Research Article **CODEN: AJPAD7** **ISSN: 2321 - 0923**

Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry

Journal home page: www.ajpamc.com

SYNTHESIS NEW ANALOGS OF 4-METHYL-1-NITROACRIDINE AND ITS BIOLOGICAL EVALUATION AS POTENTIAL ANTICANCER DRUGS

M. Gensicka-Kowalewska*[1], K. Dzierzbicka[1], M. Cichorek[2], M. Deptuła[2], A. Ronowska[3]

[1*]Department of Organic Chemistry, Gdansk University of Technology, Narutowicza St 11/12, PL 80-233 Gdansk, Poland.
[2]Department of Embryology, Medical University of Gdansk, Debinki St. 1, 80-210 Gdansk, Poland.
[3]Department of Lab Med, Medical University of Gdansk, Debinki St. 7 Bldg 27, 80-211 Gdansk, Poland.

ABSTRACT

New 4-methyl-1-nitroacridine analogues with tuftsin/retro-tuftsin derivatives were developed as potential anticancer agents. The cytotoxic activity of the novel obtained analogs were evaluated against melanotic (Ma) and amelanotic (Ab) melanoma cell line and neuroblastoma SH-SY5Y. Among the investigated derivatives only compound (8) was cytotoxic against amelanotic Ab melanoma and DC form of SH-SY5Y neuroblastoma after 72hrs incubation with IC_{50} values 88 and 116 µM, respectively.

KEYWORDS

Acridine, Anticancer, Melanoma, Neuroblastom and Tuftsin.

Author for Correspondence:

Gensicka-Kowalewska M,

Department of Organic Chemistry,

Gdansk University of Technology,

Narutowicza St 11/12, PL 80-233 Gdansk, Poland.

Email: monikagensicka@wp.pl

INTRODUCTION

Acridine/acridone analogs represent a very interesting class, displaying a lot of forms of bioactivity. They are used as anticancer drugs and cytotoxic agents, anti-bacterial drugs, anti-protozoal drugs, anti-malarial agents and anti-HIV drugs[1-11]. Moreover acridines are also reported to be useful in the treatment of Alzheimer's disease[12]. Further, acridines are applicable as dyes, fluorescent materials for visualization of biomolecules, and in laser technology[13]. These properties of acridine derivatives are attributed to the semi planar heterocyclic structure which makes, that acridines

act as DNA intercalators, and inhibit topoisomerase or telomerase enzymes[14]. In addition to clinical use, acridine derivatives are applied as pigments and dyes[15].

We report here the synthesis of a new series of 4-methyl-1-nitroacridine analogues with tuftsin/retro-tuftsin derivatives containing isopeptide bond between ε-amino group of lysine and carboxyl group of aliphatic amino acids such as Gly, Ala. In our method, synthesis of new analogues is based on the formation of carbon-nitrogen bond in the nucleophilic substitution reaction between the amine group of the respective peptide and C-9 of 4-methyl-1-nitro-9-phenoxyacridine. The newly synthesized acridine derivatives (8, 10a-c) were evaluated for their anticancer activities against melanoma and neuroblastoma tumors without satisfactory drug for the systemic treatment at metastatic stage of tumor development. Melanoma cells are used in a panel of tumor cells of the first line for testing new drugs among which for years are presented also acridine/acridone derivatives.

MATERIAL AND METHODS

Experimental

All chemicals and solvents were of reagent grade and were used without further purification. The reactions were monitored by TLC on Sigma-Aldrich F254 silica gel pre-coated plates. The following solvent systems (by vol.) were used for TLC development: $CHCl_3$-MeOH (3:1, v/v) (A), $CHCl_3$-MeOH (9:1, v/v) (B), $CHCl_3$-MeOH-NH_3 (7:1:0.01, v/v/v) (C), $CHCl_3$-MeOH-NH_3 (5:1:0.01, v/v/v) (D). MS spectra were recorded on matrix-assisted laser desorption/ionization-time on flight mass spectrometry (MALDI-TOF MS, Biflex III Bruker). The detection was carried out using UV and ninhydrin. ^1H and ^{13}C NMR spectra were measured in dimethyl sulfoxide (DMSO) or CD_3OD solutions with a Varian Unity 500 Plus spectrometer, using TMS as an internal standard. Chemical shifts (δ) are given in ppm. Multiplicity is indicated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Conditions of chromatographic HPLC separation and detection of examined compounds (8, 10a-c): column - Poroshell EC-C18 (3.0x150 mm), 2.7 µm, Agilent Technologies; column temperature - 40 °C; injection volume - 2 µL; flow rate - 0.4 mL/min; eluents: (A) 0.1 % HCOOH in water, (B) 0.1 % HCOOH in ACN/MeOH (1:1, v/v); gradient program: 0 min. 90% (A) 10% (B), 20 min. 0% (A) 100% (B), 30 min. 0% (A) 100% (B); post time - 10 min; UV-Vis detection; wavelengths UV: 254 nm; Vis: 580 nm; peak width > 0.1 min (2s); ESI MS detection.

General procedure for synthesis of acridine with retro-tuftsin/tuftsin derivatives (7) and (9a-c)

Mixture of 4-methyl-1-nitro-9-phenoxyacridine (6) (0.152 mmol) and retro-tuftsin or tuftsin derivatives (1, 2a-e) (0.304 mmol) in phenol was stirred at 50°C for 24 h under argon atmosphere. After completion, a solution of ethyl acetate (5 mL) was added and the mixture was extracted with 5% KOH (5 mL x 4). The organic phases were dried with anhydrous $MgSO_4$, and filtered. After evaporation of the solvent, the reaction mixture was purified using preparative thin layer chromatography (TLC) in solvent B to obtain compounds (7, 9a-e).

9-[Arg(NO_2)-Pro-Lys(Fmoc)-Thr-OCH_3]-4-methyl-1-nitroacridine (7)

Yield 63%; MS $[M+H]^+$ m/z calcd for $C_{51}H_{59}N_{11}O_{12}$ 1018.08, found 1018.5. R_f=0.71 (B)

9-[Thr-Lys(Fmoc)-Pro-Arg(NO_2)-OCH_3]-4-methyl-1-nitroacridine (9a)

Yield 69%; MS $[M+H]^+$ m/z calcd for $C_{51}H_{59}N_{11}O_{12}$ 1018.08, found 1018.5. R_f=0.70 (B)

9-[Thr-Lys(GlyFmoc)-Pro-Arg(NO_2)-OCH_3]-4-methyl-1-nitroacridine (9b)

Yield 61%; MS $[M+H]^+$ m/z calcd for $C_{53}H_{62}N_{12}O_{13}$ 1075.13, found 1075.4. R_f=0.72 (B)

9-[Thr-Lys(AlaFmoc)-Pro-Arg(NO_2)-OCH_3]-4-methyl-1-nitroacridine (9c)

Yield 56%; MS $[M+H]^+$ m/z calcd for $C_{53}H_{62}N_{12}O_{13}$ 1089.16, found 1089.5. R_f=0.76 (B)

General procedure for synthesis of acridine with retro-tuftsin/tuftsin derivatives (8) and (10a-c)

To a mixture of derivatives (7, 9a-c) in *N,N*-dimethylformamide (DMF) (1 mL) was added diethylamine (DEA) (0.4 mL). The mixture was left for 1h at room temperature. After completion, evaporation of the solvent, the mixture of analogues dissolved in MeOH and saturated with hydrochloric

acid in diethyl ether (Et_2O). Evaporation of the solvent followed by purification with preparative TLC in solvent C or D, provide the corresponding product (8, 10a-c).

HClx9-[Arg(NO_2)-Pro-Lys-Thr-OCH_3]-4-methyl-1-nitroacridine (8)

Yield 92%; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 1.02 (d, *J* = 6.4 Hz, 3H, γ-T4), 1.34 (m, 2H, γ-K4), 1.50 (m, 2H, δ-K5), 1.53 (m, 1H, β-K3b), 1.57 (m, 1H, β-R3b), 1.64 (m, 1H, β-K3a), 1.68 (m, 2H, γ-R4), 1.70 (m, 1H, γ-P4b), 1.73 (m, 1H, β-P3b), 1.77 (m, 1H, γ-P4a), 1.91 (m, 1H, β-P3a), 1.99 (m, 1H, β-R3a), 2.51 (s, 3H, Ar-CH_3), 2.73 (m, 2H, ε-K6), 3.03 (m, 1H, δ-P5b), 3.18 (m, 3H, δ-R5, δ-P5a), 3.60 (s, 3H, R-$COOCH_3$), 4.08 (m, 1H, β-T3), 4.11 (m, 1H, α-P2), 4.23 (m, 1H, α-T2), 4.28 (m, 1H, α-K2), 4.79 (m, 1H, α-R2), 5.04 (d, *J* = 7.5 Hz, 1H, T-OH), 7.15 (t, *J* = 7.3 Hz, 1H, 7), 7.30 (d, *J* = 7.8 Hz, 1H, 3), 7.40 (m, 1H, 5), 7.53 (t, *J* = 7.7 Hz, 1H, 6), 7.64 (d, *J* = 7.9 Hz, 1H, 2), 7.75 (d, *J* = 8.3 Hz, 1H, 8), 7.85 (d, *J* = 8.0 Hz, 1H, α-TaN), 8.05 (d, *J* = 8.0 Hz, 1H, α-KaN), 9.83 (m, 1H, α-RaN). ^{13}C NMR (400 MHz, CD_3OD) δ ppm: 173.23 (C-K1), 173.09 (C-P1), 171.46 (C-T1), 170.98 (C-R1), 159.49 (C-R1), 154.07 (C-9), 147.79 (C-1), 140.74 (C-4a), 139.13 (C-5a), 130.91 (C-4), 129.74 (C-6), 127.29 (C-2), 126.24 (C-8), 120.42 (C-7), 116.96 (C-5), 116.15 (C-8a), 116.07 (C-3), 114.51 (C-1a), 66.91 (C-T3), 62.26 (C-R2), 60.46 (C-P2), 57.81 (C-T2), 52.86 (C-K2), 51.48 (C-OMe), 48.49 (C-P5), 40.84 (C-R5), 39.67 (C-K6), 30.72 (C-K3), 30.06 (C-R3), 28.98 (C-K5), 27.34 (C-P3), 24.62 (C-R5, C-P4), 22.33 (C-K4), 18.97 (C-T4), 16.62 (Ar-CH_3). R_f=0.40 (D); 99.65% pure by HPLC.

HClx9-[Thr-Lys-Pro-Arg(NO_2)-OCH_3]-4-methyl-1-nitroacridine (10a)

Yield 89%; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 1.01 (d, *J* = 6.3 Hz, 3H, γ-T4), 1.44 (m, 2H, γ-K4), 1.49 (m, 2H, δ-K5), 1.58 (m, 2H, γ-R4), 1.60 (m, 1H, β-K3b), 1.62 (m, 1H, β-R3b), 1.67 (m, 1H, β-K3a), 1.71 (m, 1H, β-R3a), 1.79 (m, 1H, β-P3b), 1.88 (m, 2H, γ-P4), 2.04 (m, 1H, β-P3a), 2.54 (s, 3H, Ar-CH_3), 2.77 (m, 2H, ε-K6), 3.12 (m, 2H, δ-R5), 3.56 (m, 1H, δ-P5b), 3.60 (s, 3H, R-$COOCH_3$), 3.67 (m, 1H, δ-P5a), 3.79 (m, 1H, β-T3), 4.15 (m, 1H, α-R2), 4.28 (m, 1H, α-P2), 4.31 (m, 1H, α-T2), 4.54 (m, 1H, α-K2), 6.84 (d, *J* = 7.7 Hz, 1H, 8), 7.13 (t, *J* = 7.6 Hz, 1H, 7), 7.35 (d, *J* = 7.8 Hz, 1H, 3), 7.47 (d, *J* = 7.9 Hz, 1H, 5), 7.58 (m, 3H, 2, 6, α-RaN), 8.27 (d, *J* = 7.2 Hz, 1H, α-KaN), 10.03 (m, 1H, α-TaN). ^{13}C NMR (400 MHz, CD_3OD) δ ppm: 173.14 (C-R1), 173.11 (C-P1), 172.12 (C-K1), 170.66 (C-T1), 159.26 (C-R6), 156.35 (C-9), 147.74 (C-1), 140.85 (C-4a), 138.79 (C-5a), 131.54 (C-4), 130.22 (C-6), 128.18 (C-2), 127.31 (C-8), 120.45 (C-7), 117.16 (C-5), 116.18 (C-8a), 115.20 (C-3), 113.50 (C-1a), 70.78 (C-T3), 70.16 (C-T2), 60.15 (C-P2), 51.90 (C-OMe), 51.41 (C-R2), 51.09 (C-K2), 48.46 (C-P5), 40.26 (C-R5), 39.16 (C-K6), 30.11 (C-K3), 29.15 (C-P3), 28.16 (C-K5), 26.74 (C-R3), 24.76 (C-R5, C-P4), 22.03 (C-K4), 19.07 (C-T4), 16.79 (Ar-CH_3). R_f=0.40 (D); 99.35% pure by HPLC.

HClx9-[Thr-Lys(Gly)-Pro-Arg(NO_2)-OCH_3]-4-methyl-1-nitroacridine (10b)

Yield 92%; ^{1}H NMR (400 MHz, DMSO) δ ppm: 1.02 (d, *J* = 6.3 Hz, 3H, γ-T4), 1.40 (m, 2H, γ-K4), 1.45 (m, 2H, δ-K5), 1.49 (m, 2H, γ-R4), 1.53 (m, 1H, β-K3b), 1.58 (m, 1H, β-R3b), 1.67 (m, 1H, β-K3a), 1.70 (m, 1H, β-R3a), 1.79 (m, 1H, β-P3b), 1.87 (m, 2H, γ-P4), 2.03 (m, 1H, β-P3a), 2.53 (s, 3H, Ar-CH_3), 3.09 (m, 2H, ε-K6), 3.11 (m, 2H, α-G2), 3.17 (m, 2H, δ-R5), 3.55 (m, 1H, δ-P5b), 3.60 (s, 3H, R-$COOCH_3$), 3.67 (m, 1H, δ-P5a), 3.78 (m, 1H, β-T3), 4.16 (m, 1H, α-R2), 4.27 (m, 1H, α-P2), 4.30 (m, 2H, α-T2), 4.53 (m, 1H, α-K2), 6.84 (d, *J* = 7.9 Hz, 1H, 8), 7.13 (t, *J* = 7.6 Hz, 1H, 7), 7.36 (d, *J* = 7.8 Hz, 1H, 3), 7.47 (d, *J* = 7.9 Hz, 1H, 5), 7.58 (m, 1H, α-RaN), 7.77 (d, *J* = 8.2 Hz, 1H, 2), 7.83 (t, *J* = 5.6 Hz, 1H, 6), 8.20 (d, *J* = 7.2 Hz, 1H, α-KaN), 9.96 (m, 1H, α-TaN). ^{13}C NMR (400 MHz, CD_3OD) δ ppm: 173.20 (C-R1), 172.96 (C-P1), 172.17 (C-K1), 171.22 (C-G1), 170.98 (C-T1), 159.27 (C-R6), 155.98 (C-9), 147.78 (C-1), 140.81 (C-4a), 138.71 (C-5a), 131.45 (C-4), 130.19 (C-6), 128.01 (C-2), 127.41 (C-8), 120.40 (C-7), 117.13 (C-5), 116.08 (C-8a), 115.19 (C-3), 113.45 (C-1a), 70.81 (C-T3), 70.24 (C-T2), 60.13 (C-P2), 51.90 (C-OMe), 51.40 (C-R2), 51.31 (C-K2), 48.47 (C-P5), 43.46 (C-G2), 40.18 (C-R5), 38.67 (C-K6), 30.38 (C-K3), 29.08 (C-P3), 28.58 (C-K5), 28.14 (C-R3), 24.76 (C-R5, C-P4), 22.69 (C-K4), 19.08

(C-T4), 16.78 (Ar-CH_3). R_f=0.43 (D); 100% pure by HPLC.

HClx9-[Thr-Lys(β-Ala)-Pro-Arg(NO_2)-OCH_3]-4-methyl-1-nitroacridine (10c)

Yield 88%; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 1.02 (d, *J* = 6.1 Hz, 3H, γ-T4), 1.39 (m, 2H, γ-K4), 1.44 (m, 2H, δ-K5), 1.51 (m, 2H, γ-R4), 1.54 (m, 1H, β-K3b), 1.59 (m, 1H, β-R3b), 1.68 (m, 1H, β-K3a), 1.70 (m, 1H, β-R3a), 1.79 (m, 1H, β-P3b), 1.85 (m, 2H, γ-P4), 2.03 (m, 1H, β-P3a), 2.29 (t, *J* = 6.5 Hz, 2H, α-A2), 2.52 (s, 3H, Ar-CH_3), 2.84 (m, 2H, β-A3), 3.07 (m, 2H, ε-K6), 3.11 (m, 2H, δ-R5), 3.55 (m, 1H, δ-P5b), 3.60 (s, 3H, R-$COOCH_3$), 3.66 (m, 1H, δ-P5a), 3.78 (m, 1H, β-T3), 4.14 (m, 1H, α-R2), 4.30 (m, 2H, α-P2, α-T2), 4.53 (m, 1H, α-K2), 6.84 (d, *J* = 6.9 Hz, 1H, 8), 7.13 (t, *J* = 7.5 Hz, 1H, 7), 7.35 (d, *J* = 7.7 Hz, 1H, 3), 7.46 (d, *J* = 7.8 Hz, 1H, 5), 7.60 (m, 2H, 6, α-RaN), 2,6 7.78 (d, *J* = 8.2 Hz, 1H, 2), 7.97 (m, 1H, β-AbN), 8.23 (d, *J* = 7.0 Hz, 1H, α-KN), 9.99 (m, 1H, α-TaN). ^{13}C NMR (400 MHz, CD_3OD) δ ppm: 172.84 (C-R1), 172.25 (C-P1), 171.16 (C-K1), 170.84 (C-A1), 169.86 (C-T1), 159.75 (C-R6), 154.27 (C-9), 147.89 (C-1), 141.20 (C-4a), 139.07 (C-5a), 132.00 (C-4), 130.90 (C-6), 128.36 (C-2), 127.83 (C-8), 121.05 (C-7), 118.09 (C-5), 116.58 (C-8a), 115.54 (C-3), 113.85 (C-1a), 71.02 (C-T3), 70.25 (C-T2), 59.29 (C-P2), 52.26 (C-OMe), 50.46 (C-R2), 49.05 (C-K2), 47.21 (C-P5), 40.16 (C-R5), 38.75 (C-K6), 37.56 (C-A3), 36.59 (C-A2), 31.23 (C-K3), 29.47 (C-P3), 29.23 (C-K5), 28.37 (C-R3), 24.86 (C-R5, C-P4), 22.83 (C-K4), 20.25 (C-T4), 18.55 (Ar-CH_3). R_f=0.49 (D); 97.99% pure by HPLC.

Transplantable melanomas

The original transplantable melanotic melanoma (Ma) derived from a spontaneous melanoma of the skin that had appeared in a bred of golden hamster in 1959[16]. The amelanotic melanoma line (Ab) originated from the Ma form by a spontaneous alteration. The loss of pigment was accompanied by changes in many biological features of Ab line - faster tumor growth rate, shorter animal survival and changes in ultra-structure of cells. Once established, these melanomas possessed a considerable degree of phenotypic stability over decades of pass aging. Since their discovery, each melanoma line is maintained *in vivo* by consecutive, subcutaneous transplantations of tumor material every 21 (Ma) or 11 (Ab) days. This melanoma model is known as Bomirski hamster's melanomas. The experiments' procedures were approved by the Animal Ethics Committee at Medical University of Gdansk and conducted in accordance with National Health and Medical Research Council's guide for the care and use of laboratory.

Melanoma cells were isolated for each experiment from solid tumors by a non-enzymatic method[17]. The suspension consisted of 95 - 98% viable cells (estimated by trypan blue test). Melanoma cells were cultured in a RPMI medium (Sigma-Aldrich) supplemented with 10% of FBS (fetal bovine serum; Sigma-Aldrich) and antibiotics (0.05 mg streptomycin and 50 U penicillin per 1 mL; Sigma-Aldrich). Each independent experiment was performed on the cells isolated from a single tumor.

Neuroblastoma

Neuroblastoma SH-SY5Y is a cloned subline of SK-N-SH of human neuroblastoma originally established from bone marrow biopsy of a neuroblastoma patient in the early 1970s.

Human SH-SY5Y neuroblastoma cells, between 10th and 25th passage, were used for experiments. Cells were seeded at density of 4 x 10^3 cells/cm^2 on 25 cm^2 tissue culture flask in DMEM medium (Sigma-Aldrich) with 10% FBS (Sigma-Aldrich) and antibiotics (0.05 mg streptomycin and 50 U penicillin per 1 mL; Sigma-Aldrich).Cholinergic differentiation of DC variant was obtained by combined addition of 1 mmol/L dibutyryl cAMP (cAMP; Sigma-Aldrich) and 0.001 mmol/L all-trans-retinoic acid (RA; Sigma-Aldrich) for 48 h[18]. At this time the medium has been replaced for the medium without differentiating agents and tested analogs were added and culture was continued for the next 72 h. Cell line was purchased from the American Type Culture Collection (ATCC).

Analogs, dacarbazine, cisplatine treatment

New synthesized analogs at concentrations 0.1-150 μM were used. All solutions used in the experiments were prepared with a suitable for cell line cultivation medium and the main stock was dissolved in water containing 5% of DMSO

(Sigma-Aldrich). For death induction cells were incubated in media without (spontaneous death) or with analog for 48 and/or 72 hrs at 37°C. After that time cells were harvested and used for analysis.

Dacarbazine (Sigma-Aldrich) as the melanoma chemotherapeutic was used against Ab and Ma melanoma lines[19]. Cisplatine (Sigma-Aldrich) as the neuroblastoma drug was used against NC and DC SHSY5 neuroblastoma lines[20].

Cells viability assay (XTT)

Cell viability was determined by XTT assay (Roche Diagnostic, USA), which measures cells ability to reduce tetrazolium salt XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide) to water-soluble formazan product. Cells were seeded at a density of $5x10^3$ neuroblastoma, Ab melanoma and $50x10^3$ Ma melanoma cells into 96-well plates with suitable cultivation media. After 24 hrs the media were exchanged and cells were stimulated with appropriate concentrations (0,1; 1; 10; 20; 40; 50; 100; 150 μM) of the tested analogs for 48 and 72 hrs. Orange-colored formazan product was quantified at 450 nm in a micro plate reader (Multiscan FC, Thermo scientific USA). Cells proliferation was normalized with respect to the non-treated control (100%) and the half maximal inhibitory concentration IC_{50} was estimated. For analogs where IC50 was not possible to determine the percentage of living cells after treatment with 150 μM concentration was calculated.

RESULTS AND DISCUSSION

Chemistry

Synthesis of adequate protected retro-tuftsin and tuftsin derivatives (1, 2a-c) (Figure No.1) were prepared by using mixed anhydride method with isobutyl chloroform ate and *N*-methylmorpholine (NMM) in anhydrous *N,N*-dimethylformamide (DMF). *Tert*-butyloxycarbonyl protecting (Boc) group was used to protected α-amino groups of amino acids, while to protect ε-amino group of lysine we used fluorenylmethyloxycarbonyl group (Fmoc)[21-23].

The synthetic route for the synthesis of 4-methyl-1-nitroacridine linked to tuftsin/retro-tuftsin derivatives (8, 10a-c) was carried out according to reaction presented in (Scheme No.2).

4-Methyl-1-nitro-9-phenoxyacridine (6) was synthesized in three steps: Ullmann reaction, cyclization of *N*-(2'-methyl-5'-nitro-phenyl) anthranilic acid (5) and conversion of 9-chloro-4-methyl-1-nitroacridine to 4-methyl-1-nitro-9-phenoxyacridine (6) (Scheme No.1)[24]. Then, acridine (6) (1 equivalent) reacted with the corresponding peptide (1, 2a, 2b or 2c) (2 equivalent) in phenol at 50°C to obtain derivatives (7, 9a-c) in 56-69% yields. Compounds (7, 9a-c) were selectively deprotected and were dissolved in MeOH and performed to the corresponding hydrochloride (9, 10a-c) by using HCl in anhydrous Et_2O in 88-92% yields. The presence of final products (8, 10a-c) were confirmed by mass spectrometry analysis and their purity by HPLC.

Biological research

Among broad scale of targets for testing new drugs we chose melanoma and neuroblastoma that have some biological variants that clinically are treated the same ways and there are still not satisfactory systemic therapies against these tumors. According to abovementioned we planned to compare two melanomas: melanotic, amelanotic and two neuroblastomas: dopaminergic and cholinergic, differing in many biological features. The Bomirski hamster melanomas model has two basic forms: melanotic Ma (produces melanin) and amelanotic Ab (lack of melanin production). The amelanotic form is more aggressive than the dominating melanotic melanoma and both forms are presented also in human melanoma. SH-SY5Y is a neuroblast-like cell line of the dopaminergic phenotype (NC variant, SH-SY5Y-NC, mainly uses the neurotransmitter dopamine) but can be differentiated to cholinergic phenotype in vitro (DC variant SH-SY5Y-DC, mainly uses the neurotransmitter acetylcholine). The cytotoxicity of synthesized analogs was estimated by XTT test. Dacarbazine and cisplatine remain the standard chemotherapies for melanoma and neuroblastoma respectively thus the cytotoxicity of these drugs against examined cells has been estimated.

Cells viability assay (XTT)

As shown in Table No.1 among synthesized analogs only (8) decreased cells viability at concentration that allowed to estimate the IC_{50} doses as 88 μM, 116 μM for amelanotic Ab melanoma and DC form of SH-SY5Y neuroblastoma respectively after 72hrs incubation. In comparison to Ab melanoma, (8) at almost twofold higher dose (150 μM) influenced only 35% of melanotic Ma melanoma cells by mitochondrial activity inhibition. Thus amelanotic melanoma was more sensitive to (8) action than melanotic melanoma. It is worth to stressed that dacarbazine (basic systemic chemotherapy agent in melanoma) IC_{50} dose against amelanotic Ab line cells was only a little lower (69 μM) than analog (8) (88 μM). Additionally analog (8) influenced the same percentage of melanotic Ma cells as dacarbazine - over 60% of cells have no signs of mitochondrial damage.

Analog (8) also was cytotoxic against dopaminergic and cholinergic neuroblastoma variants, with higher sensitivity of the last one. Analog (8) inhibited growth of 50% of cholinergic SH-SY5Y-DC neuroblastoma cells at a concentration 116 μM while 30% more (150 μM) inhibited only 18% of dopaminergic SH-SY5Y-NC neuroblastoma. Thus SH-SY5Y-DC are more sensitive to (8) than SH-SY5Y-NC neuroblastoma. Among neuroblastoma lines cholinergic SH-SY5Y-DC cells were also more sensitive to cisplatine because after 48 hrs IC_{50} reaches about 2 μM while dopaminergic SH-SY5Y-NC cells got IC_{50} dose 2.5 μM after 72 hrs. Among tested analogs only for (8) we were able to estimate IC_{50} doses as 116 μM that was much higher than for cisplatine but referring to the serious side effects of this chemotherapeutic it could be the promising biological feature. Analog (10a) seemed to influence both melanoma lines at the highest dose - over 40% of cells have inhibited mitochondrial activity. Similar sensitivity to this analog have SH-SY5Y DC neuroblastoma form.

Analogs (10c) and (10b) at 150 μM dose influenced mitochondria in over 30% and over 40% of Ab melanoma and SH-SY5Y-NC neuroblastoma respectively. While under the same circumstances only over 20% of melanotic Ma melanoma and SH-SY5Y-DC neuroblastoma reflected changes in mitochondria. Thus it seemed that among tested lines SH-SY5Y-NC neuroblastoma was the most sensitive to analogs (10c) and (10b). Thus to sum up it seems that analog (8) could be considered for further testing as the potential drug against melanoma, especially against the melanoma with inhibited melanogenesis - amelanotic melanoma that is more aggressive than the melanotic melanoma.

Table No.1: Analogs influence on melanoma and neuroblastoma cells viability by XTT assay.[a,b,c,d]

Analog	Melanoma						Neuroblastoma SHSY5Y			
	Melanotic (Ma line)		Amelanotic (Ab line)				Dopaminergic (NC line)		Cholinergic (DC) line	
	% of viability with 150 µM		IC_{50} [µM]		% of viability with 150 µM		IC_{50} [µM]	% of viability with 150 µM	IC_{50} [µM]	% of viability with 150 µM
	48h	72h	48h	72h	48h	72h	72h	72h	72h	72h
8	77.2±17.7	65.3±11.7	109.6±30.0	87.5±25.4				82.6±5.4	116±15.7	
10a	57.4±10.1	58.0±10.0			79.6±6.3	55.3±24.0		63.5±8.1		53.6±7.0
10b	75.9±8.9	75.0±18.2			73.6±17.7	65.1±20.2		54.6±25.8		79.1±23.0
10c	74.1±22.1	70.7±14.2			78.2±16.6	66.1±37.2		53.6±12.2		75.7±30.4
Dacarbazine	71.8±17.4	69.4±10.0		68.5±20.9	47.5±9.7		ne	ne	ne	ne
Cisplatine	ne	ne	ne	ne	ne	ne	2.5±0.6		1.8±0.9*	

[a]Melanotic (Ma) and amelanotic (Ab) melanoma cells and cholinergic (NC) and dopaminergic (DC) neuroblastoma SHSY5Y cells were used as targets.
[b]Values are means ± SD from at least 3 experiments.
[c]IC_{50} = half maximal inhibitory concentration; ne analog not estimated;
[d]*according to observed cells morphology changes test has been finished after 48 hrs.

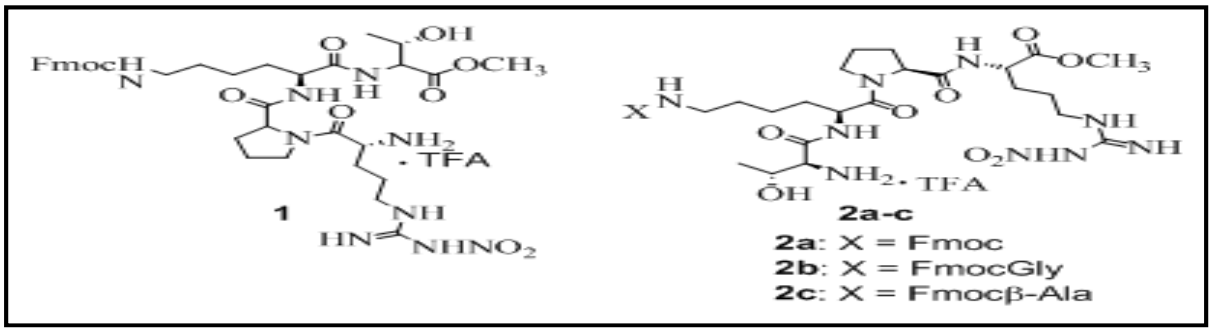
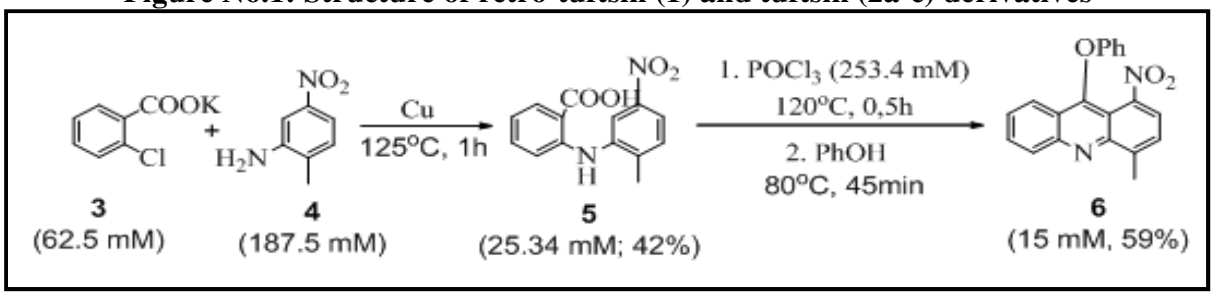
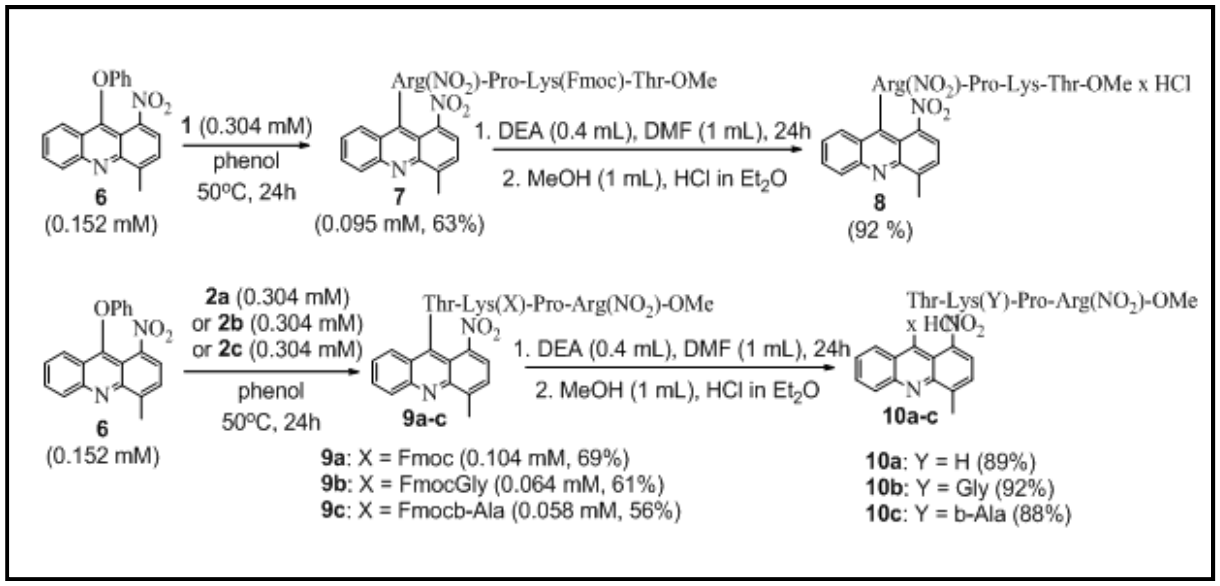


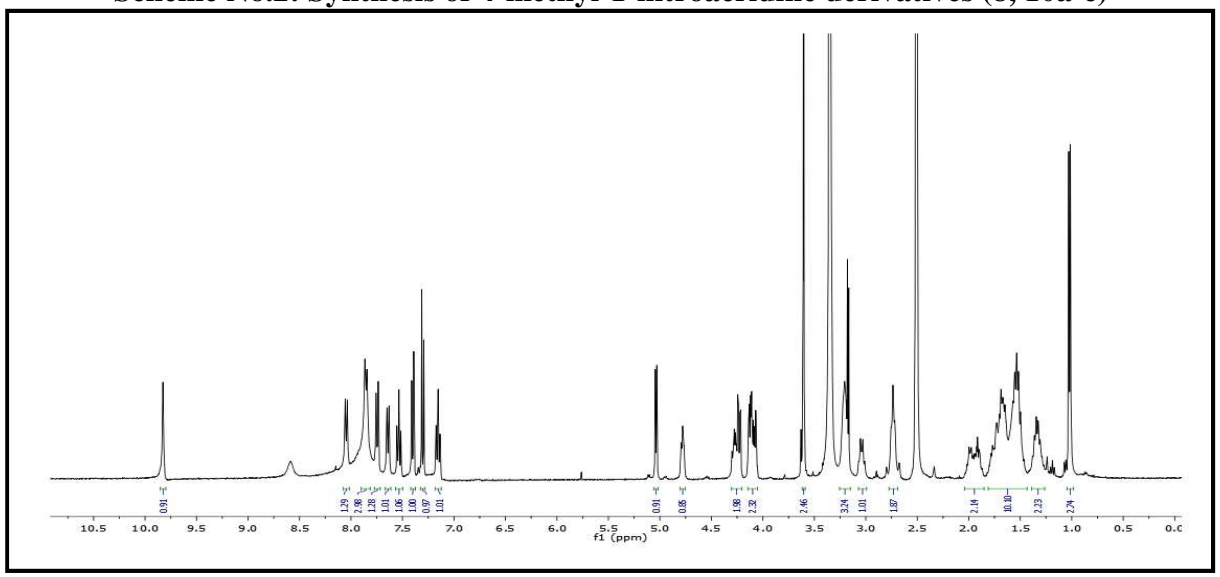
Figure No.1: Structure of retro-tuftsin (1) and tuftsin (2a-c) derivatives

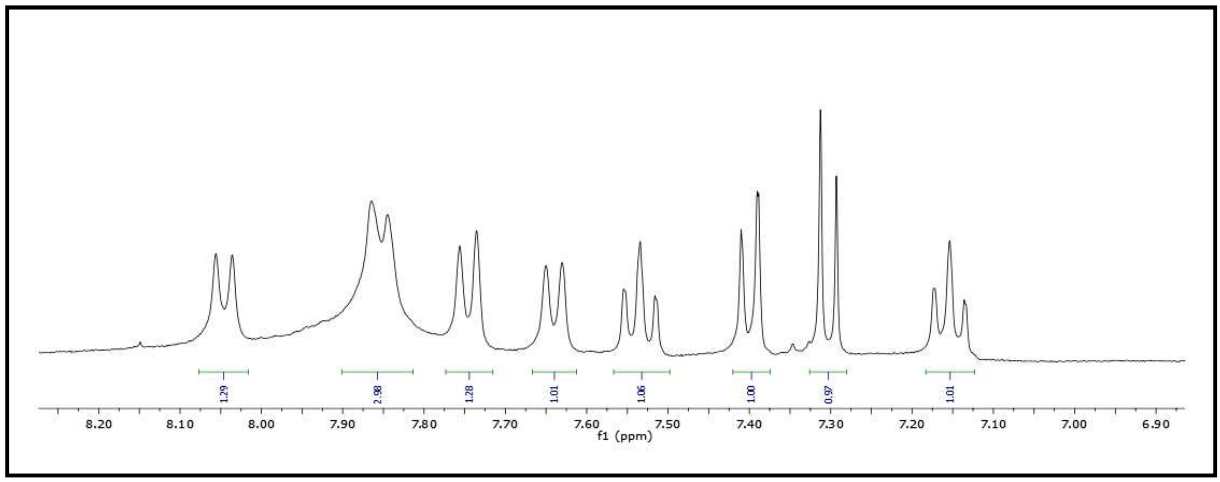


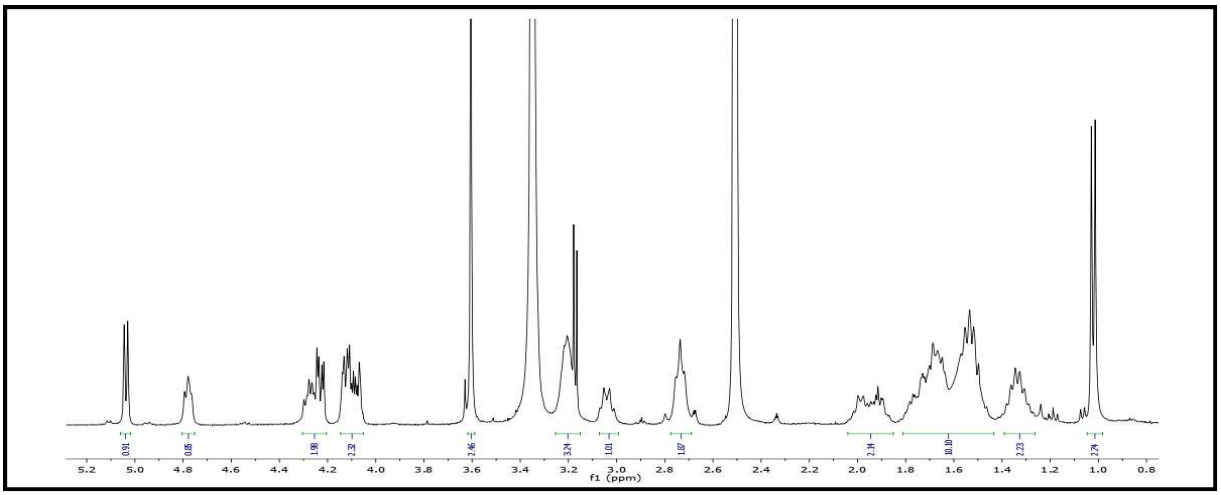
Scheme No.1: Synthesis of 4-methyl-1-nitro-9-phenoxyacridine (6)



Scheme No.2: Synthesis of 4-methyl-1-nitroacridine derivatives (8, 10a-c)



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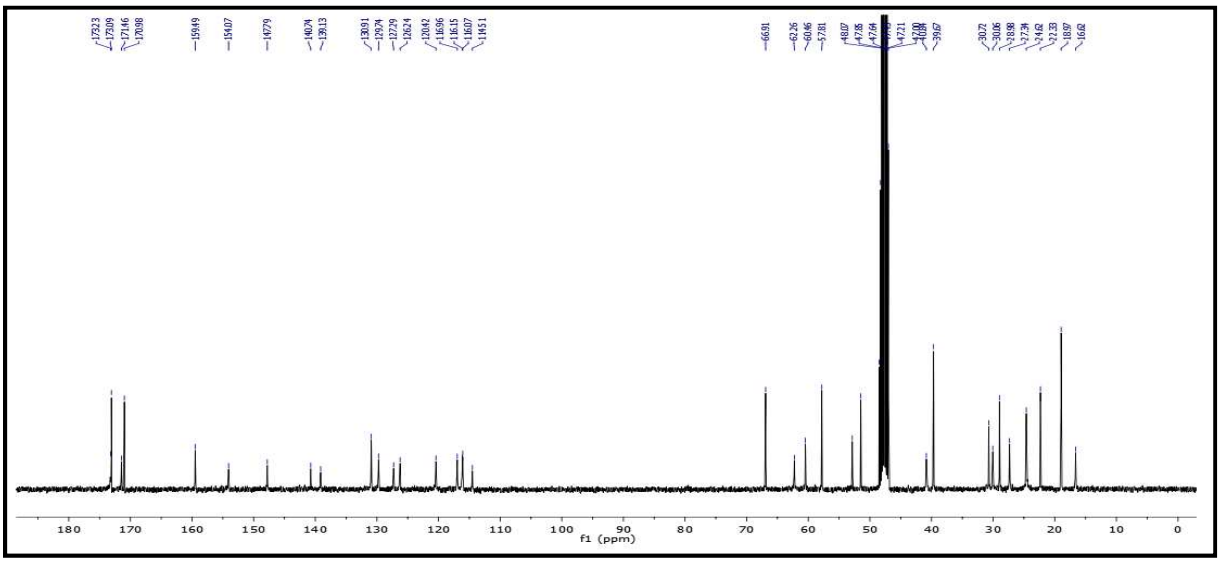
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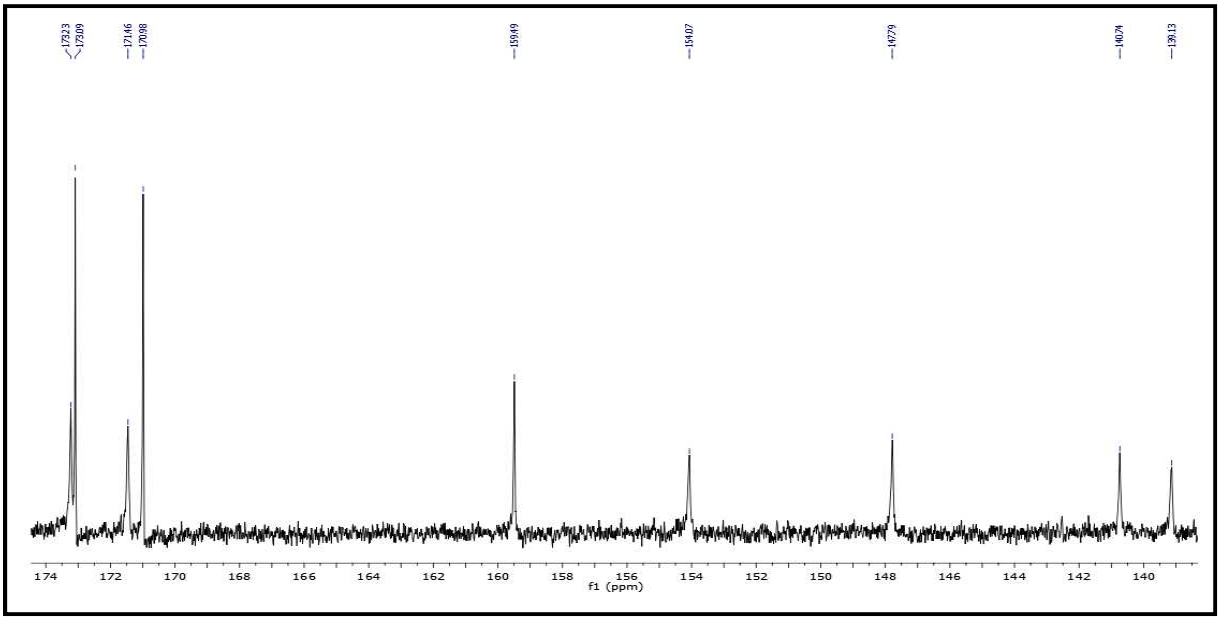
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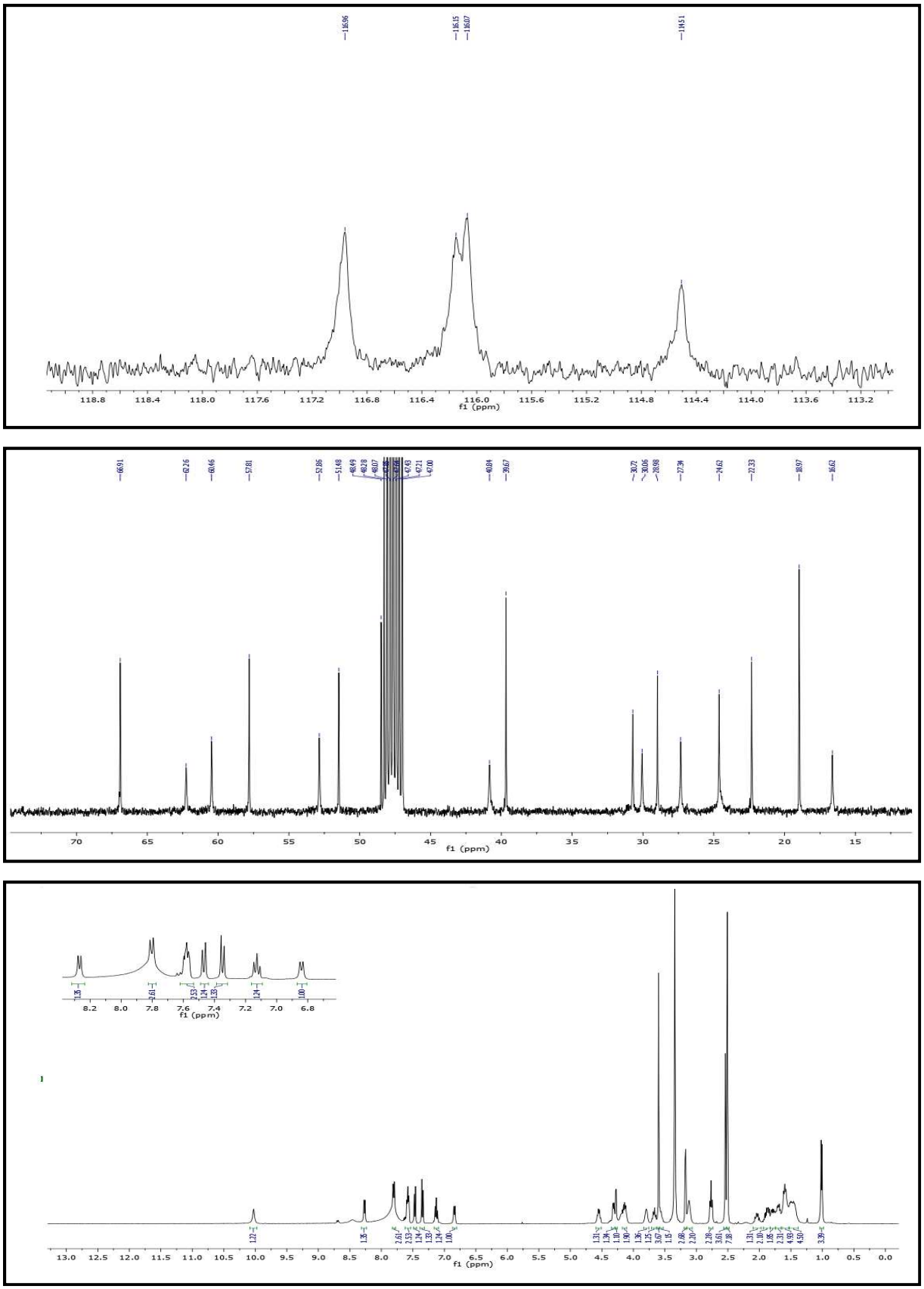
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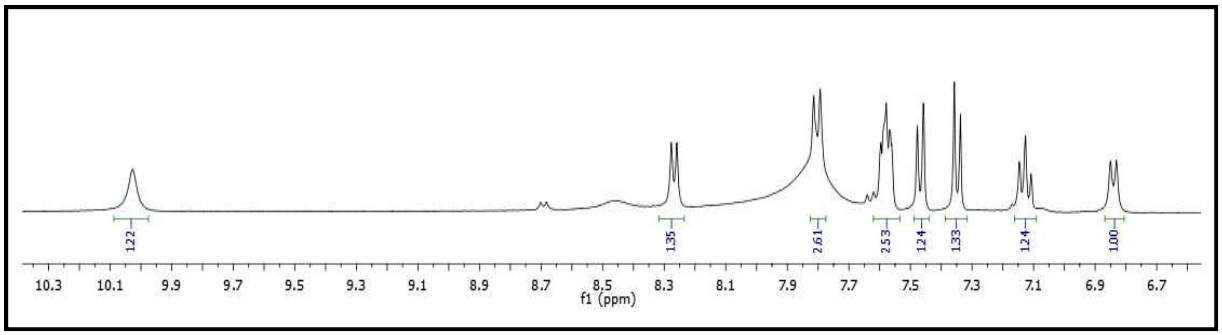
f1 (ppm)
f1 (ppm)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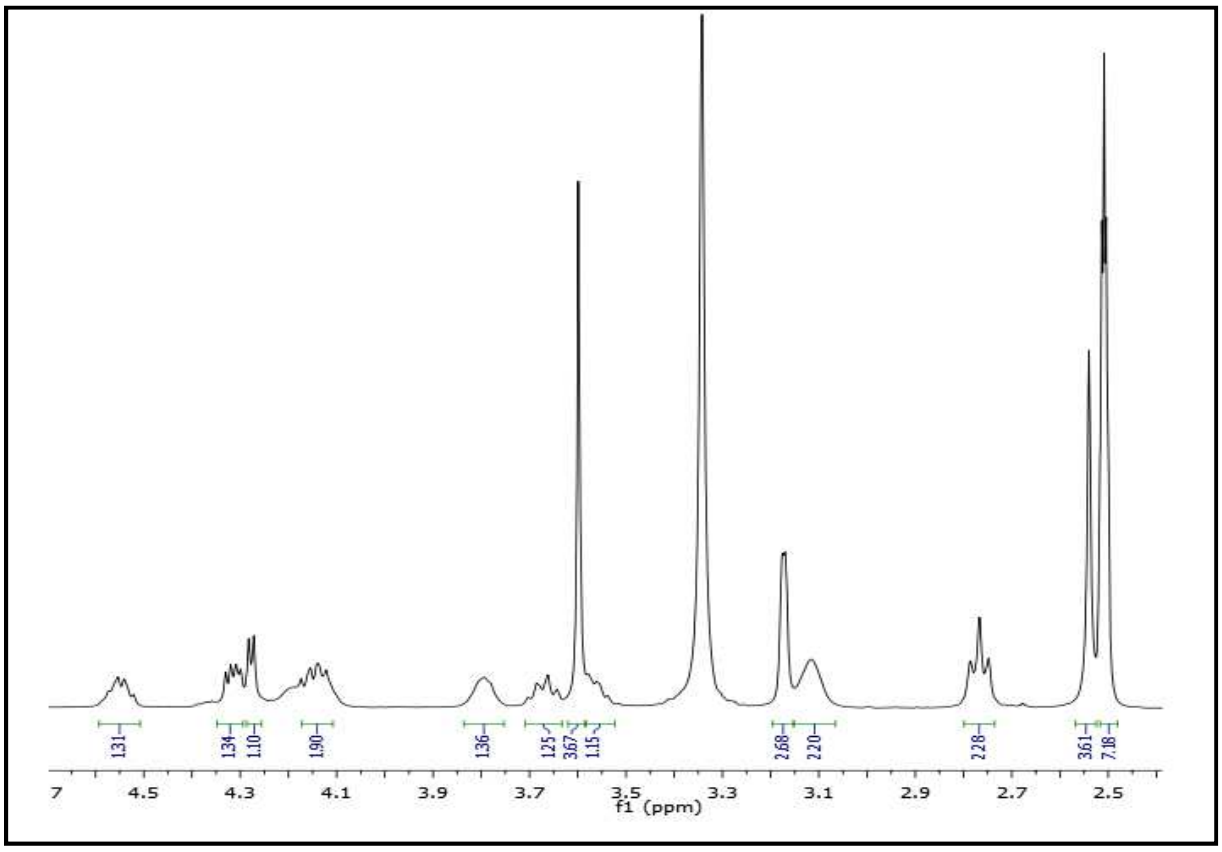
f1 (ppm)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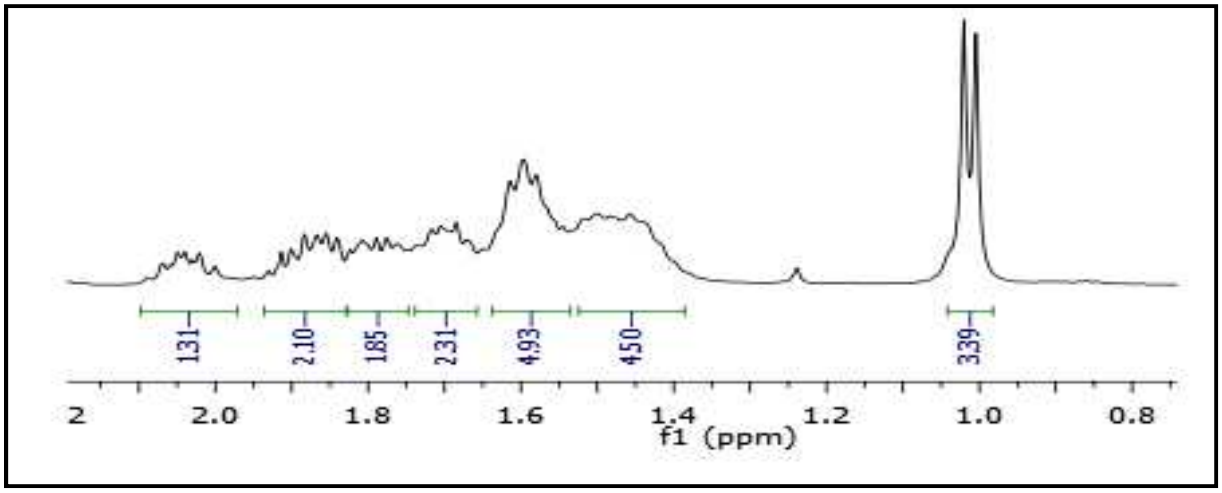
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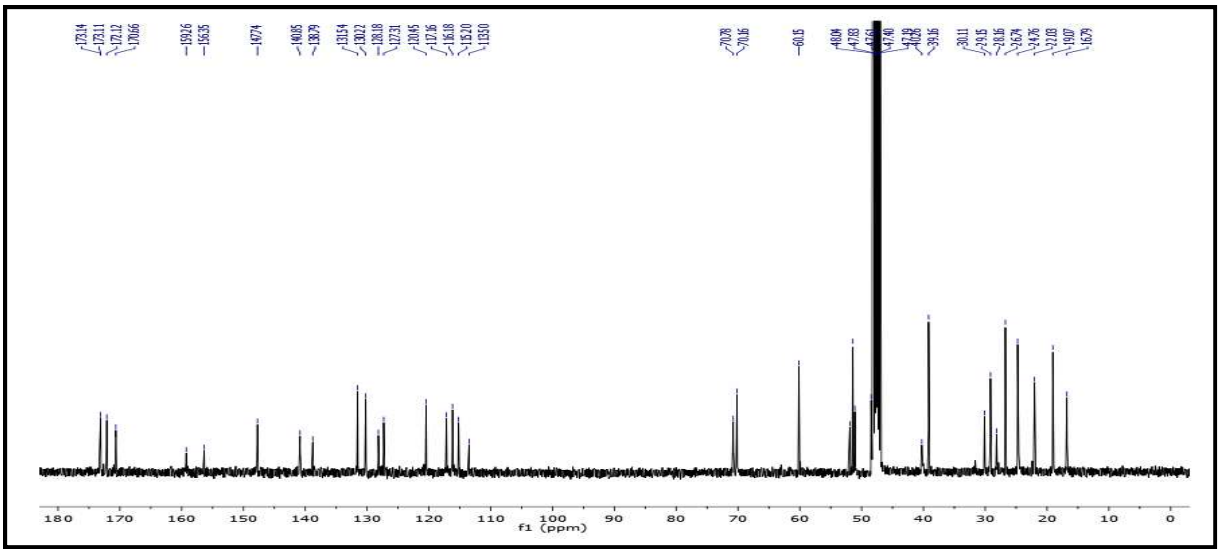
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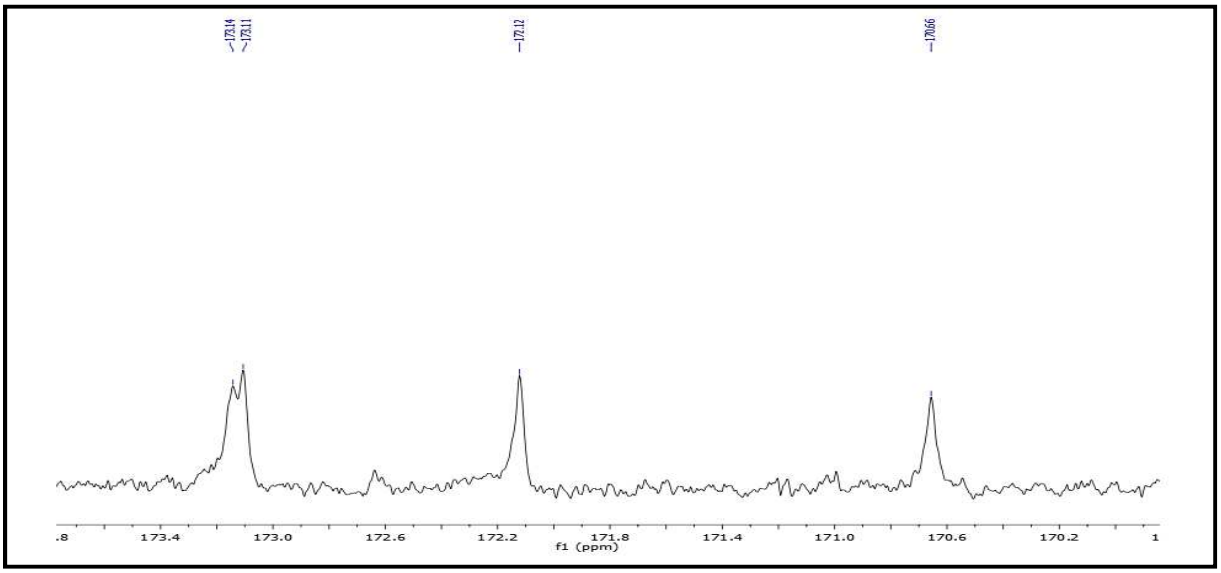
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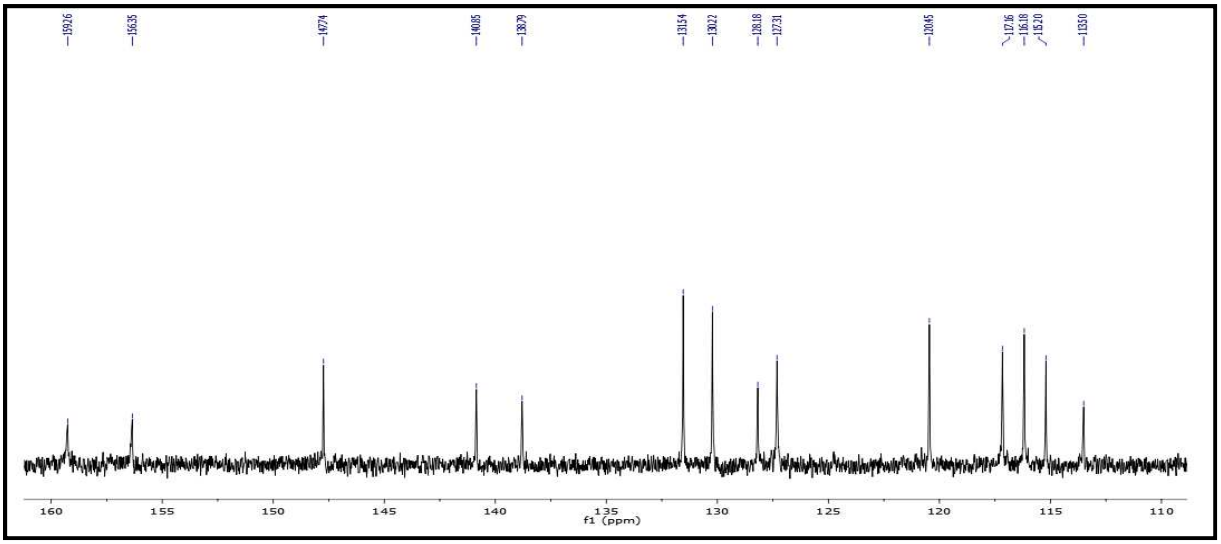
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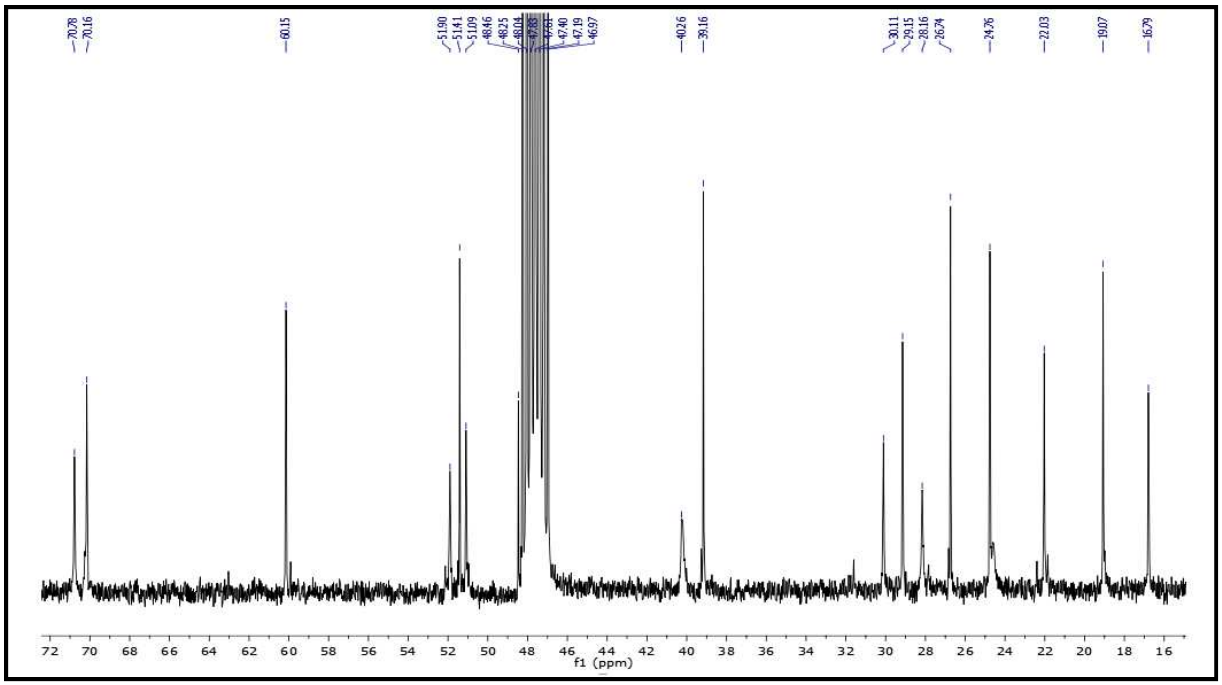
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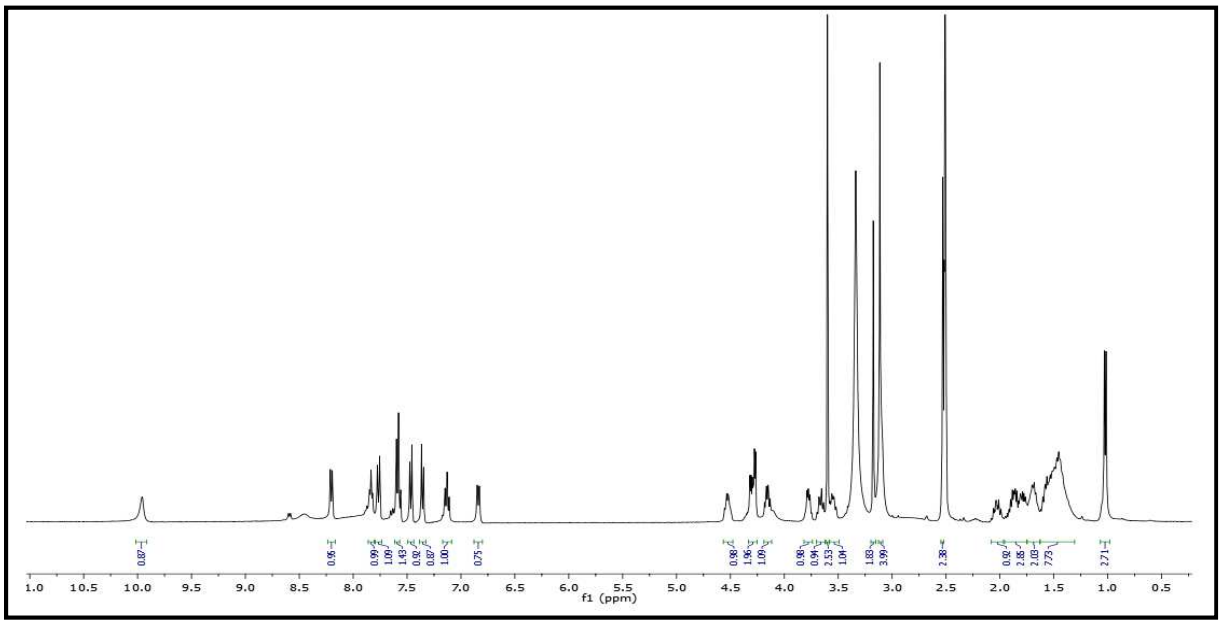
f1 (ppm)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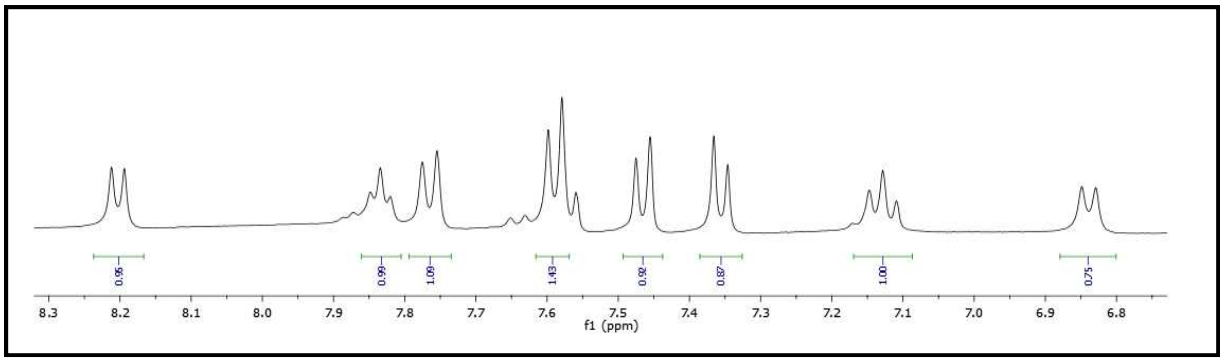
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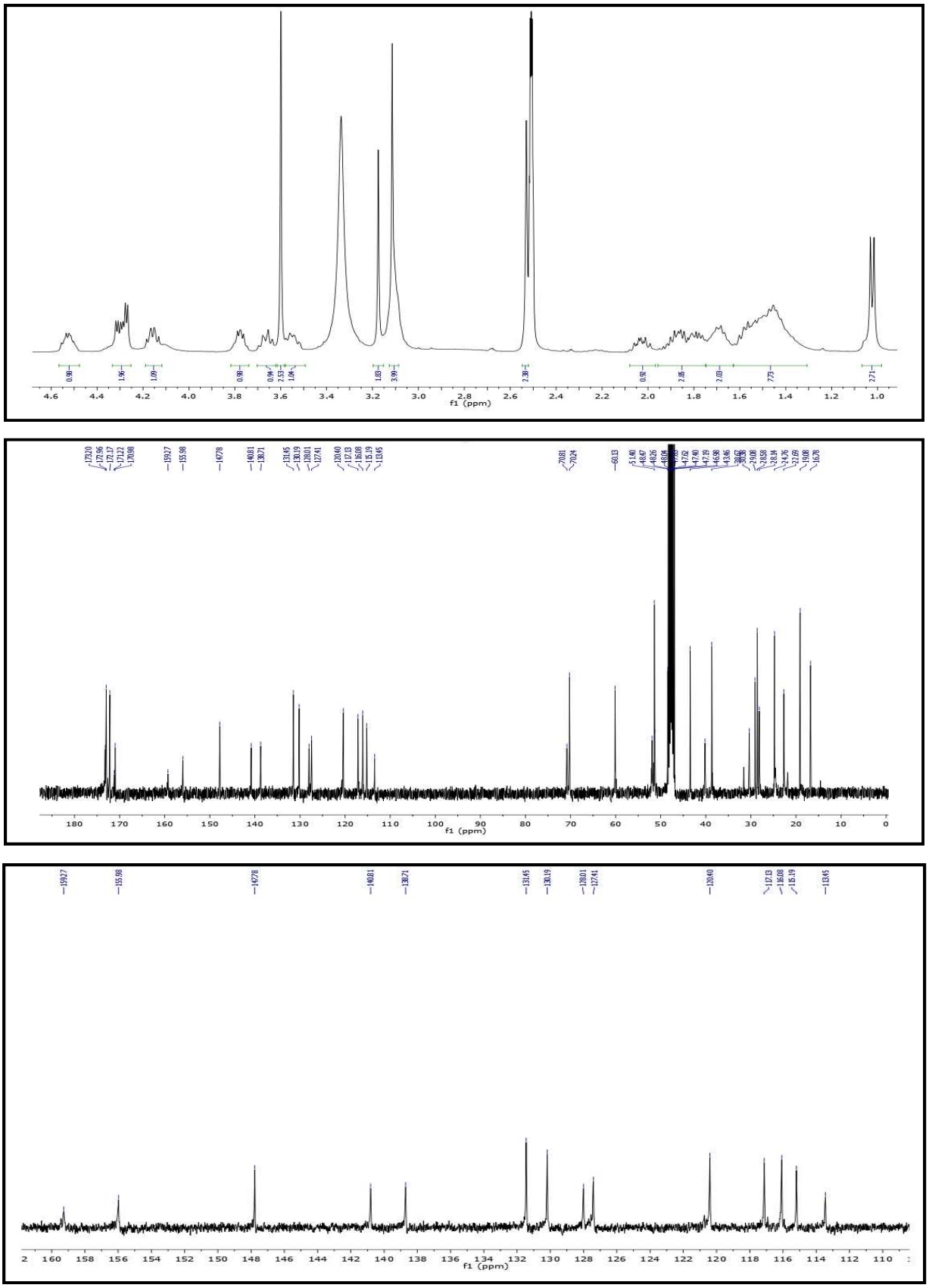
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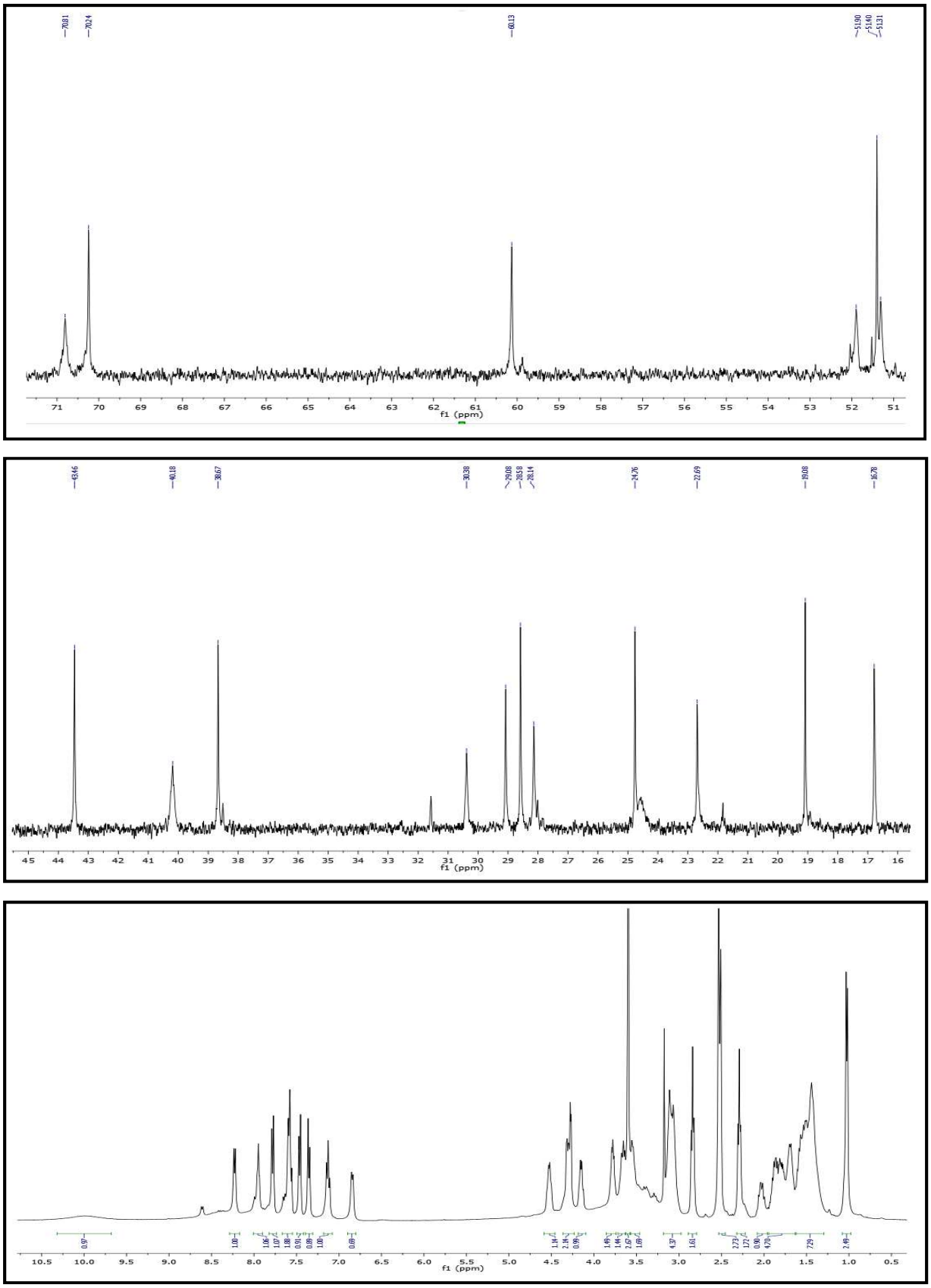
f1 (ppm)
f1 (ppm)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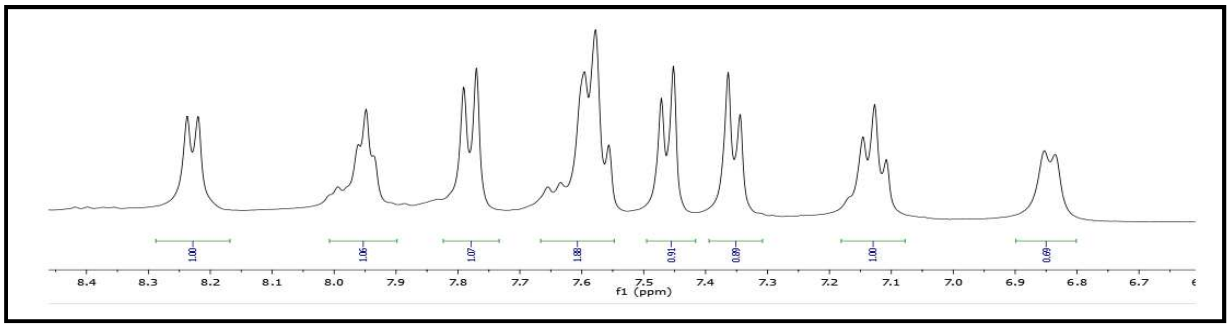
f1 (ppm)
f1 (ppm)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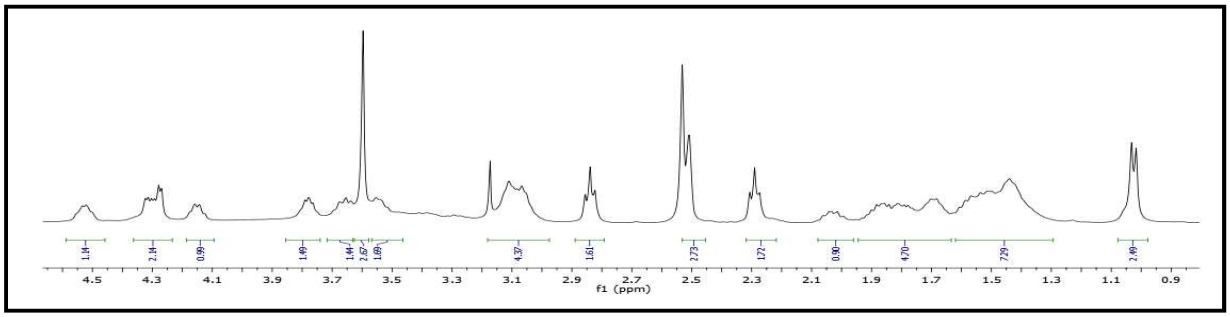
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7.29
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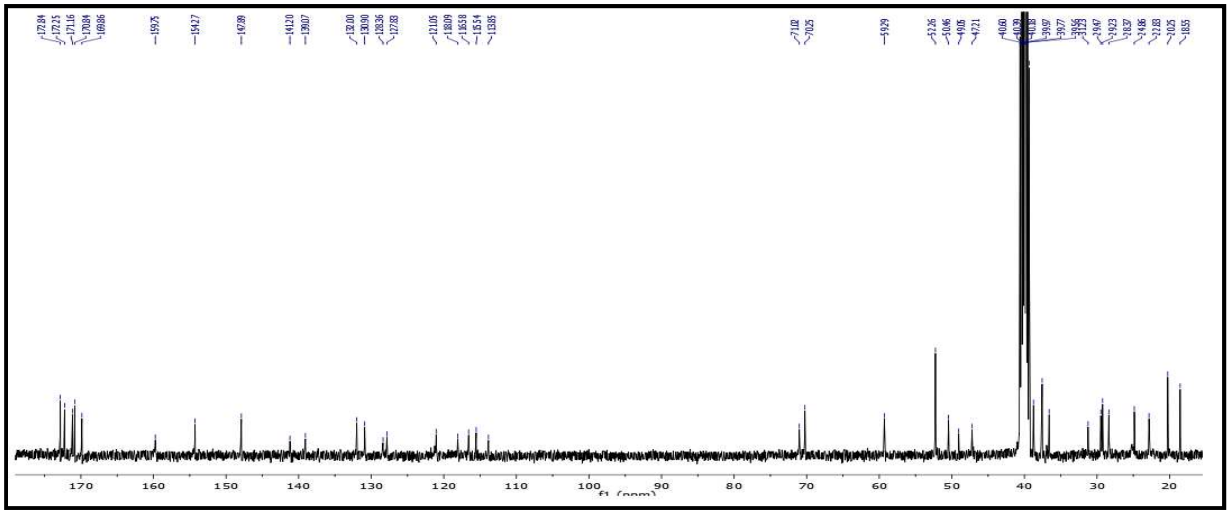
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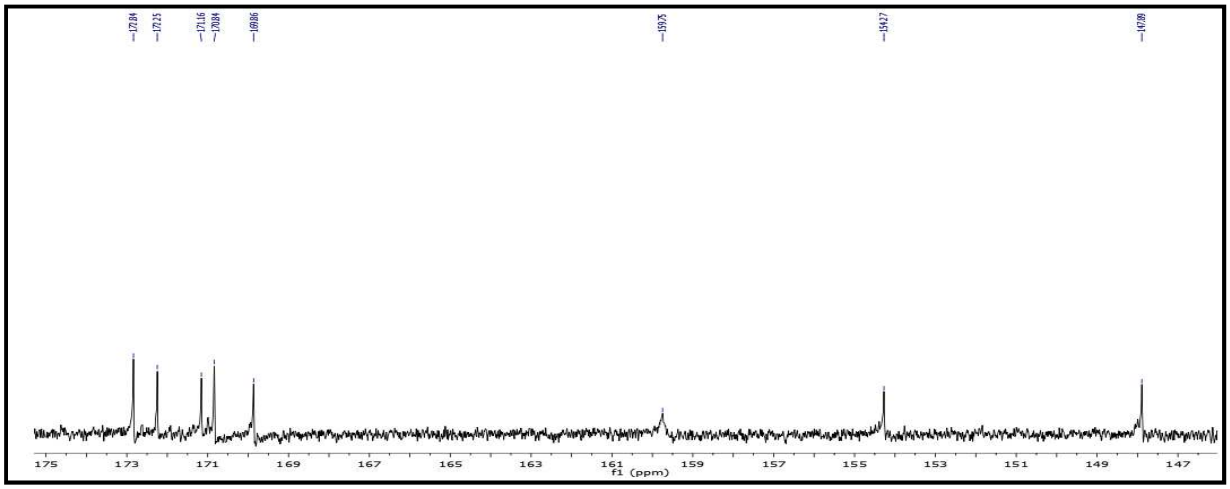
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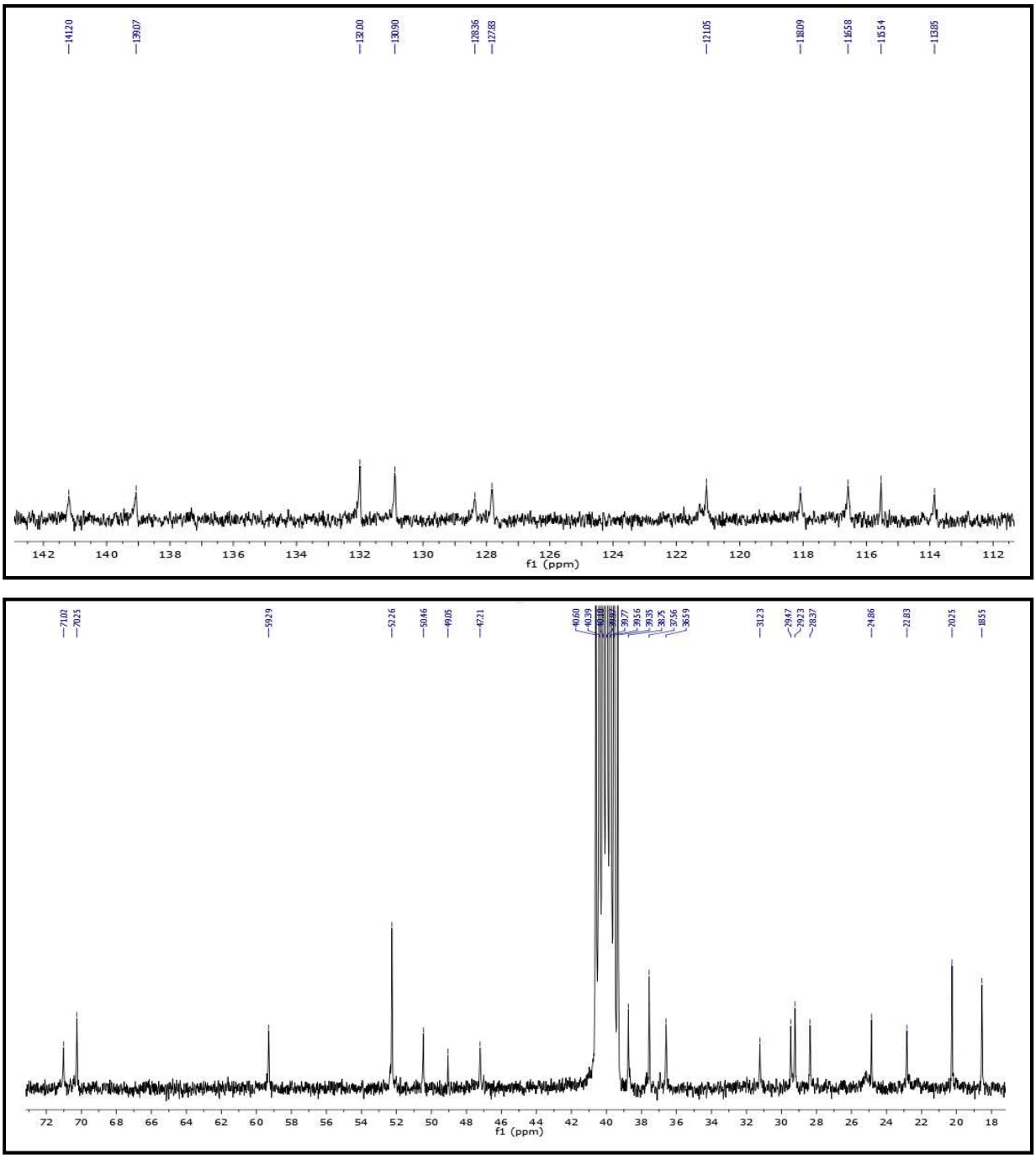

CONCLUSION

This article concerns new analogues of 4-methyl-1-nitroacridine containing tuftsin derivatives. Among the investigated analogs compound (8) exhibited the highest potency comparable to dacarbazine action for amelanotic Ab melanoma cells.

ACKNOWLEDGMENT

This work was financially supported by the National Science Center (Poland), grant no 2014/13/B/NZ7/02234.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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