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In vivo Anticonvulsant and Neurotoxicity Evaluation and Docking Study of Promising Novel [1,5]-Benzodiazepine Derivatives

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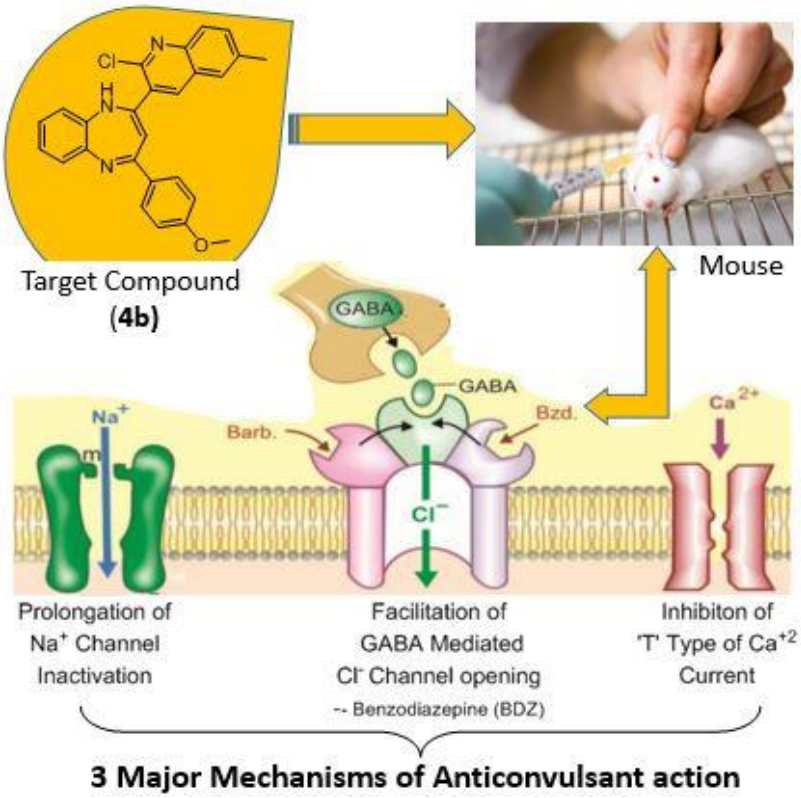
ABSTRACT

Background: Benzodiazepines (BDZs) are predominantly prescribed as sedatives, anxiolytics, and anticonvulsants throughout the world and they act on the $GABA_A$ receptor. **Objective:** New 1,4-BDZ analogues have been designed and synthesized to be evaluated for their anticonvulsant activity. **Methods:** The newly synthesized compounds have been examined by Maximal electroshock seizure (MES) test, the subcutaneous pentylenetetrazol (scPTZ) seizure test, and rotarod neurotoxicity test. Docking study was performed to justify the activity. **Results:** The most active compound that showed activity (MES Assay) at both 0.5 h and 4 h at a dose of 30 mg /kg is compound **4b**, whereas it has scored protection at a dose level of 100 mg/kg at both 0.5 h and 4 h (scPTZ assay) and did not score any neurotoxicity at maximum dose level unlike phenytoin the reference drug that scored neurotoxicity on mice at dose level of 100 mg/kg. **Conclusion:** Docking revealed that Compound **4b** is active on the three sites of the $GABA_A$ isoform $\alpha 2\beta 2\gamma$. Compared with the reference drug, phenytoin, Compound **4b** has more rapid onset and longer duration of action, is more effective, and eventually is safer**.** Furthermore, we recommend more structural modification studies to come out with much more selective BDZ derivatives to minimize the side effects.

Keywords: *1, 5-Benzodiazepine, O-Phenelendiamine, Anticonvulsant, Pentylenetetrazole,MES, rotarod, Docking.*



3 Major Mechanisms of Anticonvulsant action

1. Introduction

Epilepsy is considered as one of the most common neurological disorders that affects approximately 1-2% of the world population and characterized by recurrent unprovoked seizures (Cárdenas‐Rodríguez et al., 2020; Kaindl et al., 2006). Seizures are defined as temporary symptoms and signs ascribed to extreme or simultaneous neuronal activity of a population of neuronal cells in the brain (Sarmast, Abdullahi, & Jahan, 2020). It represents a major health problem and the recent study has reported that about 70 million people have epilepsy worldwide most of them live in the developing countries (Benlier, Ozer, & Orhan, 2020) and approximately, 10% of the affected patients are children (Gadgil et al., 2019).

Prior to the 19th century, treatment for people with epilepsy was based mainly upon spiritual and supernatural beliefs; that is, it has been referred to as "Sacred Disease"(Mervyn Eadie & Bladin, 2001). Nevertheless, potassium bromide was the first effective antiepileptic agent (MJ Eadie, 2012; Fisher & Bonner, 2018) However, after collaborative research initiatives and creative funding avenues, together with the growing knowledge about epilepsy we have seen a breakthrough in the antiepileptic therapy.

Anticonvulsant drug monotherapy is the first-line treatment of epilepsy and appeared more safe and less expensive than polytherapy (Abou-Khalil & Schmidt, 2012). Epilepsy needs long-term or even lifetime treatment which in turn, depends on the type of seizure and the used antiepileptic drugs (LoPinto-Khoury & Mintzer, 2010). The mechanism of antiepileptic or anticonvulsant drugs mainly goes either enhancement of ɤ-amino butyric acid (GABA) neurotransmitter or modulation of voltage-gated ion channels (sodium and calcium) (Saravanan, Alagarsamy, & Prakash, 2012). Benzodiazepines (BDZs), of varying potency and duration of action, are predominantly prescribed as sedatives, anxiolytics, and anticonvulsants throughout the world and they act as allosteric modulators on the $GABA_A$ receptor *via* increasing the conductance of chloride ions through the ionic channels, promoting the state of central nervous system depression (Ochoa & Kilgo, 2016). Aside from their rapid onset of action and low toxicity, benzodiazepines have some side effects such as sedation, negative effect on

cognition, and development of tolerance as an adaptive response of the body to prolonged exposure to the drug (Löscher & Schmidt, 2006). Therefore, synthesis of novel more effective and safer benzodiazepine derivatives is still an important challenge. The most commonly used 1,4-BDZs as anticonvulsants are diazepam,(Appletan, Sweeney, Choonara, Robson, & Molyneux, 1995; Faingold & Browning, 1987) lorazepam(Appletan et al., 1995), midazolam(Lahat, Aladjem, Eshel, Bistritzer, & Katz, 1992) (**Fig. 1**) clonazepam (Faingold & Browning, 1987), and clorazepate (Livingston & Pauli, 1977).

On the other hand, Li-Jun Guo *et al.* (Guo, Wei, Jia, Zhao, & Quan, 2009) reported a series of 5-alkoxy-[1,2,4]triazolo[4,3-a]quinoline derivatives were synthesized and evaluated as anticonvulsants, of which 5-(hexyloxy)-[1,2,4]triazolo[4,3-a]quinoline (**A**) was found to be the most active promising compound. Another series of 8-substituted quinolines have been reported by Muruganantham, N., *et al.* its variants, compound **B** is a representative example, were evaluated as anticonvulsants against seizures provoked by maximal electro-shock (MES), pentylenetetrazole (scMet)(Muruganantham, Sivakumar, Anbalagan, Gunasekaran, & Leonard, 2004). Compound **C** amongst a series reported by Guan, L.P., *et al.* (Guan, Jin, Tian, Chai, & Quan, 2007) displayed the strongest anticonvulsant effect in the anti-MES and anti-PTZ test with ED50 of 27.4mg/kg and 22.0 mg/kg, respectively.

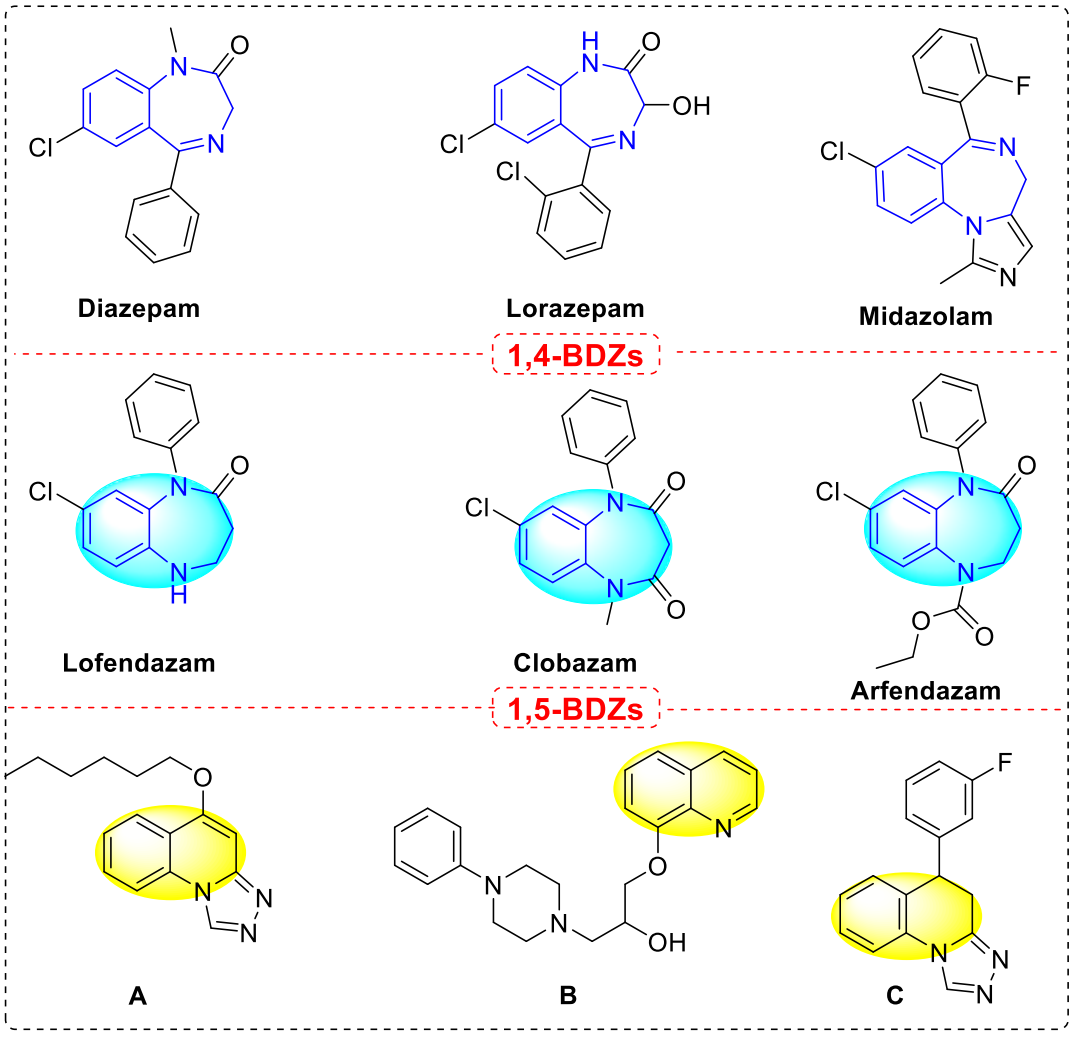


Fig. 1. Chemical structure of certain 1,4-BDZs and 1,5-BDZs anticonvulsant drugs, and quinoline-based anticonvulsant compounds.

The structure activity relationship of 1,4-BDZs and 1,5-BDZs (**Fig. 2**) indicated the effect of structural modifications of the side groups on the binding of the molecule to $GABA_A$ receptor which can modulate its pharmacological and pharmacokinetic properties .

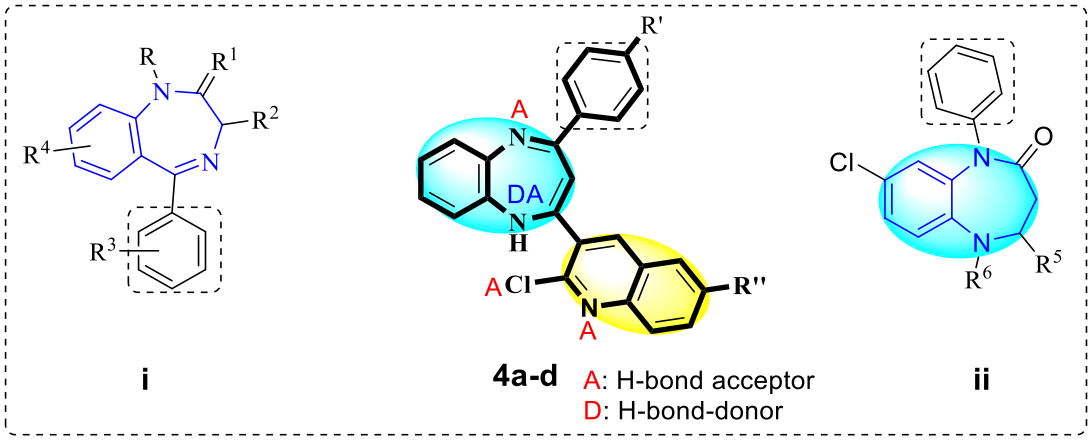


Fig. 2. General structure of 1,4-BDZs (**i**) and 1,5-BDZs **(ii)** and our target compounds (**4a-d**).

In continuation to the efforts toward design and synthesis of potential molecules as anticonvulsant agents, our aim was to synthesize new 1,5-benzodiazepine derivatives bearing 2-quinolinyl moiety in its core structure. Many studies demonstrated the diverse pharmacological activities of quinoline derivatives including the anticonvulsant activity (Ayati, Emami, & Foroumadi, 2016) and it was found interesting to synthesize a new scaffold of benzodiazepine - quinoline hybrids **4a-d (Fig. 2**). Biological evaluation of the target compounds was carried out according to the standard protocol given by the epilepsy branch of the National Institute of Neurological Disorders and Stroke (NINDS) using maximal electroshock seizure (MES) test, subcutaneous pentylynetetrazole (scPTZ) induced seizure test and rotarod neurotoxicity test in comparison with phenytoin as a reference drug. Furthermore, molecular docking simulation of the most active compound **4b** was performed to assess its binding interaction and affinity to $GABA_A$ receptor.

2. Material and methods

2.1 Chemistry

Melting points (^{0}C) were recorded on Gallenkamp melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was performed on UV fluorescent silica gel Merck 60 F254 plates and were visualized using UV lamp and n-hexane:ethyl acetate 9:1 as mobile phase. IR spectrum was carried out on Brucker-Vector - 22-FT-IR spectrophotometer (ν in cm-) using potassium bromide disc. NMR spectra (^{1}HNMR and ^{13}CNMR) spectra were performed either on a Jeol ECA (300 MHz) or Gemini 300BB (300 MHz) spectrometer, using TMS as the internal standard and DMSO-d_6/$CDCl_3$ as solvents. The chemical shifts are reported in ppm (δ), coupling constant (J) values are given in Hertz (Hz) and signal multiplicities are represented by s (singlet), d (doublet), t (triplet), q (quadruplet), and m (multiplet). The impact ionization (IE) mass spectra were recorded on AZH- Ph-AR-XO_2 at 70 ev. Elemental analysis was performed on a CHN analyzer at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. Starting materials were purchased from Aldrich Chemical Company and used without further purification. Compound 2 (Amin et al., 2004; Bhat & Bhaduri, 1986; El-Gaml, 2013; Wright, 1986) was synthesized according to the reported method

2.1.1. General procedure for the synthesis of new chalcone derivatives 3a-d

To a stirred and ice-cooled aqueous solution of sodium hydroxide (0.04 gm10 mmol) and absolute ethanol (12.5 ml), acetophenone derivatives namely (acetophenone and 4-methoxyacetophenone) (10 mmol) was added followed by 2-chloro-6-methoxyquinoline-3-carbaldehyde **2a** or 2-chloro-6-nitro-quinoline-3-carbaldehyde **2b** (10 mmol).

The reaction mixture was vigorously stirred for 3 h while the temperature was maintained below 25 ^{0}C and was left in the refrigerator overnight. The formed precipitate was filtered off and washed with a copious amount of water, washed with ice-cold ethanol (20 ml), dried and recrystallized from ethanol to afford compounds **3a-d**.

(*E*)-3-(2-Chloro-6-methylquinolin-3-yl)-1-phenylprop-2-en-1-one (3a)

Yield: 90%; yellowish white powder; m.p. 130-132 ^{0}C. **IR** (KBr, ν, cm^{-1}): 3078 (CH aromatic), 2959 (CH aliphatic), 1655 (C=O). **^{1}H-NMR** (300 MHz, DMSO-d_6) δ (ppm): 8.59 (s, 1H, C_4-H quinoline), 8.41 (d, 2H, *J* = 9.0 Hz, C_2-H, C_6-H phenyl protons), 8.27 (d, 1H, *J* = 15.0 Hz, CH alkene β proton), 7.62 (d, 1H, *J* = 15.0 Hz, CH alkene α proton), 7.56 (d, 1H,*J* = 9.0 Hz C_7-H quinoline), 7.55 (s, 1H, C_5-H quinoline), 7.45 (d, 2H, *J* = 9.0 Hz, C_3-H, C_5-H phenyl protons), 7.40(t, 1H, C_4-H phenyl proton), 2.37 (s, 3H, CH_3). **^{13}CNMR** (300 MHz, $CDCl_3$) δ (ppm): 24.1(CH_3), 126.6(2C), 127.2, 127.4, 128.1(2C), 129.8(2C), 130.0, 132.1, 134.9, 135.0, 137.8, 138.3, 144.3, 14.8, 148.1, 190.4 (C=O). **MS** (m/z): 307 (15.1%, M+), 309 (5.11%, M+2). Anal. Calc. For $C_{19}H_{14}ClNO$: C, 74.15; H, 4.59; N, 4.55. Found. C, 74.20; H, 4.33; N, 4.71.

(*E*)-3-(2-Chloro-6-methylquinolin-3-yl)-1-(4-methoxyphenyl)prop-2-en-1-one (3b)

Yield: 87%; m.p. yellowish white powder; 127-129 ^{0}C. **IR** (KBr, ν, cm^{-1}): 3103 (CH aromatic), 2952 (CH aliphatic), 1652 (C=O). **^{1}H-NMR** (300 MHz, DMSO-d_6) δ (ppm): 8.56 (s, 1H, C_4-H quinoline), 8.42 (d, 2H, *J* = 9.0 Hz, C_2-H, C_6-H phenyl protons), 8.07 (d, 1H, *J* = 15.0 Hz, CH alkene β proton), 7.93 (d, 1H, *J* = 15.0 Hz, CH alkene α proton), 7.61 (d, 1H, *J* = 9.0 Hz, C_8-H quinoline), 7.54 (d, 1H,, *J* = 9.0 Hz C_7-H quinoline), 7.52 (s, 1H, C_5-H quinoline), 7.38 (d, 2H, *J* = 9.0 Hz, C_3-H, C_5-H phenyl protons), 3.76 (s, 3H, OCH_3), 2.35 (s, 3H, CH_3). **^{13}CNMR** (300 MHz, $CDCl_3$) δ (ppm): 21.7, (CH_3), 55.4(OCH_3), 114.0(2C), 126.8(2C), 127.0, 127.8, 130.2, 130.4, 130.90(2C), 132.2, 135.2, 136.1, 144.8, 145.1, 148.3, 166.2, 190.7 (C=O). **MS** (m/z): 337 (30.7%, M+), 339 (10.24%, M+2). Anal. Calc. For $C_{20}H_{16}ClNO_2$: C, 71.11; H, 4.77; N, 4.15. Found. C, 71.32; H, 4.95; N, 4.28.

(*E*)-3-(2-Chloro-6-nitroquinolin-3-yl)-1-phenylprop-2-en-1-one (3c)

Yield: 72%; m.p. yellow powder; 149-151 ^{0}C. **IR** (KBr, ν, cm^{-1}): 3101 (CH aromatic), 2930 (CH aliphatic), 1666 (C=O). **^{1}H-NMR** (300 MHz, DMSO-d_6) δ (ppm): 8.70 (s, 1H, C_5-H quinoline), 8.30 (s, 1H, C_4-H quinoline), 8.27 (d, 1H,*J* = 9.0 Hz C_7-H quinoline), 8.13 (d, 2H, *J* = 9.0 Hz, C_2-H, C_6-H phenyl protons), 7.59 (d, 1H, *J* = 15.0 Hz, CH alkene β proton), 7.56 (d, 1H, *J* = 15.0 Hz, CH alkene α proton), 7.43 (s, 1H, C_8-H quinoline), 7.40 (t, 1H, C_4-H phenyl protons), 7.38 (d, 2H, *J* = 9.0 Hz, C_3-H, C_5-H phenyl protons). **^{13}CNMR** (300 MHz, $CDCl_3$) δ (ppm): 123.1, 124.2, 125.1, 127.1, 128.5(2C), 129.1(2C), 132.3, 134.4, 137.2, 137.7, 137.9, 145.2, 145.9, 149.2, 153.3, 189.6 (C=O). **MS** (m/z): 338 (75.9%, M+), 340 (25.4%, M+2). Anal. Calc. For $C_{18}H_{11}ClN_2O_3$: C, 63.82; H, 3.27; N, 8.27. Found. C, 63.73; H, 3.41; N, 8.33.

(*E*)-3-(2-Chloro-6-nitroquinolin-3-yl)-1-(4-methoxyphenyl)prop-2-en-1-one (3d)

Yield: 70%; m.p. yellow powder; 143-145 ^{0}C. **IR** (KBr, ν, cm^{-1}): 2954 (CH aromatic), 2824(CH aliphatic), 1676 (C=O). **^{1}H-NMR** 300 MHz, DMSO-d_6) δ (ppm): 8.80 (s, 1H, C_5-H quinoline), 8.48 (s, 1H, C_4-H quinoline), 8.45 (d, 1H,*J* = 9.0 Hz C_7-H quinoline), 8.29 (d, 2H, *J* = 9.0 Hz, C_2-H, C_6-H phenyl protons), 7.65 (d, 1H, *J* = 15.0 Hz, CH alkene β proton), 7.59 (d, 1H, *J* = 15.0 Hz, CH alkene α proton), 7.58 (d, 1H, C_8-H quinoline), 7.43 (d, 2H, *J* = 9.0 Hz, C_3-H, C_5-H phenyl protons), 3.97(s, 3H, OCH_3). **^{13}CNMR** (300 MHz, $CDCl_3$) δ (ppm): 55.4(OCH_3), 114.5(2C), 123.2, 124.5, 125.2, 127.7, 130.3, 130.80(2C), 132.3, 132.8, 137.5, 145.1, 145.8, 149.5, 153.3, 166.2, 189.9 (C=O). **MS** (m/z): 368 (51.8%, M+), 370 (17.27%, M+2). Anal. Calc. For $C_{19}H_{13}ClN_2O_4$: C, 61.88; H, 3.55; N, 7.60. Found. C, 61.90; H, 3.70; N, 7.44.

2.1.2. General procedure for the preparation of new derivatives of 2,4-substituted 1*H*-benzo[*b*][1,4]diazepine

The appropriate chalcones **3a-d** (1 mmol) and *o*-phenylenediamine (1.5mmol) in DMF (15 ml) with a few drops of piperidine was heated under reflux for 4-6 h. The progress of the reaction was monitored by using TLC. After completion of reaction, the reaction mixture was evaporated and poured into crushed ice. The crude solid product

obtained was filtered, washed with water, dried and recrystallized from ethanol to get product **4a-d** in good yields with high purity.

2-(2-Chloro-6-methylquinolin-3-yl)-4-phenyl-1*H*-benzo[*b*][1,4]diazepine (4a).

Yield: 60%; yellow crystal; m.p. 250-252 ^{0}C. **IR** (KBr, ν, cm^{-1}): 3241 (NH), 3120 (CH aromatic), 2971 (CH aliphatic). **^{1}H-NMR** (300 MHz, DMSO-d_6) δ (ppm): 10.05(s, 1H, NH, D_2O exchangeable), 8.86 (s, 1H, C_4-H quinoline), 8.33(d, 1H, C_8-H quinoline), 7.96(s, 1H, C_5-H quinoline), 7.62(d, 1H, C_7-H quinoline), 6.94-7.57(m, 9H, aromatic protons), 5.72(s, 1H, CH-benzodiazepine), 2.34 (s, 3H, CH_3). **^{13}CNMR** (300MHz, $CDCl_3$) δ (ppm): 22.1, (CH_3), 86.0, 113.3, 123.5, 124.4, 126.2(2C), 126.7, 128.3(2C), 129.5(2C), 130.2, 131.3, 132.3, 133.4, 135.3, 136.2, 137.3, 138.2, 141.2, 144.8, 148.8, 149.7, 166.9. **MS** (m/z): 395 (9.7%, M+), 397 (3.01%, M+2). Anal. Calc. For $C_{25}H_{18}ClN_3$: C, 75.85; H, 4.58; N, 10.61. Found. C, 76.08; H, 4.76; N, 10.80.

2-(2-Chloro-6-methylquinolin-3-yl)-4-(4-methoxyphenyl)-1*H*-benzo[b][1,4]-diazepine (4b).

Yield: 54%; pale yellow crystal; m.p. 218-220 ^{0}C. **IR** (KBr, ν, cm^{-1}): 3332 (NH), 3074 (CH aromatic), 2988 (CH aliphatic). **^{1}H-NMR** (300 MHz, DMSO-d_6) δ (ppm): 10.07(s, 1H, NH, D_2O exchangeable), 8.55 (s, 1H, C_5-H quinoline), 8.12(s, 1H, C_4-H quinoline), 7.43(d, 1H, C_7-H quinoline), 7.40(d, 1H, C_8-H quinoline), 6.50-7.33(m, 8H, aromatic protons), 5.60(s, 1H, CH-benzodiazepine), 3.93(s, 3H, OCH_3), 2.34 (s, 3H, CH_3). **^{13}CNMR** (300 MHz, $CDCl_3$) δ (ppm): 23.7, (CH_3), 55.0, 89.4, 90.0, 113.3, 114.6(2C), 123.2, 124.1, 125.3, 126.5(2C), 127.3, 127.7, 130.3, 130.5, 132.1, 135.5, 136.3, 138.1, 141.1, 144.4, 148.5, 149.5, 162.8, 166.7. **MS** (m/z): 425 (40.6%, M+), 427 (13.8%, M+2). Anal. Calc. For $C_{26}H_{20}ClN_3O$: C, 73.32; H, 4.73; N, 9.87. Found. C, 73.45; H, 5.03; N, 9.95.

2-(2-Chloro-6-nitroquinolin-3-yl)-4-phenyl-1*H*-benzo[*b*][1,4]diazepine (4c)

Yield: 60%; yellow crystal; m.p. 265-267 ^{0}C. **IR** (KBr, ν, cm^{-1}): 3255 (NH), 3053 (CH aromatic), 2875 (CH aliphatic). **^{1}H-NMR** (300 MHz, DMSO-d_6) δ (ppm): 10.35(s, 1H, NH, D_2O exchangeable), 8.63(d, 1H, C_8-H quinoline), 8.47 (s, 1H, C_4-H quinoline), 8.16(s, 1H, C_5-H quinoline), 8.13(d, 1H, C_7-H quinoline), 7.40-8.11(m, 9H, aromatic protons), 5.42(s, 1H, CH-benzodiazepine). **^{13}CNMR** (300 MHz, $CDCl_3$) δ (ppm): 89.8, 113.3, 123.5, 123.8, 124.1, 124.8, 125.5, 126.8, 128.8(2C), 129.3(2C), 131.8, 132.2, 132.4, 133.3, 137.7, 138.8, 141.4, 145.8, 149.2, 149.8, 154.2, 166.3. **MS** (m/z): 426 (22.3%, M+), 428 (7.4%, M+2). Anal. Calc. For $C_{24}H_{15}ClN_4O_2$: C, 67.53; H, 3.54; N, 13.13. Found. C, 67.41; H, 3.60; N, 13.21.

2-(2-Chloro-6-nitroquinolin-3-yl)-4-(4-methoxyphenyl)-1*H*-benzo[*b*][1,4]-diazepine (4d)

Yield: 35%; pale yellow crystal; m.p. 211-213 ^{0}C. **IR** (KBr, ν, cm^{-1}): 3255 (NH), 2994 (CH aromatic), 2835 (CH aliphatic). **^{1}H-NMR** (300 MHz, DMSO-d_6) δ (ppm): 10.26(s, 1H, NH, D_2O exchangeable), 8.61 (s, 1H, C_5-H quinoline), 8.57(s, 1H, C_4-H quinoline), 8.32(d, 1H, C_7-H quinoline), 8.20(d, 1H, C_8-H quinoline), 7.39-8.17(m, 8H, aromatic protons), 5.45 (s, 1H, CH-benzodiazepine), 3.83(s, 3H, OCH_3). **^{13}CNMR** (300 MHz, $CDCl_3$) δ (ppm): 55.6, 90.5, 113.3, 114.5(2C), 123.3, 123.8, 124.3, 124.9, 125.3, 125.7, 126.1, 130.3(2C), 132.3, 132.7, 137.9, 138.3, 141.1, 145.5, 149.2, 150.3, 153.2, 162.8, 164.9. **MS** (m/z): 456 (18.3%, M+), 458 (6.4%, M+2). Anal. Calc. For $C_{25}H_{17}ClN_4O_3$: C, 65.72; H, 3.75; N, 12.26. Found. C, 65.57; H, 3.81; N, 12.42.

2.2. Anticonvulsant screening

Drugs and reagents

Chemicals, drugs, and reagents were procured from Sigma Chemicals Co. St Louis, MO.

Animal

Adult albino mice of both sexes weighing (20-25 g) obtained from Animal House Facility, faculty of pharmacy, Port Said University, Egypt, were used throughout the study. Animals were housed in groups of 4-5/cage and were allowed free access to food pellets and water except for the time that animals were taken from the cage for testing. Animal housing followed the optimal standard rules. All instruments used for animal injection and drug preparation were previously sterilized. The dose for each drug was calculated thoroughly. Healthy hygiene rules were followed; the animal's cadaver was incinerated at Delta University for Science and Technology incineration. All experimental protocols were carried out with the permission from Institutional Animal Ethics committee (IAEC), form no. 07/18; registration no. is 335-sg/ 04

All newly prepared herein target compounds 1,5-benzodiazepines **4a-d** were investigated for their potential *in vivo* anticonvulsant activity. The pharmacological screening of all tested compounds was carried out according to the standard protocol given by the epilepsy branch of the National Institute of Neurological Disorders and Stroke (NINDS) following the protocol adopted by the Antiepileptic Drug Development (ADD) program. The pharmacological screening comprised maximal electroshock seizure (MES) test, subcutaneous pentylenetetrazol (scPTZ) induced seizure test and neurotoxicity assay. The groups of mice are tested at different time points (i.e., 0.5 h and 4 h) post administration of the test compounds.

Maximal electroshock seizure test (MES)

In this type of screening, a drop of anesthetic and electrolyte solution was applied to the eye of the animal before placement of the corneal electrode. The animals delivered electrical stimuli *via* the corneal electrode 60 Hz, 150 mA electrical stimuli for 0.2 s by the apparatus resembling that designated by Woodbury and Davenport.(Woodbury & Davenport, 1952) The endpoint here is to abolish the hindleg and tonic extension component of the seizure. Animals were subjected initially to different doses of the tested compounds 30, 100, 300 mg/kg body weight, through IP route. After 0.5 h and 4.0 h of drug administration, electroshocks were done *via* corneal electrodes. Absence of tonic extension suggests that the tested compounds were considered as positive criteria (Raja, Pandeya, Panda, & Stables, 2007) and the animals were considered protected.

Pentylenetetrazole -induced seizure test

The screening utilizes a dose of pentylenetetrazole (70 mg/kg in mice) subcutaneously, that produces clonic seizures lasting for a period of at least 5 s in 97% (CD97) of the animals tested. Animals were pretreated with dose levels of 30, 100, 300 mg/kg body weight. . Pentylenetetrazole was injected in the middle line of the neck. Animals were taken into an isolation cages to minimize stress. The time needed for the development of clonic seizure activity involving limbs and duration of seizure was carefully noted and taken as the end point. Animals that did not meet this manifestation were considered protected. The number of animals protected in each group was recorded and the percentage of protection was calculated.(Swinyard, 1982)

Rotarod Neurotoxicity test

This type of test was conducted primarily to assess an undesirable side effects (toxicity), by monitoring animals for overt signs of impaired neurological or muscular function. The most convenient way to carry out this measurements is through the rotarod test.(Dunham, 1957) Simply, the principle is that when a mouse is positioned on a rode that rotates at a speed of 6 rpm, the animal can maintain its equilibrium for a long time. The animal is so-called toxic if it falls down from this rotating rode three times in a 1-min period. Minimal muscular impairment may exhibit a zigzag or circular gait, tremor, hyperactivity, abnormal posture of the body, and spread of legs.

2.3. Computational Docking

In the current study, Dock module of MOE (Molecular Operating Environment) version MOE 2019.0102(2019), Chemical Computing Group Inc. , was adopted in docking studies. Our investigated compound **4b** was drawn into MarvinSketch of Marvin suite (https://chemaxon.com/products) to create its lowest energy conformer. The crystal structure of the recently reported cryo-EM structure 6HUP(Masiulis et al., 2019), was obtained from protein data bank(https://www.rcsb.org/structure/6HUP). All hydrogen atoms were added to the receptor structure to their standard geometry, followed by their energy minimization.(Siebert et al., 2018) The pocket-forming amino acids were set flexible. In the ligand conformations, the placement phase generates poses. The free energy of binding of the ligand in a given pose is estimated using the GBVI/WSA ΔG as a force field-based scoring function (Labute, 2008).

3.Results

3.1 Anticonvulsant screening

In the current study, we have evaluated four 1,4-BDZs (**4a-4d**) as anticonvulsants adopting Maximal electroshock seizure (MES), Pentylenetetrazole -induced seizures and Neurotoxicity-minimal motor impairment tests The results were summarized in **Table 1**.

Table 1: Anticonvulsant activityand neurotoxicity of compounds 4a-4d and administered IP to mice.

Compounds	MES[a] screening		scPTZ[b] screening		NT[c]screening		LogP[e]
	0.5 h[d]	4.0 h[d]	0.5 h[d]	4.0 h[d]	0.5 h[d]	4.0 h[d]	
4a	100	300	100	300	100	300	5.01± 0.98
4b	30	30	100	100	-	-	4.93± 1.21
4c	-	300	-	300	ND	ND	4.31± 0.99
4d	100	100	300	300	300	300	4.22±1.22
phenytoin	30	30	100	100	100	100	2.52± 0.38

MES; maximal electroshock test, scPTZ; subcutaneous pentylenetetrazole seizure test, ND; not determined.

[a] maximal electroshock test (administered intraperitoneally to mice at doses ranging from 30 to 300 mg/kg).

[b]Subcutaneous pentylenetetrazole test (administered IP to mice at doses ranging from 30 to 300 mg/ kg).

[c] Neurotoxicity (administered intraperitoneally to mice at doses ranging from 30 to 300 mg/kg).

[d] Time of test after drug administration.

The sign – (dash) represents an absence of activity at the maximum dose administered (300mg/kg).

[e]Log P was calculated using software ACD Labs 8.0 version

3.2 Computational Docking

We have docked the most active compound **4b** to the three binding sites of 6HUP and the docking results were shown in **Fig. 3**. The interaction energy expressed in Kcal/mol, reflects how stable is the ligand substrate binding, the lower the interaction energy the more stable the formed complex and the higher the affinity of the tested compound against the target receptor.

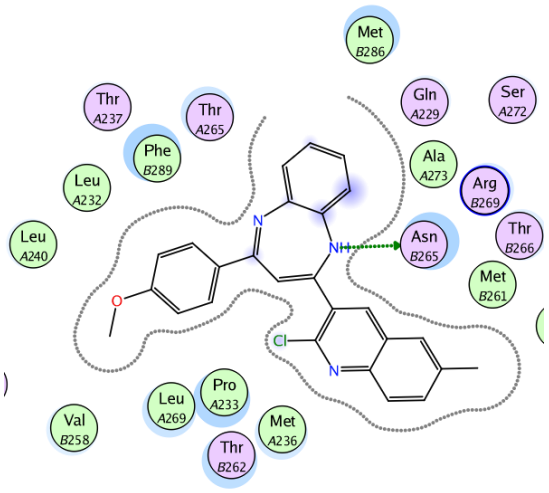
4. Discussion

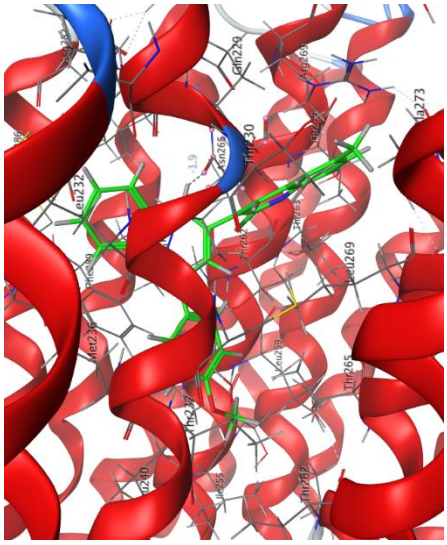
4.1 Chemistry

The synthetic routes of the new designed compounds is illustrated in scheme 1.

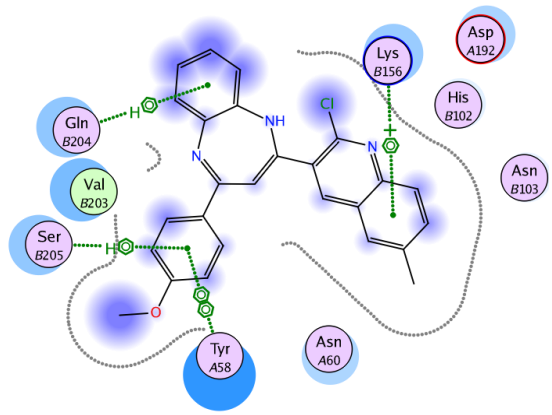
Among the most practically routes for the synthesis of 2-chlorquinoline-3-carbaldehyde is the oxidation of its corresponding alcohol utilizing a combination of diethyl diazene-1,2-dicarboxylate and catalytic $ZnBr_2$ in refluxing toluene (Cao & Grée, 2009). Moreover, Vilsmeier-Haack cyclization of acetanilides either by the traditional methods (Cohen et al., 2010; Kidwai & Negi, 1997; Mohammed, Magda, Waleed, & Basem, 2019; Ramesh, Vidhya, & Raghunathan, 2008) , microwave (Mogilaiah, Reddy, & Rao, 2002) or ultrasonic irradiation (Ali, Sana, Tasneem, Rajanna, & Saiprakash, 2002) was also reported.

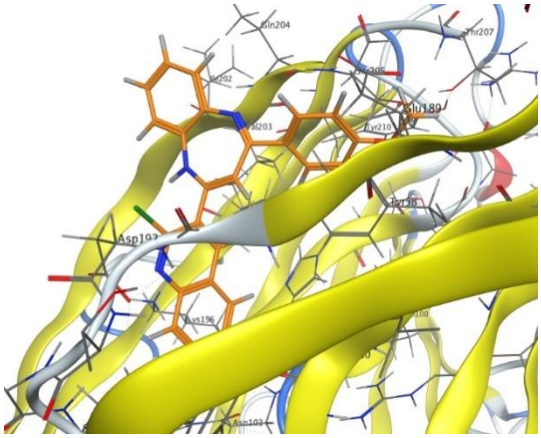
Vilsmeier-Haack reagent is an active complex obtained from the reaction of inorganic acid halides as phosphorous oxychloride ($POCl_3$) with disubstituted amides as dimethylformamide (DMF). It is considered as a versatile reagent in many synthetic transformation as formylation, chloroformylation, cyclization…etc. In the current study, the reported 2-chloro-quinoline-3-carbaldehydes **2a** and **2b** were synthesized adapting Vilsmeier-Haack reaction by cyclization of the appropriate acetanilides **1a** and **1b** with $POCl_3$ and DMF. The melting points of the separated products **2a** and **2b** agreed with the reported values.



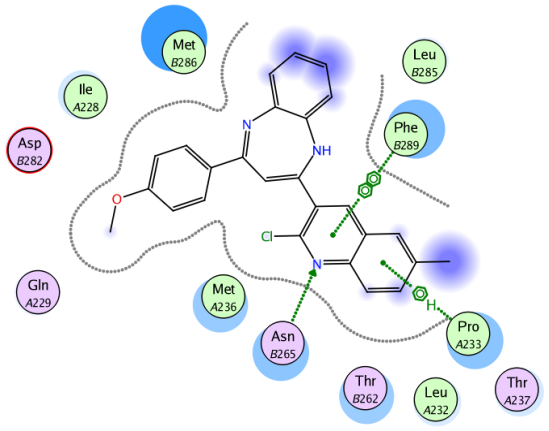


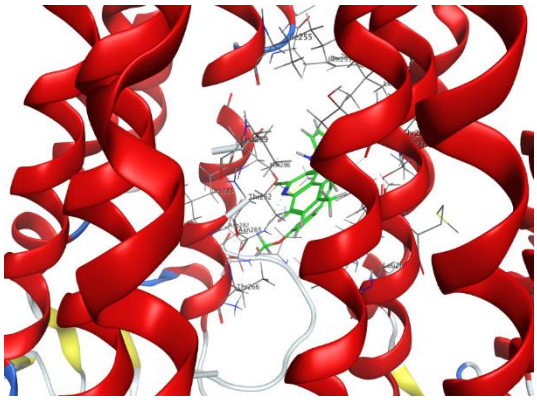
ECD;At site 1 (-12.7683687 Kcal/mol)





ECD;At site 2 (-13.5212011 Kcal/mol)





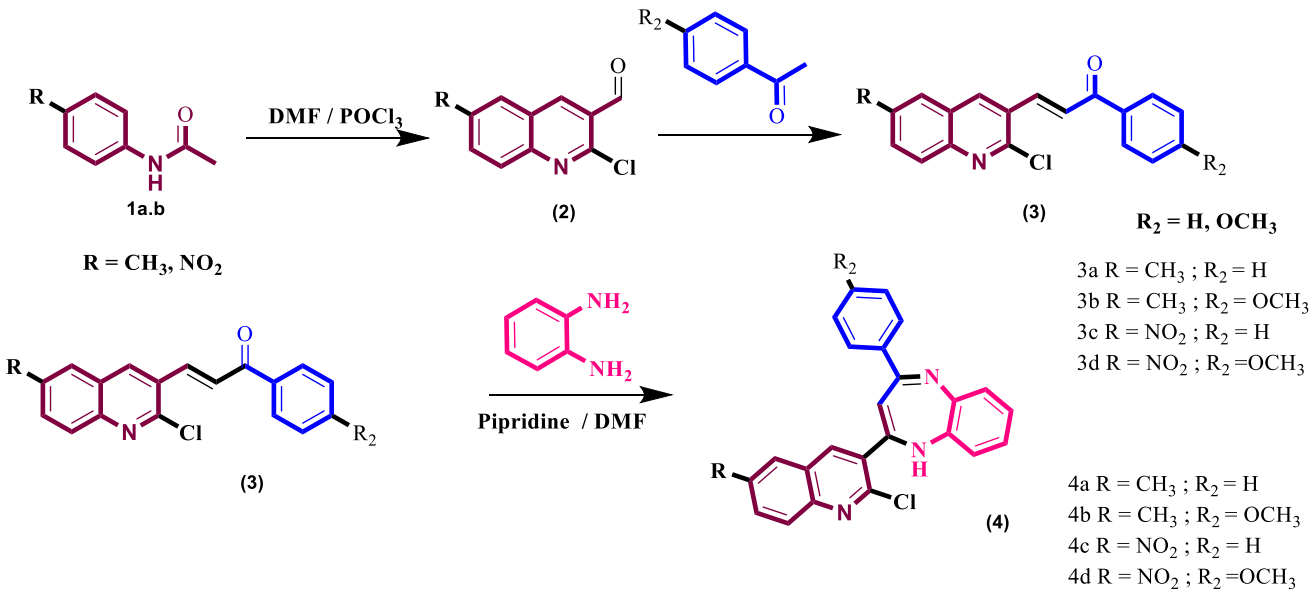
TMD;At site 3 (-11.8193426 Kcal/mol)

Fig. 3. Putative binding modes (2D & 3D) of the best poses of compound 4b in the three Pockets, with binding free energy for each in Kcal/mol.

However, the newly synthesized chalcone derivatives **3a-d** were obtained *via* condensing 2-chlorquinoline-3-carbaldehydes **2a** and **2b** with different substituted acetophenones using sodium hydroxide in ethanol following the well-known Claisen-Schmidt condensation reaction (Desai, Patel, & Dave, 2017). The IR spectra of the chalcone derivatives **3a-d** showed carbonyl stretching vibrations of the enone fragments at 1676-1652 cm^{-1}. ^{1}H NMR spectrum of compound **3b** demonstrated characteristic two singlet peaks assigned for CH_3 and OCH_3 protons of at δ 2.35 and 3.76 ppm, respectively. The vinylic protons resonated as two doublets within the aromatic region with J value 15 Hz that indicated the trans configuration of the enonechalcone structure (R. M. Silverstein, 1991). The structure of compound **3b** was further supported by ^{13}C NMR spectroscopy that showed a signal at 190.7 ppm for the carbonyl carbon, while the rest of signals appeared in the expected regions.

Synthesis of 1,5-benzodiazepines was reported to be achieved by the condensation of o-phenylenediamine with ketones, α,β-unsaturated carbonyl compounds or β-haloketones using acidic catalysts such as *p*-toluenesulfonic (Pasha & Jayashankara, 2006), silica sulfuric acid (Shaabani & Maleki, 2007) , *p*-nitrobenzoic acid (Varala, Enugala, & Adapa, 2007) which are important to enhance the condensation process (Pasha & Jayashankara, 2006). Furthermore, glacial acetic acid or piperidine catalyzed cyclization of chalcone with *o-phenelendiamines* was reported to give BDZs in DMF as a solvent. (Mahmoud, Islam, & Hasan, 2017). The aforementioned method was adopted to synthesize the target compounds **4a-d**. Hence, construction of the benzodiazepine ring was achieved by refluxing the chalcone derivatives **3a-d** and *o*-phenylenediamine in DMF with the addition of a few drops of piperidine as a catalyst for 4-6 hours.

The successful synthesis of compounds **4a-d** was verified by the micro-analytical and spectral data. IR spectra showed the disappearance of the carbonyl stretching vibration of compounds **4a-d** and the appearance of absorption bands at 3241-3332 cm^{-1} associated to NH of the benzodiazepine ring. The ^{1}H NMR spectrum of compound **4c**, as representative example, showed a D_2O exchangeable NH proton singlet at δ 10.35 ppm. Moreover, mass spectrum of compounds **4a-d** displayed molecular ion peaks in consistent with the assigned molecular weight. For compound **4c**, a molecular ion peak was observed at m/z, 426 in agreement with its molecular formula $C_{24}H_{15}ClN_4O_2$.



Scheme1: Synthesis of new chalcones **3a-d** and 1,5-BDZs **4a-d**.

4.2 Anticonvulsant screening

The anticonvulsant screening was carried out by induction of convulsion in animals either chemically using subcutaneous pentylenetetrazol (scPTZ) seizure test or maximal electroshock seizure (MES) test (Goodman, Brown, Swinyard, & Grewal, 1953; White, 2003) which are routinely used by most AEDS discovery program. scPTZ test identifies compounds that primarily effective against absence seizures, whereas MES identifies compounds that prevent seizure spread and protect against generalized tonic-clonic seizures.(Goodman et al., 1953; Piredda, Woodhead, & Swinyard, 1985; Zayed et al., 2017) additionally, rotarode test estimates the damaged motor function in rodents(Łuszczki et al., 2020).

Maximal electroshock seizure test (MES)

The antiepileptic activity of all title compounds has been presented in **Table 1**. Compound **4b** showed protection at 30 mg/kg as much as the reference drug, whereas compounds **4a** and **4d** showed protection at 100 mg/kg, meanwhile compound **4c** did not show protection at the maximum dose 300mg/kg, after 0.5 h. Furthermore, compounds **4b** and phenytoin as a positive control recorded protection at 30 mg/kg on the MES test, followed by compound **4d** that showed protection at 100 mg/kg, whereas both compounds **4a** and **4c** showed protection at 300 mg/kg, after 4 hours.

Of note, the most active compound that showed activity at both 0.5 h and 4 h at a dose of 30mg /kg is compound **4b,** indicating that this compound must have rapid onset, more effective and long acting. Compound**4d** was found to have approximately the same onset but less effective than compound **4b** as it required to raise the dose level to 100mg/kg to attain protection, but still considered as long acting as it kept protection after 4 h at the same dose 100mg/kg. Compared with compound **4d**, **4a** has the same onset, the same activity, and shorter duration of action. Lately, compound **4c** showed poor onset and low activity.

Subcutaneous pentylenetetrazole seizure test

In such chemshock assay, compounds **4a**, **4b,** and reference drug have the same potency, and each of them scored protection at the dose level of 100mg/kg, however, compound **4d** showed protection at the dose level of 300mg/kg, and compound **4c** did not exhibit protection at the maximum dose level, after 0.5 h. On the other hand, after 4 h, compound **4b** and the positive control phenytoin featured protection at the dose level of 100mg/kg, whereas compounds **4a**, **4c,** and **4d** displayed protection at a dose level of 300mg/kg.

From these results, compound **4b** is the most active anticonvulsant agent, which has rapid onset and long duration of action as it appears even after 4 h, still effective at 100mg/kg, it is as much effective as the reference drug. Despite compound **4d** has a rapid onset and long duration of action, it is still of low activity as it displayed protection at maximum dose level after 0.5 h and 4 h.

Neurotoxicity screen.

All compounds were investigated for its neurotoxicity except compound **4c** due to its poor activity as an anticonvulsant. Compounds **4b** and **4d** were not found to be neurotoxic agents even at the maximum dose level of 300mg/kg. As well, compound 4a displayed neurotoxicity 100mg/kg after 0.5 h and 300mg/kg after 4 h.

4.3. Computational docking

Indubitably, benzodiazepines act on ɤ-aminobutyric acid type A ($GABA_A$) receptor, e.g., α1β2ɤ2 *via* the key residue within the N- terminal of the α subunit, to render their anxiolytic and sedative actions.(Walters, Hadley, Morris, & Amin, 2000) Obviously, treatment of epilepsy, sleep disorder, anxiety, and anesthetics as well, target $GABA_A$ receptors.(Maja Jazvinscak & Josipa, 2015; Sieghart & Savić, 2018) Generally, ($GABA_A$) receptors are pentameric anion channels expressed extensively in the central nervous system of mammals, each isoform is formed from five homologous or identical subunits surrounding a central Cl^- ion selective channel gated by GABA.(Sigel & Steinmann, 2012) Actually, the majority of ($GABA_A$) receptors is formed of two α, two β and single ɤ subunits interfacing for example, by positive side of α (the principle) and the negative side of β or other subunit (the complementary) as shown in **Fig. 3**.

As shown if **Fig. 3**, we have docked compound **4b** onto ECD active sites; site 1 and site 2 and TMD site 3. First, we have seen the pocket in each site comprising a mixture of α (referred *A*) and β- (referred *B*) amino acids, confirming that explained by **Fig. 4**.Upon docking to ECD site 1, we found a well-built H- bond constructed between the conserved amino acid Asn265 from the β chain (*B*Asn265) and NH group of the diazepine nucleus. Added with the hydrophobic interactions, the stability and recognition of the ligand is noticeably enhanced, giving rise to a ligand/complex of the highest free binding energy -12.7683687 kcal/mol.

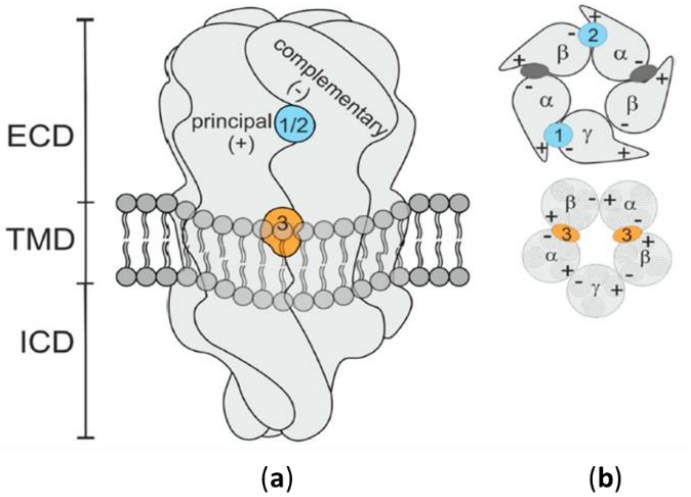


Fig. 4. (**a**) Schematic rendering $GABA_A$ receptor. Extracellular domain (ECD) comprises binding sites 1 and 2, whereas the transmembrane domain (TMD) comprises binding site 3.(Puthenkalam et al., 2016) (**b**)Schematic planes through the ECD (top) and TMD (bottom) of a canonical αβɤ receptor. In ECD; binding site 1(α+/ɤ- interface), binding site 2 (α+/β- interface), and site 3(orange) are the etomidate site.(Chiara et al., 2012)

Alternatively, upon docking of compound **4b** to ECD site 2, we noticed a characteristic cation -arene bond between the conserved amino acid Lys156 and the fused benzene ring in the quinoline moiety while Gln202 built an arene-arene bond with the fused benzene ring in benzodiazepine moiety. Moreover, Ser205 and Tyr58 constructed H-arene and arene-arene bonds with the benzene ring of 4-(4-methoxyphenyl) moiety, respectively. Additionally, the hydrophobic interactions between the ligand and the receptor amino acids, clearly noticed through the blue shadow around the ligand and the pocket amino acids, increased the total recognition of the ligand to score the highest free energy -13.5212011 kcal/mol.

Eventually, the docking results on TMD site 3 revealed that a H-bond between the nitrogen of quinoline moiety and the conserved amino acid Asn256 , a H-arene bond between the fused benzene of quinoline moiety and Pro233 of the α chain, and the aromatic amino acid Phe289 formed an arene-arene bond with the pyridine ring in quinoline moiety. Henceforth, the ligand scored the highest binding free energy -11.8193426 Kcal/mol.

Scrutinizing the docking results, different binding modes, and affinities of the ligand inside the three sites were observed, but these differences in binding affinities are too small to say that this BDZ derivative is selective to a certain site. Herein, we agree with the previous studies,(Almaghour, Zawawi, & Sherif, 2014; Auta, Kadriu, Giusti, Costa, & Guidotti, 2010; Fradley et al., 2007) we report that the compound **4b** is active on three sites of $GABA_A$, namely, the isoform α2β2ɤ. Furthermore, we recommend more structural modification studies to come out with much more selective BDZ derivatives to increase the selectivity and minimize the side effects.

5. Conclusion

Compound **4b** is found to be active on the three sites of the $GABA_A$ isoform α2β2ɤ. Compared with the reference drug, phenytoin, Compound **4b** has more rapid onset and longer duration of action, is more effective, and eventually is safer**.** Furthermore, we recommend more structural modification studies to come out with much more selective BDZs to minimize the side effects.

Conflict of interest

The authors declare no conflict of interest. The authors alone are responsible for the content and writing of the article.

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Supporting information: Contains all spectral charts (IR, ^{1}H-NMR, ^{13}C-NMR, MS) of the new compounds.

Spectral charts of the new compounds.

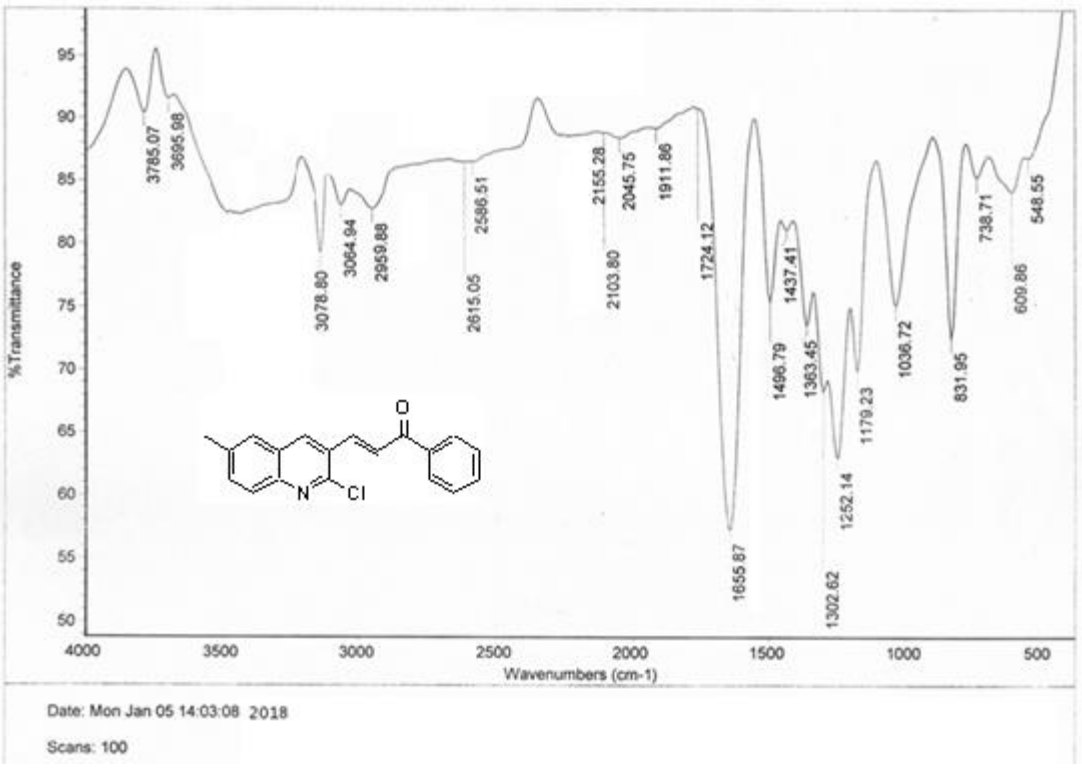

Figure 1. IR spectrum of 3a

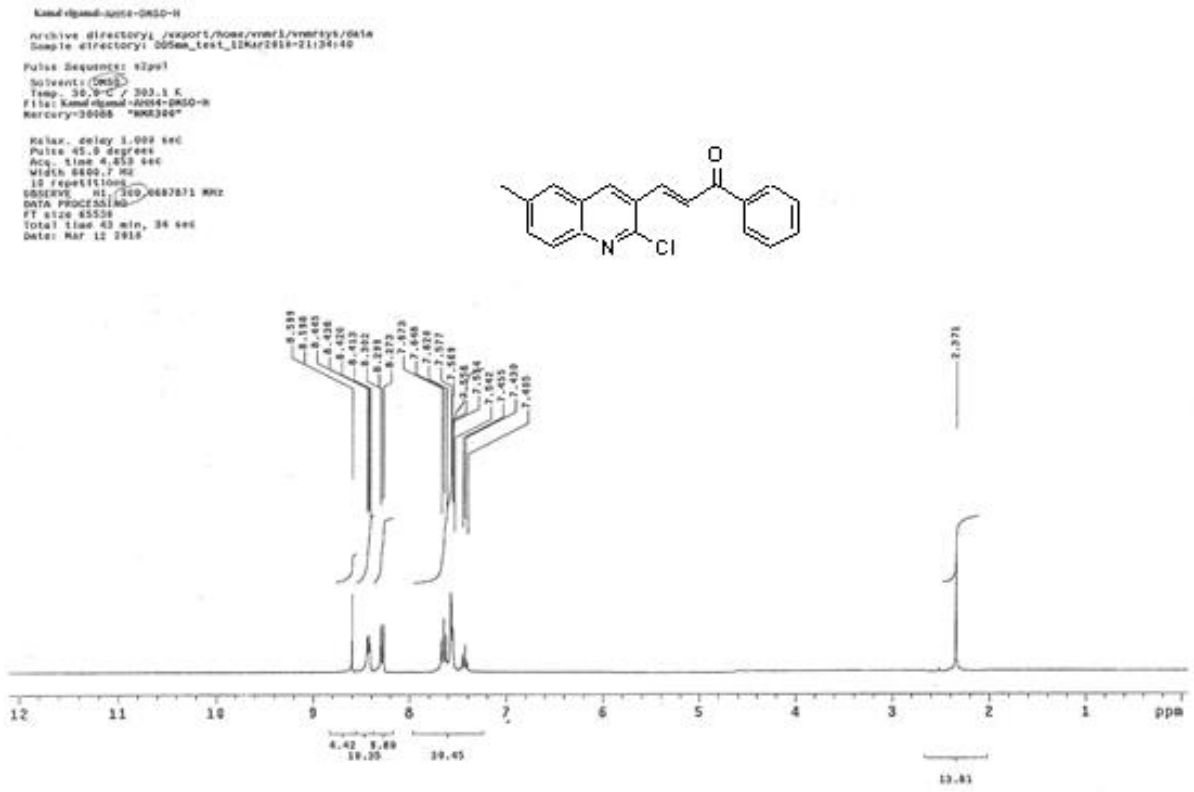

Figure 2. 1H-NMR spectrum of 3a

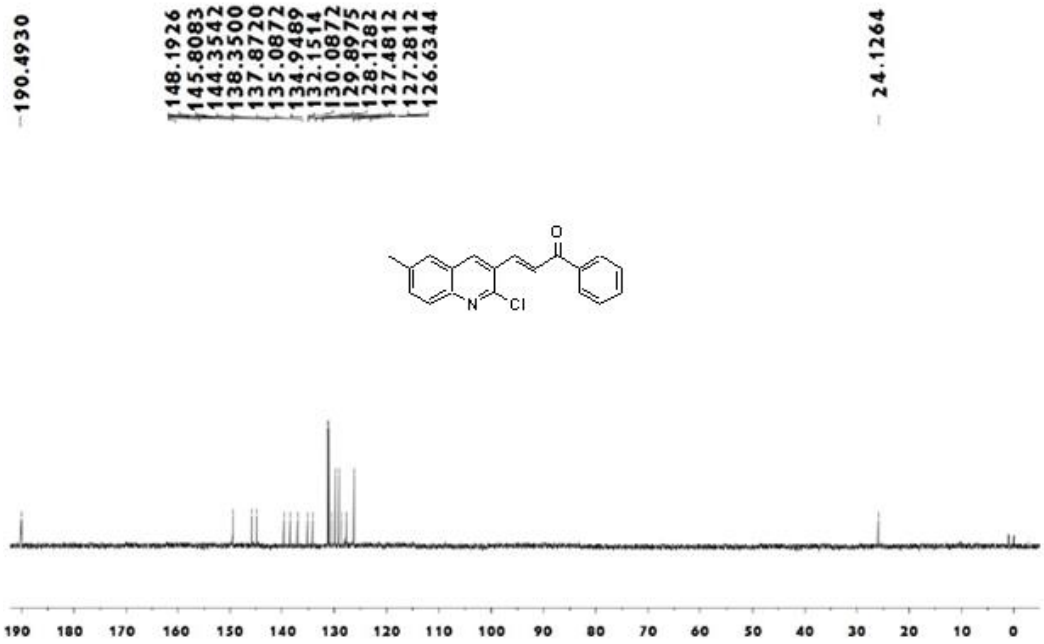

Figure 3. 13C-NMR spectrum of 3a

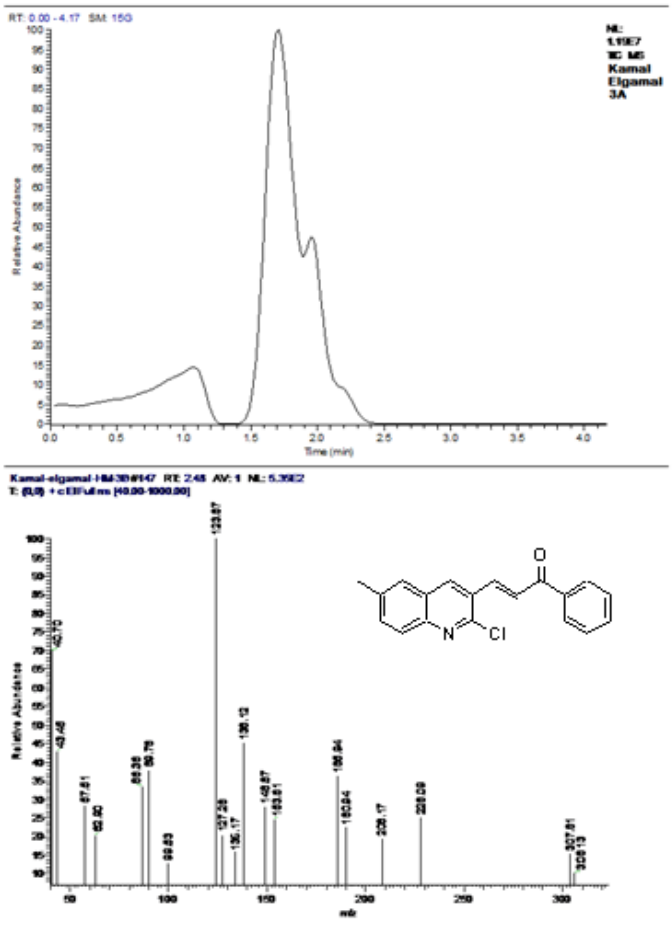

Figure 4. MS spectrum of 3a

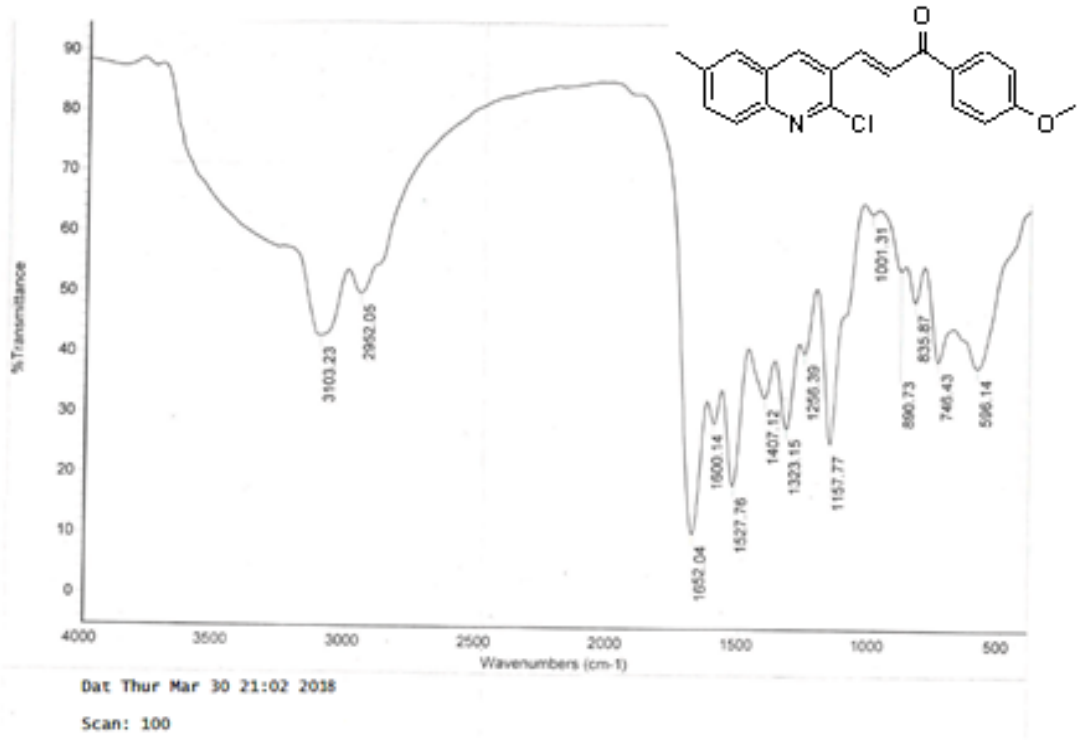

Figure 5. IR spectrum of 3b

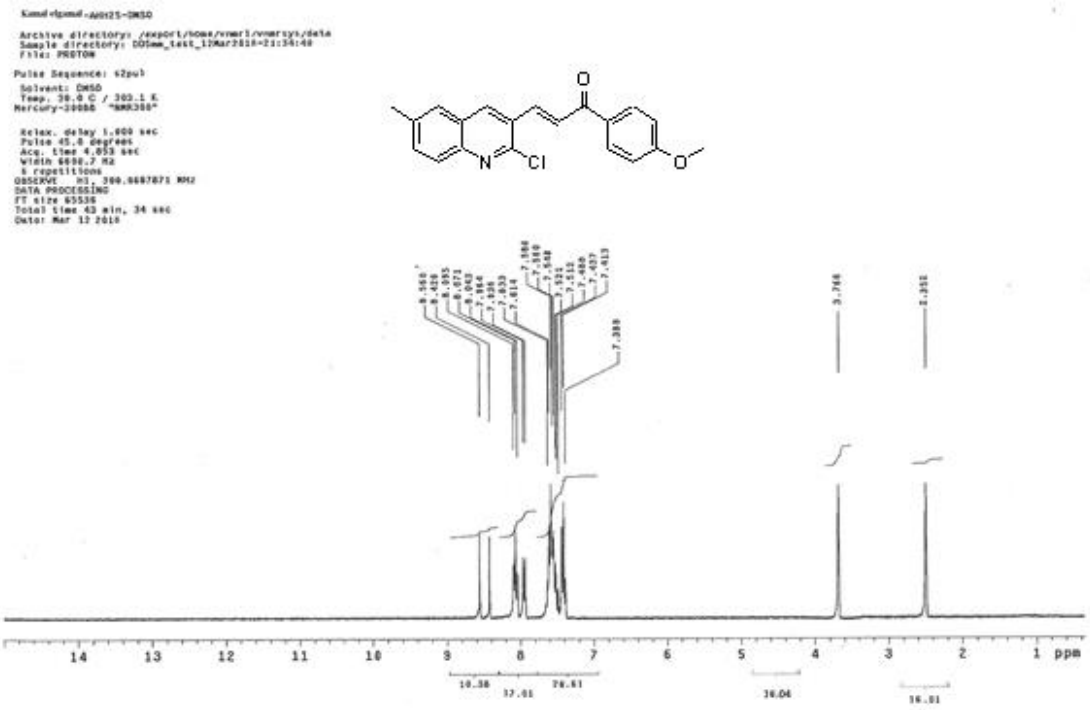

Figure 6. 1H-NMR spectrum of 3b

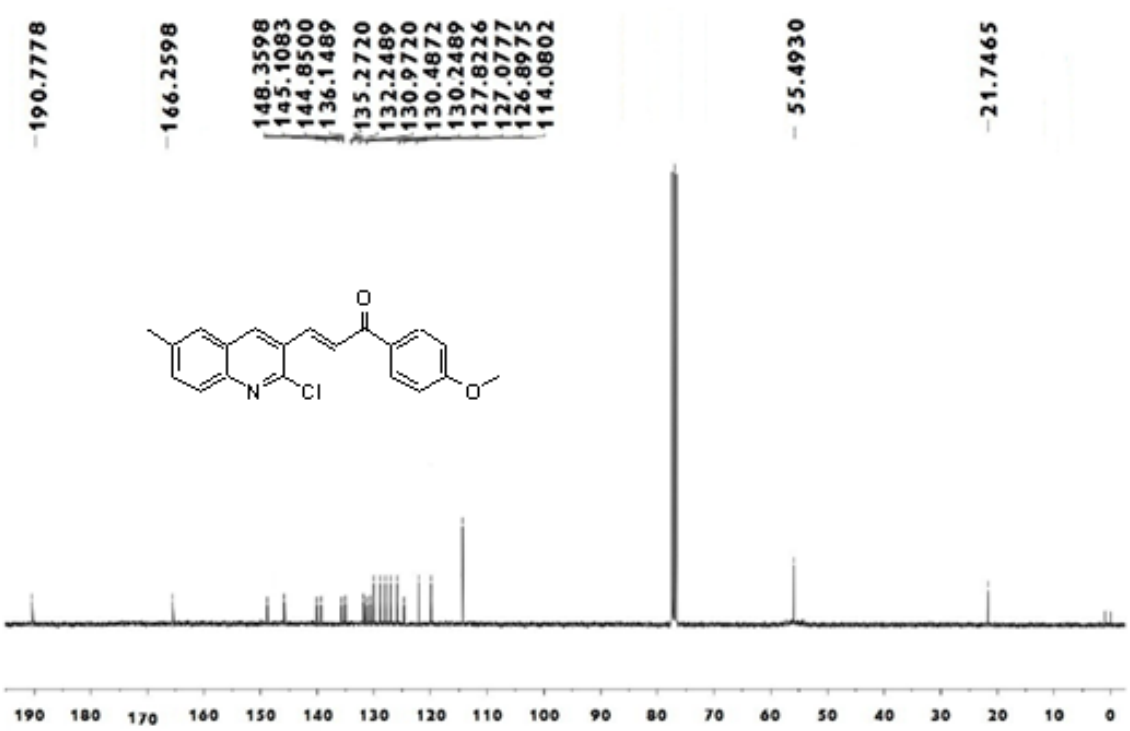

Figure 7. 13C-NMR spectrum of 3b

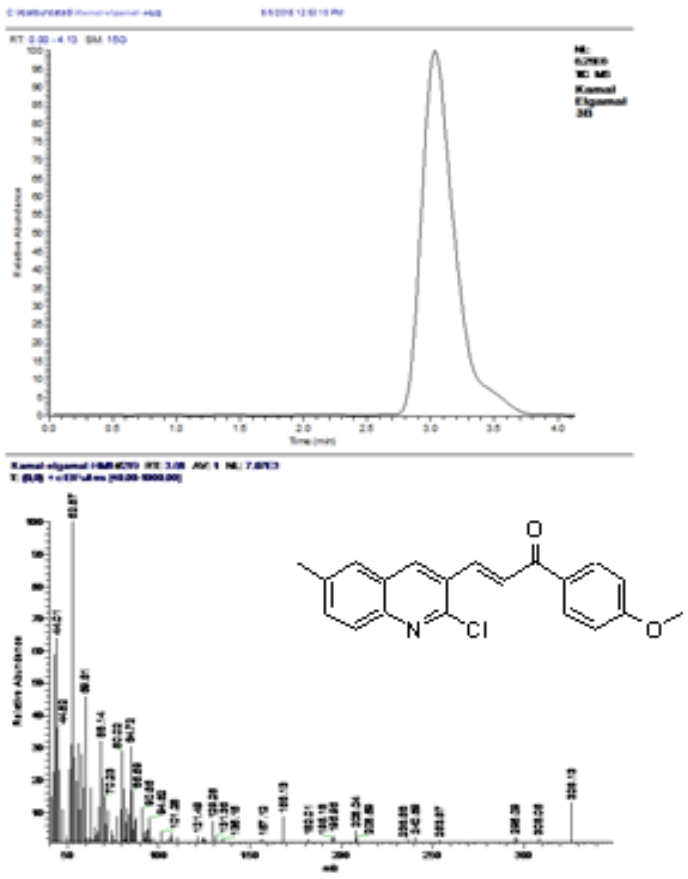

Figure 8. MS spectrum of 3b

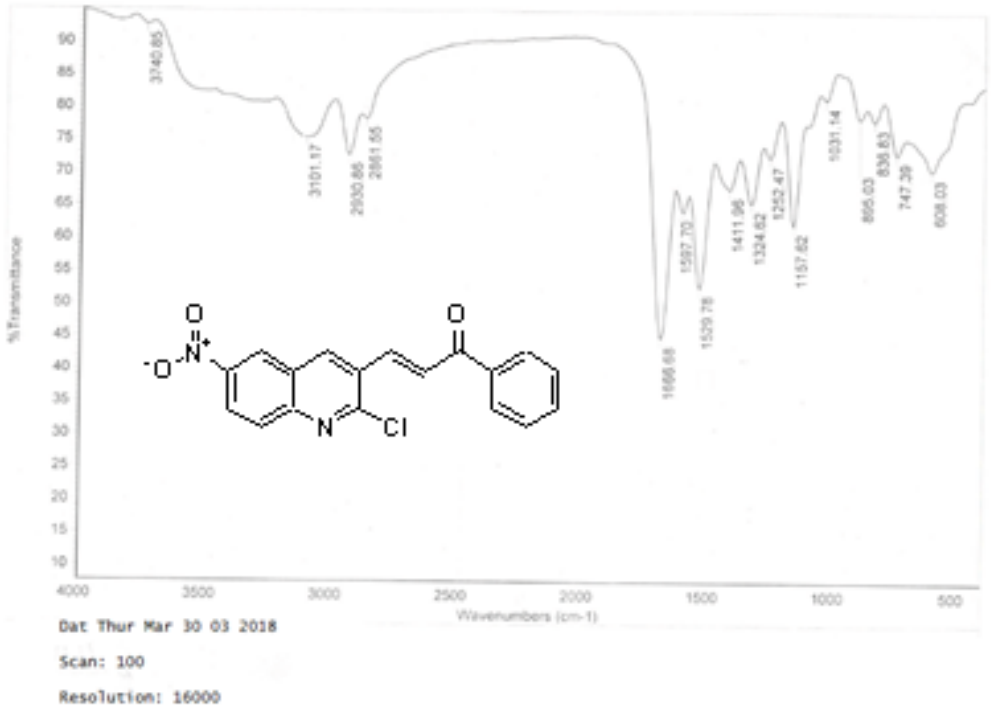

Figure 9. IR spectrum of 3c

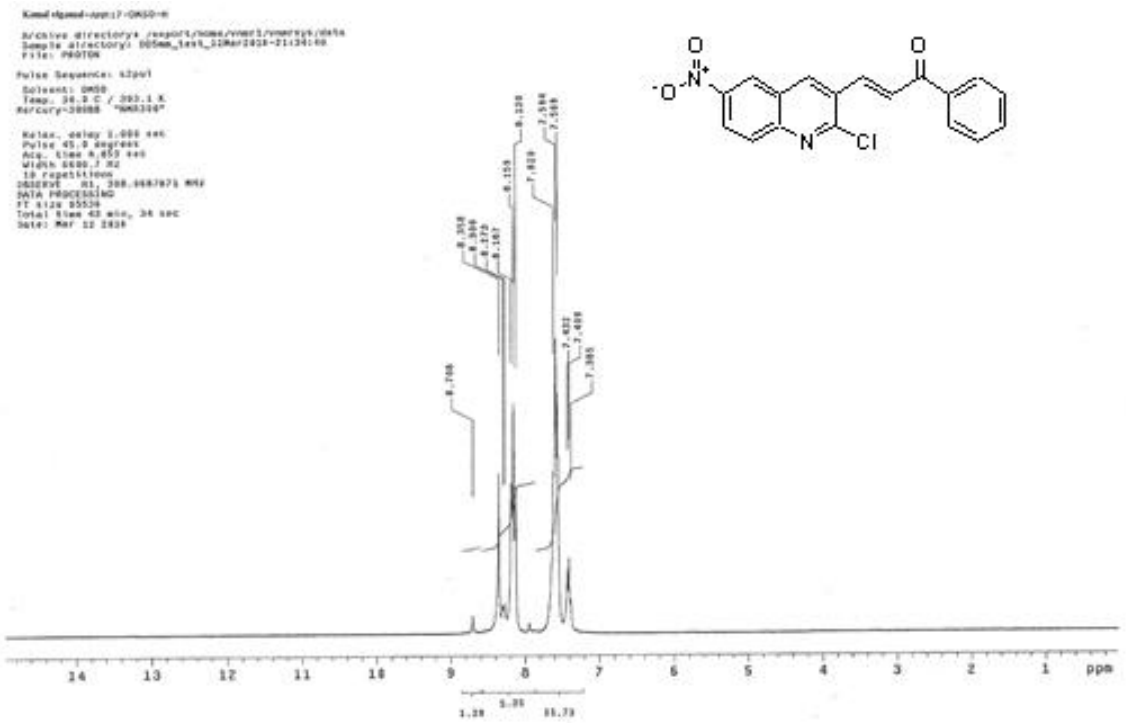

Figure 10. 1H-NMR spectrum of 3c

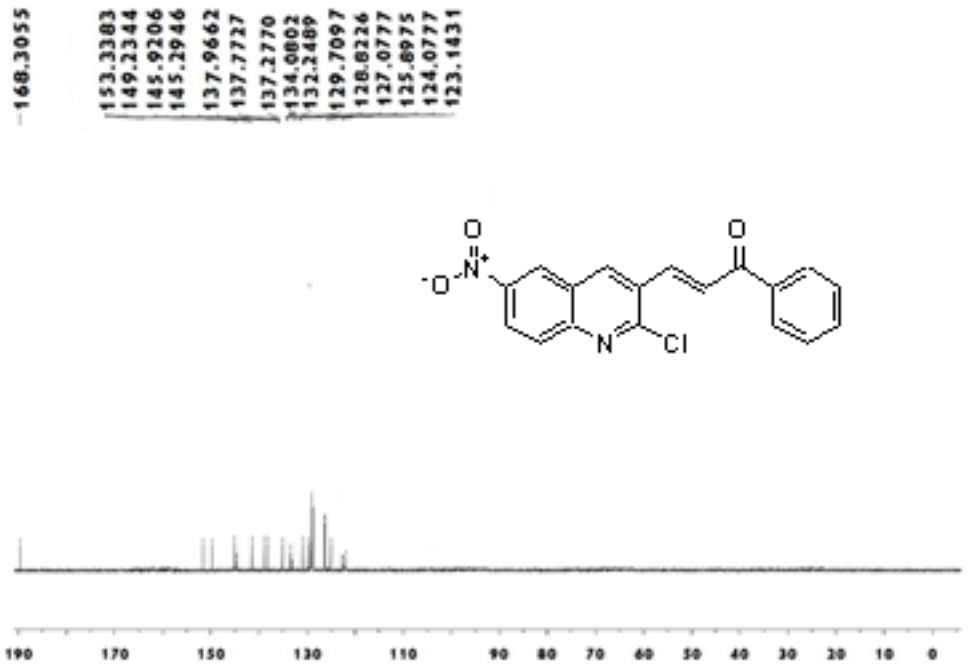

Figure 11. 13C-NMR spectrum of 3c

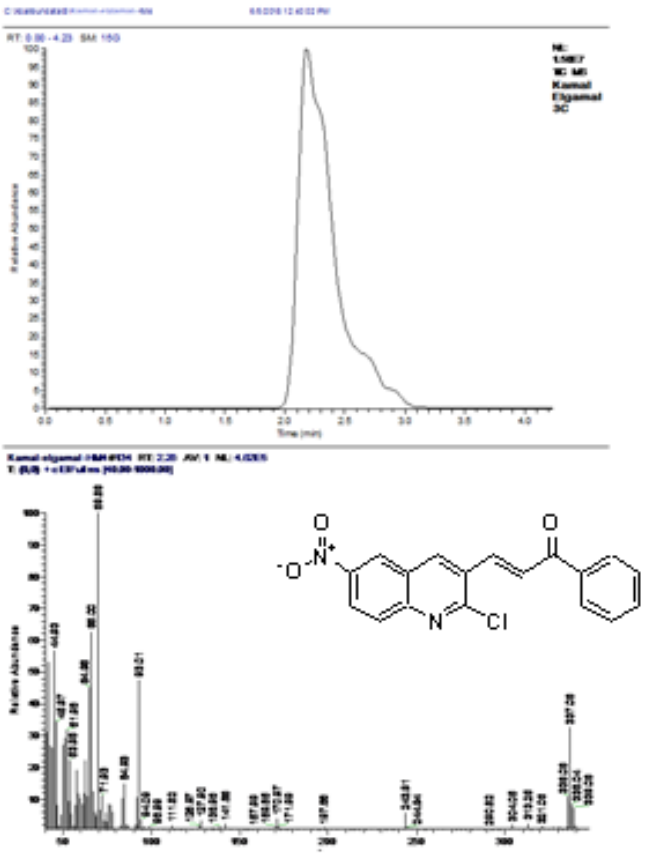

Figure 12. MS spectrum of 3c

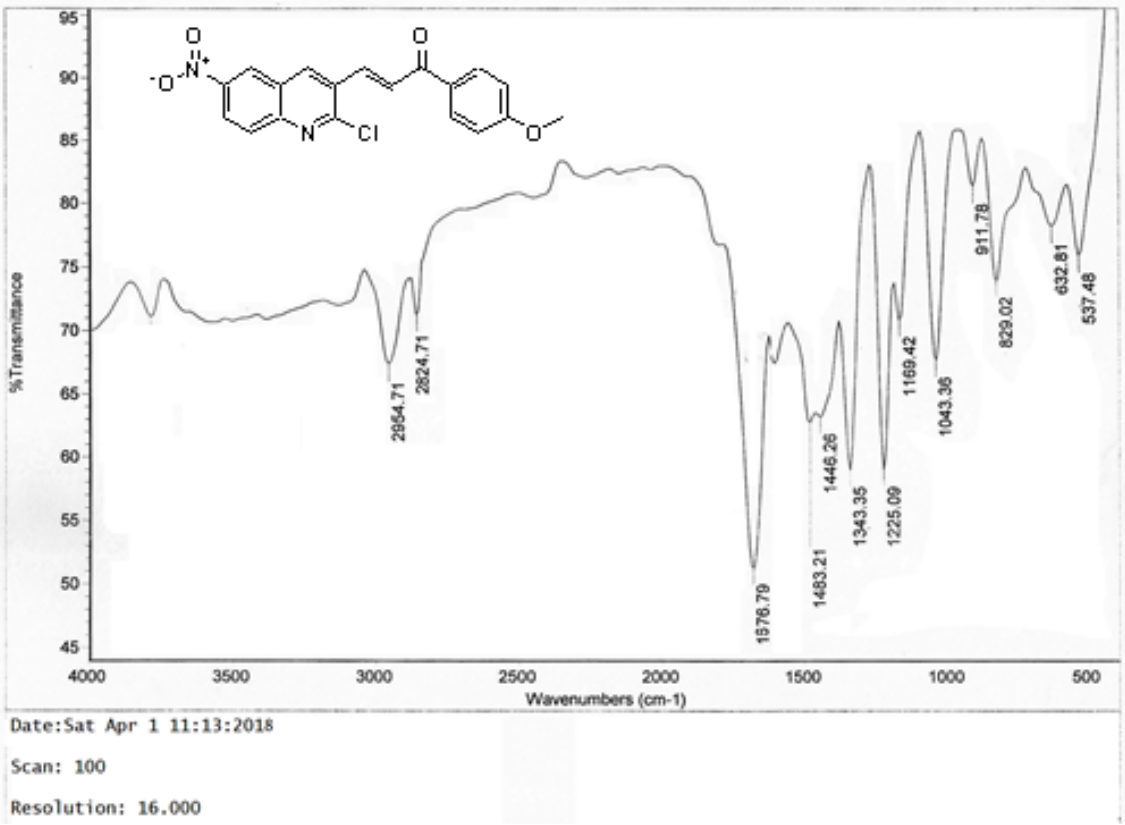

Figure 13. IR spectrum of 3d

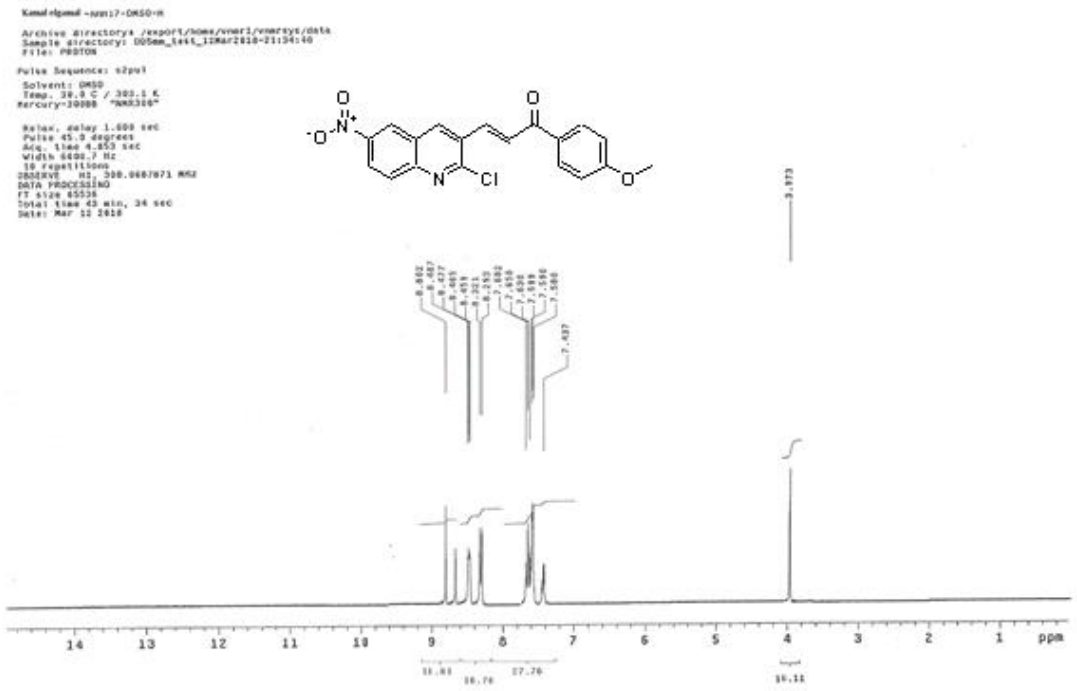

Figure 14. 1H-NMR spectrum of 3d

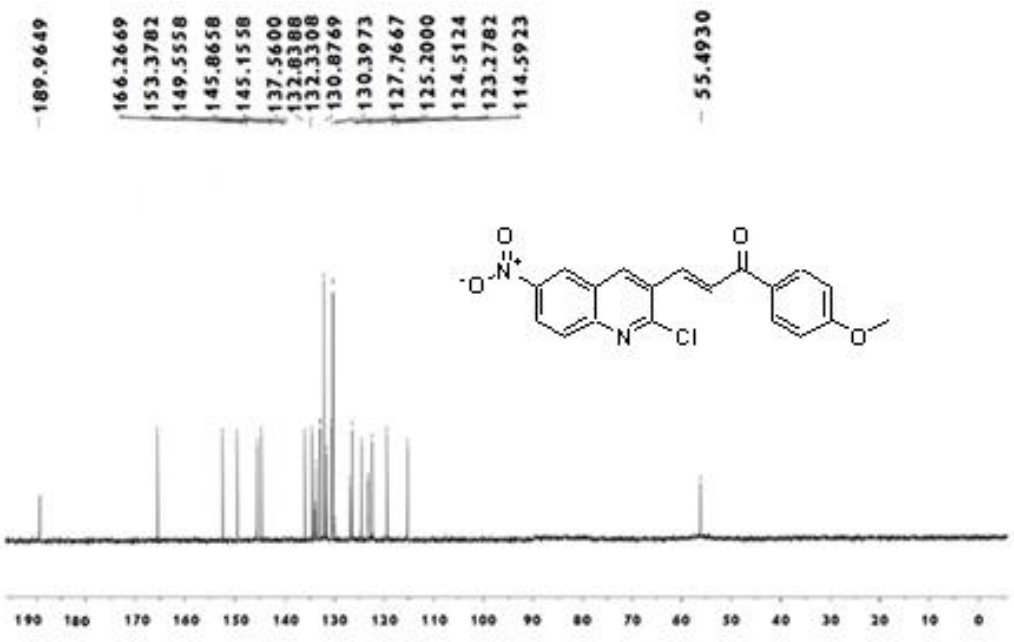

Figure 15. 13C-NMR spectrum of 3d

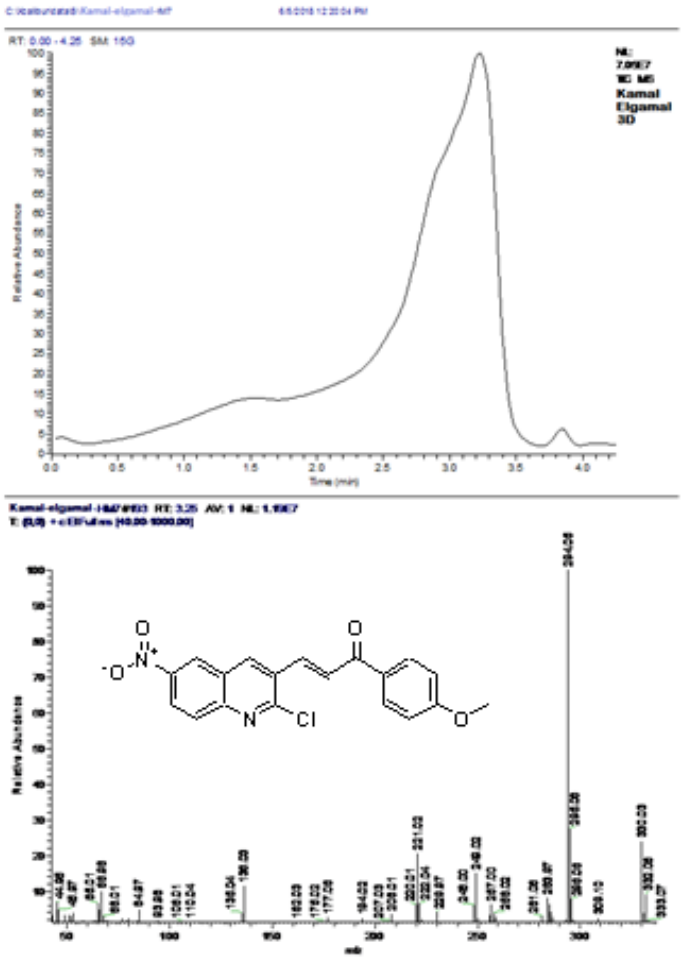

Figure 16. MS spectrum of 3d

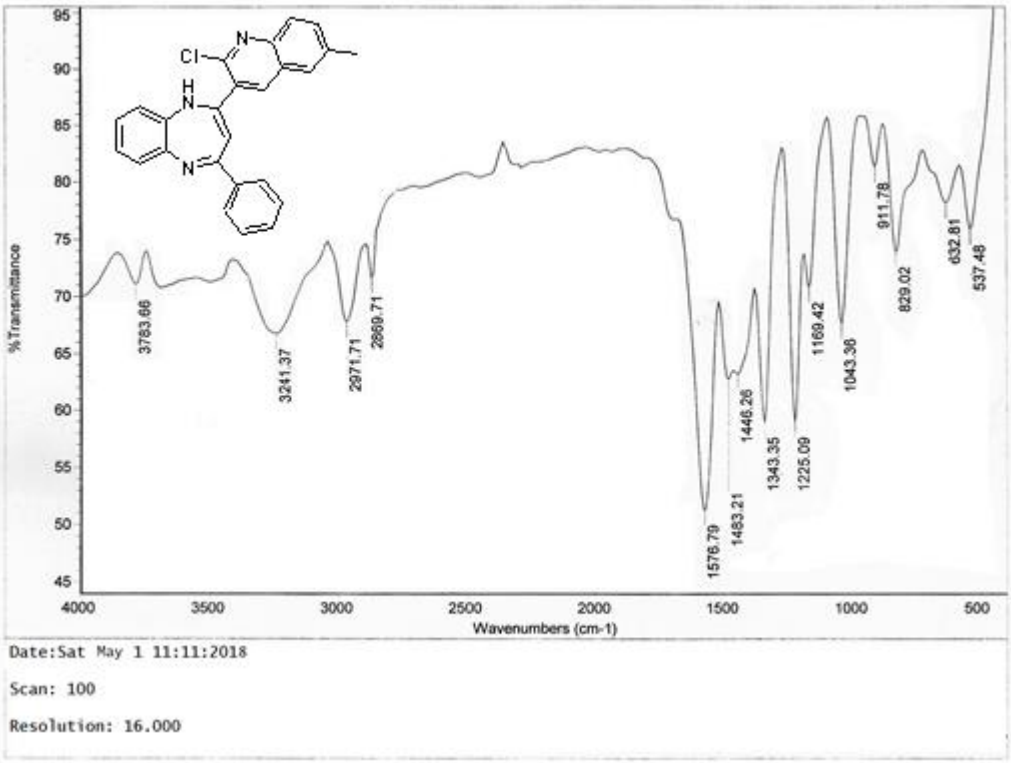

Figure 17. IR spectrum of 4a

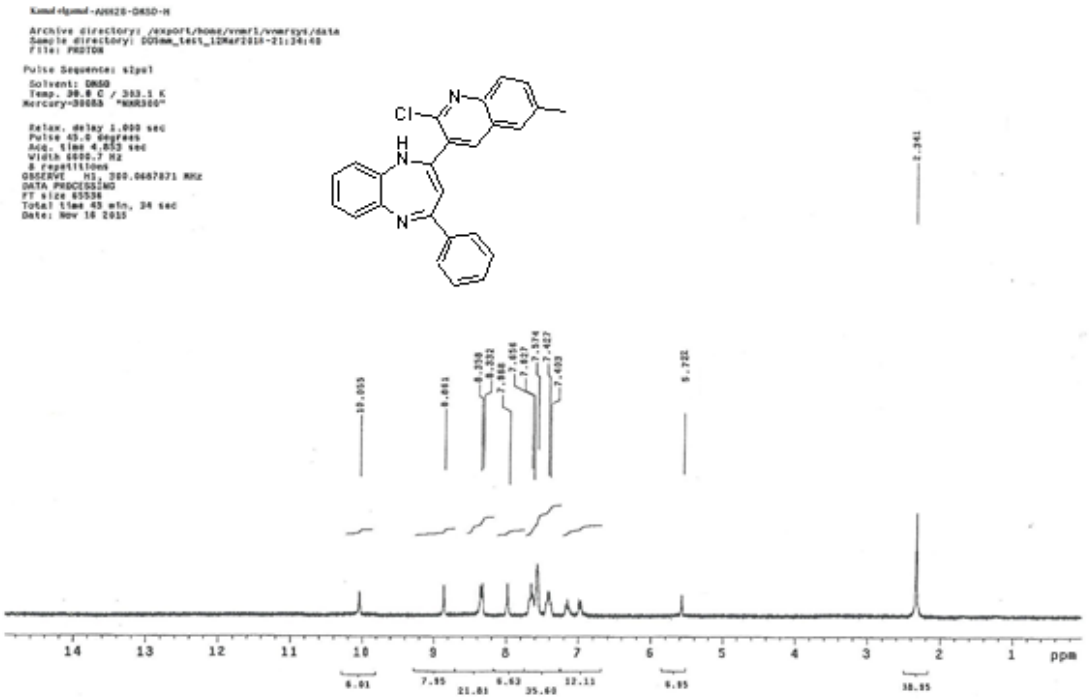

Figure 18. 1H-NMR spectrum of 4a

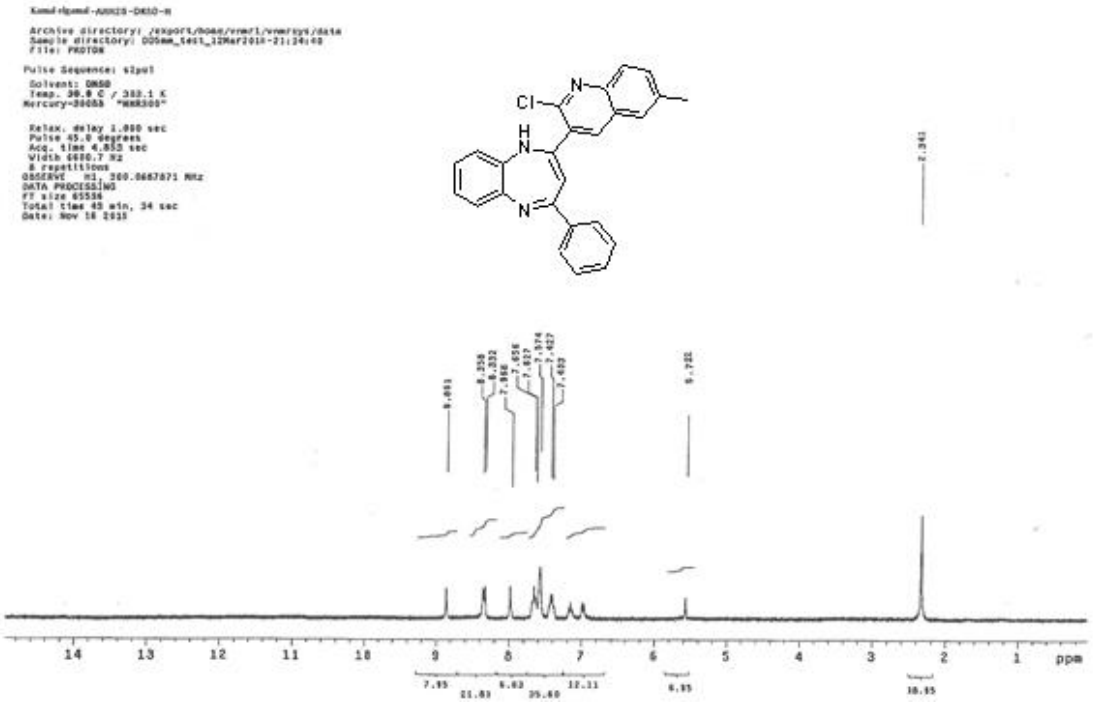

Figure 19. 1H-NMR spectrum of 4a (D2O)

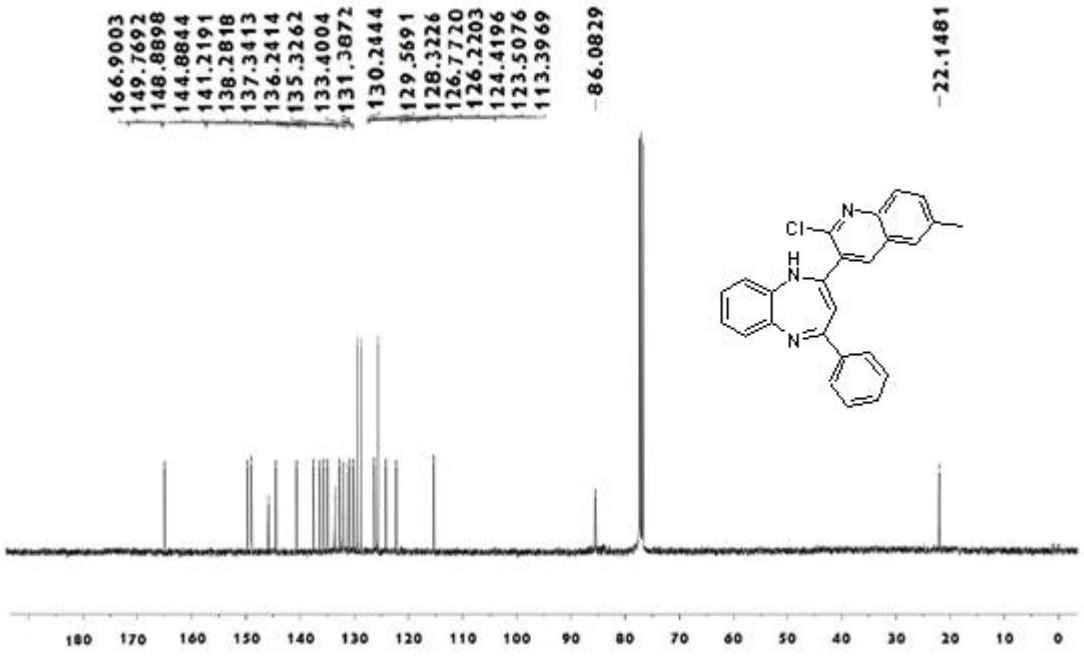

Figure 20. 13C-NMR spectrum of 4a

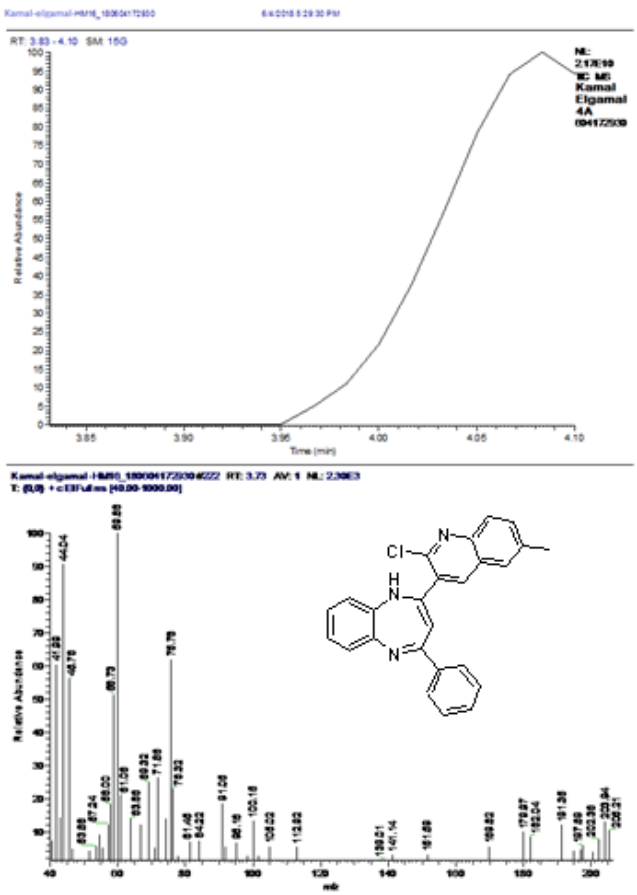

Figure 21. MS spectrum of 4a

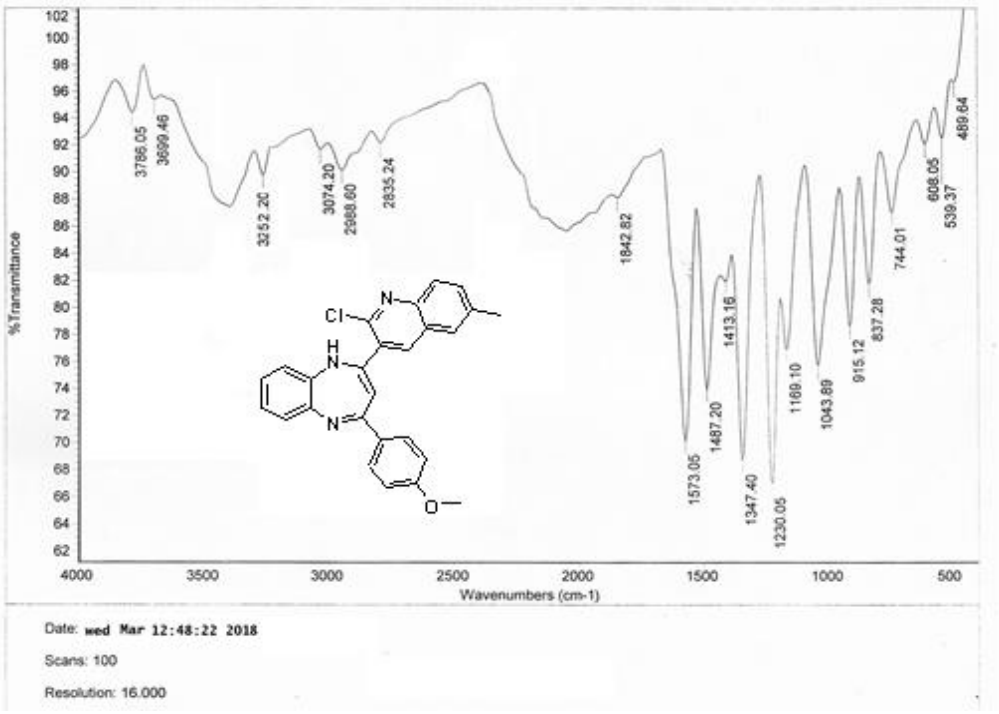

Figure 22. IR spectrum of 4b

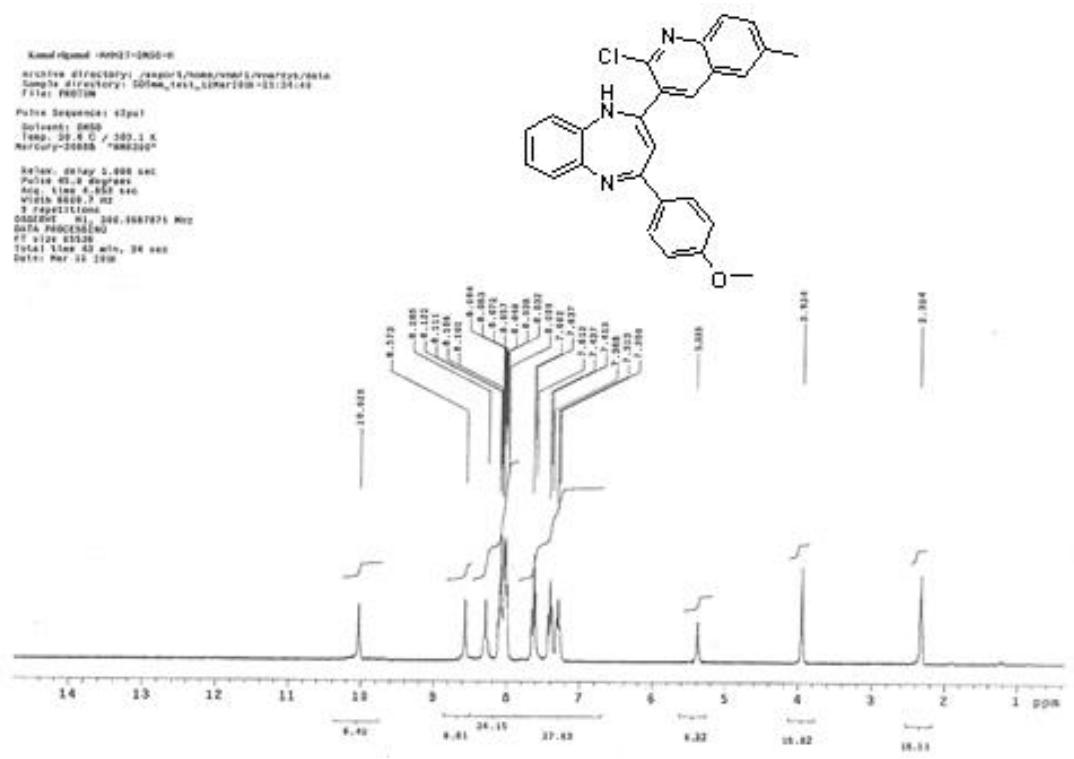

Figure 23. 1H-NMR spectrum of 4b

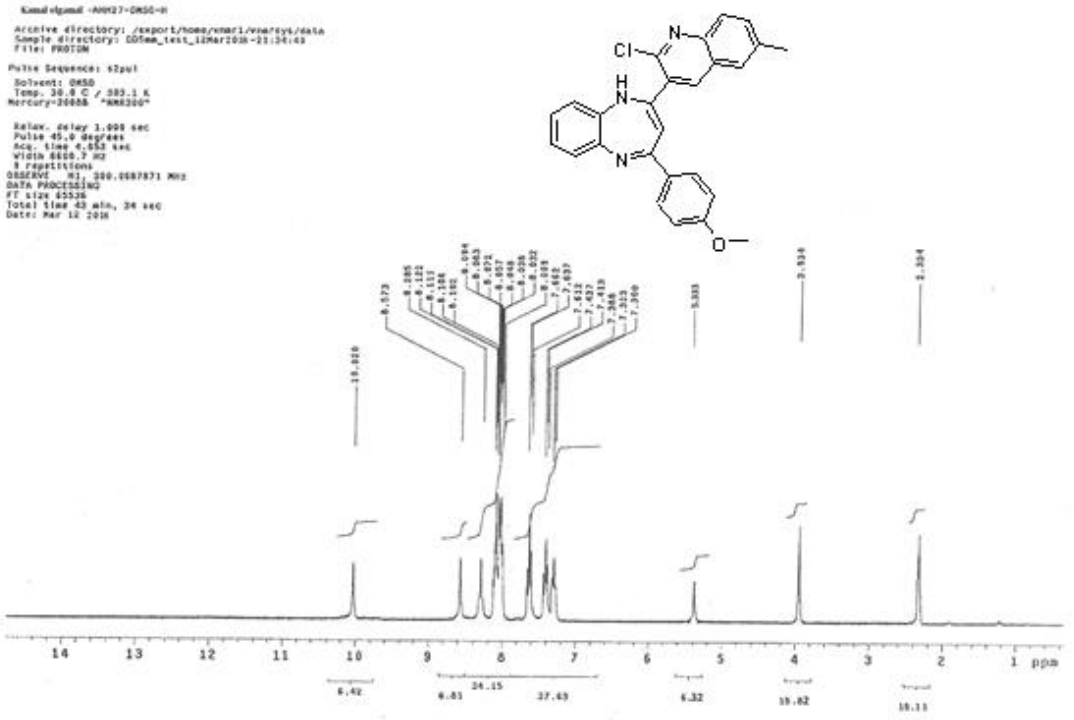

Figure 24. 1H-NMR spectrum of 4b (D2O)

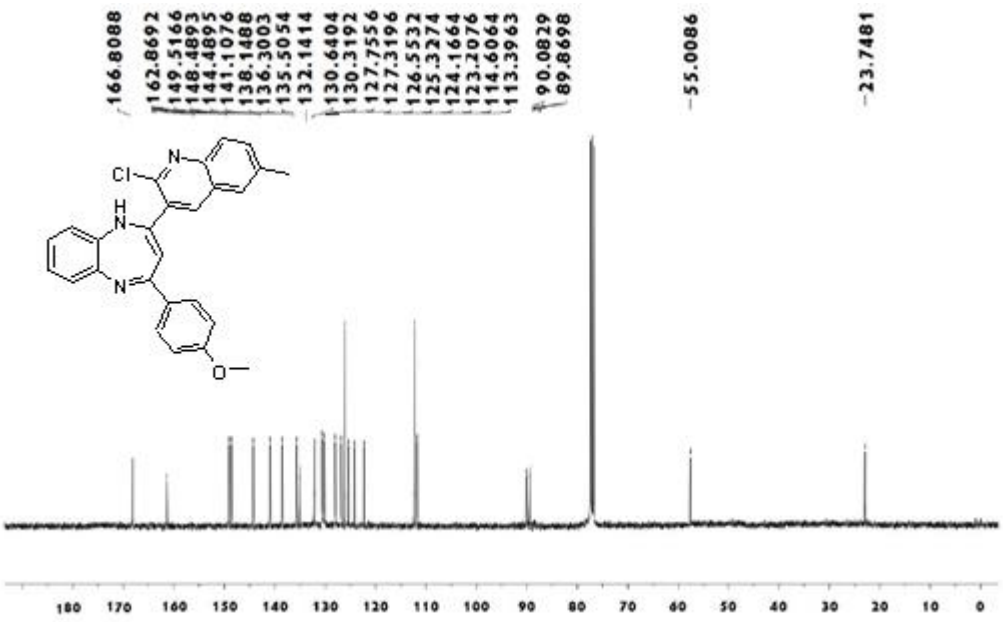

Figure 25. 13C-NMR spectrum of 4b

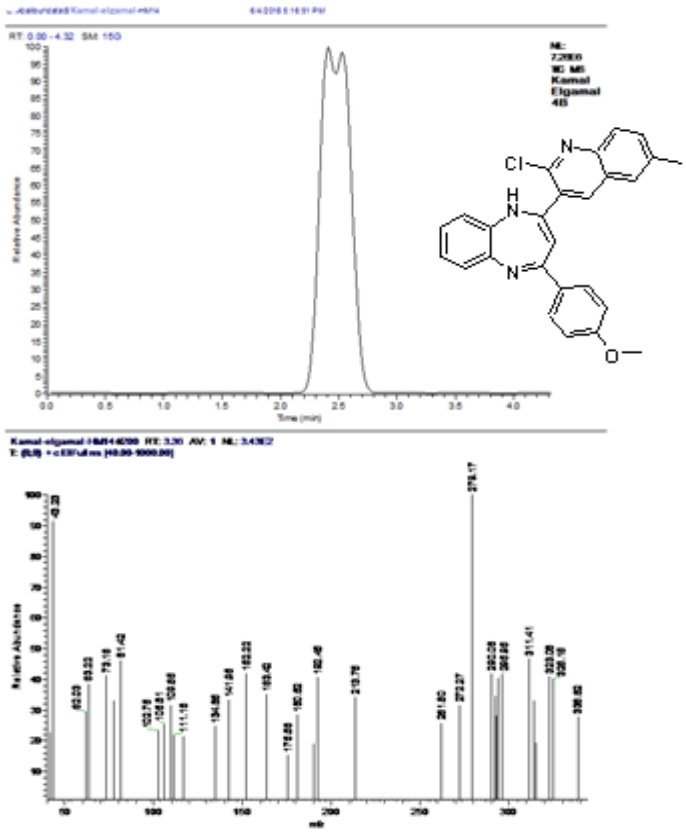

Figure 26. MS spectrum of 4b

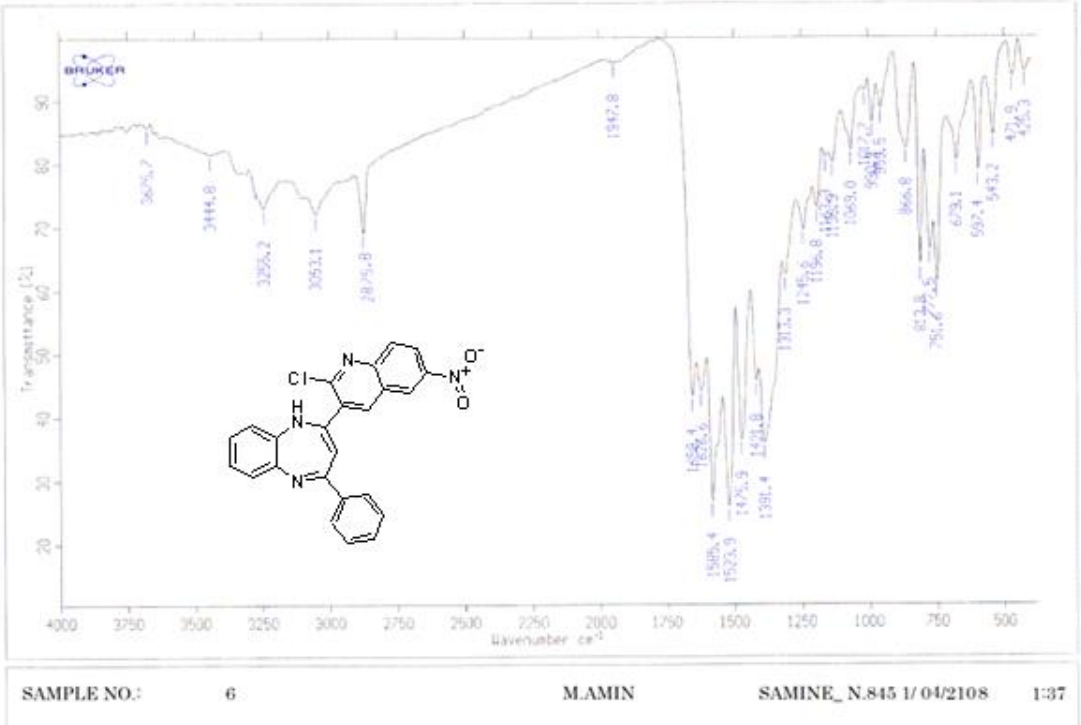

Figure 27. IR spectrum of 4c

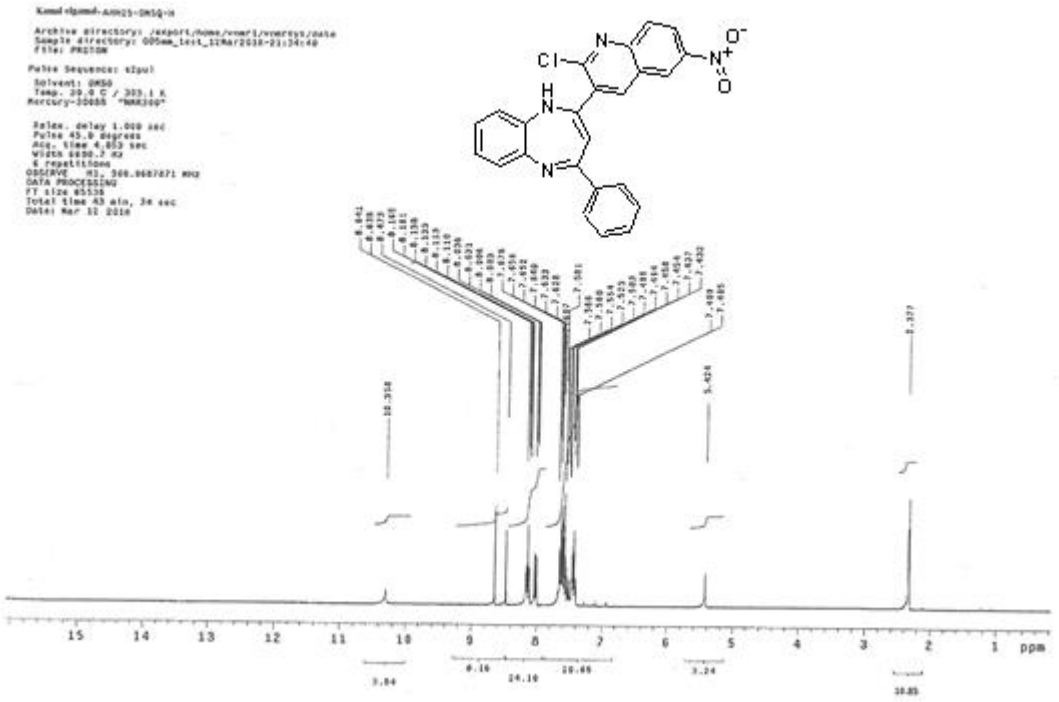

Figure 28. 1H-NMR spectrum of 4c

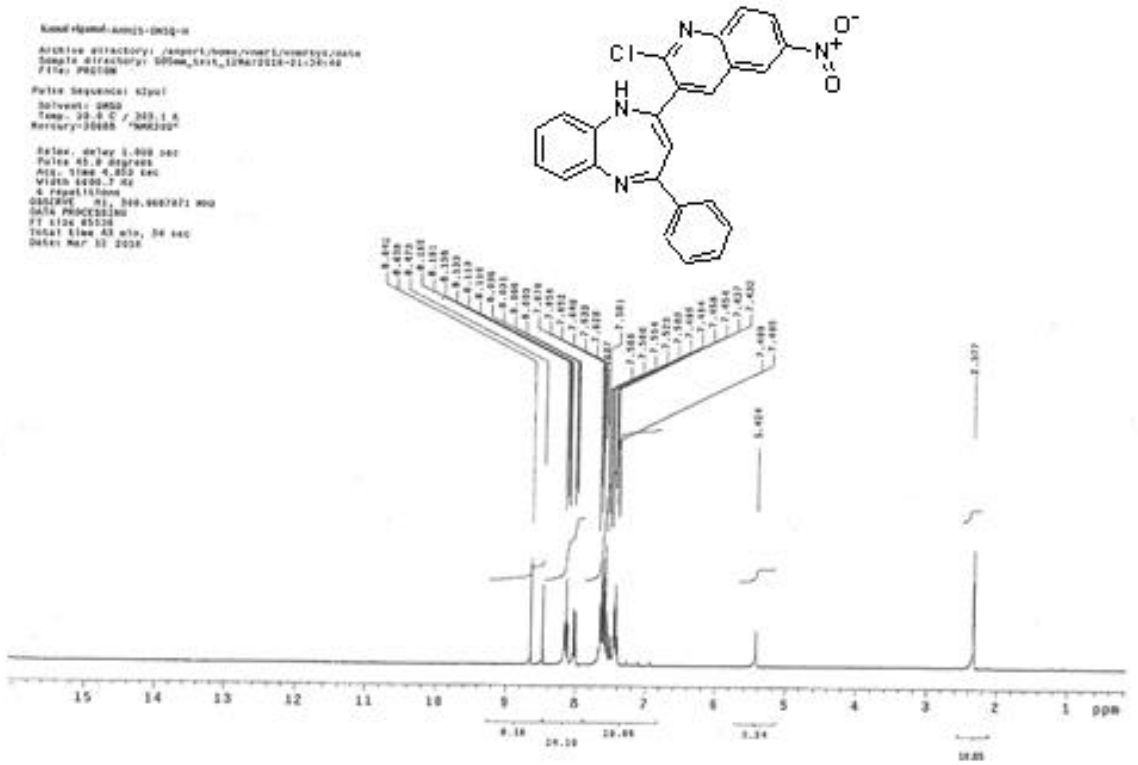

Figure 29. 1H-NMR spectrum of 4c (D2O)

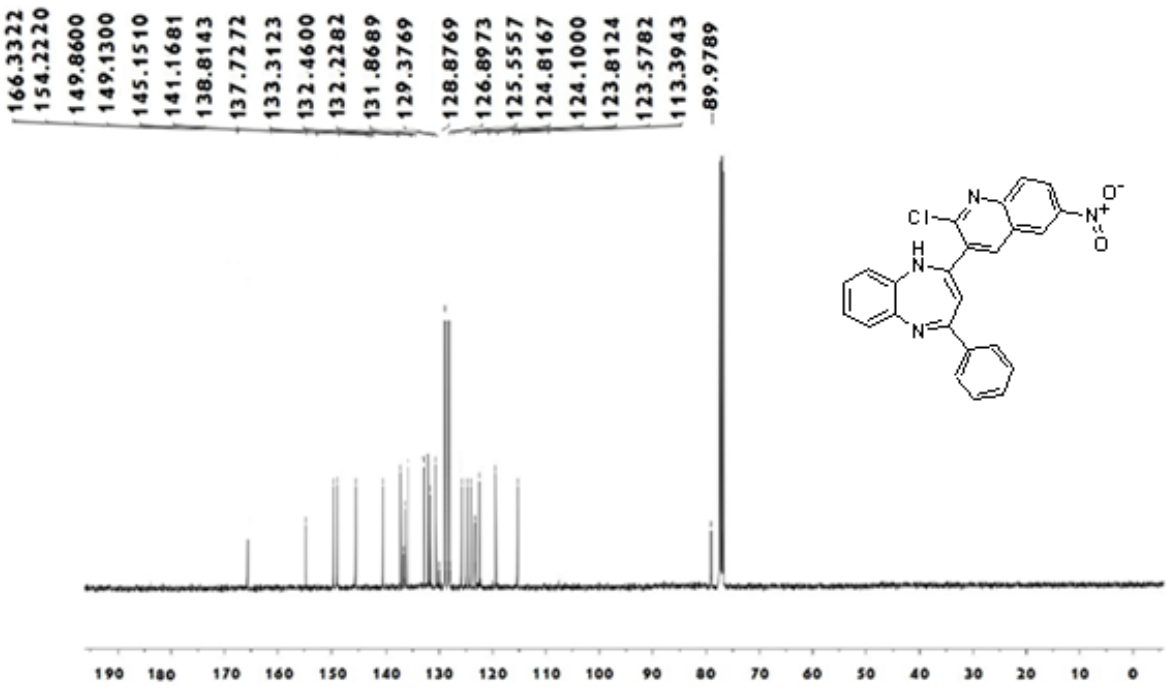

Figure 30. 13C-NMR spectrum of 4c

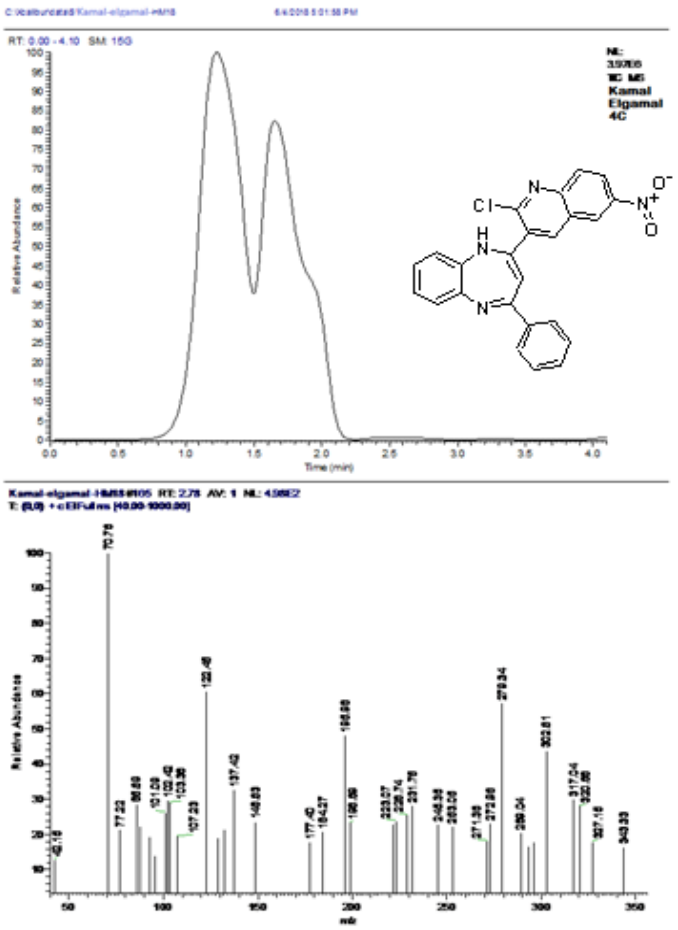

Figure 31. MS spectrum of 4c

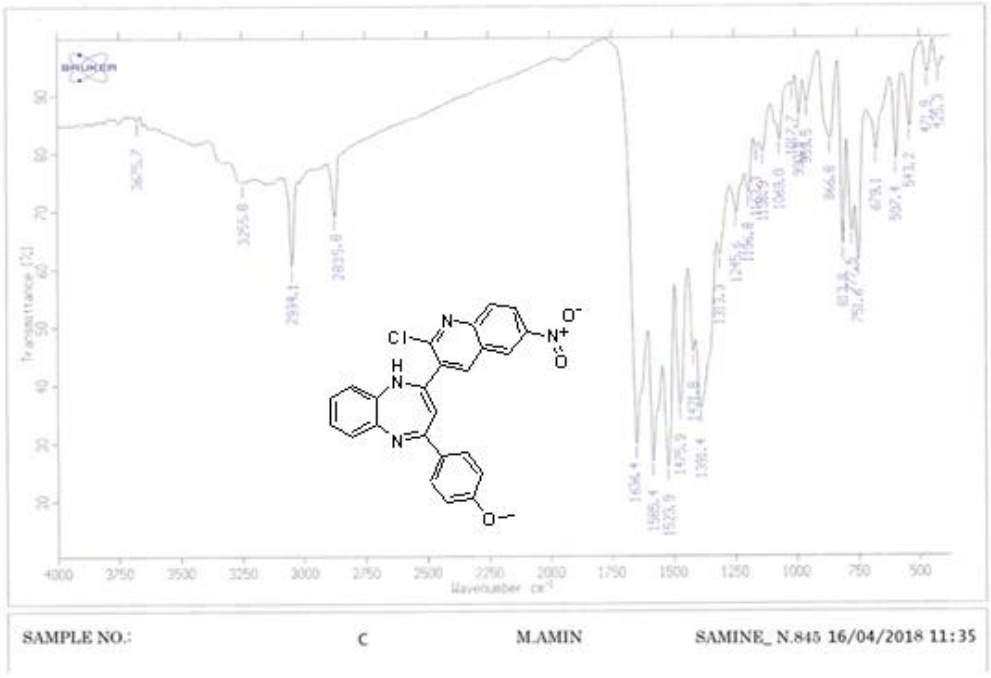

Figure 32. IR spectrum of 4d

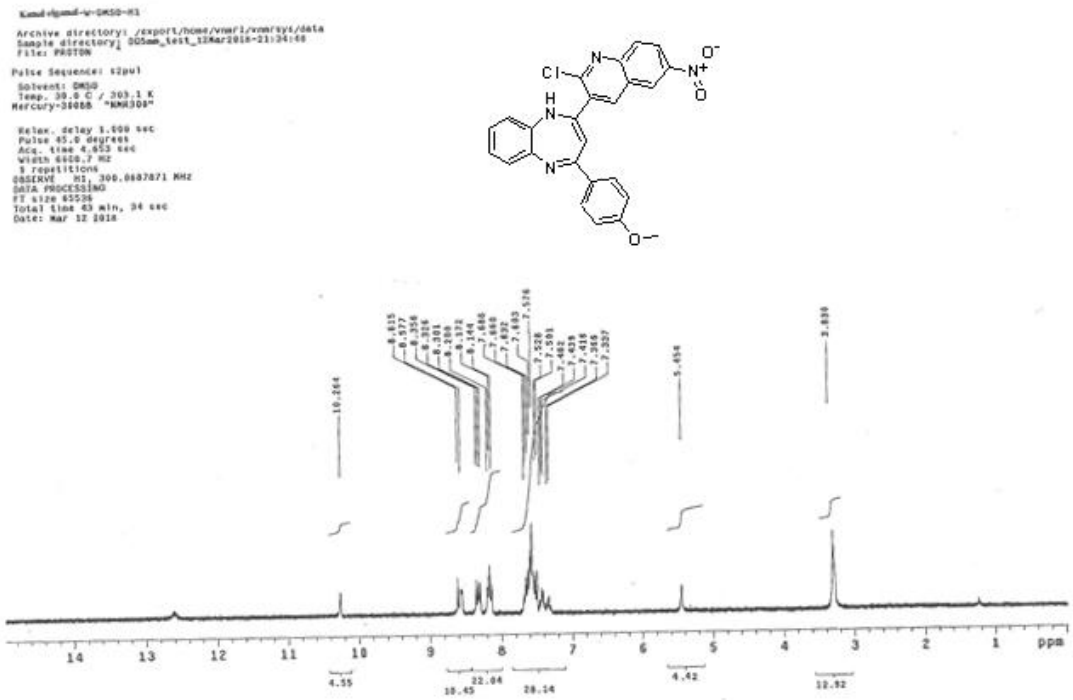

Figure 33. 1H-NMR spectrum of 4d

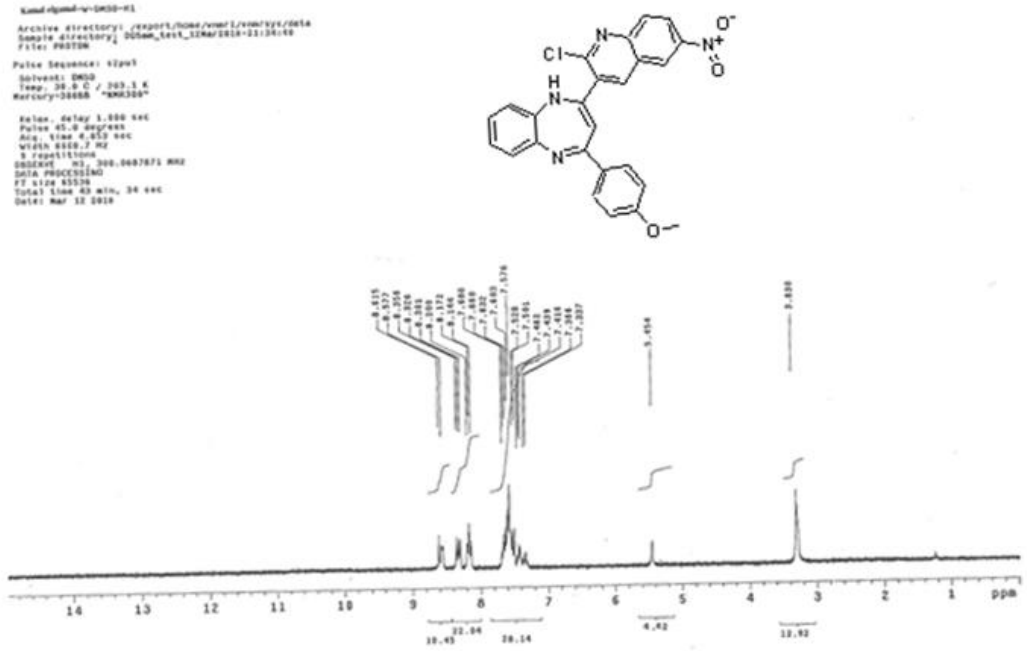

Figure 34. 1H-NMR spectrum of 4d (D2O)

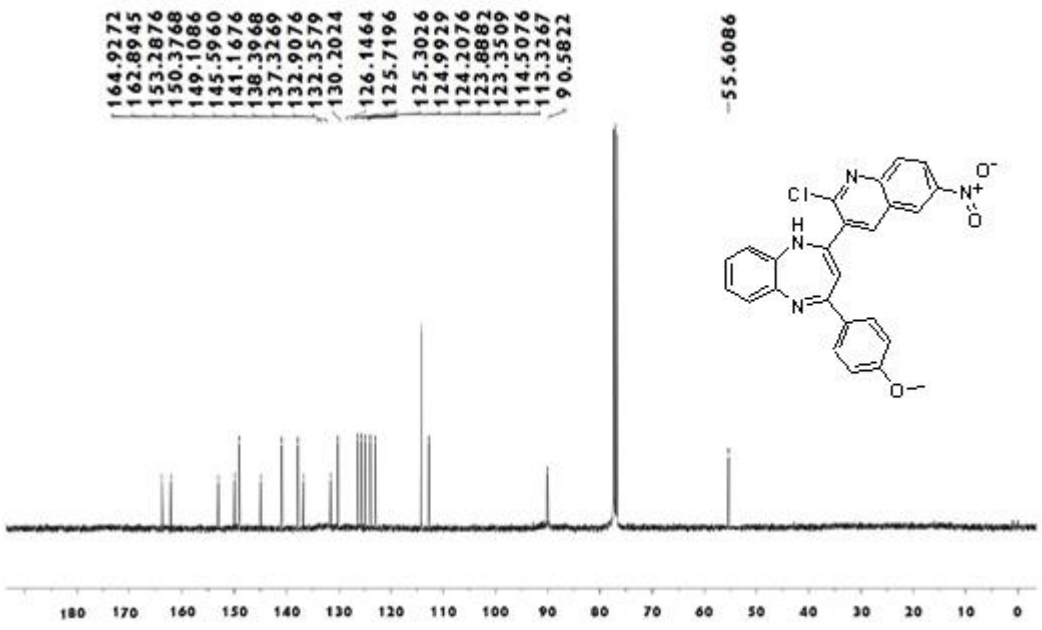

Figure 35. 13C-NMR spectrum of 4d

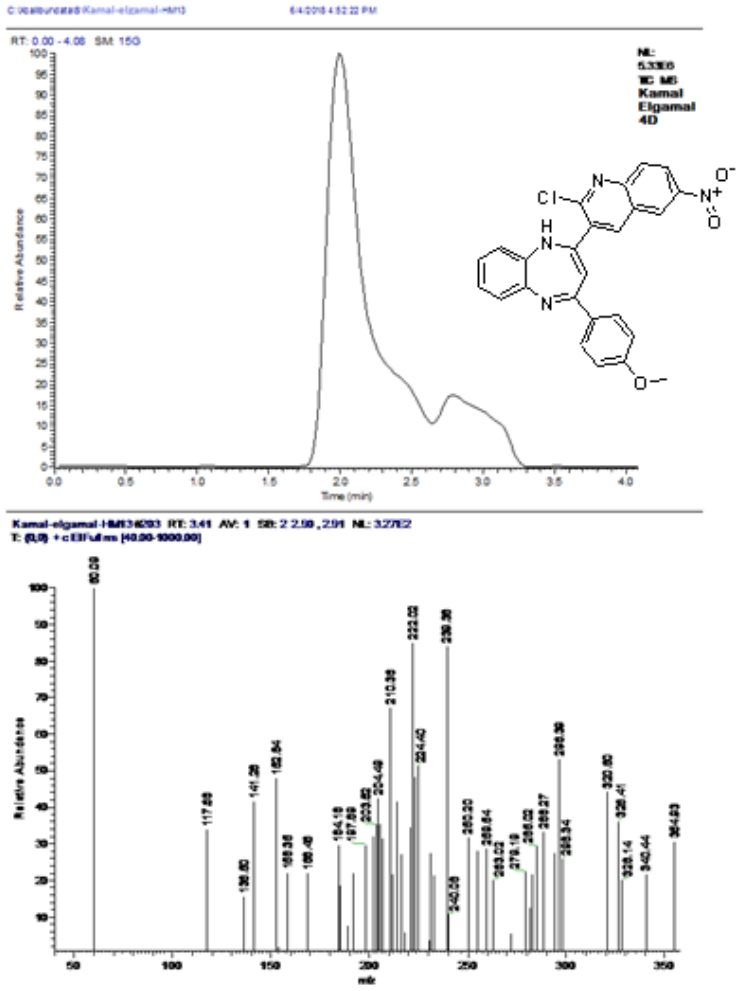

Figure 36. MS spectrum of 4d