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Muhammed Fatih POLAT
fpolat@erzincan.edu.tr

İrem Durmaz ŞAHİN
irsahin@ku.edu.tr

Rengül ATALAY
rengul@metu.edu.tr

Meral TUNÇBİLEK
tuncbile@pharmacy.ankara.edu.tr

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Exploration of novel 6,8,9-trisubstituted purine analogues: synthesis, in vitro biological evaluation, and their effect on human cancer cells

Muhammed Fatih POLAT[1,†], İrem DURMAZ ŞAHİN[2,3,4,†], Rengül ATALAY[4,5], Meral TUNÇBİLEK[*,6]

[1]Department of Pharmaceutical Basic Sciences, Faculty of Pharmacy, Erzincan Binali Yıldırım University, Erzincan, Turkiye
[2]Research Center for Translational Medicine (KUTTAM), Koç University, İstanbul, Turkiye
[3]Department of Medical Biology, School of Medicine, Koç University, İstanbul, Turkiye
[4]Cancer Systems Biology Laboratory, Graduate School of Informatics, ODTU, Ankara, Turkiye
[5]Section of Pulmonary and Critical Care Medicine, University of Chicago, Chicago, USA
[6]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ankara University, Ankara, Turkiye

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Abstract: Cancer, a leading global cause of mortality, demands continuous advancements in therapeutic strategies. This study focuses on the design and synthesis of a novel series of purine derivatives, specifically 6-(substituted phenyl piperazine)-8-(4-phenoxyphenyl)-9-cyclopentyl purine derivatives (5–11). The motivation behind this endeavor lies in addressing acquired resistance mechanisms in cancer cells, a significant hurdle in current treatment modalities. The synthesis, starting from 4,6-dichloro-5-nitropyrimidine, involves a multi-step process, resulting in seven new purine derivatives.

Biological evaluation against human liver, colon, and breast cancer cells (Huh7, HCT116, and MCF7, respectively) was performed using the SRB assay. Among the synthesized analogs, compounds 5 and 6, exhibited notable cytotoxic activity, surpassing clinically used positive controls 5-Fluorouracil and Fludarabine in terms of efficacy. This research underscores the potential of purine derivatives with a phenyl group at the C-8 position as a scaffold for developing compounds with improved anticancer properties. The findings offer insights for future exploration and development of novel agents in cancer pharmaceutical research.

Key words: 6,8,9-Trisubstituted purine analogs, synthesis, cytotoxic activity, human epithelial cancer cells

1. Introduction

Cancer, a pervasive cause of death on a global scale, accounted for approximately 10 million fatalities in the year 2020, as reported by GLOBOCAN statistics [1]. Existing treatment options encompass chemotherapy, hormone therapy, immunotherapy, and γ-radiation. However, the emergence of acquired resistance mechanisms in cancer cells poses a significant obstacle to effective disease management, compounded by the adverse effects of the drugs employed. Consequently, the exploration of novel compounds with anticancer properties becomes imperative for advancing cancer pharmaceutical research.

Analogues of purine nucleosides, and purine/pyrimidine nucleobase have found utility in the therapeutics of cancer. Purine analogues play vital roles in many cellular bioprocesses including but not limited to cell growth, proliferation, and division [2]. Consequently, these molecules assume substantial importance in cancer therapy. These analogues function as antimetabolites due to targeting nucleobases and nucleosides, which serve as precursors in the cellular metabolism of nucleic acids. Multiple nucleoside analogues have been employed in the area of cancer therapeutics. These derivatives hinder the activity of ribonucleotide reductase, impeding cellular synthesis of DNA and apoptosis or senescence induction in cancer cells [3–5]. For instance, Pentostatin, Cladribine, and Fludarabine were approved for clinical use in hematological malignancies (Figure 1) [6–8]. Fludarabine, a purine derivative employed in chronic lymphocytic leukaemia (CLL) treatment, exhibits favorable effects on melanoma, breast, and colon carcinoma [9–11]. Investigating different fludarabine complexes, platinum variants displayed enhanced cytotoxicity, particularly against cisplatin-resistant cells, B-cell lymphoma, and CLL, attributed to their ten-fold higher cellular uptake. Notably, platinum complexes exhibited greater

† These authors contributed equally to this work.

* Correspondence: tuncbile@pharmacy.ankara.edu.tr

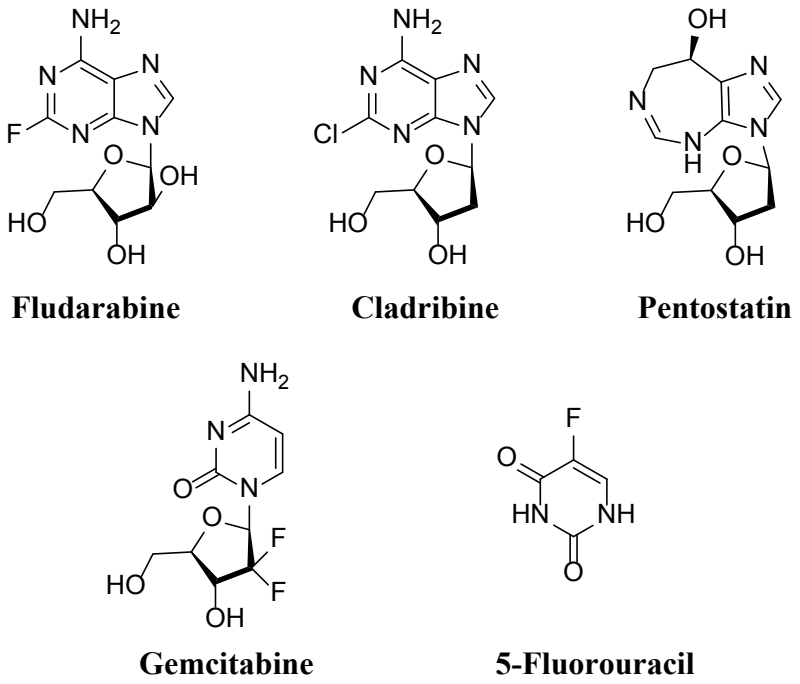


Figure 1. Structures of Fludarabine, Cladribine, Pentostatin, Gemcitabine, and 5-Fluorouracil.

selectivity for cancer cells over nonmalignant ones, contrasting with the interaction pattern of palladium complexes with isolated Calf thymus DNA [12]. Moreover, In preclinical studies, cladribine, a purine analogue, has demonstrated the ability to enhance the intracellular uptake of cytarabine in leukemic blasts, suggesting a potential synergistic effect between cladribine and cytarabine [13]. The utilization of cladribine as adjuvant therapy has been a focus of extensive research by the Polish Adult Leukemia Group, conducting several of the most significant studies on the subject [14,15].

Another notable example is Gemcitabine, which has demonstrated success in lung cancer treatment (Figure 1). This drug enters the cell through nucleoside transporters, gets activated by deoxycytidine kinase, and subsequently incorporates itself into cellular genetic material. Gemcitabine exhibits efficacy against various other cancer types, including ovarian cancer (when used alongside triapine or hydroxyurea) [16]. Furthermore, the use of gemcitabine in conjunction with lobaplatin interventional therapy was shown to have the potential to enhance the cure rate of locally advanced cervical cancer (LACC) by decreasing the levels of VEGF and MMP-9 in patients' serum [17]. Additionally, 5-Fluorouracil (5-FU) serves as another widely employed therapeutic agent for cancer. Although significant advancements have been made in enhancing its anticancer activity over the years, drug resistance remains a significant impediment associated with this molecule (Figure 1) [18].

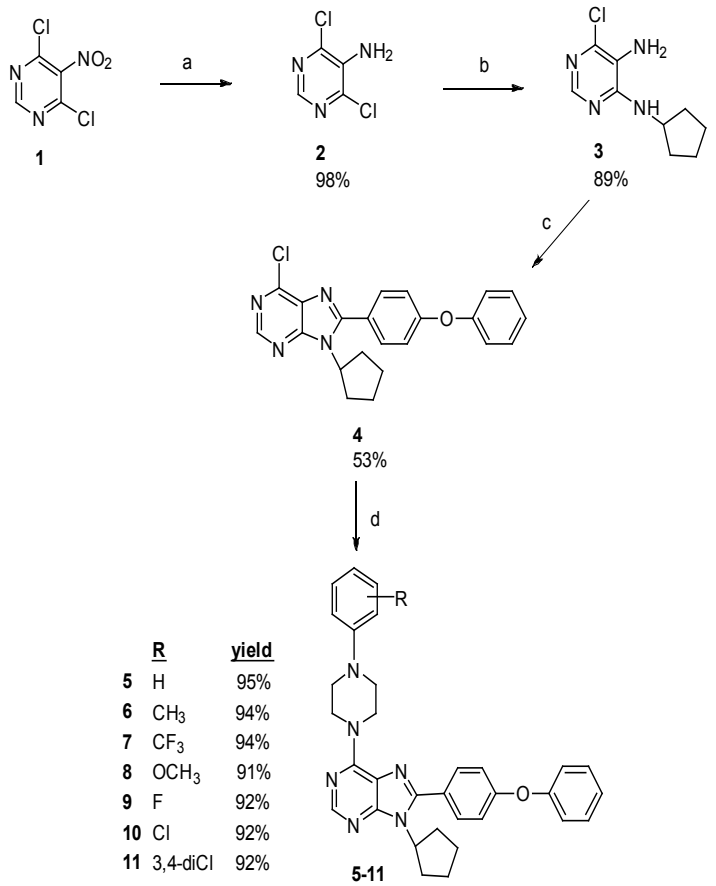
In this study, we aimed the design of newly synthesized compounds containing a phenyl at the C-8 of the purine structure. These novel derivatives were subsequently generated to produce a fresh series of 6-(substituted phenyl piperazine)-8-(4-phenoxyphenyl)-9-cyclopentyl purine derivatives (5–11), and their biological activities were evaluated against different human liver, colon, and breast cancer cells.

2. Results and discussion

2.1. Chemistry

4,6-Dichloro-5-nitropyrimidine (1) was used as the starting compound for the synthesis of 6-(substituted phenyl piperazine)-8-(4-substituted phenyl)-9-cyclopentylpurine derivatives (5–11) (Scheme). 4,6-dichloropyrimidine-5-amine (2) was obtained by reduction of 4,6-dichloro-5-nitropyrimidine (1) in the presence of tin (II) chloride ($SnCl_2$) in anhydrous medium (in step "a").

Compound 3 is intended to be obtained by the reaction of 2 and cyclopentylamine in the presence of NEt_3 via nucleophilic aromatic substitution reaction (in step "b"). By heating pyrimidine derivatives and cyclopentyl amine and 2 in ethanol at 125 °C in a sealed tube, the targeted compounds were reached as a result of nucleophilic aromatic substitution.



	R	yield
5	H	95%
6	CH_3	94%
7	CF_3	94%
8	OCH_3	91%
9	F	92%
10	Cl	92%
11	3,4-diCl	92%

Scheme. Synthesis of compounds 5–11. Reagents: (a) $SnCl_2.2H_2O$, EtOH, 2; (b) cyclopentyl amine, EtOH, 3; (c) 4-substituted benzaldehydes, p-TsOH, DMF. (d) substituted piperazines, Et_3N, EtOH.

In the next step "c", thanks to the free amine group in the structure of compound (3), when treated with 4-phenoxybenzaldehyde under p-TSA catalysis, a cyclization was obtained as a result of a second addition to the corresponding purine ring (4) on the corresponding imine structures. As a result of the reaction of 3 with 4-phenoxybenzaldehyde in the presence of p-TSA in DMF at 80 °C, compound 4 was obtained.

In the last step "d", 6,8,9-trisubstituted purine analogs (5–11) were easily prepared in high yield (91%–95%). The target compounds (5–11) were obtained by nucleophilic aromatic substitution with substituted piperazines (Scheme).

As a result, seven new purine derivatives were synthesized, and their purity checks were controlled by TLC and melting point determination. Their chemical structures were characterized by using spectroscopic techniques such as ^{1}H, ^{13}C NMR, mass, and elemental analyses.

2.2. Biological evaluation

The newly generated purine analogs (5–11) were subjected to in vitro cytotoxicity analysis using the SRB assay, performed in triplicate, against liver, breast, and colon (Huh7, MCF7, and HCT116 respectively) cancer cell lines. The purine compounds were tested at five different concentrations, ranging from 40μM to 2.5μM, over a 72-h period. Positive controls such as Fludarabine, Cladribine, and 5-Fluorouracil (5-FU) were included (Table), (Figure 2).

Among the analogs that contain substituted piperazine group at the C-6 position (5–11), specifically, the 4-methylphenyl substituted piperazine analog (6) displayed lower IC_{50} values compared to clinical positive controls 5-FU and Fludarabine, both in the micromolar concentration range. Compound 6 demonstrated superior cytotoxic activity against Huh7 cells (14.2 μM) compared to 5-FU (30.6 μM) and Fludarabine (28.4 μM). Additionally, the nonsubstituted and methoxyphenyl analogs, 5 (17.9 μM) and 8 (23.6 μM) exhibited higher cytotoxic activities than 5-FU (30.6 μM) and the clinically used nucleoside drug Fludarabine (28.4 μM) on Huh7 cells.

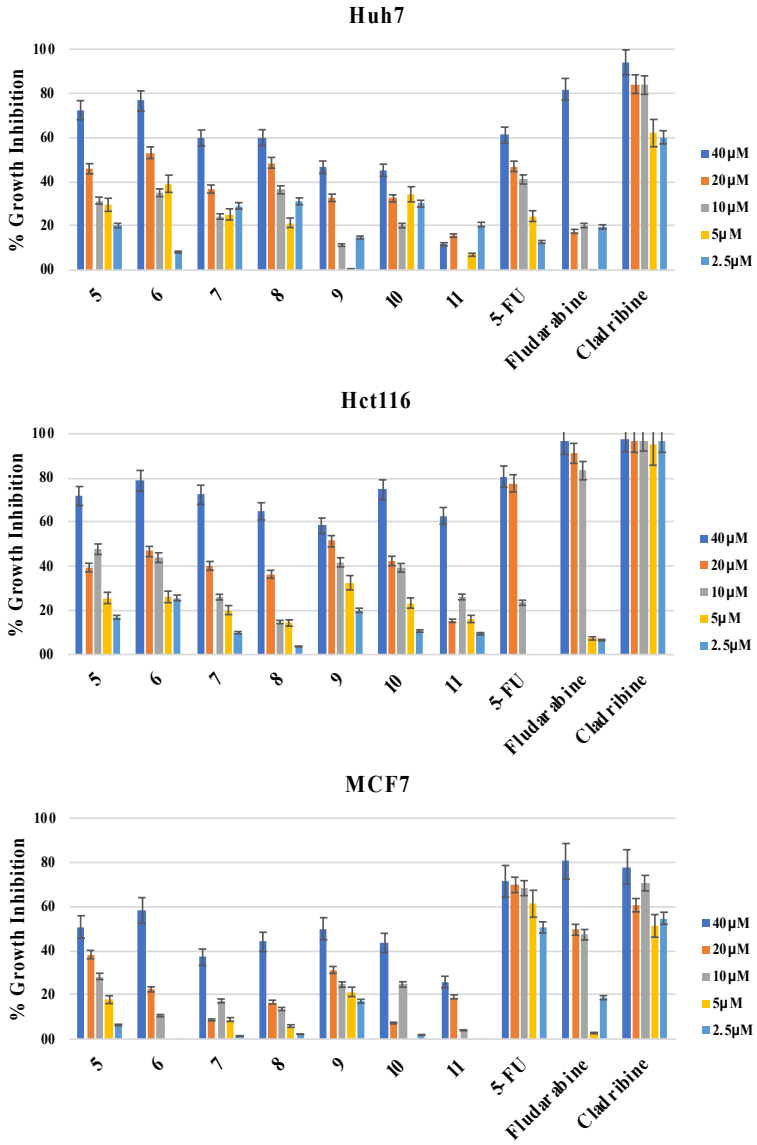
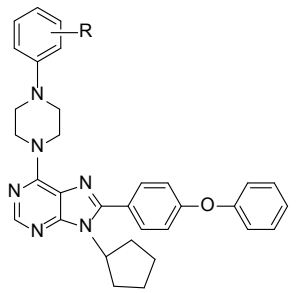


Figure 2. % Growth Inhibition of compounds 5–11 on cancer cells. Cell lines were treated with the compounds for 72 h in triplicate with increasing concentrations (40µM–2.5µM). NCI-SRB analysis was executed to explore the effect of the compounds on cellular growth.

3. Conclusion

This research involved creating new purine compounds that had a phenyl group attached to the C-8 position of the purine ring. These compounds were synthesized as a novel set of derivatives called 6-(substituted phenyl piperazine)-8-(4-phenoxyphenyl)-9-cyclopentyl purine derivatives (5–11). The researchers then tested the biotoxic effects of these compounds against various types of cancer cells (including liver, colon, and breast cancer) and the obtained cytotoxicity values were compared to the activity of 5-Fluorouracil (5-FU), Fludarabine, and Cladribine, three clinically used anticancer agents that are frequently used as positive controls in cytotoxicity assays. The results revealed that 5 and 6 exhibited auspicious cytotoxic activity on liver cancer cells with lower IC_{50} values than clinical reference drugs 5-FU and Fludarabine. These results indicated that by using these molecules as scaffolds, new compounds can be synthesized with improved performance for future perspectives.

Table. The cytotoxicity of compounds 5–11 was evaluated in vitro.



		IC_{50} (μM)		
Compound	**R**	**HUH7**	**HCT116**	**MCF7**
5 (ME69)	H	17.9 ± 0.9	17.2 ± 1.7	39.6 ± 4.8
6 (ME70)	CH_3	14.2 ± 1.4	13.7 ± 2.7	41.7 ± 3.8
7 (ME71)	CF_3	41.5 ± 8.3	21.8 ± 1.7	NI
8 (ME72)	OCH_3	23.6 ± 7.1	30.4 ± 4	NI
9 (ME73)	F	80.1 ± 16.0	19.5 ± 1.0	69.2 ± 11.8
10 (ME74)	Cl	NI	17.6 ± 5.3	NI
11 (ME75)	3,4-diCl	NI	48.2 ± 9.6	NI
5-FU		30.6 ± 1.8	4.1 ± 0.3	3.5 ± 0.7
Fludarabine		28.4 ± 19.2	8.0 ± 3.4	15.2 ± 0.1
Cladribine		0.9 ± 0.7	<0.1	2.4 ± 2.4

SRB experiment and data were expressed as means of ± SD.
NI: No inhibition

4. Experimental

4.1. General

To determine the melting points, an Electrothermal 9100 capillary melting point apparatus was employed. NMR spectra were recorded using a VARIAN Mercury 400 FT-NMR spectrometer operating at frequencies of 400 MHz for ^{1}H and 100.6 MHz for ^{13}C. TMS served as the internal standard for both ^{1}H NMR and ^{13}C NMR spectra, with chemical shifts given in ppm and coupling constants in Hz. Mass spectra were obtained using the ESI+ method on a Waters Micro-mass ZQ instrument. Elemental analyses (C, H, N) were conducted on a Leco CHNS 932 instrument, and the determined values were within ±0.4% of the theoretical values. Silica gel 60 (particle size: 40–63mm) was employed for column chromatography. Chemical reagents obtained from reputable suppliers such as Merck, Fluka, Sigma, and Aldrich were utilized for the synthesis process.

4.2. Chemistry experimental procedures

5-Amino-4,6-dichloropyrimidine (2) was synthesized according to the reduction methodology reported in the literature [19].

To a solution of 5-Amino-4,6-dichloropyrimidine (2) (6.00 g, 193.98 mmol) in 100 mL ethanol, $SnCl_2.2H_2O$ (27.90 g, 123.70 mmol) was sequentially added and refluxed for 2 h. Upon completion of the reaction, as detected by TLC, the reaction mixture was concentrated under vacuum. The reaction mixture was quenched using an aqueous saturated solution of $NaHCO_3$ until pH = 8. The water phase was extracted with EtOAc. The organic layers were combined and dried over Na_2SO_4, followed by concentration under vacuum. As a result, compound 2 was found to be (4.97 g, 98% yield) as a yellow solid. mp = 149–151 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (s, 1H), 4,52 (bs, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.90, 144.19, 145.96.

4.2.1. 6-Chloro-N^4-cyclopentylpyrimidine-4,5-diamine (3)

To a solution of 2 (1.0 g, 6.09 mmol) in MeOH, cyclopentylamine was added (2.38 g, 28.08 mmol). Then the mixture was heated in a sealed tube at 125 °C for 6 h. Afterward, the mixture was concentrated under vacuum, and the crude product was subjected to purification using silica gel column chromatography (10:1 CH_2Cl_2: MeOH). As a result, compound 3 was found to be (1.15 g, 89% yield) as a light-yellow solid. mp = 140–142 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40–1.48 (m, 2H),

1,61–1,73 (m, 4H), 2.06–2.13 (m, 2H), 3.43 (bs, 2H) 4.34–4.39 (m, 1H), 4.93 (bs, 1H), 8.06 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.71, 33.30, 53.15, 121.56, 142.88, 149.81, 155.04. MS (ESI+) m/e: 213.1 (100%) $[M + H]^+$.

4.2.2. 6-Chloro-9-cyclopentyl-8-(4-phenoxyphenyl)-9H-purine (4)

To a solution of compound 3 (4.70 mmol) in 10 mL DMF, benzaldehyde derivatives (9,40 mmol) and p-TsOH (0.17 g, 0.94 mmol) were sequentially added and stirred overnight at 80 °C. Upon completion of the reaction, as detected by TLC, the reaction mixture was concentrated under vacuum. The reaction mixture was quenched using an aqueous saturated solution of NH_4Cl, and the water phase was extracted with DCM. The organic layers were combined and dried over Na_2SO_4, followed by concentration under vacuum. The crude products were subjected to purification using silica gel column chromatography (1:5 EtOAc:Hexane) to afford 4 (0.97 g, 53% yield). mp = 146–148 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.62–1.75 (m, 2H), 2.0–2.20 (m, 4H), 2.50–2.62 (m, 2H), 4.80–4.90 (m, 1H), 7.10 (d, 2H), 7.14 (d, 2H), 7.20 (t, 1H), 7.41 (t, 2H), 7.67 (d, 2H), 8.70 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.95, 30.98, 58.51 (C-cyclopentyl), 118.29, 119.91, 123.48, 124.44, 130.05, 131.31, 131.97, 150.02 (C-phenyl), 150.63, 152.90, 155.81, 156.34, 160.04 (C-purine). MS (ESI+) *m/e*: 391.3 (100%) $[M + H]^+$, 393.0 35(%) $[M + 2]^+$. Anal. calcd for $C_{22}H_{19}ClN_4O.0.2H_2O$; C, 66.98; H, 4.96; N, 14.20. Found: C, 66.83; H, 4.80; N, 14.29.

4.2.3. General procedure for preparation of compounds 5-11

To the solution of compound 4 (1 equiv.) in absolute EtOH (10 mL) 4-substituted piperazine derivatives were added (1 equiv.) and Et_3N (3 equiv.). The resulting solution was then refluxed at 80–90 °C. After 6 h reflux period, the reaction mixture was concentrated. The crude product was subjected to purification using silica gel column chromatography (1:10/1:5 EtOAc:Hexane) to afford 5–11.

4.2.3.1. 9-Cyclopentyl-8-(4-phenoxyphenyl)-6-(4-phenylpiperazin-1-yl)-9H-purine (5)

The above procedure was followed with 1-phenylpiperazine to yield 5 (0.113 g, 95% yield). mp = 173–175 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.59–1.68 (m, 2H), 1.93–2.16 (m, 4H), 2.55–2.67 (m, 2H), 3.32 (t, 4H), 4.49 (br s, 4H), 4.70–4.78 (m, 1H), 6.88 (t, 1H), 6.97 (d, 2H), 7.14 (t, 4H), 7.19 (t, 1H), 7.28 (t, 2H), 7.40 (t, 2H), 7.61 (d, 2H), 8.36 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.73, 30.64 (C-cyclopentyl), 45.09, 49.64 (CH_2-piperazine), 57.89 (C-cyclopentyl), 116.44, 118.27, 119.79, 120.12, 120.71, 124.20, 125.13, 129.18, 129.98, 131.10, 149.48, 151.21 (C-phenyl), 151.34, 152.20, 153.68, 156.11, 159.13 (C-purine). MS (ESI+) m/e: 517.2 (100%) $[M + H]^+$. Anal. calcd for $C_{32}H_{32}N_6O.0.35H_2O$; C, 73.49; H, 6.30; N, 16.07. Found: C, 73.76; H, 6.50; N, 15.91.

4.2.3.2. 9-Cyclopentyl-8-(4-phenoxyphenyl)-6-(4-(p-tolyl)piperazin-1-yl)-9H-purine (6)

The above procedure was followed with 1-(p-tolyl)piperazine to yield 6 (0.115 g, 94% yield). mp = 170–173 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.58–1.70 (m, 2H), 1.92–2.15 (m, 4H), 2.27 (s, 3H), 2.55–2.67 (m, 2H), 3.25 (t, 4H), 4.48 (br s, 4H), 4.69–4.78 (m, 1H), 6.89 (d, 2H), 7.08–7.14 (m, 6H), 7.18 (t, 1H), 7.40 (t, 2H), 7.60 (d, 2H), 8.36 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.43 (–CH_3), 24.73, 30.63 (C-cyclopentyl), 45.11, 50.23 (CH_2-piperazin), 57.88 (C-cyclopentyl), 116.82, 118.26, 119.78, 120.69, 124.18, 125.14, 129.70, 129.98, 131.10, 149.25, 149.43 (C-phenyl), 151.21, 152.19, 153.68, 156.11, 159.11 (C-purine). MS (ESI+) m/e: 531.2 (100%) $[M + H]^+$. Anal. calcd for $C_{33}H_{34}N_6O.0.3H_2O$; C, 73.93; H, 6.50; N, 15.67. Found: C, 74.22; H, 6.56; N, 15.62.

4.2.3.3. 9-Cyclopentyl-8-(4-phenoxyphenyl)-6-(4-(4-(trifluoromethyl)phenyl) piperazin-1-yl)-9H-purine (7)

The above procedure was followed with 1-(4-(trifluoromethyl)phenyl)piperazine to yield **7** (0.126 g, 94% yield). mp = 172–174 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.59–1.70 (m, 2H), 1.92–2.16 (m, 4H), 2.54–2.67 (m, 2H), 3.41 (t, 4H), 4.49 (br s, 4H), 4.70–4.78 (m, 1H), 6.96 (d, 2H), 7.12 (t, 4H), 7.19 (t, 1H), 7.40 (t, 2H), 7.50 (d, 2H), 7.60 (d, 2H), 8.37 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.74, 30.65 (C-cyclopentyl), 44.77, 48.29 (CH_2-piperazin), 57.91 (C-cyclopentyl), 114.77, 118.26, 119.81, 120.69, 123.32, 124.24, 125.03, 126.01, 126.45 (q) (–CF_3), 129.99, 131.07, 149.65, 151.19 (C- phenyl), 152.23, 153.28, 153.59, 156.07, 159.19 (C-purine). MS (ESI+) m/e: 585.2 (100%) $[M + H]^+$. Anal. calcd for $C_{33}H_{31}F_3N_6O.0.5H_2O$; C, 66.76; H, 5.43; N, 14.16. Found: C, 66.52; H, 5.20; N, 13.95.

4.2.3.4. 9-Cyclopentyl-6-(4-(4-methoxyphenyl)piperazin-1-yl)-8-(4-phenoxy phenyl)-9H-purine (8)

The above procedure was followed with 1-(4-methoxyphenyl)piperazine to yield 8 (0.115 g, 91% yield). mp = 160–162 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.58–1.70 (m, 2H), 1.92–2.15 (m, 4H), 2.55–2.66 (m, 2H), 3.19 (t, 4H), 3.77 (s, 3H), 4.48 (br s, 4H), 4.69–4.77 (m, 1H), 6.85 (d, 2H), 6.95 (d, 2H), 7.11 (t, 4H), 7.18 (t, 1H), 7.40 (t, 2H), 7.60 (d, 2H), 8.36 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.72, 30.62 (C-cyclopentyl), 45.19, 51.16 (CH_2-piperazin), 55.54 (–OCH_3), 57.88 (C-cyclopentyl), 114.48, 118.26, 118.65, 119.78, 120.69, 124.19, 125.14, 129.98, 131.10, 145.71, 149.42, 151.21 (C-phenyl), 152.19, 153.69, 154.12, 156.11, 159.11 (C-purine). MS (ESI+) m/e: 547.3 (100%) $[M + H]^+$. Anal. calcd for $C_{33}H_{34}N_6O_2.0.1H_2O$; C, 72.26; H, 6.29; N, 15.32. Found: C, 71.96; H, 6.39; N, 15.15.

4.2.3.5. 9-Cyclopentyl-6-(4-(4-fluorophenyl)piperazin-1-yl)-8-(4-phenoxy phenyl)-9H-purine (9)
The above procedure was followed with 1-(4-fluorophenyl)piperazine to yield 9 (0.113 g, 92% yield). mp = 145–147 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.60–1.70 (m, 2H), 1.92–2.16 (m, 4H), 2.54–2.66 (m, 2H), 3.22 (t, 4H), 4.48 (br s, 4H), 4.69–4.78 (m, 1H), 6.90–7.0 (m, 4H), 7.11 (t, 4H), 7.18 (t, 1H), 7.40 (t, 2H), 7.60 (d, 2H), 8.36 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.73, 30.63 (C-cyclopentyl l), 45.09, 50.67 (CH_2-piperazin), 57.89 (C-cyclopentyl), 115.61 (d), 118.26, 119.80, 120.70, 124.21, 125.09, 129.98, 131.08, 148.02 (d), 149.51, 151.19, 152.21 (C-phenyl), 153.66, 156.08, 156.22, 158.60, 159.15 (C-purine). MS (ESI+) m/e: 535.2 (100%) $[M + H]^+$. Anal. calcd for $C_{32}H_{31}FN_6O$; C, 71.89; H, 5.84; N, 15.72. Found: C, 71.70 H, 6.00; N, 15.35.

4.2.3.6. 6-(4-(4-Chlorophenyl)piperazin-1-yl)-9-cyclopentyl-8-(4-phenoxy phenyl)-9H-purine (10)
The above procedure was followed with 1-(4-chlorophenyl)piperazine to yield 10 (0.117 g, 92% yield). mp = 168–169 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.57–1.70 (m, 2H), 1.92–2.15 (m, 4H), 2.54–2.67 (m, 2H), 3.27 (t, 4H), 4.48 (br s, 4H), 4.69–4.78 (m, 1H), 6.88 (d, 2H), 7.09–7.23 (m, 7H), 7.40 (t, 2H), 7.60 (d, 2H), 8.36 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.73, 30.64 (C-cyclopentyl), 44.93, 49.61 (CH_2-piperazin), 57.90 (C-cyclopentyl), 117.60, 118.26, 119.80, 124.21, 124.97, 125.07, 129.03, 129.98, 131.08, 149.56, 149.95 (C-phenyl), 151.19, 152.21, 153.62, 156.08, 159.15 (C-purine). MS (ESI+) m/e: 551.2 (100%) $[M]^+$, 553.3 (36%) $[M + 2]^+$. Anal. calcd for $C_{32}H_{31}ClN_6O.0.05H_2O$; C, 69.63; H, 5.68; N, 15.22. Found: C, 69.91; H, 5.88; N, 14.89.

4.2.3.7. 9-Cyclopentyl-6-(4-(3,4-dichlorophenyl)piperazin-1-yl)-8-(4-phenoxy phenyl)-9H-purine (11)
The above procedure was followed with 1-(3,4-dichlorophenyl)piperazine to yield 11 (0.124 g, 92% yield). mp = 178–179 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.58–1.70 (m, 2H), 1.92–2.16 (m, 4H), 2.54–2.65 (m, 2H), 3.28 (t, 4H), 4.47 (br s, 4H), 4.70–4.78 (m, 1H), 6.78 (dd, 1H), 6.99 (d, 1H), 7.09–7.30 (m, 6H), 7.40 (t, 2H), 7.60 (d, 2H), 8.36 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.74, 30.65 (C-cyclopentyl), 44.76, 49.06 (CH_2-piperazin), 57.91 (C-cyclopentyl), 115.63, 117.56, 118.26, 119.80, 120.72, 122.59, 124.23, 125.01, 129.99, 130.50, 131.07, 132.86, 149.60, 150.64 (C-phenyl), 151.18, 152.0, 153.46, 156.04, 159.18 (C-purine).MS (ESI+) *m/e*: 585.3 (100%) $[M]^+$, 587.2 (65%) $[M + 2]^+$, 589.3 (12%) $[M + 4]^+$. Anal. calcd for $C_{32}H_{30}Cl_2N_6O$; C, 65.64; H, 5.16; N, 14.35. Found: C, 65.60; H, 4.96; N, 14.14.
All NMR and Mass spectra are provided in the Supplementary Information.

4.3. Cytotoxicity

4.3.1. Cell culture
The human cancer cell lines were cultured in standard Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin.

4.3.2. NCI-60 Sulphorhodamine B (SRB) assay [20]
Cancer cells, (2000–5000 cells per well) were seeded into 96-well plates. After 24 h of incubation, the cells were exposed to escalating concentrations of the compounds (2.5μM to 40μM) for a duration of 72 h. Then the cells were fixed using 10% ice-cold trichloroacetic acid (TCA) in the dark at + 4 °C for one h. The plates were stained with a solution of 0.4% sulphorhodamine B (SRB) in a 1% acetic acid solution. The bound SRB stain was dissolved to measure the absorbance using a 10 mM Tris-Base solution, and the optical density (OD) values were recorded at a wavelength of 515 nm.

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Supplementary information: Spectral data

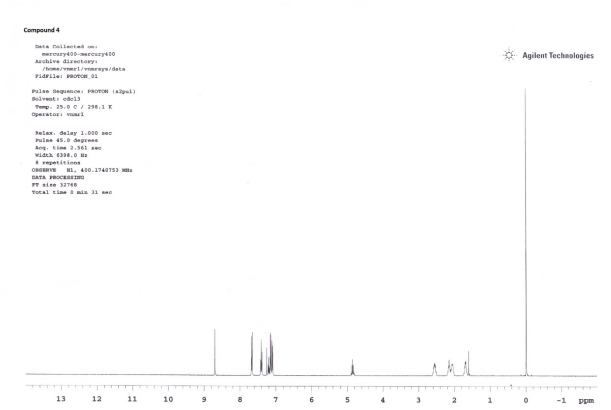

Figure S1. ^{1}H NMR spectrum of Compound 4.

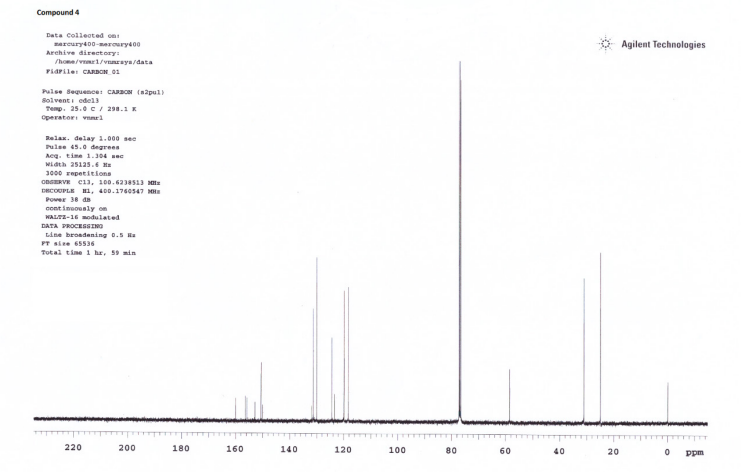

Figure S2. ^{13}C NMR spectrum of Compound 4.

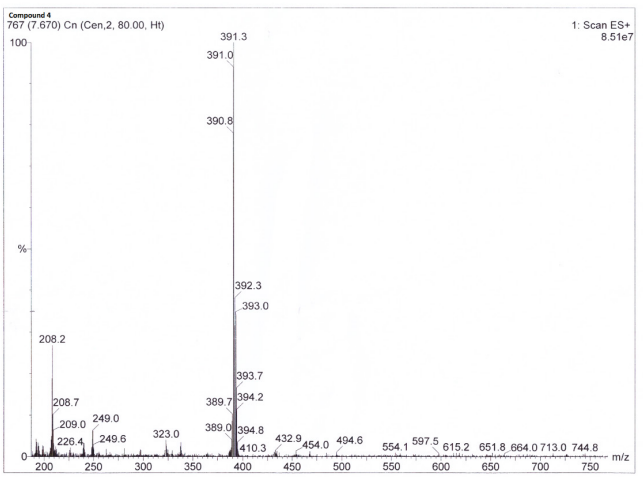

Figure S3. Mass spectrum of Compound 4.

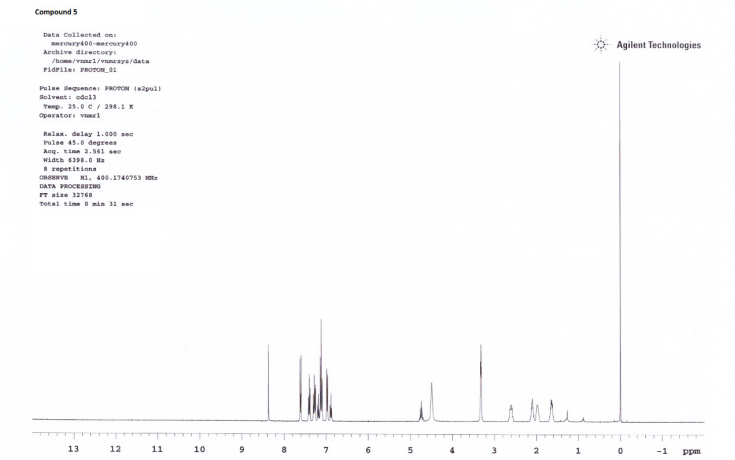

Figure S4. [1]H NMR spectrum of Compound 5.

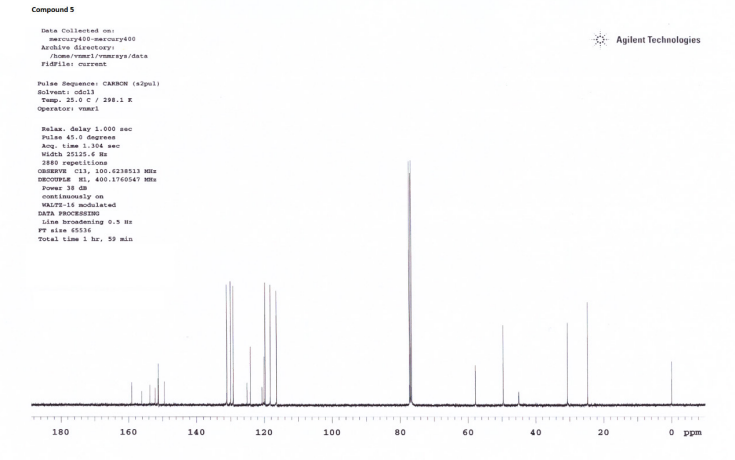

Figure S5. [13]C NMR spectrum of Compound 5.

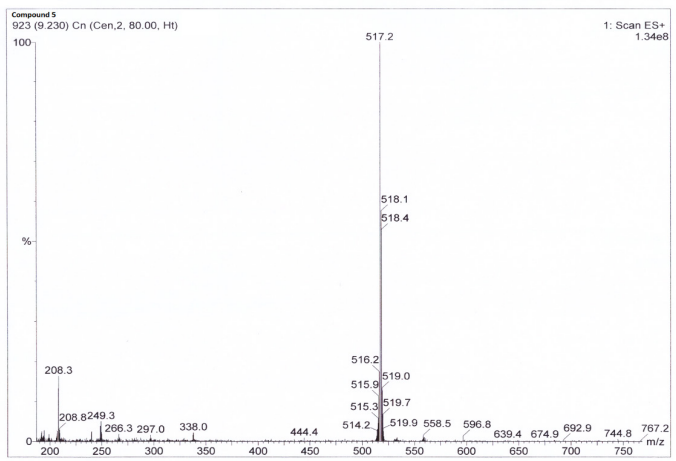

Figure S6. Mass spectrum of Compound 5.

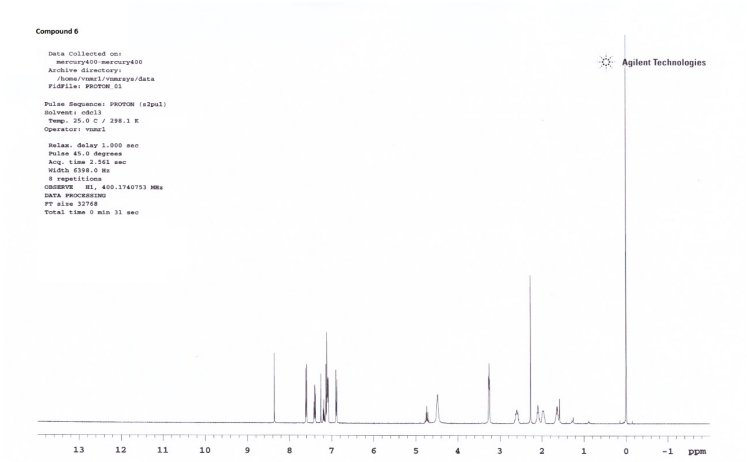

Figure S7. ^{1}H NMR spectrum of Compound 6.

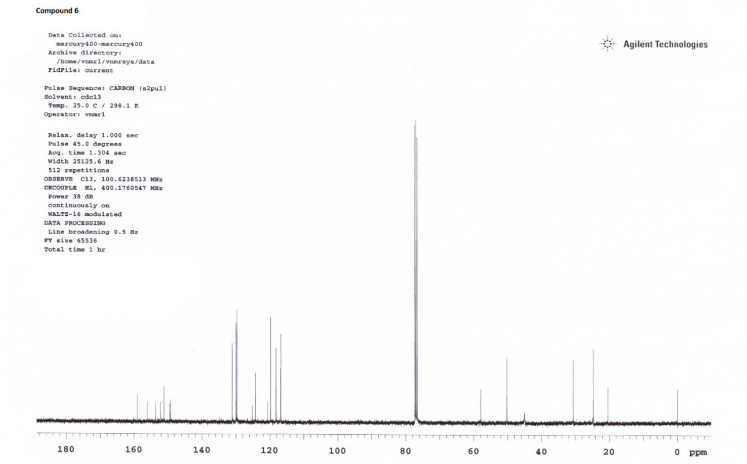

Figure S8. ^{13}C NMR spectrum of Compound 6.

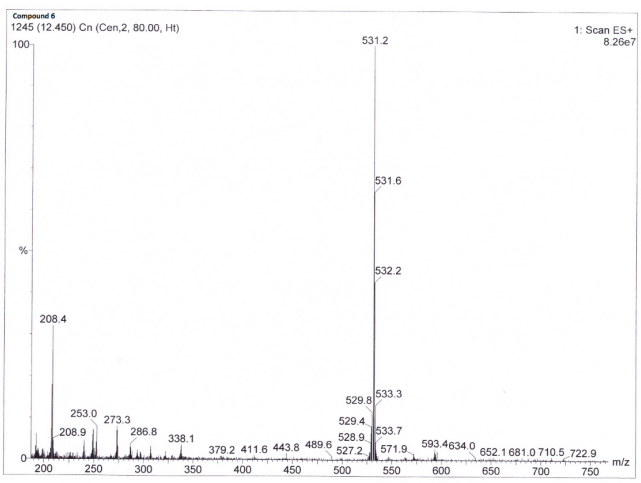

Figure S9. Mass spectrum of Compound 6.

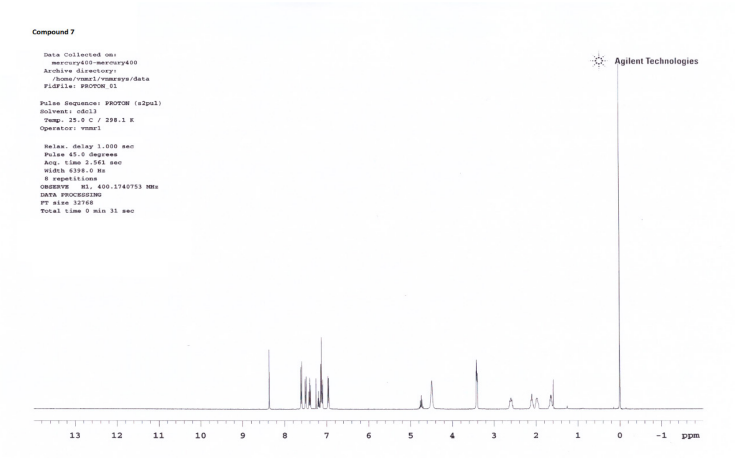

Figure S10. ^{1}H NMR spectrum of Compound 7.

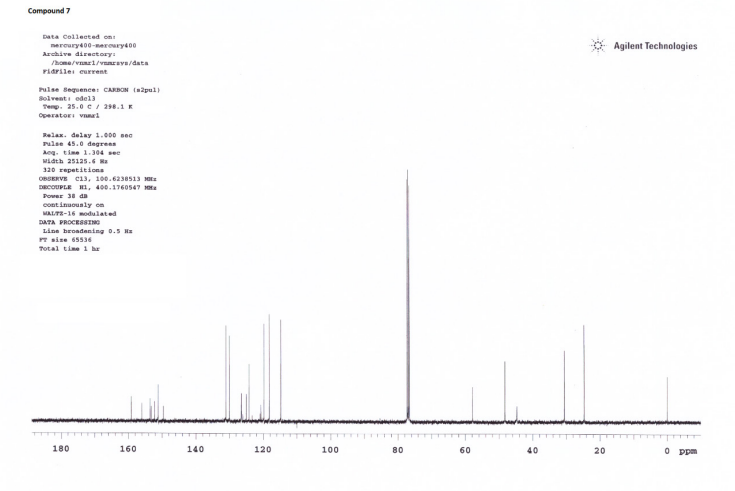

Figure S11. ^{13}C NMR spectrum of Compound 7.

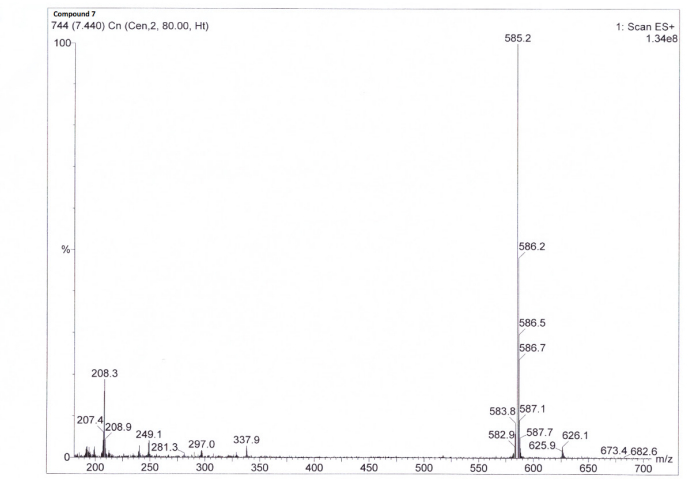

Figure S12. Mass spectrum of Compound 7.

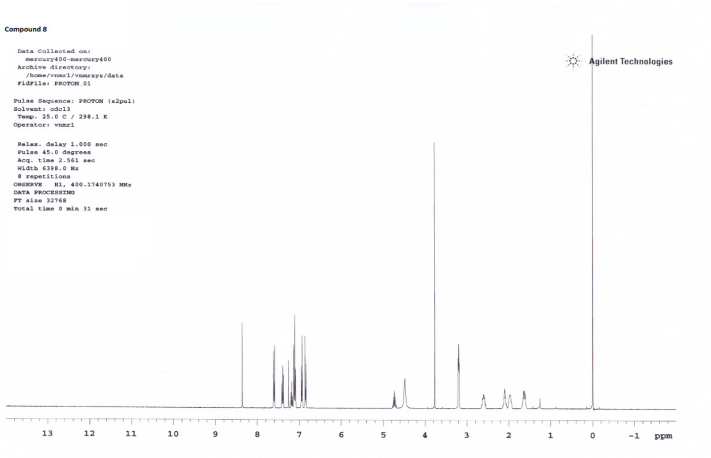

Figure S13. ^{1}H NMR spectrum of Compound 8.

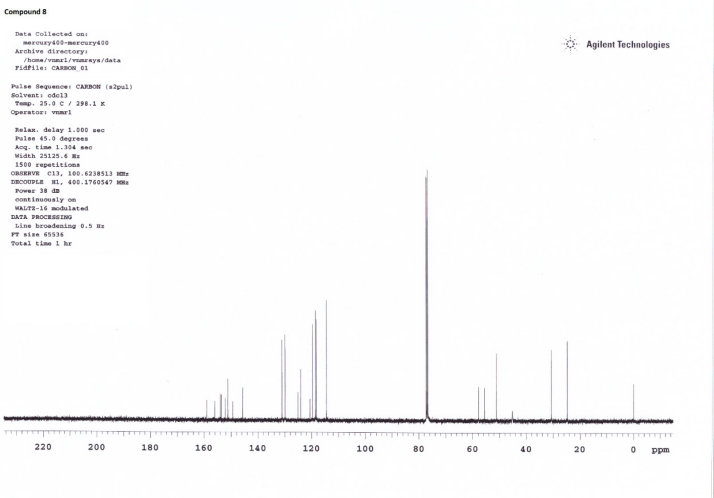

Figure S14. ^{13}C NMR spectrum of Compound 8.

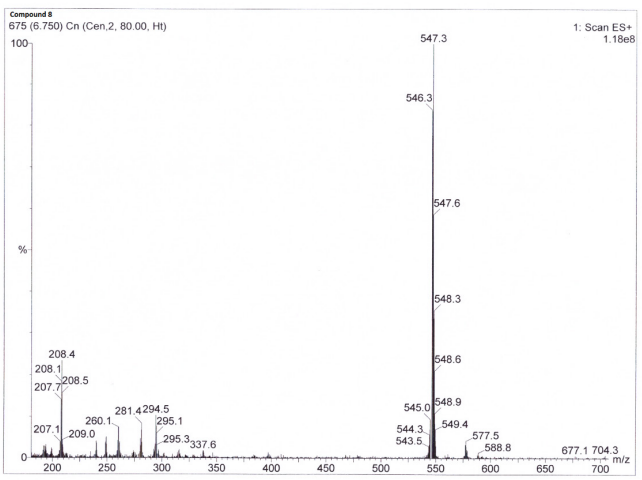

Figure S15. Mass spectrum of Compound 8.

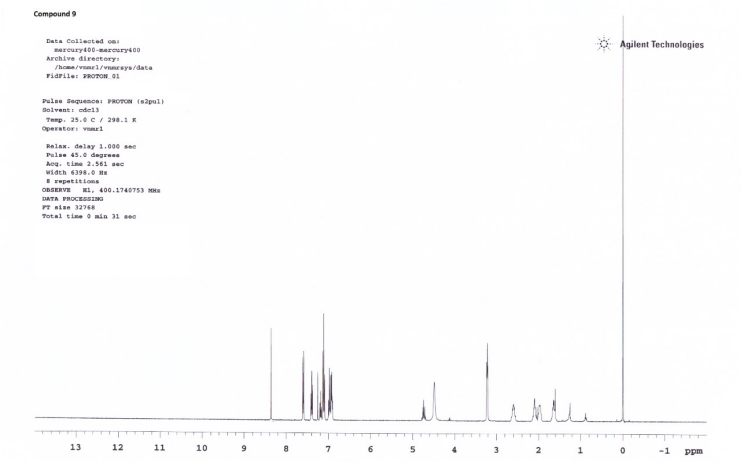

Figure S16. ^{1}H NMR spectrum of Compound 9.

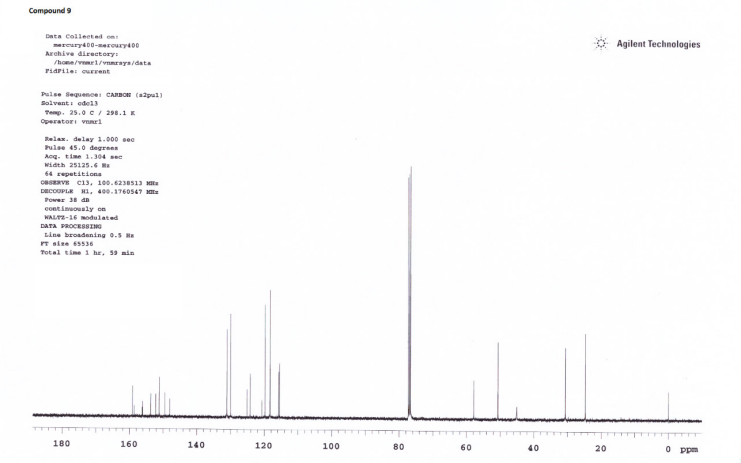

Figure S17. ^{13}C NMR spectrum of Compound 9.

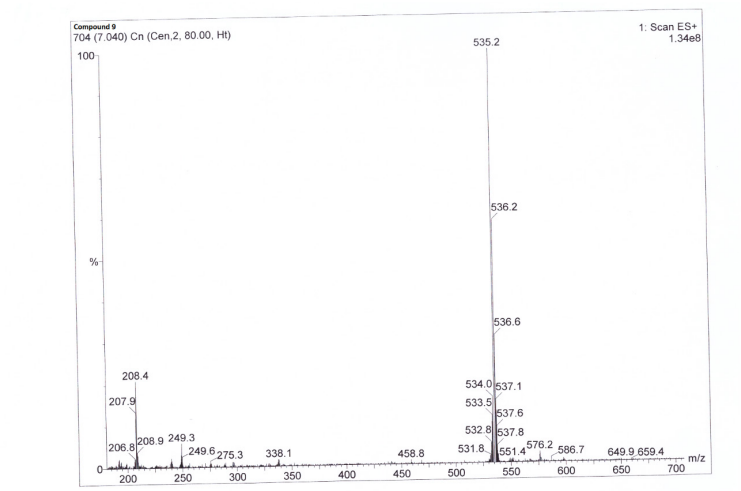

Figure S18. Mass spectrum of Compound 9.

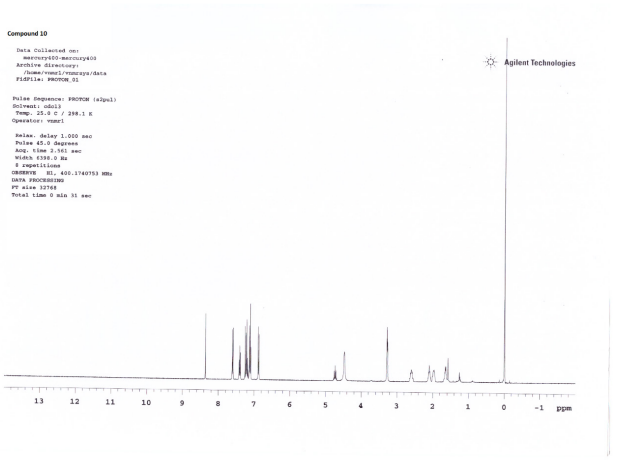

Figure S19. ^{1}H NMR spectrum of Compound 10.

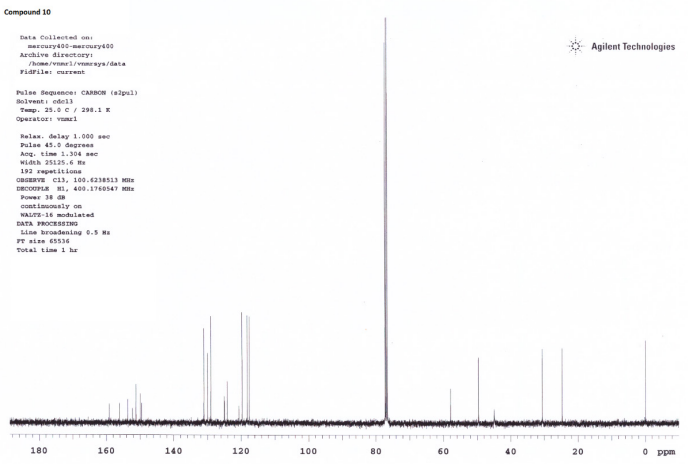

Figure S20. ^{13}C NMR spectrum of Compound 10.

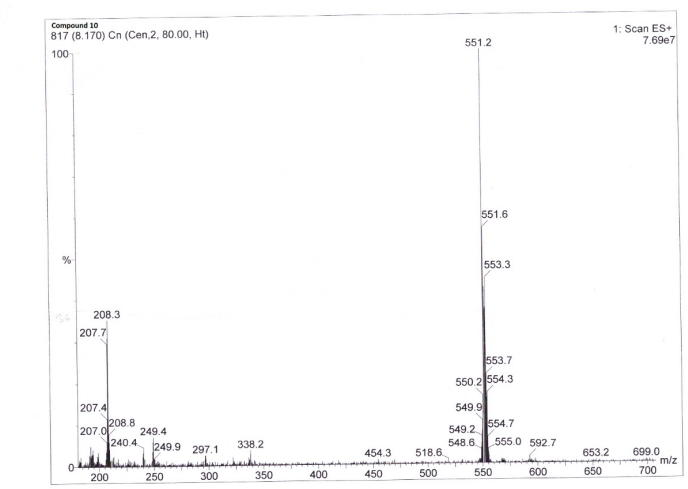

Figure S21. Mass spectrum of Compound 10.

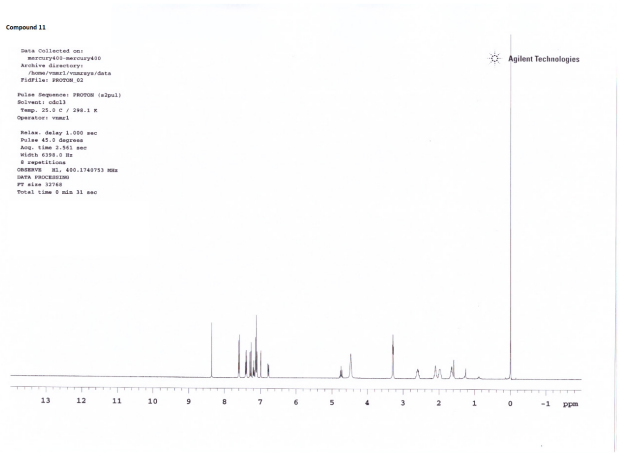

Figure S22. ^{1}H NMR spectrum of Compound 11.

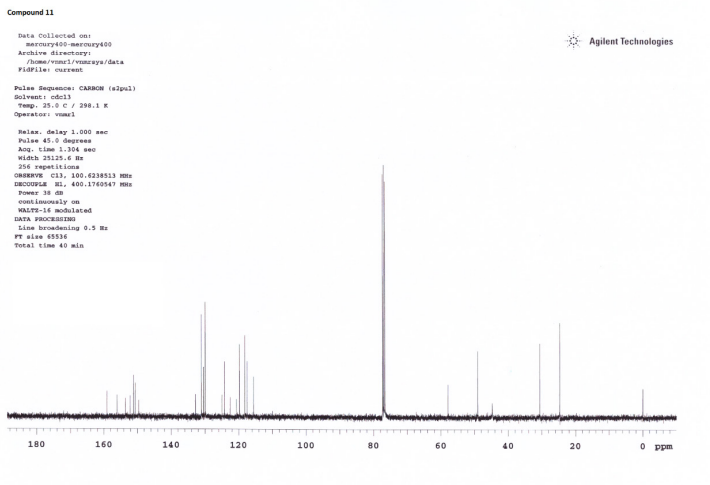

Figure S23. ^{13}C NMR spectrum of Compound 11.

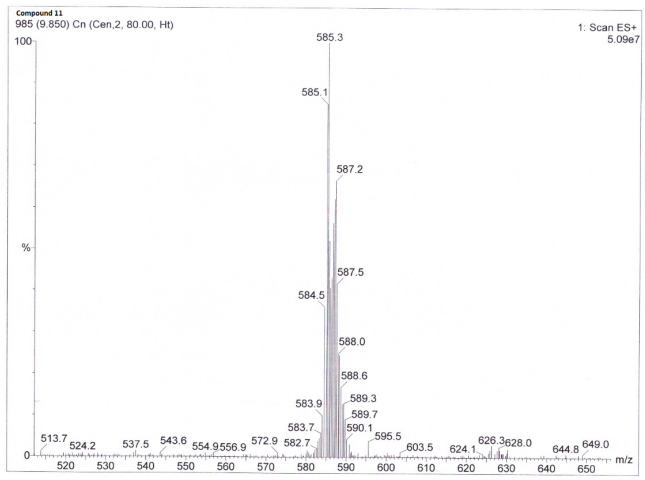

Figure S24. Mass spectrum of Compound 11.