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PURINE DERIVATIVES WITH HETEROCYCLIC MOIETIES AND RELATED ANALOGUES AS NEW ANTITUMOUR AGENTS

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Figure 1.cdx
Figure 2.cdx
Figure 3.cdx
Figure 4.cdx
Figure 5.cdx
Compound 4c at 30 uM-1.mp4

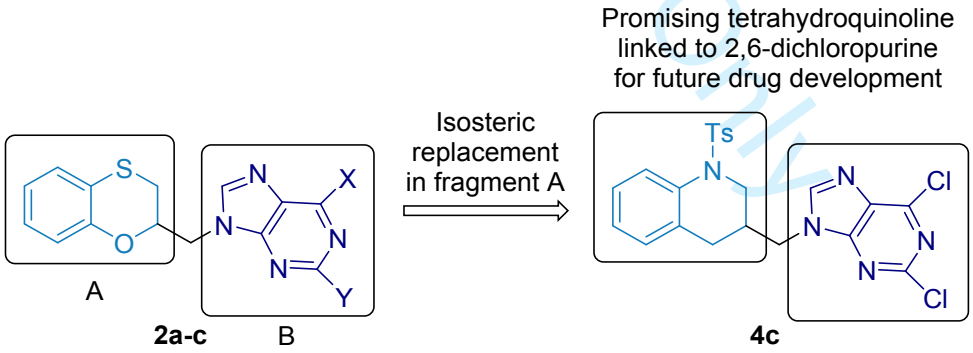
Abstract

Aim: Identification of new antiproliferative compounds.

Methodology: Four series of compounds were synthesized by the Mitsunobu reaction. Their antiproliferative activity was studied against several cancer cells and a non-cancerous fibroblast cell line. Their apoptotic activity was analyzed using a caspase 3/7 fluorescence assay.

Results & Conclusion: 9-Alkylated-6-halogenated and 2,6-dihalogenated purines show remarkable inhibition of tumour cell proliferation, with the dichloro derivatives being the most potent of all the series. The most promising compound, *tetrahydroquinoline* **4c**, exhibits significant antiproliferative activity against the cancer cells tested, while displaying a 19-fold lower potency against non-cancerous fibroblasts, a key feature that indicates potential selectivity against cancer cells. This compound produces a high percentage of apoptosis (58%) after 24h treatment in the human breast cancer MCF-7 cells.

Graphical abstract



Keywords: *benzoxazine, quinoline, pyridoxazine, Mitsunobu, antiproliferative activity, apoptosis.*

Introduction

Cancer is one of the major public health problems in the world with 17.5 million cases worldwide and 8.7 million deaths in 2015 [1]. An increase of 13.1 million cancer deaths has been estimated in 2030, according to the World Health Organization [2]. Although in recent years there have been important advances in the understanding of the underlying mechanisms leading to many cancers, some of them are still very difficult to treat and remain unmet clinical needs. Multi-drug resistance and systemic toxicity are some of the main drawbacks limiting the efficacy of current cancer treatments [3]. To tackle these limitations, the design of more effective and safer drugs is required.

It is known that many drugs activate apoptosis as a mechanism for their antitumour activity. Apoptosis is a cellular natural process whereby cells induce their own death in response to several biochemical signals that are typically triggered by an irreparable damage to DNA. It is a fundamental process of protection and maintenance of homeostasis. Generally, a group of cysteine aspartyl proteases known as caspases are responsible for the initiation (e.g. caspases 2, 8, 9 and 10) and effecting (e.g. caspases 3, 6 and 7) of apoptosis [4]. The development of drugs that induce apoptosis is an area of great activity for the discovery of new anticancer therapies [5].

Our research group has previously published several compounds that effectively induce caspase-mediated apoptosis, including a series of benzo-fused six-membered rings linked to purines through a methylene group. Substituted 9-(2,3-dihydro-1,4-benzo[*b*][1,4]oxathiin-3-ylmethyl)-9*H*-purines (**1a-c**) [6] and their isomers 9-(2,3-dihydro-1,4-benzo[*b*][1,4]oxathiin-2-ylmethyl)-9*H*-purines (**2a-c**) [7] (Figure 1) showed interesting antiproliferative activities and high apoptosis levels on the human breast adenocarcinoma MCF-7 cells.

In this article, we have introduced several structural modifications on the scaffold **2** to explore a new chemical space (Figure 1). A bioisosteric replacement was made by changing the sulfur atom of the 6-membered ring by a nitrogen one, obtaining the 2*H*-1,4-benzoxazine derivatives **3** (series A). For

synthetic reasons, in order to carry out the Mitsunobu reaction, the nitrogen atom in the heterocycle was converted into the tosylsulfonamide group. This moiety was maintained in the structure as the removal of the *o*- and *p*-nosyl group in a series of benzoxapine derivatives proved to be deleterious for the antiproliferative activity in a previous work [8] (see Figure S.1. in Supplementary Information). Additionally, the toxicity described for the NO_2 group [9, 10] led us to replace it by the *p*-CH_3 on the benzene ring (tosyl group). The influence of the distance between the heterocyclic ring and the purine was also evaluated by introducing a linker of two carbon atoms. Electron-withdrawing groups (Cl, Br, di-Cl) were used as substituents at positions 2 and 6 of the purine ring since they conferred good antiproliferative properties in previously reported derivatives **1** and **2** [6, 7].

Additionally, we decided to explore the introduction of a trifluoromethyl group (CF_3), a common substituent used in medicinal chemistry. This group offers not only high lipophilicity but also increased electron density, and is found in approved drugs such as efavirenz (for HIV disease) [11] and desoxyepothilone b (anticancer activity) [12]. Finally taking **3** as a reference scaffold, the following modifications were also introduced: (a) Elimination of the oxygen atom in the heterocycle and shortening of the side chain (derivatives **4,** *series B*); (b) change of the benzene ring for a pyridine moiety and linking the (purine-9-yl)ethyl fragment through the position 3 of the [1,4]oxazine (compounds **5**, *series C*); (c) aperture of the heterocycle to obtain open analogues, in which the tosyl group and the substituted purine moieties have been maintained (derivatives **6** and **7**, *series D*).

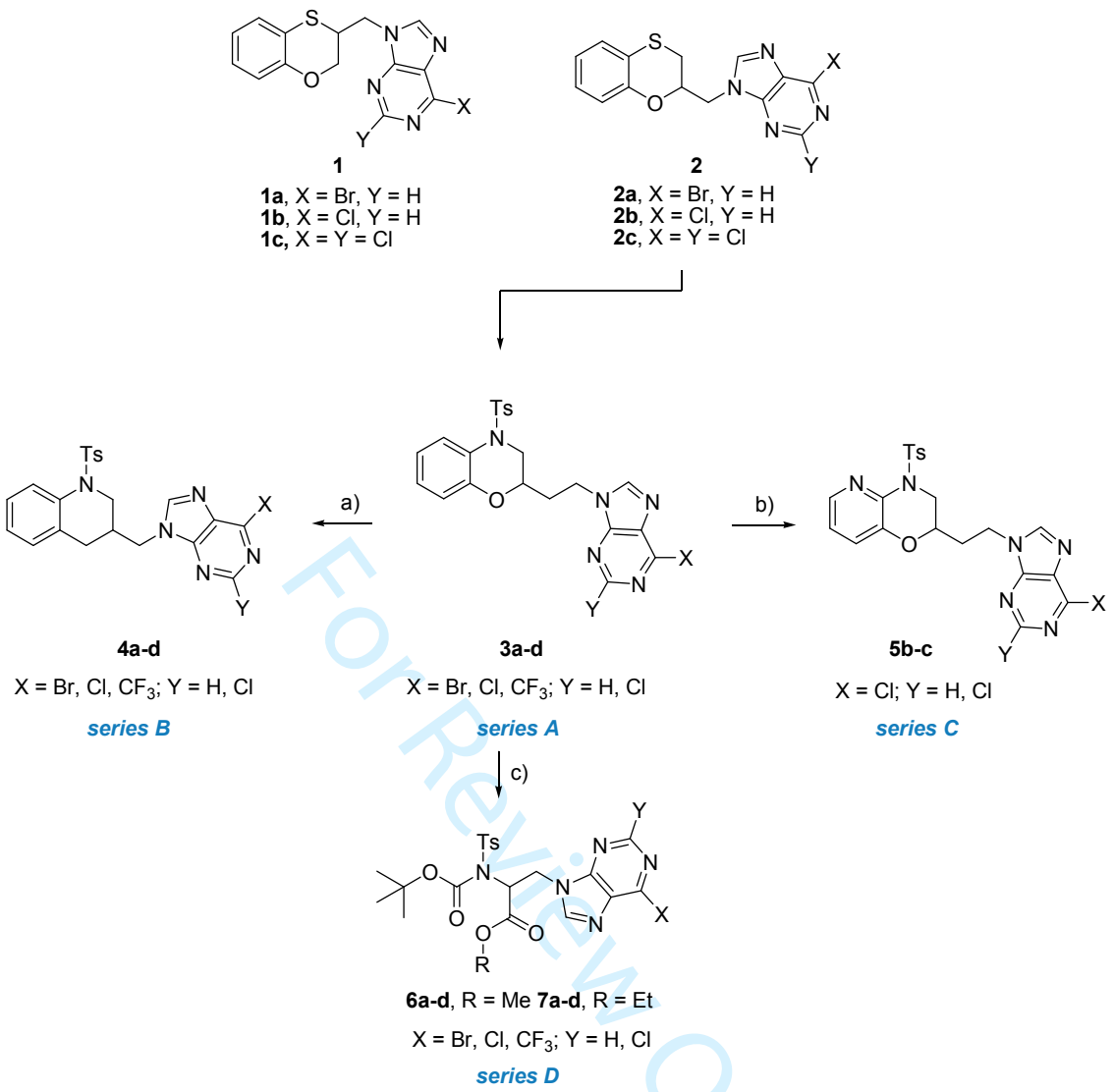


Figure 1. Benzo-fused six-membered rings linked to purines described by our research group (**1** – **2**) and chemical structure of the novel compounds objective of this article (3 – 7).

Materials and methods

Chemistry

The determination of melting points was made in open capillaries employing an Electrothermal 1A 6301 instrument and they are unrectified. Elemental analyses were designated by the element symbols, the results were not over 0.4% of the postulated values and they were carried out in a Thermo Scientific Flash 2000 analyzer. Silica (0.035-0.070 mm), 60 Å used for flash chromatography was manufactured by Acros Organic. NMR spectra were carried out on a Varian Direct Drive 400 spectrometer operating at 400 MHz for ^{1}H and 101 MHz for

^{13}C, on a Varian Direct Drive 500 spectrometer operating at 500 MHz for ^{1}H and 126 MHz for ^{13}C at room temperature (rt) in all cases. Chemicals shifts (δ) are reported in ppm and are referenced to the residual solvent peak. A VG AutoSpec Q high-resolution apparatus (Fision Instrument) was used to perform the high-resolution mass spectroscopy (HRMS). Anhydrous reactions were carried out under argon atmosphere. Reagents were purchased from Sigma-Aldrich (now Merck) and Acros Organics (part of Thermo Fisher Scientific).

Synthesis and characterization of the intermediate derivatives are presented in the Supplementary Information.

Characterization of 6-bromopurines **3a**, **4a**, **6a**, and **7a**, are described in the Supplementary Information.

Synthesis and characterization of 6-trifluoromethylpurines **3d**, **4d**, **6d** and **7d** are reported in the Supplementary Information.

General synthetic procedure of halo and dihalopurine derivatives

The tosylated derivatives **9**, **11**, **16** or **18** (0.31 mmol), Ph_3P (164.43 mg, 0.63 mmol) and the adequate halo or dihalopurine (0.34 mmol) were purged with 3 cycles of vacuum-argon exchanges in a Schlenck line previously anhydrified. Anhydrous THF was added (2 mL) under argon atmosphere and the reaction mixture was cooled to -20°C. After that, DIAD (124 µl, 0.63 mmol) was added dropwise and stirred from -20°C to rt. After 36 h the solvent was evaporated and the residue was purified by flash chromatography (EtOAc/hexane, 2:1).

Characterization of 6-chloropurines **3b***,* **4b***,* **5b***,* **6b** *and* **7b***.*

*2-(2-(6-Chloro-9*H*-purine-9-yl)ethyl)-4-tosyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine (**3b**)*

White solid (92 mg, 0.195 mmol), yield 63%, mp: 189 - 190°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.75 (s, 1H, H_{purine}), 7.96 (s, 1H, H_{purine}), 7.79 (dd, J_1 = 8.3 Hz, J_2 = 1.5 Hz, 1H, H_{benz}), 7.34 (d, J = 8.1 Hz, 2H, 2xH_{tosyl}), 7.16 – 7.00 (m, 3H, 2xH_{tosyl},H_{benz}), 7.01 – 6.88 (m, 1H, H_{benz}), 6.78 (dd, J_1 = 8.2 Hz, J_2 = 1.5 Hz, 1H, H_{benz}), 4.52 – 4.39 (m, 2H, CH_2N), 4.20 (dd, J_1 = 14.1 Hz, J_2 = 1.9 Hz, 1H, $H_{oxazine}$), 3.38 – 3.27 (m, 1H, $H_{oxazine}$), 3.21 (dd, J_1 = 14.1 Hz, J_2 = 9.7 Hz, 1H,

$H_{oxazine}$), 2.39 (s, 3H, CH_{3tosyl}), 2.35 – 2.18 (m, 1H, $C\underline{H}_2CH_2N$), 2.10 – 1.93 (m, 1H, $C\underline{H}_2CH_2N$); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 152.0 (CH_{purine}), 151.7 (C_{purine}), 151.1 (C_{purine}), 146.0 (C_{benz}), 145.2 (C_{purine}), 144.6 (C_{tosyl}), 135.3 (C_{tosyl}), 131.6 (C_{purine}), 129.7 ($2xCH_{tosyl}$), 126.9 ($2xCH_{tosyl}$), 126.3 (CH_{benz}), 124.3 (CH_{benz}), 123.5 (C_{benz}), 121.4 (CH_{benz}), 117.2 (CH_{benz}), 68.3 ($CH_{oxazine}$), 48.2 ($CH_{2oxazine}$), 40.3 (CH_2N), 31.7 ($\underline{C}H_2CH_2N$), 21.6 (CH_{3tosyl}); HRMS (ESI-TOF) (m/z) calcd. for $C_{22}H_{21}N_5O_3SCl$ $(M + H)^+$ 470.1054, found 470.1055. Anal. Calc. for $C_{22}H_{20}N_5O_3SCl$: C, 56.23; H, 4.29; N, 14.90. Found: C, 56.22; H, 4.31; N, 14.88.

*3-((6-Chloro-9*H*-purine-9-yl)methyl)-1-tosyl-1,2,3,4-tetrahydroquinoline (**4b**)*

White solid (85 mg, 0.186 mmol), yield 60%, mp: 202 - 203°C; ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.74 (s, 1H, H_{purine}), 8.04 (s, 1H, H_{purine}), 7.73 (dd, J_1 = 8.3 Hz, J_2 = 11.2 Hz, 1H, H_{benz}), 7.39 (d, J = 8.1 Hz, 2H, $2xH_{tosyl}$), 7.23 – 7.17 (m, 1H, H_{benz}), 7.12 (d, J = 8.0 Hz, 2H, $2xH_{tosyl}$), 7.10 – 7.05 (m, 1H, H_{benz}), 6.98 (dd, J_1 = 7.6 Hz, J_2 = 1.5 Hz, 1H, H_{benz}), 4.25 (dd, J_1 = 14.2 Hz, J_2 = 7.2 Hz, 1H, CH_2N), 4.15 (dd, J_1 = 14.2 Hz, J_2 = 6.7 Hz, 1H, CH_2N), 4.01 (dd, J_1 = 13.5 Hz, J_2 = 4.1 Hz, 1H, H_{pyr}), 3.45 (dd, J_1 = 13.5 Hz, J_2 = 8.9 Hz, 1H, H_{pyr}), 2.59 (dd, J_1 = 16.0 Hz, J_2 = 5.3 Hz, 1H, H_{pyr}), 2.42 – 2.37 (m, 1H, H_{pyr}), 2.36 (s, 3H, CH_{3tosyl}), 2.30 (dd, J_1 = 15.9 Hz, J_2 = 9.1 Hz, 1H, H_{pyr}); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 152.0 (CH_{purine}), 151.8 (C_{purine}), 151.3 (C_{purine}), 145.2 (CH_{purine}), 144.0 (C_{tosyl}), 136.3 (C_{benz}), 136.2 (C_{tosyl}), 131.6 (C_{purine}), 129.7 ($2xCH_{tosyl}$), 129.3 (CH_{benz}), 127.3 (C_{benz}), 127.2 (CH_{benz}), 126.8 ($2xCH_{tosyl}$), 125.2 (CH_{benz}), 124.0 (CH_{benz}), 48.5 (CH_{2pyr}), 46.7 (CH_2N), 32.8 (CH_{pyr}), 30.7 (CH_{2pyr}), 21.5 (CH_{3tosyl}); HRMS (ESI-TOF) (m/z) calcd. for $C_{22}H_{21}N_5O_2SCl$ $(M + H)^+$ 454.1104, found 454.1098. Anal. calc. for $C_{22}H_{20}N_5O_2SCl$: C, 58.21; H, 4.44; N, 15.43. Found: C, 58.19; H, 4.46; N, 15.45.

*3-(2-(6-Chloro-9*H*-purine-9-yl)ethyl)-4-tosyl-3,4-dihydro-2*H*-pyrido[3,2-b][1,4]oxazine (**5b**)*

Yellowish solid (80 mg, 0.171 mmol), yield 55%, mp: 180 - 181°C; ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.73 (s, 1H, H_{purine}), 8.30 (s, 1H, H_{purine}), 8.02 (dd, J_1 = 4.7 Hz, J_2 =1.5 Hz, 1H, H_{benz}), 7.84 (d, J = 8.1 Hz, 2H, $2xH_{tosyl}$), 7.25 (d, J = 8.0 Hz,

2H, 2xH_{tosyl}), 7.15 (dd, J_1 = 8.0 Hz, J_2 = 1.5 Hz, 1H, H_{benz}), 6.96 (dd, J_1 = 8.0 Hz, J_2 = 4.6 Hz, 1H, H_{benz}), 4.80 – 4.75 (m, 1H, $H_{oxazine}$), 4.61 – 4.43 (m, 2H, CH_2N), 4.18 (dd, J_1 = 11.4 Hz, J_2 = 1.4 Hz, 1H, $H_{oxazine}$), 3.77 (dd, J_1 = 11.4 Hz, J_2 = 2.3 Hz, 1H, $H_{oxazine}$), 2.49 – 2.39 (m, 1H, $C\underline{H}_2CH_2N$), 2.38 (s, 3H, CH_{3tosyl}), 2.20 – 2.09 (m, 1H, $C\underline{H}_2CH_2N$); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 151.8 (C_{purine}), 151.7 (CH_{purine}), 151.0 (C_{purine}), 146.0 (CH_{purine}), 144.4 (C_{tosyl}), 140.9 (CH_{benz}), 140.3 (C_{benz}), 137.1 (C_{benz}), 136.2 (C_{tosyl}), 131.8 (C_{purine}), 129.4 (2xCH_{tosyl}), 128.2 (2xCH_{tosyl}), 124.7 (CH_{benz}), 120.4 (CH_{benz}), 67.0 (CH_{2pyr}), 51.2 (C_{pyr}), 41.6 (CH_2N), 30.8 ($\underline{C}H_2CH_2N$), 21.5 (CH_{3tosyl}); HRMS (ESI-TOF) (m/z) calcd. for $C_{21}H_{20}N_6O_3SCl$ $(M + H)^+$ 471.1006, found 471.0981. Anal. calc. for $C_{21}H_{19}ClN_6O_3S$: C, 53.56; H, 4.07; N, 17.85. Found: C, 53.54; H, 4.09; N, 17.88.

*Methyl-2-(*N-ter*t-butoxycarbonyl)*-N-*tosylamino)-3-(6-chloro-9*H*-purine-9-yl)propanoate (**6b**)*

White solid (110 mg, 0.217 mmol), yield 70%, mp: 164 - 165°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.67 (s, 1H, H_{purine}), 8.20 (s, 1H, H_{purine}), 7.59 (d, J = 8.1 Hz, 2H, 2xH_{tosyl}), 7.18 (d, J = 8.1 Hz, 2H, 2xH_{tosyl}), 5.58 (dd, J_1 = 9.4 Hz, J_2 = 4.9 Hz, 1H, H_{prop}), 5.05 (dd, J_1 = 14.7 Hz, J_2 = 4.9 Hz, 1H, H_{prop}), 4.95 (dd, J_1 = 14.7 Hz, J_2 = 9.4 Hz, 1H, H_{prop}), 3.83 (s, 3H, OCH_3), 2.41 (s, 3H, CH_{3tosyl}), 1.34 (s, 9H, 3xCH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 167.8 (COOMe), 152.0 (C_{purine}), 151.9 (CH_{purine}), 150.9 (C_{purine}), 149.8 ($COBu^t$), 145.5 (CH_{purine}), 144.8 (C_{tosyl}), 135.6 (C_{tosyl}), 131.3 (C_{purine}), 129.0 (2xCH_{tosyl}), 128.1 (2xCH_{tosyl}), 86.1 ($\underline{C}(CH_3)_3$), 58.1 (CH_{prop}), 53.0 (OCH_3), 43.4 (CH_{2prop}), 27.6 ($C(\underline{C}H_3)_3$), 21.5 (CH_{3tosyl}); HRMS (ESI-TOF) (m/z) calcd. For $C_{21}H_{25}N_5O_6SCl$ $(M + H)^+$ 510.1214, found 510.1215. Anal. calc. for $C_{21}H_{24}N_5O_6SCl$: C, 49.46; H, 4.74; N, 13.73. Found: C, 49.48; H, 4.74; N, 13.71.

*Ethyl-2-(*N-tert-*butoxycarbonyl)*-N-*tosylamino)-3-(6-chloro-9*H*-purine-9-yl)propanoate (**7b**)*

White solid (146 mg, 0.279 mmol), yield 90%, mp: 175 - 176°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.71 (s, 1H, H_{purine}), 8.18 (s, 1H, H_{purine}), 7.62 (d, J = 8.2 Hz, 2H, 2xH_{tosyl}), 7.19 (d, J = 8.2 Hz, 2H, 2xH_{tosyl}), 5.55 (dd, J_1 = 9.2, J_2 = 5.0 Hz, 1H, H_{prop}), 5.03 – 4.97 (m, 2H, H_{prop}), 4.27 (q, J = 7.2 Hz, 2H, $OC\underline{H}_2CH_3$),

2.43 (s, 3H, CH_{3tosyl}), 1.33 (s, 9H, 3xCH_3), 1.26 (t, *J* = 7.3 Hz, 3H, $OCH_2C\underline{H}_3$); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 167.3 (COOEt), 152.1 (C_{purine}), 151.8 (CH_{purine}), 150.9 (C_{purine}), 149.8 ($COOBu^t$), 145.4 (CH_{purine}), 144.7 (C_{tosyl}), 135.7 (C_{tosyl}), 131.4 (C_{purine}), 128.9 (2xCH_{tosyl}), 128.0 (2xCH_{tosyl}), 85.9 ($\underline{C}(CH_3)_3$), 62.3 ($O\underline{C}H_2CH_3$), 58.1 (CH_{prop}), 43.3 (CH_{2prop}), 27.6 ($C(\underline{C}H_3)_3$), 21.4 (CH_{3tosyl}), 13.8 ($OCH_2\underline{C}H_3$); HRMS (ESI-TOF) (m/z) calcd. for$C_{22}H_{27}N_5O_6SCl$ $(M + H)^+$ 524.1371, found 524.1370. Anal. Calc. for $C_{22}H_{26}N_5O_6SCl$: C, 50.43; H, 5.00; N, 13.37. Found: C, 50.41; H, 5.03; N, 13.35.

Characterization of 2,6-dihalopurines **3c**, **4c**, **5c**, **6c** *and* **7c**.

*2-(2-(2,6-Dichloro-9*H*-purine-9-yl)ethyl)-4-tosyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine (**3c**)*

White solid (91 mg, 0.221 mmol), yield 68%, mp: 195-196°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.96 (s, 1H, H_{purine}), 7.77 (dd, J_1 = 8.3 Hz, J_2 = 1.6 Hz, 1H, H_{purine}), 7.40 (d, *J* = 8.2 Hz, 2H, 2×H_{tosyl}), 7.16 – 7.02 (m, 3H, 2×H_{tosyl}, H_{benz}), 7.01 – 6.88 (m, 1H, H_{benz}), 6.76 (dd, J_1 = 8.2 Hz, J_2 = 1.6 Hz, 1H, H_{benz}), 4.46 - 4.41 (m, 2H, CH_2N), 4.19 (dd, J_1 = 14.2 Hz, J_2 = 2.3 Hz, 1H, $H_{oxazine}$), 3.42 – 3.29 (m, 1H, $H_{oxazine}$), 3.28 – 3.13 (m, 1H, $H_{oxazine}$), 2.39 (s, 3H, CH_{3tosyl}), 2.30 – 2.14 (m, 1H, $C\underline{H}_2CH_2N$), 2.09 – 1.97 (m, 1H, $C\underline{H}_2CH_2N$); ^{13}C NMR (101 MHz, $CDCl_3$):δ (ppm) 153.2 (2×Cpurine), 151.9 (C_{Purine}) 146.1 (C_{Purine}), 146.0 (C_{benz}), 144.8 (C_{tosyl}), 135.5 (C_{tosyl}), 130.9 (C_{purine}), 129.9 (2×CH_{tosyl}), 127.1 (2×CH_{tosyl}), 126.4 (CH_{benz}), 124.3 (CH_{benz}), 123.7 (C_{benz}),, 121.6, (CH_{benz}), 117.4 (CH_{benz}), 68.7 ($CH_{oxazine}$), 48.3 ($CH_{2oxazine}$), 40.6 (CH_2N), 31.9 ($\underline{C}H_2CH_2N$), 21.8 (CH_{3tosyl}); HRMS (ESI-TOF) (m/z) calcd. for $C_{22}H_{20}N_5O_3SCl_2$ $(M + H)^+$ 504,0586, found 504,0636; Anal. Calc. for $C_{22}H_{19}N_5O_3SCl_2$: C, 52.39; H, 3.80; N, 13.89. Found: C, 52.37; H, 3.82; N, 13.91.

*3-((2,6-Dichloro-9*H*-purine-9-yl)methyl)-1-tosyl-1,2,3,4-tetrahydroquinoline (**4c**)*

White solid (102 mg, 0.232 mmol), yield 75%, mp: 207-208°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.07 (s, 1H, H_{purine}), 7.69 (dd, J_1 = 8.3 Hz, J_2 =1.1 Hz, 1H, H_{benz}), 7.38 (d, *J* = 8.0 Hz, 2H, 2×H_{tosyl}), 7.21 – 7.12 (m, 3H, H_{benz}, 2×H_{tosyl}), 7.08 – 7.03 (m, 1H, H_{benz}), 6.97 (dd, J_1 = 7.6 Hz, J_2 = 1.6 Hz, 1H, H_{benz}), 4.20 (dd, J_1

= 14.1 Hz, J_2 = 7.3 Hz, 1H, CH_2N), 4.12 (dd, J_1 = 14.2 Hz, J_2 = 6.7 Hz, 1H, CH_2N), 3.98 (dd, J_1 = 13.5 Hz, J_2 = 3.8 Hz, 1H, H_{pyr}), 3.42 (dd, J_1 = 13.5 Hz, J_2 = 8.7 Hz, 1H, H_{pyr}), 2.59 (dd, J_1 = 15.9 Hz, J_2 = 5.2 Hz, 1H, H_{pyr}), 2.39 – 2.37 (m, 1H, H_{pyr}), 2.35 (s, 3H, CH_{3tosyl}), 2.31 (dd, J_1 = 15.9 Hz, J_2 = 9.0 Hz, 1H, H_{pyr}); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 153.0 (C_{purine}), 152.9 (C_{purine}), 151.8 (C_{purine}), 146.0 (C_{purine}), 144.0 (C_{tosyl}), 136.1 (C_{benz}), 135.9 (C_{tosyl}), 130.6 (C_{purine}), 129.6 (2xCH_{tosyl}), 129.3 (CH_{benz}), 127.0 (C_{benz}), 126.9 (CH_{benz}), 126.7 (2xCH_{tosyl}), 125.1 (CH_{benz}), 123.6 (CH_{benz}), 48.2 (CH_{2pyr}), 46.7 (CH_2N), 32.3 (CH_{pyr}), 30.4 (CH_{2pyr}), 21.4 (CH_{3tosyl}); HRMS (ESI-TOF) (m/z) calcd. for $C_{22}H_{20}N_5O_2SCl_2$ $(M + H)^+$ 488.0637, found 488.0640. Anal. Calc. for $C_{22}H_{19}Cl_2N_5O_2S$: C, 54.11; H, 3.92; N, 14.34. Found: C, 54.13; H, 3.90; N, 14.32.

*3-(2-(2,6-Dichloro-9*H*-purine-9-yl)ethyl)-4-tosyl-3,4-dihydro-2*H*-pyrido[3,2-b][1,4]oxazine (**5c**)*

White solid (75 mg, 0.198 mmol), yield 64%, mp: 185 - 186°C; ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.30 (s, 1H, H_{purine}), 8.02 (dd, J_1 = 4.6 Hz, J_2 = 1.5 Hz, 1H, H_{benz}), 7.85 – 7.80 (m, 2H, 2×H_{tosyl}), 7.28 - 7.25 (m, 2H, 2×H_{tosyl}), 7.15 (dd, J_1 = 8.1 Hz, J_2 = 1.5 Hz, 1H, H_{benz}), 6.96 (dd, J_1 = 8.0 Hz, J_2 = 4.6 Hz, 1H, H_{benz}), 4.81 – 4.73 (m, 1H, $H_{oxazine}$), 4.56 – 4.49 (m, 1H, CH_2N), 4.47 – 4.40 (m, 1H, CH_2N), 4.18 (dd, J_1 = 11.4 Hz, J_2 = 1.4 Hz, 1H, $H_{oxazine}$), 3.75 (dd, J_1 = 11.4 Hz, J_2 = 2.4 Hz, 1H, $H_{oxazine}$), 2.49 – 2.39 (m, 1H, $C\underline{H}_2CH_2N$), 2.38 (s, 3H, CH_{3tosyl}), 2.16 – 2.09 (m, 1H, $C\underline{H}_2CH_2N$); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 153.1 (C_{purine}), 152.6 (C_{purine}), 151.7 (C_{purine}), 146.7 (CH_{purine}), 144.5 (C_{tosyl}), 140.9 (CH_{benz}), 140.3 (C_{benz}), 137.0 (C_{benz}), 136.0 (C_{tosyl}), 131.9 (C_{purine}), 129.4, (2×CH_{tosyl}), 128.2 (2×CH_{tosyl}), 124.8 (CH_{benz}), 120.5 (CH_{benz}), 66.9 (CH_{2pyr}), 51.1 (C_{pyr}), 41.7 (CH_2N), 30.6 ($\underline{C}H_2CH_2N$), 21.5 (CH_{3tosyl}); HRMS (ESI-TOF) (m/z) calcd. for $C_{21}H_{19}N_6O_3SCl_2$ $(M + H)^+$ 505.0859, found 505.0823; Anal. calc. for $C_{21}H_{18}Cl_2N_6O_3S$: C, 49.91; H, 3.59; N, 16.63. Found: C, 49.93; H, 3.61; N, 16.62.

*Methyl-2-(*N*-tert-butoxycarbonyl)-*N*-tosylamino)-3-(2,6-dichloro-9*H*-purine-9-yl)propanoate (**6c**)*

White solid (101 mg, 0.186 mmol), yield 60%, mp: 170-171°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.21 (s, 1H, H_{purine}), 7.59 (d, J = 8.1 Hz, 2H, 2×H_{tosyl}), 7.16 (d, J = 8.1 Hz, 2H, 2×H_{tosyl}), 5.47 (dd, J_1 = 9.4 Hz, J_2 = 4.7 Hz, 1H, H_{prop}), 4.98 – 4.81 (m, 2H, H_{prop}), 3.78 (s, 3H, OCH_3), 2.37 (s, 3H, CH_{3tosyl}), 1.31 (s, 9H, 3×CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 168.2 (COOMe), 153.9 (C_{purine}), 153.3 (C_{purine}), 151.8 (C_{purine}), 150.1 (COOBu^t), 146.7 (CH_{purine}), 145.3 (C_{tosyl}), 135.9 (C_{tosyl}), 130.9 (C_{purine}), 129.4 (2×CH_{tosyl}), 128.4 (2×CH_{tosyl}), 86.7 ($\underline{C}(CH_3)_3$), 58.7 (CH_{prop}), 53.4 (OCH_3), 43.9 (CH_{2prop}), 28.1 ($C(\underline{C}H_3)_3$), 21.9 (CH_{3tosyl}); HRMS (ESI-TOF) (m/z) calcd. for $C_{21}H_{24}N_5O_6SCl_2$ $(M + H)^+$ 544.0824, found 544.0855; Anal. calc. for $C_{21}H_{23}N_5O_6SCl_2$: C, 46.33; H, 4.26; N, 12.86. Found: C, 46.31; H, 4.27; N, 12.83.

*Ethyl-2-(*N-tert-*butoxycarbonyl)*-N-*tosylamino)-3-(2,6-dichloro-9*H-*purine-9-yl)propanoate (**7c**)*

White solid (95 mg, 0.170 mmol), yield 55%, mp: 182-183°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.19 (s, 1H, H_{purine}), 7.68 (d, J = 8.2 Hz, 2H, 2×H_{tosyl}), 7.22 (d, J = 8.2 Hz, 2H, 2×H_{tosyl}), 5.47 (dd, J_1 = 9.2 Hz, J_2 = 4.8 Hz, 1H, H_{prop}), 5.04 – 4.84 (m, 2H, H_{prop}), 4.28 (q, J = 6.8 Hz, 2H, $OC\underline{H}_2CH_3$), 2.42 (s, 3H, CH_{3tosyl}), 1.37 (s, 9H, 3×CH_3), 1.27 (t, J = 6.8 Hz, 3H, $OCH_2C\underline{H}_3$);^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 167.2 (COOEt), 153.5 (C_{purine}), 153.0 (C_{purine}), 151.6 (C_{purine}), 149.8 (COOBu^t), 146.1 (CH_{purine}), 144.8 (C_{tosyl}), 135.7 (C_{tosyl}), 130.6 (C_{purine}), 129.0 (2×CH_{tosyl}), 128.1 (2×CH_{tosyl}), 86.2, ($\underline{C}(CH_3)_3$), 62.4 ($O\underline{C}H_2CH_3$), 58.3 (CH_{prop}), 43.5 (CH_{2prop}), 27.7 ($C(\underline{C}H_3)_3$), 21.5 (CH_{3tosyl}); 13.9 ($OCH_2\underline{C}H_3$); HRMS (ESI-TOF) (m/z) calcd. for $C_{22}H_{26}N_5O_6SCl_2$ $(M + H)^+$ 558.0981, found 558.0972; Anal. calc. for $C_{22}H_{25}N_5O_6SCl_2$: C, 47.32; H, 4.51; N, 12.54. Found: C, 47.35; H, 4.50; N, 12.52.

Biology

Tissue culture

Cell lines were maintained in a tissue culture incubator with 5% CO_2 at 37ºC and grown in culture media complemented with 10% fetal bovine serum (FBS) and L-glutamine (2 mM). Human breast adenocarcinoma MCF-7 cells (purchased from ATCC), human melanoma A-375 (a kind gift from Dr Liz

Patton) and RFP TERT immortalized fibroblasts [13] were cultured in Dulbecco's Modified Eagle Media (DMEM). Human colorectal carcinoma HCT-116 cells (a kind gift from Prof Mark Arends) were cultured in McCoy's 5A Medium.

Dose−Response Viability Assay

All final products were preserved at -20ºC dissolved in DMSO. In order to obtain the desired concentrations in each experiment, the stock solutions (100 mM) were successively diluted in culture media. 96-Well plates were used to seed the cells at 1,000 cells/well for A-375; 1,500 cells/well for MCF-7 and HCT-116 and 2000 cells/well for RFP TERT immortalized fibroblasts. After 48 h of incubation, cells were treated with compounds **3a-d**, **4a**-**d**, **5b**-**c**, **6a-d** and **7a-d** (0.01 μM – 100 μM) and incubated for 5 d. Each experiment was performed in triplicate. Cells treated with DMSO (0.1 % v/v) were used as control. After 5d treatment, cell viability was determined using PrestoBlue™ reagent (10 % v/v) and results analyzed as previously described [14]. EC_{50} (half-maximal effective concentration) values are expressed as mean ± SD of 3 independent experiments.

Apoptosis Assay

Nunc black optical bottom plates acquired from Thermo Scientific were used to seed MCF-7 cells at 1500 cells/well. After 48h of incubation, NucView 488 substrate (Biotium) at 1 μM concentration in culture media was added to each well and cells treated either with DMSO or compounds **3c**, **4c**, **4d** and **5c** at 3, 10 and 30 μM. Plates were imaged in an IncuCyte™ ZOOM system for 5d and cell confluence and apoptotic counts determined with the IncuCyte software as previously described [15].

Results and discussion

Chemistry

The synthetic routes of all compounds included in four series are outlined in Figures 2 – 5. Derivatives **3a-c** enclosed in the first series have been synthesized from 2-aminofenol and ethyl 4-bromobut-2-enoate to give the ethyl 2-(3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine-2-yl)acetate [16], that was reduced with $LiAlH_4$ to give alcohol **8**. This alcohol was tosylated to give derivative **9**, which

was treated with different purines under Mitsunobu conditions to produce the final molecules **3a-c**. Finally, the 6-Br group of **3a** was substituted by 6-CF_3 to obtain **3d** [17] (Figure 2).

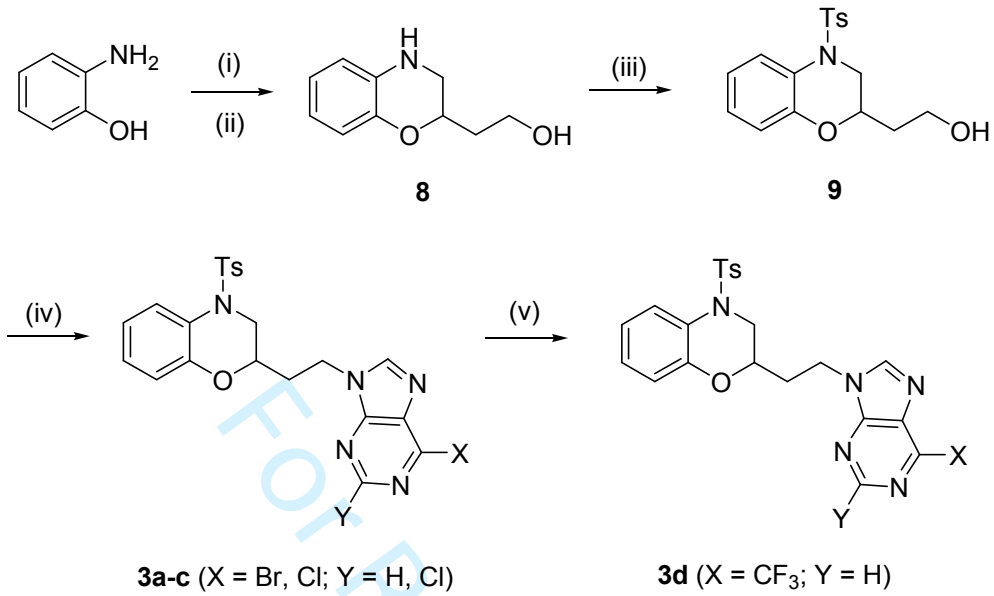


Figure 2. Synthesis of compounds included in *series A*. *Reagents and conditions*: (i) ethyl 4-bromobut-2-enoate, $NaHCO_3$, EtOH, 3 h, rt, then K_2CO_3, 30 min; (ii) $LiAlH_4$, Et_2O, 1 h, 0ºC to rt; (iii) TsCl, py, 12 h, 0ºC to rt; (iv) 6-halopurine or 2,6-dihalopurine, DIAD, Ph_3P, 36 h, -20ºC to rt; (v) MFSDA, CuI, HMAP, DMF, 12 h, 70ºC.

Figure 3 shows the synthetic route followed for compounds **4a-d** included in *series B*. These compounds have been synthesized from quinoline-3-carbonitrile, which is transformed into quinoline-3-carboxilic acid by treatment with aq. NaOH and acidified with HCl 1N [18]. The carboxylic acid is esterified to ethyl quinoline-3-carboxilate [19] and then partially reduced to the ethyl 1,2,3,4-tetrahydroquinoline-3-carboxilate using sodium cyanoborohydride [18]. Reduction of the ester function to the corresponding alcohol **10** [16] and subsequent tosylation with *p*-toluensulfonyl chloride gave (1,2,3,4-tetrahydro-1-tosylquinoline-3-yl)methanol **11**. Final compounds **4a-c** were obtained by Mitsunobu reaction with DIAD and the corresponding purines (6-bromo-, 6-chloro-, and 2,6-dichloro-purines). Finally, derivative **4d** was synthesized from bromopurine **4a**, as in the previous series, using MFSDA, CuI and HMPA [17].

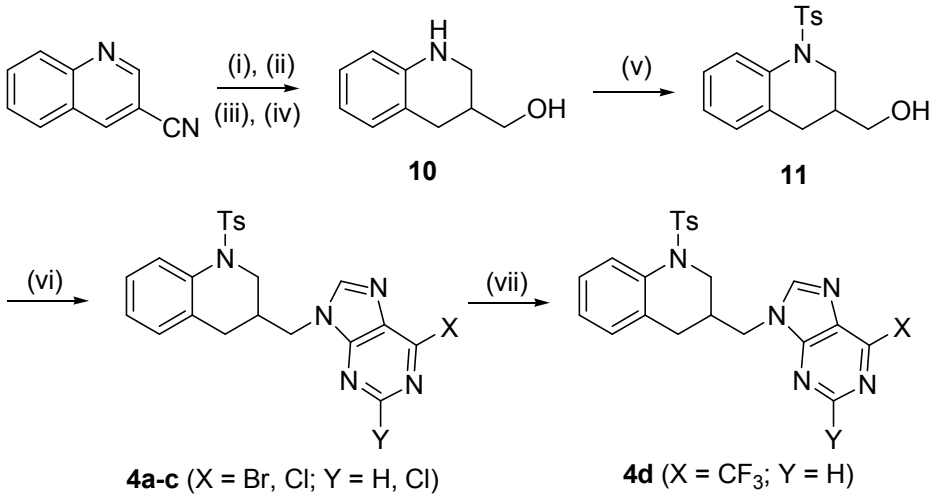


Figure 3. Synthesis of compounds included in *series B*. *Reagents and conditions*: (i) aq. NaOH, EtOH, 20h, reflux, then HCl 1N; (ii) $SOCl_2$, EtOH, 4h, reflux; (iii) $NaBH_3CN$, THF, MeOH, HCl, Et_2O, 6h, rt; (iv) $LiAlH_4$, THF, 1h, 0ºC; (v) TsCl, py, 12h, 0ºC to rt; (vi) 6-halopurine or 2,6-dihalopurine, DIAD, Ph_3P, 36h, -20ºC to rt; (vii) MFSDA, CuI, HMAP, DMF, 12h, 70ºC.

The synthesis of series C is shown in Figure 4. Firstly, 2-aminopyridine-3-ol was acetylated and then, cyclized with ethyl 4-bromobut-2-enoate to give **12**, which was reduced to the alcohol **13** and then silylated to **14**. This compound was tosylated with *p*-toluenesulfonyl chloride to **15** and the silyl group was removed to give the alcohol **16**. The last step includes the Mitsunobu reaction with 6-chloro- and 2,6-dichloro-purines to lead to **5b-c**.

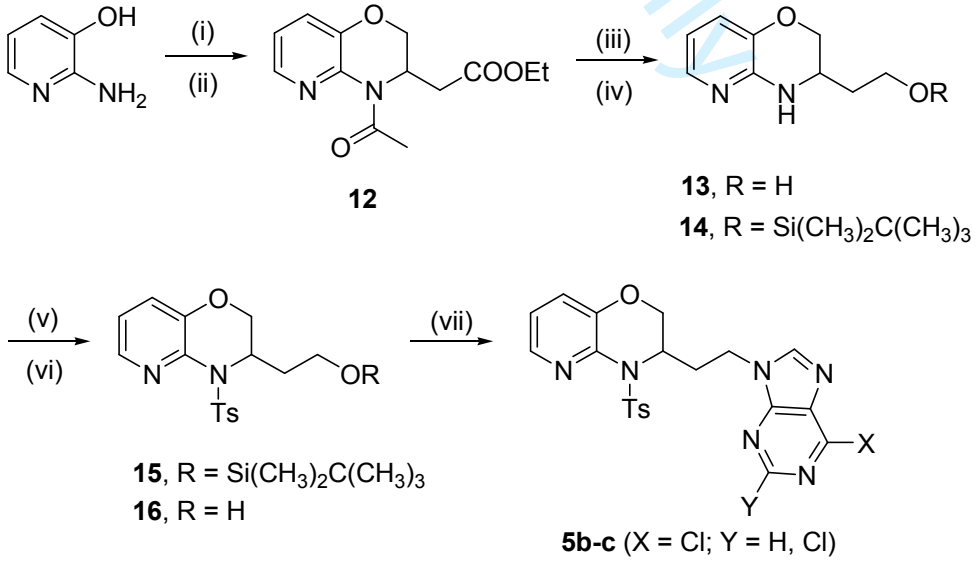


Figure 4. Synthesis of compounds included in *series C*. *Reagents and conditions*: (i) Ac_2O/py, 5min, reflux, then NaOH; (ii) ethyl 4-bromobut-2-enoate,

K_2CO_3, EtOH, 24h; (iii) $LiAlH_4$, THF, 1h, 0ºC to rt; (iv) TBDMSCl, Et_3N, DMAP, DCM, 12h, rt; (v) TsCl, Et_3N, DMAP, DCM, 12h, rt; (vi) AcOH, H_2O, THF, 12h, rt; (vii) 6-chloropurine or 2,6-dichloropurine, DIAD, Ph_3P, -20ºC to rt, 36h.

Finally, compounds **6a-d** and **7a-d** were synthesized as shown in Figure 5 from D/L-serine, which is tosylated [20], then esterified with methanol or ethanol [21] and silylated to compounds **17a-b** [22]. Subsequent protection of the amine with Boc anhydride [23], followed by removal of the silyl group [24] leads to the alcohols **18a-b**, which are transformed into the last derivatives **6a-c** and **7a-c** by the Mitsunobu reaction with the appropriate purines. Finally, **6a** and **7a** were converted into **6d** and **7d** as previously described.

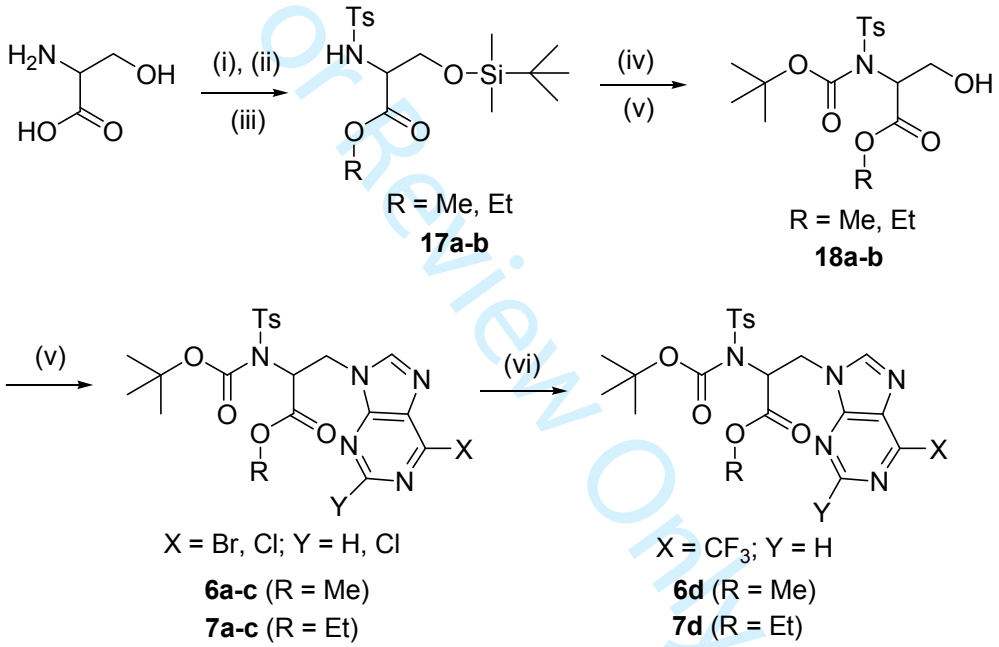


Figure 5. Synthesis of compounds included in *series D*. *Reagents and conditions*: (i) TsCl, 2N NaOH, 12h, rt; (ii) $SOCl_2$, ROH, 1h, 0ºC, then 12h rt; (iii) TBDMSCl, Et_3N, DMAP, DCM, 2h, rt; (iv) $(Boc)_2O$, DMAP, Et_3N, DCM, 2h; (v) AcOH, H_2O, THF, 12h, rt; (vi) 6-halo- or 2,6-dihalo-purines, DIAD, Ph_3P, 36h, -20ºC to rt; (vii) MFSDA, CuI, HMAP, DMF, 12h, 70ºC.

Biological evaluation

Breast adenocarcinoma MCF-7 [25], colorectal carcinoma HCT-116 [26] and melanoma A-375 [27] are human cell lines that have been widely used as experimental cell models in drug discovery. These 3 types of cancers are

nowadays in the top-20 of the most deadly ones, therefore the identification of new treatments is required.

We first analysed the antiproliferative properties of the 18 synthesized compounds against MCF-7 cell line. Those structures that exhibited an EC_{50} value lower than 50 μM were further investigated in A-375 and HCT-116 cells. The results of this assessment are shown in Table 1. 5-Fluorouracil (5-FU) has been used as a control for the breast and colon carcinoma cell lines. Today this drug and its derivatives such as capecitabine, are widely used in different solid tumours despite its high toxicity and side effects [28, 29].

In general, 6-halo, 2,6-dihalo and 6-trifluoromethyl groups on the 9-alkylated purine show a notable inhibition on cell proliferation, with the dichloro analogue being the most interesting in each of the series and tumour cell lines. This data are in agreement with the results previously published by our research group where the highest antiproliferative activity were obtained with electron-withdrawing groups in the purine ring [7], specifically derivatives bearing two chloro atoms at 2 and 6 positions. Accordingly, the most active compounds in MCF-7 cell line are the 9-alkylated 2,6-disubstituted purines with two chlorine atoms, with **4c** being the most potent inhibitor (EC_{50} = 4.26 ± 0.15 μM), only slightly less active than the positive control 5-FU. Compounds **4c-d** (within *series B*), which feature a tetrahydroquinoline heterocycle, exhibited higher activity than their isosteric benzoxazines **3c-d** (*series A*). The open structures included in *series D* (**6a-d** and **7a-d**) mediated no or low inhibition of cell proliferation. Only the most lipophilic derivative, **7c** (R = Et; X = Cl, Y = Cl), showed some level of activity. This might be due to a reduced capacity of the open structures to penetrate the cell membrane.

Regarding the A-375 cell line, derivatives with a tetrahydroquinoline (**4c**) and pyridoxazine (**5c**) heterocycle show similar inhibition values (EC_{50} = 5.54 ± 0.64 and 5.66 ± 0.65 μM, respectively). In this cell line there is not much difference of activity between the 6-chloro (**3b**) and 2,6-dichloro (**3c**) derivatives from *series A*.

The best inhibition values were obtained in the carcinoma colon line HCT-116 where the 2,6-dicloro derivatives **3c** (7.06 ± 0.80 μM), **4c** (2.80 ± 0.31 μM) and **5c** (3.13 ± 0.35 μM) mediated high activity. **4c**, the most potent derivative of the series, showed an activity comparable to the reference compound 5-FU. Interestingly, in this tumour cell line the open derivative with a methyl carboxylate moiety (**6c**) shows a better inhibition than the one with an ethyl carboxylate group, unlike the other two cell lines.

Generally, in all derivatives it is observed that the oxygen atom of the benzoxazine ring (derivatives **3**) is not essential for the antitumour activity since when it was eliminated (derivatives **4**) compounds show better inhibition values. Furthermore, if we compare derivatives **3** and **5**, the substitution of the benzene ring by its isosteric pyridine, as well as the change of the side chain position in the condensate system, have led to an improvement in the activity. Finally, the presence of a heterocyclic system is very important for the antitumour inhibition since compounds are less active when it is not present (open derivatives **6** and **7**).

Table 1. In vitro anticancer activity of final compounds against MCF-7, A-375 and HCT-116 tumour cells.

Comp	X	Y	R	Cancer cell lines		
				MCF-7 EC_{50} (μM)[a]	A-375 EC_{50} (μM)[a]	HCT-116 EC_{50} (μM)[a]
3a	Br	H	-	27.45 ± 0.18	26.11 ± 3.01	17.30 ± 2.67
3b	Cl	H	-	37.63 ± 4.85	13.32 ± 0.43	17.54 ± 0.86
3c	Cl	Cl	-	13.00 ± 0.11	10.85 ± 1.40	7.06 ± 0.80
3d	CF_3	H	-	33.61 ± 3.62	34.59 ± 2.80	56.06 ± 2.66
4a	Br	H	-	> 100	-	-
4b	Cl	H	-	> 100	-	-
4c	Cl	Cl	-	4.26 ± 0.15	5.54 ± 0.64	2.80 ± 0.31
4d	CF_3	H	-	21.95 ± 1.7	61.57 ± 0.67	60.14 ± 5.35
5b	Cl	H	-	> 100	-	-
5c	Cl	Cl	-	11.56 ± 0.28	5.66 ± 0.65	3.13 ± 0.35
6a	Br	H	Me	> 100	-	-
6b	Cl	H	Me	> 100	-	-

6c	Cl	Cl	Me	45.96 ± 5.69	20.48 ± 2.40	22.20 ± 1.57
6d	CF_3	H	Me	> 100	-	-
7a	Br	H	Et	> 100	-	-
7b	Cl	H	Et	> 100	-	-
7c	Cl	Cl	Et	26.15 ± 3.22	17.62 ± 1.90	38.32 ± 2.01
7d	CF_3	H	Et	> 100	-	-
5-FU				1.5 ± 0.31	-	2.40 ± 0.62

[a]Cell viability was measured after 5 days treatment using the PrestoBlue™ reagent. Experiments were conducted in triplicate. Data are the mean ± SD of 3 independent determinations.

To analyze if the observed growth inhibition was due to an apoptotic effect, NucView™ 488 caspase-3 substrate was used to assess the rate of caspase-3/7 mediated apoptosis in MCF-7 cells once treated with derivatives **3c**, **4c**, **4d** and **5c**. This fluorescent probe contains a peptide sequence (DEVD) that after cleaved by caspase-3/7 activity releases a DNA-binding dye which stains the cell nucleus bright green.

As shown in Figure 6, image-based measurement of caspase 3/7 activity demonstrated significant levels of apoptotic cell death in a time-dependent manner in comparison with the control DMSO.

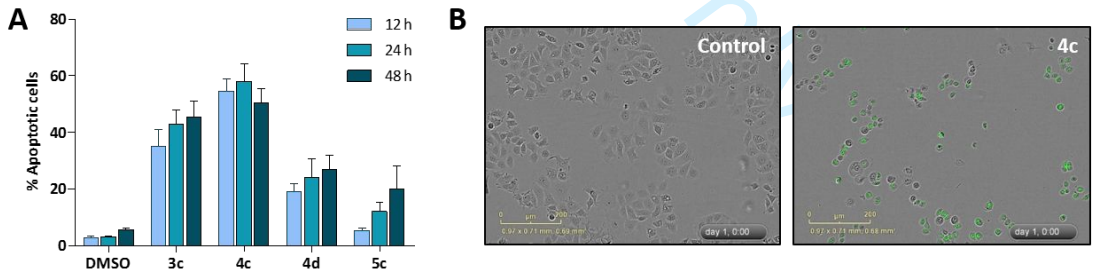


Figure 6. (A) Percentage of apoptotic cells after treatment with compounds **3c**, **4c**, **4d** and **5c** (30 μM) for 12h (blue), 24 h (light green) and 48h (dark green). **(B)** Representative images of MCF-7 cells stained using caspase 3/7-detecting reagent after 24 h treatment with compound **4c** (30 μM).

The most interesting compound was the tetrahydroquinoline **4c** that induces 58% apoptosis after 24 h treatment at 30 μM. This structure also displayed strong apoptotic activity at lower concentrations (see Figure S.2. of the

Supplementary Information). On the contrary, compound **5c** (which showed moderate activity in MCF-7 with an EC_{50} value of 11.56 ± 0.28 μM) did not produce an important increase in apoptotic cells (18% at 48 h). This fact could indicate a different mechanism of action (e.g. cell cycle inhibitor, cytotoxic, etc.) for this pyridoxazine derivative. In addition, the change of the 2,6-dichloropurine moiety (**4c**) to 6-trifluoromethylpurine (**4d**) has produced a significant decrease of apoptosis (from 58 to 25% after 24h) as we observed with the antiproliferative activity. The benzoxazine **3c** has shown moderate apoptosis after 48h of treatment (45%).

Furthermore, as a preliminary assessment of the safety profile of compound **4c**, a cell proliferation study was performed in non-tumour cells (RFP TERT immortalized fibroblasts). As shown in Figure 7, treatment of non-cancerous fibroblasts with compound **4c** resulted in a significantly lower antiproliferative activity, with a reduction of activity of up to 19-fold respect to the HCT-116 tumour cell line, indicating promising preferential activity towards cancerous cells.

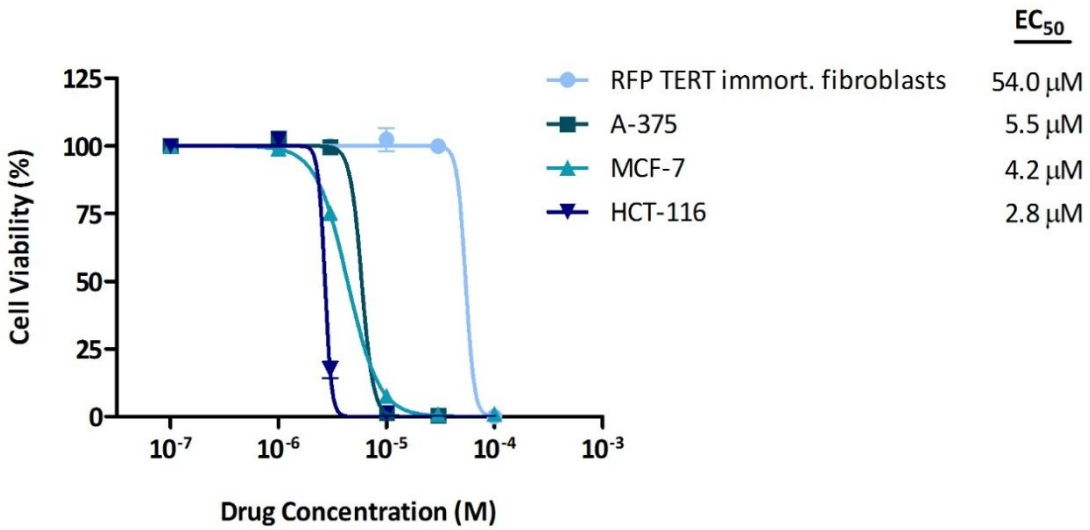


Figure 7. Semilog dose-response curves and EC_{50} values for compound **4c** against RFP TERT immortalized fibroblasts, A-375, MCF-7 and HCT-116 cells. Error bars: ± SD from n = 3.

Conclusion

In an attempt to find new antitumour agents, eighteen purine molecules linked to different heterocycles (benzoxazine, quinoline and pyridoxazine) and open analogues (methyl and ethyl propanoate derivatives) were synthesized. We evaluated their antiproliferative activity against three cancerous cell lines (MCF-7, A-375 and HCT-116) and the induction of apoptosis in MCF-7. In this study we have shown the importance of the substitution in the purine rings since compounds with a 2,6-dichloropurine moiety are consistently more active than other members of the series. In addition, compounds with the complete heterocyclic systems have been more active than open analogues, highlighting the compound with the quinoline ring. In addition, an increase in the distance between the purine and the heterocycle leads to a decrease of antiproliferative activity. **4c** is the most active compound in the cancer cell lines tested and an apoptotic inducer through activation of caspases 3/7, with a low cytotoxicity in non-cancerous cells. In conclusion, this derivative can be considered as a promising antiproliferative lead compound for future optimization campaigns.

Future prespective

One of the main hallmarks of cancer is the ability of malignant cells to evade programed cell death. Therefore, the development of chemical structures able to effectively induce apoptosis represents an interesting approach in the finding of new anticancer treatments. In the search for improved therapies, chemical moieties such as purines and related heterocycles are privileged structures in medicinal chemistry. This work reports an interesting derivative **4c** (3-((2,6-dichloro-9*H*-purine-9-yl)methyl)-1-tosyl-1,2,3,4-tetrahydroquinoline) that was easily prepared and had a promising activity against three cancer cells lines. Moreover, this compound induces apoptosis through activation of caspases, and shows a good safety profile. This quinoline could be an interesting starting point for further structural optimization to obtain new promising antitumour agents.

Executive summary

Purine derivatives linked to heterocycles as a promising scaffold

• 18 Novel purine compounds incorporating different heterocycles and their open analogues were designed, synthesized and evaluated for their antiproliferative activity.

• In the 4 series of derivatives the most interesting molecules have a moiety of 2,6-dichloropurine.

Antiproliferative and apoptosis activity

• Compound **4c** with a quinoline ring shows the lowest EC_{50} values against MCF-7 (4.26 μM), A-375 (5.54 μM) and HCT-116 (2.80 μM).

• **4c** induced apoptosis by activation of caspases 3/7 in a time-dependent manner. It shows a pronounced selectivity against cancer cells.

• **4c** is therefore a leading structure for future anticancer drug development due to its straightforward synthesis and relevant bioactivity.

Supplementary data

See online the supplementary data with the synthesis and characterization of intermediate compounds, 6-bromo and 6-trifluoromethylpurines derivatives and a two-day time-lapse motion picture of MCF-7 cell proliferation under treatment with 30 μM of **4c** with Nucview 488 (apoptosis fluorescent marker).

Financial & competing interest disclosure

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The authors have no other relevant affiliations or financial involvement with any organization or entity with financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

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Table 1. In vitro anticancer activity of final compounds against MCF-7, A-375 and HCT-116 tumour cells.

Comp	X	Y	R	Cancer cell lines		
				MCF-7 EC_{50} (μM)[a]	A-375 EC_{50} (μM)[a]	HCT-116 EC_{50} (μM)[a]
3a	Br	H	-	27.45 ± 0.18	26.11 ± 3.01	17.30 ± 2.67
3b	Cl	H	-	37.63 ± 4.85	13.32 ± 0.43	17.54 ± 0.86
3c	Cl	Cl	-	13.00 ± 0.11	10.85 ± 1.40	7.06 ± 0.80
3d	CF_3	H	-	33.61 ± 3.62	34.59 ± 2.80	56.06 ± 2.66
4a	Br	H	-	> 100	-	-
4b	Cl	H	-	> 100	-	-
4c	Cl	Cl	-	4.26 ± 0.15	5.54 ± 0.64	2.80 ± 0.31
4d	CF_3	H	-	21.95 ± 1.7	61.57 ± 0.67	60.14 ± 5.35
5b	Cl	H	-	> 100	-	-
5c	Cl	Cl	-	11.56 ± 0.28	5.66 ± 0.65	3.13 ± 0.35
6a	Br	H	Me	> 100	-	-
6b	Cl	H	Me	> 100	-	-
6c	Cl	Cl	Me	45.96 ± 5.69	20.48 ± 2.40	22.20 ± 1.57
6d	CF_3	H	Me	> 100	-	-
7a	Br	H	Et	> 100	-	-
7b	Cl	H	Et	> 100	-	-
7c	Cl	Cl	Et	26.15 ± 3.22	17.62 ± 1.90	38.32 ± 2.01
7d	CF_3	H	Et	> 100	-	-
5-FU				1.5 ± 0.31	-	2.40 ± 0.62

[a]Cell viability was measured after 5 days treatment using PrestoBlue™ reagent. Experiments were conducted in triplicate. Data are the mean ± SD of 3 independent determinations.

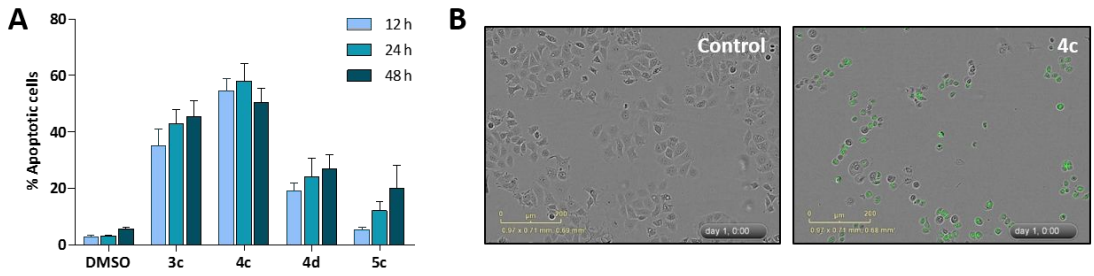

Figure 6. (A) Percentage of apoptotic cells after treatment with compounds **3c**, **4c**, **4d** and **5c** (30 μM) for 12 h (blue), 24 h (light green) and 48 h (dark green). **(B)** Representative images of MCF-7 cells stained using caspase 3/7-detecting reagent after 24 h treatment with compound **4c** (30 μM).

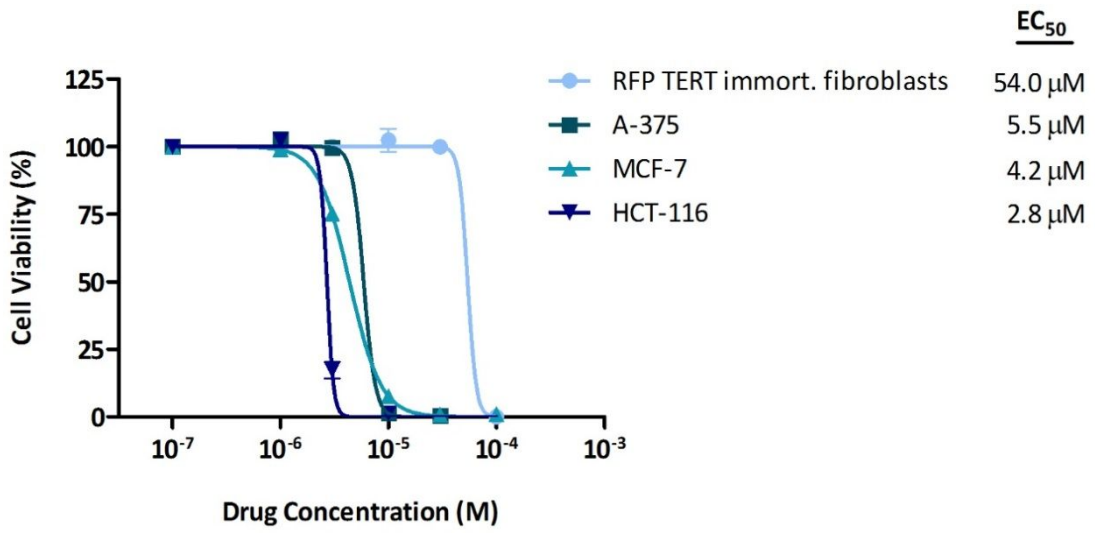

Figure 7. Dose−response curves and calculated EC_{50} values determined by PrestoBlue cell viability assay after incubation of RFP TERT immortalized fibroblasts, A-375, MCF-7 and HCT-116 cells with compound **4c**. Error bars: ± SD from n = 3.

Supplementary Information

PURINE DERIVATIVES WITH HETEROCYCLIC MOIETIES AND RELATED ANALOGUES AS NEW ANTITUMOUR AGENTS

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Preparation of intermediate derivatives

*2-(3,4-Dihydro-2*H*-1,4-benzo[*b*][1,4]oxazine-2-yl)ethanol (**8**)*

To a solution of $LiAlH_4$ 1M (1.79 mL, 1.79 mmol) in anhydrous diethyl ether (7.5 mL) at 0ºC, ethyl 2-(3,4-dihydro-2*H*-1,4-benzo[*b*][1,4]oxazine-2-yl)acetate (396 mg, 1.79 mmol) in anhydrous diethyl ether (7.5 mL) was added dropwise and stirred for 1h. After this time, fresh water (15 mL) was added and the resulting mixture was extracted with DCM. The combined organic layers were washed with brine and dried over Na_2SO_4, filtered, and evaporated. The resulting residue was purified by flash chromatography (EtOAc/hexane, 1:1) to afford **8**. White solid (240 mg, 1.34 mmol), yield 89%, mp: 55 - 56°C;^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 6.80 – 6.74 (m, 2H, H_{benz}), 6.66 (dd, J_1 = 7.7 Hz, J_2 = 1.5 Hz, 1H, H_{benz}), 6.60 (dd, J_1 = 7.7 Hz, J_2 = 1.5 Hz, 1H, H_{benz}), 4.33 – 4.27 (m, 1H, $H_{oxazine}$), 3.91 – 3.83 (m, 2H, CH_2OH), 3.36 (dd, J_1 = 11.6 Hz, J_2 = 2.4 Hz, 1H, $H_{oxazine}$), 3.17 (dd, J_1 = 11.6 Hz, J_2 = 7.7 Hz, 1H, $H_{oxazine}$), 2.99 (s, 1H, NH), 2.02 – 1.89 (m, 1H), 1.90 – 1.77 (m, 1H, CH_2CH_2OH); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 143.3 (C_{benz}), 132.9 (C_{benz}), 121.2 (CH_{benz}), 118.7 (CH_{benz}), 116.6 (CH_{benz}), 115.3 (CH_{benz}), 72.2 ($CH_{oxazine}$), 59.4 (CH_2OH), 45.3 ($CH_{2oxazine}$), 35.2 (CH_2CH_2OH); HRMS (ESI-TOF) (m/z) calcd. for $C_{10}H_{14}NO_2$ $(M + H)^+$ 180.0946, found 180.0878.

*(3,4-Dihydro-4-tosyl-2*H*-1,4-benzo[*b*][1,4]oxazine-2-yl)ethanol (**9**)*

A solution of (3,4-dihydro-2*H*-1,4-benzoxazine-2-yl)ethanol **8** (292 mg, 1.63 mmol) and pyridine (396 µL, 4.89 mmol) in DCM (17 mL) was prepared under argon atmosphere and cooled to 0°C, then *p*-toluenesulfonyl chloride (1.34 mg, 1.63 mmol) was added. The reaction mixture was stirred at rt. after 12h, cold water was added, the reaction was extracted with DCM, and the organic layer was washed with aq. 1N HCl and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash chromatography (EtOAc/hexane, 1:3). White solid (429 mg, 1.288 mmol), yield 79%, mp: 130 - 131°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.83 (dd, J_1 = 8.3 Hz, J_2 = 1.6 Hz, 1H, H_{benz}), 7.56 – 7.51 (m, 2H, H_{tosyl}), 7.26 – 7.20 (m, 2H, H_{tosyl}), 7.04 (ddd, J_1 = 8.2 Hz, J_2 = 7.3 Hz, J_3 = 1.6 Hz, 1H, H_{benz}), 6.92 (ddd, J_1 = 8.3 Hz, J_2 = 7.3 Hz, J_3 = 1.5 Hz, 1H, H_{benz}), 6.79 (dd, J_1 = 8.2 Hz, J_2 = 1.6 Hz, 1H, H_{benz}), 4.32 (dd, J_1 = 14.3 Hz, J_2 = 2.4 Hz, 1H, $H_{oxazine}$), 3.80 – 3.73 (m, 2H, CH_2OH), 3.63 – 3.54 (m, 1H, $H_{oxazine}$), 3.19 (dd, J_1 = 14.3, J_2 = 9.9 Hz, 1H, $H_{oxazine}$), 2.38 (s, 3H, CH_{3tosyl}), 1.83 – 1.72 (m, 2H, CH_2CH_2OH); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 146.4 (C_{benz}), 144.1 (C_{tosyl}), 135.4 (C_{tosyl}), 129.7 (2xCH_{tosyl}), 127.1 (2xCH_{tosyl}), 125.8 (CH_{benz}), 124.0 (CH_{benz}), 123.5 (C_{benz}), 120.8 (CH_{benz}), 117.2 (CH_{benz}), 70.0 ($CH_{oxazine}$), 58.7 (CH_2OH), 48.5 ($CH_{2oxazine}$), 34.9

($\underline{C}H_2CH_2OH$), 21.4 (CH_{3tosyl}); HRMS (ESI-TOF) (m/z) calcd. for $C_{17}H_{19}NO_4NaS$ $(M + Na)^+$ 356.0932, found 356.0919.

*(1,2,3,4-Tetrahydro-1-tosylquinoline-3-yl)methanol (**11**)*

Following the same procedure used for the synthesis of **9**, and starting from (1,2,3,4-tetrahydroquinoline-3-yl)methanol **10** (266 mg, 1.63 mmol), a residue was obtained and was purified by flash chromatography (EtOAc/hexane, 1:2). Yellow solid (414 mg, 1.30 mmol), yield 80%, mp: 172 - 173°C;^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.68 (d, J = 8.3 Hz, 1H, H_{benz}), 7.51 (d, J = 8.0 Hz, 2H, H_{tosyl}), 7.21 (d, J = 8.0 Hz, 2H, H_{tosyl}), 7.18 – 7.13 (m, 1H, H_{benz}), 7.08 – 7.01 (m, 2H, H_{benz}), 4.12 (dd, J_1 = 13.4 Hz, J_2 = 4.2 Hz, 1H, H_{pyr}), 3.57 – 3.47 (m, 2H, $C\underline{H}_2OH$), 3.42 (dd, J_1 = 13.3 Hz, J_2 = 9.4 Hz, 1H, H_{pyr}), 2.59 (dd, J_1 = 16.5 Hz, J_2 = 6.0 Hz, 1H, H_{pyr}), 2.38 (s, 3H, CH_{3tosyl}), 2.24 (dd, J_1 = 16.5 Hz, J_2 = 9.1 Hz, 1H, H_{pyr}),1.98 – 1.90 (m, 1H, H_{pyr}); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 143.8 (C_{tosyl}), 137.0 (C_{benz}), 136.9 (C_{tosyl}), 129.8 ($2xCH_{tosyl}$), 129.5 (CH_{benz}), 129.3 (C_{benz}), 127.1 ($2xCH_{tosyl}$), 126.6 (CH_{benz}), 124.9 (C_{benz}), 123.8 (CH_{benz}), 64.3 ($\underline{C}H_2OH$), 48.5 (CH_{2pyr}), 35.2 (CH_{pyr}), 29.5 (CH_{2pyr}), 21.6 (CH_{3tosyl}); HRMS (ESI-TOF) (m/z) calcd. for $C_{17}H_{20}NO_3S$ $(M + H)^+$ 318.1086, found 318.1103.

*Ethyl (4-acetyl-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]oxazine-3-yl)acetate (**12**)*

To a K_2CO_3 solution (1.089 g, 7.88 mmol) in EtOH (20 mL), *N*-(3-hydroxypyridine-2-yl)acetamide (300 mg, 1.97 mmol) and ethyl 4-bromobut-2-enoate (0.54 mL, 2.96 mmol) were added. The reaction mixture was stirred at rt for 24h. After this time, the solvent was removed and the residue was purified by flash chromatography (EtOAc/hexane, 2:1) to provide **12**. Yellow solid (264 mg, 0.99 mmol), yield 51%, mp: 59 - 60°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.96 (dt, J_1 = 4.7 Hz, J_2 = 1.3 Hz, 1H, H_{pyr}), 7.20 (dt, J_1 = 8.1 Hz, J_2 = 1.3 Hz, 1H, H_{pyr}), 7.00 (ddd, J_1 = 8.0 Hz, J_2 = 4.6 Hz, J_3 = 0.9 Hz, 1H, H_{pyr}), 5.44 – 5.38 (m, 1H, $H_{oxazine}$), 4.47 (dd, J_1 = 11.4 Hz, J_2 = 1.2 Hz, 1H, $H_{oxazine}$), 4.16 – 4.09 (m, 2H, $C\underline{H}_2CH_3$), 4.07 (dd, J_1 = 11.4 Hz, J_2 = 2.9 Hz, 1H, $H_{oxazine}$), 2.59 (s, 3H, $COC\underline{H}_3$), 2.51 – 2.45 (m, 2H, $C\underline{H}_2COO$), 1.22 (t, J = 7.2 Hz, 3H, $CH_2C\underline{H}_3$); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 170.2 (COO), 169.7 ($\underline{C}OCH_3$), 140.7 (C_{pyr}), 139.5 (CH_{pyr}), 138.5 (C_{pyr}), 124.3 (CH_{pyr}), 120.6 (CH_{pyr}), 67.2 ($CH_{2oxazine}$), 60.7 ($\underline{C}H_2CH_3$), 44.6 ($CH_{oxazine}$), 33.6 ($\underline{C}H_2COO$), 25.7 ($CO\underline{C}H_3$), 13.9 ($CH_2\underline{C}H_3$); HRMS (ESI-TOF) (m/z) calcd. for $C_{13}H_{17}N_2O_4$ $(M + H)^+$ 265.1188, found 265.1182.

*2-(3,4-Dihydro-2*H*-pyrido[3,2-*b*][1,4]oxazine-3-yl)ethanol (**13**)*

To a solution of **12** (950 mg, 3.59 mmol) in anhydrous THF (36 mL) at 0ºC, $LiAlH_4$ 1M (4.5 mL, 4.5 mmol) was added dropwise and stirred for 1h. After this time, ethyl acetate (3 mL) and sodium potassium tartrate (3 mL) were added. The resulting precipitate was filtered over celite. The filtrated solvent was extracted with ethyl acetate. The organic layer was dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash chromatography (EtOAc/hexane, 5:1). Yellow solid (455 mg, 2.51 mmol), yield 70%, mp: 90 - 91°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.57 (dt, J_1 = 5.1 Hz, J_2 = 1.4 Hz, 1H, H_{pyr}), 6.93 (dt, J_1 = 7.8 Hz, J_2 = 1.3 Hz, 1H,H_{pyr}), 6.49 (ddd, J_1 = 7.8 Hz, J_2 = 5.0 Hz, J_3 = 1.1 Hz, 1H, H_{pyr}), 6.14 (bs, 1H, OH), 5.12 (bs, 1H, NH), 4.15 (ddd, J_1 = 10.3 Hz, J_2 = 2.8 Hz, J_3 = 1.3 Hz, 1H, $H_{oxazine}$), 3.99 – 3.95 (m, 1H, C$\underline{H}_2$OH), 3.88 – 3.85 (m, 1H, C$\underline{H}_2$OH), 3.82 (ddd, J_1 = 10.3 Hz, J_2 = 7.6 Hz, J_3 = 1.3 Hz, 1H, $H_{oxazine}$), 3.78 – 3.74 (m, 1H, $H_{oxazine}$), 1.78 – 1.61 (m, 2H, C$\underline{H}_2$$CH_2$OH); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 147.0 (C_{pyr}), 139.1 (C_{pyr}), 139.0 (CH_{pyr}), 121.7 (CH_{pyr}), 113.2 (CH_{pyr}), 68.7 ($CH_{2oxazine}$), 60.1 (CH_2OH), 49.2 ($CH_{oxazine}$), 33.3 ($\underline{C}H_2CH_2$OH); HRMS (ESI-TOF) (m/z) calcd. for $C_9H_{13}N_2O_2$ $(M + H)^+$ 181.0977, found 181.0965.

*3-((((*tert-*Butyldimethyl)silyl)oxy)ethyl)-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]oxazine (**14**)*

To a suspension of DMAP (14 mg, 0.11 mmol), Et_3N (427µL, 2,22 mmol), **13** (200 mg, 1.11 mmol) in anhydrous DCM (5 mL), TBDMSCl (250 mg, 1,66 mmol) dissolved in anhydrous DCM (1 mL) was added under argon atmosphere and stirred at rt. After 12h the reaction mixture was poured into aq. AcOH (5%) and extracted with DCM. The organic layer was dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash chromatography (EtOAc/hexane, 1:5). Yellow oil (278 mg, 0.94 mmol), yield 86%; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.66 (d, J = 5.0 Hz, 1H, H_{pyr}), 6.94 (d, J = 7.7 Hz, 1H, H_{pyr}), 6.52 (dd, J_1 = 7.7 Hz, J_2 = 4.9 Hz, 1H, H_{pyr}), 5.32 (bs, 1H, NH), 4.21 (dd, J_1 = 10.7 Hz, J_2 = 2.9 Hz, 1H, $H_{oxazine}$), 3.87 (dd, J_1 = 10.6 Hz, J_2 = 7.0 Hz, 1H, $H_{oxazine}$), 3.84 – 3.77 (m, 2H, C$\underline{H}_2$O), 3.77 – 3.70 (m, 1H, $H_{oxazine}$), 1.76 – 1.65 (m, 2H, C$\underline{H}_2$$CH_2$O), 0.91 (s, 9H, (C$\underline{H}_3$)$_3$), 0.07 (s, 6H, (C$\underline{H}_3$)$_2$Si); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 147.5 (C_{pyr}), 140.5 (CH_{pyr}), 139.6 (C_{pyr}), 122.1 (CH_{pyr}), 114.2 (CH_{pyr}), 69.3 ($CH_{2oxazine}$), 60.7 ($\underline{C}H_2$O), 48.6 ($CH_{oxazine}$), 35.3 ($\underline{C}H_2CH_2$O), 26.4 ((CH_3)$_3$), 18.7 ($\underline{C}$(CH_3)$_3$), -4.9 (CH_3Si), -5.0 (CH_3Si); HRMS (ESI-TOF) (m/z) calcd. for $C_{15}H_{27}N_2O_2Si$ $(M + H)^+$ 295.1842, found 295.1863.

*3-(2-((*tert-*Butyldimethylsilyl)oxy)ethyl)-4-tosyl-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]oxazine (**15**)*

To a suspension of DMAP (73.30 mg, 0.6 mmol), Et_3N (812µL, 5.82 mmol) and **14** (857 mg, 2.91 mmol) in dry DCM (10 mL) under argon atmosphere, *p*-toluenesulfonyl chloride was added (1.165 g, 6.11 mmol) at 0°C and stirred at rt. After 36h the reaction mixture was poured into fresh water. The precipitate was filtered off and the solvent was purified by flash chromatography (EtOAc/hexane, 1:10). Yellow sirup (391 mg, 0.87 mmol), yield 30%; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.01 (d, *J* = 8.3 Hz, 2H, H_{tosyl}), 7.94 (dd, J_1 = 4.7 Hz, J_2 = 1.6 Hz, 1H, H_{pyr}), 7.28 (d, *J* = 5.9 Hz, 2H, H_{tosyl}), 7.12 (dd, J_1 = 8.0 Hz, J_2 = 1.6 Hz, 1H, H_{pyr}), 6.90 (dd, J_1 = 8.0 Hz, J_2 = 4.7 Hz, 1H, H_{pyr}), 5.01 - 4.96 (m, 1H, $H_{oxazine}$), 4.37 (dd, J_1 = 11.1 Hz, J_2 = 1.6 Hz, 1H, $H_{oxazine}$), 4.02 (dd, J_1 = 11.1, J_2 = 2.5 Hz, 1H, $H_{oxazine}$), 3.72 – 3.62 (m, 2H, CH_2O), 2.40 (s, 3H, CH_{3tosyl}), 1.73 (q, *J* = 6.4 Hz, 2H, CH_2CH_2O) 0.90 (s, 9H, $(CH_3)_3$), 0.04 (s, 3H, $(CH_3)_2Si$), 0.05 (s, 3H, $(CH_3)_2Si$); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 143.4 (C_{tosyl}), 140.7 (C_{pyr}), 140.1 (CH_{pyr}), 138.0 (C_{pyr}), 137.9 (C_{tosyl}), 128.9 ($2xCH_{tosyl}$), 128.3 ($2xCH_{tosyl}$), 123.9 (CH_{pyr}), 119.6 (CH_{pyr}), 67.4 ($CH_{2oxazine}$), 59.4 (CH_2O), 50.9 ($CH_{oxazine}$), 33.3 (CH_2CH_2O), 25.7 ($(CH_3)_3$), 21.4 (CH_{3tosyl}), 18.0 ($C(CH_3)_3$), -5.5 (CH_3Si), -5.7 (CH_3Si); HRMS (ESI-TOF) (m/z) calcd. for $C_{22}H_{33}N_2O_4SiS$ $(M + H)^+$ 449.1930, found 449.1964.

*2-(4-Tosyl-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]oxazine-3-yl)etanol (**16**)*

The silyl derivative **15** (200 mg, 0.44 mmol) was added to a mixture of THF/H_2O (1:1) (6.6 mL) and glacial acetic acid (16 mL) was added. After stirred for 22h the mixture was poured into sat. $NaHCO_3$ (25 ml). The aqueous fractions were extracted with EtOAc. The organic layers were combined, dried over Na_2SO_4, filtered and evaporated. The residue was purified by flash chromatography (EtOAc/hexane, 1:2). Colourless oil (74 mg, 0.22 mmol), yield 50 %; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.97 (dd, J_1 = 4.7 Hz, J_2 = 1.6 Hz, 1H, H_{pyr}), 7.93 (d, *J* = 8.3 Hz, 2H, H_{tosyl}), 7.26 (d, *J* = 8.1 Hz, 2H, H_{tosyl}), 7.14 (dd, J_1 = 8.1 Hz, J_2 = 1.5 Hz, 1H, H_{pyr}), 6.92 (dd, J_1 = 8.1 Hz, J_2 = 4.7 Hz, 1H, H_{pyr}), 4.95 (m, 1H, $H_{oxazine}$), 4.27 (dd, J_1 = 11.2 Hz, J_2 = 1.5 Hz, 1H, $H_{oxazine}$), 3.86 (dd, J_1 = 11.2 Hz, J_2 = 2.5 Hz, 1H, $H_{oxazine}$), 3.84 – 3.79 (m, 1H, CH_2OH), 3.74 – 3.68 (m, 1H, CH_2OH), 2.38 (s, 3H, CH_{3tosyl}), 1.89 - 1.71 (m, 2H, CH_2CH_2OH); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 144.0 (C_{tosyl}), 140.5 (C_{pyr}), 140.2 (CH_{pyr}), 137.4 (C_{pyr}), 136.7 (C_{tosyl}), 129.2 ($2xCH_{tosyl}$), 128.2 ($2xCH_{tosyl}$), 124.5 (CH_{pyr}), 119.8 (CH_{pyr}), 67.4 ($CH_{2oxazine}$), 58.3 (CH_2OH), 50.8 ($CH_{oxazine}$), 33.4 (CH_2CH_2OH), 21.7 (CH_{3tosyl}); HRMS (ESI-TOF) (m/z) calcd. for $C_{16}H_{19}N_2O_4S$ $(M + H)^+$ 335.0987, found 335.0878.

*Methyl/Ethyl 2-(*N*-(*tert*-butoxycarbonyl)-*N*-tosylamino)-3-hydroxypropanoate (**18a,b**)*

To a suspension of DMAP (11.4 mg, 0.1 mmol), Et_3N (156.3 µl, 1.12 mmol) and **17a,b** (360 mg, 0.95 mmol) in anhydrous DCM (3 ml) under argon atmosphere, $(Boc)_2O$ was added (244 mg, 1.12 mmol), dissolved in anhydrous DCM (3 ml) and stirred at rt. After 2h the reaction mixture was poured into aq. AcOH (5%) and extracted with DCM. The organic layer was dried over Na_2SO_4, filtered, and evaporated. The crude obtained without isolation was added to a mixture of THF/H_2O (1:1) (10 ml) and glacial acetic acid was added (40 ml). After stirring for 22h the mixture reaction was poured into brine and sat. $NaHCO_3$. The aqueous fraction was extracted with EtOAc, and the organic layer was dried, filtered and evaporated. The residue was purified by flash chromatography (EtOAc/hexane, 1:7).

(***18a***): White solid (262 mg, 0.846 mmol), yield 89%, mp: 102 - 103°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.93 – 7.90 (m, 2H, H_{tosyl}), 7.34 – 7.30 (m, 2H, H_{tosyl}), 5.22 (t, J = 6.5 Hz, 1H, H_{prop}), 4.31 (dd, J_1 = 11.6 Hz, J_2 = 6.4 Hz, 1H, H_{prop}), 3.96 (dd, J_1 = 11.6 Hz, J_2 = 6.5 Hz, 1H, H_{prop}), 3.76 (s, 3H, OCH_3), 2.45 (s, 3H, CH_{3tosyl}), 1.31 (s, 9H, 3xCH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 169.6 (COOMe), 150.2 (COOBu^t), 144.4 (C_{tosyl}), 136.5 (C_{tosyl}), 129.1 (2xCH_{tosyl}), 128.3 (2xCH_{tosyl}), 85.4 ($C(CH_3)_3$), 61.8 (CH_{2prop}), 59.9 (CH_{prop}), 52.4 (OCH_3), 27.7 ($C(CH_3)_3$), 21.5 (CH_{3tosyl}); HRMS (ESI-TOF) (m/z) calcd. for $C_{16}H_{24}NO_7S$ $(M + H)^+$ 374.1273, found 374.1278.

(***18b***): White solid (282 mg, 0.874 mmol), yield 92%, mp: 119 - 120°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.94 – 7.90 (m, 2H, H_{tosyl}), 7.33 – 7.30 (m, 2H, H_{tosyl}), 5.19 (t, J = 6.5 Hz, 1H, H_{prop}), 4.32 (dd, J_1 = 11.5 Hz, J_2 = 6.6 Hz, 1H, H_{prop}), 4.21 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 3.95 (dd, J_1 = 11.5 Hz, J_2 = 6.4 Hz, 1H, H_{prop}), 2.45 (s, 3H, CH_{3tosyl}), 1.31 (s, 9H, 3xCH_3), 1.23 (t, J = 7.1 Hz, 3H, OCH_2CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 169.7 (COOEt), 150.6 (COOBu^t), 144.9 (C_{tosyl}), 137.1 (C_{tosyl}), 129.5 (2xCH_{tosyl}), 128.8 (2xCH_{tosyl}), 85.9 ($C(CH_3)_3$), 62.3 (OCH_2CH_3), 62.2 (CH_{2prop}), 60.3 (CH_{prop}), 28.2 ($C(CH_3)_3$), 22.1 (CH_{3tosyl}), 14.4 (OCH_2CH_3); HRMS (ESI-TOF) (m/z) calcd. for $C_{17}H_{26}NO_7S$ $(M + H)^+$ 388.1430, found 388.1444.

Synthesis and characterization of 6-bromopurines 3a, 4a, 6a, and 7a

For the preparation of these compounds the general synthetic procedure of halopurine derivatives is followed.

*2-(2-(6-Bromo-9*H*-purine-9-yl)ethyl)-4-tosyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine (**3a**)*

Yellow solid (120 mg, 0.232 mmol), yield 75%, mp: 192 - 193°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.69 (s, 1H, H_{purine}), 7.98 (s, 1H, H_{purine}), 7.77 (dd, J_1 = 8.3 Hz, J_2 = 1.6

Hz, 1H, H_{benz}), 7.44 – 7.31 (m, 2H, H_{tosyl}), 7.12 – 7.01 (m, 3H, 2xH_{tosyl}, H_{benz}), 6.93 (ddd, J_1 = 8.8 Hz, J_2 =7.3 Hz, J_3 =1.5 Hz, 1H, H_{benz}), 6.77 (dd, J_1 = 8.2 Hz, J_2 = 1.5 Hz, 1H, H_{benz}), 4.54 – 4.38 (m, 2H, CH_2N), 4.19 (dd, J_1 = 14.2 Hz, J_2 = 2.3 Hz, 1H, $H_{oxazine}$), 3.36 – 3.32 (m, 1H, $H_{oxazine}$), 3.21 (dd, J_1 = 14.2 Hz, J_2 = 9.7 Hz, 1H, $H_{oxazine}$), 2.39 (s, 3H, CH_{3tosyl}), 2.30 – 2.26 (m, 1H, C$\underline{H}_2$$CH_2N$), 2.10 – 1.96 (m, 1H, C$\underline{H}_2$$CH_2N$); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 151.8 (CH_{purine}), 150.4 (C_{purine}), 145.9 (C_{benz}), 145.0 (CH_{purine}), 144.5 (C_{tosyl}), 143.1 (C_{purine}), 135.2 (C_{tosyl}), 134.1 (C_{purine}), 129.6 (2xCH_{tosyl}), 126.8 (2xCH_{tosyl}), 126.2 (CH_{benz}), 124.1 (CH_{benz}), 123.4 (C_{benz}), 121.3 (CH_{benz}), 117.2 (CH_{benz}), 68.3 ($CH_{oxazine}$), 48.1 ($CH_{2oxazine}$), 40.3 (CH_2N), 31.7 ($\underline{C}H_2CH_2N$), 21.6 (CH_{3tosyl}); HRMS (ESI-TOF) (m/z) calcd. for $C_{22}H_{21}N_5O_3SBr$ $(M + H)^+$ 514.0548, found 514.0565. Anal. calc. for $C_{22}H_{20}N_5O_3SBr$: C, 51.37; H, 3.92; N, 13.61. Found: C, 51.35; H, 3.90; N, 13.63.

*3-((6-Bromo-9*H*-purine-9-yl)methyl)-1-tosyl-1,2,3,4-tetrahydroquinoline (**4a**)*

White solid (115 mg, 0.23 mmol), yield 73%, mp: 204 - 205°C; ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.76 (s, 1H, H_{purine}), 8.70 (s, 1H, H_{purine}), 7.61 (d, J = 8.3 Hz, 1H, H_{benz}), 7.25 (d, J = 7.9 Hz, 2H, 2xH_{tosyl}), 7.21 – 7.12 (m, 3H, 2xH_{tosyl}, H_{benz}), 7.07 (d, J = 4.5 Hz, 2H, 2xH_{benz}), 4.26 (dd, J_1 = 8.0 Hz, J_2 = 7.6 Hz, 2H, CH_2N), 4.05 (dd, J_1 = 13.5, J_2 = 4.0 Hz, 1H, H_{pyr}), 3.35 (dd, J_1 = 9.4 Hz, J_2 = 4.3 Hz, 2H, 2xH_{pyr}) 2.55 (dd, J_1 = 16.5 Hz, J_2 = 5.3 Hz, 1H, H_{pyr}), 2.30 (s, 3H, CH_{3tosyl}), 2.15 – 2.04 (m, 1H, H_{pyr}); ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm) 151.8 (CH_{purine}), 150.9 (C_{purine}), 147.6 (CH_{purine}), 144.0 (C_{tosyl}), 142.0 (C_{purine}), 136.0 (C_{benz}), 135.7 (C_{tosyl}), 133.6 (C_{purine}), 129.9 (2xCH_{tosyl}), 129.7 (CH_{benz}), 128.8 (C_{benz}), 126.7 (CH_{benz}), 126.6 (2xCH_{tosyl}), 125.1 (CH_{benz}), 123.5 (CH_{benz}), 48.5 (CH_{2pyr}), 46.1 (CH_2N), 32.6 (CH_{pyr}), 30.3 (CH_{2pyr}), 21.1 (CH_{3tosyl}); HRMS (ESI-TOF) (m/z) calcd. for $C_{22}H_{21}N_5O_2SBr$ $(M + H)^+$ 498.0599, found 498.0619. Anal. calc. for $C_{22}H_{20}N_5O_2SBr$: C, 53.02; H, 4.04; N, 14.05. Found: C, 53.05; H, 4.01; N, 14.02.

*Methyl-2-(*N*-tert-butoxycarbonyl)-*N*-tosylamino)-3-(6-bromo-9*H*-purine-9-yl)propanoate (**6a**)*

White solid (139 mg, 0.251 mmol), yield 81%; mp: 160 - 161°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.56 (d, J = 6.4 Hz, 1H, H_{purine}), 8.19 (s, 1H, H_{purine}), 7.53 (d, J = 8.0 Hz, 2H, 2xH_{tosyl}), 7.14 (d, J = 8.0 Hz, 2H, 2xH_{tosyl}), 5.54 (dd, J_1 = 9.4 Hz, J_2 = 4.9 Hz, 1H, H_{prop}), 4.98 – 4.93 (m, 2H, H_{prop}), 3.78 (s, 3H, OCH_3), 2.36 (s, 3H, CH_{3tosyl}), 1.30 (s, 9H, 3xCH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 168.0 (COOMe), 151.9 (C_{purine}), 151.0 (CH_{purine}), 149.9 (COOBu^t), 145.6 (CH_{purine}), 144.9 (C_{tosyl}), 143.0 (C_{purine}), 135.7 (C_{tosyl}), 134.1 (C_{purine}), 129.1(2xCH_{tosyl}), 128.1 (2xCH_{tosyl}), 86.2 ($\underline{C}(CH_3)_3$), 58.2 (CH_{prop}), 53.1

(OCH$_3$), 43.5 (CH$_{2prop}$), 27.8 (C(CH$_3$)$_3$), 21.7 (CH$_{3tosyl}$); HRMS (ESI-TOF) (m/z) calcd. for $C_{21}H_{25}N_5O_6SBr$ $(M + H)^+$ 554.0709, found 554.0728. Anal. calc. for $C_{21}H_{24}N_5O_6SBr$: C, 45.49; H, 4.36; N, 12.63. Found: C, 45.51; H, 4.34; N, 12.61.

*Ethyl-2-(*N-tert-*butoxycarbonyl)*-N-*tosylamino)-3-(6-bromo-9*H*-purine-9-yl)propanoate (**7a**)*

White solid (140 mg, 0.248 mmol), yield 80%, mp: 171 - 172°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.63 (s, 1H, H$_{purine}$), 8.19 (s, 1H, H$_{purine}$), 7.60 (d, *J* = 8.1 Hz, 2H, 2xH$_{tosyl}$), 7.19 (d, *J* = 8.1 Hz, 2H, 2xH$_{tosyl}$), 5.55 (dd, J_1 = 9.3 Hz, J_2 = 5.0 Hz, 1H, H$_{prop}$), 5.04 (dd, J_1 = 14.7 Hz, J_2 = 5.0 Hz, 1H, H$_{prop}$), 4.95 (dd, J_1 = 14.7 Hz, J_2 = 9.3 Hz, 1H, H$_{prop}$), 4.28 (q, *J* = 7.4 Hz, 2H, OCH$_2$CH$_3$), 2.41 (s, 3H, CH$_{3tosyl}$), 1.33 (s, 9H, 3xCH$_3$), 1.27 (t, *J* = 7.4 Hz, 3H, OCH$_2$CH$_3$); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 167.6 (COOEt), 152.1 (CH$_{purine}$), 151.1 (C$_{purine}$), 150.1 (COOBu^t), 145.6 (CH$_{purine}$), 145.0 (C$_{tosyl}$), 143.3 (C$_{purine}$), 136.0 (C$_{tosyl}$), 134.3 (C$_{purine}$), 129.2 (2xCH$_{tosyl}$), 128.3 (2xCH$_{tosyl}$), 86.3 (C(CH$_3$)$_3$), 62.6 (OCH$_2$CH$_3$), 58.4 (CH$_{prop}$), 43.7 (CH$_{2prop}$), 27.9 (C(CH$_3$)$_3$), 21.8 (CH$_{3tosyl}$), 14.1 (OCH$_2$CH$_3$); HRMS (ESI-TOF) (m/z) calcd. for $C_{22}H_{27}N_5O_6SBr$ $(M + H)^+$ 568.0865, found 568.0867; Anal. calc. for $C_{22}H_{26}N_5O_6SBr$: C, 46.49; H, 4.61; N, 12.32. Found: C, 46.51; H, 4.60; N, 12.34.

Synthesis and characterization of 6-trifluoromethylpurines 3d, 4d, 6d and 7d

A mixture of $FSO_2CF_2CO_2Me$ (MFSDA, 32µl, 0.25 mmol), CuI (32 mg, 0.17 mmol), HMPA (30.5 µl, 0.175 mmol) and the appropriate bromopurines (**3a**, **4a**, **6a** and **7a**) (0.14 mmol) in anhydrous DMF was stirred for 13h at 70°C. After this time, the reaction was cooled, dissolved in EtOAc/hexane (7:3), washed with sat. aq. NH_4Cl, sat. aq. $NaHCO_3$, water and brine, dried, filtered and the solvent was evaporated off. The residue was purified by flash chromatography using EtOAc/hexane as eluent.

*2-(2-(6-Trifluromethyl-9*H*-purine-9-yl)ethyl)-4-tosyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine* (**3d**)

(EtOAc/hexane, 1:1), white solid, (47 mg, 0.094 mmol), yield 67%, mp: 178 - 179°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 9.08 (s, 1H, CH$_{purine}$), 8.13 (s, 1H, CH$_{purine}$), 7.76 (dd, J_1 = 8.3 Hz, J_2= 1.6 Hz, 1H, CH$_{benz}$), 7.46 – 7.35 (m, 2H, 2xCH$_{tosyl}$), 7.10 (d, *J* = 8.0 Hz, 2H, 2xCH$_{tosyl}$), 7.08 – 7.03 (m, 1H, CH$_{purine}$), 6.95 – 6.90 (m, 1H, CH$_{purine}$), 6.74 (dd, J_1 = 8.2 Hz, J_2= 1.5 Hz, 1H, CH$_{purine}$), 4.55 – 4.49 (m, 2H, CH$_2$CH$_2$N), 4.20 (dd, J_1 = 14.2 Hz, J_2 = 2.4 Hz, 1H, CH$_{2oxazine}$), 3.46 – 3.42 (m, 1H, CH$_{oxazine}$), 3.23 (dd, J_1 = 14.3 Hz, J_2 =

9.6 Hz, 1H,$CH_{2oxazine}$), 2.37 (s, 3H, CH_{3tosyl}), 2.30 – 2.25 (m, 1H, $C\underline{H}_2CH_2N$), 2.13 – 2.05 (m, 1H, $C\underline{H}_2CH_2N$); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 153.6 (C_{purine}), 152.4 (C_{purine}),151.8 (CH_{purine}), 145.8 (C_{benz}), 145.2 (q, *J* = 37.1 Hz, C_{purine}),144.6 (C_{tosyl}), 135.3 (C_{tosyl}), 129.9 (C_{purine}), 129.7 (2xCH_{tosyl}), 126.8 (2xCH_{tosyl}), 126.2 (CH_{benz}), 124.0, (CH_{benz}), 123.4 (C_{benz}), 121.4 (CH_{benz}), 120.69 (q, *J* = 274.9 Hz, CF_3), 117.1 (CH_{benz}), 68.6 ($CH_{oxazine}$), 48.1 ($CH_{2oxazine}$), 40.1 ($CH_2\underline{C}H_2N$), 31.8 ($\underline{C}H_2CH_2N$), 21.4 (CH_{3tosyl}); HRMS (ESI-TOF) (m/z) calcd. for $C_{23}H_{21}N_5O_3SF_3$ $(M + H)^+$ 504.1317, found 504.1345. Anal. Calc. for $C_{23}H_{20}N_5O_3SF_3$: C, 54.87; H, 4.00; N, 13.91. Found: C, 54.86; H, 4.03; N, 13.94.

*3-((6-Trifluoromethyl-9*H*-purine-9-yl)methyl)-1-tosyl-1,2,3,4-tetrahydroquinoline* (**4d**)

(EtOAc/hexane, 1:1), yellowish solid, (41 mg, 0.105 mmol), yield 75%, mp: 195 - 196°C;^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 9.08 (s, 1H, CH_{purine}), 8.21 (s, 1H, CH_{purine}), 7.72 (d, *J* = 8.3 Hz, 1H), CH_{benz}, 7.40 (d, *J* = 7.9 Hz, 2H, 2xCH_{tosyl}), 7.20 (t, *J* = 7.8 Hz, 1H, CH_{benz}), 7.16 – 7.09 (m, 2H, 2xCH_{tosyl}), 7.07 (d, *J* = 7.3 Hz, 1H, CH_{benz}), 6.99 (d, *J* = 7.6 Hz, 1H, CH_{benz}), 4.32 (dd, J_1= 14.3 Hz, J_2 = 6.6 Hz, 1H, CH_2N), 4.21 (dd, J_1 = 14.4 Hz, J_2 = 6.4 Hz, 1H, CH_2N), 4.02 (dd, J_1 = 13.5 Hz, J_2 = 3.7 Hz, 1H, CH_{2pyr}), 3.48 (dd, J_1 = 13.5 Hz, J_2 = 8.5 Hz, 1H, CH_{2pyr}), 2.61 (dd, J_1 = 15.9 Hz, J_2 = 5.1 Hz, 1H. CH_{2pyr}), 2.49 – 2.38 (m, 1H, CH_{pyr}), 2.35 (s, 3H, CH_{3tosyl}), 2.31 (d, *J* = 8.6 Hz, 1H, CH_{2pyr}); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 154.2 (C_{purine}), 152.4 (CH_{purine}), 147.9 (CH_{purine}), 145.8 (q, *J* = 37.7 Hz, C_{purine}),144.4 (C_{tosyl}), 136.7 (C_{benz}), 136.6 (C_{tosyl}), 130.4 (C_{purine}), 130.1 (2xCH_{tosyl}), 129.8 (CH_{benz}), 127.6 (CH_{benz}), 127.5 (C_{benz}), 127.2 (2xCH_{tosyl}), 125.6 (CH_{benz}), 124.3 (CH_{benz}), 120.8 (q, *J* = 275.9 Hz, CF_3), 48.9 (CH_{2pyr}), 47.0 (CH_2N), 33.2 (CH_{pyr}), 31.1 (CH_{2pyr}), 21.9 (CH_{3tosyl}); HRMS (ESI-TOF) (m/z) calcd. for $C_{23}H_{21}N_5O_2SF_3$ $(M + H)^+$ 488.1368, found 488.1415. Anal. Calc. for $C_{23}H_{20}N_5O_2SF_3$: C, 56.67; H, 4.14; N, 14.37. Found: C, 56.65; H, 4.15; N, 14.40.

*Methyl-2-(*N-tert-*butoxycarbonyl)*-N-*tosylamino)-3-(6-trifluoromethyl-9*H*-purine-9-yl)propanoate* (**6d**)

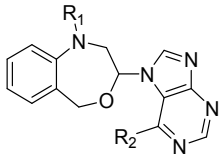
(EtOAc/hexane, 1:1), white solid, (41 mg, 0.075 mmol), yield 50%, mp: 156 - 157°C; ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) δ 9.03 (s, 1H, CH_{purine}), 8.36 (s, 1H, CH_{purine}), 7.63 (d, *J* = 8.1 Hz, 2H, 2xCH_{tosyl}), 7.19 (d, *J* = 8.1 Hz, 2H, 2xCH_{tosyl}), 5.61 (dd, J_1 = 9.2 Hz, J_2 = 4.9 Hz, 1H, H_{prop}), 5.12 (dd, J_1 = 14.7 Hz, J_2 = 4.8 Hz, 1H, H_{prop}), 5.01 (dd, J_1 = 14.7 Hz, J_2 = 9.2 Hz, 1H, H_{prop}), 3.85 (s, 3H, OCH_3), 2.40 (s, 3H, CH_{3tosyl}), 1.28 (s, 9H, 3xCH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 168.1 (COOMe), 154.3 (C_{purine}), 152.1 (CH_{purine}), 150.1 ($COOBu^t$), 148.0 (CH_{purine}), 145.3 (C_{tosyl}), 145.1 (q, *J* = 37.2 Hz, C_{purine}), 135.9

(C_{tosyl}), 130.1 (C_{purine}), 129.3 ($2xCH_{tosyl}$), 128.4 ($2xCH_{tosyl}$), 120.6 (q, *J* = 275.9 Hz, CF_3),86.5 ($\underline{C}(CH_3)_3$), 58.1 (CH_{prop}), 53.3 (OCH_3), 43.7 (CH_{2prop}), 27.8 ($C(\underline{C}H_3)_3$), 21.7 (CH_{3tosyl}); HRMS (ESI-TOF) (m/z) calcd. for $C_{22}H_{25}N_5O_6SF_3$ $(M + H)^+$ 544.1478, found 544.1480. Anal. calc. for $C_{22}H_{24}N_5O_6SF_3$: C, 48.62; H, 4.45; N, 12.89. Found: C, 48.63; H, 4.42; N, 12.91.

*Ethyl-2-(*N-tert-*butoxycarbonyl)*-N-*tosylamino)-3-(6-trifluoromethyl-9*H*-purine-9-yl)propanoate* (**7d**)

(EtOAc/hexane, 1:1), white solid, (47 mg, 0.084 mmol), yield 61%, mp: 160 - 161°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 9.03 (s, 1H, CH_{purine}), 8.36 (s, 1H, CH_{purine}), 7.65 (d, *J* = 8.2 Hz, 2H, $2xCH_{tosyl}$), 7.21 (t, *J* = 8.2 Hz, 2H, $2xCH_{tosyl}$), 5.57 (dd, J_1 = 9.0 Hz, J_2 = 5.1 Hz, 1H, H_{prop}), 5.07 – 5.03 (m, 2H, H_{prop}), 4.29 (q, *J* = 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 2.41 (d, *J* = 8.9 Hz, 3H, CH_{3tosyl}), 1.30 (s, 9H, $3xCH_3$), 1.27 (t, *J* = 7.2 Hz, 3H, $OCH_2C\underline{H}_3$); ^{13}C NMR (101 MHz, $CDCl_3$): δ (ppm) 167.9 (COOEt), 154.7 (C_{purine}), 152.4 (CH_{purine}), 150.4 ($COOBu^t$), 148.4 (CH_{purine}), 145.2 (q, *J* = 37.4 Hz, C_{purine}), 144.9 (C_{tosyl}), 136.3 (C_{tosyl}), 130.4 (C_{purine}), 129.5 ($2xCH_{tosyl}$), 128.7 ($2xCH_{tosyl}$), 120.6(q, *J* = 275.9 Hz, CF_3), 86.7 ($\underline{C}(CH_3)_3$), 63.0 ($O\underline{C}H_2CH_3$), 58.5 (CH_{prop}), 43.9 (CH_{2prop}), 28.2 ($C(\underline{C}H_3)_3$), 22.0 (CH_{3tosyl}), 14.4 ($OCH_2\underline{C}H_3$); HRMS (ESI-TOF) (m/z) calcd. for $C_{23}H_{27}N_5O_6SF_3$ $(M + H)^+$ 558.1634, found 558.1634. Anal. calc. for $C_{23}H_{26}N_5O_6SF_3$: C, 49.55; H, 4.70; N, 12.56. Found: C, 49.57; H, 4.69; N, 12.56.

Figure S.1. Chemical structure of benzoxazepine derivatives [Díaz-Gavilán, M. *et al. Bioorg. Med. Chem. Lett.* 18, 1457-1460 (2008)]



a R_1 = SO_2-C_6H_4-*o*NO_2, R_2 = SPh
b R_1 = SO_2-C_6H_4-*p*NO_2, R_2 = SPh
c R_1 = H, R_2 = SPh

Figure S.1. Benzoxazepine derivatives previously published by our group.

Figure S.2. Apoptosis after treatment with 4c at lower concentrations

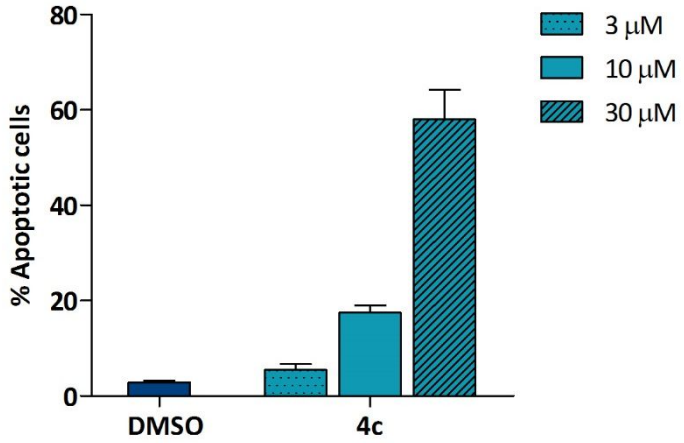
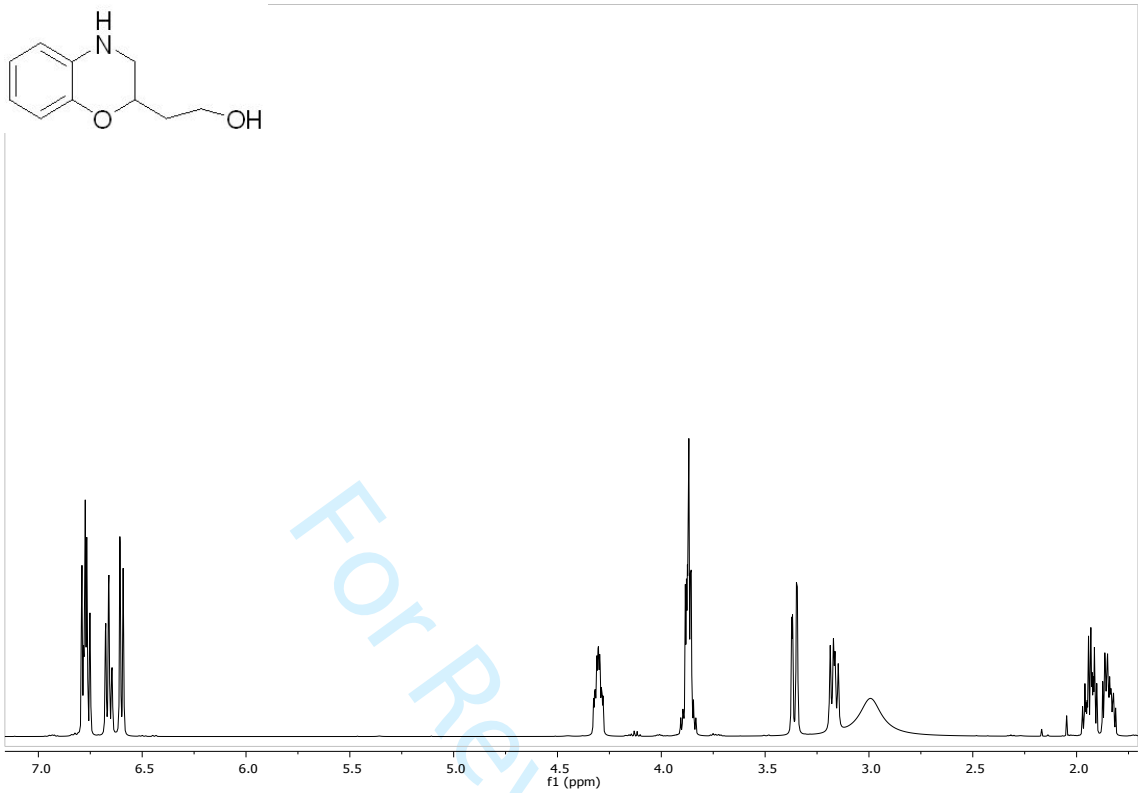


Figure S.2. Percentage of apoptotic cells after treatment with compound **4c** at 3, 10 and 30 μM.

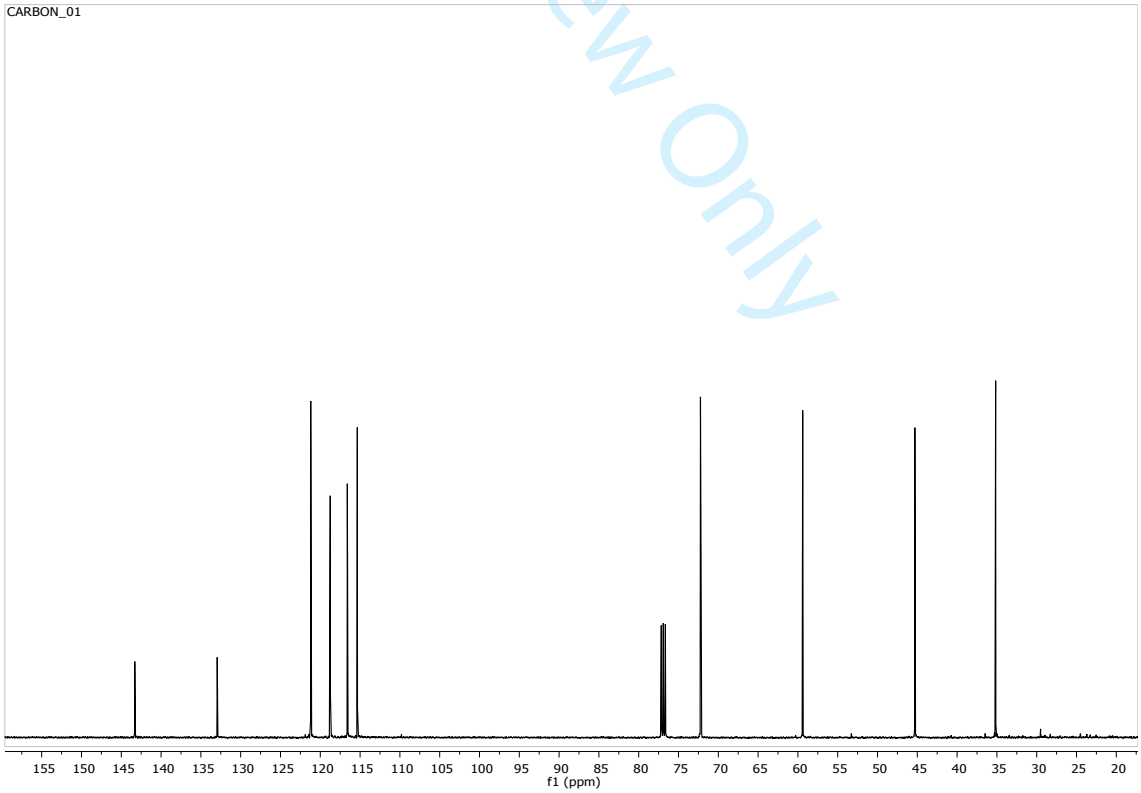
Supplementary video: two-day time-lapse motion picture of MCF-7 cell proliferation under treatment with 30 μM of **4c** with Nucview488 (apoptosis fluorescent marker)

^{1}H and ^{13}C-RMN spectra of intermediate derivatives

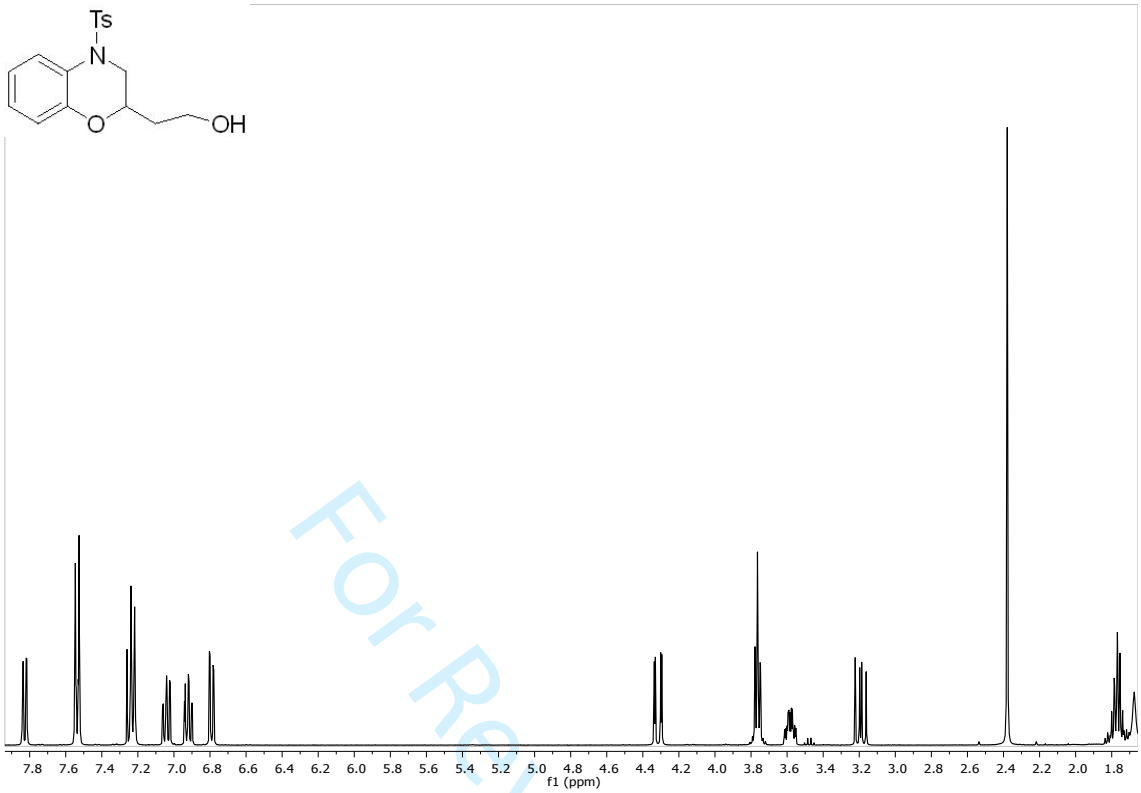
^{1}H NMR *2-(3,4-Dihydro-2*H*-1,4-benzo[*b*][1,4]oxazine-2-yl)ethanol (**8**)*



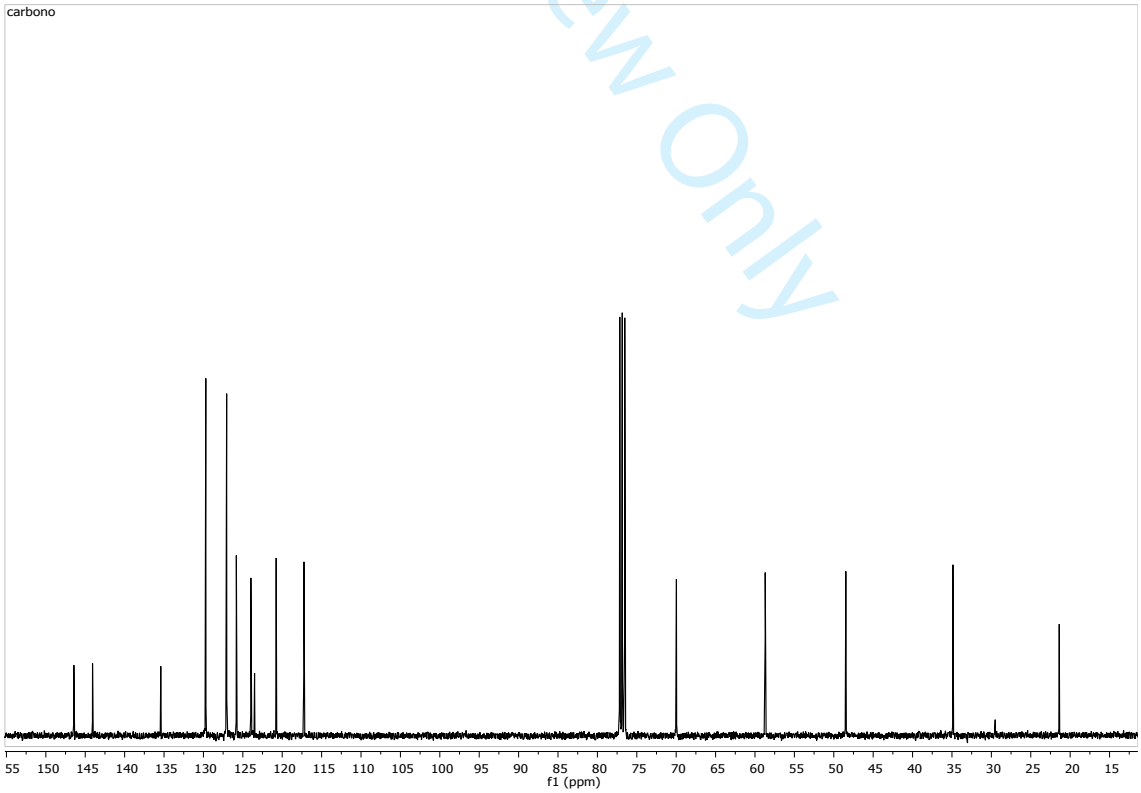
^{13}C NMR



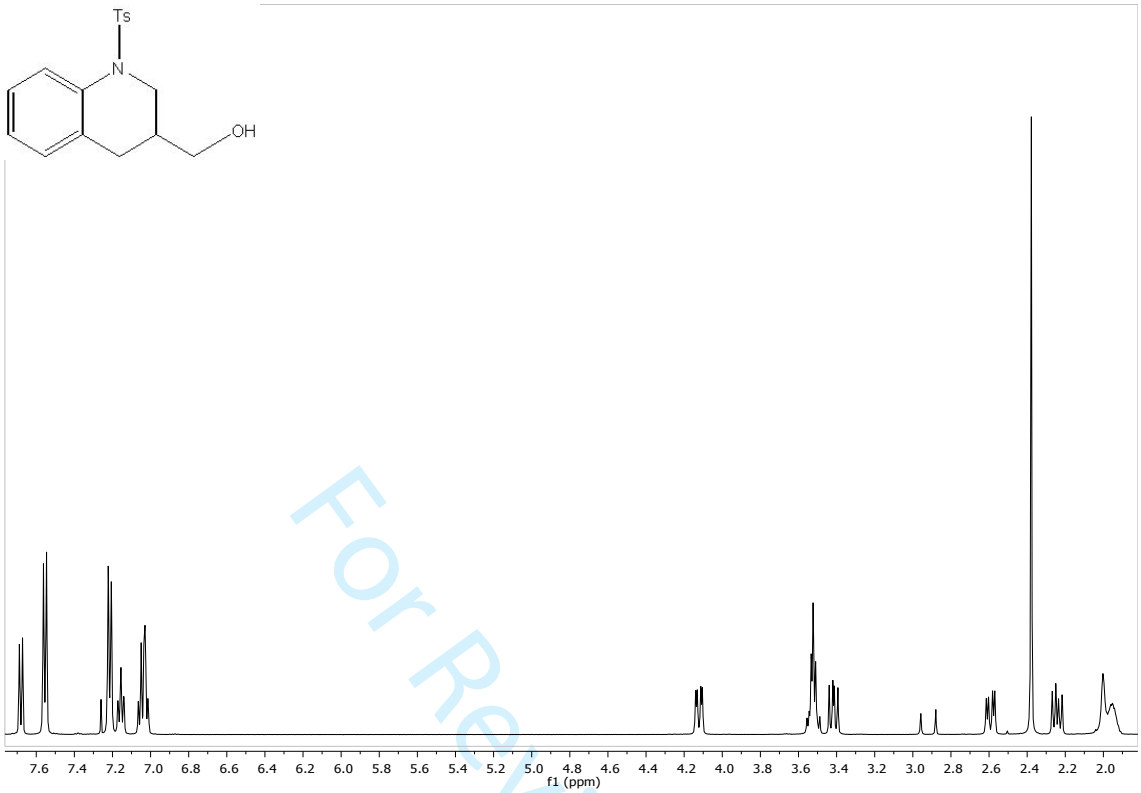
^{1}H NMR *(3,4-Dihydro-4-tosyl-2*H*-1,4-benzo[*b*][1,4]oxazine-2-yl)ethanol (**9**)*



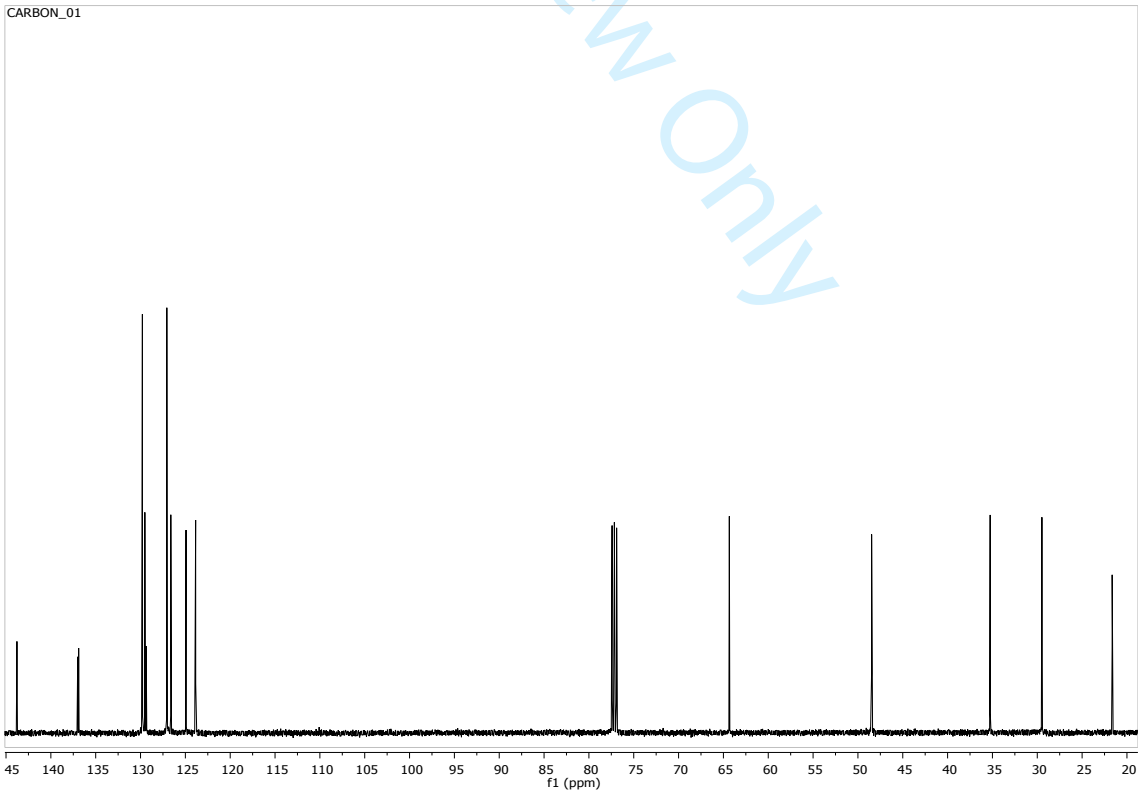
^{13}C NMR



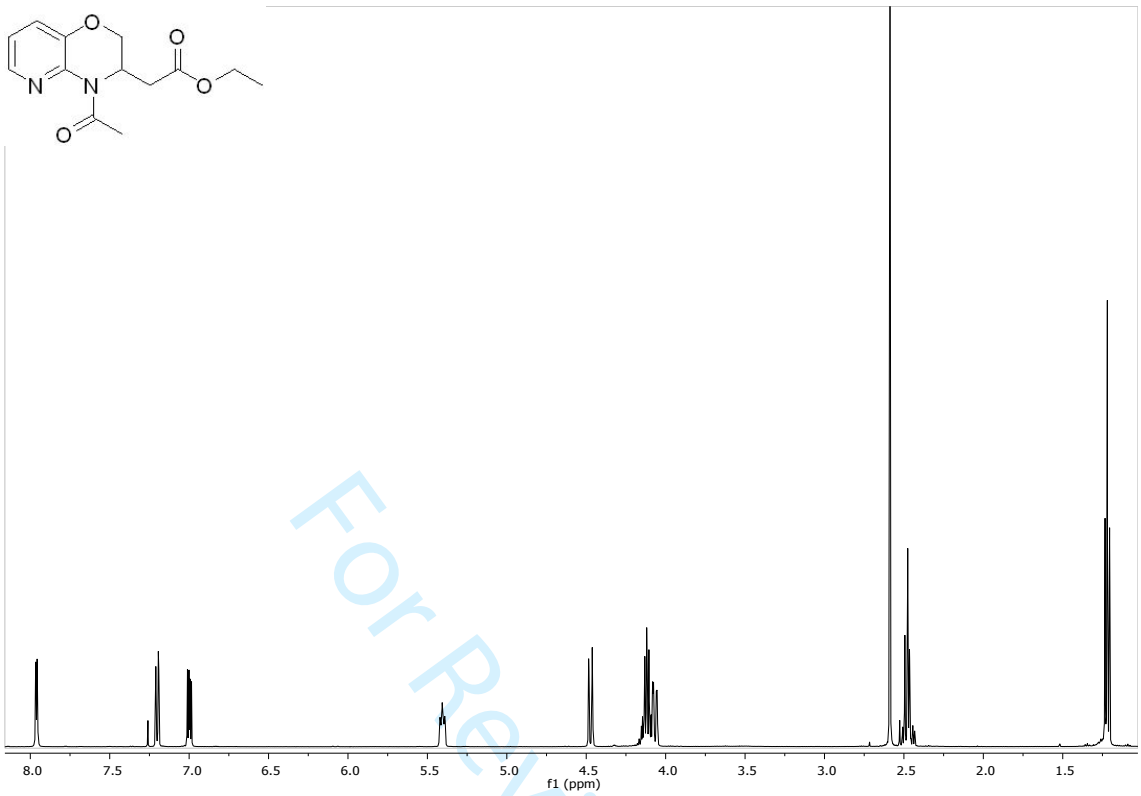
^{1}H NMR *(1,2,3,4-Tetrahydro-1-tosylquinoline-3-yl)methanol (**11**)*



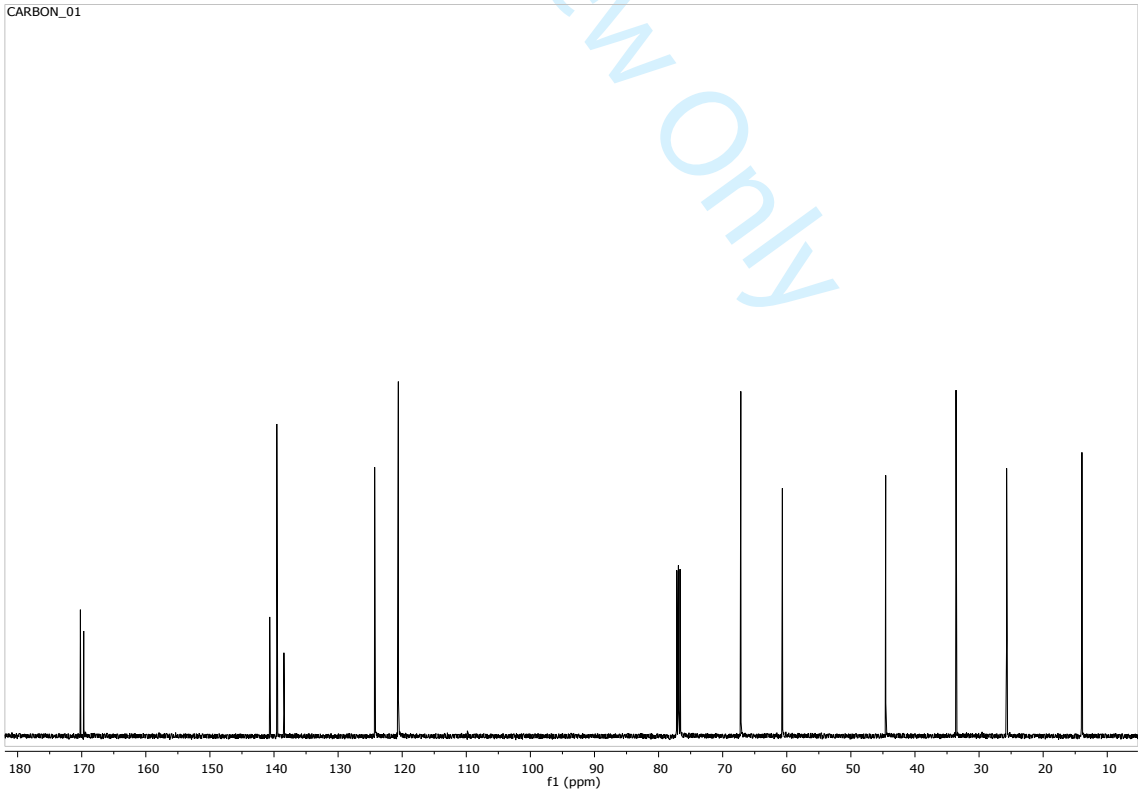
^{13}C NMR



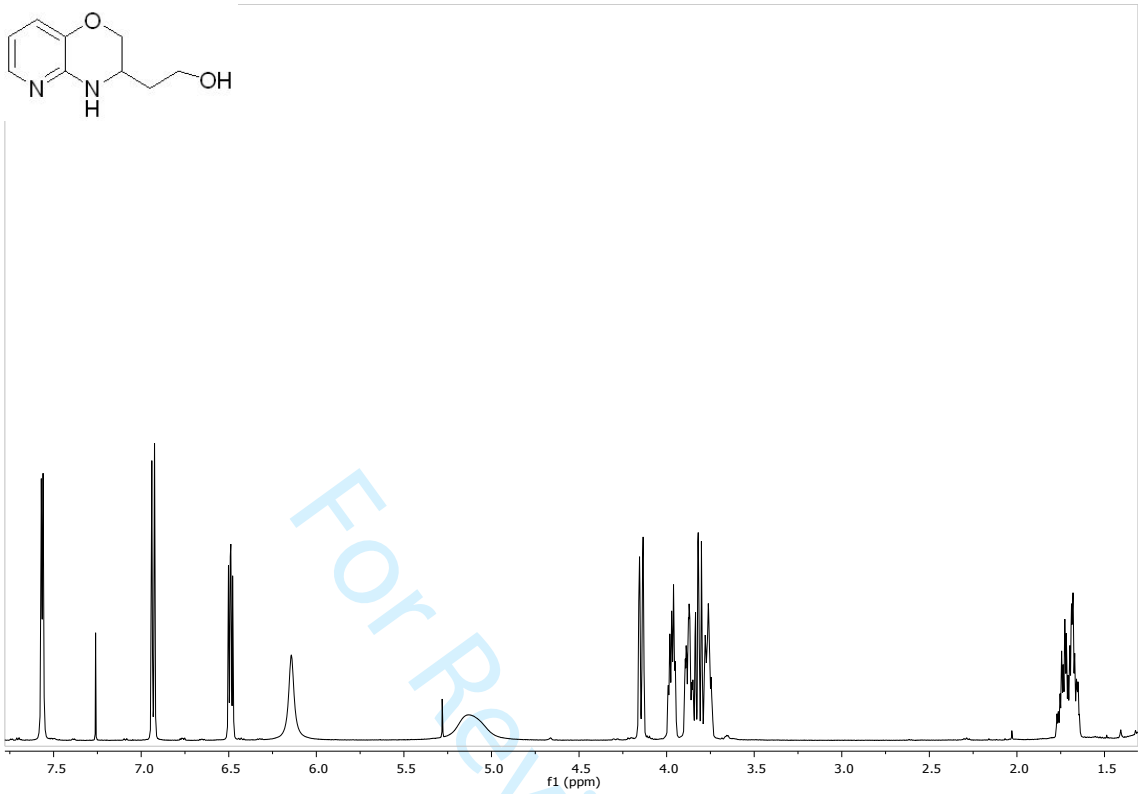
^{1}H NMR *Ethyl (4-acetyl-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]oxazine-3-yl)acetate (**12**)*



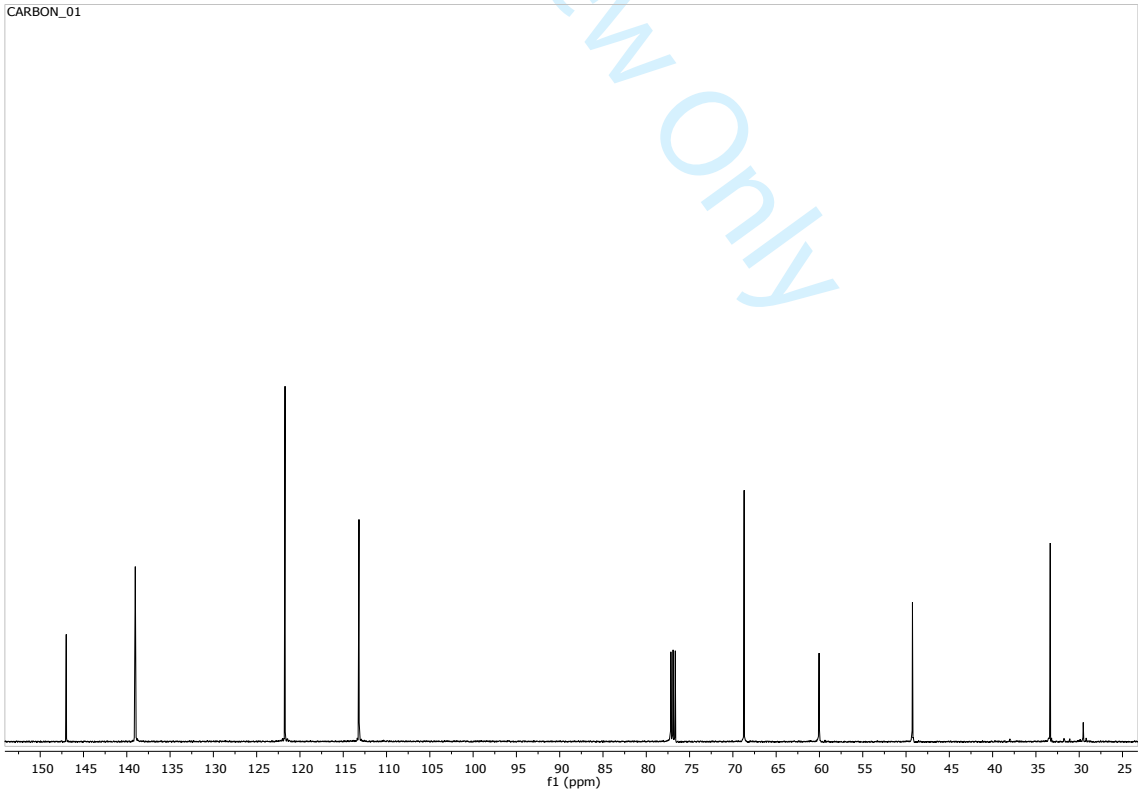
^{13}C NMR



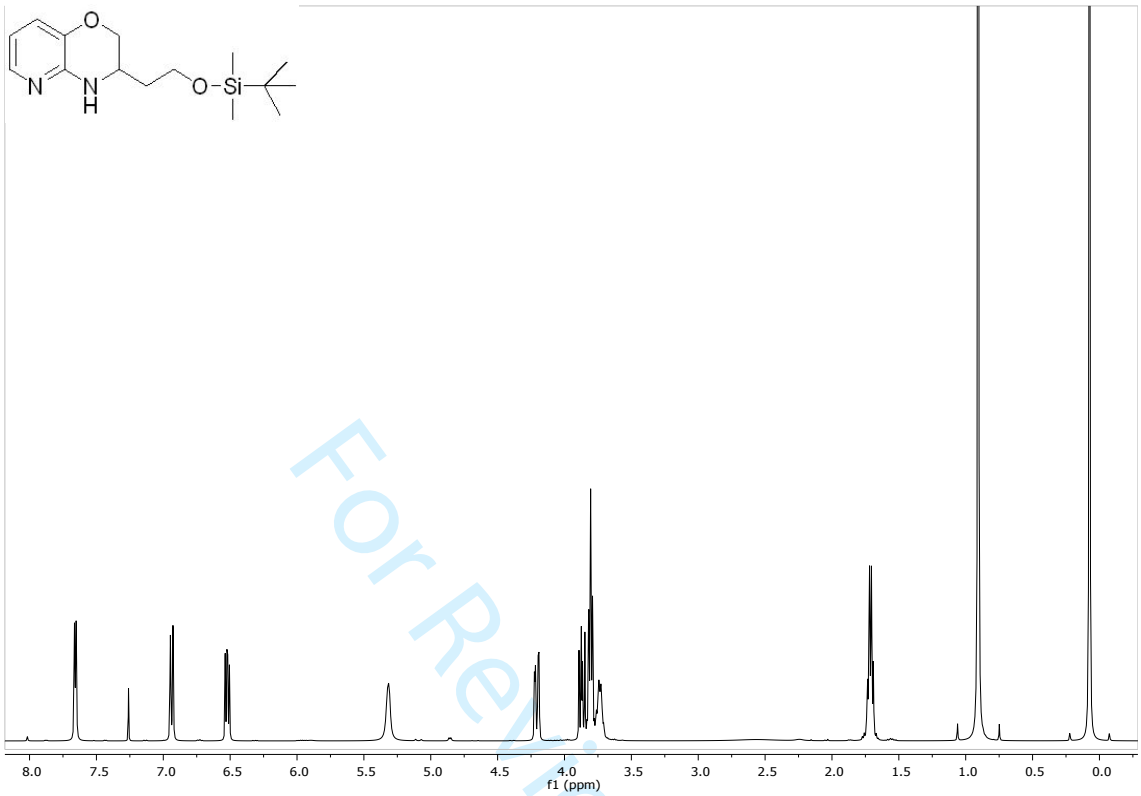
^{1}H NMR *2-(3,4-Dihydro-2*H*-pyrido[3,2-*b*][1,4]oxazine-3-yl)ethanol (**13**)*



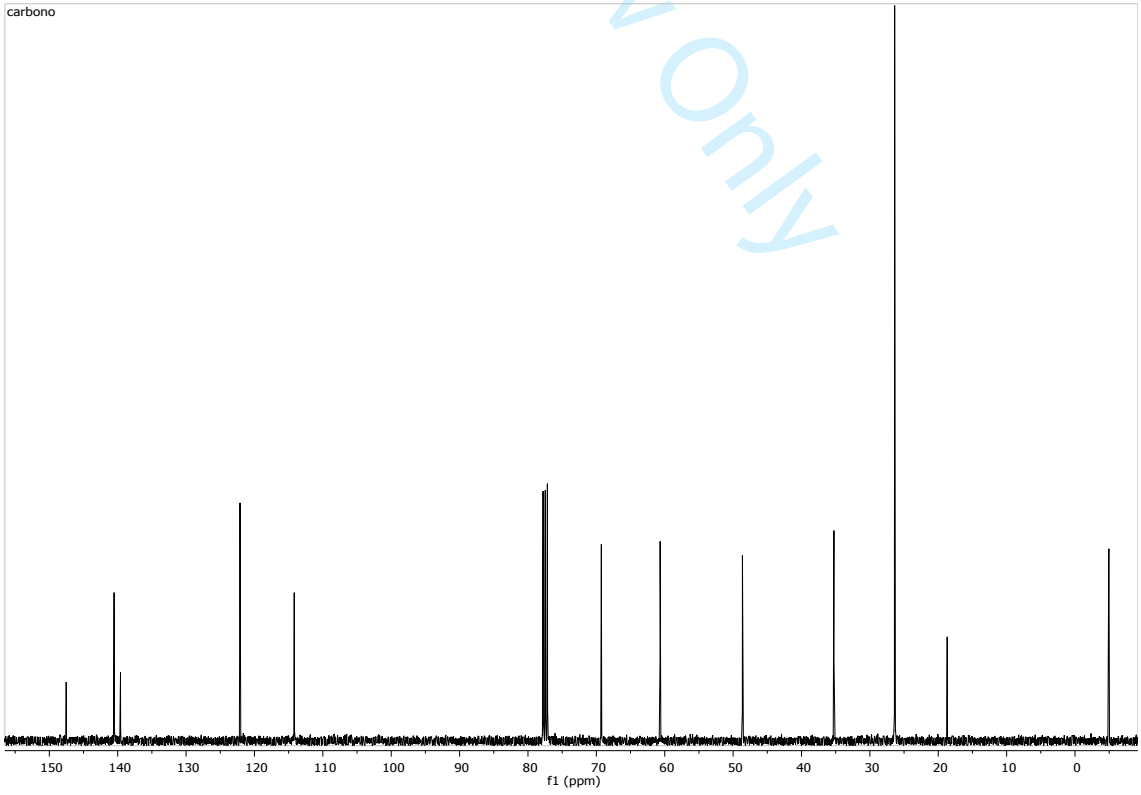
^{13}C NMR



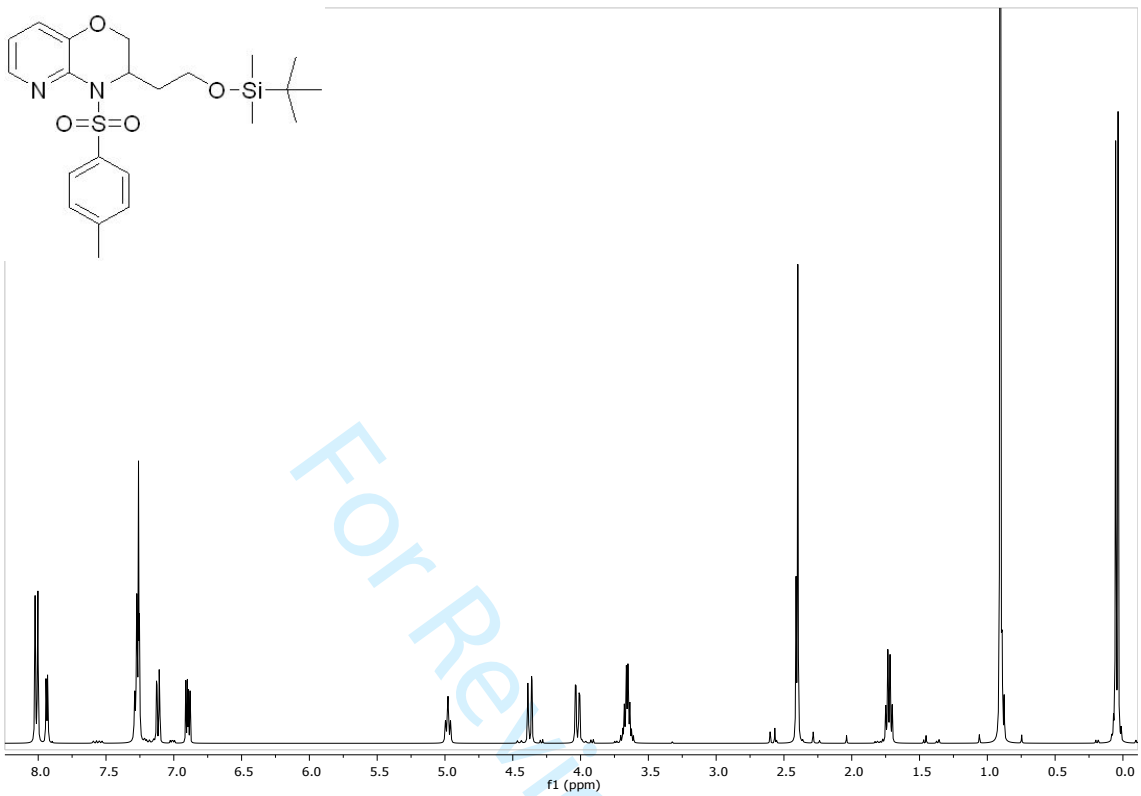
^{1}H NMR *3-((((*tert*-Butyldimethyl)silyl)oxy)ethyl)-3,4-dihydro-2*H*-pyrido[3,2-b][1,4]oxazine (**14**)*



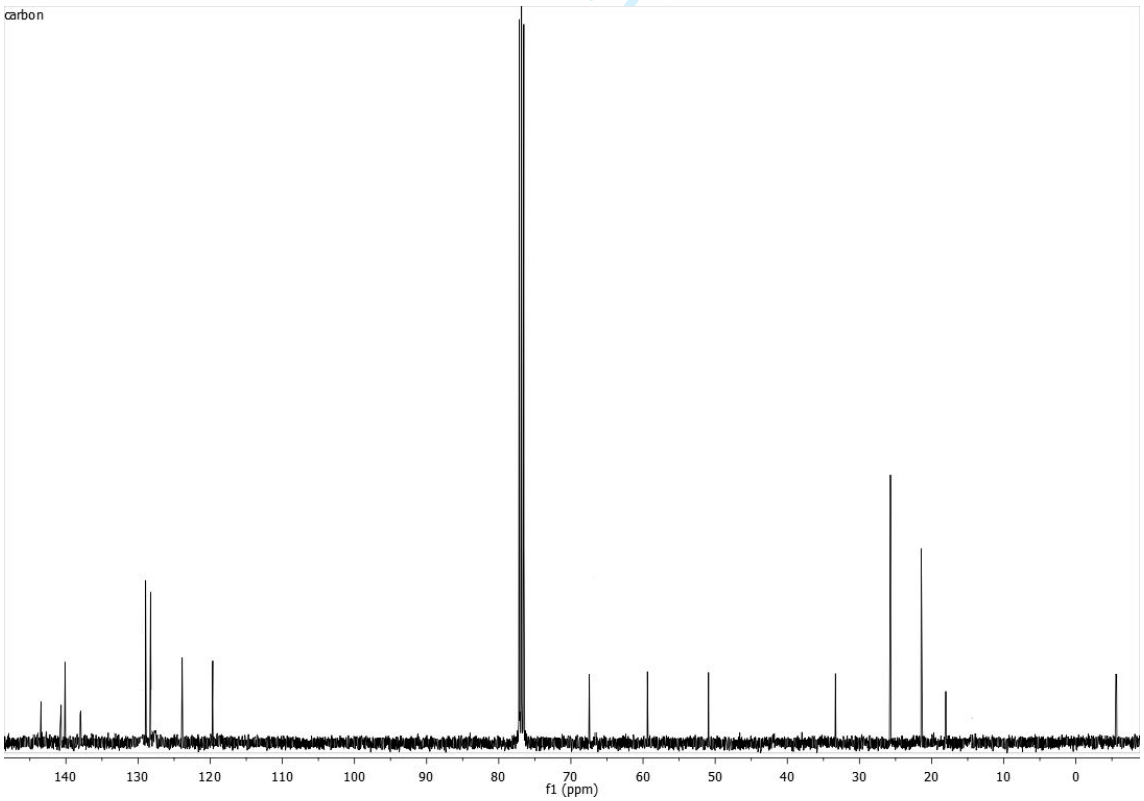
^{13}C NMR



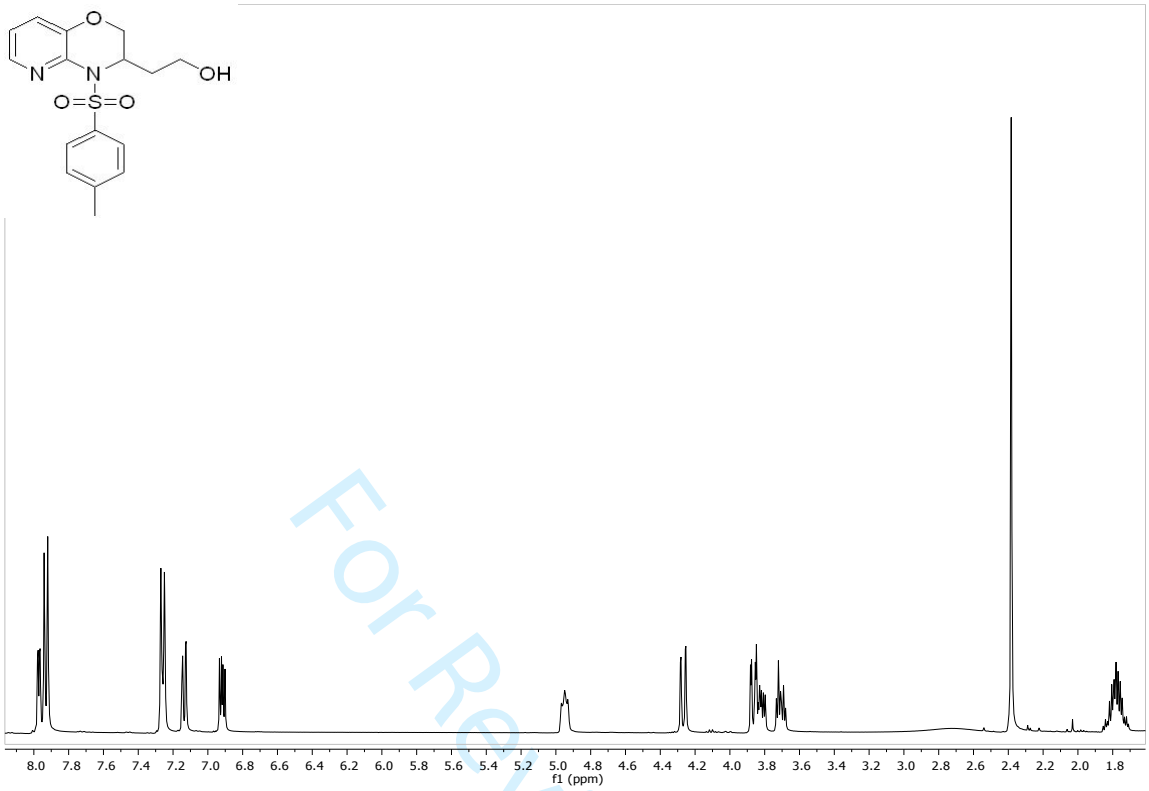
^{1}H NMR *3-(2-((*tert-*Butyldimethylsilyl)oxy)ethyl)-4-tosyl-3,4-dihydro-2*H-*pyrido[3,2-b][1,4]oxazine (**15**)*



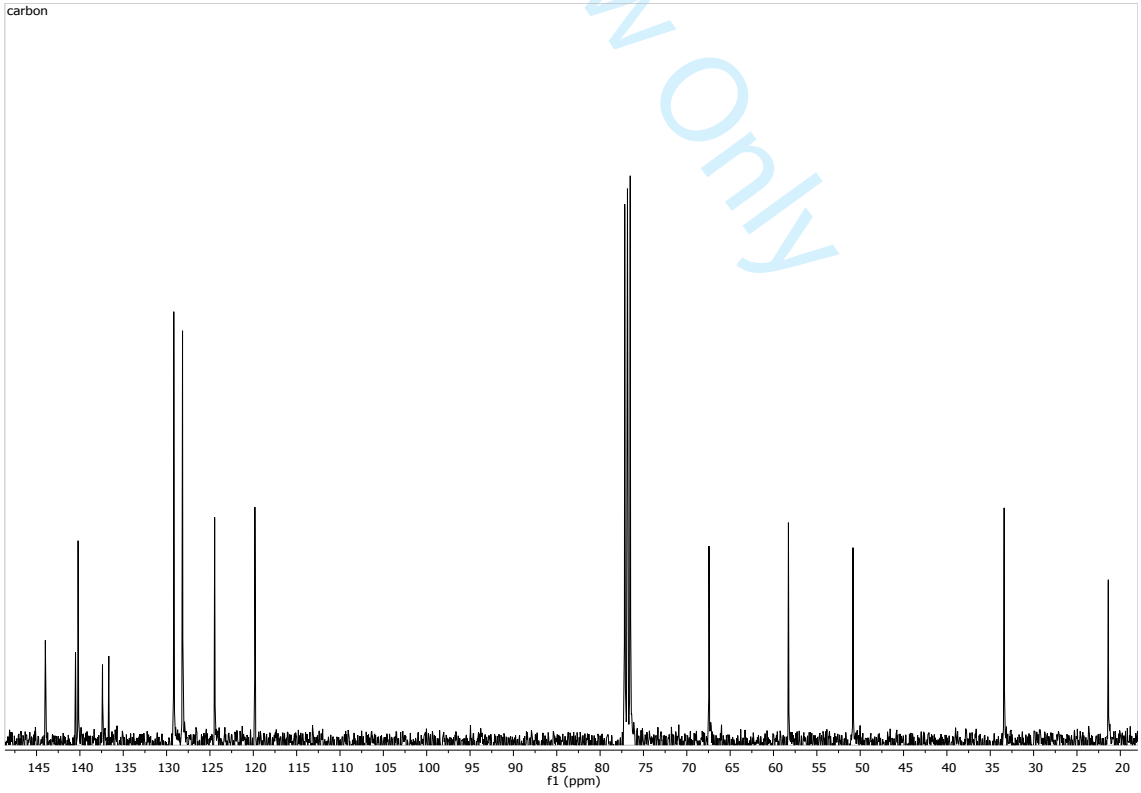
^{13}C NMR



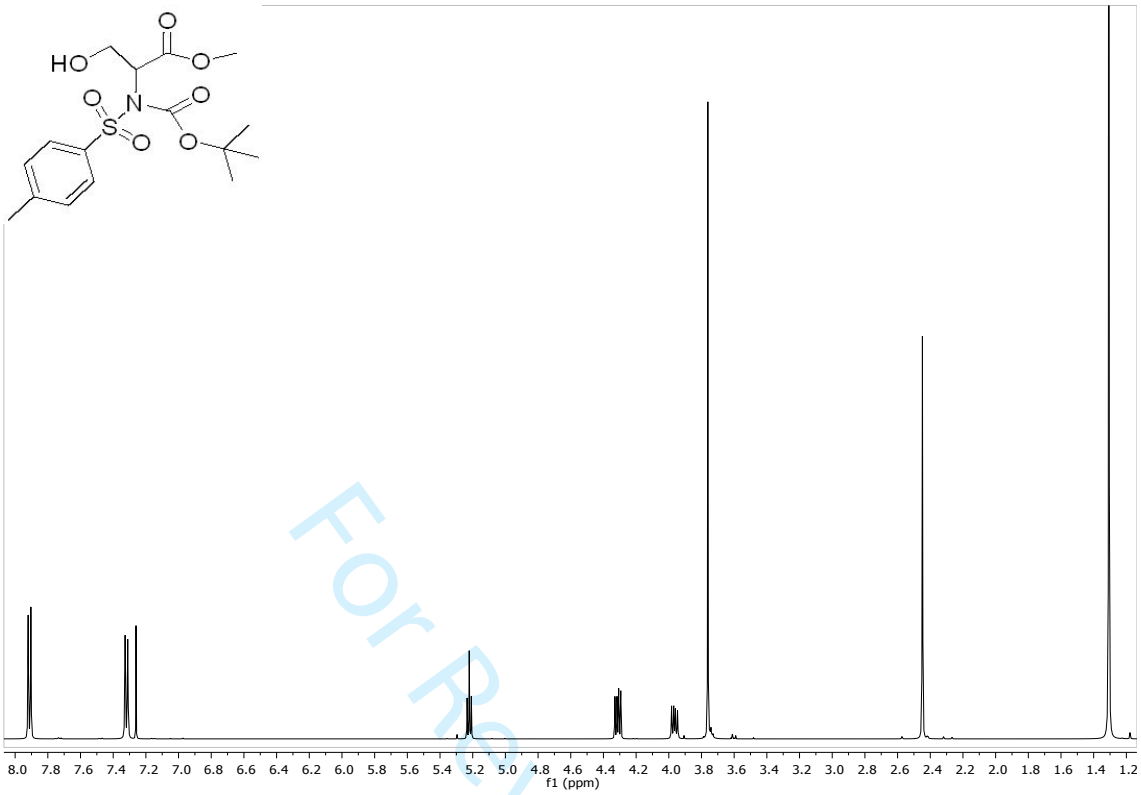
^{1}H NMR *2-(4-Tosyl-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]oxazine-3-yl)etanol (**16**)*



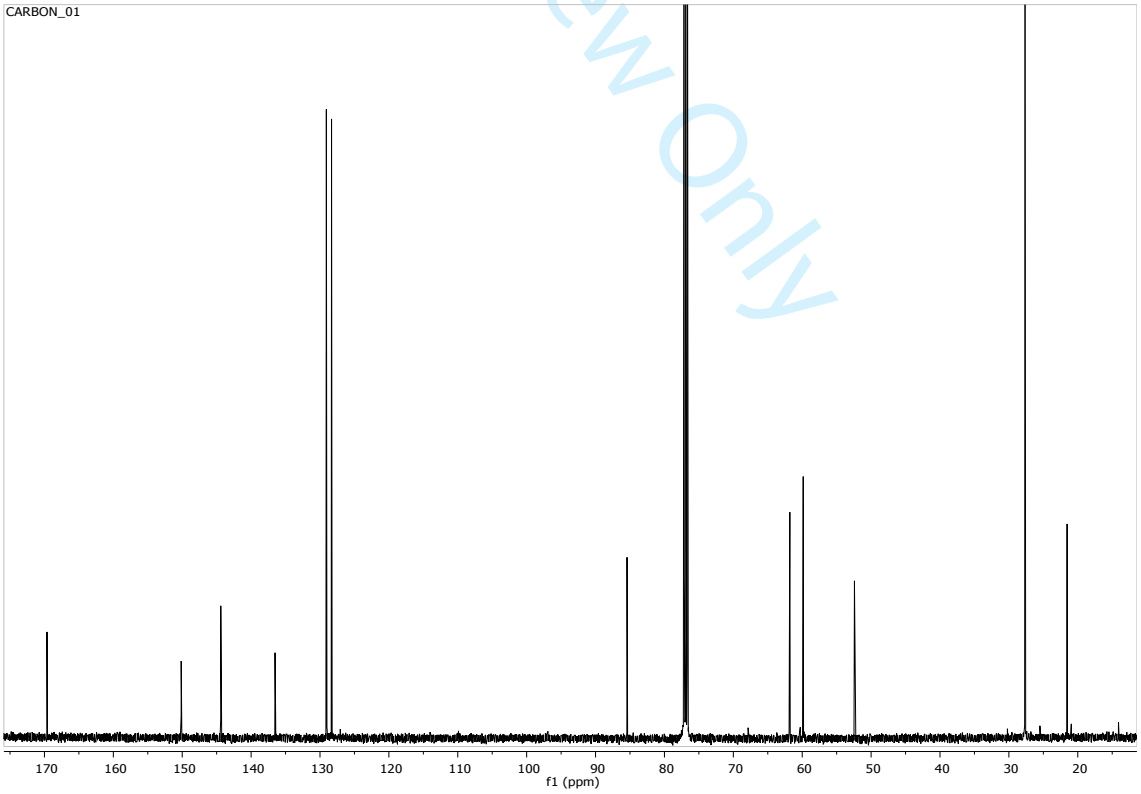
^{13}C NMR



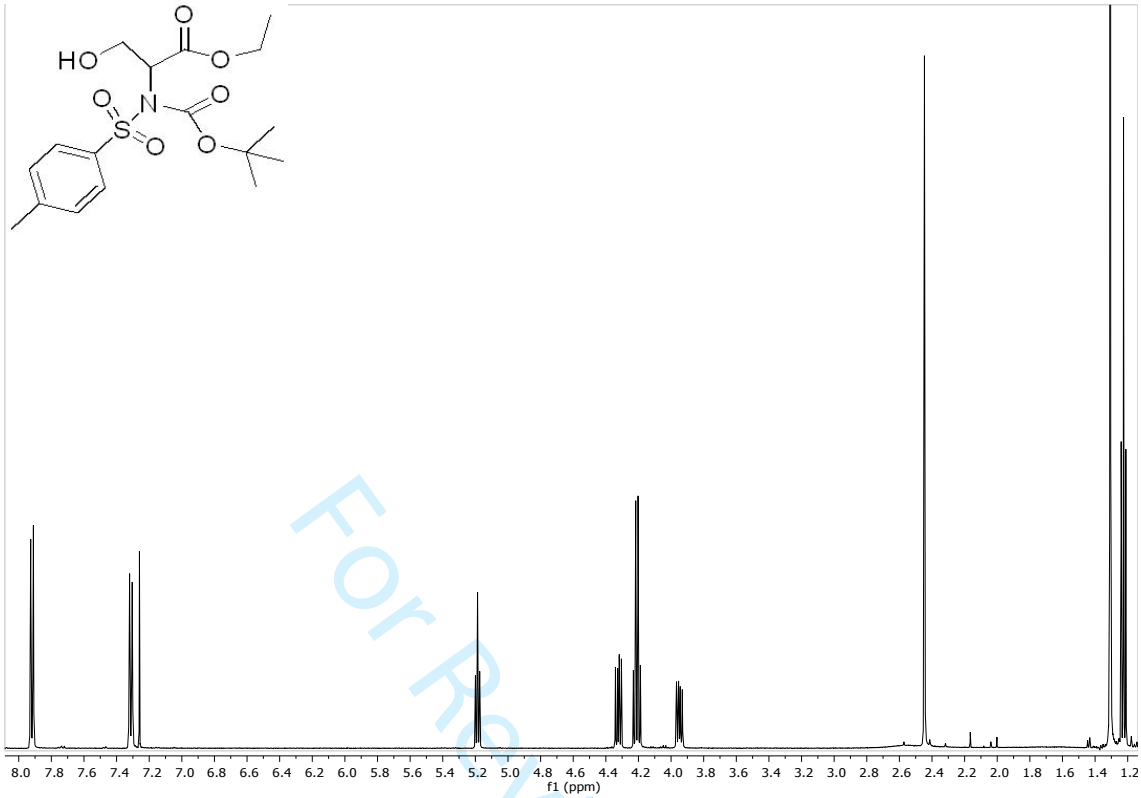
^{1}H NMR *Methyl 2-(*N-*(*tert-*butoxycarbonyl)*-N-*tosylamino)-3-hydroxypropanoate (**18a**)*



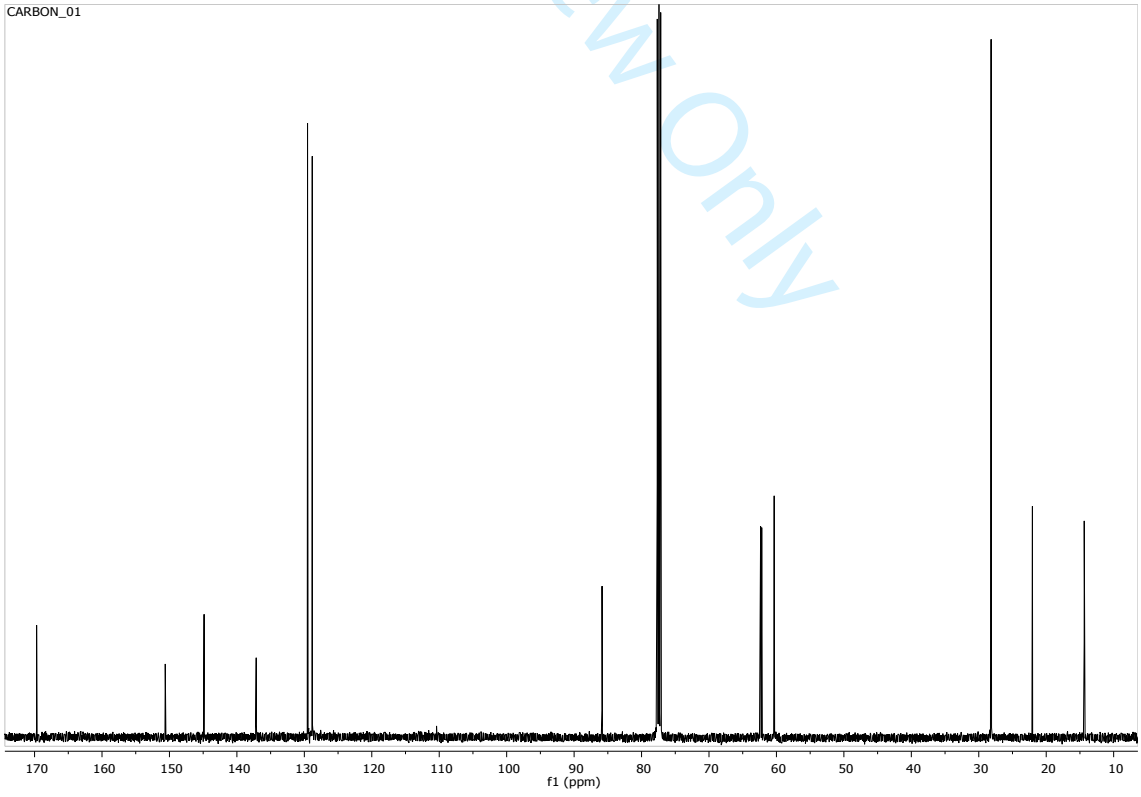
^{13}C NMR



^{1}H NMR *Ethyl 2-(*N-*(*tert-*butoxycarbonyl)*-N-*tosylamino)-3-hydroxypropanoate (**18b**)*

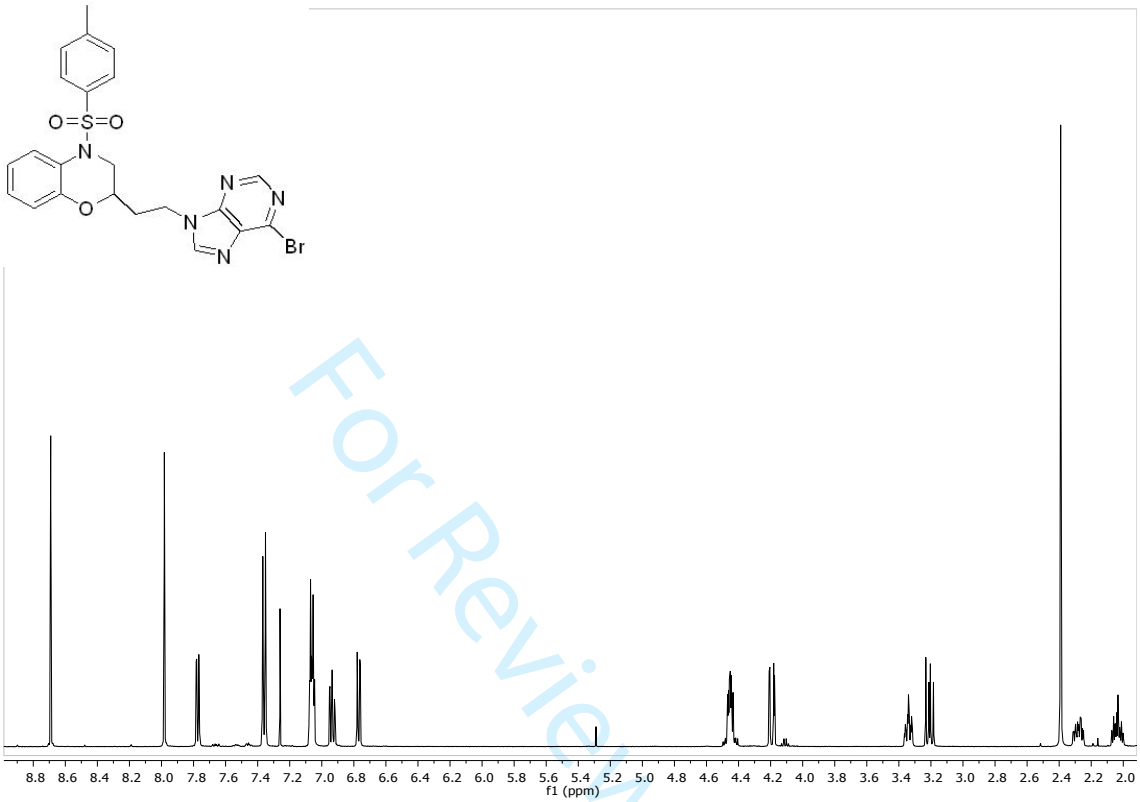


^{13}C NMR

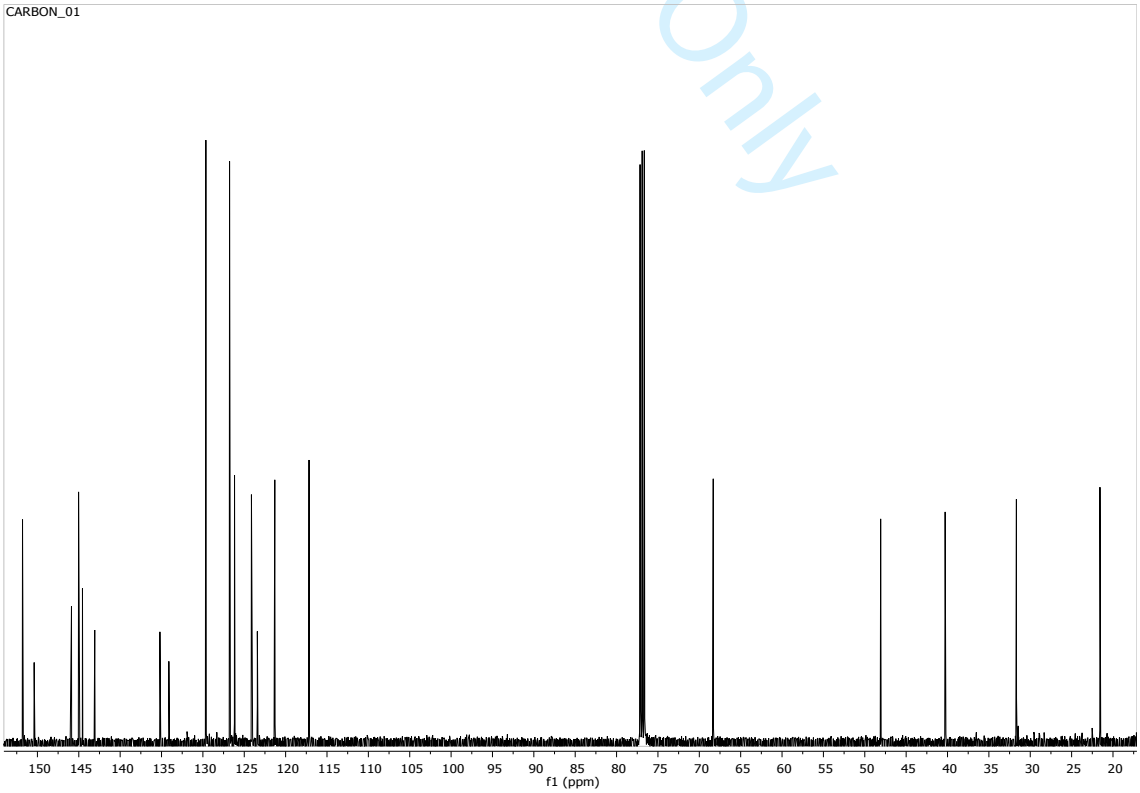


^{1}H and ^{13}C-RMN spectra of 6-halo and 2,6-dihalopurines

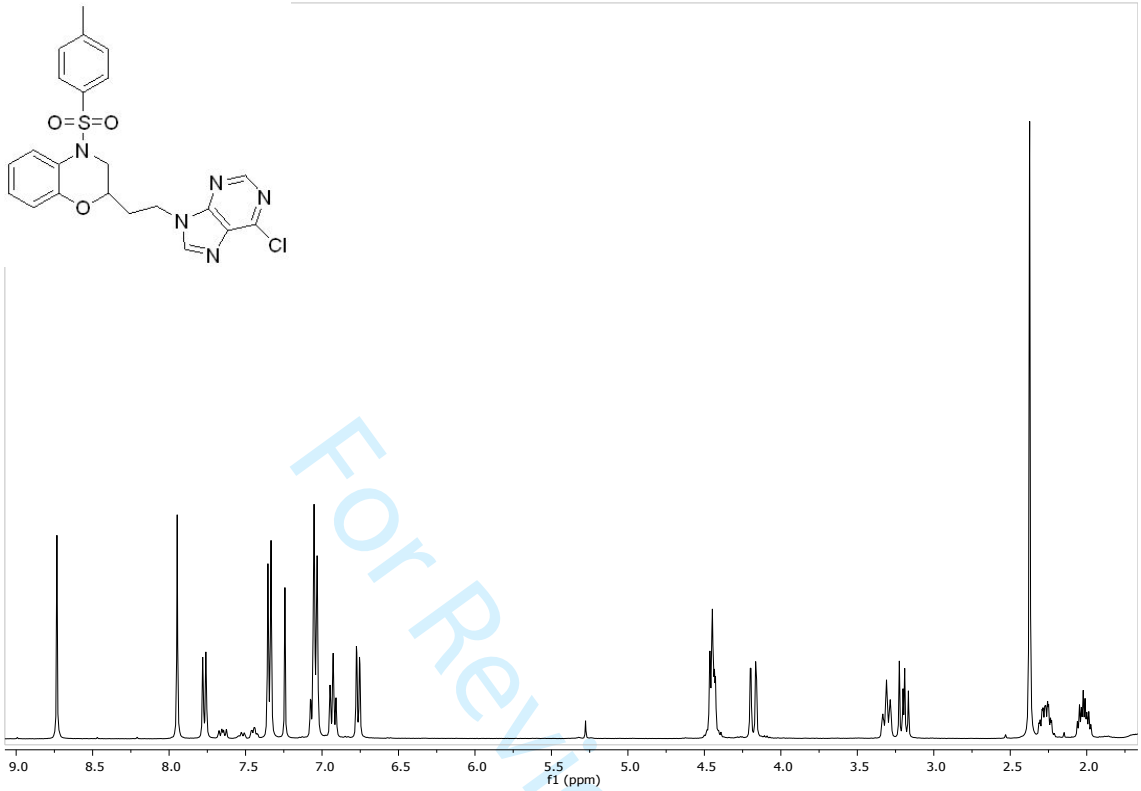
^{1}H NMR *2-(2-(6-Bromo-9*H*-purine-9-yl)ethyl)-4-tosyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine (**3a**)*



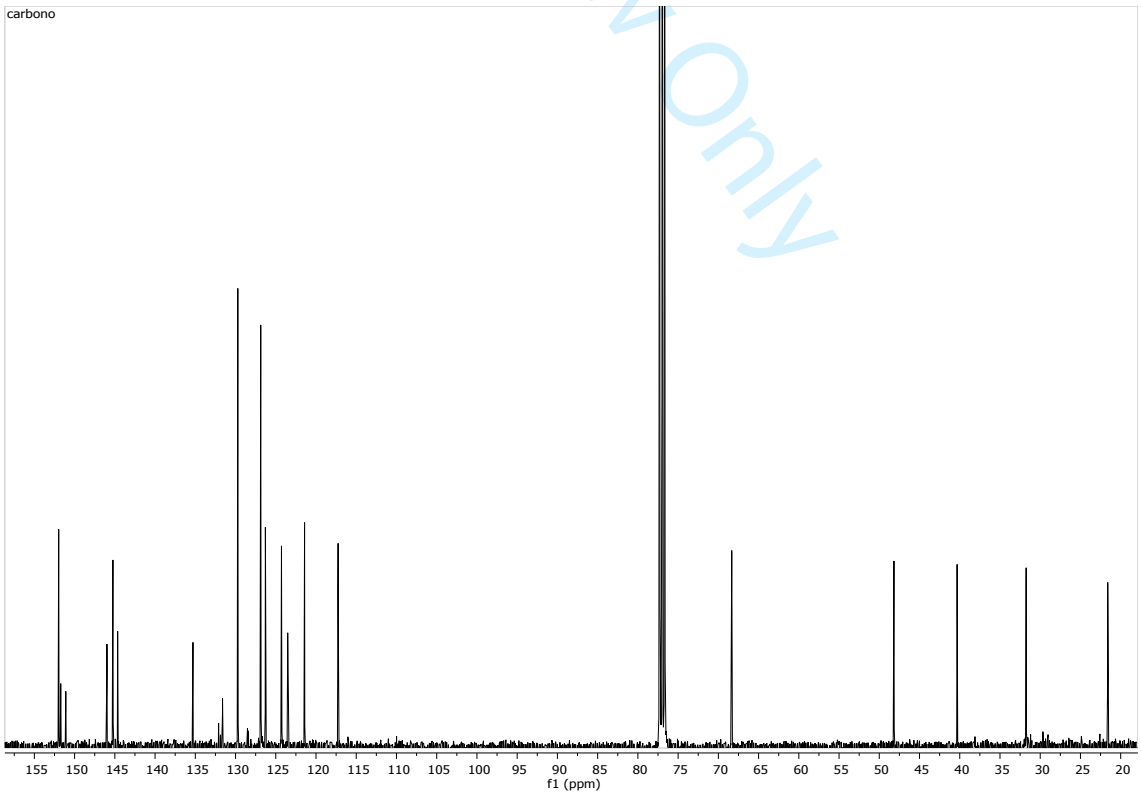
^{13}C NMR



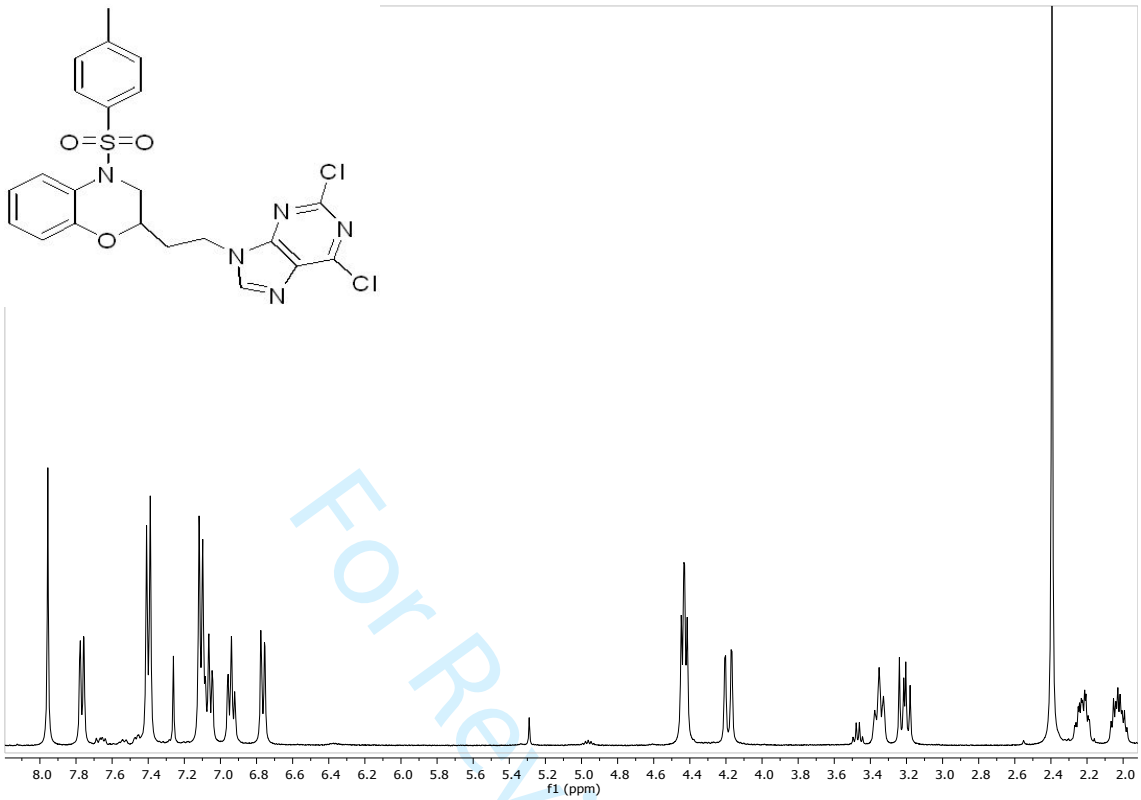
^{1}H NMR *2-(2-(6-Chloro-9*H*-purine-9-yl)ethyl)-4-tosyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine (**3b**)*



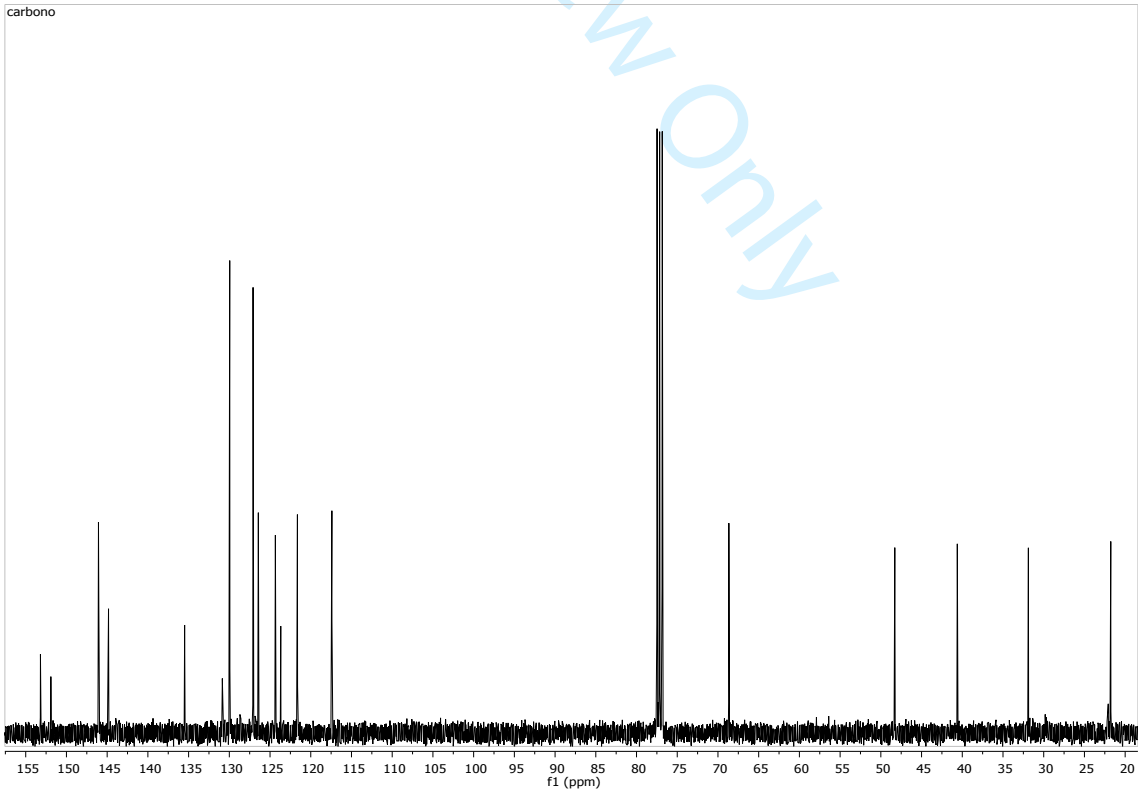
^{13}C NMR



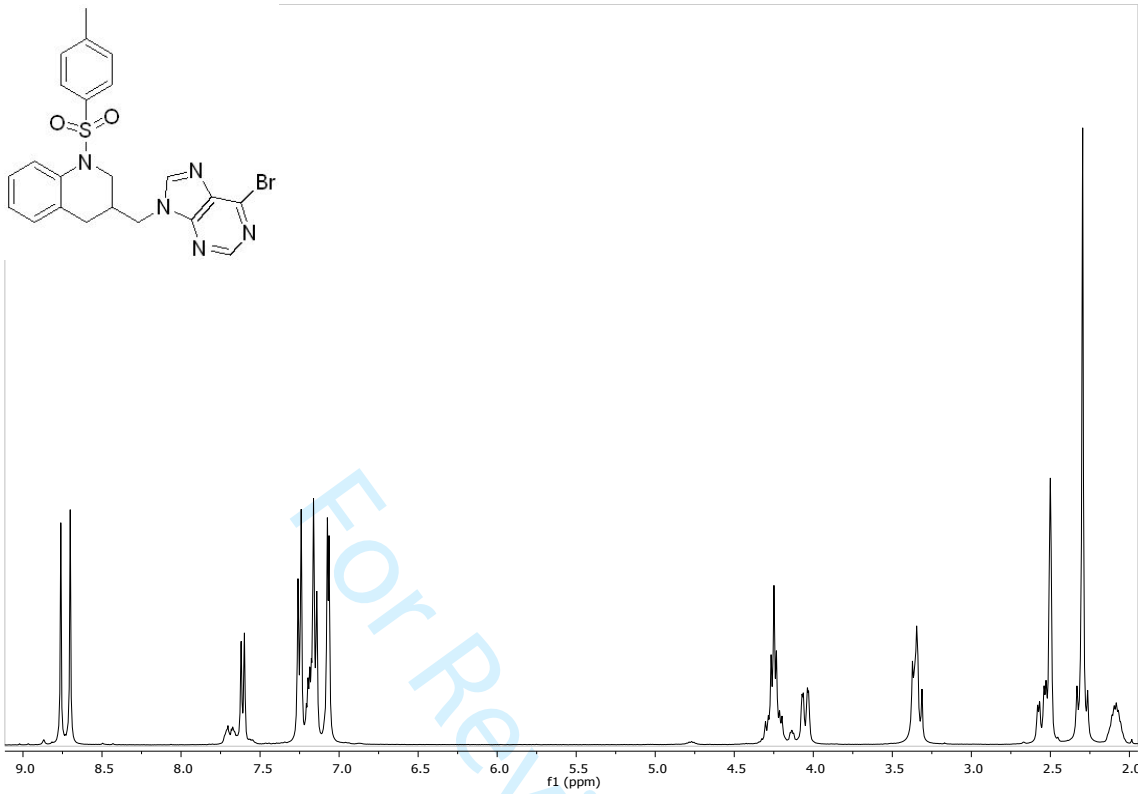
^{1}H NMR *2-(2-(2,6-Dichloro-9*H*-purine-9-yl)ethyl)-4-tosyl-3,4-dihydro-2*H*-benzo[b][1,4]oxazine (**3c**)*



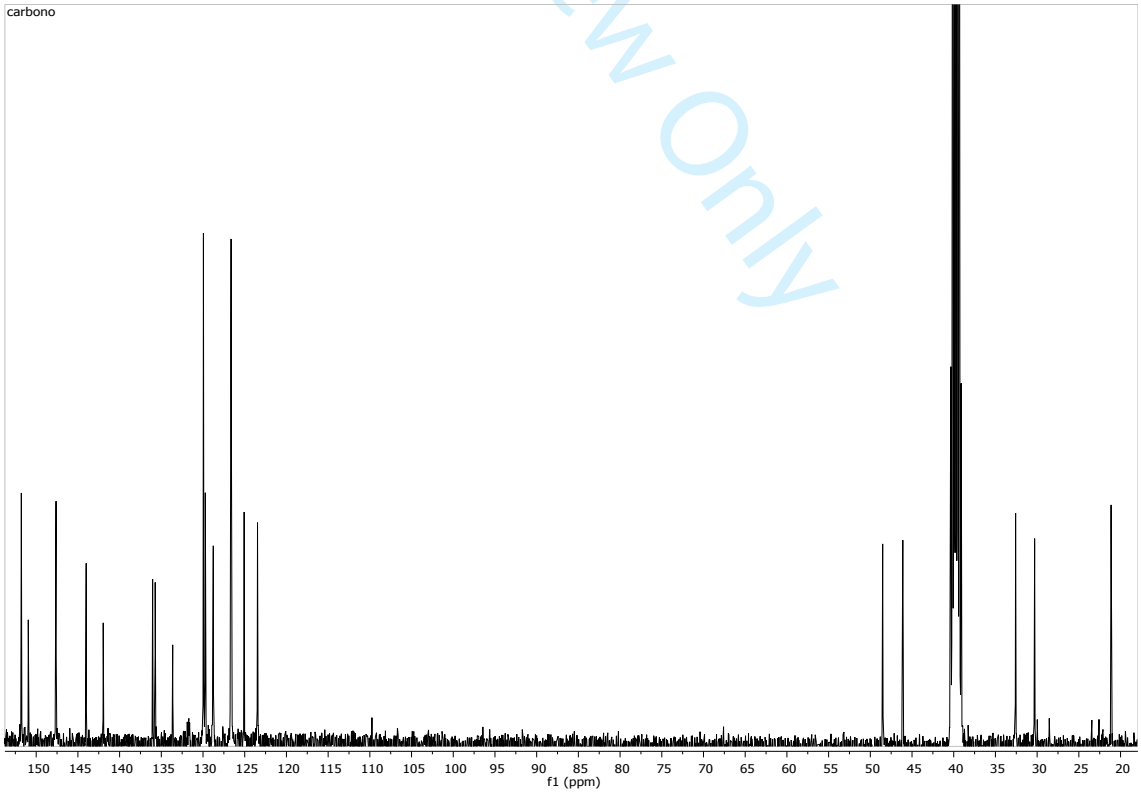
^{13}C NMR



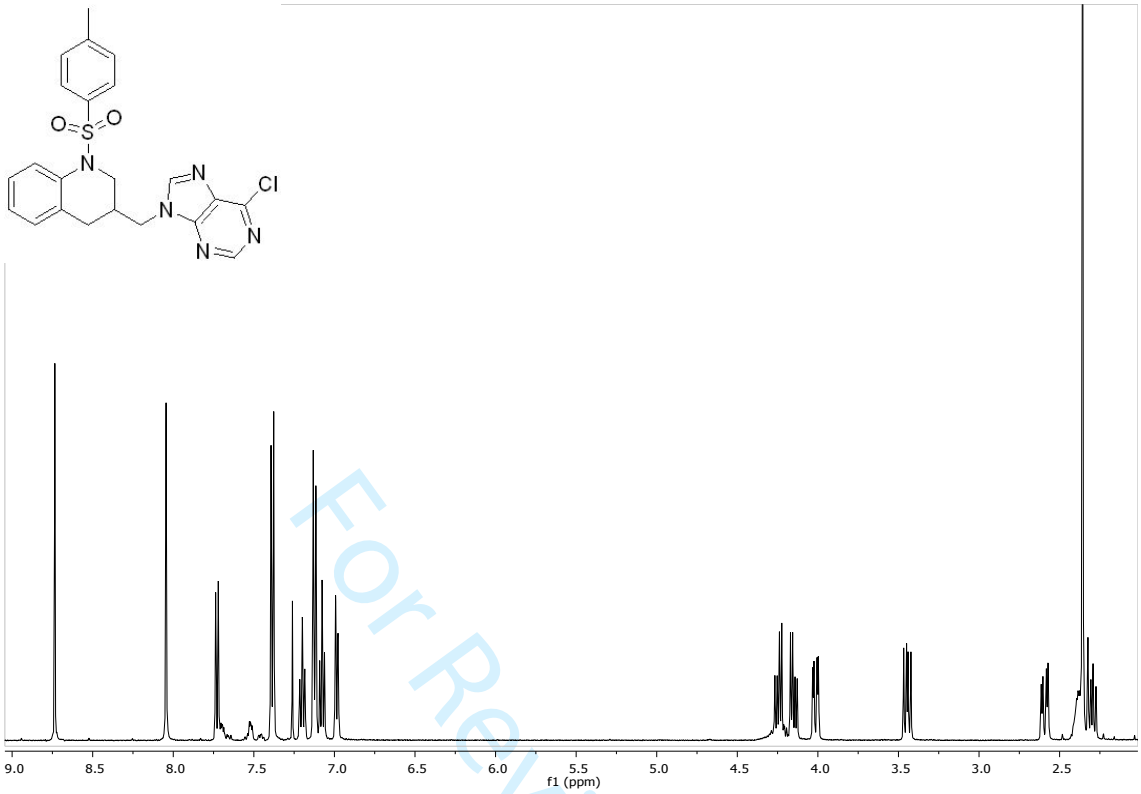
^{1}H NMR *3-((6-Bromo-9*H*-purine-9-yl)methyl)-1-tosyl-1,2,3,4-tetrahydroquinoline (**4a**)*



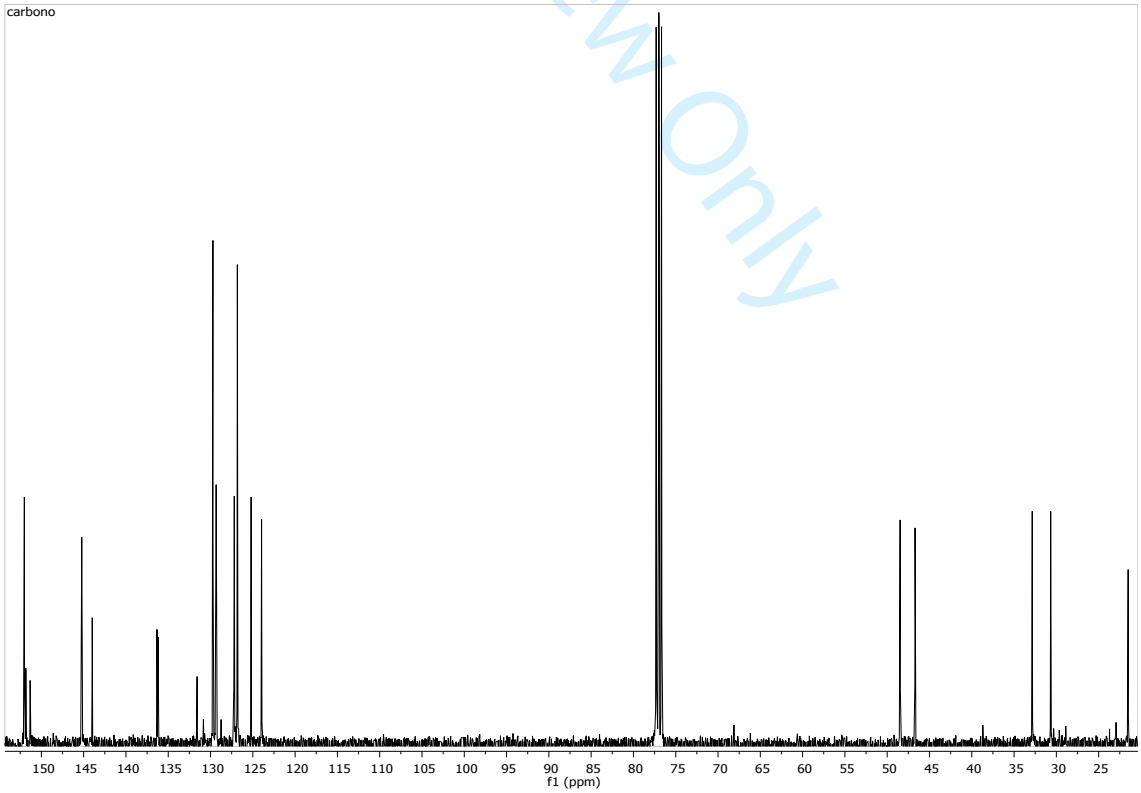
^{13}C NMR



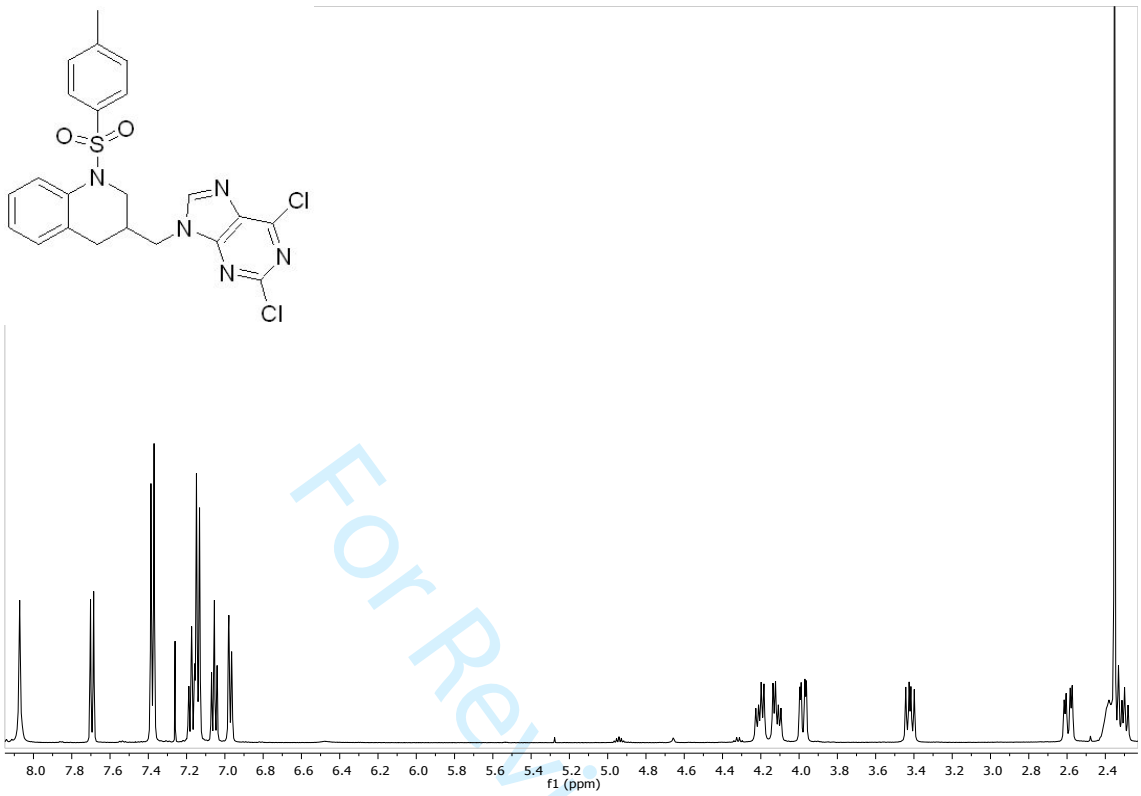
^{1}H NMR *3-((6-Chloro-9*H*-purine-9-yl)methyl)-1-tosyl-1,2,3,4-tetrahydroquinoline (**4b**)*



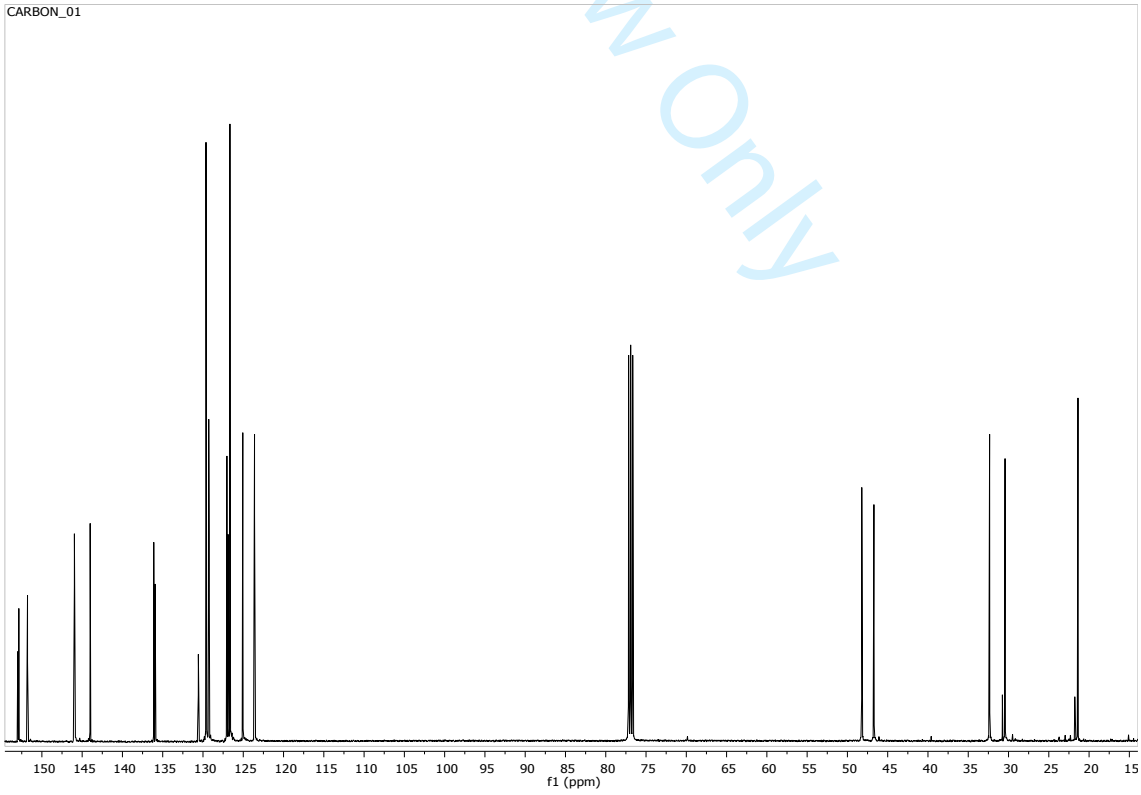
^{13}C NMR



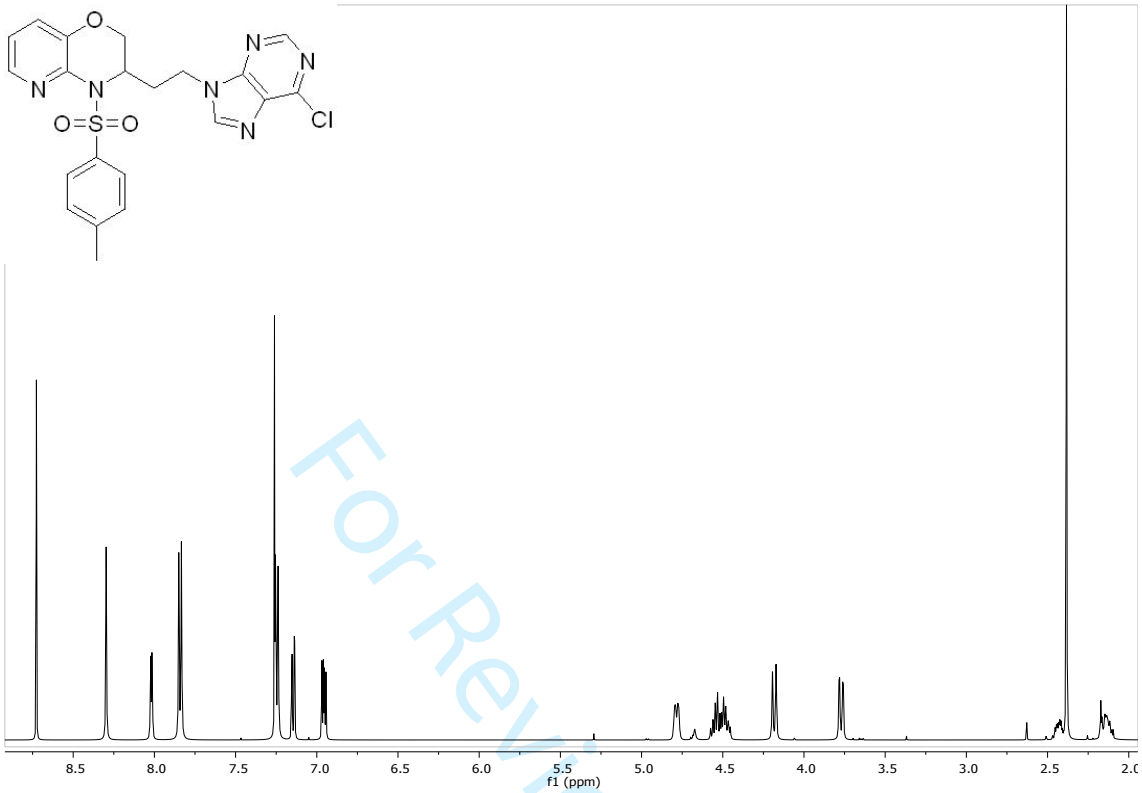
^{1}H NMR *3-((2,6-Dichloro-9*H*-purine-9-yl)methyl)-1-tosyl-1,2,3,4-tetrahydroquinoline (**4c**)*



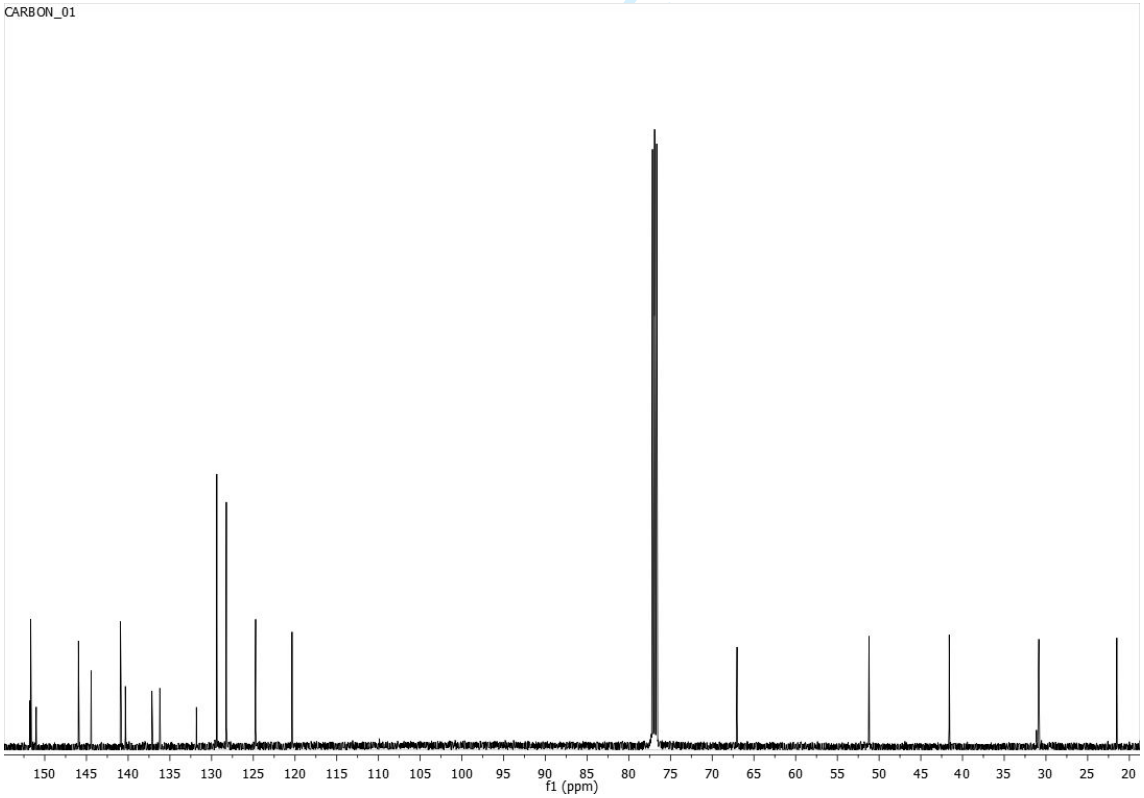
^{13}C NMR



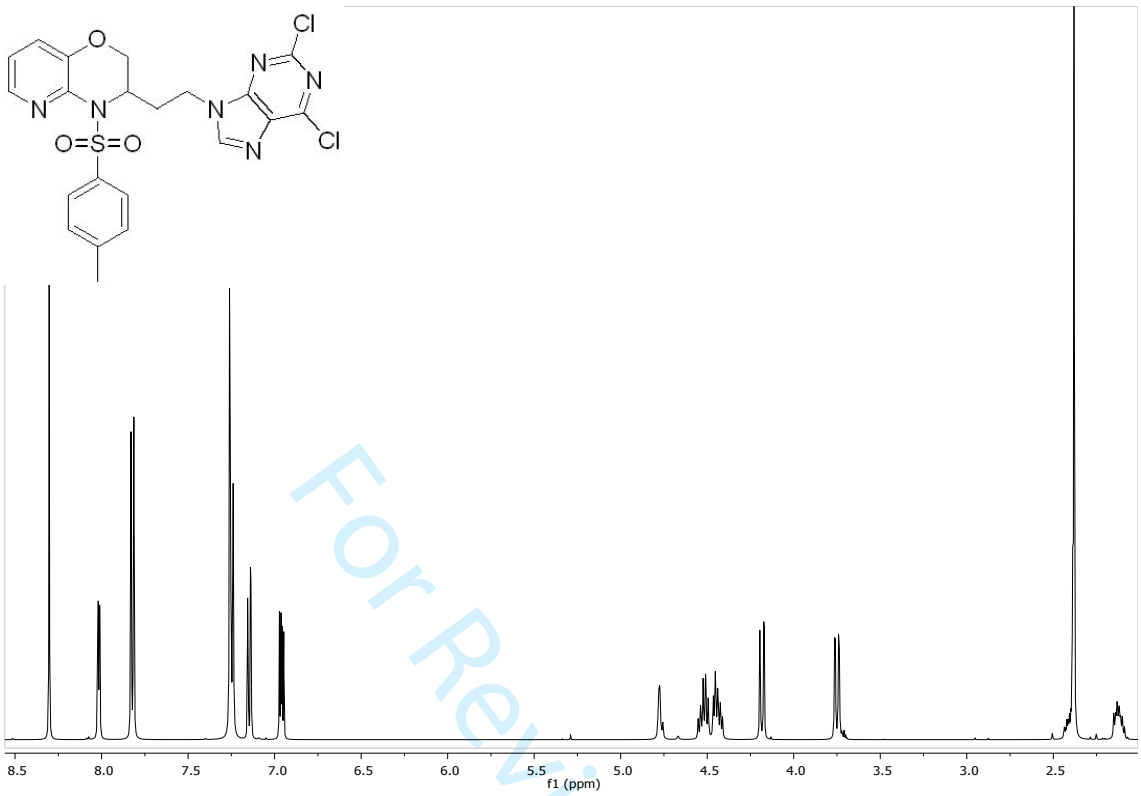
^{1}H NMR *3-(2-(6-Chloro-9*H*-purine-9-yl)ethyl)-4-tosyl-3,4-dihydro-2*H*-pyrido[3,2-b][1,4]oxazine (**5b**)*



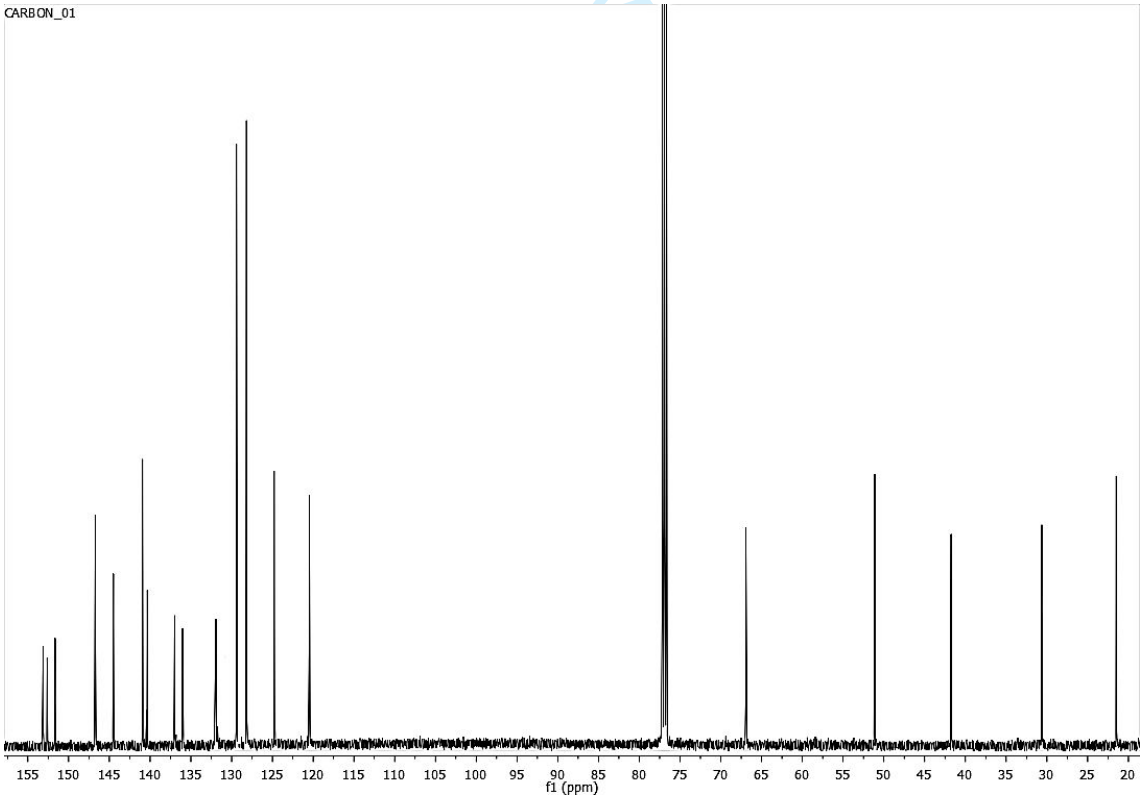
^{13}C NMR



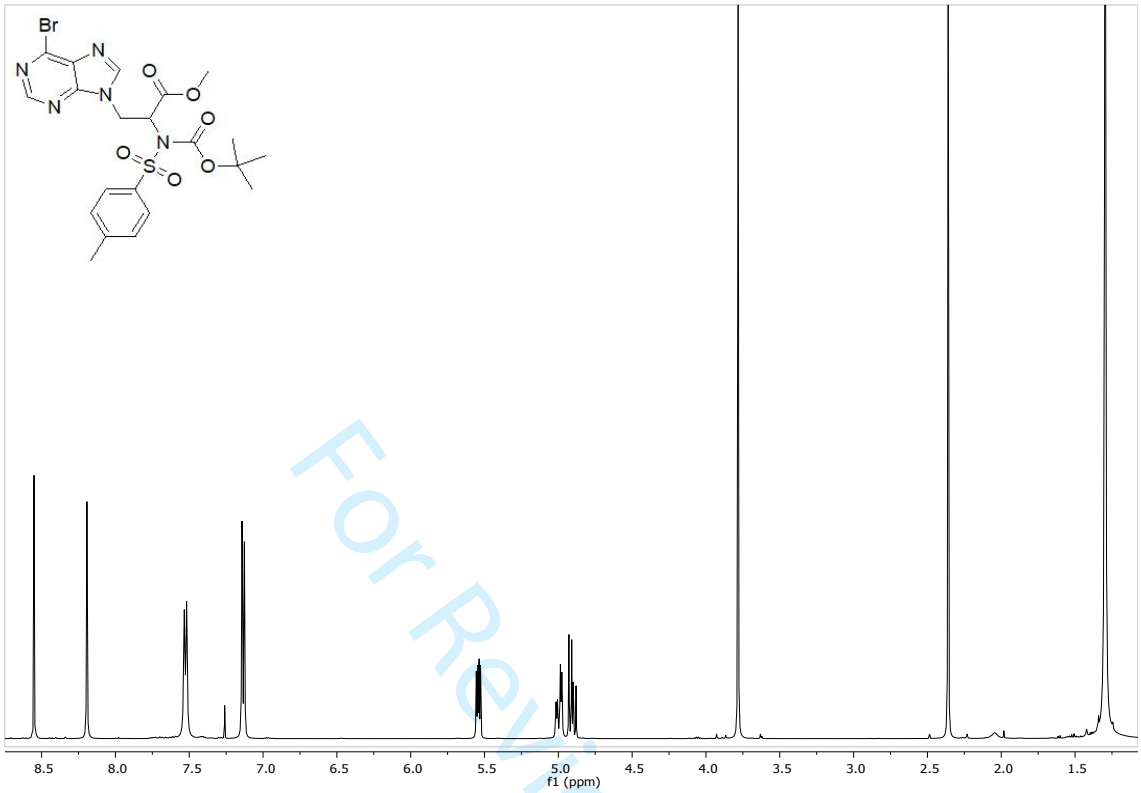
^{1}H NMR *3-(2-(2,6-Dichloro-9*H*-purine-9-yl)ethyl)-4-tosyl-3,4-dihydro-2*H*-pyrido[3,2-b][1,4]oxazine (**5c**)*



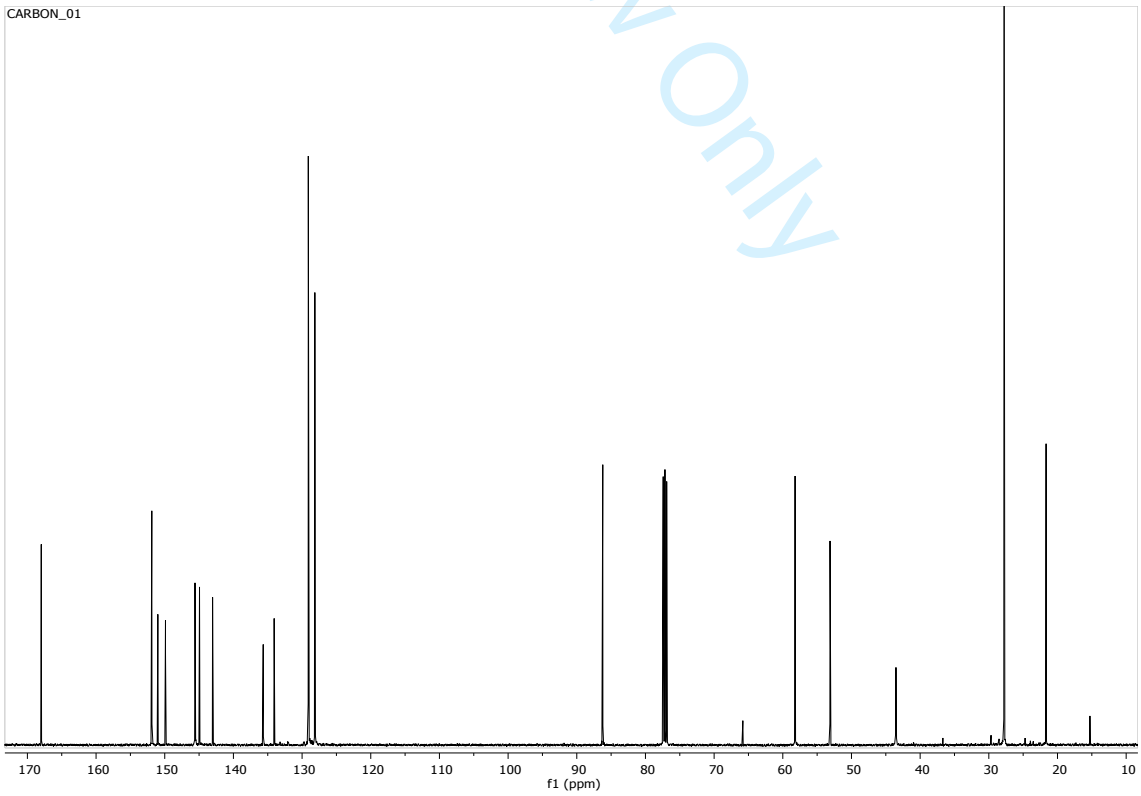
^{13}C NMR



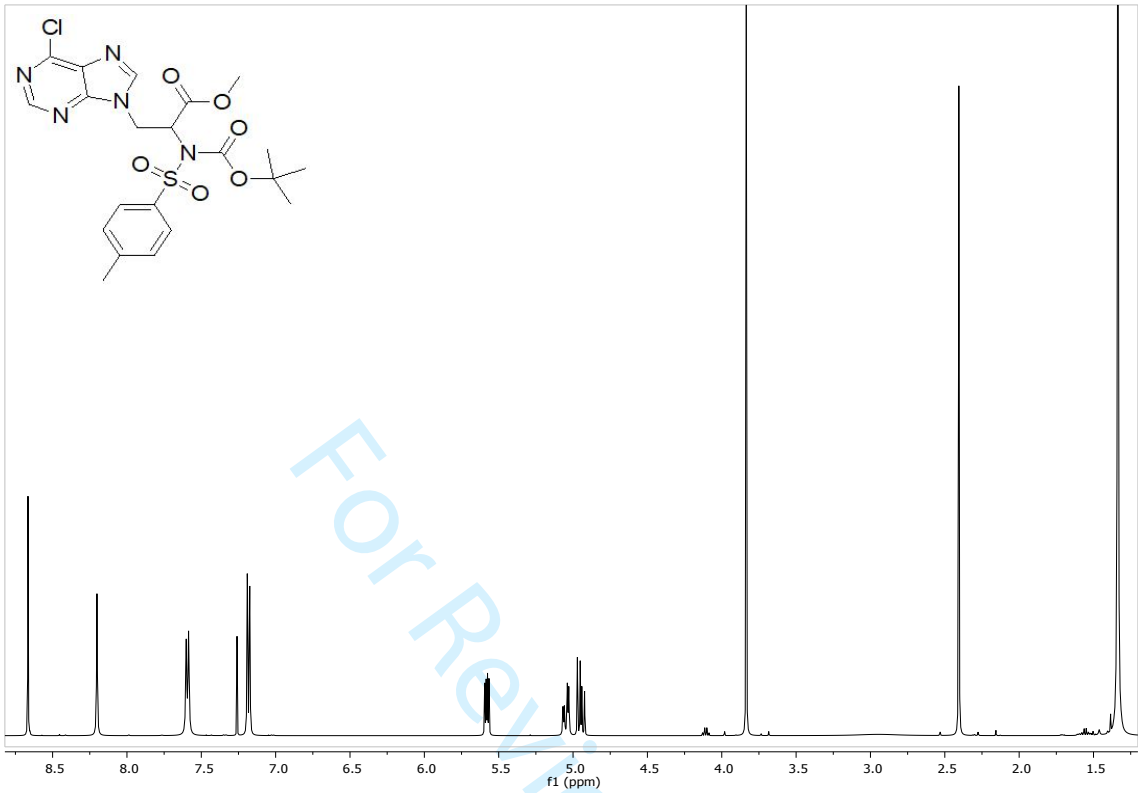
^{1}H NMR *Methyl-2-(*N-tert-*butoxycarbonyl)*-N-*tosylamino)-3-(6-bromo-9*H-*purine-9-yl)propanoate (**6a**)*



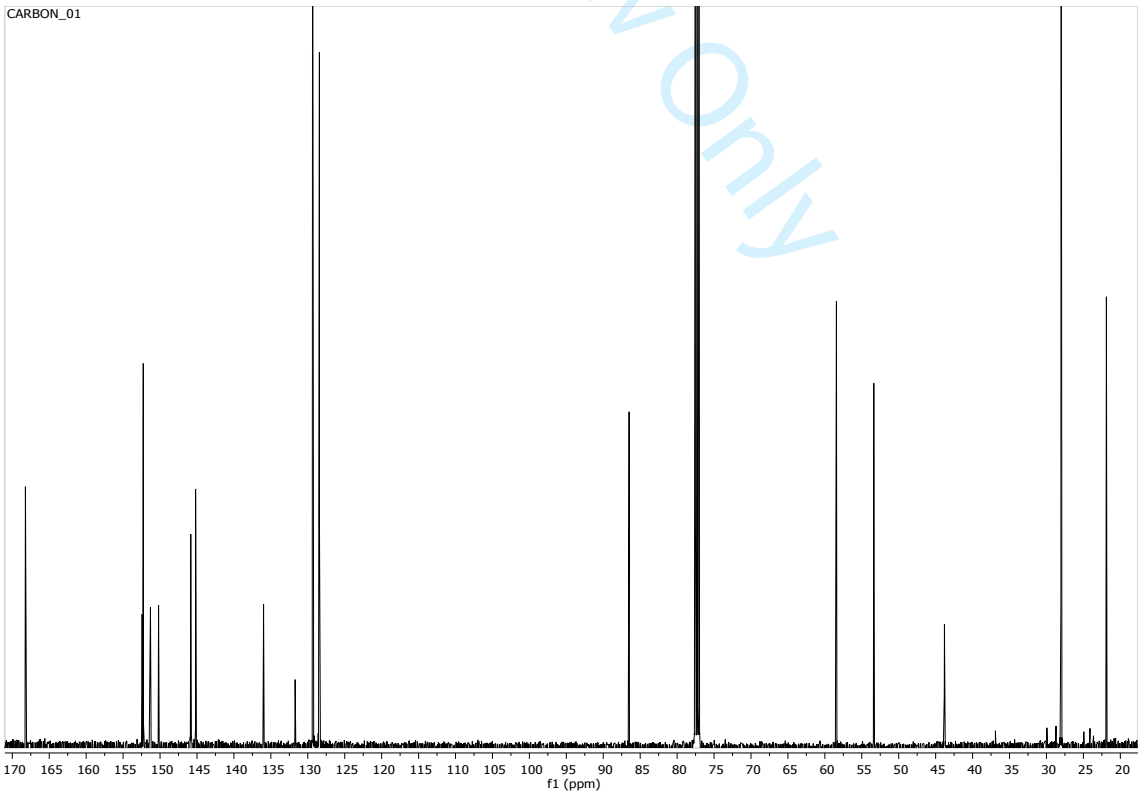
^{13}C NMR



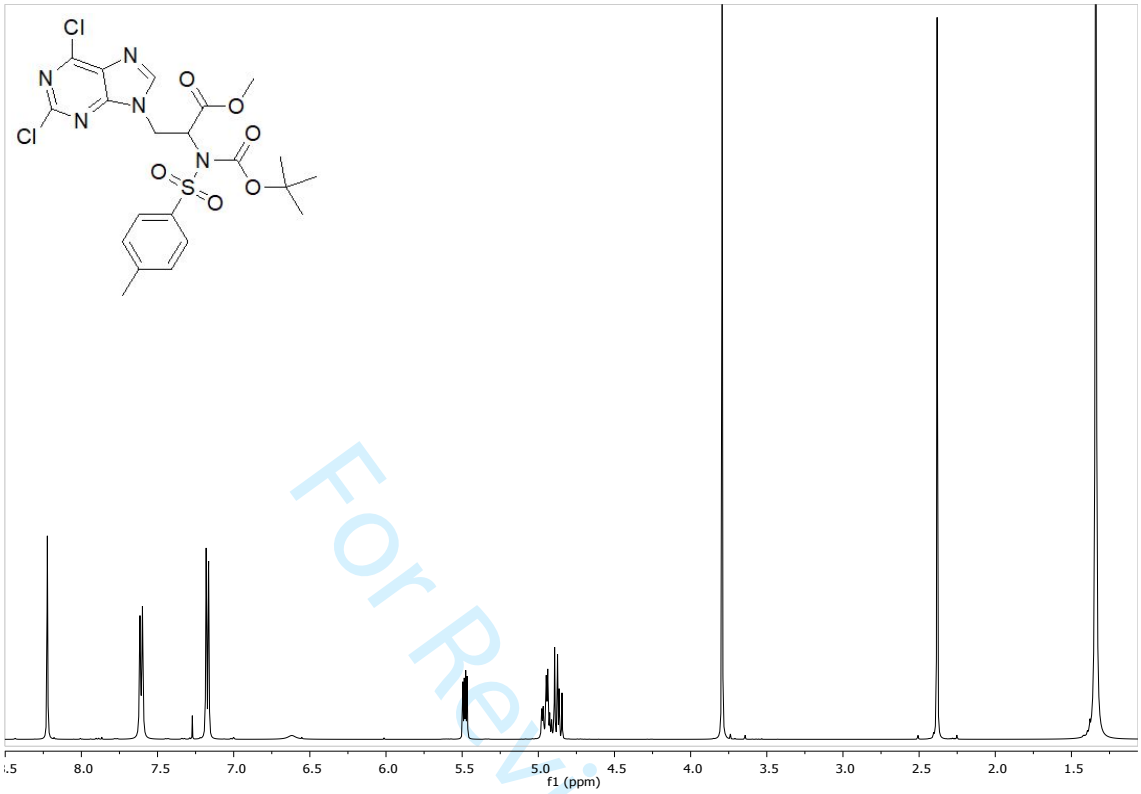
^{1}H NMR *Methyl-2-(*N-tert*-butoxycarbonyl)*-N-*tosylamino)-3-(6-chloro-9*H*-purine-9-yl)propanoate (**6b**)*



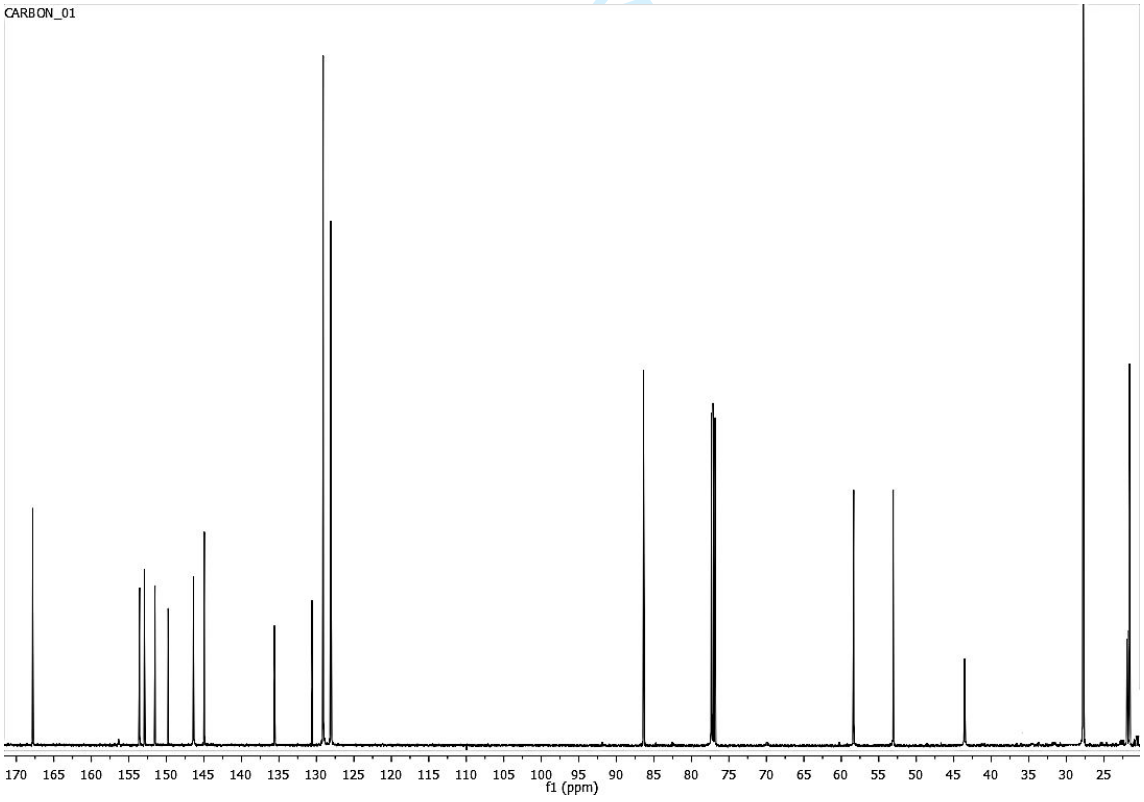
^{13}C NMR



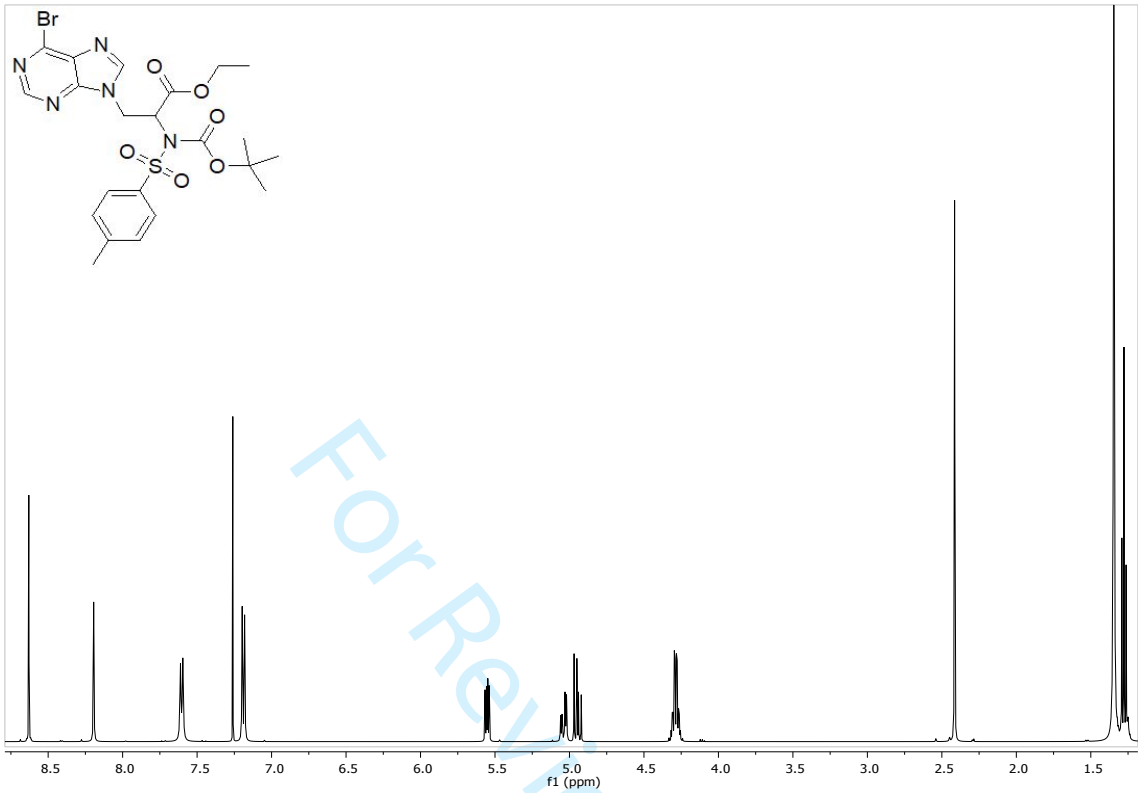
^{1}H NMR *Methyl-2-(*N-tert-*butoxycarbonyl)*-N-*tosylamino)-3-(2,6-dichloro-9*H-*purine-9-yl)propanoate (**6c**)*



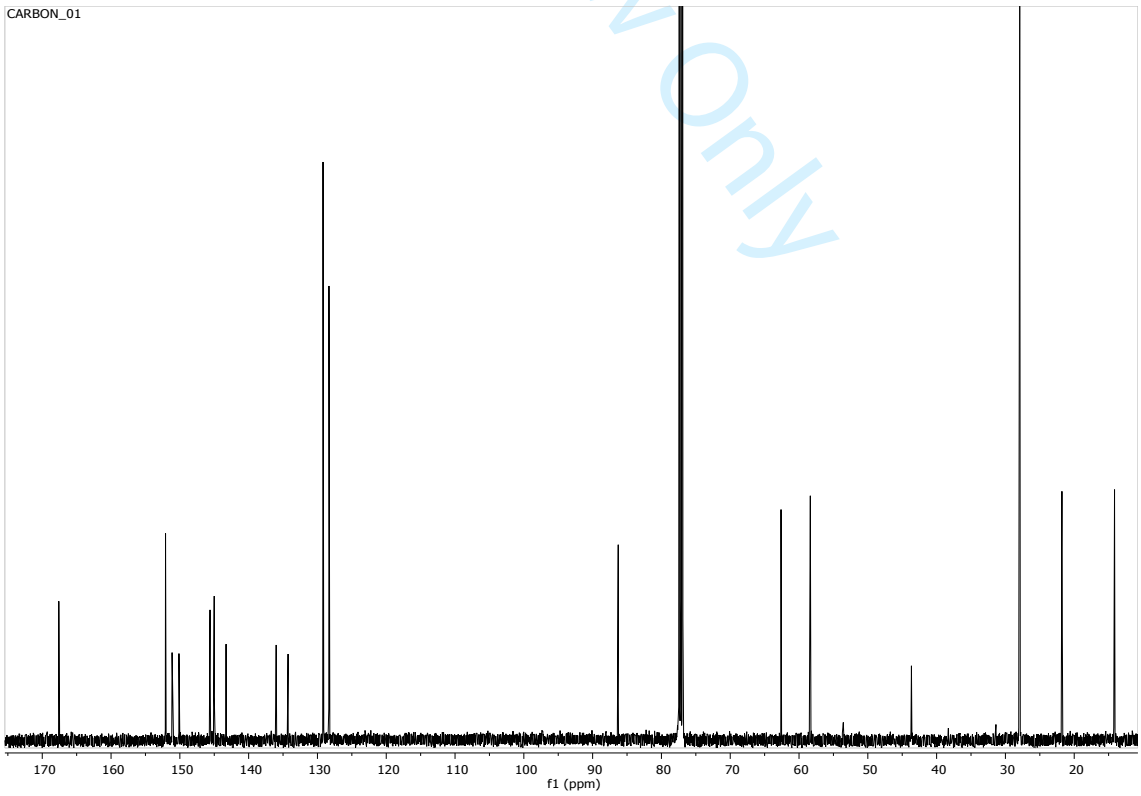
^{13}C NMR



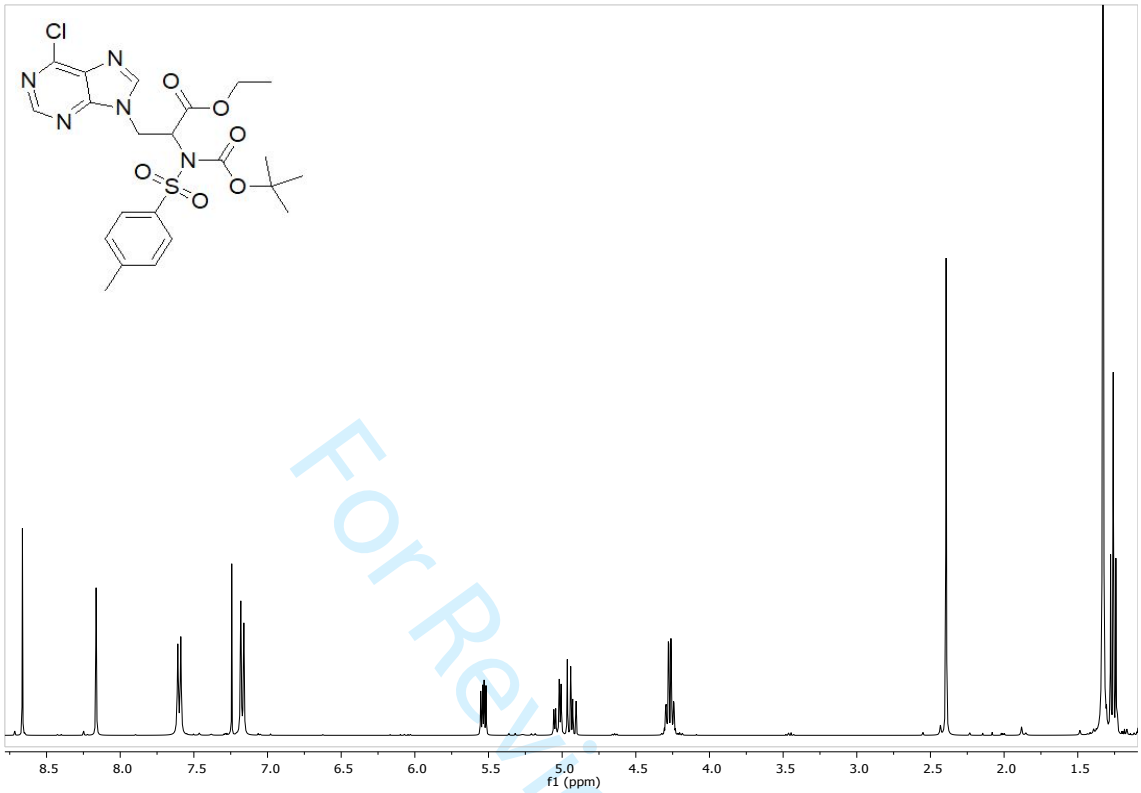
^{1}H NMR *Ethyl-2-(*N-tert-*butoxycarbonyl)*-N-*tosylamino)-3-(6-bromo-*9H-*purine-9-yl)propanoate (**7a**)*



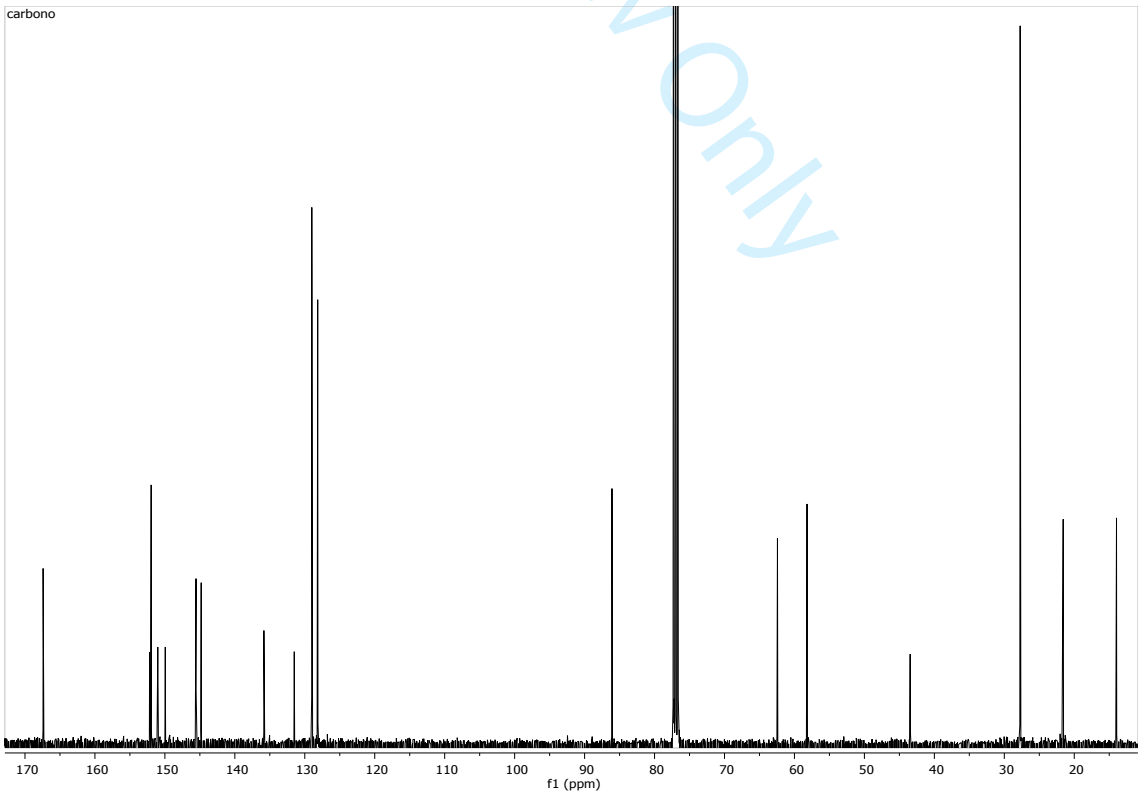
^{13}C NMR



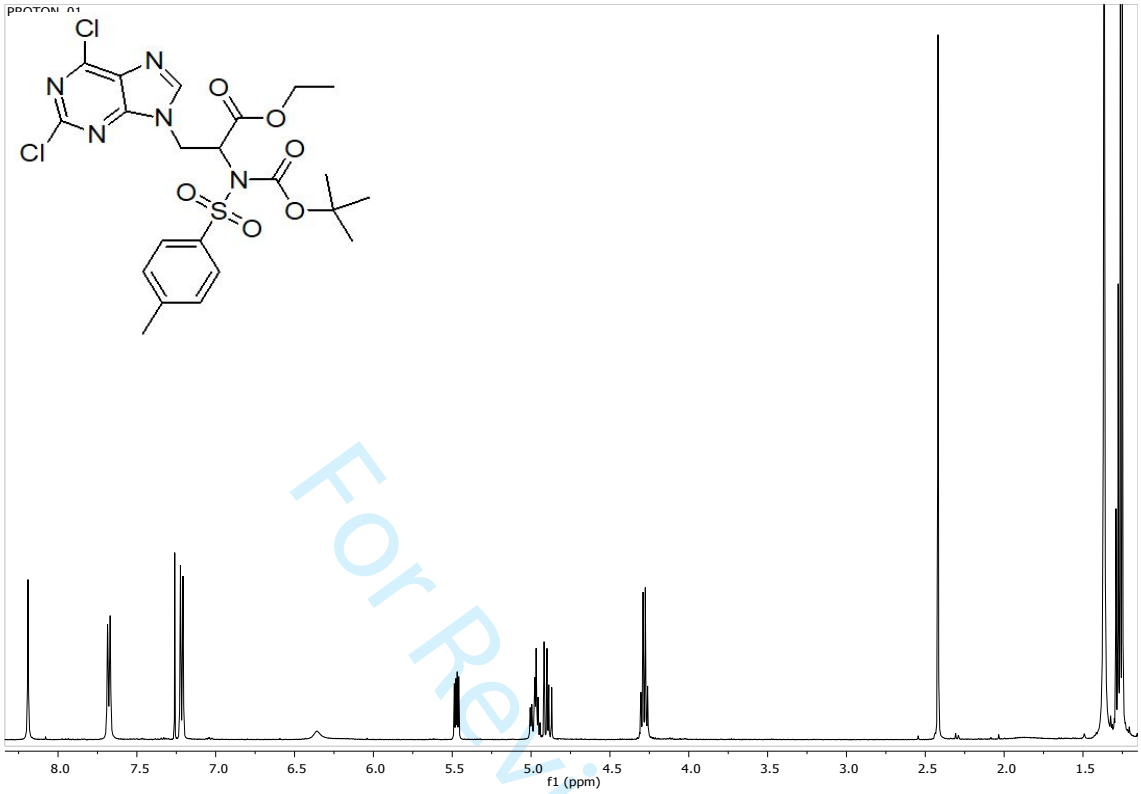
^{1}H NMR *Ethyl-2-(*N-tert-*butoxycarbonyl)*-N-*tosylamino)-3-(6-chloro-9*H-*purine-9-yl)propanoate (**7b**)*



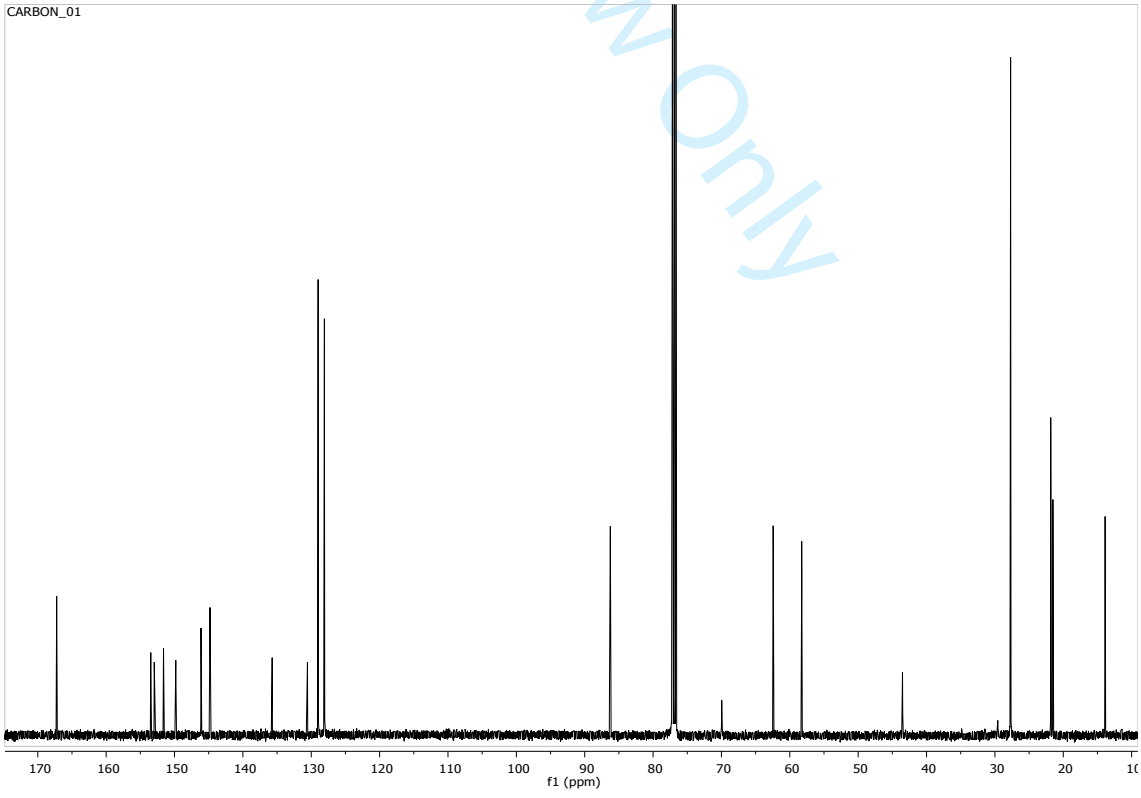
^{13}C NMR



^{1}H NMR *Ethyl-2-(*N-tert-*butoxycarbonyl)*-N-*tosylamino)-3-(2,6-dichloro-9*H-*purine-9-yl)propanoate (**7c**)*

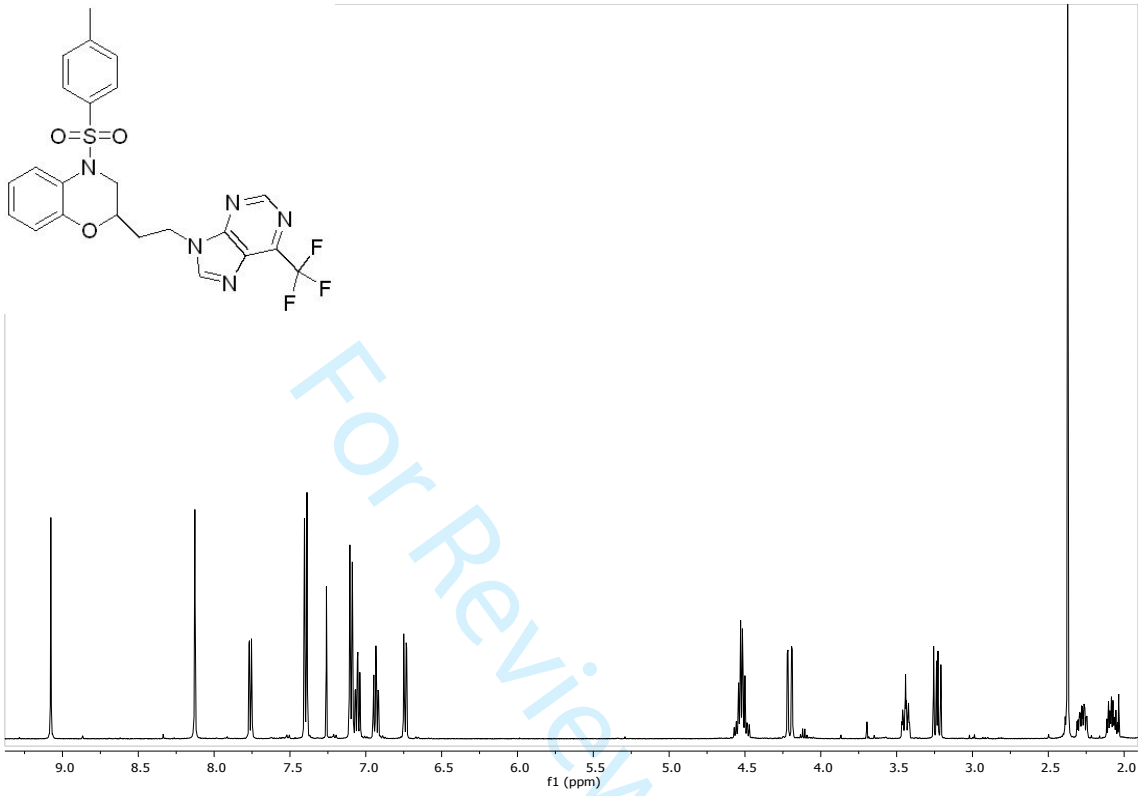


^{13}C NMR

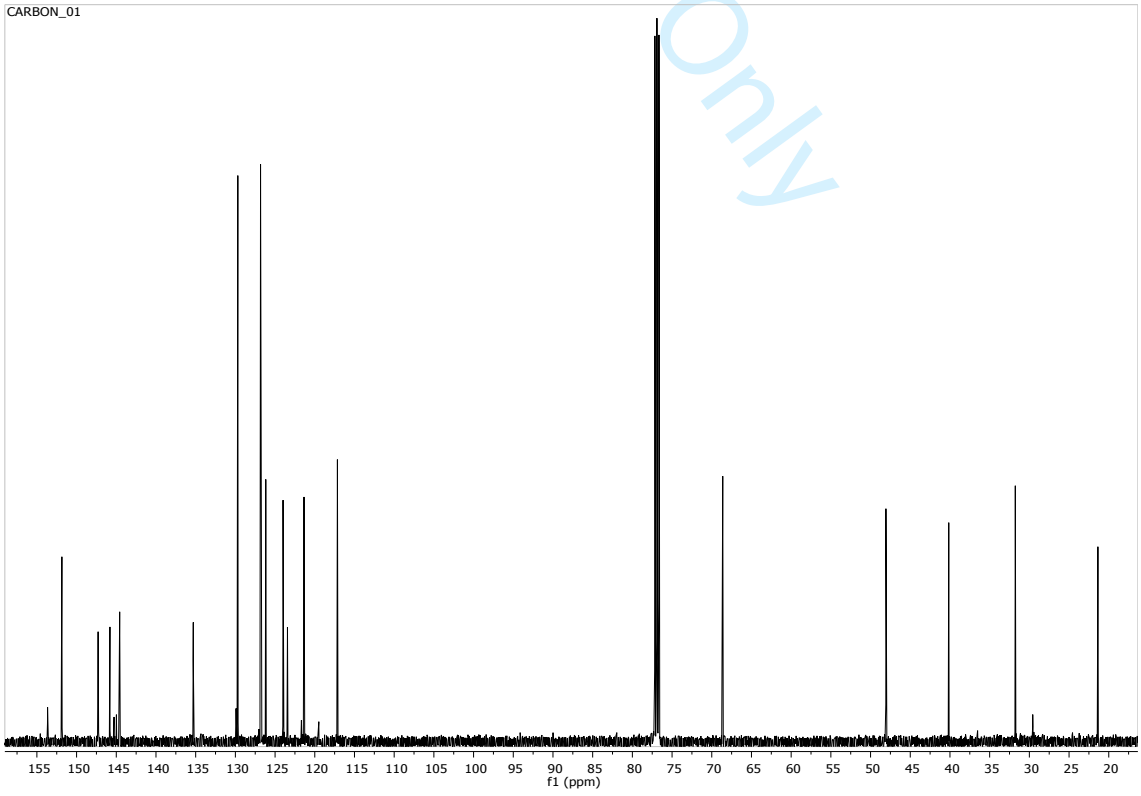


^{1}H and ^{13}C-RMN spectra of 6-trifluoromethylpurines

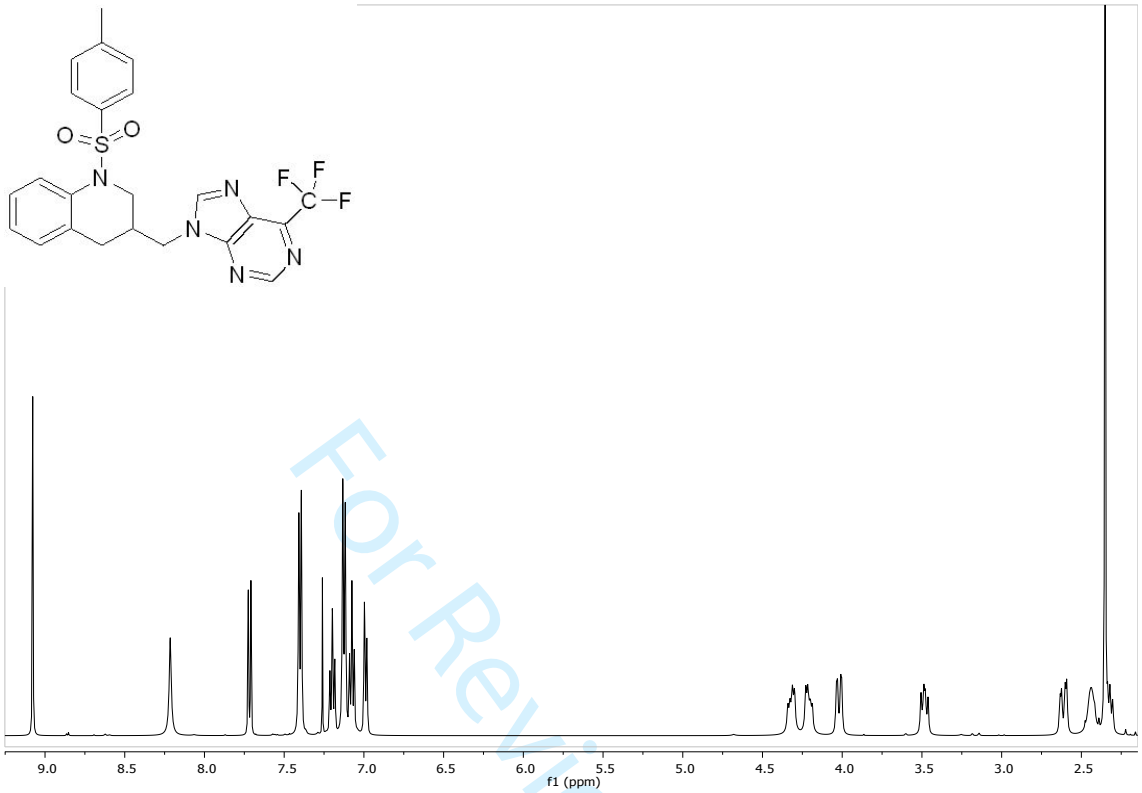
^{1}H NMR *2-(2-(6-Trifluromethyl-9*H*-purine-9-yl)ethyl)-4-tosyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine* (**3d**)



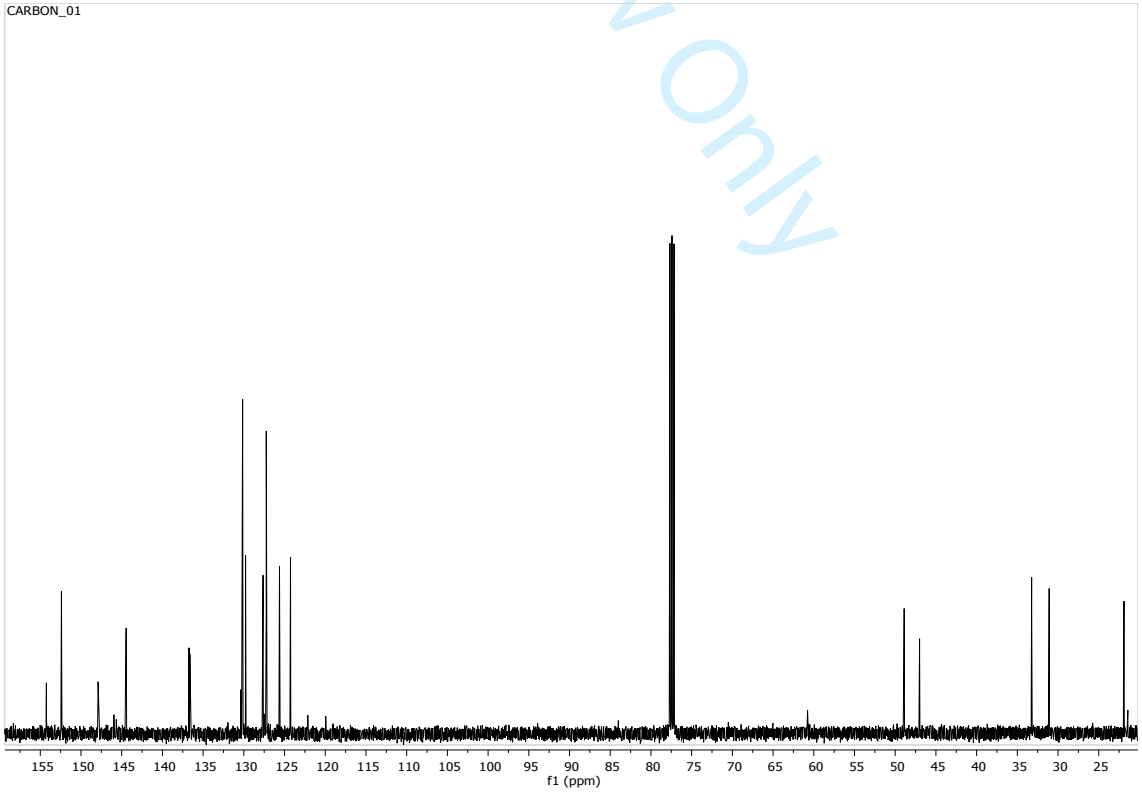
^{13}C NMR



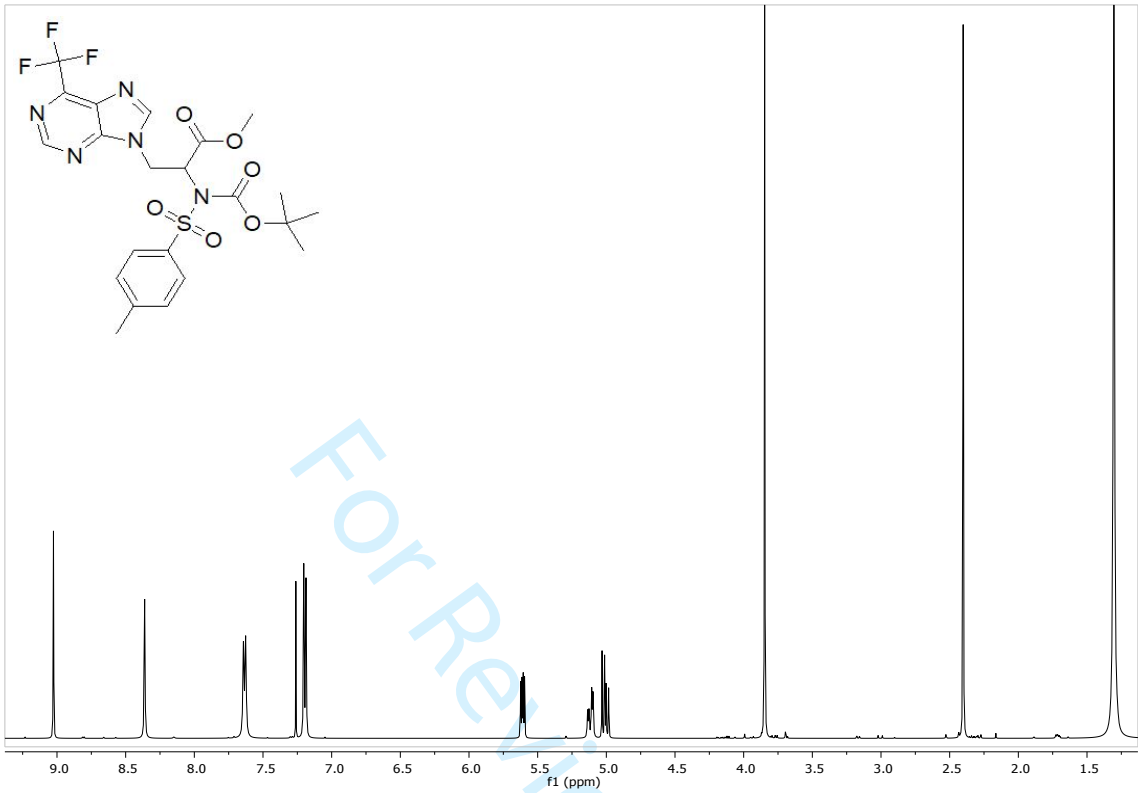
^{1}H NMR *3-((6-Trifluoromethyl-9*H*-purine-9-yl)methyl)-1-tosyl-1,2,3,4-tetrahydroquinoline* (**4d**)



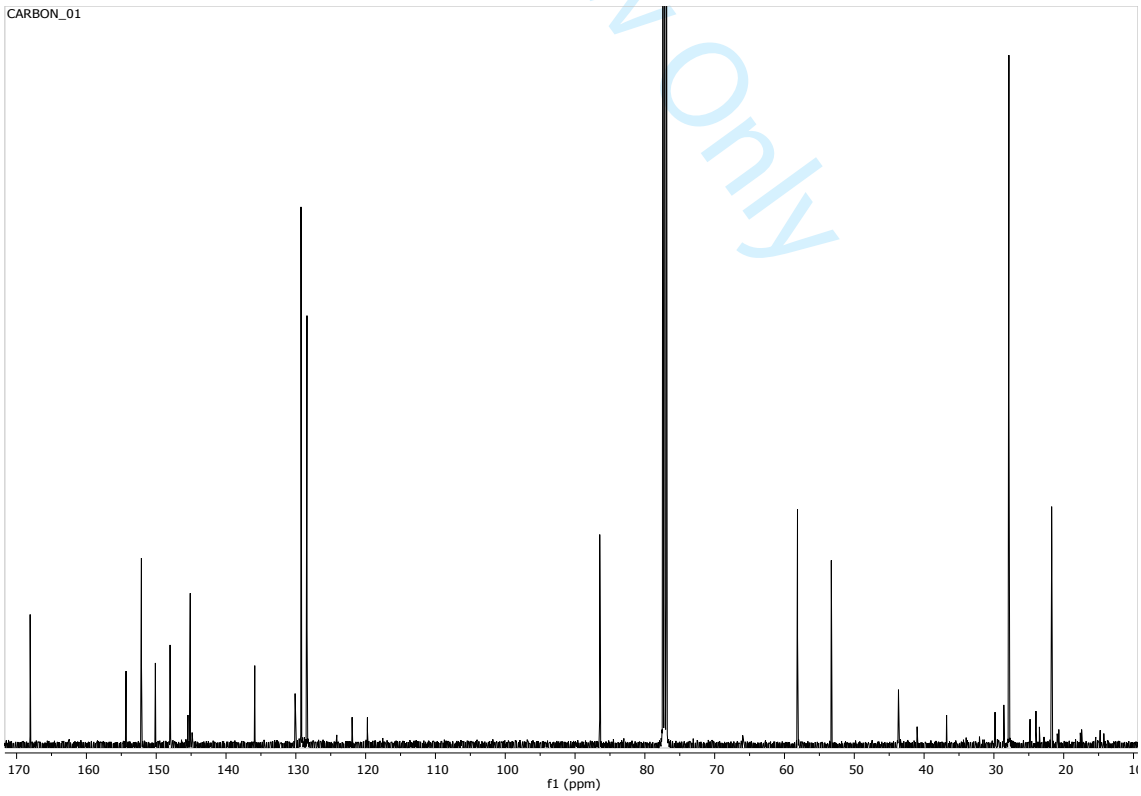
^{13}C NMR



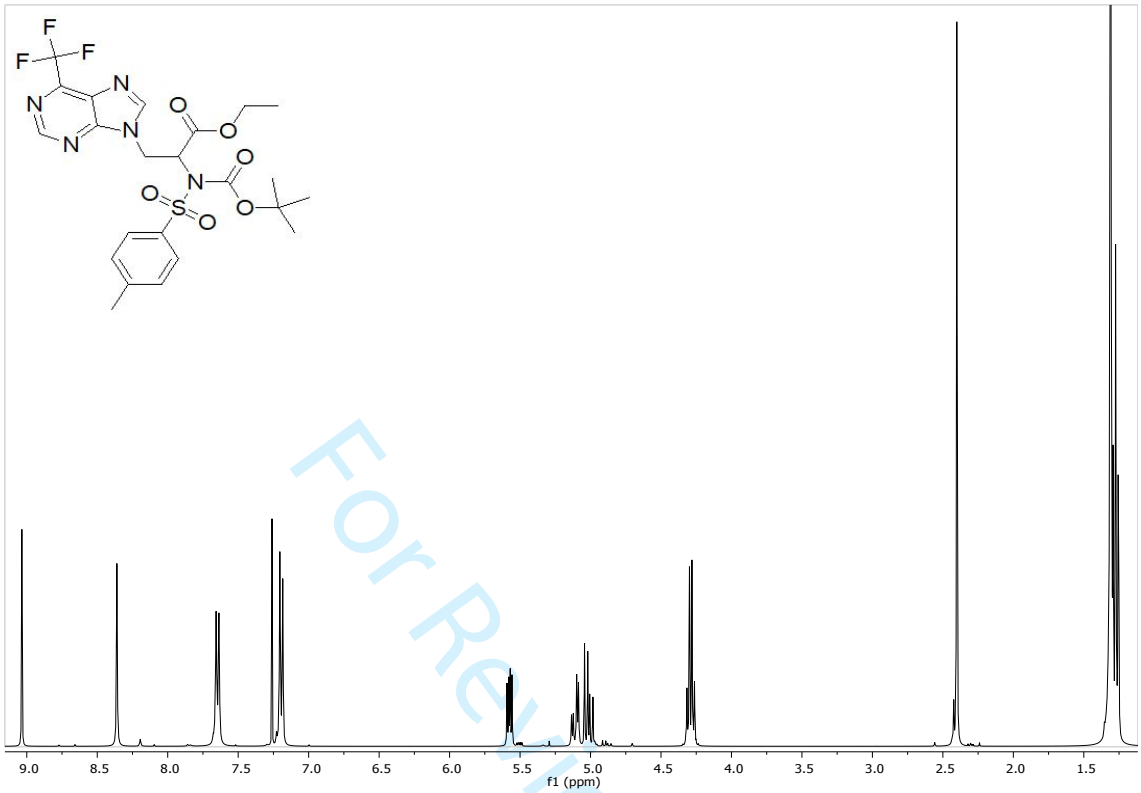
^{1}H NMR *Methyl-2-(*N-tert-*butoxycarbonyl)*-N-*tosylamino)-3-(6-trifluoromethyl-9*H-*purine-9-yl)propanoate*(**6d**)



^{13}C NMR



^{1}H NMR *Ethyl-2-(*N-tert-*butoxycarbonyl)*-N-*tosylamino)-3-(6-trifluoromethyl-9*H-*purine-9-yl)propanoate*(**7d**)



^{13}C NMR