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NMR studies of new heterocycles tethered to purine moieties with anticancer activity

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KEYWORDS: ^1H and ^{13}C 1D NMR; $^1\text{H}/^{13}\text{C}$ 2D NMR (HSQC, HMBC); 2D NOESY; benzoxazine, quinoline, pyridoxazine.

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/mrc.4871

Introduction

Cancer is one of the greatest threats of our society because is one of the leading causes of death. Many active compounds have been developed to face this problem in recent years.^[1-3]

Some purine derivatives with an interesting anti-proliferative activity have been previously synthesized by combining benzo-fused heterocycles linked to substituted purines.^[4-10] In order to improve the activity and have a deeper idea of the structure-activity relationship, new compounds have been obtained. Such changes include some bioisosteric replacements as elimination of oxygen atom of the heterocycle or elongation of the linking chain between the six-membered heterocycles and the substituted purines.

In this way the synthesis and biological evaluation of these novel families of purine derivatives linked to six-membered heterocyclic moieties **3a-d**, **5a-d** and **11a-b**, which were designed and evaluated as anticancer agents, have been described.^[11]

The structures of these new compounds have been determined by ¹H and ¹³C NMR and mass spectrometry (MS). Some of them have been studied more detailed in order to corroborate their skeleton by using two-dimensional techniques, and an elemental analysis have been performance to all the final compounds.

The present study reports the unambiguous assignment of each signal in the ¹H and ¹³C NMR spectra in benzoxazine derivatives (**3a-d**), including elongation of the side chain, tetrahydroquinolines (**5a-d**) and pyridoxazine derivatives (**11a-b**) in which a pyridine ring is merged to the oxazine heterocycle, using one- and two-dimensional resonance techniques. The assignment of derivatives **1**, **2**, **4**, **6-10**, the precursors in their synthetic pathway, are also included.

Experimental

NMR spectra

Nuclear magnetic resonance (NMR) spectra were made on a 400-MHz ¹H and 101 MHz ¹³C NMR Agilent Varian Direct Drive, and a 500-MHz ¹H and 125-MHz ¹³C NMR Agilent Varian Innova Unity spectrometers at 298 K. In DEPT experiments the employed parameters

were the following: pulse width (135°), 9.0 ms; recycle time, 1 s; $\frac{1}{2} J$ (CH) = 4 ms; 65 536 data points acquired and transformed from 1024 scans; spectral width, 15 KHz; and line broadening, 1.3 Hz. Chemical shifts (δ) are quoted in parts per million (ppm) and are referenced to the residual solvent peak: CDCl_3 , $\delta = 7.26$ ppm (^1H), $\delta = 77.4$ ppm (^{13}C) or $(\text{CD}_3)_2\text{SO}$, $\delta = 2.50$ ppm (^1H), $\delta = 39.52$ ppm (^{13}C). Spin multiplicities are given as s (singlet), bs (broad singlet), d (doublet), dd (doublet of doublet), ddd (doublet of doublet of doublet), td (triplet of doublet), t (triplet), tt (triplet of triplet), q (quadruplet), and m (multiplet). Coupling constant (J) are given in Hz.

The HSQC spectra were calculated with a pulse sequence gc2hsqcse (Standard sequence, Agilent Vnmrj_3.2A software). The HMBC spectra were determined with a pulse sequence gc2hmbc (standard sequence, Agilent Vnmrj_3.2A software) optimized for 8 Hz (inter-pulse delay for the evolution of long-range couplings: 62.5 ms).

Nuclear Overhauser spectra were recorded on a Agilent Varian Direct Drive spectrometer, operating at 500 MHz, with a spectral width of 8.01 KHz at 16K complex points (acquisition time 2s). The mixing time in 1D-NOESY experiment was 0.5 s. Data processing with zero filling at 64K and apodization with exponential function (LB = 0.5 Hz).

Results and Discussion

Schemes 1-3 depict the synthetic route carried out in the preparation of final compounds **3a-d**, **5a-d** and **11a-b**, previously reported^[11] and Tables 1-4 show the spectroscopic data for both the intermediate and final synthesized compounds.

Schemes 1-3 may be inserted here

Table 1-2 may be inserted here

The structure of these compounds has been elucidated by routine ^1H and ^{13}C NMR techniques. Nevertheless, a definitive assignment of all signals was accomplished with the help of NMR techniques. Such procedures include the following: i) DEPT experiments for determining the presence of primary, secondary and tertiary carbon atoms; ii) HSQC spectra to assign the ^{13}C resonances of the primary, secondary and tertiary carbons; iii) HMBC

sequences to corroborate the signals of quaternary carbons via two-bond and three-bond interactions.

Tables 1 and 2 show the ^1H NMR signals of each proton for molecules **1-11**, whereas Tables 3 and 4 show the corresponding ^{13}C NMR chemical shifts for the same compounds. The NMR spectra of the intermediates were carried out in CDCl_3 solutions. The NMR spectra of the final compounds were performed in CDCl_3 solutions, except for **5c**, which was accomplished in $\text{DMSO}-d_6$ for solubility reasons. As a consequence, some significant variations are observed in the chemical shifts depending of the solvent.

In relation to the benzoxazine family, chemical shifts of ^1H and ^{13}C NMR of the tosylated intermediate **2** and the final derivatives **3a-d** are similar for H-2, H-3a, H-3b, H-5, H-6, H-7 and H-8; nevertheless, in the untosylated intermediate **1**, H-2 appears at a greater chemical shift than H-3a. In the same way, C-2 of the intermediate **1** appears at a higher shift than C-2 in both compounds, the tosylated intermediate **2** and the tosylated purines **3a-d**. Finally, the presence of trifluoromethyl group in **3d** is justified due to the following C-F couplings: 145.20 (q, $J_{\text{C-CF}_3} = 37.1$, C-6') and 120.69 (q, $J_{\text{CF}} = 274.9$, CF_3).

In addition, in the dihydroquinoline series, the ^1H NMR signals are similar in both, the intermediate **4** and the final purines **5a-d**, except for the CH_2 signal of the side chain. In derivative **4** the CH_2 linked to the OH group appears as a multiplet at $\delta = 3.53$ ppm, while in **5a-d** the CH_2 peak, which is linked to the purine moiety, appears as two doublet of doublets between $\delta 4.12 - 4.21$ ppm and $\delta 4.20 - 4.32$ ppm respectively. Nevertheless, the methylene group of **5c** appears as multiplet centered at $\delta 4.26$ ppm. Moreover, in this family, the main difference in ^{13}C is the CH_2 aliphatic signal, which appears at $\delta 64.34$ ppm in the intermediate **4** and between $\delta 46.10 - 47.00$ in purines **5a-d**. Otherwise, the trifluoromethyl group presents in **5d** is corroborated by the two quadruplets in the ^{13}C NMR spectrum which appear at $\delta 145.80$ ppm ($J_{\text{C-CF}_3} = 37.7$, C-6') and $\delta 120.80$ ppm ($J_{\text{CF}} = 275.9$, CF_3), respectively.

In the last pyridoxazine family, the intermediate **6**, structurally different from derivatives **7 - 10**, does not follow the same coupling pattern of the latter, being similar the signals of the remaining intermediates except for H-3. This proton appears between $\delta 3.74 - 3.76$ ppm in the untosylated derivatives (**7, 8**) and at $\delta 4.95 - 4.98$ ppm in the tosylated ones (**9, 10**),

respectively. In addition, target molecules **11a** and **11b** show similar chemical shifts with respect to their tosylated intermediates, except for the CH₂X and CH₂CH₂X aliphatic signals, due to the different nature of the heteroatom linked to the aliphatic chain (X = O in the intermediate compounds; X = N in the final derivatives).

Table 3 may be inserted here

Table 4 may be inserted here

HSQC and HMBC experiments were performed on an intermediate and a final molecule of each family. Table 5 shows the HSQC correlations for compounds **2**, **4**, **7**, **3c**, **5b** and **11a**, whereas Figure 1 illustrates the more important connectivities found in the HMBC spectra of the same molecules.

Table 5 may be inserted here

HSQC experiments performed on compounds **2**, **4**, and **7** allows the assignment of the secondary carbon atoms chemical shifts C-3, CH₂OR and CH₂CH₂OR, and the assignment of the chemical shifts for tertiary carbon atoms C-5, C-6, C-7 and C-8 in the intermediate derivatives **1**, **2**, **4**, **6-8** and **10**. These atoms show signals in ranges of δ 48.47- 72.24 (C-2), δ 35.25 - 50.92 (C-3), δ 33.60 - 64.34 (CH₂OR), δ 33.33 - 35.26 (CH₂CH₂OR), δ 115.35 - 129.52 (C-5), δ 120.76-140.52 (C-6), δ 113.18 - 125.82 (C-7) and δ 116.60 - 124.46 (C-8).

Similar HSQC experiments performed on **3c**, **5b** and **11a** indicate that the ¹³C NMR signals for the secondary and tertiary carbon atoms in the purine derivatives **3a-d**, **5a-d** and **11a-b** are in similar ranges. Such chemical shifts are the following: δ 48.22 - 68.66 (C-2), δ 32.35 - 51.21 (C-3), δ 40.15- 47.00 (CH₂N), δ 30.63 - 31.88 (CH₂CH₂N), δ 30.30 - 31.09 (C-4), δ 117.15 - 129.79 (C-5), δ 121.31 - 140.94 (C-6), δ 120.31 - 126.44 (C-7), δ 123.47 -124.79 (C-8), δ 151.02-153.19 (C-2') (except for **3a**, **5a** and **11a** where C-2' is a quaternary carbon atom) and δ 145.03 - 147.92 (C-8').

The quaternary carbon signals were confirmed by HMBC spectra on the intermediate (**2**, **4** and **7**) and final (**3c**, **5b** and **11a**) compounds (Figure 1). In **2**, correlation between H-6 (δ 6.92) and the ¹³C at 123.51 ppm, allows the unequivocal assignment of C-4a; another correlation between H-7 (δ 7.04) and the peak at 146.44 ppm allows the identification of C-

8a. In the same way, the H-2' and H-6' signals (δ 7.54) correlate with the ^{13}C peak at 144.07 ppm, making possible to assign this peak to C-4', and the H-3' and H-5' signals (δ 7.23) correlate to peak at δ 135.41 ppm, being identified as C-1' accordingly. In **4**, correlations between H-6 (δ 7.15) and H-5 (δ 7.04) with the ^{13}C signal which appears at 137.01 ppm allow to identify it as C-4a. Assignment of C-8a has been possible due to the interactions between the signals at δ 7.06 ppm (H-7) and δ 7.68 ppm (H-8) and the ^{13}C peak at 129.27 ppm. Correlation between H-4 (δ 2.24 and δ 2.59 ppm) and the same ^{13}C peak permits us to assign this peak to C-8a. Furthermore, the quaternary carbons of the tosyl group C-1' and C-4' were assigned by the observed correlation among δ 7.21 ppm (H-3' and H-5') and the ^{13}C atom at 136.90 ppm (C-1'). Correlation between δ 7.51 ppm (H-2' and H-6') and δ 143.78 ppm allows us to assign unequivocally this signal to C-4'. Finally, in **7**, correlations of H-2a (δ 4.14), H-2b (δ 3.82) and H-7 (δ 6.49) and the ^{13}C at δ 139.08 ppm enables the assignment of C-8a. In addition, the assignment of C-4a is possible due to the observed correlation between H-6 (δ 7.57) and H-8 (δ 6.93) with the peak at δ 146.99 ppm.

The HMBC experiment performed on **3c** indicates correlations between H-3 (δ 4.19) and H-6 (δ 6.93) with the signal δ 123.42 ppm (C-4a). Other correlation exists between H-7 (δ 7.06) and the peak which appears at δ 145.88 ppm (C-8a). In the same way, the ^{13}C at δ 150.39 ppm that correlates with H-2' (δ 8.69), H-8' (δ 7.98) and CH_2N (δ 4.44) can be identified as C-4'. A correlation of H-2' (δ 8.69) and the ^{13}C peak at δ 143.07 ppm allows to identify C-6' and finally, correlation of H-8' (δ 7.98) and the signal at 135.20 ppm permits us to identify it as C-5'. C-1'' corresponds to a signal appearing at δ 134.11 ppm due to its correlation with H-3'' and H-5'' (δ 7.06). C-4'' is assigned to the ^{13}C peak at δ 144.55 ppm because is correlated with H-2'' and H-6'' (δ 7.36).

Figure 1 may be inserted here

In **5b**, the assignment of the C-4a and C-8a signals of the quaternary carbons included in the tetrahydroquinoline heterocycle has been deduced through the correlations between H-6 (δ 7.20) and the ^{13}C at 136.33 ppm (C-4a). In the same way, correlations between δ 7.08 (H-7), δ 6.98 (H-5) and H-4 (δ 2.30 and 2.59) with δ 127.23, permits the latter to be assigned to C-8a. In addition, the quaternary carbons of the purine moiety and the tosyl group have been assigned by similar correlations to those of their benzoxazine isosteres, being these signals C-

4' (δ 151.84 ppm), C-5' (δ 131.59 ppm), C-6' (δ 151.33 ppm), C-1'' (δ 136.21 ppm) and C-4'' (δ 143.99 ppm).

The HMBC experiment performed on **11a**, shows that H-6 (δ 8.02) and H-8 (δ 7.15) are correlated with the ^{13}C signal at δ 137.00 ppm, being assigned this to C-4a. H-7 (δ 6.96) and H-2a (δ 4.18) are correlated with the peak at δ 140.34 ppm (C-8a). Furthermore, the signal appearing at δ 153.13 ppm is assigned to C-4' due to its correlation with H-8' (δ 8.30) and CH_2N (δ 4.44 and δ 4.52). In addition, H-8' is correlated with a signal at δ 130.95, which can be assigned to C-5'. Moreover, the quaternary carbon atoms identification of the tosyl moiety has been deduced similarly as in their two isosteric families, being C-1'' the peak at δ 136.01 ppm and C-4'' the δ 144.51 ppm signal. At last, the characterization of quaternary atoms in the purine skeleton C-2' and C-6' has been deduced by analogy to the corresponding purine chemical shifts already justify in the two previous isosteric families (see Figure 1).

Intermediate **6**, precursor of the pyridoxazine family **11a-b**, has been studied in detail to prove its structure. In this way, to obtain only the desired 3-regioisomer we have started from 2-aminopyridin-3-ol, followed by acylation of its amino group by treatment with acetic anhydride and pyridine, to decrease the nucleophilicity of the amino group. Subsequently, cyclization of this intermediate with 4-bromobut-2-enoate (ethyl crotonate) in ethanol and K_2CO_3 only gave **6**, but not its 2-regioisomer **12** (Scheme 4). This is different to that described by other authors who obtained a mixture of two pyridoxazine regioisomers (ethyl-4-acetyl-3,4-dihydro-2H-pyrido[3,2-b][1,4]oxazine-2-carboxylate and ethyl-4-acetyl-3,4-dihydro-2H-pyrido[3,2-b][1,4]oxazine-3-carboxylate), by reaction between *N*-(3-hydroxypyridin-2-yl)acetamide and ethyl 2,3-dibromopropionate in the presence of K_2CO_3 , employing CH_3CN as solvent [12].

Scheme 4 may be inserted here

In order to unequivocally determine the structure of **6**, 2D NMR (HSQC and HMBC) experiments have been carried out. HSQC (Table 5) allows the unequivocal assignment of the tertiary carbon in the pyrido[3,2-b][1,4]oxazine ring C-3, C-6, C-7 and C-8, and the methyl group of both, acetyl and ethyl carboxylate moieties. These atoms show signals at δ 44.57, 139.56, 120.60, 124.30, 25.70 and 13.95 ppm, respectively. In addition, the secondary

carbons C-2 (δ 67.17 ppm), the CH₂ linker carbon (δ 33.60 ppm) and the CH₂ of the ethyl carboxylate group (δ 60.69 ppm) have also been assigned.

The HMBC experiment to determine the quaternary carbons (Figure 1) shows that H-2a (δ 4.47 ppm) and H-8 (δ 6.93 ppm) are correlated with the ¹³C at δ 140.67 ppm (C-8a). H-3 (δ 5.41 ppm) and H-6 (δ 7.96 ppm) peaks are correlated with the signal at δ 138.46 (C-4a). In addition, both ester and amide CO groups show very similar chemical shifts (δ 170.21 and δ 169.69 ppm). The signal at δ 4.12 ppm of the CH₂ ethyl carboxylate group is correlated with a peak at δ 170.21 ppm (CO ester group). Furthermore, a correlation between the CH₃ amide group (δ 2.59 ppm) and the ¹³C at δ 169.69 ppm indicates that this peak is the CO amide moiety.

Scheme 4 shows two possible structures (**6** and **12**) that could be formed in the cyclization reaction. In our case, we have only obtained **6**, as demonstrated by the ¹H/¹³C HMBC study. This experiment corroborates that H-3 (δ 5.41 ppm) is correlated with the ¹³C chemical shift of the amide group (δ 169.69). This correlation confirms the proximity between these groups and proves that **6** but not **12** is the obtained regioisomer.

Due to **6** is the intermediate of the final purines **11a-b**, it is demonstrated that the side chain links through the 3-position of the heterocycle, unlike derivatives **3a-d**, whose purine residue binds through its 2-position.

Nuclear Overhauser Spectroscopy experiments performed on compound **3c** (X = Br, Y = H) shows the existence of a NOESY effect between H-2 and H-8' and within both multiplet hydrogen atoms CH₂CH₂N of the linear chain and H-8', A correlation with CH₂N and the same hydrogen of the purine skeleton H-8' is observed, but no NOESY effects are noticed among H-3a or H-3b and H-8'. These facts are compatible with a preferred conformational arrangement in which the imidazole ring of the purine is located below that of the benzoxazine moiety (Figure 2a).

Figure 2 may be inserted here

Acknowledgements

This work was partially supported by the Junta de Andalucía (project nº CS2016.1).

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Table 1. ¹H NMR signal assignments of intermediate compounds **1**, **2**, **4**, **6-10**

Compound	H-2a	H-2b	H-3a	H-3b	H-4	H-5	H-6	H-7	H-8	CH ₂ OR	CH ₂ CH ₂ OR
1	4.30 (m)	-	3.36 (dd, 11.6, 2.4)	3.17 (dd, 11.6, 7.7)	2.99 (bs)	6.60 (dd, 7.7, 1.5)	6.66 (dd, 7.7, 1.5)	6.77 (m)	6.77 (m)	3.87 (m)	1.84 (m), 1.94 (m)
2	3.58 (m)	-	4.32 (dd, 14.3, 2.4)	3.19 (dd, 14.3, 9.9)	-	6.79 (dd, 8.1, 1.6)	6.92 (ddd, 8.1, 7.3, 1.6)	7.04 (ddd, 8.2, 7.3, 1.6)	7.83 (dd, 8.2, 1.6)	3.76 (m)	1.77 (m)
4	4.12 (dd, 13.4, 4.2)	3.42 (dd, 13.3, 9.4)	1.94 (m)	-	2.24 (dd, 16.5, 9.1), 2.59 (dd, 16.5, 6.0)	7.04 (m)	7.15 (m)	7.06 (m)	7.68 (d, 8.3)	3.53 (m)	-
6	4.47 (dd, 11.4, 1.2)	4.07 (dd, 11.4, 2.9)	5.41 (m)	-	-	-	7.96 (dd, 4.6, 1.4)	7.00 (dd, 8.1, 4.6)	7.20 (dd, 8.1, 1.4)	-	-
7	4.15 (dd, 10.3, 2.8)	3.82 (dd, 10.3, 1.3)	3.76 (m)	-	5.12 (bs)	-	7.57 (dd, 5.1, 1.4)	6.49 (dd, 7.8, 5.1)	6.93 (dd, 7.8, 1.4)	3.87 (m), 3.97(m)	1.70 (m)
8	4.21 (dd, 10.7, 2.9)	3.87 (dd, 10.6, 7.0)	3.74 (m)	-	5.32 (bs)	-	7.66 (dd, 5.0, 1.4)	6.52 (dd, 7.7, 5.0)	6.94 (dd, 7.7, 1.4)	3.80 (m)	1.70 (m)
9	4.37 (dd, 11.1, 1.6)	4.02 (dd, 11.1, 2.5)	4.98 (m)	-	-	-	7.94 (dd, 4.7, 1.6)	6.90 (dd, 8.0, 4.7)	7.12 (dd, 8.0, 1.6)	3.65 (m)	1.73 (q, 6.4)
10	4.27 (dd, 11.2, 1.5)	3.86 (dd, 11.2, 2.5)	4.95 (m)	-	-	-	7.97 (dd, 4.7, 1.6)	6.92 (dd, 8.1, 4.7)	7.14 (dd, 8.1, 1.6)	3.71 (m), 3.82 (m)	1.79 (m)

Solvent used CDCl₃.Chemical shifts (in CDCl₃) are reported in δ (ppm) relative to CDCl₃; multiplicities and coupling constants (Hz) are given in parentheses.¹H signals for the tosyl substituent: **2**, H-2', H-6': 7.54 (m), H-3', H-5': 7.23 (m), CH₃: 2.38 (s); **4**, H-2', H-6': 7.51 (d, 8.0), H-3', H-5': 7.21 (d, 8.0), CH₃: 2.38 (s); **9**, H-2', H-6': 8.01 (d, 8.3), H-3', H-5': 7.28 (d, 5.9), CH₃: 2.40 (s); **10**, H-2', H-6': 7.93 (d, 8.3), H-3', H-5': 7.26 (d, 8.1), CH₃: 2.38 (s).¹H signals for the silyl substituent: **8**: (CH₃)₃: 0.91 (s), (CH₃)₂: 0.07 (s); **9**: (CH₃)₃: 0.90 (s), (CH₃)₂: 0.05 (s).Other signals of compound **6**: COOCH₂CH₃: 4.12 (m), CH₂COOCH₂CH₃: 2.47 (m), COCH₃: 2.59 (s), COOCH₂CH₃: 1.22 (t, 7.2).

s, singlet; bs, broad singlet; d, doublet; dd, doublet doublet; ddd, doublet doublet doublet; t, triplet; q, quadruplet; m, multiplet.

Table 2. ¹H NMR signal assignments of final compounds **3a-d**, **5a-d**, **11a-b**.

Compound	H-2a	H-2b	H-3a	H-3b	H-4	H-5	H-6	H-7	H-8	H-2'	H-8'	CH ₂ N	CH ₂ CH ₂ N
3a	3.35 (m)	-	4.19 (dd, 14.2, 2.3)	3.21 (dd, 14.2, 9.6)	-	6.76 (dd, 8.2, 1.6)	6.94 (m)	7.08 (m)	7.77 (dd, 8.3, 1.6)	-	7.96 (s)	4.43 (dd, 7.7, 5.5)	2.02 (m), 2.23 (m)
3b	3.31 (m)	-	4.20 (dd, 14.1, 1.9)	3.21 (dd, 14.1, 9.7)	-	6.78 (dd, 8.2, 1.5)	6.93 (m)	7.05 (m)	7.79 (dd, 8.3, 1.5)	8.75 (s)	7.96 (s)	4.44 (m)	2.27 (m), 2.02 (m)
3c	3.34 (m)	-	4.19 (dd, 14.2, 2.3)	3.21 (dd, 14.2, 9.7)	-	6.77 (dd, 8.2, 1.5)	6.93 (ddd, 8.8, 7.3, 1.5)	7.06 (m)	7.77 (dd, 8.3, 1.6)	8.69 (s)	7.98 (s)	4.44 (m)	2.04 (m), 2.28 (m)
3d	3.44 (tt, 9.7, 2.8)	-	4.20 (dd, 14.2, 2.4)	3.23 (dd, 14.3, 9.6)	-	6.74 (dd, 8.2, 1.5)	6.93 (ddd, 8.6, 7.3, 1.5)	7.05 (m)	7.76 (dd, 8.3, 1.6)	9.08 (s)	8.13 (s)	4.52 (m)	2.08 (m), 2.28 (m)
5a	3.98 (dd, 13.5, 3.8)	3.42 (dd, 13.5, 8.7)	2.38 (m)	-	2.31 (dd, 15.9, 9.0), 2.59 (dd, 15.9, 5.2)	6.98 (dd, 7.6, 1.5)	7.16 (m)	7.06 (td, 7.5, 1.2)	7.69 (dd, 8.3, 1.1)	-	8.07 (s)	4.12 (dd, 14.2, 6.7), 4.20 (dd, 14.2, 7.3)	-
5b	4.01 (dd, 13.5, 4.1)	3.45 (dd, 13.5, 8.9)	2.39 (m)	-	2.30 (dd, 15.9, 9.1), 2.59 (dd, 15.9, 5.3)	6.98 (dd, 7.6, 1.5)	7.2 (td, 7.9, 1.5)	7.08 (td, 7.5, 1.2)	7.73 (dd, 8.3, 1.1)	8.74 (s)	8.04 (s)	4.15 (dd, 14.2, 6.7), 4.25 (dd, 14.2, 7.2)	-
5c	4.05 (dd, 13.5, 4.0)	3.35 (m)	2.09 (m)	-	2.30 (m), 2.55 (dd, 16.5, 5.3)	7.07 (d, 7.5)	7.17 (m)	7.07 (d, 7.5)	7.61 (d, 8.3)	8.76 (s)	8.70 (s)	4.26 (m)	-
5d	4.02 (dd, 13.5, 3.7)	3.48 (dd, 13.5, 8.5)	2.48 (m)	-	2.31 (d, 8.6), 2.61 (dd, 15.9, 5.1)	6.99 (d, 7.6)	7.20 (t, 7.8)	7.07 (d, 7.3)	7.72 (d, 8.3)	9.08 (s)	8.21 (s)	4.21 (dd, 14.4, 6.4), 4.32 (dd, 14.3, 6.6)	-
11a	4.18 (dd, 11.4, 1.4)	3.75 (dd, 11.4, 2.4)	4.77 (d, 10.2)	-	-	-	8.02 (dd, 4.6, 1.5)	6.96 (dd, 8.1, 4.6)	7.15 (dd, 8.1, 1.5)	-	8.30 (s)	4.44 (m), 4.52 (m)	2.13 (m)
11b	4.18 (dd, 11.4, 1.4)	3.77 (dd, 11.4, 2.3)	4.78 (d, 9.8)	-	-	-	8.02 (dd, 4.7, 1.5)	6.96 (dd, 8.0, 4.6)	7.15 (dd, 8.0, 1.5)	8.73 (s)	8.30 (s)	4.51 (m)	2.44 (m), 2.15 (m)

Solvent used CDCl₃. *Solvent used DMSO-*d*₆.Chemical shifts (in CDCl₃) are reported in δ (ppm) relative to CDCl₃; multiplicities and coupling constants (Hz) are given in parentheses.

¹H signals for the tosyl substituent: **3a**, H-2'': 7.40 (d, 8.2), H-3'': 7.08 (m), CH₃: 2.39 (s); **3b**, H-2'': 7.35 (d, 8.1), H-3'': 7.05 (m), CH₃: 2.39 (s); **3c**, H-2'': 7.36 (m), H-3'': 7.06 (m), CH₃: 2.39 (s); **3d**, H-2'': 7.39 (m), H-3'': 7.10 (d, 8.0), CH₃: 2.37 (s); **5a**, H-2'': 7.38 (d, 8.0), H-3'': 7.16 (m), CH₃: 2.35 (s); **5b**, H-2'': 7.39 (d, 8.1), H-3'': 7.12 (d, 8.1), CH₃: 2.36 (s); **5c**, H-2'': 7.25 (d, 7.9), H-3'': 7.16 (m), CH₃: 2.30 (s); **5d**, H-2'': 7.40 (d, 7.9), H-3'': 7.12 (m), CH₃: 2.35 (s); **11a**, H-2'': 7.82 (m), H-3'': 7.25 (d, 10.3), CH₃: 2.38 (s); **11b**, H-2'': 7.84 (d, 8.1), H-3'': 7.25 (d, 8.1), H-5'': 7.25 (d, 8.1), CH₃: 2.38 (s).

s, singlet; d, doublet; dd, doublet doublet; ddd, doublet doublet doublet; t, triplet; td, triplet doublet; tt, triplet triplet; m, multiplet.

Table 3. ¹³C NMR signal assignments of intermediate compounds **1**, **2**, **4**, **6-10**.

Compound	C-2	C-3	C-4	C-4a	C-5	C-6	C-7	C-8	C-8a	<u>C</u> H ₂ OR	<u>C</u> H ₂ CH ₂ OR
1	72.24	45.31	-	132.95	115.35	121.20	118.75	116.60	143.30	59.40	35.16
2	69.97	48.47	-	123.51	117.25	120.76	125.82	123.98	146.44	58.70	34.88
4	48.47	35.25	29.48	137.01	129.52	126.62	124.93	123.60	129.37	64.34	-
6	67.17	44.57	-	138.46	-	139.56	120.60	124.30	140.67	33.60	-
7	68.70	49.25	-	146.99	-	139.02	113.18	121.72	139.08	60.06	33.35
8	69.27	48.64	-	147.53	-	140.52	114.18	121.11	139.59	60.67	35.26
9	67.75	50.92	-	140.66	-	140.11	119.64	123.88	137.96	59.39	33.33
10	67.44	50.83	-	140.50	-	140.21	119.82	124.46	137.39	58.28	33.40

Solvent used CDCl₃.
 Chemical shifts (in CDCl₃) are reported in δ (ppm) relative to CDCl₃; ¹³C signals for the tosyl substituents: **2**, C-1': 135.41, C-2', C-6': 127.05, C-3', C-5': 129.70, C-4': 144.07, CH₃: 21.39; **4**, C-1': 136.90, C-2', C-6': 127.07, C-3', C-5': 129.79, C-4': 143.78, CH₃: 21.65; **9**, C-1': 137.98, C-2', C-6': 128.26, C-3', C-5': 128.95, C-4': 143.98, CH₃: 21.39; **10**, C-1': 136.66, C-2', C-6': 128.16, C-3', C-5': 129.17, C-4': 143.98, CH₃: 21.39.
¹³C signals for the silyl substituent: **8**: C: 18.69, (CH₃)₃: 26.38, (CH₃)₂: 4.96; **9**: C: 18.03, (CH₃)₃: 25.76, (CH₃)₂: 5.65.
 Other signals of compound **6**: COOCH₂CH₃: 170.21, COCH₃: 169.69, COOCH₂CH₃: 60.69, COCH₃: 25.70, COOCH₂CH₃: 13.95.

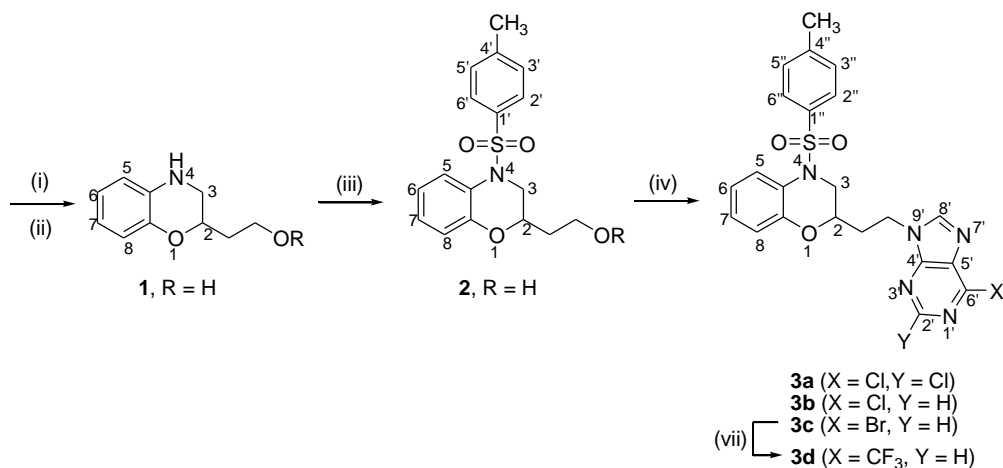
Table 4. ¹³C NMR signal assignments of final compounds **3a-d**, **5a-d**, **11a-b**.

Compound	C-2	C-3	C-4	C-4a	C-5	C-6	C-7	C-8	C-8a	CH ₂ N	CH ₂ CH ₂ N	C-2'	C-4'	C-5'	C-6'	C-8'
3a	68.66	48.31	-	123.68	117.40	121.60	126.44	124.35	145.98	40.61	31.88	153.19	153.17	130.86	151.91	146.07
3b	68.33	48.18	-	123.52	117.25	121.42	126.29	124.29	145.98	40.33	31.75	151.97	151.72	131.62	151.09	145.24
3c	68.32	48.08	-	123.42	117.17	121.31	126.18	124.13	145.88	40.29	31.67	151.80	150.39	135.20	143.07	145.03
3d	68.62	48.07	-	123.44	117.15	121.36	126.17	124.00	145.80	40.15	31.77	152.40	153.65	129.92	145.20 (q, 37.1)	147.29
5a	48.22	32.35	30.43	136.11	129.29	127.03	125.06	123.60	126.87	46.71	-	153.00	152.86	130.58	151.78	145.96
5b	48.47	32.85	30.67	136.33	129.34	127.19	125.20	123.97	127.23	46.70	-	152.01	151.84	131.59	151.33	145.22
5c^a	48.51	32.58	30.30	136.04	129.73	126.71	125.07	123.47	128.77	46.10	-	151.78	150.95	133.63	141.96	147.62
5d	48.90	33.24	31.09	136.73	129.79	127.64	125.62	124.27	127.51	47.00	-	152.40	154.25	130.36	145.80 (q, 37.7)	147.92
11a	66.92	51.13	-	137.00	-	140.94	120.46	124.79	140.34	41.74	30.63	152.63	153.13	130.95	151.66	146.72
11b	67.02	51.21	-	137.15	-	140.93	120.36	124.72	140.34	41.60	30.85	151.02	151.81	131.82	151.68	145.96

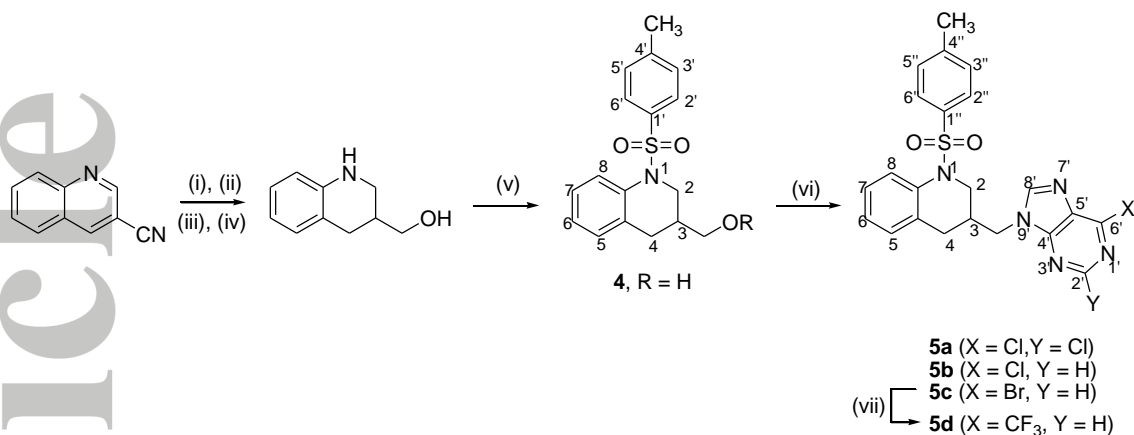
Solvent used CDCl₃. ^aSolvent used DMSO-*d*₆.
 Chemical shifts (in CDCl₃) are reported in δ (ppm) relative to CDCl₃; multiplicities and coupling constants (Hz) are given in parentheses.
¹³C signals for the tosyl substituents: **3a**, C-1'': 135.49, C-2'': 127.08, C-6'': 129.95, C-3'': 144.83, CH₃: 21.77; **3b**, C-1'': 135.29, C-2'': 126.87, C-3'': 129.72, C-4'': 144.65, CH₃: 21.61; **3c**, C-1'': 134.11, C-2'': 126.78, C-3'': 129.64, C-4'': 144.55, CH₃: 21.57; **3d**, C-1'': 135.30, C-2'': 126.83, C-3'': 129.69, C-4'': 144.57, CH₃: 21.37. **5a**, C-1'': 135.94, C-2'': 126.66, C-3'': 129.63, C-4'': 144.01, CH₃: 21.38; **5b**, C-1'': 136.21, C-2'': 126.83, C-3'': 129.72, C-4'': 143.99, CH₃: 21.51; **5c**, C-1'': 135.76, C-2'': 126.63, C-3'': 129.95, C-4'': 144.43, CH₃: 21.13; **5d**, C-1'': 136.63, C-2'': 127.25, C-3'': 130.15, C-4'': 144.45, CH₃: 21.90; **11a**, C-1'': 136.01, C-2'': 128.18, C-3'': 129.41, C-4'': 144.51, CH₃: 21.49; **11b**, C-1'': 136.21, C-2'': 128.23, C-3'': 129.37, C-4'': 144.43, CH₃: 21.50.
¹³C signals for the CF₃ substituent: **3d**: 120.69 (q, 274.9); **5d**: 120.80 (q, 275.9).

Table 5. HSQC correlations found for compounds **2**, **4**, **6**, **7**, **3c**, **5b** and **11a**

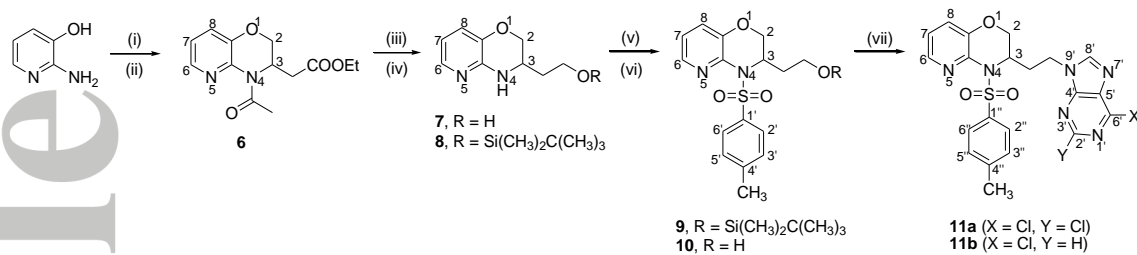
¹ H/ ¹³ C	2	4	6	7	3c	5b	11a
H-2	3.58	4.12, 3.42	4.47, 4.07	4.15, 3.82	3.34	4.01, 3.45	4.18, 3.75
C-2	69.97	48.47	67.17	68.7	68.32	48.47	66.92
H-3	4.32, 3.19	1.94	5.41	3.76	4.19, 3.21	2.39	4.77
C-3	48.47	35.25	44.57	49.25	48.08	32.85	51.13
H-4	-	2.24	-	5.12	-	2.30	-
C-4	-	29.48	-	-	-	30.67	-
H-5	6.79	7.04	-	-	6.77	6.98	-
C-5	115.35	129.52	-	-	117.17	129.34	-
H-6	6.92	7.15	7.96	7.57	6.93	7.2	8.02
C-6	120.76	126.62	139.56	139.02	121.31	127.19	140.34
H-7	7.04	7.04	7.00	6.49	7.06	7.08	6.96
C-7	125.82	124.93	120.60	113.18	126.18	125.2	120.46
H-8	7.83	7.8	7.20	6.93	7.77	7.73	7.15
C-8	123.98	123.60	124.30	121.72	124.13	123.97	124.79
H-2'	-	-	-	-	8.69	8.74	-
C-2'	-	-	-	-	151.8	152.01	153.13
H-8'	-	-	-	-	7.98	8.04	8.30
C-8'	-	-	-	-	145.03	145.22	146.72
CH ₂ X	3.76	3.53	2.47	3.87, 3.97	4.44	4.15, 4.25	4.44, 4.52
CH ₂ X	58.7	64.34	33.60	60.06	40.29	46.7	41.74
CH ₂ CH ₂ X	1.77	-	-	1.70	2.04, 2.28	-	2.13
CH ₂ CH ₂ X	34.88	-	-	33.35	31.67	-	30.63
COOCH ₂ CH ₃	-	-	4.12	-	-	-	-
COOCH ₂ CH ₃	-	-	60.69	-	-	-	-
COOCH ₂ CH ₃	-	-	1.22	-	-	-	-
COOCH ₂ CH ₃	-	-	13.95	-	-	-	-
COH ₃	-	-	2.59	-	-	-	-
COH ₃	-	-	25.70	-	-	-	-



Scheme 1. Synthesis of compounds 3a-d included in the benzoxazine family. Reagents and conditions: (i) ethyl 4-bromobut-2-enoate, NaHCO₃, EtOH, 3 h, rt, then K₂CO₃, 30 min; (ii) LiAlH₄, Et₂O, 1 h, 0°C to rt; (iii) TsCl, py, 12 h, 0°C to rt; (iv) 2,6-dihalopurine or 6-halopurine, DIAD, Ph₃P, 36 h, -20°C to rt; (v) MFSDA, CuI, HMAP, DMF, 12 h, 70°C.

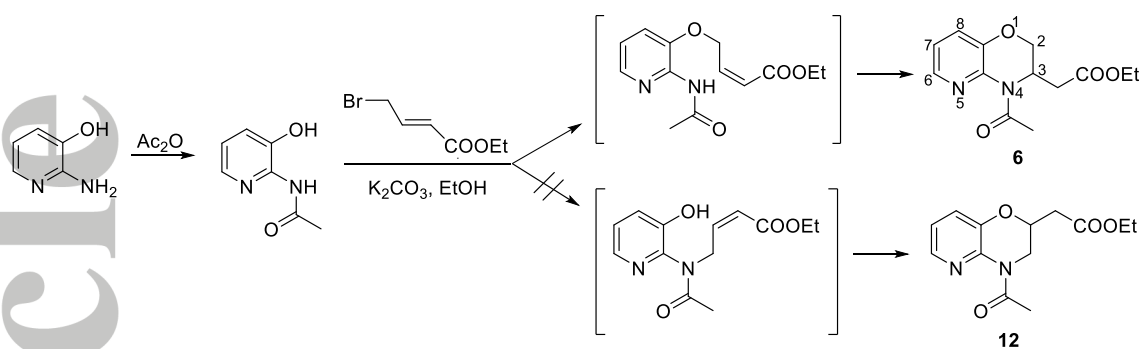


Scheme 2. Synthesis of the tetrahydroquinoline family compounds (5a-d). *Reagents and conditions:* (i) aq. NaOH, EtOH, 20 h, reflux, then HCl 1N; (ii) SOCl₂, EtOH, 4 h, reflux; (iii) NaBH₃CN, THF, MeOH, HCl, Et₂O, 6 h, rt; (iv) LiAlH₄, THF, 1 h, 0°C; (v) TsCl, py, 12 h, 0°C to rt; (vi) 2,6-dihalopurine or 6-halopurine, DIAD, Ph₃P, 36 h, -20°C to rt; (vii) MFSDA, CuI, HMAP, DMF, 12 h, 70°C.



Scheme 3. Synthesis of compounds belonging to the pyridoxazine family (11a-b).

Reagents and conditions: (i) Ac₂O/py, 5 min, reflux, then NaOH; (ii) ethyl 4-bromobut-2-enoate, K₂CO₃, EtOH, 24 h; (iii) LiAlH₄, THF, 1 h, 0°C to rt; (iv) TBDMSCl, Et₃N, DMAP, DCM, 12 h, rt; (v) TsCl, Et₃N, DMAP, DCM, 12 h, rt; (vi) AcOH, H₂O, THF, 12 h, rt; (vii) 2,6-dichloropurine or 6-halopurine, DIAD, Ph₃P, -20°C to rt, 36 h.



Scheme 4. Detailed route leading to the intermediate regioisomer **6** from 2-aminopyridin-3-ol.

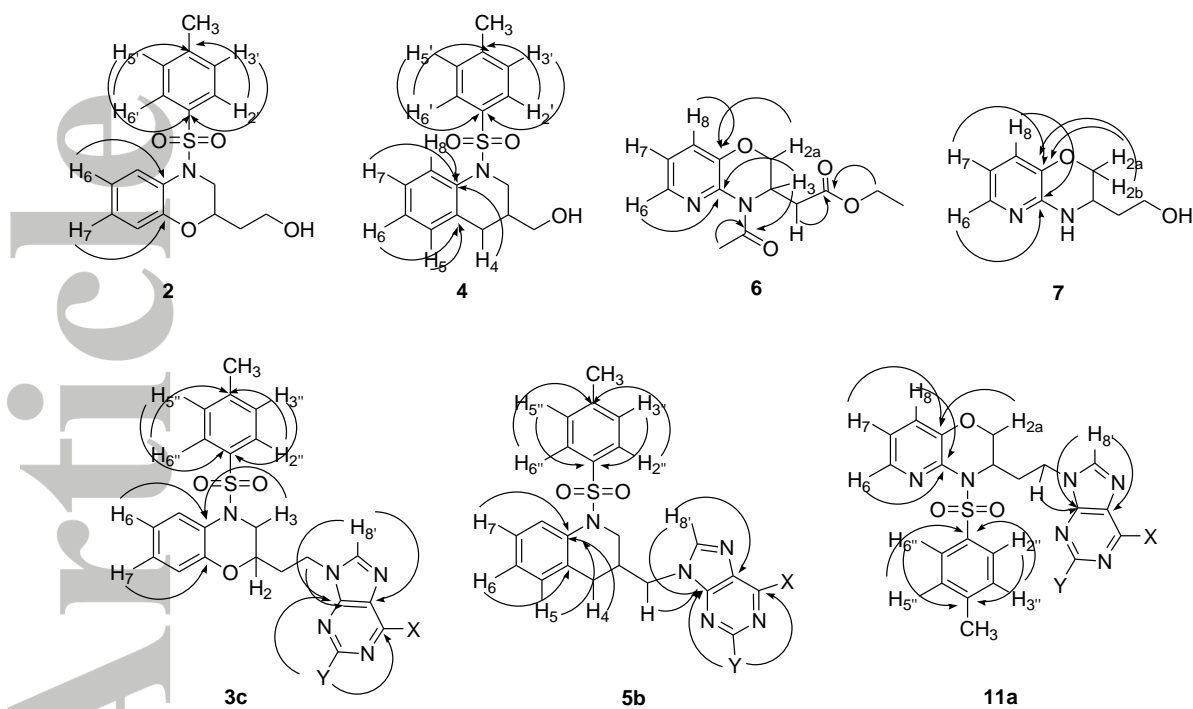


Figure 1. Main connectivities found in the HMBC $^1\text{H}/^{13}\text{C}$ spectra of intermedia compounds (2, 4, 6, 7) and final derivatives (3c, 5b and 11a).

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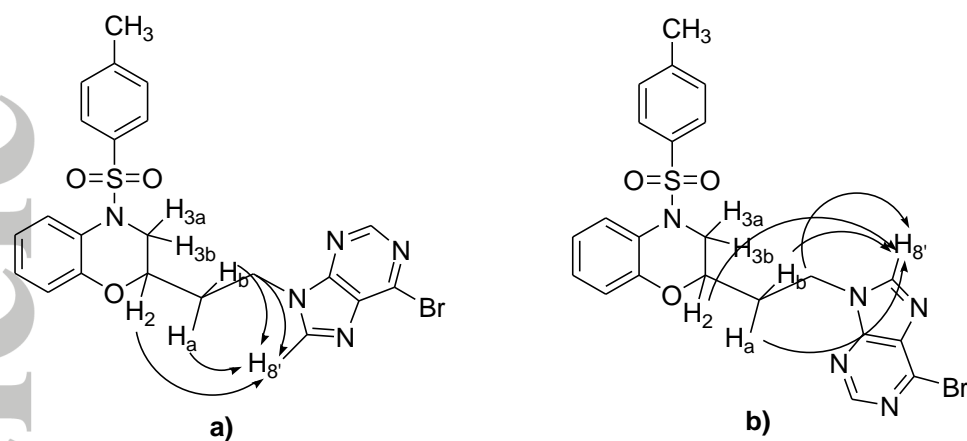
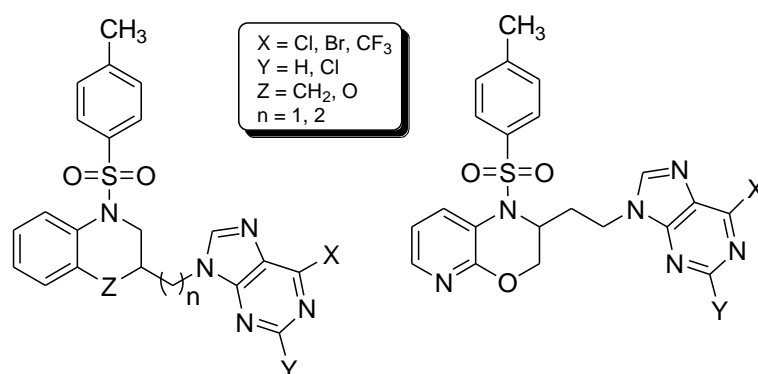


Figure 2. Selected NOESY correlations for compound 3c.

Graphical table of Contents



The NMR assignment of several heterocyclic families linked to purine moieties has been unequivocally established by using the concerted application of one- and two-dimensional NMR experiments (DEPT, HSQC and HMBC).

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