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Synthesis of novel 1-(2,3-dihydro-5*H*-4,1-benzoxathiepin-3-yl)-uracil and -thymine, and their corresponding *S*-oxidized derivatives

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Abstract—On the basis of molecular variations on isosteric replacements from the prototype 1-(2,3-dihydro-5*H*-1,4-benzodioxepin-3-yl)-5-fluorouracil a series of 3-(2,3-dihydro-5*H*-4,1-benzoxathiepin-3-yl)-uracil or -thymine *O*,*N*-acetals was prepared. The nature of the *cis*- and *trans*-sulfoxide isomers was established by means of their conformational analyses carried out with Sybyl and after comparing the theoretical results with the ^{1}H NMR responses of the target molecules. (*RS*)-3-(1,1-Dioxo-2,3-dihydro-5*H*-4,1-benzoxathiepin-3-yl)thymine and (1*S**, 3*S**)-1-(1-oxo-3,5-dihydro-2*H*-4,1-benzoxathiepin-3-yl)thymine were found to be inhibitors of the MCF-7 cell growth.

1. Introduction

1-(2,3-Dihydro-5*H*-1,4-benzodioxepin-3-yl)-5-fluorouracils (**1**–**3**) have proved to be good antiproliferative agents against the MCF-7 human breast cancer cell line (Fig. 1).[1] Such compounds accumulate the cancerous cells in the G_0/G_1 phase. On the other hand, **4**[2] that shows an $IC_{50}=5.4$ μM against the MCF-7 cell line, acts in a similar way to Ftorafur, a known prodrug of 5-fluorouracil (5-FU) (Fig. 1), because both gather together the cancerous cells in the synthesis phase (S). In contrast to 5-FU,[3] the benzannelated 5-FU *O*,*N*-acetals[1] and corresponding open analogues[2] have proved to be non-toxic.

With all this background it seems that compounds **1**–**3** are drugs per se, whilst **4** is a prodrug of 5-FU. Therefore, following our ongoing Anticancer Drug Programme we have planned in this paper the synthesis of compounds **5**–**10** (Fig. 2) to be tested subsequently against the human breast cancer cell line MCF-7.

On the one hand, all these compounds bear a natural pyrimidine base (uracil for compounds **5**, **7** and **9** and thymine for **6**, **8** and **10**) and on the other, the oxygen atom at position 1 of the seven-membered cycle is replaced by its isosteric sulfur atom (**5** and **6**), and its oxidized states, that is sulfoxide (**7** and **8**) and sulfones (**9** and **10**). Should these compounds show antiproliferative activities, new avenues of anticancer research would be opened based on the non-toxic natural bases uracil and thymine and, very probably, with a mechanism of action different from that of 5-FU. *O*,*N*-Acetalic benzoxathiepins are compounds not referred to in bibliography, and with this paper we will try to fill the gap.

2. Results and discussion

2.1. Synthesis

The synthesis of the targets is depicted in Scheme 1. We have previously reported the preparation of the cyclic acetal (*RS*)-3-methoxy-2,3-dihydro-5*H*-1,4-benzodioxepin.[1] We applied the same procedure for the synthesis of **12**, starting from the commercially available *o*-(hydroxymethyl)thiophenol, that is selective alkylation using bromoacetaldehyde dimethyl acetal (1 mol in the presence of 1 mol of sodium hydride in dry dimethylformamide (DMF) under conditions published by Crombie et al.[4] gave **11** (79%). Subsequent cyclization of the resulting hydroxyacetal using catalytic amounts of *p*-toluenesulfonic acid in dry toluene for 3 h

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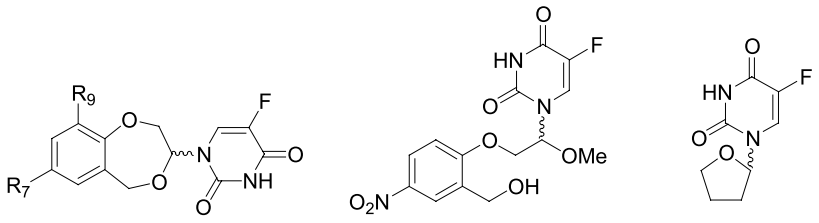


Figure 1.

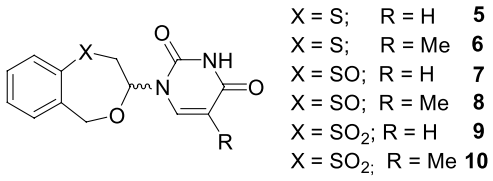


Figure 2. Novel benzo-fused seven-membered sulfur-containing *O,N*-acetals with natural pyrimidine nucleobases. In all the cases the aminalic bond between the C-3 atom of the seven-membered moiety and the nucleobase is through its *N*-1 atom.

produced the sulfur-containing acetal **12** (95%). The last step was the condensation reaction between the seven-membered acetal **12** and the natural pyrimidine bases uracil and thymine. For this procedure we used a 1.0 M solution of tin(IV) chloride in dichloromethane, trimethylchlorosilane (TCS) and 1,1,1,3,3,3-hexamethyldisilazane (HMDS) in dry acetonitrile, and the experimental conditions were optimised (unpublished results) by warming the reaction mixture up to 45 °C for 20–24 h. Under these conditions **5** and **6** were obtained with 73 and 58% yields, respectively.

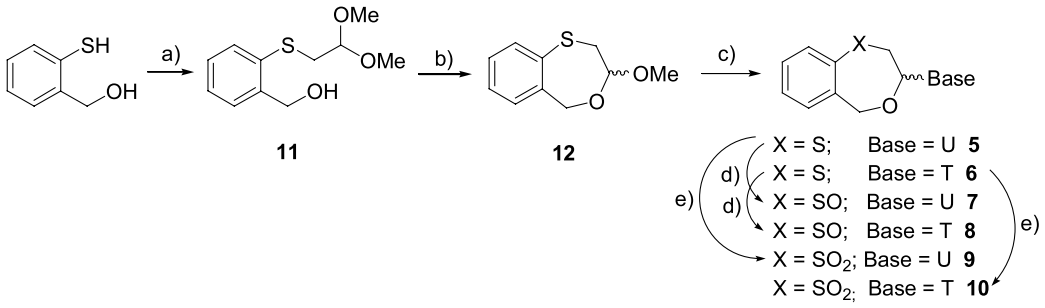
The oxidation of sulfides is one of the most important and straightforward methods for the preparation of sulfones, which are utilized for biologically active substances and substrates for the synthesis of drugs.[5] Sulfoxides **7** and **8** were obtained using the procedure used by Matteucci et al.[6] who used catalytic scandium trifluoromethansulfonate that greatly increases the efficiency of hydrogen peroxide mediated monooxidation of alkyl–aryl sulfides and methyl cysteine-containing peptides. When applied to our sulfides a mixture of *cis*-**7** and *trans*-**7** (77%) was obtained, but after using the flash 40 equipment a fast-moving spot was isolated, that will be characterized as *cis*-**7** (vide infra). When the oxidation of **6** was carried out without catalyst, *trans*-**8** was the only product produced (50%). Finally, the oxidation of sulfide **5** (and **6**) with potassium peroxymonosulfate (OXONE™) under previously reported conditions[7] yielded **9** (and **10**) with 66 (and 55%) yields.

2.2. Spectroscopic analysis of 5

The ^{1}H and ^{13}C NMR spectra were recorded using DMSO-d_6 as solvent. Herein we will mention only the NMR spectroscopic data that explain the conformational preference for **5**. The hydrogen atoms of the seven-membered framework of **5** appear between δ 5.98 ppm and δ 3.18 ppm. The chemical shift found for the aminalic hydrogen H-3 is δ 5.98 ppm with a double doublet multiplicity (dd), and the respective couplings to the vicinal hydrogen atoms (H-2) are 9.1 and 2.2 Hz. In fact, the two H-2 atoms appear as two dd's with a $J_{gem}=14.1$ Hz and the two coupling constants observed at δ 5.98 ppm (9.6 and 1.8 Hz). Finally, each of the two benzylic H-5 atoms resonates as two doublets at δ 5.02 and δ 4.96 ppm with a $J_{gem}=13.6$ Hz, showing a diastereotopic character.

The most important consequence of the ^{1}H NMR study is that it unequivocally proves through the three following data that the linkage between the seven-membered moiety and uracil is through *N*-1′:

(a) At δ 5.60 ppm one hydrogen resonates as a dd ($J=8.0$ and 2.2 Hz), the former coupling constant being typical of an aromatic hydrogen atom, and this resonance could be assigned to H-5′[8] of the uracil base. H-6′ appears as a doublet (7.69 ppm, d, $J=8.0$ Hz) downfield in relation to H-5′. The coupling pattern for H-5′ is a dd due to its coupling with H-6′ ($J=8.0$ Hz) and a small one ($J_{H5'-NH_{3'}}=$ 2.2 Hz) through a 1–3 bond relationship, and a doublet for H-6′. Nevertheless, had the aminalic bond been established with N–$H_{3'}$ the coupling pattern would have been more



Scheme 1. Synthetic route for the preparation of the target molecules. Reagents and conditions: (a) $BrCH_2CH(OMe)_2$, NaH, anhydrous DMF; (b) *p*-TsOH, anhydrous toluene, 3 h; (c) pyrimidine base, HMDS, TCS, $SnCl_4$, MeCN, 45 °C; (d) H_2O_2, $Sc(OTf)_3$, CH_2Cl_2/10% EtOH; (e) OXONE™, MeOH, H_2O.

complicated due to the simultaneous coupling of H-6′ with H-5′ and N–$H_{1'}$ (a dd for each of the three hydrogen atoms involved, that is, N–$H_{1'}$, H-5′ and H-6′).

(b) H-3 exhibits connectivity through three bonds to C-6′ (HMBC[9]).

(c) The NOEDIFF effect observed between H-6′ and H-2β justifies the conformational preference of the uracil moiety in which H-6′ is near in space to H-2β. Accordingly, the carbonyl group C-2′ is far from the referred hydrogen atom and this obviously implies the linkage through N-1′. Had the aminalic bond been established through C-3 and N-3′, no NOEDIFF effect would have been detected between some hydrogen atoms of the seven-membered moiety and those of the uracil fragment. H-3 shows a NOEDIFF effect with the more deshielded H-5 atom (δ 5.02 ppm), and with the more shielded H-2 one (δ 3.18 ppm) and these facts strongly support a chair conformation of the seven-membered moiety in which the aminalic hydrogen atom adopts an axial disposition (as H-5 at δ 5.02 ppm). Hence, the uracil moiety occupies an equatorial position (Fig. 3).

2.3. Conformational analysis of *cis*-7 and *trans*-7, and *cis*-8 and *trans*-8

Compounds **7** and **8** can both exist as geometric isomers and accordingly, the question is immediately posed as to which are the *cis*- or the *trans*- ones. In the case of **7**, during the oxidation reaction of the sulfur atom, both isomers were obtained (in a ratio of 3:1) and the pure minor compound was isolated by flash chromatography. In the case of the mixture, the ^{1}H signals did not overlap and this fact allowed us the assignment of all the protons for both isomers. Nevertheless, the following question still remains to be answered: Which is the *cis*- and which is the *trans*- isomer?

In our case the seven-membered sulfoxides are scarcely reported in the literature and hence the support from bibliographic sources is null. To solve the identification of both isomers we decided to tackle the conformational analysis of the target molecules, trying to clarify the conformational differences between both isomers and their possible responses in their ^{1}H NMR spectra.

The conformational study of *cis*-**7** and *trans*-**7** was carried out in two well differentiated phases. In the first one, the study of the base ring, without the uracil moiety, was tackled to identify the most stable conformation of this compound. In the second one, starting from the less energetic conformations of this compound, the uracil fragment was added to give rise to the *cis*-**7** and the *trans*-**7** and the conformational analysis on both isomers was carried out. The whole process was accomplished as previously reported by us.[2]

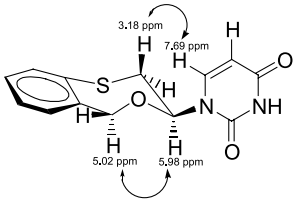


Figure 3. Preferred conformation of **5** showing the NOEDIFF effects by the double arrows.

Figure 4 shows the four most stable conformations of the *cis*-**7** isomer and Figure 5 shows the four most stable conformations of the *trans*-**7** isomer. In both isomers the energetic differences between the different conformations are important. These energy differences are sufficient to consider the upper left-handed conformation of *cis*-**7** in Figure 4, the only one existing in solution at 25 °C, its conformational population being calculated as 94.1%, considering a Boltzman distribution. For the *trans*-**7**, the most stable conformation represents 94.6% at 25 °C of the total population [upper left conformation in Figure 5 (*trans*-**7**)]. Therefore, it can be stated that the most stable conformations of both *cis*-**7** and *trans*-**7** are practically the only ones in the conformational equilibrium in solution, and the molecule properties could be explained from these conformers.

Figure 6 displays the most stable conformations of *cis*-**7** and *trans*-**7**. In both cases the uracil moiety adopts an equatorial disposition but the most interesting interactions are those established between the sulfinyl group (S═O) with the hydrogen atoms of the seven-membered fragment.

In the former case (*cis*-**7**) the equatorial S═O group bisects both H-2 atoms, whilst in *trans*-**7** the axial S═O group is antiperiplanar in relation to H-2α and accordingly, synperiplanar in relation to H-2β (see Fig. 6). Therefore, the H-2 atoms of both isomers must show a completely different behaviour in NMR. Moreover, the S═O group interacts with the axial H-3 atom and with the axial H-5 atom *trans*-**7**. Nevertheless such interactions are not observed in *cis*-**7** and, therefore, such interactions must result in different NMR responses of the hydrogen atoms of the seven-membered moieties of both isomers. In fact, the experimental data confirmed the conformational analysis data in the following way: in *trans*-**7**, the differences in chemical shifts of H-2 are higher than those of *cis*-**7** (3.62 ppm for H-2α and 3.30 ppm for H-2β for *trans*-**7**, whilst the chemical shifts are 3.47 ppm for H-2α and 3.34 ppm for H-2β for *cis*-**7**). The same holds true for H-5 of both isomers (4.84 ppm for H-5α and 5.61 ppm for H-5β for *trans*-**7**, being 4.67 ppm for H-5α and 4.83 ppm for H-5β for *cis*-**7**). Figure 6 shows that the distance between the sulfinyl group and the H-5α group (2.44 Å) adequately explains the chemical shift differences between H-5α and H-5β for *trans*-**7**. Moreover, H-3 is more deshielded in *trans*-**7** (δ 6.65 ppm) than in *cis*-**7** (δ 6.40 ppm). The whole ^{1}H NMR spectroscopic data are collected in the Section 4.

The conformational analysis of *cis*-**8** and *trans*-**8** was nearly identical to that of *cis*-**7** and *trans*-**7**, with only a slight variation in the energy of the several conformations (data not shown).

We have assigned the isomerism of the unique product in the reaction between **6** and H_2O_2 as being *trans*-**8**, based on the difference between the chemical shift of its H-2 atoms (0.36 ppm), which is closer to the 0.32 ppm difference

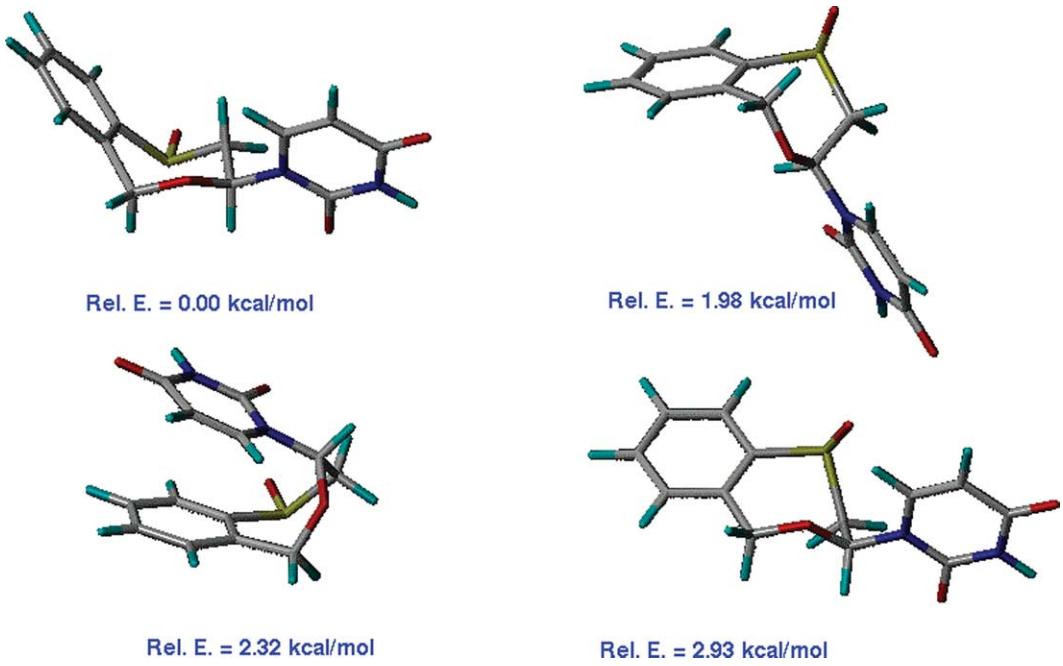


Figure 4. Representation of the four most stable conformations of compound *cis*-**7**. The relative energy values were calculated by means of molecular mechanics (tripos).

shown by *trans*-**7** and very different from the 0.13 ppm value of *cis*-**7**.

2.4. Antiproliferative activities

The MCF-7 human breast cancer cell line had been used as an excellent experimental model to improve the efficacy of different therapies before its use in patients.[10,11] Compounds **5**–**10** were assayed for their in vitro antiproliferative activity against the MCF-7 cell line.

The two antiproliferative compounds are **10** ($IC_{50}=12.74\pm4.79$ μM) and *trans*-**8** ($IC_{50}=30.05\pm0.71$ μM). The rest of the compounds are inactive ($IC_{50}>100$ μM). Therefore, it has to be pointed out that **10** and *trans*-**8** show an interesting antiproliferative activity with the natural base thymine in its structure. This is an outstanding fact that has not being previously reported in scientific bibliography, and this compound (or a related one) may serve as a prototype for the development of even more potent structures, probably endowed with a new mechanism of action. At present we are

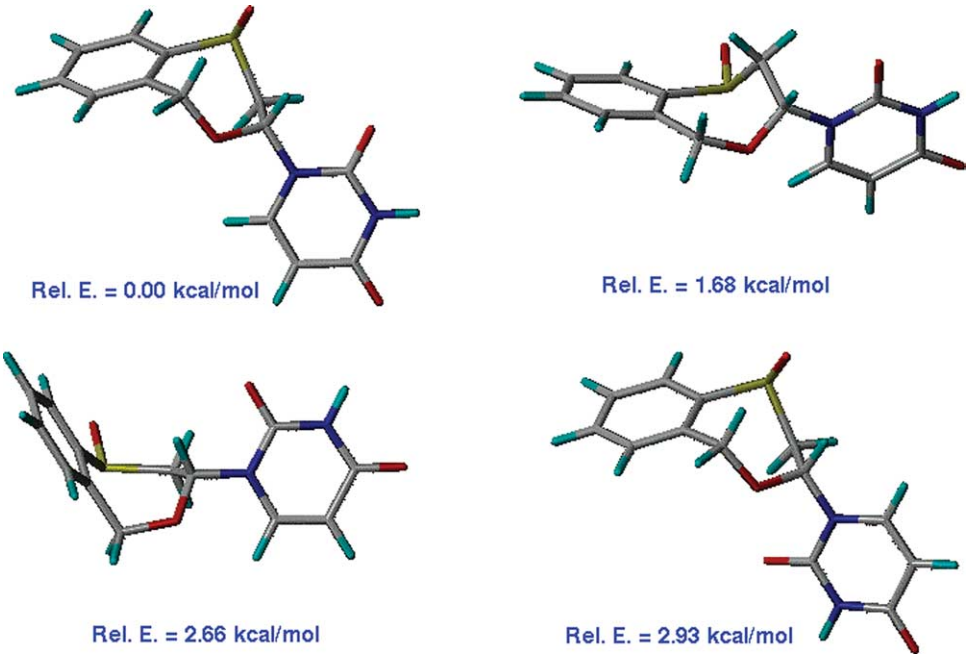


Figure 5. Representation of the four most stable conformations of compound *trans*-**7**. The relative energy values were calculated by means of molecular mechanics (tripos).

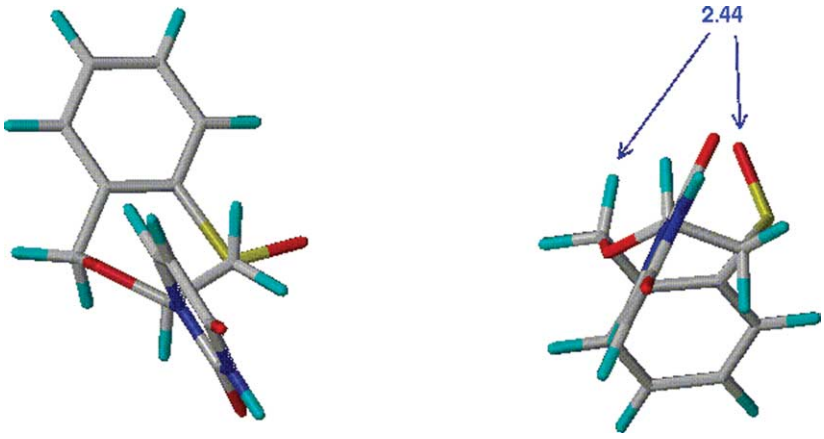


Figure 6. The most stable conformations of *cis*-**7** (left) and *trans*-**7** (right). The distance between the sulfinyl group and the H-5β group (2.44 Å) is shown for the *trans*-**7** conformer.

studying several antiproliferative markers in order to solve the mechanism of action at the molecular level of such a benzannelated seven-membered *O*,*N*-acetal.

3. Conclusions

Six final *O*,*N*-acetals have been obtained and screened for their anticancer activity. The modifications carried out affect two parts of the molecule: (a) on the one hand, the pyrimidine bases are always natural ones (uracil and thymine), and on the other (b) they affect the nature of the heteroatom of the seven-membered moiety that is linked directly to the benzene ring (sulfur, and several oxidation states of the sulfur atom: sulfinyl and sulfonyl). The NOEDIFF data for (*RS*)-3-(2,3-dihydro-5*H*-4,1-benzoxathiepin-3-yl)uracil **5** are compatible with a chair conformation for the seven-membered ring and an equatorial orientation of the uracil moiety on the C-3 position. The nature of the *cis*- and *trans*-sulfoxide isomers of **7** and **8** has been established by means of their conformational analyses and after comparing the theoretical results with the ^{1}H NMR responses of the target molecules. (*RS*)-3-(1,1-Dioxo-2,3-dihydro-5*H*-4,1-benzoxathiepin-3-yl)thymine **10** and (1*S**,3*S**)-1-(1-oxo-2,3-dihydro-5*H*-4,1-benzoxathiepin-3-yl)thymine (*trans*-**8**) were found to be inhibitors of the MCF-7 cell growth. To our knowledge, to date there have been no reports on nucleobase *O*,*N*-acetals with antitumour activities. At present, studies are being carried out to determine the mechanism of action at the molecular level of this compound.

4. Experimental

4.1. Chemistry

The general methods were the same as those previously described.[12,13]

4.1.1. Starting materials.

4.1.1.1. *o*-Hydroxymethylphenylthioacetaldehyde dimethyl acetal (11). 2-Mercaptobenzyl alcohol (1.62 mL, 14 mmol) was added dropwise to a stirred mixture of 547 mg (14 mmol) of a 60% (w/w) suspension of NaH in mineral oil and 40 mL of anhydrous DMF, under argon and at 0 °C; the resulting solution was left at rt until it became clear (45 min). To this clear solution was added dropwise 1.65 mL (14 mL) of bromoacetaldehyde dimethyl acetal and the resulting solution was left overnight at rt and then evaporated to dryness under vacuum. The residue was treated with water (20 mL), then extracted with dichloromethane (4×30 mL) and treated with brine: the combined organic phases were then dried (Na_2SO_4), filtered and evaporated under reduced pressure to yield a colourless oil, which after flash chromatography (EtOAc/hexane 1:4) gave pure **11** (2.9 g, 92% yield) as a clear dense liquid. The procedure previously reported used bromoacetaldehyde diethyl acetal[4] instead of bromoacetaldehyde dimethyl acetal. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 (m, 1H, H-arom); 7.38 (m, 1H, H-arom); 7.25 (m, 2H, H-arom); 4.77 (s, 2H, CH_2OH); 4.44 (t, J=5.6 Hz, 1H, CH(OMe)$_2$); 3.26 (s, 6H, O*Me*); 3.07 (d, J=5.6 Hz, 2H, SCH_2). ^{13}C NMR (75 MHz) δ 142.33 (C-1); 134.18 (C-2); 132.17, 129.12, 128.58, 127.62 (C-arom); 102.71 (*C*H(OMe)$_2$); 63.85 (*C*H_2OH); 53.04 (O*Me*); 37.09 (S*C*H_2). Anal. Calcd for $C_{11}H_{16}O_3S$: C, 57.87; H, 7.06; S, 14.05. Found: C, 57.90; H, 6.94; S, 14.26.

4.1.1.2. (*RS*)-3-Methoxy-2,3-dihydro-5*H*-4,1-benzoxathiepin (12). A mixture of 1.90 g (8.33 mmol) of **11**, 75 mg (0.436 mmol) of *p*-toluenesulfonic acid and 100 mL of anhydrous toluene was heated to reflux (110 °C) during 2 h; the solution was then brought back to rt and evaporated to dryness. The residue was taken up in diethyl ether (50 mL) and the resulting solution was dried (K_2CO_3): it was then filtered and evaporated to dryness, yielding an oil, which upon flash chromatography (EtOAc/hexane 1:2) gave 1.55 g of pure **12** (95% yield) as a clear oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 (m, 1H, H-arom); 7.23 (m, 3H, H-arom); 5.22 (d, J=13.5 Hz, 1H, H-5); 4.85 (t, J=4.1 Hz, 1H, H-3); 4.50 (d, J=13.5 Hz, 1H, H-5); 3.52 (s, 3H, O*Me*). ^{13}C NMR (75 MHz) δ 142.26 (C-9a); 136.61, 131.78, 129.59, 128.09, 127.58 (C-arom); 102.74 (C-3); 66.01 (C-5); 55.60 (O*Me*); 37.93 (C-2). HR LSIMS (NOBA matrix) calcd for $C_{10}H_{12}O_2S$: 196.0558; found: 196.0558. Anal. Calcd for $C_{10}H_{12}O_2S$: C, 61.20; H, 6.16; S, 16.34. Found: C, 61.56; H, 6.15; S, 16.68.

4.1.2. Final compounds.

4.1.2.1. (*RS*)-1-(2,3-Dihydro-5*H*-4,1-benzoxathiepin-3-yl)uracil (5). A 1.0 M solution of $SnCl_4/CH_2Cl_2$ (2.45 mL, 2.44 mmol) was added dropwise with stirring under argon to a suspension of **12** (400 mg, 2.04 mmol), uracil (250 mg, 2.23 mmol), which contained TCS (0.26 mL, 2.04 mmol) and HMDS (0.43 mL, 2.04 mmol) in dry acetonitrile (10 mL) at −25 °C. After 10 min at −20 °C, the suspension was left to reach rt and then warmed to 45 °C for 24 h. After cooling, the reaction was quenched by the addition of a concentrated aqueous solution of Na_2CO_3. The solvent was removed with a rotary evaporator and the residue was triturated with CH_2Cl_2 (5 mL), filtered and **5** was obtained as a creamy solid (410 mg, 73%); mp: 223–224 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.48 (s, 1H, NH); 7.69 (d, 1H, J=8.0 Hz, H-6′); 7.66 (m, 1H, H-arom); 7.52 (m, 1H, H-arom); 7.39 (m, 1H, H-arom); 5.98 (dd, 1H, J=9.1, 2.2 Hz, H-3α); 5.60 (dd, 1H, J=8.0, 2.2 Hz, H-5′); 5.02 (d, 1H, J=13.6 Hz, H-5α); 4.96 (d, 1H, J=13.6 Hz, H-5β); 3.27 (dd, 1H, J=14.1, 9.1 Hz, H-2α); 3.18 (dd, 1H, J=14.1, 2.3 Hz, H-2β). ^{13}C NMR (75 MHz) δ 162.93 (C-4′); 149.85 (C-2′); 142.14; 141.07 (C-6′); 135.76, 132.12, 130.13, 128.73, 128.22 (C-arom); 101.66 (C-5′); 86.86 (C-3); 72.90 (C-5); 36.74 (C-2). Anal. Calcd for $C_{13}H_{12}N_2O_3S$ C, 56.51; H, 4.38; N, 10.14; S, 11.60. Found: C, 56.36; H, 4.20; N, 9.86; S, 11.72.

4.1.2.2. (*RS*)-1-(2,3-Dihydro-5*H*-4,1-benzoxathiepin-3-yl)thymine (6). A 1.0 M solution of $SnCl_4/CH_2Cl_2$ (1.22 mL, 1.22 mmol) was added dropwise with stirring under argon to a suspension of **12** (200 mg, 1.02 mmol), thymine (140 mg, 1.15 mmol), which contained TCS (0.13 mL, 1.02 mmol) and HMDS (0.22 mL, 1.02 mmol) in dry acetonitrile (6 mL) at −25 °C. After 10 min at −20 °C, the suspension was left to reach rt and then warmed to 45 °C for 40 h. After cooling, the reaction was quenched by the addition of a concentrated aqueous solution of Na_2CO_3. The solvent was removed with a rotary evaporator and the residue was purified by flash chromatography (CH_2Cl_2/MeOH 9.99:0.01) and **6** was obtained as a white solid (343 mg, 58%); mp: 203–204 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.35 (s, 1H, NH); 7.59 (m, 1H, H-arom); 7.49 (s, 1H, H-6′); 7.44 (m, 1H, H-arom); 7.31 (m, 2H, H-arom); 5.89 (dd, J=9.7, 1.4 Hz, 1H, H-3); 4.89 (d, J=13.5 Hz, 1H, H-5β); 4.86 (d, J=13.5 Hz, 1H, H-5α); 3.20 (dd, J=14.1, 9.8 Hz, 1H, H-2α); 3.05 (dd, J=14.1, 1.4 Hz, 1H, H-2β); 1.68 (s, 3H, *Me*). ^{13}C NMR (100 MHz) δ 163.63 (C-4′); 149.84 (C-2′); 142.32 (C-arom); 136.59 (C-6′); 136.83, 132.18, 130.15, 128.72, 128.26 (C-arom); 109.40 (C-5′); 86.59 (C-3); 72.89 (C-5); 35.79 (C-2); 11.85 (*Me*). HR LSIMS (NOBA matrix) calcd for $C_{14}H_{14}N_2O_3SNa$ $(M+Na)^+$: 313.0623; found: 313.0624. Anal. Calcd for $C_{14}H_{14}N_2O_3S$: C, 57.92; H, 4.86; N, 9.65; S, 11.04. Found: C, 57.83; H, 5.10; N, 9.46; S, 11.32.

4.1.2.3. (1*R,3*S**)- and (1*S**,3*S**)-1-(2,3-Dihydro-5*H*-4,1-benzoxathiepin-3-yl)uracil (*cis*-7 and *trans*-7).** Hydrogen peroxide, 50 wt% solution in water (0.05 mL, 0.087 mmol) was added to a solution of $Sc(OTf)_3$ (71 mg, 0.145 mmol) in a mixture of 0.7 mL of EtOH and 6.3 mL of CH_2Cl_2. Compound **5** (200 mg, 0.724 mmol) was added after 5 min and the resulting solution was left at rt for 3.5 h. After this, more H_2O_2 was added (0.05 mL) and the reaction was left during 3.5 h more. The mixture was diluted with CH_2Cl_2 (20 mL) and washed with H_2O (2×15 mL), the organic layer was dried (Na_2SO_4), concentrated and purified by flash chromatography using a mixture of CH_2Cl_2/MeOH 9.5:0.5 to yield 164 mg, which were characterized as a mixture of *cis*-**7** and *trans*-**7** (164 mg, 77%). 130 mg of this mixture were purified by flash 40 chromatography using a gradient elution (EtOAc/hexane 3:2→EtOAc/hexane 4:1→EtOAc) and *cis*-**7** was obtained pure. *cis*-**7** ^{1}H NMR (300 MHz, $CDCl_3$ +2 drops of CD_3OD) δ 7.86 (dd, J=7.8, 1.3 Hz, 1H, H-arom); 7.63 (dt, J=7.7, 1.3 Hz, 1H, H-arom); 7.50 (dt, J=7.4, 1.4 Hz, 1H, H-arom); 7.32 (dd, J=7.4, 1.1 Hz, 1H, H-arom); 7.21 (d, J=8.2 Hz, 1H, H-6′); 6.40 (dd, J=10.6, 1.8 Hz, 1H, H-3); 5.67 (d, J=8.2 Hz, 1H, H-5′); 4.83 (d, J=14.3 Hz, 1H, H-5β); 4.67 (d, J=14.3 Hz, 1H, H-5α); 3.47 (dd, J=12.1, 1.8 Hz, 1H, H-2α); 3.34 (dd, J=12.0, 10.6 Hz, 1H, H-2β). *trans*-**7** ^{1}H NMR (300 MHz, $CDCl_3$ +2 drops of CD_3OD) δ 7.86 (dd, J=7.1, 1.8 Hz, 1H, H-arom); 7.50 (dt, J=7.6, 1.5 Hz, 1H, H-arom); 7.50 (dt, J=7.6, 1.5 Hz, 1H, H-arom); 7.40 (d, J=8.2 Hz, 1H, H-6′); 6.65 (dd, J=9.5, 2.4 Hz, 1H, H-3); 5.67 (d, J=8.2 Hz, 1H, H-5′); 5.61 (d, J=14.0 Hz, 1H, H-5β); 4.84 (d, J=14.0 Hz, 1H, H-5α); 4.84 (d, J=8.2 Hz, 1H, H-5′); 3.62 (dd, J=13.4, 2.4 Hz, 1H, H-2); 3.30 (dd, J=13.4, 6.7 Hz, 1H, H-2). Anal. Calcd for $C_{13}H_{12}N_2O_4S$: C, 53.42; H, 4.14; N, 9.58; S, 10.97. Found: C, 53.40; H, 4.20; N, 9.46; S, 10.72.

4.1.2.4. (1*S,3*S**)-1-(1-Oxo-2,3-dihydro-5*H*-4,1-benzoxathiepin-3-yl)thymine (*trans*-8).** Compound **6** (0.06 mL, 1.04 mmol) was dissolved in a H_2O_2 50 wt% solution in water, and the resulting solution was warmed under stirring for 2 h. After cooling, the mixture was diluted with H_2O (10 mL) and was extracted with EtOAc (3× 10 mL), the organic layer was dried (Na_2SO_4), concentrated and purified by flash chromatography using a gradient elution (CH_2Cl_2/MeOH 9.8:0.2→9.5:0.5→9:1) to yield *trans*-**8** (104 mg, 50%); mp: 271–273 °C. *trans*-**8** ^{1}H NMR (300 MHz, $CDCl_3$+DMSO-d_6) δ 10.85 (br s, 1H, N*H*); 7.66 (dd, J=7.6, 1.5 Hz, 1H, H-arom); 7.25 (dt, J=undet., 1.5 Hz, 1H, H-arom); 7.19 (dt, J=7.6, 1.5 Hz, 1H, H-arom); 7.06 (d, J=7.3, 1.3 Hz, 1H, H-arom); 6.88 (d, J=1.2 Hz, 1H, H-6′); 6.03 (dd, J=10.5, 1.6 Hz, 1H, H-3); 4.95 (d, J=14.0 Hz, 1H, H-5); 4.51 (d, J=14.0 Hz, 1H, H-5); 3.57 (dd, J=14.3, 10.4 Hz, 1H, H-2); 3.21 (dd, J=14.3, 1.6 Hz, 1H, H-2); 1.40 (d, J=1.2 Hz, 1H, *Me*). ^{13}C NMR (75 MHz) δ 165.02 (C-4′); 151.13 (C-2′); 140.71 (C-arom); 136.45, 135.31, 132.28, 130.25, 128.75 (C-arom+C-6′); 112.27 (C-5′); 83.58 (C-3); 72.59 (C-5); 60.66 (C-2); 13.38 (*Me*). LSIMS (NOBA matrix) calcd for $C_{14}H_{14}N_2O_4SNa$ $(M+Na)^+$: 329.0572; found: 329.0572. Anal. Calcd for $C_{14}H_{14}N_2O_5S$: C, 52.17; H, 4.38; N, 8.69; S, 9.95. Found: C, 52.40; H, 4.40; N, 8.36; S, 9.72.

4.1.2.5. (*RS*)-3-(1,1-Dioxo-2,3-dihydro-5*H*-4,1-benzoxathiepin-3-yl)uracil (9). Potassium peroxymonosulfate (OXONE™, 709 mg, 1.15 mmol) in H_2O (3 mL) was added to a solution of **5** in MeOH (10 mL) and the resulting suspension was left at rt for 2 h. After filtration and washing with H_2O and CH_2Cl_2, the residue was recrystallized from acetone to yield **9** (118 mg, 66%) as a white solid; mp: 245–247 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.98 (d, J=7.6, 1.5 Hz, 1H, H-arom); 7.66 (d, J=8.2 Hz, 1H, H-6′); 7.61 (m, 3H, H-arom); 6.28 (d, J=10.6 Hz, 1H, H-5′); 5.58 (d,

$J=8.0$ Hz, 1H, H-3); 5.10 (d, $J=14.1$ Hz, 1H, H-5); 4.99 (d, $J=14.1$ Hz, 1H, H-5); 4.37 (dd, $J=14.3$, 10.7 Hz, 1H, H-2); 4.04 (d, $J=14.3$ Hz, 1H, H-2). ^{13}C NMR (75 MHz) δ 162.85 (C-4′); 149.90 (C-2′); 141.00 (C-6′); 139.60 (C-arom); 136.13, 136.29, 131.41, 129.29, 127.00 (C-arom); 102.20 (C-5′); 82.18 (C-3); 70.57 (C-5); 58.16 (C-2). Anal. Calcd for $C_{13}H_{12}N_2O_5S$: C, 50.64; H, 3.92; N, 9.09; S, 10.40. Found: C, 50.60; H, 3.80; N, 9.34; S, 10.75.

4.1.2.6. (*RS*)-3-(1,1-Dioxo-2,3-dihydro-5*H*-4,1-benzoxathiepin-3-yl)thymine (10). The procedure was similar to Section 4.1.2.5, but starting from **6**. Yield (58%) as a white solid; mp: 272–274 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.55 (s, 1H, NH); 8.07 (dd, $J=7.6$, 1.4 Hz, 1H, H-6′); 7.74 (m, 1H, H-arom); 6.37 (dd, $J=10.6$, 1.4 Hz, 1H, H-3); 5.20 (d, $J=14.1$ Hz, 1H, H-5); 5.07 (d, $J=14.1$ Hz, 1H, H-5); 4.42 (dd, $J=14.5$, 10.7 Hz, 1H, H-2); 4.11 (dd, $J=14.5$, 1.4 Hz, 1H, H-2); 1.76 (s, 3H, *Me*). ^{13}C NMR (75 MHz) δ 163.34 (C-4′); 149.72 (C-2′); 139.48 (C-arom); 136.36, 136.02, 134.08, 131.22, 129.08, 126.79 (C-6′+C-arom); 109.66 (C-5′); 81.73 (C-3); 70.32 (C-5); 58.22 (C-2); 11.67 (*Me*). Anal. Calcd for $C_{14}H_{14}N_2O_5S$: C, 52.17; H, 4.38; N, 8.69; S, 9.95. Found: C, 52.40; H, 4.58; N, 8.44; S, 9.75.

4.2. Biological activity

The biological methods were the same as those previously described.[12]

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