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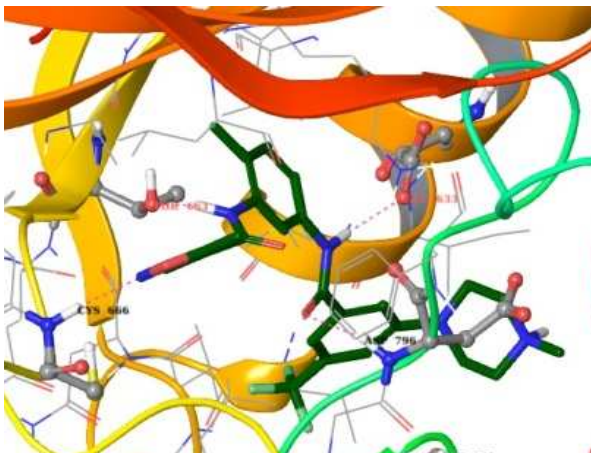
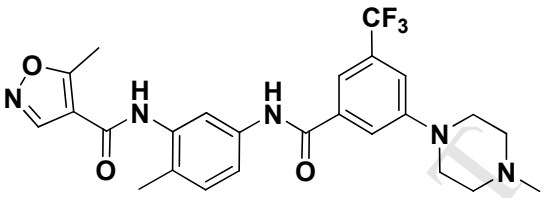
Graphical abstract





IC_{50} / FMS = 9.95 nM
GI_{50}/ U937 = 16.0 nM

Discovery of 4-Arylamido 3-methyl isoxazole derivatives as novel FMS kinase inhibitors

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Abstract – A series of 4-Arylamido 3-methyl isoxazoles were synthesized and evaluated for their antiproliferative activities against the A375P melanoma and U937 hematopoietic cell lines. Most compounds showed selective antiproliferative activity toward the U937 cell line and the activities were better than that of sorafenib, the reference standard. Derivatives were made as amide **5a-b, 6a-o** and urea **7a-n, 8a-g** with hydrophobic moieties, and one of the most potent inhibitor **6a,** 5-methyl-*N*-(2-methyl-5-(3-(4-methylpiperazin-1-yl)-5-(trifluoromethyl)benzamido)phenyl)isoxazole-4-carboxamide was found to be very potent inhibitor of FMS kinase (GI_{50} = 0.016 μM, IC_{50} = 9.95 nM) with excellent selectivity profiles and is a promising candidate for further development in therapeutics for cancer.

Keywords: 4-arylamido 3-methyl isoxazoles; antiproliferative activity; hematopoietic cell line; kinase inhibitor; kinase selectivity.
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1. Introduction

Since the discovery of a protein kinase in 1954 [1], the field of protein kinase drug discovery has advanced enormously [2-5]. Early studies were focused on receptor tyrosine kinases including EGFR, VEGFR, and PDGFR, especially as therapeutic targets for cancer. Receptor tyrosine kinases have been shown not only to be key regulators of normal cellular processes but also to have critical roles in the development and progression of many types of cancer [6]. The impact of RTK inhibition on cancer cells can be illustrated by the assortment of kinase drugs that have been approved by the FDA [7].

Figure 1. FDA approved RTK inhibitors.

Among all the receptor tyrosine kinases known as drug targets, FMS kinase [8-10] has not been given a lot of attention as a therapeutic target. FMS, first discovered as the oncogene responsible for Feline McDonough Sarcoma, is a type III receptor tyrosine kinase that binds to macrophage or monocyte colony-stimulating factor (M-CSF or CSF-1). Ligand stimulated FMS tyrosine phosphorylation regulates the survival, proliferation, and differentiation of monocyte/macrophage lineages [11]. Macrophages, microglia, and osteoclasts play important roles in inflammatory processes [12], and tumor-associated macrophages are increasingly recognized as a driving force for tumor progression and metastasis [13] as well as resistance to chemo- and radiotherapy [14,15]. Thus, the inhibition of

FMS kinase activity has great potential in the treatment of bone osteolysis and inflammation as well as cancers promoted by macrophages [16-20].

Here we report the discovery of a highly selective and potent FMS inhibitor through screening of our in-house protein kinase inhibitor library on A375P [21] and U937 cell lines [22]. During the cellular screening of protein kinase inhibitors, several type II chemical scaffolds were identified as possessing high antiproliferative activities toward the two cell lines, but the compound with a methyl isoxazole scaffold as the hinge binder showed potent antiproliferative activity only for the U937 cell line, prompting us to investigate its kinase profiling. The following experiments showed that 2-methyl-*N*-(5-methylisoxazol-4-yl)benzamide is a selective FMS inhibitor (IC_{50} = 216 nM) with negligible activities toward the other 45 kinases tested (Supplementary information). Subsequently, we decided to synthesize more 2-methyl-*N*-(5-methylisoxazol-4-yl)benzamide derivatives with various aromatic tails.

Figure 2. Initial investigations of various hinge binder and its preference toward cancer cell lines

2. Results and discussion

2.1 Chemistry

The route that enabled the synthesis of the 4-arylamido 3-methyl isoxazoles derivatives is outlined in **Scheme 1**. The synthesis of these 4-arylamido 3-methyl isoxazoles started from commercially available 5-methylisoxazle-carboxylic acid (**1**). The carboxylic acid was converted to acid chloride (**2**) using $SOCl_2$ and subsequently coupled with 2-methyl-4/5-nitroaniline. Then, the nitro group of 5-methyl-*N*-(2-methyl-4-nitrophenyl) isoxazole-4-carboxamide (**3a** and **3b**) was reduced to aniline (**4a** and **4b)** using $SnCl_2$ in acidic conditions. The *N*-(5-amino-2-methylphenyl)-5-methylisoxazole-4/5-carboxamide was then linked with various aromatic acids by using HATU in DMF condition or aromatic carboxylic acid chloride to give amide analogues (**5a-5b** ,**6a-6o),** and coupled with propane-1-sulfonyl chloride to yield **9a-9b.** Urea analogues (**7a**–**7n**, **8a-8g**) were synthesized by directly reacting with various aromatic isocyanates.

Scheme 1.

2.2. Biological evaluation

2.2.1 Antiproliferation assays on cancer cell lines

All the synthesized compounds **5a~5b**, **6a~6o**, **7a~7g**, **8a~8g,** and **9a~9b** were evaluated for their anticancer activity against U937 (human histiocytic lymphoma) and A375P (human melanoma) cell lines by MTT assay. They were also evaluated for cytotoxicity on a human normal cell line, HS27. The results are summarized in **Table 1**. The results of this assay showed that all the tested compounds had selective potency against U937 rather than against A375P (data not shown for A375P because the measured activity for all compounds was negligible). The results also indicated that these compounds exhibited weak cytotoxic activity against normal cell line HS27.

Among the compounds tested, amide link analogue **6a** showed the most potent activity against U937, with an IC_{50} value of 16 nM, which was much better than that of the positive control sorafenib. Structure activity relationships (SARs) were inferred from the data of cell proliferative experiments reported in **Table 1**. First of all, amide link analogues showed more potent activities against U937 than did the urea link analogues. Urea analogues (**7a**~**7g**, **8a**~**8g**), generally, were not as active toward U937, for both the 3- and 4-substituted patterns of the middle phenyl ring.

Secondly, out of these amide analogues, the effects of the substitution pattern of the middle phenyl ring was also very clear, and 3-amide was absolutely more potent, while 4-amide analogues (**5a**~**5b**) showed only weak inhibitory activities. The sulfonamide analogues with a simple propyl chain were not active at all (**9a**~**9b**). The type, number, and position of substituents on the phenyl ring linking the middle phenyl moiety also played important roles in the antiproliferative activities. The inhibitory activities of compounds **6a**~**6o** against U937 varied depending on the different hydrophobic substituents included in the carboxylic acid moiety. Based on the IC_{50} values, compounds could be grouped by activity from most to least potent as follows : compounds with a 1,3,5-substituted benzoic acid tail (**6a, 6m, 6n, 6o**) exhibited potent inhibitory activities with IC_{50}s of 0.016, 0.019, 0.026 and 0.049 μM, respectively; compounds with a 1,3,4-bulky substituents on aromatic acid tail or in similar patterns of imidazole substituent (**6b, 6j**) displayed slightly weaker inhibitory activities with IC_{50} of 0.465 and 0.139 μM; compounds with simpler mono-substitution on a benzene tail (**6d, 6k**) or compounds with a bulky aromatic tail (**6e, 6f, 6g, 6h**) showed even weaker activities.

The above results indicated that the introduction of a bulky aromatic acid substituent through direct amide connection caused dramatic decrease in activity while introduction of 3, 5-disubstituted benzoic acid resulted in high activity, especially when one substituent was a bulky morpholine, alkylpiperazine, or alkylimidazole. Comparing the activities of compounds **6a**, **6m**, **6n**, and **6o** against U937, we found that compound **6a** with $–CF_3$ and methylpiperazinyl substituents at the 3 and 5 positions exhibited the most potent activity (IC_{50} = 0.016 μM).

Table1. Antiproliferative activities of *N*-(4 or 5-amino-2-methylphenyl)-5-methylisoxazole-4-carboxamide derivatives.

2.2.2. Protein Kinase Profiling Assay

As shown in **Table 2**, the representative compound **6a** was screened against a selected panel of 30 different kinases at a single dose concentration of 10 μM and it was revealed that the compound has an excellent selectivity profile. While this compound effectively inhibited the activities of FMS, V600E B-Raf, C-Raf, and Lyn, the activity level of inhibition exerted on most of the other kinases tested was below 10 %. Furthermore, we evaluated the *in vitro* enzymatic inhibition of the compounds, **6a** and **8a** toward FMS, V600E B-Raf, C-Raf, and Lyn. As shown in **Table 3**, compound **6a** demonstrated superb activity on FMS kinase (9.95 nM), but only good potency on V600E B-Raf, C-Raf, and Lyn (5.96 μM, 668 nM, 65.9 nM, and 44.8 nM).

Table 2. Percentages of enzymatic inhibitions by compound **6a** (10 μM) on selected protein kinase panel.

Table 3. IC_{50} for enzymatic inhibitory activity of the selected compounds.

2.3. Molecular docking studies

In order to better understand the interaction between the synthesized compounds and FMS kinase, molecular docking of the potent compound **6a** into the ATP binding site of FMS kinase (PDB: 3LCO) was performed using Glide (SCHRODINGER software package Version 14.2). The binding model is depicted in Fig. 3. In this binding model, compound **6a** is nicely bound to the ATP-binding site of FMS *via* four hydrogen bonds, two π-cation interactions, and an ion interaction.

Figure 3. Docking structures of designed 4-arylamido 3-methyl isoxazoles scaffold amide derivatives (thin, cyan) in FMS (PDB: 3LCO) [27]

The nitrogen atom of 5-methylisoxazole forms a hydrogen bond (N/HeN: 2.0Å) with the amino hydrogen atom of Cys666 and another hydrogen bond (2.43 Å) between oxygen atom of Thr663 with N-H of amide bond, which suggests that the isoxazole group plays an important role in the combination of the receptor and ligand. The middle phenyl ring of **6a** forms a π-cation interaction with the amino group of Lys 616 and there is another π-cation interaction between the positively charged nitrogen atom of the piperazine ring and His776 (shown in light green). Two more hydrogen bonds were identified at the amide bond, one between the *N*-H of the amide bond and the oxygen atom of the GLU 633 residue, and the other between the carbonyl oxygen of the amide bond and hydrogen atom (2.4 Å) of the Asp796 backbone. Furthermore, the strong ion interaction between the protonated nitrogen of the piperazine ring (1.6 Å) and the negative charge of Asp796 might enhance the binding affinity, resulting in the increased antiproliferative activity of this compound. The light blue color shows the hydrophobic regions around the hydrophobic tail.

3. Conclusions

A series of 4-arylamido 3-methyl isoxazole derivatives containing various aromatic moieties were synthesized and verified as novel FMS inhibitors that displayed good antiproliferative activities against tumor cell lines (U937) and weak cytotoxic activities against a human normal cell line and melanoma cell line A375P. Compound **6a** exhibited the most potent inhibitory activity against FMS with an IC_{50} of 9.95 nM, and also showed the greatest inhibitory activities against the hematopoietic cancer cell line U937 with a GI_{50} of 16 nM. Molecular docking of the most potent inhibitor **6a** into the ATP binding site of FMS kinase was performed and the result suggested that compound **6a** could bind well with the FMS active site. The above findings provided a theoretical basis for further structural optimization of 4-arylamido 3-methyl isoxazole derivatives as FMS inhibitors and demonstrated that compound **6a** could be a promising scaffold for new therapeutics for cancer, due to its strong kinase profiling.

4. Materials and methods

4.1. General chemistry

All chemicals (reagent grade) used were purchased from Aldrich (USA). Separation of the compounds by column chromatography was carried out with silica gel 60 (200–300 mesh ASTM, E. Merck, Germany). The quantity of silica gel used was 50–100 times the weight charged on the column. Thin layer chromatography (TLC) was run on the silica gel coated aluminum sheets (silica gel 60 GF254, E. Merck, Germany) and visualized in ultraviolet (UV) light (254 nm). ^{1}H NMR and ^{13}C NMR spectra were recorded on Brucker model digital AVANCE III 400 MHz spectrometer at 25 ^{o}C, using tetramethylsilane (TMS) as the internal standard. High-resolution MS (HR/MS) experiments were conducted with a Finnigan LTQ Orbitrap mass spectrometer (Thermo Fisher Scientific Inc, MA, USA) operated in positive-ion electrospray mode.

4.1.1. Synthesis of 5-methylisoxazole-4-carbonyl chloride (2)

The 5-methylisoxazole-4-carboxylic acid (**1**) (1g, 7.86 mmol) in $SOCl_2$ (3 mL) was heated at 50^{o}C until compound **1** disappeared in TLC. After reaction was terminated, the mixture was cooled to ambient temperature and solvent was evaporated under reduced pressure. 5-methylisoxazole-4-carbonyl chloride, a crude yellow oil (**2**) (96%) was used for the next step without additional purification; ^{1}H NMR (400 MHz, DMSO) δ 8.77 (1H, s), 2.64 (3H, s).

4.1.2. General Syntheses of 5-methyl-*N*-(2-methyl-4-or 5-nitrophenyl) isoxazole-4-carboxamide (3a-3b)

The mixture of 5-methylisoxazole-4-carbonyl chloride (**2**) (1.1 g, 7.55mmol) and 2-methyl-4-nitroaniline (1.15g, 7.55mmol) in THF (75 ml) was heated at 65 ^{o}C until compound **2** disappeared in TLC. After completion of the reaction, the mixture was cooled to ambient temperature and solvent was removed *in vacuo*. The concentrated crude product was purified by flash column chromatography with EA/Hex (1:3) as the eluent to give product

4.1.2.1. 5-methyl-*N*-(2-methyl-4-nitrophenyl)isoxazole-4-carboxamide (3a) : The title compound was isolated as a yellow solid (100%); ^{1}H NMR (400 MHz, DMSO) δ 9.91 (1H, s), 9.06 (1H, s), 8.19 (1H, s), 8.11 (1H, t, *J* = 2.8 Hz), 7.79 (1H, t, *J* = 4.4), 2.68 (3H, s), 2.38 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 173.12, 159.36, 149.29, 144.26, 142.15, 133.75, 125.56, 125.44, 121.55, 111.55, 17.89, 12.13; HRMS (ESI) calcd for $C_{12}H_{11}N_3O_4$ [M+H]+: 262.2334, found 261.9872. 261.0187.; m.p. 169-170^{o}C; IR(ATR) ν_{max}/cm^{-1}: 3437, 1698, 1586, 1543, 1499, 1336, 1276, 1103.

4.1.2.2. 5-methyl-*N*-(2-methyl-4-nitrophenyl)isoxazole-4-carboxamide (3b): The title compound was isolated as a yellow solid (100%); ^{1}H NMR (400 MHz, DMSO) δ 9.94 (1H, s), 9.05 (1H, s), 8.33 (1H, s), 8.04 (1H, t, *J* = 4.0 Hz), 7.58 (1H, d, *J* = 8), 2.68 (3H, s), 2.37 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 173.01, 159.53, 149.30, 145.70, 141.31, 136.57, 131.59, 120.46, 120.33, 111.50, 18.22, 12.16; HRMS (ESI) calcd for $C_{12}H_{11}N_3O_4$ [M+H]+:

262.2334, found 261.9872. 261.0187 ; m.p.: 173-174^{o}C.; IR(ATR) ν_{max}/cm^{-1}: 3340, 3113, 3095, 2928, 2854, 1646, 1532, 1344, 1074

4.1.3. General Synthesis of *N*-(4 or 5-amino-2-methylphenyl)-5-methylisoxazole-4-carboxamide (4a-4b)

5-methyl-*N*-(2-methyl-4 or 5-nitrophenyl) isoxazole-4-carboxamide (**3a** or **3b**) (460 mg, 1.76mmol) and 35% HCl (0.69 mL), $SnCl_2{\cdot}2H_2O$ (1.98 g, 8.80mmol) in EtOH (3.5 mL) was stirredat 80^{o}C. Stirring was continued for 7h and the clear solution was cooled to room temperature. Solvent was removed *in vacuo* and was then poured into ice. The pH was made slightly basic (pH 7–8) by addition of saturated aqueous sodium bicarbonate before being extracted with ethyl acetate. The organic phase was thoroughly washed with brine, and dried over sodium sulfate. The concentrated crude product was purified by flash column chromatography with MC:MeOH (10:1) as the eluent to yield product.

4.1.3.1. *N*-(4-amino-2-methylphenyl)-5-methylisoxazole-4-carboxamide (4a): The title compound was isolated as a yellow solid (93%); ^{1}H NMR (400 MHz, DMSO) δ 9.42 (1H, s), 9.01 (1H, s), 6.87 (1H, d, *J* = 8.4 Hz), 6.44 (1H, s), 6.40 (1H, dd, *J*=2.4, *J*=2.4), 5.06 (2H, –NH, br, s), 2.64 (3H, s), 2.04(3H, s); ^{13}C NMR (101 MHz, DMSO) δ 172.05, 159.29, 149.05, 147.16, 134.55, 127.72, 123.87, 115.19, 111.83, 111.47, 17.98, 11.97.; HRMS (ESI) calcd for $C_{12}H_{13}N_3O_4$ [M+H]+: 231.2505, found 231.9919. 232.0234.; m.p.: 155-156^{o}C.; IR(ATR) ν_{max}/cm^{-1}: 3419, 3337, 3248, 2925, 1656, 1613, 1506, 1485, 1239, 1228.

4.1.3.2 *N*-(5-amino-2-methylphenyl)-5-methylisoxazole-4-carboxamide (4b) : The title compound was isolated as a yellow solid (91%); ^{1}H NMR (400 MHz, DMSO) δ 9.47 (1H, s), 9.00 (1H, s), 6.89 (1H, d, *J* = 8.4 Hz), 6.56 (1H, d, *J* = 2.0 Hz), 6.40 (1H, dd, *J*=2.4, *J*=2.4), 4.94 (2H, –NH, br, s), 2.65 (3H, s), 2.03 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 172.28, 158.92, 149.14, 146.36, 135.69, 130.50, 120.40, 112.51, 112.36, 111.89, 16.97, 12.03; HRMS (ESI) calcd for $C_{12}H_{13}N_3O_4$ [M+H]+: 232.0234, found 232.0549.; mp:169-170^{o}C; IR(ATR) ν_{max}/cm^{-1}: 3436, 3358, 3245, 3204, 2919, 1645, 1526, 1494, 1236, 1151.

4.1.4. General Syntheses of *N*-(4-Arylamido-2-methylphenyl)-5-methylisoxazole-4-carboxamide (5a-5b)

The aryl carboxylic acid (0.18 mmol, 1.2 eq.) in $SOCl_2$ (0.3 mL) was heated at 50^{o}C until aryl carboxylic acid disappeared in TLC. After reaction was terminated, the mixture was cooled to ambient temperature and solvent was evaporated under reduced pressure. Aryl carboxylic acid chloride, a crude yellow oil was diluted with THF and poured into *N*-(4-amino-2-methylphenyl)-5-methylisoxazole-4-carboxamide (**4a**) (34.6 mg, 0.15 mmol, 1eq.) in THF (1.5 mL), was heated at 65^{o}C until compound **4a** disappeared in TLC. Purification of column chromatography with MC/MeOH eluents was performed to afford compound **5.**

4.1.4.1. 5-methyl-*N*-(2-methyl-4-(3-(4-methylpiperazin-1-yl)-5-(trifluoromethyl)benzamido)phenyl) isoxazole -4-carboxamide (5a) : The title compound was isolated as a white solid (16%); ^{1}H NMR (400 MHz, DMSO) δ 10.35 (1H, s), 9.78 (1H, s), 9.03 (1H, s), 7.81 (1H, d, J=2.0 Hz), 7.71 (1H, s), 7.61-7.57 (2H, m), 7.37 (1H, s), 7.27 (1H, d, J=8.4 Hz), 3.38-3.29 (4H, m), 2.67 (3H, s), 2.53-2.50(4H, m), 2.24-2.21 (6H, m); ^{13}C NMR (101 MHz, DMSO) δ 172.49, 164.34, 159.26, 151.26, 149.16, 137.04, 136.79, 136.58, 133.97, 131.48, 131.38, 126.82, 122.80, 122.41, 118.45, 117.33, 113.50, 111.71, 54.27, 47.31, 45.58, 18.14, 12.07.; HRMS (ESI) calcd for $C_{25}H_{26}F_3N_5O_3$ [M+H]+: 502.1988, found 502.3596. 502.1993.; m.p.: 222-230^{o}C. IR(ATR) ν_{max}/cm^{-1}: 3253, 2922, 2844, 1648, 1604, 1507, 1452, 1375 1251, 1166, 1120.

4.1.4.2. 5-methyl-*N*-(2-methyl-4-(3-morpholino-5-(trifluoromethyl)benzamido)phenyl)isoxazole-4-carboxamide (5b) : The title compound was isolated as a white solid (69%); ^{1}H NMR (400 MHz, DMSO) δ 10.33 (1H, s), 9.76 (1H, s), 9.05(1H, s), 8.24-8.21 (2H, m), 7.81 (1H, d, *J*=2.0 Hz), 7.65 (1H, d, *J* = 8.0 Hz), 7.58 (1H, s), 7.25 (1H, d, *J*=8.4 Hz), 3.73-3.71 (4H, m), 2.95-2.93 (4H, m), 2.68 (3H,s), 2.15(3H, s); ^{13}C NMR (101 MHz, DMSO) δ 172.46, 163.68, 159.23, 154.40, 153.93, 149.12, 136.88, 133.96, 132.90, 131.27, 130.61, 126.92, 126.87, 126.82, 124.46, 124.17, 123.83, 122.18, 118.25, 111.69, 66.41, 53.09, 18.12, 12.04.; HRMS (ESI) calcd for $C_{24}H_{23}F_3N_4O_4$ [M+H]+: 489.1671, found 489.3348. 489.1672; m.p.: 176-180^{o}C; IR(ATR) ν_{max}/cm^{-1}: 3273, 2969, 2856, 1645, 1611, 1500, 1298, 1237, 1114, 1060, 1039.

4.1.5. General syntheses of 5-Methyl-isoxazole-4-carboxylic acid (2-methyl-5-aryl-phenyl)-amide (6a-6o)

Method (A) The aryl carboxylic acid (0.18 mmol, 1.2 eq.) in $SOCl_2$ (0.3 mL) was heated at 50^{o}C until aryl carboxylic acid disappeared in TLC. After reaction was terminated, the mixture was cooled to ambient temperature and solvent was evaporated under reduced pressure. Aryl carboxylic acid chloride, a crude yellow oil was diluted with THF and poured into *N*-(4-amino-2-methylphenyl)-5-methylisoxazole-4-carboxamide (**4b**) (34.6 mg, 0.15 mmol, 1eq.) in THF (1.5 mL), was heated at 65^{o}C until compound **4b** disappeared in TLC. Purification of column chromatography with MC/MeOH eluents was performed to afford compound **6.**

Method (B) *N*-(5-amino-2-methylphenyl)-5-methylisoxazole-4-carboxamide (**4b**) (25 mg, 0.11mmol), Aryl acid (1 eq., 0.11mmol), and HATU (61.75 mg, 0.16mmol) in DMF (1.1 mL) was heated at 45^{o}C overnight. The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate. The organic layer was dried over Na_2SO_4. Purification was performed by column chromatography with MC/MeOH = 20:1 to afford compound **6.**

4.1.5.1. 5-Methyl-isoxazole-4-carboxylic acid {2-methyl-5-[3-(4-methyl-piperazin-1-yl)-5-trifluoromethyl-benzoylamino]-phenyl}-amide (6a) : Method (A) The title compound was isolated as a white solid (24%); ^{1}H NMR (400 MHz, DMSO) δ 10.36 (1H, s), 9.78 (1H, s), 9.06 (1H, s), 7.81 (1H, d, *J*=2.0 Hz), 7.71 (1H, s), 7.61-7.57 (2H, m), 7.37 (1H, s), 7.27 (1H, d, *J*=8.4 Hz), 3.38-3.29 (4H, m), 2.68 (3H, s), 2.53-2.50 (4H, m), 2.24-2.21 (6H, m); ^{13}C NMR (101 MHz, DMSO) δ 172.58, 164.31, 159.16, 151.24, 149.16, 136.85, 136.56, 135.46, 130.28,

129.96, 128.99, 125.50, 122.79, 118.72, 118.51, 117.31, 113.50, 111.69, 54.26, 47.31, 45.57, 17.42, 12.06.; HRMS (ESI) calcd for $C_{25}H_{26}F_3N_5O_3$ [M+H]+: 502.1988, found 502.2390. 502.1995.; m.p.: 208-210^oC; IR(ATR) ν_{max}/cm^{-1}: 3255, 2923, 2845, 1651, 1598, 1526, 1450, 1304, 1278, 1244, 1167, 1119.

4.1.5.2. 5-Methyl-isoxazole-4-carboxylic acid [2-methyl-5-(4-morpholin-4-yl-3-trifluoromethyl-benzoyl amino)-phenyl]-amide (6b) : Method (B) The title compound was isolated as a white solid (19%); ^1H NMR (400 MHz, DMSO) δ 10.37 (1H, s), 9.76 (1H, s), 9.05(1H, s), 8.24-8.21 (2H, m), 7.81 (1H, d, *J*=2.0 Hz), 7.65 (1H, d, *J*=8.0 Hz), 7.58-7.55 (1H, m), 7.25 (1H, d, *J*=8.4 Hz), 3.73-3.71 (4H, m), 2.95-2.93 (4H, m), 2.67 (3H,s), 2.19 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 172.56, 163.65, 159.13, 154.38, 149.15, 136.93, 135.45, 132.90, 130.59, 130.28, 128.90, 126.90, 126.85, 124.44, 123.83, 118.50, 118.32, 111.67, 66.41, 53.08, 17.40, 12.05.; HRMS (ESI) calcd for $C_{24}H_{23}F_3N_4O_4$ [M+H]+: 489.1671, found 489.2651. 489.1680.; m.p.: 205-206^oC.; IR(ATR) ν_{max}/cm^{-1}: 3247, 3128, 2956, 2864, 1641, 1609, 1520, 1502, 1486, 1291, 1275, 1240, 1131, 1111.

4.1.5.3. 5-Methyl-isoxazole-4-carboxylic acid [5-(4-chloro-3-trifluoromethyl-benzoylamino)-2-methyl-phenyl]-amide (6c) : Method (A) The title compound was isolated as a white solid (64%); ^1H NMR (400 MHz, DMSO) δ 10.53 (1H, s), 9.77 (1H, s), 9.05 (1H, s), 8.39 (1H, s), 8.38-8.24 (1H, m), 7.93 (1H, d, *J*=8.4 Hz), 7.83 (1H, d, *J*=2 Hz), 7.59 (1H, dd, *J*=2.4 Hz), 7.28 (1H, d, *J* = 8.4 Hz), 2.68 (3H, s), 2.21 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 173.91, 163.44, 159.64, 149.64, 137.15, 135.99, 134.53, 134.36, 133.86, 132.41, 130.85, 129.64, 127.47, 127.25, 126.94, 119.01, 118.84, 112.16, 17.92, 12.55.; HRMS (ESI) calcd for $C_{20}H_{15}ClF_3N_3O_3$ [M+H]+: 438.0754, found 438.2612; m.p.: 179-180^oC.; IR(ATR) ν_{max}/cm^{-1}: 3227, 3119, 3069, 1638, 1607, 1524, 1490, 1332, 1308, 1279, 1255, 1246, 1110, 1091, 1036.

4.1.5.4. 5-Methyl-isoxazole-4-carboxylic acid {5-[2-(2-fluoro-phenyl)-acetylamino]-2-methyl-phenyl}-amide (6d) : **Method (A)** The title compound was isolated as a white solid (79%); ^1H NMR (400 MHz, DMSO) δ 10.22 (1H, s), 9.70 (1H, s), 9.03 (1H, s), 7.67 (1H, d, *J*=1.6 Hz), 7.40-7.36 (2H, m), 7.34-7.28 (1H, m), 7.19-7.14 (3H, m), 2.66 (3H, s), 2.16 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 172.14, 167.50, 158.66, 148.73, 136.74, 135.09, 131.53, 131.49, 129.96, 127.71, 123.80, 123.77, 116.73, 116.54, 114.69, 114.47, 111.26, 35.86, 16.94, 11.63. ; HRMS (ESI) calcd for $C_{20}H_{18}FN_3O_3$ [M+H]+: 368.1332, found 368.1450. 368.1332 ; m.p.: 171-174^oC.; IR (ATR) ν_{max}/cm^{-1}: 3236, 3042, 1666, 1638, 1606, 1521, 1308, 1291, 1242, 1180, 1130.

4.1.5.5. 5-Methyl-isoxazole-4-carboxylic acid {5-[2,2-bis-(4-chloro-phenyl)-acetylamino]-2-methyl-phenyl}-amide (6e) : Method (A) The title compound was isolated as a white solid (88%); ^1H NMR (400 MHz, DMSO) δ 10.47 (1H, s), 9.69 (1H, s), 9.02 (1H, s), 7.71 (1H, s), 7.41 (4H, d, *J*=8.4 Hz), 7.35 (5H, d, *J*=7.2 Hz), 7.19 (1H, d, *J*=8.4 Hz), 5.17 (1H, s), 2.65 (3H, s), 2.16 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 172.60, 169.11, 159.11, 149.18, 138.51, 136.84, 135.60, 131.80, 130.47, 130.35, 128.53, 128.48, 117.29, 117.14, 111.69, 55.76, 17.41, 12.08.; HRMS (ESI) calcd for $C_{26}H_{21}Cl_2N_3O_3$ [M+H]+: 494.0960, found 494.0594. 494.0989.; m.p.: 178-181^oC.; IR (ATR) ν_{max}/cm^{-1}: 3230, 3063, 1639, 1607, 1521, 1489, 1241, 1092.

4.1.5.6. 5-Methyl-isoxazole-4-carboxylic acid {5-[(6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carbonyl)-amino]-2-methyl-phenyl}-amide (6f) : Method (B) The title compound was isolated as a white solid (51%); ^{1}H NMR (400 MHz, DMSO) δ 9.72 (1H, s), 9.21 (1H, s), 9.02 (1H, s), 7.67 (1H, d, *J*=2.0 Hz), 7.52 (1H, s), 7.36 (1H, dd, *J*=8.4 Hz, J=2.4 Hz), 7.18 (1H, d, *J*=8.4 Hz), 2.68 (3H, s), 2.16 (6H, d, *J*=7.6 Hz), 2.07 (3H, s), 2.17-1.50 (4H, m), 1.99 (3H, d, *J*=7.2Hz) 1.50 (3H, d, *J*=4.8 Hz); ^{13}C NMR (101 MHz, DMSO) δ 172.59, 172.11, 159.11, 149.17, 146.03, 143.90, 136.30, 135.45, 130.22, 128.92, 122.79, 121.43, 120.35, 118.15, 118.08, 117.05, 111.66, 77.43, 29.35, 23.80, 20.13, 17.43, 12.81, 12.17, 12.08, 11.83.; HRMS (ESI) calcd for $C_{26}H_{29}N_3O_5$ [M+H]+: 464.2107, found 464.3754. 464.2116.; m.p.: 155-156^{o}C. ; IR (ATR) ν_{max}/cm^{-1}: 3395, 3292, 2923, 2854, 1655, 1610, 1519, 1449, 1415, 1240, 1126, 1087.

4.1.5.7. 5-Methyl-isoxazole-4-carboxylic acid [5-(2-1H-indol-3-yl-acetylamino)-2-methyl-phenyl]-amide (6g) : Method (B) The title compound was isolated as a white solid (17%); ^{1}H NMR (400 MHz, DMSO) δ 10.88 (1H, s), 10.06 (1H, s), 9.67 (1H, s), 9.01 (1H, s), 7.66-7.58 (2H, m), 7.33 (2H, s), 7.24 (1H, s), 7.17 (1H, s), 7.06 (1H, s), 6.99 (1H, d, *J*=7.2 Hz), 3.71 (2H, s), 2.66 (3H, s), 2.15 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 172.57, 169.63, 159.07, 149.18, 137.45, 136.12, 135.48, 130.34, 128.00, 127.20, 123.86, 121.00, 118.71, 118.39, 117.12, 116.98, 111.70, 111.38, 108.59, 33.84, 17.39, 12.08.; HRMS (ESI) calcd for $C_{22}H_{20}N_4O_3$ [M+H]+: 389.1535, found 389.3299.389.1539 ; m.p.: 169-171^{o}C.; IR(ATR) ν_{max}/cm^{-1}: 3267, 2922, 1561, 1606, 1523, 1488, 1455, 1287, 1131, 1093.

4.1.5.8. 5-Methyl-isoxazole-4-carboxylic acid {5-[2-(2-cyano-phenylsulfanyl)-benzoylamino]-2-methyl-phenyl}-amide (6h): Method (B) The title compound was isolated as white solid (52%); ^{1}H NMR (400 MHz, DMSO) δ 10.51 (1H, s), 9.80 (1H, s), 9.03 (1H, s), 7.91(1H, s), 7.81(1H, s), 7.72-7.65 (2H, m), 7.52-7.39 (5H, m), 7.24 (1H, d, *J*=8.0 Hz), 7.18-7.15 (1H, m), 2.67 (3H, s), 2.18 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 172.53, 165.69, 159.09, 149.16, 139.10, 138.48, 136.97, 135.54, 134.25, 134.03, 132.81, 132.22, 131.63, 131.12, 130.31, 129.01, 128.51, 128.40, 127.79, 117.83, 117.74, 116.85, 114.05, 111.67, 17.47, 12.05.; HRMS (ESI) calcd for $C_{26}H_{20}N_4O_3S$ [M+H]+: 469.1256, found 469.0677. 469.1261.; m.p.: 218-220^{o}C. IR(ATR) ν_{max}/cm^{-1}: 3227, 3119, 2220, 1643, 1633, 1607, 1595, 1519, 1319, 1242.

4.1.5.9. 5-Methyl-isoxazole-4-carboxylic acid [5-(2-biphenyl-4-yl-acetylamino)-2-methyl-phenyl]-amide (6i) : Method (B) The title compound was isolated as white solid (30%); ^{1}H NMR (400 MHz, DMSO) δ 10.21 (1H, s), 9.70 (1H, s), 9.03 (1H, s), 7.67-7.61(5H, m), 7.47-7.33 (6H, m), 7.19 (1H, d, *J*=8.0 Hz), 3.66 (2H, d, *J*=8.0 Hz), 2.68 (3H, d, *J*=8.0 Hz), 2.16 (3H, d, *J*=7.6 Hz); ^{13}C NMR (101 MHz, DMSO) δ 172.59, 168.96, 159.10, 149.19, 139.99, 138.52, 137.25, 135.53, 135.31, 130.40, 129.66, 128.94, 128.15, 127.34, 126.66, 126.59, 117.17, 117.00, 111.71, 42.98, 17.40, 12.09.; HRMS (ESI) calcd for $C_{26}H_{23}N_3O_3$ [M+H]+: 426.1739, found 426.1245. 426.1740.; m.p.: 135-138^{o}C.; IR(ATR) ν_{max}/cm^{-1}: 3234, 3047, 1643, 1622, 1613, 1601, 1543, 1240, 1134.

4.1.5.10. 5-Methyl-isoxazole-4-carboxylic acid {2-methyl-5-[(1-phenyl-5-trifluoromethyl-1H-pyrazole-4-carbonyl)-amino]-phenyl}-amide (6j) : Method (B) The title compound was isolated as white solid (74%); ^{1}H

NMR (400 MHz, DMSO) δ 10.53 (1H, s), 9.79 (1H, s), 9.05 (1H, s), 8.31 (1H, s), 7.80 (1H, s), 7.62-7.60 (3H, m), 7.54-7.53 (2H, m), 7.47 (1H, d, *J* = 8.0 Hz), 7.26 (1H, d, *J* = 8.4 Hz), 2.68 (3H, s), 2.19 (3H, d, *J* = 8.0 Hz); ^{13}C NMR (101 MHz, DMSO) δ 172.61, 159.16, 158.85, 149.20, 139.75, 138.74, 136.75, 135.62, 130.45, 130.09, 129.57, 129.43, 129.15, 126.01, 121.50, 120.69, 117.83, 117.71, 111.68, 17.49, 12.09.; HRMS (ESI) calcd for $C_{23}H_{18}F_3N_5O_3$ [M+H]+: 470.1362, found 470.0538. 470.1365.; m.p.: 173-176^{o}C.; IR(ATR) ν_{max}/cm^{-1}: 3252, 3027, 1646, 1608, 1531, 1499, 1488, 1297, 1242, 1177, 1149, 1135

4.1.5.11. 5-Methyl-isoxazole-4-carboxylic acid [5-(3,5-dimethyl-benzoylamino)-2-methyl-phenyl]-amide (6k) : Method (B) The title compound was isolated as white solid (53%); ^{1}H NMR (400 MHz, DMSO) δ 10.16 (1H, d, *J* = 7.6 Hz), 9.78 (1H, s), 9.06 (1H, s), 7.83 (1H, d, *J* = 6.0 Hz), 7.57-7.54 (3H, m), 7.24 (2H, m), 2.68 (3H, s), 2.35 (6H, d, *J* = 8.4 Hz), 2.19 (3H, d, *J* =8.0 Hz); ^{13}C NMR (101 MHz, DMSO) δ 172.57, 165.61, 159.13, 149.18, 137.51, 137.31, 135.41, 134.91, 132.79, 130.22, 128.67, 125.33, 118.39, 118.21, 111.70, 20.86, 17.44, 12.07; HRMS (ESI) calcd for $C_{21}H_{21}N_3O_3$ [M+H]+: 364.1583, found 364.1226. 364.1587.; m.p.: 215-216^{o}C.; IR(ATR) ν_{max}/cm^{-1}: 3225, 3047, 1650, 1635, 1621, 1597, 1537, 1238, 1135.

4.1.5.12. 5-Methyl-isoxazole-4-carboxylic acid {5-[(biphenyl-4-carbonyl)-amino]-2-methyl-phenyl}-amide (6l) : Method (B) The title compound was isolated as white solid (14%); ^{1}H NMR (400 MHz, DMSO) δ 10.31 (1H, s), 9.79 (1H, s), 9.06 (1H, s), 8.05 (2H, s), 7.85-7.83 (3H, m), 7.75 (2H, m), 7.59 (1H, s), 7.50 (2H, d), 7.42 (1H, s), 7.24 (1H, s), 2.68 (3H, d, *J* = 3.2 Hz), 2.20 (3H, d, *J* = 2.8 Hz); ^{13}C NMR (101 MHz, DMSO) δ 172.58, 165.01, 159.15, 149.19, 143.08, 139.11, 137.25, 135.45, 133.63, 130.27, 129.07, 128.78, 128.35, 128.15, 126.92, 126.58, 118.46, 118.30, 111.71, 17.46, 12.08.; HRMS (ESI) calcd for $C_{25}H_{21}N_3O_3$ [M+H]+: 412.1583, found 412.1126. 412.1590.; m.p.: 182-186^{o}C.; IR(ATR) ν_{max}/cm^{-1}: 3284, 1639, 1607, 1530, 1486, 1326, 1240.

4.1.5.13. 5-Methyl-isoxazole-4-carboxylic acid {2-methyl-5-[3-(2-methyl-imidazol-1-yl)-5-trifluoromethyl-benzoylamino]-phenyl}-amide (6m) : Method (A) The title compound was isolated as white solid (60.5%) ^{1}H NMR (400 MHz, DMSO) δ 10.82 (1H, s), 9.90 (1H, s), 9.15 (1H, s), 8.54 (2H, s), 8.31 (1H, d, s), 7.99 (1H, s), 7.85 (1H, s), 7.71 (1H, s), 7.65-7.63 (1H, m), 7.28 (1H, d, *J*=8.4 Hz), 2.67 (3H, s), 2.55 (3H, s), 2.21 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 172.65, 162.76, 159.21, 149.20, 138.56, 137.16, 136.62, 135.57, 130.69, 130.43, 129.27, 128.34, 127.68, 124.90, 124.87, 123.67, 123.63, 121.13, 118.61, 118.41, 111.71, 17.46, 13.58, 12.10.; HRMS (ESI) calcd for $C_{24}H_{20}F_3N_5O_3$ [M+H]+: 484.1518, found 483.3688. 483.1524.; m.p.: 210-214^{o}C.; IR(ATR) ν_{max}/cm^{-1}: 3292, 3118, 2928, 2853, 2811, 1674, 1651, 1601, 1336, 1314, 1287, 1243, 1171, 1164, 1042

4.1.5.14. 5-Methyl-isoxazole-4-carboxylic acid {2-methyl-5-[3-(5-methyl-imidazol-1-yl)-5-trifluoromethyl-benzoylamino]-phenyl}-amide (6n) : Method (B) The title compound was isolated as white solid (22%); ^{1}H NMR (400 MHz, DMSO) δ 10.79 (1H, s), 9.88 (1H, s), 9.46 (1H, s), 9.14 (1H, s), 8.60 (2H, d, *J* = 10.8 Hz), 8.41 (1H, s), 7.85 (1H, s), 7.65 (2H, s), 7.29 (1H, d, *J* = 8.0 Hz), 2.67 (3H, s), 2.26 (3H, s) 2.21 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 172.66, 162.46, 159.23, 149.32, 137.43, 136.60, 136.37, 135.60, 134.50, 130.97, 130.72, 130.39, 130.33, 130.20, 129.57, 124.56, 121.85, 118.86, 118.53, 117.30, 111.67, 17.55, 12.12, 9.26.; HRMS (ESI) calcd for

$C_{24}H_{20}F_3N_5O_3$ [M+H]+: 484.1518, found 484.0913. 484.1525.; m.p.: 225-226°C.; IR(ATR) ν_{max}/cm^{-1}: 3328, 3206, 3109, 3047, 2981, 2719, 2649, 1672, 1661, 1614, 1538, 1524, 1375 1168, 1128.

4.1.5.15. 5-Methyl-isoxazole-4-carboxylic acid {2-methyl-5-[3-(4-methyl-imidazol-1-yl)-5-trifluoromethyl-benzoylamino]-phenyl}-amide (6o) : Method (B) The title compound was isolated as white solid (32%); ^{1}H NMR (400 MHz, DMSO) δ 10.88 (1H, s), 9.88 (1H, s), 9.84 (1H, s), 9.14 (1H, s), 8.77 (1H, s), 8.43 (1H, s), 8.28 (2H, s), 7.89 (1H, s), 7.70 (1H, d, *J* = 8.0 Hz), 7.29 (1H, d, *J* = 8.0 Hz), 2.68 (3H, s), 2.36 (3H, s) 2.22 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 172.68, 162.55, 159.24, 149.31, 137.47, 136.66, 135.76, 135.57, 134.58, 130.90, 130.84, 130.57, 130.31, 129.57, 125.23, 124.86, 124.61, 118.83, 118.54, 117.43, 111.68, 17.56, 12.13, 9.89.; HRMS (ESI) calcd for $C_{24}H_{20}F_3N_5O_3$ [M+H]+: 484.1518, found 483.0598. 484.1517.; m.p.: 239-240°C.; IR(ATR) ν_{max}/cm^{-1}: 3233, 1665, 1613, 1541, 1523, 1495, 1378, 1275, 1122, 1094.

4.1.6. General Syntheses of *N*-(5-(3-Aryl ureido)-2-methylphenyl)-5-methylisoxazole-4-carboxamide (7a-7g)

The mixture of compound **4b** (10 mg, 0.043 mmol), and aryl isocyanate (6.7 mg, 0.043 mmol), in THF (0.4 ml) was stirred at r.t overnight. After completion of the reaction, solvent was removed *in vacuo* and the concentrated crude product was purified by flash column chromatography with MC: MeOH = 20:1 as the eluent.

4.1.6.1. 5-Methyl-isoxazole-4-carboxylic acid {5-[3-(4-chloro-phenyl)-ureido]-2-methyl-phenyl}-amide (7a) : The title compound was isolated as white solid (85%); ^{1}H NMR (400 MHz, DMSO) δ 9.75 (1H, s), 9.35 (1H, s), 9.11 (1H, s), 9.03 (1H, s), 8.06 (1H, s), 7.84-7.58 (5H, m), 7.23 (1H, s), 2.67 (3H, s), 2.15 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 172.55, 170.38, 161.41, 159.11, 152.42, 149.22, 138.75, 137.56, 135.67, 130.48, 128.64, 126.90, 125.31, 119.70, 116.29, 111.77, 59.79, 20.79, 17.33, 14.11, 12.10.; HRMS (ESI) calcd for $C_{19}H_{17}ClN_4O_3$ [M+H]+: 385.0989, found 385.1133. 407.0978. (Na^+); m.p.: 205-207°C.; IR(ATR) ν_{max}/cm^{-1}: 3381, 1698, 1642, 1596, 1537, 1490, 1281, 1206, 1134, 1094, 1042.

4.1.6.2. 5-Methyl-isoxazole-4-carboxylic acid {2-methyl-5-[3-(3-trifluoromethyl-phenyl)-ureido]-phenyl}-amide (7b) : The title compound was isolated as pale yellow solid (37%); ^{1}H NMR (400 MHz, DMSO) δ 9.75 (1H, s), 9.38 (1H, s), 9.11 (1H, s), 9.06 (1H, s), 8.01 (1H, s),7.94 (2H, s), 7.55 (1H, s), 7.51-7.48 (1H, m), 7.30 (1H, d, J=7.6 Hz), 7.23-7.15 (1H, m),2.67 (3H, s), 2.15 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 172.52, 159.10, 152.48, 149.20, 140.60, 137.36, 135.67, 130.45, 129.90, 129.67, 129.35, 127.07, 125.57, 121.78, 118.03, 116.44, 114.09, 111.75, 17.32, 12.07.; HRMS (ESI) calcd for $C_{20}H_{17}F_3N_4O_3$ [M+H]+: 419.1253, found 419.1513. 419.1258.; m.p.: 204-205°C. IR(ATR) ν_{max}/cm^{-1}: 3320, 3227, 2914, 1644, 1613, 1533, 1336, 1170, 1123.

4.1.6.3. 5-Methyl-isoxazole-4-carboxylic acid {5-[3-(4-chloro-3-trifluoromethyl-phenyl)-ureido]-2-methyl-phenyl}-amide (7c) : The title compound was isolated as pale yellow solid (90%); ^{1}H NMR (400 MHz, DMSO) δ 9.71 (1H, s), 9.10 (1H, s), 9.03 (1H, s), 8.85 (1H, s), 8.12 (1H, s), 7.60-7.56 (3H, m), 7.19 (2H, s), 2.67 (3H, s), 2.16 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 172.14, 167.50, 158.66, 148.73, 136.74, 135.09, 131.53, 131.49, 129.96, 127.71, 123.80, 123.77, 116.73, 116.54, 114.69, 114.47, 111.26, 35.86, 16.94, 11.63.; HRMS (ESI) calcd

for $C_{20}H_{16}ClF_3N_4O_3$ [M+H]+: 453.0863, found 453.1885. 453.0866.; m.p.: 215-216^oC. IR(ATR) ν_{max}/cm^{-1}: 3227, 3110, 2930, 1678, 1656, 1610, 1598, 1540, 1231, 1180, 1131, 1112.

4.1.6.4. 5-Methyl-isoxazole-4-carboxylic acid {5-[3-(2-fluoro-phenyl)-ureido]-2-methyl-phenyl}-amide (7d) : The title compound was isolated as pale yellow solid (76%); ^1H NMR (400 MHz, DMSO) δ 9.71 (1H, s), 9.05 (1H, s), 8.93 (1H, s), 8.80 (1H, s), 7.65-7.02 (7H, m), 2.67 (3H, s), 2.16 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 172.55, 159.11, 152.15, 150.76, 149.22, 137.46, 135.75, 130.55, 127.64, 127.54, 126.91, 124.54, 122.43, 122.35, 120.43, 116.00, 111.77, 17.33, 12.09.; HRMS (ESI) calcd for $C_{19}H_{17}FN_4O_3$ [M+H]+: 369.1285, found 369.1312. 369.1291.; m.p.: 215-216^oC. IR(ATR) ν_{max}/cm^{-1}: 3322, 3222, 1685, 1633, 1599, 1547, 1312, 1287, 1255, 1234, 1185.

4.1.6.5. 5-Methyl-isoxazole-4-carboxylic acid {5-[3-(3-chloro-phenyl)-ureido]-2-methyl-phenyl}-amide (7e) : The title compound was isolated as white solid (36%); ^1H NMR (400 MHz, DMSO) δ 9.70 (1H, s), 9.03 (1H, s), 8.84 (1H, s), 8.76 (1H, s), 7.70 (1H, s), 7.55 (1H, s), 7.30-7.23 (3H, m), 7.18 (1H, d, *J* = 5.6Hz), 7.01 (1H, d, *J* = 6Hz), 2.67 (3H, s), 2.15 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 173.00, 159.56, 152.82, 149.67, 141.76, 139.05, 137.89, 136.13, 133.66, 130.93, 130.86, 127.46, 121.86, 117.97, 117.06, 116.80, 112.22, 17.79, 12.55.; HRMS (ESI) calcd for $C_{19}H_{17}ClN_4O_3$ [M+H]+: 385.0989, found 385.0453. 385.0984.; m.p.: 218-221^oC. IR(ATR) ν_{max}/cm^{-1}: 3298, 3223, 2922, 1638, 1586, 1557, 1281, 1228, 1173, 1130, 1103.

4.1.6.6. 5-Methyl-isoxazole-4-carboxylic acid {5-[3-(3,5-dichloro-phenyl)-ureido]-2-methyl-phenyl}-amide (7f) : The title compound was isolated as white solid (16%); ^1H NMR (400 MHz, DMSO) δ 9.75 (1H, s), 9.52 (1H, s), 9.22 (1H, s), 9.07 (1H, s), 7.53-7.52 (3H, m), 7.22 (1H, dd, *J* = 8.3, 2.2 Hz), 7.17 (1H, d, *J* = 8.4 Hz), 7.14 (1H, t, *J* = 1.9 Hz), 2.68 (3H, s), 2.16 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 172.55, 159.10, 152.32, 149.23, 142.43, 137.26, 135.69, 134.10, 130.49, 127.24, 120.70, 116.43, 116.35, 116.01, 111.74, 17.35, 12.10.; HRMS (ESI) calcd for $C_{19}H_{16}Cl_2N_4O_3$ [M+H]+: 419.0599, found 419.0153. 441.0591(Na^+); m.p.: 212-213^oC. IR(ATR) ν_{max}/cm^{-1}: 3302, 3241, 2920, 2853, 1652, 1584, 1538, 1504, 1306, 1239.

4.1.6.7. 5-Methyl-isoxazole-4-carboxylic acid {5-[3-(3,4-dichloro-phenyl)-ureido]-2-methyl-phenyl}-amide (7g) : The title compound was isolated as white solid (33%); ^1H NMR (400 MHz, DMSO) δ 9.70 (1H, s), 9.13-8.81 (3H, m), 7.87 (1H, d, J=6.4 Hz), 7.53(2H, d, *J*=9.6 Hz), 7.33 (2H, d, *J*=8.0Hz), 7.18 (1H, d, *J*=2.0Hz), 2.66 (3H, s), 2.15 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 172.56, 159.11, 152.30, 149.21, 140.01, 137.30, 135.68, 131.04, 130.57, 130.49, 127.14, 123.05, 119.26, 118.33, 116.48, 116.46, 111.76, 17.34, 12.10.; HRMS (ESI) calcd for $C_{19}H_{16}Cl_2N_4O_3$ [M+H]+: 419.0599, found 419.0493. 419.0595.; m.p.: 209-210^oC.; IR(ATR) ν_{max}/cm^{-1}: 3366, 3273, 3181, 3113, 2906, 1669, 1608, 1582, 1544, 1474, 1320, 1133, 1125, 1027.

4.1.7. General Syntheses of *N*-(4-(3-Aryl ureido)-2-methylphenyl)-5-methylisoxazole-4-carboxamide (8a-8f)

The mixture of compound **4a** (10 mg, 0.043 mmol), aryl isocyanate (1 eq., 0.043 mmol), in THF (0.4 ml) was stirred at r.t for overnight. After completion of the reaction, solvent was removed by vacuo and the concentrated crude product was purified by flash column chromatography with MC: MeOH = 20:1 as the eluent.

4.1.7.1. 5-Methyl-isoxazole-4-carboxylic acid {4-[3-(4-chloro-3-trifluoromethyl-phenyl)-ureido]-2-methyl-phenyl}-amide (8a): The title compound was isolated as white solid (99%); ^{1}H NMR (400 MHz, DMSO) δ 9.63 (1H, s), 9.15 (1H, s), 9.02 (1H, s), 8.81 (1H, s), 8.12 (1H, s), 7.61 (2H, s), 7.38 (1H, s), 7.29 (1H, d, *J*=6.0Hz), 7.20 (1H, s), 2.66 (3H, s), 2.18 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 172.44, 159.28, 152.42, 149.16, 139.40, 137.29, 134.38, 132.02, 129.95, 127.19, 126.56, 123.05, 122.25, 120.25, 116.76, 116.70, 116.41, 111.73, 18.14, 12.07.; HRMS (ESI) calcd for $C_{20}H_{16}ClF_3N_4O_3$ [M+H]+: 453.0863, found 453.0186. 453.0865.; m.p.: 217-219^{o}C. IR(ATR) ν $_{max}$/cm^{-1}: 3283, 3214, 3063, 1646, 1632, 1609, 1548, 1293, 1257, 1230, 1170, 1136, 1122, 1113.

4.1.7.2. 5-Methyl-isoxazole-4-carboxylic acid {2-methyl-4-[3-(3-trifluoromethyl-phenyl)-ureido]-phenyl}-amide (8b) : The title compound was isolated as white solid (64%); ^{1}H NMR (400 MHz, DMSO) δ 9.64 (1H, s), 9.04 (2H, d, *J*=4.8 Hz), 8.77 (1H, s), 8.03 (1H, s), 7.57-7.49 (2H, m), 7.39(1H, d, *J* = 2.4 Hz), 7.31-7.28 (2H, m), 7.20 (1H, d, *J* = 8.4Hz), 2.66 (3H, s), 2.19 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 172.88, 159.74, 152.98, 149.62, 141.08, 137.91, 134.83, 130.39, 130.27, 130.15, 127.65, 123.35, 122.27, 120.56, 118.49, 116.72, 114.53, 112.20, 18.60, 12.53.; HRMS (ESI) calcd for $C_{20}H_{17}F_3N_4O_3$ [M+H]+: 419.1253, found 419.1173. 419.1252.; m.p.: 206-208^{o}C. IR(ATR) ν $_{max}$/cm^{-1}: 3286, 3241, 3041, 1639, 1561, 1338, 1125.

4.1.7.3. 5-Methyl-isoxazole-4-carboxylic acid {4-[3-(3-chloro-phenyl)-ureido]-2-methyl-phenyl}-amide (8c) : The title compound was isolated as white solid (66%); ^{1}H NMR (400 MHz, DMSO) δ 9.63 (1H, s), 9.03 (1H, s), 8.93 (1H, s), 8.76 (1H, s), 7.71 (1H, s), 7.37 (1H, s), 7.29 (2H, d, *J* = 6.4 Hz), 7.19 (2H, d, *J* = 4.8Hz), 7.01 (1H, d, *J* = 4.0Hz), 2.66 (3H, s), 2.18 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 172.43, 159.30, 152.52, 149.20, 141.43, 137.65, 134.40, 133.23, 130.42, 129.65, 127.25, 121.32, 119.82, 117.36, 116.45, 115.99, 111.76, 18.17, 12.09.; HRMS (ESI) calcd for $C_{19}H_{17}ClN_4O_3$ [M+H]+: 385.0989, found 385.0793. 407.0981(Na^{+}); m.p.: 217-219^{o}C.; IR(ATR) ν $_{max}$/cm^{-1}: 3313, 3270, 1638, 1622, 1587, 1548, 1305, 1221.

4.1.7.4. 5-Methyl-isoxazole-4-carboxylic acid {4-[3-(2-fluoro-phenyl)-ureido]-2-methyl-phenyl}-amide (8d) : The title compound was isolated as white solid (53%); ^{1}H NMR (400 MHz, DMSO) δ 9.64 (1H, s), 9.05 (2H, d, J=5.2 Hz), 8.55 (1H, s), 8.15 (1H, s), 7.36 (1H, s), 7.28 (1H, s), 7.23 (2H, d, J=9.2 Hz), 7.13 (1H, s), 7.00 (1H, s), 2.67 (3H, s), 2.19 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 172.43, 159.28, 152.19, 150.79, 149.16, 137.56, 134.46, 129.71, 127.64, 127.53, 127.29, 124.56, 122.46, 120.50, 119.70, 115.86, 115.08, 114.89, 111.75, 18.16, 12.07.; HRMS (ESI) calcd for $C_{19}H_{17}FN_4O_3$ [M+H]+: 369.1285, found 369.0972. 369.1280.; m.p.: 240-245^{o}C.; IR(ATR) ν $_{max}$/cm^{-1}: 3353,3281, 1651, 1638, 1619, 1596, 1565, 1460, 1308, 1233.

4.1.7.5. 5-Methyl-isoxazole-4-carboxylic acid {4-[3-(4-chloro-phenyl)-ureido]-2-methyl-phenyl}-amide (8e) : The title compound was isolated as white solid (63%); ^{1}H NMR (400 MHz, DMSO) δ 9.68 (1H, s), 9.38 (1H, s),

9.20 (1H, s), 9.07 (1H, s), 7.49 (2H, dd, *J* = 2.4 Hz, *J* = 2.4 Hz), 7.34-7.28 (4H, m), 7.17 (1H, d, *J* = 8.8 Hz), 2.65 (3H, s), 2.17 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 172.38, 159.26, 152.43, 149.13, 138.73, 137.63, 134.33, 128.61, 127.18, 125.30, 119.91, 119.82, 119.70, 116.07, 111.73, 18.14, 12.05.; HRMS (ESI) calcd for $C_{19}H_{17}ClN_4O_3$ [M+H]+: 385.0989, found 385.1133. 407.0982(Na^+); m.p.: 230-240^{o}C.; IR(ATR) ν_{max}/cm^{-1}: 3295, 2922, 2855, 1637, 1559, 1520, 1489, 1305, 1221.

4.1.7.6. 5-Methyl-isoxazole-4-carboxylic acid {4-[3-(3,4-dichloro-phenyl)-ureido]-2-methyl-phenyl}-amide (8f) : The title compound was isolated as white solid (42%); ^{1}H NMR (400 MHz, DMSO) δ 9.63 (1H, s), 9.02 (2H, s), 8.80 (1H, s), 7.88 (1H, s), 7.51 (1H, d, *J* = 7.2 Hz), 7.36(1H, s), 7.32-7.27 (2H, m), 7.19 (1H,s), 2.65 (3H, s), 2.18 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 172.43, 159.28, 152.35, 149.16, 140.03, 137.39, 134.37, 131.05, 130.59, 129.84, 127.20, 123.03, 120.09, 119.22, 118.30, 116.24, 111.73, 18.15, 12.07.; HRMS (ESI) calcd for $C_{19}H_{16}Cl_2N_4O_3$ [M+H]+: 419.0599, found 419.0493. 419.0596.; m.p.: 235-237^{o}C. IR(ATR) ν_{max}/cm^{-1}: 3305, 3272, 1651, 1637, 1621, 1586, 1550, 1305, 1224, 1131.

4.1.7.7. 5-Methyl-isoxazole-4-carboxylic acid {4-[3-(3,5-dichloro-phenyl)-ureido]-2-methyl-phenyl}-amide (8g) : The title compound was isolated as white solid (38%); ^{1}H NMR (400 MHz, DMSO) δ 9.87 (1H, s), 9.70 (1H, s), 9.38 (1H, s), 9.08 (1H, s), 7.52 (2H, s), 7.35 (1H, s), 7.28 (1H, s), 7.19 (1H,s), 7.14 (1H,s), 2.66 (3H, s), 2.18 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 172.38, 159.24, 152.20, 149.11, 142.28, 137.20, 134.32, 134.06, 129.95, 127.14, 120.83, 120.22, 116.37, 116.27, 111.70, 18.10, 12.03.; HRMS (ESI) calcd for $C_{19}H_{16}Cl_2N_4O_3$ [M+H]+: 419.0599, found 419.2533. 419.0591.; m.p.: 230-231^{o}C.; IR (ATR) ν_{max}/cm^{-1}: 3300, 3266, 2922, 1638, 1584, 1543, 1517, 1304, 1240.

4.1.8. General Syntheses of 5-Methyl-isoxazole-4-carboxylic acid [2-methyl-5 or 4-(propane-1-sulfonylamino)-phenyl]-amide (9a-9b)

The mixture of compound **4** (20 mg, 0.086 mmol), propane-1-sulfonyl chloride (13.85 mg, 97%, 0.095 mmol), in MC (0.4 ml) was stirred at r.t for overnight. After the reaction was completed, solvent was removed by evaporation, and the concentrated crude product was purified by flash column chromatography with EA/Hex (1:1) as the eluent to produce compound **9.**

4.1.8.1. 5-Methyl-isoxazole-4-carboxylic acid [2-methyl-5-(propane-1-sulfonylamino)-phenyl]-amide (9a) : The title compound was isolated as yellow solid (99%); ^{1}H NMR (400 MHz, DMSO) δ 9.75 (1H, s), 9.66 (1H, s), 9.01 (1H, s), 7.24 (1H, d, *J*=8.4 Hz), 7.09-7.04 (2H, m), 3.07-3.04 (2H, m), 2.65 (3H, s), 2.17 (3H, s), 1.69 (2H, d, *J*=7.2Hz), 0.93 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 173.09, 159.57, 149.68, 136.78, 136.58, 131.52, 129.37, 117.98, 117.94, 112.10, 52.49, 17.82, 17.28, 13.04, 12.56.; HRMS (ESI) calcd for $C_{15}H_{19}N_3O_4S$ [M+H]+: 338.1096, found 338.2801. 338.1090.; m.p.: 34-36^{o}C.; IR(ATR) ν_{max}/cm^{-1}: 3247, 2963, 2917, 2878, 1650, 1610, 1525, 1484, 1383, 1320, 1240, 1132.

4.1.8.2. 5-Methyl-isoxazole-4-carboxylic acid [2-methyl-4-(propane-1-sulfonylamino)-phenyl]-amide (9b) : The title compound was isolated as yellow solid (64%); ^{1}H NMR (400 MHz, DMSO) δ 9.74 (2H, s), 9.02 (1H, s), 7.20 (2H, s), 7.03 (1H, d, *J* = 8.0 Hz), 3.03 (2H, s), 2.66 (3H, s), 2.15 (3H, s), 1.68-1.66 (2H, m), 0.93 (3H, s); ^{13}C NMR (101 MHz, DMSO) δ 172.48, 159.26, 149.13, 136.34, 134.88, 131.35, 127.57, 121.21, 117.29, 111.66, 52.29, 18.11, 16.85, 12.57, 12.04.; HRMS (ESI) calcd for $C_{15}H_{19}N_3O_4S$ [M+H]+: 338.1096, found 338.1524. 338.1094.; m.p.: 38-43^{o}C.; IR(ATR) ν_{max}/cm^{-1}: 3248, 2967, 2922, 1649, 1613, 1500, 1482, 1384, 1320, 1240, 1141, 1092.

4.2 Determinations of antiproliferation assays on cancer cell lines

A375P cells were purchased from American Type Culture Collection (ATCC, Rockville, MD, USA) and were maintained in DMEM medium (Welgene, Daegu, Korea) supplemented with 10% FBS (Welgene) and 1% penicillin/streptomycin (Welgene) in a humidified atmosphere with 5% CO_2 at 37 ^{o}C. A375P cells were taken from the culture substrate with 0.05% trypsin-0.02% EDTA and plated at a density of 5×10^3 cells/well in 96 well plates and then incubated at 37 ^{o}C for 24 h in a humidified atmosphere with 5% CO_2 prior to treatment with test compounds at various concentrations (3-fold serial dilution, 12 points). The A357P cell viability was assessed by the conventional 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) reduction assay. MTT assays were carried out with CellTiter 96® (Promega) according to the manufacturer's instructions. The absorbance at 590 nm was recorded using EnVision 2103 (Perkin Elmer; Boston, MA, USA). The IC_{50} was calculated using GraphPad Prism 4.0 software.

U937 cells were purchased from American Type Culture Collection (ATCC, Rockville, MD, US) and maintained in RPMI 1640 medium (Welgene, Daegu, Korea) supplemented with 10% FBS (Welgene), 1% penicillin/streptomycin (Welgene) and 25 mM HEPES (Welgene) in a humidified atmosphere with 5% CO_2 at 37 ^{o}C. U937 cells were taken from the culture substrate and plated at a density of 5×10^3 cells/well in 96 well plates and then incubated at 37℃ for 24 h in a humidified atmosphere with 5% CO_2 prior to test compounds at various concentration (3-fold serial dilution, 12 points). The U937 cell viability was assessed by the conventional 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay. MTT assays were carried out with Thiazolyl Blue Tetrazolium Bromide (SIGMA) according to the manufacturer's instructions. The absorbance at 570 nm was recorded using Multiskan EX (Thermo; Waltham, MA, USA). The IC50 was calculated using GraphPad Prism 4.0 software

4.3 Docking Simulations

Molecular docking of compound **6a** into the 3D x-ray structure of FMS (PDB code: 3LCO) was carried out using Glide (Schrodinger software package Version 14.1). The 3D x-ray protein structure of FMS as a complex with ligand was obtained from the PDB and prepared using Protein Preparation Wizard of the Schrodinger Maestro program. The structures of new designed inhibitors were drawn using Chemdraw, and their 3D conformations were generated using the Schrodinger LigPrep program with the OPLS 2005 force field. When making an optimal grid

file, a grid box was manually adjusted from a cube into a cuboid, constraints (hydrogen bonding and hydrophobic cube) were added, and unwanted pockets were treated as excluded volume. Molecular docking of compound **6a** into the structure of FMS produced predictive docking pose, (1) SP (standard precision) and XP (extra precision) docking of compound **6a**, (2) revision of the docking poses through substructure energy minimization using Schrodinger Macro Model, and (3) scoring of revised the docking pose.

4.4 Selected Kinases Profiling

We used Reaction Biology Corp. *Kinase HotSpot*^{SM} service (www.reactionbiology.com) for screening of **6a**, and IC_{50} *Profiler Express* for IC_{50} measurement. Their assay protocol was as follows: in a final reaction volume of 25 μL FMS (h) (5-10 mU) was incubated with 25 mM Tris pH 7.5, 0.02 mM EGTA, 0.66 mg/mL myelin basic protein, 10 mM magnesium acetate and [γ-^{33}P-ATP] (specific activity approx. 500 cpm/pmol, concentration as required). The reaction is initiated by the addition of the Mg-ATP mix. After incubation for 40 minutes at room temperature, the reaction was stopped by the addition of 5 μL of a 3% phosphoric acid solution. 10 μL of the reaction was then spotted onto a P30 filtermat and washed three times for 5 minutes in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting.

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Supplementary data

Supplementary data related to this article can be found at

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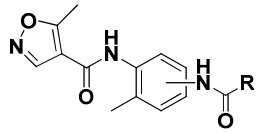
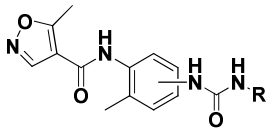
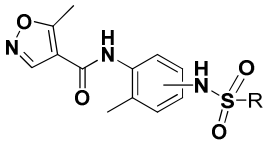
Table1. Antiproliferative activities of *N*-(4 or5-amino-2-methylphenyl)-5-methylisoxazole-4-carboxamide derivatives.



5a~5b, 6a~6y



7a~7n



8a~8b

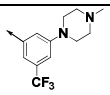
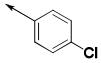
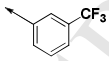
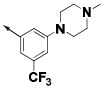
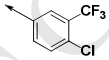
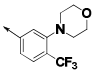
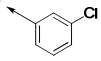
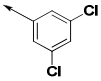
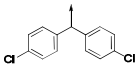
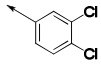
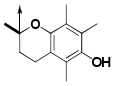
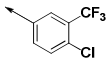
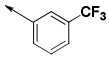
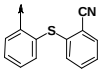
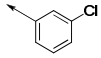
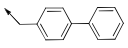
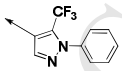
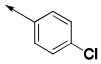
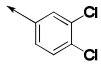
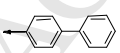
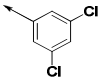
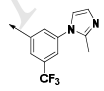
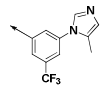
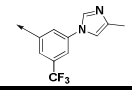
No	Substit-ution	R	U937 (GI_{50}, μM)	HS27 (GI_{50}, μM)	No	Substit-ution	R	U937 (GI_{50}, μM)	HS27 (GI_{50}, μM)
5a	*4*-amide		> 30	> 30	**7a**	*3*-urea		> 30	> 30
5b	*4*-amide		> 30	> 30	**7b**	*3*-urea		> 30	> 30
6a	*3*-amide		0.016 ± 0.006	> 30	**7c**	*3*-urea		> 30	> 30
6b	*3*-amide		0.465 ± 0.022	> 30	**7d**	*3*-urea		> 30	> 30
6c	*3*-amide		> 30	> 30	**7e**	*3*-urea		> 30	> 30
6d	*3*-amide		> 30	> 30	**7f**	*3*-urea		> 30	> 30
6e	*3*-amide		> 30	> 30	**7g**	*3*-urea		1.89 ± 0.31	> 30
6f	*3*-amide		> 30	> 30	**8a**	*4*-urea		16.5 ± 1.44	> 30
6g	*3*-amide		> 30	> 30	**8b**	*4*-urea		> 30	> 30
6h	*3*-amide		> 30	> 30	**8c**	*4*-urea		> 30	> 30
6i	*3*-amide		> 30	> 30	**8d**	*4*-urea		> 30	> 30
6j	*3*-amide		0.139 ± 0.019	> 30	**8e**	*4*-urea		> 30	> 30
6k	*3*-amide		3.1 ± 0.05	> 30	**8f**	*4*-urea		> 30	> 30
6l	*3*-amide		> 30	> 30	**8g**	*4*-urea		> 30	> 30
6m	*3*-amide		0.019 ± 0.007	> 30	**9a**	*3-sulfone amide*		> 30	> 30
6n	*3*-amide		0.026 ± 0.003	> 30	**9b**	*4-sulfon eamide*		> 30	> 30
6o	*3*-amide		0.049 ± 0.040	> 30		**Sorafenib**		2.74 ± 0.05	> 30

Table 2. Percentages of enzymatic inhibitions by compound **6a** (10 μM) on selected Protein Kinases panel.

Kinase	% Inhibition	Staurosporine IC_{50} (nM)
AKT1	5.73	4.62
ALK	0	< 1.00
Aurora A	0	< 1.00
BRAF	61.2	14.0
BRAF (V599E)	87.9	4.50
c-Kit	7.5	84.2
c-MET	0	179
CDK1/cyclin B	0	2.52
CDK2/cyclin E	1.85	< 1.00
EGFR	4.68	283
ERK1	7.54	6540
FAK/PTK2	0	13.2
FGFR2	3.60	1.77
FGFR3	0	10.3
FLT3	2.12	< 1.00
FMS	98.7	1.14
GSK3b	0	2.62
IGF1R	0	43.3
JAK3	0	< 1.00
JNK3	15.3	1100[b]
KDR/VEGFR2	8.27	10.7
Lyn	97.8	< 1.00
MEK1	0	12.1
mTOR/FRAP1	1.14	9900[c]
PKA	0	< 1.00
PLK1	0	192
RAF1	95.9	2.87[a]
RON/MST1R	0	326
ROS/ROS1	0	< 1.00
SYK	3.81	< 1.00

a. Data of GW5074 [23]
b. Data of JNKi VIII [24-25]
c. Data of LY294002 [26]

Table 3. IC_{50} for enzymatic inhibitory activity of the selected compounds.

	IC_{50}				
	B-Raf	B-Raf V600E	FMS	Lyn	C-Raf
6a	5.96 μM	668 nM	9.95 nM	65.9 nM	44.8 nM

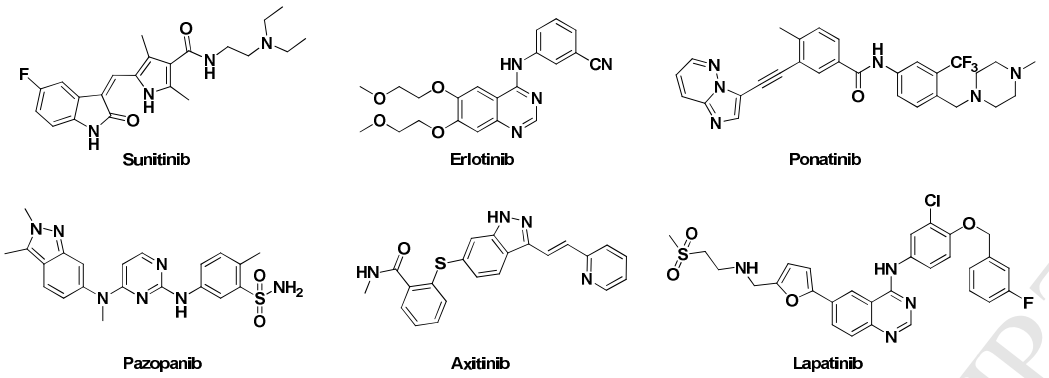
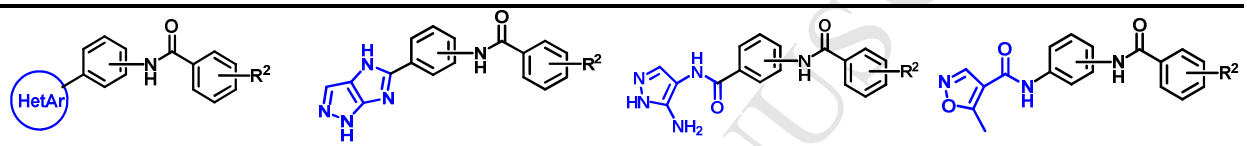
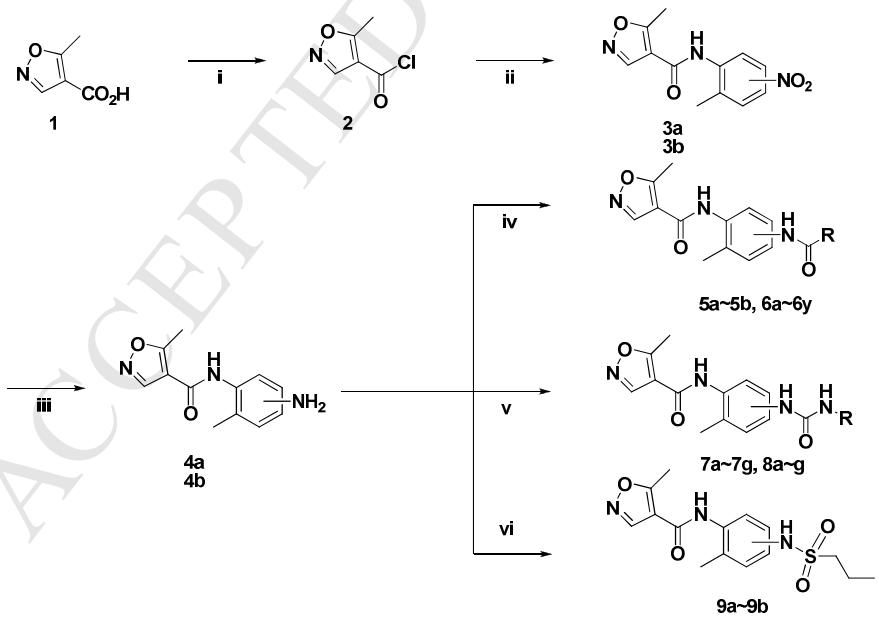


Figure 1. FDA approved RTK inhibitors.



Antiproliferative activity	A375P	+++	+++	
	U937	-	+	+++

Figure 2. Initial investigations of various hinge binder and its preference toward cancer cell lines



Scheme 1. (i) 5-methylisoxazole-4-carboxylic acid, $SOCl_2$, 50°C; (ii) 2-methyl-4-nitroaniline, 2-methyl-5-nitroaniline, THF, 65°C, (iii) $SnCl_2$, HCl, EtOH, 7h, 80°C (iv) (a) RCOOH, HATU, DMF, rt, overnight (b) RCOCl, THF, 65°C (v) RNCO,THF, rt, overnight (vi) propane-1-sulfonyl chloride, pyridine, M,C , rt

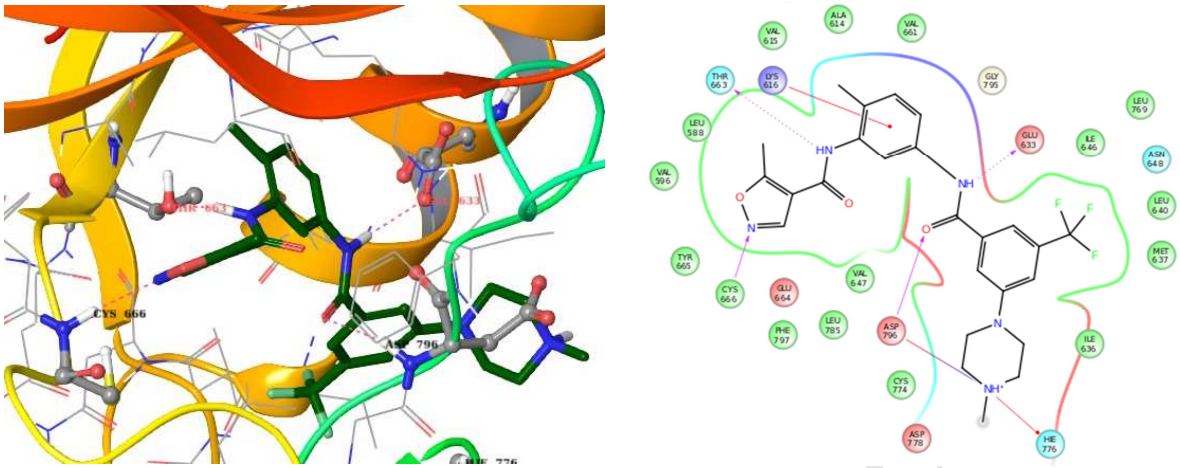


Figure 3. Docking structures of designed 4-arylamido 3-methyl isoxazoles scaffold amide derivatives (thin, cyan) in FMS (PDB: 3LCO) [27]

We synthesized 4-arylamido 3-methyl isoxazoles as potential protein kinase inhibitor.

We found their potent antiproliferative activities on U937 hematopoietic cell line.

The most potent inhibitor was found to be a potent and selective FMS inhibitor.

Supplementary material

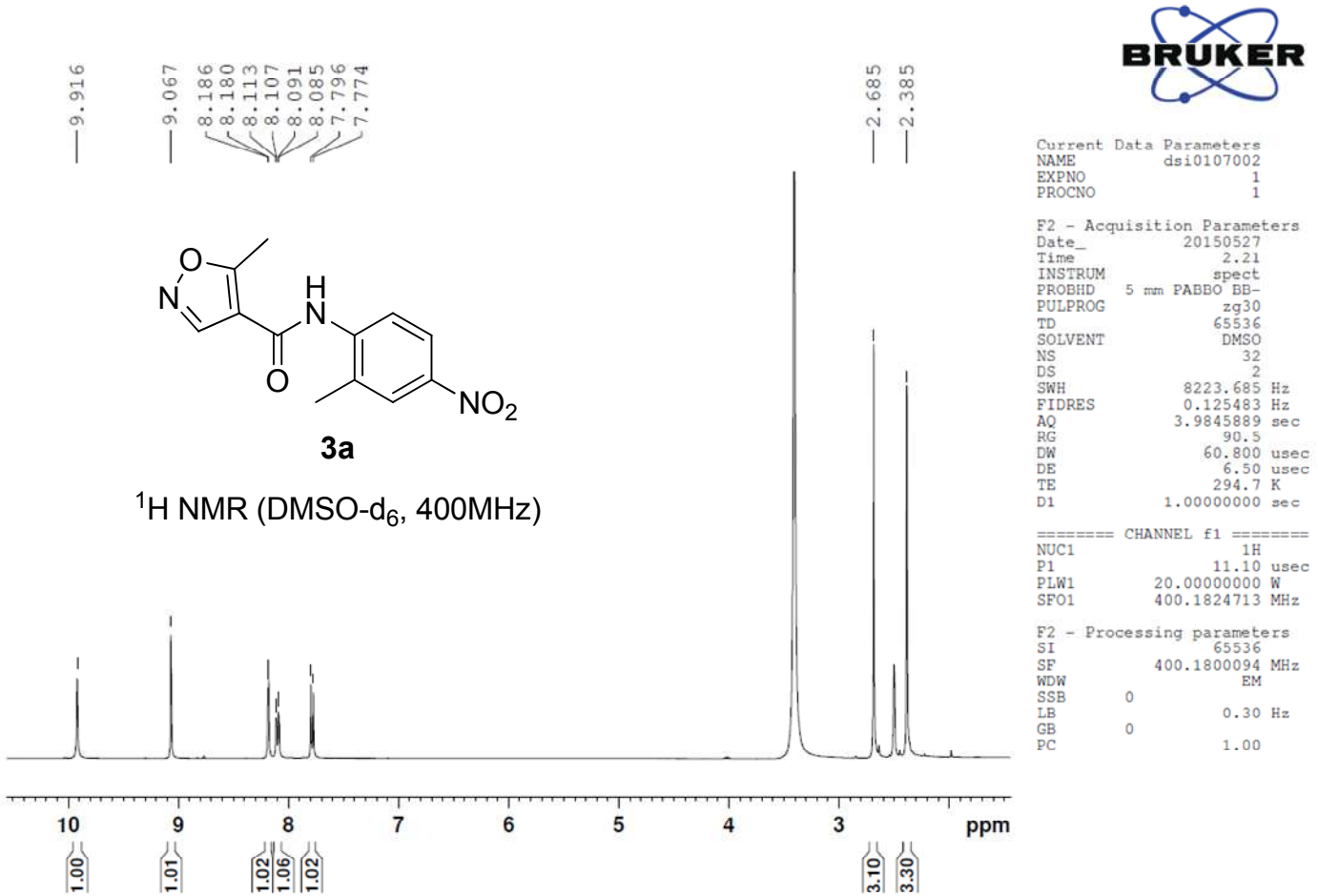
Discovery of 4-Arylamido 3-methyl isoxazole derivatives as novel FMS kinase inhibitors

Daseul Im, Kyungjin Jung, Songyi Yang, Waqar Aman, and Jung-Mi Hah*

Department of Pharmacy, College of Pharmacy, & Institute of Pharmaceutical Science and Technology, Hanyang University 55 Hanyangdaehak-ro, Sangnok-gu, Ansan Kyeonggi-do, 426-791, Korea

[1] **The list of kinases tested** for the first hit compound 2-methyl-*N*-(5-methylisoxazol-4-yl)benzamide analogue (10 μM) in figure 2 is ABL1, AKT1, ALK, Aurora A, BRAF, BRAF (V599E), c-Kit, c-MET, c-Src, CDK1/cyclin B, CDK2/cyclin E, EGFR, ERK1, FAK/PTK2, FGFR1, FGFR2, FGFR3, FLT1, FLT3, FLT4, GSK3b, Hck, IGF1R, ITK, JAK1, JAK3, JNK2, JNK3, KDR/VEGFR2, Lck, Lyn, MEK1, mTOR, PDGFRa, PDGFRb, PI3K, PKA, PKC, PLK1, RAF1, Ret, RON/MST1R, ROS/ROS1, SYK, Tie2.

[2] ^{1}H and ^{13}C NMR Spectra of representative compounds

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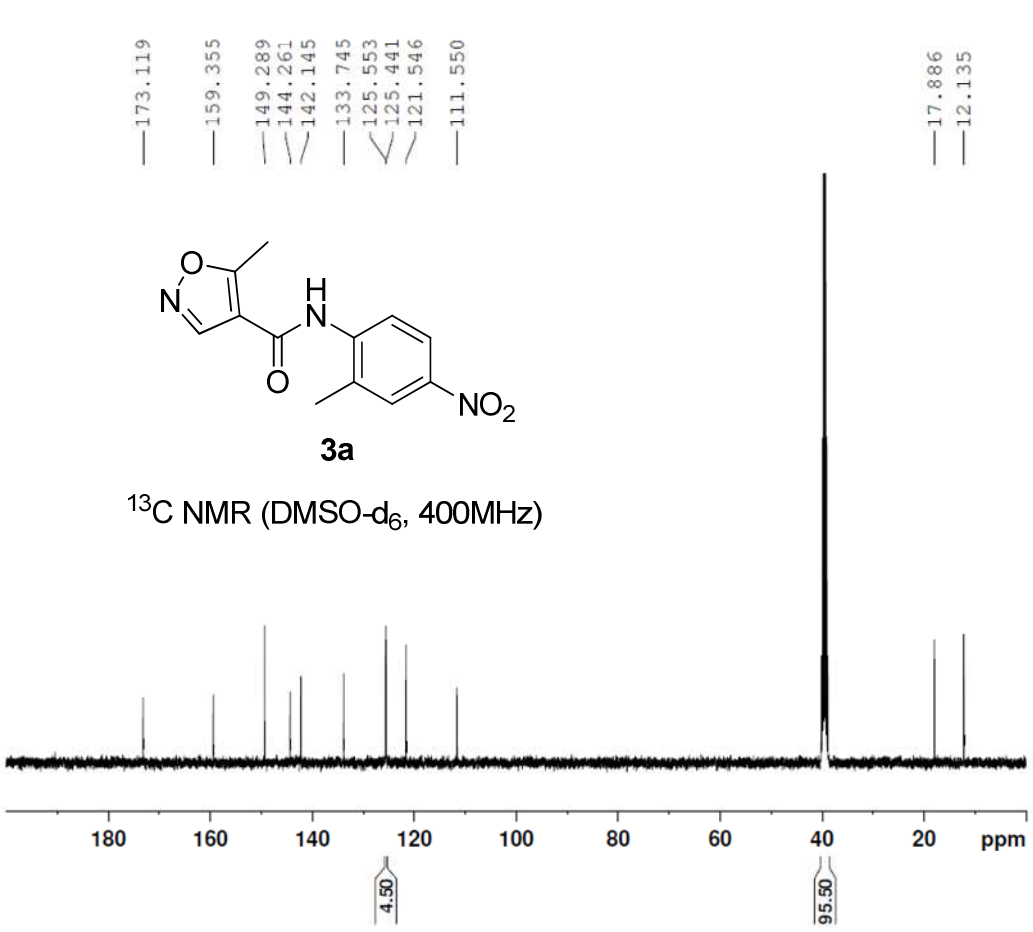


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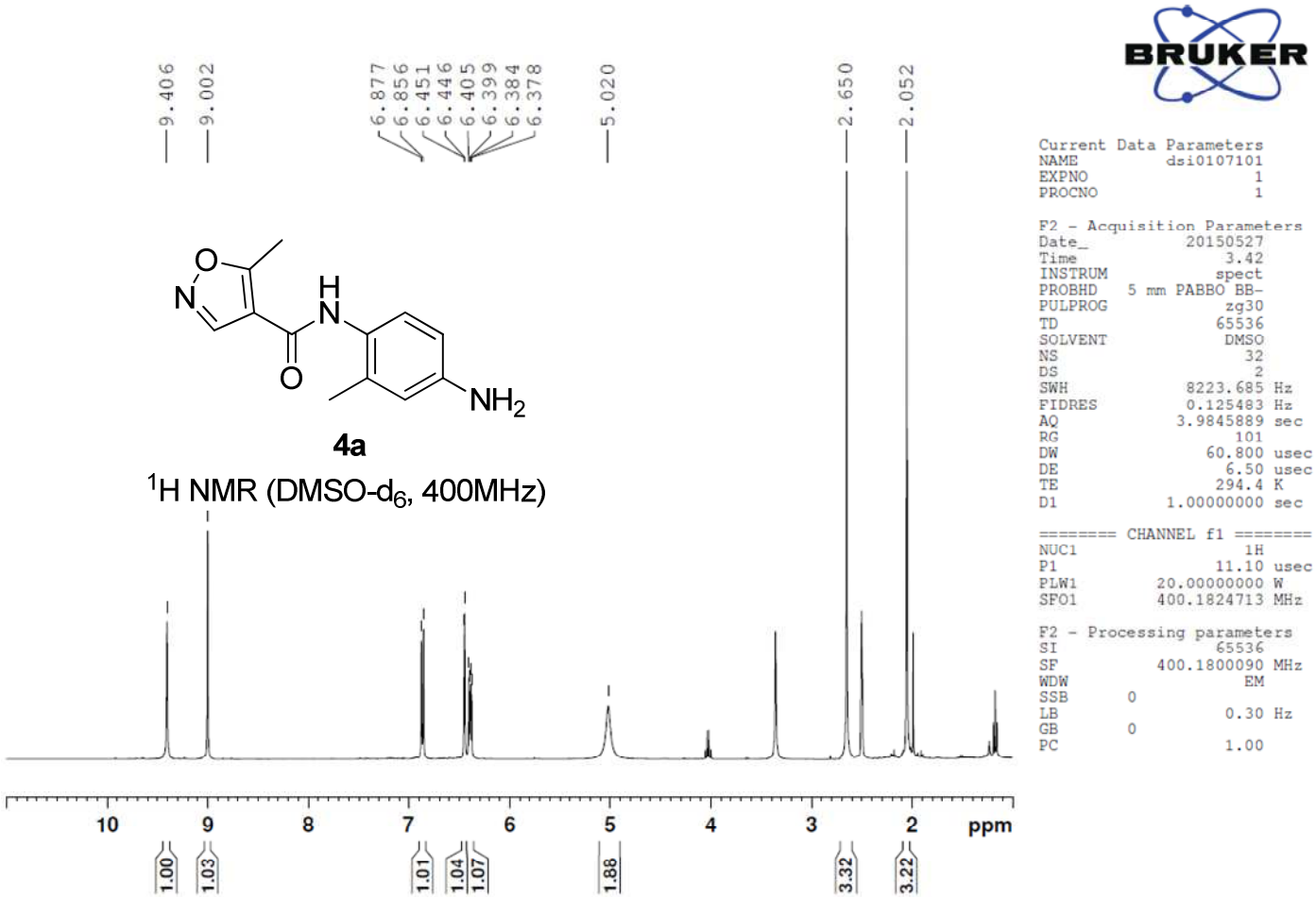


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AQ                  3.9845889 sec
RG                       101
DW                    60.800 usec
DE                     6.50 usec
TE                    294.4 K
D1                  1.00000000 sec

======== CHANNEL f1 ========
NUC1                       1H
P1                      11.10 usec
PLW1              20.00000000 W
SFO1              400.1824713 MHz

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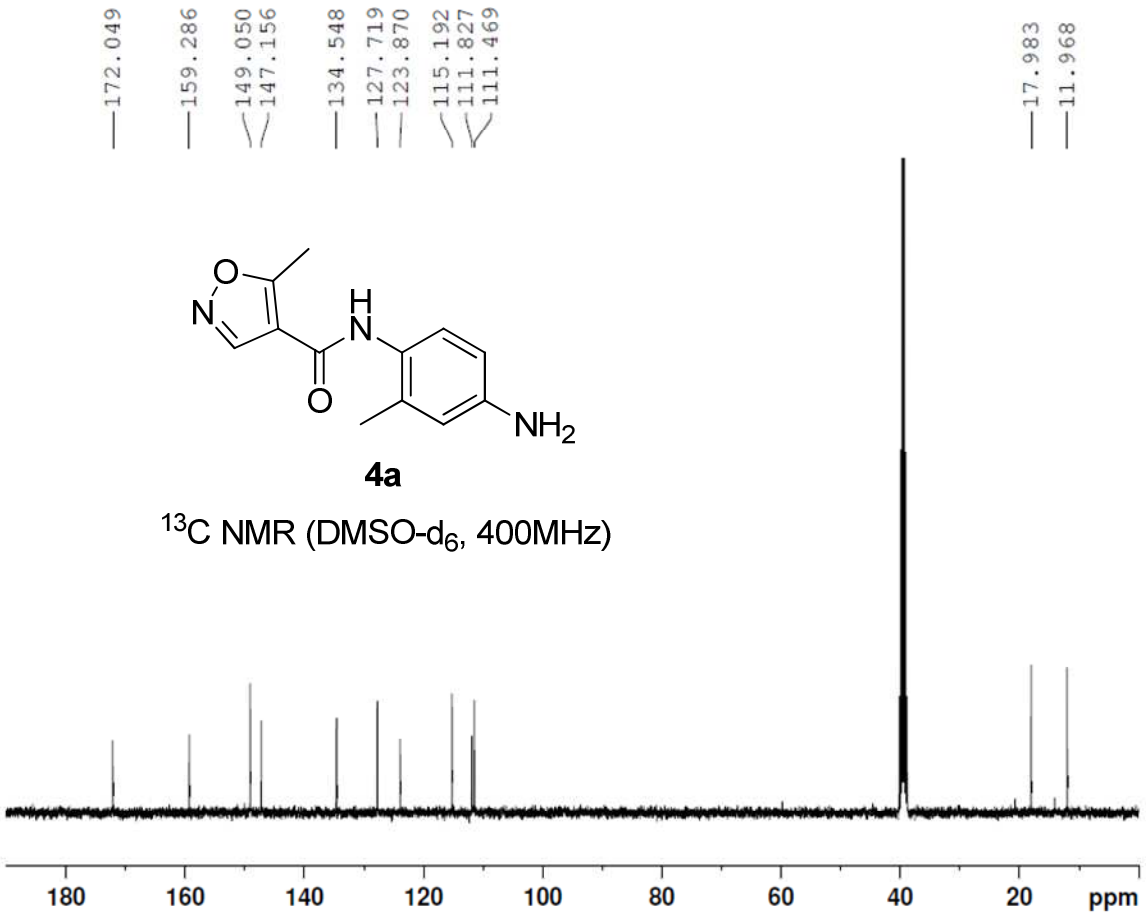


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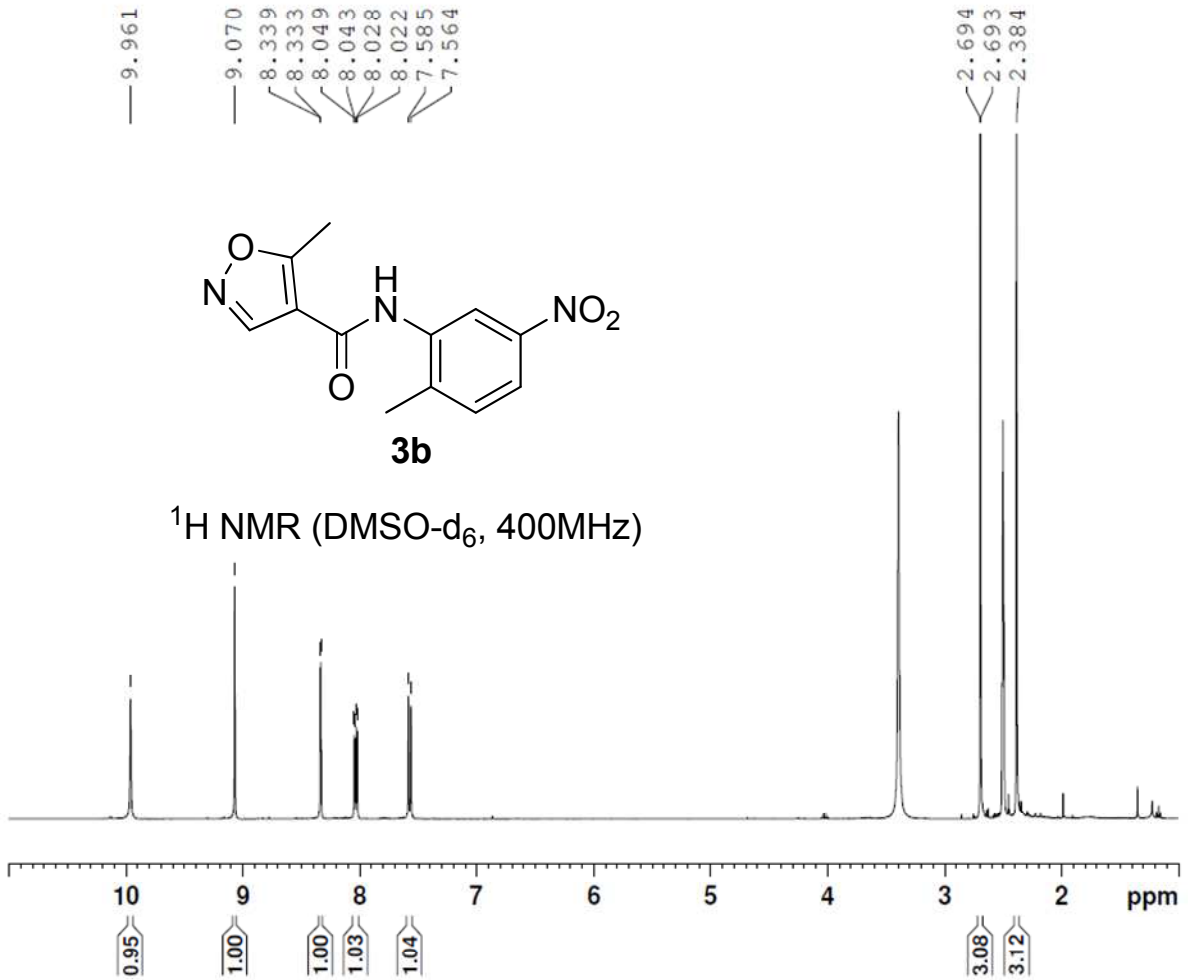
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150527
Time               3.49
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG         zgpg30
TD               65536
SOLVENT           DMSO
NS                 138
DS                   4
SWH          24038.461 Hz
FIDRES        0.366798 Hz
AQ            1.3631488 sec
RG                 512
DW              20.800 usec
DE               6.50 usec
TE              294.5 K
D1           2.00000000 sec
D11          0.03000000 sec

======== CHANNEL f1 ========
NUC1               13C
P1               10.70 usec
PLW1       50.00299835 W
SFO1      100.6354031 MHz

======== CHANNEL f2 ========
CPDPRG[2        waltz16
NUC2               1H
PCPD2            89.00 usec
PLW2       20.00000000 W
PLW12       0.29999000 W
PLW13       0.23762000 W
SFO2      400.1816007 MHz

F2 - Processing parameters
SI               32768
SF        100.6253885 MHz
WDW                EM
SSB      0
LB               1.00 Hz
GB       0
PC               1.40
```



3b

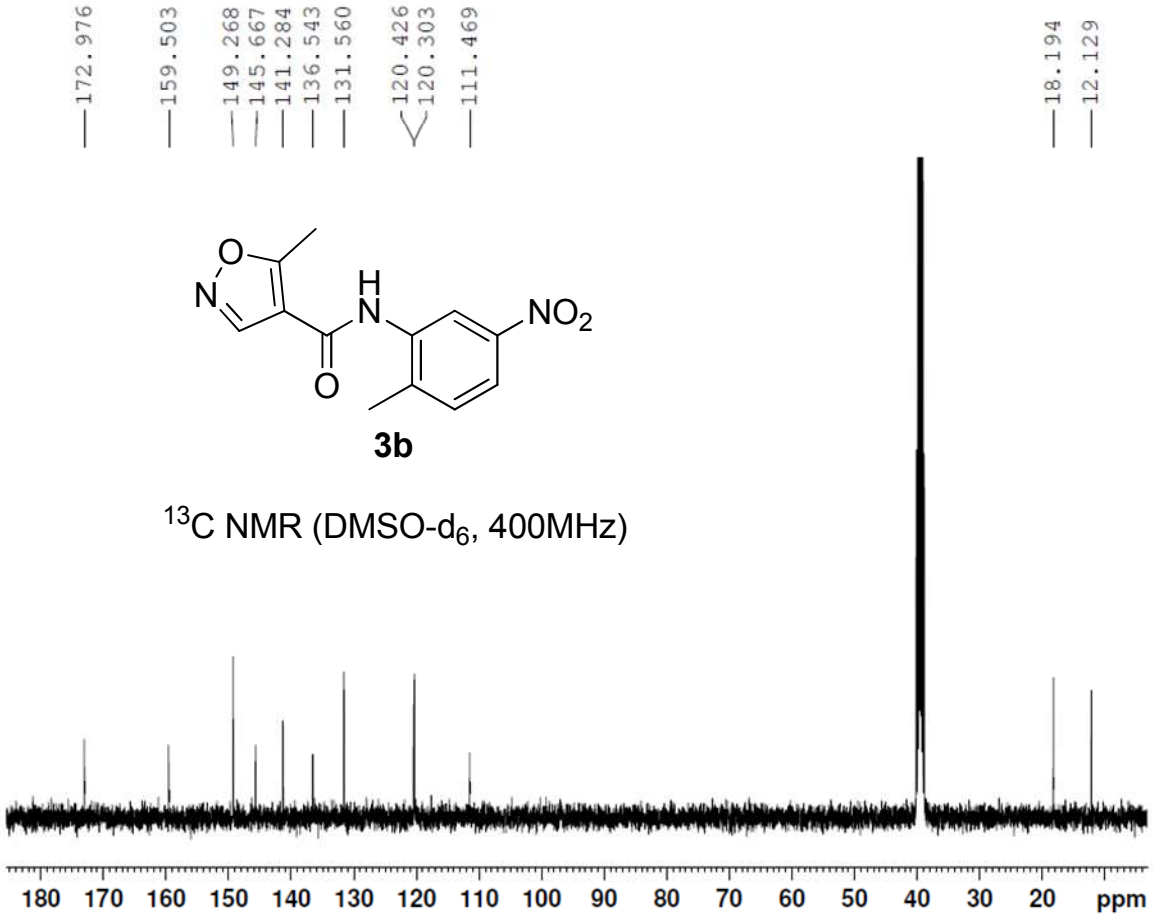
^{1}H NMR (DMSO-d_6, 400MHz)

```
Current Data Parameters
NAME          DSI0107602
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150630
Time                2.25
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT             DMSO
NS                    32
DS                     2
SWH             8223.685 Hz
FIDRES          0.125483 Hz
AQ              3.9845889 sec
RG                   181
DW                60.800 usec
DE                 6.50 usec
TE                294.9 K
D1             1.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                 11.10 usec
PLW1         20.00000000 W
SFO1         400.1824713 MHz

F2 - Processing parameters
SI                 65536
SF           400.1800096 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```



3b

^{13}C NMR (DMSO-d_6, 400MHz)

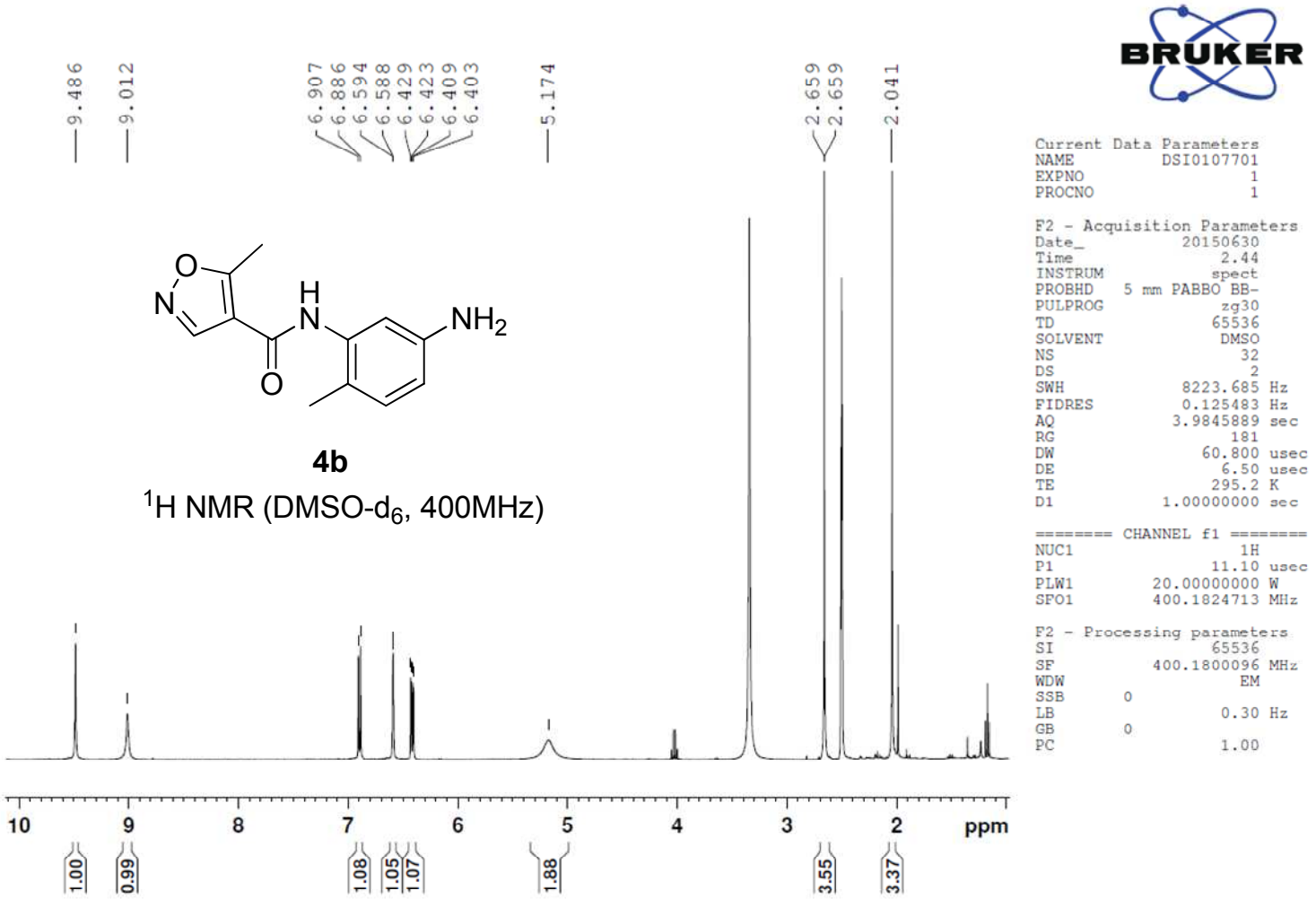
```
Current Data Parameters
NAME      DSI0107602_C13
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150630
Time                2.34
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                   108
DS                     4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ             1.3631488 sec
RG                   724
DW                20.800 usec
DE                 6.50 usec
TE                295.2 K
D1           2.00000000 sec
D11          0.03000000 sec

======== CHANNEL f1 ========
NUC1                 13C
P1                10.70 usec
PLW1         50.00299835 W
SFO1        100.6354031 MHz

======== CHANNEL f2 ========
CPDPRG[2          waltz16
NUC2                 1H
PCPD2             89.00 usec
PLW2         20.00000000 W
PLW12         0.29999000 W
PLW13         0.23762000 W
SFO2        400.1816007 MHz

F2 - Processing parameters
SI                  32768
SF           100.6253894 MHz
WDW                    EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```



4b

^{1}H NMR (DMSO-d_6, 400MHz)

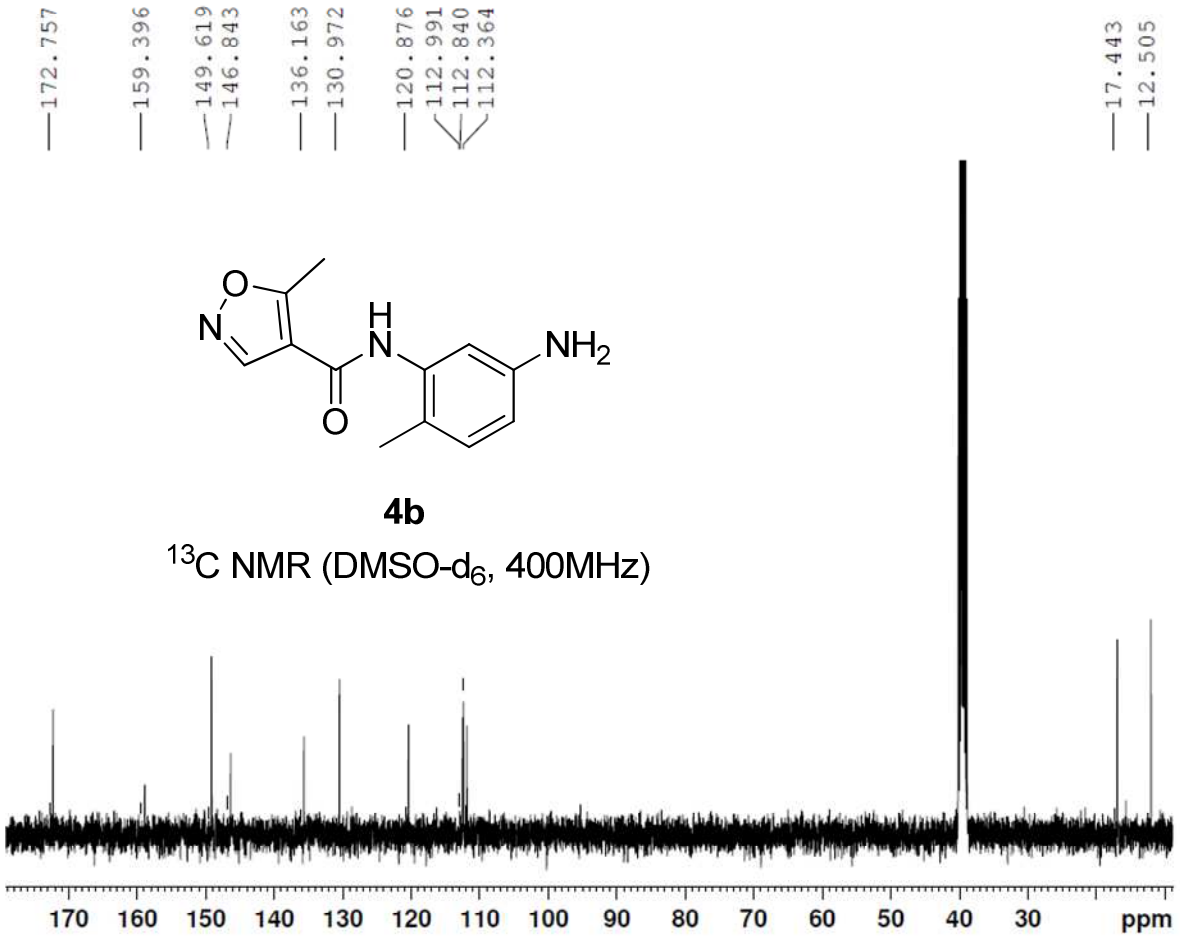
BRUKER

```
Current Data Parameters
NAME          DSI0107701
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150630
Time                2.44
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT             DMSO
NS                    32
DS                     2
SWH             8223.685 Hz
FIDRES          0.125483 Hz
AQ              3.9845889 sec
RG                   181
DW                60.800 usec
DE                 6.50 usec
TE                295.2 K
D1             1.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                 11.10 usec
PLW1         20.00000000 W
SFO1         400.1824713 MHz

F2 - Processing parameters
SI                 65536
SF           400.1800096 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```



4b

^{13}C NMR (DMSO-d_6, 400MHz)

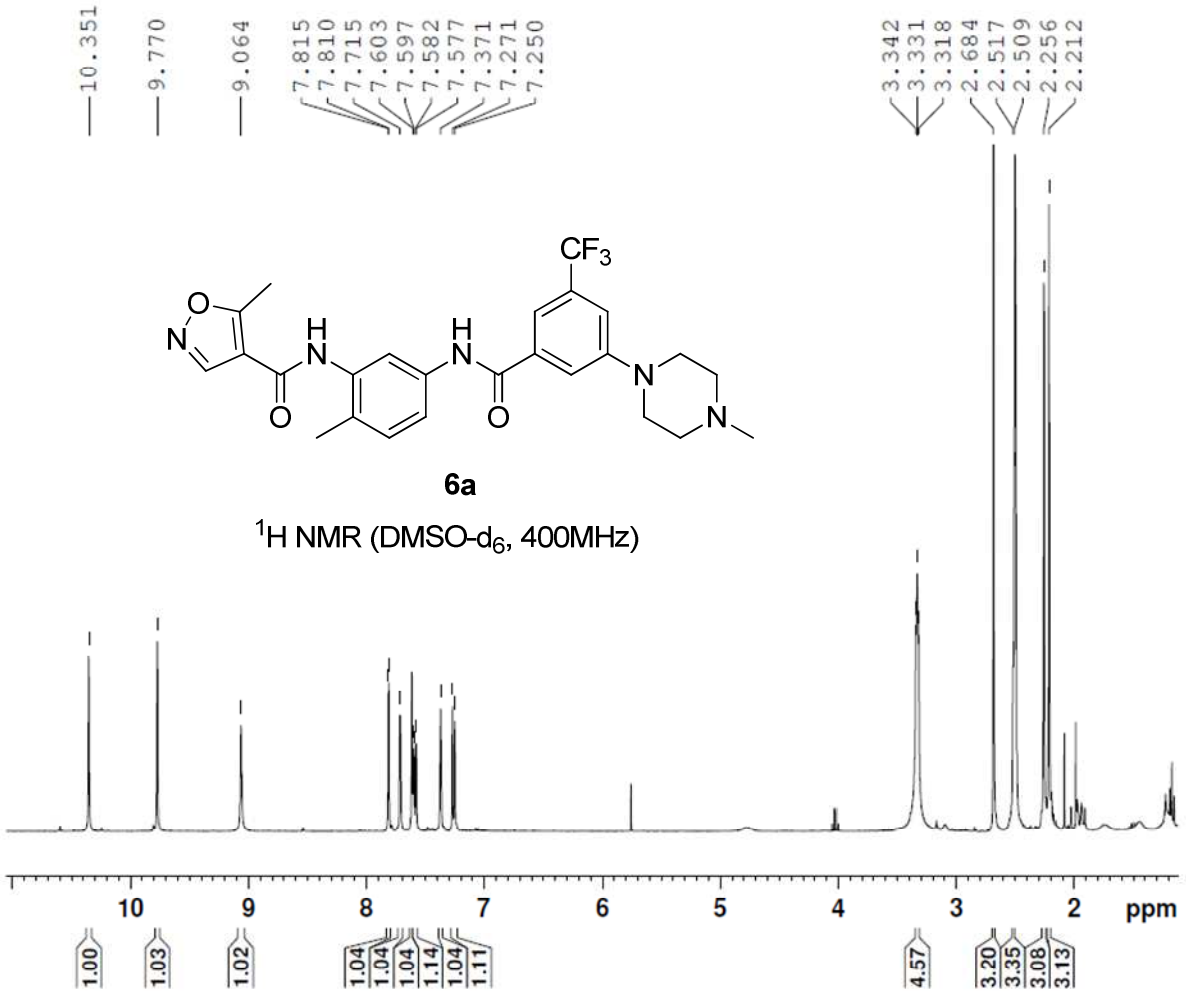
```
Current Data Parameters
NAME      DSI0107701_C13
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150630
Time                2.53
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                   242
DS                     4
SWH          24038.461 Hz
FIDRES        0.366798 Hz
AQ           1.3631488 sec
RG                 724
DW              20.800 usec
DE               6.50 usec
TE              295.5 K
D1           2.00000000 sec
D11          0.03000000 sec

======== CHANNEL f1 ========
NUC1               13C
P1              10.70 usec
PLW1       50.00299835 W
SFO1      100.6354031 MHz

======== CHANNEL f2 ========
CPDPRG[2        waltz16
NUC2               1H
PCPD2           89.00 usec
PLW2       20.00000000 W
PLW12       0.29999000 W
PLW13       0.23762000 W
SFO2      400.1816007 MHz

F2 - Processing parameters
SI                32768
SF         100.6253899 MHz
WDW                EM
SSB       0
LB              1.00 Hz
GB        0
PC              1.40
```



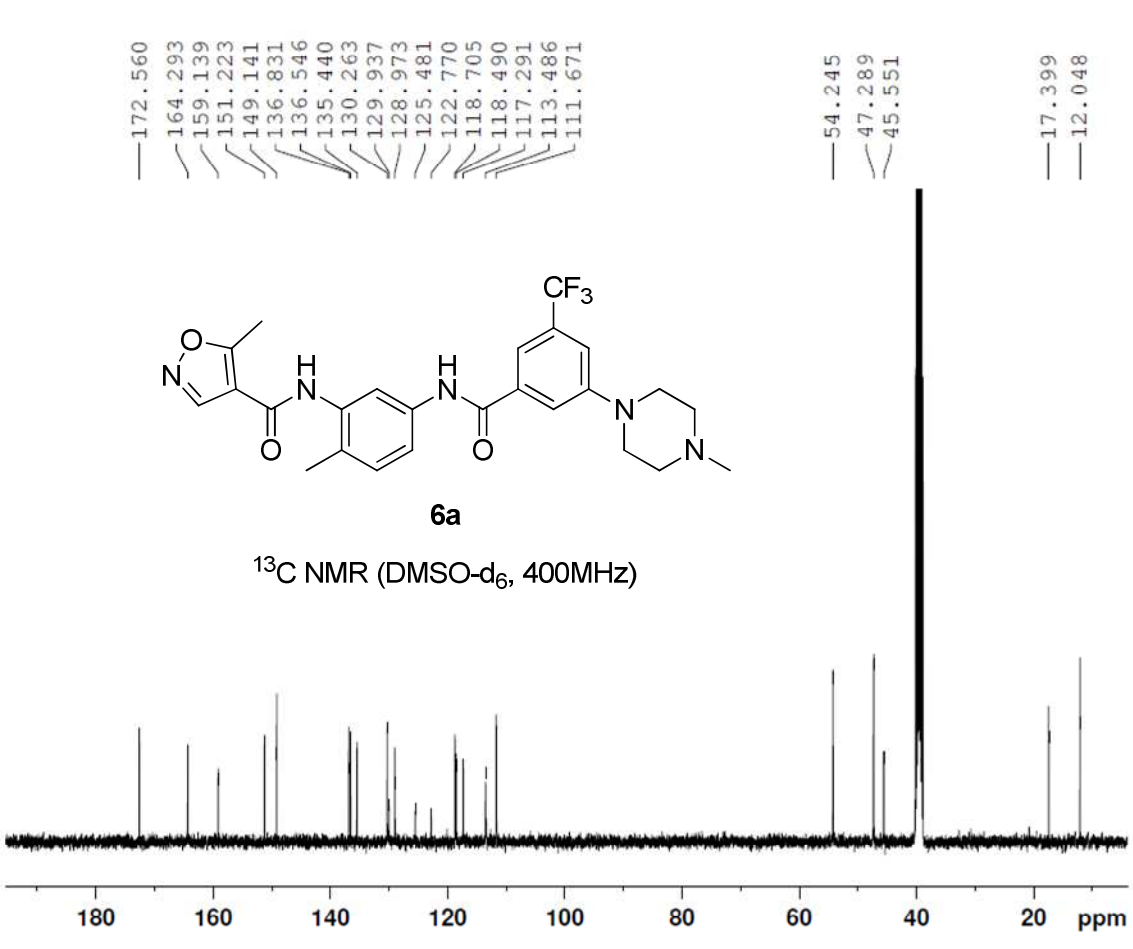
BRUKER

```
Current Data Parameters
NAME                  JKJ0206201
EXPNO                          1
PROCNO                         1

F2 - Acquisition Parameters
Date_                   20150608
Time                        6.19
INSTRUM                    spect
PROBHD    5 mm PABBO BB-
PULPROG                     zg30
TD                         65536
SOLVENT                     DMSO
NS                            32
DS                             2
SWH                     8223.685 Hz
FIDRES                  0.125483 Hz
AQ                      3.9845889 sec
RG                           144
DW                        60.800 usec
DE                         6.50 usec
TE                        295.2 K
D1                      1.00000000 sec

======== CHANNEL f1 ========
NUC1                          1H
P1                         11.10 usec
PLW1                 20.00000000 W
SFO1                 400.1824713 MHz

F2 - Processing parameters
SI                         65536
SF                   400.1800090 MHz
WDW                           EM
SSB                0
LB                          0.30 Hz
GB                 0
PC                          1.00
```



6a

^{13}C NMR (DMSO-d_6, 400MHz)

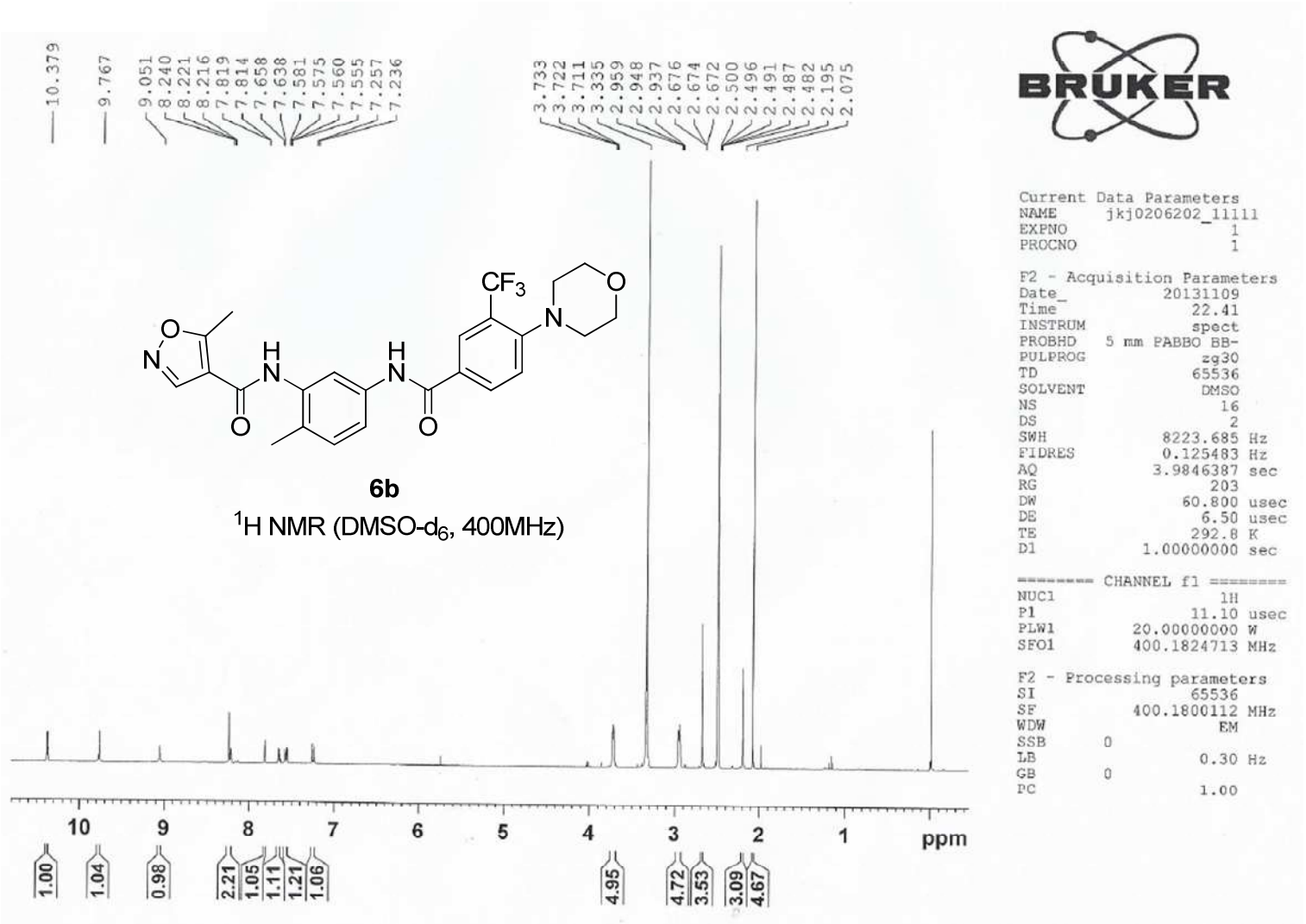
```
Current Data Parameters
NAME      JKJ0206201_C13
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150608
Time                6.36
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                 1315
DS                    4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ             1.3631488 sec
RG                  322
DW               20.800 usec
DE                6.50 usec
TE                295.7 K
D1           2.00000000 sec
D11          0.03000000 sec

======== CHANNEL f1 ========
NUC1                13C
P1               10.70 usec
PLW1        50.00299835 W
SFO1       100.6354031 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                1H
PCPD2            89.00 usec
PLW2        20.00000000 W
PLW12        0.29999000 W
PLW13        0.23762000 W
SFO2       400.1816007 MHz

F2 - Processing parameters
SI                 32768
SF          100.6253906 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```



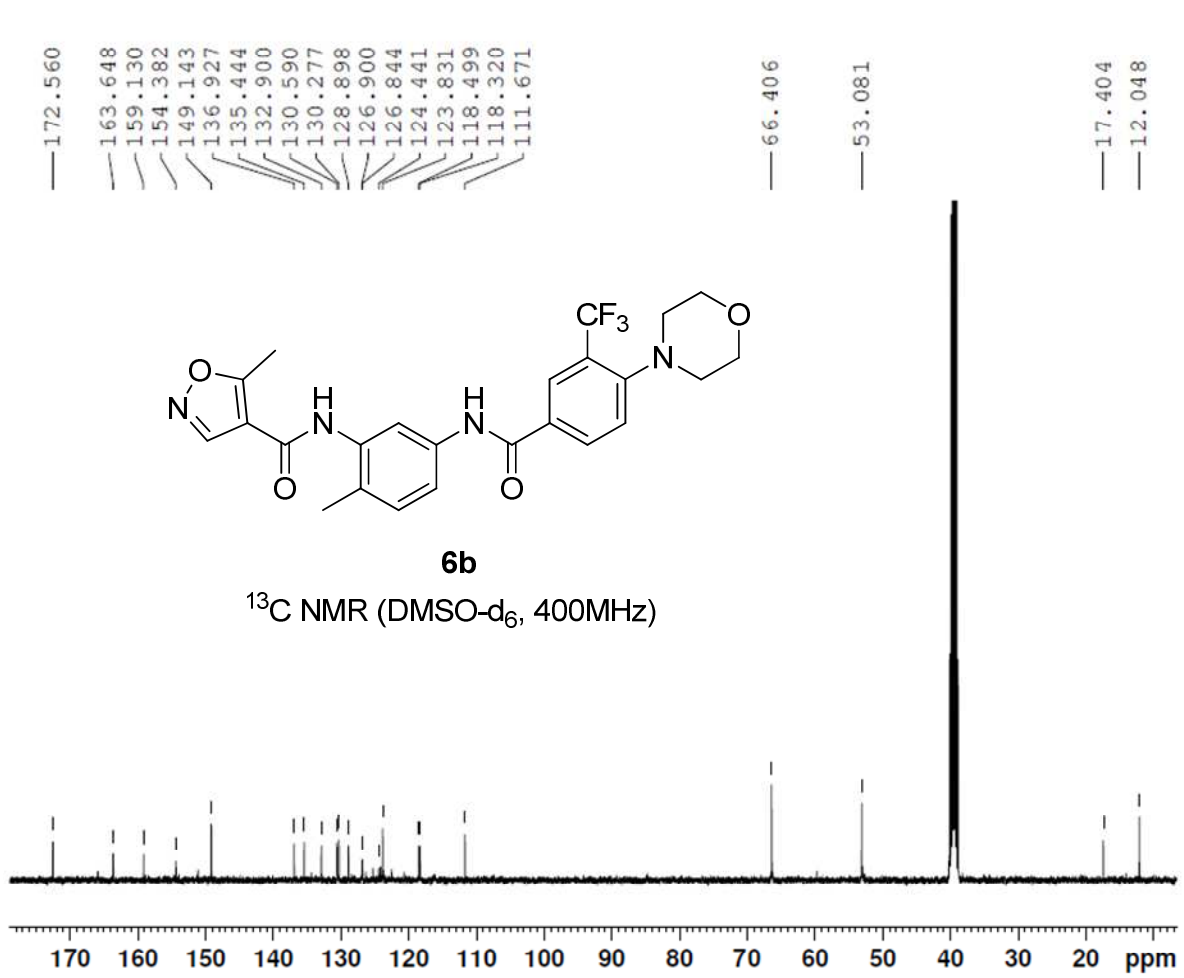
BRUKER

```
Current Data Parameters
NAME      jkj0206202_11111
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_              20131109
Time                 22.41
INSTRUM              spect
PROBHD   5 mm PABBO BB-
PULPROG              zg30
TD                  65536
SOLVENT              DMSO
NS                      16
DS                       2
SWH             8223.685 Hz
FIDRES          0.125483 Hz
AQ              3.9846387 sec
RG                     203
DW                60.800 usec
DE                 6.50 usec
TE                292.8 K
D1             1.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                  11.10 usec
PLW1          20.00000000 W
SFO1          400.1824713 MHz

F2 - Processing parameters
SI                  65536
SF            400.1800112 MHz
WDW                    EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```



6b

^{13}C NMR (DMSO-d_6, 400MHz)

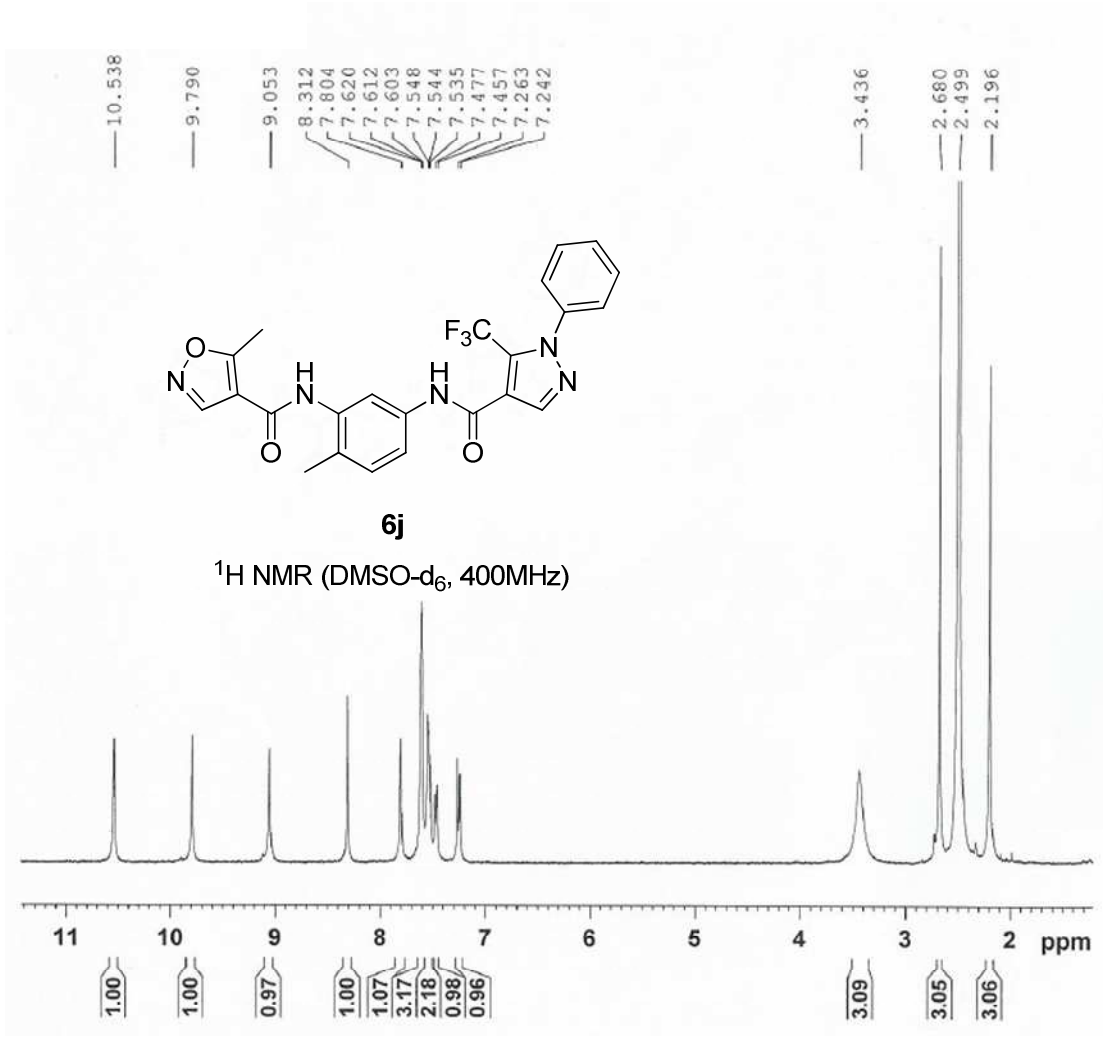
```
Current Data Parameters
NAME      JKJ0206202_C13
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150529
Time                6.03
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG          zqpg30
TD                65536
SOLVENT            DMSO
NS                 1036
DS                    4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ             1.3631488 sec
RG                  456
DW                20.800 usec
DE                 6.50 usec
TE                295.7 K
D1           2.00000000 sec
D11          0.03000000 sec

======== CHANNEL f1 ========
NUC1                13C
P1                10.70 usec
PLW1        50.00299835 W
SFO1       100.6354031 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                1H
PCPD2            89.00 usec
PLW2        20.00000000 W
PLW12        0.29999000 W
PLW13        0.23762000 W
SFO2       400.1816007 MHz

F2 - Processing parameters
SI                 32768
SF          100.6253923 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```



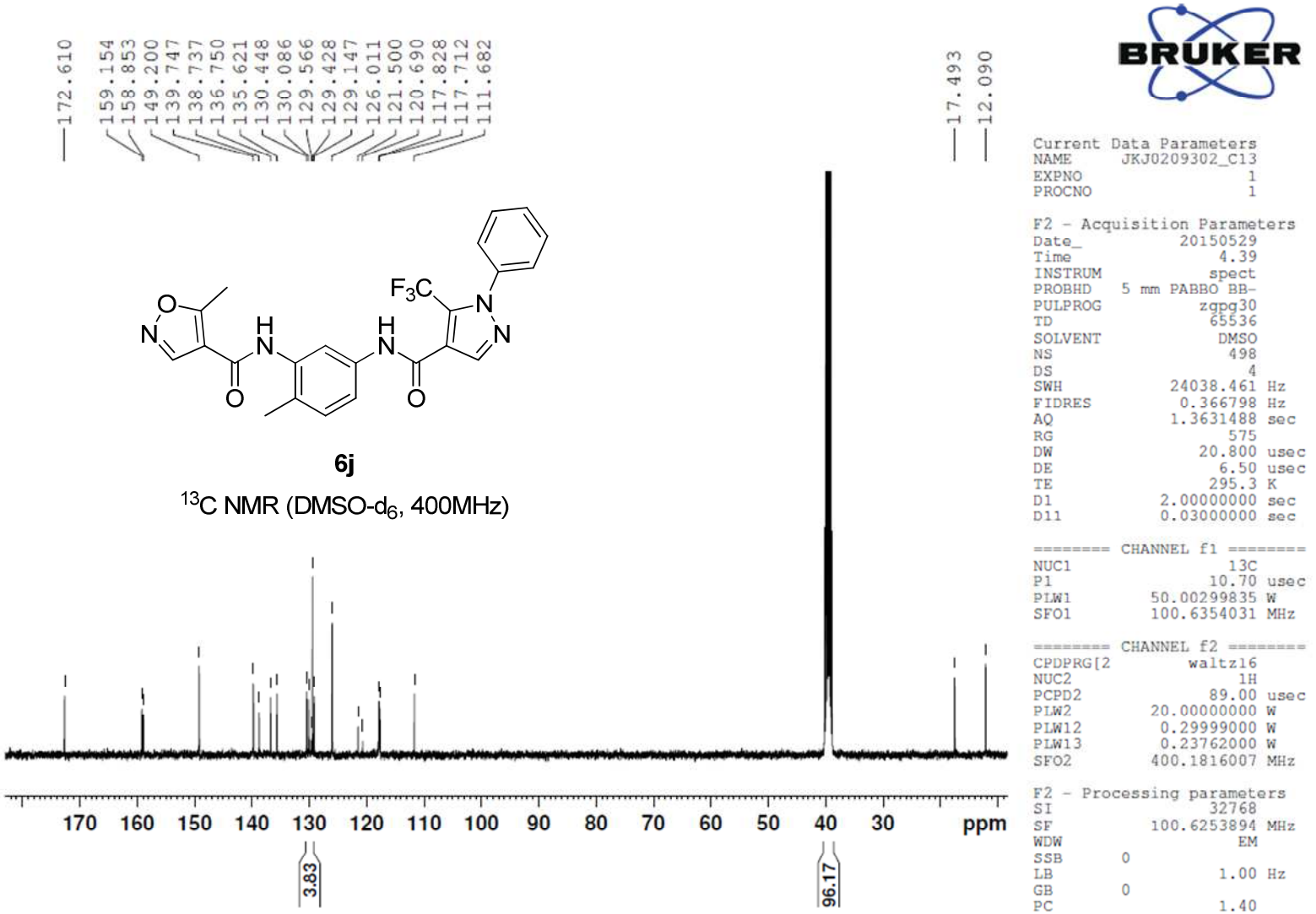
BRUKER

```
Current Data Parameters
NAME          jkj0209302
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20130303
Time              14.29
INSTRUM           spect
PROBHD   5 mm PABBO BB-
PULPROG            zg30
TD                65536
SOLVENT            DMSO
NS                   32
DS                    2
SWH            8223.685 Hz
FIDRES        0.125483 Hz
AQ            3.9846387 sec
RG                  203
DW               60.800 usec
DE                6.50 usec
TE               295.1 K
D1            1.00000000 sec

======== CHANNEL f1 ========
NUC1                1H
P1                11.10 usec
PLW1       20.00000000 W
SFO1       400.1824713 MHz

F2 - Processing parameters
SI                65536
SF         400.1800091 MHz
WDW                 EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```



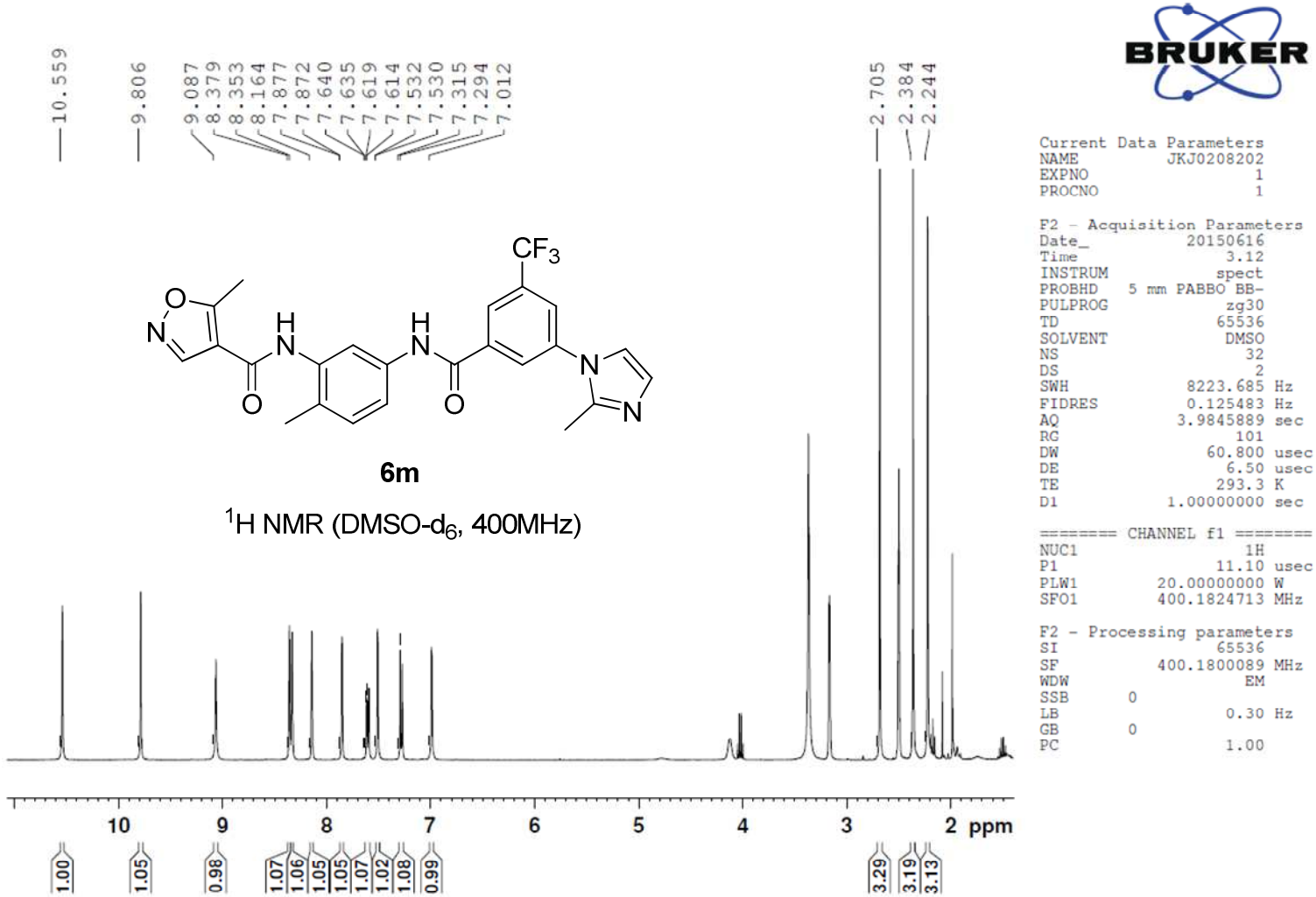

```
Current Data Parameters
NAME      JKJ0209302_C13
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150529
Time                4.39
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT             DMSO
NS                   498
DS                     4
SWH            24038.461 Hz
FIDRES          0.366798 Hz
AQ              1.3631488 sec
RG                   575
DW                20.800 usec
DE                 6.50 usec
TE                295.3 K
D1             2.00000000 sec
D11            0.03000000 sec

======== CHANNEL f1 ========
NUC1                 13C
P1                10.70 usec
PLW1         50.00299835 W
SFO1        100.6354031 MHz

======== CHANNEL f2 ========
CPDPRG[2          waltz16
NUC2                 1H
PCPD2             89.00 usec
PLW2         20.00000000 W
PLW12         0.29999000 W
PLW13         0.23762000 W
SFO2        400.1816007 MHz

F2 - Processing parameters
SI                  32768
SF          100.6253894 MHz
WDW                    EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```

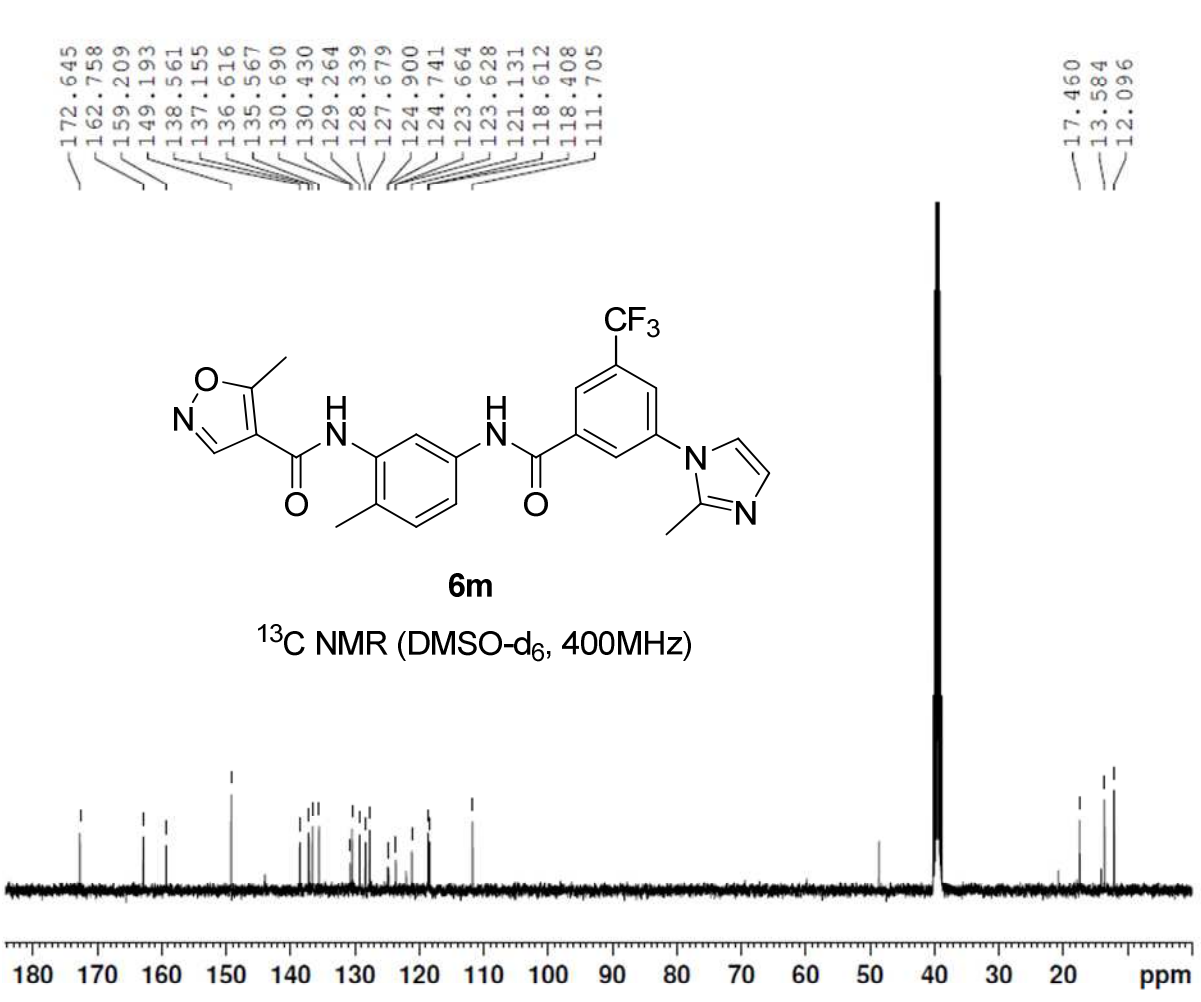



```
Current Data Parameters
NAME          JKJ0208202
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150616
Time                3.12
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG             zg30
TD                 65536
SOLVENT             DMSO
NS                    32
DS                     2
SWH             8223.685 Hz
FIDRES          0.125483 Hz
AQ              3.9845889 sec
RG                   101
DW                60.800 usec
DE                 6.50 usec
TE                293.3 K
D1             1.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                 11.10 usec
PLW1         20.00000000 W
SFO1         400.1824713 MHz

F2 - Processing parameters
SI                 65536
SF           400.1800089 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```



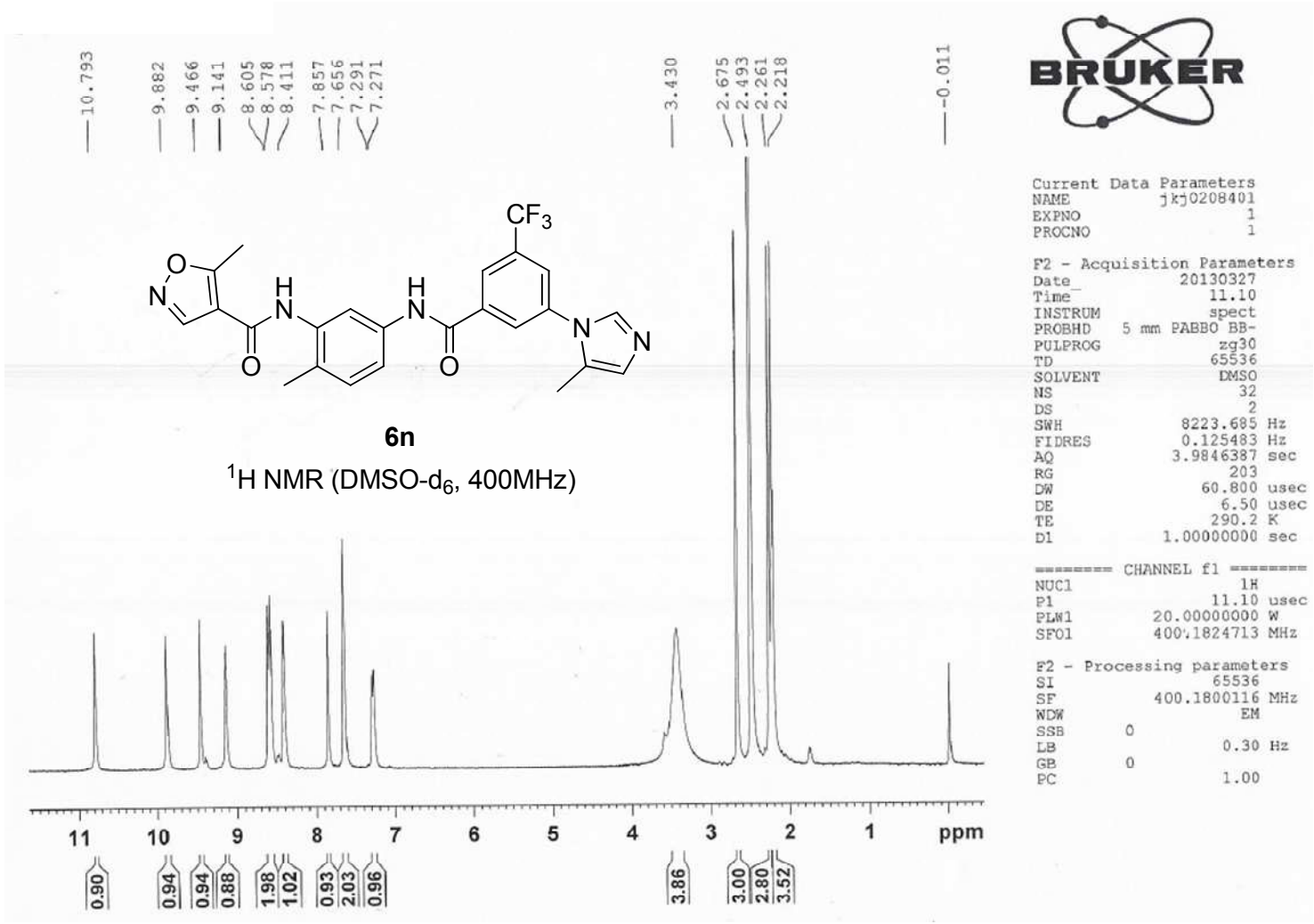

```
Current Data Parameters
NAME      JKJ0208202_C13
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150616
Time                3.20
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                  267
DS                    4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ             1.3631488 sec
RG                  362
DW                20.800 usec
DE                 6.50 usec
TE                293.6 K
D1            2.00000000 sec
D11           0.03000000 sec

======== CHANNEL f1 ========
NUC1                13C
P1                10.70 usec
PLW1         50.00299835 W
SFO1        100.6354031 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                1H
PCPD2            89.00 usec
PLW2         20.00000000 W
PLW12         0.29999000 W
PLW13         0.23762000 W
SFO2        400.1816007 MHz

F2 - Processing parameters
SI                 32768
SF          100.6253874 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```



6n

^{1}H NMR (DMSO-d_6, 400MHz)

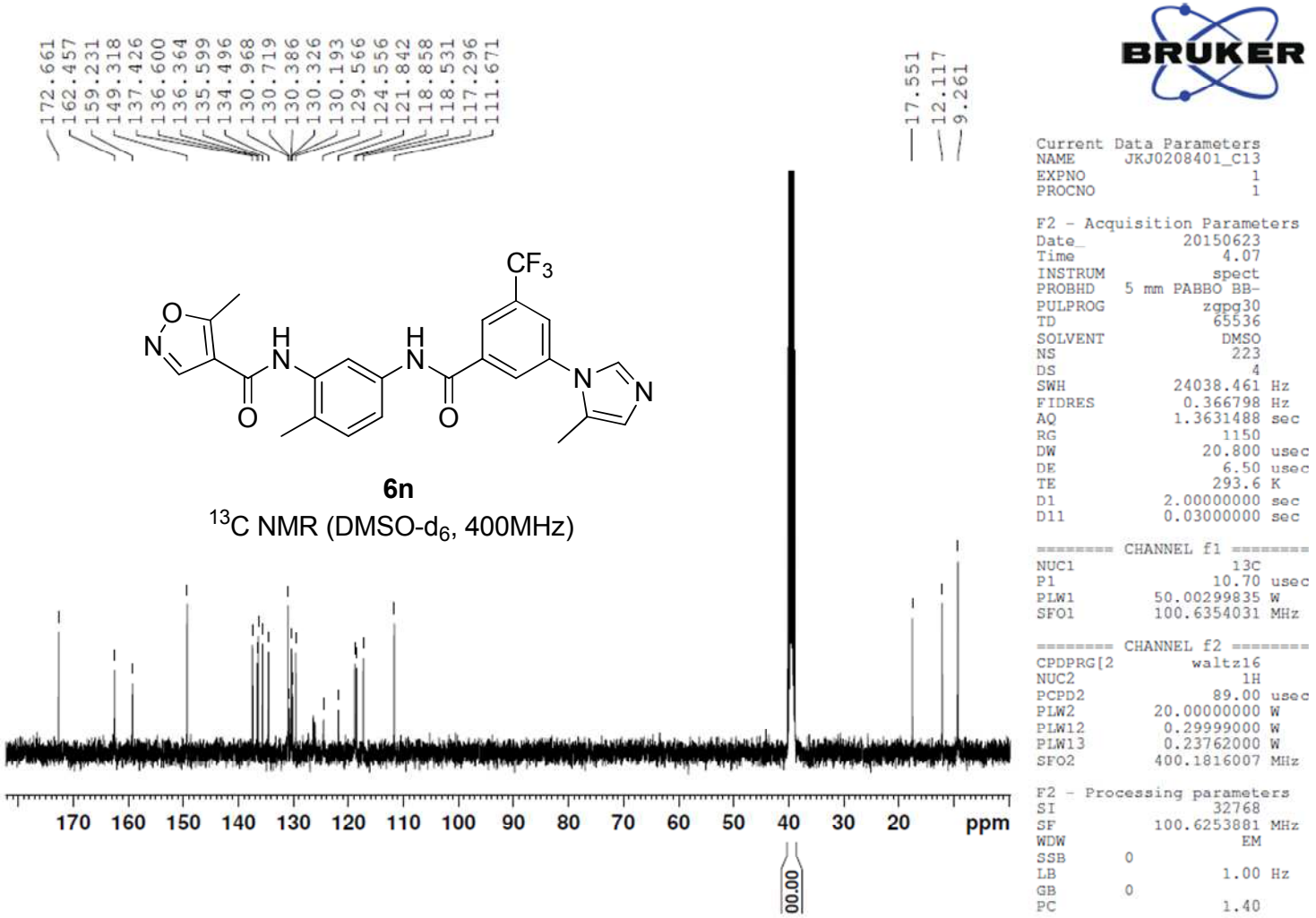
BRUKER

Current Data Parameters
NAME jkj0208401
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20130327
Time 11.10
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 32
DS 2
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 203
DW 60.800 usec
DE 6.50 usec
TE 290.2 K
D1 1.00000000 sec

======== CHANNEL f1 ========
NUC1 1H
P1 11.10 usec
PLW1 20.00000000 W
SFO1 400.1824713 MHz

F2 - Processing parameters
SI 65536
SF 400.1800116 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



BRUKER

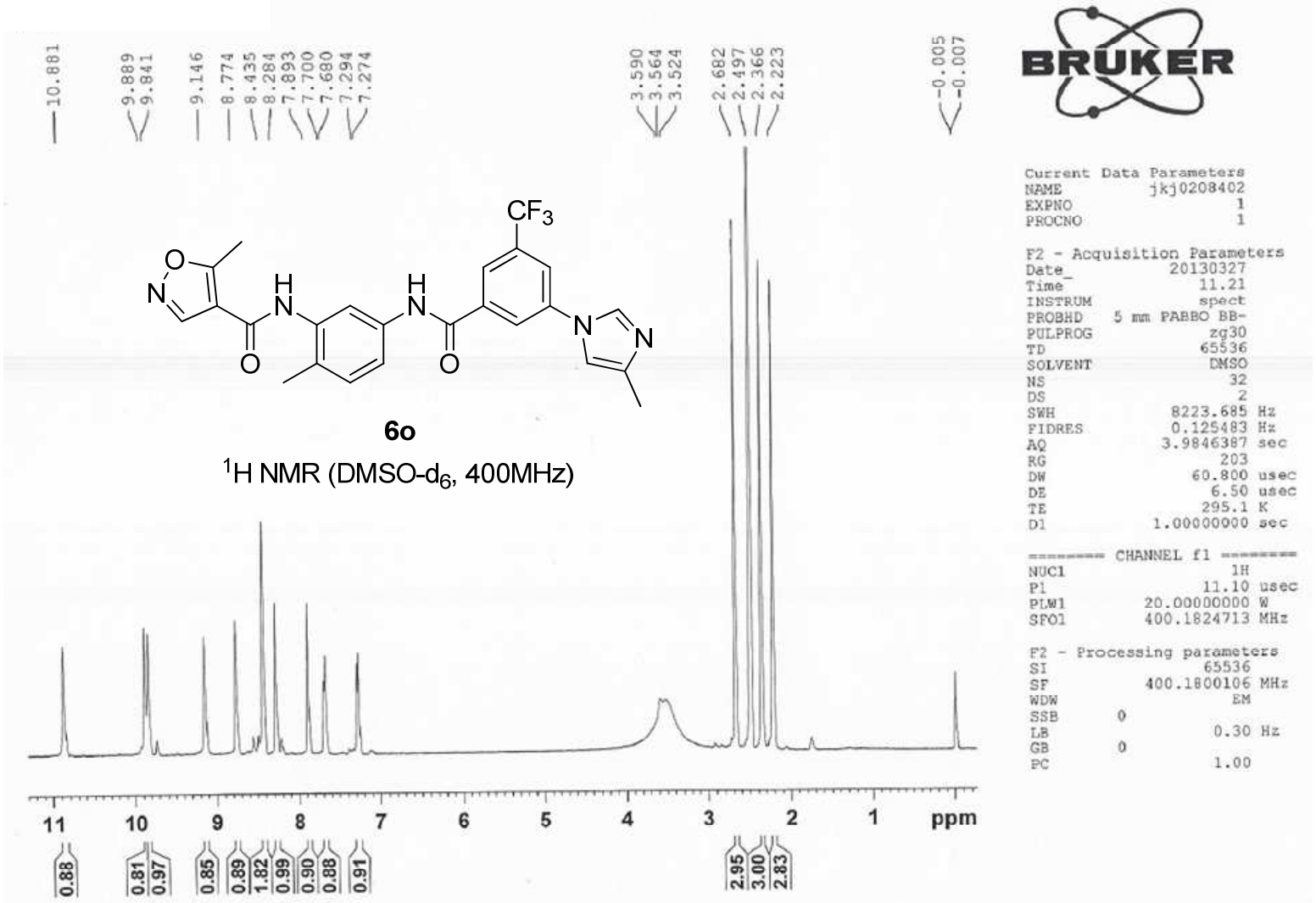
```
Current Data Parameters
NAME      JKJ0208401_C13
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150623
Time                4.07
INSTRUM            spect
PROBHD   5 mm PABBO BB-
PULPROG          zgpg30
TD                65536
SOLVENT            DMSO
NS                  223
DS                    4
SWH           24038.461 Hz
FIDRES         0.366798 Hz
AQ             1.3631488 sec
RG                 1150
DW               20.800 usec
DE                6.50 usec
TE               293.6 K
D1            2.00000000 sec
D11           0.03000000 sec

======== CHANNEL f1 ========
NUC1                13C
P1                10.70 usec
PLW1         50.00299835 W
SFO1        100.6354031 MHz

======== CHANNEL f2 ========
CPDPRG[2         waltz16
NUC2                1H
PCPD2             89.00 usec
PLW2         20.00000000 W
PLW12         0.29999000 W
PLW13         0.23762000 W
SFO2        400.1816007 MHz

F2 - Processing parameters
SI                 32768
SF          100.6253881 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.40
```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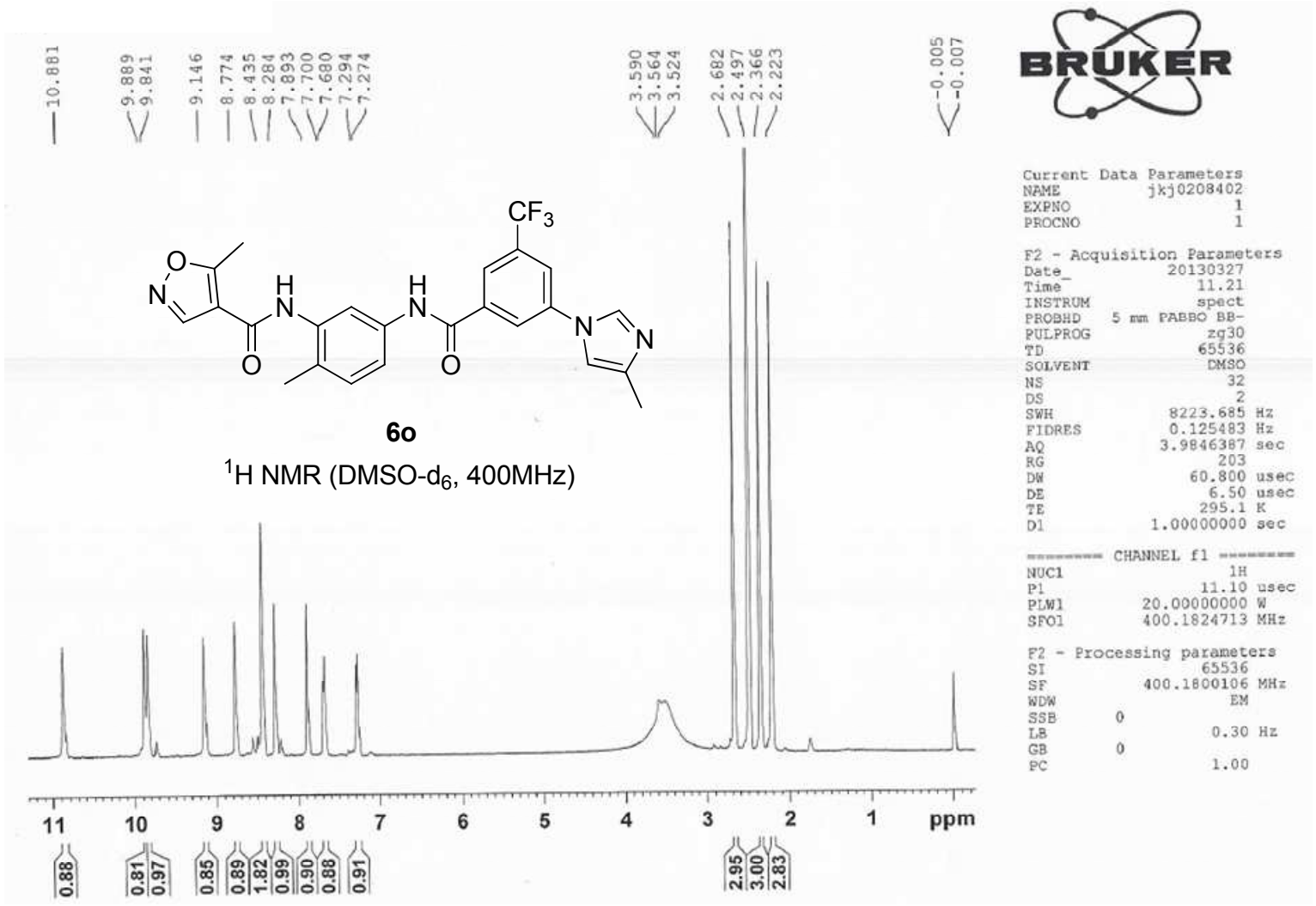
9.889
9.841
9.146
8.774
8.435
8.284
7.893
7.700
7.680
7.294
7.274
3.590
3.564
3.524
2.682
2.497
2.366
2.223
-0.005
-0.007
CF3
O
N
H
N
H
N
O
O
N
N
6o
1H NMR (DMSO-d6, 400MHz)
11
10
9
8
7
6
5
4
3
2
1
ppm
0.88
0.81
0.97
0.85
0.89
1.82
0.99
0.90
0.88
0.91
2.95
3.00
2.83
BRUKER
Current Data Parameters
NAME jkj0208402
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20130327
Time 11.21
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 32
DS 2
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 203
DW 60.800 usec
DE 6.50 usec
TE 295.1 K
D1 1.00000000 sec
======== CHANNEL f1 ========
NUC1 1H
P1 11.10 usec
PLW1 20.00000000 W
SFO1 400.1824713 MHz
F2 - Processing parameters
SI 65536
SF 400.1800106 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



BRUKER

Current Data Parameters
NAME jkj0208402
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20130327
Time 11.21
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 32
DS 2
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 203
DW 60.800 usec
DE 6.50 usec
TE 295.1 K
D1 1.00000000 sec

======== CHANNEL f1 ========
NUC1 1H
P1 11.10 usec
PLW1 20.00000000 W
SFO1 400.1824713 MHz

F2 - Processing parameters
SI 65536
SF 400.1800106 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00