Synthesis and antitumor activities of 2-(piperidin-4-yl)-thiazole-4-carboxamides analogues of tubulysins

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Abstract: Tubulysins are a family of natural products that exhibit potent antitumor activities, which have attracted much attention from synthetic and medicinal chemists. Previous attempts to aromatize the Tuv part of tubulysin analogues led to a loss in antitumor activity, which suggested that the Ile and Tuv fragments of these analogues were adopting different conformations than the natural products. In this study, a series of 2-(piperidin-4-yl)-thiazole-4-carboxamides have been prepared to investigate whether an intramolecular H-bond between key NH and OH groups is responsible for the conformational control. The antitumor activities of these analogues have been screened using MDA-MB-231-breast, Siha-cervical, MCF7-breast, and PC3-prostate cell lines, with ureas 5m and 5k shown to exhibit moderate antitumor activities (IC50 values of 0.2 and 0.6 µM against MCF7 cells, respectively).

Key words: Tubulysin analogues, antitumor activity, 2-(piperidin-4-yl)-thiazole-4-carboxamides, synthesis

1. Introduction

Tubulysins are a family of antimitotic tetrapeptides that were isolated from Angiococcus disciformis in 2000,1 which exhibit potent antiproliferative activity. The structures of tubulysins can be subdivided into four parts, namely Mep, Ile, Tuv, and Tup. The mode of action for tubulysins is the result of inhibition of tubulin polymerization.1 Consequently, a large number of reports have appeared describing their total synthesis and the antitumor activities2–5 of structural analogues6–8 and conjugates.9–11 These structurally challenging natural products contain multiple chiral centers, which potentially hinder their commercial development. Therefore, a number of studies have been directed towards simplifying their structures while maintaining or enhancing their antitumor potencies. Cyclizative aromatization strategies are often used in drug discovery campaigns to decrease the number of chiral centers of a drug candidate while rigidifying its structure and increasing its lipophilicity.12,13 Most of the chiral centers present in tubulysins originate from nonnatural amino acid fragments (Tuv and Tup) that are not easy to synthesize without epimerization and side reactions occurring.14

In our previous work, we reported that aromatization of the Tuv part of tubulysin (I, Figure) led to a dramatic loss in antitumor activity.15 Initially, this loss of biological activity was attributed to the introduction of conformational restraint, which led to weaker interactions from the Tup fragment of tubulin. Therefore, we proposed that an intramolecular H-bond between the OH group of Tuv and the NH group of Ile might be forming a 6-membered ring intramolecular H-bond. To verify this hypothesis, a series of 2-(piperidin-4-yl)-
thiazole-4-carboxamides (II, Figure) have been synthesized and their antitumor activities were screened using the MTT method.

![Chemical structures](image)

**Figure.** Retrosynthesis of 2-substituted-thiazole-4-carboxamides I and II.

### 2. Results and discussion

#### 2.1. Chemistry

A three-step synthesis was employed to transform commercially available N-Boc-4-piperidine-carboxylic acid 1 into thiazole compound 2 (Scheme). The first step involved converting the acid functionality of 1 into an amide using an amine, dicyclohexylcarbodiimide (DCC), and ammonium chloride; the second step used Lawesson’s reagent to convert the amide functionality into a thioamide group; and the third step involved refluxing with ethyl bromopyruvate in ethanol to afford a thiazole ring. The ester group of 2 was then hydrolyzed under alkaline conditions, and the resultant acid was converted into a series of amides 3a–3c using traditional peptide coupling chemistry with three amines. Methylamine (MeNH₂), benzylamine (PhCH₂NH₂), and phenylethylamine (PhCH₂CH₂NH₂) were chosen as nucleophiles because a previous structure-activity relationship study had shown that these amines could be used to replace Tup with little effect on antitumor activity.¹⁶,¹⁷ N-Boc deprotection of 3a–3c with trifluoroacetic acid (TFA) was coupled with N-Boc-protected amino acids valine (Val), leucine (Leu), and phenylalanine (Phe) to afford six relatively hydrophobic peptides, 4a–4f. N-Boc deprotection of 4a–4f with TFA was followed by peptide coupling with (D)-1-Me-piperidine-2-carboxylic acid or 4-pyridine-carboxylic acid to give amides 5a–5e, or reaction with a series of isocyanates to afford ureas 5f–5m in moderate yields.

#### 2.2. MTT cell proliferation assay

##### 2.2.1. Cell lines and cell culture

Human cancer cell lines were cultured in RPMI-1640 media supplemented with 10% fetal calf serum, penicillin (100 U/mL), and streptomycin (100 µg/mL) (GIBCO BRL, NY, USA). These cells were then incubated at 37 °C in a humidified air atmosphere containing 5% CO₂, with all cells harvested in their exponential growth phase.

##### 2.2.2. Cell viability assay

Cell viability was measured using the MTT assay. Briefly, cells were seeded in multiple 96-well plates at a density of 4 × 10⁴/mL. After incubation overnight, triplicate wells were exposed to screening compounds for 72 hours.
h. MTT solution (5 mg/mL) was then added to each well and incubated for 4 h. DMSO was added to dissolve the MTT formazan product and its absorbance was measured at 570 nm using a Molecular Devices (USA) SpectraMax M5 spectrophotometer. Relative cell viability rates were calculated versus untreated controls, with 50% inhibitory concentration (IC$_{50}$) values calculated using Graph Pad Prism 5 (Graph Pad Software Inc., USA).

2.3. Conclusions

The structures of all compounds and their antitumor activities are listed in the Table. This series of compounds had relatively low molecular weights (between 500 and 550) and fewer stereocenters (1 or 2) when compared to tubulysins, with their antitumor activities found to be 10-fold less than that of taxol. Intermediate 4a had been shown to exhibit moderate antitumor activity in previous screening studies. Due to the N-Boc group in 4a being unstable, we tried to replace the O- in the Boc group with N- to generate a relatively stable urea group. The more stable ureas 5k and 5m showed the best potent antitumor activities, with IC$_{50}$ values of 0.2 and 0.6 µM against MCF7 cells, respectively. Comparing the three R$_1$ groups of these analogues, those containing phenylethylamine fragments generally possessed better activity than those with methylamine and benzylamine fragments (e.g., 5k and 5m vs. 5f and 5j). The presence of the nitro groups on the aryl rings of 5l and 5i resulted in analogues with a significant reduction in antitumor activity. We have previously shown that aromatization of the Tuv fragment led to a dramatic loss in its antitumor activity, with Ryu$^{18}$ reporting similar observations for tetrahydropyran isosteres in Tuv N-methyl tubulysin. Although the 2-(piperidin-4-yl)-thiazoles were less potent than taxol (and many other tubulysin derivatives), they were more potent than our previously prepared aromatic analogues. The observed loss of biological activity in these cyclic analogues suggested that the conformation and orientation of the central Tuv core is crucial in maintaining the antitumor activity of tubulysins.

A series of 2-(piperidin-4-yl)-thiazole-4-carboxamides have been synthesized and their antitumor activities were screened in the MTT proliferation assay, with ureas 5m and 5k showing the best antitumor activity against the MCF7 cancer cell line.

3. Experimental

3.1. General

The NMR spectra of the intermediates and final products of 2-(piperidin-4-yl)-thiazole-4-carboxamides in deuterated solvent were detected on a Bruker 400 or 600 MHz spectrometer (see Supporting information). High-resolution mass spectra (HRMS) were recorded on an Agilent 6210 ESI/TOF mass spectrometer. Melting points (mp) were recorded on a Büchi B-540 melting point apparatus and are uncorrected. Flash column chromatographic separation was achieved using a silica gel from Qingdao Ocean Chemical Co. (200 to 300 mesh) with particle size from 54 to 74 µm using ethyl acetate and hexane (or petroleum ether) as the eluent. Analytical TLC was carried out on Merck precoated silica gel 60 GF-254 using 0.25-mm-thick TLC plates.

3.2. General procedure for synthesis of 2-(piperidin-4-yl)-thiazole-4-carboxamides

3.2.1. tert-Butyl 4-(4-(ethoxycarbonyl)thiazol-2-yl)piperidine-1-carboxylate (2)

Step 1: DCC (16.21 g, 0.079 mol) was added to a solution of 1-(tert-butoxycarbonyl)piperidine-4-carboxylic acid (12.03 g, 0.052 mol) in dichloromethane (DCM) (200 mL). The reaction mixture was allowed to stir at
Table. Structures and antitumor activities of 2-(piperidin-4-yl)-thiazole-4-carboxamides.

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<th>Compound</th>
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<th>R&lt;sub&gt;2&lt;/sub&gt;</th>
<th>R&lt;sub&gt;3&lt;/sub&gt;</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;(µM)</th>
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<td></td>
<td></td>
<td></td>
<td>&lt; 0.01 0.006 0.003 /</td>
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</table>

Reagents and conditions: (a). DCC, NH<sub>4</sub>Cl, TEA, r.t.; (b). L. R. 50 °C; (c). Ethyl bromopyruvate, EtOH, reflux; (d). NaOH, THF/H<sub>2</sub>O, r. t.; (e). EDC, HOBT, DIPEA, R<sub>1</sub>NH<sub>2</sub>, r.t.; (f). 1). TFA, DCM, r.t. 2). EDC, HOBT, DIPEA, Boc-L-aa, r.t.; (g). 1). TFA, DCM, r.t. 2). EDC, HOBT, DIPEA, R<sub>3</sub>COOH, or R<sub>3</sub>NCO, DCM, r.t.;

Scheme. Synthetic route to 2-(piperidin-4-yl)-thiazole-4-carboxamides.

r.t. for 3 h, triethylamine (TEA) (21 mL) and ammonium chloride (NH<sub>4</sub>Cl) (4.16 g, 0.079 mol) were then added, and the resultant mixture was stirred for 12 h at r.t. Water (100 mL) was added and the aqueous layer
was extracted with DCM (3 × 100 mL) with organic layers combined, dried over anhydrous sodium sulfate (Na$_2$SO$_4$), filtered, and concentrated to afford a crude product that was purified by column chromatography (DCM:MeOH = 20:1) to afford an amide (10.11 g, 85%) as a white solid. Mp: 154–156 °C.

Step 2: Lawesson’s reagent (3.27 g, 8.1 mmol) was added to a solution of the above amide (3.71 g, 16 mmol) in 1,4-dioxane (50 mL) at r.t. and the reaction mixture was stirred at 50 °C for 2 h. The reaction mixture was cooled to r.t., sodium bicarbonate (NaHCO$_3$) (0.80 g, 9.6 mmol) was added, and the solvent was removed under reduced pressure to afford a crude residue that was poured into a mixture of crushed ice and water. The resultant aqueous solution was extracted with DCM (3 × 50 mL) and the organic layers were combined, dried (Na$_2$SO$_4$), filtered, and concentrated to afford a crude product that was purified by column chromatography (DCM:MeOH = 10:1) to afford thioamide (3.52 g, 89%) as a white solid. Mp: 131–132 °C.

Step 3: Ethyl bromopyruvate (10.05 g, 0.051 mol) was added to a solution of the above thioamide (8.04 g, 0.048 mol) in absolute ethanol (EtOH) (50 mL) and the reaction mixture was refluxed for 5 h. The solvent was removed under reduced pressure, and the resultant residue was dissolved in hot EtOH (40 mL), with cooling to r.t. resulting in formation of a precipitate. This solid was filtered off and washed with cold EtOH to afford the title compound (11.13 g, 88%) as a yellow solid. Mp: 69–71 °C.

1H NMR (400 MHz, CDCl$_3$): H 8.03 (s, 1H, ArH), 4.35 (q, J = 7.1 Hz, 2H, CH$_2$CH$_3$), 4.16 (dd, J = 7.7, 3.4 Hz, 2H, CH$_2$), 3.30–3.12 (m, 1H, CH), 2.81 (d, J = 11.0 Hz, 2H, CH$_2$), 2.06 (d, J = 12.7 Hz, 2H, CH$_2$), 1.66 (dd, J = 12.5, 4.1 Hz, 2H, CH$_2$), 1.40 (s, 9H, (CH$_3$)$_3$), 1.33 (t, J = 7.1 Hz, 3H, CH$_2$CH$_3$).

13C NMR (100 MHz, CDCl$_3$): 28.4, 28.7, 35.6, 36.5, 40.9, 43.7, 79.7, 123.6, 127.0, 128.6, 129.2, 139.5, 149.6, 157.7, 160.8, 175.2; HRMS calcd for C$_{16}$H$_{24}$N$_2$O$_4$S [M+H]$^+$ 341.1457, found 341.1443.

3.2.2. tert-Butyl 4-(4-(phenylethylcarbamoyl)thiazol-2-yl)piperidine-1-carboxylate (3a)

Step 1. Sodium hydroxide (NaOH) (2.16 g, 0.054 mol) was added to a solution of the above ester (6.21 g, 0.018 mol) in a 1:1 mixture of THF/H$_2$O (50 mL) and the reaction mixture was stirred at r.t. for 10 h. The reaction was concentrated under reduced pressure and the resultant aqueous solution was adjusted to pH 2 using 10% HCl. Extraction with ethyl acetate (3 × 50 mL) resulted in combined organic layers that were dried (Na$_2$SO$_4$), filtered, and concentrated to afford a crude product that was used in the next step without further purification.

Step 2. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (EDC) (2.35 g, 12.0 mmol) and 1-hydroxybenzotriazole (HOBt) (1.08 g, 8.0 mmol) were added to a solution of the above acid (2.52 g, 8.0 mmol) in DCM (100 mL) at 0 °C. The reaction mixture was stirred at r.t. for 3 h, with phenylethylamine (0.97 g, 8.0 mmol) and DIPEA (1 mL) then added. The resultant reaction mixture was stirred at r.t. for 10 h before water (100 mL) was added to quench the reaction. The aqueous solution was extracted with DCM (3 × 50 mL) and the combined organic layers were then dried (Na$_2$SO$_4$), filtered, and concentrated to afford a crude product that was purified by column chromatography (PE:EA = 5:1) to afford the title compound (2.81 g, 85.1% over two steps) as a white wax. 1H NMR (400 MHz, CDCl$_3$): δ H 7.98 (s, 1H, ArH), 7.40–7.42 (m, 1H, NH), 7.30–7.34 (m, 2H, ArH), 4.16–4.19 (m, 2H, NCH$_2$), 3.68 (t, J = 6.8 Hz, 2H, CH$_2$), 3.06–3.12 (m, 1H, CH), 2.87–2.94 (m, 4H, 2NCH$_2$), 2.04–2.08 (m, 2H, CH$_2$), 1.70–1.74 (m, 2H, CH$_2$), 1.48 (s, 9H, (CH$_3$)$_3$).

13C NMR (100 MHz, CDCl$_3$): 28.4, 28.7, 35.6, 36.5, 40.9, 43.7, 79.7, 123.6, 127.0, 128.6, 129.2, 139.5, 149.6, 157.7, 160.8, 175.2; HRMS calcd for C$_{22}$H$_{29}$N$_3$O$_3$S [M+H]$^+$ 416.1938, found 416.1938.
3.2.3. tert-Butyl 4-(4-(benzylcarbamoyl)thiazol-2-yl)piperidine-1-carboxylate (3b)
Prepared using the same method described for 3a. Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ H 8.02 (s, 1H, ArH), 7.63 (s, 1H, NH), 7.26–7.36 (m, 5H, ArH), 4.64 (d, $J = 6.08$ Hz, 2H, CH$_2$), 4.16–4.19 (m, 2H, CH$_2$), 3.06–3.11 (m, 1H, CH), 2.83–2.89 (m, 2H, CH$_2$), 2.05–2.08 (m, 2H, CH$_2$), 1.69–1.74 (m, 2H, CH$_2$), 1.46 (s, 9H, (CH$_3$)$_3$). $^{13}$C NMR (100 MHz, CDCl$_3$): 28.4, 29.3, 36.2, 43.8, 44.4, 79.6, 123.8, 126.8, 127.0, 128.7, 142.7, 149.2, 157.7, 161.1, 175.4; HRMS calcd for C$_{21}$H$_{27}$N$_3$O$_3$S [M+H]$^+$ 402.1773, found 402.1779.

3.2.4. tert-Butyl 4-(4-(methylcarbamoyl)thiazol-2-yl)piperidine-1-carboxylate (3c)
Prepared using the same method described for 3a. Colorless oil. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ H 8.02 (s, 1H, ArH), 7.37 (s, 1H, NH), 4.19–4.22 (m, 2H, NCH$_2$), 3.10–3.16 (m, 1H, CH), 3.01 (s, 3H, NCH$_3$), 2.88–2.94 (m, 2H, CH$_2$), 2.05–2.11 (m, 2H, CH$_2$), 1.75–1.78 (m, 2H, CH$_2$), 1.49 (s, 9H, (CH$_3$)$_3$). $^{13}$C NMR (100 MHz, CDCl$_3$): 25.9, 28.4, 32.1, 40.3, 43.2, 79.6, 121.8, 149.6, 154.6, 161.7, 174.4; HRMS calcd for C$_{15}$H$_{23}$N$_3$O$_3$S [M+H]$^+$ 326.1460, found 326.1468.

3.3. General method A for carrying out peptide coupling reactions
3.3.1. (S)-tert-Butyl 4-methyl-1-oxo-1-(4-(4-(phenethylcarbamoyl)thiazol-2-yl)piperidin-1-yl)pentan-2-ylcarbamate (4a)
EDC (0.68 g, 4.36 mmol), HOBt (0.39 g, 2.89 mmol), and diisopropylethylamine (DIPEA) (1 mL) were added to a solution of L-Boc-Leu (0.67 g, 2.89 mmol) in DCM (20 mL) at 0 °C. The reaction mixture was stirred at r.t. for 1 h and then cooled to 0 °C using an ice-water bath. Amide 3a (1.20 g, 2.89 mmol) was dissolved in 4 mL of TFA and the reaction mixture was stirred for 3 h. Excess TFA was then removed under reduced pressure to afford a residue that was diluted with DCM (25 mL) and treated with excess DIPEA in the required volume. This solution was then added to the activated acid solution at 20 °C, with the resultant reaction mixture then stirred at r.t. for 18 h. Water (100 mL) was added to the reaction mixture and the aqueous solution was extracted with DCM (3 × 100 mL). The organic layers were combined, dried (Na$_2$SO$_4$), filtered, and concentrated to afford a crude product that was purified by column chromatography (EA:PE = 1:3) to afford the title compound (1.35 g, 89%) as a white wax. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ H 8.01 (m, 1H, ArH), 7.38–7.39 (m, 1H, NH), 7.23–7.34 (multiple, 6H, ArH+NH), 5.31–5.33 (m, 1H, CH), 4.68–4.73 (m, 1H, CH), 4.53–4.63 (m, 1H, CH), 3.95–4.07 (m, 1H, CH), 3.66–3.72 (m, 2H, CH$_2$), 3.17–3.31 (m, 2H, CH$_2$), 2.91–2.95 (m, 2H, CH$_2$), 2.11–2.25 (m, 2H, CH$_2$), 1.70–1.80 (multiple, 4H), 1.44 (s, 9H, (CH$_3$)$_3$), 0.93–1.02 (m, 6H, (CH$_3$)$_2$).

3.3.2. (S)-tert-Butyl 3-methyl-1-oxo-1-(4-(4-(phenethylcarbamoyl)thiazol-2-yl)piperidin-1-yl)butan-2-ylcarbamate (4b)
Prepared from Boc-L-Val using general method A. White wax, (1.29 g, 89.1%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$H 8.01 (m, 1H, ArH), 7.40 (s, 1H, NH), 7.34–7.33 (m, 5H, ArH), 5.37 (d, $J = 9.0$ Hz, 1H, CH), 4.51–4.67 (m, 2H, CH+NH), 4.01–4.13 (m, 1H, CH), 3.70 (q, $J = 6.3$ Hz, 2H, CH$_2$), 3.22–3.26 (m, 2H, CH$_2$), 2.89–2.97 (m, 3H, CH+CH$_2$), 2.15–2.22 (m, 2H, CH$_2$), 1.76–1.96 (m, 2H, CH$_2$), 1.45 (s, 9H, (CH$_3$)$_3$), 0.96 [dt, $J = 11.8$, 6.9 Hz, 6H, (CH$_3$)$_2$].
3.3.3. (S)-tert-Butyl1-oxo-1-(4-(4-(phenethylcarbamoyl)thiazol-2-yl)piperidin-1-yl)-3-phenylpropan-2-yl carbamate (4c)
Prepared from Boc-L-Phe using general method A. White wax (1.20 g 88.8%). 1H NMR (400 MHz, CDCl3): δH 7.96–7.99 (m, 1H, ArH), 7.10–7.35 (multiple, 12H, ArH+NH), 5.40–5.49 (m, 1H, CH), 4.86–4.90 (m, 1H, CH), 4.48–4.60 (m, 1H, CH2), 2.88–3.08 (multiple, 6H), 2.66–2.72 (m, 1H, CH), 2.00–2.02 (m, 1H, CH), 1.65–1.74 (multiple, 2H), 1.45 (s, 9H, 3CH3).

3.3.4. (S)-tert-Butyl4-methyl-1-(4-(4-(methylcarbamoyl)thiazol-2-yl)piperidin-1-yl)-1-Oxo-pentan-2-ylcarbamate (4d)
Prepared from Boc-L-Leu and 3c using general method A. Colorless oil (2.14 g, 87.5%). 1H NMR (400 MHz, CDCl3): δH 7.99 (s, 1H, ArH), 7.27–7.30 (m, 1H, NH), 5.31–5.35 (m, 1H, NH), 4.63–4.72 (m, 2H, NCH2), 4.05–4.12 (m, 1H, CH), 3.20–3.28 (m, 2H, CH2), 3.02 (s, 3H, NCH3), 2.81–2.96 (m, 1H, CH), 2.04–2.24 (m, 2H, CH2), 1.72–1.82 (multiple, 3H, CH2), 1.40–1.43 (multiple, 11H, 3Me+CH2), 0.99 (dd, 6H, J = 6.4 Hz, 2CH3). 13C NMR (100 MHz, CDCl3): 23.1, 25.5, 26.1, 28.5, 29.8, 35.8, 41.0, 43.9, 52.4, 80.5, 125.1, 152.9, 157.8, 165.4, 170.4, 173.6; HRMS calcd for C21H34N4O4S [M+H]+ 439.2301, found 439.2306.

3.3.5. (S)-tert-Butyl3-methyl-1-(4-(4-(methylcarbamoyl)thiazol-2-yl)piperidin-1-yl)-1-oxobutan-2-ylcarbamate (4e)
Prepared from Boc-L-Val and 3c using general method A. Colorless oil (2.06 g, 85.5%). 1H NMR (400 MHz, CDCl3): δH 7.99 (s, 1H, ArH), 7.27–7.30 (m, 1H, NH), 5.34–5.37 (m, 1H, NH), 4.65–4.68 (m, 1H, CH), 4.49–4.52 (m, 1H, CH), 4.01–4.15 (m, 1H, CH), 3.20–3.28 (m, 2H, CH2), 3.02 (s, 3H, NCH3), 2.83–2.88 (m, 1H, CH), 2.13–2.24 (m, 2H, CH2), 1.74–1.96 (multiple, 4H), 1.45 (s, 9H, 3Me), 0.99 (dd, 6H, J = 6.4 Hz, 2CH3).

3.3.6. (S)-tert-Butyl1-(4-(4-(benzylcarbamoyl)thiazol-2-yl)piperidin-1-yl)-4-methyl-1-oxopentan-2-ylcarbamate (4f)
Prepared from Boc-L-Leu and 3b using general method A. White wax (2.45 g 85.5%). 1H NMR (400 MHz, CDCl3): δH 7.99 (m, 1H, ArH), 7.61 (s, 1H, NH), 7.26–7.36 (m, 5H, ArH), 5.27–5.36 (m, 1H, CH), 4.57–4.68 (multiple, 4H), 3.96–4.00 (m, 1H, CH), 3.16–3.23 (m, 2H, NCH2), 2.74–2.88 (m, 1H, CH), 2.11–2.26 (m, 2H, CH2), 1.98–2.01 (m, 1H, CH), 1.71–1.87 (multiple, 4H), 1.45 (s, 9H, 3Me), 0.99 (dd, 6H, J = 6.4 Hz, 2CH3).

3.3.7. (R)-1-Methyl-N-((S)-4-methyl-1-oxo-1-(4-(4-(phenethylcarbamoyl)thiazol-2-yl)piperidin-1-yl)pentan-2-yl)piperidine-2-carboxamide (5a)
Prepared from D-N-Methyl-pipecolinic acid and compound 4a using general method A. White wax (0.18 g, 85.7%). 1H NMR (400 MHz, CD3OD): δH 8.07 (d, J = 3.6, 1H, ArH), 7.33–7.18 (m, 5H, ArH), 4.99 (J = 9.5, 4.4, 1H, dd, CH), 4.51 (dd, J = 31.6, 13.3, 1H, CH), 4.14 (dd, J = 36.2, 13.6, 1H, CH), 3.62 (t, J = 7.4, 2H, CH2), 3.39 (dd, J = 16.1, 8.4, 2H, CH2), 3.36 (s, 3H, NCH3), 3.04–2.85 (m, 4H, CH2), 2.58 (dd, J = 11.1, 2.3, 1H, CH), 2.33–2.03 (m, 6H, CH2), 1.88–1.44 (multiple, 8H, CH2), 0.99 [d, J = 4.2, 6H, (CH3)2]. 13C NMR (100 MHz, CDCl3): 174.7, 173.7, 170.8, 165.8, 152.7, 139.9, 130.1, 126.7, 124.9, 66.6, 53.1, 51.3,
3.3.8. \((R)-1\text{-Methyl}-N-((S)-3\text{-methyl-1-oxo-1-}(4-(4-(phenethylcarbamoyl)thiazole-2-yl)piperidin-1-yl)butan-2-yl)piperidine-2-carboxamide (5b)\)

Prepared from \(D\)-N-Methyl-pipecolinic acid and compound 4b using general method A. White wax (0.18 g, 88.7%). 

\(1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta H 8.04 (d, J = 6.5, 1H, ArH), 7.51 (s, 1H, NH), 7.47-7.37 (m, 1H, NH), 7.36-7.22 (m, 5H, ArH), 4.85 (dd, \(J = 8.7, 6.3, 1H, CH\)), 4.60 (dd, \(J = 44.9, 13.4, 1H, CH\)), 4.28-4.01 (m, 1H, CH), 3.81-3.62 (m, 2H, CH\(_2\)), 3.39-3.06 (m, 2H, CH\(_2\)), 3.04-2.78 (m, 4H, CH\(_2\)), 2.39 (d, \(J = 4.3\) Hz, 3H, CH\(_3\)), 2.22 (dd, \(J = 24.6, 11.4, 2H, CH\)), 2.14-2.02 (m, 6H, CH\(_2\)), 2.00-1.55 (multiple, 5H), 1.09-0.92 (m, 6H, (CH\(_3\))\(_2\)). 

\(13\)C NMR (100 MHz, CDCl\(_3\)): 173.6, 173.3, 172.1, 152.3, 139.8, 129.3, 128.6, 126.6, 124.4, 66.7, 55.9, 53.1, 43.6, 43.3, 42.7, 35.8, 35.5, 30.1, 28.3, 27.4, 24.7, 22.3, 18.8; HRMS calcd for C\(_{29}\)H\(_{41}\)N\(_5\)O\(_3\)S [M+H]+ 554.3087, found 554.3083.

3.3.9. \((R)-1\text{-Methyl-N-}((S)-1\text{-oxo-1-}(4-(4-(phenethylcarbamoyl) thiazol-2-yl) thiazol-2-yl) piperidin-1-yl)-3-phenylpropan-2-yl)piperidine-2-carboxamide (5c)\)

Prepared from \(D\)-N-methyl-pipecolinic acid and compound 4c using general method A. White wax (0.14 g, 87.5%). 

\(1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta H 8.09-8.00 (m, 1H, ArH), 7.33-7.15 (multiple, 10H, ArH), 5.24-5.09 (m, 1H, CH), 4.56-4.42 (m, 1H, CH), 4.13-3.92 (m, 1H, CH), 3.60 (dt, \(J = 10.7, 7.5, 2H, CH_2\)), 3.29-2.73 (m, 9H, (CH\(_2\))\(_4\), CH), 2.57-2.44 (m, 1H, CH), 2.08 (d, \(J = 3.4\) Hz, 3H, CH\(_3\)), 1.28-1.73 (multiple, 8H), 1.32-1.17 (m, 2H, CH\(_2\)). 

\(13\)C NMR (100 MHz, CDCl\(_3\)): 175.3, 173.5, 171.4, 160.6, 149.3, 139.7, 138.8, 129.8, 129.5, 128.6, 128.0, 126.5, 126.1, 124.1, 62.5, 53.5, 51.4, 42.4, 40.9, 40.1, 41.4, 37.6, 36.1, 35.5, 29.8, 27.5, 25.6 23.0; HRMS calcd for C\(_{33}\)H\(_{41}\)N\(_5\)O\(_3\)S [M+H]+ 588.2930, found 588.2932.

3.3.10. \((S)-1\text{-Oxo-1-}(4-(4-(phenethylcarbamoyl) thiazol-2-yl) piperidin-1-yl)-3-phenylpropan-2-yl)isonicotinamide (5d)\)

Prepared from isonicotinic acid and compound 4c using general method A. White wax (0.22 g, 41%). 

\(1\)H NMR (400 MHz, MeOD): \(\delta H 8.66-8.70 (m, 2H), 8.04 (d, J = 12.4, 1H), 7.75-7.79 (m, 2H), 7.13-7.32 (m, 10H), 5.29-5.34 (m, 1H), 4.51 (t, \(J = 11.9, 1H\)), 4.16-3.97 (m, 1H), 3.60 (dt, \(J = 19.4, 7.4, 2H, CH_2\)), 3.29-3.04 (m, 4H, CH\(_2\)), 2.86 (m, 4H), 2.15-1.92 (m, 2H), 1.86 (m, 1H). 

\(13\)C NMR (100 MHz, CD\(_3\)OD): 173.6, 171.8, 167.3, 165.8, 153.5, 143.1, 139.8, 137.7, 129.6, 129.3, 128.9, 128.7, 127.2, 126.6, 124.7, 120.3, 43.9, 42.7, 38.2, 35.8, 35.2, 29.8; HRMS calcd for C\(_{32}\)H\(_{33}\)N\(_5\)O\(_3\)S [M+H]+ 568.2304, found 568.2303.

3.3.11. \((S)-1\text{-Oxo-1-}(4-(methylcarbamoyl) thiazol-2-yl)piperidin-1-yl)-3-phenylpropan-2-yl)isonicotinamide (5e)\)

White wax (0.15 g, 39%). 

\(1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta H 8.71 (d, J = 6.0, 2H, ArH), 8.07 (d, J = 13.4, 1H, ArH), 7.81 (d, J = 6.0, 2H, ArH), 4.95 (d, J = 8.3, 1H, CH), 4.54-4.67 (m, 1H, CH), 4.36-4.44 (m, 1H, CH), 3.35-3.50 (m, 2H, CH\(_2\)), 2.93-2.95 (multiple, 4H, CH\(_3\)+CH), 2.17-2.30 (m, 2H, CH\(_2\)), 1.75-1.81 (m, 2H, CH\(_2\)), 1.04 [dd, \(J = 12.3, 6.7, 6H, (CH_3)_2\)]. 

\(13\)C NMR (100 MHz, CDCl\(_3\)) 173.6, 172.7, 167.6, 165.2, 153.5,
152.3, 143.6, 126.0, 120.3, 55.6, 43.9, 35.6, 30.1, 28.9, 26.2, 19.3; HRMS calcd for C_{21}H_{27}N_{5}O_{3}S $[M+H]^+$ 430.1835, found 430.1837.

3.3.12. (S)-1-(4-methoxyphenyl)-3-(4-methyl-1-(4-(4-(methylcarbamoyl)thiazol-2-yl)piperidin-1-yl)-1-oxopentan-2-yl)urea (5f)

White wax (0.23 g, 76%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$H 8.04 (d, $J = 16$ Hz, 1H, NH), 7.51–7.52 (m, 1H, NH), 7.20–7.38 (m, 3H, ArH), 6.80 (d, $J = 12$ Hz, 2H, ArH), 6.42–6.43 (m, 1H, NH), 5.05–5.08 (m, 1H, CH), 4.57–4.64 (m, 1H), 4.14-4.23 (m, 1H), 3.77 (s, 3H, OCH$_3$), 3.25–3.31 (m, 2H), 3.02–3.05 (multiple, 4H, NCH$_3$, CH), 2.07–2.24 (m, 2H), 1.79–1.85 (m, 2H), 1.44–1.56 (m, 2H), 1.27–1.30 (m, 1H, CH), 0.97–0.99 [m, 6H, (CH$_3$)$_2$]. $^{13}$C NMR (100 MHz, CDCl$_3$): 174.8, 171.2, 165.2, 155.3, 154.2, 150.5, 128.1, 125.1, 122.7, 114.3, 55.9, 51.7, 42.4, 41.2, 36.1, 29.5, 26.2, 22.6, 21.7; HRMS calcd for C_{24}H_{33}N_{5}O_{4}S $[M+H]^+$ 488.2253, found 488.2257.

3.3.13. (S)-1-(1-(4-(4-(Benzylcarbamoyl)thiazol-2-yl)piperidin-1-yl)-4-methyl-1-oxopentan-2-yl)-3-(4-methoxyphenyl)urea (5g)

White wax (0.18 g, 73%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$H 8.11–8.12 (s, 1H, ArH), 8.01–8.04 (m, 1H, NH), 7.32–7.35 (m, 1H, ArH), 7.28–7.32 (m, 2H, ArH), 7.21–7.28 (multiple, 4H, ArH), 6.93–6.98 (m, 1H, NH), 6.83–6.89 (m, 2H, ArH), 5.99–6.01 (m, 1H, NH), 5.04–5.05 (m, 1H, CH), 4.70 (s, 1H), 4.51–4.65 (m, 1H), 4.09–4.15 (m, 1H), 3.77 (s, 3H, OCH$_3$), 3.71–3.73 (m, 2H), 3.23–3.28 (m, 2H), 2.87–3.00 (m, 2H), 2.04–2.15 (m, 4H), 1.40–1.46 (m, 1H), 1.05 [m, $J = 8.0$ Hz, 6H, 2(CH$_3$)]. $^{13}$C NMR (100 MHz, CDCl$_3$): 173.7, 170.8, 168.6, 155.7, 154.9, 152.3, 140.3, 132.3, 128.2, 127.9, 127.7, 125.1, 122.5, 113.7, 56.5, 52.8, 44.5, 43.3, 41.1, 35.9, 29.3, 25.6, 23.0; HRMS calcd for C_{30}H_{37}N_{5}O_{4}S $[M+H]^+$ 564.2566, found 564.2569.

3.3.14. (S)-1-(4-Methoxyphenyl)-3-(4-methyl-1-oxo-1-(4-(4-(phenethylcarbamoyl)thiazol-2-yl)piperidin-1-yl)pentan-2-yl)urea (5h)

White wax (0.14 g, 65%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$H 8.11–8.12 (m, 1H, NH), 8.01–8.04 (m, 1H, NH), 7.31–7.35 (m, 1H, ArH), 7.28–7.32 (m, 2H, ArH), 7.21–7.28 (multiple, 4H, ArH), 6.93–6.98 (m, 1H, NH), 6.83–6.89 (m, 2H, ArH), 5.99–6.01 (m, 1H, NH), 4.70 (s, 1H), 4.52–5.65 (m, 1H), 4.13–4.17 (m, 1H), 3.77 (s, 3H, OCH$_3$), 3.71–3.73 (m, 2H), 3.23–3.28 (m, 2H), 2.87–3.00 (m, 2H), 2.04–2.15 (m, 4H), 1.40–1.46 (m, 1H), 1.05 [m, $J = 8.0$ Hz, 6H, 2(CH$_3$)]. $^{13}$C NMR (100 MHz, CDCl$_3$): 174.9, 171.1, 162.8, 156.5, 154.5, 149.7, 139.7, 129.7, 129.1, 128.6, 126.5, 123.7, 122.5, 114.2, 55.9, 51.3, 42.2, 42.1, 40.4, 36.5, 35.6, 29.3, 25.6, 23.9; HRMS calcd for C_{31}H_{39}N_{5}O_{4}S $[M+H]^+$ 578.2723, found 578.2728.

3.3.15. (S)-1-(4-Nitrophenyl)-3-(4-methyl-1-oxo-1-(4-(4-(phenethylcarbamoyl)thiazol-2-yl)piperidin-1-yl)pentan-2-yl)urea (5i)

White wax, 78%. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$H 8.41 (s, 1H, NH), 8.01–8.04 (m, 1H, NH), 7.95–7.98 (m, 2H, ArH), 7.41–7.48 (m, 1H, ArH), 7.23–7.35 (multiple, 7H, ArH), 7.01–7.09 (m, 1H, NH), 5.01–5.04 (m, 1H), 4.57–4.68 (m, 1H), 4.09–4.23 (m, 1H), 3.68–3.74 (m, 2H), 3.23–3.55 (m, 2H), 2.89–3.16 (multiple, 5H), 2.22–2.40 (m, 2H), 1.80–1.91 (m, 2H), 1.45–1.56 (m, 2H), 0.97–1.02 (m, 6H, 2CH$_3$). $^{13}$C NMR (100 MHz, CDCl$_3$): 175.4,
172.8, 168.1, 154.5, 149.4, 144.7, 142.0, 139.5, 128.5, 127.8, 126.3, 123.8, 122.7, 121.2, 51.3, 42.1, 41.0, 40.3, 36.3, 35.9, 29.7, 22.4, 21.6; HRMS calcd for C_{30}H_{36}N_{5}O_{6}S [M+H]^+ 593.2468, found 593.2466.

3.3.16. (S)-1-(4-Chlorophenyl)-3-(4-methyl-1-(4-(4-(methylcarbamoyl)thiazol-2-yl)piperidin-1-yl)-1-oxopentan-2-yl)urea (5j)

White wax, yield: 73%. \(^1^H\) NMR (400 MHz, CDCl\(_3\)): \(\delta_H\) 8.01–8.04 (m, 1H, ArH), 7.71–7.80 (m, 1H, NH), 7.25–7.28 (m, 1H, NH), 7.07–7.08 (m, 4H, ArH), 6.62–6.69 (m, 1H, NH), 4.95–5.04 (m, 1H, CH), 4.56–4.66 (m, 2H), 4.11–4.21 (m, 1H), 3.21–3.42 (m, 2H), 2.98 (s, 3H, NCH\(_3\)), 2.22–2.35 (m, 2H), 1.95–2.05 (m, 1H), 1.72–1.85 (m, 2H), 1.41–1.52 (m, 1H), 1.2–1.3 (m, 2H), 0.97–1.02 (m, 6H, 2CH\(_3\)). \(^1^3^C\) NMR (100 MHz, CDCl\(_3\)): 174.9, 172.6, 168.1, 154.5, 149.4, 134.9, 129.9, 129.4, 123.9, 123.0, 51.4, 42.4, 41.2, 36.4, 29.2, 26.9, 23.0, 21.2; HRMS calcd for C_{31}H_{39}N_{5}O_{4}S [M+H]^+ 578.2723, found 578.2728.

3.3.17. (S)-1-(4-Chlorophenyl)-3-(4-methyl-1-oxo-1-(4-(4-(phenethylcarbamoyl)thiazol-2-yl)piperidin-1-yl)pentan-2-yl)urea (5k)

White wax, yield: 75%. \(^1^H\) NMR (400 MHz, CDCl\(_3\)): \(\delta_H\) 8.0–8.02 (m, 1H, ArH), 7.12–7.37 (multiple, 11H, ArH + NH), 6.36–6.44 (m, 1H, NH), 4.98–5.07 (m, 1H, NCH), 4.52–4.69 (m, 1H), 4.02–4.19 (m, 1H), 3.67–3.73 (m, 2H), 3.23–3.44 (m, 2H), 2.91–2.95 (m, 3H), 2.13–2.36 (m, 2H), 1.74–1.85 (multiple, 3H), 1.33–1.48 (m, 2H), 1.03–1.05 (d, \(J = 6.5\) Hz, 3H, CH\(_3\)), 0.97–0.98 (m, 3H, CH\(_3\)). \(^1^3^C\) NMR (100 MHz, CDCl\(_3\)): 23.3, 26.5, 29.1, 34.6, 35.4, 41.3, 42.7, 44.9, 55.2, 121.5, 124.7, 128.1, 128.2, 129.0, 139.0, 139.2, 152.3, 155.2, 168.7, 170.9, 173.5; HRMS calcd for C_{30}H_{37}ClN_{5}O_{3}S [M+H]^+ 582.1565, found 582.1569.

3.3.18. (S)-1-(3-Methyl-1-oxo-1-(4-(4-(phenethylcarbamoyl)thiazol-2-yl)piperidin-1-yl)butan-2-yl)-3-(4-nitrophenyl)urea (5l)

White wax, 77%. \(^1^H\) NMR (400 MHz, CDCl\(_3\)): \(\delta_H\) 8.31 (s, 1H, NH), 8.06–8.08 (m, 3H, ArH), 7.41–7.43 (m, 3H, ArH), 7.25–7.28 (m, 6H, ArH + NH), 6.86–6.91 (m, 1H, NH), 4.83–4.89 (m, 1H, NCH), 4.62–4.70 (m, 1H), 4.26–4.28 (m, 1H, CH), 3.71–3.74 (m, 2H), 3.25–3.48 (m, 2H), 3.01–3.09 (m, 1H), 2.93–2.96 (m, 2H), 2.15–2.35 (m, 4H), 1.77–1.89 (m, 1H, CH), 1.01–1.11 (m, 6H, 2CH\(_3\)). \(^1^3^C\) NMR (100 MHz, CDCl\(_3\)): 173.3, 172.0, 165.9, 155.7, 152.7, 145.4, 144.1, 139.4, 129.1, 128.2, 126.7, 124.9, 121.1, 119.2, 57.6, 43.7, 42.8, 35.8, 35.5, 30.2, 29.4, 20.3; HRMS calcd for C_{29}H_{34}N_{6}O_{5}S [M+H]^+ 579.2311, found 579.2318.

3.3.19. (S)-1-(4-chloro-3-(trifluoromethyl)phenyl)-3-(3-methyl-1-oxo-1-(4-(4-(phenethylcarbamoyl)thiazol-2-yl)piperidin-1-yl)butan-2-yl)urea (5m)

White wax, 61%. \(^1^H\) NMR (400 MHz, CDCl\(_3\)): \(\delta_H\) 8.04–8.06 (m, 1H, ArH), 8.01–8.04 (m, 1H, NH), 7.57 (m, 1H, ArH), 7.46 (m, 1H, ArH), 7.31–7.34 (m, 1H, ArH), 7.24–7.28 (m, 6H, ArH), 6.68–6.76 (m, 1H), 4.80–4.89 (m, 1H), 4.60–4.70 (m, 1H), 4.26–4.28 (m, 1H), 3.68–3.75 (m, 2H), 3.31 (m, 1H), 2.92–2.98 (m, 2H), 2.15–2.34 (m, 2H), 2.02–2.07 (m, 2H), 1.64–1.88 (m, 2H), 1.40–1.46 (m, 1H), 1.02–1.06 (m, 6H, 2CH\(_3\)). \(^1^3^C\) NMR (100 MHz, CDCl\(_3\)): 172.5, 160.5, 155.9, 154.8, 149.3, 138.4, 137.8, 131.2, 128.4, 128.3, 128.2, 128.1, 126.1, 126.0, 122.0, 117.4, 116.9, 54.1, 45.3, 41.8, 40.2, 39.2, 35.4, 31.9, 30.9, 29.2, 19.3, 17.4; HRMS calcd for C_{30}H_{33}ClF_{3}N_{5}O_{3}S [M+H]^+ 636.1945, found 636.1948.
Acknowledgment

The authors would like to thank the Doctoral Foundation of Yantai University (No. YX13B04) and the Talent Development Project of the Blue Economic Zone of Shandong Province (No. RS11YX) for the financial support.

References

Supporting information includes

**Chemical Structure:**

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**Diagram:**

- A spectrogram showing chemical shifts from 0.0 to 7.5 ppm.
- Notable resonances at 4.0, 2.0, and 0.8 ppm.
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d11 0.00000000 sec
MCREST 0.00000000 sec
MCWRK 0.01500000 sec

======== CHANNEL f1 ========
NUC1 13C
P1 7.40 usec
PL1 -2.00 dB
SFO1 100.6246344 MHz

======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -4.00 dB
PL12 10.26 dB
SFO2 400.1316005 MHz

F2 - Processing parameters
SI 32768
SF 100.6127770 MHz
WW 1.40
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
Boc-NH₂

Parameter Value
1 Solvent CDCl₃
2 Temperature 295.5 K
3 Pulse Sequence
4 Experiment ID
5 Number of 16 Scans
6 Receiver Gain
7 Relaxation 1.0000
8 Pulse Width 15.5000 μs
9 Acquisition 2.7329 s
10 Acquisition Date 2016-10-25 10:26:22
11 Modification Date 2016-10-25 10:26:30
12 Spectrometer Frequency 400.13 MHz
13 Spectral Width 11990.4 Hz
14 Lowest Frequency -4488.7 Hz
15 Nucleus ¹H
16 Acquisition Size 32768
17 Spectral Size 65536
BXF-3770010-2-1H

Current Data Parameters
NAME: Proton-s
EXPNO: 9210
PROCNO: 1

F2 - Acquisition Parameters
Date: 20180302
Time: 11:38
INSTRUM: av400
PROBHD: 5 mm DUL 13C-1
PULPROG: zg30
TD: 65536
SOLVENT: CDCl3
NS: 16
DS: 0
SWH: 11990.407 Hz
FIDRES: 0.182959 Hz
AQ: 2.7329011 sec
RG: 64
DW: 41.700 usec
DE: 6.00 usec
TE: 292.8 K
DT: 1.00000000 sec
MCREST: 0.00000000 sec
MCWRK: 0.01500000 sec

------- CHANNEL f1 -------
NUC1: 1H
P1: 15.50 usec
PL1: -4.00 dB
SFO1: 400.1315084 MHz

F2 - Processing parameters
SI: 32768
SF: 400.1300041 MHz
WDW: EM
SSB: 0
LB: 0.30 Hz
GB: 0
PC: 1.00
BXF-3a-1H

Parameter | Value
---|---
Comment | BXF-3a-1H
Spectrometer | av400
Solvent | CDCl3
Temperature | 298.2
Pulse Sequence | zg30
Experiment | 1D
NS | 16
RG | 64
Relaxation Delay | 1.0000
Pulse Width | 15.5000
Acquisition Date | 2016-11-02T09:56:00
Spectrometer Frequency | 400.13
Spectral Width | 11990.4
Lowest Frequency | -4488.7
Nucleus | 1H
Acquired Size | 32768
Spectral Size | 65536
Parameter | Value
--- | ---
Comment | BXF-XBYA-1H
Spectrometer | av400
Solvent | MeOD
Temperature | 292.4
Pulse Sequence | zg30
Experiment | 1D
NS | 16
RG | 64
Relaxation Delay | 1.0000
Pulse Width | 15.5000
Acquisition Date | 2017-01-06T10:23:48
Spectrometer Frequency | 400.13
Spectral Width | 11990.4
Lowest Frequency | -4495.3
Nucleus | 1H
Acquired Size | 32768
Spectral Size | 65536
Current Data Parameters
NAME       Proton-s
EXPNO      9271
PROCNO     1

F2 - Acquisition Parameters
Date       20180316
Time       10.59
INSTRUM    av400
PROBHD     5 mm DUL 13C-1
PULPROG    zg30
TD          65536
SOLVENT    CDCl3
NS          16
DS          0
SWH         11990.407 Hz
FIDRES     0.182959 Hz
AQ          2.7329011 sec
RG          64
DW          41.700 usec
DE          6.00 usec
TE          294.3 K
DT          1.00000000 sec
MCREST      0.00000000 sec
MCWRK       0.01500000 sec

-------- CHANNEL f1 --------
NUC1       1H
P1          15.50 usec
PL1         -4.00 dB
SFO1       400.1315084 MHz

F2 - Processing parameters
SI          32768
SF          400.1300074 MHz
WDW         EM
SSB         0
LB          0.30 Hz
GB          0
PC          1.00
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### Current Data Parameters

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#### F2 - Acquisition Parameters

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<td>INSTRUM</td>
<td>av400</td>
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<tr>
<td>PROBHD</td>
<td>5 mm DUL 13C-1</td>
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<tr>
<td>PULPROG</td>
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<td>TD</td>
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<td>SOLVENT</td>
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<td>NS</td>
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<td>DS</td>
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<td>SWH</td>
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<tr>
<td>FIDRES</td>
<td>0.182959 Hz</td>
</tr>
<tr>
<td>AQ</td>
<td>2.7329011 sec</td>
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<tr>
<td>RG</td>
<td>64</td>
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<tr>
<td>DW</td>
<td>41.700 usec</td>
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<tr>
<td>DE</td>
<td>6.00 usec</td>
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<tr>
<td>TE</td>
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<tr>
<td>DT</td>
<td>1.00000000 sec</td>
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<tr>
<td>MCREST</td>
<td>0.00000000 sec</td>
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<tr>
<td>MCWRK</td>
<td>0.01500000 sec</td>
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#### CHANNEL f1

<table>
<thead>
<tr>
<th>NUC1</th>
<th>1H</th>
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</thead>
<tbody>
<tr>
<td>P1</td>
<td>15.50 usec</td>
</tr>
<tr>
<td>PL1</td>
<td>-4.00 dB</td>
</tr>
<tr>
<td>SFO1</td>
<td>400.1315084 MHz</td>
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#### F2 - Processing parameters

| SI              | 32768           |
| SF              | 400.1300092 MHz |
| WDW            | EM              |
| SSB            | 0               |
| LB             | 0.30 Hz         |
| GB             | 0               |
| PC             | 1.00            |

---

**BXF377-0012-4-1H**

![NMR Spectrum](image-url)