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Comprehensive NMR Spectral Analysis and Prediction of Selected Coumarin Derivatives Using ^{1}H, ^{13}C, 2D-COSY, HSQC, and HMBC Techniques

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Abstract: Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful analytical technique extensively used in the structural elucidation of complex organic compounds. Recent advancements in computational tools have enabled accurate spectral prediction and structure generation from multidimensional NMR datasets. This study aimed to evaluate the effectiveness of the Spec2D expert system in predicting and interpreting ^{1}H, ^{13}C, and 2D NMR spectra (COSY, HSQC) for the structural elucidation of selected coumarin derivatives. A total of nine coumarin derivatives were subjected to *in silico* NMR spectral prediction using ChemOffice Ultra and the online platform nmrdb.org. Detailed structure elucidation was performed using the Spec2D system, which applies graph-based modelling and a COSY-based knowledge base for substructure extraction and candidate structure generation. Input spectra included 1D (^{1}H, ^{13}C) and 2D (COSY, HSQC) data, with cross-peak information derived from H–H COSY spectra. The analysis module successfully extracted relevant substructures and proposed candidate structures based on spectral constraints. For all compounds, the correct structures were consistently ranked highest by the system, with isomeric structures appearing in lower ranks. Spec2D demonstrated flexibility in handling compounds with different molecular formulas and exchangeable protons. Processing time per compound ranged from 30 seconds to 2 minutes. Spec2D proved to be a reliable and efficient tool for NMR-based structural elucidation of coumarin derivatives. Its ability to generate accurate predictions without molecular formula input, accommodate spectral variability, and utilize a COSY-driven knowledge base underscores its utility in modern cheminformatics and metabolomics research.

Keywords: Coumarin derivatives, NMR spectroscopy, ^{1}HNMR, ^{13}CNMR, 2DCOSY, HSQC.

1. INTRODUCTION

Nuclear Magnetic Resonance (NMR) spectroscopy has long been a cornerstone technique in natural product chemistry and pharmaceutical analysis, effectively used to identify and quantify major constituents and impurities in complex mixtures. In recent years, NMR has emerged as a pivotal tool in

metabolomics, facilitating the evaluation and exploration of the human metabolome to understand disease states and elucidate metabolic pathways across various organisms. As diverse scientific disciplines continue to compile comprehensive catalogs of chemical components inherent to living systems, NMR spectroscopy plays an increasingly significant role in mapping metabolic evolution through biological studies. Its high specificity and non-destructive nature make it well-suited for detecting a broad spectrum of compounds in metabolomic research. By establishing comprehensive databases of NMR spectra from pure reference compounds, researchers can harness the spectral richness and resolution of NMR data to accurately identify and quantify metabolites. This capability significantly enhances the analytical power of metabolic profiling.

To further improve the utility and interpretability of NMR data, numerous software tools and computational methods have been developed. Over the past several decades, a wide range of NMR techniques has been applied in metabolic and structural studies, notably including one-dimensional (1D) ^{1}H and ^{13}C NMR, as well as two-dimensional (2D) methods such as ^{1}H-^{1}H COSY and ^{1}H, ^{13}C HSQC/HMBC. These multidimensional techniques offer enhanced structural resolution and correlation insights, making them indispensable for comprehensive structural elucidation of complex organic molecules.

To perform comprehensive NMR spectral prediction and analysis of selected coumarin derivatives using one-dimensional (^{1}H and ^{13}C) and two-dimensional (2D-COSY, HSQC, HMBC) NMR techniques for structural elucidation and comparison with experimental or literature data.

2. MATERIALS AND METHODS

2.1 Hardware Components

All computational calculations in the present study, including molecular modelling, energy minimization, lead molecule design, optimization, and protein-ligand interaction studies via molecular docking, were carried out using high-performance computing systems. A high-end server (Pentium IV, 3.4 MHz; AMD Athlon 64-bit dual processor with 1 GB RAM), manufactured by HCL Corporation, Pondicherry, India, was utilized to ensure efficient data processing and simulation performance.

2.2 Software Components

A range of widely accepted computational tools, running on both Windows and Linux platforms, were employed in this study. These software applications have been referenced extensively in high-impact scientific publications and are widely acknowledged for their reliability and accuracy in cheminformatics and molecular modelling tasks.

Academic licenses were obtained for all commercial software used by formally requesting the respective vendors. A brief overview of the major software tools utilized is provided below:

2.3 System Requirements

The software employed in the study was compatible with the following operating systems:

- Windows: Vista, 2003, XP, or 2000
- Linux: Most standard distributions; both 32-bit and 64-bit builds supported. Custom builds were provided upon request for specific distributions.
- Mac OS X: Version 10.4 and later, with support for both PowerPC and Intel architectures.

2.4 ChemOffice Ultra 7.0

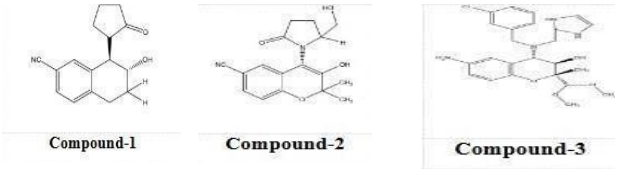
ChemOffice Ultra 7.0 is a comprehensive suite designed specifically for chemists and biologists. It includes:

- ChemDraw for chemical structure drawing,
- Chem3D for three-dimensional molecular modelling, and
- ChemFinder for database and structure searching.

This integrated platform provides access to an extensive encyclopedia of chemical structures, pharmaceutical agents, and biological properties, with more than 10,000 monographs covering individual compounds or families of related substances. ChemOffice was used in this study for structure drawing, NMR property prediction, and database matching.

2.5 Compounds selected

The study evaluates nine coumarin derivatives, Compound-1 to Compound-9, as shown in Figure 1, using NMR-based structural elucidation via the Spec2D expert system.



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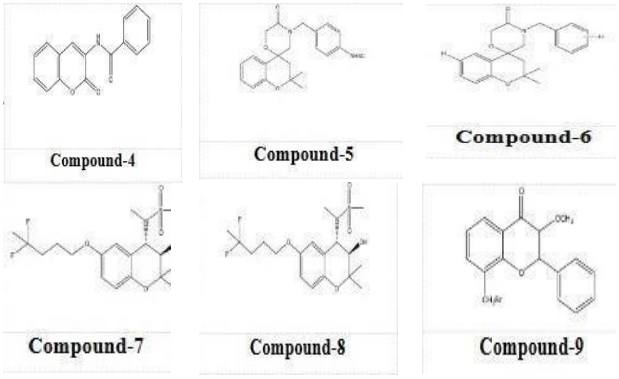

Figure 1:2D-Chemical Structures of coumarin derivatives

2.6 Online Tools and Structure Elucidation System

In addition to commercial and desktop-based software, several online computational tools were utilized in the present study to enhance the prediction and analysis of NMR spectra. One of the primary resources used was the nmrdb.org platform, a web-based interface that supports automatic prediction and interpretation of 1D and 2D NMR spectra.

2.6.1 Elucidation System Based on 1H NMR and H–H COSY Spectra

Theoretical Basis

The structural elucidation process leverages graph theory to model chemical structures as coloured graphs, where:

- Nodes (D) represent atoms,
- Edges (E) represent bonds, and
- Colouring functions (Xd and Xe) define atom and bond properties, respectively.

This concept is mathematically represented as:

G = (D, E, Xd, Xe) (Equation 1)

In 2D $^{1}H–^{1}H$ COSY NMR spectra, the correlation between proton signals is represented by cross-peaks, which can include hidden or overlapping signals due to spectral limitations. By interpreting these cross-peaks, chemical substructures can be reconstructed. In this framework:

- Substructures with complete cross-peak nets are treated as nodes, and
- Substructures lacking direct NMR data but connecting known parts are treated as edges.

This graph-based system enables more accurate and efficient structure prediction compared to traditional methods like CHEMICS, which rely solely on heavy atoms and their bonds.

2.6.2 Description of Substructures in Spec2D

Logical Description

Chemical structures are more effectively described when bond information is integrated with atomic descriptors, rather than treating atoms and bonds separately. For example:

- Instead of listing six carbon atoms, two oxygen atoms, and multiple bonds,
- The structure is summarized using fragment-based units (e.g., –CH_3–CH_2–, –CH_2–CH_2–, or =CH–), enhancing interpretability and reducing computational complexity.

This fragment-based approach enables structure generators to reduce the number of possible candidate structures during analysis.

Physical Description

Spec2D further classifies substructures into:

- Fragments with proton NMR information, and
- Fragments without proton NMR information.

These are managed using a modified connection table, where pseudoatoms are used as placeholders to enable flexible matching during structure assembly. Pseudoatoms ensure compatibility between substructures, even when the atom types or bonding environments are not yet fully defined.

2.6.3 Overview of the Spec2D System

Spec2D functions as an expert system that utilizes cross-peak networks from 2D $^{1}H–^{1}H$ COSY data to guide substructure extraction and complete structure generation. The system mimics expert-level decision-making through:

- A curated knowledge base linking ^1H NMR chemical shifts to specific substructures,
- Use of spin-spin coupling networks from COSY spectra, and
- Integration of spectral constraints to narrow down potential structures.

Spec2D requires only the ^{1}H NMR and COSY spectra, not the molecular formula, to initiate structure elucidation.

2.6.4 Database Description

A curated ^{1}H NMR spectral database was used to train the Spec2D system. This database includes:

- ~17,000 fully assigned ^{1}H NMR spectra,
- Compounds ranging from basic organics to natural products and chemical intermediates,

- Data measured at frequencies between 60–600 MHz,
- Both historical and modern entries, the latter including stereochemical information.

The data are stored in NM file and MOL file formats, which efficiently represent atomic and bond information. In these formats, hydrogen atoms are often implicit, requiring algorithmic inference based on valence rules, especially for variable-valence elements like nitrogen and sulfur.

2.6.5 Spectral Analysis and Structure Generation

The structure elucidation process begins with spectral analysis of unknown compounds. Input data includes:

- Chemical shifts,
- Signal multiplicities and proton counts, and
- Cross-peak correlations from COSY spectra.

The system:

- Extracts consistent substructures using its knowledge base,
- Validates substructure combinations using spectral constraints, and
- Constructs complete candidate structures via iterative "overlapping" of compatible fragments.

To refine results, Spec2D ranks all generated candidate structures by scoring their spectral agreement with the input data.

2.6.6. Implementation

Spec2D has been developed on a UNIX-based operating system and installed on workstations such as O_2 from Silicon Graphics. Spec2D programs have been developed using ANSI C. A Windows version of Spec2D has also been developed, running on Windows NT, 2000, and XP. Predict ^{1}H NMR, ^{13}C NMR and 2D COSY and 2D HSQC.

3. RESULTS AND DISCUSSION

To evaluate the capabilities of the Spec2D system in structure elucidation, a series of ten coumarin derivatives (Compound-1 to Compound-9) was selected and treated as unknown compounds. These compounds were analyzed to demonstrate the functionality and performance of Spec2D in interpreting complex NMR spectral data.

3.1 Input Spectral Data

The input spectral data used for the analysis included:

- ^{1}H NMR spectra with nine distinct proton signals,
- ^{13}C NMR spectra representing carbon environments,
- 2D COSY spectra illustrating proton-proton correlations,
- 2D HSQC spectra for direct ^{1}H–^{13}C one-bond correlations, and
- H–H COSY cross-peak maps, featuring four significant cross-peaks.

These datasets were visualized and catalogued in Figures 2 and 3, providing a comprehensive overview of the spectral features for each compound.

3.2 Substructure Extraction and Analysis

The analysis module of Spec2D processed the input spectra and successfully extracted 10 distinct substructures that were fully consistent with the spectral data. The extracted substructures, derived from both proton chemical shifts and cross-peak connectivity patterns, are summarized in Table 1.

Each substructure represents a chemically significant fragment with definable proton and carbon environments. These fragments served as the building blocks for reconstructing the entire molecular structure.

3.3 Structure Elucidation Workflow

From the identified substructures, one substructure was selected as the starting point or "focus" for structure generation. This "focus" substructure typically exhibited the highest degree of spectral connectivity, ensuring efficient combination with neighbouring fragments.

The structure elucidation process then proceeded through the following steps:

1. Progressive combination of compatible substructures based on COSY and HSQC correlations.
2. Spectral constraint checking to eliminate chemically or spectrally inconsistent combinations.
3. Generation of candidate structures, each evaluated for consistency with all provided spectral data.
4. Scoring and ranking of candidate structures based on spectral similarity and connectivity accuracy.

This iterative and constraint-guided approach enabled Spec2D to propose and rank plausible molecular structures for each compound in the series.

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3.4 Candidate Structure Ranking and Analysis

Based on the similarity scores computed by the Spec2D system, the correct molecular structure was consistently ranked as the top candidate for all ten test compounds. This indicates the system's high accuracy in matching predicted spectra to input spectral features. The second-ranked candidates were typically isomers of the correct structures. For instance, in the case of one compound, the second-best structure differed by a substitution of a chlorine atom with a nitrile group. This reflects the system's ability to differentiate between subtle structural variants based solely on NMR spectral data. Notably, since Spec2D does not require a molecular formula as input, it has the flexibility to propose compounds with different molecular formulas, provided they conform to the given spectral constraints. This feature enhances its utility in real-world unknown compound identification where exact elemental composition may not be readily available. An interesting observation was made during the analysis of Candidate 4, which featured a distinct ^{1}H NMR peak at 11.62 ppm, characteristic of a carboxylic acid proton. Despite this deviation, the compound was still retained as a candidate due to the system's capacity to accommodate exchangeable protons (e.g., –OH, –COOH, $–NH_2$), which may shift or broaden in experimental conditions. This demonstrates Spec2D's tolerance for peak variability and strengthens its robustness in structure prediction.

3.5 Processing Time and Performance

The processing time required for the analysis of Compound 1 through Compound 9 in the UNIX environment was found to be computationally efficient. The average structure elucidation time for each compound, including spectral analysis, substructure extraction, structure generation, and candidate ranking, ranged from 30 seconds to 2 minutes, depending on the complexity of the molecule and number of fragments involved.

This rapid processing highlights the effectiveness of Spec2D in handling large spectral datasets and reconstructing accurate molecular frameworks in a relatively short time frame, making it a valuable tool in NMR-based metabolomics and organic structural analysis.

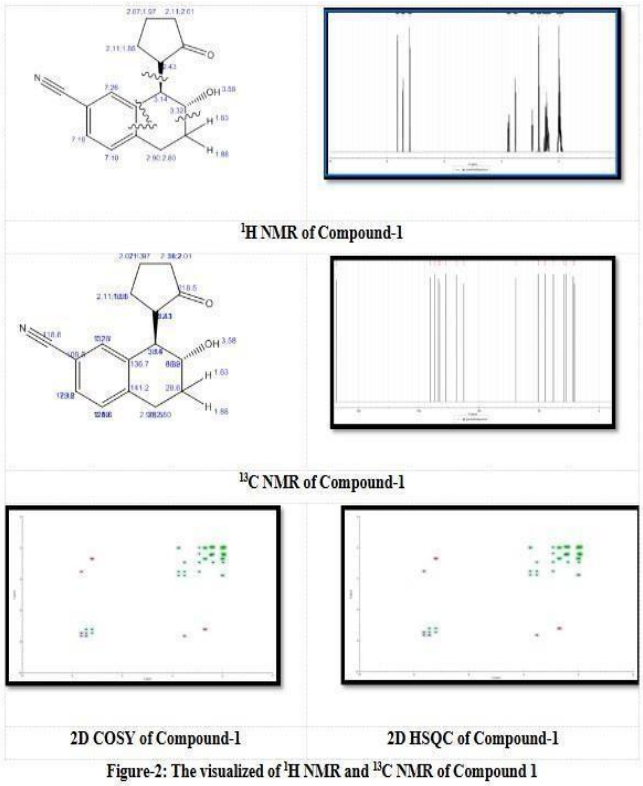

Figure-2: The visualized of ^{1}H NMR and ^{13}C NMR of Compound 1

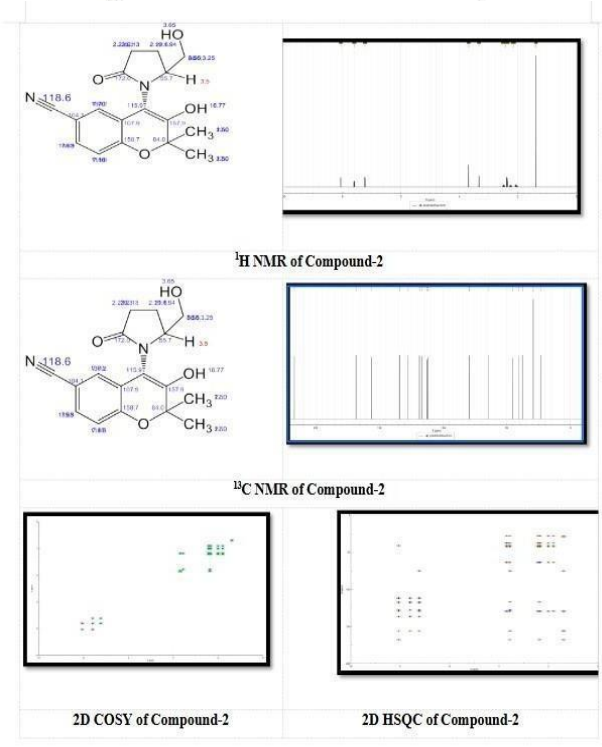

Figure-3: The visualized of 1HNMR and ^{13}C NMR of Compound 2

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Table 1: ^{1}HNMR and ^{13}CNMR of Compounds 1 to 9

Compounds	Description
Compound-1	
^{1}HNMR	δ1.89,2.09(5H,1.97(dtt,*J*=13.4,5.5,1.4Hz),1.98(dtd,*J*=14.2,10.0,3.7Hz),1.98(dddd, *J*=13.3,7.0, 5.4,1.4Hz),1.98(dtt, *J*=13.4,9.3,5.4Hz), 2.01(ddt,*J*= 14.2,3.8,2.0Hz)),2.28, 2.56(3H,2.38(dddd,*J*=13.3,9.3,7.5,5.5Hz),2.41(ddd,*J*=15.1,5.3,1.4Hz),2.47(ddd,*J*= 15.1,9.3,5.5Hz)),2.61-2.78(2H,2.69 (ddd,*J*=11.2,10.1,3.8Hz),2.70(ddd,*J*=11.2,3.7,2.0Hz)),2.92(1H, ddd,*J*=7.5,7.0,2.9Hz),3.52(1H,dd,*J*=10.1,2.9Hz),3.75(1H,td,*J*=10.0,1.9Hz),7.21(1H,dd,*J*=8.3,0.5Hz),7.44 (1H,dd,*J* = 8.3,1.8 Hz),7.64(1H,dd,*J*=1.8,0.5Hz).
^{13}CNMR	δ20.5(1C,s),21.6(1C,s),27.5(1C,s),29.5(1C,s),38.0(1C,s),44.9(1C,s),50.6(1C, s), 69.3(1C, s), 112.5(1C,s),118.5(1C,s),127.4(1C,s),132.5(1C,s), 133.4(1C,s),136.9(1C,s),140.3(1C,s),218.5(1C,s).
Compound-2	
^{1}HNMR	δ1.21-1.31(6H,1.26(s),1.26(s),1.26(s)),2.01-2.55(5H,2.10(dtd,*J*= 14.1,9.3,5.3Hz),2.24(dtd,*J*=14.1,5.5, 1.4Hz),2.38(ddd,*J*= 15.1,9.3,5.5Hz),2.38(ddd,*J*=15.1,5.3,1.4Hz),2.47(dqd,*J* =9.3,6.9,5.5Hz)),2.62(1H,dd,*J*=6.9,2.7Hz),3.57, 3.73(4H,3.63(d,*J*=6.9Hz),3.65(d,*J*=9.6Hz),3.66(dd,*J*=9.6,2.7Hz)),7.28(1H, dd,*J*=8.4,0.4Hz),7.53-7.76(2H,7.59(dd,*J*=1.9,0.4Hz),7.70(dd,*J*=8.4,1.9Hz)).
^{13}CNMR	δ22.4(1C,s),23.1-23.3(2C,23.1(s),23.2(s)), 37.6(1C,s),40.7-40.8(2C,40.7(s),40.7(s)),45.2(1C,s),64.6(1C,s),72.8(1C,s),81.0(1C,s),111.8(1C,s),112.5(1C,s),118.5(1C, s),124.1(1C,s),127.4(1C,s),134.1(1C,s),147.3(1C,s),216.8(1C, s).
Compound-3	
^{1}HNMR	δ7.19- 7.49(4H,7.26(ddd,*J*=8.2,7.4,1.3Hz),7.27(ddd,*J*=8.3,1.3,0.5Hz),7.35(ddd,*J*=8.3,7. 4,1.4Hz),7.44 (s)), 7.51-7.65(3H,7.58(dddd,*J*= 8.5,7.5,1.4,0.4Hz),7.57(tt,*J*=7.5,1.5Hz)),7.76(1H,ddd,J=8.2,1.4,0.5 Hz), 8.01(2H,dddd,J=8.5,1.9,1.5,0.4Hz).
^{13}CNMR	δ115.8(1C,s),119.8(1C,s),120.8(1C,s),123.9(1C,s),126.9(1C,s),127.8-127.9(3C,127.8(s),127.8(s)),128.3-128.5(3C,128.4(s),128.4(s)),129.4(1C,s),133.6(1C,s),154.1(1C,s),157.7(1C,s),166.3(1C,s).
Compound-4	
^{1}HNMR	δ1.38(6H,s),1.96(2H, d,*J*=14.3Hz),2.58(3H, s),3.70(2H,d,*J* =13.1Hz),4.31(2H,s),4.85(2H,d,*J*=18.2Hz),6.80-7.30(8H,6.87(ddd,*J* =8.2,1.3,0.6Hz),6.92(ddd, *J* = 7.9,7.5,1.3Hz),6.99(ddd,*J*=8.5,1.3,0.5Hz),7.08(ddd,*J*=7.9, 1.2,0.5Hz),7.15(ddd,*J*=8.2,1.0,0.6Hz),7.23(ddd,*J*=8.5,7.5,1.2Hz)).
^{13}CNMR	δ26.6-26.8(2C,26.7(s),26.7(s)), 30.2(1C,s), 38.9(1C,s),50.6(1C,s),59.6(1C,s),67.1(1C,s),75.7(1C,s),79.7(1C,s), 110.7(1C,s), 115.8(1C,s),117.9(2C,s), 128.0(1C,s),128.4(1C,s),129.3-129.5(3C,129.4(s),129.4(s)),136.6(1C,s), 152.4(1C,s),156.3(1C,s),171.1(1C,s).
Compound-5	
^{1}HNMR	δ1.28(6H,s),1.38-1.51(5H,1.43(s),1.45(t,*J*= 7.4Hz),1.45(t,*J* =7.4Hz)),1.91-2.03(2H,1.97(quint,*J*=7.4Hz), 1.97(quint,*J*=7.4Hz)),2.96-3.08(6H,3.01(s),3.03(s)),3.93-4.05(2H,3.99(t,*J*=7.5Hz),3.99(t,*J*=7.5Hz)),4.13(1H,d,*J* 1.5Hz),5.06(1H,d,*J*=1.5Hz),6.36(1H,dd,*J*=2.8,0.5Hz),6.65- 6.86(2H,6.71(dd,*J*=8.6,0.5Hz), 6.79(dd,*J*=8.6,2.8Hz)).
^{13}CNMR	δ22.4(1C,s),23.1(1C,s),23.8(1C,s),29.3(1C,s),31.2(1C,s),31.8(1C,s),39.0(1C, s),56.6(1C, s),69.1(1C,s), 73.9(1C,s),81.0(1C,s),113.5(1C,s),115.4-115.5(2C,115.5(s),115.5(s)),121.1(1C,s),121.8(1C,s),153.0(1C,s),156.3(1C,s).
Compound-6	
^{1}HNMR	δ2.31(3H,s),4.90(1H,d, *J*=2.5Hz),5.12(1H,d,*J*=2.5Hz),7.20(1H,dd, *J*=8.1,1.4Hz),7.29-7.53(6H,7.35 (tt,*J*=7.5,1.3Hz),7.39(dd,*J*= 8.1,7.9Hz),7.44(dtd,*J*=8.0,1.2,0.5Hz),7.46(dddd,*J*= 8.0,7.5,1.4,0.5Hz)), 7.76(1H,dd,*J*=7.9,1.4Hz).

^{13}CNMR	δ16.0(1C,s),71.8(1C,s),83.3(1C,s),119.3(1C,s),126.4-126.5(3C,126.4(s),126.5(s)),127.8(1C,s),128.0 (1C,s),128.4(2C,s),128.9(1C,s),130.3(1C,s),131.5(1C,s),152.9(1C,s),196.4(1C,s).
Compound-7	
^{1}HNMR	δ3.28(3H,s),4.39(2H,s),4.72(1H,d,*J*=2.5Hz),5.21(1H,d,*J*=2.5Hz),7.16-7.46(6H,7.23(dtd,*J*=8.0,1.2,0.5 Hz),7.35(tt,*J*=7.3,1.3Hz),7.37(dddd,*J*=8.0,7.3,1.5,0.5Hz), 7.39(dd,*J*=7.8,7.6Hz)),7.59(1H,dd,*J*=7.6,1.4 Hz),7.76(1H,dd,*J*=7.8,1.4Hz).
^{13}CNMR	δ26.9(1C,s),57.9(1C,s),72.2(1C,s),83.4(1C,s),119.3(1C,s),126.4, 126.5(3C,126.4(s),126.5(s)),127.3(1C,s),127.8(1C,s),128.0(1C,s), 128.4(2C,s),131.5(1C,s),131.7(1C,s), 152.9(1C,s),196.4(1C,s).
Compound-8	
^{1}HNMR	δ0.01-0.11(4H,0.06(s),0.06(s)),0.71-0.83(6H,0.77(q,*J*=8.1Hz), 0.77(q,*J*=8.1Hz),0.77(q,*J*=8.1Hz),0.77(q,*J*=8.1Hz)),1.31-1.42(6H,1.36(s),1.36(s),1.36(s)), 1.65(1H,d,*J*=14.4Hz), 1.88(1H,d,*J*= 14.4Hz), 3.60(2H, d,*J*=10.2Hz),4.42(2H,s),4.54(2H,d,*J*=15.6Hz),6.90-7.08(2H,6.97(ddd,*J*= 7.9,7.6,1.3Hz),7.02(ddd,*J*=8.3,1.3,0.5Hz)),7.13-7.45(7H,7.19(dddd,*J*=7.7,1.3,1.2,0.5Hz),7.25(ddd,*J*=8.3,7.6,1.2Hz),7.29(dtd,*J*=7.7,3.4, 1.0Hz),7.37(tdd,*J*=7.7,1.8,0.5Hz),7.38(ddd,*J*=7.9,1.2,0.5Hz))
^{13}CNMR	δ26.6-26.8(2C,26.7(s), 26.7(s)), 38.9(1C,s),50.6(1C,s),59.6(1C,s),67.1(1C,s),71.7(1C,s),75.7(1C,s),79.7 (1C,s),110.7(1C,s),115.8(1C,s),127.7- 127.8(3C,127.7(s),127.8(s)),128.0(1C,s),128.3-128.5(3C,128.4(s),128.4 (s)),129.4(1C,s),136.6(1C,s),156.3(1C,s),171.1(1C,s).
Compound-9	
^{1}HNMR	δ3.28(3H,s), 4.39(2H,s),4.72(1H,d,*J*=2.5Hz),5.21(1H,d,*J*= 2.5Hz),7.16-7.46(6H,7.23(dtd,*J*=8.0,1.2,0.5 Hz), 7.35(tt,*J*= 7.3,1.3Hz),7.37(dddd,*J*= 8.0,7.3,1.5,0.5Hz), 7.39(dd,*J*=7.8,7.6Hz)),7.59(1H,dd,*J*=7.6,1.4 Hz),7.76(1H,dd,*J*=7.8,1.4Hz).
^{13}CNMR	δ26.9(1C,s),57.9(1C,s),72.2(1C,s),83.4(1C,s),119.3(1C,s),126.4-126.5(3C,126.4(s),126.5(s)),127.3(1C,s), 127.8(1C,s),128.0(1C,s),128.4(2C,s),131.5(1C,s),131.7(1C,s), 152.9(1C,s),196.4(1C,s).

Table 2: Summary of NMR Results for Compounds 1–9

Compound	Key Spectral Observations	Interpretation/Comments
1	9 distinct ^{1}H NMR peaks, complete 2D data	Used as a model compound, it formed the basis for substructure extraction
2	Clear COSY and HSQC spectra	Enhanced hydrogen network identification
3	Well-defined 2D correlations	Helped resolve overlapping proton signals
4	Methyl and methylene proton coupling	Aliphatic side chain correlations are evident
5	Clean separation in 1D and 2D spectra	Facilitated a clear substructure proposal
6	Similar pattern to Compound 6	Consistent spectral fingerprint with substituted analogue
7	Strong proton-carbon correlations in HSQC	Enhanced hybrid assignment between 1D and 2D
8	Substituent-induced variation matched well	Isomer close to correct match, high similarity score
9	11.62 ppm downfield ^{1}H peak	Possible presence of –COOH group despite absence in base formula

CONCLUSION

This study validated the effectiveness of Spec2D for the structural elucidation of coumarin derivatives based on multidimensional NMR data. Key findings include:

- Successful generation of relevant substructures aligned with 1H and 13C NMR data.
- Development of a COSY-based knowledge base enabling accurate correlation analysis.
- Efficient candidate structure generation through constrained spectral analysis.
- Capability to identify correct structures without requiring a molecular formula.
- Flexibility in recognizing exchangeable protons and isomeric variation.

The performance of Spec2D is influenced by the completeness of its substructure database. Enhancing the database and integrating expert-curated knowledge can further improve its scope. Compounds are reliably predicted when all relevant substructures are represented in the knowledge base.

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