

Structure-Activity Relationship of Neplanocin A Analogues for the Identification of Novel Anti-HBV Leads

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1. Antiviral Assay

All the synthesized compounds were evaluated for their antiviral activity against Hepatitis B virus and toxicity profile despite in table 1. HepG2.2.15.7 cells (1 × 10⁴ cells/well) were inoculated into a microtiter plate. After incubation for 24 h, the cells were cultured in the presence of various concentrations of test compounds. Then, every 3 days, the culture medium was replaced by fresh one containing an appropriate concentration of the compounds. After 9 days of incubation, the culture supernatants were collected and examined for their HBV DNA levels by real-time PCR. The cells were examined for their viability by the tetrazolium dye method. EC₅₀ and CC₅₀ were calculated, and the results are shown in below table 1. EC₅₀: 50% Effective concentration based on the inhibition of HBV DNA levels in culture supernatants. CC₅₀: 50% Cytotoxic concentration based on the reduction of viable cell number.

2. *General Methods*

All reactions were carried out in oven-dried glassware under nitrogen atmosphere. The chemicals and solvents were purchased from Spectrochem, Across, Rankem or Sigma-Aldrich. Melting points were recorded on Veego melting point apparatus. Analytical thin layer chromatography (TLC) was performed on precoated plates (silica gel 60 F-254) purchased from Merck Inc. Purification by gravity column chromatography was carried out on silica gel (100-200 mesh). Eleco UV/Visible is spectrophotometer was used for recording the UV spectra. $^1\text{H}/^{13}\text{C}$ NMR were obtained from a Varian (400 MHz) and Bruker (400 MHz) spectrometers using $\text{CDCl}_3/\text{DMSO}-d_6/\text{CD}_3\text{OD}$, as solvents. Peaks are recorded with the following abbreviations: s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet; J, coupling constant (hertz).

3. $^1\text{H}/^{19}\text{F}/^{13}\text{C}$ NMR, HPLC and HR-MS Characterization:

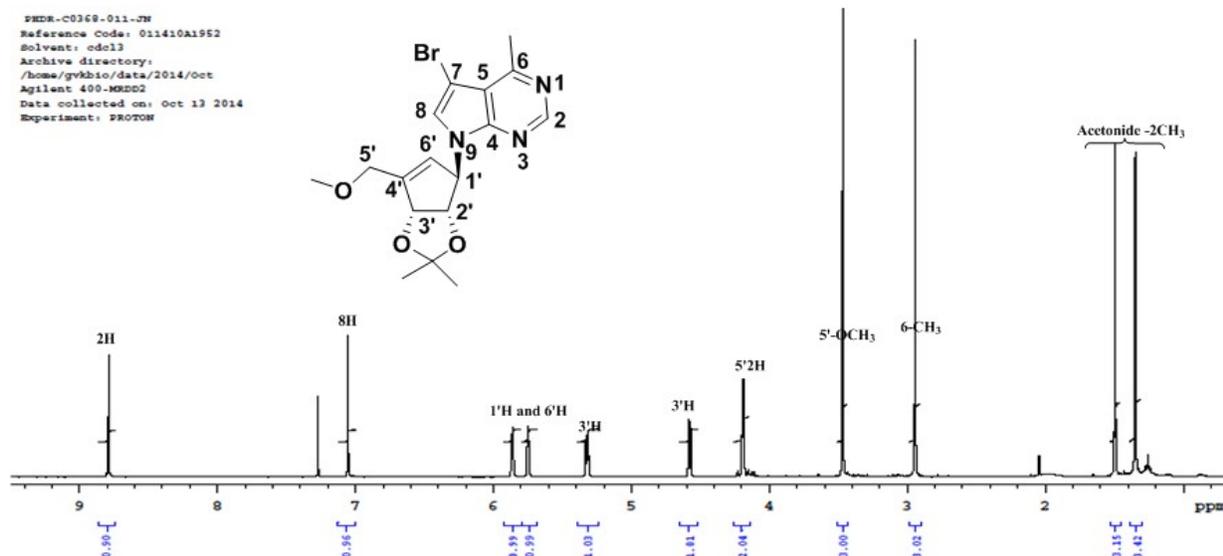


Figure S1: ^1H -NMR (400 MHz, CDCl_3) of the compound **3a**

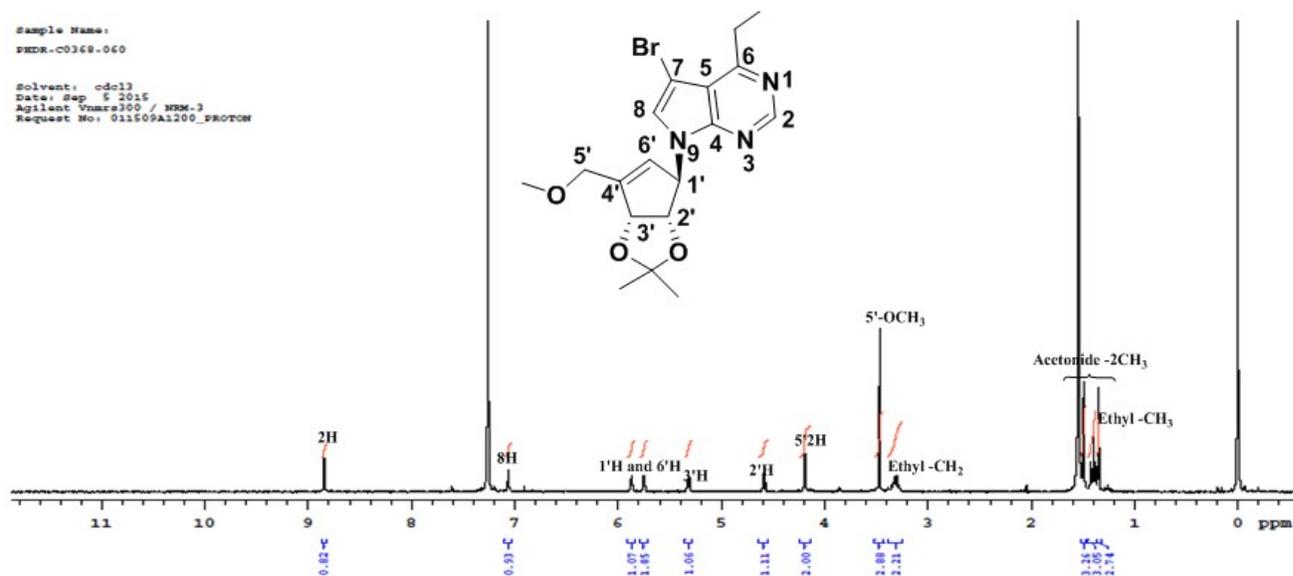
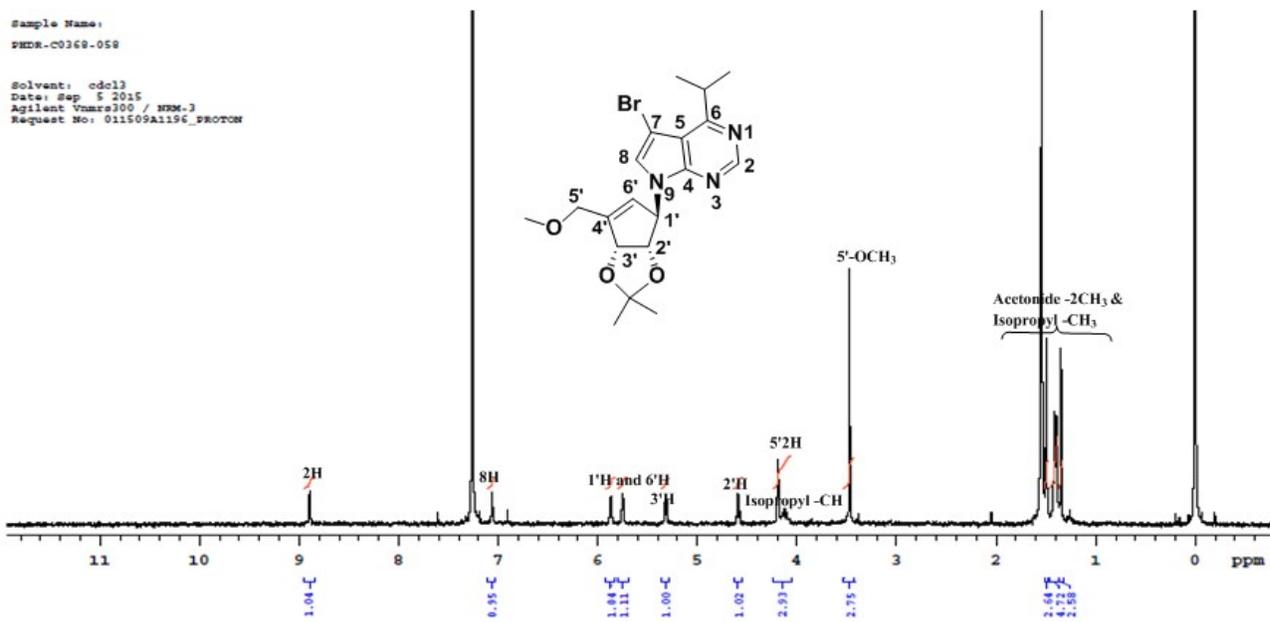
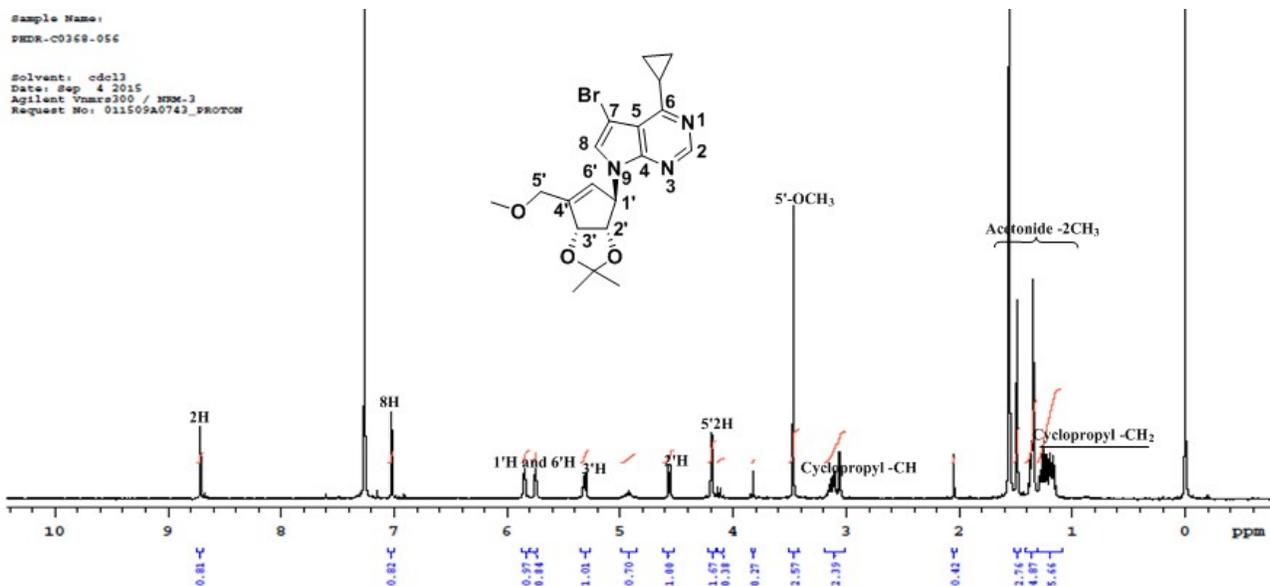


Figure S2: ^1H -NMR (300 MHz, CDCl_3) of the compound **2b**



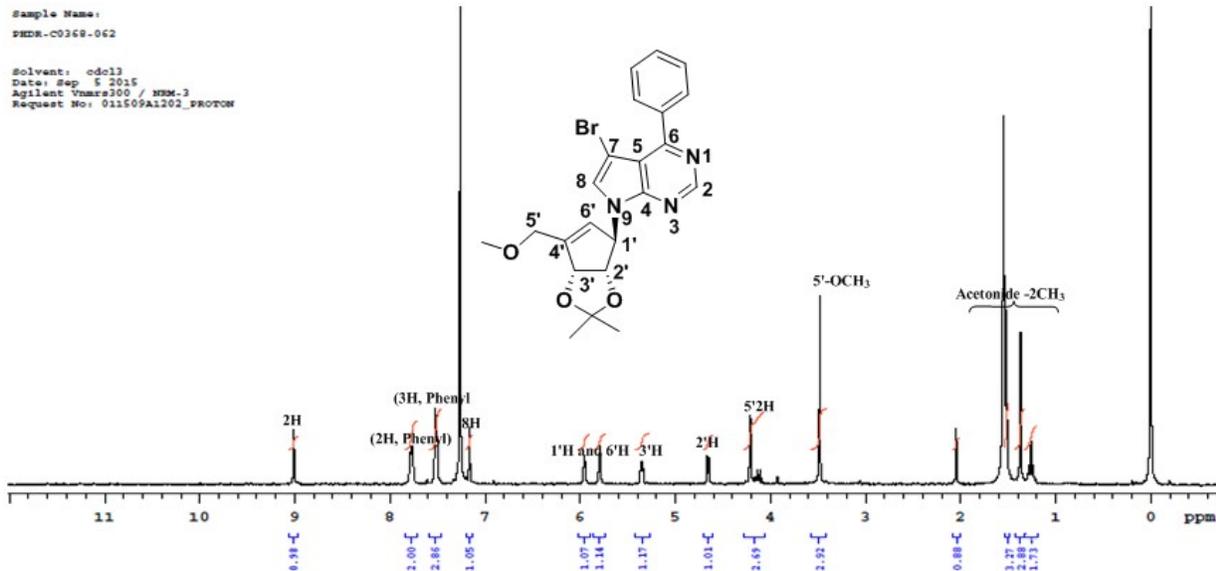


Figure S5: $^1\text{H-NMR}$ (300 MHz, CDCl_3) of the compound **3e**

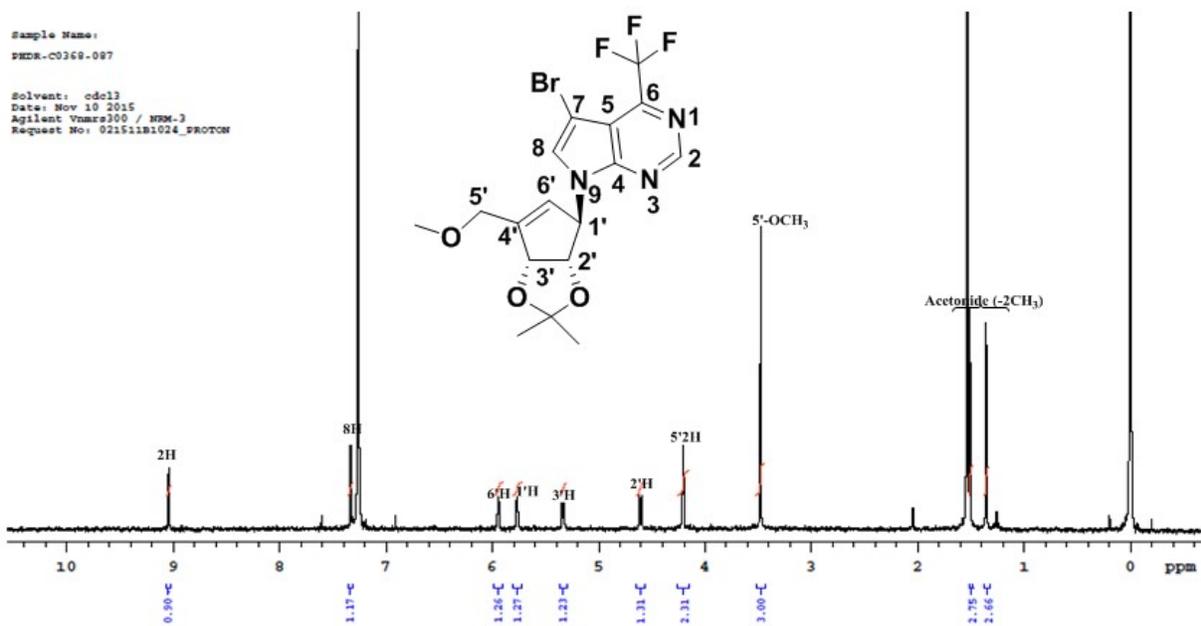


Figure S6: $^1\text{H-NMR}$ (300 MHz, CDCl_3) of the compound **3f**

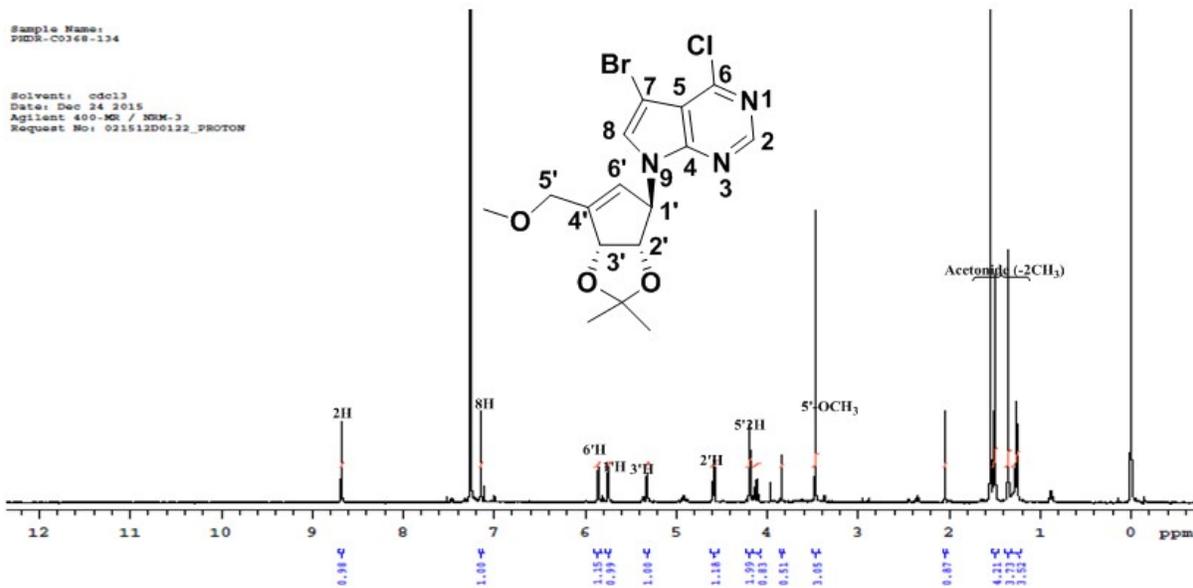


Figure S7: ¹H-NMR (400 MHz, CDCl₃) of the compound 3g

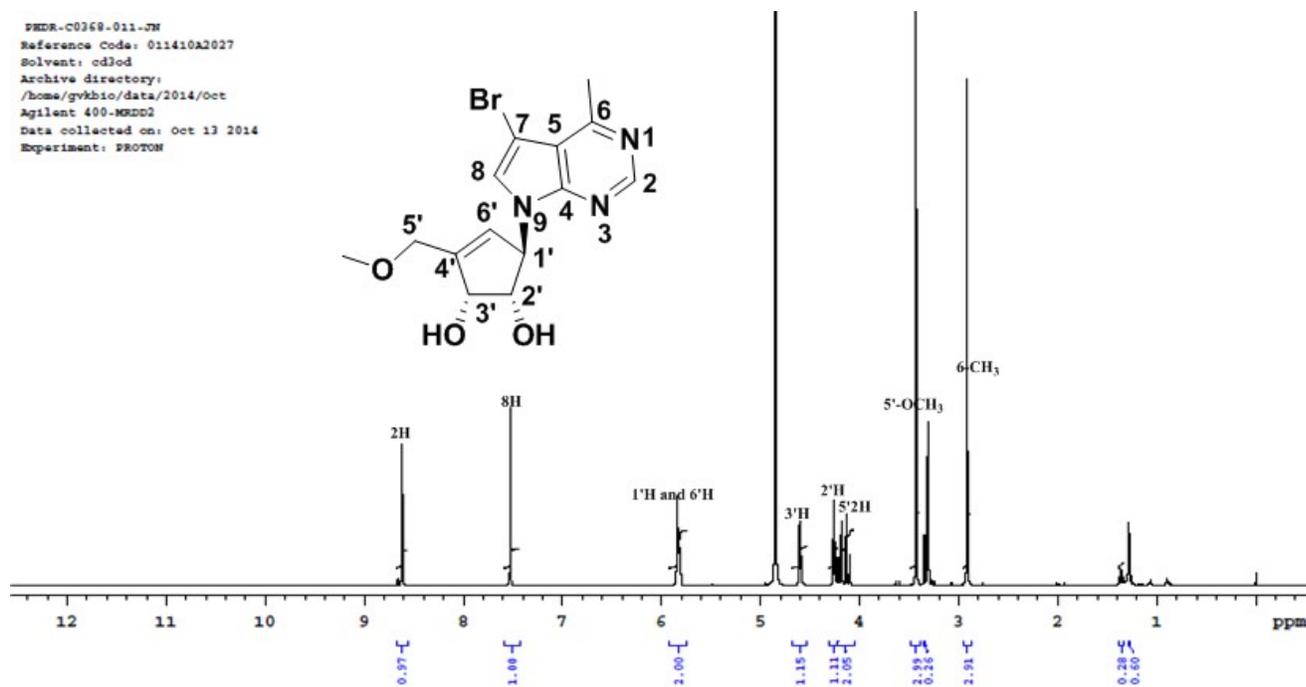


Figure S8: ¹H-NMR (400 MHz, CD₃OD) of the compound 4a

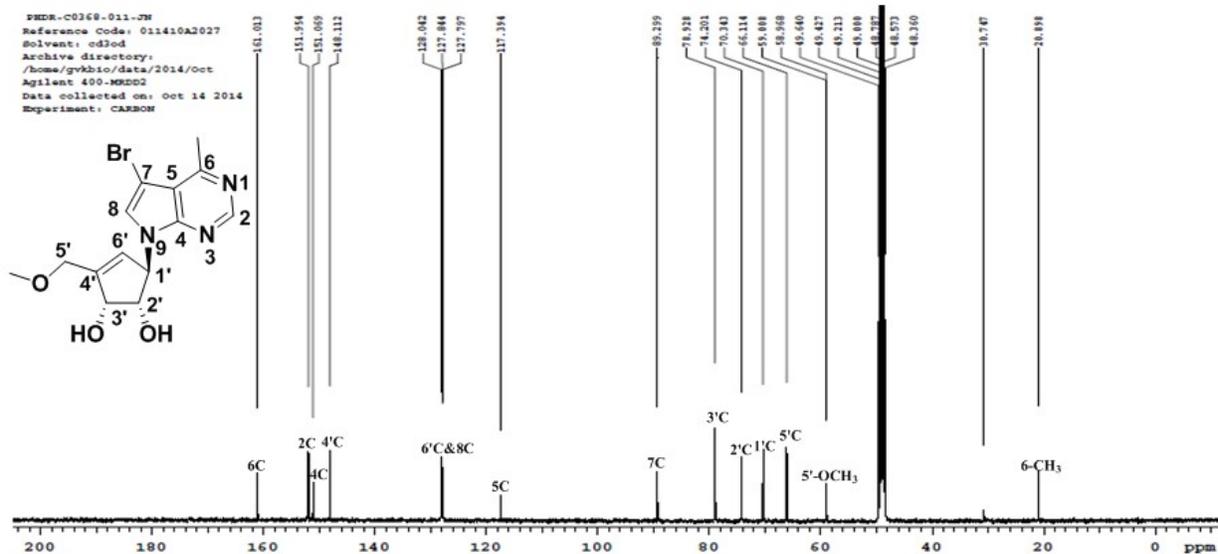


Figure S9: ^{13}C -NMR (100 MHz, CD_3OD) of the compound **4a**

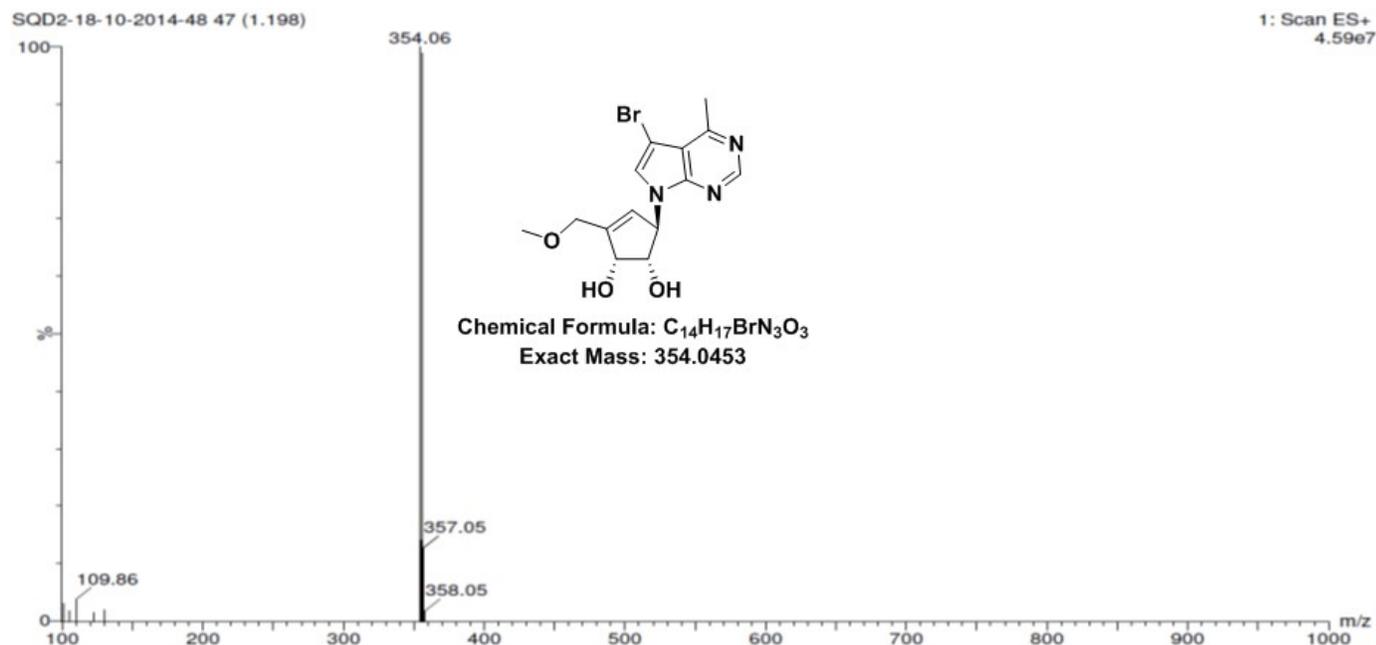


Figure S10: HRMS data of the compound **4a**

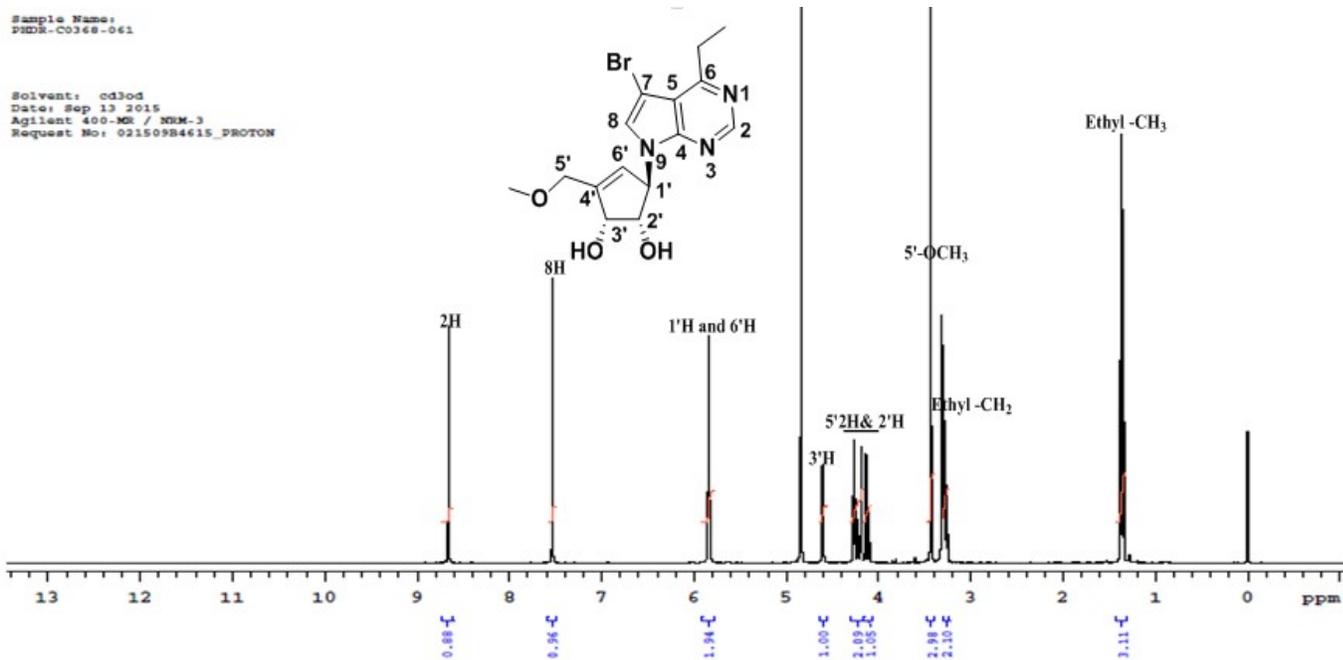


Figure S11: $^1\text{H-NMR}$ (400 MHz, CD_3OD) of the compound 4b

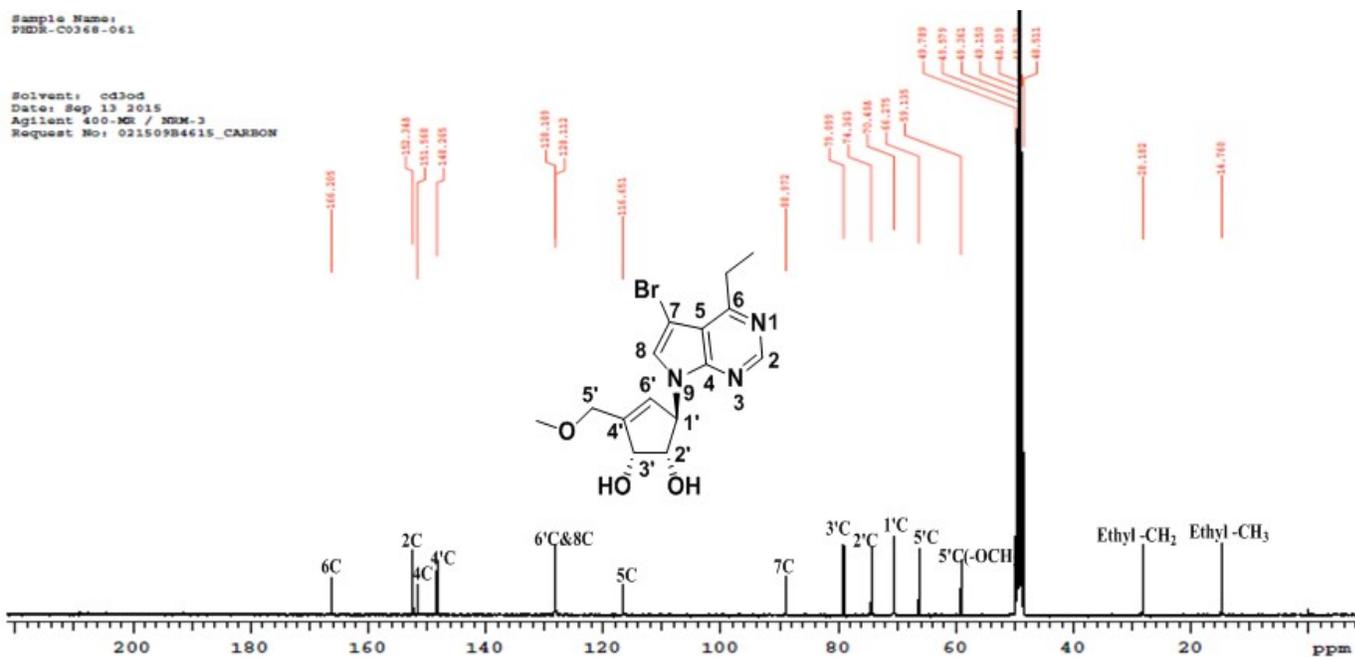


Figure S12: $^{13}\text{C-NMR}$ (100 MHz, CD_3OD) of the compound 4b

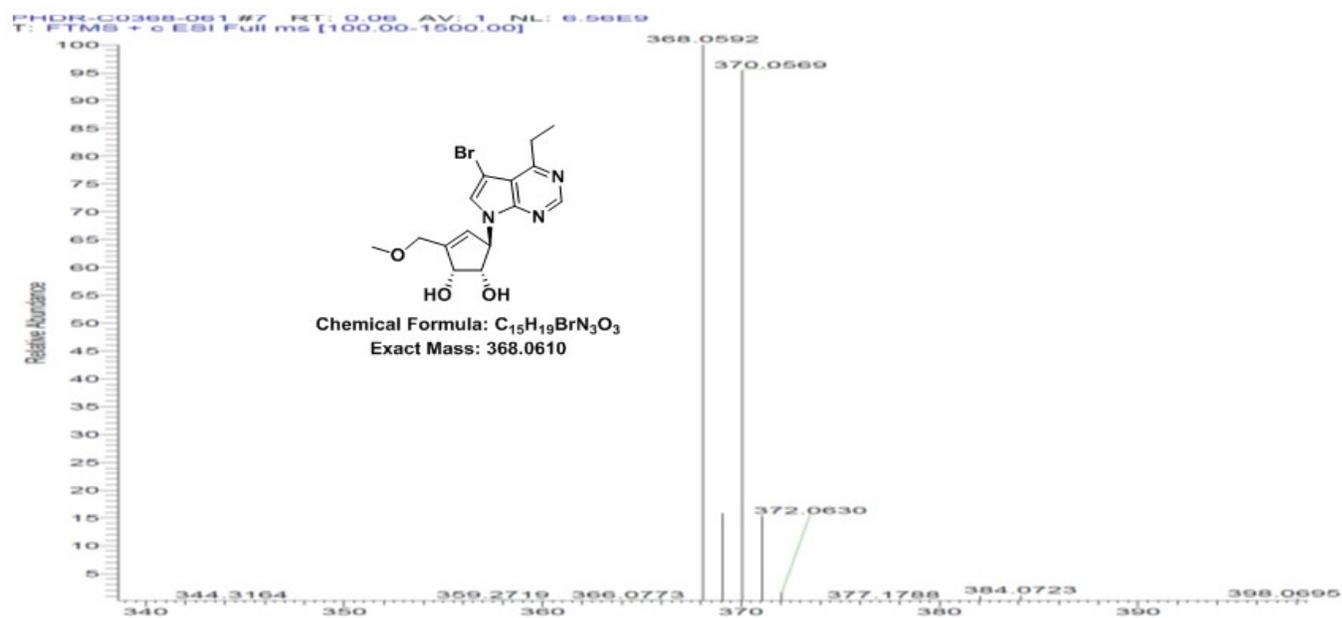


Figure S13: HRMS data of the compound **4b**

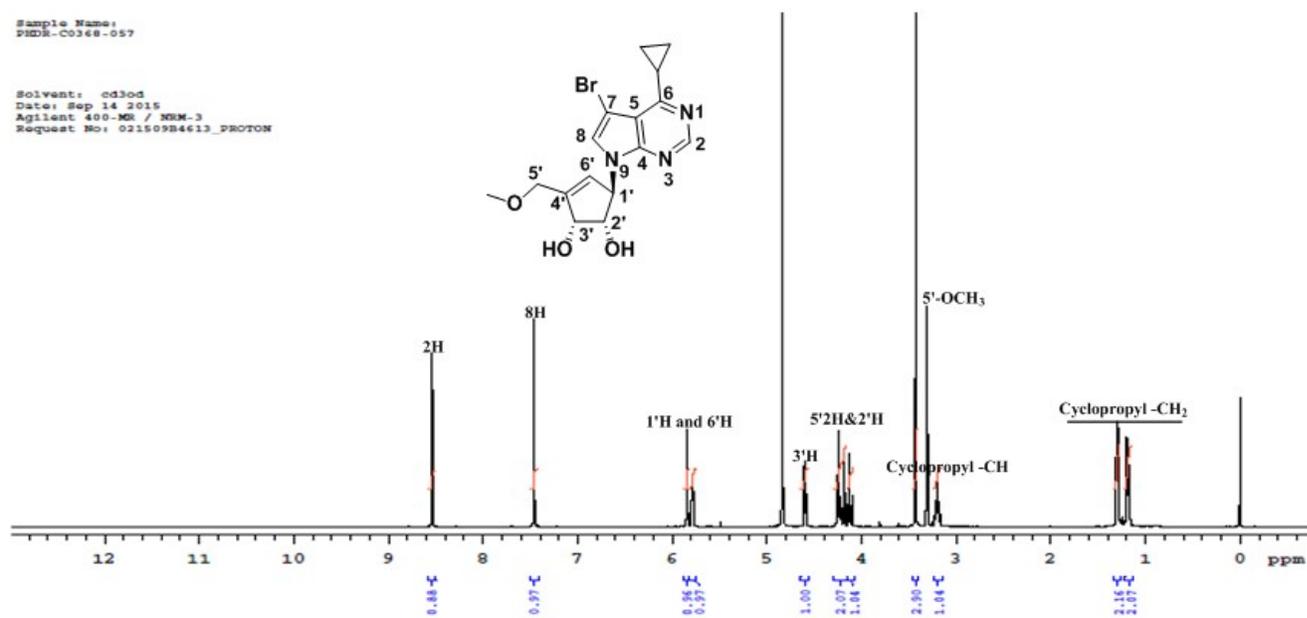


Figure S14: $^1\text{H-NMR}$ (400 MHz, CD_3OD) of the compound **4c**

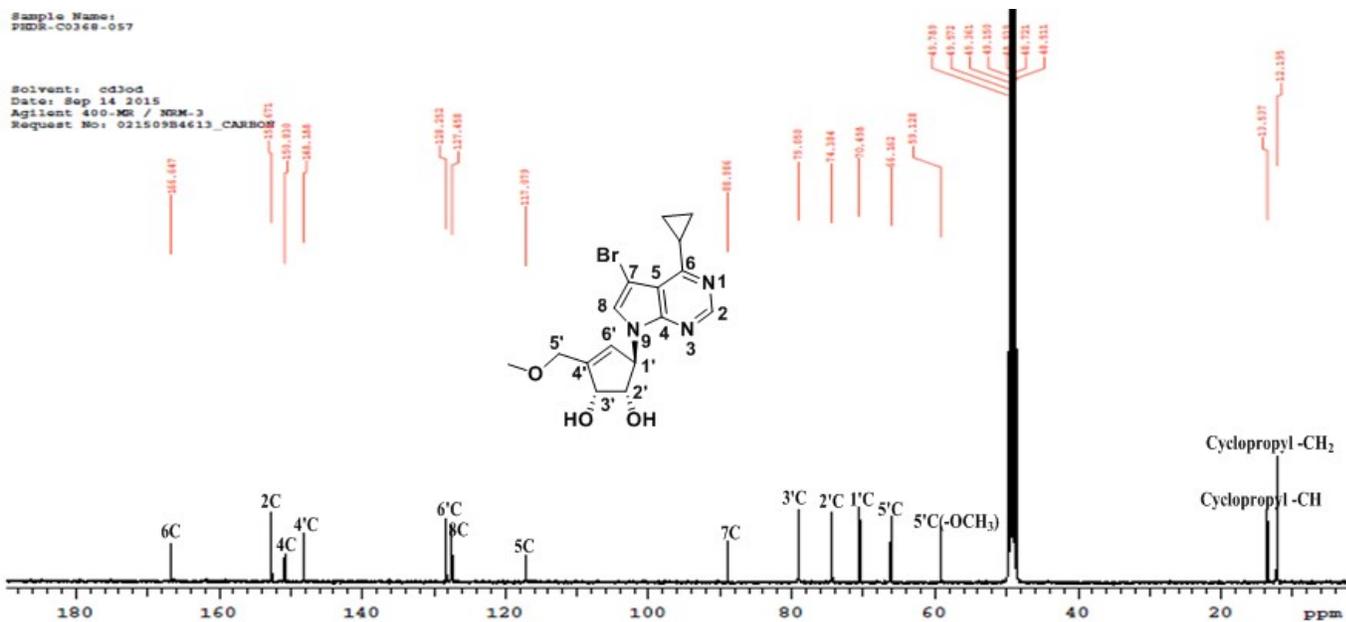


Figure S15: ¹³C-NMR (100 MHz, CD₃OD) of the compound 4c

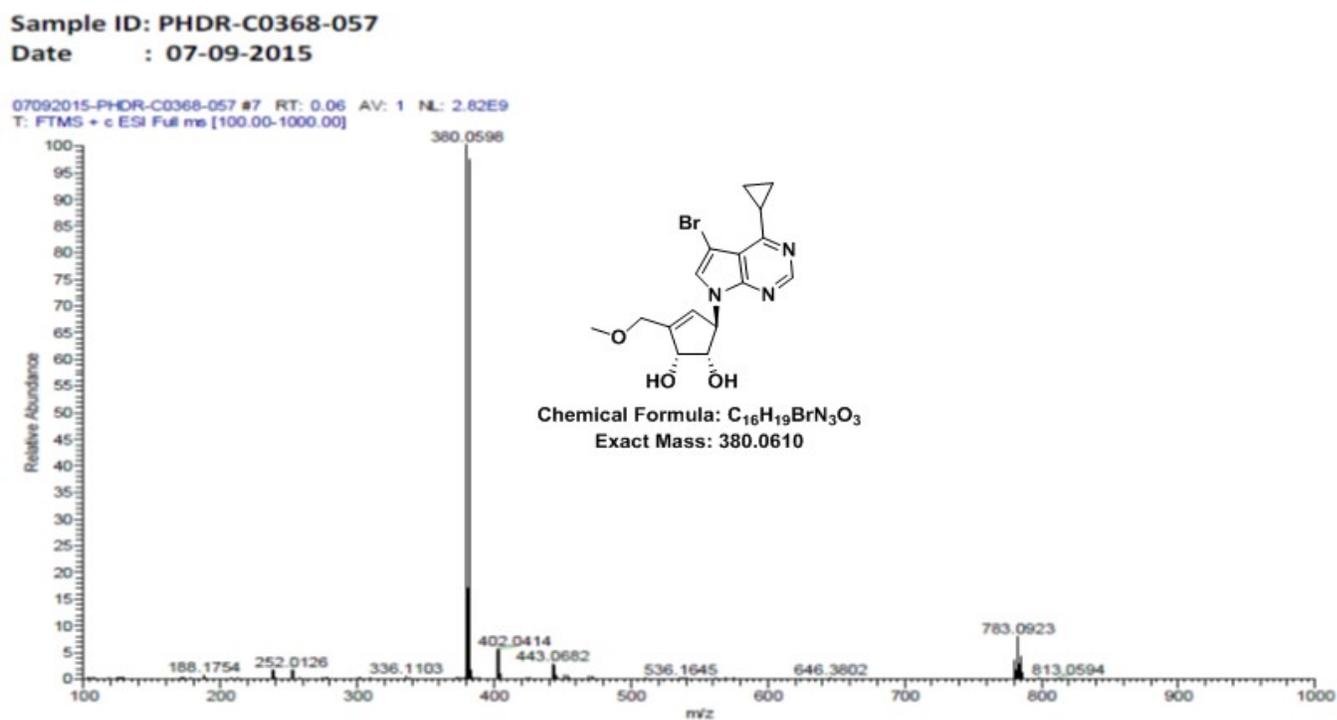


Figure S16: HRMS data of the compound 4c

Sample Name:
PMDR-C0368-059
Solvent: cd3od
Date: Sep 8 2015
Agilent 400-MR / NMR-3
Request No: 021509A8761_PROTON

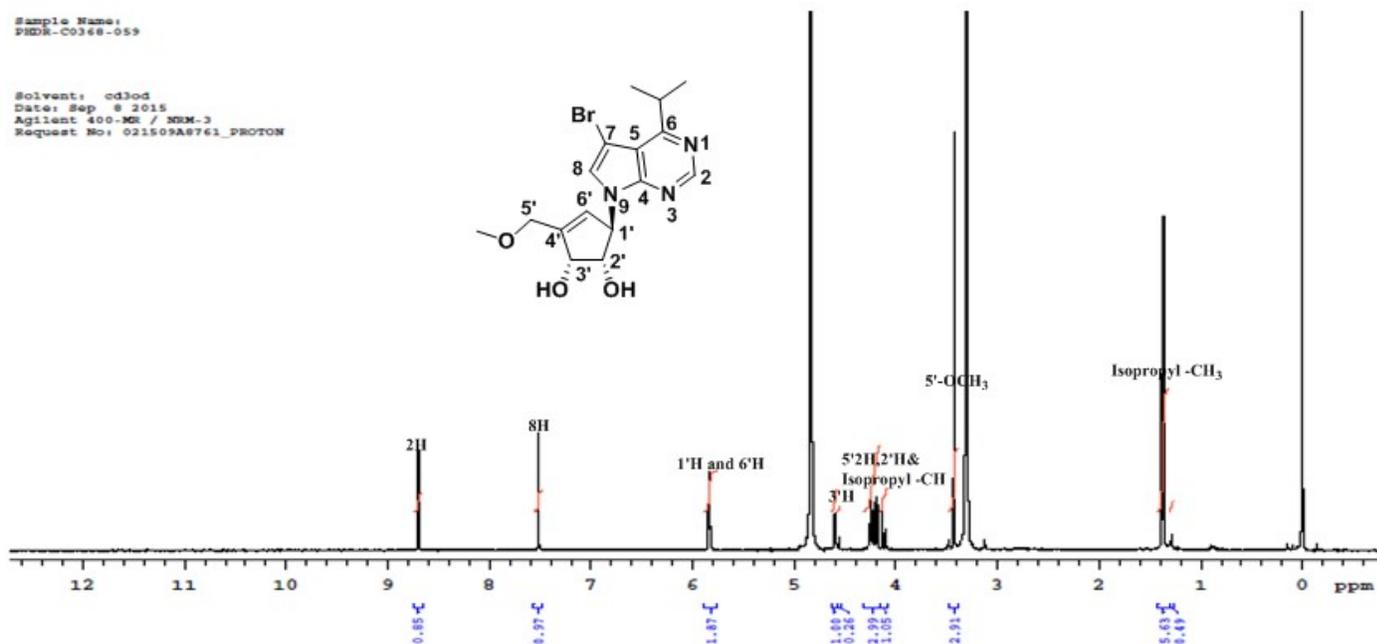


Figure S17: ¹H-NMR (400 MHz, CD₃OD) of the compound 4d

Sample Name:
PMDR-C0368-059
Solvent: cd3od
Date: Sep 13 2015
Agilent 400-MR / NMR-3
Request No: 021509B4614_CARBON

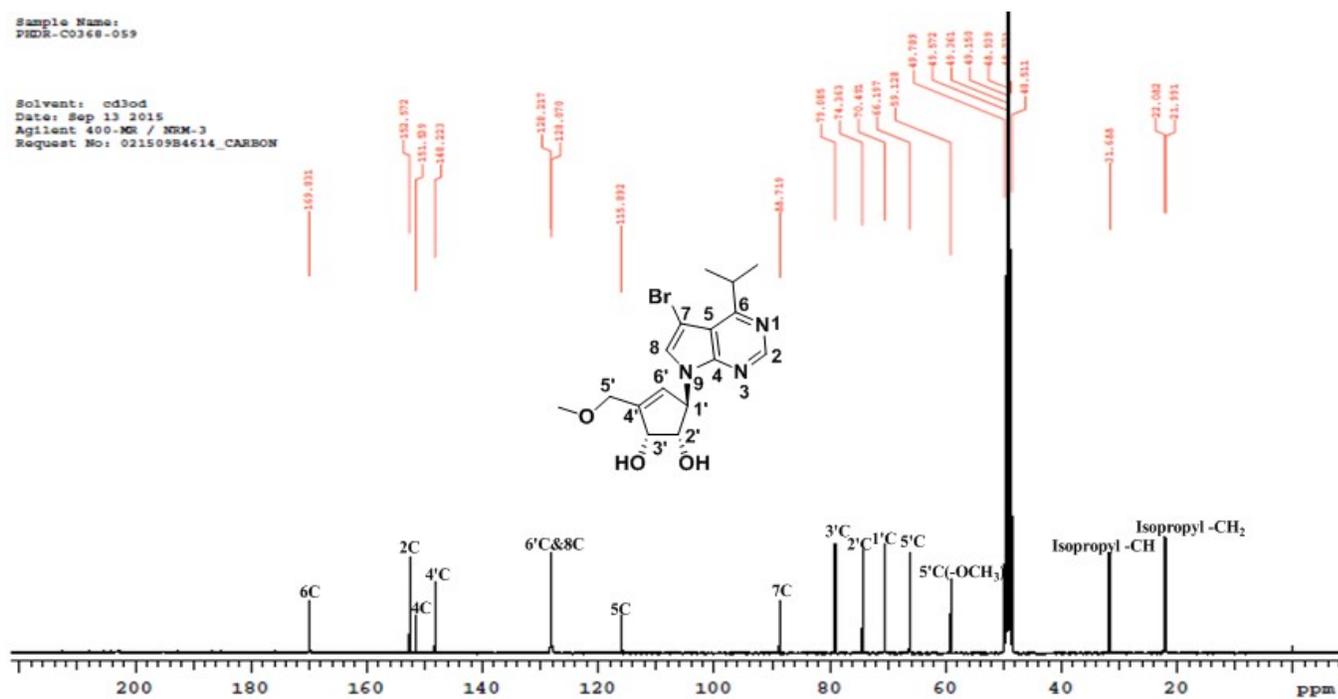


Figure S18: ¹³C-NMR (100 MHz, CD₃OD) of the compound 4d

Sample ID: PHDR-C0368-059

Date : 09-09-2015

08092015-15-PHDR-C0368-059 #9 RT: 0.08 AV: 1 NL: 4.93E9
T: FTMS + c ESI Full ms [100.00-1250.00]

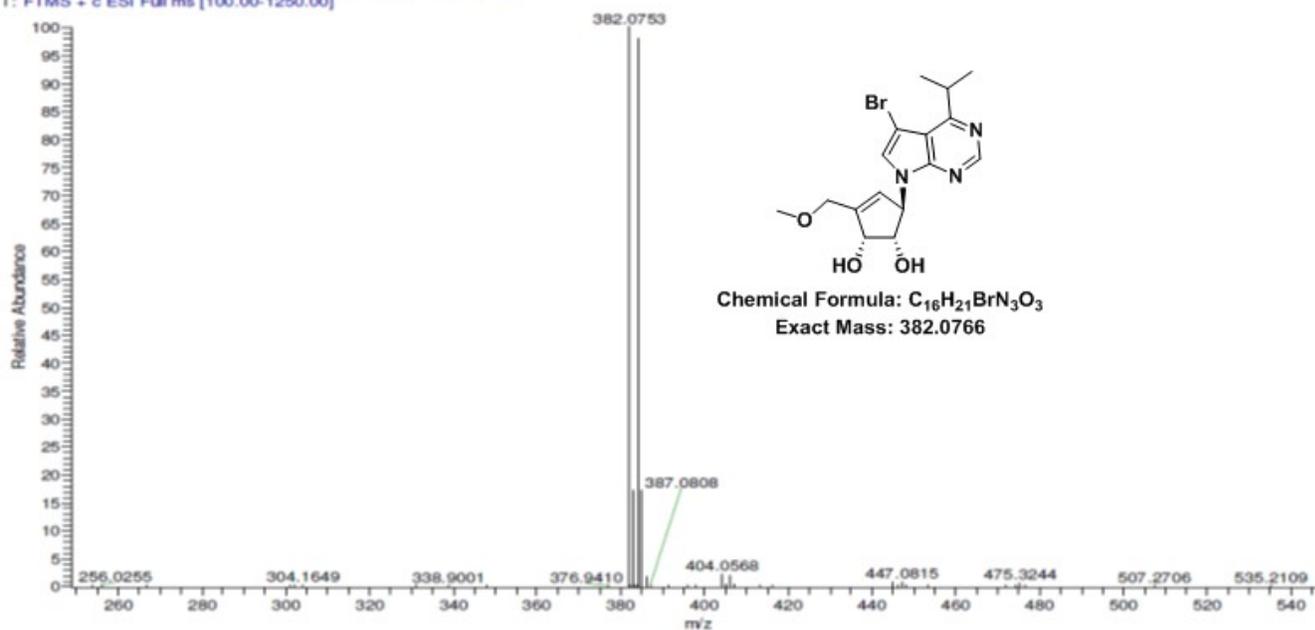


Figure S19: HRMS data of the compound **4d**

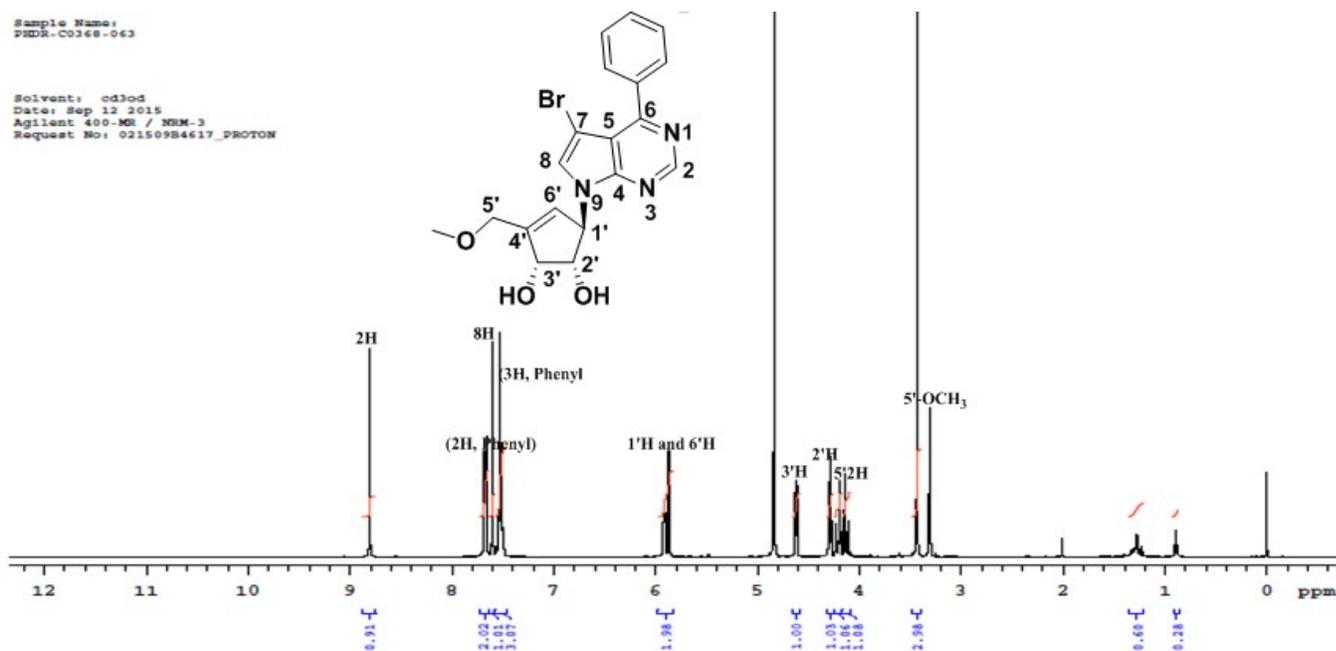


Figure S20: 1H -NMR (400 MHz, CD_3OD) of the compound **4e**

Sample Name:
PDR-C0368-063

Solvent: cd3od
Date: Sep 12 2015
Agilent 400-MR / MRM-3
Request No: 021509B4617 CARBON

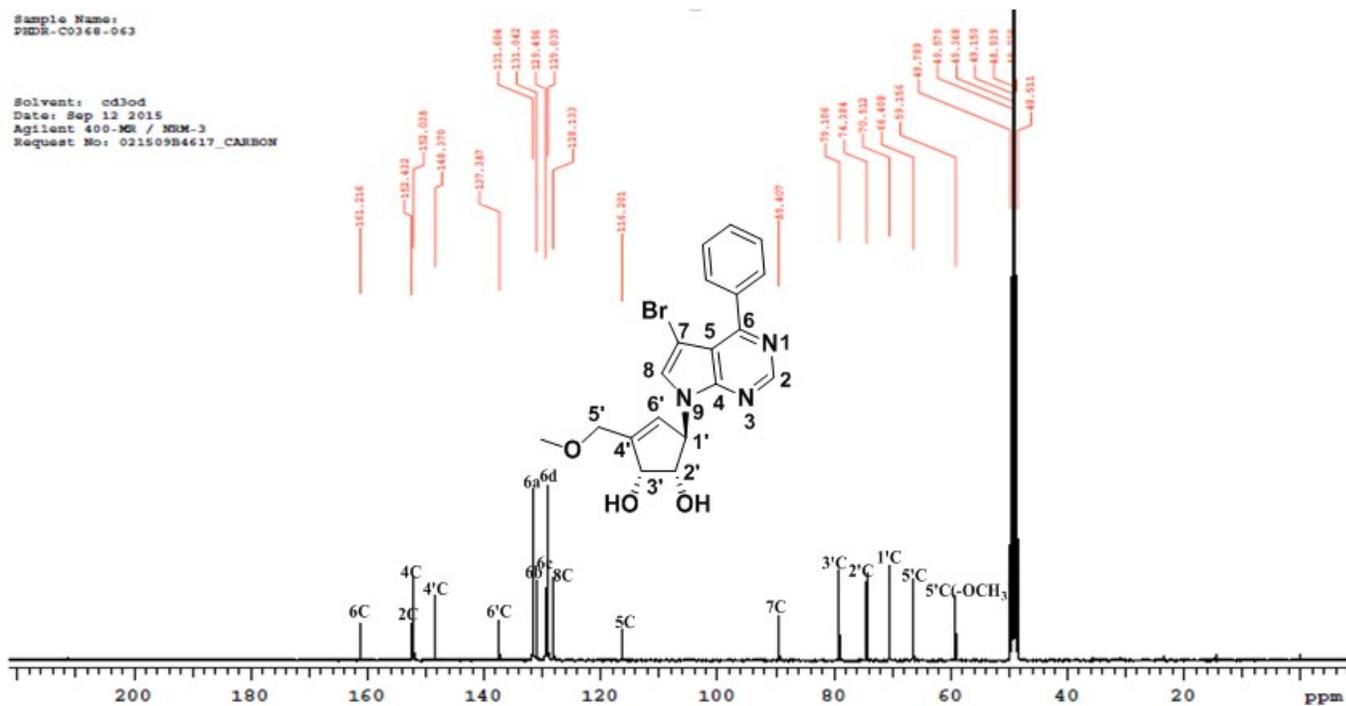


Figure S21: ^{13}C -NMR (100 MHz, CD_3OD) of the compound 4e

Sample ID: PHDR-C0368-063

Date : 09-09-2015

08092015-15-PHDR-C0368-063 #9 RT: 0.08 AV: 1 NL: 5.65E9
T: FTMS + c ESI Full ms [100.00-1250.00]

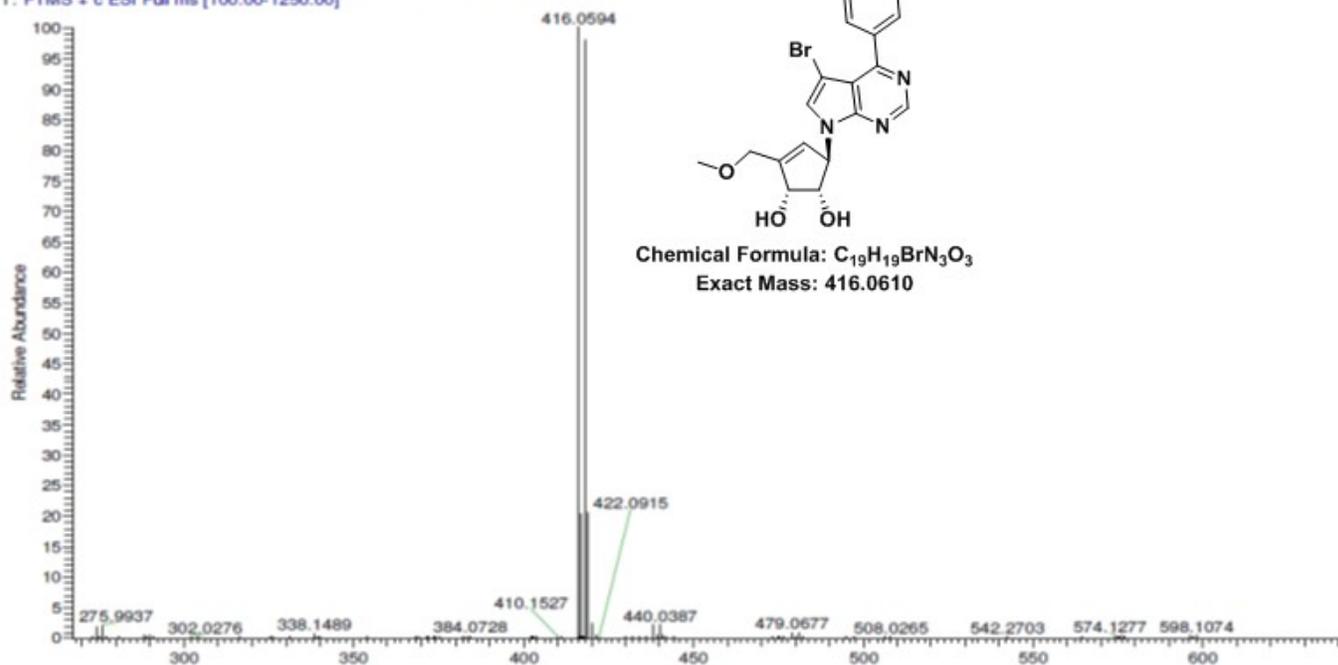


Figure S22: HRMS data of the compound 4e

Sample Name:
PHDR-C0368-089

Solvent: cd3od
Date: Nov 28 2015
Agilent 400-MR / NMR-3
Request No: 021511D4567_PROTON

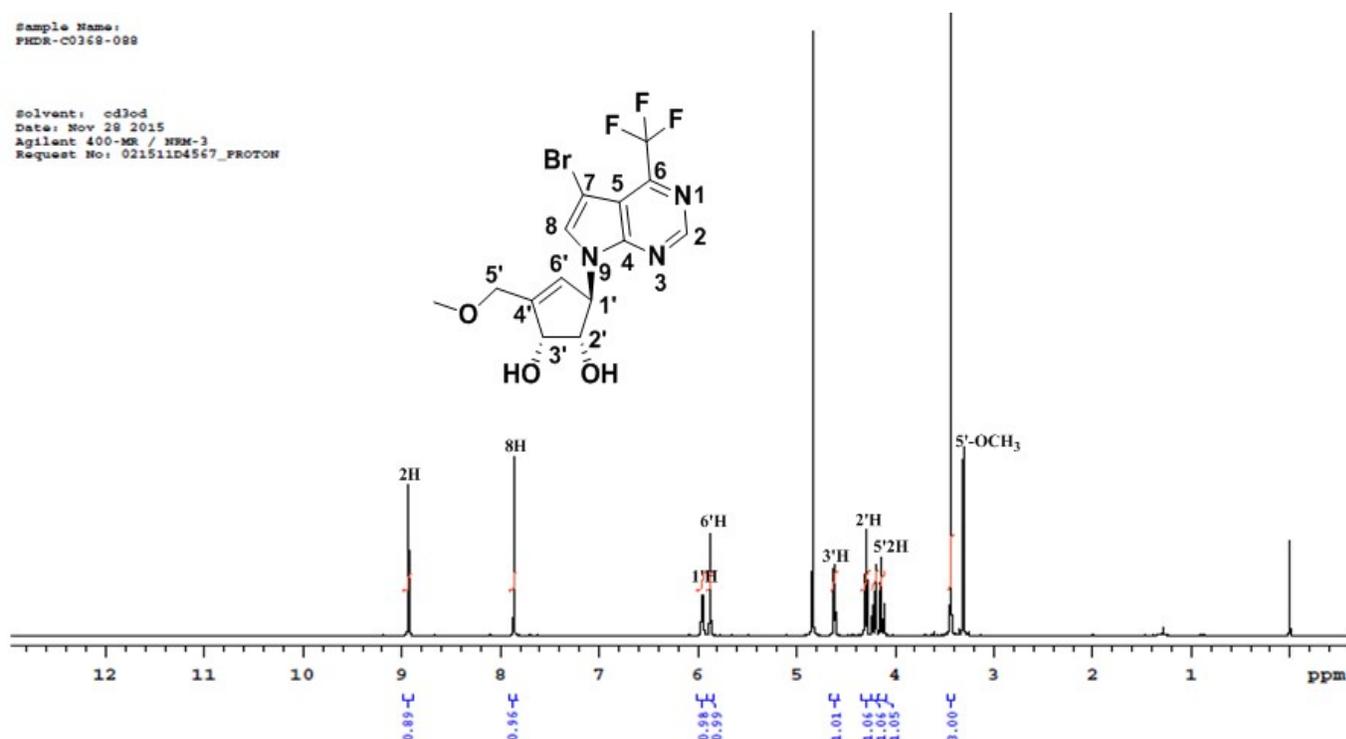


Figure S23: 1H -NMR (400 MHz, CD_3OD) of the compound 4f

Sample Name:
PHDR-C0368-088

Solvent: cd3od
Date: Nov 28 2015
Agilent 400-MR / NMR-3
Request No: 021511D4567 CARBON

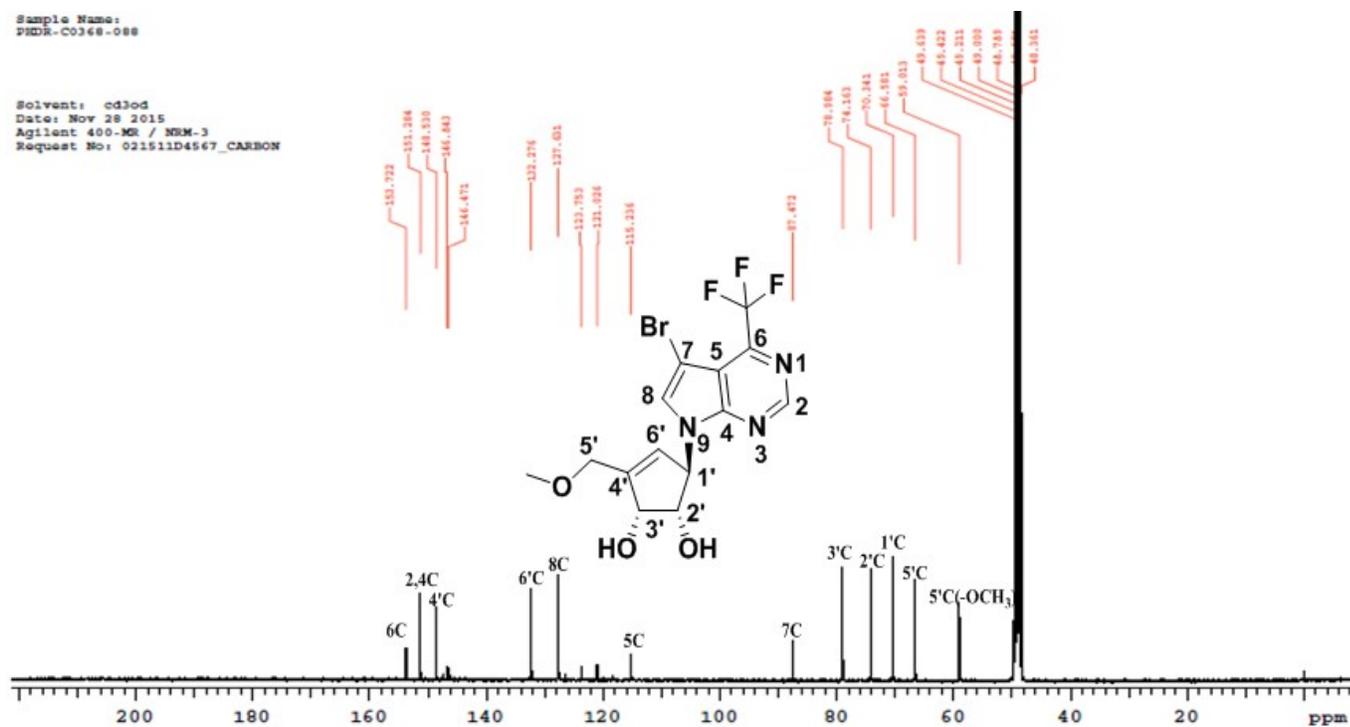


Figure S24: ¹³C-NMR (100 MHz, CD₃OD) of the compound 4f

Sample Name:
PHDR-C0368-088

Solvent: cd3od
Date: Nov 28 2015
Agilent 400-MR / NMR-3
Request No: 021511D4567 FLUORINE

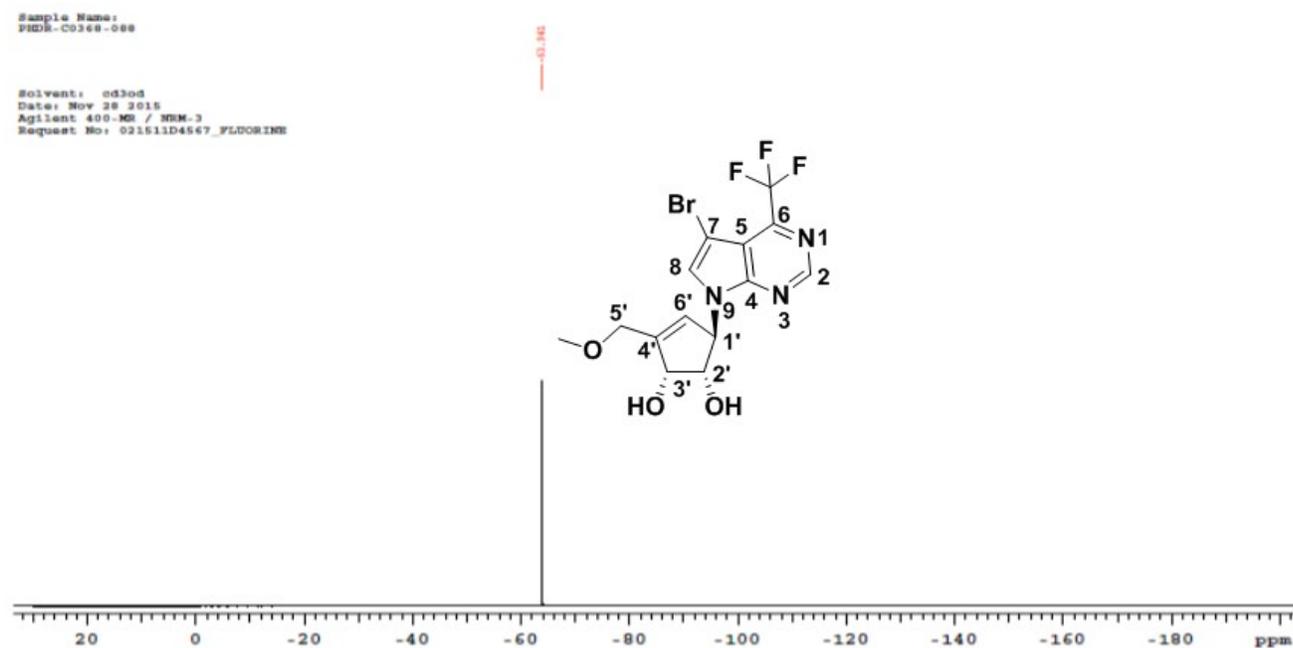


Figure S25: ¹⁹F-NMR (376 MHz, CD₃OD) of the compound 4f

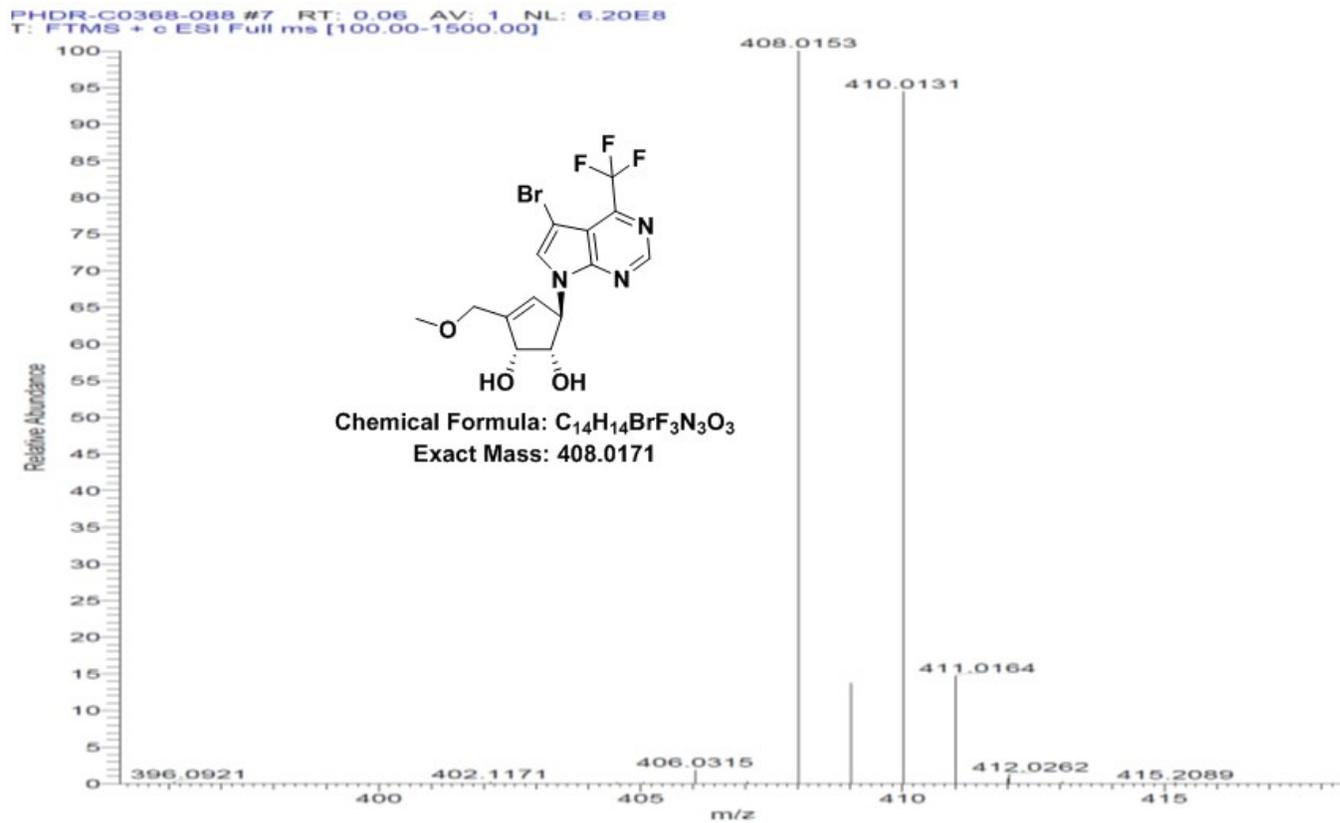


Figure S26: HRMS data of the compound 4f

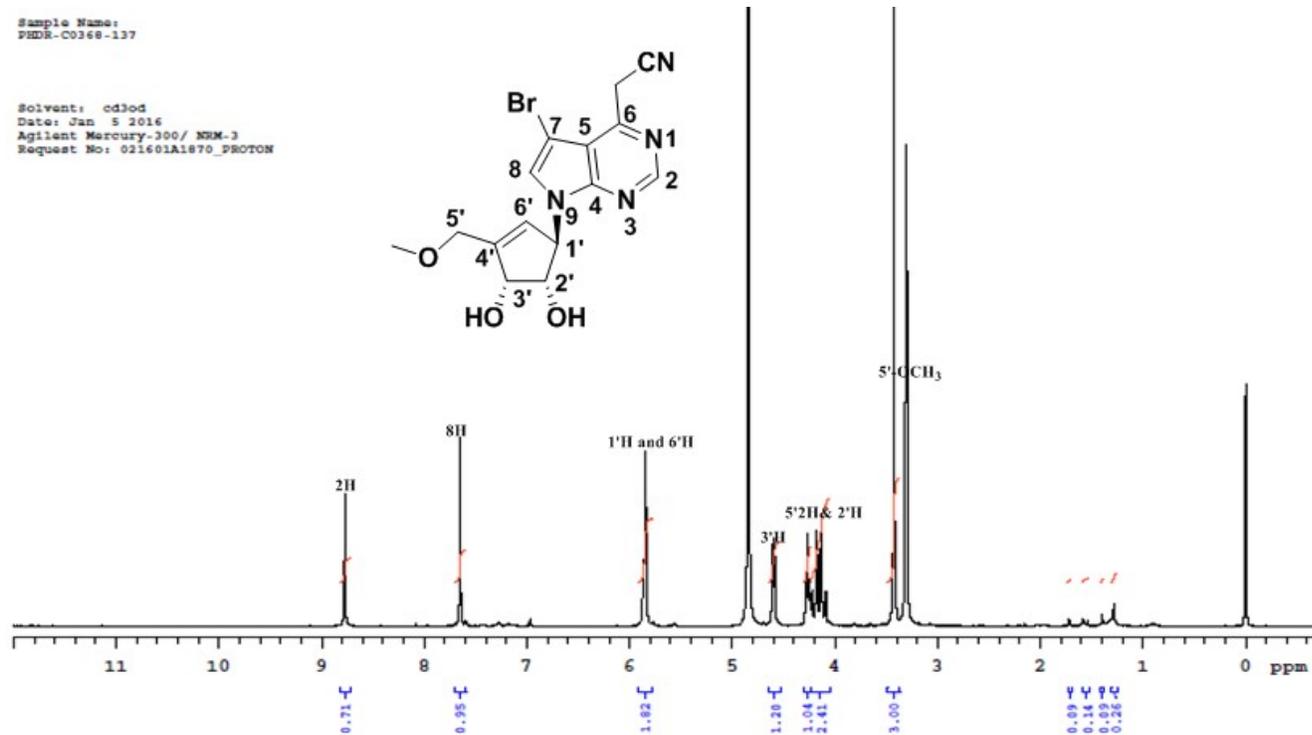


Figure S27: 1H -NMR (300 MHz, CD_3OD) of the compound 4g

Sample Name:
PHDR-C0368-137

Solvent: dmsd
Date: Jan 9 2016
Agilent 400-MR / NMR-3
Request No: 021601A8656 CARBON

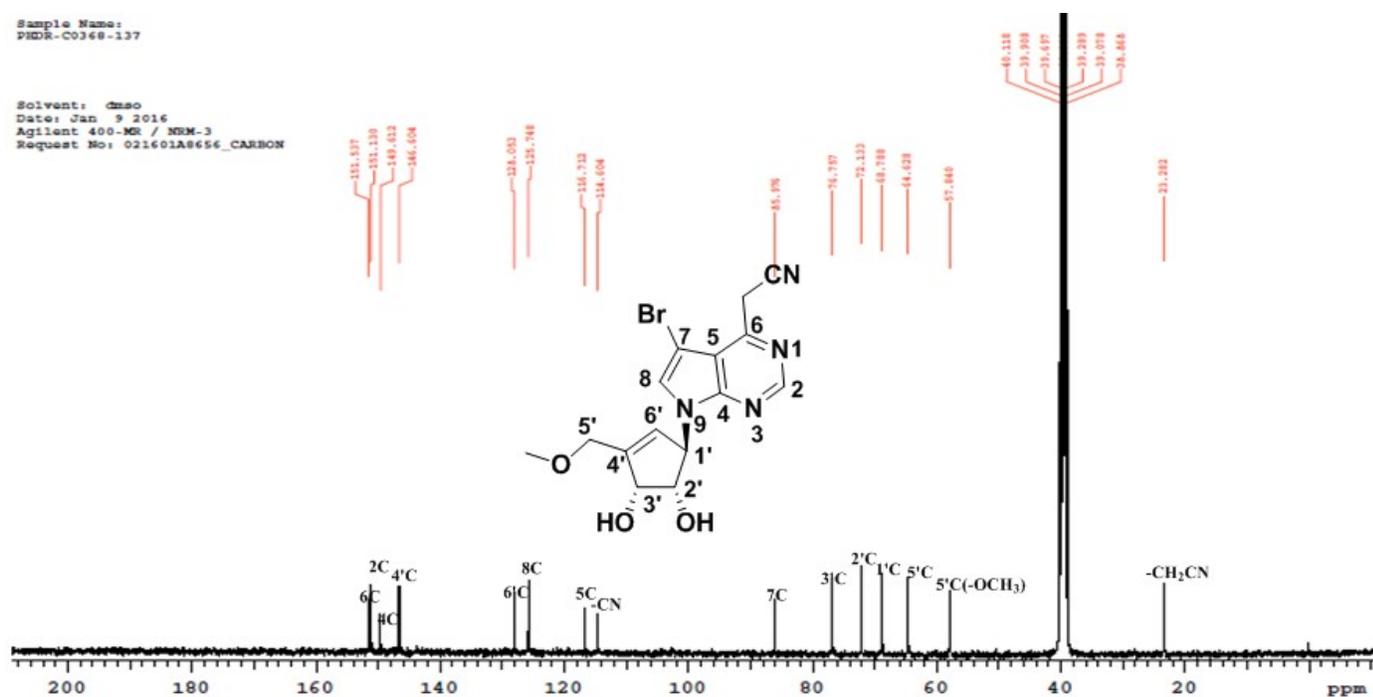


Figure S28: ^{13}C NMR (100 MHz, CD_3OD) of the compound **4g**

Sample ID: PHDR-C0368-137
Date : 08-01-2016

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T: FTMS + e ESI Full ms [133.40-2000.00]

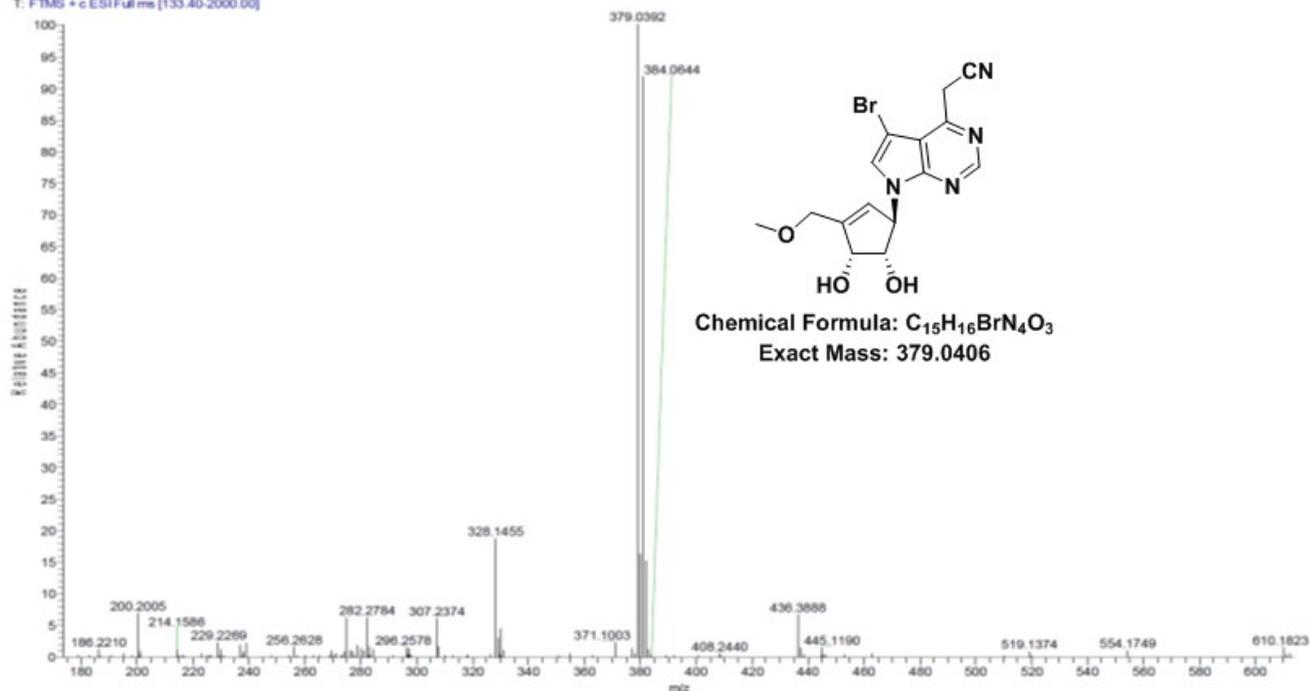


Figure S29: HRMS data of the compound **4g**

Sample Name:
PMDR-C0368-113

Solvent: cd3od
Date: Dec 5 2015
Agilent 400-MR / NMR-3
Request No: 021512A6794_PROTON

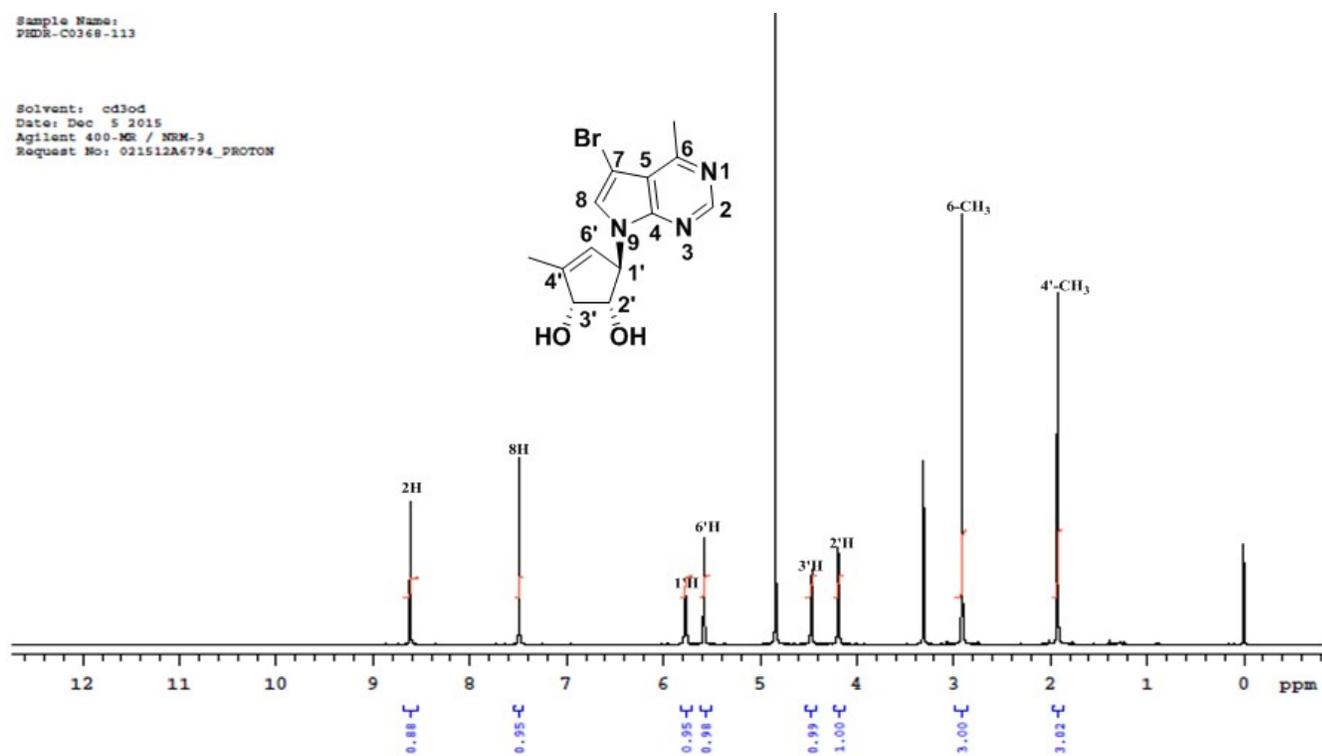


Figure S30: $^1\text{H-NMR}$ (400 MHz, CD_3OD) of the compound 7a

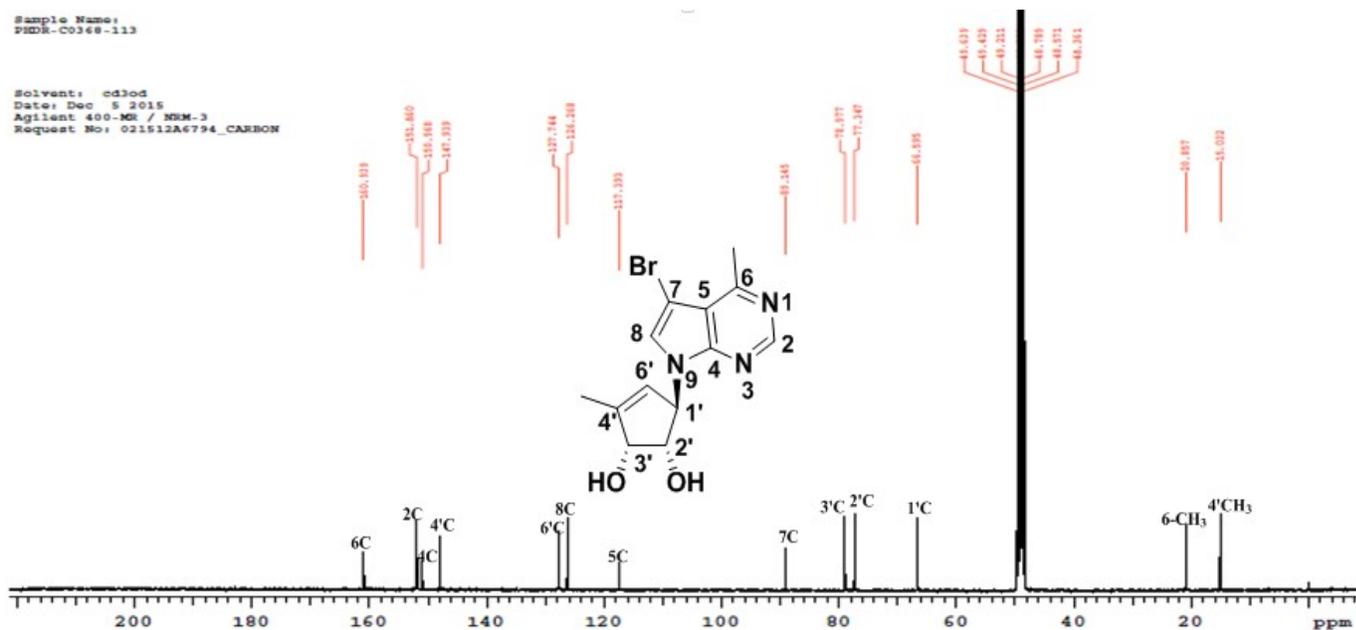


Figure S31: $^{13}\text{C-NMR}$ (100 MHz, CD_3OD) of the compound 7a

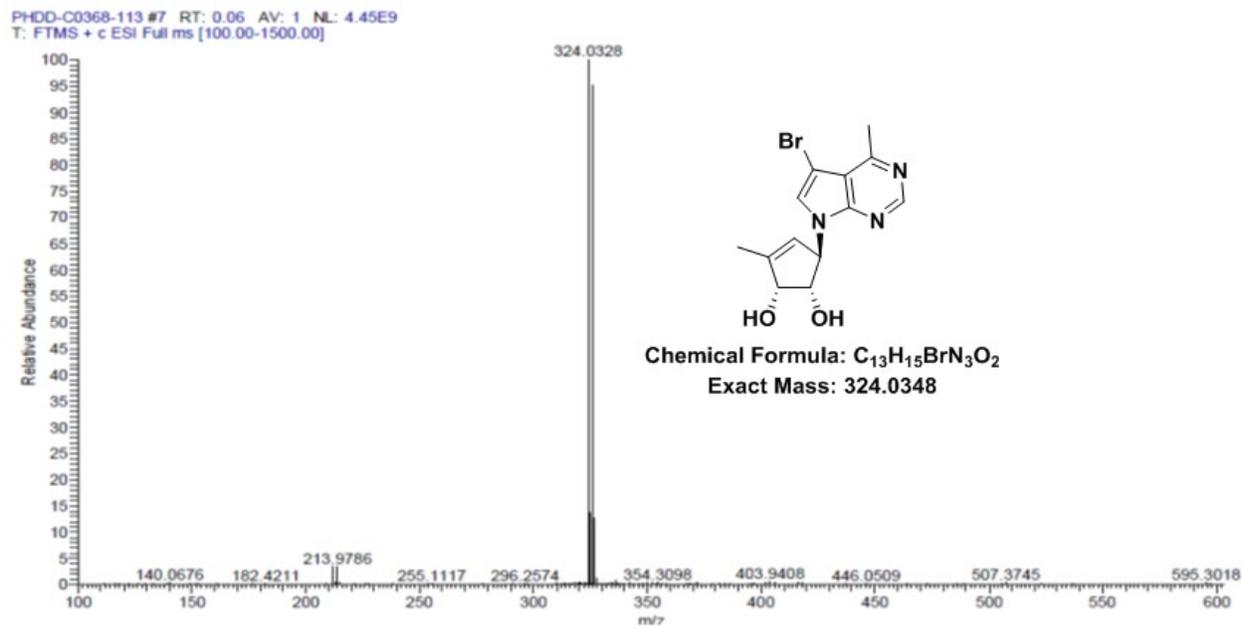


Figure S32: HRMS data of the compound **7a**

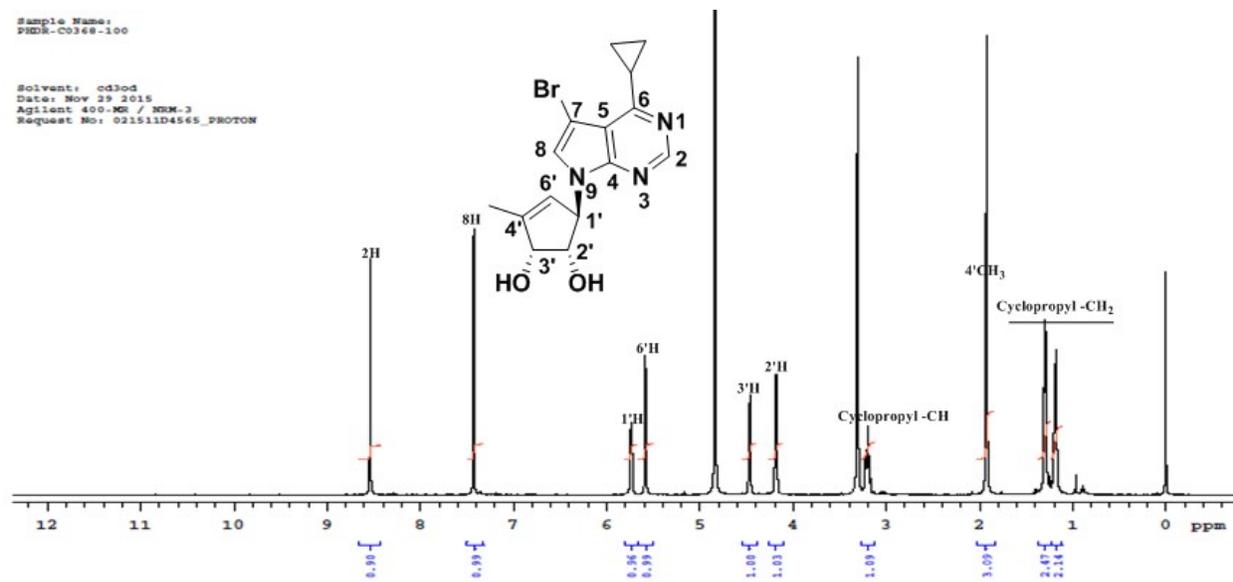


Figure S33: $^1\text{H-NMR}$ (400 MHz, CD_3OD) of the compound **7b**

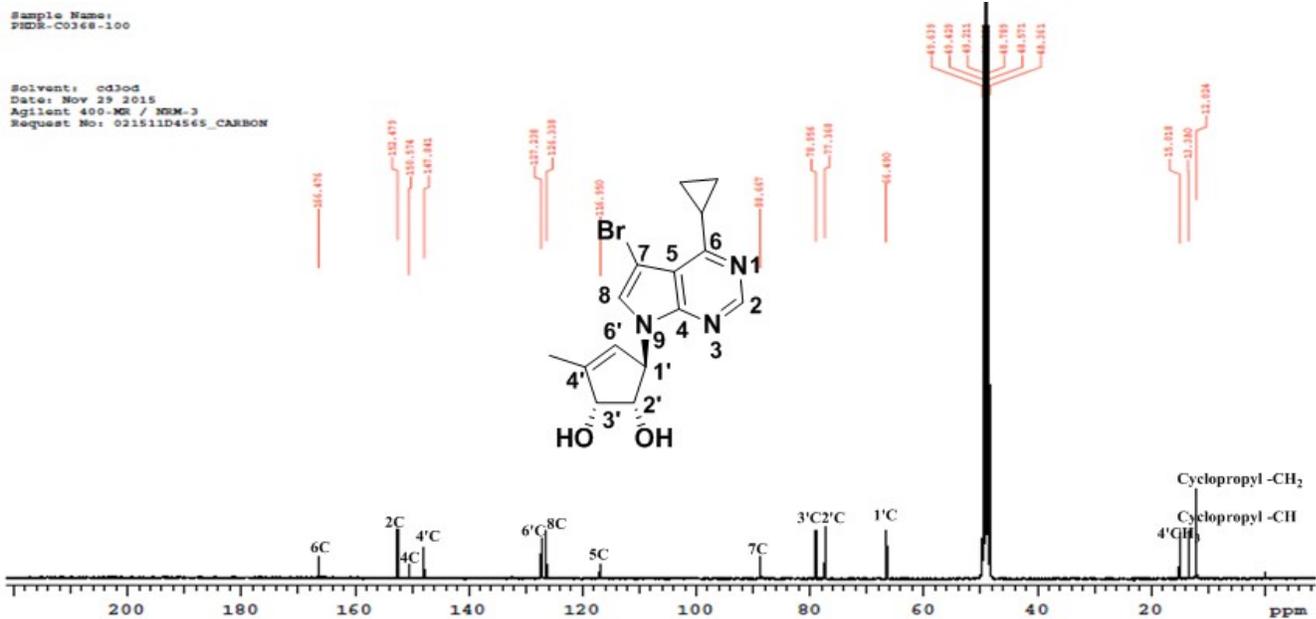


Figure S34: ^{13}C -NMR (100 MHz, CD_3OD) of the compound 7b

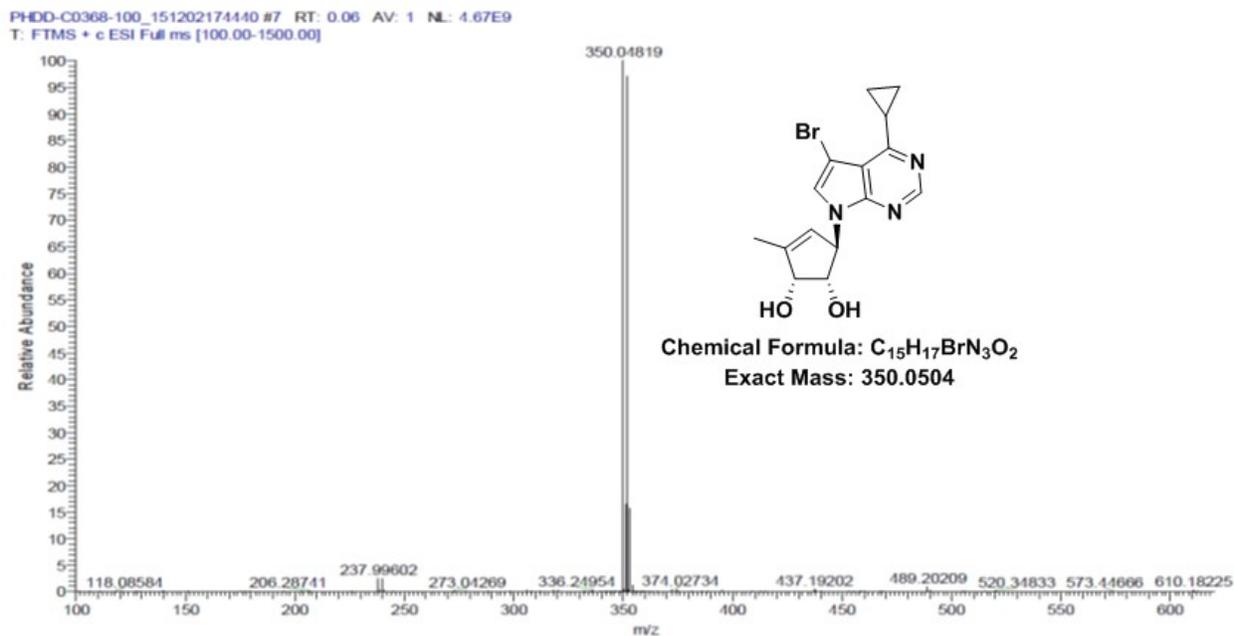


Figure S35: HRMS data of the compound 7b

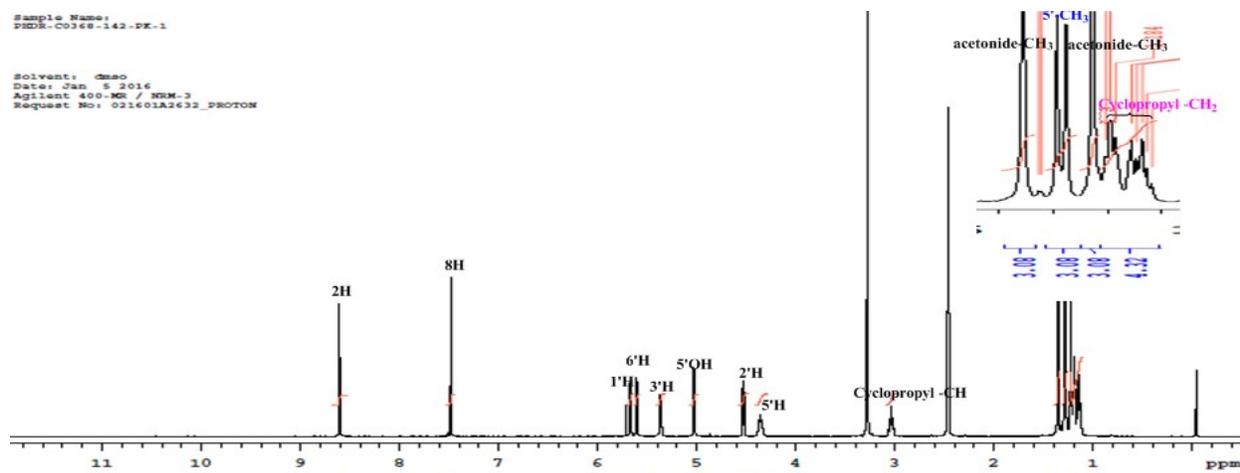


Figure S36: $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$) of the compound **9a** with proton assignment

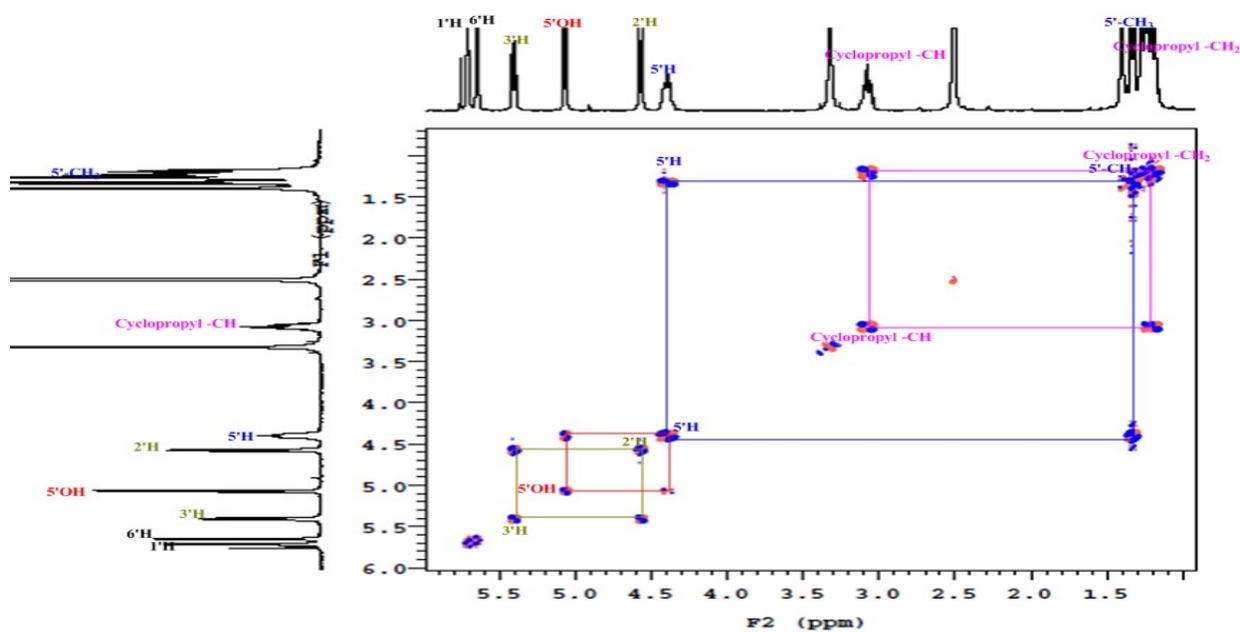


Figure S37: $^1\text{H-}^1\text{H}$ COSY spectrum of **9a** showing the correlation of adjacent protons in the cross-diagonal peaks.

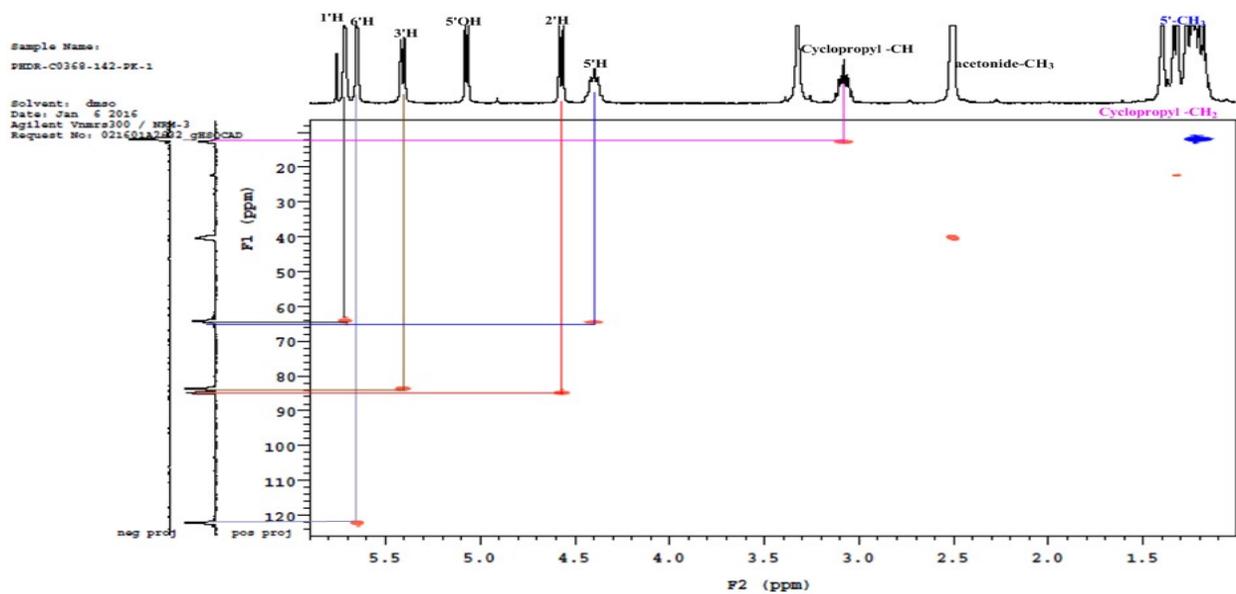


Figure S38: HSQC (^1H - ^{13}C single bond correlation) spectrum of **9a**

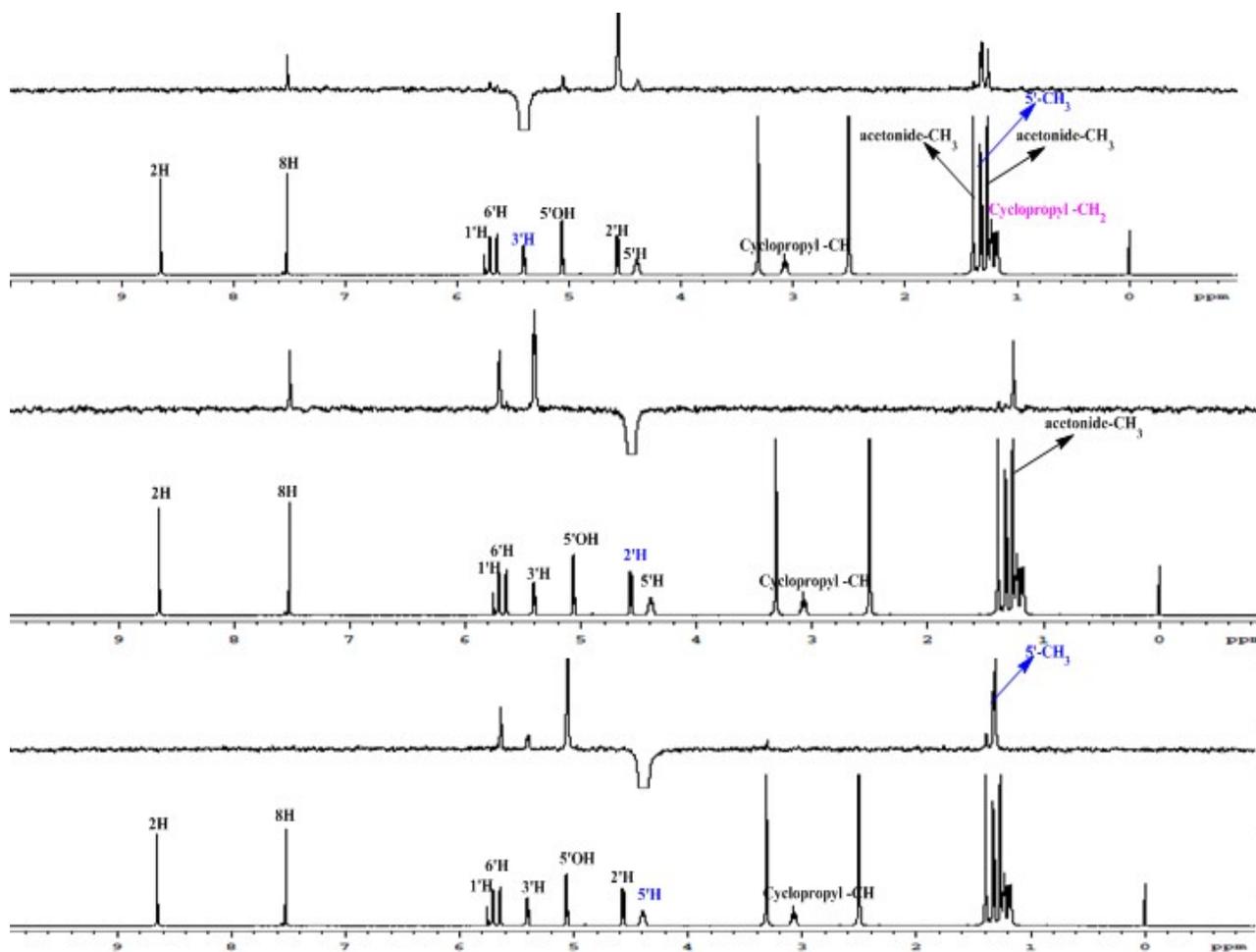


Figure S39: The NOE correlation between 5'-H and 3'-H was not clearly observed for compound **9a**, which may indicate a relatively distant spatial orientation between these protons. However, the NOE data do not allow the assignment of the configuration of the **9a** diastereomer.

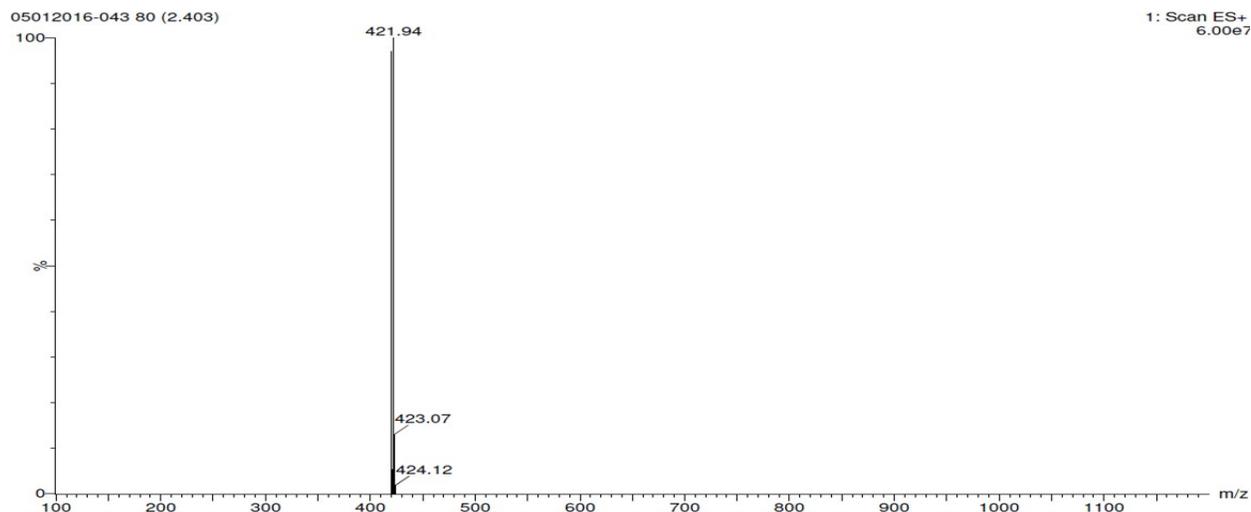


Figure S40: Mass spectrum (MS) of **9a** [M+1] is 421.79 [421.94]

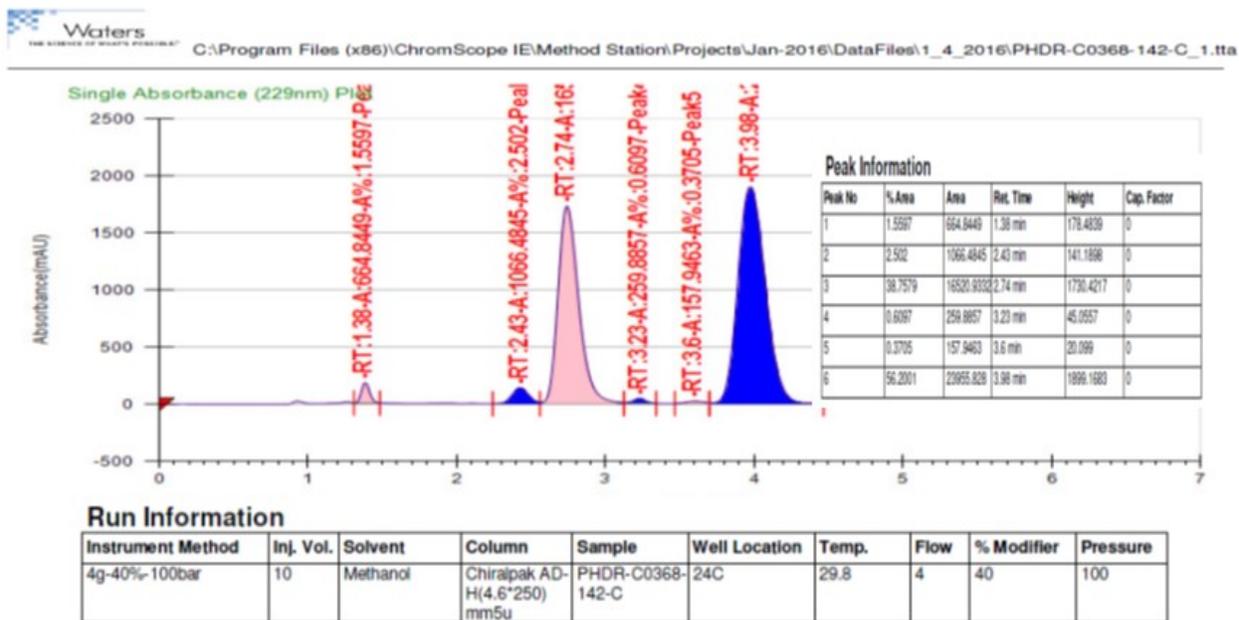


Figure S41: Chiral HPLC chromatogram with column ChiralPAK AD-H showing the mixture of two isomers **9a** and **9b**.

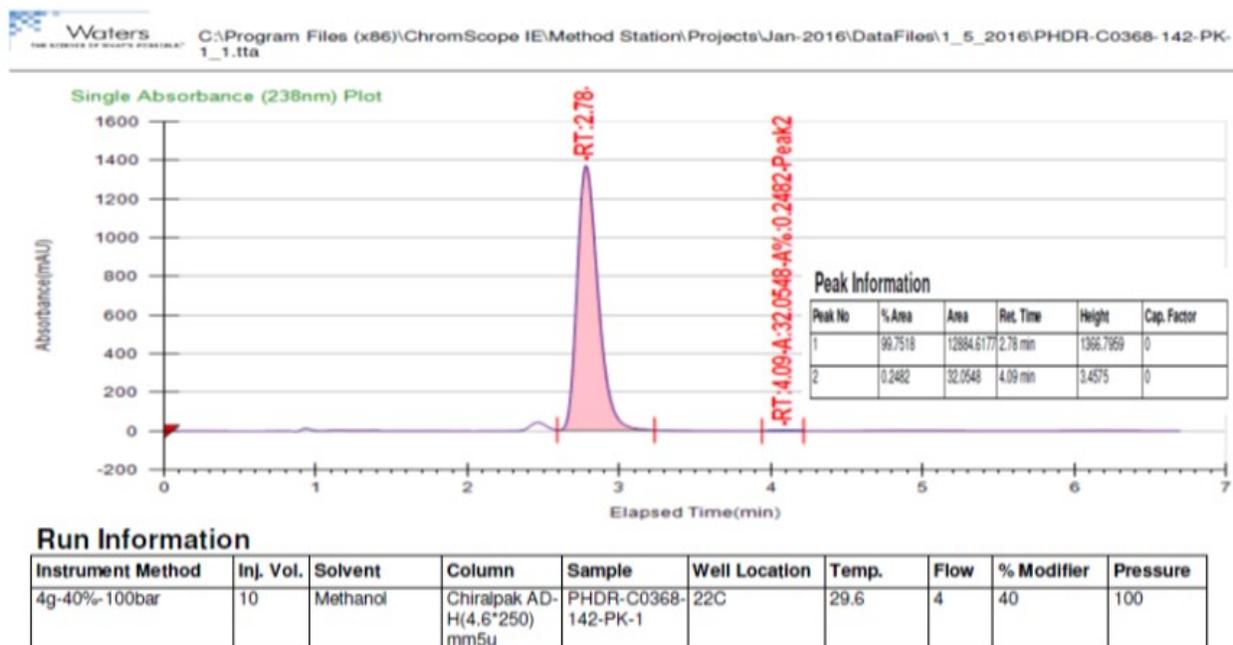


Figure S42: Chiral HPLC chromatogram **9a** with column ChiralPAK AD-H showing 99.75% chiral purity (single isomer)

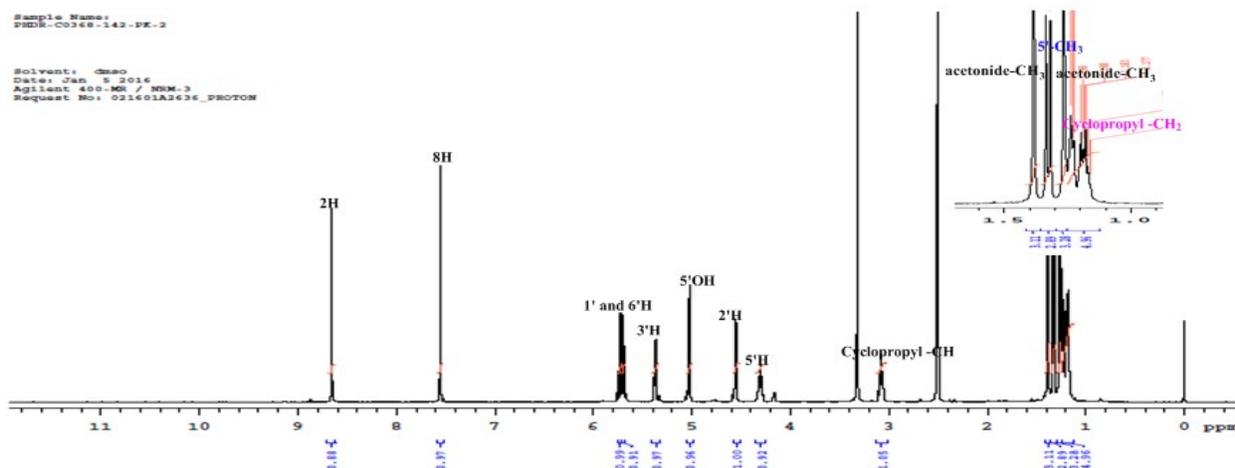


Figure S43: ^1H -NMR (400 MHz, $\text{DMSO}-d_6$) of the compound **9b** with proton assignment

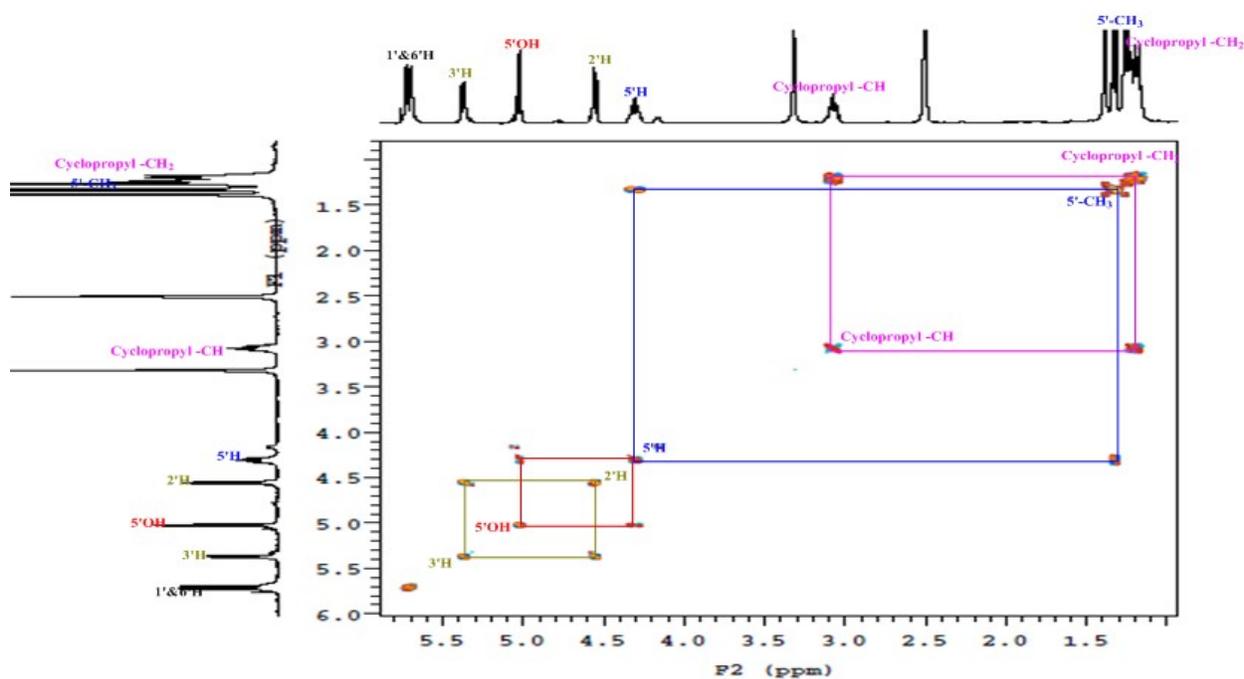


Figure S44: ^1H - ^1H COSY spectrum of **9b** showing the correlation of adjacent protons in the cross-diagonal peaks.

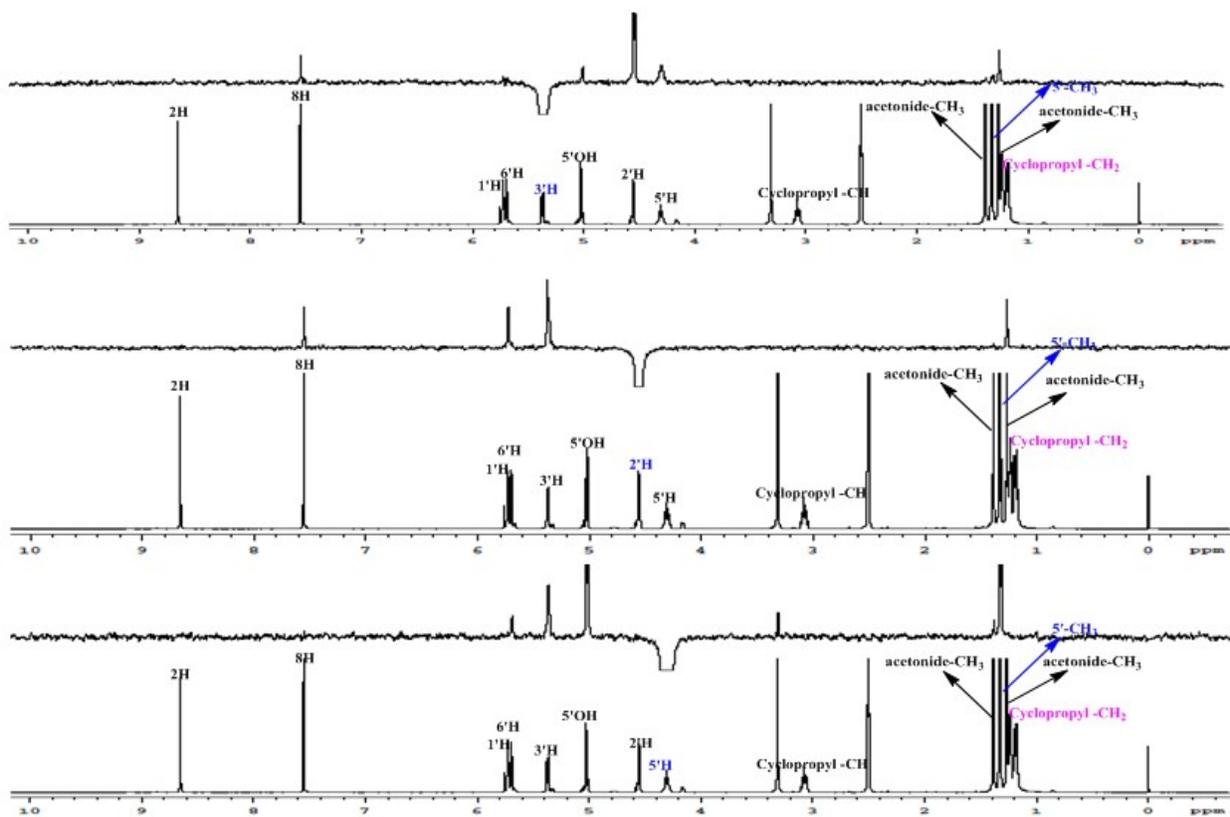


Figure S45: Irradiation of 5'-H in **9b** produced an NOE enhancement of 3'-H, which may likely result from their close spatial proximity. However, free rotation about the C4'-C5' bond in **9b** prevents a reliable configurational assignment.

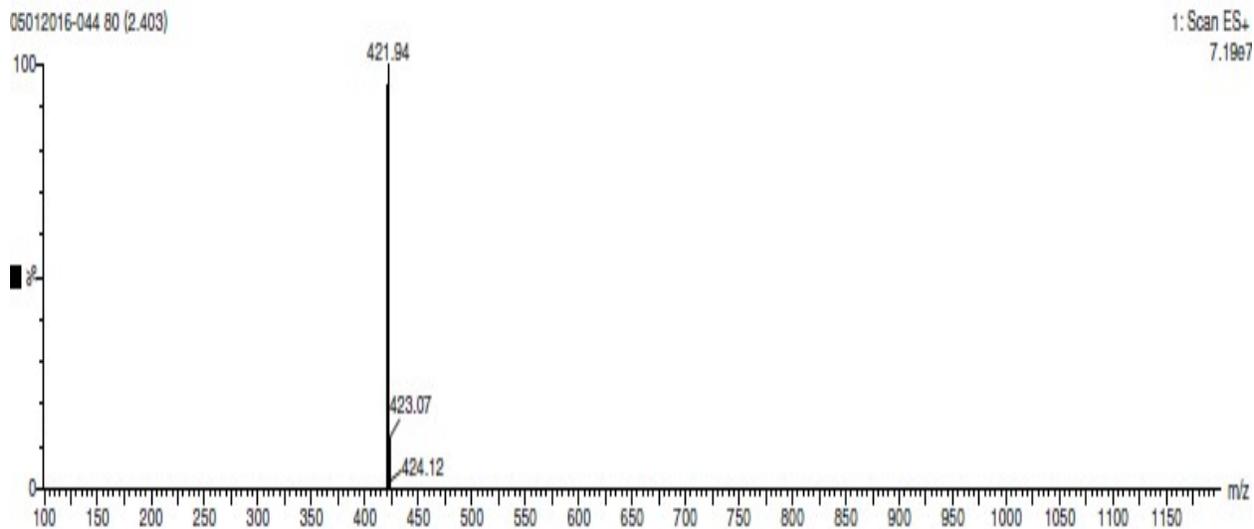


Figure 46: Mass spectrum (MS) of **9b** [M+1] is 421.79 [421.94]

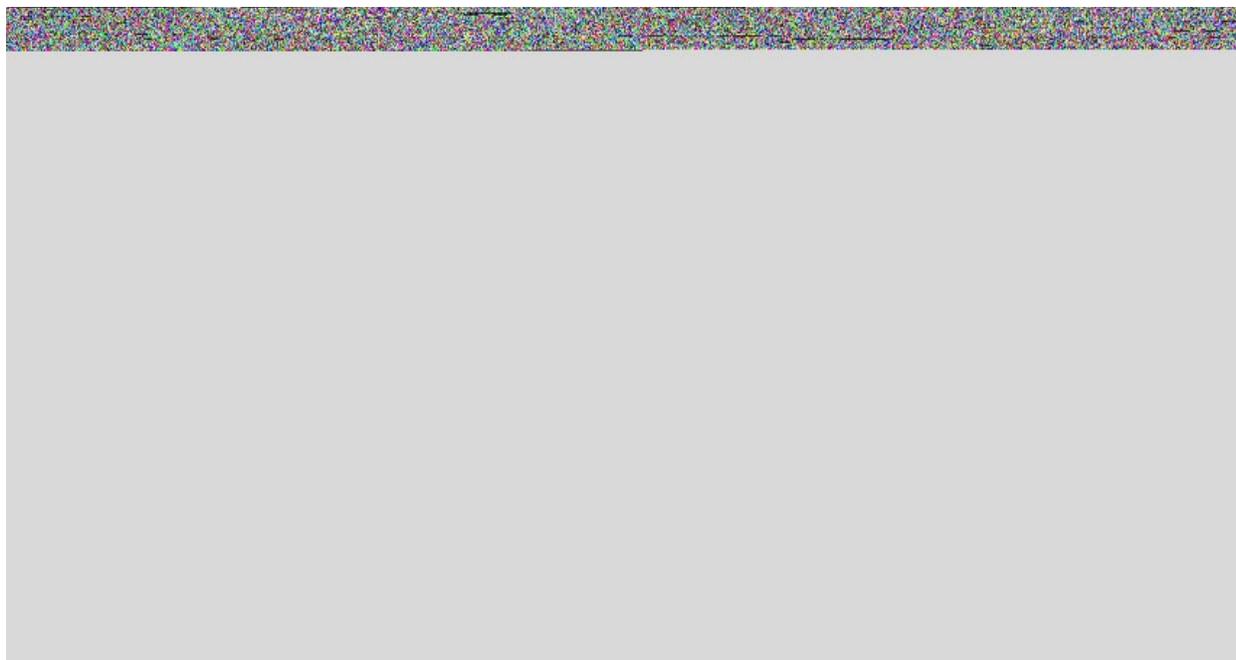


Figure S47: Chiral HPLC chromatogram **9b** with column ChiralPAK AD-H showing a mixture of two isomers

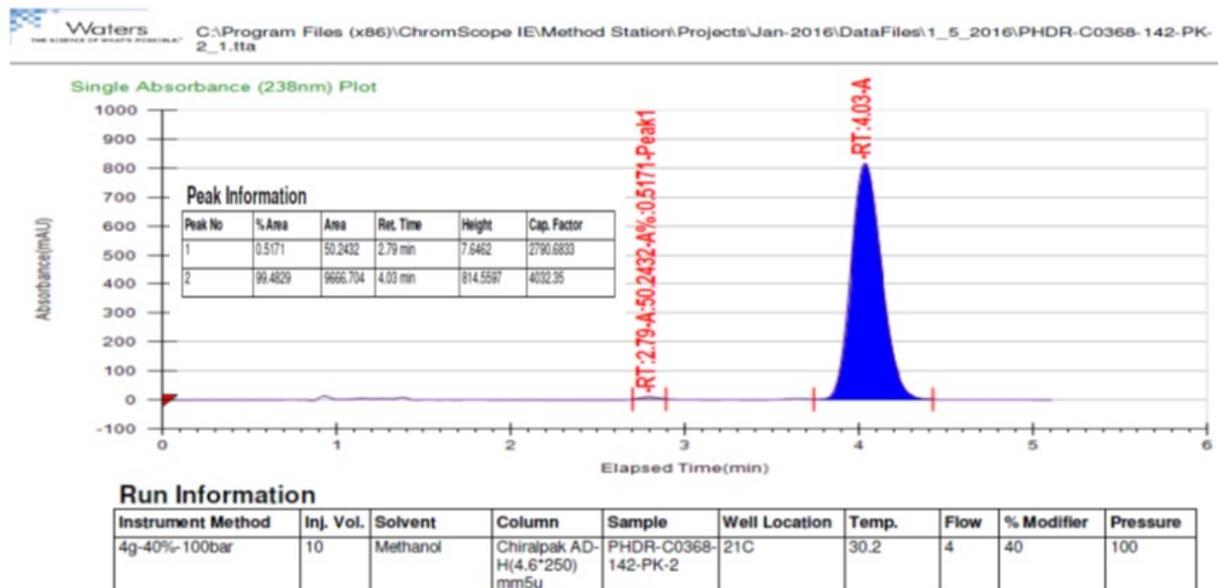


Figure S48: Chiral HPLC chromatogram **9b** with column ChiralPAK AD-H showing 99.75% chiral purity (single isomer)

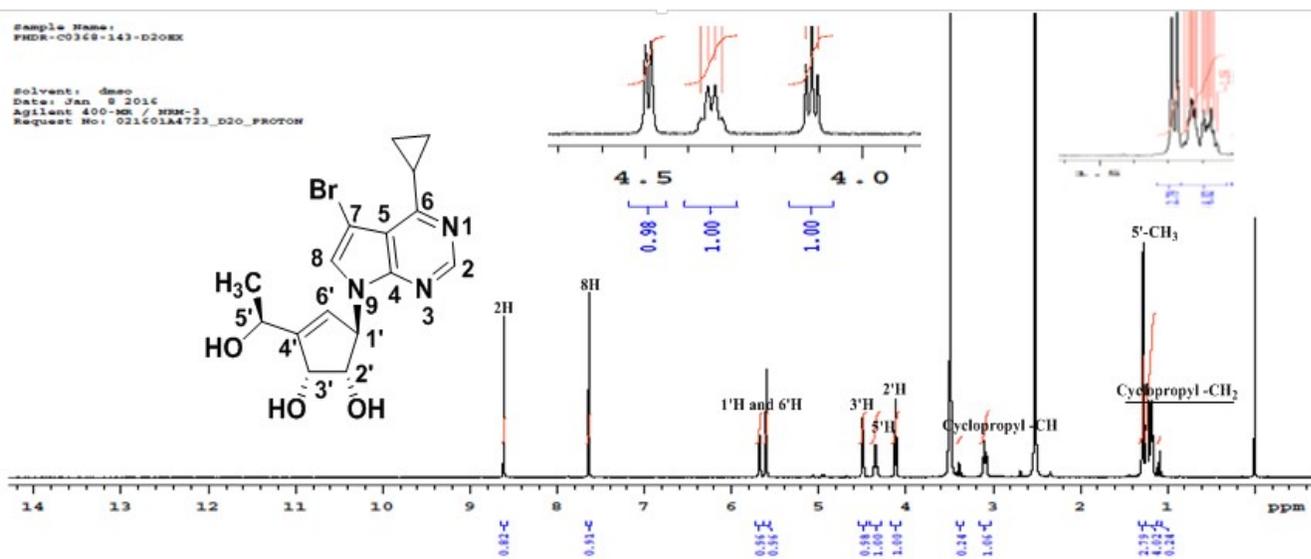


Figure S49: $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$) of the compound 10a

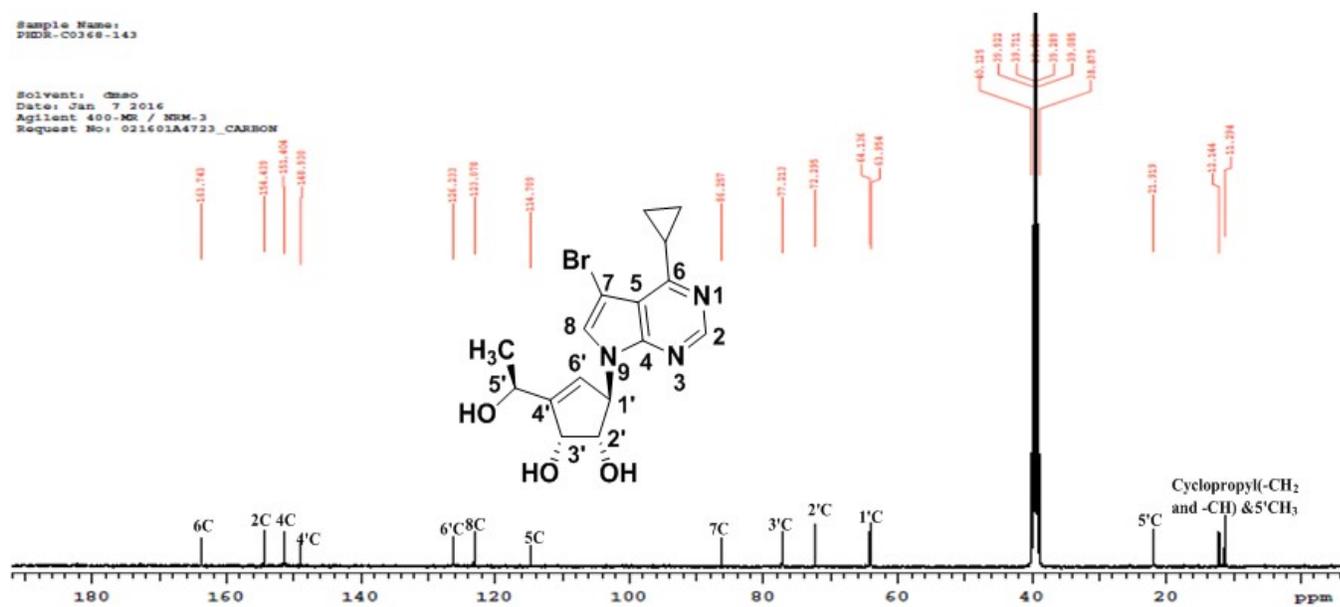


Figure S50: $^{13}\text{C-NMR}$ (100 MHz, $\text{DMSO-}d_6$) of the compound 10a

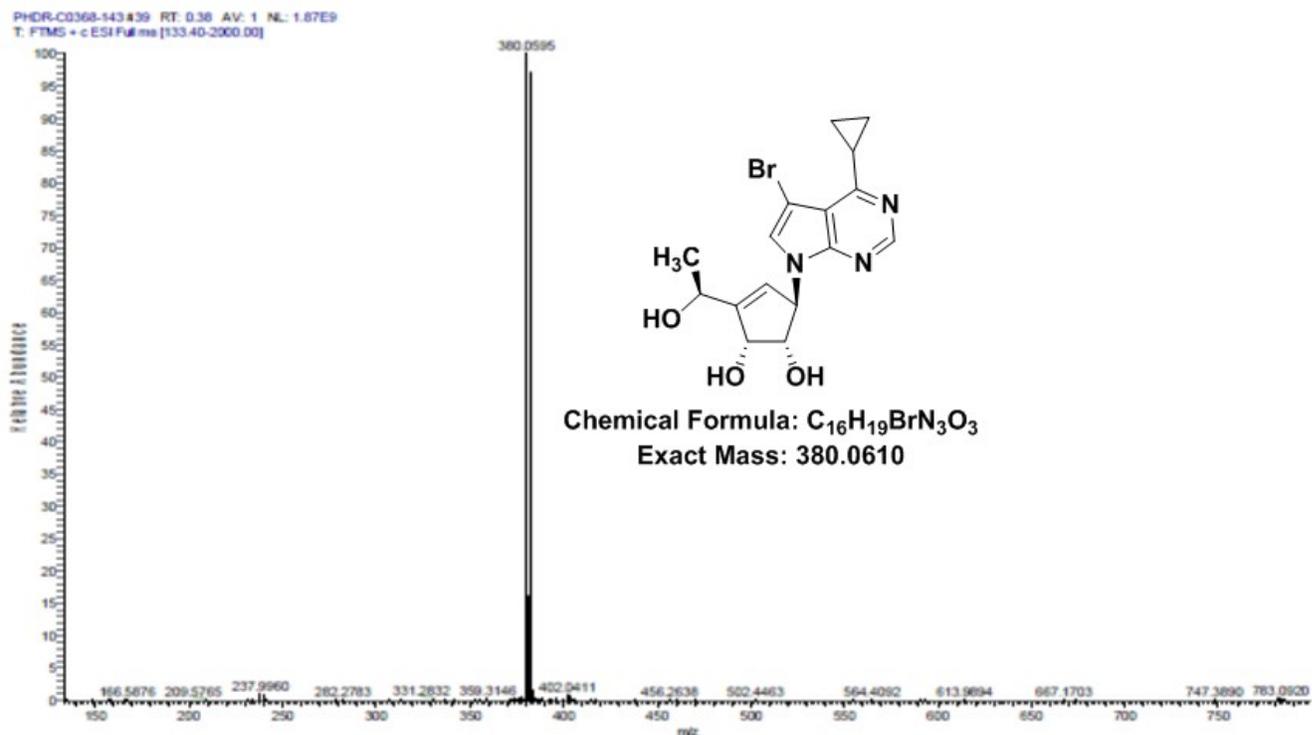


Figure S51: HRMS data of the compound 10a

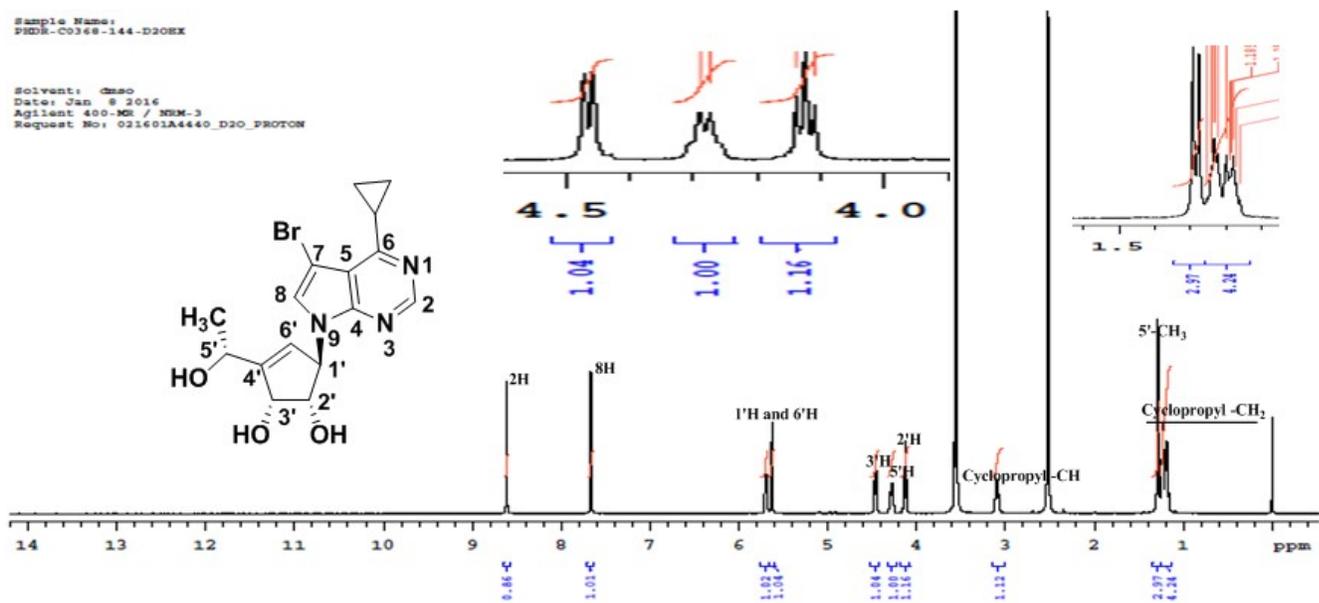


Figure S52: $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$) of the compound 10b

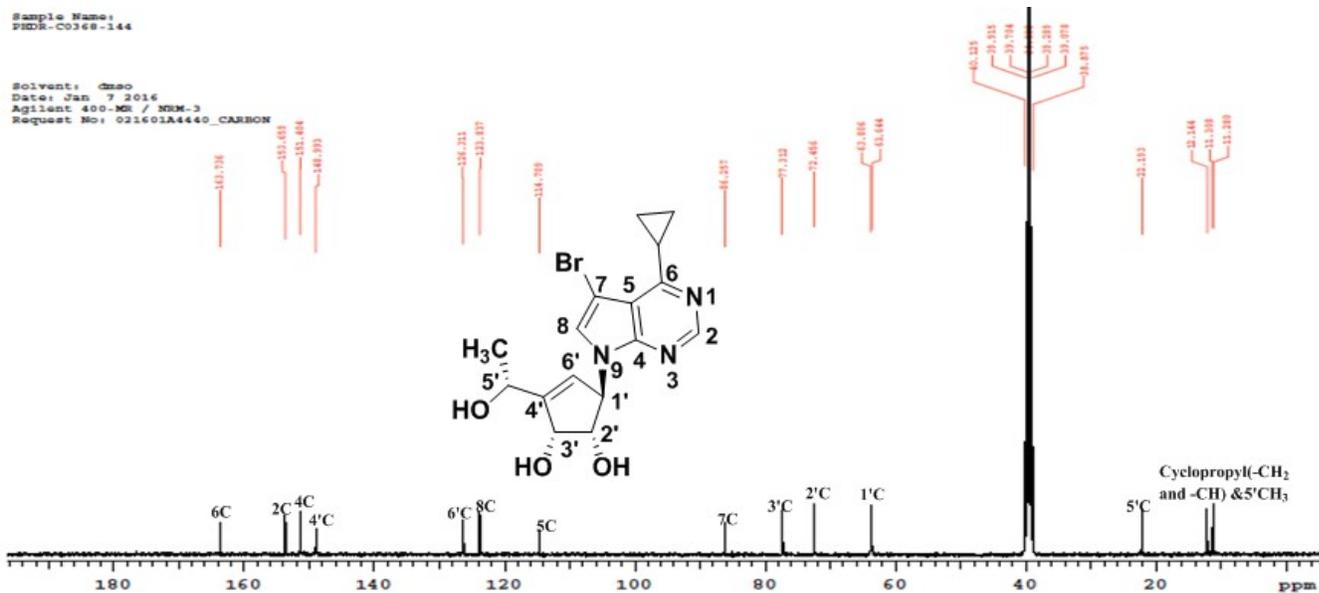


Figure S53: ^{13}C -NMR (100 MHz, $\text{DMSO-}d_6$) of the compound 10b

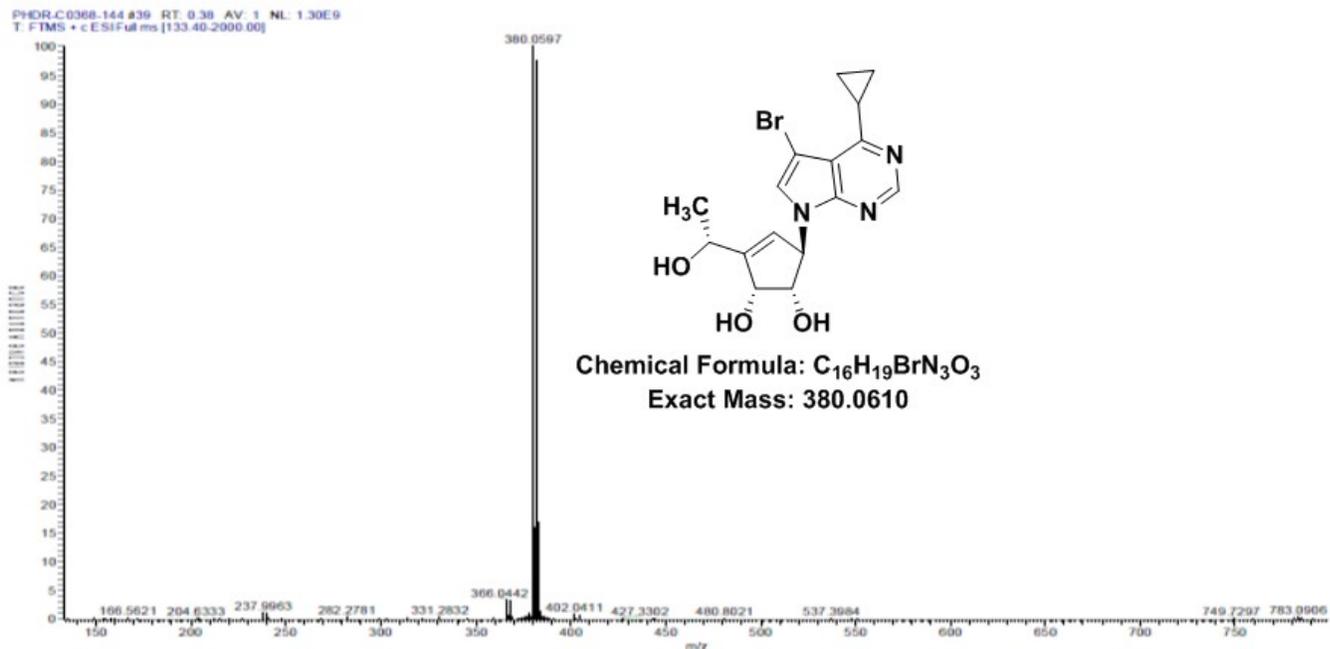
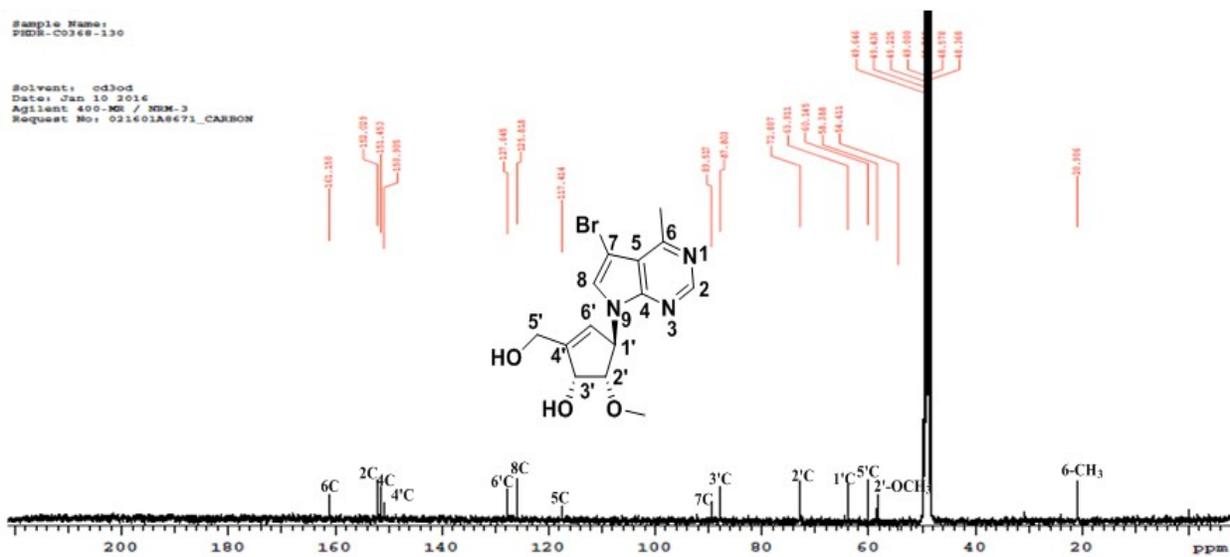
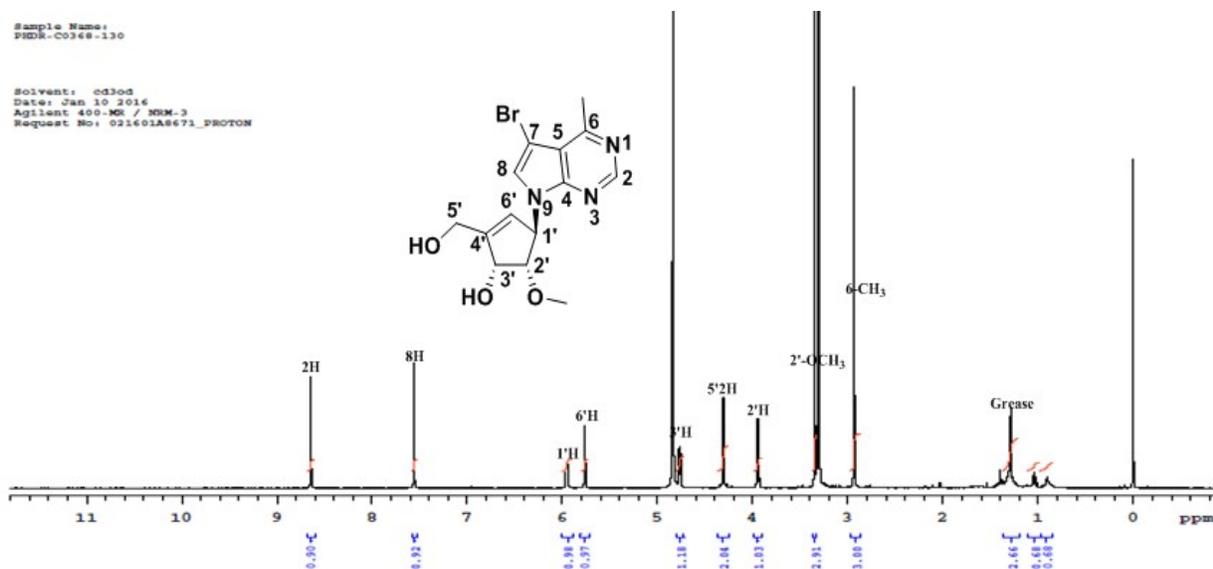


Figure S54: HRMS data of the compound 10b



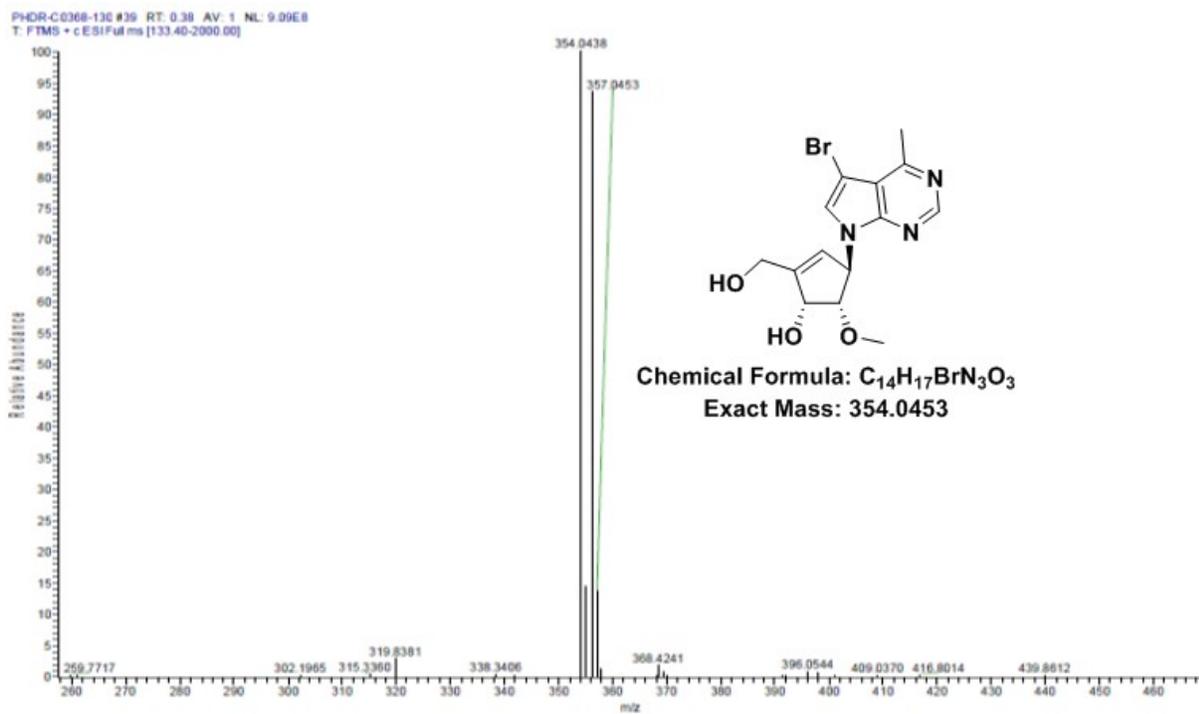


Figure S57: HRMS data of the compound 13