Supplemental Materials

Novel Ethyl 2-Hydrazineylidenethiazolidin-5-ylidene Acetate Clubbed With Coumarinylthiazolyl Pyrazole System As Potential VEGFR-2 Inhibitors and Apoptosis Inducer: Synthesis, Cytotoxic Evaluation, Cell Cycle, Autophagy, In Silico ADMET and Molecular Docking Studies

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1. Experimental and characterization of the synthesized compounds 5a-h.

General Marks

The melting points were determined in an open capillary tube on a digital Stuart SMP-3 apparatus. IR spectra were measured on FT-IR (Nicolet IS10) spectrophotometer using ATR technique. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker spectrometer (400 and 100 MHz), using DMSO-d6 as a solvent and TMS (δ) as an internal standard. Mass spectra were recorded on direct probe controller inlet part to single quadropole mass analyzer in (thermo scientific GCMS). Elemental microanalyses were performed Perkin-Elmer 2400II at the Chemical War department, Ministry of Defense. The purity of the synthesized compounds was checked by thin layer chromatography (TLC) and elemental microanalysis.

The synthesis of 3-(2-oxo-2*H*-chromen-3-yl)-1-(4-phenylthiazol-2-yl)-1*H*-pyrazole-4-carboxaldehyde (3).

A mixture of 3-acetylcoumarin (**1**) (0.94 g, 5 mmol), thiosemicarbazide (0.46 g, 5 mmol) and phenacyl bromide (5 mmol) in absolute ethanol (20 ml) containing a few drops of glacial acetic acid, was heated under reflux for 4 h. The formed hydrazone **2** was filtered off and dried. A solution of DMF (15 ml) and $POCl_3$ (1.5 ml, 16 mmol) was allowed to stir at 0−5 ^{o}C for 15 minutes. After that the hydrazones **2** (4 mmol) was added at room temperature. The reaction mixture was warmed under stirring at 50 ^{o}C for 6 hours. The reaction mixture was poured into beakers containing crushed ice or ice-cold water and neutralized with sodium acetate. The formed solid was filtered off and washed several times with water. The crude product was dried and crystallized from acetic acid to afford the target aldehyde **3** as beige solid in 77% yield, mp 226−228 ^{o}C [43]. IR (KBr), (*v* max, cm^{-1}): 3093 (C–H_{arom}), 2987, 2899 (C−$H_{aldehyde}$), 1716 (C=$O_{coumarin}$), 1694 (C=$O_{aldehyde}$), 1605, 1538 (C=C), 1572 (C=N). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.38–7.52 (m, 5H, Ph–H, H–$6_{coumarin}$ and H–$8_{coumarin}$), 7.71 (t, 1H, *J*=8.0 Hz, H–$7_{coumarin}$), 7.90 (d, 1H, *J*=7.2 Hz, H–$5_{coumarin}$), 8.02 (d, 2H, *J*=8.0 Hz, Ph–H), 8.10 (s, 1H, H–$5_{thiazole}$), 8.44 (s, 1H, H–$4_{coumarin}$), 9.37 (s, 1H, H–$5_{pyrazole}$), 9.98 (s, 1H, CHO). ^{13}C-NMR (100 MHz, DMSO-

d_6): δ 113.5 (C–$5_{thiazole}$), 119.2 (C–$8_{coumarin}$), 119.7 (C–$3_{coumarin}$), 120.8 (C–$4a_{coumarin}$), 124.4 (C–$4_{pyrazole}$), 125.5 (C–$6_{coumarin}$), 126.5 (C–4_{phenyl}), 127.7 (C–$2,6_{phenyl}$), 128.6 (C–$5_{coumarin}$), 129.1 (C–1_{phenyl}), 129.5 (C–$3,5_{phenyl}$), 133.9 (C–$7_{coumarin}$), 146.9 (C–$4_{coumarin}$), 149.1 (C–$5_{pyrazole}$), 150.0 (C–$4_{thiazole}$), 152.3 (C–$3_{pyrazole}$), 154.2 (C–$8a_{coumarin}$), 159.5 (C=$O_{coumarin}$), 166.9 (C–$2_{thiazole}$), 189.9 (C=$O_{aldehyde}$). MS (*m/z*, I %): 399 (M^+, 17%). Anal. Calcd for $C_{22}H_{13}N_3O_3S$ (399.42): C, 66.16%; H, 3.28%; N, 10.52%; S, 8.03%. Found: C, 66.03%; H, 3.16%; N, 10.35%; S, 7.89%.

General procedure for the synthesis of products 5a-h.

A mixture of 3-(2-oxo-2*H*-chromen-3-yl)-1-(4-phenylthiazol-2-yl)-1*H*-pyrazole-4-carboxaldehyde (**3**) (0.39 g, 1.0 mmol), thiosemicarbazide derivative **4a-h** (1 mmol) and diethyl acetylene dicarboxylate (1.1 mmol) in acetic acid (20 ml) was heated under reflux for 5 h. The reaction mixtures were cooled to room temperature. The formed solids were filtered off and crystallized from ethanol to afford the target compounds.

Ethyl 2-{4-oxo-2-(2-((3-(2-oxo-2*H*-chromen-3-yl)-1-(4-phenylthiazol-2-yl)-1*H*-pyrazol-4-yl)methylene)hydrazineyl)thiazol-5(4*H*)-ylidene}acetate (5a). Yellow solid in 67% yield, mp > 300 °C. IR (KBr), (*ν* max, cm^{-1}): 3116 (NH), 3002 (C–H_{arom}), 2946, 2938 (C–H_{aliph}), 1726 (C=$O_{thiazolidinone}$), 1716 (C=$O_{coumarin}$), 1691 (C=O_{ester}), 1644 (CH=$N_{exocyclic}$), 1609, 1578, 1544 (C=N, C=C). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.25 (t, 3H, *J*=6.8 Hz, CH_3), 4.19 (q, 2H, *J*=6.8 Hz, OCH_2), 6.54 (s, 1H, =$CH_{exocyclic}$), 7.40 (t, 2H, *J*=6.8 Hz, Ph–H), 7.47–7.52 (m, 3H, Ph–H, H–$6_{coumarin}$ and H–$8_{coumarin}$), 7.67 (t, 1H, *J*=7.2 Hz, H–$7_{coumarin}$), 7.86 (d, 1H, *J*=7.2 Hz, H–$5_{coumarin}$), 8.00–8.02 (m, 3H, Ph–H and H–$5_{thiazole}$), 8.36 (s, 1H, CH=$N_{exocyclic}$), 8.54 (s, 1H, H–$4_{coumarin}$), 9.09 (s, 1H, H–$5_{pyrazole}$), 12.69 (brs, 1H, NH). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 14.5 (CH_3), 61.6 (CH_2), 112.7 (C–$5_{thiazole}$), 117.2 (C–$8_{coumarin}$), 119.0 (C–$3_{coumarin}$), 119.8 (C–$4a_{coumarin}$), 120.7 (C–$4_{pyrazole}$), 122.5 (=$CH_{exocyclic}$), 125.1 (C–4_{phenyl}), 126.4 (C–$2,6_{phenyl}$), 128.9 (C–$6_{coumarin}$), 129.3 (C–$3,5_{phenyl}$), 129.9 (C–$5_{coumarin}$), 130.4 (C–1_{phenyl}), 133.8 (C–$7_{coumarin}$), 137.4 (CH=$N_{exocyclic}$), 143.2 (C–$5_{thiazolidinone}$), 143.6 (C–$4_{coumarin}$), 148.6 (C–$5_{pyrazole}$), 151.3

(C–$4_{thiazole}$), 152.2 (C–$3_{pyrazole}$), 154.2 (C–$8a_{coumarin}$), 159.2 (C=$O_{thiazolidinone}$), 159.6 (C=$O_{coumarin}$), 160.2 (C=O_{ester}), 165.5 (C–$2_{thiazole}$), 166.2 (C–$2_{thiazolidinone}$). MS (*m/z*, I%): 596 (M^+, 25%). Anal. Calcd for $C_{29}H_{20}N_6O_5S_2$ (596.64): C, 58.38%, H, 3.38%, N, 14.09%, S, 10.75%. Found: C, 58.30%, H, 3.24%, N, 14.01%, S, 10.66%.

Ethyl 2-{3-methyl-4-oxo-2-(2-((3-(2-oxo-2*H*-chromen-3-yl)-1-(4-phenylthiazol-2-yl)-1*H*-pyrazol-4-yl)methylene)hydrazineyl)thiazol-5(4*H*)-ylidene}acetate (5b). Yellow solid in 84% yield, mp 277-278 °C. IR (KBr), (*v* max, cm^{-1}): 3092 (C–H_{arom}), 2986, 2948, 2899 (C–H_{aliph}), 1717 (C=$O_{thiazolidinone}$), 1706 (C=$O_{coumarin}$), 1699 (C=O_{ester}), 1632 (CH=$N_{exocyclic}$), 1617, 1606, 1578 (C=N, C=C). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.26 (t, 3H, *J*=6.8 Hz, CH_3), 3.27 (s, 3H, NCH_3), 4.21 (q, 2H, *J*=7.2 Hz, OCH_2), 6.65 (s, 1H, =$CH_{exocyclic}$), 7.42 (t, 2H, *J*=7.2 Hz, Ph–H), 7.49–7.54 (m, 3H, Ph–H, H–$6_{coumarin}$ and H–$8_{coumarin}$), 7.69 (t, 1H, *J*=6.8 Hz, H–$7_{coumarin}$), 7.88 (d, 1H, *J*=8.4 Hz, H–$5_{coumarin}$), 8.04 (d, 2H, *J*=7.6 Hz, Ph–H), 8.06 (s, 1H, H–$5_{thiazole}$), 8.40 (s, 1H, CH=$N_{exocyclic}$), 8.64 (s, 1H, H–$4_{coumarin}$), 9.15 (s, 1H, H–$5_{pyrazole}$). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 14.2 (CH_3), 36.2 (NCH_3), 62.3 (OCH_2), 112.8 (C–$5_{thiazole}$), 117.4 (C–$8_{coumarin}$), 119.3 (C–$3_{coumarin}$), 119.6 (C–$4a_{coumarin}$), 120.6 (C–$4_{pyrazole}$), 122.1 (=$CH_{exocyclic}$), 125.4 (C–4_{phenyl}), 127.2 (C–$2,6_{phenyl}$), 128.4 (C–$6_{coumarin}$), 129.4 (C–$5_{coumarin}$), 130.1 (C–$3,5_{phenyl}$), 131.4 (C–1_{phenyl}), 132.8 (C–$7_{coumarin}$), 137.0 (CH=$N_{exocyclic}$), 142.0 (C–$5_{thiazolidinone}$), 143.0 (C–$4_{coumarin}$), 148.2 (C–$5_{pyrazole}$), 151.2 (C–$4_{thiazole}$), 152.8 (C–$3_{pyrazole}$), 153.7 (C–$8a_{coumarin}$), 159.1 (C=$O_{thiazolidinone}$), 159.4 (C=$O_{coumarin}$), 160.7 (C=O_{ester}), 165.9 (C–$2_{thiazole}$), 166.7 (C–$2_{thiazolidinone}$). MS (*m/z*, I%): 610 (M^+, 7%). Anal. Calcd for $C_{30}H_{22}N_6O_5S_2$ (610.66): C, 59.01%, H, 3.63%, N, 13.76%, S, 10.50%. Found: C, 58.86%, H, 3.49%, N, 13.59%, S, 10.39%.

Ethyl 2-{3-ethyl-4-oxo-2-(2-((3-(2-oxo-2*H*-chromen-3-yl)-1-(4-phenylthiazol-2-yl)-1*H*-pyrazol-4-yl)methylene)hydrazineyl)thiazol-5(4*H*)-ylidene}acetate (5c). Yellow solid in 82% yield, mp 252-253 °C. IR (KBr), (*v* max, cm^{-1}): 3132 (C–H_{arom}), 2984, 2937, 2905

(C–H_{aliph}), 1754 (C=$O_{thiazolidinone}$), 1720 (C=$O_{coumarin}$), 1704 (C=O_{ester}), 1636 (CH=$N_{exocyclic}$), 1624, 1607, 1574 (C=N, C=C). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.20 (t, 3H, *J*=7.2 Hz, CH_3), δ 1.26 (t, 3H, *J*=7.2 Hz, CH_3), 3.84 (q, 2H, *J*=6.8 Hz, NCH_2), 4.21 (q, 2H, *J*=7.2 Hz, OCH_2), 6.64 (s, 1H, =$CH_{exocyclic}$), 7.41 (t, 2H, *J*=7.2 Hz, Ph–H), 7.48–7.54 (m, 3H, Ph–H, H–$6_{coumarin}$ and H–$8_{coumarin}$), 7.69 (t, 1H, *J*=8.0 Hz, H–$7_{coumarin}$), 7.87 (d, 1H, *J*=7.6 Hz, H–$5_{coumarin}$), 8.02 (d, 2H, *J*=7.6 Hz, Ph–H), 8.05 (s, 1H, H–$5_{thiazole}$), 8.39 (s, 1H, CH=$N_{exocyclic}$), 8.63 (s, 1H, H–$4_{coumarin}$), 9.12 (s, 1H, H–$5_{pyrazole}$). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 13.0 (CH_3), 15.1 (CH_3), 44.7 (NCH_2), 61.6 (OCH_2), 113.7 (C–$5_{thiazole}$), 116.5 (C–$8_{coumarin}$), 119.6 (C–$3_{coumarin}$), 120.1 (C–$4a_{coumarin}$), 120.6 (C–$4_{pyrazole}$), 122.4 (=$CH_{exocyclic}$), 125.4 (C–4_{phenyl}), 127.2 (C–$2,6_{phenyl}$), 128.7 (C–$6_{coumarin}$), 129.3 (C–$5_{coumarin}$), 130.1 (C–$3,5_{phenyl}$), 130.8 (C–1_{phenyl}), 133.2 (C–$7_{coumarin}$), 138.1 (CH=$N_{exocyclic}$), 141.9 (C–$5_{thiazolidinone}$), 143.6 (C–$4_{coumarin}$), 148.2 (C–$5_{pyrazole}$), 150.7 (C–$4_{thiazole}$), 152.8 (C–$3_{pyrazole}$), 154.8 (C–$8a_{coumarin}$), 159.0 (C=$O_{thiazolidinone}$), 159.4 (C=$O_{coumarin}$), 161.2 (C=O_{ester}), 165.9 (C–$2_{thiazole}$), 166.9 (C–$2_{thiazolidinone}$). MS (*m/z*, I%): 624 (M^+, 7%). Anal. Calcd for $C_{31}H_{24}N_6O_5S_2$ (624.69): C, 59.60%, H, 3.87%, N, 13.45%, S, 10.26%. Found: C, 59.43%, H, 3.79%, N, 13.29%, S, 10.09%.

Ethyl 2-{3-allyl-4-oxo-2-(2-((3-(2-oxo-2*H*-chromen-3-yl)-1-(4-phenylthiazol-2-yl)-1*H*-pyrazol-4-yl)methylene)hydrazineyl)thiazol-5(4*H*)-ylidene}acetate (5d). Yellow solid in 79% yield, mp 256-257 ^{o}C. IR (KBr), (*v* max, cm^{-1}): 3102 (C–H_{arom}), 2977, 2941, 2902 (C–H_{aliph}), 1737 (C=$O_{thiazolidinone}$), 1719 (C=$O_{coumarin}$), 1703 (C=O_{ester}), 1631 (CH=$N_{exocyclic}$), 1622, 1607, 1574 (C=N, C=C). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.27 (t, 3H, *J*=6.4 Hz, CH_3), 4.22 (q, 2H, *J*=6.4 Hz, OCH_2), 4.43 (s, 2H, CH_2), 5.13–5.17 (m, 2H, =CH_2), 5.85–5.92 (m, 1H, =CH), 6.68 (s, 1H, =$CH_{exocyclic}$), 7.42 (t, 2H, *J*=6.4 Hz, Ph–H), 7.49–7.54 (m, 3H, Ph–H, H–$6_{coumarin}$ and H–$8_{coumarin}$), 7.69 (t, 1H, *J*=7.2 Hz, H–$7_{coumarin}$), 7.87 (d, 1H, *J*=6.4 Hz, H–$5_{coumarin}$), 8.02–8.06 (m, 3H, Ph–H and H–$5_{thiazole}$), 8.39 (s, 1H, CH=$N_{exocyclic}$), 8.62 (s, 1H, H–$4_{coumarin}$), 9.14 (s, 1H, H–$5_{pyrazole}$). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 14.2 (CH_3), 51.3

(CH_2), 61.2 (CH_2), 112.8 (C–$5_{thiazole}$), 116.9 (C–$8_{coumarin}$), 117.9 (=CH_2), 119.3 (C–$3_{coumarin}$), 120.0 (C–$4a_{coumarin}$), 120.6 (C–$4_{pyrazole}$), 122.1 (=$CH_{exocyclic}$), 125.1 (C–4_{phenyl}), 127.2 (C–$2,6_{phenyl}$), 128.3 (C–$6_{coumarin}$), 130.1 (C–$3,5_{phenyl}$), 129.4 (C–$5_{coumarin}$), 131.4 (C–1_{phenyl}), 132.7 (=CH), 134.5 (C–$7_{coumarin}$), 138.7 (CH=$N_{exocyclic}$), 142.7 (C–$5_{thiazolidinone}$), 143.6 (C–$4_{coumarin}$), 148.2 (C–$5_{pyrazole}$), 151.7 (C–$4_{thiazole}$), 152.9 (C–$3_{pyrazole}$), 155.4 (C–$8a_{coumarin}$), 159.0 (C=$O_{thiazolidinone}$), 159.5 (C=$O_{coumarin}$), 160.6 (C=O_{ester}), 164.9 (C–$2_{thiazole}$), 166.9 (C–$2_{thiazolidinone}$). MS (*m/z*, I%): 636 (M^+, 8%). Anal. Calcd for $C_{32}H_{24}N_6O_5S_2$ (636.70): C, 60.37%, H, 3.80%, N, 13.20%, S, 10.07%. Found: C, 60.19%, H, 3.69%, N, 13.06%, S, 9.91%.

Ethyl 2-{3-(adamantyl-1-yl)-4-oxo-2-(2-((3-(2-oxo-2*H*-chromen-3-yl)-1-(4-phenyl-thiazol-2-yl)-1*H*-pyrazol-4-yl)methylene)hydrazineyl)thiazol-5(4*H*)-ylidene}acetate (5e). Yellow solid in 77% yield, mp 284-285 °C. IR (KBr), (*v* max, cm^{-1}): 3076 (C–H_{arom}), 2984, 2908, 2846 (C–H_{aliph}), 1728 (C=$O_{thiazolidinone}$), 1716 (C=$O_{coumarin}$), 1698 (C=O_{ester}), 1652 (CH=$N_{exocyclic}$), 1608, 1548 (C=N, C=C). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.26 (t, 3H, *J*=6.8 Hz, CH_3), 1.53–1.62 (m, 6H, $CH_{2adamantyl}$), 1.74 (s, 6H, $CH_{2adamantyl}$), 1.98 (s, 3H, $CH_{adamantyl}$), 4.23 (q, 2H, *J*=6.8 Hz, OCH_2), 6.70 (s, 1H, =$CH_{exocyclic}$), 7.41 (t, 2H, *J*=6.8 Hz, Ph–H), 7.48–7.52 (m, 3H, Ph–H, H–$6_{coumarin}$ and H–$8_{coumarin}$), 7.70 (t, 1H, *J*=7.6 Hz, H–$7_{coumarin}$), 7.87 (d, 1H, *J*=7.6 Hz, H–$5_{coumarin}$), 8.05 (d, 2H, *J*=7.6 Hz, Ph–H), 8.08 (s, 1H, H–$5_{thiazole}$), 8.41 (s, 1H, CH=$N_{exocyclic}$), 9.23 (s, 1H, H–$4_{coumarin}$), 9.26 (s, 1H, H–$5_{pyrazole}$). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 14.3 (CH_3), 28.5 ($3CH_{admantyl}$), 34.9 ($3CH_{2admantyl}$), 37.3 ($3CH_{2admantyl}$), 61.7 (CH_2), 62.7 (C–$1_{admantyl}$), 112.7 (C–$5_{thiazole}$), 117.2 (C–$8_{coumarin}$), 119.3 (C–$3_{coumarin}$), 119.6 (C–$4a_{coumarin}$), 120.6 (C–$4_{pyrazole}$), 122.1 (=$CH_{exocyclic}$), 125.1 (C–4_{phenyl}), 127.3 (C–$2,6_{phenyl}$), 128.4(C–$6_{coumarin}$), 129.3 (C–$5_{coumarin}$), 130.1 (C–$3,5_{phenyl}$), 131.5 (C–1_{phenyl}), 132.7 (C–$7_{coumarin}$), 137.4 (CH=$N_{exocyclic}$), 141.5 (C–$5_{thiazolidinone}$), 143.7 (C–$4_{coumarin}$), 148.3 (C–$5_{pyrazole}$), 150.5 (C–$4_{thiazole}$), 153.8 (C–$3_{pyrazole}$), 155.9 (C–$8a_{coumarin}$), 159.1 (C=$O_{thiazolidinone}$), 159.4 (C=$O_{coumarin}$), 161.2 (C=O_{ester}), 165.8 (C–$2_{thiazole}$), 166.8 (C–$2_{thiazolidinone}$). MS (*m/z*, I%): 729 (M-1, 72%).

Anal. Calcd for $C_{39}H_{34}N_6O_5S_2$ (730.86): C, 64.09%, H, 4.69%, N, 11.50%, S, 8.77%. Found: C, 63.92%, H, 4.53%, N, 11.39%, S, 8.66%.

Ethyl 2-{4-oxo-2-(2-((3-(2-oxo-2*H*-chromen-3-yl)-3-phenyl-1-(4-phenylthiazol-2-yl)-1*H*-pyrazol-4-yl)methylene)hydrazineyl)thiazol-5(4*H*)-ylidene}acetate (5f). Yellow solid in 80% yield, mp > 300 ^{o}C. IR (KBr), (ν max, cm^{-1}): 3064 (C–H_{arom}), 2984, 2850 (C–H_{aliph}), 1716 (C=$O_{thiazolidinone}$), 1704 (C=$O_{coumarin}$), 1698 (C=O_{ester}), 1626 (CH=$N_{exocyclic}$), 1607, 1573 (C=N, C=C). ^{1}H-NMR (400 MHz, DMSO-d$_6$): δ 1.29 (t, 3H, *J*=6.8 Hz, CH_3), 4.25 (q, 2H, *J*=6.8 Hz, OCH_2), 6.71 (s, 1H, =$CH_{exocyclic}$), 7.46–7.54 (m, 9H, Ph–H, H–$6_{coumarin}$ and H–$8_{coumarin}$), 7.67–7.71 (m, 2H, Ph–H and H–$7_{coumarin}$), 7.86 (d, 1H, *J*=8.4 Hz, H–$5_{coumarin}$), 8.02 (d, 2H, *J*=8.4 Hz, Ph–H), 8.05 (s, 1H, H–$5_{thiazole}$), 8.37 (s, 1H, CH=$N_{exocyclic}$), 8.45 (s, 1H, H–$4_{coumarin}$), 9.06 (s, 1H, H–$5_{pyrazole}$). ^{13}C-NMR (100 MHz, DMSO-d$_6$): δ 14.2 (CH_3), 61.6 (CH_2), 111.7 (C–$5_{thiazole}$), 116.6 (C–$8_{coumarin}$), 119.2 (C–$3_{coumarin}$), 119.3 (C–$4a_{coumarin}$), 119.6 (C–$4_{pyrazole}$), 122.1 (=$CH_{exocyclic}$), 125.8 (C–2`,6`$_{phenyl}$), 125.2 (C–4_{phenyl}), 127.2 (C–$2,6_{phenyl}$), 128.0 (C–$6_{coumarin}$), 128.4 (C–4`$_{phenyl}$), 129.4 (C–$5_{coumarin}$), 130.1 (C–$3,5_{phenyl}$), 131.5 (C–$1_{phenyl}$), 128.7 (C–3`,5`$_{phenyl}$), 133.7 (C–$7_{coumarin}$), 137.0 (CH=$N_{exocyclic}$), 139.6 (C–1`$_{phenyl}$), 143.0 (C–$5_{thiazolidinone}$), 143.7 (C–$4_{coumarin}$), 148.2 (C–$5_{pyrazole}$), 150.7 (C–$4_{thiazole}$), 152.8 (C–$3_{pyrazole}$), 155.3 (C–$8a_{coumarin}$), 159.5 (C=$O_{thiazolidinone}$), 160.2 (C=$O_{coumarin}$), 161.2 (C=O_{ester}), 164.9 (C–$2_{thiazole}$), 166.6 (C–$2_{thiazolidinone}$). MS (*m/z*, I%): 672 (M^+, 16%). Anal. Calcd for $C_{35}H_{24}N_6O_5S_2$ (672.73): C, 62.49%, H, 3.60%, N, 12.49%, S, 9.53%. Found: C, 62.38%, H, 3.49%, N, 12.21%, S, 9.39%.

Ethyl 2-{3-benzyl-4-oxo-2-(2-((3-(2-oxo-2*H*-chromen-3-yl)-1-(4-phenylthiazol-2-yl)-1*H*-pyrazol-4-yl)methylene)hydrazineyl)thiazol-5(4*H*)-ylidene}acetate (5g). Yellow solid in 81% yield, mp 259-260 ^{o}C. IR (KBr), (ν max, cm^{-1}): 3069 (C–H_{arom}), 2984 (C–H_{aliph}), 1722 (C=$O_{thiazolidinone}$), 1714 (C=$O_{coumarin}$), 1688(C=O_{ester}), 1627 (CH=$N_{exocyclic}$), 1608, 1569 (C=N, C=C). ^{1}H-NMR (400 MHz, DMSO-d$_6$): δ 1.27 (t, 3H, *J*=7.2 Hz, CH_3), 4.22 (q, 2H, *J*=7.2 Hz,

OCH_2), 5.01 (s, 2H, CH_2), 6.70 (s, 1H, $=CH_{exocyclic}$), 7.29–7.34 (m, 5H, Ph–H), 7.41 (t, 2H, *J*=7.2 Hz, Ph–H), 7.49–7.54 (m, 3H, Ph–H, H–$6_{coumarin}$ and H–$8_{coumarin}$), 7.69 (t, 1H, *J*=8.0 Hz, H–$7_{coumarin}$), 7.87 (d, 1H, *J*=7.2 Hz, H–$5_{coumarin}$), 8.02 (d, 2H, *J*=7.6 Hz, Ph–H), 8.05 (s, 1H, H–$5_{thiazole}$), 8.39 (s, 1H, $CH=N_{exocyclic}$), 8.62 (s, 1H, H–$4_{coumarin}$), 9.13 (s, 1H, H–$5_{pyrazole}$). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 13.6 (CH_3), 61.2 (CH_2), 63.3 (CH_2), 113.1 (C–$5_{thiazole}$), 117.2 (C–$8_{coumarin}$), 118.8 (C–$3_{coumarin}$), 119.3 (C–$4a_{coumarin}$), 119.6 (C–$4_{pyrazole}$), 121.8 ($=CH_{exocyclic}$), 124.9 (C–4_{phenyl}), 125.4 (C–4_{benzyl}), 127.2 (C–$2,6_{phenyl}$), 128.0 (C–$2,6_{benzyl}$), 128.4 (C–$6_{coumarin}$), 129.0 (C–$3,5_{benzyl}$), 129.4 (C–$5_{coumarin}$), 130.1 (C–$3,5_{phenyl}$), 130.8 (C–1_{phenyl}), 134.1 (C–$7_{coumarin}$), 137.4 ($CH=N_{exocyclic}$), 139.6 (C–1_{benzyl}), 142.3 (C–$5_{thiazolidinone}$), 143.3 (C–$4_{coumarin}$), 148.9 (C–$5_{pyrazole}$), 151.1 (C–$4_{thiazole}$), 152.6 (C–$3_{pyrazole}$), 154.8 (C–$8a_{coumarin}$), 159.1 ($C=O_{thiazolidinone}$), 159.6 ($C=O_{coumarin}$), 160.5 ($C=O_{ester}$), 165.6 (C–$2_{thiazole}$), 167.6 (C–$2_{thiazolidinone}$). MS (*m/z*, I%): 686 (M^+, 39%). Anal. Calcd for $C_{36}H_{26}N_6O_5S_2$ (686.76): C, 62.96%, H, 3.82%, N, 12.24%, S, 9.34%. Found: C, 62.79%, H, 3.71%, N, 12.09%, S, 9.21%.

Ethyl 2-{3-(4-chlorophenyl)-4-oxo-2-(2-((3-(2-oxo-2*H*-chromen-3-yl)-1-(4-phenyl-thiazol-2-yl)-1*H*-pyrazol-4-yl)methylene)hydrazineyl)thiazol-5(4*H*)-ylidene}acetate (5h). Yellow solid in 87% yield, mp > 300 °C. IR (KBr), (*v* max, cm^{-1}): 3059 (C–H_{arom}), 2986, 2989, 2912 (C–H_{aliph}), 1729 ($C=O_{thiazolidinone}$), 1712 ($C=O_{coumarin}$), 1703 ($C=O_{ester}$), 1627 ($CH=N_{exocyclic}$), 1611, 1575 (C=N, C=C). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.29 (t, 3H, *J*=6.8 Hz, CH_3), 4.25 (q, 2H, *J*=7.6 Hz, OCH_2), 6.71 (s, 1H, $=CH_{exocyclic}$), 7.42 (t, 2H, *J*=6.8 Hz, Ph–H), 7.49–7.54 (m, 5H, Ph–H, Ar–H, H–$6_{coumarin}$ and H–$8_{coumarin}$), 7.61 (d, 2H, *J*=7.6 Hz, Ar–H), 7.69 (t, 1H, *J*=7.6 Hz, H–$7_{coumarin}$), 7.87 (d, 1H, *J*=7.6 Hz, H–$5_{coumarin}$), 8.03 (d, 2H, *J*=7.6 Hz, Ph–H), 8.06 (s, 1H, H–$5_{thiazole}$), 8.38 (s, 1H, $CH=N_{exocyclic}$), 8.45 (s, 1H, H–$4_{coumarin}$), 9.08 (s, 1H, H–$5_{pyrazole}$). ^{13}C-NMR (100 MHz, DMSO-d_6): δ 14.2 (CH_3), 61.2 (CH_2), 113.2 (C–$5_{thiazole}$), 116.3 (C–$8_{coumarin}$), 119.3 (C–$3_{coumarin}$), 119.6 (C–$4a_{coumarin}$), 120.6 (C–$4_{pyrazole}$), 122.1 ($=CH_{exocyclic}$), 125.4(C–4_{phenyl}), 127.2 (C–$2,6_{phenyl}$), 128.0 (C–$6_{coumarin}$), 129.4 (C–$5_{coumarin}$), 130.1 (C–$3,5_{phenyl}$),

130.8 (C–1_{phenyl}), 131.1 (C–2`,$6`_{aryl}$), 131.3 (C–3`,$5`_{aryl}$), 133.2 (C–$4`_{aryl}$), 134.1 (C–$7_{coumarin}$), 138.7 (CH=$N_{exocyclic}$), 139.2 (C–$1`_{aryl}$), 143.6 (C–$5_{thiazolidinone}$), 144.9 (C–$4_{coumarin}$), 149.1 (C–$5_{pyrazole}$), 151.7 (C–$4_{thiazole}$), 152.8 (C–$3_{pyrazole}$), 155.2 (C–$8a_{coumarin}$), 159.8 (C=$O_{thiazolidinone}$), 160.1 (C=$O_{coumarin}$), 161.1 (C=O_{ester}), 165.6 (C–$2_{thiazole}$), 166.9 (C–$2_{thiazolidinone}$). MS (*m/z*, I%): 708 (M+2, 20%), 706 (M^+, 63%). Anal. Calcd for $C_{35}H_{23}ClN_6O_5S_2$ (707.18): C, 59.45%, H, 3.28%, N, 11.88%, S, 9.07%. Found: C, 59.32%, H, 3.20%, N, 11.69%, S, 8.92%.

In *Vitro* Cytotoxicity

The American type of culture collection (ATCC) provided human cell lines for human liver cancer cells (Huh-7 and HepG2). A humidified, 5% (v/v) CO_2 atmosphere was used to culture the cells at 37 ^oC in RPMI-1640 supplemented with (100 µg/mL); penicillin (100 units/mL); and heat-inactivated fetal bovine serum (10% v/v) [45]. Using the sulforhodamine B (SRB) assay, the cytotoxicity of the synthesized compounds against Huh-7 and HepG2 human tumor cells was assessed before being treated with the synthesized compounds, cells that were growing at 80% confluency, trypsinized and cultured in a 96-well tissue culture plate for 24 h. Cells were subjected to six different doses of each chemical (0.01, 0.1, 1, 10, and 1000 µg/mL), with untreated cells added as a control. Before the cells were fixed with TCA (10% w/v) for an hour at 4 ^oC, they were exposed to the concentrations for 72 h. After multiple washings, cells were stained with a 0.4% (w/v) SRB solution for 10 min in the dark. The surplus stain was eliminated using 1% (v/v) glacial acetic acid. The SRB-stained cells were dissolved in Tris-HCl buffer after drying overnight. A microplate reader was used to gauge the color intensity at 540 nm. Sigma Plot 12.0 software was used to examine the association between each tumor cell line's viability percentage and compound concentrations in order to determine the IC_{50} (drug dose that reduces survival to 50%) [45].

Apoptosis Analysis

The Huh-7 and HepG2cancer cells were treated for 48 h with the products **5d** and **5g** before being trypsinized and subjected to two PBS washes. According to the manufacturer, apoptosis was evaluated using Alexa Fluor-488/PI staining Apoptosis Detection Kit, Cell Signaling Technology (CST Briefly, cells were gently mixed with 0.5 ML of binding buffer for 15 min at room temperature in a dark area after being resuspended in 5 μL of Alexa Fluor-488 of PI (staining solution), and 5 μL of binding buffer [46]. The cells were then subjected to a FACS analysis using a Cytek®Northern Lights 2000 spectral flow cytometer and SpectroFloTM Software version 2.2.0.3 (Cytek Biosciences, Fremont, CA, USA).

Cell Cycle Analysis

The IC_{50} values for the products **5d** and **5g** were pre-calculated and administered to Huh-7 and HepG2 cells for 48 h. The cells were then fixed in ice-cold 60% ethanol at 40 ^{o}C and trypsinized before being washed twice in phosphate-buffered saline. After resuspending, the cells were incubated for 15 min in 500 L of Cell Signaling Technology's (CST) propidium iodide with RNase staining buffer. In order to evaluate the data from 10,000 cells and the distribution of cell cycle phases for each sample, FACS analysis was completed using a Cytek®Northern Lights 2000 spectral flow cytometer (Cytek Biosciences, Fremont, CA, USA) and SpectroFloTM Software version 2.2.0.3 (Cytek Biosciences, Fremont, CA, USA), both of which are available from the United States [47].

Autophagy Assessment

Autophagic cell death is quantitatively assessed using acridine orange lysosomal stain. coupled with flowcytometric analysis. After treatment with test compounds for the specified duration, cells (105 cells) are collected by trypsinization and washed twice with ice-cold PBS (pH 7.4). Cells are stained with acridine orange (10 uM) and incubated in dark at 37°C for 30 minutes. After staining, the cells were then subjected to a FACS analysis using a

Cytek®Northern Lights 2000 spectral flow cytometer and SpectroFloTM Software version 2.2.0.3 (Cytek Biosciences, Fremont, CA, USA) [48].

Molecular Docking

The bioactive compounds were subject to docking study to explore their binding mode towards vascular endothelial growth factor (VEGF) (PDB ID:4ASE) protein which was downloaded from protein data bank. The ligand and receptor were prepared for docking with rigid protein geometry using Auto Dock Tools version 1.5.6 [59]. The docking cavities were defined according to the interactions of protein with the co-crystalized ligands which are also used as reference ligands. The grid box with dimensions of 14× 16 × 14, with 1.0 Å spacing were placed to make the entire binding cavities involved. The co-crystalized ligands were redocked to the receptor to validate the docking parameters. Docking was performed using AutoDockVina [60]. The 2D images were generated by Discovery Studio and Chimera [61,62].

2. Figures S1-S32: Copies of the IR, MS, ^{1}H- and ^{13}C-NMR spectra for the synthesized compounds **5a-h**.

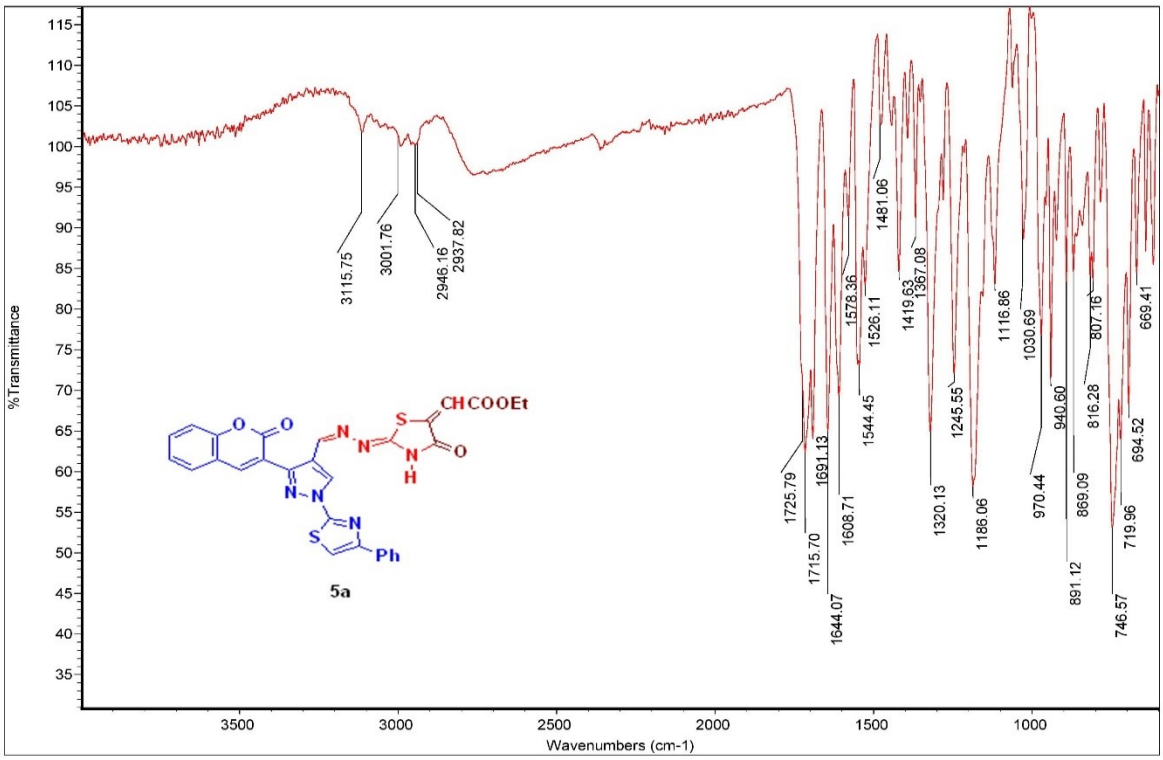

Figure S1: The IR spectrum of compound **5a.**

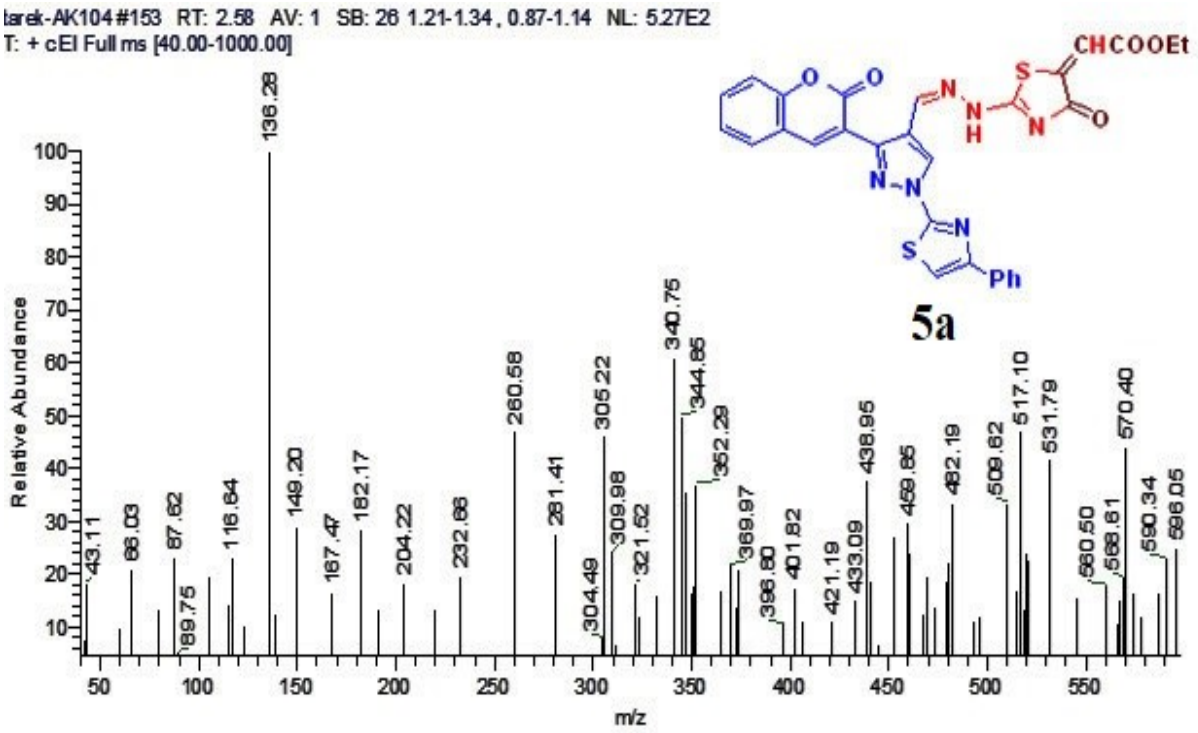

Figure S2: The mass spectrum of compound **5a.**

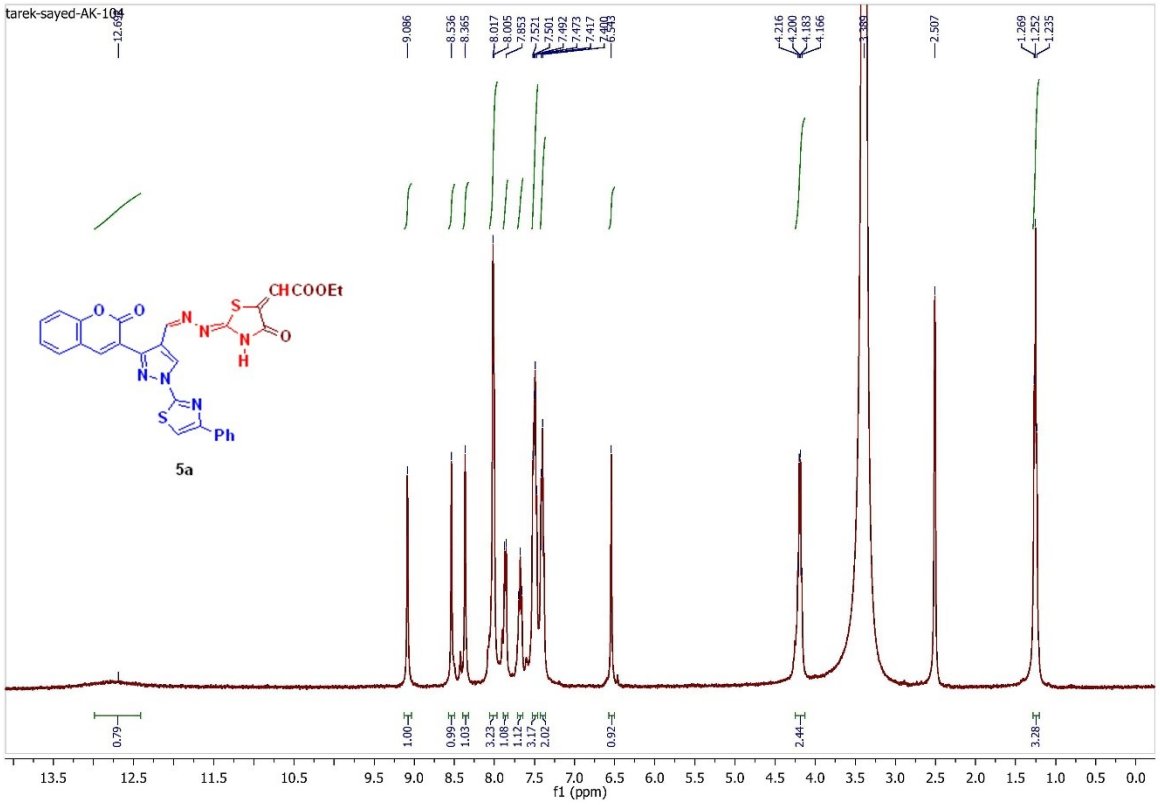

Figure S3: The ^{1}H-NMR spectrum of compound **5a.**

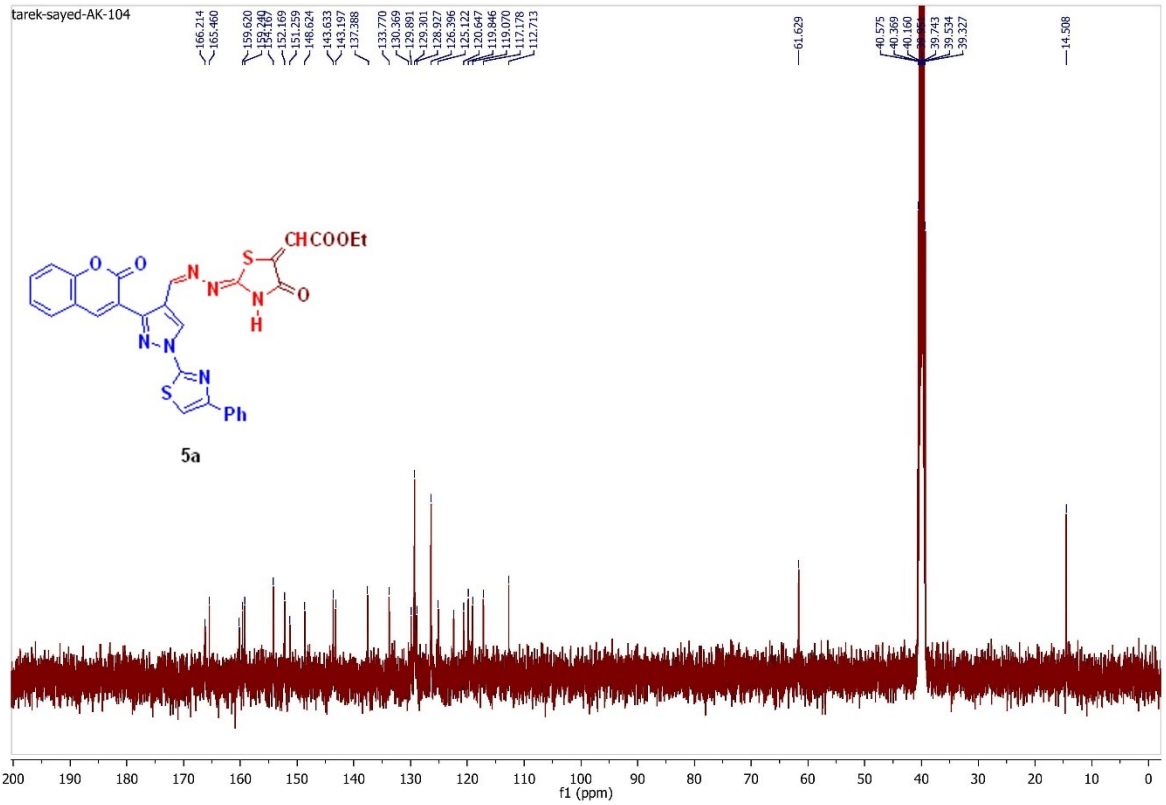

Figure S4: The ^{13}C-NMR spectrum of compound **5a.**

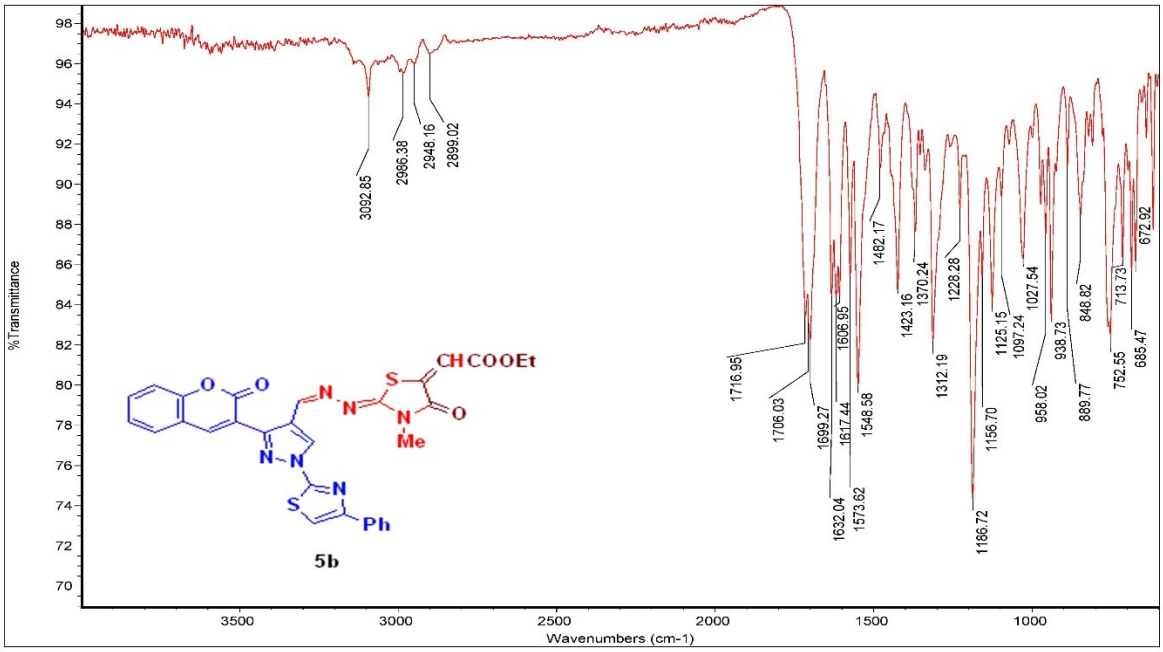

Figure S5: The IR spectrum of compound **5b.**

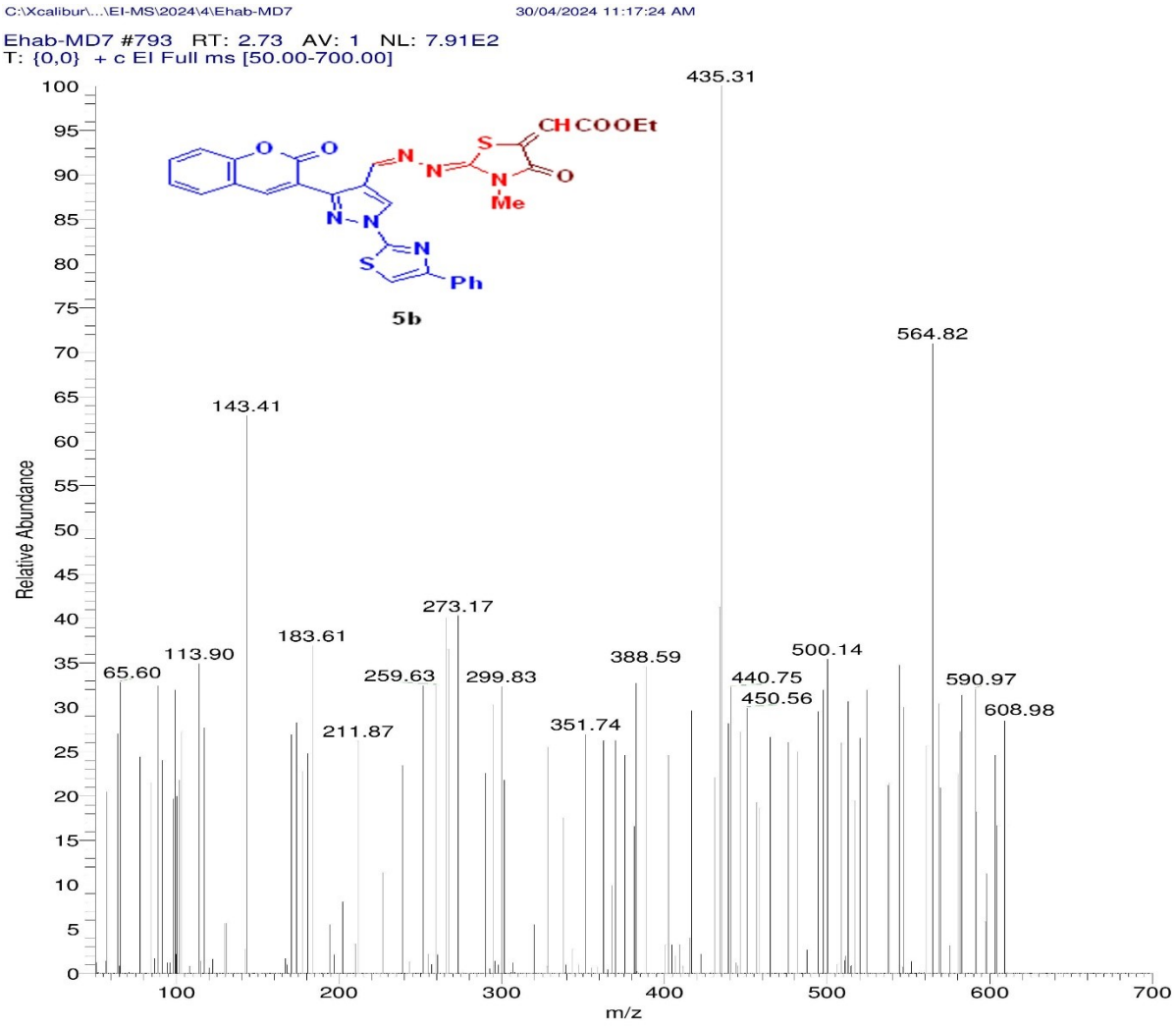

Figure S6: The mass spectrum of compound **5b.**

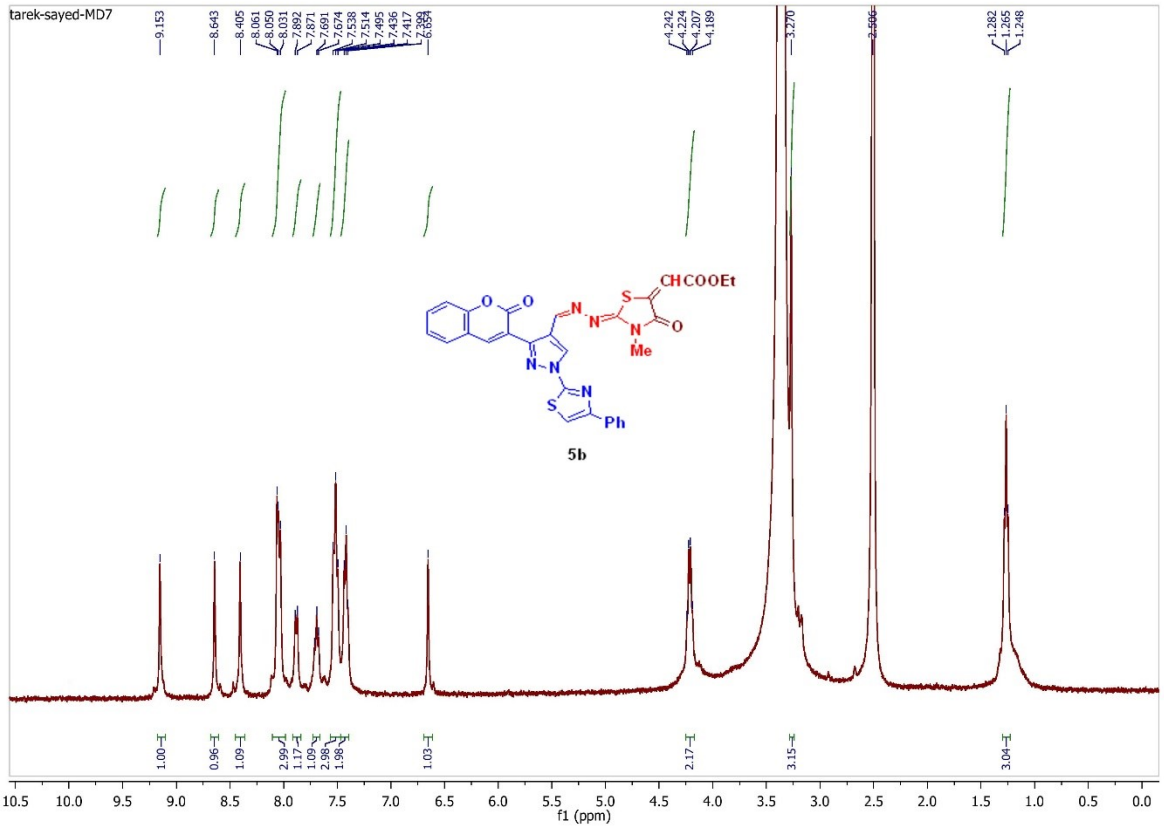

Figure S7: The ^{1}H-NMR spectrum of compound **5b.**

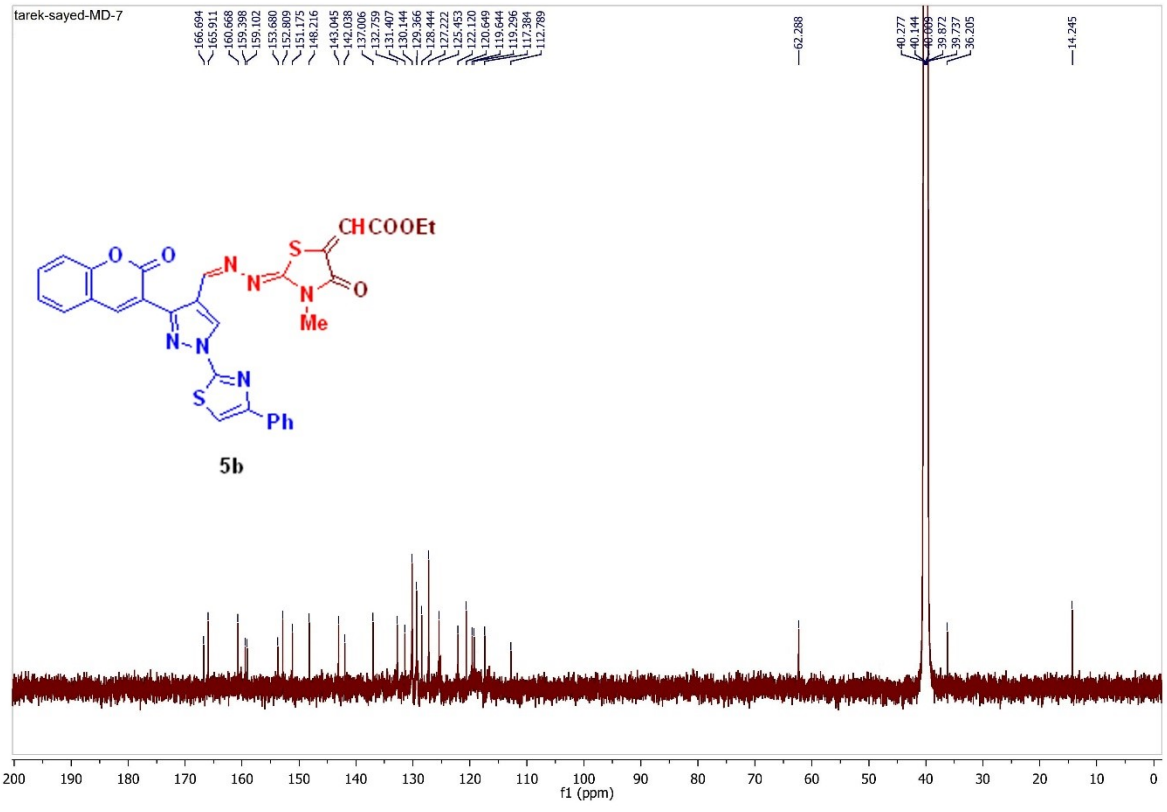

Figure S8: The ^{13}C-NMR spectrum of compound **5b.**

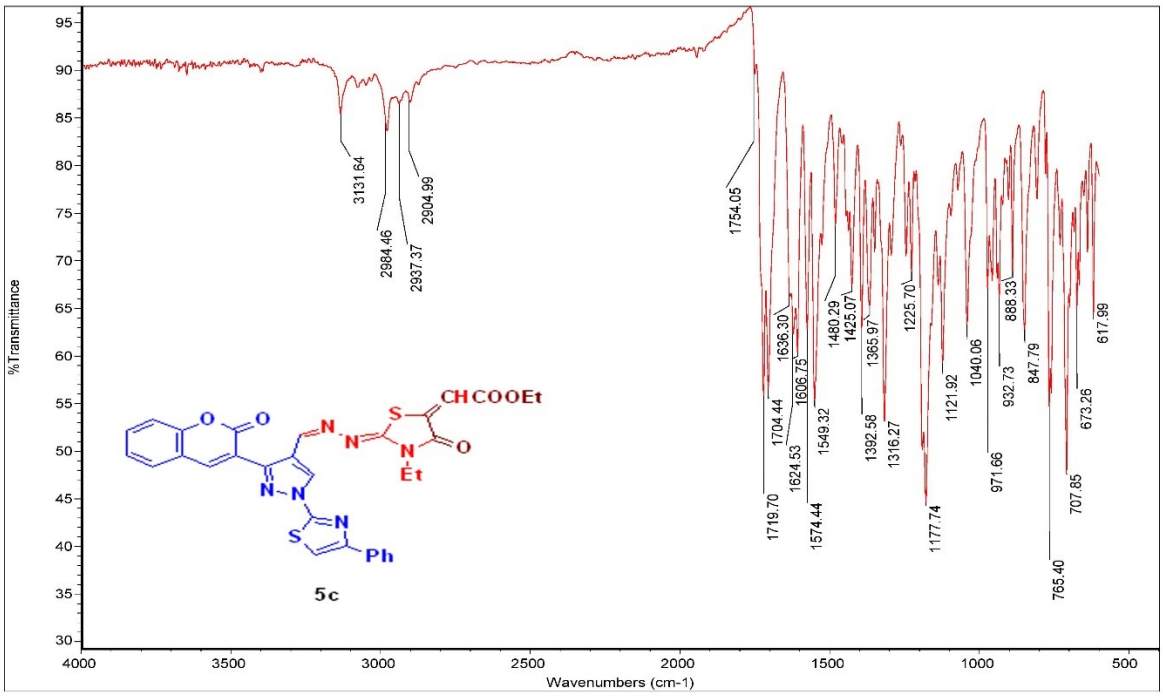

Figure S9: The IR spectrum of compound **5c.**

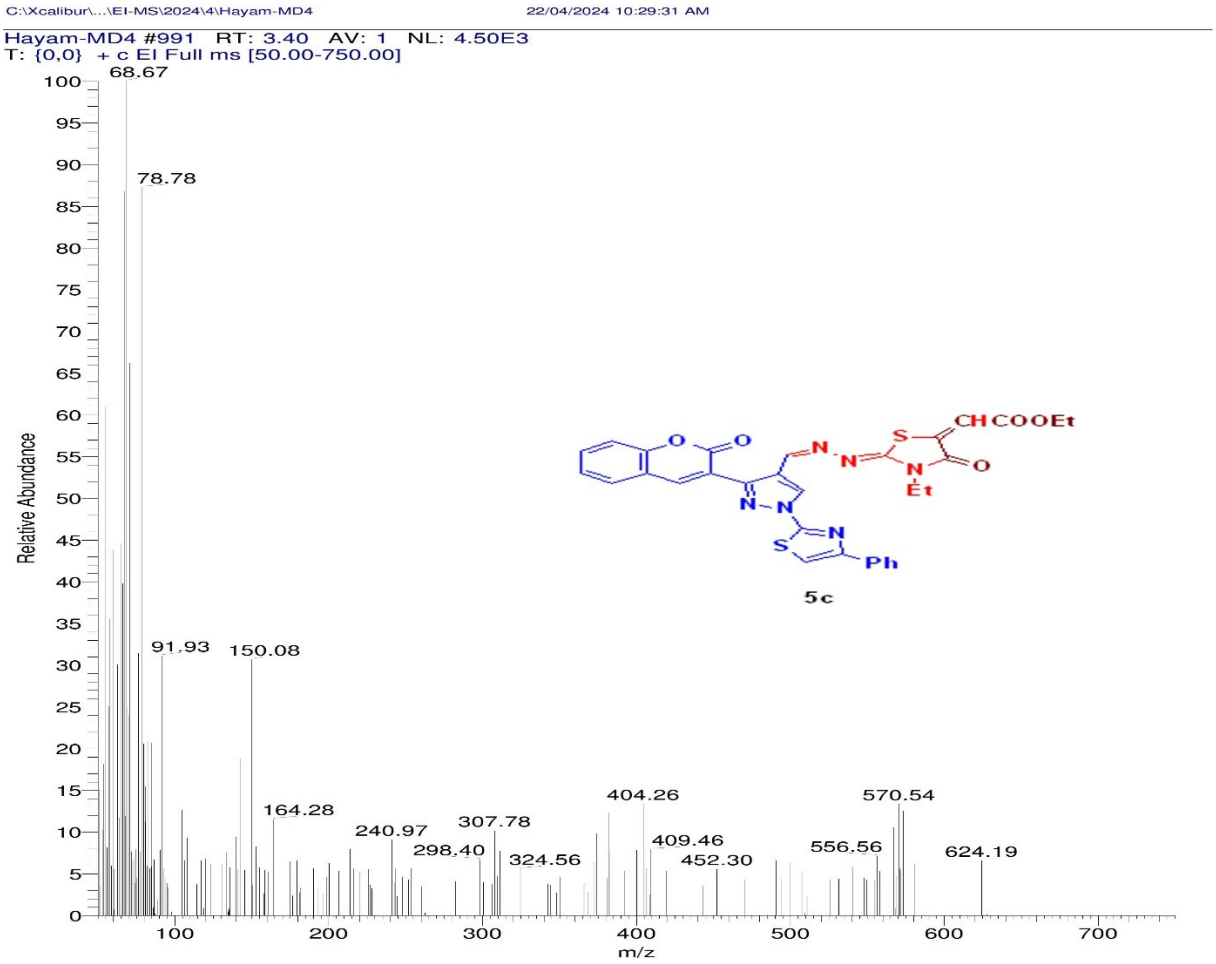

Figure S10: The mass spectrum of compound **5c.**

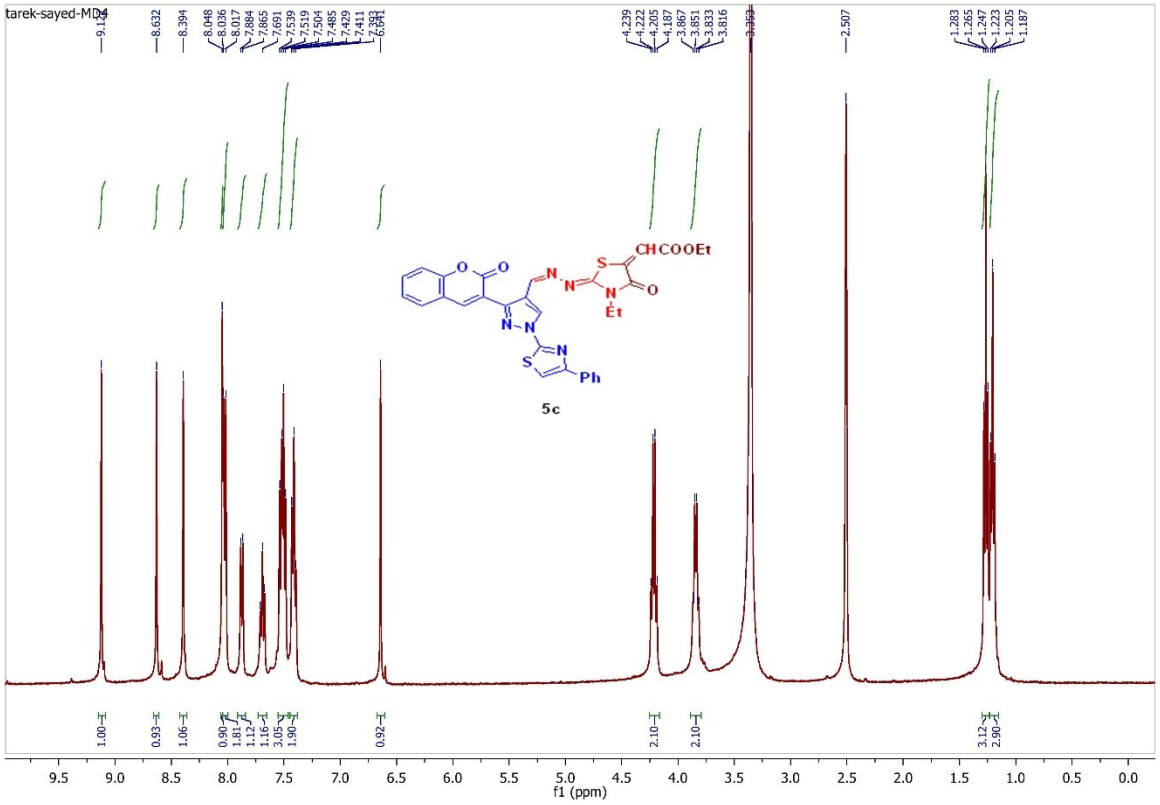

Figure S11: The ^{1}H-NMR spectrum of compound **5c.**

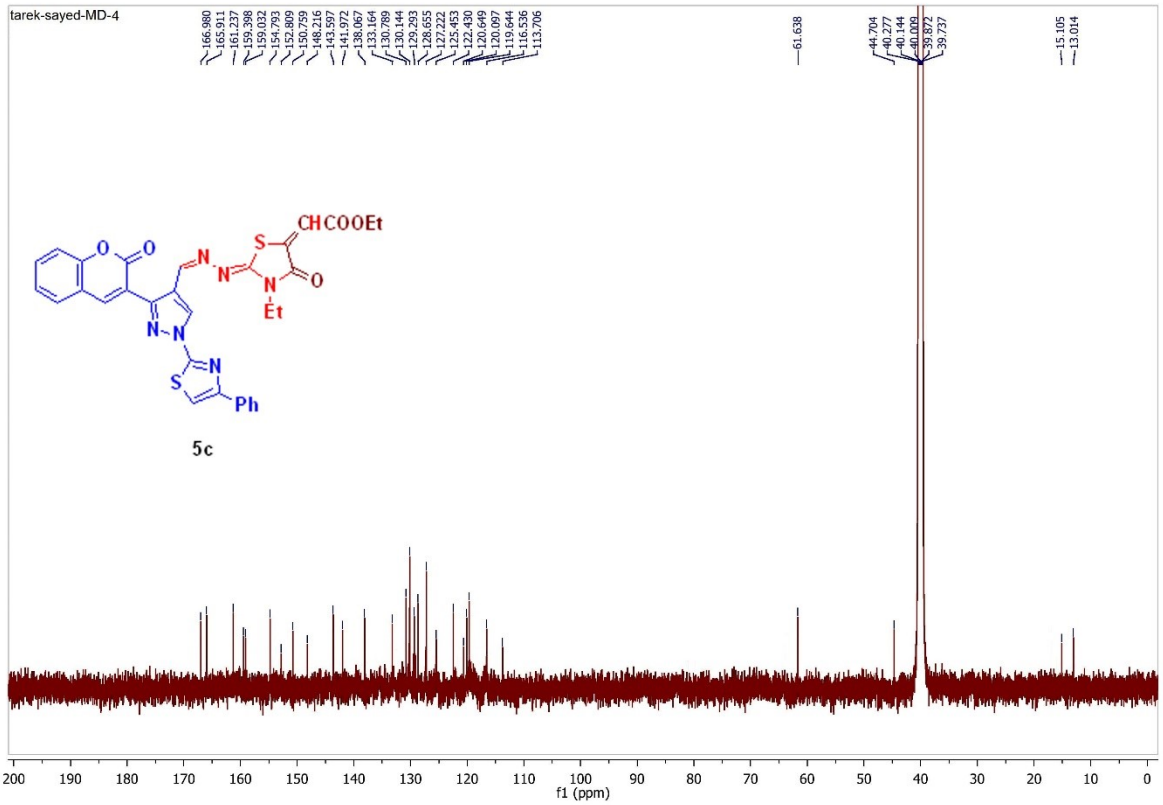

Figure S12: The ^{13}C-NMR spectrum of compound **5c.**

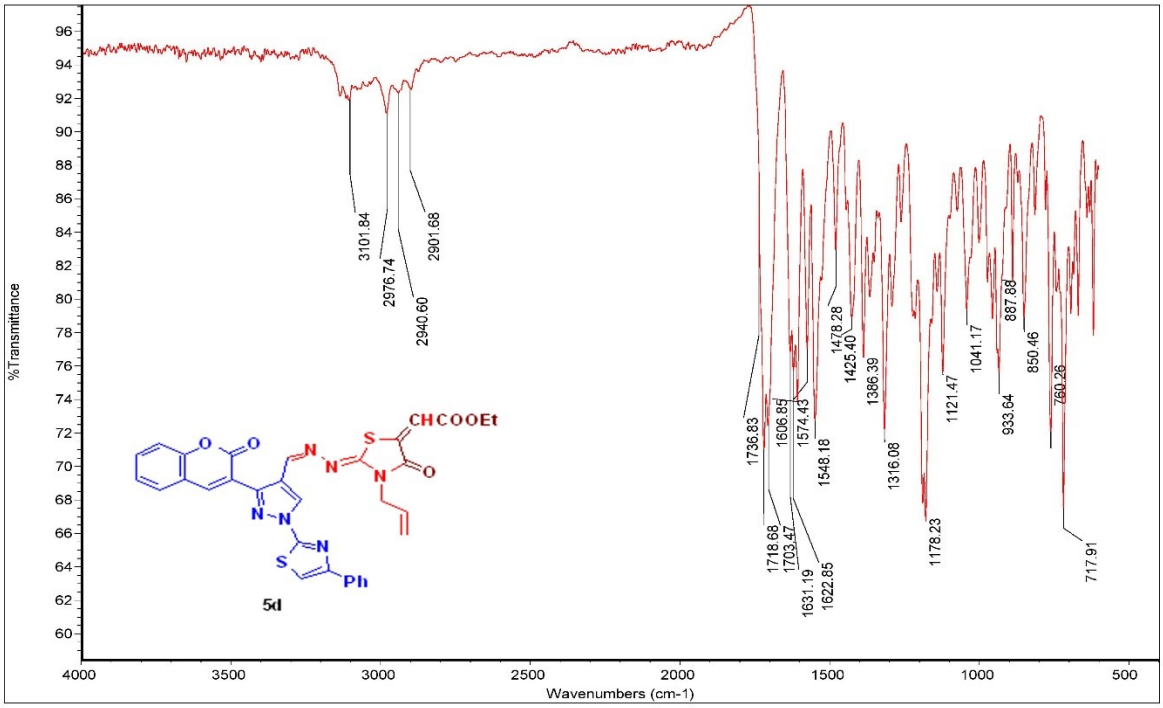

Figure S13: The IR spectrum of compound **5d.**

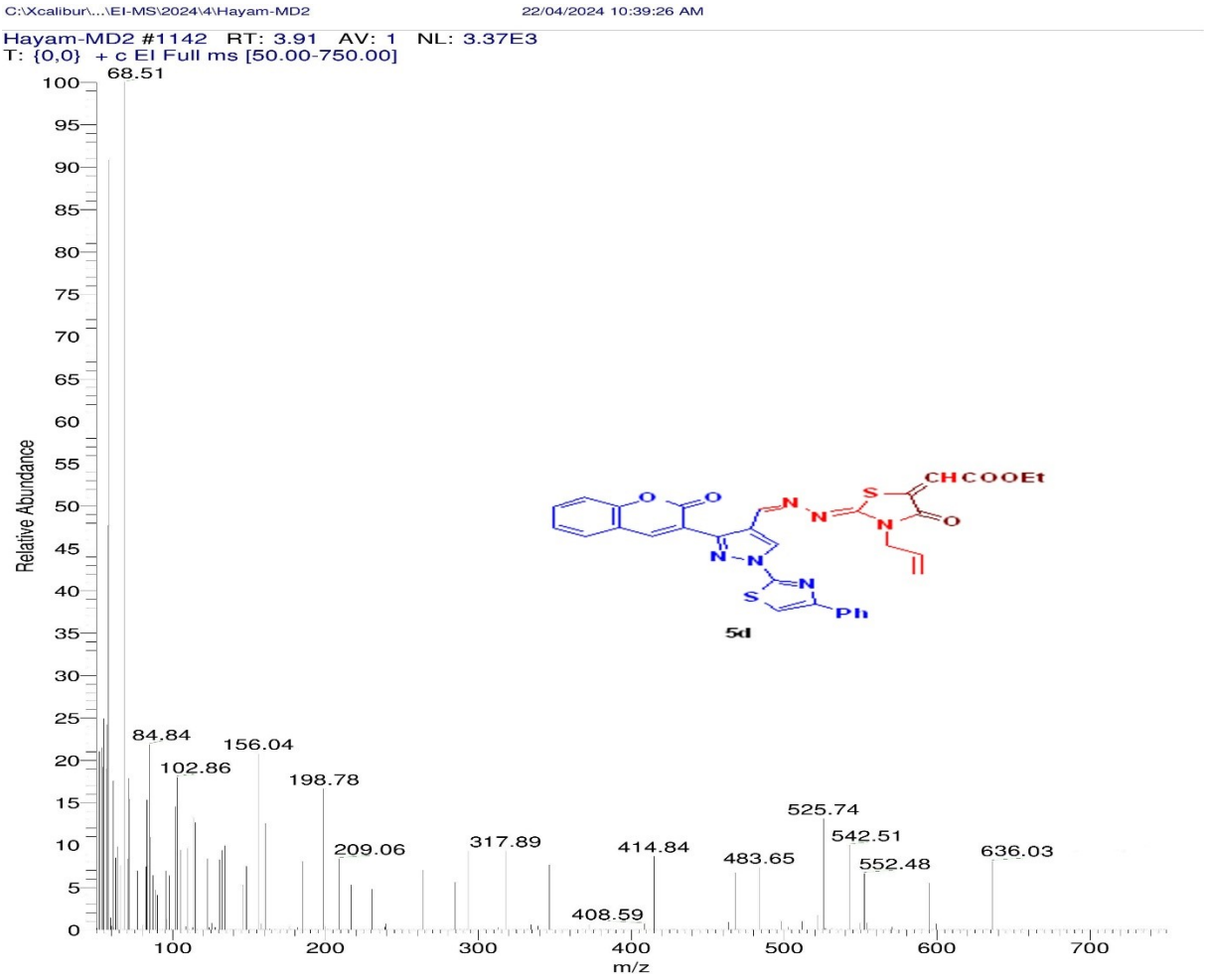

Figure S14: The mass spectrum of compound **5d.**

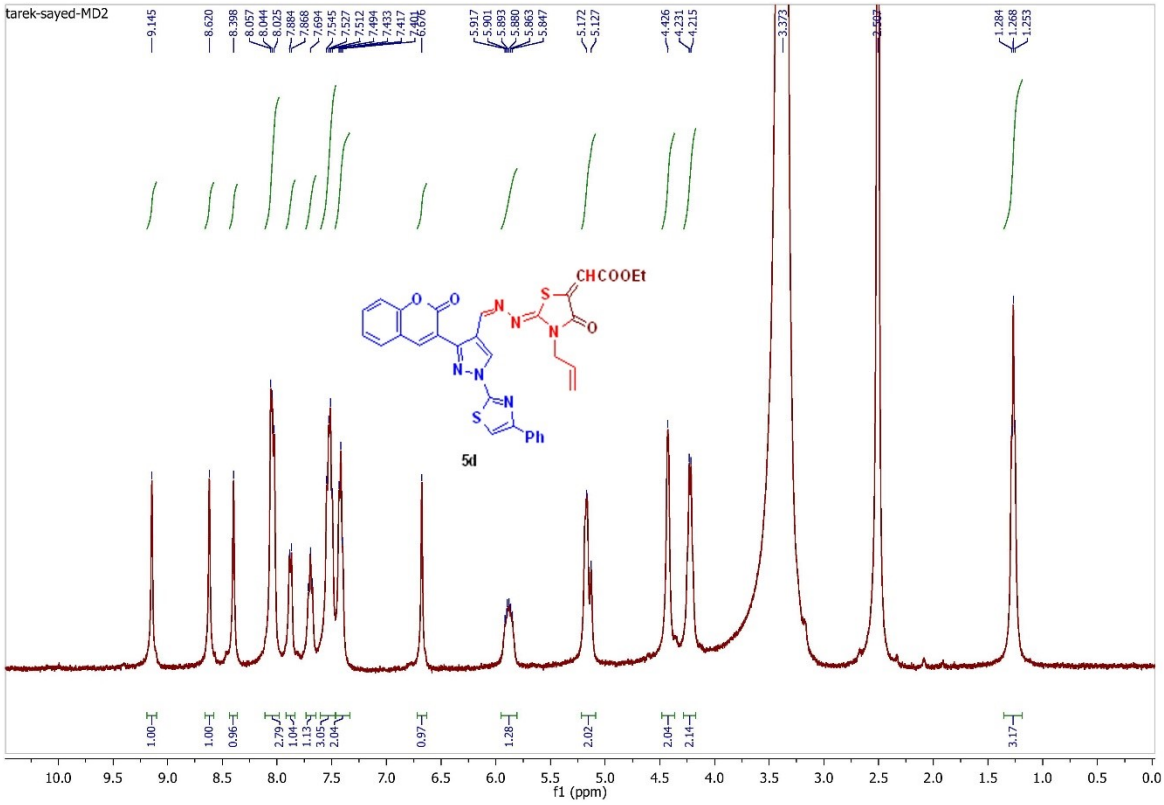

Figure S15: The ^{1}H-NMR spectrum of compound **5d.**

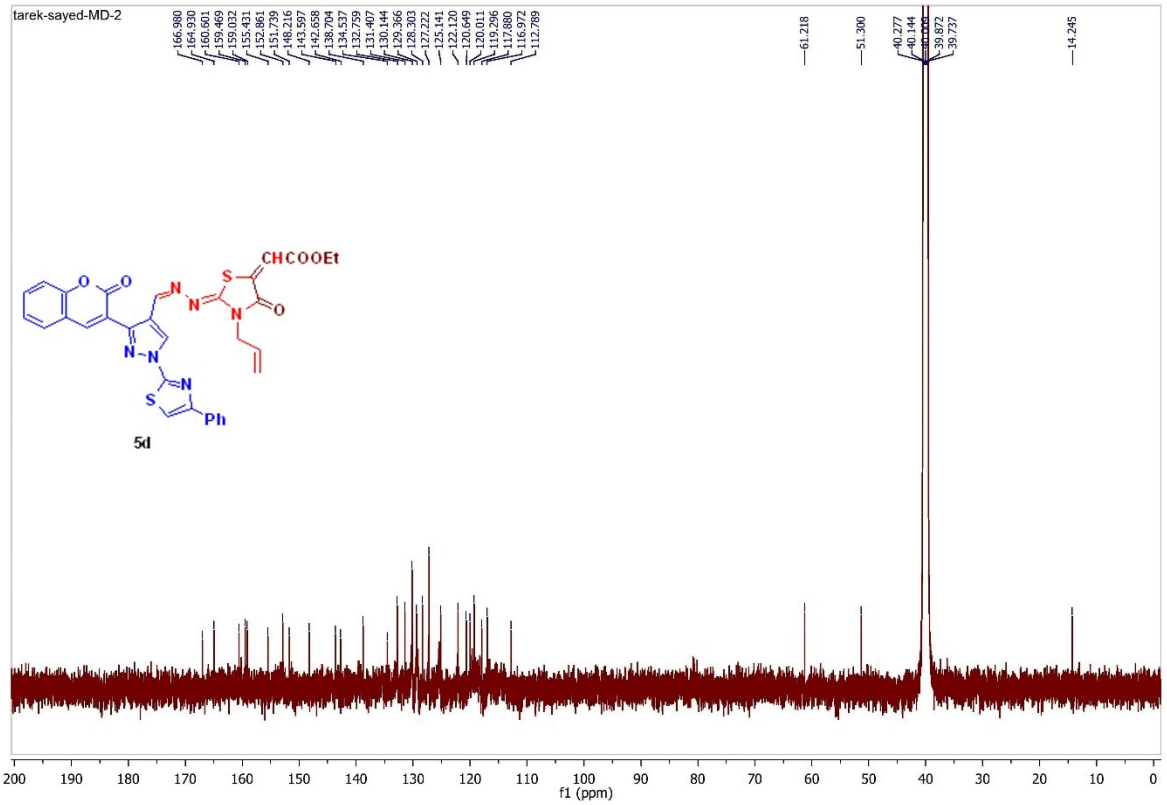

Figure S16: The ^{13}C-NMR spectrum of compound **5d.**

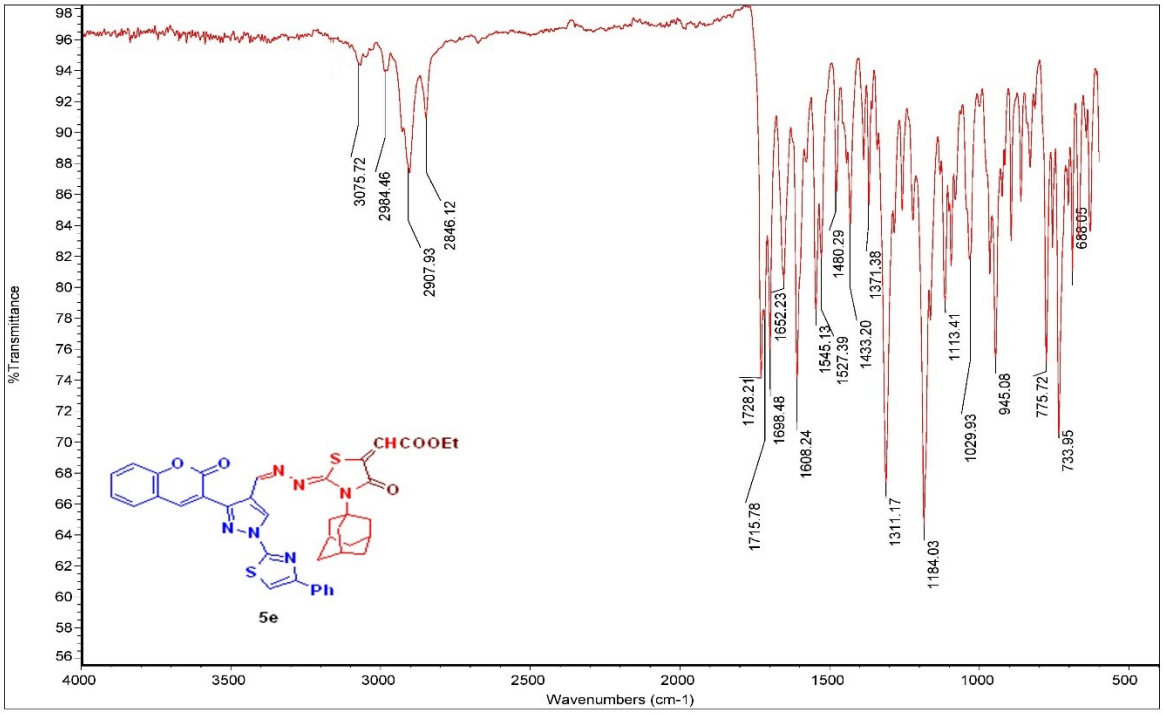

Figure S17: The IR spectrum of compound **5e.**

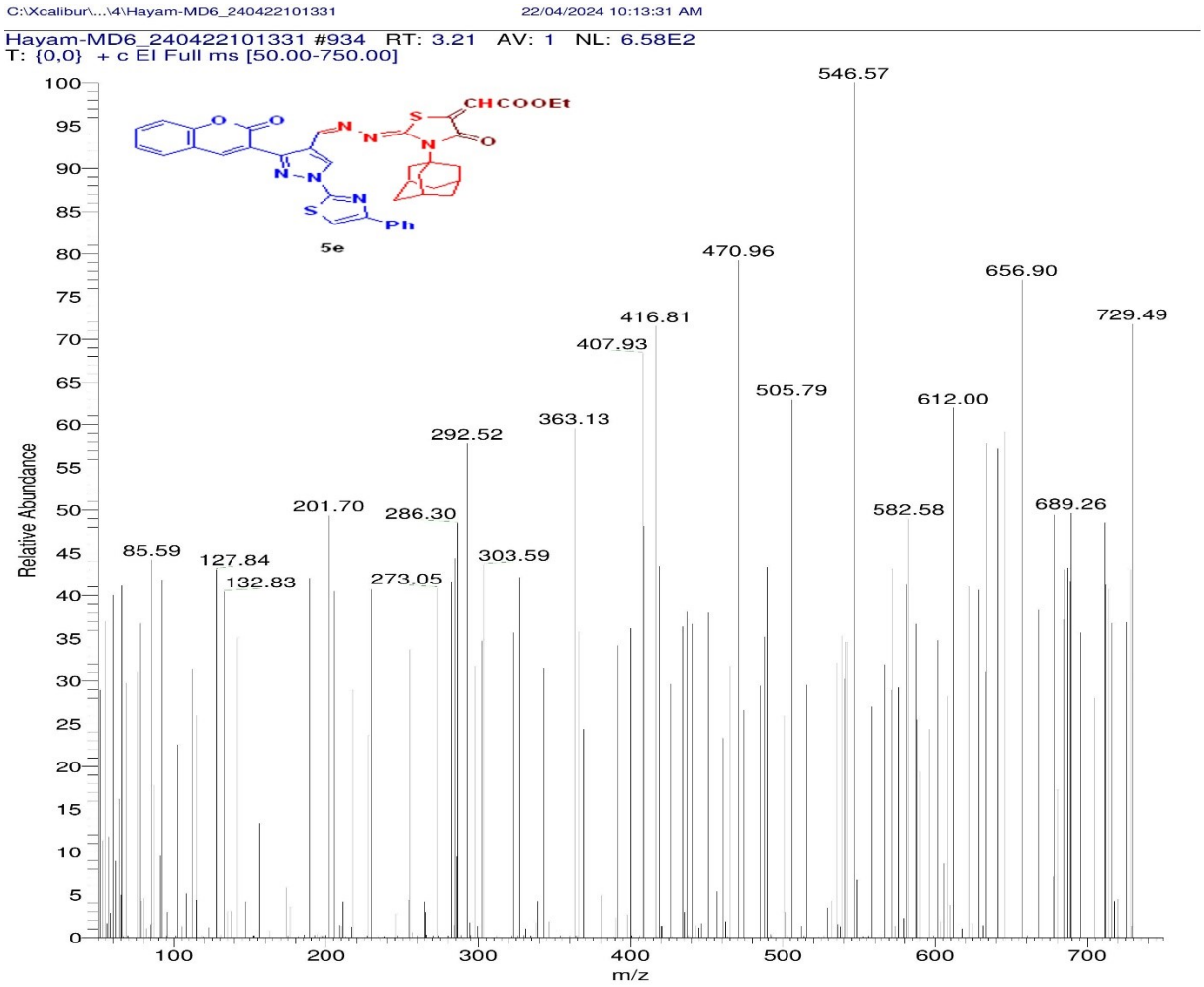

Figure S18: The mass spectrum of compound **5e.**

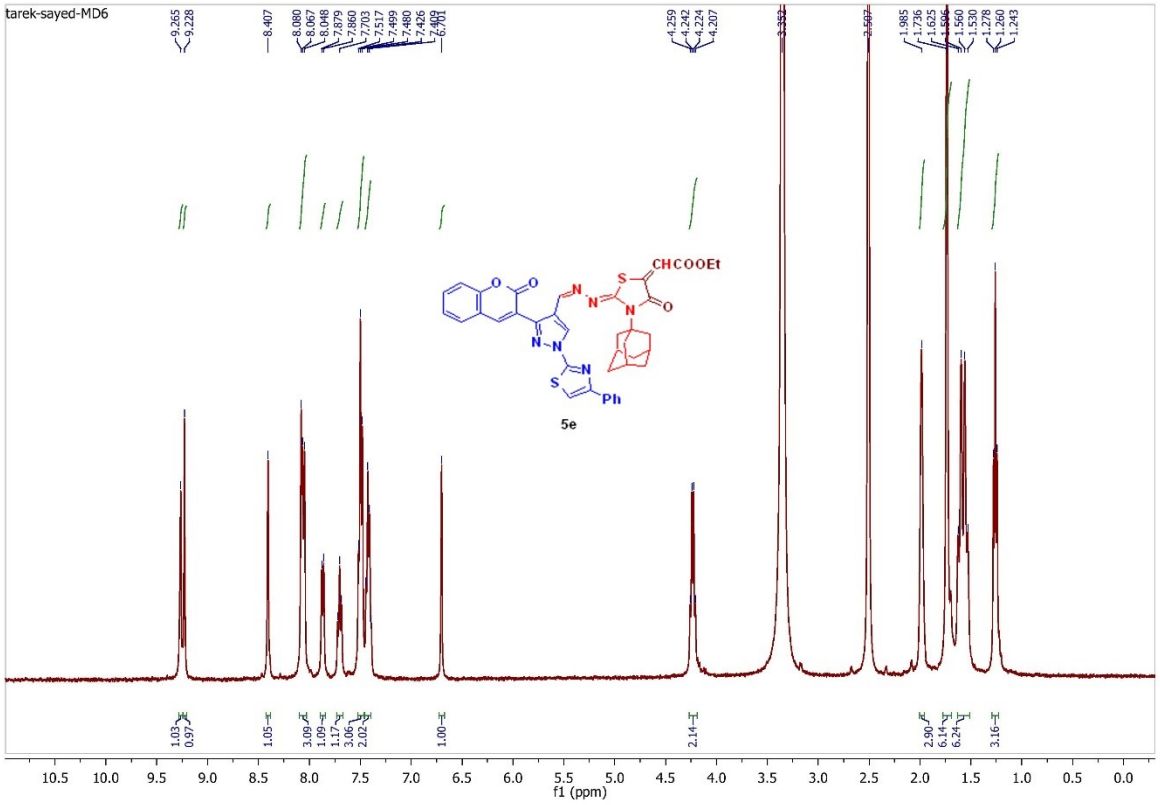

Figure S19: The ^{1}H-NMR spectrum of compound **5e.**

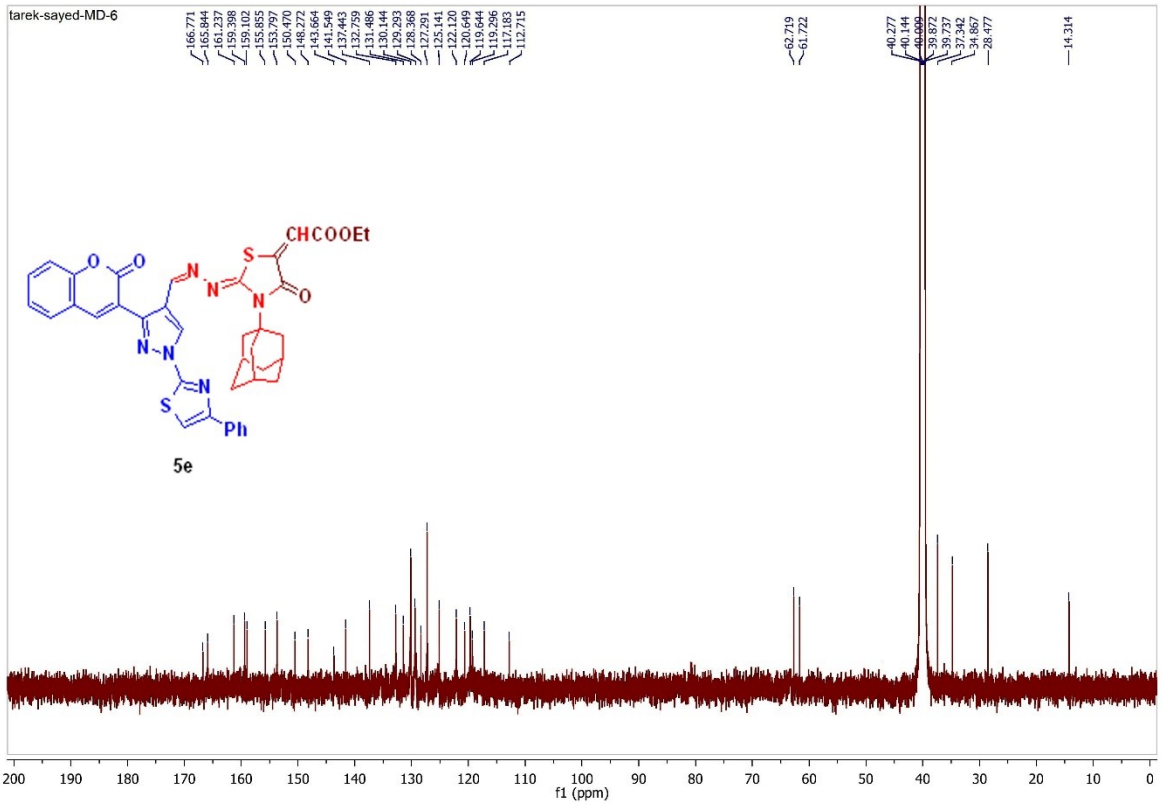

Figure S20: The ^{13}C-NMR spectrum of compound **5e.**

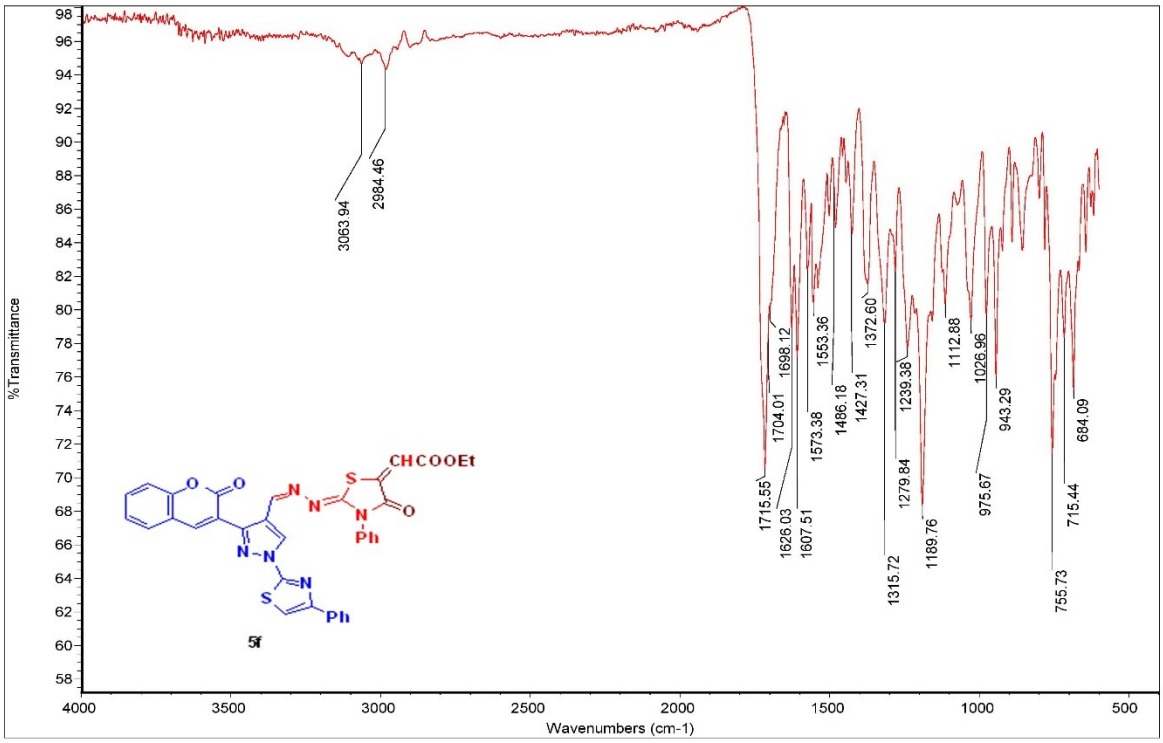

Figure S21: The IR spectrum of compound **5f.**

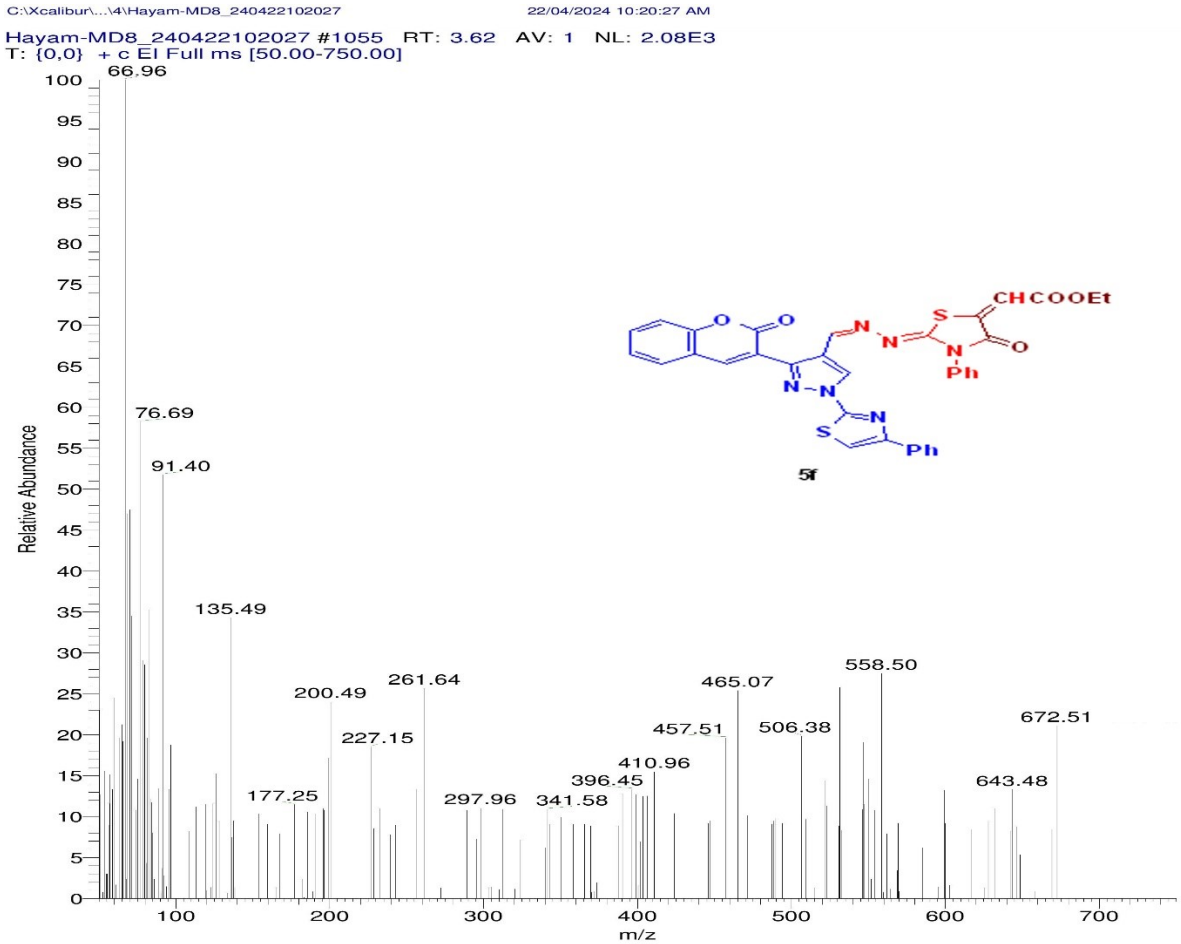

Figure S22: The mass spectrum of compound **5f.**

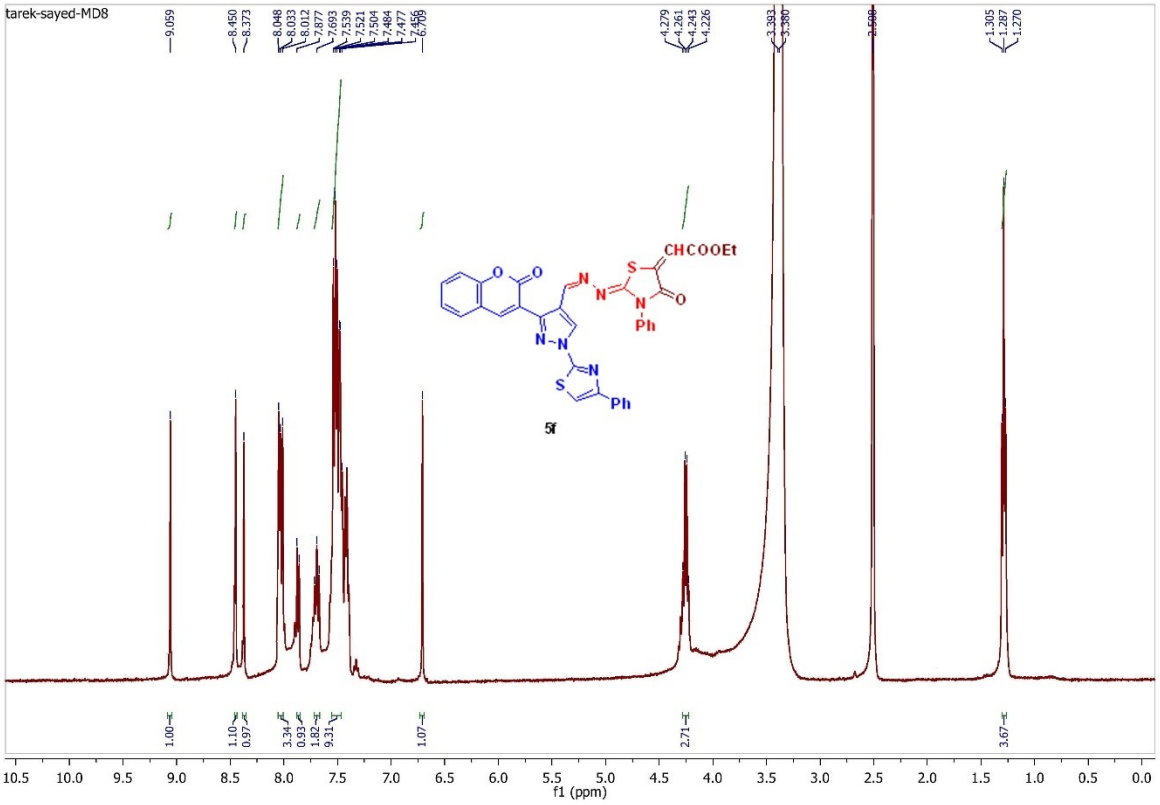

Figure S23: The ^{1}H-NMR spectrum of compound **5f.**

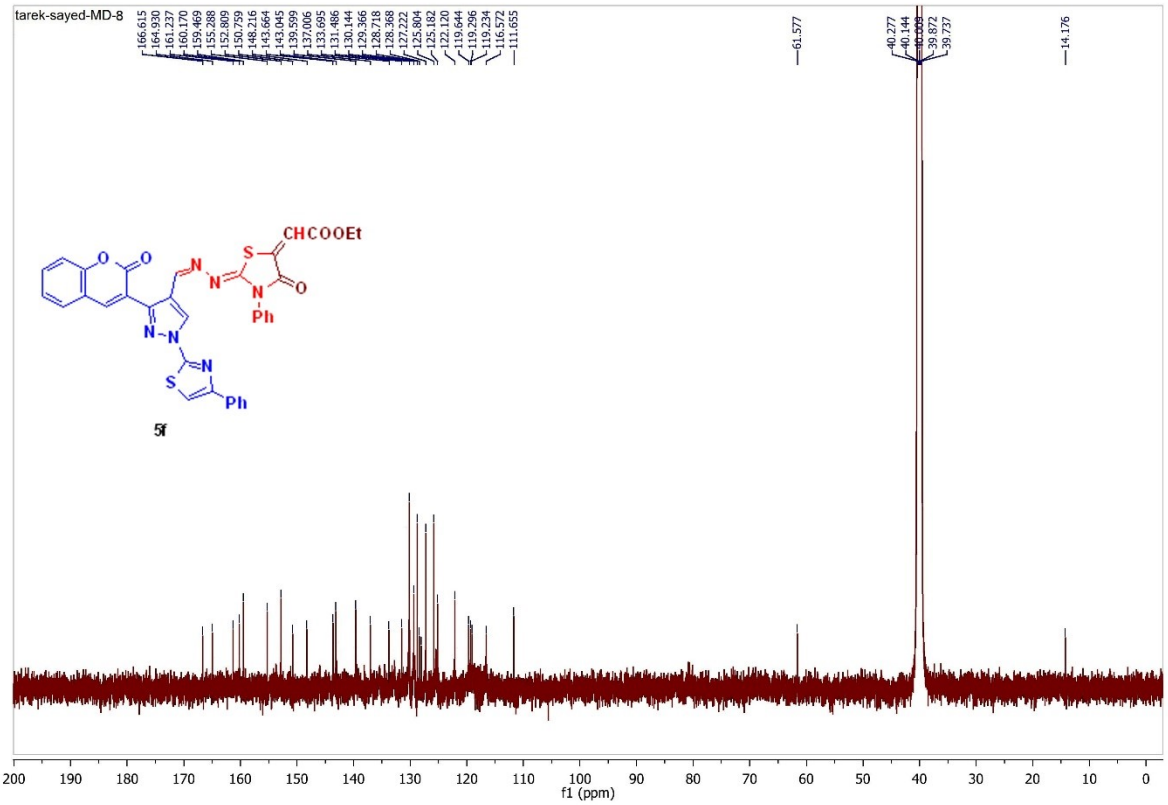

Figure S24: The ^{13}C-NMR spectrum of compound **5f.**

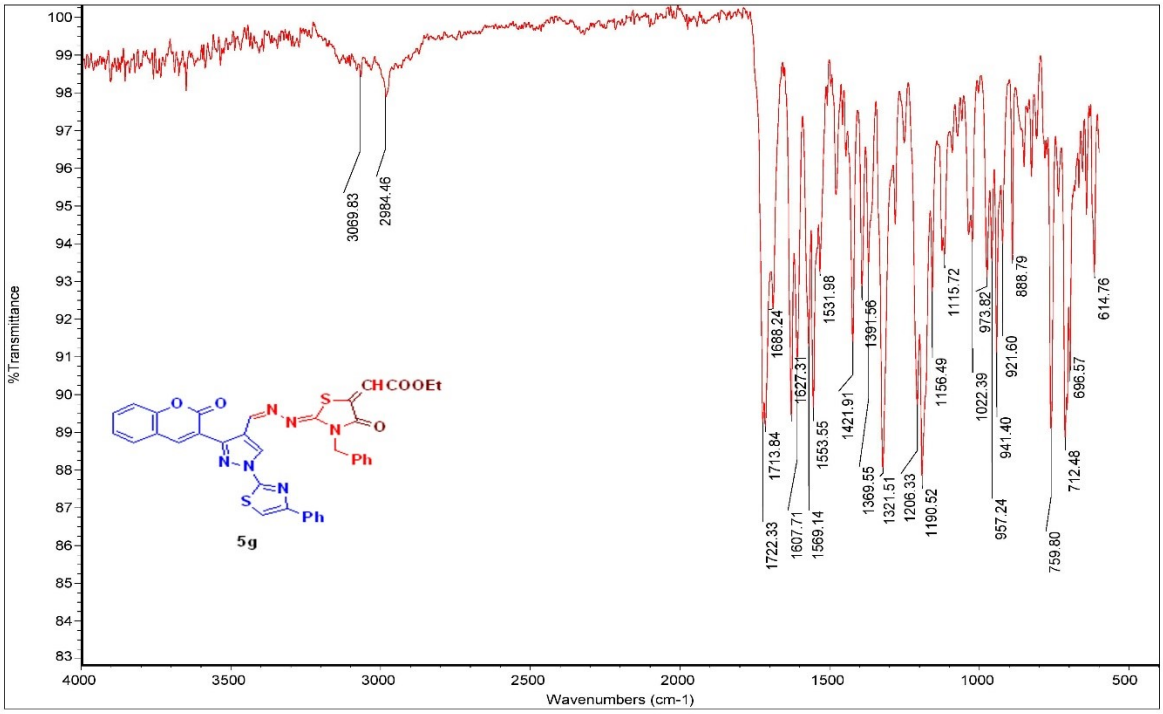

Figure S25: The IR spectrum of compound **5g.**

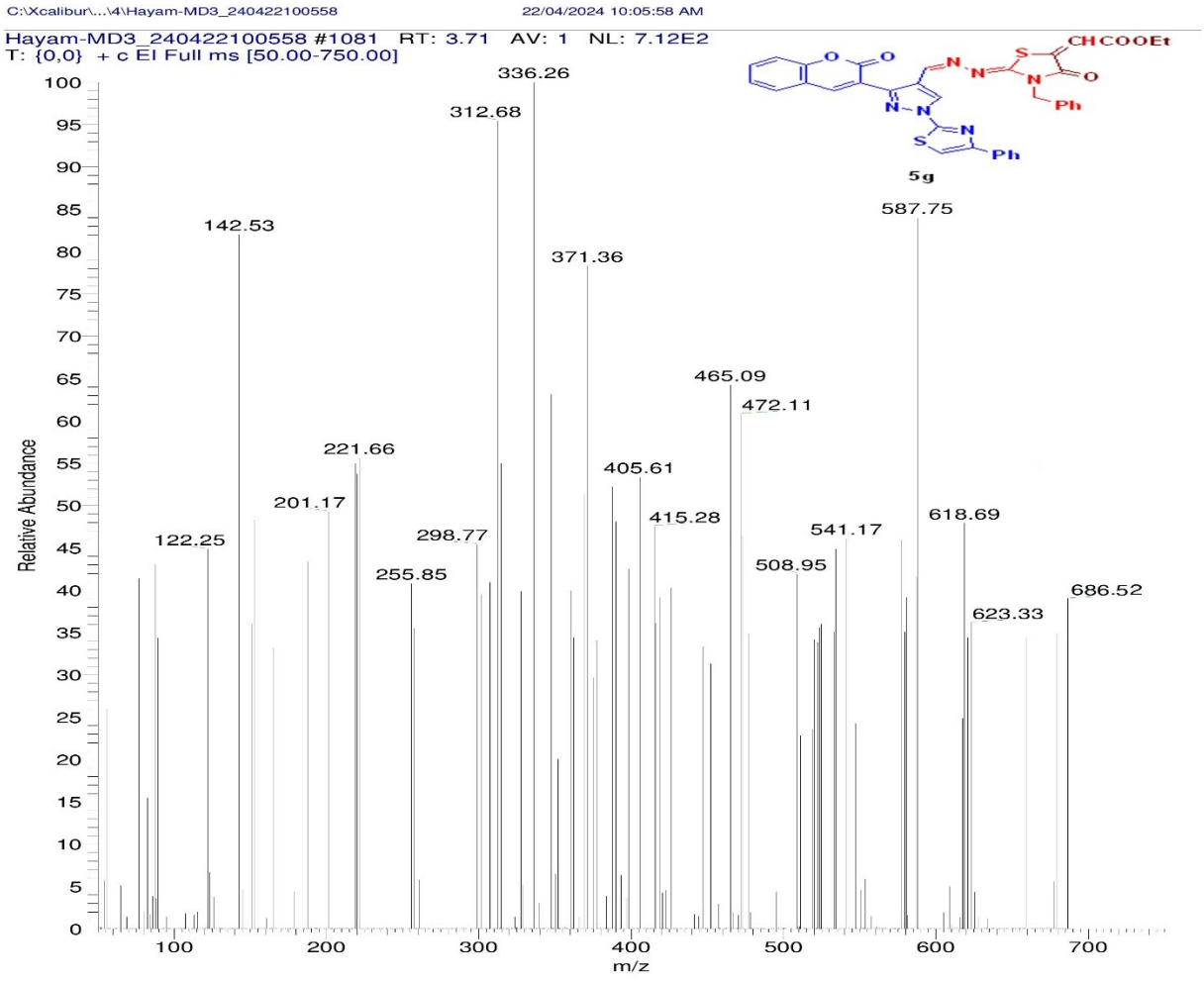

Figure S26: The mass spectrum of compound **5g.**

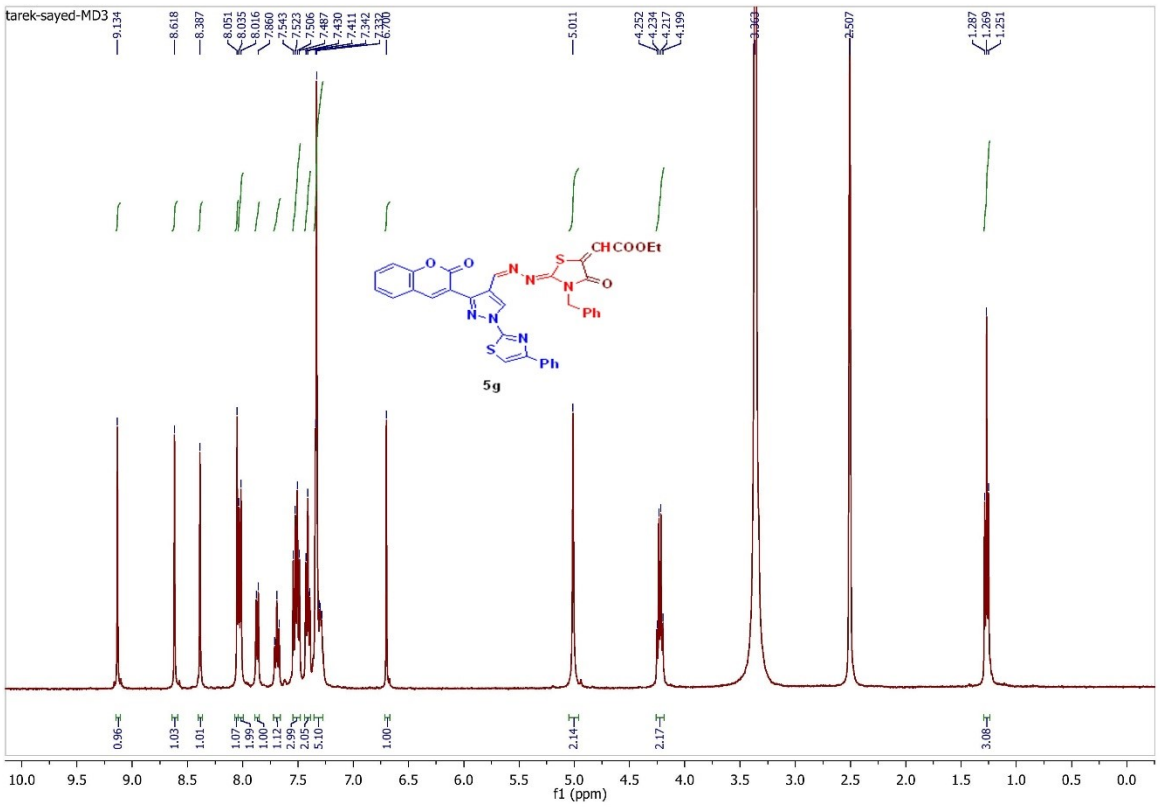

Figure S27: The ^{1}H-NMR spectrum of compound **5g.**

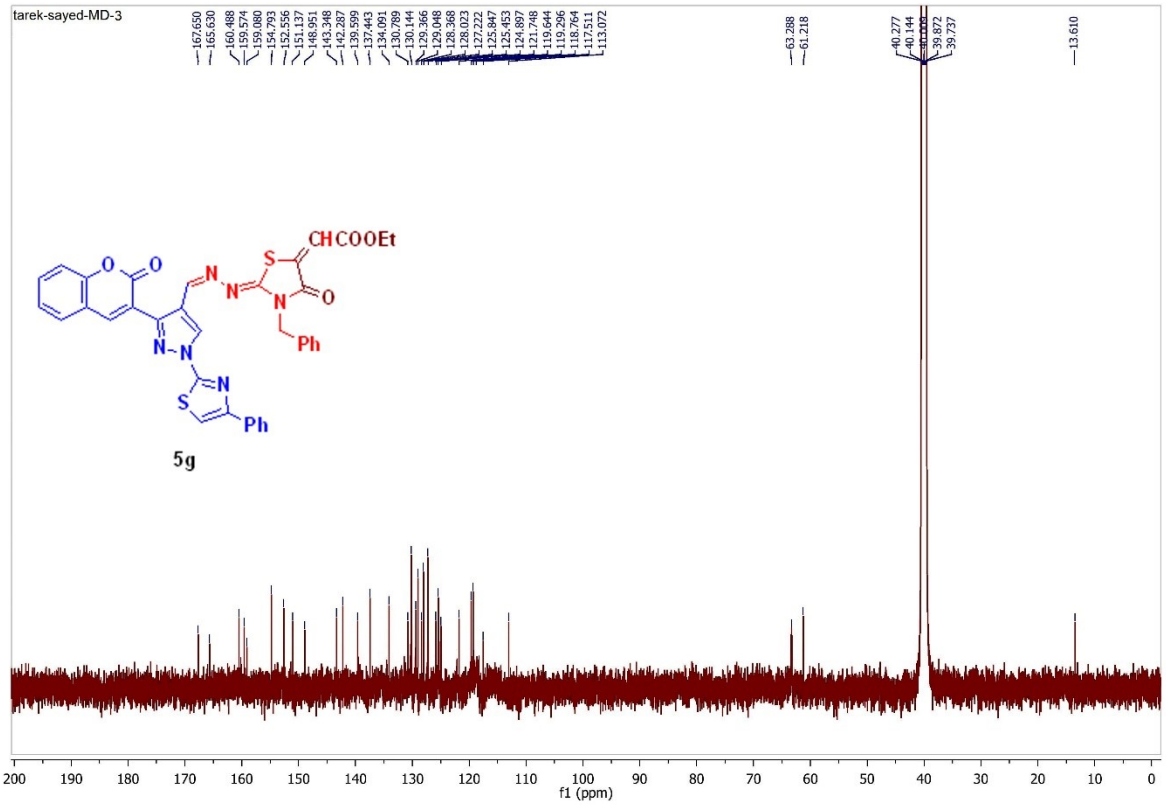

Figure S28: The ^{13}C-NMR spectrum of compound **5g.**

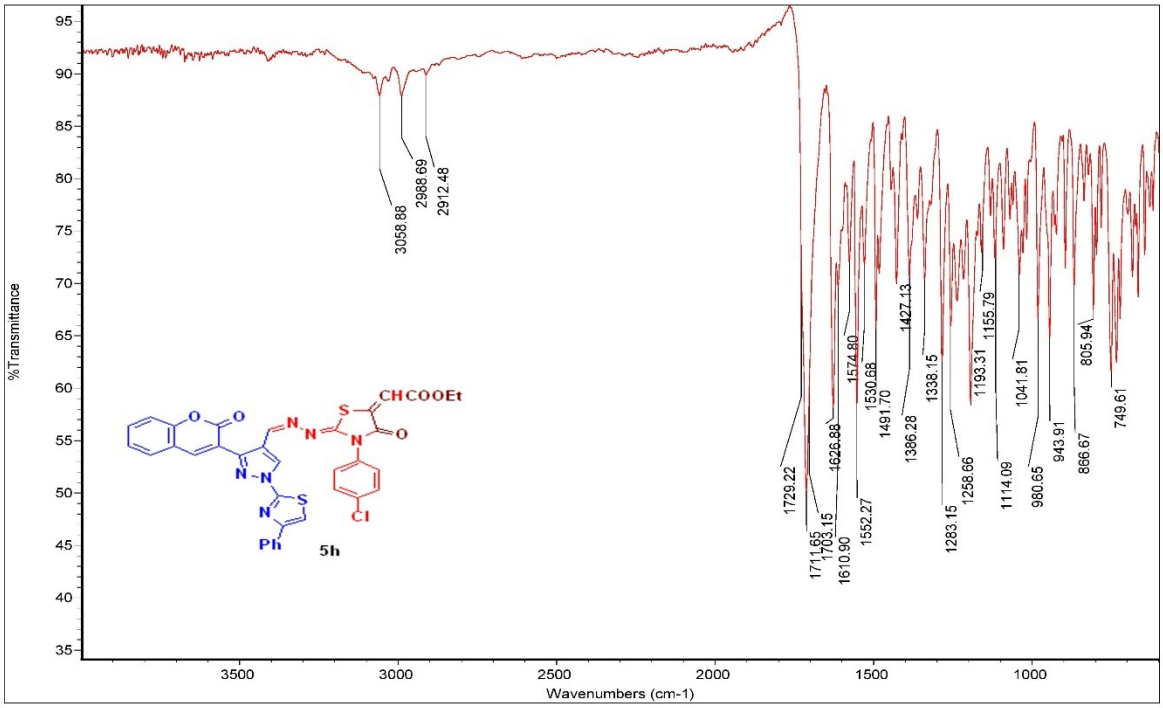

Figure S29: The IR spectrum of compound **5h.**

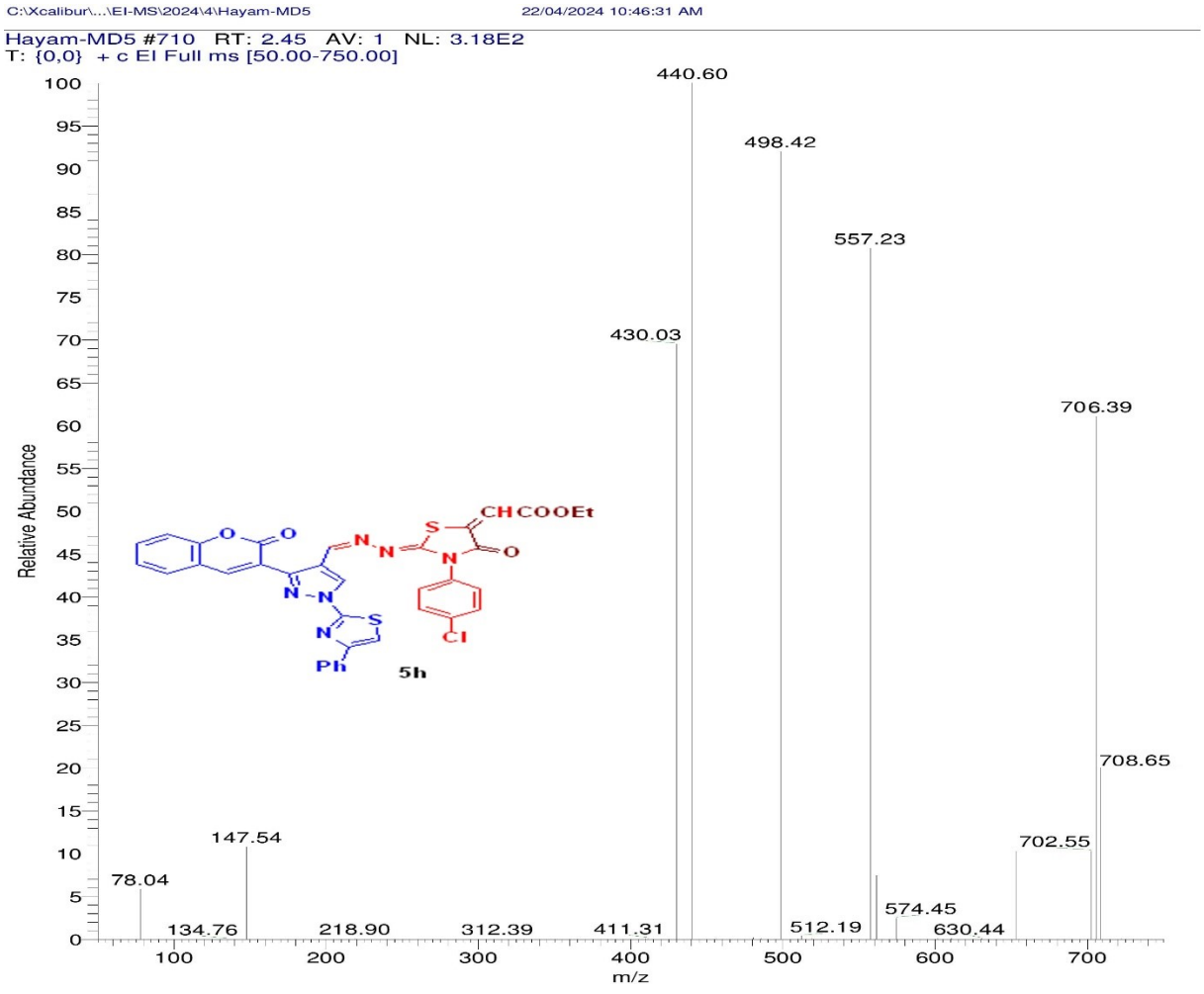

Figure S30: The mass spectrum of compound **5h.**

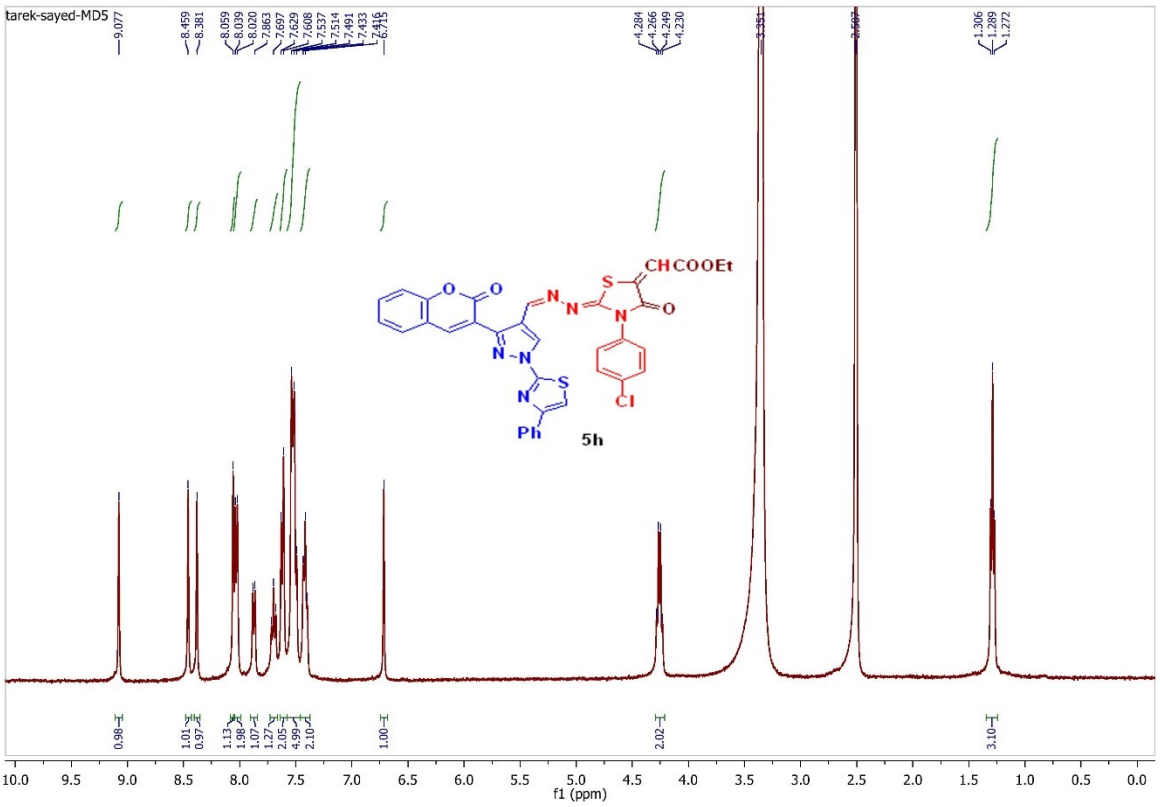

Figure S31: The ^{1}H-NMR spectrum of compound **5h.**

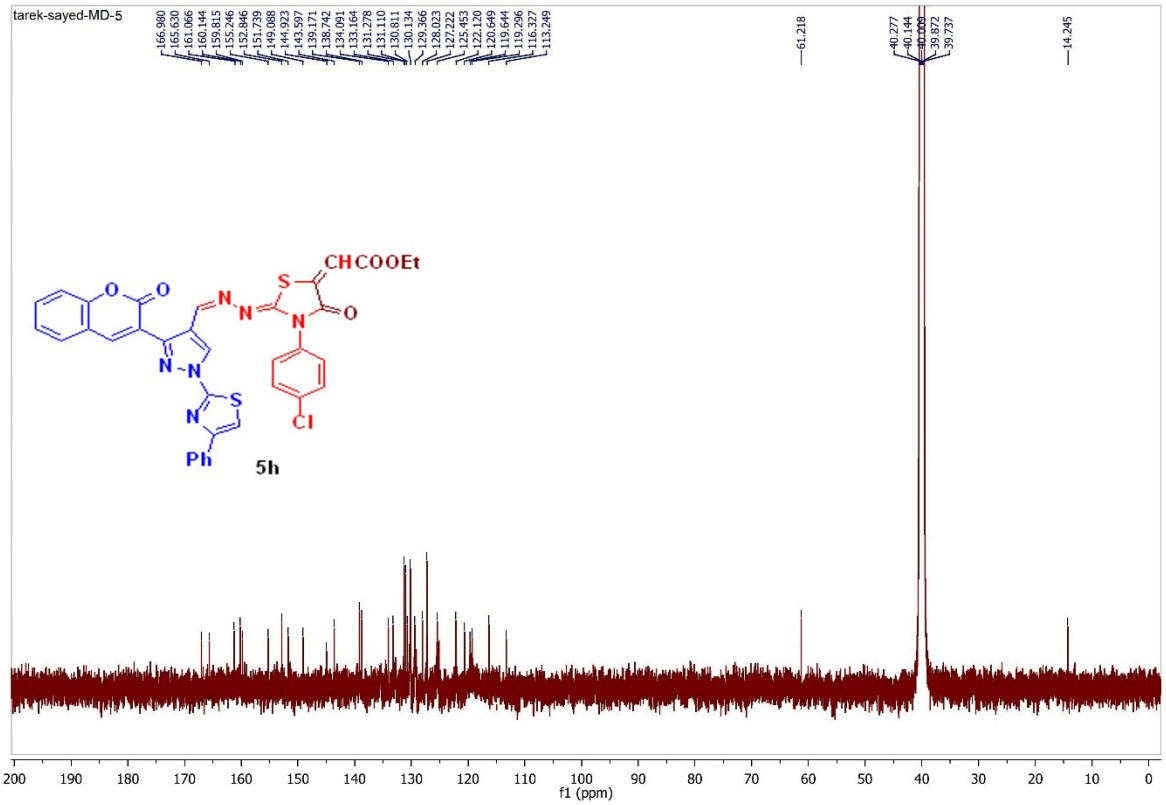

Figure S32: The ^{13}C-NMR spectrum of compound **5h.**

Table S1: The interactions of **Tivozanib** (**AV-951**), **5d** and **3g** with VEGFR-2 Receptor (PDB ID:4ASE) and their binding energies.

Compound	Binding Affinity (kcal/mol)	Amino Acid	Interaction Types	Distance (A°)
AV951	-11.4	CYS 919	Hydrogen bond	1.96
		GLU 885	Hydrogen bond	3.23
		ASP 1046	Hydrogen bond	2.06
		CYS 1045	Carbon Hydrogen bond	2.54
		LEU 840	Pi–Sigma	3.74
		PHE 1047	Pi–Pi T shaped	4.96
		LEU 1035	Pi–Alkyl	4.59
		ALA 866	Pi–Alkyl	3.53
5d	-8.4	ASN 923	Hydrogen bond	2.82
		ARG 1051	Hydrogen bond	2.76
		CYS 919	Hydrogen bond	2.48
		GLY 841	Carbon Hydrogen bond	2.37
		LEU 840	Pi–Sigma	2.47
		CYS 1045	Pi–Sulfur	5.23
		LYS 868	Pi–Cation	5.11
		PHE 1047	Pi–Pi Stacked	4.15
		LEU 1035	Pi–Alkyl	5.22
		VAL 848	Pi–Alkyl	4.06
		ALA 866	Pi–Alkyl	5.05
		VAL 899	Pi–Alkyl	4.61
		VAL 916	Pi–Alkyl	4.95
5g	-7.5	CYS 919	Hydrogen bond	2.50
		ARG 1051	Hydrogen bond	3.71
		LEU 840	Carbon Hydrogen bond	4.94
		ASN 923	Pi-Doner Hydrogen bond	3.08
		PHE 918	Pi–Pi Stacked	5.03
		LEU 1035	Pi–Alkyl	5.01, 5.26
		ALA 866	Pi–Alkyl	4.29
		VAL 848	Pi–Alkyl	3.94
		VAL 916	Pi–Alkyl	4.83
		VAL 899	Pi–Alkyl	4.73, 4.08
		CYS 1045	Pi–Alkyl	4.65, 4.68

Compd. No.	Molecular formula (MW[a])	TPSA (Å²)[b]	nRB[c]	nHBA[d]	nHBD[e]	MLog *P*[f]	Violations[g]
5d	$C_{32}H_{24}N_6O_5S_2$ (636.7)	185.79	10	9	0	3.3	2 (Lipinski's and Veber rule)
5g	$C_{36}H_{26}N_6O_5S_2$ (686.76)	185.79	10	9	0	3.9	2 (Lipinski's and Veber rule)
Doxorubicin	$C_{27}H_{29}NO_{11}$ (543.52)	206.07	5	12	6	-2.10	3 (Lipinski's and Veber rule)

Table S2. The physicochemical features of the synthesized compounds **5d, 5g** and doxorubicin

[a]Molecular weight. [b]Topological plar surface area. [c]Number of the rotatable bond. [d]Number of the hydrogen bond acceptor, [e]Number of the hydrogen bond donor. [f]Calcaluated lipophilicity (MLog *P*). [g]Violations from Lipniski and Veber rules.

Table S3. The pharmacokinetic features of the synthesized compounds **5d, 5g** and doxorubicin.

Compd. No.	GIT absorption	BBB permeability	P-gp substrate	Bioavailability Score	Pains alert
5d	Low	No	No	0.17	0
5g	Low	No	No	0.17	0
Doxorubicin	Low	No	Yes	0.17	1

Table S4. The toxicity properties of the synthesized **5d, 5g** and doxorubicin.

Compd No.	hERG Blockers	AMES Toxicity	Acute oral toxicity	Carcinogenicity (three-class)
5d	0.237 (Weak inhibitor)	0.790 (Non-toxic)	0.487 (III)	0.839 (Non-required)
5g	0.473 (Weak inhibitor)	0.798 (Non-toxic)	0.381 (III)	0.768 (Non-required)
Doxorubicin	0.169 (Weak inhibitor)	0.994 (Non-toxic)	0.940 (III)	0.976 (Non-required)