Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron
Tetrahedron 63 (2007) 183–190

6′-Chloro-7- or 9-(2,3-dihydro-5*H*-4,1-benzoxathiepin-3-yl)-7*H*- or 9*H*-purines and their corresponding sulfones as a new family of cytotoxic drugs

María C. Núñez,[a] Fernando Rodríguez-Serrano,[b] Juan A. Marchal,[b] Octavio Caba,[c] Antonia Aránega,[c] Miguel A. Gallo,[a] Antonio Espinosa[a] and Joaquín M. Campos[a,*]

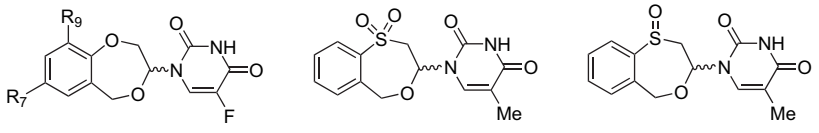
[a]*Departamento de Química Farmacéutica y Orgánica, Facultad de Farmacia, Universidad de Granada, c/ Campus de Cartuja s/n, 18071 Granada, Spain*
[b]*Departamento de Ciencias de la Salud, Facultad de Ciencias Experimentales y de la Salud, Paraje de las Lagunillas s/n, 23071 Jaén, Spain*
[c]*Departamento de Anatomía y Embriología Humana, Facultad de Medicina, Avenida de Madrid s/n, 18071 Granada, Spain*

Received 11 August 2006; revised 28 September 2006; accepted 10 October 2006
Available online 27 October 2006

Abstract—A series of 1-(2,3-dihydro-5*H*-4,1-benzoxathiepin-3-yl)pyrimidine derivatives were synthesized and two of them (**8** and **9**) showed a modest antiproliferative activity against the MCF-7 breast cancer cell line. We then decided to change the pyrimidine base for the more lipophilic 6′-chloropurine, and the *N*-9′ purine (**15**) and *N*-7′ purine (**17**) were obtained. The sulfone *N*-7′-alkylated-6-chloropurine **18** was the most active derivative. Compound **17** was found to be slightly more active than its regioisomer **15**, with an activity similar to that of 5-fluorouracil as a reference drug. Encouraged by these values, we tested these compounds against both the HT-29 human colon cancer cell line and the IEC-6 normal rat intestinal epithelial cell line, and **15** was found to be 12.7-fold more active against HT-29 than versus IEC-6.

1. Introduction

Despite major breakthroughs in many areas of modern medicine over the past 100 years, the successful treatment of cancer remains a significant challenge at the start of the 21st century. Therefore, the development of new drugs against cancer remains among the priorities of the development of science and fundamental research. Because it is difficult to discover novel agents that selectively kill tumour cells or inhibit their proliferation without general toxicity, the use of traditional cancer chemotherapy is still very limited. We have reported the synthesis and anticancer activities of compounds **1**–**3**,[1,2] (Fig. 1). A fundamental approach that has guided the design of novel drugs is bioisosterism, which we have carried out as suitable structural modifications of the seven-membered building block, such as the modification O-1→S (at different oxidation states). Moreover, the unnatural 5-fluorouracil (5-FU) base was changed by naturally occurring bases. In this way, we have recently reported that compounds **3** and **4**, with a thymine linked to a sulfur-containing seven-membered ring (Fig. 1), exhibited in vitro antiproliferative activities (IC_{50}=12.74±4.79 and 30.05± 0.71 μM, respectively) against the MCF-7 human breast cancer cell line,[3] which had been used as an excellent experimental model to improve the efficacy of different therapies before their use in patients.[4,5] Although compounds **3** and **4**



1 R_7= R_9= H
2 R_7= OMe; R_9= H
3 R_7 = H; R_9= OMe

3

trans-**4**

Figure 1. Several benzo-fused seven-membered alkylated pyrimidines that show antiproliferative activities against the human breast cancer cell line MCF-7.

Keywords: Antitumour compounds; Benzoxathiepins; Purines; Pyrimidines.
* Corresponding author. Tel.: +34 958 243848; fax: +34 958 243845; e-mail: jmcampos@ugr.es

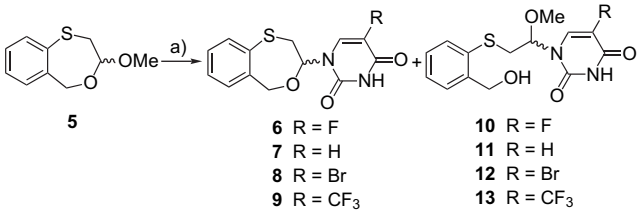
0040–4020/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2006.10.023

showed modest antiproliferative activities, this fact was worth highlighting due to the presence of the natural base thymine in the *O*,*N*-acetal[6] framework. Up to now we have found no reports on hemiaminalic[7] compounds with natural bases endowed with antiproliferative activities. A nucleoside containing a dihydrooxepin ring at the sugar moiety was the only reported case with a seven-membered ring, although devoid of any anticancer and anti-HIV activity.[8] In this paper, we describe details and discussions of structure–activity relationships (SARs) and antitumour activities that have been revealed during the study. Therefore, these compounds may serve as prototypes for the development of even more potent structures.

2. Results and discussion

During our ongoing research we have planned the synthesis of pyrimidine and purine 4,1-benzoxathiepin derivatives, and the corresponding acyclic ones to be tested subsequently against the human breast cancer cell line MCF-7.

The synthesis of the targets is depicted in Scheme 1. The preparation of the pyrimidine *O*,*N*-acetals was carried out between the seven-membered acetal **5**[3] and the unnatural bases such as 5-FU, 5-bromouracil and 5-trifluorouracil, and the natural one, uracil. We have used several Lewis acids, such as $Sc(OTf)_3$ and a 1.0 M solution of tin(IV) chloride ($SnCl_4$) in dichloromethane. The results are summarized in Table 1.



Scheme 1. Synthetic route for the preparation of the pyrimidine target molecules. Reagents and conditions: (a) pyrimidine base, HMDS, TCS, Lewis acid, anhydrous MeCN.

Treatment of **5** with 5-FU and tin(IV) chloride, 1.0 M solution in dichloromethane, at room temperature during 20 h in acetonitrile produced the cyclic seven-membered alkylated pyrimidine **6** with a 14% yield (Table 1, entry 1). When the reaction was carried out with uracil in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) at room temperature, the reaction was selective towards the acyclic alkylated uracil **11**, which was obtained with a 50% yield (Table 1, entry 2). In this case, the relatively low rate of silylation with 1,1,1,3,3,3-hexamethyldisilazane (HMDS) was accelerated by adding catalytic amounts of an acidic catalyst such as $(NH_4)_2SO_4$ (Table 1, entry 2)[9] or trimethylchlorosilane (TCS) (entries 1, 3–10). When the reaction time was increased up to 72 h, but the other experimental conditions of entry 1 kept the same, in addition to **7** (15%), the acyclic derivative **11** was obtained (55%, Table 1, entry 3). When the reaction mixture was warmed at 45 °C[10] for 20 h, the only product formed was the cyclic compound **7** (96%, Table 1, entry 4). In this case, the hypothesis that the cyclic structure could be formed from the corresponding acyclic one is supported by the reactivity of 5-FU 1,4-dioxepane hemiaminals.[11,12] The use of a Lewis acid (e.g., TMSOTf), whose role is solely to generate an oxonium ion[13] from the acetalic OMe group, should favour the formation of the acyclic structure and should not result in any formation of the corresponding cyclic one (Table 1, entry 2). When the Lewis acid was changed [$SnCl_4$ by $Sc(OTf)_3$] approximately equal amounts of both the cyclic seven-membered alkylated uracil **7** and the acyclic alkylated uracil **11** were obtained (Table 1, entry 5, 35% and 42%, respectively). Finally, the cyclic seven-membered alkylated-5-bromouracil and 5-(trifluoromethyl)uracil derivatives (Table 1, entries 6 and 7, respectively) were obtained under the optimal conditions carried out with uracil (Table 1, entry 4).

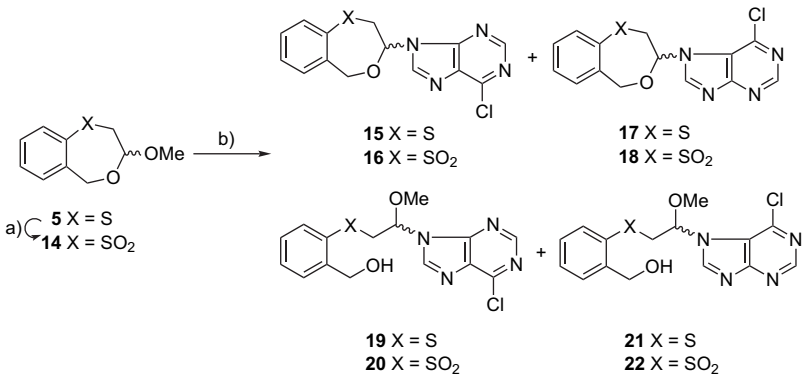
Table 1. Conditions studied for the reaction between **5** and several pyrimidine bases

Entry	Base	Time (h)	Lewis acid	Temp (°C)	Cyclic alkylated pyrimidine (%)	Acyclic alkylated pyrimidine (%)
1	5-FU	20	$SnCl_4$	rt	**6** (14)	**10** (—)
2	U	24	TMSOTf[a]	rt	**7** (—)	**11** (50)
3	U	72	$SnCl_4$	rt	**7** (15)	**11** (55)
4	U	20	$SnCl_4$	45	**7** (96)	**11** (—)
5	U	24	$Sc(OTf)_3$	45	**7** (35)	**11** (42)
6	5-BrU	20	$SnCl_4$	45	**8** (51)	**12** (—)
7	5-F_3CU	20	$SnCl_4$	45	**9** (38)	**13** (—)

[a] $(NH_4)_2SO_4$ was used instead of TCS, according to Ref. 8.

Once the *N*-1′-alkylated pyrimidine derivatives had been studied, we decided to shift to the more lipophilic purine ones (Scheme 2). The condensation reaction between the seven-membered acetal **5** and 6-chloropurine was accomplished using TMSOTf, TCS and HMDS in dry acetonitrile for 24 h (Table 2, entry 1). It produced the *N*-9′-seven-membered 6′-chloropurine (**15**, 56%) and the *N*-7′-acyclic alkylated-6′-chloropurine (**19**, 13%) regioisomers, which were separated by flash chromatography. When tin(IV) chloride was employed, the seven-membered alkylated *N*-7′ isomer **17** (8%) was produced, together with the acyclic *N*-9′-alkylated-6′-chloropurine **19** (4%, Table 2, entry 2). The *N*-7′ purine nucleosides are produced, presumably due to prior complexation between the tin and nitrogen atoms (*N*-9′).[14,15] When the starting reactant was the sulfone **14** (obtained from **5**, using Oxone® as oxidizing agent, under previously reported conditions[16]), with $SnCl_4$ as Lewis acid, the major product was the acyclic *N*-7′-alkylated-6′-chloropurine **22** (19%), together with equimolecular amounts of both *N*-7′-seven-membered alkylated-6′-chloropurine **18** (12%) and *N*-7′-acyclic alkylated-6′-chloropurine **20** (Table 2, entry 3, 12%).

Sulfoxide **23** (Scheme 3) was obtained by the procedure used by Matteucci et al.[17] who used catalytic scandium trifluoromethanesulfonate that greatly increases the efficiency of hydrogen-peroxide-mediated monooxidation of alkyl-aryl sulfides and methyl cysteine-containing peptides. When applied to sulfide **15**, the reaction proved to be stereospecific and *trans*-**23** (96%) was the only product obtained, according to Nuñez et al.:[3] the chemical shift differences (CSDs) between both the H-5 protons were δ 0.77 ppm for *trans*-1-(1-oxo-2,3-dihydro-5*H*-4,1-benzoxathiepin-3-yl)uracil and δ 0.16 ppm for its cis isomer,[3] whilst the CSD was

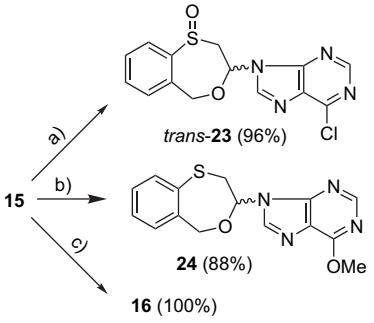


Scheme 2. Synthetic route for the preparation of the alkylated 6′-chloropurines. Reagents and conditions: (a) Oxone®, MeOH, H_2O; (b) 6-chloropurine, HMDS, TCS, Lewis acid, anhydrous MeCN, 45 °C, reaction time: 20 h.

Table 2. Comparison of the products obtained in the condensation reaction of **5** (and **14**) with 6-chloropurine

Entry	Starting *O,O*-acetal	Lewis acid	Cyclic *N*-9′-alkylated purine (%)	Cyclic *N*-7′-alkylated purine (%)	Acyclic *N*-9′-alkylated purine (%)	Acyclic *N*-7′-alkylated purine (%)
1	**5**	TMSOTf	**15** (56)	**17** (—)	**19** (13)	**21** (—)
2	**5**	$SnCl_4$	**15** (—)	**17** (8)	**19** (4)	**21** (—)
3	**14**	$SnCl_4$	**16** (—)	**18** (12)	**20** (12)	**22** (19)

δ 0.46 ppm for *trans*-**23**. Moreover, H-3 was more deshielded in *trans*-1-(1-oxo-2,3-dihydro-5*H*-4,1-benzoxathiepin-3-yl)uracil (δ 6.65 ppm) than in its cis analogue (δ 6.40 ppm), whilst *trans*-**23** showed a chemical shift of δ 6.60 ppm for its hemiaminalic proton.



Scheme 3. Several modifications on **15**. Reagents and conditions: (a) H_2O_2 50%, $Sc(OTf)_3$; (b) NaH/MeOH; (c) Oxone®, MeOH, H_2O.

The oxidation of sulfide **15** with Oxone® (Scheme 3)[16] produced the sulfone derivative **16** (*N*-9′ isomer) with 100% yield to obtain the regioisomer of **18** (*N*-7′) and later on to assay its antiproliferative activity and compare their biological properties as a function of their isomerism. Finally, the substitution of the chlorine atom of **15** by the methoxy group (**24**, Scheme 3) will give us information about the relationship between the nature of the functional group at position 6′ and the biological activity.

2.1. Spectroscopic analysis of the *N*-9′- and the *N*-7′-alkylated purines

The structures of the final compounds have been determined by ^1H and ^{13}C NMR, DEPT, HMBC (Heteronuclear Multiple Quantum Connectivity) experiments, HR LSIMS and elemental analyses. The chemical shift patterns of the alkylated pyrimidine and purine in both ^1H and ^{13}C spectra are consistent with those observed for related pyrimidine[3,18] and purine derivatives.[19]

The discrimination between *N*-9′- and *N*-7′-alkylated purines relies on the correlation between H-3 of the seven-membered moiety and C-4′ and C-5′, respectively. Very important is the correlation between H-3 (δ 6.29 ppm, dd, J=3.3 and 8.1 Hz) and the quaternary carbon at δ 151.42 ppm, which has the following two consequences: (a) this signal can be assigned to C-4′ and (b) this correlation proves unequivocally that the linkage between the seven-membered moiety and the purine base takes place through *N*-9′ in compound **15**. Moreover, assignments of *N*-9′ versus *N*-7′ isomers can be readily made from the ^{13}C NMR signal of the C-4′ peaks; for the *N*-9′ isomers: δ 151.85 ppm (**16**), δ 150.19 ppm (**20**), δ 149.51 ppm (*trans*-**23**) and δ 150.70 ppm (**24**); for the *N*-7′ isomers: δ 162.11 ppm (**17**), δ 161.81 ppm (**18**) and δ 162.24 ppm (**22**). These values agree with previous findings on acyclic alkylated adenine derivatives.[18]

2.2. Antiproliferative activity of the target molecules

Table 3 shows the antiproliferative activities against the MCF-7 human breast cancer cell line for the target compounds, including 5-FU as reference drug. As a rule the following can be stated: (a) the most active compounds are the

Table 3. Antiproliferative activities against the MCF-7 cell line for 5-FU, alkylated pyrimidine and purine compounds

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
5-FU	4.32±0.02	**11**	27.5±0.18	**19**	40.0±3.90
6	>100	**15**	5.46±0.02	**20**	63.8±6.42
8	44.1±4.23	**16**	16.5±1.59	**22**	81.9±11.9
9	34.9±3.96	**17**	3.55±1.10	*trans*-**23**	74.9±3.76
10	>100	**18**	2.58±0.08	**24**	44.6±0.50

Table 4. Antiproliferative activities against several cancerous and normal cell lines for 5-FU, **15**, **17** and **18**

Compound	IC_{50} MCF-7	IC_{50} MCF-10A	In vitro TI[a] (breast)	IC_{50} HT-29	IC_{50} IEC-6	In vitro TI[b] (intestinal)
5-FU	4.32±0.02	0.73±0.12	0.17	1.08±0.15	0.66±0.10	0.61
15	5.46±0.02	1.85±0.10	0.34	0.92±0.04	11.7±2.40	12.7
17	3.55±1.10	1.68±0.12	0.47	2.85±0.26	5.46±0.34	1.90
18	2.58±0.08	5.84±2.48	2.26	10.48±4.03	10.36±0.01	0.99

[a] In vitro TI=IC_{50} MCF-10A/IC_{50} MCF-7.
[b] In vitro TI=IC_{50} IEC-6/IC_{50} HT-29.

4,1-benzoxathiepin (**15**, **17**) and 1,1-dioxo-4,1-benzoxathiepin-6′-chloropurine derivatives (**16**, **18**); (b) the *N*-7′ purine derivatives present a better activity (**17**, **18**) than their *N*-9′ (**15**, **16**) regioisomers; (c) the most active compound is the *N*-7′-6′-chlorosubstituted purine (**18**), which is nearly 2-fold more potent than 5-FU.

Encouraged by these results, we decided to extend our studies to other cancerous cell lines, such as the colon human-derived cell line HT-29. This cell line was established from a colon adenocarcinoma, one of the most frequent solid human cancers that is also mainly resistant to chemotherapy,[20] making these cells appropriate for the search for new antitumour drugs. Moreover, we established a comparison of the three most active compounds (**15**, **17** and **18**) between the cancerous lines (MCF-7 and HT-29) and the corresponding normal ones, such as MCF-10 (non-cancerous human mammary epithelial cells) and IEC-6 (a rat intestinal epithelial cell line), in an intent to define the in vitro therapeutic index as a measure of the selectivity. The in vitro therapeutic index (TI) of a drug is defined as the ratio of the toxic dose to the therapeutic dose. Table 4 shows the antiproliferative activities for 5-FU, **15**, **17** and **18**, and the corresponding in vitro TIs. Compound **18** is the most selective compound against the cancerous breast cancer cell line (TI=2.26) in relation to the normal one, while **15** is against the cancerous colon cell line in relation to normal intestinal cells (TI=12.7). It has to be pointed out that, although in a different therapeutic area, the selective cyclooxygenase-2 (COX-2) inhibitors, celecoxib and rofecoxib, which are in clinical use, are 7.3- and 8.4-fold more potent as in vitro COX-2 than COX-1 inhibitors.[21] The unnatural pyrimidine base 5-FU is not selective at all, with TI<1 in both epithelial non-cancerous cells [in vitro TI (breast)=0.17 and in vitro TI (intestinal)=0.61].

3. Conclusion

In short, we have synthesized 14 *N*-alkylated pyrimidine and purine derivatives and tested their preliminary in vitro anticancer activities. These results provide promising information for the further development of potent antiproliferative agents. It has been observed that the antiproliferative activity of **15** is drastically increased in the HT-29 human colon tumour compared to the normal tissue. The subject of our current research[22] is to overcome the shortcomings of the present cancer chemotherapy, i.e., the synthesis of antitumour drugs with a new mechanism of action, capable of discriminating tumour cells from normal proliferative cells and which exhibit selective toxicity against cancer. After having described both the chemistry and preliminary biological activities of the alkylated pyrimidine and purine derivatives, we plan to carry out microarray studies to recognize the genes implicated in the anticarcinogenic activities of the three most potent compounds (**15**, **17** and **18**).

4. Experimental

4.1. Chemistry

The general methods were the same as those previously described.[1,3] The HMBC spectra[23] were measured using a pulse sequence optimized for 10 Hz (inter-pulse delay for the evolution of long-range couplings: 50 ms) and a 5:3:4 gradient combination. In this way, direct responses (^1J couplings) were not completely removed.

4.2. Starting material

4.2.1. Synthesis of (*RS*)-1,1-dioxo-3-methoxy-2,3-dihydro-5*H*-4,1-benzoxathiepin (14). Potassium peroxymonosulfate (Oxone®, 941 mg, 1.53 mmol) in H_2O (4 mL) was added to a solution of **5** in MeOH (12 mL) and the resulting suspension was left at room temperature for 2 h. After filtration and washing (H_2O and CH_2Cl_2) the residue was purified by gradient flash chromatography (CH_2Cl_2/MeOH, 9.8/0.2→9.5/0.5) to yield **14** (160 mg, 91%) as an amorphous white solid; mp: 131–133 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 8.07 (dd, J=1.5, 7.6 Hz, 1H, C*H*-arom); 7.58 (dt, J=1.5, 7.3 Hz, 1H, C*H*-arom); 7.51 (dt, J=1.5, 7.6 Hz, 1H, C*H*-arom); 7.37 (dd, J=1.5, 7.3 Hz, 1H, C*H*-arom); 5.11 (dd, J=2.3, 8.3 Hz, 1H, C*H*-3); 5.06 (d, J=14.2 Hz, 1H, CH_2-5); 4.97 (d, J=14.2 Hz, 1H, CH_2-5); 3.59 (d, J=2.3, 14.7 Hz, 1H, CH_2-2); 3.53 (s, 1H, O*Me*); 3.42 (d, J=8.3, 14.7 Hz, 1H, CH_2-2). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 139.88; 137.13; 133.78; 130.40; 128.84; 127.71; 101.62 (*C*H-3); 67.17 (*C*H_2-5); 60.27 (*C*H_2-2); 56.55 (O*Me*). Anal. for $C_{10}H_{12}O_4S$: calcd: C 52.62; H 5.30; N 14.05. Found: C 52.83; H 5.15; N 13.89.

4.3. Final compounds

4.3.1. General procedure for the reaction between (*RS*)-3-methoxy-2,3-dihydro-5*H*-4,1-benzoxathiepin (5) [or (*RS*)-1,1-dioxo-3-methoxy-2,3-dihydro-5*H*-4,1-benzoxathiepin (15)] and pyrimidine bases or 6-chloropurine.

4.3.1.1. Method A. Synthesis of (*RS*)-1-(2,3-dihydro-5*H*-4,1-benzoxathiepin-3-yl)-5-fluorouracil (6). A 1.0 M solution of $SnCl_4/CH_2Cl_2$ (1.53 mL, 1.53 mmol) was added dropwise with stirring to a suspension of **5**[3] (250 mg, 1.27 mmol), 5-fluorouracil (181 mg, 1.39 mmol), 1,1,1,3,3,3-hexamethyldisilazane (HMDS, 0.27 mL, 1.27 mmol) and trimethylchlorosilane (TCS, 0.16 mL, 1.27 mmol) in dry acetonitrile (4 mL) at 0 °C, under argon. After 20 h of stirring at room temperature, the reaction was

quenched by the addition of a concentrated aqueous solution of K_2CO_3, then concentrated under diminished pressure, the resulting residue was suspended (H_2O) and extracted with CH_2Cl_2 (3×20 mL). The organic layers were pooled, dried (Na_2SO_4), filtered and the solvent removed under vacuum. The residue was purified by flash chromatography using a mixture of CH_2Cl_2/MeOH (9.9/0.1), and **6** (53 mg, 14%) was obtained as an amorphous white solid; mp 213–215 °C. ^{1}H NMR (acetone-d_6, 300 MHz) δ (ppm) 10.50 (s, 1H, N*H*); 7.81 (d, 1H, C*H*-arom); 7.62 (m, 1H, C*H*-arom); 7.48 (m, 1H, C*H*-arom); 7.36 (m, 2H, C*H*-arom); 6.01 (dt, *J*=5.8 Hz, 1H, C*H*-3); 5.06 (d, *J*=13.5 Hz, 1H, CH_2-5); 4.98 (d, *J*=13.5 Hz, 1H, CH_2-5); 3.14 (d, *J*=5.8 Hz, 2H, CH_2-2). ^{13}C NMR (acetone-d_6, 100 MHz): δ (ppm) 155.87 (*C*=O); 147.79 (*C*=O); 141.87; 139.95 (d, *J*=230.8 Hz, *C*-5′); 135.57; 131.73; 129.51; 128.24; 127.80; 124.05 (d, *J*=34.5 Hz, *C*H-6′); 86.90 (*C*H-3); 72.97 (*C*H_2-5); 36.80 (*C*H_2-2). HR LSIMS calcd for $C_{13}H_{12}N_2O_3FS$ $(M+H)^+$ 295.0554, found 295.0553. Anal. for $C_{13}H_{11}FN_2O_3S$: calcd: C 53.05; H 3.77; N 9.52. Found: C 52.89; H 4.05; N 9.81.

4.3.1.2. Method B. Synthesis of (*RS*)-1-[2-(2-hydroxymethylphenylthio)-1-methoxyethyl]uracil (11). A suspension of uracil (72 mg, 0.64 mmol), HMDS (3 mL, 14 mmol) and $(NH_4)_2SO_4$ (6.4 mg, 0.048 mmol) in anhydrous acetonitrile (4 mL) under argon was heated to 130 °C for 2 h. The resulting solution was cooled to room temperature, concentrated under diminished pressure and more anhydrous acetonitrile (4 mL) was added. Trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.12 mL, 0.67 mmol) and then **5**[3] (250 mg, 1.27 mmol) dissolved in anhydrous acetonitrile (3 mL) were added dropwise at −45 °C and the reaction mixture was left at room temperature for 24 h. After the usual workup and flash chromatography (CH_2Cl_2/MeOH, 9.8/0.2), **11** (88 mg, 50%) was obtained as an amorphous white solid; mp 130–131 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 8.75 (m, 1H, N*H*); 7.45 (m, 2H, C*H*-arom); 7.27 (m, 3H, C*H*-arom); 5.71 (dd, *J*=8.1, 2.0 Hz, 1H, C*H*-5′); 5.67 (t, *J*=5.9 Hz, 1H, C*H*); 4.72 (s, 2H, CH_2OH); 3.22 (d, *J*=14.5 Hz, 1H, CH_2S); 3.15 (d, *J*=14.5 Hz, 1H, CH_2S); 2.68 (s, 1H, O*H*). ^{13}C NMR ($CDCl_3$+some drops of CD_3OD, 75 MHz) δ (ppm) 163.30 (*C*=O); 151.41 (*C*=O); 142.20; 138.40; 132.36; 131.52; 128.95; 128.40; 127.90; 103.40 (*C*H-5′); 85.92 (*C*H); 62.83 (*C*H_2OH); 57.01 (O*Me*); 38.60 (*C*H_2S). Anal. for $C_{14}H_{16}N_2O_4S$: calcd: C 54.53; H 5.23; N 9.08. Found: C 54.76; H 5.35; N 9.30.

4.3.1.3. Method C. Syntheses of 7 and 11. A suspension of **5**[3] (400 mg, 2.04 mmol), uracil (250 mg, 2.23 mmol), HMDS (0.43 mL, 2.04 mmol) and TCS (0.26 mL, 2.04 mmol) in dry acetonitrile (6 mL) was prepared under argon. After 15 min at room temperature it was cooled to −30 °C and a 1.0 M solution of $SnCl_4/CH_2Cl_2$ (2.45 mL, 2.45 mmol) in anhydrous acetonitrile (5 mL) was added dropwise while stirring the suspension, and after 10 min at −30 °C, the corresponding solution was left at room temperature for 72 h. After the usual workup and flash chromatography (CH_2Cl_2/MeOH, 9.9/0.1), **7** (86 mg, 15%) and **11** (310 mg, 55%) were obtained.

4.3.1.4. Method D. Synthesis of 7. A suspension of **5**[3] (400 mg, 2.04 mmol), uracil (250 mg, 2.23 mmol), HMDS (0.43 mL, 2.04 mmol) and TCS (0.26 mL, 2.04 mmol) in dry acetonitrile (6 mL) was prepared under argon. After 15 min at room temperature, the reaction mixture was cooled to −25 °C, and a 1.0 M solution of $SnCl_4/CH_2Cl_2$ (2.45 mL, 2.45 mmol) in anhydrous acetonitrile (5 mL) was added dropwise with stirring. After 10 min at −20 °C the reaction mixture was left to reach room temperature and then warmed at 45 °C for 20 h. After the usual workup the crude was triturated with CH_2Cl_2 (5 mL), filtered and **7** was obtained (540 mg, 96%).

4.3.1.5. Method E. Syntheses of 7 and 11. Compound **5**[3] (110 mg, 0.56 mmol) was dissolved in anhydrous acetonitrile (4 mL) under argon. After cooling the solution at −20 °C, uracil (63 mg, 0.56 mmol), TCS (72 μL, 0.56 mmol), HMDS (0.117 mL, 0.56 mmol) and $Sc(OTf)_3$ (276 mg, 0.56 mmol) were added. After 10 min at room temperature, the reaction mixture was warmed to 45 °C for 24 h. After the usual workup and flash chromatography (CH_2Cl_2/MeOH, 9.9/0.1), **7** (51 mg, 35%) and **11** (65 mg, 42%) were obtained.

4.3.1.6. Synthesis of (*RS*)-1-(2,3-dihydro-5*H*-4,1-benzoxathiepin-3-yl)-5-bromouracil (8). Following Method D, but starting from **5**[3] (200 mg, 1.02 mmol), 5-bromouracil (213 mg, 1.12 mmol), HMDS (0.22 mL, 1.02 mmol) and TCS (0.13 mL, 1.02 mmol), and a 1.0 M solution of $SnCl_4/CH_2Cl_2$ (1.22 mL, 1.22 mmol) in anhydrous acetonitrile (6 mL), **8** was obtained as an amorphous white solid (132 mg, 51%); mp 239–240 °C. ^{1}H NMR ($CDCl_3$+some drops of DMSO-d_6, 300 MHz) δ (ppm) 11.78 (s, 1H, N*H*); 7.69 (m, 1H, C*H*-arom); 7.51 (m, 1H, C*H*-arom); 7.26 (m, 3H, C*H*-arom); 5.88 (t, *J*=5.5 Hz, 1H, C*H*-3); 4.95 (d, *J*=13.3 Hz, 1H, CH_2-5); 4.84 (d, *J*=13.4 Hz, 1H, CH_2-5); 2.93 (d, *J*=5.6 Hz, 2H, CH_2-2). ^{13}C NMR ($CDCl_3$+some drops of DMSO-d_6, 75 MHz) δ (ppm) 158.20 (*C*=O); 148.19 (*C*=O); 140.68; 138.32; 134.77; 131.39; 128.86; 127.71; 127.23; 95.66 (*C*-5′); 86.25 (*C*H-3); 72.67 (*C*H_2-5); 36.88 (*C*H_2-2). Anal. for $C_{13}H_{11}BrN_2O_3S$: calcd: C 43.96; H 3.12; N 7.89. Found: C 43.86; H 3.43; N 7.60.

4.3.1.7. Synthesis of (*RS*)-1-(2,3-dihydro-5*H*-4,1-benzoxathiepin-3-yl)-5-trifluoromethyluracil (9). Following Method D, but starting from **5**[3] (200 mg, 1.02 mmol), 5-trifluoromethyluracil (201 mg, 1.12 mmol), HMDS (0.22 mL, 1.02 mmol) and TCS (0.13 mL, 1.02 mmol), and a 1.0 M solution of $SnCl_4/CH_2Cl_2$ (1.22 mL, 1.22 mmol) in anhydrous acetonitrile (6 mL), **9** was obtained as an amorphous white solid (133 mg, 52%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 8.91 (s, 1H, N*H*); 7.86 (m, 1H, C*H*-arom); 7.63 (m, 1H, C*H*-arom); 7.35 (m, 3H, C*H*-arom); 6.02 (dd, *J*=1.7, 9.5 Hz, 1H, C*H*-3); 5.10 (d, *J*=13.4 Hz, 1H, CH_2-5); 4.95 (d, *J*=13.4 Hz, 1H, CH_2-5); 3.09 (dd, *J*=1.7, 14.1 Hz, 1H, CH_2-2); 2.79 (dd, *J*=9.5, 14.1 Hz, 1H, CH_2-2). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 157.96 (*C*=O); 148.61 (*C*=O); 141.40 (*C*-arom); 140.82; 135.78; 132.94; 130.12; 129.27; 128.73; 121.74 (d, *J*=268.9 Hz); 105.64 (d, *J*=33.4 Hz); 87.79 (*C*H-3); 74.29 (*C*H_2-5); 38.72 (*C*H_2-2). HR LSIMS calcd for $C_{14}H_{11}N_2O_3F_3NaS$ $(M+Na)^+$ 367.0344; found 367.03444. Anal. for $C_{14}H_{11}F_3N_2O_3S$: calcd: C 48.84; H 3.22; N 8.14. Found: C 48.98; H 3.42; N 8.54.

4.3.1.8. Syntheses of (*RS*)-6′-chloro-9-(2,3-dihydro-5*H*-4,1-benzoxathiepin-3-yl)-9*H*-purine (15) and (*RS*)-6′-chloro-9-[2-(2-hydroxymethylphenylthio)-1-methoxy-

ethyl]-9*H*-purine (19). Starting from **5**[3] (0.500 g, 2.55 mmol), 6-chloropurine (394 mg, 2.55 mmol), HMDS (0.53 mL, 2.55 mmol), TCS (0.33 mL, 2.55 mmol), $Sc(OTf)_3$ (1.25 g, 2.55 mmol), anhydrous acetonitrile (20 mL), and following Method D a residue was obtained and purified by gradient flash chromatography (EtOAc/hexane, 3/7→4/6). A first fraction identified as **15** (0.456 g, 56%) was obtained as an amorphous white solid; mp 185–187 °C. HMBC experiments were carried out on **15**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 8.76 (s, 1H, C*H*-2′); 8.28 (s, 1H, C*H*-8′); 7.65 (m, 1H, C*H*-arom); 7.36 (m, 3H, C*H*-arom); 6.29 (dd, *J*=3.3, 8.1 Hz, 1H, C*H*-3); 5.18 (d, *J*=13.3 Hz, 1H, CH_2-5); 5.01 (d, *J*=13.3 Hz, 1H, CH_2-5); 3.27 (m, *J*=indet., 6.1 Hz, 2H, CH_2-2); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 152.23 (*C*H-2′); 151.42 (*C*-4′); 150.60 (*C*-6′); 142.79 (*C*H-8′), 141.75 (*C*-5a); 135.82 (*C*-9a); 132.84; 131.77 (*C*-5′); 130.18; 129.20; 128.75; 87.77 (*C*H-3); 73.93 (CH_2-5); 39.19 (CH_2-2). Anal. for $C_{14}H_{11}ClN_4OS$: calcd: C 52.75; H 3.48; N 17.58. Found: C 52.65; H 3.63; N 17.12.

The second fraction, as an amorphous white powder was identified as **19** (117 mg, 13%) as a brown dense oil; HMBC experiments were carried out on **19**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 8.69 (s, 1H, C*H*-2′); 8.21 (s, 1H, C*H*-8′); 7.34 (dd, *J*=1.6, 7.6 Hz, 1H, C*H*-arom); 7.22 (dd, *J*=1.4, 7.6 Hz, 1H, C*H*-arom); 7.15 (dt, *J*=1.4, 7.6 Hz, 1H, C*H*-arom); 7.05 (dt, *J*=1.6, 7.6 Hz, 1H, C*H*-arom); 5.73 (dd, *J*=5.0, 7.8 Hz, 1H, C*H*-3); 4.72 (dd, *J*=12.8 Hz, 2H, CH_2OH); 3.69 (dd, *J*=7.1, 14.5 Hz, 1H, CH_2S); 3.57 (dd, *J*=5.0, 14.5 Hz, 1H, CH_2S); 3.31 (s, 3H, O*Me*). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 152.19 (*C*H-2′); 151.70 (*C*-6′); 151.39 (*C*-4′); 143.23 (*C*H-8′); 142.13; 132.12; 131.99; 131.87 (*C*-5′); 129.06; 128.37; 128.24; 87.10 (*C*H); 63.37 (CH_2OH); 57.34 (O*Me*); 39.45 (CH_2S). Anal. for $C_{15}H_{15}ClN_4O_2S$: calcd: C 51.35; H 4.31; N 15.97. Found: C 51.00; H 4.16; N 15.87.

4.3.1.9. Syntheses of (*RS*)-6′-chloro-7-(2,3-dihydro-5*H*-4,1-benzoxathiepin-3-yl)-7*H*-purine (17) and 19. Starting from **5**[3] (0.252 g, 1.28 mmol), 6-chloropurine (216 mg, 1.40 mmol), HMDS (0.27 mL, 1.28 mmol), TCS (0.16 mL, 1.28 mmol) and a 1.0 M solution of $SnCl_4$/CH_2Cl_2 (1.53 mL, 1.28 mmol) in anhydrous acetonitrile (7 mL), and following Method D a residue was obtained and purified by flash chromatography (CH_2Cl_2/MeOH, 9.9/0.1). A first fraction identified as **17** (33 mg, 8%) was obtained as an amorphous white solid. HMBC experiments were carried out on **17**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 8.89 (s, 1H, C*H*-2′); 8.43 (s, 1H, C*H*-8′); 7.66 (m, 1H, C*H*-arom); 7.35 (m, 3H, C*H*-arom); 6.55 (dd, *J*=1.8, 9.4 Hz, 1H, C*H*-3); 5.18 (d, *J*=13.4 Hz, 1H, CH_2-5); 5.02 (d, *J*=13.4 Hz, 1H, CH_2-5); 3.33 (dd, *J*=1.8, 14.0 Hz, 1H, CH_2-2); 3.18 (dd, *J*=9.4, 14.0 Hz, 1H, CH_2-2). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 162.11 (*C*H-4′); 152.83 (*C*-2′); 145.97 (*C*-8′); 142.91 (*C*H-6′); 141.71 (*C*-5a); 135.42 (*C*-9a); 133.01; 130.19; 129.25; 128.90; 121.50 (*C*-5′); 89.36 (*C*H-3); 73.95 (CH_2-5); 39.80 (CH_2-2). Anal. for $C_{14}H_{11}ClN_4OS$: calcd: C 52.75; H 3.48; N 17.58. Found: C 52.87; H 3.54; N 17.74.

The second fraction, as an amorphous white powder was identified as **19** (18 mg, 4%).

4.3.1.10. Syntheses of (*RS*)-6′-chloro-7-(1,1-dioxo-2,3-dihydro-5*H*-4,1-benzoxathiepin-3-yl)-7*H*-purine (18), (*RS*)-6′-chloro-9-[1,1-dioxo-2-(2-hydroxymethylphenylthio)-1-methoxyethyl]-9*H*-purine (20) and (*RS*)-6′-chloro-7-[1,1-dioxo-2-(2-hydroxymethylphenylthio)-1-methoxyethyl]-7*H*-purine (22). Starting from **5**[3] (137 mg, 0.60 mmol), 6-chloropurine (101 mg, 0.65 mmol), HMDS (0.12 mL, 0.60 mmol), TCS (0.08 mL, 0.60 mmol) and a 1.0 M solution of $SnCl_4$/CH_2Cl_2 (0.72 mL, 0.72 mmol) in anhydrous acetonitrile (4 mL), and following Method D a residue was obtained and purified by flash chromatography (CH_2Cl_2/MeOH, 9.8/0.2). A first fraction identified as **18** (29 mg, 12%) was obtained as an amorphous white solid; mp 205–206 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 8.89 (s, 1H, C*H*-2′); 8.41 (s, 1H, C*H*-8′); 8.13 (d, *J*=7.7 Hz, 1H, C*H*-arom); 7.67 (t, *J*=7.4 Hz, 1H, C*H*-arom); 7.61 (t, *J*=7.7 Hz, 1H, C*H*-arom); 7.43 (d, *J*=7.4 Hz, 1H, C*H*-arom); 6.97 (t, *J*=6.0 Hz, 1H, C*H*-3); 5.55 (d, *J*=14.0 Hz, 1H, CH_2-5); 4.99 (d, *J*=14.0 Hz, 1H, CH_2-5); 3.98 (d, *J*=6.0 Hz, 2H, CH_2-2). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 161.81 (*C*H-4′); 153.16 (*C*-2′); 145.89 (*C*-8′); 143.36 (*C*H-6′); 138.93 (*C*-9a); 135.53 (*C*-5a); 134.79; 131.28; 129.68; 128.46; 121.50 (*C*-5′); 84.41 (*C*H-3); 71.81 (CH_2-5); 60.60 (CH_2-2). Anal. for $C_{14}H_{11}ClN_4O_3S$: calcd: C 47.94; H 3.16; N 15.97. Found: C 47.97; H 3.40; N 16.08.

The second fraction, as an amorphous white powder was identified as **20** (28 mg, 12%); mp 113–114 °C. ^{1}H NMR (CD_3OD, 300 MHz) δ (ppm) 8.67 (s, 1H, C*H*-2′); 8.62 (s, 1H, C*H*-8′); 7.67 (m, 2H, C*H*-arom); 7.59 (dt, *J*=1.4, 7.6 Hz, 1H, C*H*-arom); 7.33 (dt, *J*=1.2, 7.6 Hz, 1H, C*H*-arom); 6.18 (t, *J*=6.1 Hz, 1H, C*H*-3); 5.02 (d, *J*=5.7 Hz, 2H, CH_2OH); 4.61 (dd, *J*=6.5, 14.8 Hz, 1H, CH_2S); 4.56 (t, *J*=5.7 Hz, 1H, O*H*); 4.46 (dd, *J*=6.1, 14.8 Hz, 1H, CH_2S); 3.23 (s, 3H, *Me*). ^{13}C NMR (CD_3OD, 75 MHz) δ (ppm) 151.99 (*C*-6′); 151.84 (*C*H-2′); 150.19 (*C*-4′); 145.02 (*C*H-8′); 141.79 (*C*-arom); 136.59 (*C*-arom); 134.09 (*C*H-arom); 131.83 (*C*-5′); 129.47 (*C*H-arom); 129.42 (*C*H-arom); 127.46 (*C*H-arom); 82.07 (*C*H); 60.91 (CH_2OH); 58.34 (CH_2S); 56.11 (O*Me*). Anal. for $C_{15}H_{15}ClN_4O_4S$: calcd: C 47.06; H 3.95; N 14.64. Found: C 46.96; H 3.71; N 14.92.

The third fraction, as an amorphous white powder was identified as **22** (43 mg, 19%); mp 131–132 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 8.99 (s, 1H, C*H*-2′); 8.82 (s, 1H, C*H*-8′); 7.77 (m, 3H, C*H*-arom); 7.40 (dt, *J*=1.5, 7.6 Hz, 1H, C*H*-arom); 6.38 (dd, *J*=5.0, 7.1 Hz, 1H, C*H*-3); 5.52 (t, *J*=5.5 Hz, 1H, O*H*); 4.88 (d, *J*=5.5 Hz, 2H, CH_2OH); 4.50 (dd, *J*=7.2, 14.8 Hz, 1H, CH_2S); 4.40 (dd, *J*=5.0, 14.8 Hz, 1H, CH_2S); 3.16 (s, 3H, *Me*). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm) 162.24 (*C*-4′); 152.37 (*C*H-2′); 149.68 (*C*H-8′); 142.55 (*C*-6′); 136.40; 134.77; 129.81; 129.56; 127.94; 122.51 (*C*-5′); 83.23 (*C*H); 60.18 (CH_2OH); 59.87 (CH_2S); 56.72 (O*Me*). Anal. for $C_{15}H_{15}ClN_4O_4S$: calcd: C 47.06; H 3.95; N 14.64. Found: C 47.12; H 3.62; N 14.50.

4.3.1.11. Synthesis of (*RS*)-6′-chloro-9-(1,1-dioxo-2,3-dihydro-5*H*-4,1-benzoxathiepin-3-yl)-9*H*-purine (16). Following the experimental procedure for the preparation of **14** (Section 4.2.1) and using Oxone® (578 mg,

0.94 mmol), H_2O (3 mL), **5** (150 mg, 0.47 mmol) and MeOH (7 mL), compound **16** (165 mg, 100%) was obtained as an amorphous white solid; mp 230–231 °C. HMBC experiments were carried out on **16**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 8.77 (s, 1H, C*H*-2′); 8.27 (s, 1H, C*H*-8′); 8.18 (dd, *J*=1.0, 7.6 Hz, 1H, C*H*-arom); 7.56 (dt, *J*=1.2, 7.6 Hz, 1H, C*H*-arom); 7.64 (dt, *J*=1.0, 7.6 Hz, 1H, C*H*-arom); 7.46 (d, *J*=7.6 Hz, 1H, C*H*-arom); 6.66 (dd, *J*=1.5, 10.5 Hz, 1H, C*H*-3); 5.58 (d, *J*=14.0 Hz, 1H, CH_2-5); 5.01 (d, *J*=14.0 Hz, 1H, CH_2-5); 4.31 (dd, *J*=10.5, 14.0 Hz, 1H, CH_2-2); 3.98 (dd, *J*=1.5, 14.0 Hz, 1H, CH_2-2). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm) 152.55 (*C*H-2′); 151.85 (*C*-4′); 150.76 (*C*-6′); 143.01 (*C*H-8′); 139.32 (*C*-9a); 135.58 (*C*-5a); 134.63; 131.95 (*C*-5′); 131.32; 129.65; 128.43; 83.69 (*C*H-3); 72.09 (*C*H_2-5); 59.97 (*C*H_2-2). Anal. for $C_{14}H_{11}ClN_4O_3S$: calcd: C 47.94; H 3.16; N 15.97. Found: C 48.30; H 3.34; N 16.14.

4.3.1.12. Synthesis of (1*R,3*S**)-6′-chloro-9-(1-oxo-2,3-dihydro-5*H*-4,1-benzoxathiepin-3-yl)-9*H*-purine (23, *trans*-23).** H_2O_2, 50 wt % solution in water (0.03 mL, 0.56 mmol) was added to a solution of $Sc(OTf)_3$ (46 mg, 0.09 mmol) in a mixture of EtOH (0.5 mL) and CH_2Cl_2 (4.5 mL). Compound **15** was added after 5 min and the resulting solution was left at room temperature for 3.5 h. After this, more H_2O_2 was added (0.05 mL) and the reaction was left for 20.5 h. The mixture was diluted with CH_2Cl_2 (15 mL) and washed with H_2O (2×10 mL), the organic layer was dried (Na_2SO_4), concentrated and purified by flash chromatography using a mixture of EtOAc/MeOH, 9.9/0.1 to produce *trans*-**23** (150 mg, 96%) as an amorphous white solid; mp: 271–273 °C. HMBC and NOEdiff experiments were carried out on *trans*-**23**. ^{1}H NMR (CD_3OD, 300 MHz) δ (ppm) 8.22 (s, 1H, C*H*-8′); 8.11 (dd, *J*=1.5, 7.5 Hz, 1H, C*H*-9); 8.07 (s, 1H, C*H*-2′); 7.72 (dt, *J*=1.5, 7.2 Hz, 1H, C*H*-7); 7.65 (dt, *J*=1.5, 7.6 Hz, 1H, C*H*-8); 7.55 (dd, *J*=1.5, 7.5 Hz, 1H, C*H*-6); 6.60 (dd, *J*=1.6, 10.6 Hz, 1H, C*H*-3); 5.47 (d, *J*=14.1 Hz, 1H, CH_2-5β); 5.01 (d, *J*=14.1 Hz, 1H, CH_2-5α); 4.58 (dd, *J*=10.6, 14.5 Hz, 1H, CH_2-2α); 4.08 (dd, *J*=1.6, 14.5 Hz, 1H, CH_2-2β). ^{13}C NMR (CD_3OD, 75 MHz) δ (ppm) 158.8 (*C*-6′); 149.51 (*C*-4′); 147.18 (*C*H-2′); 141.20 (*C*-9a); 140.27 (*C*H-8′); 137.79 (*C*-5a); 135.52 (*C*H-7); 132.35 (*C*H-6); 130.37 (*C*H-8); 128.69 (*C*H-9); 125.46 (*C*-5′); 84.10 (*C*H-3); 72.43 (*C*H_2-5); 60.40 (*C*H_2-2). Anal. for $C_{14}H_{11}ClN_4O_2S$: calcd: C 50.23; H 3.31; N 16.74. Found: C 50.12; H 3.36; N 16.49.

4.3.1.13. Synthesis of (*RS*)-6′-methoxy-9-(2,3-dihydro-5*H*-4,1-benzoxathiepin-3-yl)-9*H*-purine (24). Anhydrous MeOH (2 mL) at 0 °C and under argon was added to a suspension of NaH in mineral oil (10 mg, 0.24 mmol), and the resulting solution was left at room temperature for 30 min. To this clear solution, **15** (75 mg, 0.24 mmol) was added dropwise and the mixture boiled at 90 °C for 5 h. After removing the solvent, the residue was treated with water (10 mL), then extracted with dichloromethane (3×15 mL). The combined organic phases were then dried (Na_2SO_4), filtered and evaporated under reduced pressure to yield a residue, which after flash chromatography (CH_2Cl_2/MeOH, 9.8/0.2) gave pure **24** (65 mg, 88% yield) as an amorphous white solid; mp 205–206 °C. HMBC experiments were carried out on **24**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm) 8.55 (s, 1H, C*H*-2′); 8.06 (s, 1H, C*H*-8′); 7.64 (m, 1H, C*H*-arom); 7.34 (m, 3H, C*H*-arom); 6.26 (dd, *J*=3.9, 7.5 Hz, 1H, C*H*-3); 5.16 (d, *J*=13.3 Hz, 1H, CH_2-5); 4.99 (d, *J*=13.3 Hz, 1H, CH_2-5); 4.18 (s, 3H, O*Me*); 3.27 (m, 2H, CH_2-2). ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm) 161.27 (*C*H-6′); 152.40 (*C*-2′); 150.70 (*C*H-4′); 141.98 (*C*-5a); 139.69 (*C*-8′); 135.99 (*C*-9a); 132.77; 130.10; 129.01; 128.58; 121.53 (*C*-5′); 87.47 (*C*H-3); 73.78 (*C*H_2-5); 54.34 (O*Me*); 39.19 (*C*H_2-2). Anal. for $C_{15}H_{14}N_4O_3S$: calcd: C 54.53; H 4.27; N 16.96. Found: C 54.59; H 4.51; N 16.79.

4.4. Biological activity

The biological methods were the same as those previously described.[11,24]

4.4.1. Cell lines. The cell lines IEC-6 (reference ECACC No. 88071401), HT-29 (reference ECACC No. 91072201), MCF-7 (reference ATCC No. CRL 1592) y MCF-10A (reference ATCC No. CRL 10317) were selected for the development of the different assays. All were obtained from the cell culture service of the Scientific Instrumentation Centre of the University of Granada. MCF-7 and HT-29 cells were grown at 37 °C in an atmosphere containing 5% CO_2, with Dubelcco's modified Eagle Medium (DMEM) (Gibco, Grand Island, NY) supplemented with 10% heat-inactivated foetal bovine serum (FBS) (Gibco), 2% L-glutamine, 2.7% sodium bicarbonate, 1% Hepes buffer, 40 mg/L gentamicin and 500 mg/L ampicillin. For the IEC-6 the previous medium supplemented with 0.1 IU/mL of insulin of bovine pancreas was used. For the MCF-10A, the DMEM-F12 was used, supplemented with decomplemented horse serum, 10 μg/mL of insulin of bovine pancreas, 100 ng/mL of choleric toxin, 0.5 μg/mL of hydrocortisone, 20 ng/mL of epidermis growth factor and 100 μg/mL of penicillin/streptomycin.

Acknowledgements

This study was supported by the Instituto de Salud Carlos III (Fondo de Investigación Sanitaria) through Projects No. 01/1092 and 01/928, and by the Junta de Andalucía through the Excellence Research Project No. 00636.

References and notes

1. Saniger, E.; Campos, J.; Entrena, A.; Marchal, J. A.; Súarez, I.; Aránega, A.; Choquesillo, D.; Niclós, J.; Gallo, M. A.; Espinosa, A. *Tetrahedron* **2003**, *59*, 5457.
2. Campos, J.; Saniger, E.; Marchal, J. M.; Aiello, S.; Suárez, I.; Boulaiz, H.; Aránega, A.; Gallo, M. A.; Espinosa, A. *Curr. Med. Chem.* **2005**, *12*, 1423.
3. Núñez, M. C.; Entrena, A.; Rodríguez-Serrano, F.; Marchal, J. A.; Aránega, A.; Gallo, M. A.; Espinosa, A.; Campos, J. M. *Tetrahedron* **2005**, *61*, 10363.
4. Matsuo, S.; Tanako, S.; Yamashita, J.; Ogawa, M. *Anticancer Res.* **2000**, *12*, 1575.
5. Trouet, A.; Passioukov, A.; Derpooten, K. V.; Fernandez, A. M.; Abarca-Quinones, J.; Baurian, R.; Lobl, T. J.; Oliyai, C.; Dubois, V. *Cancer Res.* **2001**, *61*, 2843.

6. For the numbering of the compounds, the atoms of the benzoxathiepin ring are tagged with numbers without primes, while those of the pyrimidine and purine bases are numbered with primes (′).
7. Functional groups possessing an sp^3 hybridized carbon bonded to oxygen and to nitrogen are known as *O,N*-acetals or hemiaminals.
8. Nomura, M.; Endo, K.; Shuto, S.; Matsuda, A. *Tetrahedron* **1999**, *55*, 14847.
9. Danel, K.; Larsen, E.; Pedersen, E. B.; Vestergaard, B. F.; Nielsen, C. *J. Med. Chem.* **1996**, *39*, 2427.
10. Bohringer, M.; Roth, H.-J.; Hunziker, J.; Gobel, M.; Rishman, R.; Giger, A.; Schweizer, B.; Schreiber, J.; Leumann, C.; Eschenmoser, A. *Helv. Chim. Acta* **1992**, *75*, 1416.
11. Gómez, J. A.; Trujillo, M. A.; Campos, J.; Gallo, M. A.; Espinosa, A. *Tetrahedron* **1998**, *54*, 13295.
12. Campos, J.; Domínguez, J. F.; Gallo, M. A.; Espinosa, A. *Curr. Pharm. Des.* **2000**, *6*, 1797.
13. Choi, W.-B.; Wilson, L. J.; Yeola, S.; Liotta, D. C. *J. Am. Chem. Soc.* **1991**, *113*, 9377.
14. Vorbrüggen, H.; Ruh-Pohlenz, C. Synthesis of Nucleosides. In *Organic Reactions*; Paquette, L. A., Ed.; Wiley: New York, NY, 2000; Vol. 55, pp 1–630.
15. Garner, P.; Ramakanth, S. *J. Org. Chem.* **1988**, *53*, 1294; *N*-7 Guanine nucleosides were obtained from silylated purines using $SnCl_4$ and MeCN at room temperature, while the *N*-9 glycosylated products were obtained from the same purines, using TMSOTf and refluxing 1,2-dichloroethane.
16. Khanapure, S. P.; Garvey, D. S.; Young, D. V.; Ezawa, M.; Earl, R. A.; Gaston, R. D.; Fang, X.; Murty, M.; Martino, A.; Shumway, M.; Trocha, M.; Marek, P.; Tam, S. W.; Janero, D. R.; Letts, L. G. *J. Med. Chem.* **2003**, *46*, 5484.
17. Matteucci, M.; Bhalay, G.; Bradley, M. *Org. Lett.* **2003**, *5*, 235.
18. Díaz-Gavilán, M.; Gómez-Vidal, J. A.; Entrena, A.; Gallo, M. A.; Espinosa, A.; Campos, J. M. *J. Org. Chem.* **2006**, *71*, 1043.
19. Gómez, J. A.; Campos, J.; Marchal, J. A.; Trujillo, M. A.; Melguizo, C.; Prados, J.; Gallo, M. A.; Aránega, A.; Espinosa, A. *Tetrahedron* **1997**, *53*, 7319.
20. Schnall, S.; Macdonald, J. S. *Manual of Oncologic Therapeutics*; Macdonald, J. S., Haller, D. G., Mayer, R. J., Eds.; J.B. Lippincott: Philadelphia, PA, 1995; pp 170–184.
21. Palomer, A.; Cabré, F.; Pascual, J.; Campos, J.; Trujillo, M. A.; Entrena, A.; Gallo, M. A.; García, L.; Mauleón, D.; Espinosa, A. *J. Med. Chem.* **2002**, *45*, 1402.
22. Campos, J. M.; Espinosa, A.; Gallo, M. A.; Gómez-Vidal, J. A.; Núñez, M. C.; Aránega, A.; Marchal, J. A.; Rodríguez-Serrano, F. Spanish Patent Application No. P200601538, 2006.
23. Wilker, W.; Leibfritz, D.; Kerssebaum, R.; Bermel, W. *Magn. Reson. Chem.* **1993**, *31*, 287.
24. Díaz-Gavilán, M.; Rodríguez-Serrano, F.; Gómez-Vidal, J. A.; Marchal, J. A.; Aránega, A.; Gallo, M. A.; Espinosa, A.; Campos, J. M. *Tetrahedron* **2004**, *60*, 11547.