CHAPTER 4

SYNTHESIS OF DIAMIDES DERIVATIVES OF GLYCINE AS DIPEPTIDYL PEPTIDASE-IV INHIBITORS

4.1 Introduction

In recent years, diabetes has become a severe and increasingly prevalent disease due to urbanization and lifestyle changes [1]. The symptoms of this chronic disease being less marked often leads to late diagnosis until the microvascular or macrovascular complications set to show [2-6]. Treatment includes various therapies, acting through different pathways, including the dipeptidyl peptidase IV (DPP-IV) inhibition.

Dipeptidyl peptidase IV (EC 3.4.14.5) is a highly specific, cell surface, serine protease which is responsible for rendering the incretin hormones like the GLP-1 and GIP inactive, *in-vivo*, by cleaving the N-terminal dipeptides with L-proline or L-alanine at the penultimate position [7-9].

Glucagon-like peptide-1 (GLP-1), secreted by the L-cells of intestine in response to the food intake, acts as a stimulator of endogenous insulin release while inhibiting the glucagon secretion in a glucose dependent manner, thereby reducing the risk of hypoglycemia [10-13]. Continuous infusion of GLP-1 has been reported to significantly reduce the blood glucose level in patients with T2D [14]. This active form of GLP-1[7-36]amide is rapidly degraded by DPP-IV in about a minute, to its inactive form GLP-1[9-36]amide which has no therapeutic effect [15-16]. Thus inhibition of DPP-IV will help to increase the half-life of GLP-1 *in-vivo*, thereby increasing its bio-activity.

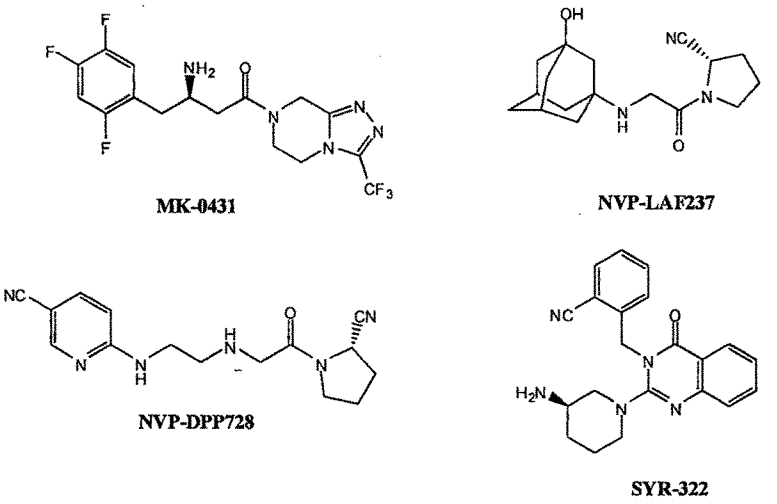


Figure 4.1: DPP-IV inhibitors

Various classes of DPP-IV inhibitors (Figure 4.1) have been reported from many laboratories, and most of them are derived from α- and β-amino acids aping the N-terminal dipeptide residues of the incretin hormones. Amongst them sitagliptin (MK-0431) and alogliptin (SYR-322) are exceptions [17-18]. Some of the potent DPP-IV inhibitors reported so far, have sulfonamide at the P-2 position.

So far, effect of substitution of sulphonamide (Chapter 2) and coumarin derivatives (Chapter 3) at the P-1 site on DPP-IV inhibition have been studied.

From both these studies it can be concluded that substitution of sulphonamide at the P2 site resulted in better enzyme inhibition. Taking into account all these structure activity relationship studies, diamide derivatives of glycine with 1-(phenylsulfonyl)piperidine-3-carboxylic acid condensed at the N-terminus of glycine while condensing various 1^{o} or 2^{o} amines at the C-terminus have been designed (Figure 4.2), synthesized and studied for their anti-diabetic activity. All these synthesized molecules were then screened for *in-vitro* DPP-IV inhibition.

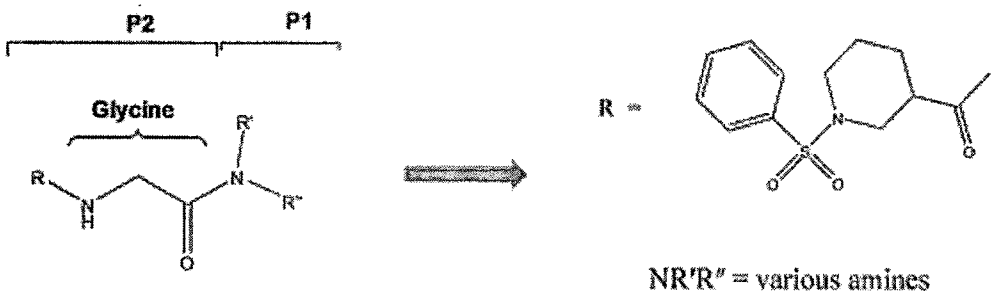
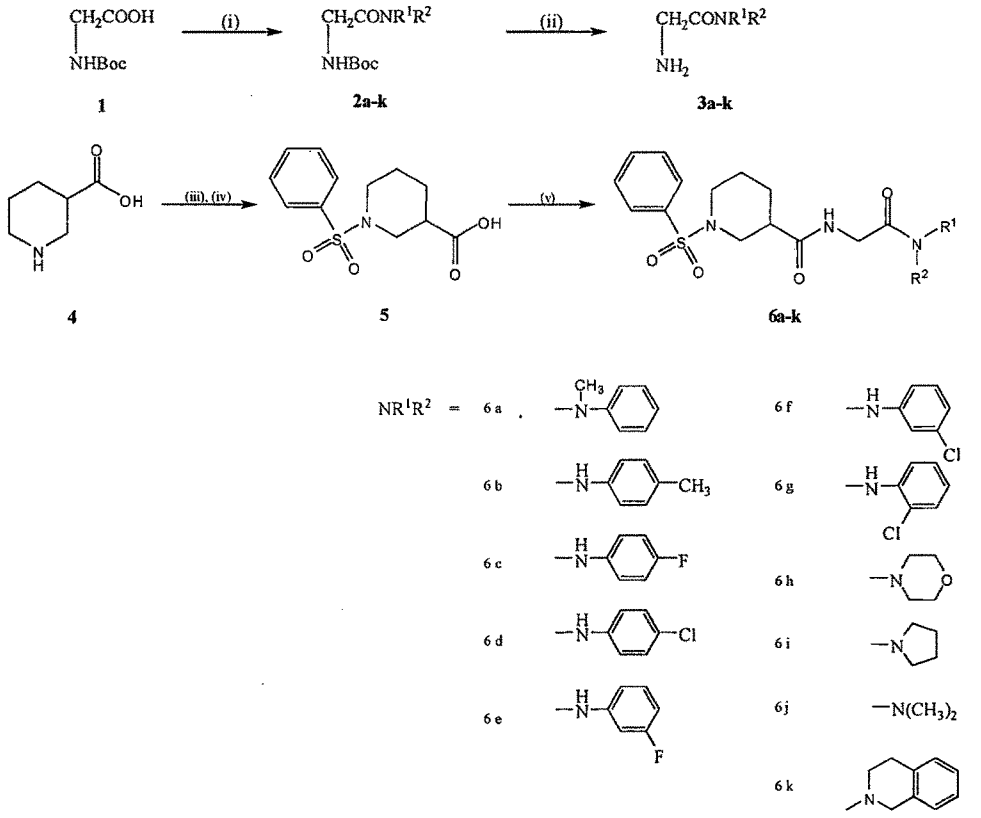


Figure 4.2: Design of diamides derivatives of glycine as DPP-IV inhibitors.

4.2 Results and Discussion

4.2.1 Chemistry

In order to synthesize diamide derivatives of glycine, commercially available boc-protected glycine **1** was at first reacted with various amines in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI), 1-hydroxybenzotriazole (HOBt), 4-dimethylaminopyridine (DMAP), to yield corresponding C-substituted amide derivatives of glycine **2a-k** as shown in Scheme 4.1. The structures of few intermediates from **2a-k** have been proved in Chapter 5 (compounds **5a-f**). Figure 5.3.1 to 5.8.4 shows IR, ^{1}H NMR, ^{13}C NMR and ESI-MS spectra of few boc-glycylamides **2a-k**. On the other hand, reaction of piperidine-3-carboxylic acid **4** with benzenesulfonyl chloride, in the presence of sodium carbonate as a base, in a mixture of dichloromethane : water (1:1) gave 1-(phenylsulfonyl)piperidine-3-carboxylic acid **5** on acidification. IR spectrum of **5** (**Figure 2.5.1**, **Chapter 2**) showed bands at 1693 and 1349 cm^{-1} for the carbonyl group of carboxylic acid and sulfonamide group respectively while the ^{1}H NMR (**Figure 2.5.2**, **Chapter 2**) showed multiplet from δ 7.56-7.79 for the five aromatic protons and a broad singlet at δ 8.98 indicating the proton of the carboxylic acid group thereby confirming the formation of **5.** Free bases **3a-k** were obtained on stirring boc-protected glycine derivatives **2a-k** in 10% TFA in DCM, which on further reaction with 1-(phenylsulfonyl)piperidine-3-carboxylic acid **5** in the presence of peptide coupling agents EDCI, HOBt, DMAP gave the desired diamide derivatives of glycine **6a-k** as shown in Scheme 4.1.



Scheme 4.1 : Reagents: (i) EDCI, DMAP, DCM, primary or secondary amine; (ii) TFA, DCM; (iii) $PhSO_2Cl$, Na_2CO_3, DCM, H_2O; (iv) HCl; (v) EDCI, HOBt, DMAP, DCM, **3a-k**.

The structures of **6a-k** were confirmed by their IR, ^1H NMR, ^{13}C NMR and ESI-MS analysis. The IR spectrum of compounds **6d** (Figure 4.6.1) exhibited two strong band at 3329, 3303 cm^{-1} for the two amide –NH protons, another two strong bands 1679 and 1644 cm^{-1} for amide carbonyl groups and a strong band at 1355 cm^{-1} for sulfonamide group. In the ^1H NMR spectrum of **6d** (Figure 4.6.2), the methylene group of glycine showed a multiplet at δ 3.84-3.86 due to the interactions with the neighbouring amide groups and multiplet form δ 7.36-7.74 represented the aromatic protons while in the ^{13}C NMR spectrum (Figure 4.6.3), two peaks at 168.26 and 173.16 for the carbonyl

carbons of the amide groups, six peaks from 24.14 to 48.63 for the piperidyl and glycyl carbons and eight peaks ranging from 121.10-138.25 for the aromatic carbons thereby confirming the formation of **6d** which is also supported by its ESI-MS spectrum (Figure 4.6.4) with a peak at *m/z* 435.9 for $[M+H]^+$.

Figures from 4.3.1 onwards, upto 4.13.4 show IR, ^1H NMR, ^{13}C NMR and ESI-MS spectra of compounds **6a-k** thus confirming the structure of all the synthesised compounds.

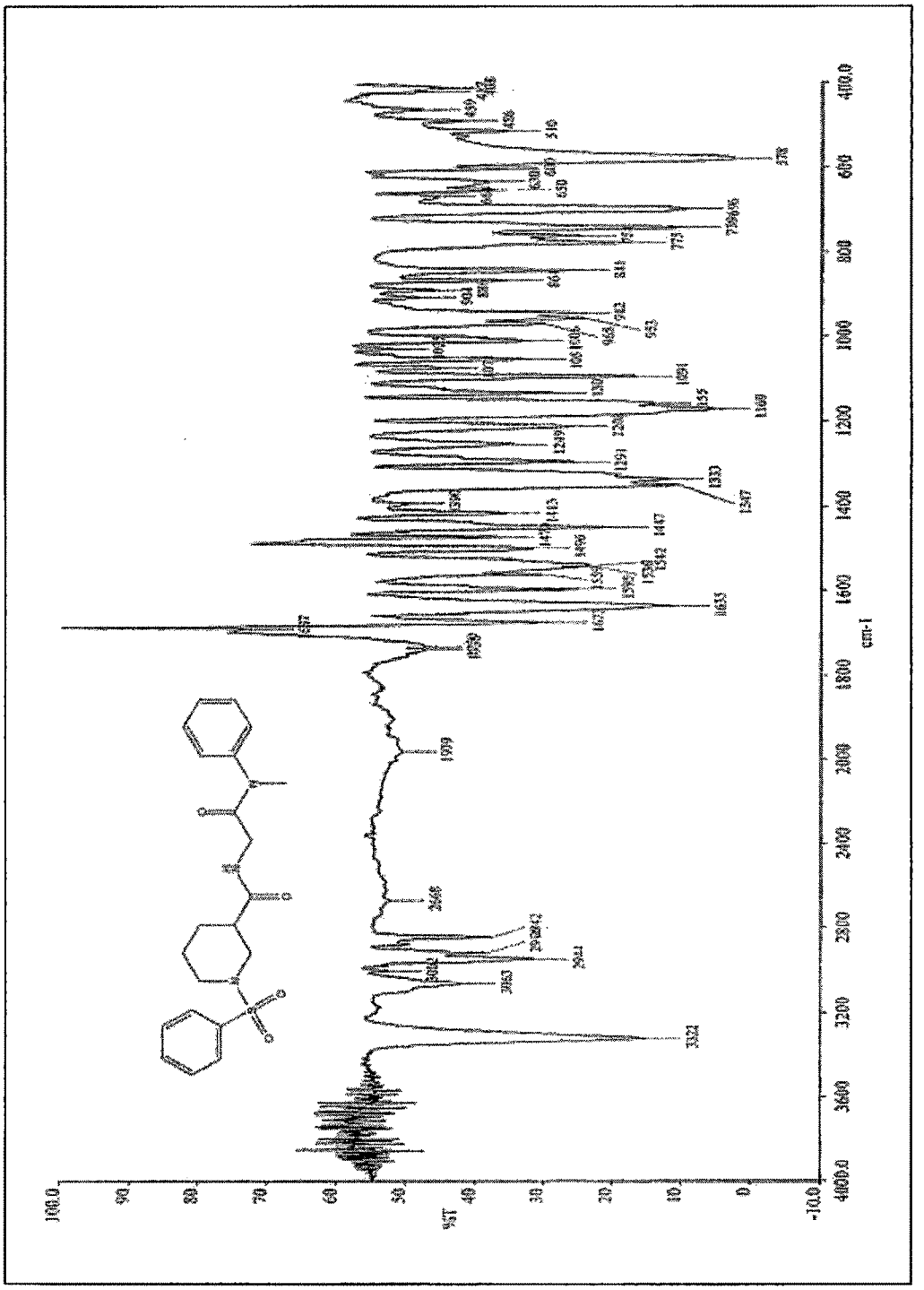


Figure 4.3.1: IR spectrum of N-(2-(methyl(phenyl)amino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6a**

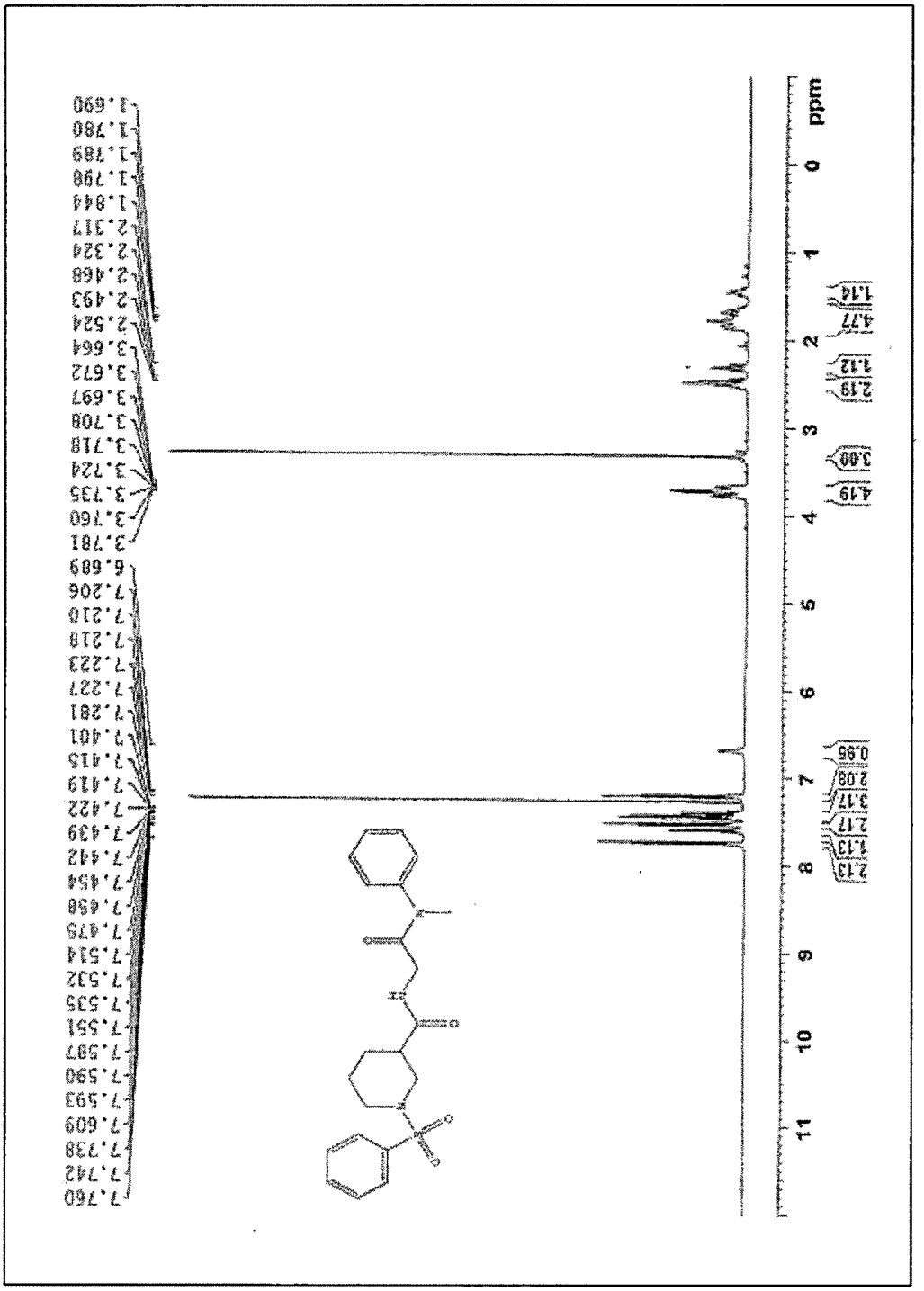


Figure 4.3.2: ^{1}H NMR spectrum of N-(2-(methyl(phenyl)amino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6a**

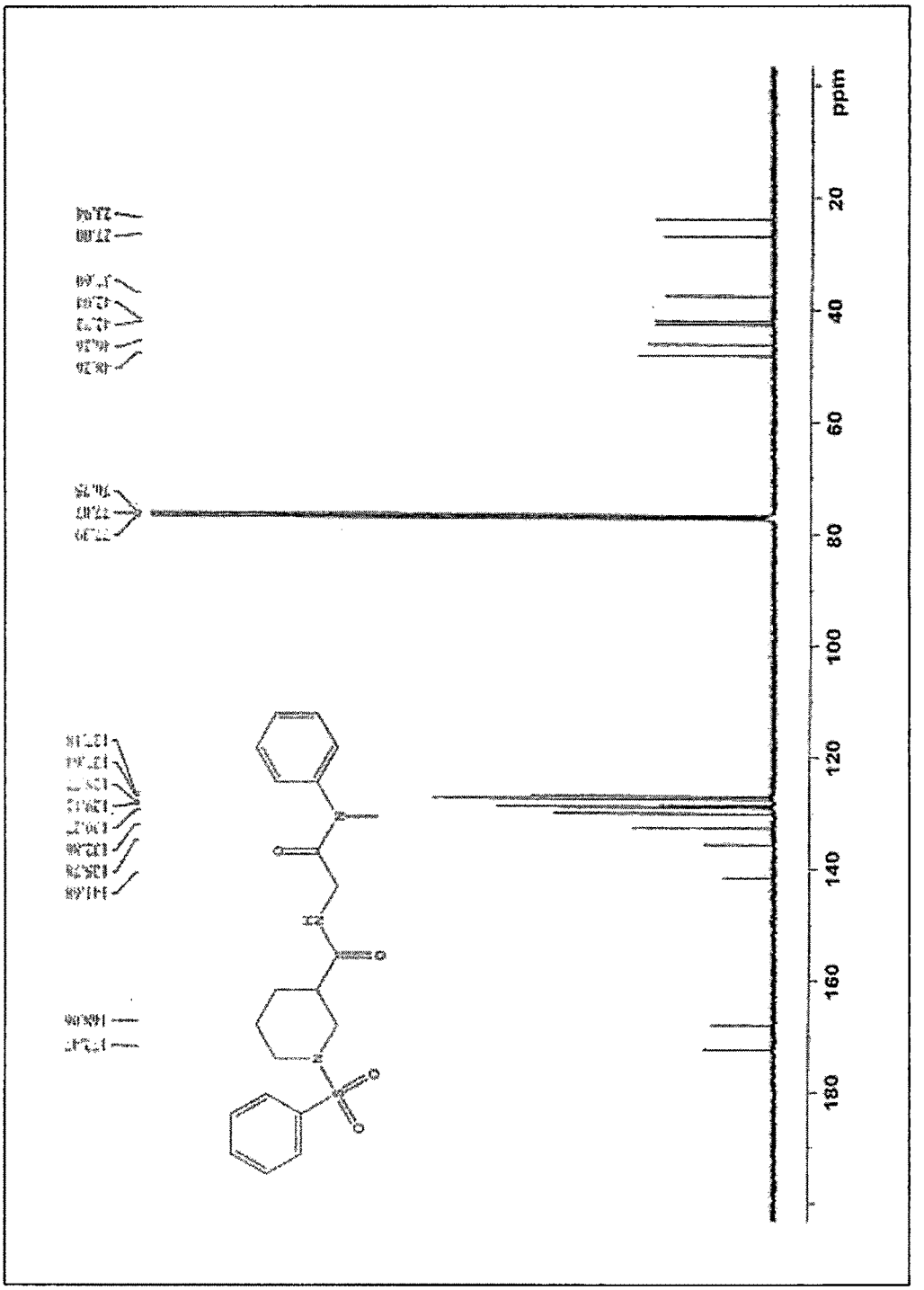


Figure 4.3.3: ^{13}C NMR spectrum of N-(2-(methyl(phenyl)amino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6a**

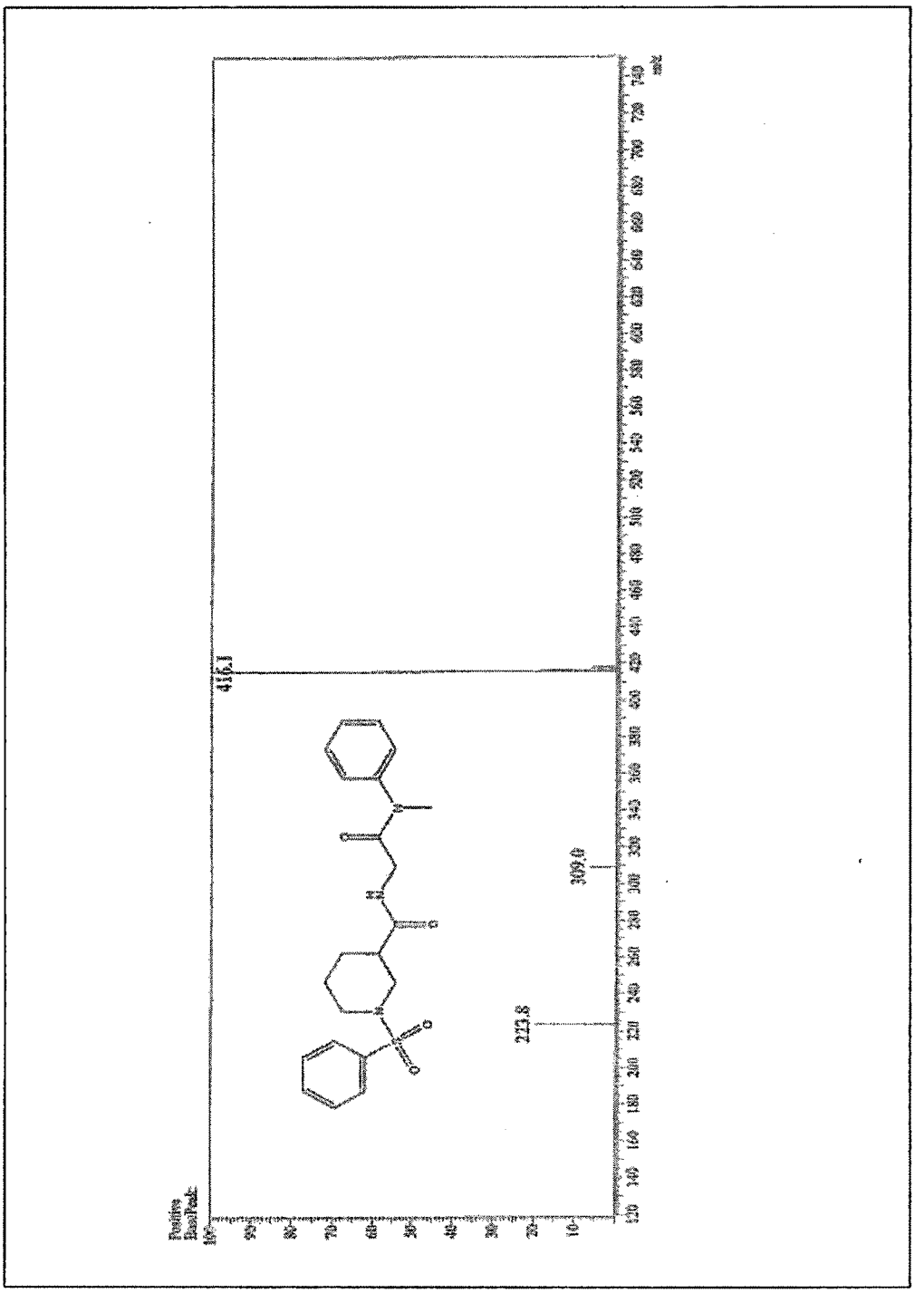


Figure 4.3.4: ESI-MS spectrum of N-(2-(methyl(phenyl)amino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6a**

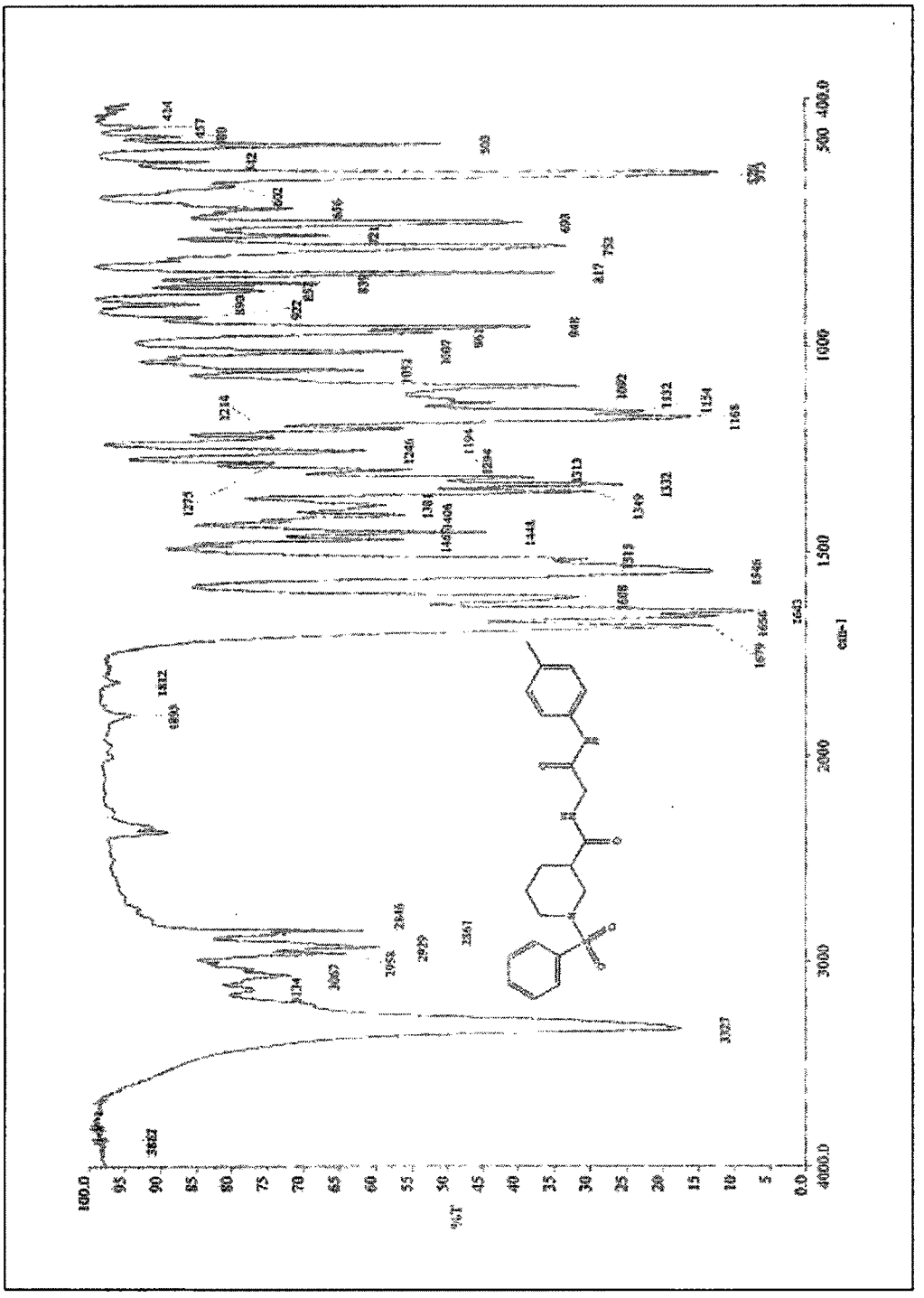


Figure 4.4.1: IR spectrum of N-(2-oxo-2-(p-tolylamino)ethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6b**

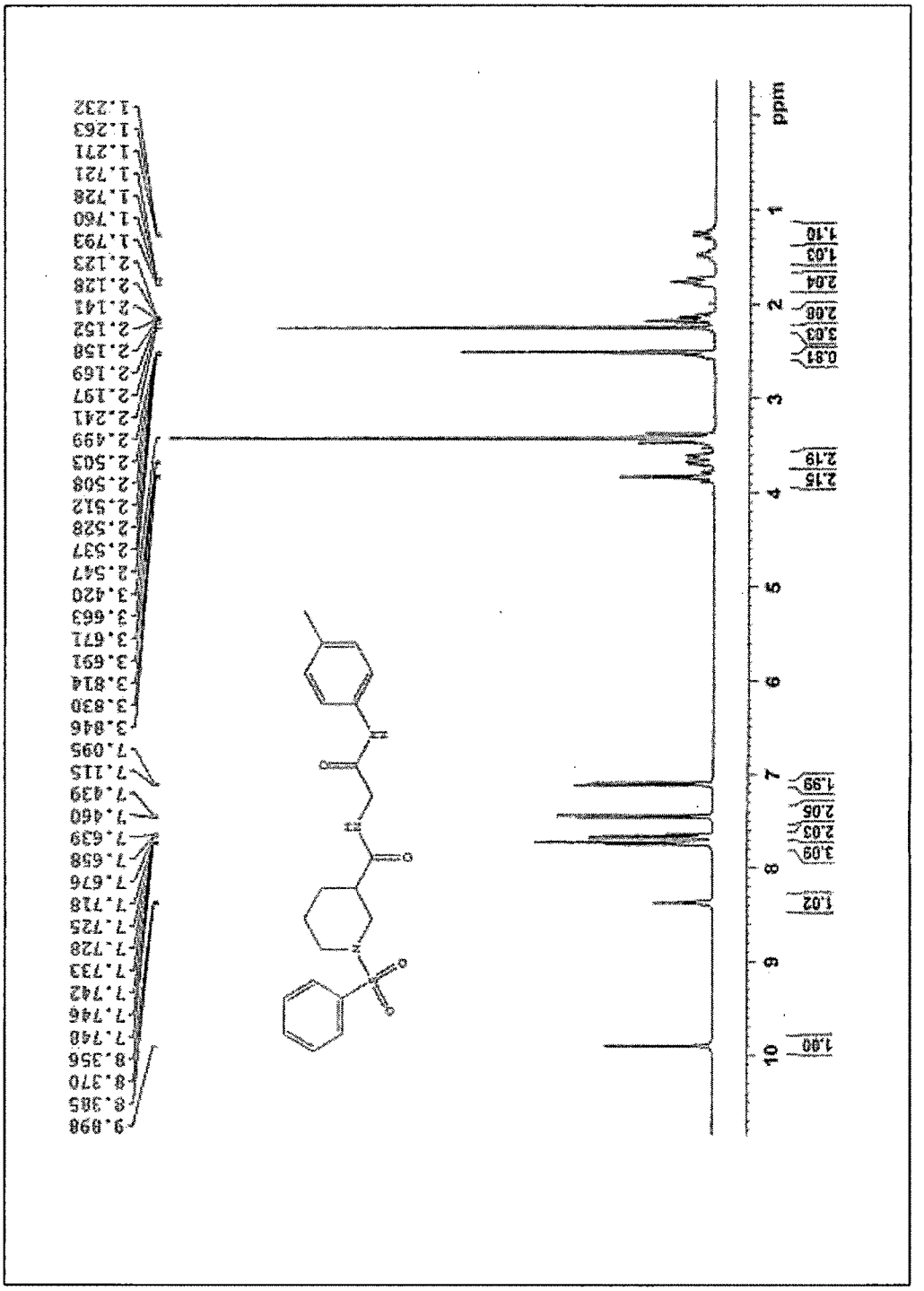


Figure 4.4.2: ^{1}H NMR spectrum of N-(2-oxo-2-(p-tolylamino)ethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6b**

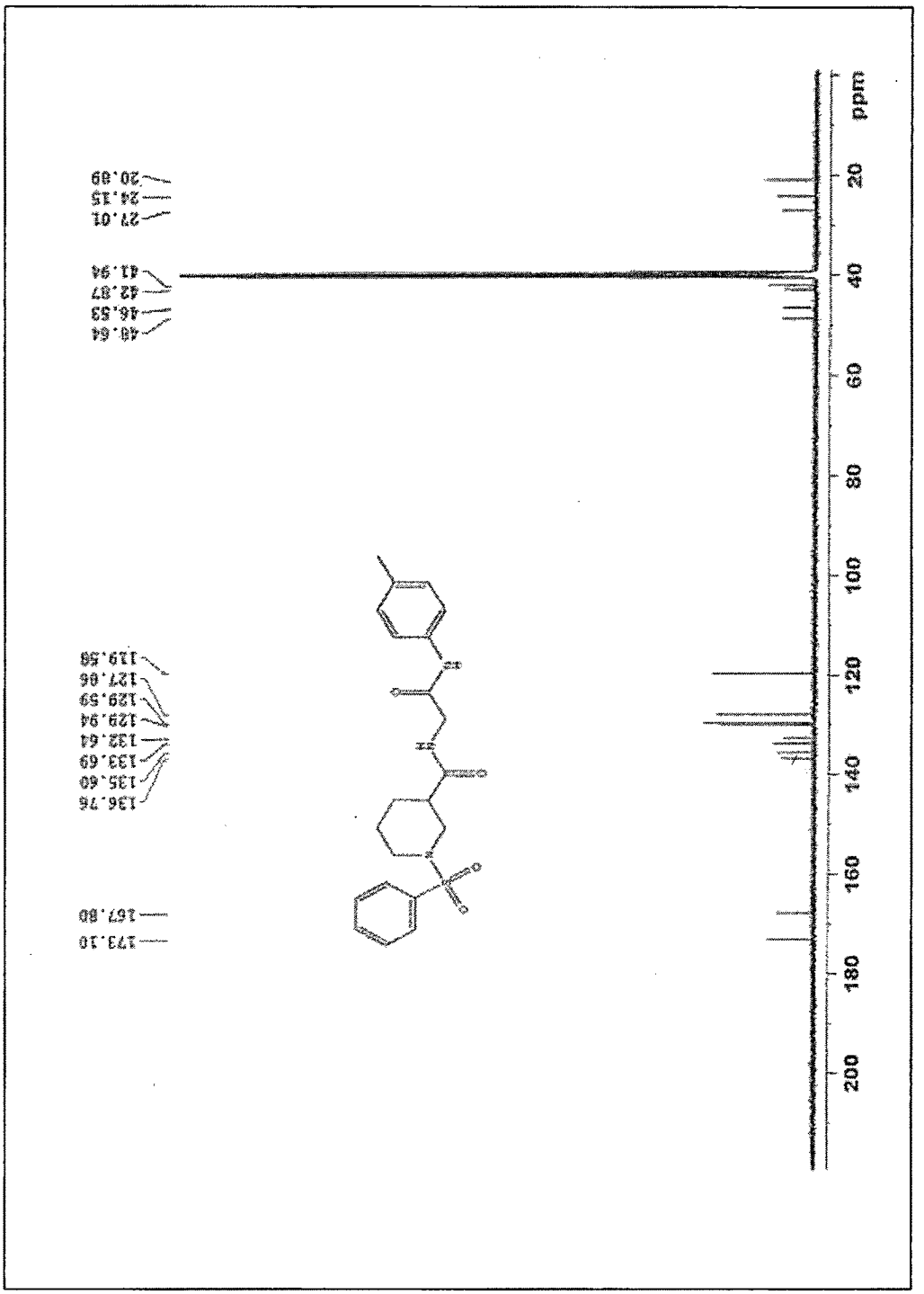


Figure 4.4.3: ^{13}C NMR spectrum of N-(2-oxo-2-(p-tolylamino)ethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6b**

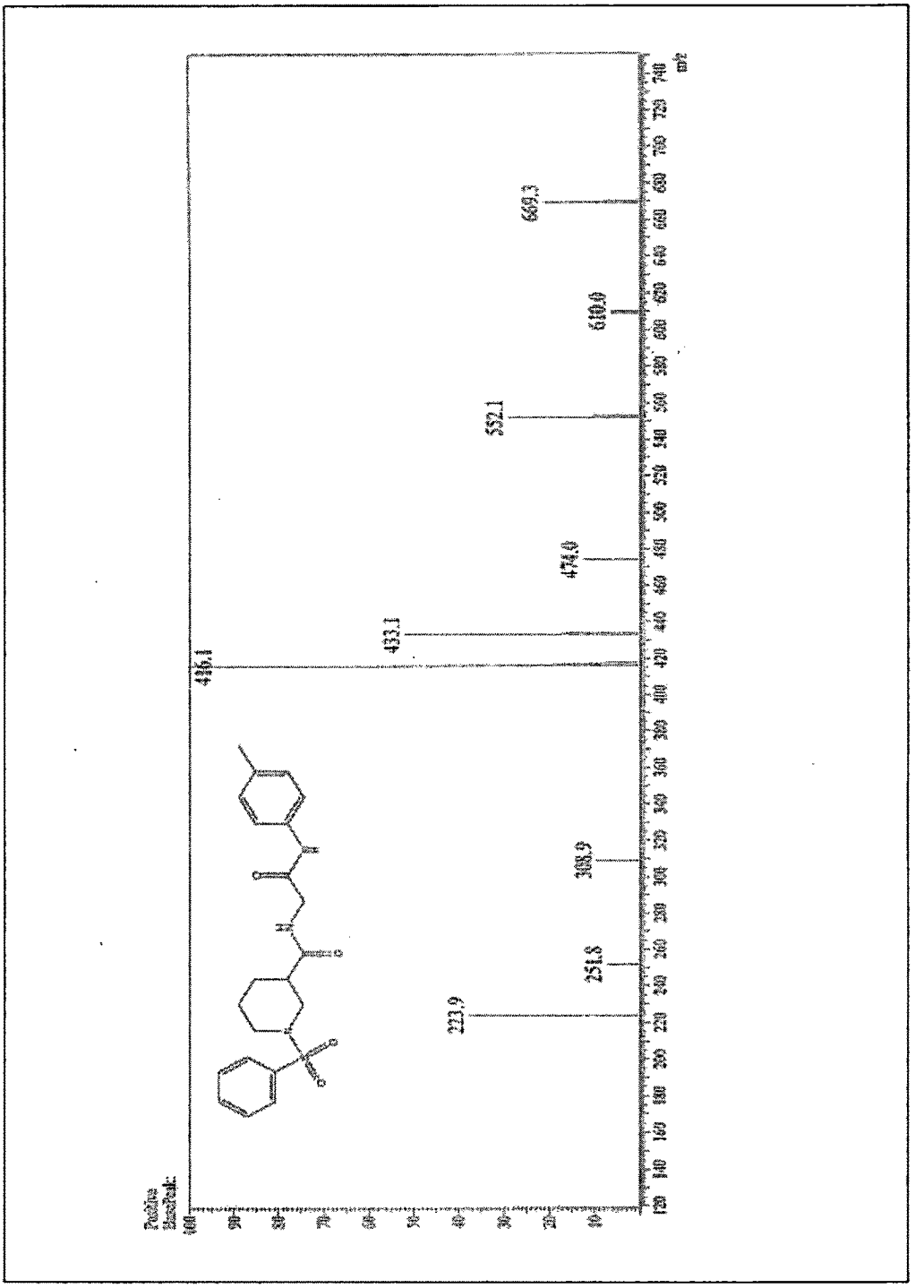


Figure 4.4.4: ESI-MS spectrum of N-(2-oxo-2-(p-tolylamino)ethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6b**

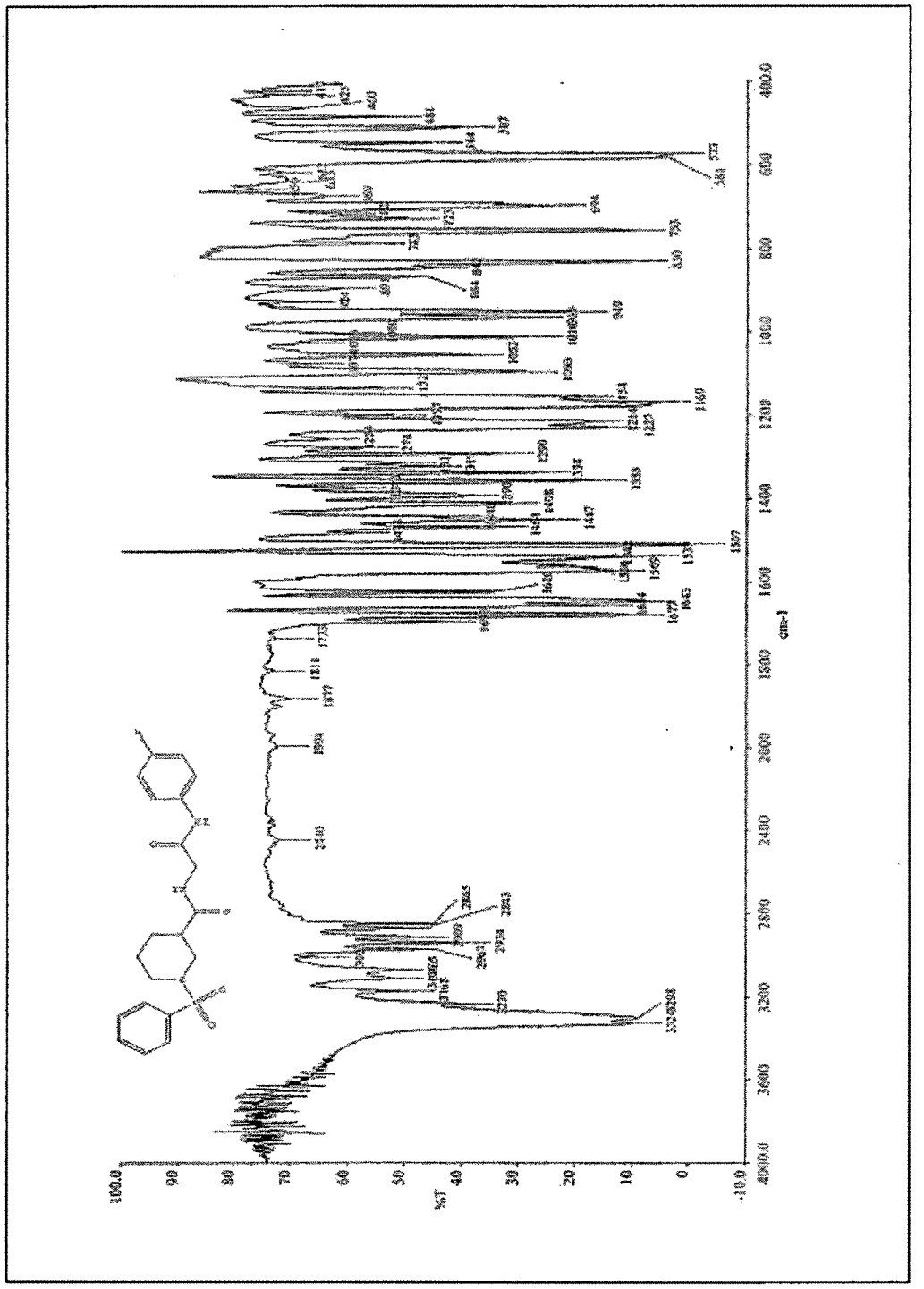


Figure 4.5.1: IR spectrum of N-(2-(4-fluorophenylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6c**

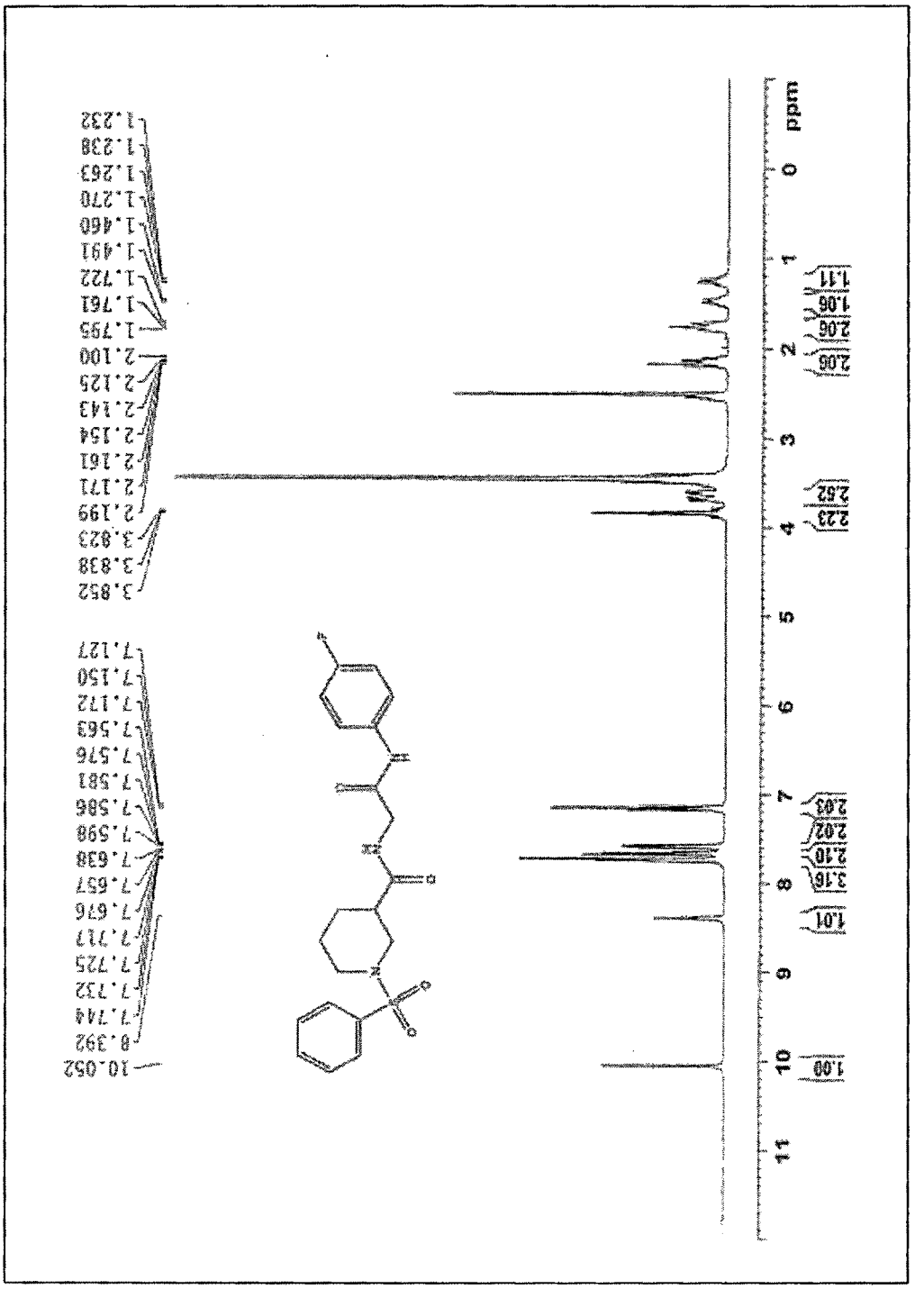


Figure 4.5.2: ^{1}H NMR spectrum of N-(2-(4-fluorophenylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6c**

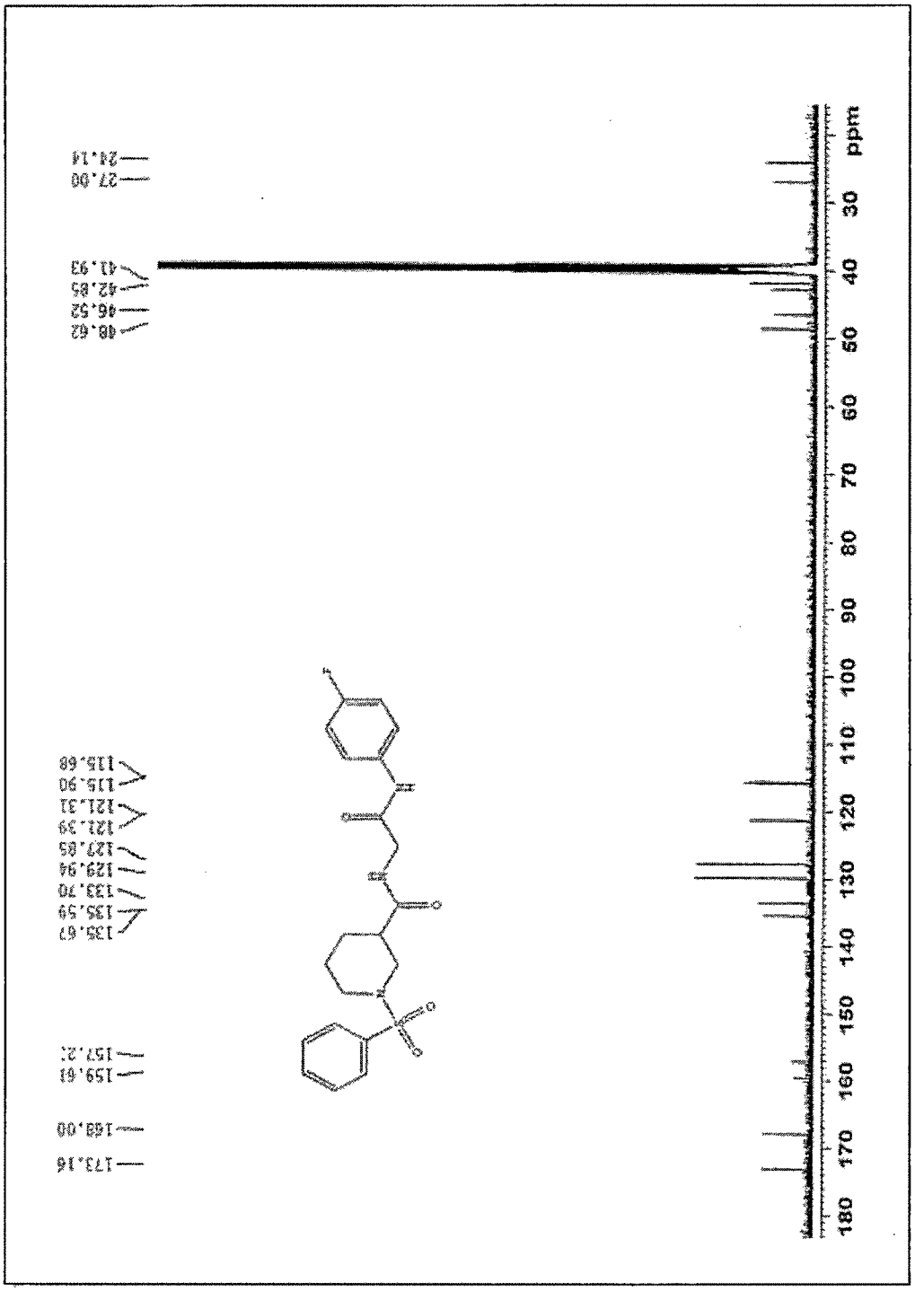


Figure 4.5.3: ^{13}C NMR spectrum of N-(2-(4-fluorophenylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6c**

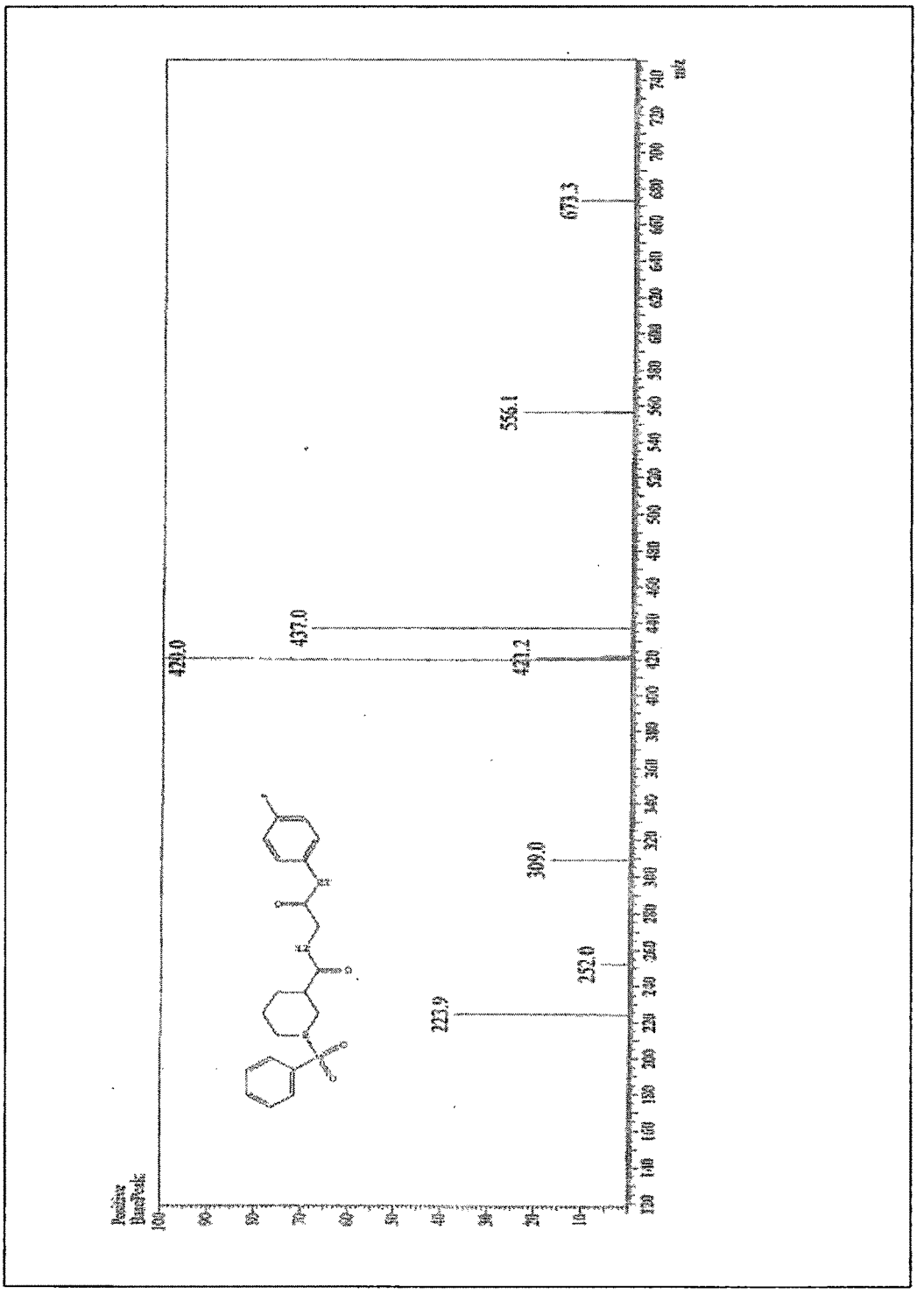


Figure 4.5.4: ESI-MS spectrum of N-(2-(4-fluorophenylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6c**

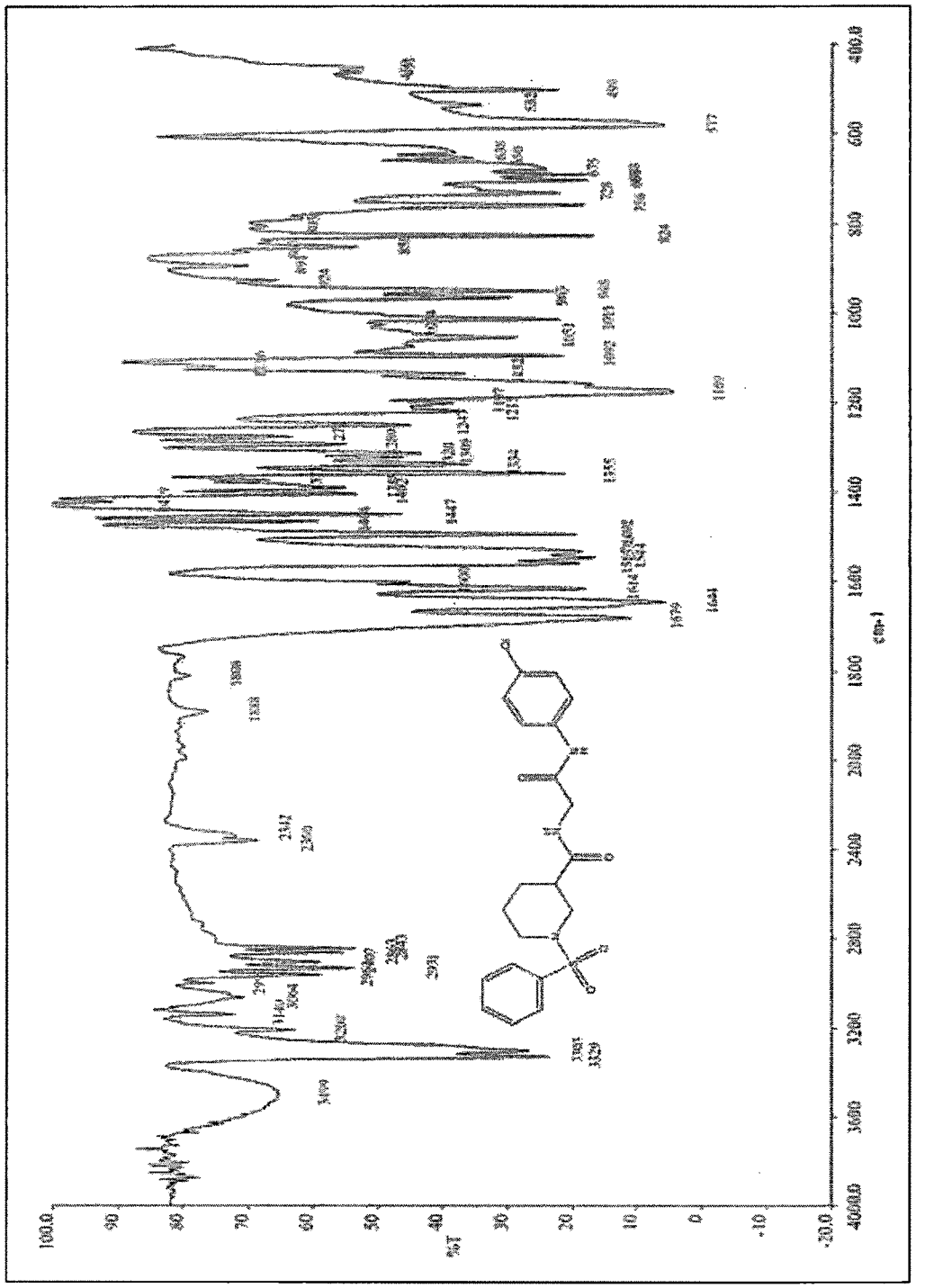


Figure 4.6.1: IR spectrum of N-(2-(4-chlorophenylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6d**

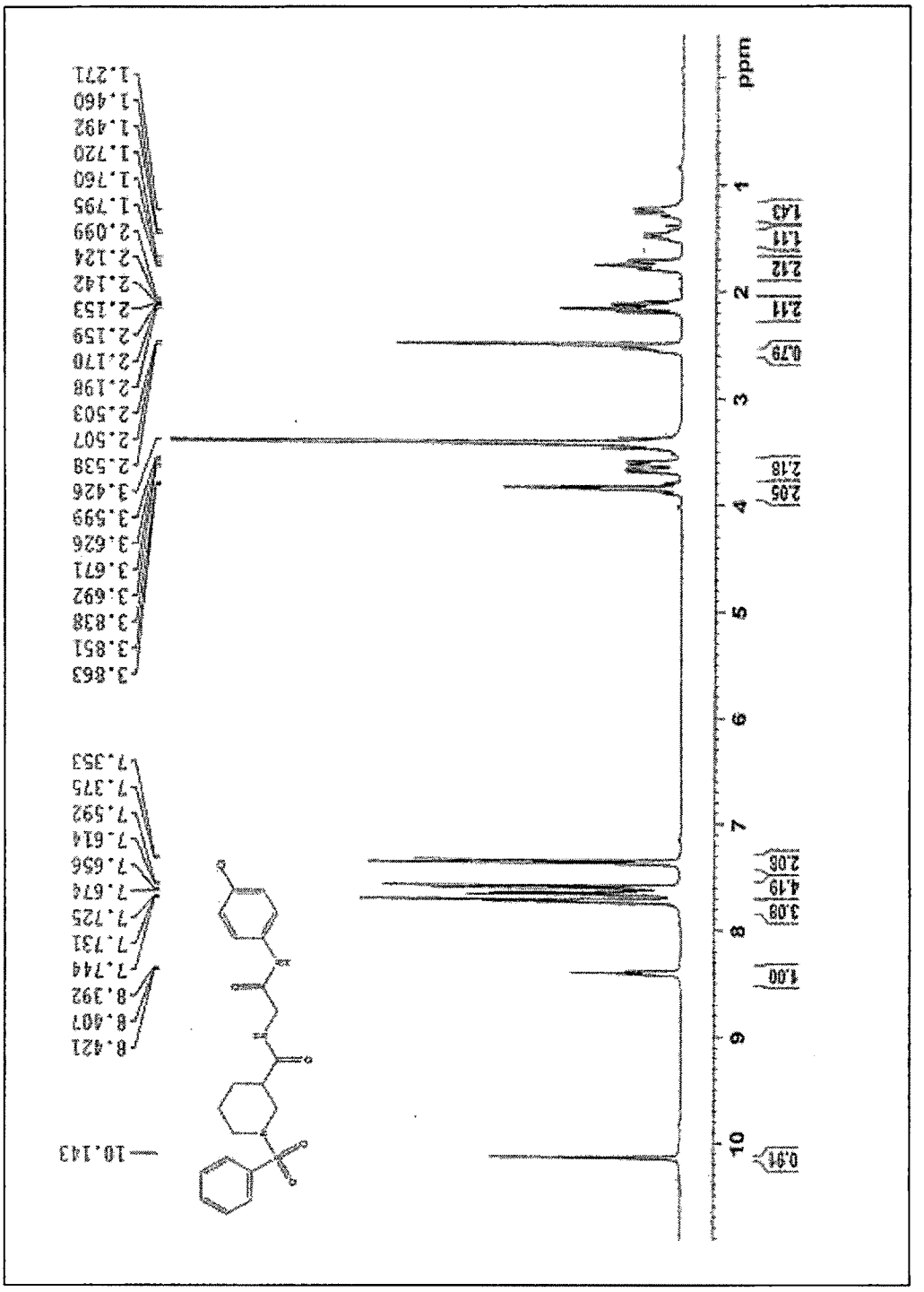


Figure 4.6.2: ^{1}H NMR spectrum of N-(2-(4-chlorophenylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6d**

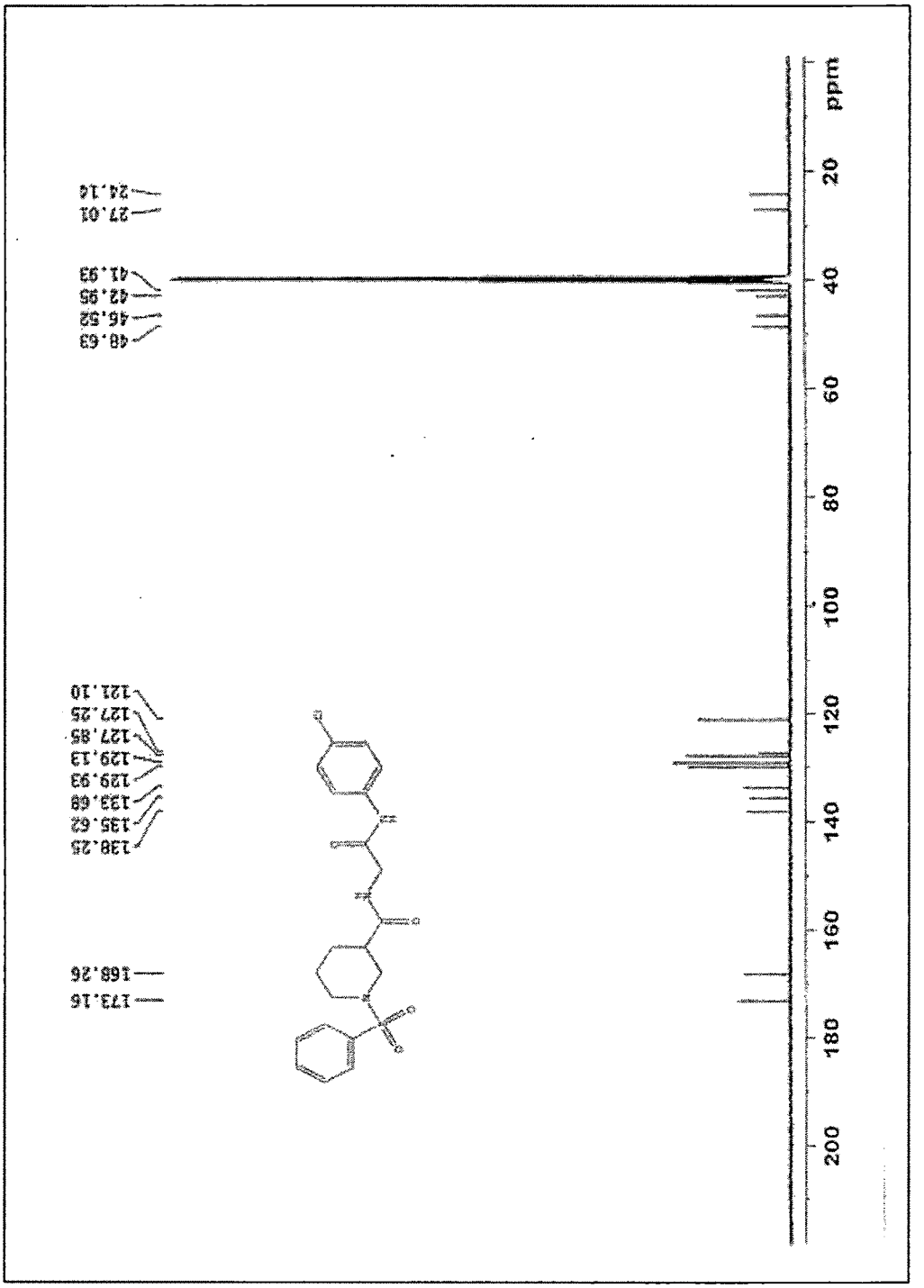


Figure 4.6.3: ^{13}C NMR spectrum of N-(2-(4-chlorophenylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6d**

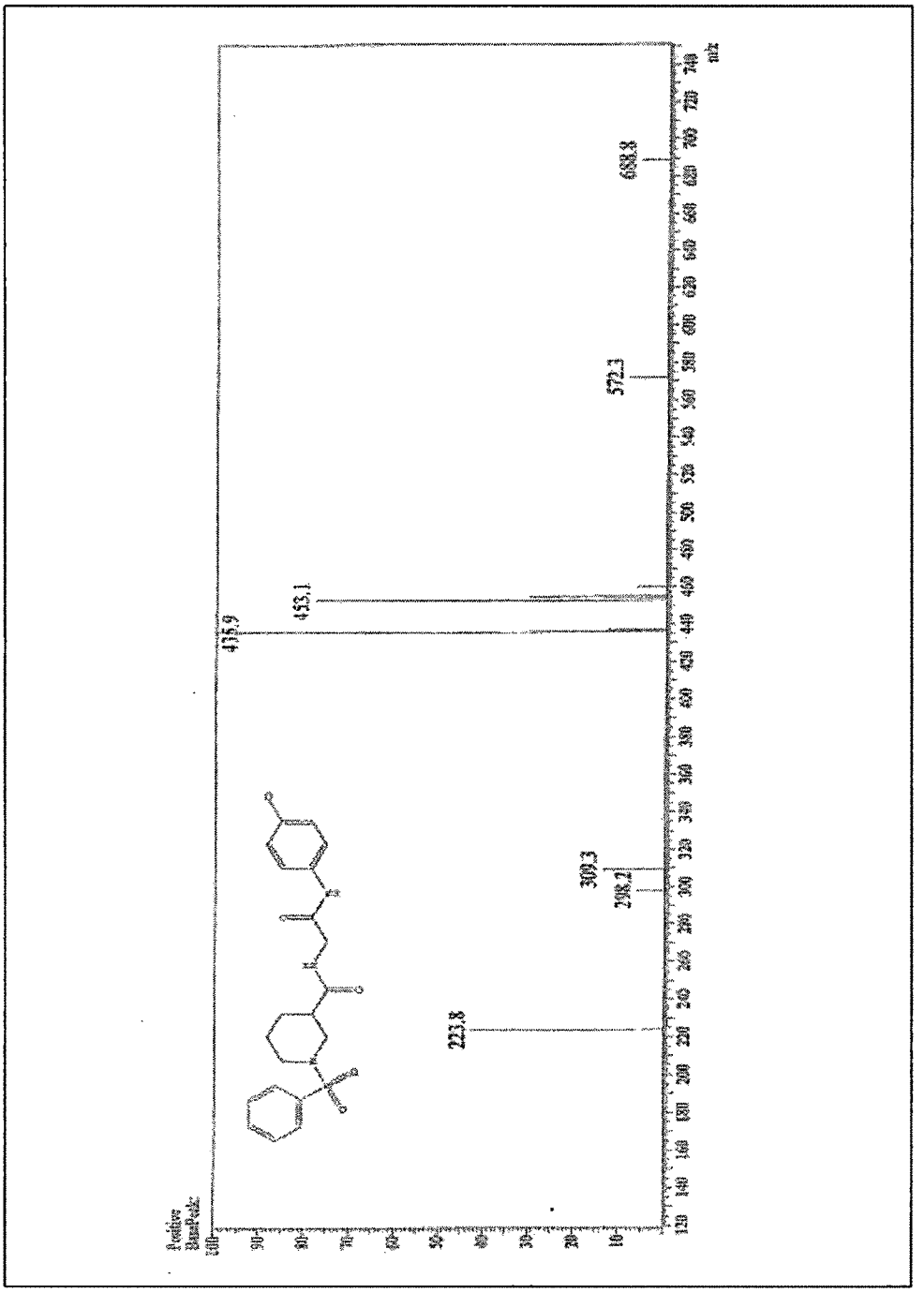


Figure 4.6.4: ESI-MS spectrum of N-(2-(4-chlorophenylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6d**

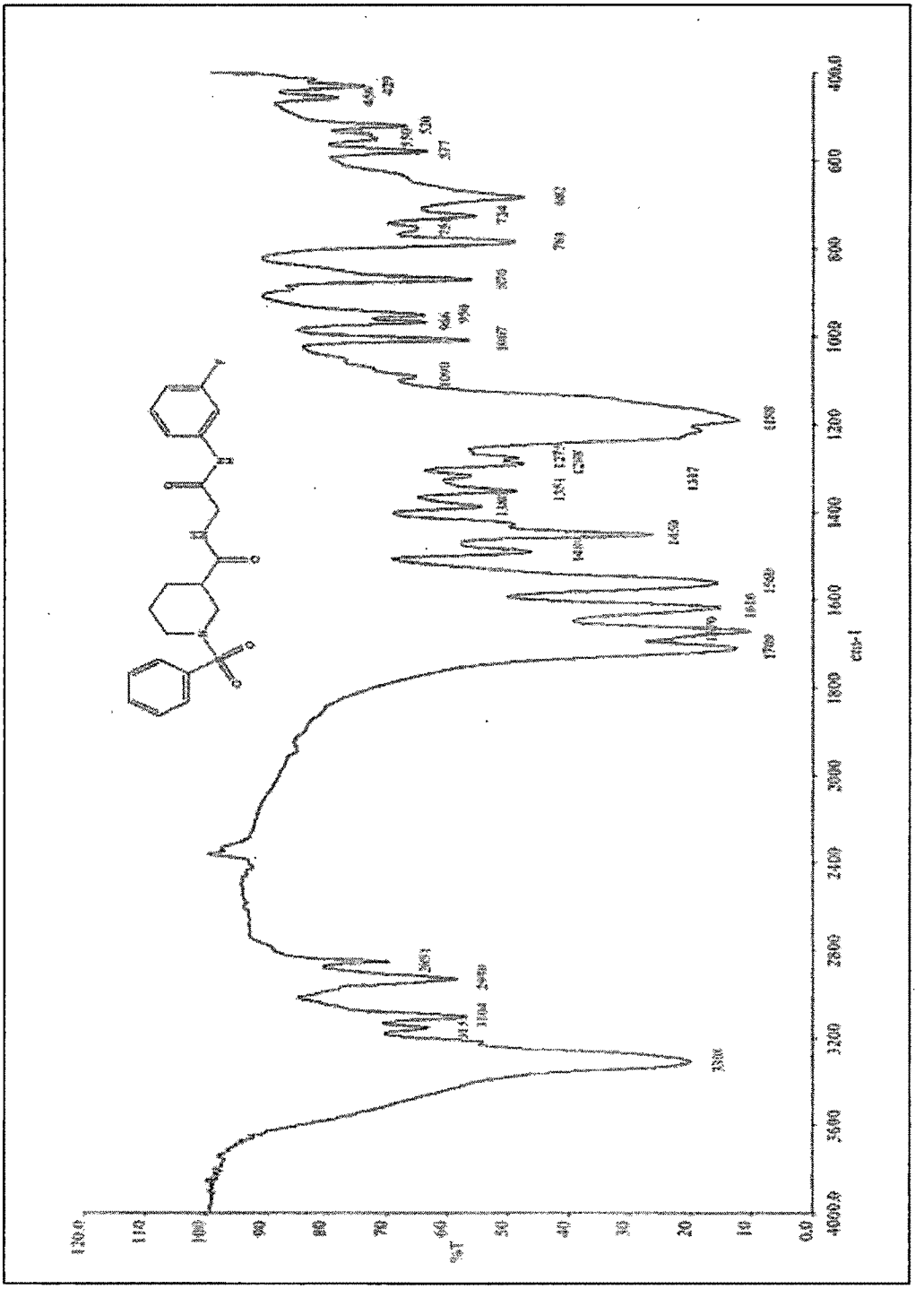


Figure 4.7.1: IR spectrum of N-(2-(3-fluorophenylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6e**

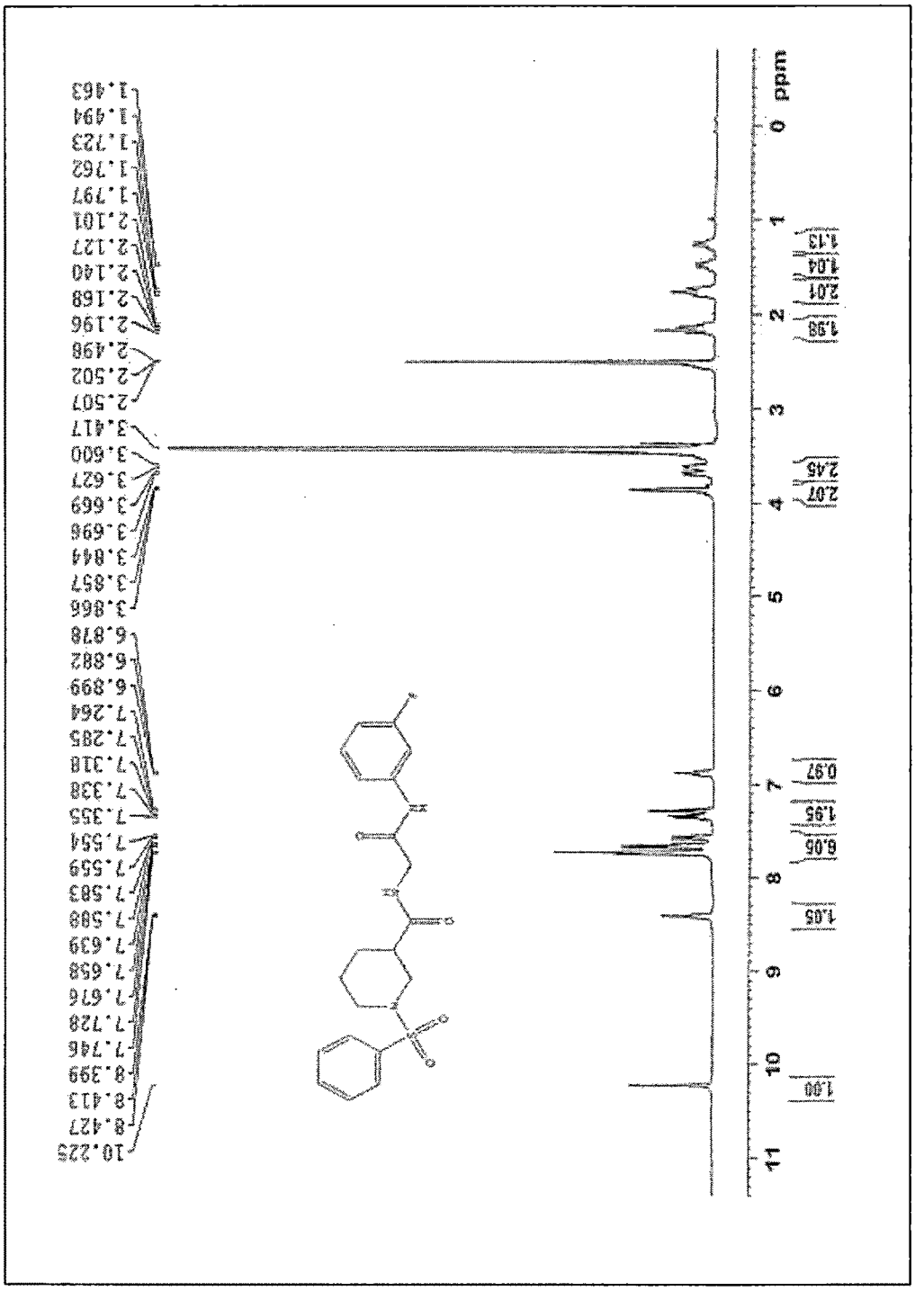


Figure 4.7.2: ^{1}H NMR spectrum of N-(2-(3-fluorophenylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6e**

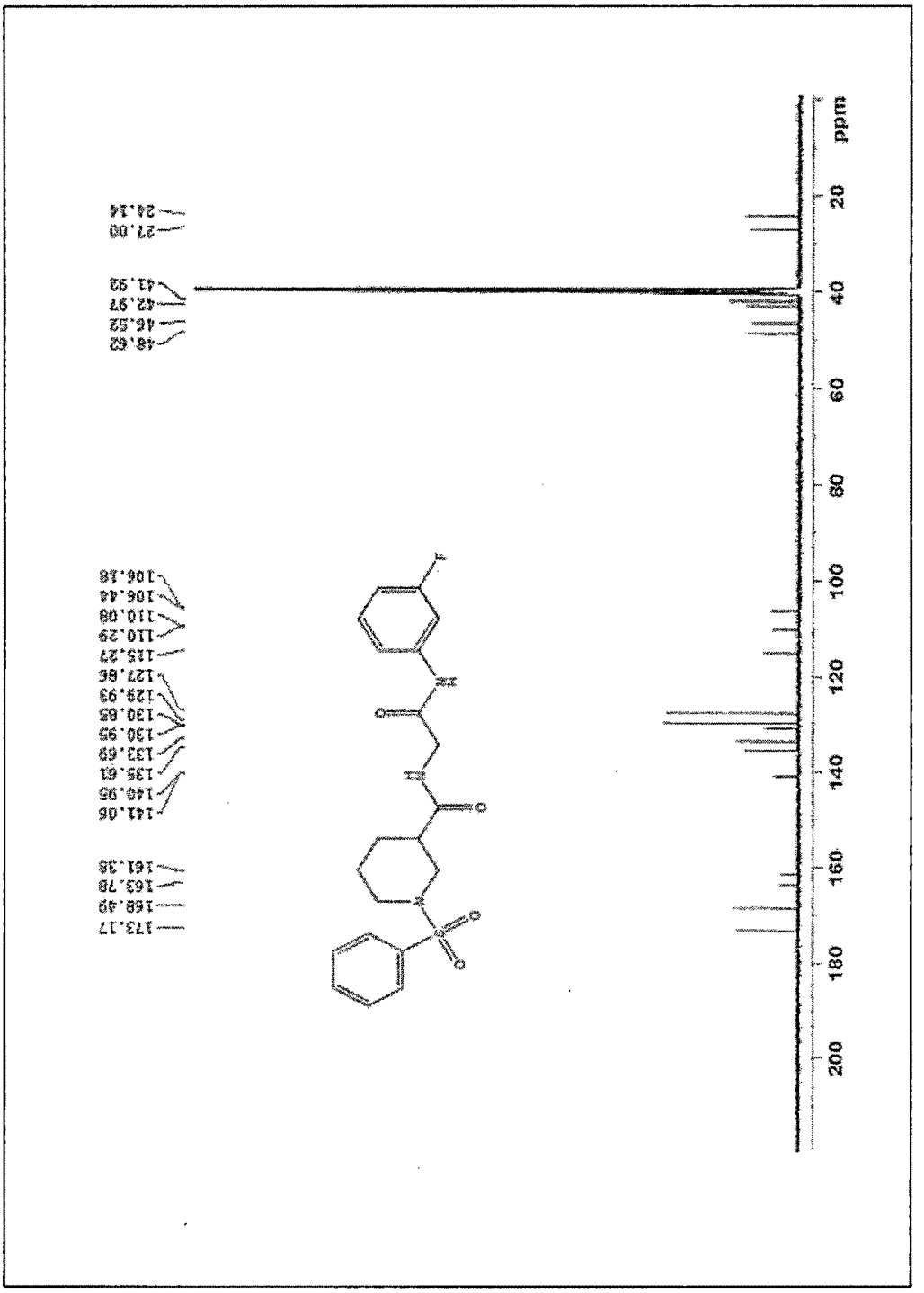


Figure 4.7.3: ^{13}C NMR spectrum of N-(2-(3-fluorophenylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6e**

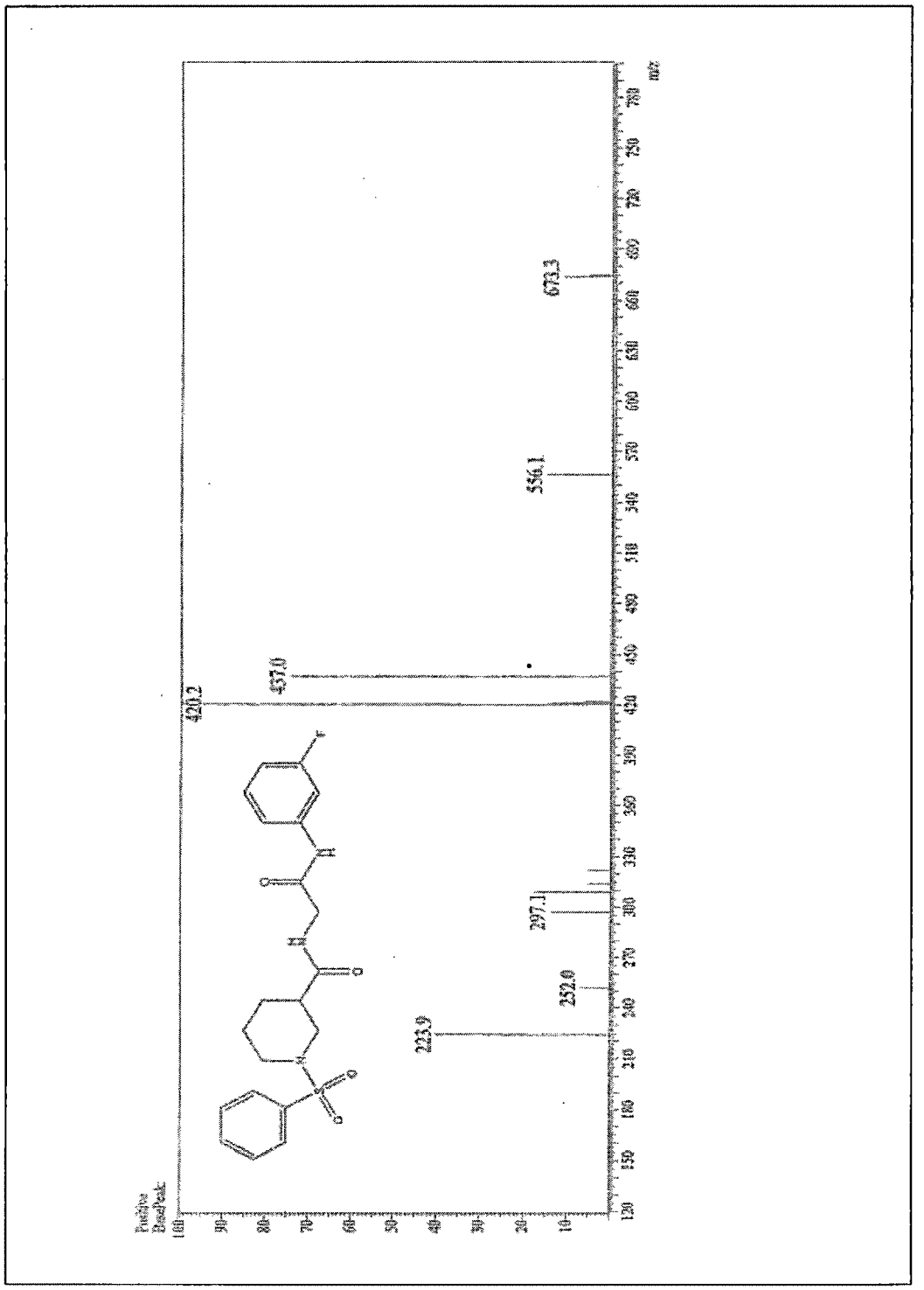


Figure 4.7.4: ESI-MS spectrum of N-(2-(3-fluorophenylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6e**

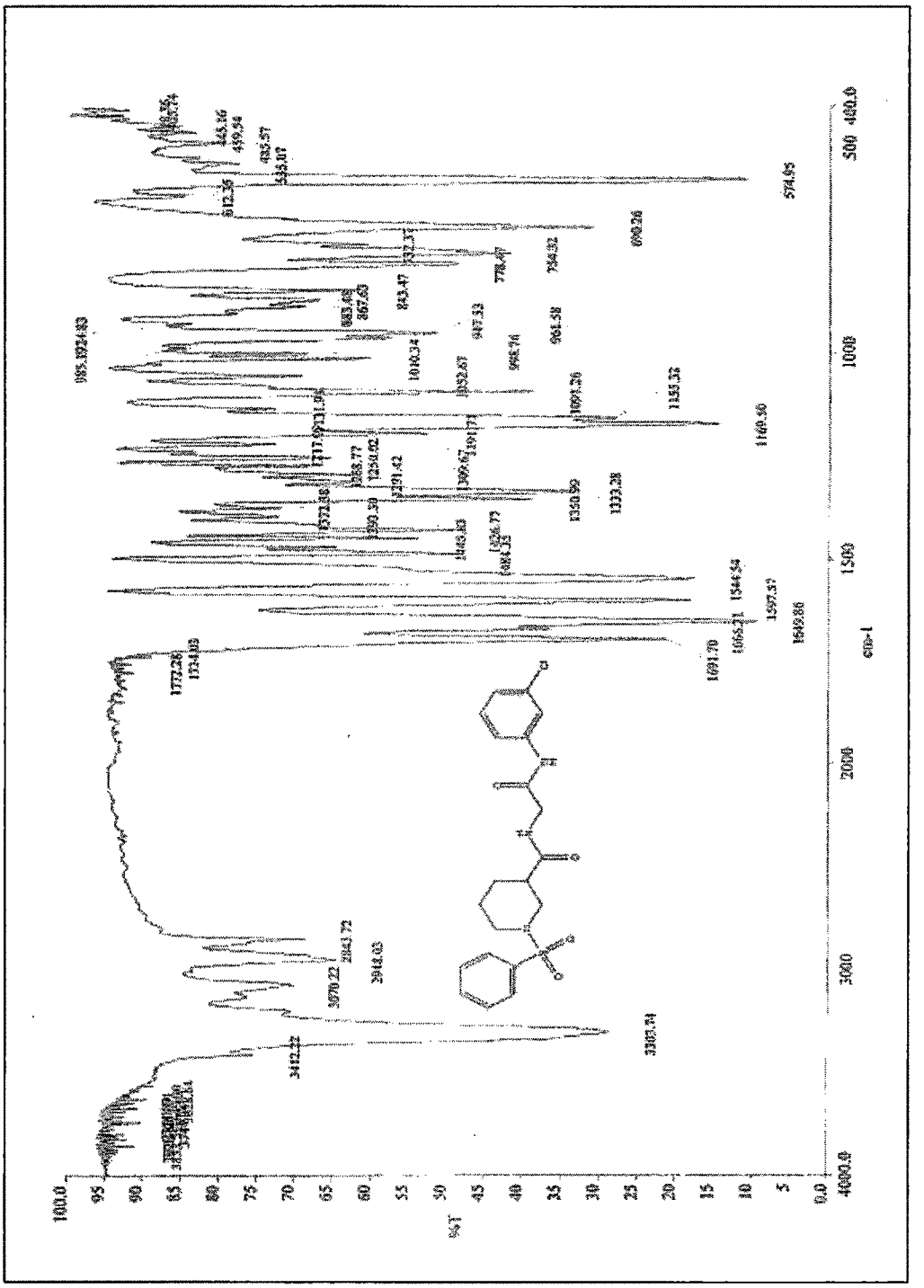


Figure 4.8.1: IR spectrum of N-(2-(3-chlorophenylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6f**

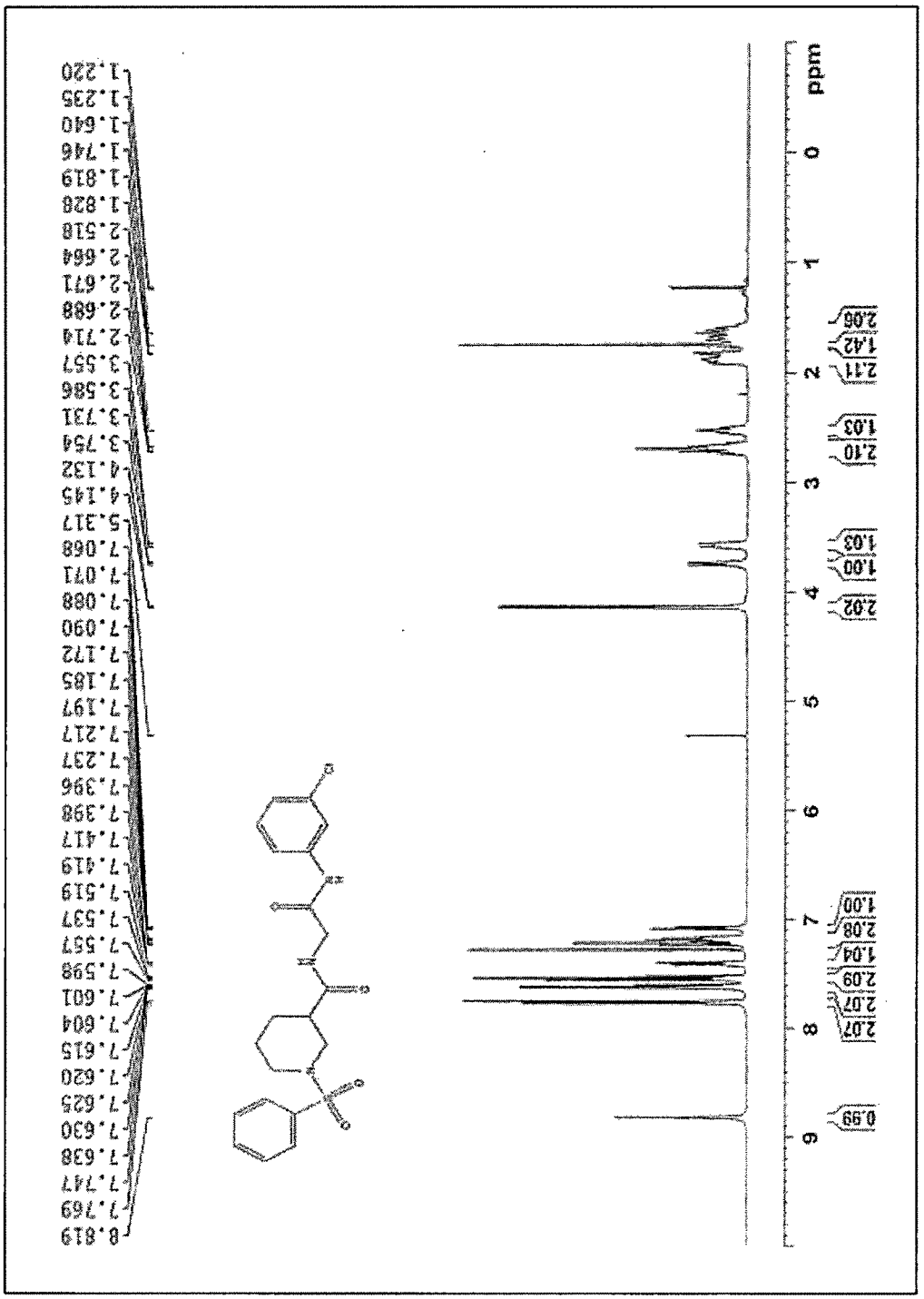


Figure 4.8.2: ^{1}H NMR spectrum of N-(2-(3-chlorophenylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6f**

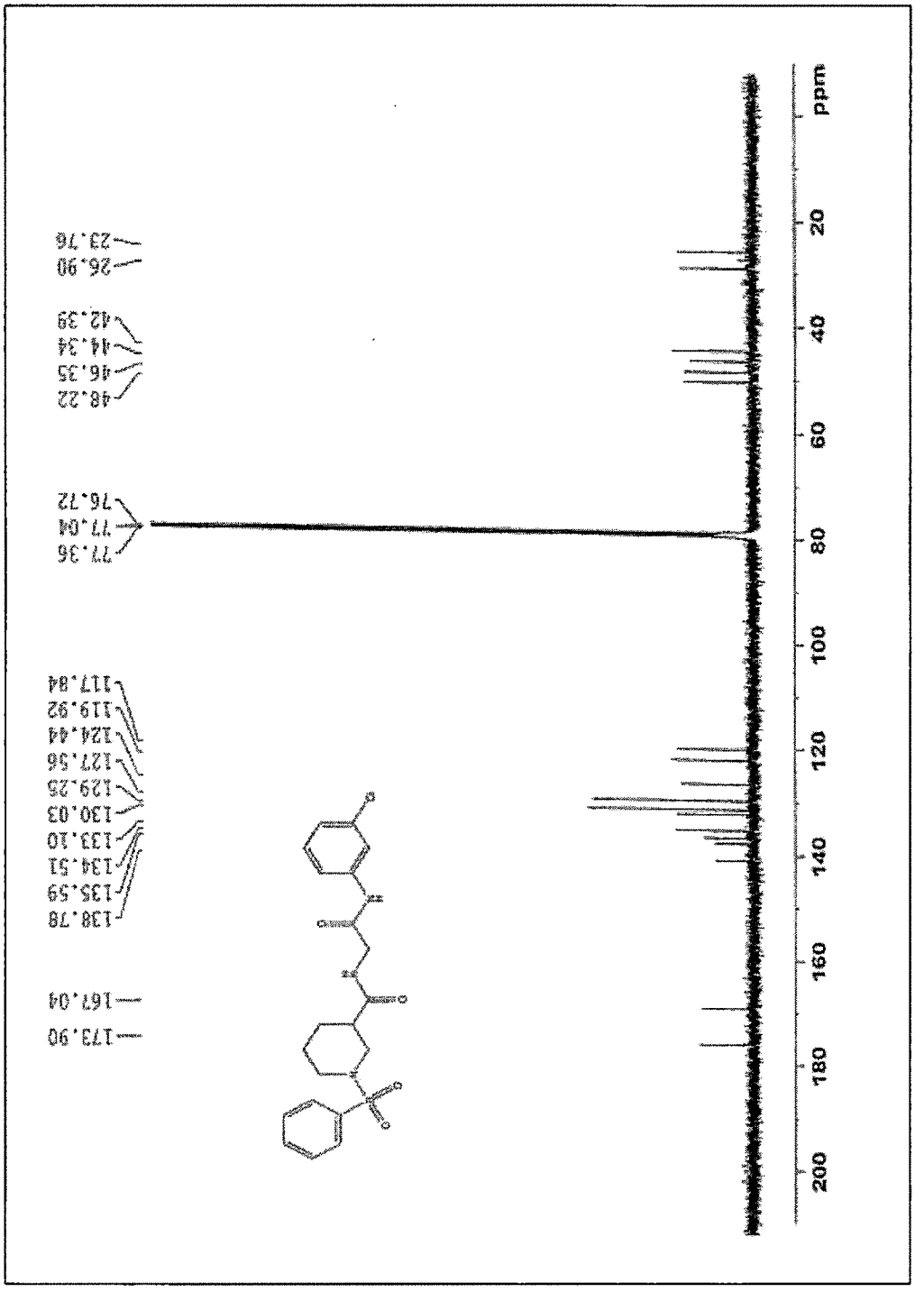


Figure 4.8.3: ^{13}C NMR spectrum of N-(2-(3-chlorophenylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6f**

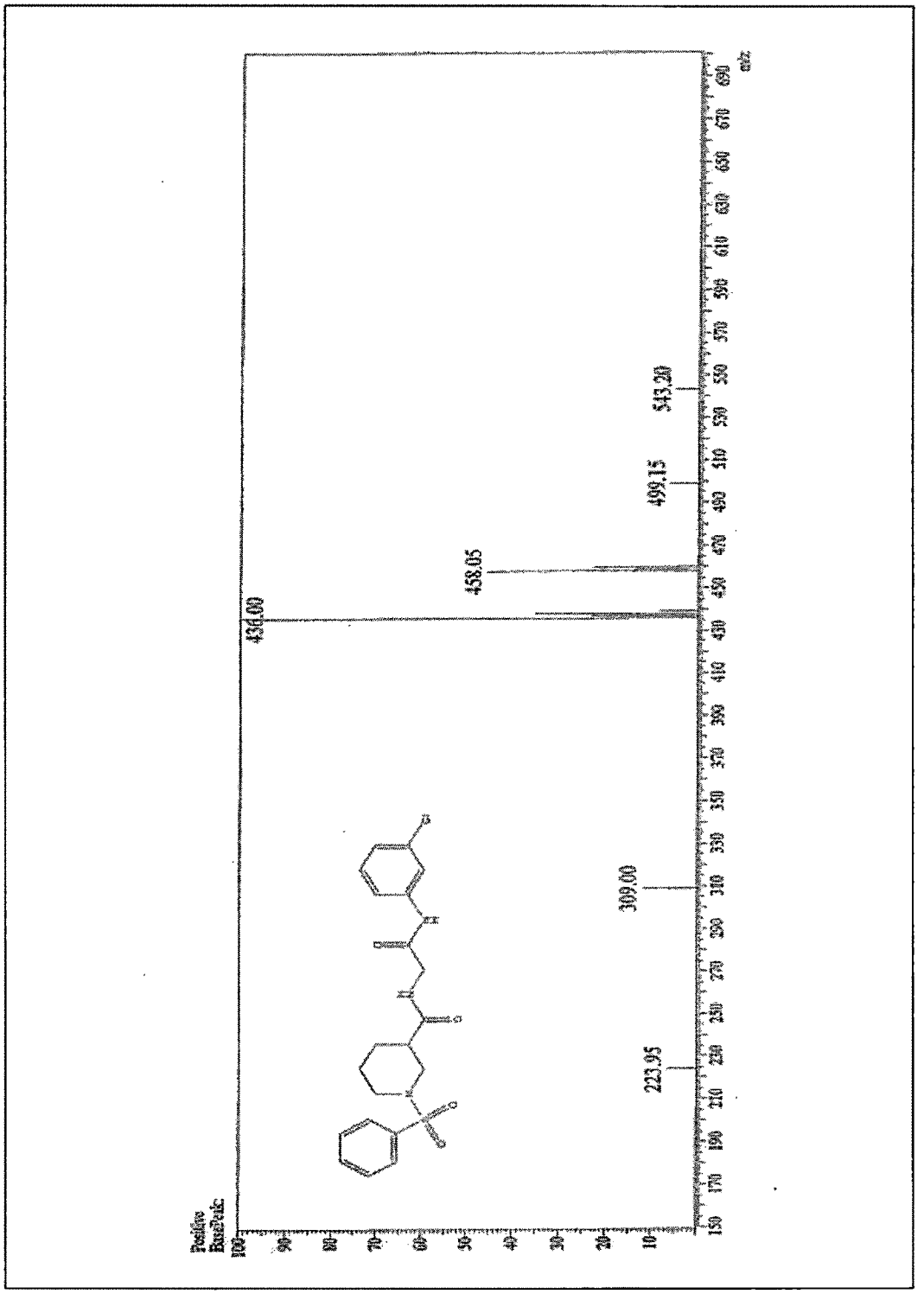


Figure 4.8.4: ESI-MS spectrum of N-(2-(3-chlorophenylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6f**

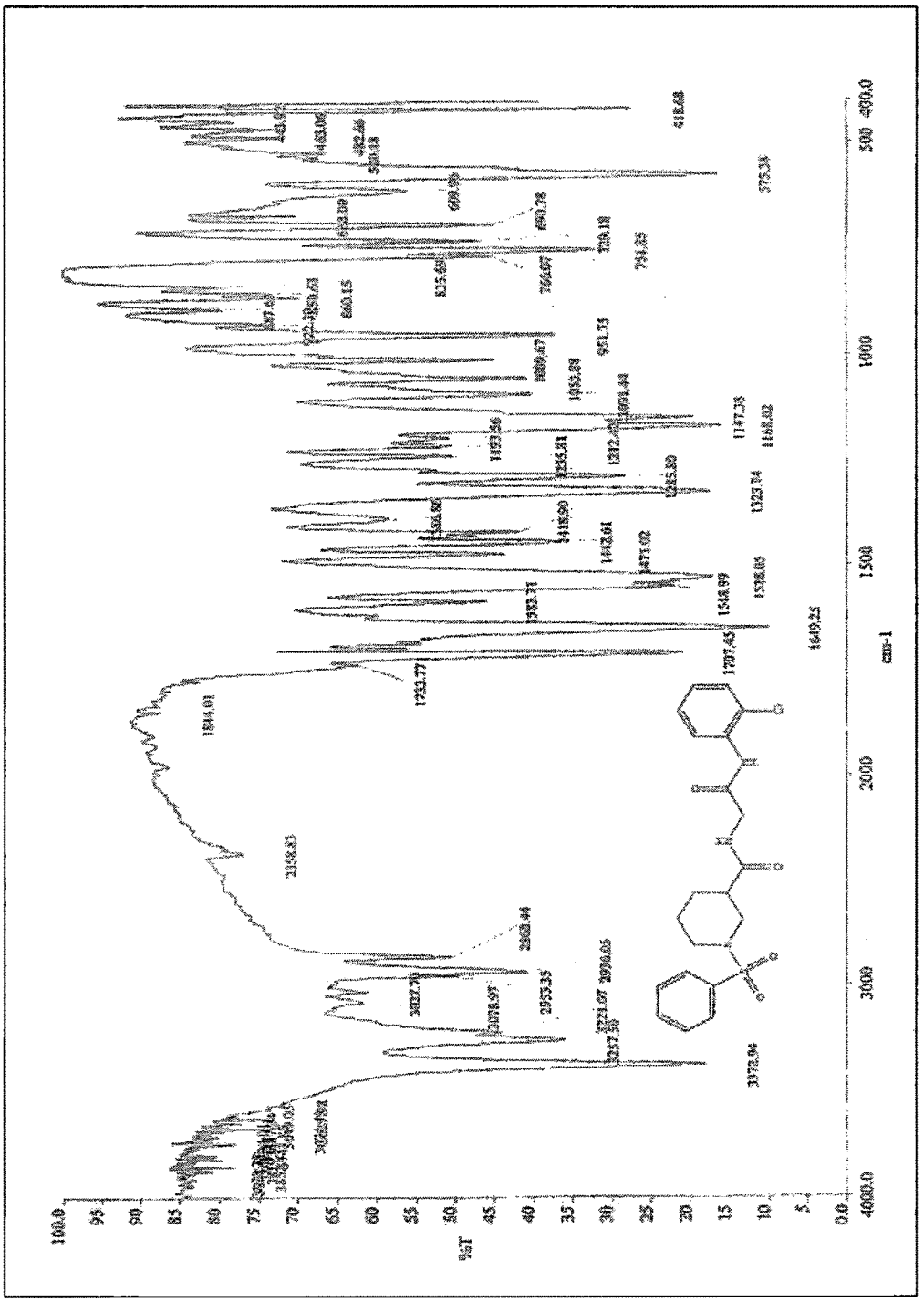


Figure 4.9.1: IR spectrum of N-(2-(2-chlorophenylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6g**

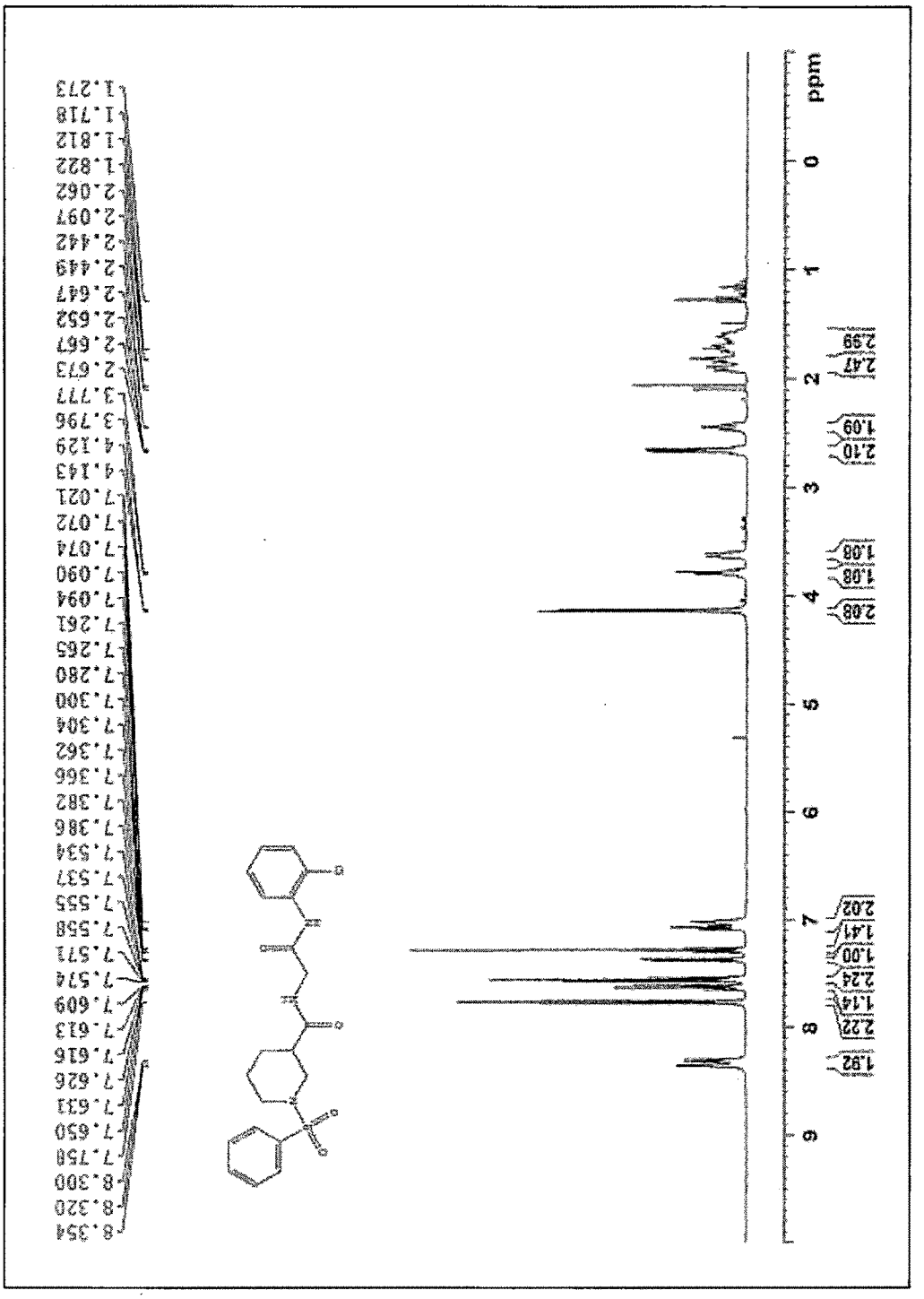


Figure 4.9.2: ^{1}H NMR spectrum of N-(2-(2-chlorophenylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6g**

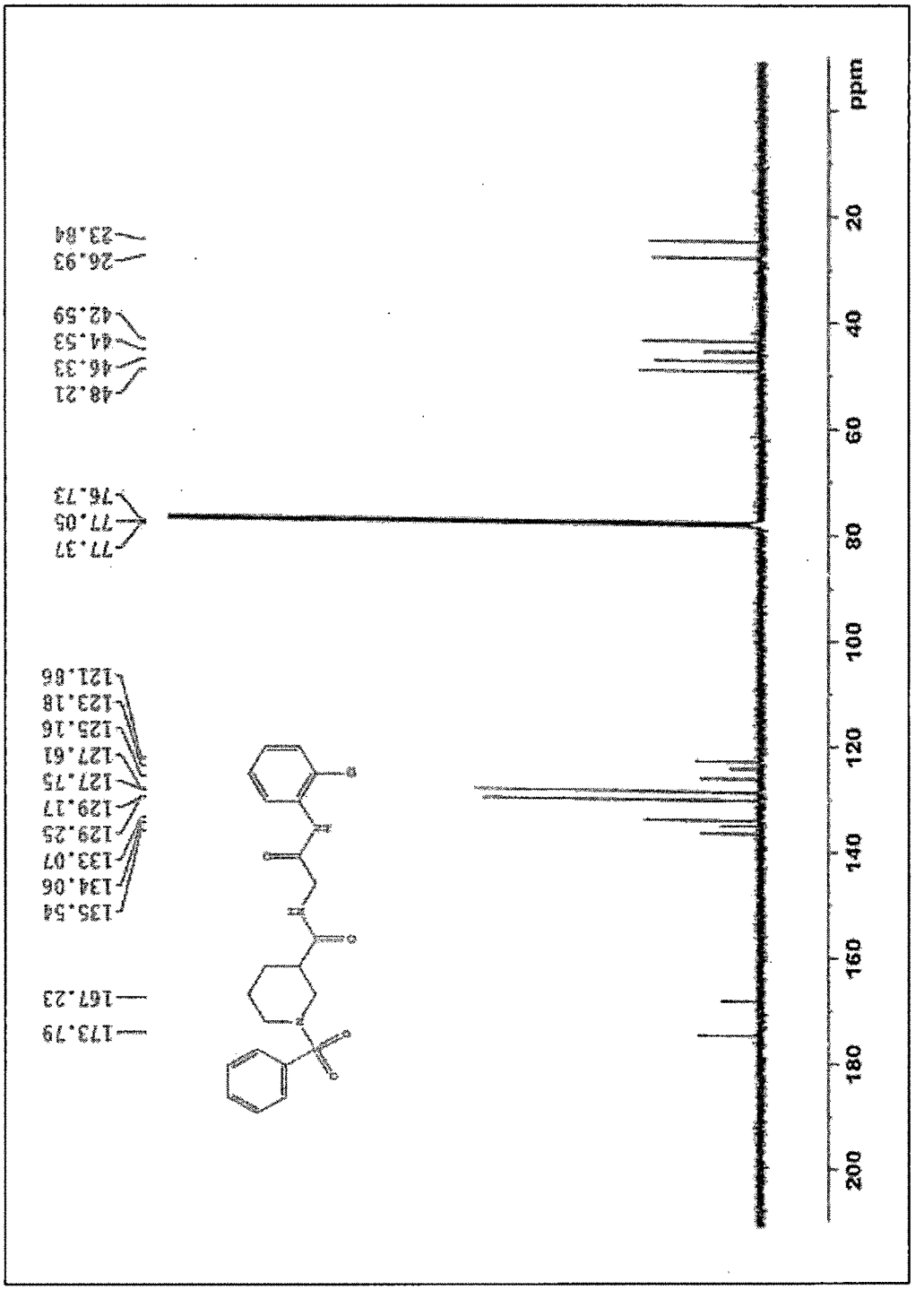


Figure 4.9.3: ^{13}C NMR spectrum of N-(2-(2-chlorophenylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6g**

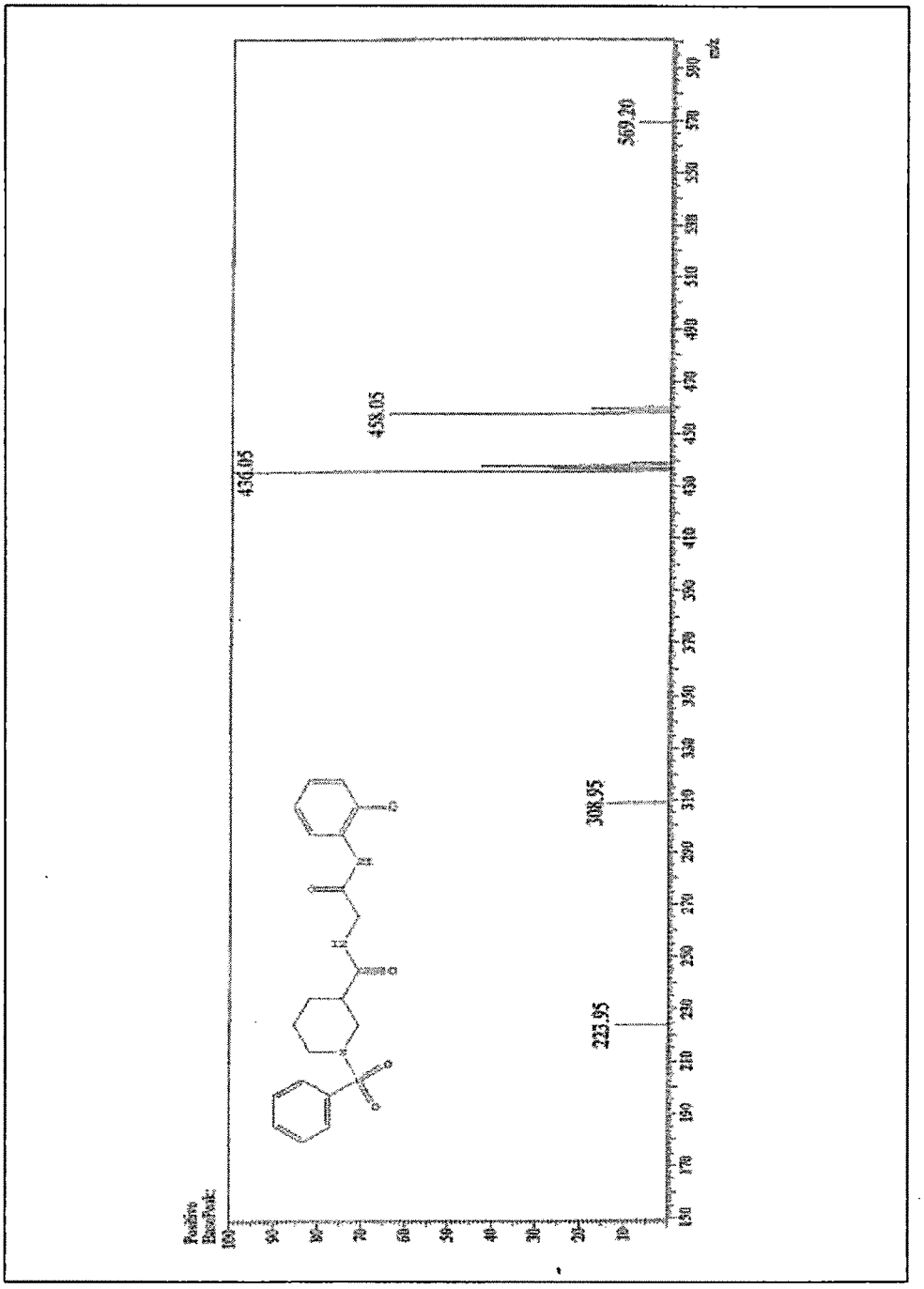


Figure 4.9.4: ESI-MS spectrum of N-(2-(2-chlorophenylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6g**

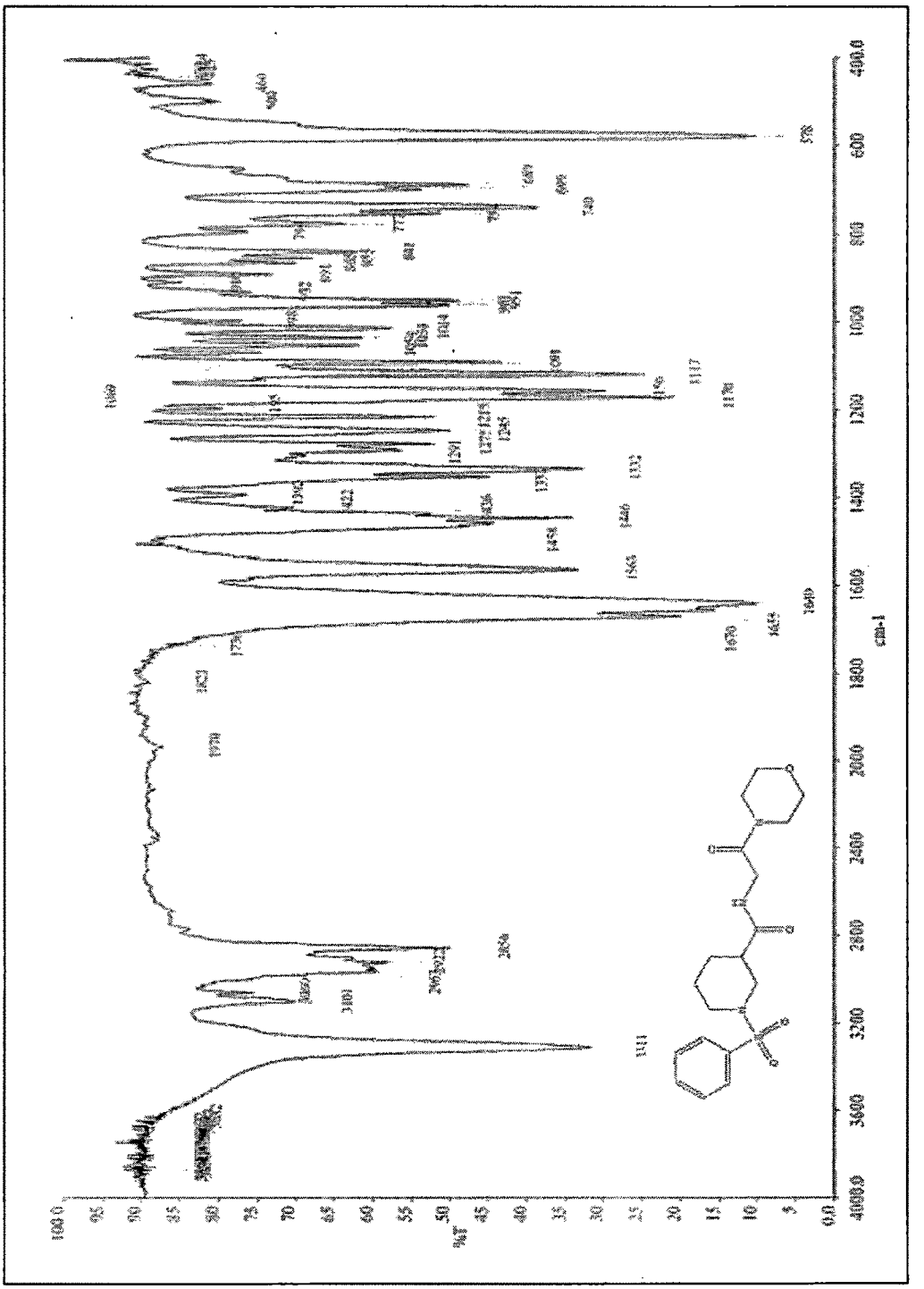


Figure 4.10.1: IR spectrum of N-(2-morpholino-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6h**

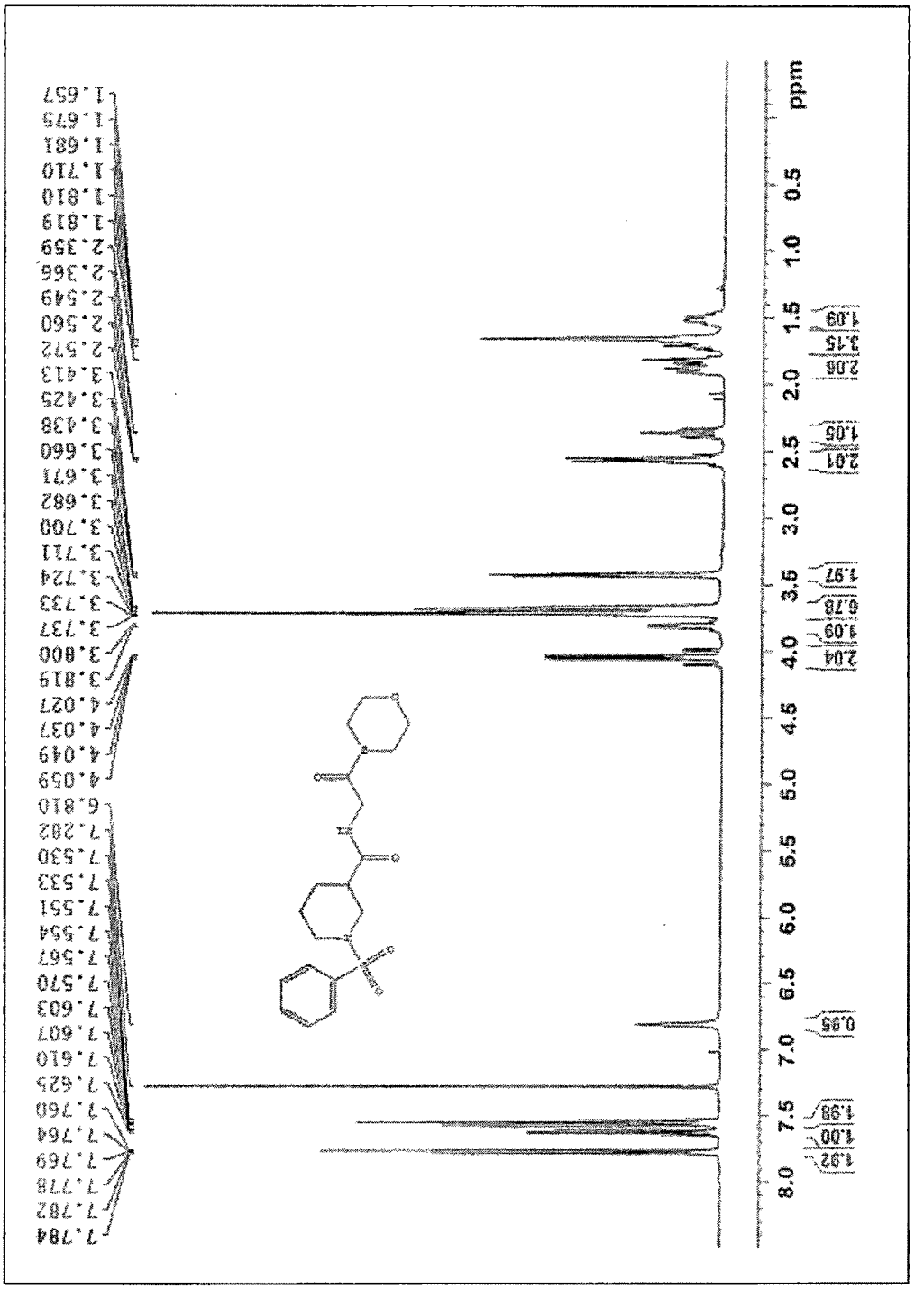


Figure 4.10.2: ^{1}H NMR spectrum of N-(2-morpholino-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6h**

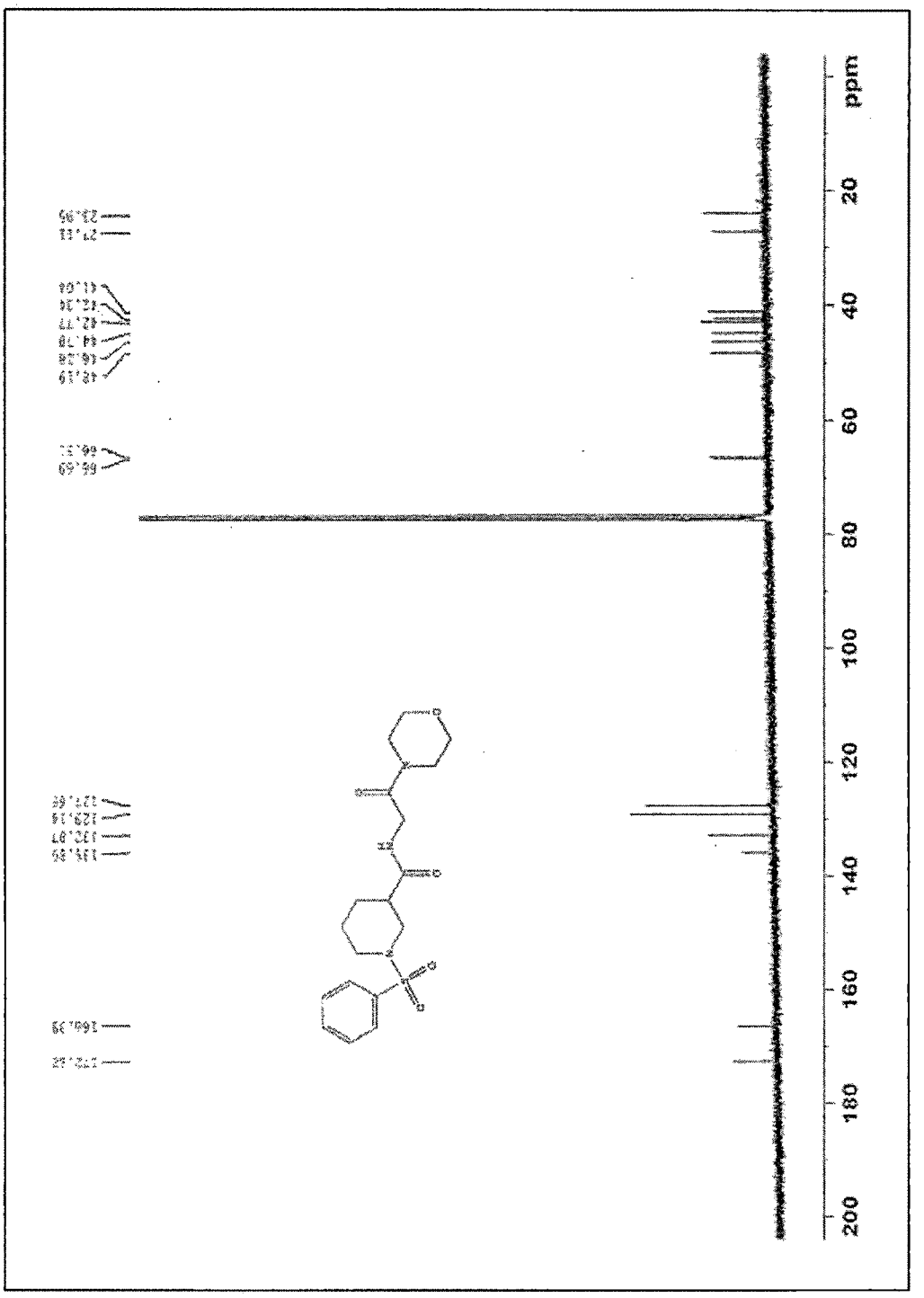


Figure 4.10.3: ^{13}C NMR spectrum of N-(2-morpholino-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6h**

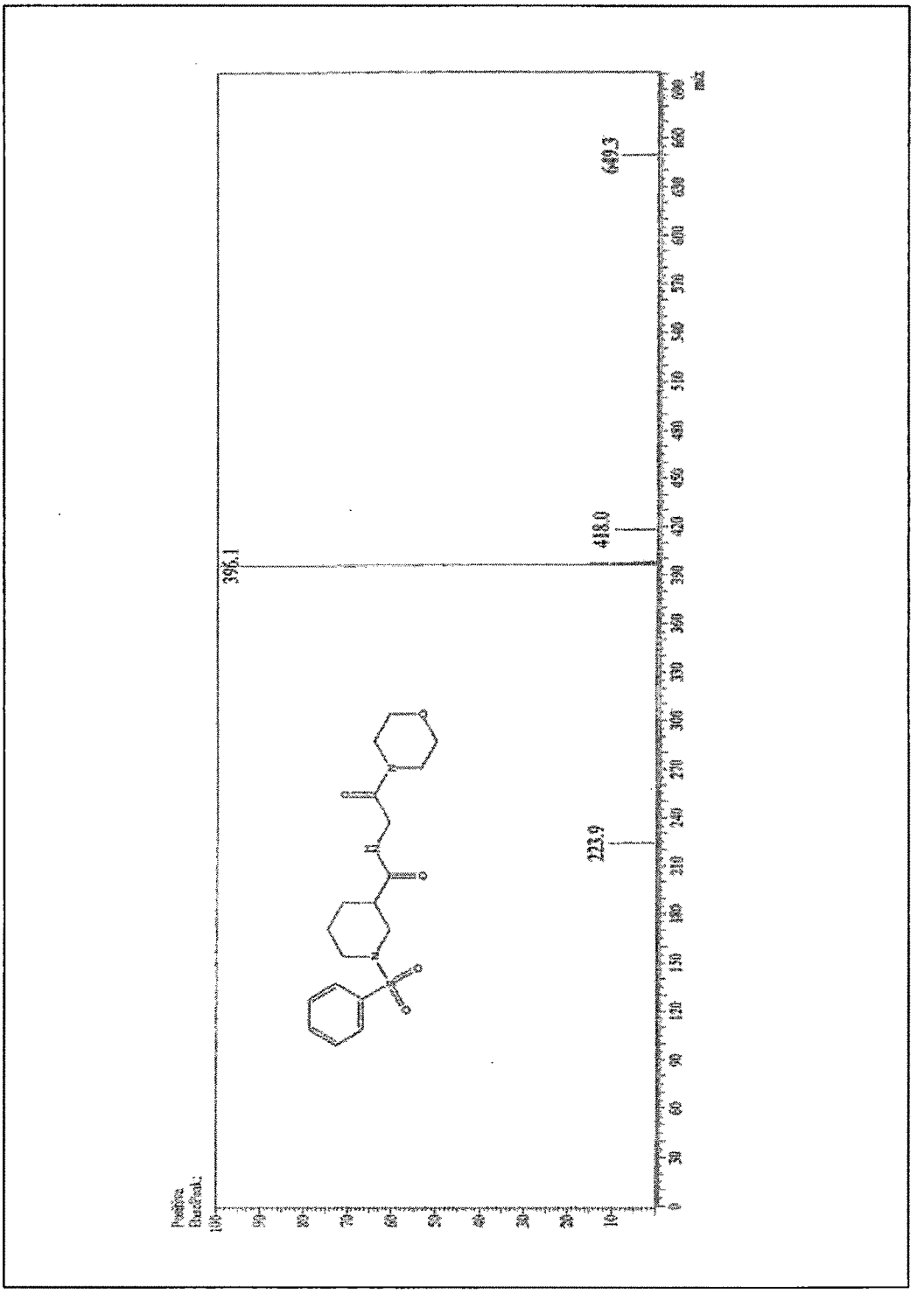


Figure 4.10.4: ESI-MS spectrum of N-(2-morpholino-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6h**

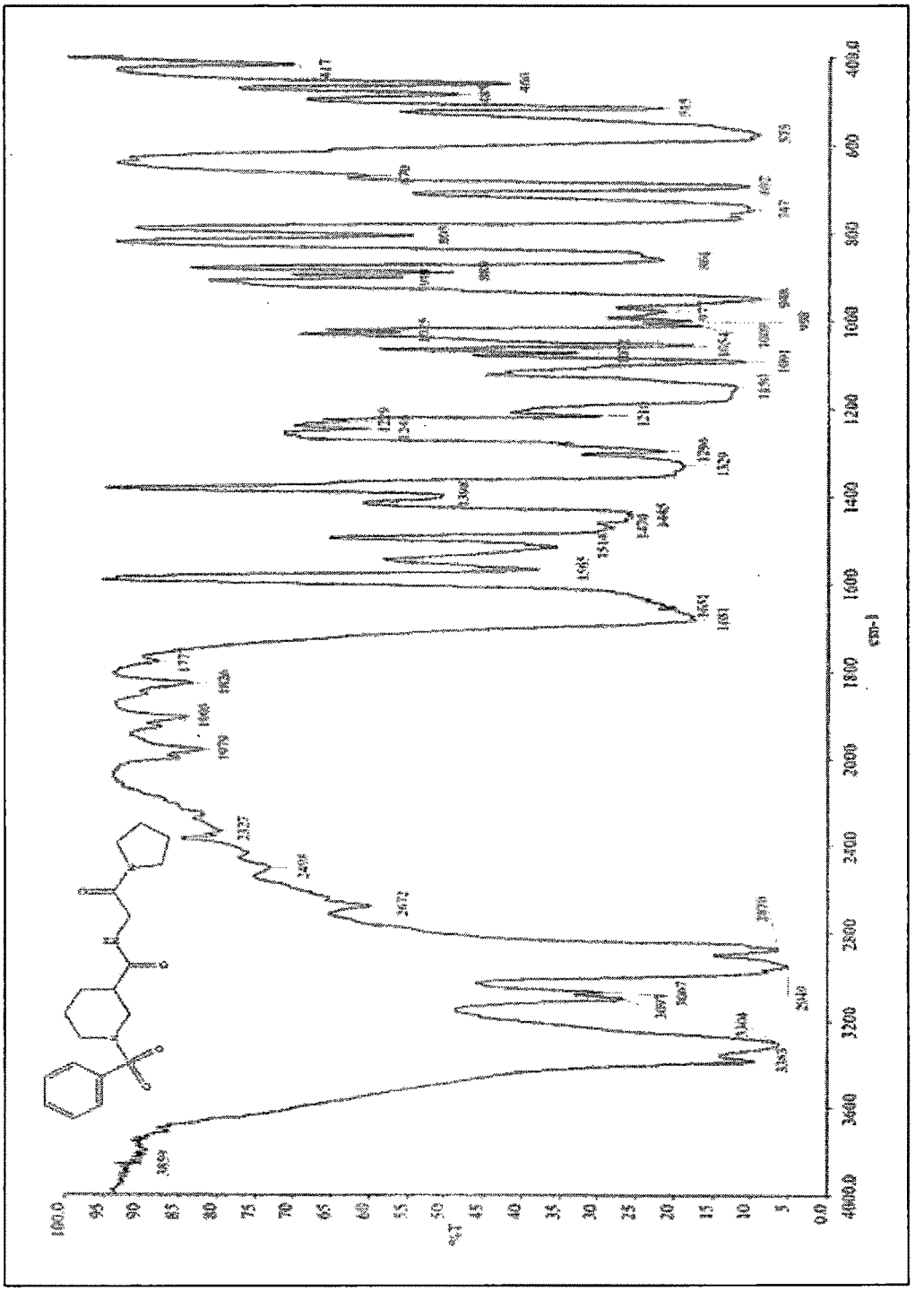


Figure 4.11.1: IR spectrum of N-(2-oxo-2-(pyrrolidin-1-yl)ethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6i**

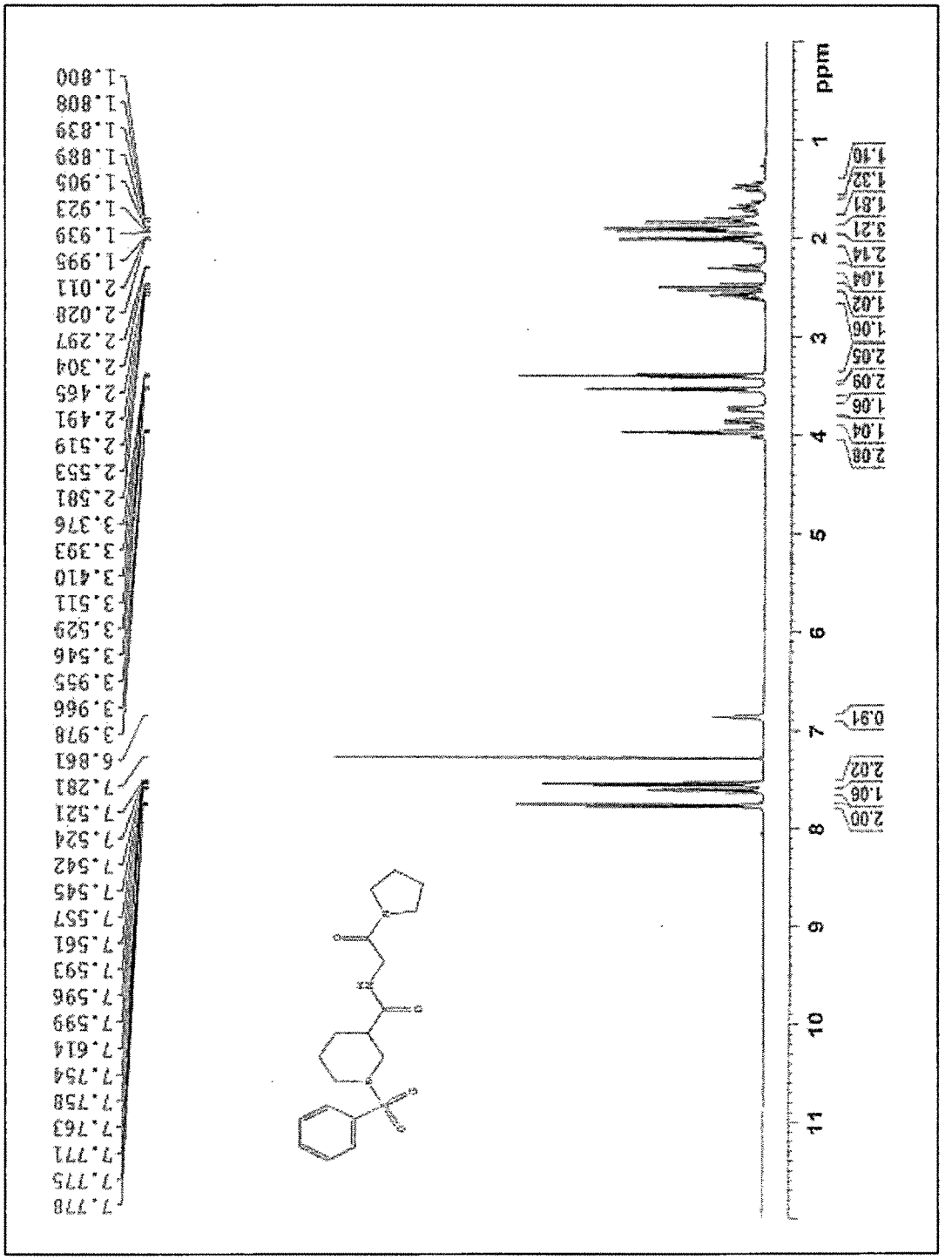


Figure 4.11.2: ^{1}H NMR spectrum of N-(2-oxo-2-(pyrrolidin-1-yl)ethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6i**

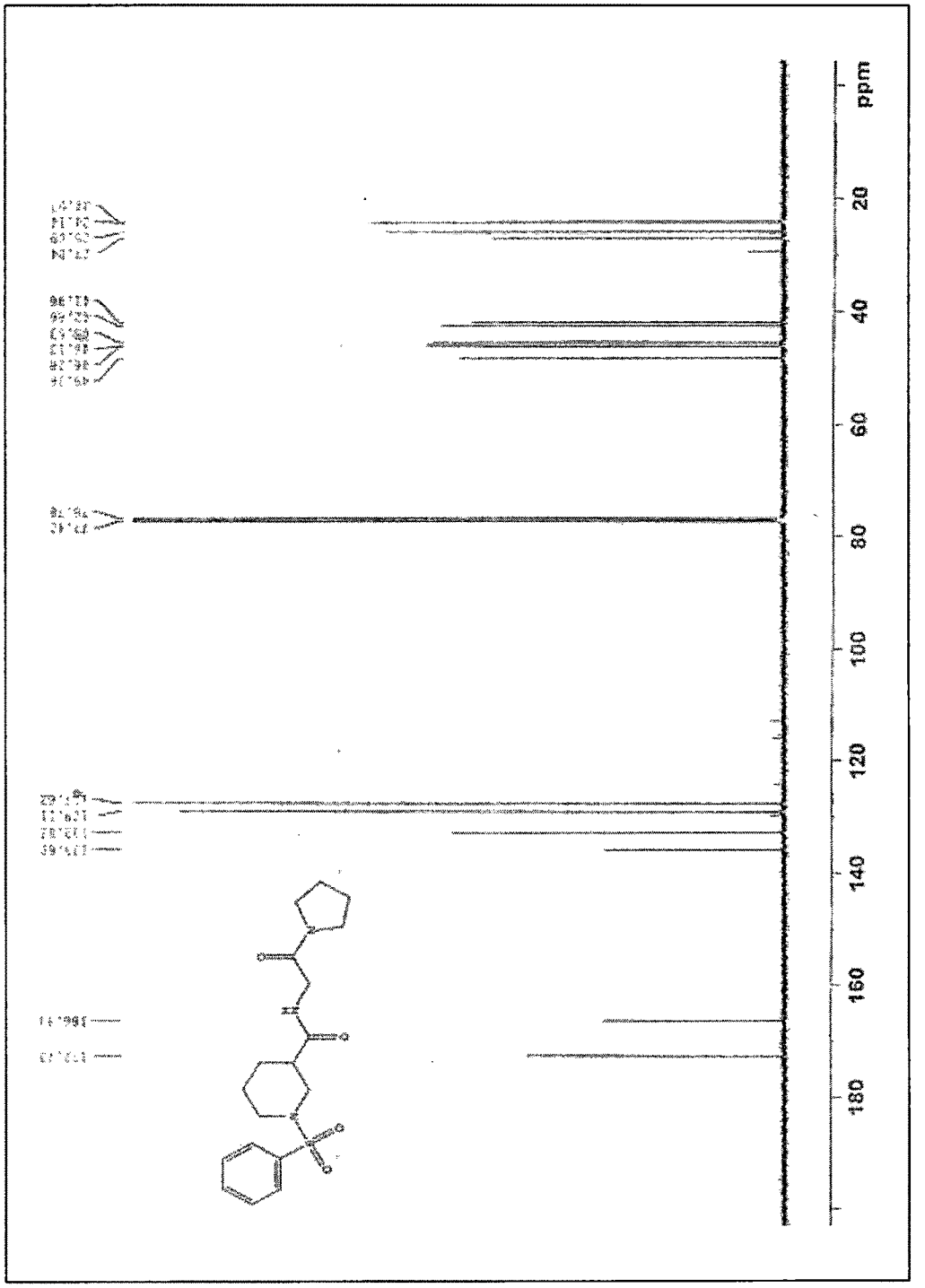


Figure 4.11.3: ^{13}C NMR spectrum of N-(2-oxo-2-(pyrrolidin-1-yl)ethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6i**

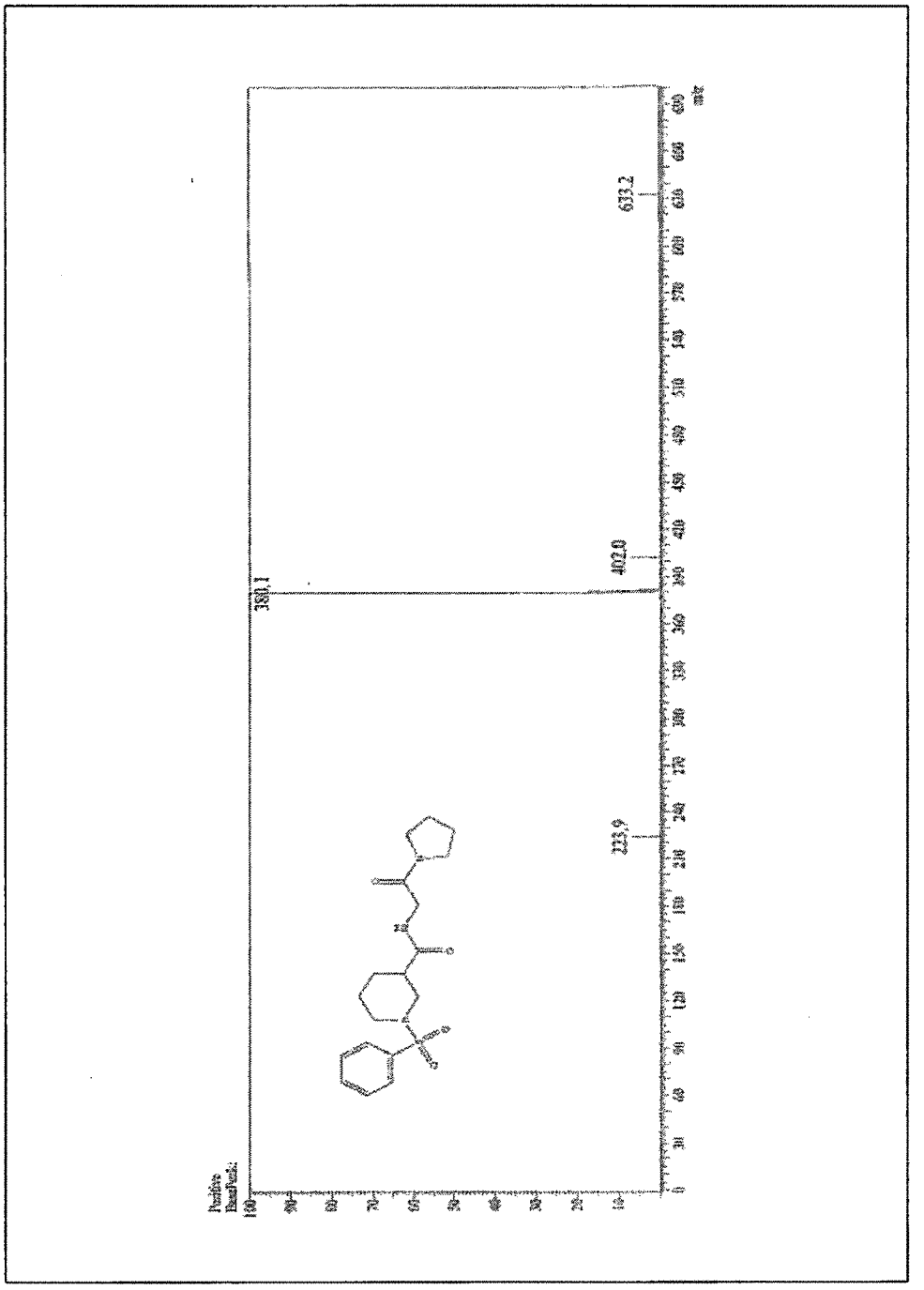


Figure 4.11.4: ESI-MS spectrum of N-(2-oxo-2-(pyrrolidin-1-yl)ethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6i**

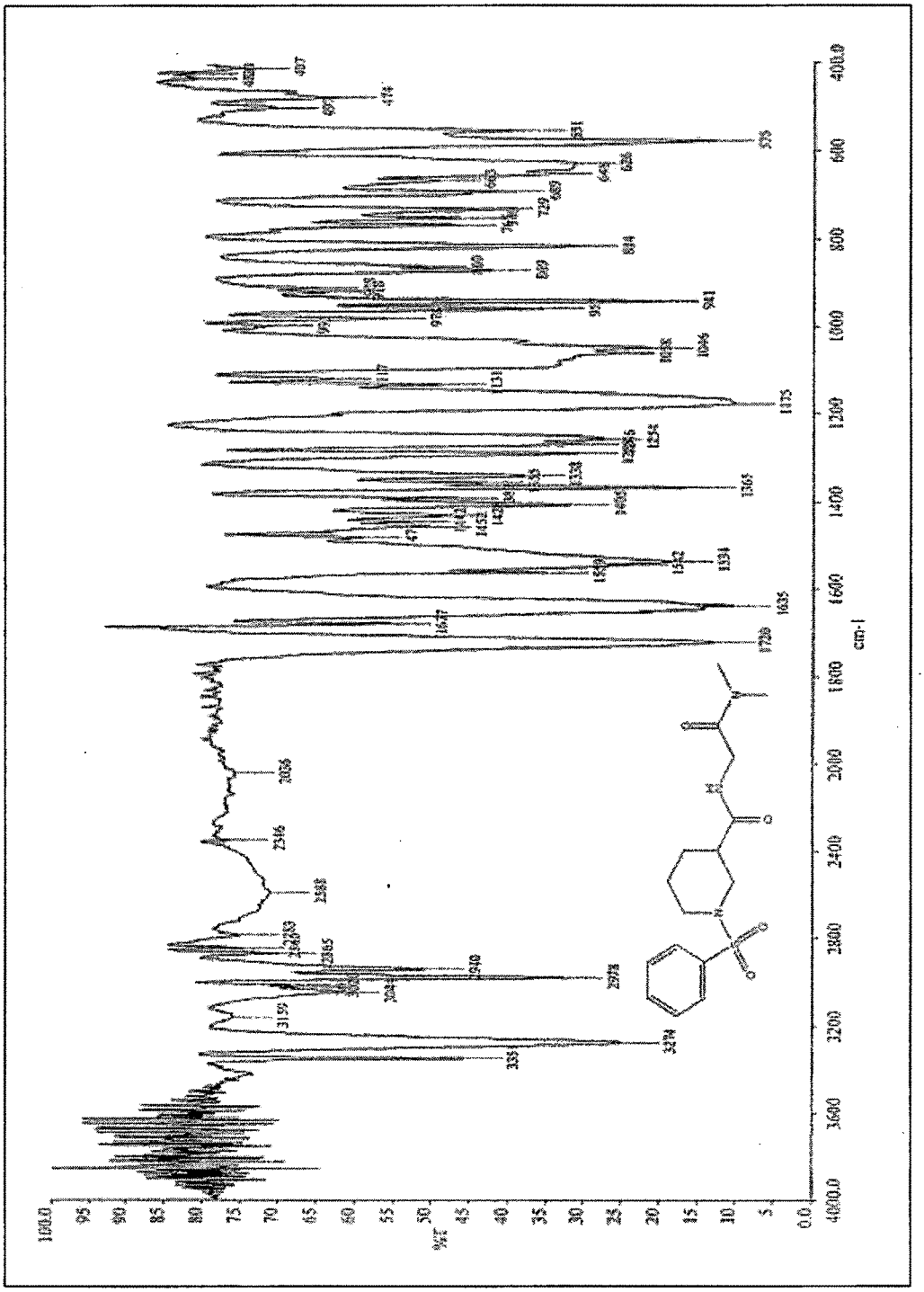


Figure 4.12.1: IR spectrum of N-(2-(dimethylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6j**

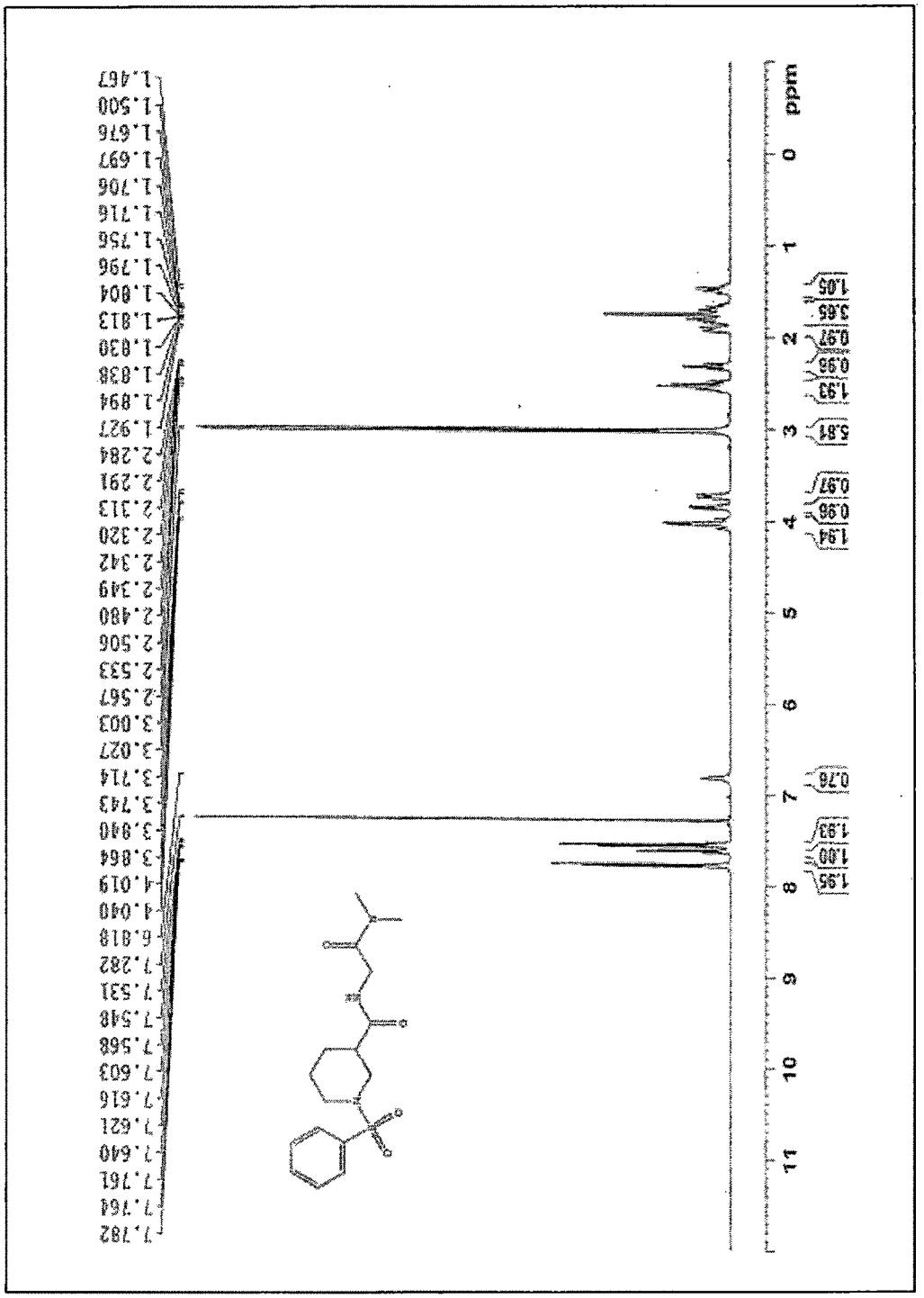


Figure 4.12.2: ^{1}H NMR spectrum of N-(2-(dimethylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6j**

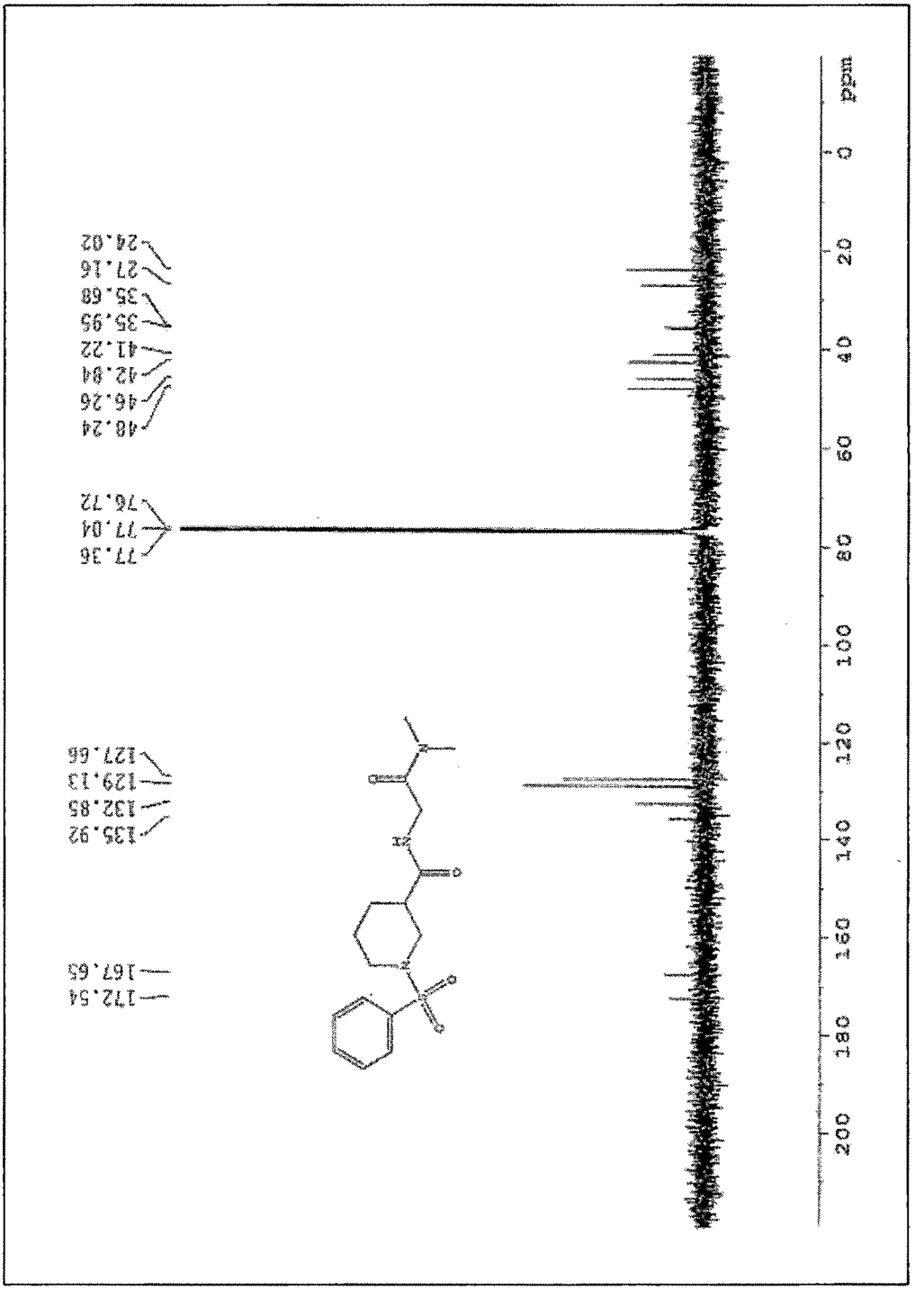


Figure 4.12.3: ^{13}C NMR spectrum of N-(2-(dimethylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6j**

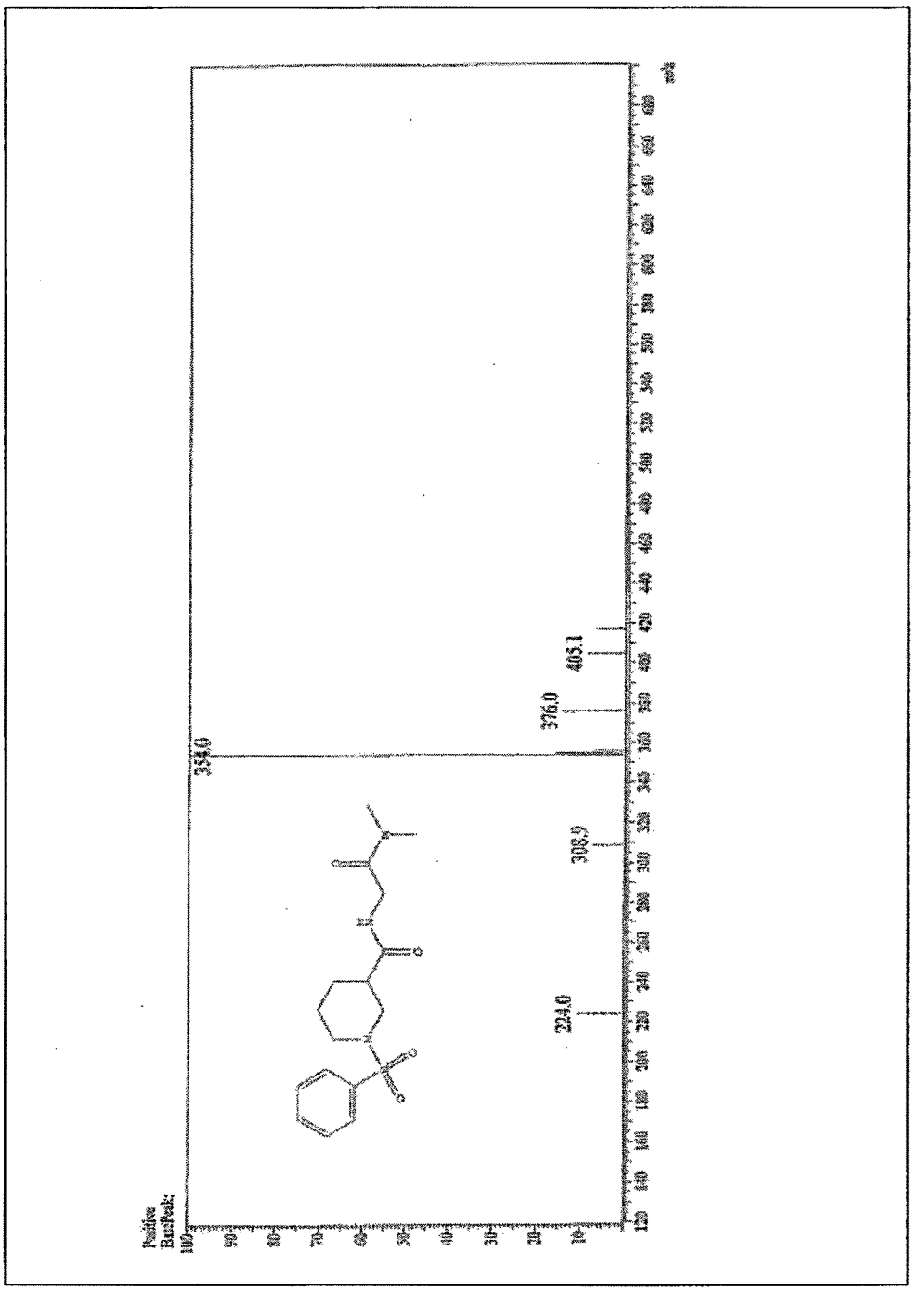


Figure 4.12.4: ESI-MS spectrum of N-(2-(dimethylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6j**

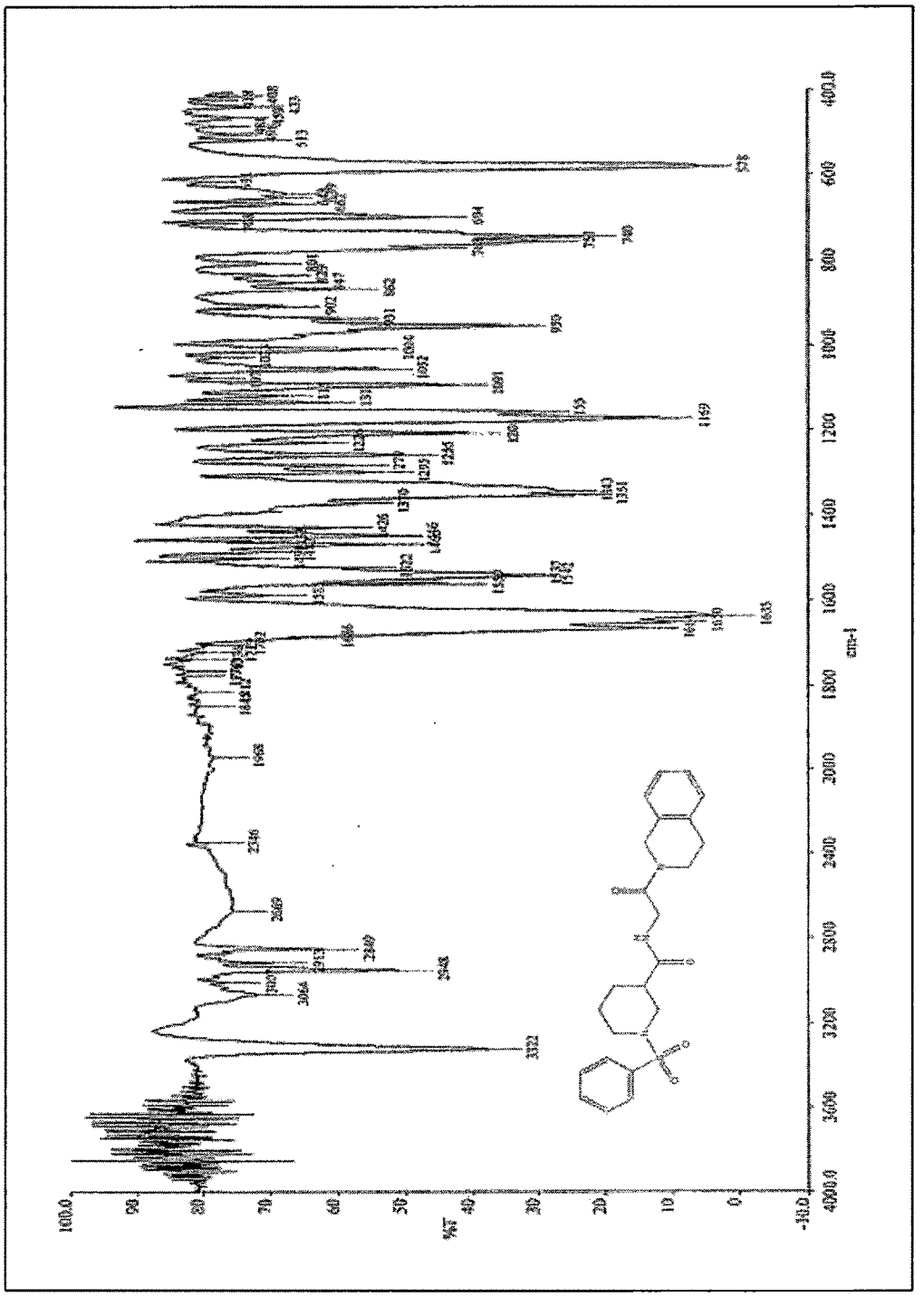


Figure 4.13.1: IR spectrum of N-(2-(3,4-dihydroisoquinolin-2(1H)-yl)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6k**

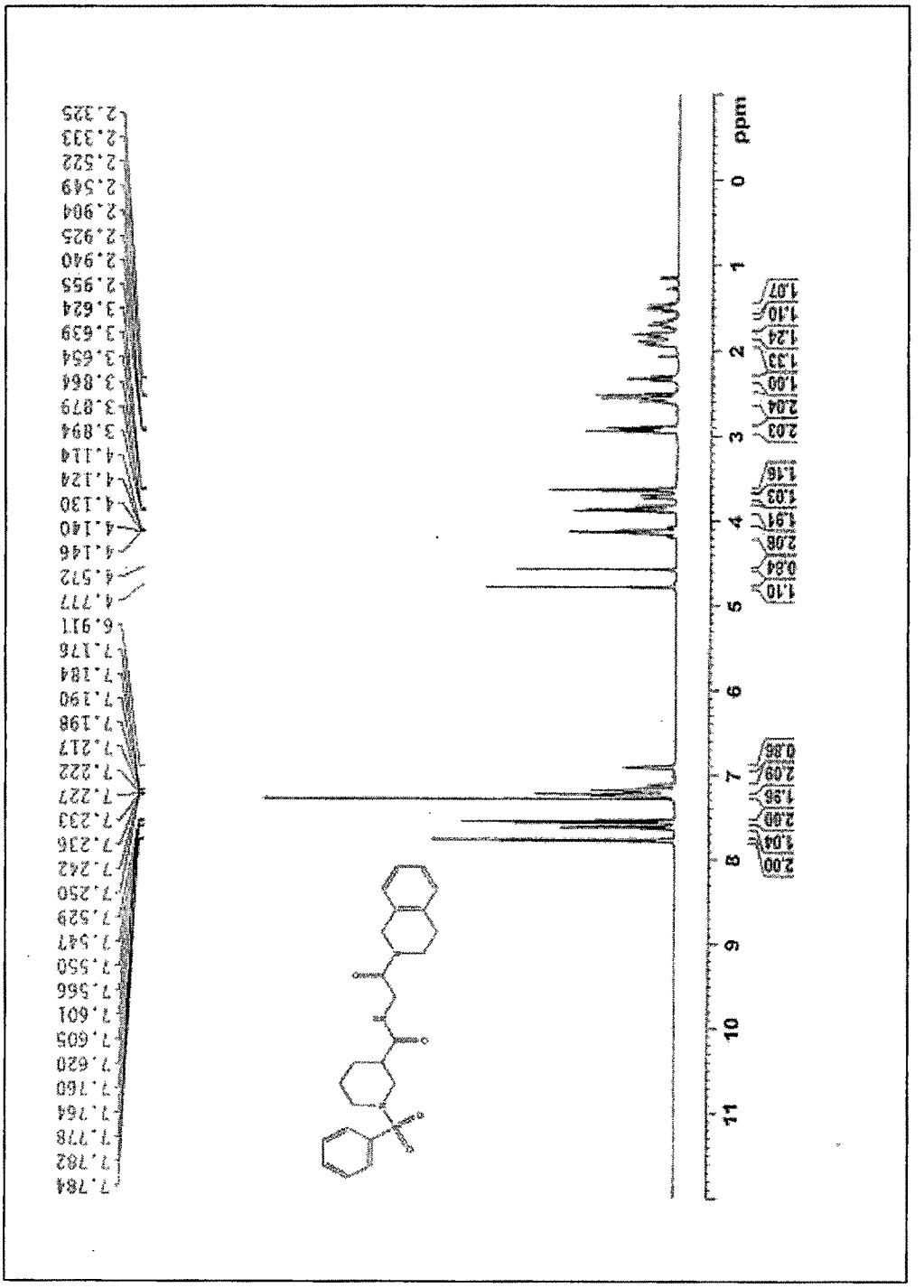


Figure 4.13.2: ^{1}H NMR spectrum of N-(2-(3,4-dihydroisoquinolin-2(1H)-yl)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6k**

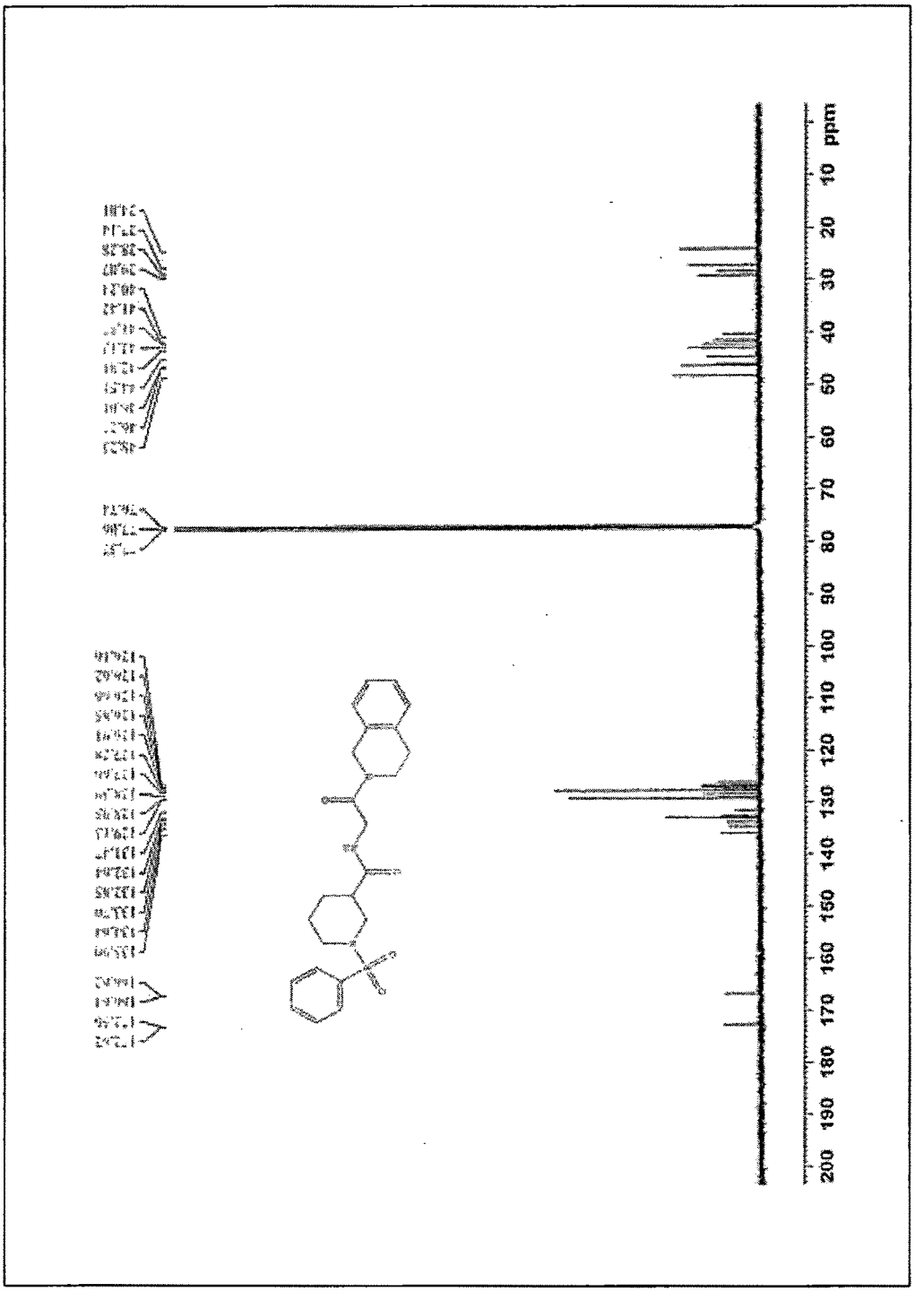


Figure 4.13.3: ^{13}C NMR spectrum of N-(2-(3,4-dihydroisoquinolin-2(1H)-yl)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6k**

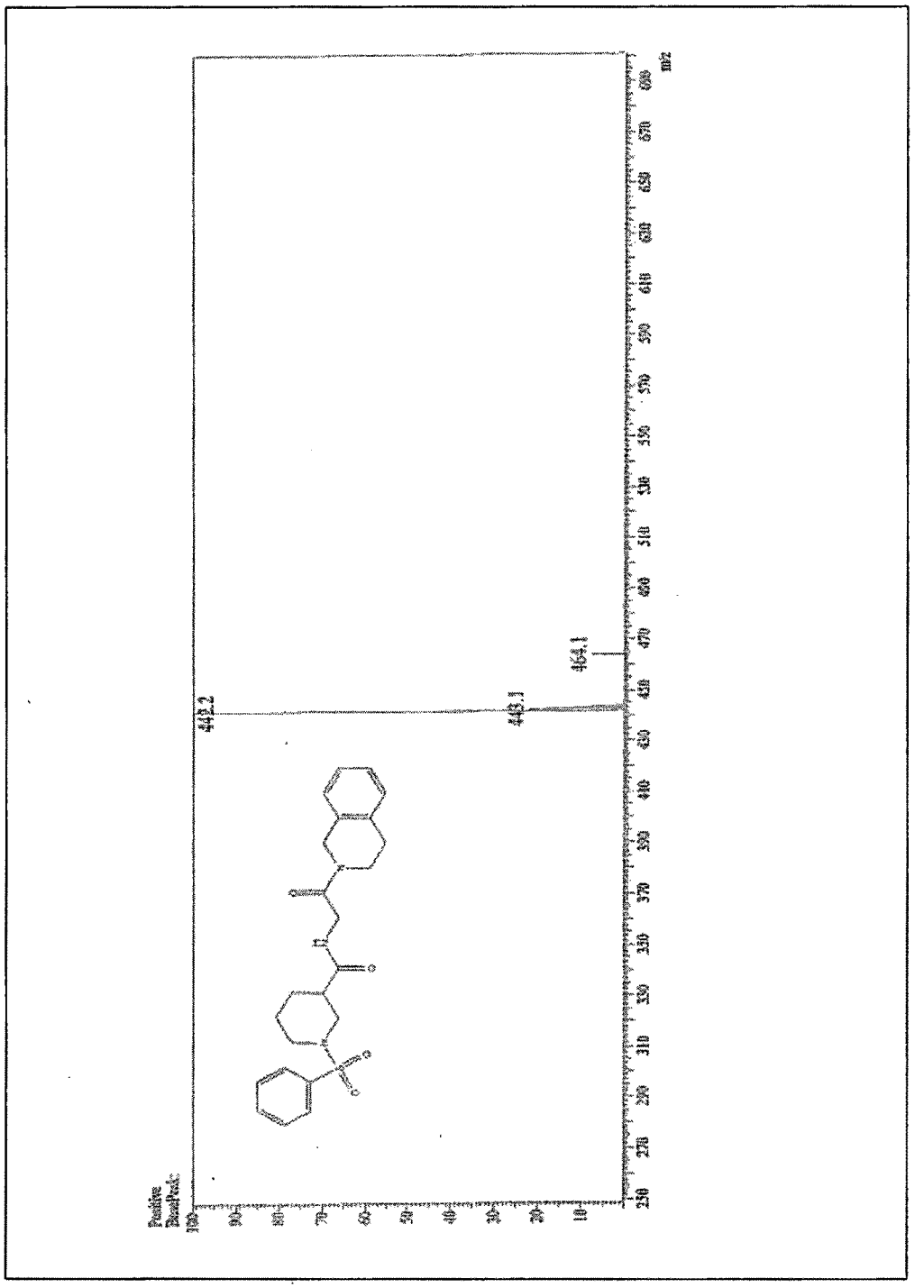


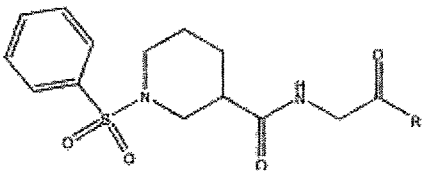
Figure 4.13.4: ESI-MS spectrum of N-(2-(3,4-dihydroisoquinolin-2(1H)-yl)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide **6k**

4.2.2 Biological evaluation

DPP-IV inhibition assay uses fluorogenic substrate, Gly-Pro-Aminomethylcoumarin (AMC), to measure DPP-IV activity. Cleavage of the peptide bond by DPP-IV releases the free AMC group, resulting in fluorescence that is analyzed using an excitation wavelength of 350-360 nm and emission wavelength of 450-465 nm. Human recombinant DPP-IV enzyme procured from Enzo life science (batch no BML-SE434-9091), substrate, H-Gly-Pro-AMC procured from Enzo life science (batch no BML-P189-9091) and assay buffer, prepared in-house containing HEPES (25 mM), NaCl(140 mM), $MgCl_2$ (80 mM) and BSA (1 % v/v) in deionized water having pH. 7.8 were used in the assay.

DPP-IV activity was measured by mixing reagents in 96-well plate (order of addition of reagents: assay buffer, enzyme, solvent/inhibitor and finally substrate). Both the enzyme and 96-well plate were incubated for 30 min and the resulting fluorescence was measured using Spectra Max fluorometer (Molecular Devices, Sunnyvale CA) by exciting at 360 nm and emission at 460 nm with the excitation filter at 360 nm and emission filter at 460 nm at sensitivity of 45.

The IC_{50} values were determined for test compounds using Graph Pad prism software.



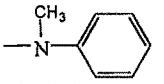
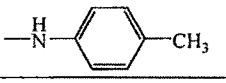
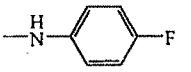
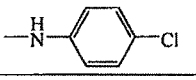
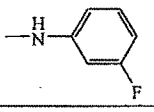
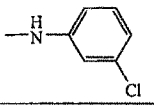
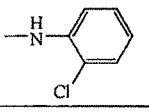
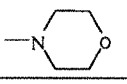
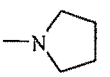
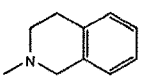
Compound	R	% Inhibition of DPP-IV at 3μM
6 a		50.5
6 b		14.4
6 c		55.0
6 d		69.5
6 e		58.5
6 f		14.1
6 g		14.4
6 h		10.1
6 i		12.4
6 j	$-N(CH_3)_2$	51.3
6 k		51.6

Table 4.1: *DPP-IV inhibition by compounds* ***6a-k*** *at 3μM concentrations.*

Preliminary DPP-IV inhibition assay was performed to test compounds **6a-k** for their inhibition potential at 3μM concentration and sitagliptin phosphate was used as a standard exhibiting 91.7% inhibition at the same concentration. Compounds showing greater than 50% inhibition at 3μM, qualified for IC_{50} determination.

Compound	IC_{50} (nM)
6 a	592.56
6 c	573.74
6 d	94.82
6 e	205.40
6 j	188.97
6 k	448.60

Table 4.2: *Inhibition of DPP-IV (IC_{50} nM) of selected compounds.*

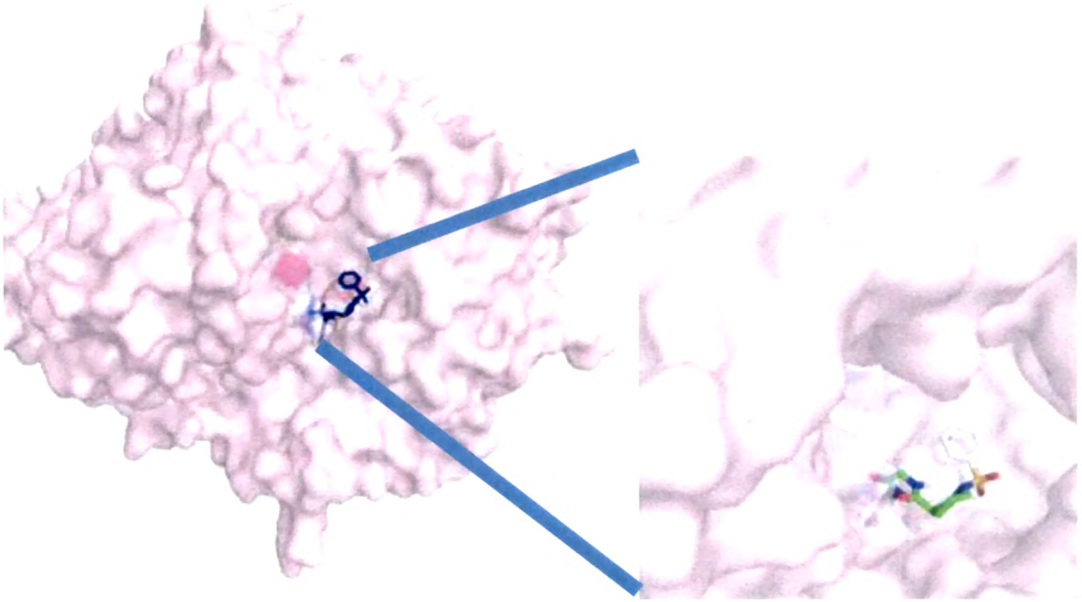
4.2.3 Structure activity relationship

From the *in-vitro* assay it was observed that substitution of secondary amines at the C-terminus of glycine did not show good inhibition. Substitution of cyclic aliphatic secondary amines like morpholine **6h** and pyrrolidine **6i** did not show good inhibition while substitution of N-methyl aniline **6a** and cyclic aliphatic aromatic amine 1,2,3,4-tetrahydroisoquinoline **6k** and dimethyl amine **6j** showed better DPP-IV inhibition, latter exhibited the best enzyme inhibition with an IC_{50} of 188.97 nM. Further, effect of substitution of halogens and methyl on the aniline at the P1 site was studies and it was found that *para-* substituted aniline showed better inhibition than the *meta-* or

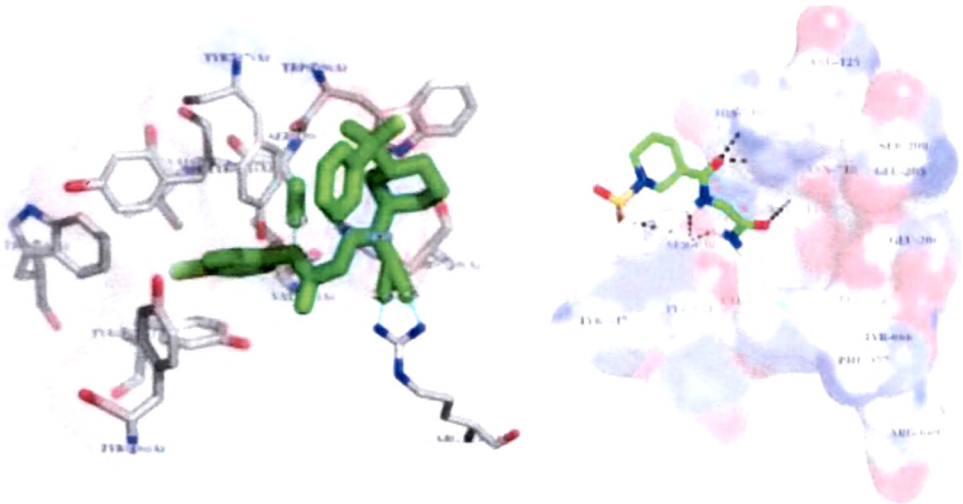
ortho-substituted anilines. As seen in table 4.2, it was observed that *m*-fluoro aniline **6e** is twice more potent than *p*-fluoro aniline derivative **6c** but similar trend was not observed on substitution of chlorine instead of fluorine. Compound **6d** with *p*-chloro aniline substituted at the C-terminus amide of glycine was found to be the most potent of all the compounds synthesized in the series with an IC_{50} of 94.82 nM.

4.2.4 Molecular Docking Study

For docking studies, binding site residues of the A chain of DPP-4 (PDB ID: 3W2T) [19] at a distance of 4.5 A from vildagliptin were selected. AutoDock Vina [20] was used for carrying out docking studies. Initial docking studies showed higher affinity of diamides as compared to the standard and so these molecules were synthesised. The affinity for the compound **6d** was -8.5 kcal/mol while that of NVP-LAF237 (vildagliptin) was shown to be -6.7 kcal/mol. LigPlot [21] was used to observe the interaction of the ligand with the binding site residues as seen in Figure 4.14. Pymol [22] was used to visualize the protein and the docked compound **6d** as seen in Figure 4.15.



A



B C

Figure 4.14: Binding of **6d** at the active site of DPP-IV

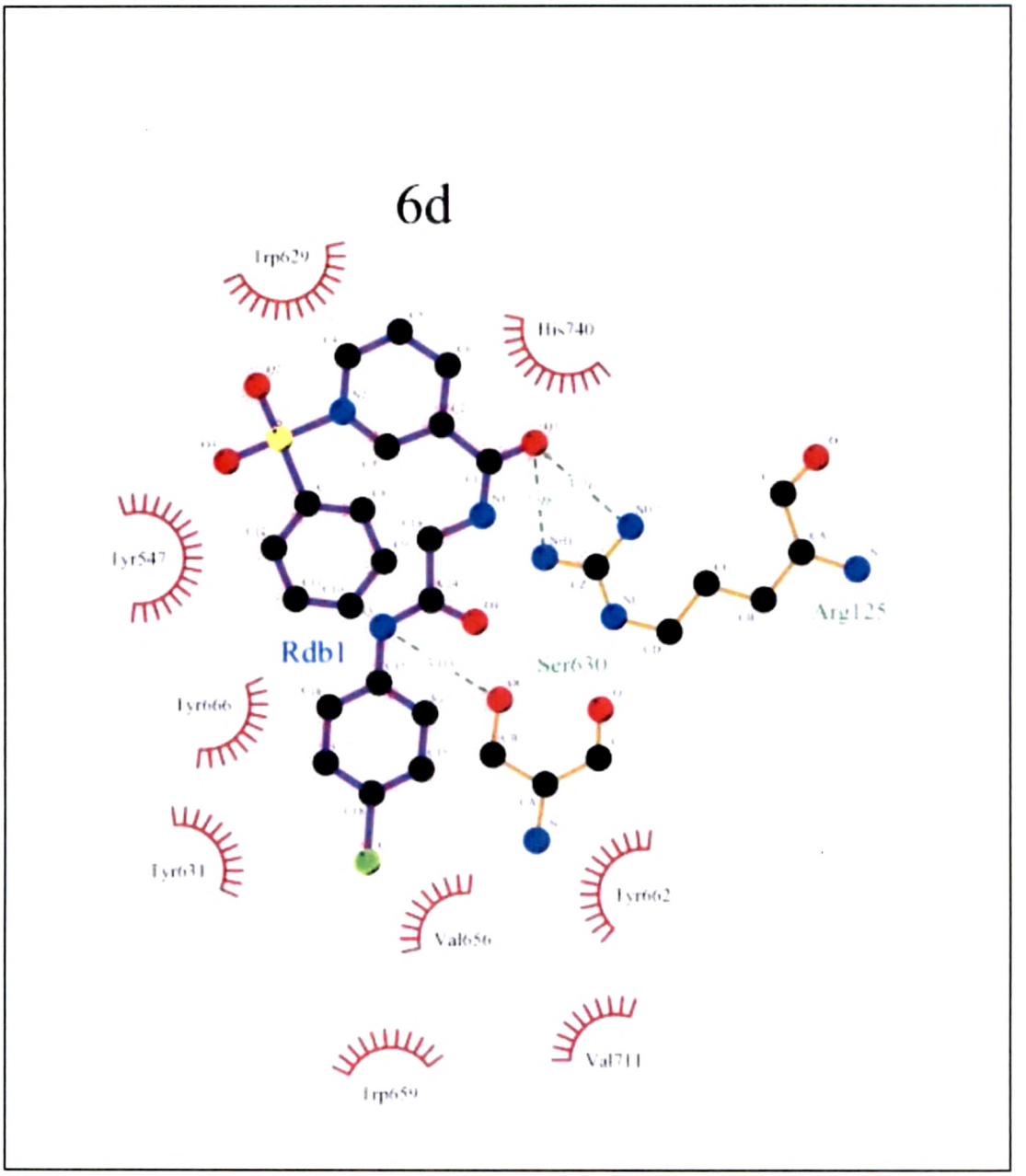


Figure 4.15: Interaction of **6d** with the binding site residues of DPP-IV

4.3 Conclusion

Thus it has been observed that cyclic secondary amines like pyrrolidine and morpholine are not good substituents for the DPP-IV inhibition. It was also observed that aliphatic amine, dimethyl amine, when substituted at the P1 site shows good enzyme inhibition. Substitution of chlorine at the *para* position of aniline, at the P1 site renders the compound more potent than any other substitution. This study was further supported by molecular modelling of **6d** at the active site of DPP-IV which suggested H-bonding interactions with SER630, ARG125 and TYR547 as seen in Figure 4.15.

4.4 Experimental

4.4.1 Chemistry

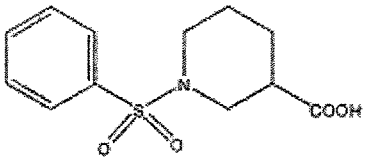
Reagent grade chemicals and solvents were purchased from commercial supplier and used after purification. TLC was performed on silica gel F254 plates (Merck). Acme's silica gel (60-120 mesh) was used for column chromatographic purification. All reactions were carried out in nitrogen atmosphere. Melting points are uncorrected and were measured in open capillary tubes, using a Rolex melting point apparatus. IR spectra were recorded as KBr pellets on Perkin Elmer RX 1 spectrometer. ^{1}H NMR and ^{13}C NMR spectral data were recorded on Advance Bruker 400 spectrometer (400 MHz) with $CDCl_3$ or DMSO-d_6 as solvent and TMS as internal standard. *J* values are in Hz. Mass spectra were determined by ESI-MS, using a Shimadzu LCMS 2020 apparatus. Elemental analyses were recorded on Thermosinnigan Flash 11-12 series EA. All the reactions were carried out under nitrogen atmosphere.

General procedure for the preparation of compounds 2a-k

A mixture of boc-glycine **1** (1.11 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.67 mmol) (EDCI), 1-hydroxybenzotriazole (1.11 mmol) (HOBt), 4-dimethylaminopyridine (1.34mmol) (DMAP) and amine (1^0 and 2^0) (1.22 mmol) in dichloromethane (50 mL) (DCM) was stirred at room temperature for 16 hours. The reaction was monitored using TLC. On completion of the reaction, it was washed with water (2X20 mL), brine (1X10 mL), dried over anhydrous sodium sulphate and the solvent evaporated under reduced pressure to give the crude product which was then purified by column chromatography using silica

gel as stationary phase and methanol:dichloromethane (5:95) as eluent to yield desired N-boc glycine amide **3a-k**, as white solid.

1-(phenylsulfonyl)piperidine-3-carboxylic acid 5:



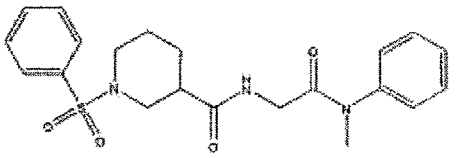
To a mixture piperidine-3-carboxylic acid **4** (1.0 mmol) and sodium carbonate (3.0 mmol) in 25 mL DCM:water (1:1) benzene sulphonyl chloride (1.1 mmol) was added and the reaction mixture stirred at room temperature for 16 hours or till the completion of reaction, as monitored by TLC. On completion of reaction, the reaction mixture was washed with petroleum ether (20 mL) and then acidified with conc. HCl, till pH 2. The white solid thus separated was filtered, washed with water several times and then dried to yield the desired product as white solid.

Yield : 91%; white solid; m.p. : 115-117 °C; IR (KBr) : 3100-2500 (b), 2940, 1812, 1693, 1352 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) : δ 1.41-1.50 (m, 1H), 1.65-1.73 (m, 1H), 1.80-1.85 (m, 1H), 1.99-2.04 (m, 1H), 2.41 (dt, 1H, J_1 = 2.8 Hz, J_2 = 11.2 Hz), 2.57 (t, 1H, J = 10.8 Hz), 2.65-2.71 (m, 1H), 3.59 (br d, 1H, J = 11.6 Hz), 3.83 (dd, 1H, J_1 = 3.2 Hz, J_2 = 7.2 Hz), 7.54-7.58 (m, 2H), 7.61-7.63 (m, 1H), 7.77-7.79 (m, 2H), 8.98 (br s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) : δ 23.88, 26.21, 40.73, 46.26, 47.35, 127.62, 129.18, 132.94, 135.96, 178.63; $C_{12}H_{15}NO_4S$; ESI-MS: *m/z* 292.0 $[M+Na]^+$

General procedure for the preparation of compounds 6a-k

Compounds **3a-k** were de-protected by stirring it in 10% trifluoroacetic acid (TFA) in DCM. On completion of the reaction after an hour or as monitored by TLC, the solvent was evaporated under reduced pressure and once again the product was dissolved in DCM to give solution of compounds **3a-k**. To a solution of compound **5** (1.0 mmol), in DCM (20 mL), EDCI (1.5 mmol), HOBt (1.0 mmol) and DMAP (1.0 mmol) were added at 0-5 °C, followed by the solution of compound **3a-k** (1.1 mmol) in DCM (5 mL) and the reaction mixture was then stirred at room temperature for 10 hours or till the completion of the reaction as detected by TLC. After completion of the reaction, it was washed with water (2X20 mL), brine (1X10 mL), dried over anhydrous sodium sulphate and the solvent evaporated under reduced pressure to give the crude product which was then purified by column chromatography using silica gel, employing ethylacetate : petroleum ether (70:30) as eluent to give pure product **6a-k** as white solid.

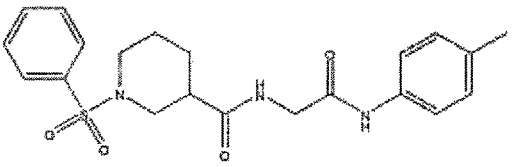
N-(2-(methyl(phenyl)amino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide 6a:



Yield: 65%; white solid; m.p.: 156-158 °C; IR (KBr): 3322, 2944, 1673, 1635, 1347, 1333cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.44-1.50 (m, 1H), 1.66-1.89 (m, 3H), 2.29-2.35 (m, 1H), 2.44-2.56 (m, 2H), 3.31 (s, 3H), 3.66-3.78 (m, 4H), 6.69 (br s, 1H), 7.21-7.23 (m, 2H), 7.38-7.48 (m, 3H), 7.51-7.55 (m, 2H), 7.59-7.63 (m, 1H), 7.73-7.76 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 23.94, 27.00, 37.60, 42.04, 42.72, 46.26, 48.26,

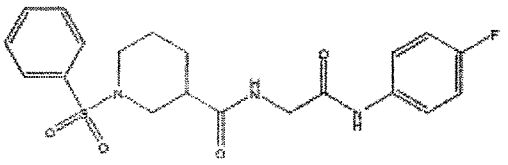
127.18, 127.64, 128.77, 129.12, 130.27, 132.86, 135.78, 141.68, 168.06, 172.47; Anal. Calc. for $C_{21}H_{25}N_3O_4S$: C, 60.70; H, 6.06; N, 10.11; found: C, 60.71; H, 5.85; N, 10.12%; ESI-MS: *m/z* 416.1 $[M+H]^+$.

N-(2-oxo-2-(p-tolylamino)ethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide 6b:



Yield: 85%; white solid; m.p.: 194-196 °C; IR (KBr): 3331, 3327, 2956, 2929, 2846, 1678, 1657, 1644, 1358, 1332 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 1.23-1.27 (m, 1H), 1.46-1.49 (m, 1H), 1.72-1.79 (m, 2H), 2.10-2.20 (m, 2H), 2.24 (s, 3H), 2.50-2.56 (m, 1H), 3.66-3.69 (m, 2H), 3.77-3.85 (m, 2H), 7.10 (d, 2H, *J* = 8.0 Hz), 7.45 (d, 2H, *J* = 8.0 Hz), 7.64-7.68 (m, 2H), 7.72-7.75 (m, 3H), 8.36 (m, 1H,), 9.90 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) : δ 20.89, 24.15, 27.01, 41.94, 42.87, 46.53, 48.64, 119.58, 127.86, 129.59, 129.94, 132.64, 133.69, 135.60, 136.76, 167.80, 173.10; Anal. Calc. for $C_{21}H_{25}N_3O_4S$: C, 60.70; H, 6.06; N, 10.11; found: C, 60.78; H, 6.18; N, 9.90%; ESI-MS: *m/z* 416.1 $[M+H]^+$.

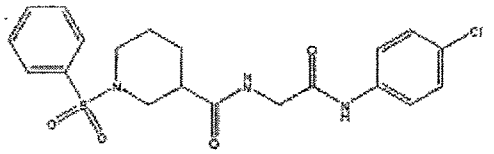
N-(2-(4-fluorophenylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide 6c:



Yield: 70%; white solid; m.p.: 196-198 °C; IR (KBr): 3324, 3298, 2967, 2934, 2843, 2865, 1677, 1654, 1643, 1355 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 1.23-1.27

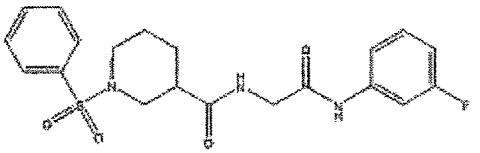
(m, 1H), 1.46-1.49 (m, 1H), 1.72-1.79 (m, 2H), 2.10-2.20 (m, 2H), 2.50-2.51 (m, 1H), 3.58-3.63 (m, 1H), 3.66-3.72 (m, 1H), 3.82-3.85 (m, 2H), 7.15 (t, 2H, J = 8.8 Hz), 7.56-7.60 (m, 2H), 7.66 (t, 2H, J = 8.4 Hz), 7.72-7.74 (m, 3H), 8.39 (br s, 1H), 10.05 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6): δ 24.14, 27.00, 41.93, 42.85, 46.52, 48.62, 115.68, 115.90, 121.31, 121.39, 127.85, 129.94, 133.70, 135.59, 135.67, 157.23, 159.61, 168.00, 173.16; Anal. Calc. for $C_{20}H_{22}FN_3O_4S$: C, 57.27; H, 5.29; N, 10.02; found: C, 57.42; H, 4.90; N, 9.97%; ESI-MS: *m/z* 420.0 $[M+H]^+$.

N-(2-(4-chlorophenylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide 6d:



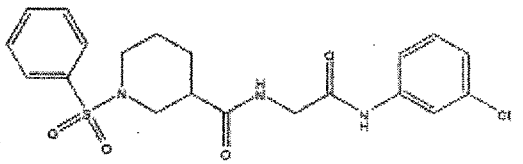
Yield: 55%; white solid; m.p.: 178-180 °C; IR (KBr): 3329, 3303, 2931, 2863, 2843, 1679, 1644, 1614, 1355, 1334 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.22-1.27 (m, 1H), 1.46-1.49 (m, 1H), 1.75 (t, 2H, J = 15 Hz), 2.10-2.20 (m, 2H), 2.50-2.54 (m, 1H), 3.60-3.69 (m, 2H), 3.84-3.86 (m, 2H), 7.36 (d, 2H, J = 8.8 Hz), 7.59-7.67 (m, 4H), 7.72-7.74 (m, 3H), 8.41 (t, 1H, J = 8.0 Hz), 10.14 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6): δ 24.14, 27.01, 41.93, 42.95, 46.52, 48.63, 121.10, 127.25, 127.85, 129.13, 129.93, 133.68, 135.62, 138.25, 168.26, 173.16; $C_{20}H_{22}ClN_3O_4S$ ESI-MS: *m/z* 435.9 $[M+H]^+$.

N-(2-(3-fluorophenylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide 6e:



Yield: 62%; white solid; m.p.: 186-188 °C; IR (KBr) : 3308, 3104, 2930, 2851, 1709, 1670, 1616, 1351, 1317 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 1.23-1.27 (m, 1H), 1.46-1.49 (m, 1H), 1.72-1.79 (m, 2H), 2.10-2.20 (m, 2H), 2.50-2.51 (m, 1H), 3.61 (d, 1H, J = 10.8 Hz), 3.68 (d, 1H, J = 10.8 Hz), 3.84-3.87 (m, 2H), 6.88-6.90 (m, 1H), 7.26-7.36 (m, 2H), 7.55-7.75 (m, 6H), 8.41 (br s, 1H), 10.22 (s, 1H); ^{13}C NMR (400 MHz, DMSO-d_6) : δ 24.14, 27.00, 41.92, 42.97, 46.52, 48.62, 106.18, 106.44, 110.08, 110.29, 115.27, 127.86, 129.93, 130.85, 130.95, 133.69, 135.61, 140.95, 141.06, 161.38, 163.78, 168.49, 173.17; Anal. Calc. for $C_{20}H_{22}FN_3O_4S$: C, 57.27; H, 5.29; N, 10.02; found: C, 57.42; H, 4.90; N, 9.97%. ESI-MS: *m/z* 420.2 $[M+H]^+$.

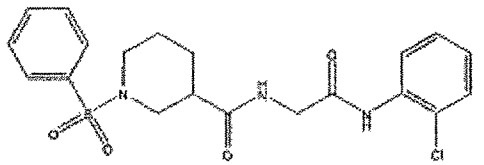
N-(2-(3-chlorophenylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide 6f:



Yield: 59%; white solid; m.p.: 140-142 °C; IR (KBr): 3412, 3303, 2948, 2843, 1692, 1666, 1650, 1350, 1333 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.67-1.71 (m, 1H), 1.75 (s, 1H), 1.82-1.91 (m, 2H), 2.45-2.55 (m, 1H), 2.64-2.74 (m, 2H), 3.57 (d, 1H, J = 11.6 Hz),3.74 (d, 1H, J = 9.2 Hz), 4.14 (d, 2H, J = 5.2 Hz), 7.01-7.10 (m, 1H), 7.16-7.24 (m, 2H), 7.40-7.42 (m, 1H), 7.52-7.56 (m, 2H), 7.60-7.64 (m, 2H), 7.75-7.77 (m, 2H),

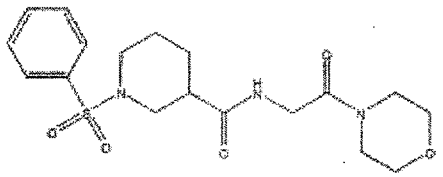
8.82 (s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 23.76, 26.90, 42.39, 44.34, 46.35, 48.22, 117.84, 119.92, 124.44, 127.56, 129.25, 130.03, 133.10, 134.51, 135.59, 138.78, 167.04, 173.90; $C_{20}H_{22}ClN_3O_4S$; ESI-MS: *m/z* 436.00 $[M+H]^+$

N-(2-(2-chlorophenylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide 6g:



Yield: 48%; white solid; m.p.: 162-164 °C; IR (KBr): 3373, 3257, 2953, 2936, 2863, 1707, 1649, 1583, 1386, 1323 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.58-1.76 (m, 2H), 1.80-1.93 (m, 2H), 2.41-2.48 (m, 1H), 2.63-2.70 (m, 2H), 3.61-3.64 (m, 1H), 3.75-3.82 (m, 1H), 4.13-4.14 (m, 2H), 7.01-7.10 (m, 2H), 7.26-7.30 (m, 1H), 7.36-7.39 (m, 1H), 7.53-7.57 (m, 2H), 7.61-7.65 (m, 1H), 7.76-7.78 (m, 2H), 8.30-8.35 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 23.84, 26.93, 42.59, 44.53, 46.33, 48.21, 121.86, 123.18, 125.16, 127.61, 127.75, 129.17, 129.25, 133.07, 134.06, 135.54, 167.23, 173.79; $C_{20}H_{22}ClN_3O_4S$; ESI-MS: *m/z* 436.05 $[M+H]^+$.

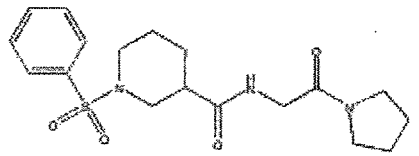
N-(2-morpholino-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide 6h:



Yield: 79%; white solid; m.p.: 178-180 °C; IR (KBr): 3311, 2963, 2922, 2856, 1670, 1655, 1640, 1351, 1332 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.49-1.56 (m, 1H),

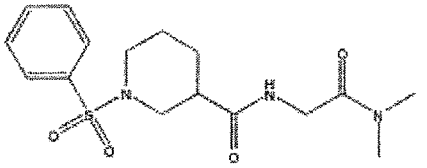
1.66-1.74 (m, 1H), 1.80-1.91 (m, 2H), 2.33-2.39 (m, 1H), 2.52-2.58 (m, 2H), 3.41-3.44 (m, 2H), 3.66-3.74 (m, 7H), 3.81 (d, 1H, J = 8.0 Hz), 4.03-4.10 (m, 2H), 6.81 (br s, 1H), 7.53-7.57 (m, 2H), 7.60-7.64 (m, 1H), 7.76-7.78 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 23.95, 27.11, 41.04, 42.34, 42.77, 44.78, 46.28, 48.19, 66.31, 66.69, 127.65, 129.14, 132.87, 135.89, 166.39, 172.62; Anal. Calc. for $C_{18}H_{25}N_3O_5S$: C, 54.67; H, 6.37; N, 10.63; found: C, 54.75; H, 5.86; N, 10.32%. ESI-MS: *m/z* 396.1 $[M+H]^+$.

N-(2-oxo-2-(pyrrolidin-1-yl)ethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide 6i:



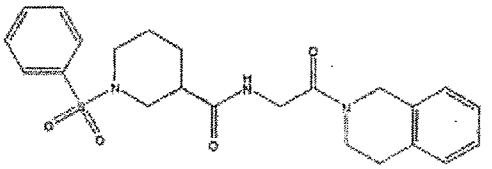
Yield: 66%; white solid; m.p.: 138-140 °C; IR (KBr): 3383, 3304, 2949, 2870, 1681, 1651, 1398 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.43-1.53 (m, 1H), 1.66-1.73 (m, 1H), 1.79-1.84 (m, 2H), 1.90-1.94 (m, 2H), 1.98-2.03 (m, 2H), 2.27-2.33 (m, 1H), 2.49 (t, 1H, J = 10.4 Hz) 2.55-2.60 (m, 1H), 3.39 (t, 2H, J = 6.8 Hz), 3.53 (t, 2H, J = 6.8 Hz), 3.84-3.85 (m, 1H), 3.86-3.87 (m, 1H), 3.95-3.99 (m, 2H), 6.86 (br s, 1H), 7.52-7.56 (m, 2H), 7.59-7.63 (m, 1H), 7.75-7.78 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 24.07, 24.14, 25.89, 27.04, 41.96, 42.66, 45.53, 46.13, 46.26, 48.36, 127.62, 129.11, 132.82, 135.88, 166.33, 172.73. Anal. Calc. for $C_{18}H_{25}N_3O_4S$: C, 56.97; H, 6.64; N, 11.07; found: C, 57.00; H, 6.82; N, 11.30%. ESI-MS: *m/z* 380.1 $[M+H]^+$.

N-(2-(dimethylamino)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide 6j:



Yield: 80%; white solid; m.p.: 128-130 °C; IR (KBr): 3351, 3274, 2978, 2940, 1720, 1677, 1635, 1365, 1338 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.46-1.50 (m, 1H), 1.65-1.85 (m, 2H), 1.89-1.93 (m, 1H), 2.28-2.35 (m, 1H), 2.48-2.60 (m, 2H), 3.02 (d, 6H, *J* = 9.6 Hz), 3.73 (d, 1H, *J* = 11.6 Hz), 3.85 (d, 1H, *J* = 9.6 Hz), 4.03 (d, 2H, *J* = 8.4 Hz), 6.82 (br s, 1H), 7.53-7.57 (m, 2H), 7.60-7.64 (m, 1H), 7.77 (d, 2H, *J* = 7.2 Hz); ^{13}C NMR (400 MHz, $CDCl_3$) : δ 24.02, 27.16, 35.68, 35.95, 41.22, 42.84, 46.26, 48.24, 127.66, 129.13, 132.85, 135.92, 167.65, 172.54; $C_{16}H_{23}N_3O_4S$; ESI-MS: *m/z* 354.0 $[M+H]^+$.

N-(2-(3,4-dihydroisoquinolin-2(1H)-yl)-2-oxoethyl)-1-(phenylsulfonyl)piperidine-3-carboxamide 6k:



Yield: 62%; white solid; m.p.: 142-144 °C; IR (KBr): 3322, 2948, 2913, 2849, 1664, 1650, 1635, 1351, 1343 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.48-1.51 (m, 1H), 1.67-1.70 (m, 1H), 1.80-1.85 (m, 1H), 1.90-1.93 (m, 1H), 2.30-2.36 (m, 1H), 2.50-2.60 (m, 2H), 2.90-2.96 (m, 2H), 3.64 (t, 1H, *J* = 6.0 Hz), 3.72 (d, 1H, *J* = 11.2 Hz), 3.83-3.89 (m, 2H), 4.11-4.16 (m, 2H), 4.57 (s, 1H), 4.78 (s, 1H), 7.11-7.13 (m, 1H), 7.16-7.20

(m, 2H), 7.22-7.25 (m, 2H), 7.53-7.57 (m, 2H), 7.60-7.64 (m, 1H), 7.76-7.78 (m, 2H); ^{13}C NMR (400 MHz, $CDCl_3$): δ 24.01, 27.14, 28.28, 29.07, 40.24, 41.42, 41.57, 42.13, 42.81, 44.51, 46.01, 46.27, 48.23, 126.16, 126.62, 126.66, 126.85, 126.91, 127.28, 127.66, 128.39, 128.95, 129.13, 131.47, 132.64, 132.85, 133.70, 134.64, 135.90, 166.62, 166.64, 172.56, 172.62. Anal. Calc. for $C_{23}H_{27}N_3O_4S$: C, 62.56; H, 6.16; N, 9.52; found: C, 62.46; H, 6.07; N, 9.49%; ESI-MS: *m/z* 442.2 $[M+H]^+$.

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