**SUPPLEMENTARY MATERIALS**

# *In vitro* Cytotoxicity Evaluation of Thiourea derivatives bearing *Salix sp.* Constituent against HK-1 Cell Lines

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**Abstract**

In searching for drugs from natural product scaffolds has gained interest among researchers. In this study, a series of twelve halogenated thiourea (**ATX 1-12)** *via* chemical modification of aspirin (a natural product derivative) and evaluated for cytotoxic activity against nasopharyngeal carcinoma (NPC) cell lines, HK-1 via MTS-based colorimetric assay. The cytotoxicity studies demonstrated that halogens at *meta* position of **ATX** showed promising activity against HK-1 cells ($IC_{50}$ value $\leq$ 15 µM) in comparison to cisplatin, a positive cytotoxic drug ($IC_{50}$ value = 8.9±1.9 µM). **ATX 11**, bearing iodine at *meta* position, showed robust cytotoxicity against HK-1 cells with an $IC_{50}$ value of 4.7±0.7 µM. Molecular docking interactions between **ATX 11** and cyclooxygenase-2 demonstrated a robust binding affinity value of -8.1 kcal/mol as compared to aspirin's binding affinity value of -6.4 kcal/mol. The findings represent a promising lead molecule from natural product with excellent cytotoxic activity against NPC cell lines.

**Keywords**: aspirin; thiourea; cytotoxicity; molecular docking; cyclooxygenase-2

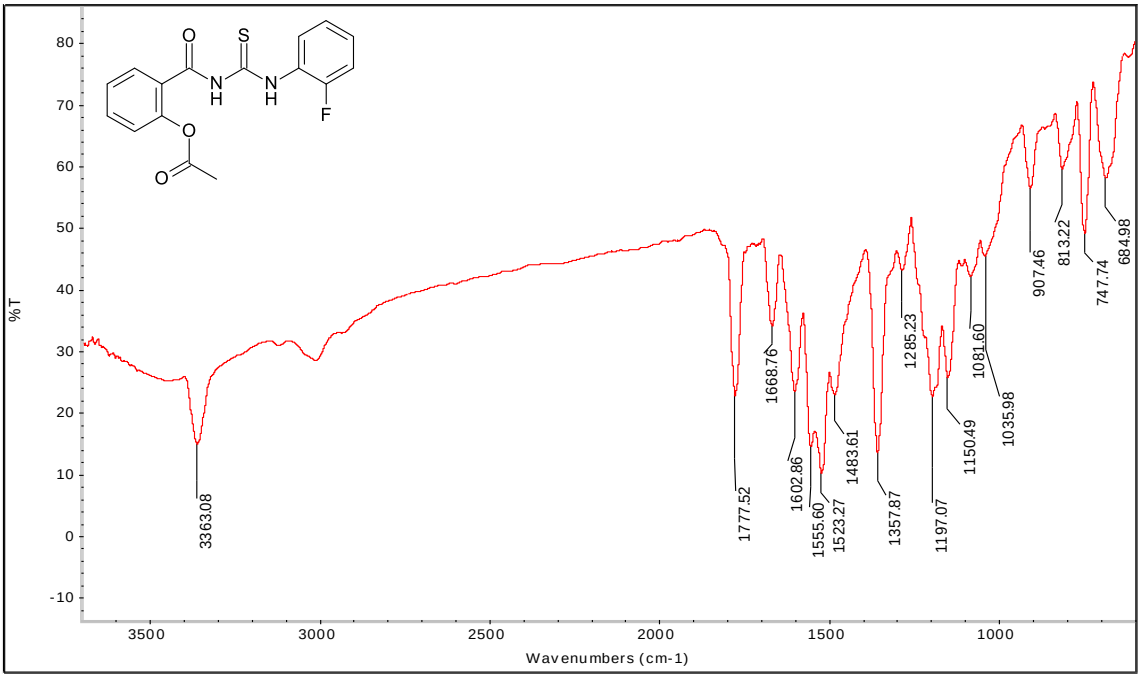


**Figure S1:** FTIR spectrum of **ATX 1**

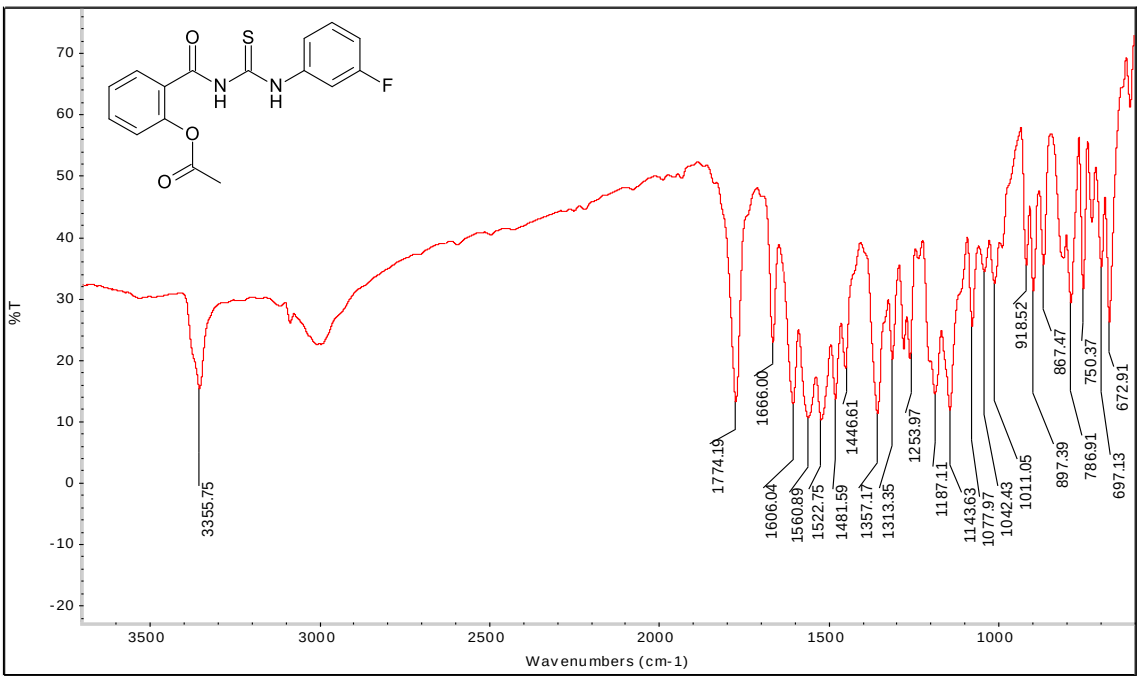


**Figure S2:** FTIR spectrum of **ATX 2**

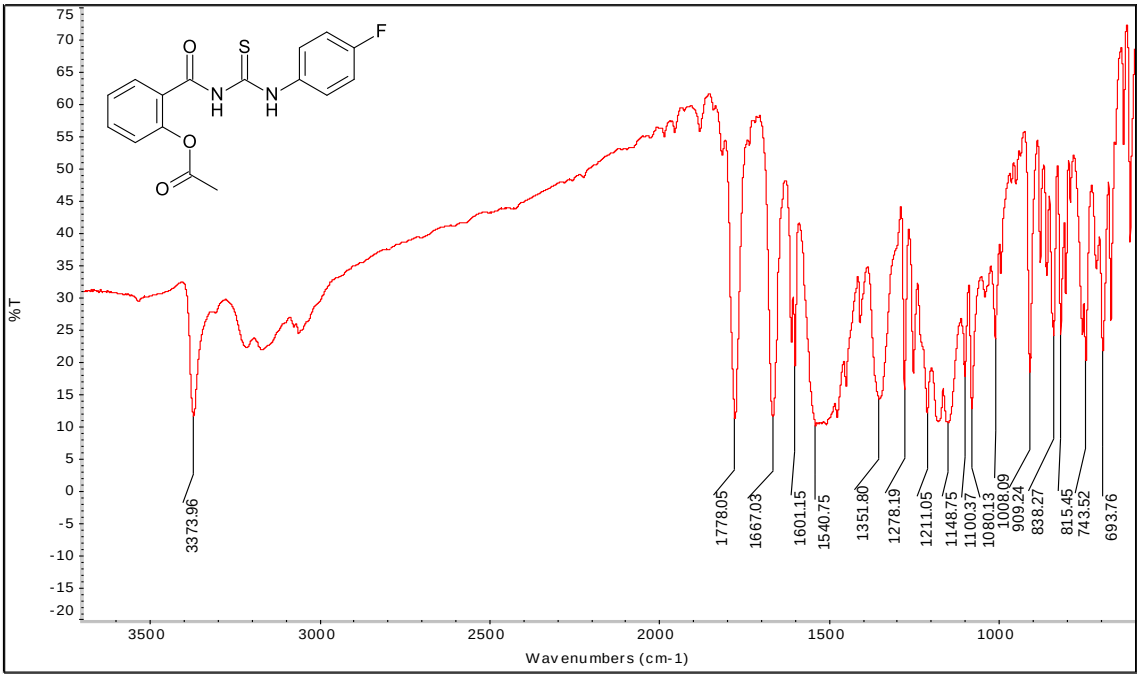


**Figure S3:** FTIR spectrum of **ATX 3**

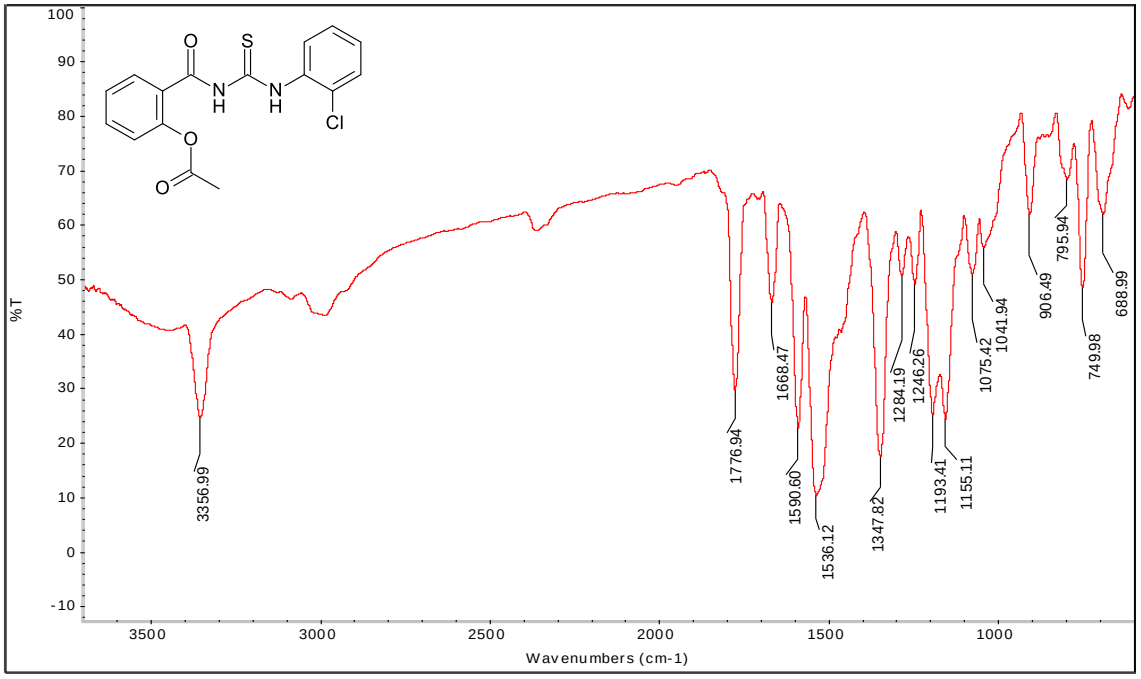


**Figure S4:** FTIR spectrum of **ATX 4**

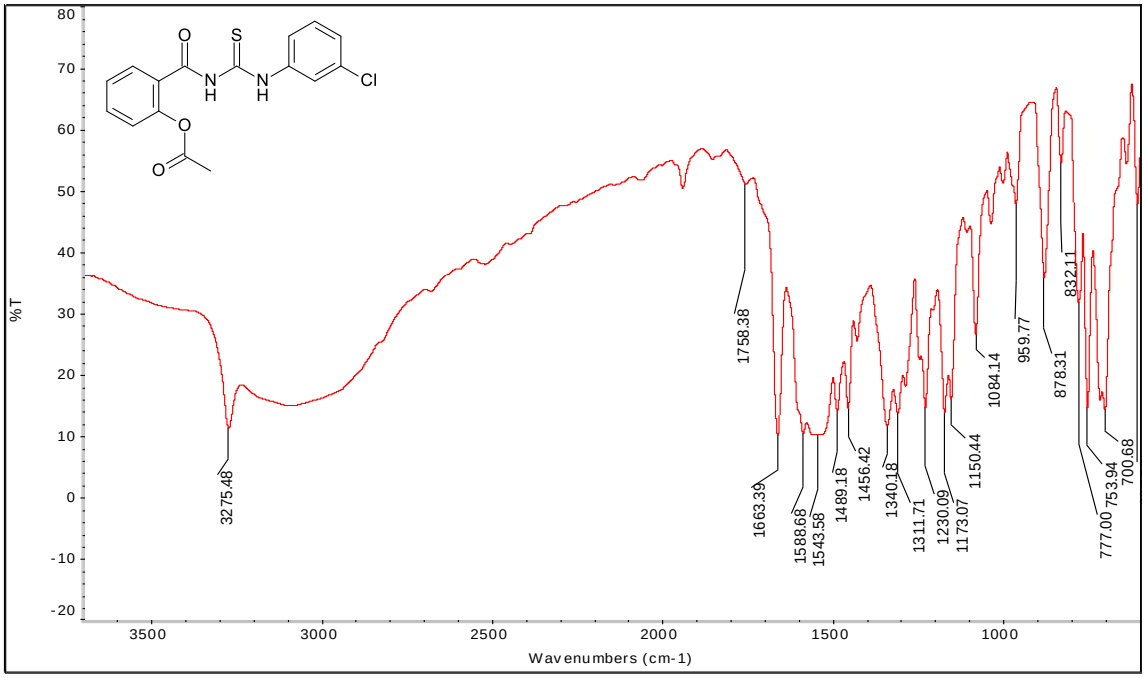


**Figure S5:** FTIR spectrum of **ATX 5**

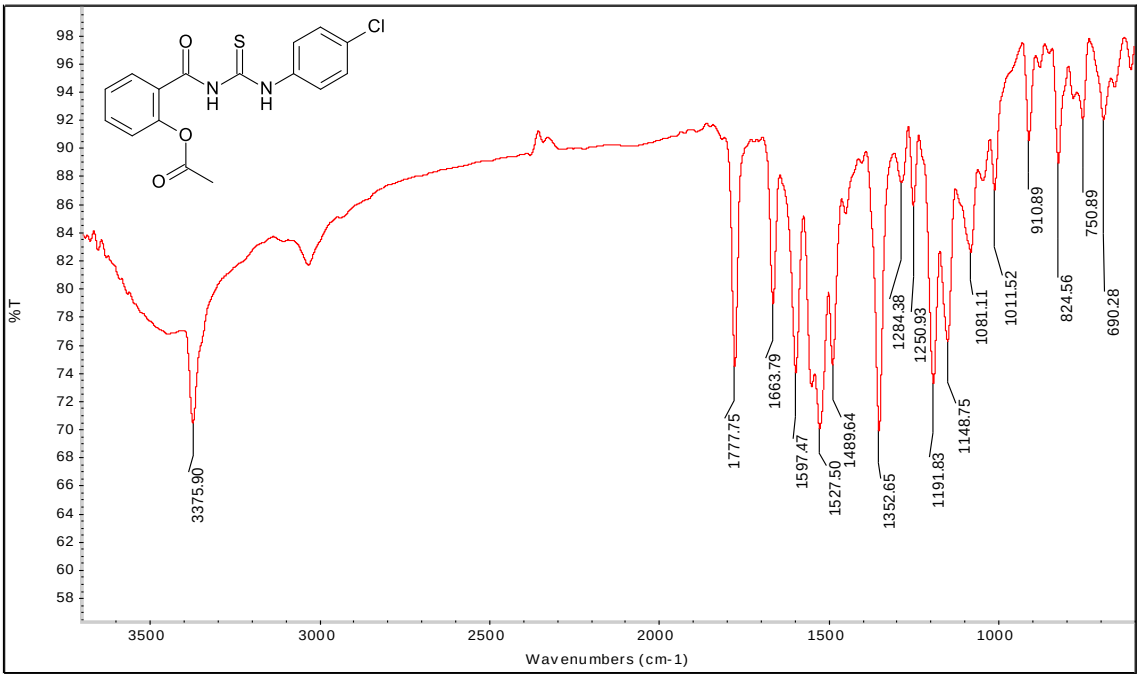


**Figure S6:** FTIR spectrum of **ATX 6**

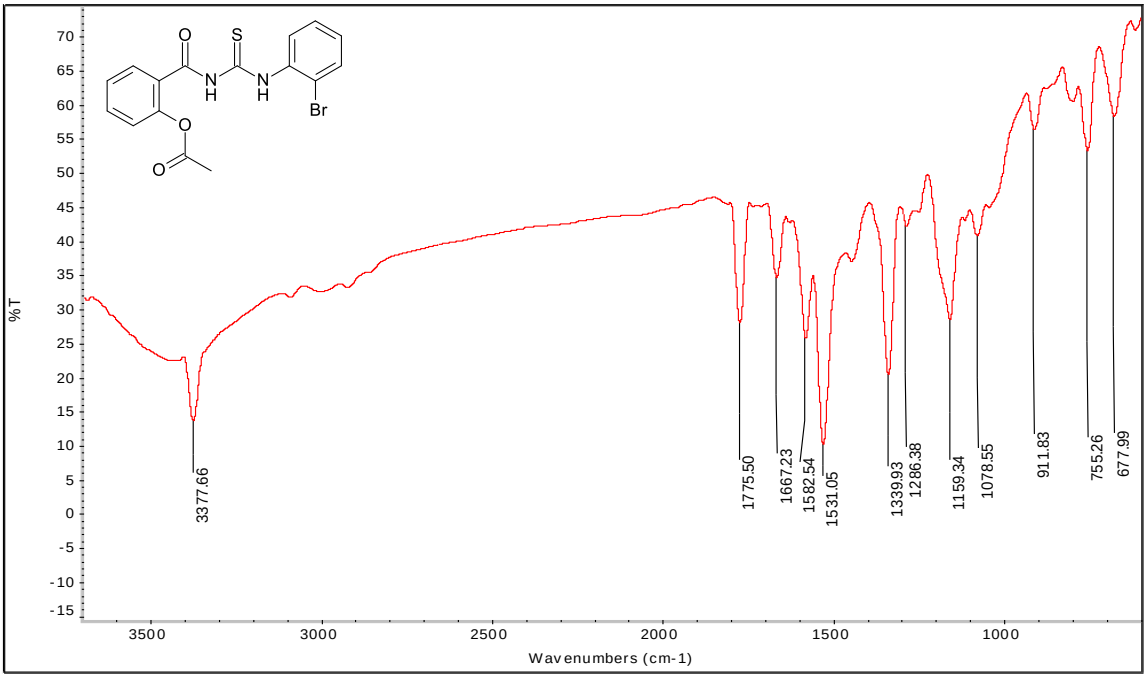


**Figure S7:** FTIR spectrum of **ATX 7**

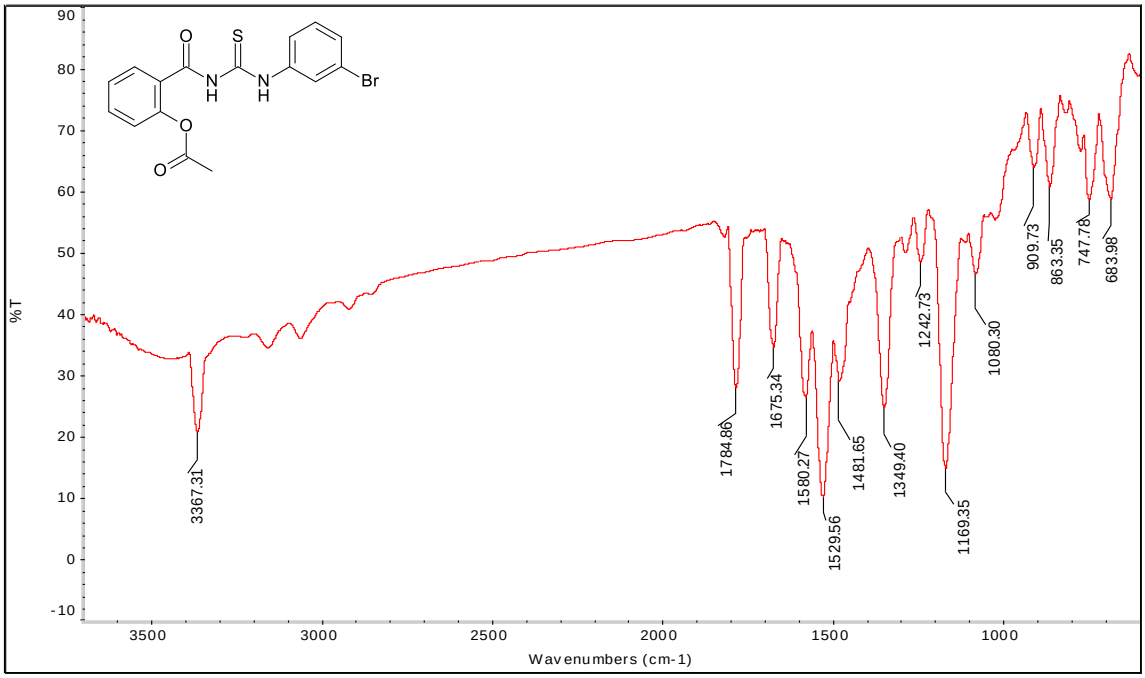


**Figure S8:** FTIR spectrum of **ATX 8**

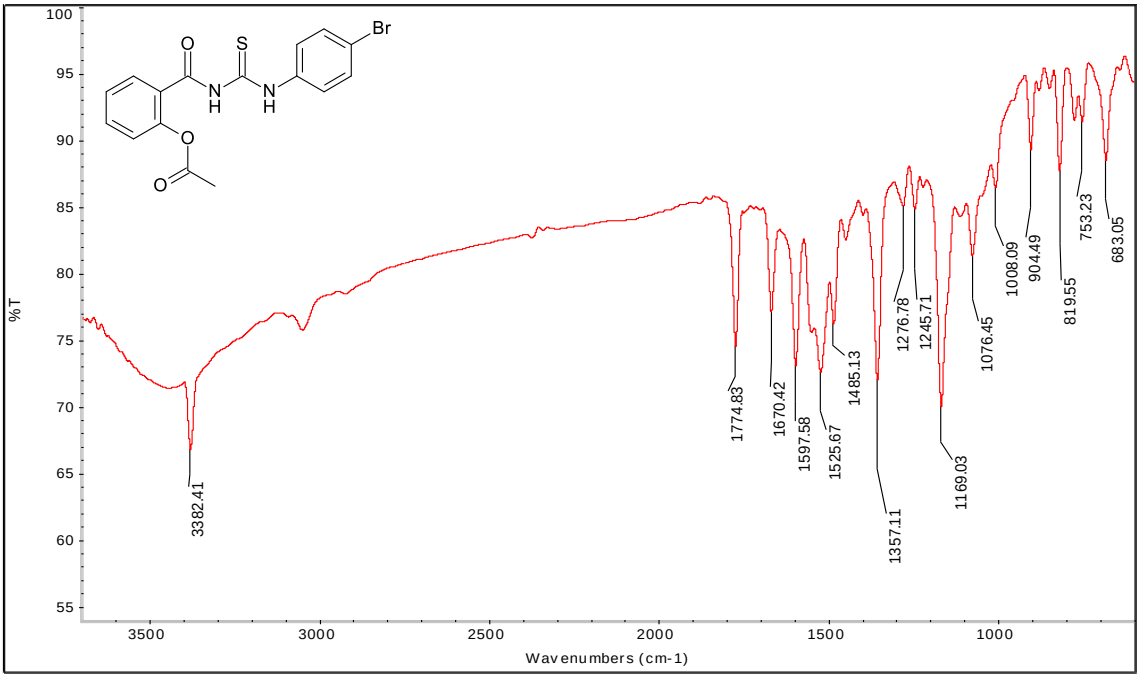


**Figure S9:** FTIR spectrum of **ATX 9**

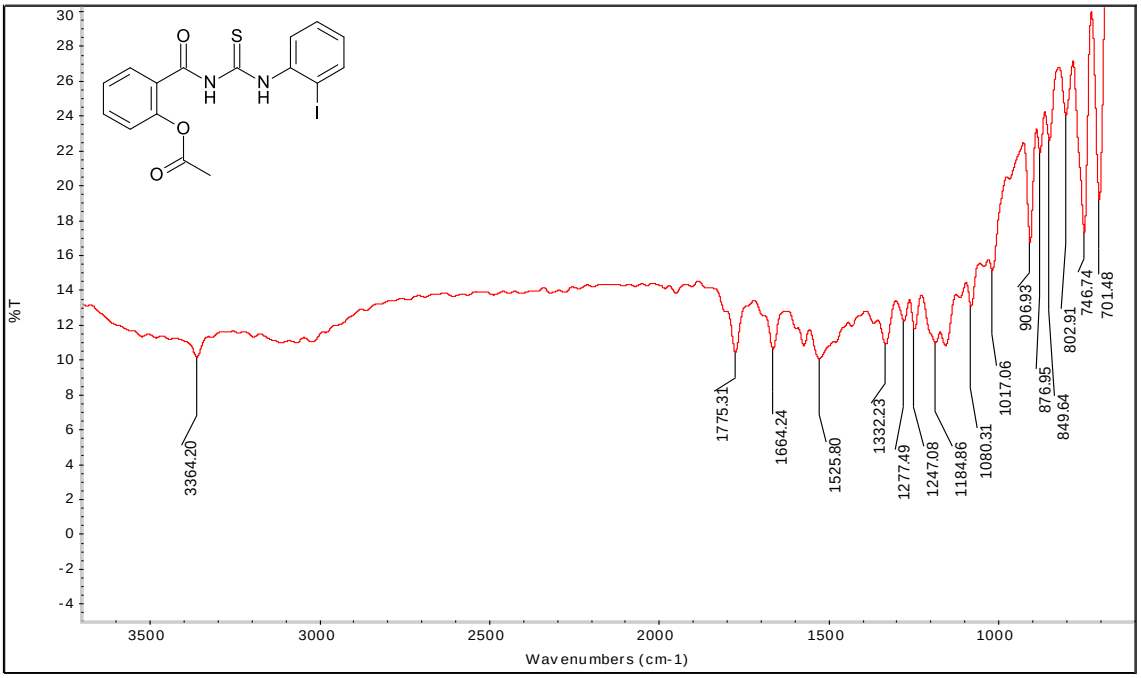


**Figure S10:** FTIR spectrum of **ATX 10**

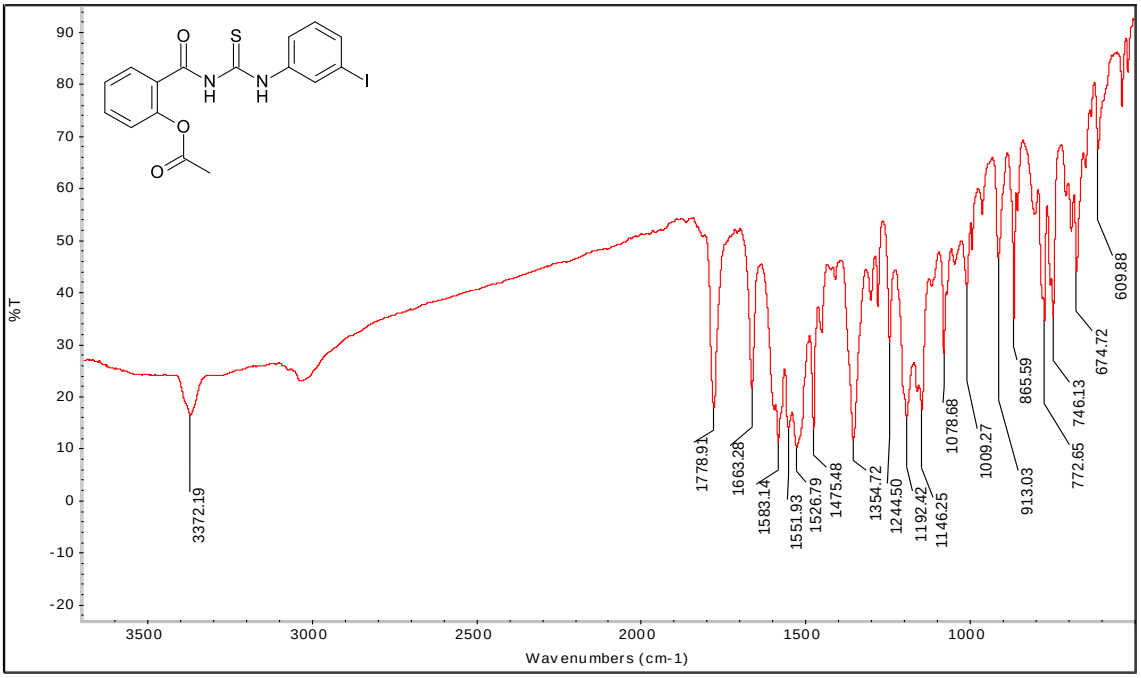


**Figure S11:** FTIR spectrum of **ATX 11**

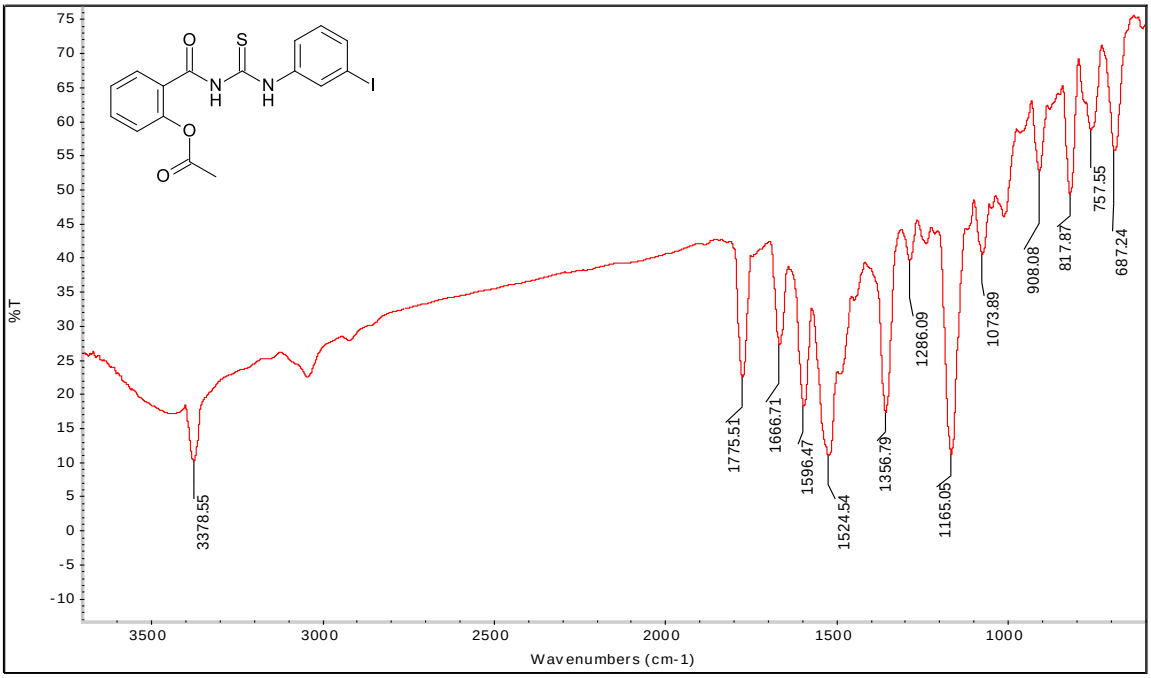


**Figure S12:** FTIR spectrum of **ATX 12**

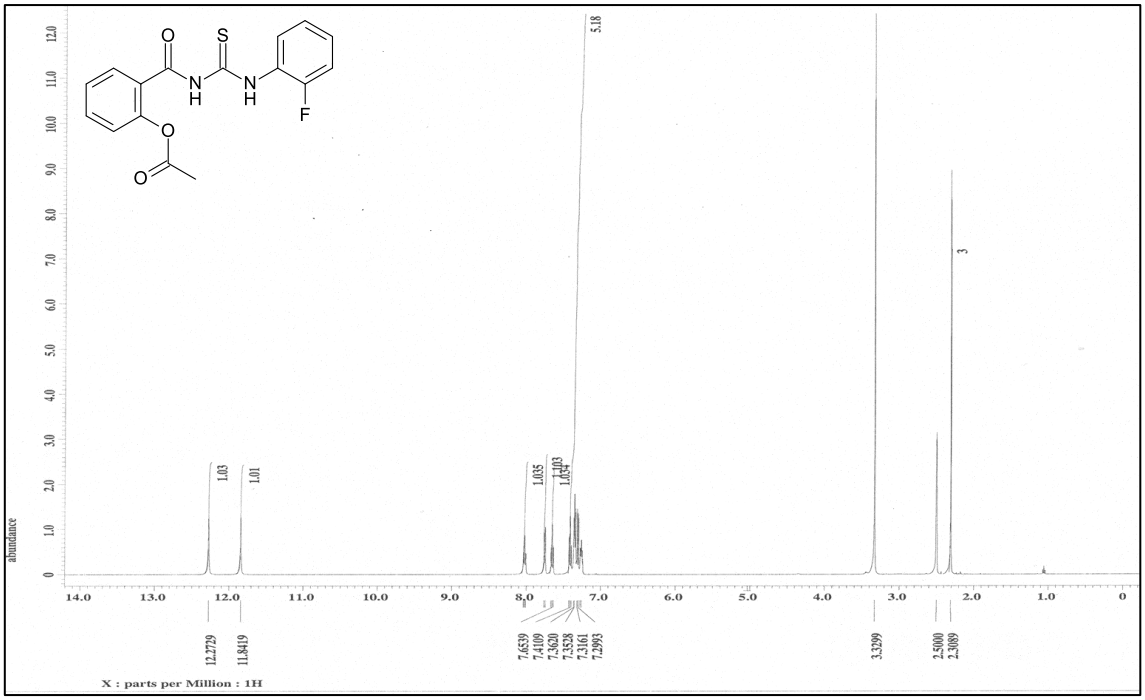


**Figure S13:** $^{1}$H NMR spectrum of **ATX 1**

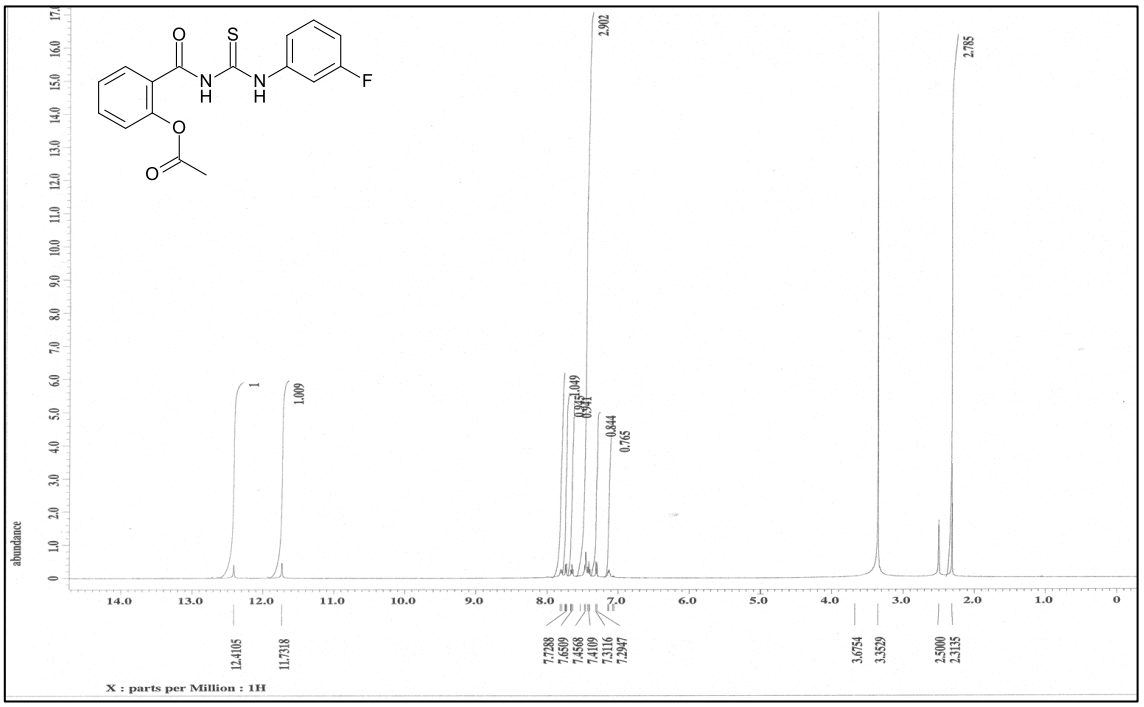


**Figure S14:** $^{1}$H NMR spectrum of **ATX 2**

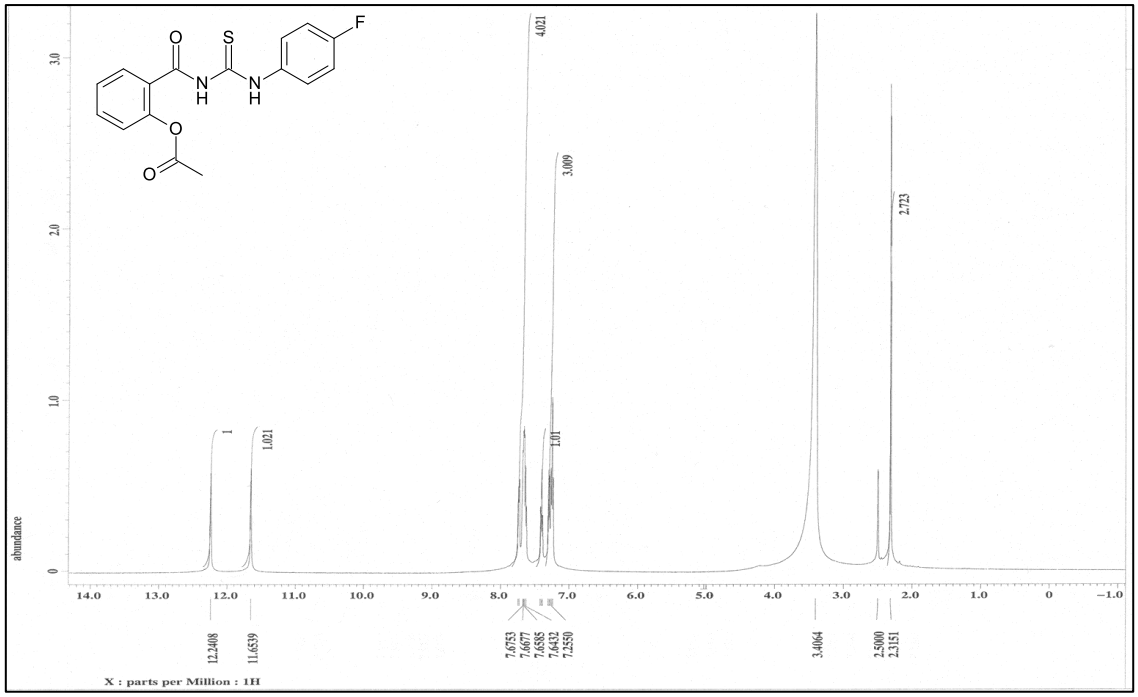


**Figure S15:** $^{1}$H NMR spectrum of **ATX 3**


**Figure S16:** $^{1}$H NMR spectrum of **ATX 4**

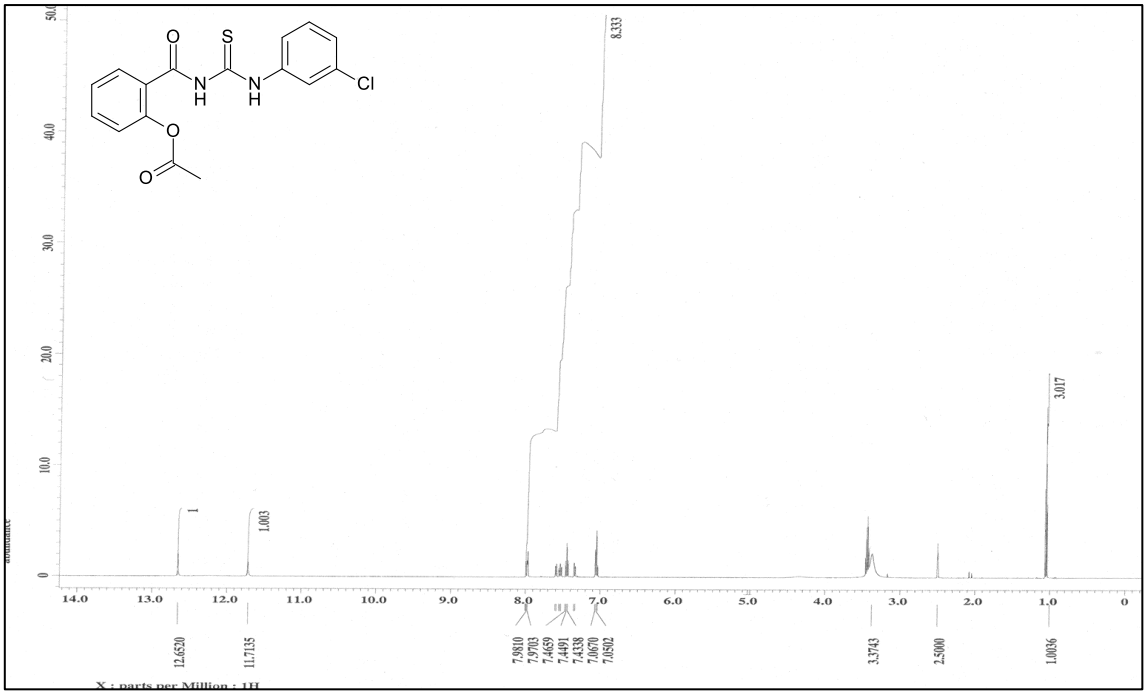


**Figure S17:** $^{1}$H NMR spectrum of **ATX 5**

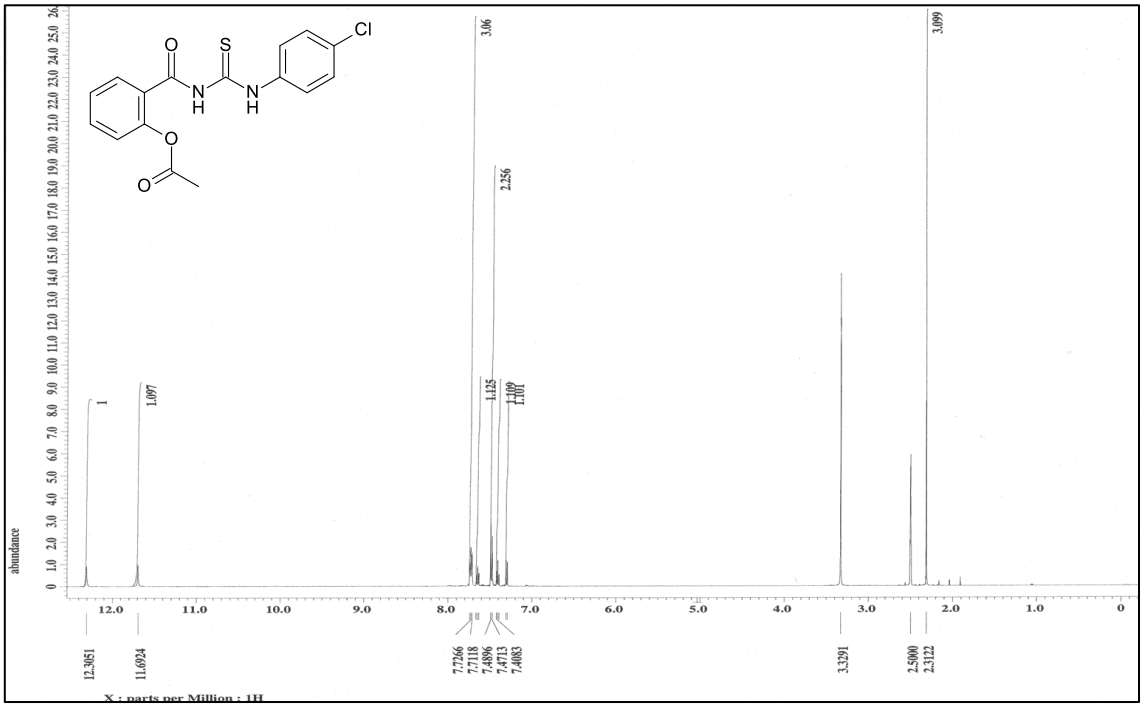


**Figure S18:** $^{1}$H NMR spectrum of **ATX 6**

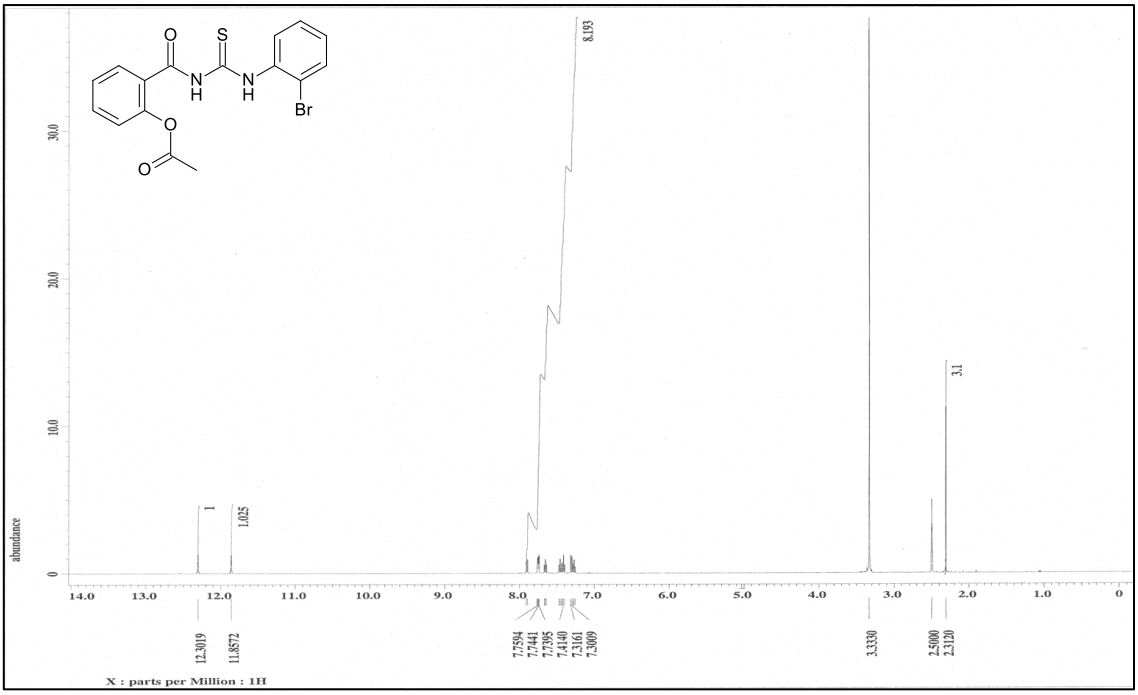


**Figure S19:** $^{1}$H NMR spectrum of **ATX 7**

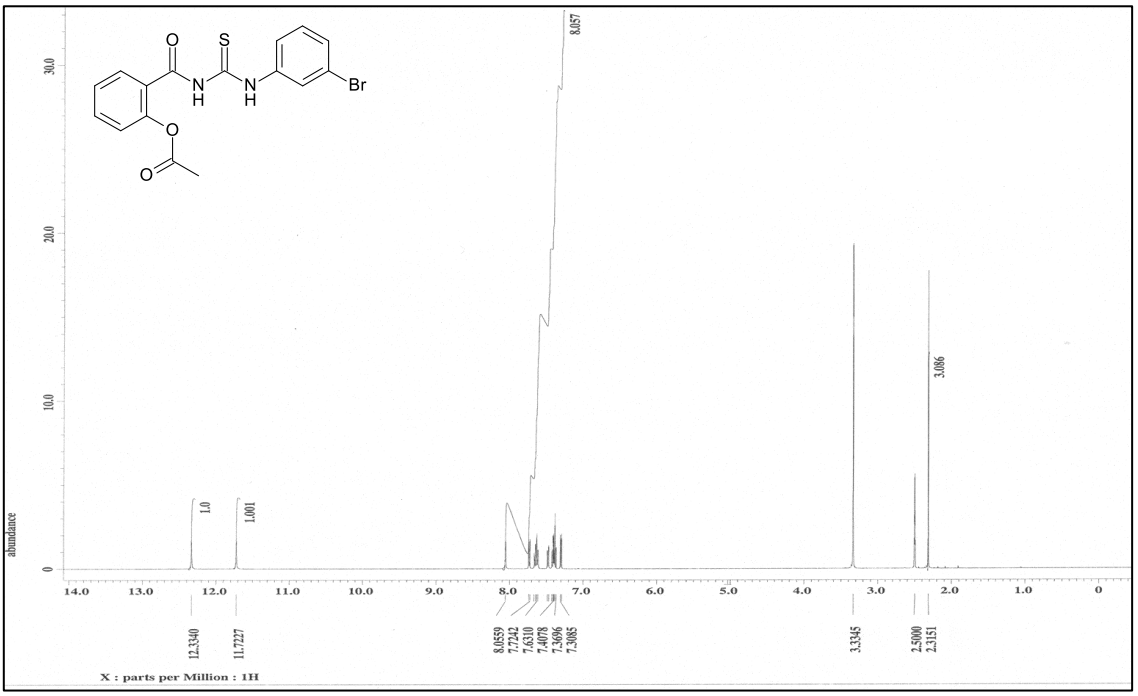


**Figure S20:** $^{1}$H NMR spectrum of **ATX 8**

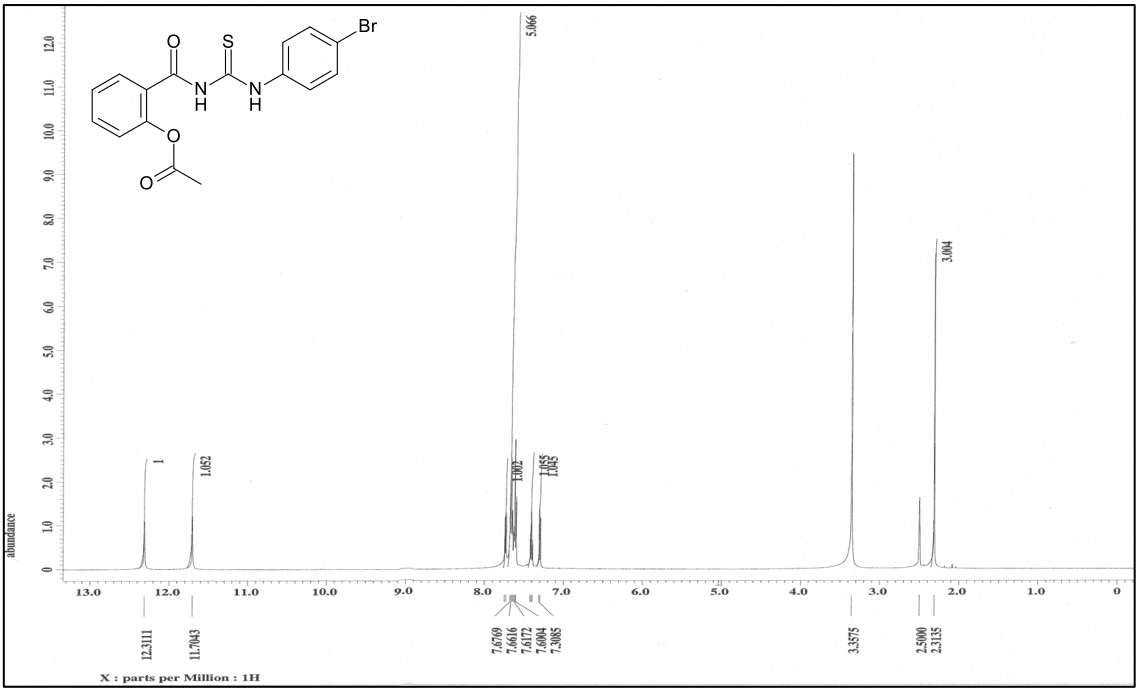


**Figure S21:** $^{1}$H NMR spectrum of **ATX 9**

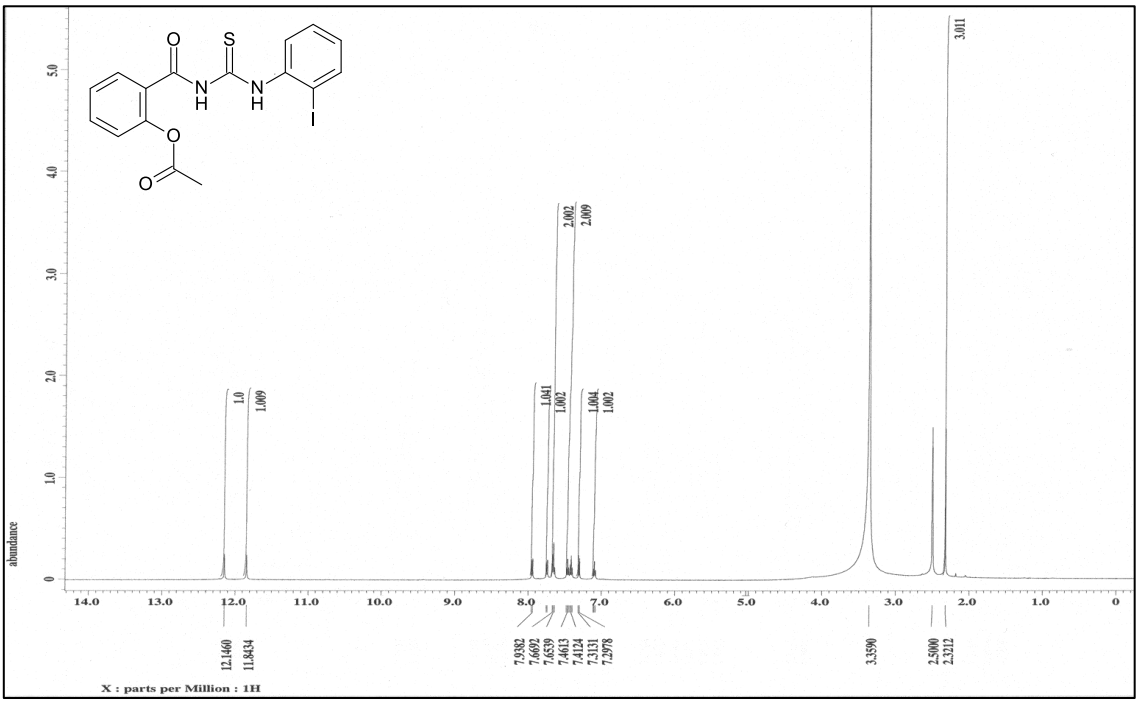


**Figure S22:** $^{1}$H NMR spectrum of **ATX 10**

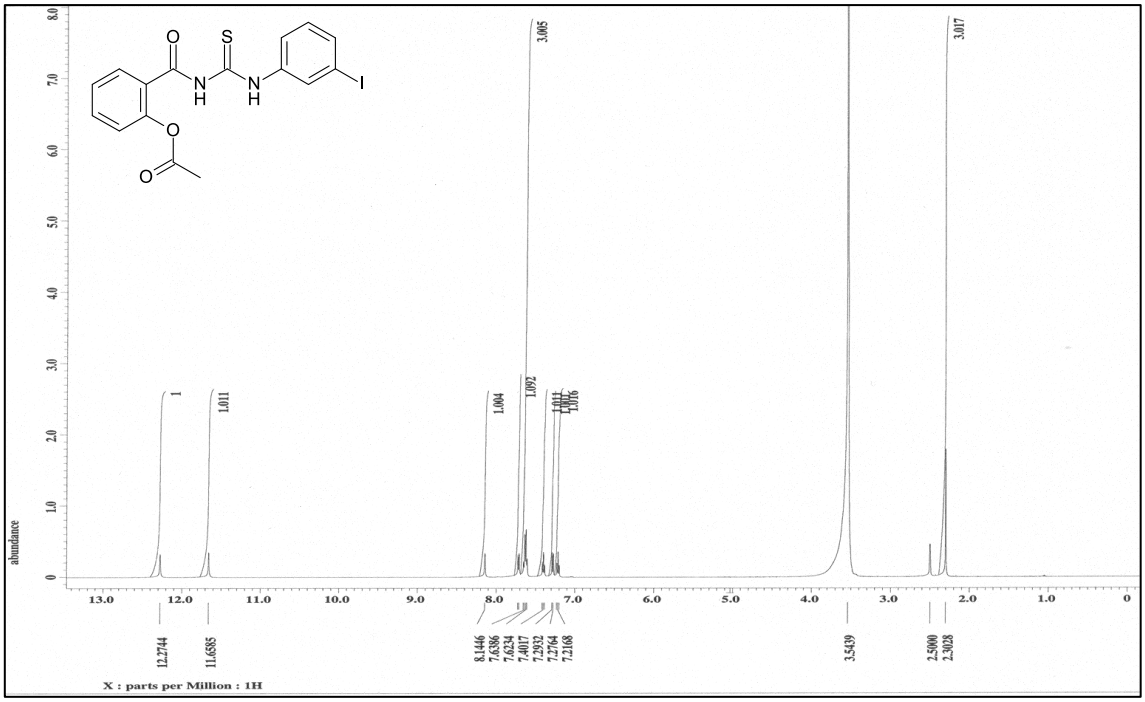


**Figure S23:** $^{1}$H NMR spectrum of **ATX 11**

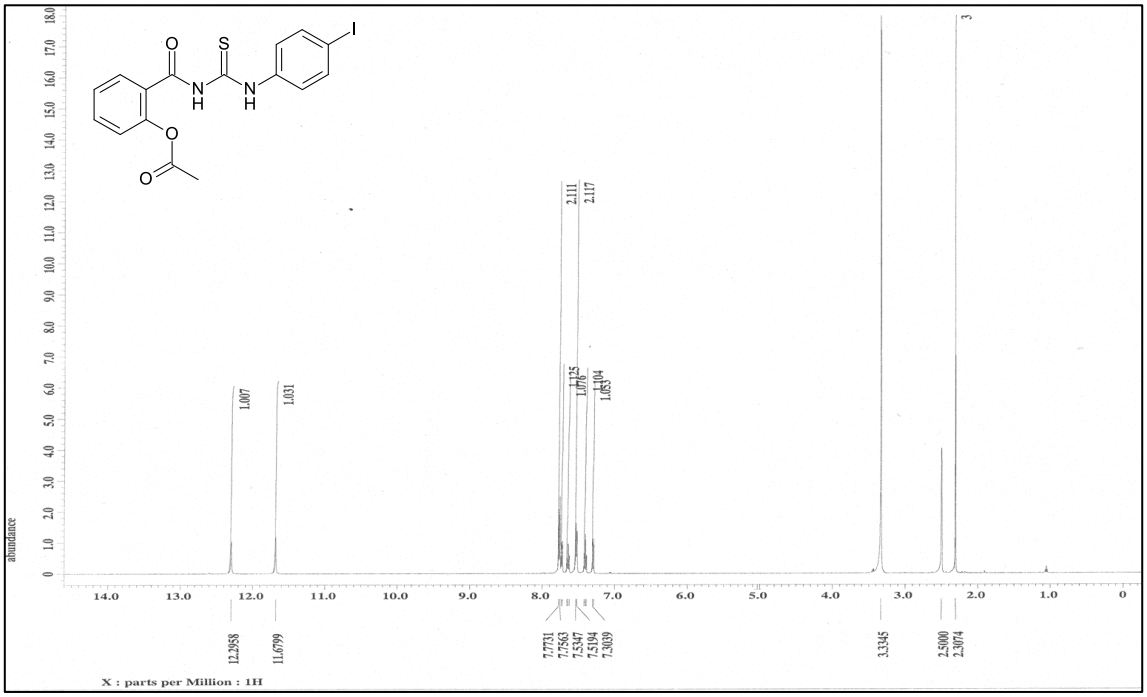


**Figure S24:** $^{1}$H NMR spectrum of **ATX 12**

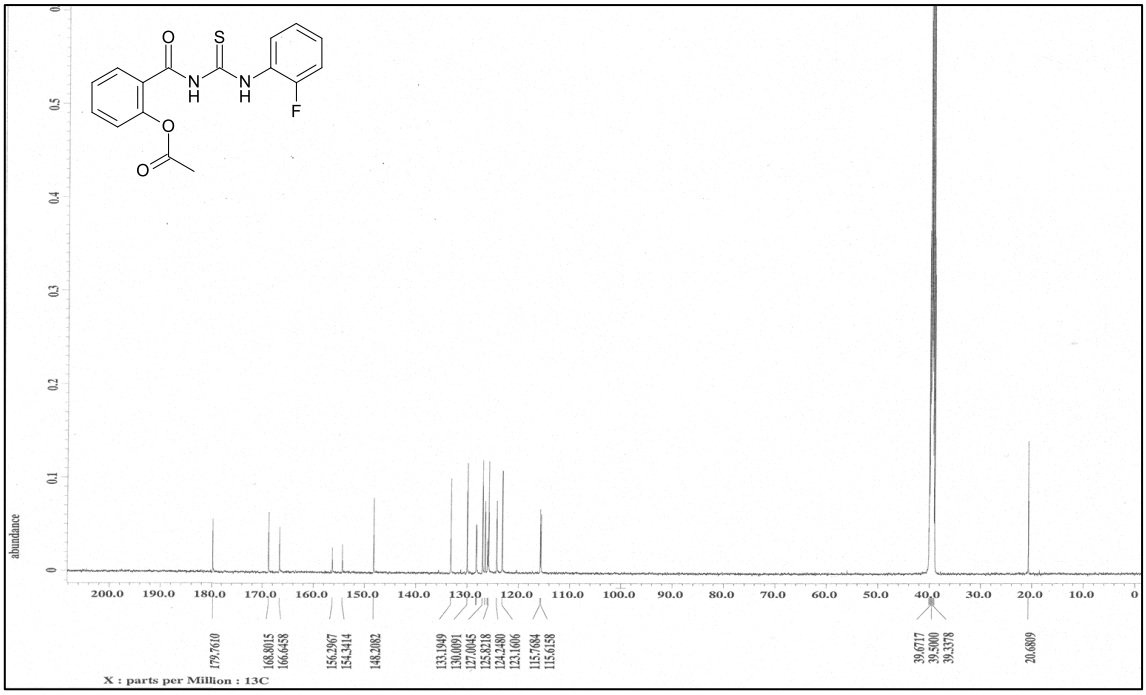


**Figure S25:** $^{13}$C NMR spectrum of **ATX 1**

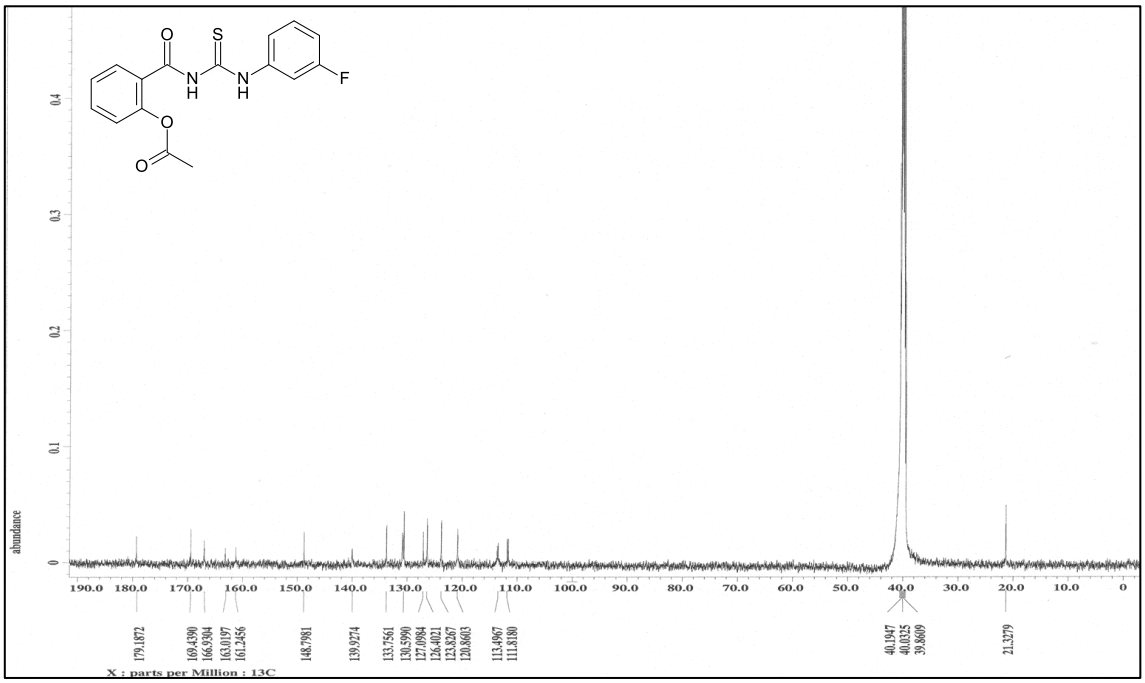


**Figure S26:** $^{13}$C NMR spectrum of **ATX 2**

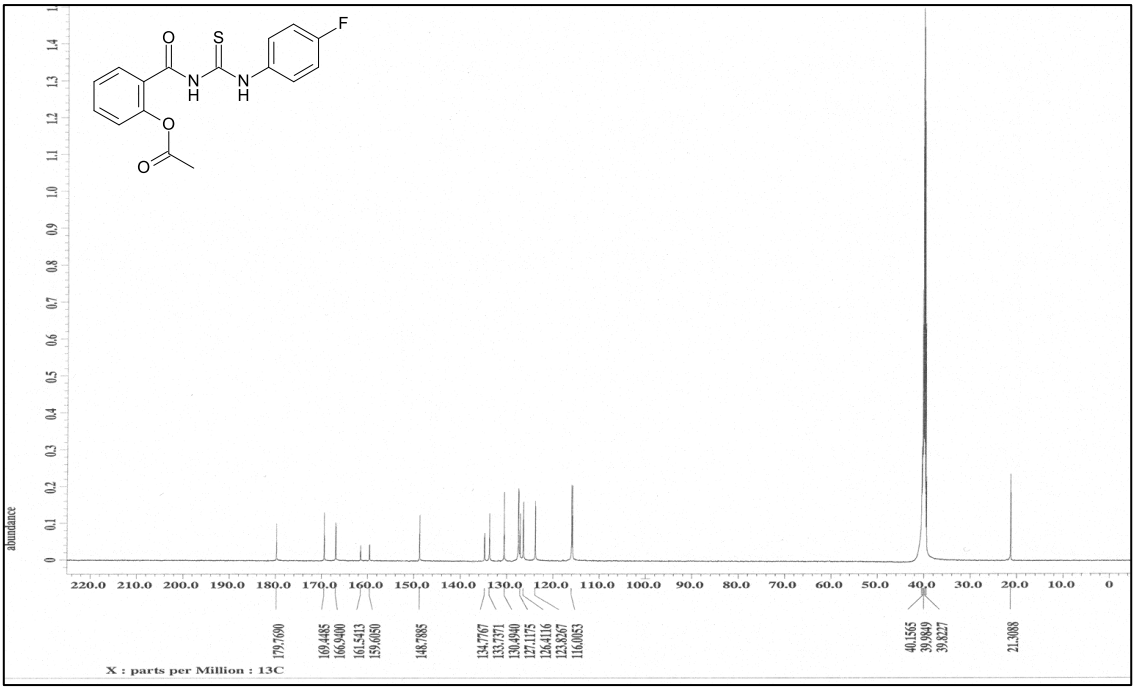


**Figure S27:** $^{13}$C NMR spectrum of **ATX 3**

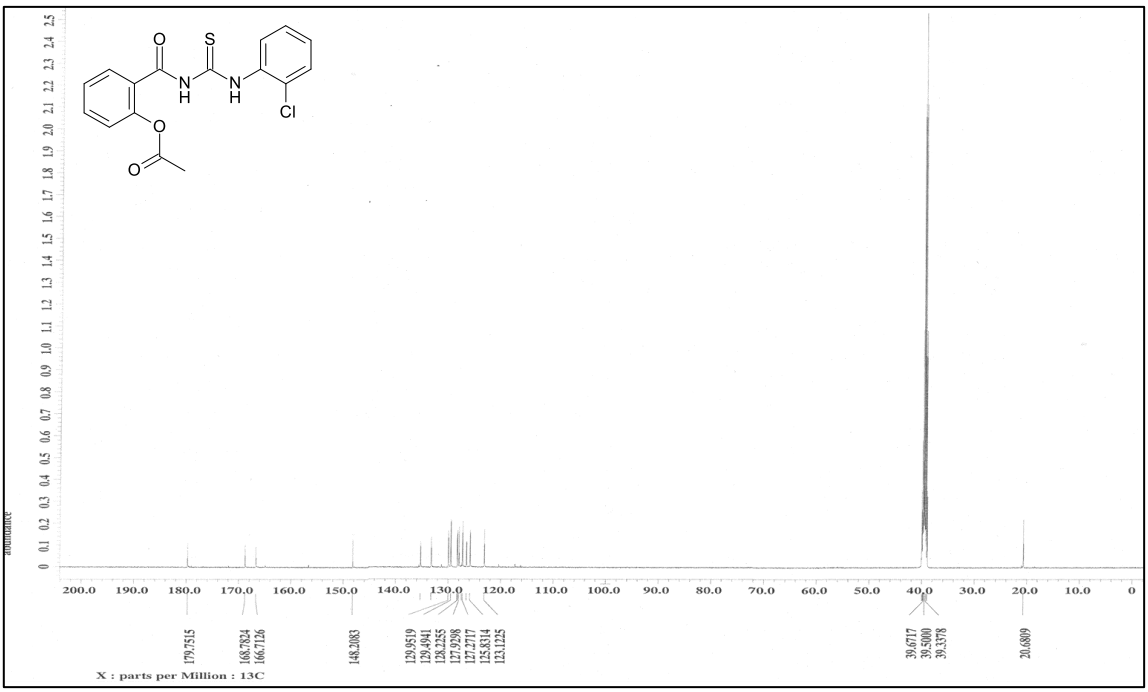


**Figure S28:** $^{13}$C NMR spectrum of **ATX 4**

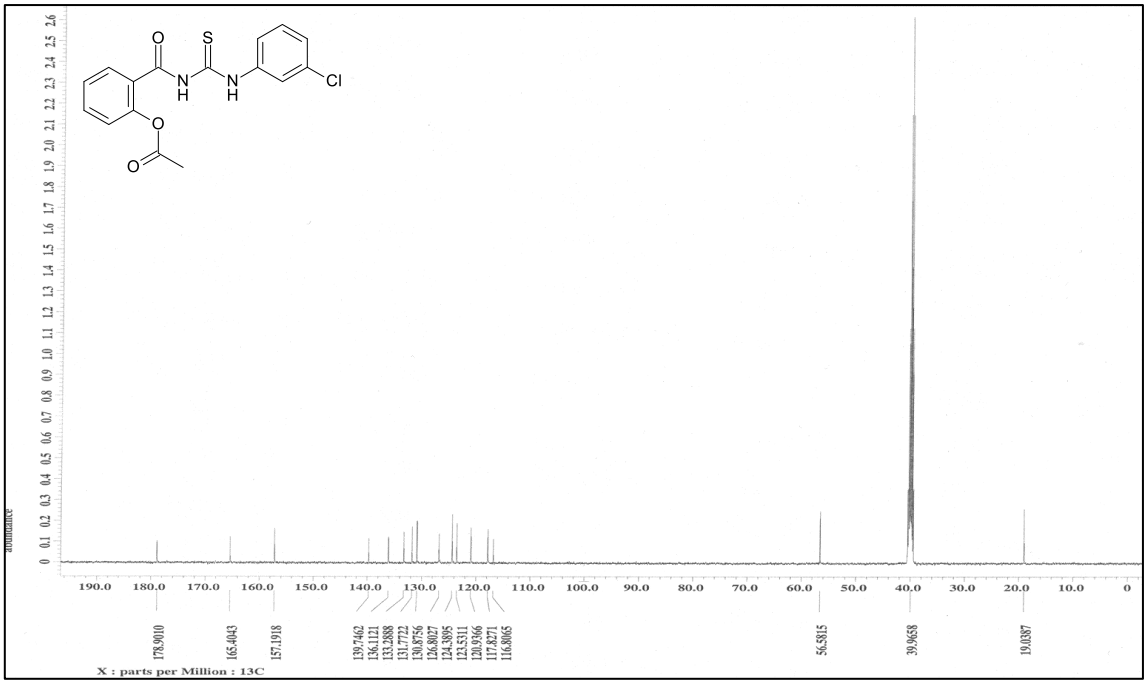


**Figure S29:** $^{13}$C NMR spectrum of **ATX 5**

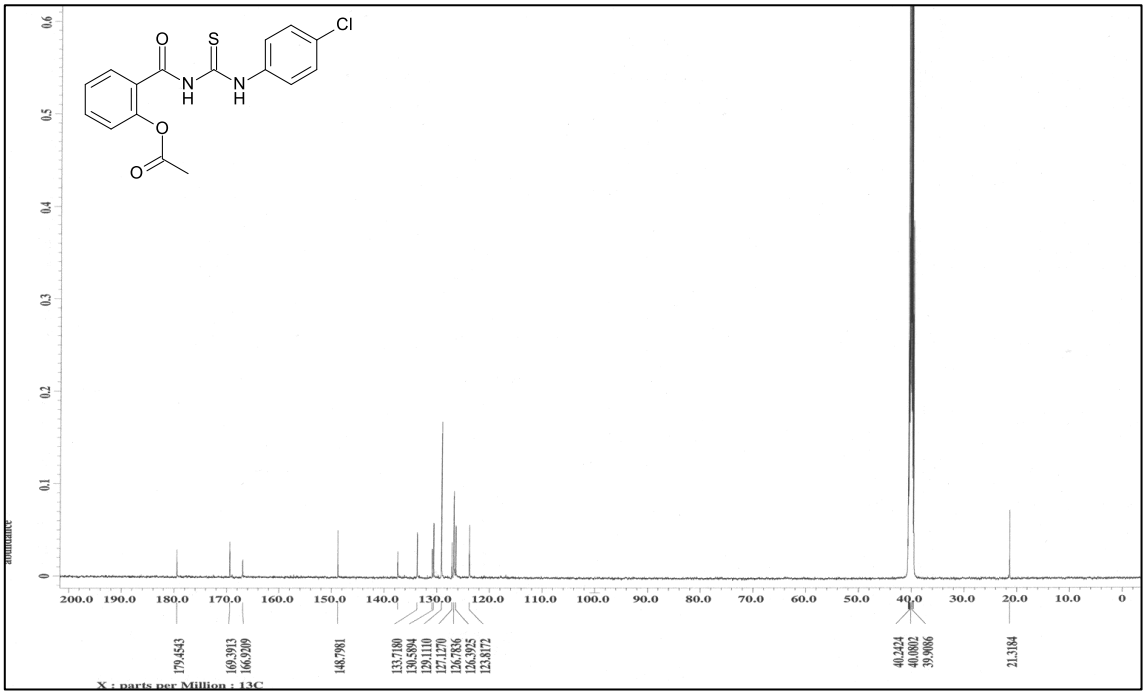


**Figure S30:** $^{13}$C NMR spectrum of **ATX 6**

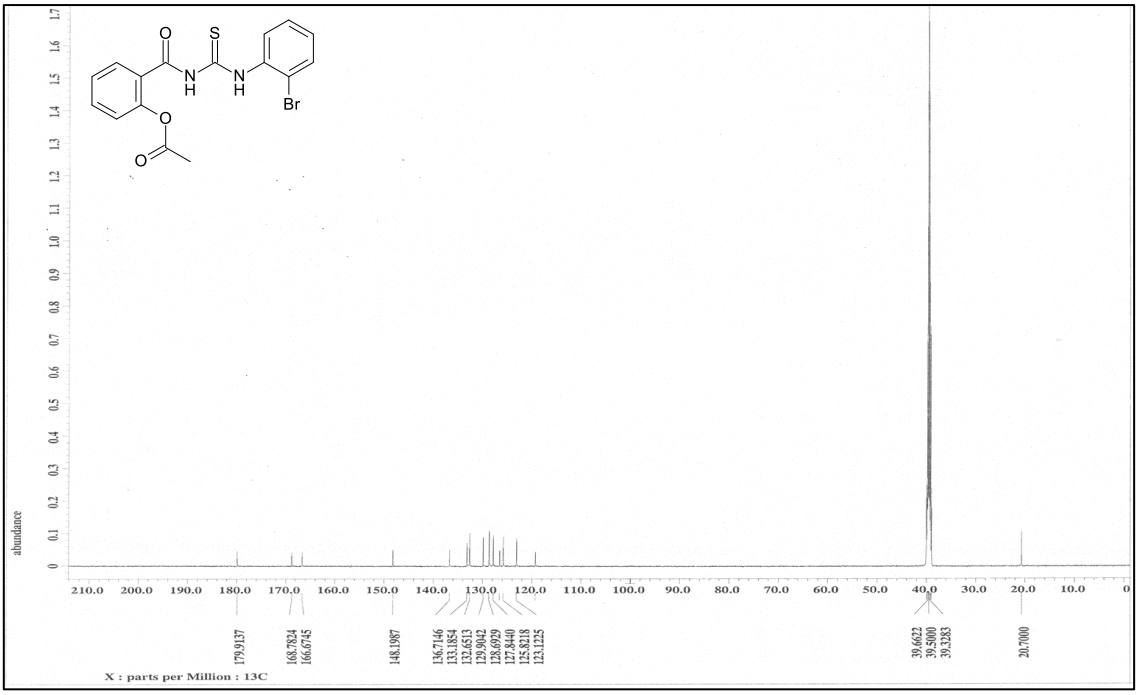


**Figure S31:** $^{13}$C NMR spectrum of **ATX 7**

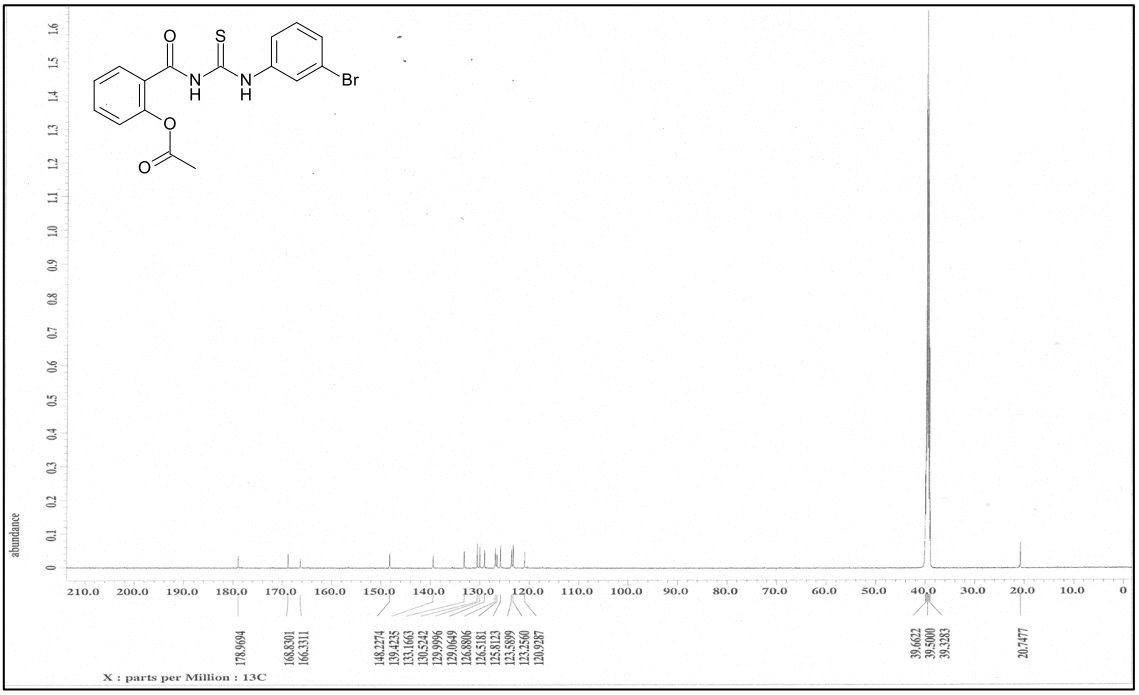


**Figure S32:** $^{13}$C NMR spectrum of **ATX 8**

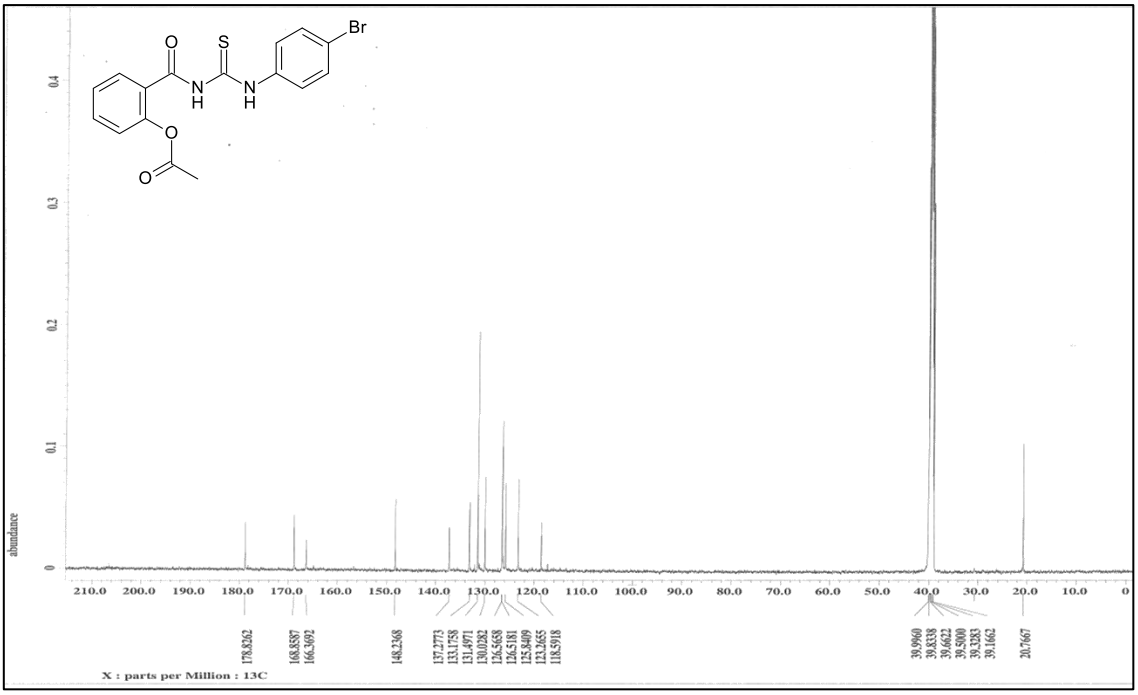


**Figure S33:** $^{13}$C NMR spectrum of **ATX 9**

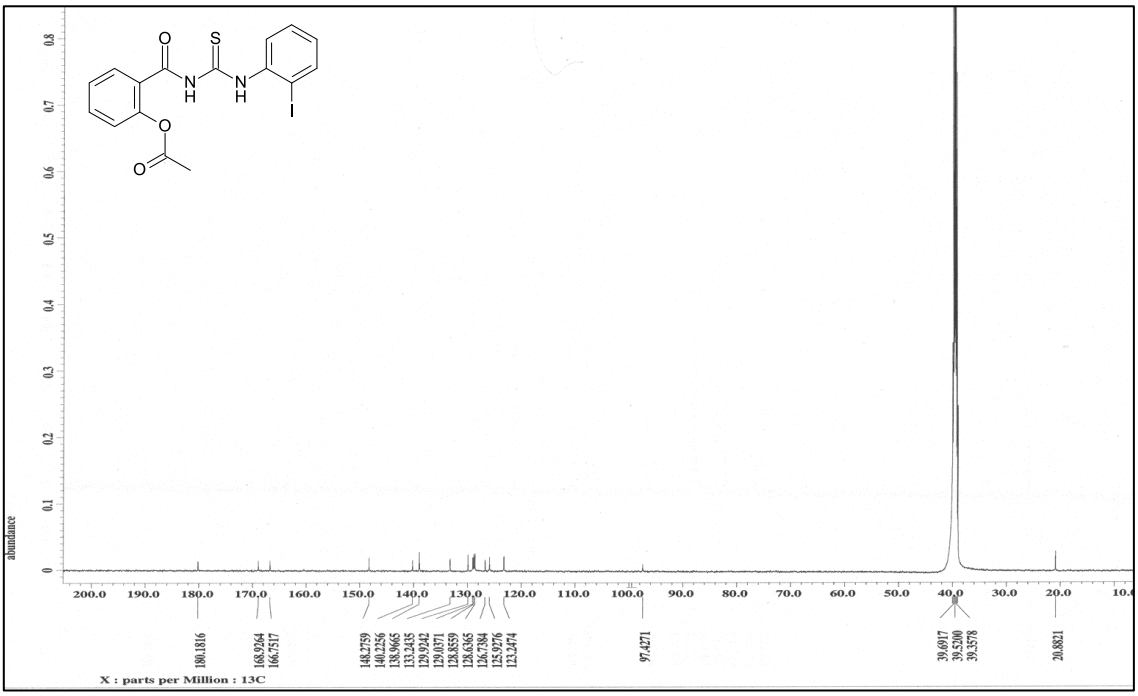


**Figure S34:** $^{13}$C NMR spectrum of **ATX 10**

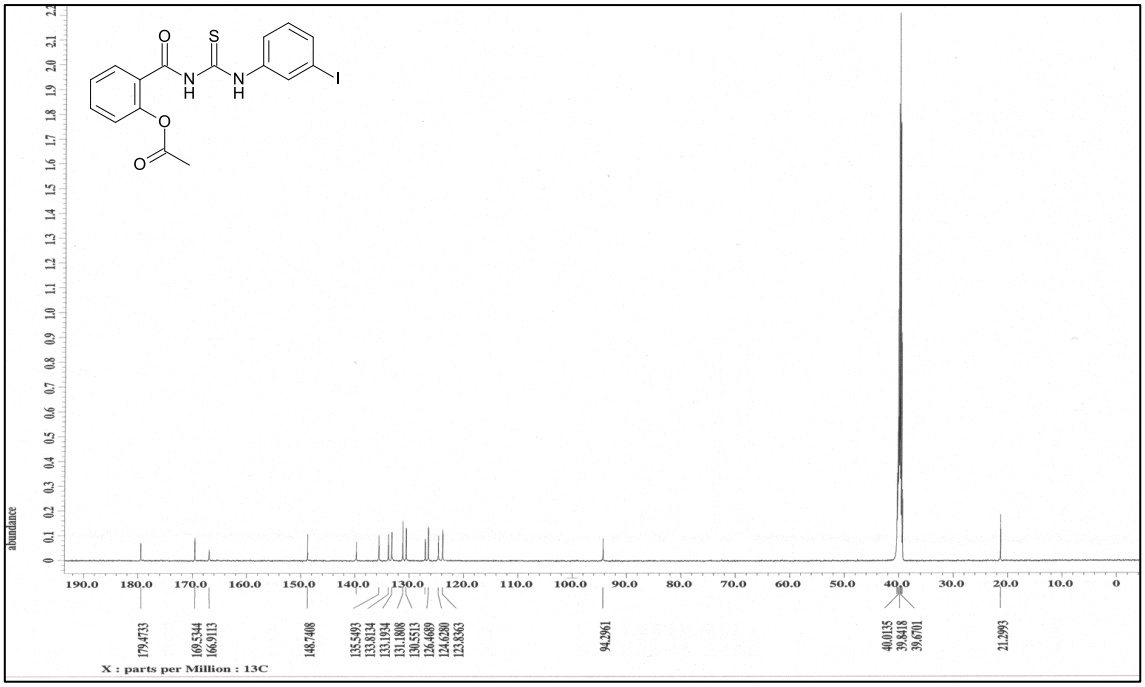


**Figure S35:** $^{13}$C NMR spectrum of **ATX 11**

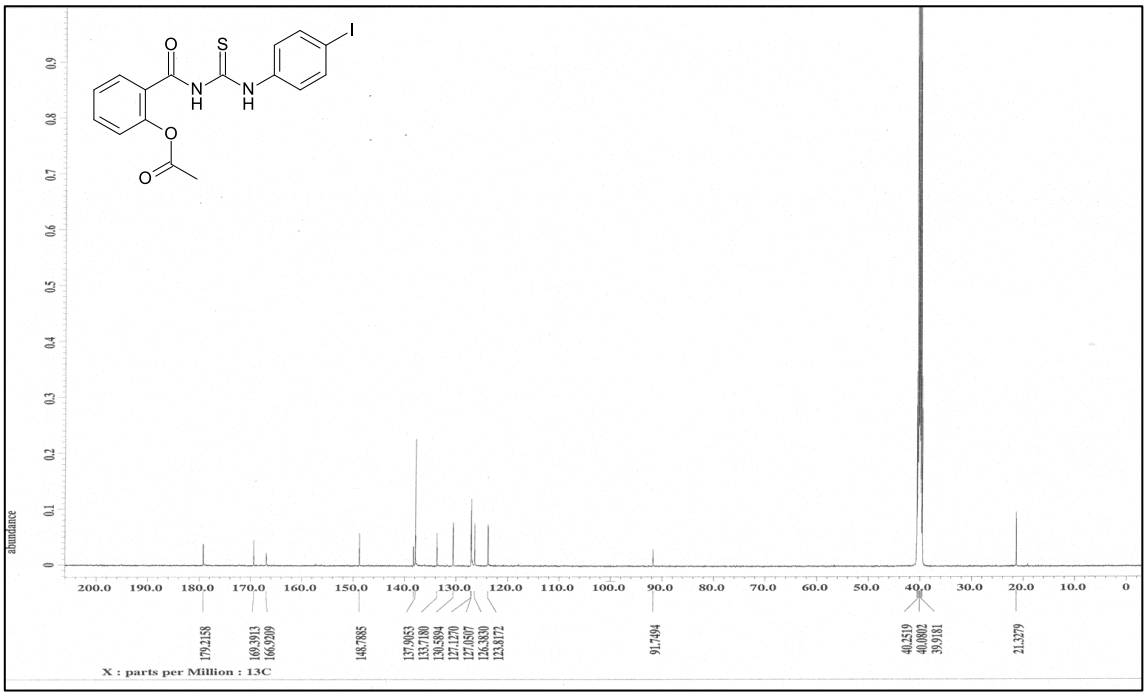


**Figure S36:** $^{13}$C NMR spectrum of **ATX 12**

**Table S1:** $IC_{50}$ values of **ATX 1-12**, cisplatin and aspirin (in µM) against NPC cell lines, HK1.

| **Compounds** | **ATX** | **$IC_{50}$ (µM)** |
|---|---|---|
| 1 | 1 (*o*-F) | 12.6 ± 1.5 |
| 2 | 2 (*m*-F) | 7.0 ± 1.4 |
| 3 | 3 (*p*-F) | 12.3 ± 3.7 |
| 4 | 4 (*o*-Cl) | 12.4 ± 3.4 |
| 5 | 5 (*m*-Cl) | 5.6 ± 0.8 |
| 6 | 6 (*p*-Cl) | 8.6 ± 1.4 |
| 7 | 7 (*o*-Br) | 10.8 ± 1.6 |
| 8 | 8 (*m*-Br) | 14.6 ± 3.3 |
| 9 | 9 (*p*-Br) | 5.7 ± 1.6 |
| 10 | 10 (*o*-I) | 13.9 ± 1.2 |
| 11 | 11 (*m*-I) | 4.7 ± 0.7 |
| 12 | 12 (*p*-I) | 8.3 ± 1.4 |
| 13 | Cisplatin | 8.9 ± 1.9 |
| 14 | Aspirin | >50 |
| 15 | Aniline (*m*-I) | >50 |

Note: All the values are expressed as mean ± SEM**,** significant difference ($P$<0.05)

**Table S2**: 95% confidence interval for mean statistical analysis on cytotoxicity of **ATX 1-12**.

| | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| 1 | 3 | 12.6000 | 2.60000 | 1.50111 | 6.1412 | 19.0588 |
| 2 | 3 | 7.0000 | 2.38956 | 1.37961 | 1.0640 | 12.9360 |
| 3 | 3 | 12.3333 | 6.40104 | 3.69564 | -3.5677 | 28.2344 |
| 4 | 3 | 12.3667 | 5.80460 | 3.35128 | -2.0527 | 26.7861 |
| 5 | 3 | 5.5667 | 1.44684 | .83533 | 1.9725 | 9.1608 |
| 6 | 3 | 8.6000 | 2.40624 | 1.38924 | 2.6226 | 14.5774 |
| 7 | 3 | 10.7667 | 2.82902 | 1.63333 | 3.7390 | 17.7943 |
| 8 | 3 | 14.6333 | 5.78475 | 3.33983 | .2632 | 29.0034 |
| 9 | 3 | 5.6667 | 2.77909 | 1.60451 | -1.2370 | 12.5703 |
| 10 | 3 | 4.6667 | 1.17189 | .67659 | 1.7555 | 7.5778 |
| 11 | 3 | 8.2667 | 2.37136 | 1.36910 | 2.3759 | 14.1574 |
| 12 | 3 | 13.9333 | 2.11975 | 1.22384 | 8.6676 | 19.1991 |
| Total | 36 | 9.7000 | 4.52510 | .75418 | 8.1689 | 11.2311 |

**Table S3**: ANOVA statistical analysis on cytotoxicity of **ATX 1-12**.

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 405.280 | 11 | 36.844 | 2.840 | .016 |
| Within Groups | 311.400 | 24 | 12.975 | | |
| Total | 716.680 | 35 | | | |

**Table S4**: Homogeneous subset statistical analysis on cytotoxicity of **ATX 1-12**.

| Compound | N | Subset for alpha = 0.05 |
|---|---|---|
| | | 1 |
| 10 | 3 | 4.6667 |
| 5 | 3 | 5.5667 |
| 9 | 3 | 5.6667 |
| 2 | 3 | 7.0000 |
| 11 | 3 | 8.2667 |
| 6 | 3 | 8.6000 |
| 7 | 3 | 10.7667 |
| 3 | 3 | 12.3333 |
| 4 | 3 | 12.3667 |
| 1 | 3 | 12.6000 |
| 12 | 3 | 13.9333 |
| 8 | 3 | 14.6333 |
| Sig. | | .079 |

**Table S5**: T-test (one-sample statistics) statistical analysis on cytotoxicity of **ATX 1-12**.

| | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|
| IC50 | 36 | 9.7000 | 4.52510 | .75418 |

**Table S6**: T-test (one-sample test) statistical analysis on cytotoxicity of **ATX 1-12**.

| | Test Value = 0 | | | | | |
|---|---|---|---|---|---|---|
| | t | df | Sig. (2-tailed) | Mean Difference | 95% Confidence Interval of the Difference | |
| | | | | | Lower | Upper |
| IC50 | 12.862 | 35 | .000 | 9.70000 | 8.1689 | 11.2311 |

**Data S1**: Data Analysis of **ATX 1-12**

*2-(((2-fluorophenyl)carbamothioyl)carbamoyl) phenyl acetate (**ATX 1**)*

Yield 0.1068 g, 32.2% as white solid, m.p.: 151-153 °C, Elemental analysis (Found: C, 57.67; H, 3.62; N, 8.01; S, 9.42. $C_{16}H_{13}FO_3N_2S$ Requires C, 57.82; H, 3.94; N, 8.43; S, 9.65%); $\nu_{max}$ FTIR (KBr/cm$^{-1}$) 3364 (N-H), 1778 (C=O ester), 1670 (C=O amide), 1524 (Ar-C), 1152 (C-N), 749 (C=S). $\delta_H$ $^1$H NMR (500 MHz, DMSO-$d_6$) 2.31 (3H, s, $CH_3$), 7.24-7.43 (5H, m, Ar-H), 7.64 (1H, t, Ar-H), 7.75 (1H, d, *J*=7.7 Hz, Ar-H), 8.01 (1H, t, Ar-H), 11.84 (1H, s, NH), 12.27 (1H, s, NH). $\delta_C$ $^{13}$C NMR (125 MHz, DMSO-$d_6$) 20.7 ($CH_3$), 115.8 (Ar-C), 123.2 (Ar-C), 124.2 (Ar-C), 125.8 (Ar-C), 126.5 (Ar-C), 127.0 (Ar-C), 128.2 (Ar-C), 130.0 (Ar-C), 133.2 (Ar-C), 148.2 (Ar-C), 154.3 (Ar-C), 156.3 (Ar-C), 166.6 (C=O), 168.8 (C=O) and 179.8 (C=S).

*2-(((3-fluorophenyl)carbamothioyl)carbamoyl) phenyl acetate (**ATX 2**)*

Yield 0.1033 g, 31.1% as white solid, m.p.: 115-116 °C, (Found: C, 57.06; H, 3.82; N, 8.30; S, 9.52. $C_{16}H_{13}FO_3N_2S$ Requires C, 57.82; H, 3.94; N, 8.43; S, 9.65%); $\nu_{max}$ FTIR (KBr/cm$^{-1}$) 3356 (N-H), 1774 (C=O ester), 1666 (C=O amide), 1561 (Ar-C), 1144 (C-N), 787 (C=S). $\delta_H$ $^1$H NMR (500 MHz, DMSO-$d_6$) 2.31 (3H, s, $CH_3$), 7.13 (1H, t, Ar-H), 7.29 (1H, d, *J*=8.5 Hz, Ar-H), 7.40-7.47 (3H, m, Ar-H), 7.64 (1H, t, Ar-H), 7.73 (1H, d, *J*=7.6 Hz, Ar-H), 7.79 (1H, d, *J*=10.7 Hz, Ar-H), 11.73 (1H, s, NH), 12.41 (1H, s, NH). $\delta_C$ $^{13}$C NMR (125 MHz, DMSO-$d_6$) 21.3 ($CH_3$), 111.8 (Ar-C), 113.5 (Ar-C), 120.9 (Ar-C), 123.8 (Ar-C), 126.4 (Ar-C), 127.1 (Ar-C), 130.6 (Ar-C), 133.8 (Ar-C), 139.9 (Ar-C), 148.8 (Ar-C), 161.2 (Ar-C), 163.0 (Ar-C), 166.9 (C=O), 169.4 (C=O) and 179.2 (C=S).

*2-(((4-fluorophenyl)carbamothioyl)carbamoyl) phenyl acetate (**ATX 3**)*

Yield 0.1020 g, 30.7% as yellowish crystals, m.p.: 141-143 °C, (Found: C, 57.75; H, 3.70; N, 8.78; S, 9.78. $C_{16}H_{13}FO_3N_2S$ Requires C, 57.82; H, 3.94; N, 8.43; S, 9.65%); $\nu_{max}$ FTIR

(KBr/cm$^{-1}$) 3374 (N-H), 1778 (C=O ester), 1667 (C=O amide), 1541 (Ar-C), 1149 (C-N), 744 (C=S). $\delta_H$ $^1$H NMR (500 MHz, DMSO-$d_6$) 2.32 (3H, s, $CH_3$), 7.24-7.31 (3H, m, Ar-H), 7.39 (1H, t, Ar-H), 7.63-7.74 (4H, m, Ar-H), 11.65 (1H, s, NH), 12.24 (1H, s, NH). $\delta_C$ $^{13}$C NMR (125 MHz, DMSO-$d_6$) 21.3 ($CH_3$), 116.0 (Ar-C), 123.8 (Ar-C), 126.4 (Ar-C), 127.1 (Ar-C), 130.5 (Ar-C), 133.7 (Ar-C), 134.8 (Ar-C), 148.8 (Ar-C), 159.6 (Ar-C), 161.5 (Ar-C), 166.9 (C=O), 169.4 (C=O) and 179.8 (C=S).

*2-(((2-chlorophenyl)carbamothioyl)carbamoyl) phenyl acetate (**ATX 4**)*

Yield 0.1155 g, 33.1% as white solid, m.p.: 140-143 °C, (Found: C, 55.40; H, 3.92; N, 8.94; S, 9.13. $C_{16}H_{13}ClO_3N_2S$ Requires C, 55.09; H, 3.76; N, 8.03; S, 9.19%); $\nu_{max}$ FTIR (KBr/cm$^{-1}$) 3357 (N-H), 1777 (C=O ester), 1668 (C=O amide), 1536 (Ar-C), 1155 (C-N), 750 (C=S). $\delta_H$ $^1$H NMR (500 MHz, DMSO-$d_6$) 2.31 (3H, s, $CH_3$), 7.30-7.34 (2H, m, Ar-H), 7.40-7.43 (2H, m, Ar-H), 7.59 (1H, d, *J*=7.7 Hz, Ar-H), 7.64 (1H, t, Ar-H), 7.75 (1H, d, *J*=7.7 Hz, Ar-H), 8.03 (1H, d, *J*=6.2 Hz, Ar-H), 11.79 (1H, s, NH), 12.40 (1H, s, NH). $\delta_C$ $^{13}$C NMR (125 MHz, DMSO-$d_6$) 20.7 ($CH_3$), 123.1 (Ar-C), 125.8 (Ar-C), 126.5 (Ar-C), 127.3 (Ar-C), 127.9 (Ar-C), 128.2 (Ar-C), 128.3 (Ar-C), 129.5 (Ar-C), 130.0 (Ar-C), 133.2 (Ar-C), 135.2 (Ar-C), 148.2 (Ar-C), 166.7 (C=O), 168.8 (C=O) and 179.8 (C=S).

*2-(((3-chlorophenyl)carbamothioyl)carbamoyl) phenyl acetate (**ATX 5**)*

Yield 0.1188 g, 34.1% as yellowish solid, m.p.: 167-177 °C, (Found: C, 54.90; H, 3.57; N, 7.84; S, 9.00. $C_{16}H_{13}ClO_3N_2S$ Requires C, 55.09; H, 3.76; N, 8.03; S, 9.19%); $\nu_{max}$ FTIR (KBr/cm$^{-1}$) 3275 (N-H), 1663 (C=O amide), 1588 (Ar-C), 1173 (C-N), 754 (C=S). $\delta_H$ $^1$H NMR (500 MHz, DMSO-$d_6$) 1.00 (3H, s, $CH_3$), 7.03 (1H, t, Ar-H), 7.34 (1H, d, *J*=6.9 Hz, Ar-H), 7.43 (1H, t, Ar-H), 7.52 (1H, t, Ar-H), 7.59 (1H, d, *J*=6.9 Hz, Ar-H), 7.97-8.00 (3H, m, Ar-H), 11.71 (1H, s, NH), 12.65 (1H, s, NH). $\delta_C$ $^{13}$C NMR (125 MHz, DMSO-$d_6$) 19.0 ($CH_3$), 116.8 117.8 (Ar-C), 120.9 (Ar-C), 123.5 (Ar-C), 124.4 (Ar-C), 126.8 (Ar-C), 130.9

(Ar-C), 131.8 (Ar-C), 133.3 (Ar-C), 136.1 (Ar-C), 139.7 (Ar-C), 157.2 (C=O), 165.4 (C=O) and 178.9 (C=S).

*2-(((4-chlorophenyl)carbamothioyl)carbamoyl) phenyl acetate (**ATX 6**)*

Yield 0.1213 g, 34.8% as white solid, m.p.: 140-141 °C, (Found: C, 54.87; H, 3.85; N, 8.66; S, 9.11. $C_{16}H_{13}ClO_3N_2S$ Requires C, 55.09; H, 3.76; N, 8.03; S, 9.19%); $\nu_{max}$ FTIR (KBr/$cm^{-1}$) 3376 (N-H), 1778 (C=O ester), 1664 (C=O amide), 1528 (Ar-C), 1149 (C-N), 751 (C=S). $\delta_H$ $^1$H NMR (500 MHz, DMSO-$d_6$) 2.31 (3H, s, $CH_3$), 7.29-7.31 (1H, d, *J*=7.7 Hz, Ar-H), 7.39 (1H, t, Ar-H), 7.47 (2H, d, *J*=9.2 Hz, Ar-H), 7.63 (1H, t, Ar-C), 7.71-7.74 (3H, m, Ar-H), 11.70 (1H, s, NH), 12.31 (1H, s, NH). $\delta_C$ $^{13}$C NMR (125 MHz, DMSO-$d_6$) 20.7 ($CH_3$), 123.2 (Ar-C), 125.8 (Ar-C), 126.2 (Ar-C), 126.5 (Ar-C), 128.5 (Ar-C), 130.0 (Ar-C), 130.3 (Ar-C), 133.1 (Ar-C), 136.8 (Ar-C), 148.2 (Ar-C), 166.3 (C=O), 168.8 (C=O) and 178.9 (C=S).

*2-(((2-bromophenyl)carbamothioyl)carbamoyl) phenyl acetate (**ATX 7**)*

Yield 0.1365 g, 34.7% as white solid, m.p.: 130-131 °C, (Found: C, 48.61; H, 3.09; N, 7.02; S, 8.18. $C_{16}H_{13}BrO_3N_2S$ Requires C, 48.87; H, 3.33; N, 7.12; S, 8.15%); $\nu_{max}$ FTIR (KBr/$cm^{-1}$) 3378 (N-H), 1776 (C=O ester), 1667 (C=O amide), 1531 (Ar-C), 1159 (C-N), 755 (C=S). $\delta_H$ $^1$H NMR (500 MHz, DMSO-$d_6$) 2.31 (3H, s, $CH_3$), 7.25-7.32 (2H, m, Ar-H), 7.40-7.47 (2H, m, Ar-H), 7.64 (1H, t, Ar-H), 7.74-7.76 (2H, m, Ar-H), 7.89 (1H, d, *J*=10.0 Hz, Ar-H), 11.86 (1H, s, NH), 12.30 (1H, s, NH). $\delta_C$ $^{13}$C NMR (125 MHz, DMSO-$d_6$) 20.7 ($CH_3$), 119.3 (Ar-C), 123.1 (Ar-C), 125.8 (Ar-C), 126.5 (Ar-C), 127.8 (Ar-C), 128.7 (Ar-C), 129.9 (Ar-C), 132.7 (Ar-C), 133.2 (Ar-C), 136.7 (Ar-C), 148.2 (Ar-C), 166.7 (C=O), 168.8 (C=O) and 179.9 (C=S).

*2-(((3-bromophenyl)carbamothioyl)carbamoyl) phenyl acetate (**ATX 8**)*

Yield 0.1353 g, 34.4% as white solid, m.p.: 113-114 °C, (Found: C, 48.60; H, 3.05; N, 7.09; S, 7.94. $C_{16}H_{13}BrO_3N_2S$ Requires C, 48.87; H, 3.33; N, 7.12; S, 8.15%); $\nu_{max}$ FTIR (KBr/cm$^{-1}$) 3367 (N-H), 1785 (C=O ester), 1675 (C=O amide), 1529 (Ar-C), 1169 (C-N), 863 (C=S). $\delta_H$ $^1$H NMR (500 MHz, DMSO-$d_6$) 2.32 (3H, s, $CH_3$), 7.29-7.31 (1H, d, *J*=8.4 Hz, Ar-H), 7.37-7.42 (2H, m, Ar-H), 7.47 (1H, d, *J*=10.0 Hz, Ar-H), 7.62-7.67 (2H, m, Ar-H), 7.72 (1H, d, *J*=6.9 Hz, Ar-H), 8.06 (1H, s, Ar-H), 11.72 (1H, s, NH), 12.33 (1H, s, NH). $\delta_C$ $^{13}$C NMR (125 MHz, DMSO-$d_6$) 20.8 ($CH_3$), 120.9 (Ar-C), 123.3 (Ar-C), 123.6 (Ar-C), 125.8 (Ar-C), 126.5 (Ar-C), 126.9 (Ar-C), 129.1 (Ar-C), 130.0 (Ar-C), 130.5 (Ar-C), 133.2 (Ar-C), 139.4 (Ar-C), 148.2 (Ar-C), 166.3 (C=O), 168.8 (C=O) and 179.0 (C=S).

*2-(((4-bromophenyl)carbamothioyl)carbamoyl) phenyl acetate (**ATX 9**)*

Yield 0.1285 g, 32.7% as white solid, m.p.: 157-159 °C, (Found: C, 48.31; H, 3.20; N, 7.41; S, 8.37. $C_{16}H_{13}BrO_3N_2S$ Requires C, 48.87; H, 3.33; N, 7.12; S, 8.15%); $\nu_{max}$ FTIR (KBr/cm$^{-1}$) 3382 (N-H), 1775 (C=O ester), 1670 (C=O amide), 1526 (Ar-C), 1169 (C-N), 820 (C=S). $\delta_H$ $^1$H NMR (500 MHz, DMSO-$d_6$) 2.31 (3H, s, $CH_3$), 7.29 (1H, d, *J*=7.6 Hz, Ar-H), 7.39 (1H, t, Ar-H), 7.60-7.67 (4H,m, Ar-H), 7.72 (2H, d, *J*=6.9 Hz, Ar-H), 11.69 (1H, s, NH), 12.30 (1H, s, NH). $\delta_C$ $^{13}$C NMR (125 MHz, DMSO-$d_6$) 20.8 ($CH_3$), 118.6 (Ar-C), 123.3 (Ar-C), 125.8 (Ar-C), 126.5 (Ar-C), 130.0 (Ar-C), 131.5 (Ar-C), 137.3 (Ar-C), 148.2 (Ar-C), 166.4 (C=O), 168.9 (C=O) and 178.8 (C=S).

*2-(((2-iodophenyl)carbamothioyl)carbamoyl) phenyl acetate (**ATX 10**)*

Yield 0.1691 g, 38.4% as yellowish solid, m.p.: 121-122 °C, (Found: C, 43.15; H, 2.65; N, 5.99; S, 7.56. $C_{16}H_{13}IO_3N_2S$ Requires C, 43.65; H, 2.98; N, 6.36; S, 7.28%); $\nu_{max}$ FTIR (KBr/cm$^{-1}$) 3364 (N-H), 1775 (C=O ester), 1665 (C=O amide), 1526 (Ar-C), 1154 (C-N), 747 (C=S). $\delta_H$ $^1$H NMR (500 MHz, DMSO-$d_6$) 2.32 (3H, s, $CH_3$), 7.08 (1H, t, Ar-H), 7.30 (1H, d, *J*=7.7 Hz, Ar-H), 7.40-7.48 (2H, m, Ar-H), 7.64-7.67 (2H, m, Ar-H), 7.74 (1H, d, *J*=7.6 Hz,

Ar-H), 7.94 (1H, d, *J*=7.7 Hz, Ar-H), 11.84 (1H, s, NH), 12.15 (1H, s, NH). $\delta_C$ $^{13}$C NMR (125 MHz, DMSO-$d_6$) 20.9 ($CH_3$), 97.4 (Ar-C), 123.2 (Ar-C), 125.9 (Ar-C), 126.7 (Ar-C), 128.6 (Ar-C), 128.9 (Ar-C), 129.0 (Ar-C), 129.9 (Ar-C), 133.2 (Ar-C), 139.0 (Ar-C), 140.2 (Ar-C), 148.3 (Ar-C), 166.8 (C=O), 168.9 (C=O) and 180.2 (C=S).

*2-(((3-iodophenyl)carbamothioyl)carbamoyl) phenyl acetate (**ATX 11**)*

Yield 0.1850 g, 42% as brownish solid, m.p.: 120-122 °C, (Found: C, 43.77; H, 2.58; N, 6.16; S, 6.90. $C_{16}H_{13}IO_3N_2S$ Requires C, 43.65; H, 2.98; N, 6.36; S, 7.28%); $\nu_{max}$ FTIR (KBr/cm$^{-1}$) 3372 (N-H), 1779 (C=O ester), 1663 (C=O amide), 1527 (Ar-C), 1146 (C-N), 773 (C=S). $\delta_H$ $^1$H NMR (500 MHz, DMSO-$d_6$) 2.32 (3H, s, $CH_3$), 7.20 (1H, t, Ar-H), 7.29 (1H, d, *J*=7.7 Hz, Ar-H), 7.39 (1H, t, Ar-H), 7.63-7.74 (4H, m, Ar-H), 8.16 (1H, s, Ar-H), 11.71 (1H, s, NH), 12.60 (1H, s, NH). $\delta_C$ $^{13}$C NMR (125 MHz, DMSO-$d_6$) 21.4 ($CH_3$), 94.5 (Ar-C), 123.8 (Ar-C), 124.6 (Ar-C), 126.4 (Ar-C), 127.1 (Ar-C), 130.6 (Ar-C), 131.1 (Ar-C), 133.2 (Ar-C), 133.7 (Ar-C), 135.5 (Ar-C), 139.8 (Ar-C), 148.8 (Ar-C), 166.9 (C=O), 169.4 (C=O) and 179.5 (C=S).

*2-(((4-iodophenyl)carbamothioyl)carbamoyl) phenyl acetate (**ATX 12**)*

Yield 0.1567 g, 35.6% as yellowish solid, m.p.: 153-154 °C, (Found: C, 44.01; H, 2.78; N, 6.22; S, 7.06. $C_{16}H_{13}IO_3N_2S$ Requires C, 43.65; H, 2.98; N, 6.36; S, 7.28%); $\nu_{max}$ FTIR (KBr/cm$^{-1}$) 3379 (N-H), 1776 (C=O ester), 1667 (C=O amide), 1525 (Ar-C), 1165 (C-N), 818 (C=S). $\delta_H$ $^1$H NMR (500 MHz, DMSO-$d_6$) 2.31 (3H, s, $CH_3$), 7.29 (1H, d, *J*=8.4 Hz, Ar-H), 7.39 (1H, t, Ar-H), 7.52 (2H, d, *J*=7.7 Hz, Ar-H), 7.63 (1H, t, Ar-H), 7.72 (1H, d, *J*=7.7 Hz, Ar-H), 7.76 (2H, d, *J*=8.4 Hz, Ar-H), 11.68 (1H, s, NH), 12.30 (1H, s, NH). $\delta_C$ $^{13}$C NMR (125 MHz, DMSO-$d_6$) 21.3 ($CH_3$), 91.7 (Ar-C), 123.8 (Ar-C), 126.4 (Ar-C), 127.0 (Ar-C), 127.1 (Ar-C), 130.6 (Ar-C), 133.7 (Ar-C), 137.9 (Ar-C), 138.3 (Ar-C), 148.8 (Ar-C), 166.9 (C=O), 169.4 (C=O) and 179.2 (C=S).