous DMF; (ii) $BF_3{\cdot}Et_2O$ in anhydrous Et_2O; (iii) 5-FU, HMDS, TCS, $SnCl_4/CH_2Cl_2$, CH_3CN.



Scheme 2. *Reagents*: (i) See Ref. 10; (ii) 2-hydroxybenzaldehyde; (iii) $BF_3{\cdot}Et_2O$; (iv) 5-FU, HMDS, TCS, $SnCl_4/CH_2Cl_2$, CH_3CN.



Scheme 3. *Reagents*: (i) K_2CO_3, anhydrous acetonel, $BrCH_2CH_2CH(OMe)_2$; (ii) K_2CO_3, anhydrous acetone, $BrCH_2CH_2CH(OCH_2)_2$; (iii) anhydrous MeOH, 1% H_2SO_4; (iv) $BF_3{\cdot}Et_2O$ in anhydrous Et_2O.

ways, as is depicted in Scheme 3:

(a) directly by treatment with potassium carbonate in anhydrous acetone, using 3-bromopropanal dimethyl acetal as a reactant (**21**, 23%),
(b) through **22** by alkylation with 2-(2-bromoethyl)-1,3-dioxolane in anhydrous acetone and potassium carbonate as base (**21**, 21%) and subsequent transacetalization reaction with anhydrous methanol in 1% sulfuric acid (**21**, 60%).

Finally, the intramolecular cyclization reaction catalyzed by boron trifluoride diethyl etherate gave 4-methoxy-8-chromanol (**20**, 51%) instead of the expected 2-methoxy-3,4-dihydro-2*H*-1,5-benzodioxepin **23**. The spectroscopic characterization of the supposed compound (**23**) did not result concordant with the proposed structure in spite of the fact that the HR LSIMS gave a compatible molecular ion. The chemical shift of H-4, the presence of three aromatic protons (H-5, H-6 and H-7), the proton of the phenolic group in ^{1}H NMR, and also the corresponding ^{13}C NMR signals, were over-riding arguments to reject structure **23** as the correct one. The most important signal of **20** is the H-4a (δ, 4.33), which appears as a double doublet with coupling constants of 3.3 and 6.7 Hz with H-3a and H-3b, respectively. The ^{1}H NMR chemical shift of H-4a confirms that this proton is not acetalic (for compounds **14**–**16** and **19** the range for acetalic hydrogen atoms is $\approx\delta$ 4.8 ppm). The proton of the phenoxy group appears at δ 5.56 ppm. After a thorough study of the ^{1}H NMR spectrum, it has been possible to determine the coupling constants of H-4a with H-3a and H-3b being 3.3 Hz and 6.7 Hz, respectively. $J_{2a,3b}$ is 9.1 Hz and $J_{2b,3a}$ and $J_{2b,3b}$ are 6.6 and 3.5 Hz, respectively. Both H-2a and H-2b resonate at the same field as a multiplet, which eliminates the geminal constant. The determination of the other coupling constants has been possible on the basis of the coupling constants of H-3. Regarding H-3a and H-3b, the former appears at δ 2.16 ppm as a ddd and the latter at δ 2.04 ppm as a dddd with the following coupling constants: $J_{3a,4a}$=3.3 Hz, $J_{3a,2b}$=6.6 Hz, $J_{3a,3b}$=14.2 Hz and $J_{3b,2b}$=3.5 Hz, $J_{3b,4a}$=6.7 Hz, $J_{3b,2a}$=9.1 Hz, $J_{3b,3a}$=14.2 Hz. It is worth mentioning that proton H-3a forms a 90° angle with H-2a and, accordingly, $J_{3a,2a}$=0 Hz. Finally, the ^{13}C NMR chemical shift of C-4 is δ 71.23 ppm, which confirms that this carbon atom is not acetalic (for compounds **14**–**16** and **19** the range for acetalic carbons is δ 100–101 ppm).

One interpretation of the unexpected formation of **20** could be the following (Scheme 4).

Boron trifluoride coordinated one of the methoxy groups of **21** to give the oxocarbenium ion **24**, which resulted from the loss of the methoxy group, and which could progress in the two following manners:

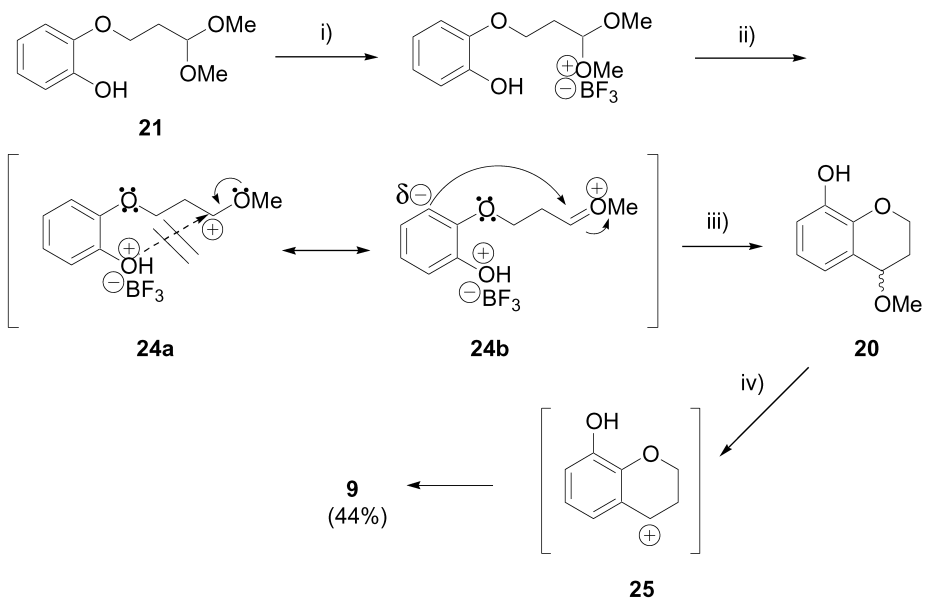
(a) Intramolecular cyclization with the phenolic hydroxyl group with formation of **23**. This process was highly improbable due to the poor nucleophilicity of the hydroxy phenolic group on being complexed to the Lewis acid BF_3.
(b) Intramolecular cyclization with the aromatic ring, whose non-substituted position adjacent to the ethereal oxygen atom was exceedingly nucleophilic so as to form the non-acetalic chromane **20**.

The substitution of the methoxy moiety was carried out by the reaction of **20** with 5-FU under the above-mentioned conditions to yield the chromane analogue **9** (44%). The substitution of the benzylic methoxy group, although non-acetalic, was possible under the conditions described for the reaction due to the formation of the stable carbenium ion **25**. In this sense, the reactivity of the benzylic methoxy group of **20** is similar to that of an acetalic one.

It is worth mentioning that the signal of H-6 for compound **9** appears as a sharp doublet (J=6.1 Hz) at δ 6.96 ppm, due to the coupling with the adjacent F atom. This fact, together with the elemental analysis data, demonstrates unambiguously that the 5-FU fragment is linked to the aminalic carbon through *N*-1 and not through *N*-3.[13] Regarding the ^{13}C NMR spectrum, the C-4, C-5 and C-6 signals (of the pyrimidine base) show couplings with the F atom of 25.7 for C-4, 229.1 for C-5 and 33.4 Hz for C-6, all appearing as doublets, whilst C-2 appears as a singlet (δ 149.89 ppm).

2.2. Molecular structure and inter-molecular interactions of 5

Single crystals of **5** were obtained by the slow diffusion of



Scheme 4. *Reagents*: (i) $BF_3{\cdot}Et_2O$ in anhydrous Et_2O; (ii) $-MeO{\cdot}BF_3^{-}$[1]; (iii) H_2O; (iv) 5-FU, HMDS, TCS, $SnCl_4/CH_2Cl_2$, MeCN.

ether in its methanol solution. The crystal structure reveals the following interesting facts: (a) the seven-membered ring of the 2,3-dihydro-1,4-benzodioxepin moiety shows a chair conformation, and (b) the 5-FU fragment is in an equatorial orientation (Fig. 1). In the crystal, pairs of molecules related by the symmetry transformation ($1=-x+1, -y+2, -z+2$ are linked by two symmetrical and rather linear hydrogen bonding interactions of the type $N(3')-H(3')\cdots O(2')$(1 (d(N···O) 2.84 Å, ∠(N–H···O) 173°). It is noteworthy that the O atoms of the dioxepin ring are not involved in this kind of intermolecular interactions. In addition, pairs of 5-fluorouracil rings (symmetry code ($2=-x, -y, -z$) and of benzene rings (symmetry transformation ($3=1-x, 1-y, 1-z$) from adjacent molecules build up centro-symmetric π,π-stacking interactions (parallel rings, with dihedral inter-planar angles α=0.00°). In these stacks, the centroid–centroid distance d_{c-c} and the inter-planar $d_{\pi-\pi}$ distance are 3.77 and 3.53 Å or 3.84 and 3.64 Å, respectively. These data are consistent with slipping angles $\beta=\gamma$=20.72 or 19.02°, respectively. Such ring–ring stacking interactions co-operate with the referred hydrogen bonds to give a corrugate 2D molecular framework (Fig. 2) where the C_6-benzene ring and the 5-fluoruracil moiety of the existing conformer define an open dihedral angle of 64.1°. These layered structures interact by Van der Waals forces to build up the crystal.

2.3. Conformational analysis

We have used theoretical methods at semiempirical and ab initio levels to compare the results with the data obtained by X-ray crystallography. In view of the biological importance

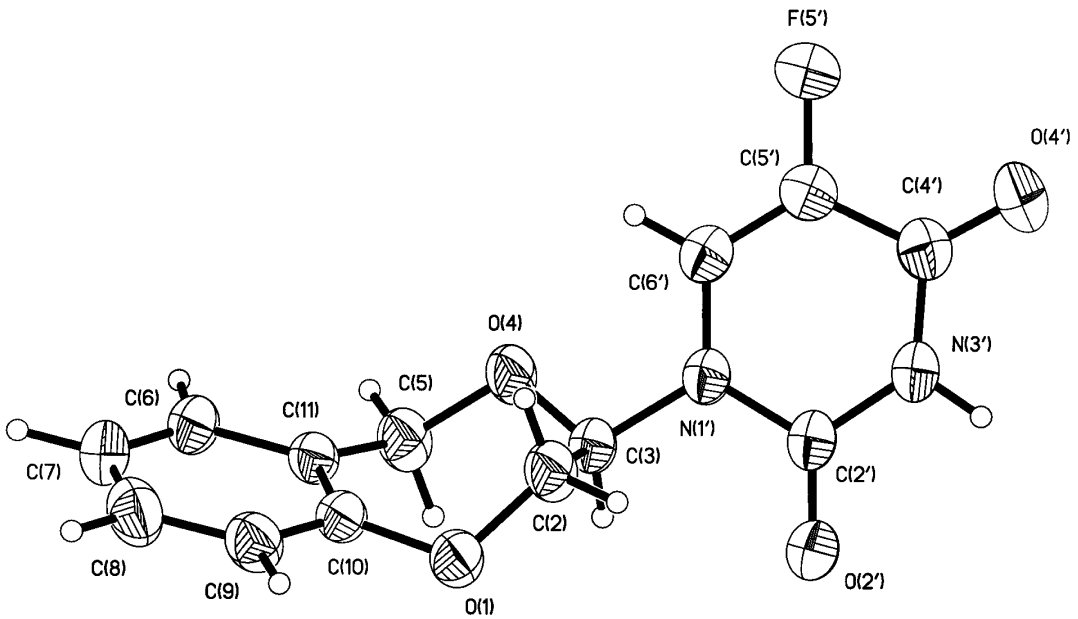


Figure 1. ORTEP drawing of **5** at 50% probability.

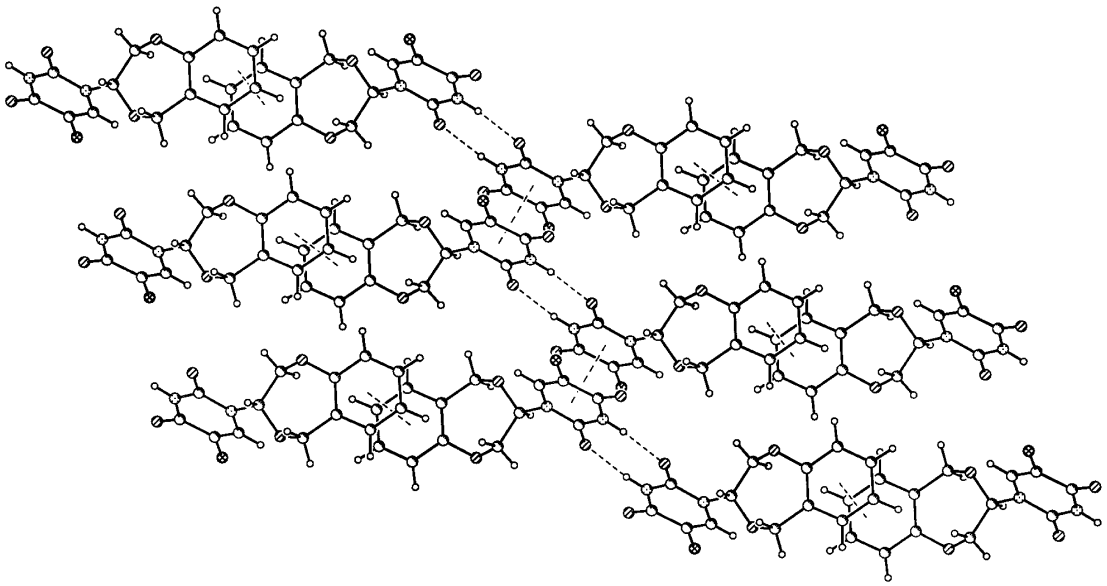


Figure 2. Packing of the molecules of **5** in the unit cell showing their hydrogen bond network and π,π-stacking interactions.

of compounds **5**–**7** (see below), we have carried out a detailed conformational analysis of the simplest compound of the series (**5**) to gain more insight into the molecular structure prior to exploring further structure–activity relationships. The conformational analysis has been carried out by means of the Tripos force field,[14] implemented in the Sybyl calculation package.[15] The initial geometries have been generated from standard fragments of the program libraries and their optimization have been carried out by means of the Powell method,[16] up to the point where the energy gradient is less than 0.01 kcal/mol. The electrostatic component has been calculated by means of the atomic charges generated according to the Gasteiger method,[17] using a distance-dependent dielectric constant equal to the unity (ε=1). The conformational analysis has been carried out in two phases: in the first one, the possible conformations of the base ring **5** have been identified and used as starting points, and in the second one, the hydrogens of the C-3 atom of the ring have been substituted by the 5-FU moiety and the possible rotamers around the exocyclic C–N bond have been investigated.

2.3.1. Conformational analysis of 2,3-dihydro-5*H*-1,4-benzodioxepin. A chair form has been defined as a starting conformation for the seven-membered ring and, once optimized, conformational searches have been carried out by means of the Sybyl gridsearch utility by rotating each of the 1,4-dioxepane ring bonds, except the one that is common to the benzene system. The conformations thus obtained have been subsequently minimized once the imposed restrictions to the torsional angles were removed, and have been compared. The final result has led to only six conformations: two chair, two boat and two twist-boat conformations.

The most stable conformation is the chair, followed by the twist-boat and the boat, the conformations of the same family being isoenergetic. The relative energy values of the chair and twist-boat conformations coincide with the reported values[9] for such a compound, calculated from ^{13}C NMR data at a very low temperature (−120°C). At such a temperature, the observed conformational population for the chair conformation is higher than 98%, other conformations not being detectable. Moreover, an ab initio calculation at the 6-31G* level (Gaussian98[18]) of such conformations has been undertaken. The same order of stability has been found, although there exists a slightly higher energetic difference between the three studied conformations. Figure 3 shows the conformers and their described energy values.

2.3.2. Conformational analysis of 5. In the second phase, the six conformations found for the 2,3-dihydro-1,4-benzodioxepin have been used as starting conformations,

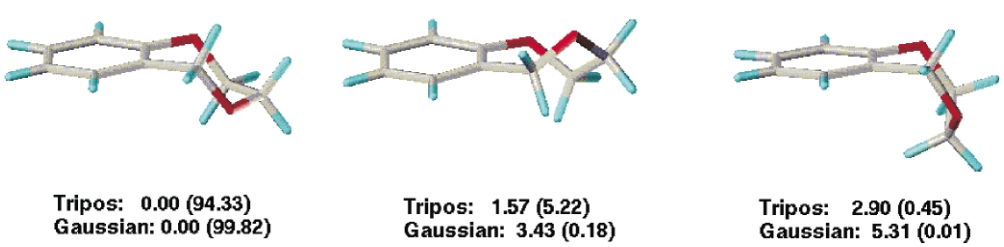


Figure 3. The only three conformations of 1,4-benzodioxepin, together with their relative energy values (kcal/mol) calculated by means of molecular mechanics (Tripos) and ab initio (Gaussian). The calculated conformational populations at 25°C are shown in parentheses. The three remaining conformations are the inverted ones of these.

substituting the 3-β hydrogen atom for a 5-fluorouracil-1-yl fragment. Each of the new molecules were minimized as described previously and, once optimized, a conformational search by means of the gridsearch Sybyl utility has been carried out, rotating the exocyclic C–N bond at 10° intervals. Subsequently, the restrictions of the different geometries obtained in each conformational search were released and a new minimization of all the conformations was carried out. After such a minimization, the conformations obtained in all the conformational searches were compared with each other to identify those which are different from a geometric and energetic point of view.

Twenty conformations for the compound **5** were obtained, relative energies being lower than 10 kcal/mol in all cases. The 10 most stable conformations have been optimized by means of ab initio calculations at level 6-31G*, reducing them to eight energetically and geometrically distinct conformations. Figure 4 shows the four most stable conformations found for this compound.

The most stable conformation is a chair for the 1,4-benzodioxepin fragment with the 5-FU group in an equatorial orientation, while the second one is a twist-boat with 5-FU in an isoclinal orientation.[19] The third one in importance is a boat with the substituent equatorial and, finally, the fourth one is a chair with the substituent in an axial orientation.

If we pay attention to the calculated conformational populations, the first of the above-mentioned is the only significant conformation because it represents more than 93% of the equilibrium of such a compound. Actually, X-ray crystallography shows a conformation in the solid phase that agrees perfectly with the one calculated as the most stable in vacuum.

The ^{1}H NMR studies in solution show the existence of NOE effects between protons 3α and 5α on one hand, and between 2β and 6′ on the other (Fig. 5). Both NOE effects are compatible with the calculated distances for the most stable conformation of this compound: 2.29 and 2.87 Å, respectively. If the twist-boat conformation were more important than the calculated one, a NOE effect would have been observed between the 2α and 5α hydrogen atoms, because they are located at a 2.37 Å distance; nevertheless, such a NOE effect is not experimentally observable, which confirms that the chair is the most important conformation, practically the only one for compound **5**.

2.4. Antiproliferative activities and apoptosis induction

The IC_{50} values[20] of compounds are shown in Table 1. The most active compounds are **5** and **6**. Compound **4** shows less antiproliferative activity (IC_{50}=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_{50}=14±1.02 μM). On comparing structures **8** and **5**, it is worth emphasizing that the bioisosteric change of carbon for oxygen and the saturation of the double bond in compound **5** increases the antiproliferative activity twice (IC_{50}=7±0.61 μM). The introduction of a methoxy group into the benzene ring of **5** provokes different influences on the antiproliferative activities. Thus, the C-7 substitution produces an increase of the antiproliferative activity (**6**, IC_{50}=4.5±0.33 μM), whilst if C-9 is the substituted position it gives rise to a

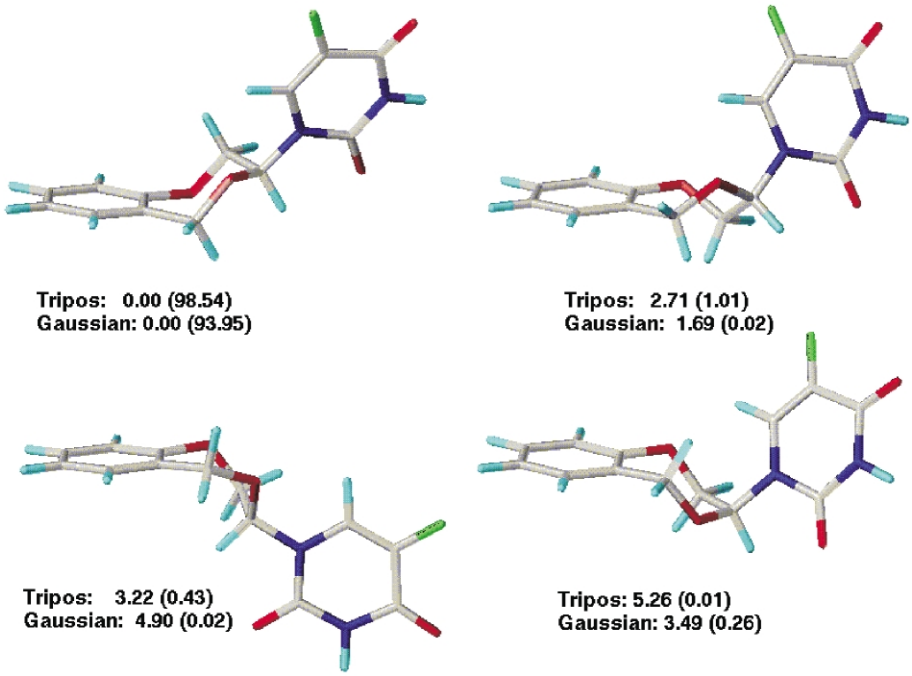


Figure 4. Representation of the four most stable conformations of compound **5**. The relative energy values calculated by means of molecular mechanics (Tripos) and ab initio (Gaussian) are expressed in kcal/mol. The four remaining conformations are the inverted ones of these, and the calculated conformational populations at 25°C are shown in parentheses considering the eight more stable conformations of this compound.

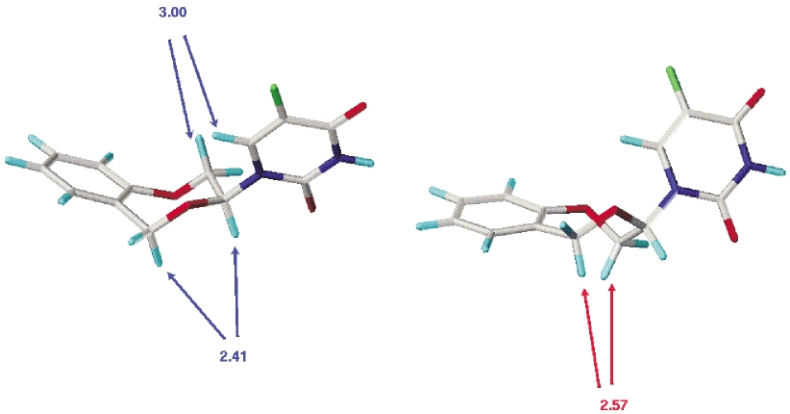


Figure 5. Calculated distances in Å in the chair and twist-chair conformations of **5**. The distances of 2.41 and 3.00 Å are compatible with the experimentally observed NOE effects. The 2.57 Å distance of the twist-boat conformation is compatible with a NOE effect that is not experimentally observed, and it may thus be concluded that the chair conformation is the only existing one in solution.

decrease in the antiproliferative activity (**7**, IC_{50}=22±0.93 μM).

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 needs 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.[21,22] Quantitation of apoptotic cells was done by monitoring the binding of fluorescein isothiocyanate (FITC)-labelled annexin V (a phosphatidyl-serine-binding protein) to cells in response to our title compounds as described.[23] The apoptosis study shows that compounds **4**, **7**, **8** and **9**, at the IC_{50} concentration, provoke early apoptosis in the cells treated for 24 and 48 h. It is worth pointing out that compound **7** induces greater apoptosis at 48 h (46.73%) than at 24 h (40.08%) and so does compound **4** [48 h (53.92%) and 24 h (46.63%)]. The compounds that show the most important apoptotic indexes at 24 h are **8** (57.33%) and **9** (54.33%), whereas at 48 h are **4** (53.92%) and **8** (51.37%). These compounds are more potent as apoptosis inductors against the MCF-7 human breast cancer cells than paclitaxel (Taxol®), which induced programmed cell death up to 43% of cell population.[24] Accordingly, the early apoptotic inductions and the low IC_{50} values give rise to a significant antitumoral activity. Our recent findings suggest that compounds **5**–**9** provoke a G_0/G_1-phase cell cycle arrest, whereas Ftorafur [1-(2-tetrahydrofuranyl)-5-fluorouracil], a known prodrug† of 5-FU, induces a S-phase cell cycle arrest and, accordingly, the title compounds are drugs per se. Also compounds **5**–**9** administered intravenously twice a week (with a 50 mg/kg dose each time) induce neither toxicity nor death in mice after a one-month treatment (results not shown).

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

Comp.	IC_{50} (μM)[a]	% Apoptosis[b]	
		24 h	48 h
Control		1.24	1.24
4	23±0.88	46.63	53.92
5	7±0.61	8.45	12.17
6	4.5±0.33	1.50	3.50
7	22±0.93	40.08	46.73
8	14±1.02	57.33	51.37
9	69±2.31	54.33	35.49

[a] See Ref. 20. The data are means±SEM of three independent determinations.

[b] Apoptosis was determined using an annexin V-based assay (Ref. 23). The data indicate the percentage of cells undergoing apoptosis in each sample. All experiments were conducted in duplicate and gave similar results.

3. Conclusion

We have completed the synthesis and biological evaluation of novel benzannelated medium oxacyclic 5-FU *O*,*N*-acetals. The synthesized compounds exhibited good anti-proliferative activities against the breast cancer cell line MCF-7, among which derivative **6** was found to be the most cytotoxic. Our investigation suggest that **8** may be particularly useful in stimulating the apoptotic process in breast cancers. At present, studies are being carried out to determine the mechanism of action at the molecular level of the title compounds.

4. Experimental

4.1. Chemistry

Melting points (mp) were taken in open capillaries on an Electrothermal melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a 400.1 MHz ^1H and 100.03 MHz ^{13}C NMR Bruker ARX 400 or 300.13 MHz ^1H and 75.78 MHz ^{13}C NMR Bruker AMX-300 spectrometers, and chemical shifts (ppm) are reported relative to the solvent peak ($CHCl_3$ in $CDCl_3$ at δ 7.24 and 77.1 ppm; DMSO in DMSO-d_6 at δ 2.50 and 39.5 ppm). Signals are designated as follows: s, singlet; bs,

† Prodrugs have been described as the chemical modification of a biologically active compound to form a new structure, which will liberate the parent drug upon either in vivo enzymatic or non-enzymatic attack.

broad singlet; d, doublet; dd, doublet of doublet; ddd, double doublet of doublet; dddd, double double doublet of doublet; t, triplet; q, quadruplet; m, multiplet. Coupling constants (*J*) are expressed in hertz. The NMR, MS, and IR spectral data of all compounds were consistent with the assigned structures. All final products had satisfactory (within ±0.4%) C, H, and N analyses. Liquid secondary ion mass spectra (LSIMS), electron impact (EI) and high-resolution mass spectra (HR) were carried out on a VG AutoSpec Q high-resolution mass spectrometer (Fisons Instruments). Analytical thin-layer chromatography (TLC) was performed using Merck Kieselgel 60F-254 plates as the solvent system. Chromatograms were visualized under a UV lamp (254 nm), by placing the air-dried plates in a tank of I_2 vapour, or charring with a 20% sulfuric acid/methanol solution. Preparative separations were performed by flash chromatography on silica gel (Merck; 230–400 mesh) using mixtures of diethyl ether/hexane or CH_2Cl_2/MeOH as eluents. Acetonitrile was dried by refluxing and distilling from calcium hydride and anhydrous methanol was prepared by the magnesium drying procedure. Dimethylformamide was distilled from phosphorus pentoxide and stored over molecular sieves (4 Å). All reactions were carried out in dry glassware and under dry argon. Evaporations were carried out in vacuo with a rotary evaporator.

4.1.1. Starting materials

4.1.1.1. Synthesis of 1-(hydroxymethyl)-2-phenoxyacetaldehyde dimethyl acetals 11–13. The preparation of **11** was previously reported[9] and we proceeded with the same method to obtain **12** and **13** but changing the solvent of the reaction DMSO by DMF, starting from 2-hydroxy-5-methoxybenzaldehyde[8] and the commercially available 2-hydroxy-3-methoxybenzaldehyde, respectively. The purification of the liquid acyclic acetals **12** and **13** was performed by flash chromatography using a diethyl ether/hexane (1/2) mixture as eluant.

1-(*Hydroxymethyl*)-5-*methoxy*-2-*phenoxyacetaldehyde dimethyl acetal* **12**. Yield: 72%. R_f (4/1, diethyl ether/hexane): 0.4. R_f (3/1, diethyl ether/hexane): 0.2. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.82 (d, 1H, J=2.9 Hz, H-6), 6.78 (d, 1H, J=8.8 Hz, H-3), 6.72 (dd, 1H, J=2.9, 8.8 Hz, H-4), 4.66 (t, 1H, J=5.2 Hz, H-1′), 4.60 (s, 2H, CH_2OH), 3.98 (d, 2H, J=5.2 Hz, H-2′), 3.72 (s, 3H, 5-O*Me*), 3.40 (s, 6H, (O*Me*)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.13 (C-5), 150.62 (C-2), 131.34 (C-1), 114.78 (C-4), 113.55 (C-3), 113.19 (C-6), 101.80 (C-1′), 66.42 (C-2′), 61.83 (CH_2OH), 55.64 (5-O*Me*), 53.94 (O*Me*)$_2$). HR LSIMS calcd for $C_{12}H_{18}O_5Na$ (M + Na)$^+$ 265.1051, found 265.1050. Anal. for $C_{12}H_{18}O_5$: calcd: C 59.49; H 7.49. Found: C 59.35;H 7.22.

1-(*Hydroxymethyl*)-3-*methoxy*-2-*phenoxyacetaldehyde dimethyl acetal* **13**. Yield: 64%. R_f (4/1, diethyl ether/hexane): 0.4. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.99 (t, 1H, J=7.9 Hz, H-5), 6.87 (d, 1H, J=2.3 Hz, H-6 or H-4), 6.84 (dd, 1H, J=2.3 Hz, 4.0, H-4 or H-6), 4.69 (t, 1H, J=5.1 Hz, H-1′), 4.60 (d, 2H, J=7 Hz, CH_2OH), 4.14 (d, 2H, J=5.1 Hz, H-2′), 3.83 (s, 3H, 3-O*Me*), 3.42 (s, 6H, OMe_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.05 (C-2), 146.10 (C-3), 134.77 (C-1), 123.98 (C-5), 121.52 (C-6), 112.28 (C-4), 102.51 (C-1′), 71.53 (C-2′), 61.50 (CH_2OH), 55.75 (3-O*Me*), 54.00 (O*Me*)$_2$). HR LSIMS calcd for $C_{12}H_{18}O_5Na$ (M+Na)$^+$ 265.1051, found: 265.1052. Anal. for $C_{12}H_{18}O_5$: calcd: C 59.49; H 7.49. Found: C 59.53; H 7.45.

4.1.1.2. Synthesis of (*RS*)-3-methoxy-2,3-dihydro-5*H*-1,4-benzodioxepins 14–16. The preparation of **14** was previously reported[9] but we used the Lewis acid $BF_3{\cdot}Et_2O$ in anhydrous diethyl ether, formerly established by us,[6] and we proceeded with the same method to obtain **15** and **16** (as liquids), starting from the acyclic acetals **11**–**13**, respectively. In the case of **14**, we found that the distillation of **11** in vacuo on a Kugelrohr apparatus (150°C/5 mm Hg)‡ produced the required cyclization to yield the final cycloacetal **14** in an one-pot process (40%).§

(*RS*)-3-*Methoxy*-2,3-*dihydro*-5*H*-1,4-*benzodioxepin* **14**. Yield: 40%.

(*RS*)-3,7-*Dimethoxy*-2,3-*dihydro*-5*H*-1,4-*benzodioxepin* **15**. Yield: 45%. R_f (2/1, diethyl ether/hexane): 0.37. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.91 (d, 1H, J=8.7 Hz, H-9), 6.70 (dd, 1H, J=3.0, 8.7 Hz, H-8), 6.63 (d, 1H, J=3.0 Hz, H-6), 5.12 (d, 1H, J_{gem}=14.1 Hz, H-5β), 4.81 (dd, 1H, J=3.3, 5.6 Hz, H-3α), 4.36 (d, 1H, J_{gem}=14.1 Hz, H-5α), 4.01 (m, 2H, H-2α, H-2β), 3.74 (s, 3H, 7-O*Me*), 3.49 (s, 3H, 3-O*Me*). ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.10 (C-7), 152.91 (C-10), 131.15 (C-11), 120.86 (C-8), 113.82 (C-9), 113.62 (C-6), 101.54 (C-3), 73.00 (C-2), 63.23 (C-5), 55.71 (7-O*Me*), 55.63 (3-O*Me*). HR LSIMS calcd for $C_{11}H_{14}O_4Na$ (M+Na)$^+$ 233.0789, found: 233.0788. Anal. for $C_{11}H_{14}O_4$: calcd: C 62.85; H 6.71. Found: C 62.48; H 6.89.

(*RS*)-3,9-*Dimethoxy*-2,3-*dihydro*-5*H*-1,4-*benzodioxepin* **16**. Yield: 80%. R_f (2/1, diethyl ether/hexane): 0.3 ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.88 (pt, 1H, J=7.6 Hz, H-7), 6.79 (dd, 1H, J=1.6 Hz, 7.6, H-6 Or H-8), 6.65 (dd, 1H, J=1.6, 7.5 Hz, H-8 Or H-6), 5.22 (D, 1H, J=14.4 Hz; H-5β), 4.87 (dd, 1H, J=3.3, 7.0 Hz; H-3α), 4.36 (D, 1H, J=14.4 Hz, H-5α), 4.20 (dd, 1H, J=3.3 Hz, 13.0, H-2α), 4.1 (dd, 1H, J=7.0, 13.0 Hz, H-2β), 3.82 (S, 3H, 9-O*Me*), 3.46 (S, 3H, 3-O*Me*). ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.50 (C-9), 147.90 (C-10), 130.49 (C-11), 122.61 (C-7), 120.20 (C-6), 111.44 (C-8), 101.25 (C-3), 72.37 (C-2), 62.85 (C-5), 56.08 (9-O*Me*), 55.48 (3-O*Me*). HR LSIMS calcd for $C_{11}H_{14}O_4Na$ (M+Na)$^+$ 233.0789, found 233.0789. Anal. for $C_{11}H_{14}O_4$: calcd: C 62.85; H 6.71. Found: C, 62.88; H, 6.68.

4.1.1.3. (*Z*)-4-(2-Hydroxyphenyl)-3-butenal dimethyl acetal 18. Potassium *t*-butoxide (39.2 g, 0.35 mol) was added in three portions to a mechanically stirred mixture of crude phosphonium chloride **17**[10] (from 0.3 mol of acrolein) in THF (400 mL) at 0°C. The orange mixture was stirred at room temperature during 2 h and cooled in ice. 2-Hydroxybenzaldehyde (16.5 mL, 0.31 mol) was added in 2 min and the reaction mixture was stirred for 16 h at room temperature. Extractive workup gave a syrup from which most of the Ph_3PO was removed by crystallization from diethyl ether. The filtrate was concentrated and purified by flash chromatography using a diethyl ether/hexane (1/9) mixture as eluant, giving (*Z*)-**18** (9.36 g, 15%) as a

‡ According to Ref. 9 the distillation under a better vacuum, and therefore a lower temperature of distillation (110°C/1 mm Hg), yielded the acyclic acetal (62% yield).

§ The combined yield of **14** is ≈35% for the two-step process (see Ref. 9).

colourless oil. R_f (2/1, diethyl ether/hexane): 0.66. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15 (dt, 1H, J=1.7, 7.6 Hz, H_{Ar}), 7.04 (dd, 1H, J=1.7, 7.5 Hz, H_{Ar}), 6.87 (m, 2H, H_{Ar}), 6.47 (d, 1H, J=11.3 Hz, H-4′), 6.11 (s, 1H, O*H*), 5.87 (dt, 1H, J=7.6, 11.3 Hz, H-3′), 4.47 (t, 1H, J=5.3 Hz, H-1′), 3.35 (s, 6H, O*Me*), 2.42 (ddd, 2H, J=1.6, 5.3, 7.6 Hz, H-2′). ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.77 (C-1), 129.91 (C-4′), 129.33 (C-5), 128.76 (C-3), 126.71 (C-3′), 123.33 (C-2), 120.18 (C-4), 116.22 (C-6), 103.83 (C-1′), 53.91 (O*Me*), 32.93 (C-2′). HR LSIMS calcd for $C_{12}H_{16}O_3Na$ (M+Na)$^+$ 231.0997, found 231.0996. Anal. for $C_{12}H_{16}O_3$: calcd: C, 69.21; H, 7.74. Found: C, 68.99; H, 7.82.

4.1.1.4. (*RS*)-2-Methoxy-2,3-dihydrobenzoxepin 19. Compound **18** (2.3 g, 7.5 mmol) was dissolved in dry diethyl ether (30 mL), and a few drops of $BF_3{\cdot}OEt_2$ were added. The solution was kept at room temperature for 7 days, washed with an aqueous solution of K_2CO_3 (10%), and the organic layer was dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography with diethyl ether/hexane (1/9), yielding 1.32 g (34%) of **19** as a colourless oil. R_f (2/1, diethyl ether/hexane): 0.7. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.1 (m, 4H, H_{Ar}), 6.32 (d, 1H, J=11.9 Hz, H-5), 5.74 (ddd, 1H, J=3.7, 5.5, 11.9 Hz, H-4), 4.94 (dd, 1H, J=2.3, 7.4 Hz, H-2a), 3.55 (s, 3H, O*Me*), 2.80 (ddd, 1H, 13.6, H-3a), 2.72 (dddd, 1H, J=1.8, 5.5, 7.4, 13.6 Hz, H-3b). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.67 (C-10), 131.86 (C-5), 128.45 (C-8), 128.24 (C-6), 128.33 (C-11), 128.24 (C-6), 125.21 (C-4), 123.33 (C-7), 120.52 (C-9), 104.6 (C-2), 56.23 (O*Me*), 38.65 (C-3). HR LSIMS calcd for $C_{11}H_{12}O_2Na$ (M + Na)$^+$ 199.0837, found 199.0837. Anal. for $C_{11}H_{12}O_2$: calcd: C, 74.98; H, 6.86. Found: C, 75.23; H 7.12.

4.1.1.5. 2-(2-Hydroxyphenoxy)ethyl-1,3-dioxolane 22. 2-(2-Bromoethyl)-1,3-dioxolane (16.44 g, 90.8 mmol) was added in small portions to a suspension of catechol (10 g, 90.8 mmol) and K_2CO_3 (7.53 g, 54.5 mmol) in anhydrous acetone (250 mL) after stirring at room temperature for 30 min under argon. The resulting suspension was refluxed during 6 h, then brought back to room temperature, filtered and diethyl ether (220 mL) was added and the resulting solution washed with a NH_4Cl saturated solution. The organic phase was dried ($MgSO_4$), filtered and evaporated under reduced pressure to yield a colourless oil which, upon flash chromatography using diethyl ether/hexane (1/6) as eluant, gave pure **22** (3.90 g, 21%). R_f (4/1, diethyl ether/hexane): 0.57. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.84 (m, 4H, H_{Ar}), 5.06 (t, 1H, J=4.5 Hz, H-3′), 4.20 (t, 2H, 5.8, H-1′), 4.00 (m, 2H, (OCH_2)$_2$); 3.90 (m, 2H, (OCH_2)$_2$), 2.15 (q, 2H, J=5.8 Hz, H-2′). ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.21 (C-1), 146.10 (C-2), 122.70 (C-5), 120.07 (C-4), 115.12 (C-6), 114.62 (C-3), 103.07 (C-1′), 66.36 (C-3′), 65.04 (OCH_2)$_2$), 33.49 (C-2′). HR (LSIMS) calcd for $C_{11}H_{14}O_4$Na (M+Na)$^+$ 233.0789, found: 233.0789. Anal. for $C_{11}H_{14}O_4$: calcd.: C, 62.85; H, 6.71. Found: C, 62.88; H, 6.60.

4.1.1.6. 2-(2-Hydroxyphenoxy)acetaldehyde dimethyl acetal 21. The following two methods have been carried out.

Method A. Following the procedure described for the obtention of **22**, but changing 2-(2-bromoethyl)-1,3-dioxolane by 3-bromopropanal dimethyl acetal, gave pure **21** (1.2 g, 23%).

Method B. Anhydrous MeOH (27 mL) containing H_2SO_4 (0.13 mL) was added to **21** (3.84 g, 18.5 mmol) to give a final 1% concentration of the acid. The solution was kept at room temperature for 72 h, neutralized with a methanolic solution of KOH, filtered and concentrated to dryness. After adding $CHCl_3$ (35 mL) and washing with H_2O (2 Å 25 mL), the organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo, and the residue was purified by flash chromatography using a diethyl ether/hexane (1/6) mixture, yielding **21** (4.19 g, 60%) as a colourless liquid. R_f (2/1, diethyl ether/hexane): 0.5. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.87 (m, 4H, H_{Ar}), 6.37 (bs, 1H, O*H*), 4.65 (t, 2H, J=5.5 Hz, H-1′), 4.10 (t, 2H, J=6 Hz, H-3′), 3.38 (s, 6H, (O*Me*)$_2$), 2.10 (q, 2H, J=5.8 Hz, H-2′). ^{13}C NMR (75 MHz, $CDCl_3$) δ 146.64 (C-1), 145.92 (C-2), 122.35 (C-5), 120.10 (C-4), 115.07 (C-6), 113.87 (C-3), 102.47 (C-1′), 66.15 (C-3′), 53.23 (OMe_2), 32.37 (C-2′). HR LSIMS calcd for $C_{11}H_{16}O_4Na$ (M+Na)$^+$ 235.0946, found 235.0945. Anal. for $C_{11}H_{16}O_4$: calcd: C, 62.25; H, 7.60. Found: C, 62.28; H, 7.65.

4.1.1.7. (*RS*)-4-Methoxychroman-8-ol 20. The procedure was the same applied for the preparation of **19**, but using **21** (4.19 g, 19.7 mmol), anhydrous diethyl ether (85 mL) containing $BF_3{\cdot}Et_2O$ (1.42 mL). The residue was purified by flash chromatography with diethyl ether/hexane (1/6), yielding 1.81 g (51%) of **20** as a colourless liquid. R_f (2/1, diethyl ether/hexane): 0.49. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.85 (q, 2H, J=4.8, 9.6 Hz, H_{Ar}), 6.79 (d, 1H, J=4.8 Hz, H_{Ar}), 5.06 (m, 1H, H_{Ar}), 5.56 (bs, 1H, O*H*), 4.33 (dd, 1H, J=3.3, 6.7 Hz, H-4a), 4.31 (m, 2H, J=3.5, 6.6, 9.1 Hz, H-2a, H-2b), 3.42 (s, 3H, O*Me*), 2.16 (dddd, 1H, J=3.3, 6.6, 14.2 Hz, H-3a), 2.04 (dddd, 1H, J=3.5, 6.7, 9.1, 14.2 Hz, H-3b). ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.92 (C-9), 144.39 (C-8), 120.42 (C-10), 119.95 (C-6), 119.95 (C-5), 114.48 (C-7), 71.23 (C-4), 62.57 (C-2), 55.95 (O*Me*), 27.43 (C-3). HR LSIMS calcd for $C_{10}H_{12}O_3Na$ (M + Na)$^+$ 203.0694. found 203.0694. Anal. for $C_{10}H_{12}O_3$: calcd: C, 66.60; H, 6.71. Found: C, 66.30; H, 6.68.

4.1.2. Final products

4.1.2.1. Reaction between the benzoannelated seven- or six-membered compounds 14–16, 19–20 and 5-fluorouracil. General procedure. A 1.0 M solution of $SnCl_4/CH_2Cl_2$ (1.25 mmol) was added dropwise with stirring under argon at rt to a suspension of **14**–**16**, **19**–**20** (1 mmol), 5-fluorouracil (1 mmol), which contains trimethylchlorosilane (TCS, 0.8 mmol) and 1,1,1,3,3,3-hexamethyldisilazane (HMDS, 0.8 mmol) in dry acetonitrile (10 mL/mmol of **14**–**16**, **19**–**20**). After 24–144 h of stirring, the reaction was quenched by the addition of a concentrated aqueous solution of Na_2CO_3. The solvent was removed with a rotary evaporator. The sticky residue was dissolved in MeOH, and silica gel was added. The solvent was removed with a rotary evaporator, and the residue was applied to the top of a flash chromatography column packed with CH_2Cl_2/MeOH (100/1). Elution with mixtures of CH_2Cl_2/MeOH by gradient elution (100/1→100/5) and concentration with a rotary evaporator gave the target molecules **5**–**9**.

(*RS*)-1-(2,3-*Dihydro*-5*H*-1,4-*benzodioxepin*-3-*yl*)-5-*fluorouracil* **5**. Reaction time: 24 h. Yield: 43%. White solid, mp 196–198°C (decomp.). R_f (9.5/0.5, CH_2Cl_2/MeOH): 0.61; R_f (9.9/0.1, CH_2Cl_2/MeOH): 0.32. ^{1}H NMR (400 MHz,

DMSO-d_6) δ 11.96 (s, 1H, NH), 7.95 (d, 1H, J_{H-F}=7 Hz, $H_{5\text{-FU}}$), 7.27 (m, 2H, AA′ part of an AA′BB′ system, H-6 and H-7 or H-8 and H-9), 7.06 (m, 2H, BB′ part of an AA′BB′ system, H-8 and H-9 or H-6 and H-7), 5.91 (dd, 1H, J=1.6, 7.2 Hz, H-3α), 4.92 (d, 1H, J_{gem}=14.2 Hz, H-5α), 4.87 (d, 1H, J_{gem}=14.2 Hz, H-5β), 4.46 (dd, 1H, J=2.2, 12.9 Hz, H-2α), 4.13 (dd, 1H, J=7.4, 12.9 Hz, H-2β). ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.26 (C-10), 156.96 (d, J=25.81 Hz, C-$4_{5\text{-FU}}$), 148.71 (s, C-$2_{5\text{-FU}}$), 139.96 (d, J=229.57 Hz, C-$5_{5\text{-FU}}$), 130.47 (C-11), 129.41 and 129.23 (C-6 and C-8), 125.78 (d, J=34.21 Hz, C-$6_{5\text{-FU}}$), 123.45 (C-9), 120.19 (C-7), 83.99 (C-3), 72.56 (C-2), 68.65 (C-5). HR LSIMS calcd for $C_{13}H_{11}N_2O_4FNa$ $(M+Na)^+$ 301.0600, found 301.0600. Anal. for $C_{13}H_{11}N_2O_4F$: calcd: C, 56.33; H, 3.79; N, 10.13. Found: C, 56.35; H, 3.87; N, 10.01.

(*RS*)-1-(7-*Methoxy*-2,3-*dihydro*-5*H*-1,4-*benzodioxepin*-3-yl)-5-*fluorouracil* **6**. Reaction time: 144 h. Yield: 40%. White solid, mp 220–222°C. R_f (9/1, CH_2Cl_2/MeOH): 0.49. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.16 (s, 1H, NH), 7.86 (d, 1H, J_{H-F}=5.4 Hz, $H_{5\text{-FU}}$), 6.97 (d, 1H, J=8.7 Hz, H-9), 6.88 (d, 1H, J=3.1 Hz, H-6), 6.80 (dd, 1H, J=3.1, 8.7 Hz, H-8), 6.05 (d, 1H, J=7.9 Hz, H-3α), 4.81 (d, 1H, J=13.7 Hz, H-5α), 4.65 (d, 1H, J=13.7 Hz, H-5β), 4.59 (dd, 1H, J=3.4, 12.3 Hz, H-2β), 4.35 (dd, 1H, J=1.5, 12.3 Hz, H-2α), 3.7 (s, 3H, 7-O*Me*). ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.06 (d, J=25.4 Hz, C-$4_{5\text{-FU}}$), 154.96 (s, C-$2_{5\text{-FU}}$), 152.71 (C-7), 149.32 (C-10), 139.15 (d, J=224.5 Hz, C-$5_{5\text{-FU}}$), 132.41 (C-11), 125.88 (d, J=31.5 Hz, C-$6_{5\text{-FU}}$), 121.15 (C-8), 114.48 (C-9), 114.14 (C-6), 84.88 (C-3), 73.49 (C-2), 70.28 (C-5), 55.41 (7-O*Me*). HR LSIMS calcd for $C_{14}H_{13}N_2O_5FNa$ $(M+Na)^+$ 331.0706, found 331.0706. Anal. for $C_{14}H_{13}N_2O_5F$: C, 54.55; H, 4.25; N, 9.09. Found: C, 54.87; H, 4.33; N, 8.79.

(*RS*)-1-(9-*Methoxy*-2,3-*dihydro*-5*H*-1,4-*benzodioxepin*-3-yl)-5-*fluorouracil* **7**. Reaction time: 24 h. Yield: 16%. White solid, mp 130–132°C. R_f (9/1, CH_2Cl_2/MeOH): 0.5. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.25 (s, 1H, NH), 7.59 (d, 1H, J_{H-F}=6 Hz, $H_{5\text{-FU}}$), 7.04 (d, 1H, J=8.0 Hz, H-5), 6.92 (dd, 1H, J=1.4, 8.0 Hz, H-6), 6.76 (dd, 1H, J=1.4, 7.6 Hz, H-4), 6.12 (d, 1H, J=5.9 Hz, H-3α), 5.07 (d, 1H, J=14.2 Hz, H-5α), 4.79 (d, 1H, J=14.2 Hz, H-5β), 4.59 (dd, 1H, J=2.3, 13 Hz, H-2α), 3.97 (dd, 1H, J=5.9, 13 Hz, H-2β), 3.88 (s, 3H, 9-O*Me*). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.82 (d, J=26.5 Hz, C-$4_{5\text{-FU}}$), 151.04 (s, C-$2_{5\text{-FU}}$), 148.72 (C-8), 147.39 (C-10), 140.61 (d, J=236.7 Hz, C-$5_{5\text{-FU}}$), 130.72 (C-11), 125.03 (d, J=34.2 Hz, C-$6_{5\text{-FU}}$), 124.08 (C-7), 120.42 (C-6), 112.59 (C-8), 84.20 (C-3), 73.32 (C-2), 69.43 (C-5), 56.20 (9-O*Me*). HR (LSIMS) calcd for $C_{14}H_{13}N_2O_5FNa$ $(M+Na)^+$ 331.0706, found: 331.0706. Anal. for $C_{14}H_{13}N_2O_5F$: calcd: C, 54.55; H, 4.25; N, 9.09. Found: C, 54.88; H, 4.37; N, 9.45.

(*RS*)-1-(2,3-*Dihydrobenzoxepin*-2-yl)-5-*fluorouracil* **8**. Reaction time: 24 h. Yield: 10%. White solid, mp 215–217°C. R_f (9/0.5, CH_2Cl_2/MeOH): 0.60. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.81 (d, 1H, J=5.2 Hz, NH), 7.20 (d, 1H, J=5.6 Hz, $H_{5\text{-FU}}$), 7.11 (m, 1H, H-8 or H-7), 7.06 (m, 1H, H-7 or H-8), 6.96 (d, 1H, J=7.4 Hz, H-9 or H-6), 6.5 (d, 1H, J=9.9 Hz, H-6 or H-9), 6.4 (d, 1H, J=11.9 Hz, H-5), 6.3 (ddd, 1H, J=11.9, 0 Hz, H-4), 5.88 (ddd, 1H, J=2.7, 6.4, 9.1 Hz, H-2a), 4.14 (m, 1H, H-3b), 2.61 (dd, 1H, J=6.4 Hz, 18.1 Hz, H-3a). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.88 (d, J=24.7 Hz, C-$4_{5\text{-FU}}$), 155.47 (C-$2_{5\text{-FU}}$), 150.86 (C-10), 140.41 (d, J=233.9 Hz, C-$5_{5\text{-FU}}$), 132.47 (C-5), 132.47 (d, J=29 Hz, C-$6_{5\text{-FU}}$), 126.92 (C-11), 126.63 (C-8), 123.89 (C-6), 123.64 (C-4), 123.32 (C-7), 120.26 (C-9), 83.68 (C-2), 35.85 (C-3). HR (EI) calcd for $C_{14}H_{11}N_2O_3FNa$ 274.0753, found 274.0753. Anal. for $C_{14}H_{11}N_2O_3F$: calcd: C, 61.31; H, 4.04; N, 6.93. Found: C, 61.68; H, 5.32; N, 7.24.

(*RS*)-1-(8-*Hydroxychroman*-4-*yl*)-5-*fluorouracil* **9**. Reaction time: 24 h. Yield: 44%. White solid, mp 138–140°C. R_f (9/1, $CHCl_3$/MeOH): 0.62. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.08 (s, NH), 6.96 (d, 1H, J_{H-F}=6.1 Hz, $H_{5\text{-FU}}$), 5.08 (s, 1H, O*H*), 5.7 (t, 1H, J=5.6 Hz, H-4a), 4.28 (ddd, 1H, J=3.3, 7.2, 11.4 Hz, H-2a), 4.16 (ddd, 1H, J=3.2, 8.0, 11.4 Hz, H-2b), 2.29 (dddd, 1H, J=3.3, 5.6, 8.0, 11.3 Hz, H-3a), 2.37 (dddd, 1H, J=3.2, 7.2, 11.3 Hz, H-3b). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.10 (d, J=25.7 Hz, C-$4_{5\text{-FU}}$), 149.89 (s, C-$2_{5\text{-FU}}$), 145.92 (C-11), 144.39 (C-10), 139.81 (d, J=229.1 Hz, C-$5_{5\text{-FU}}$), 127.09 (d, J=33.4 Hz, C-$6_{5\text{-FU}}$), 120.43 (C-6), 120.33 (C-8), 117.80 (C-7), 115.10 (C-9), 63.65 (C-4), 50.02 (C-2), 27.16 (C-3). HR LSIMS calcd for $C_{13}H_{11}N_2O_4FNa$ 278.0702, found: 278.0703. Anal. for $C_{13}H_{11}N_2O_4F$: calcd: C, 56.12; H, 3.98; N, 10.07. Found: C, 55.99; H, 4.12; N, 10.02.

4.1.3. X-Ray crystallographic study. Crystallographic data were collected at 293 K using graphite monochromated Mo Kα radiation (λ=0.71073 Å) on a Siemens P4 diffractometer. The structure was solved by direct methods using the program SHELXS97[25] and all non-hydrogen atoms were refined with anisotropic thermal parameters by full-matrix least squares techniques of F^2 using the program SHELXL97.[26] Hydrogen atoms were inserted in calculated positions and refined isotropically. Molecular graphics and geometric calculations were obtained from SHELXTL[27] and PLATON.[28] Relevant crystal data: formula $C_{13}H_{11}FN_2O_4$, formula weight 278.24, T=293(2) K, crystal system triclinic, space group P-1, unit cell dimensions a=6.548(2), b=7.794(2) and c=12.504(3) Å, and α=90.33(2), β=92.24(2) and γ=107.55(2)°, Z=2, D=1.520 mg m^3, μ(Mo Kα)=0.124 mm^{-1}, measured/unique reflections 4395/3535 (R(int) 0.051), refined parameters 225, refinement method=full-matrix lest-squares on F^2, final R_1 ($I>2\sigma(I)$)=0.066 and wR_2=0.176, and GOF=1.051. CCDC reference number 197927. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccd.cam.acuk].

4.1.4. Conformational analysis. The study of the possible conformations has been carried out by the Sybyl[15] program on a Silicon Graphics workstation. The different molecules have been constructed from standard fragments of the libraries of the programme. The force field Amber 4.1,[29] implemented in the Sybyl programme, has been used in the energy calculations. Once the initial geometries generated, we proceeded to their optimization using the Powell[16] method. The atomic charges were calculated by means of the AM1[30] hamiltonian implemented in the MOPAC 6.0[31] programme. A distance-dependent dielectric constant with a value of ε=1 was used and the optimization was continued

until the energy gradient was less than 0.01 kcal/mol Å^2. Conformational searches were carried out by means of molecular dynamics, using the 'simulated annealing' technique, heating the molecule up to 1000 K for 1000 ps and cooling it down later exponentially to 200 K, maintaining it for another 1000 ps. Five hundred heating–cooling cycles were carried out on each molecule and the geometries obtained at the end of each cooling period were kept. These 500 conformations were optimized under the same conditions described before and were compared with each other to remove those which were geometrically and energetically equal. Such conformations were optimized using ab initio (3-21G) calculations using the Gaussian98[18] program. After this, those conformations that were energetically and geometrically different were selected for their subsequent study.

4.2. Biological activity

The antiproliferative activities[20] and apoptosis induction[23] in the MCF-7 human breast cancer cell line were followed in accordance with the protocols previously reported. The results are recorded in Table 1.

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References

1. Harris, J. R.; Lippman, M. E.; Morrow, M.; Hellman, S. *Diseases of the Breast*; Lippincott-Raven: New York, 1996; p 159.
2. Hickman, J. A. *Cancer Metastasis Rev.* **1992**, *11*, 121–139.
3. Rasbridge, S. A.; Gillet, C. E.; Seymour, A. M.; Patel, K.; Richards, M. A.; Rubens, R. D.; Millis, R. R. R. *Brit. J. Cancer* **1994**, *70*, 335–341.
4. Campos, J.; Pineda, M. J.; Gómez, J. A.; Entrena, A.; Trujillo, M.Á.; Gallo, M.Á.; Espinosa, A. *Tetrahedron* **1996**, *52*, 8907–8924.
5. Gómez, J. A.; Trujillo, M.Á.; Campos, J.; Gallo, M.Á.; Espinosa, A. *Tetrahedron* **1998**, *54*, 13295–13312.
6. Trujillo, M.Á.; Gómez, J. A.; Campos, J.; Espinosa, A.; Gallo, M.Á. *Tetrahedron* **2001**, *57*, 3951–3961.
7. Campos, J.; Gómez, J. A.; Trujillo, M.Á.; Gallo, M.Á.; Espinosa, A. *Il Farmaco* **1997**, *52*, 263–269.
8. Hammond, M. L.; Zambias, R. A.; Chang, M. N.; Jensen, N. P.; McDonald, J.; Thompson, K.; Boulton, D. A.; Kopka, I. E.; Hand, K. M.; Opas, E. E.; Luell, S.; Bach, T.; Davies, P.; MacIntyre, D. E.; Bonney, R. J.; Humes, J. L. *J. Med. Chem.* **1990**, *33*, 908–918.
9. Lachapelle, A.; St-Jacques, M. *Tetrahedron* **1988**, *44*, 5033–5044.
10. Wanner, M. J.; Koomen, G.-J. *Eur. J. Org. Chem.* **1998**, 889–895.
11. Schlosser, M. *Top. Stereochem.* **1970**, *5*, 1–30.
12. Pretsch, E.; Bühlmann, P.; Affolter, C. *Structure Determination of Organic Compounds*; Springer: Berlin, 2000.
13. Osaki, S.; Watanabe, Y.; Hoshiko, T.; Nagase, T.; Ogawawara, T.; Furukawa, H.; Uemura, A.; Ishikawi, K.; Mori, A.; Iigok, M.; Tokuzen, R. *Chem. Pharm. Bull.* **1986**, *34*, 150–157.
14. Clark, M.; Cramer, R. D. *J. Comp. Chem.* **1989**, *10*, 982–1012.
15. SYBYL Molecular Modeling Software, Tripos Inc. 1699 S. Hanley Road, St. Louis MO 63144-2913, www.tripos.com.
16. Powell, M. J. D. *Math. Program.* **1997**, *12*, 241–254.
17. (a) Gasteiger, J.; Marsili, M. *Tetrahedron* **1980**, *36*, 3219–3228. (b) Marsili, M.; Gasteiger, J. *Croat. Chem. Acta* **1980**, *53*, 601–614. (c) Gasteiger, J.; Marsili, M. *Org. Magn. Reson.* **1981**, *15*, 353–360.
18. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E., Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Peterson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Lomaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. Gaussian, Inc.: Pittsburgh, PA, 1998.
19. Entrena Guadix, A.; Gallo Mezo, M. Á.; Campos Rosa, J.; Gómez Vidal, J. A.; Espinosa Úbeda, A. *J. Org. Chem.* **1997**, *62*, 337–349.
20. Campos, J.; Núñez, M. C.; Rodríguez, V.; Gallo, M.Á.; Espinosa, A. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 767–770.
21. Meyn, R. E.; Stephens, L. C.; Hunter, N. R.; Milas, L. *Int. J. Cancer* **1995**, *60*, 725–729.
22. Milas, L.; Hunter, N. R.; Kurdoglu, B.; Mason, K. A.; Meyn, R. E.; Stephens, L. C.; Peters, L. J. *Cancer Chemother. Pharm.* **1995**, *35*, 297–303.
23. Chadderton, A.; Villeneuve, D. J.; Gluck, S.; Kirwan-Rhude, A. F.; Gannon, B. R.; Blais, D. E.; Parissenti, A. M. *Breast Cancer Res. Tr.* **2000**, *59*, 231–244.
24. Saunders, D. E.; Lawrence, W. D.; Christensen, C.; Wappler, N. L.; Ruan, H.; Deppe, G. *Int. J. Cancer* **1997**, *70*, 214–220.
25. Sheldrick, G. M. *SHELXS97: Program for the solution of crystal structures*; University of Göttingen: Germany, 1997.
26. Sheldrick, G. M. *SHELXL97: Program for the refinement of crystal structures*; University of Göttingen: Germany, 1997.
27. Bruker, *SHELXTL: Integrated system for the determination of crystal structures*; Bruker AXS Inc.: Madison, WI, USA, 2000.
28. Spek, A. L. *PLATON: A Multipurpose Crystallographic Tool*; Utrecht University: The Netherlands, 2002.
29. Weiner, P. K.; Kollman, P. A. *J. Comput. Chem.* **1981**, *2*, 287–303.
30. Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902–3909.
31. Stewart, J. J. P. *MOPAC 6.0* QCPE No. 455, 1990.