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ACUTE TOXICITY, SPECTROSCOPIC AND MOLECULAR MODELING STUDIES OF SCHIFF BASE DERIVED FROM L-METHIONINE AND 2-HYDROXY-1-NAPHTHALDEHYDE

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ABSTRACT

Condensation 2-hydroxy-1-naphthaldehyde **1** with L-methionine **2** yielded *E*-((2)-(((2-hydroxynapthalene-1-yl)ethylene)amino)-4-(methylthio)butanoic acid **3** in a good yield. The synthesized compound was characterized by elemental analysis, IR and ^{1}H, ^{13}C, HSQC, HMBC and ROSY NMR spectroscopy. The toxicity study of the synthesized analogue **3** was assayed for its LD_{50} value by using Dixon's up and down method, which exhibited value of 477 mg / kg of body weight. Molecular modeling studies were performed, showing the hydrogen bending and hydrophobic interactions.

Keywords: Acute toxicity, 2-Hydroxy-1- napthaldehyde, L-Methionine, Schiff base, Molecular modeling study

INTRODUCTION

The chemistry of imine derivatives plays a vital role in progresses of chemistry science[1]. Schiff bases have been known since 1864 when Hugo Schiff reported the condensation of primary amines with carbonyl compounds[2]. Schiff bases and their complexes have a variety of applications in biological, clinical, analytical and pharmacological areas[3,4]. They are used as starting materials for the synthesis of various bioactive heterocyclic compounds like 4-thiazolidinones, 2-azetidinones, benzoxazines and formazans. Furthermore, they have been used as fine chemicals and medical substrates[5]. In addition, they possess various pharmacological activity such as antibacterial, antifungal, antitumor and anticancer activities[6-9]. Amino acid Schiff bases are an important class of ligands because, since their metal complexes have a variety of applications including biological, clinical, analytical and industrial interest, in addition to their important role in catalysis and organic synthesis[10]. Condensation of L-amino acids with several aldehydes furnished various Schiff bases as reported in the literatures[11-13].

In the present work, we describe the synthesis and spectral characterization of Schiff base analogue from condensation of 2-hyroxy-1-naphthaldehyde **1** and L-methionin **2**, in addition to evaluation of the LD_{50} value by using Dixon's up and down method, with the aim to develop a new drug which can be use in treatment of several animal diseases, in addition to study of the theoretical molecular modeling of this analogue.

EXPERIMANTAL

Materials and methods

a-Physical measurements

Melting points were measured by a Philip Harris melting point apparatus and uncorrected. ^{1}H, ^{13}C, ROESY, HSQC and HMBC NMR spectra were measured on a Brucker at 600 MHz, with TMS as internal reference at Konstanz University, Germany. Infrared spectra (IR) were recorded as KBr discs in the range of 4000-400 cm^{-1} using FT-IR spectrophotometer Shimadzu model IR. Affinity-1 at the department of Chemistry, College of Education for pure sciences, University of Basrah, Iraq. Microanalysis were carried out by a Perkin-Elmer

240B Elemental Analyzer at the department of Chemistry, College of Science, University of Basrah-Iraq.

b- Acute toxicity (LD50)

Animals. All experiments were performed on 10-14-weak old male and female ratus-ratus/rats weighing 200-250 gm at the time of treatment by using up-and-down method, Dixon 1980[14].

Male and female rats were injected intraperitonially with different doses of the Amoxicillin derivative after conducting series of test levels. With equal spacing between doses, a series of trails were carried out using this method: increased dose following a negative response and decreased dose following a positive response. Testing continued until chosen "nominal" sample size was reached. LD_{50} were determined after reading final result (response-dead (X) or non response alive (O), then the following equation was applied $LD_{50} = XF + Kd$.

The estimate of LD_{50} is XF + Kd, where (XF) is the final test level and (K) is the interval between dose levels, where (d) is the tabulated value (Table 1).

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Table 1: represents Dixon values.

	K represented serial tests started with :-				
	O	OO	OOO	OOOO	
XOOO	0.157-	0.154-	0.154-	0.154-	OXXX
XOOX	0.878-	0.861-	0.860-	0.860-	OXXO
XOXO	0.701	0.747	0.741	0.741	OXOX
XOXX	0.084	0.169	0.181	0.182	OXOO
XXOO	0.305	0.372	0.380	0.381	OOXX
XXOX	0.305-	0.169	0.144-	0.142-	OOXO
XXXO	1.288	1.500	1.544	1.549-	OOOX
XXXX	0.555	0.0897	0.985	1.000	OOOO
	X	XX	XXX	XXXX	
	K represented serial tests startedwith :-				

LD_{50} =X*f* +Kd; **LD_{50}**:= Median Lethal Dose; **xf**: Last dose used in the experiment ; **k**: Factor of change from the table; **d**: Difference between doses.

C- Synthesis of Schiff-base of L-methionine derivative

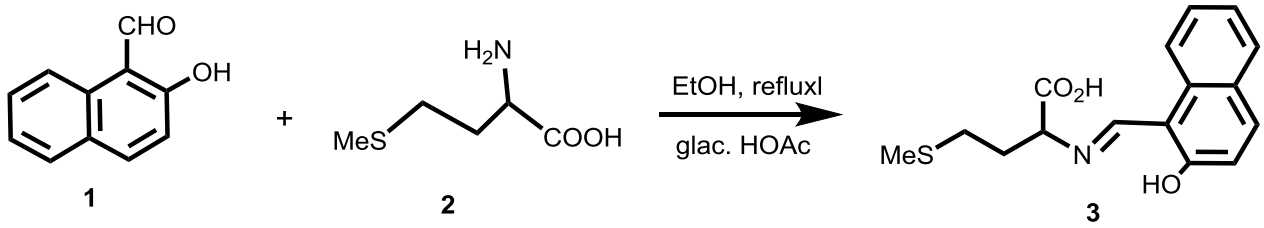
***E-((2)-(((2-hydroxynapthalene-1-yl)ethylene) amino)-4-(methylthio)butanoic* (3)**

To a stirred solution of L-methionine **2**(1.49 g, 10mmol) in EtOH(10 mL) was added to a solution of 2-hydroxy-1-naphthaledyne**1** (1.72 g, 10mmol) in EtOH (10 ml) and the mixture was refluxed for 5 h. After cooling at low temperature for 24 h, the yellow precipitate was separated by filtration, and dried. Recrystallization from EtOH afforded the pure analogue **3** (2.39 g, 79%) as a pale-yellow crystals. M.P.:176-181ºC. FT-IR(KBr, cm^{-1}), 3250(OH), 3082(C-H, aromatic), 2900,2883(C-H, aliphatic), 1870(C=O carboxylic),1622(C=N), 1602(C=C), 1556, 1323 (CO_2H). ^{1}H NMR(DMSO-d_6):δ14.43(s,1H,CO_2H),10.83(s,1H, OH-Ar), 9.20(s,1H,CH=N),7.94-6.82(m,6H, $H_{arom.}$),4.56(s,1H,*CH*-CO_2H),2.65-2.50 (m,2H, SCH_2(cis+ trans)),2.27-2.14(m, 2H,CH_2(cis+trans)), 2.07(s,3H, SCH_3).^{13}C NMR(DMSO-d_6): 194.9(CO_2H), 172.5 (*C*-Ar-OH), 160.6 (*C*-CH=N);134-112.9 ($C_{arom.}$), 63.8 (*CH*-CO_2H)), 39.6 (C-*CH_2*), 31.7(*C*-SCH_2), 15.0(*C*-SCH_3). Anal. calc. for $C_{16}H_{17}NO_3S$(303.38): C, 63.35; H, 5.65; N, 4.62. Found: C,62.89; H, 5.31; N, 4.73.

RESULTS AND DISCUSSION

Chemistry

The present work describes the synthesis of Schiff base of L-methionine with an potent aldehyde, aiming to develop a new analogue for treatment of some animal diseases. Thus, the reaction of 2-hydroxy -1-naphthaldehyde **1** with L-methionine **2** in a 1:1 mole ratio gave **3** (79%) (Scheme 1).



Scheme 1. Synthesis of schiff base derivative of naphthalene conjugated L-methionine

The structure of **3** was identified from the IR, ^{1}H, ^{13}C and 2D NMR spectra. The IR spectrum displayed common features in certain regions and characteristic bands in the fingerprint and other regions. The strong and broad band at 3250cm^{-1} was assigned to ν(O-H) stretching vibration, and this value was lower than free OH because of the hydrogen bonding with carboxylic group. It characterized also with disappearance of the band assigned to the primary amine stretching vibration ν (NH). The band at 1622 cm^{-1} was attibuted to the azomethine group ν (-HC=N-) stretching vibrations, while the band at 1870 cm^{-1} was due to ν (C=O) cm^{-1} stretching vibration for CO_2H group. The bands at 1556 and 1323 cm^{-1} were assigned to asymmetric and symmetric stretching vibration of CO_2H moiety, respectively. In addition, the bands at 1602, 3082, 2900-2883 cm^{-1} were assigned to ν (C=C), aromatic, ν (C–H) aromatic and ν (C–H) aliphatic residues, respectively[15]. In the ^{1}H NMR spectrum of **3**, the two singlets at δ14.43 and 10.83 ppm were assigned to CO_2H and OH protons, exchangeable with D_2O, while the singlet at δ9.20 ppm was attributed to the azomethine proton (CH=N). The multiplet at the region δ7.94-6.82 ppm was belonged to the aromatic protons, whereas the singlet at δ4.56 ppm was assigned to *CH*-CO_2H proton[16]. The spectrum was characterized by the presence of a mixture of *cis* and *trans* isomer of **3**, since the methylene protons SCH_2 and SCH_2CH_2 were appeared as two multiplets at the regions δ2.65-2.50 and 2.27-2.14 ppm, respectively, and identified as a mixture of *cis* and *trans* isomers, Figs.2-4. In the ^{13}C NMR spectrum of the analogue **3**, the lower field resonance at □□194.9 ppm was atributed to CO_2H, whereas the phenolic carbon atom (C-OH) appeared at δ172.5 ppm. In addition, the signal at δ160.6 ppm was attributed to CH=N carbon atom, while the signals at δ143.0-112.9 ppm were assigned to the aromatic carbon atoms. The high field signal at δ63.8 ppm was due to ***CH***CO_2H carbon atom, whereas the singlets at δ32.9 and 29.7 ppm were attributed to SCH_2***CH₂*** and SCH_2 carbon atoms, respectively. Furthermore, the resonance at δ15.1 ppm was assigned to SMe carbon atom[17,18], Fig.5. Compund **3** was further identified by ^{1}H,^{13}C HSQC[19], ^{1}H,^{13}C HMBC [20] and ^{1}H,^{1}H ROESY[21] NMR spectra. The HSQC spectrum of **3** showed eleven crosses peak, these entire crosses peak due to the aromatic protons and carbons (Figs. 6 and7). In the HMBC NMR gradient spectrum of **3**, a $^{2}J_{CH}$ coupling between CH=N proton at δ 9.20 ppm and aromatic carbon atom (C-1) at δ112.9 ppm, in addition to a $^{3}J_{CH}$ coupling between the same proton (CH=N) and carbon atom (*CH*-CO_2H) at δ63.8 ppm were observed as shown in Fig.8. In the ROESY spectrum of **3**, CH=N proton at δ 9.20 ppm was correlated with *CH*CO_2H proton at δ 4.56 ppm, and such correlation is indicative for the existence of **3** predominately in a *cis* configuration, as shown in Fig.9.

Median lethal dose (LD_{50})

Acute toxicity (LD_{50})

Determination of the 50% of lethal dose (LD_{50}) of the studied compound *in- vivo* was detected in the rats by using the "up-and-down" procedure described by (Dixon, 1980)[14]. In the experiment we using 10 animals of white rats 10-14 weeks in age, Graded doses of injection to each one animal, a series of concentrations (200, 250, 300, 350, 400 and 450) mg/k.gb.w) in 0.1 mL of dimethyl sulphoxide (DMSO), were administered and chosen with equal spacing (concentrations) between doses. Mortality was recorded after 24 h that each one animal treated with one dose and after 24 h was recorded as O if the animal lives and then increased the treated dose. While X recorded for the death of animal and then

decreased the dose according for the result of the animal the code which formed as being (OOOX) and according for Dixon value was get and the LD_{50} was determined according to the formula employed by Dixon (1980).

LD50 =X*f*+ Kd

LD_{50} = 400 + 1.544) x 50

LD_{50} = 477 mg / kg b.w

1/10 LD_{50}= 47.70 mg / kg (1 kg = 6 rats) depending on the weight rat about 175 g**.**

1/10 LD_{50}= 7.95 mg /rat, depending on the weight rat 175 g.

Molecular modeling study

Our theoretical molecular docking analysis of the new analogue **3** is based on the modeling studies which were performed to understand the binding mode of this analogue with the HIV-RT binding pocket (NNIBP) (PDB code: 3DLG)[22]. The molecular docking was performed using SYBYL-X 1.1, and the results were visualized with PYMOL[23].

The binding energy score for **3** is -8.4 kcal mol^{-1}, indicating selectivity and potency profiles of this analogue to bind the active site of HIV-RT pocket. As shown in Fig. 1, the aromatic rings of **3** fitted into an aromatic-rich subpocket surrounded by the aromatic side chains of Tyr176, and Tyr181.

Detailed analysis of the binding mode showed that the aromatic rings point toward the aromatic rings of Tyr176 and Tyr181 residues apparently developing □-□□stacking interactions with the two residues. The schiff base backbone is located in the middle of the binding pocket, anchoring the CH=N group in a favorable position for hydrogen bonding with the OH proton of Tyr181 of the reverse transcriptase (RT) enzyme, as well as the Lys216 with the sulphur atom of L-methionine residue. Overall, the combination of hydrophobic interaction and □-stacking appears to govern the binding of **3** with HIV RT (binding energy -8.4 kcal mol^{-1}).

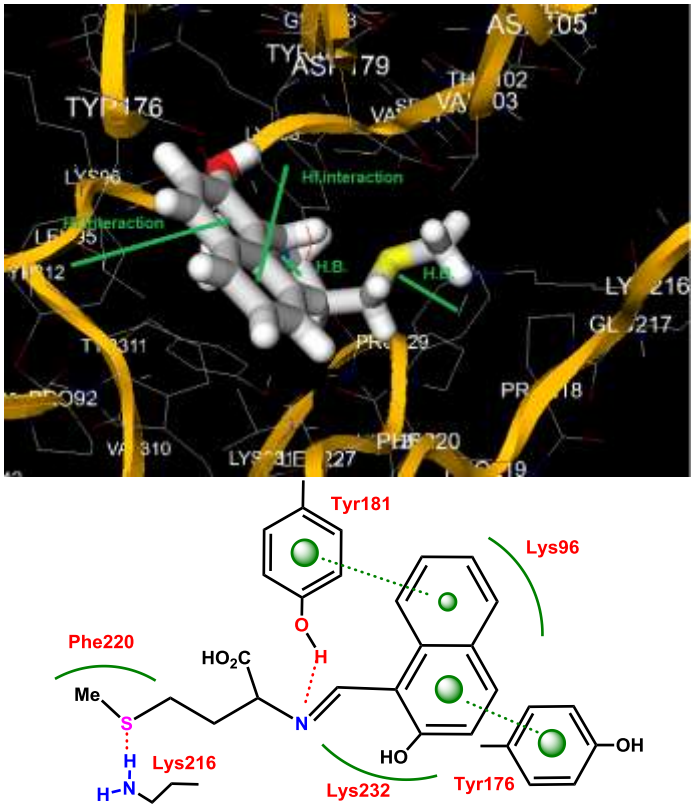


Fig. 1. Docked conformation of **3** showing two hydrogen bonds: OH proton of Tyr181 with *N* atom of CH=N group as well as NH_2 terminal proton of Lys216 with S atom of L-methionin. In addition, two hydrophobic interactions between the aromatic ring of Tyr181 and aromatic ring B, as well as aromatic ring A, carrying the CH=N group and Tyr176 of the reverse transcriptase (RT) enzyme residues were observed.

NMR spectra:

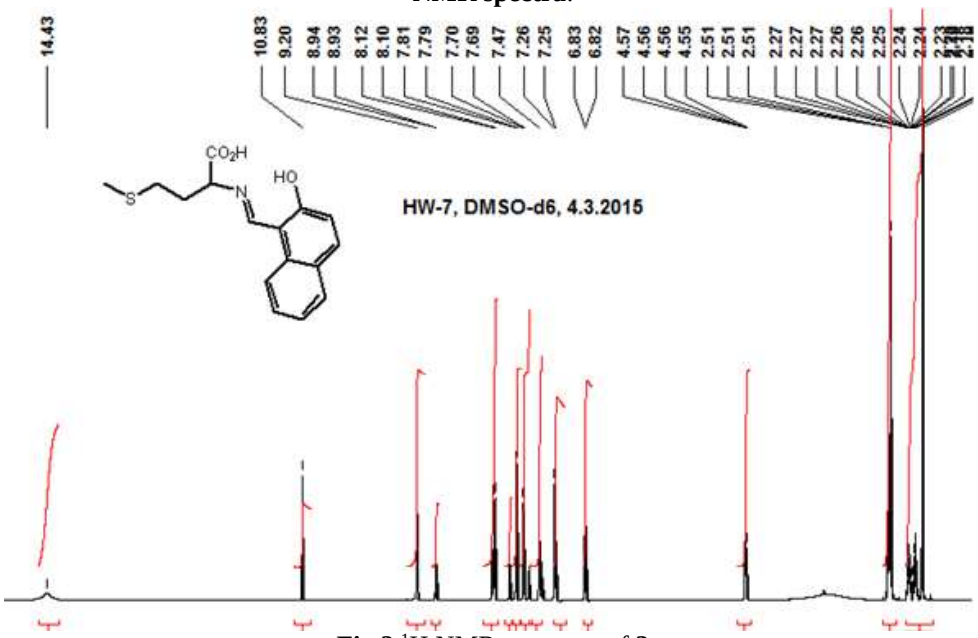


Fig 2.[1]H NMR spectrum of **3**

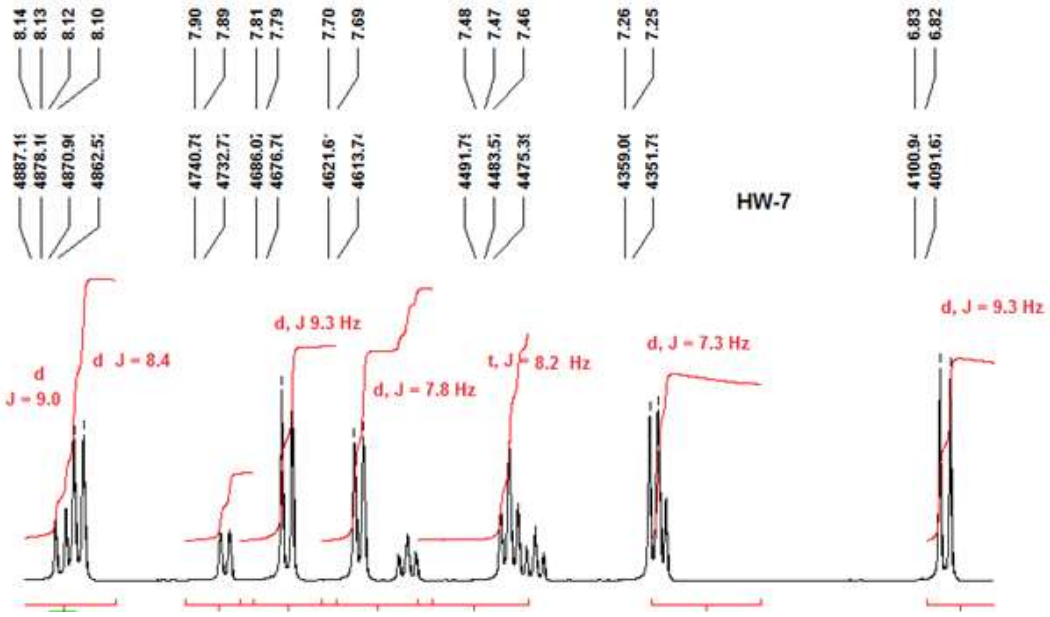


Fig 3.Expansion-1 of [1]H NMR spectrum of **3**

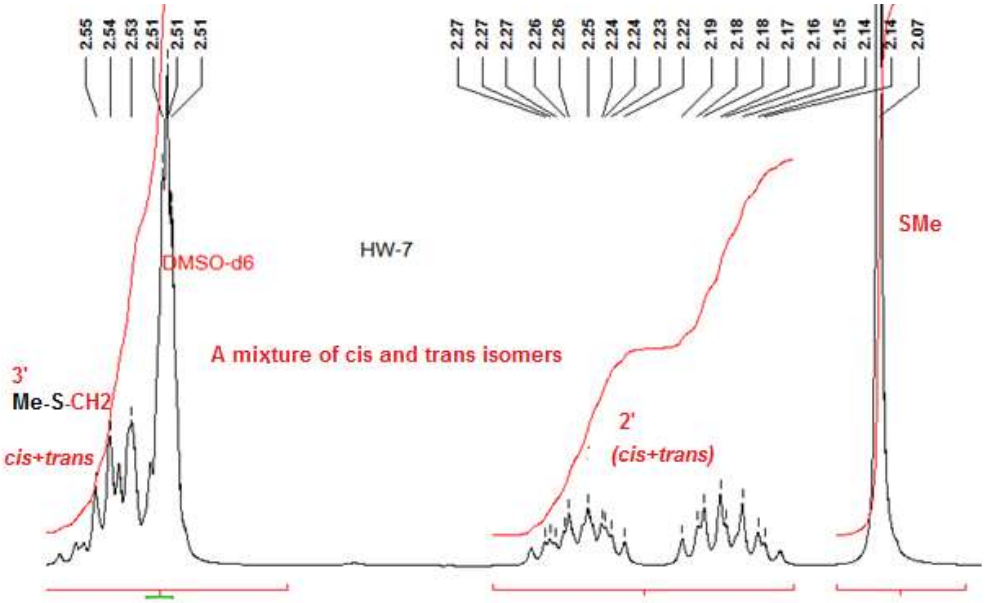


Fig. 4. Expansion-2 of ^{1}H NMR spectrum of **3**

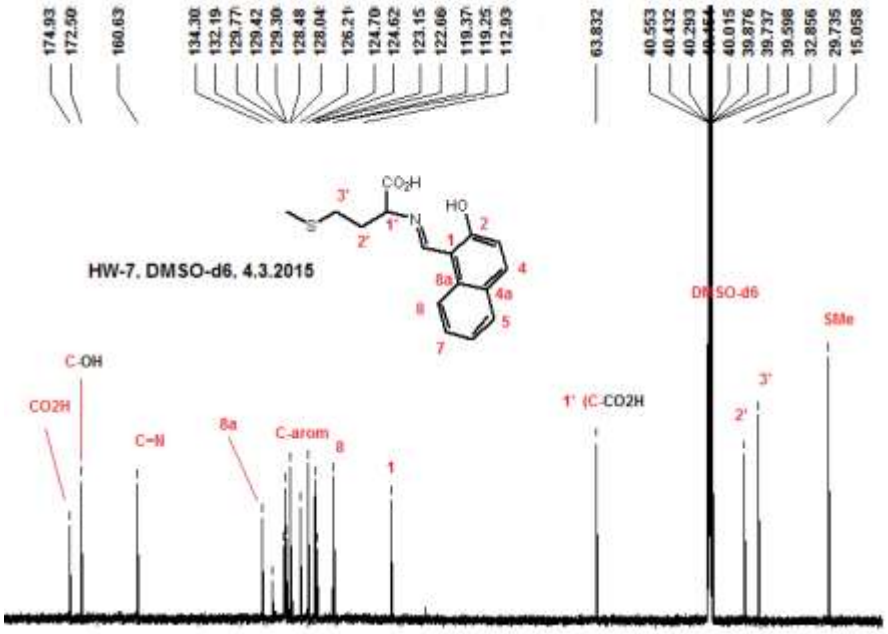


Fig. 5.^{13}C NMR spectrum of **3**

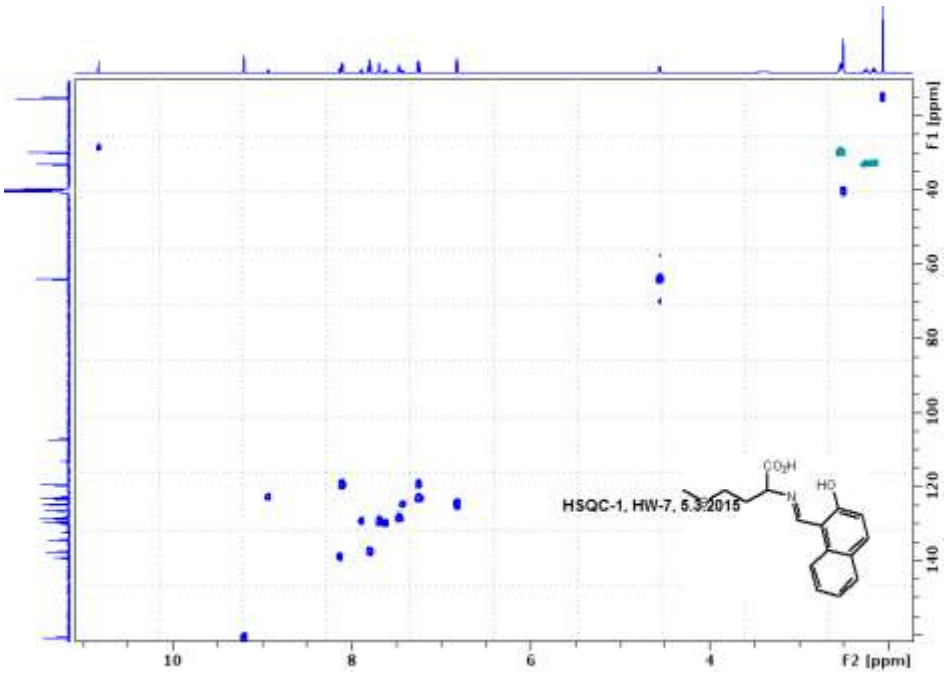


Fig. 6. HSQC NMR spectrum of **3**

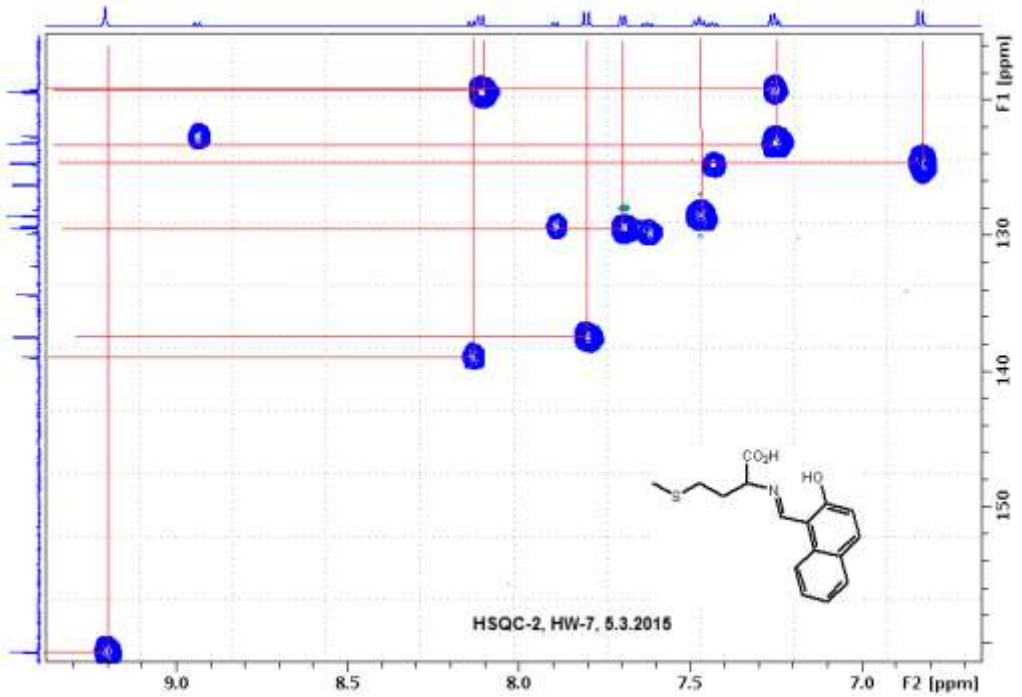


Fig. 7. Expansion of HSQCNMR spectrum of **3**

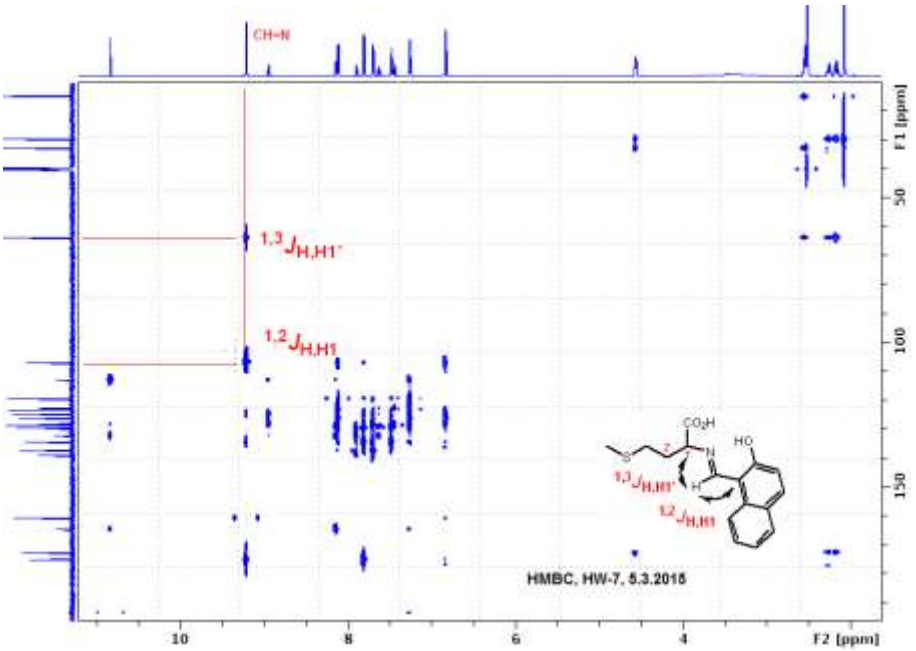


Fig. 8. HMB NMR spectrum of **3**

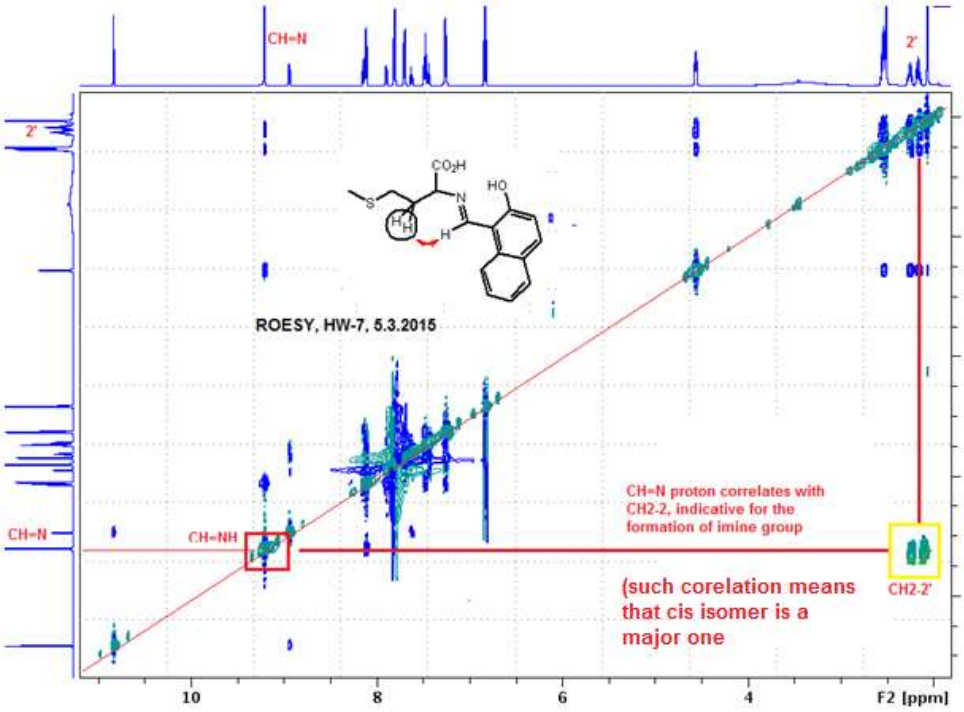


Fig.9. ROESY NMR spectrum of **3**

CONCLUSION

In conclusion, the present study reported the synthesis of methionine analogue namely 2-{[(2-hydroxynaphthalen-1-yl)methylidene]amino}-4-(methylsulfanyl)butanoic acid which revealed moderate *in vivo* toxic effects by LD_{50} measurment. In addition, the *in silico*, molecular modeling study of the synthesized Schiff's base has been studies

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