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Research Article

Synthesis, Chemical Characterization, and Biological Screening for Cytotoxic and Antitumor Activity of Novel p-Chlorophenyl Maleanilic Acid and Its Corresponding Chelates

M. A. Zayed,[1] Fatma S. M. Hassan,[2] Adila E. Mohamed,[2] and Khloud R. Oraby[2]

[1] *Chemistry Department, Faculty of Science, Cairo University, Giza 12613, Egypt*
[2] *Chemistry Department, Faculty of Science, Aswan University, Aswan 81528, Egypt*
Address correspondence to M. A. Zayed, mazayed429@yahoo.com

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Abstract A novel p-chlorophenyl maleanilic acid ligand (L) was prepared from condensation reaction of maleic anhydride with p-chloroaniline. New synthesized organometallic chelates of the proposed general formula $[M(CO)_4L]$, where M = Cr, Mo or W, were prepared by reaction of metal hexa carbonyls condensation with bidentate maleanilic acid ligand in inert nitrogen atmosphere. The prepared novel ligand and its organometallic chelates were characterized by elemental analyses, FT-IR, mass spectra, ^{1}H-NMR, and thermal analyses. The results obtained refer to an octahedral structure of the prepared organometallic chelates. The free ligand and its chromium chelate were screened for antitumor activity in vitro against cell lines of HCT-116 (human colon carcinoma), hepG-2 (human hepatocellular carcinoma), and MCF-7 (human breast carcinoma). The results obtained referred to a high antitumor activity of both novel ligand and its chromium chelate.

Keywords p-chlorophenyl maleanilic acid; transition metal chelates; spectroscopic analysis; thermal analysis; anticancer activity

1. Introduction

Metal carbonyls are coordination complexes of transition metals with carbon monoxide ligands. Metal carbonyls are useful in organic synthesis and as catalysts or catalyst precursors in homogeneous catalysis, such as hydroformylation and Reppe chemistry. In organometallic chemistry, metal carbonyls serve as precursors for the preparation of other organometallic complexes. Metal carbonyls are toxic by skin contact, inhalation or ingestion, in part because of their ability to carbonylate hemoglobin to give carboxyhemoglobin, which prevents the binding of O_2 [1, 2]. The nomenclature of the metal carbonyls depends on the charge of the complex, the number and type of central atoms, and the number and type of ligands and their binding modes. They occur as neutral complexes, as positively charged metal carbonyl cations or as negatively charged metal carbonylates. The carbon monoxide ligand may be bounded terminally to a single metal atom or bridging to two or more metal atoms. These complexes may be homoleptic, that is containing only CO ligands, such as nickel carbonyl $Ni(CO)_4$, but more commonly metal carbonyls are heteroleptic and contain a mixture of ligands. The number of carbon monoxide ligands in a metal carbonyl complex is described by a Greek numeral, followed by the word carbonyl. Carbon monoxide has different binding modes in metal carbonyls. They differ in the hapticity and the bridging mode. The hapticity describes the number of carbon monoxide ligands, which are directly bonded to the central atom. The denomination shall be made by the letter η^n, which is prefixed to the name of the complex. The superscript n indicates the number of bounded atoms. In monohapto coordination, such as in terminally bonded carbon monoxide, the hapticity is 1 and it is usually not separately designated. If carbon monoxide is bound via the carbon atom and via the oxygen to the metal, it will be referred to as dihapto coordinated η^2 [3,4]. On studying physical characteristics of most mononuclear carbonyl complexes, they are colorless or pale yellow volatile liquids or solids that are flammable and toxic vanadium hexacarbonyl, a uniquely stable 17-electron metal carbonyl, is a blue-black solid [1], while di- and polymetallic carbonyls tend to be more deeply colored.

The most important technique for characterizing metal carbonyls is infrared spectroscopy. The C−O vibration, typically called υCO, occurs at 2,143 cm^{-1} for CO gas. The positions of the υCO band(s) for the metal carbonyls are inversely correlated with the strength of the π-bonding between the metal and the carbon [5]. The carbonyl groups can have two stretching modes: symmetric and asymmetric stretching. Since both of these modes result in a change in dipole moment, in the infrared spectra of a terminally ligated carbon monoxide two bands are expected. The infrared and Raman spectroscopy together can be used to determine the geometry of the metallic carbonyls and

helps in determination of the ligated carbon monoxide bond order. The C−O bond order and the frequency related to its absorption are directly proportional. Thus, it can be predicted that the frequencies of absorption are in the following order: free CO > metal carbonyl cation > neutral metal carbonyl > metal carbonyl anion. It is also used to distinguish the terminal and bridging carbonyl groups. The C−O bonding in terminal carbonyl groups is stronger than the bridged carbonyl groups. Therefore, it is possible to differentiate the terminal carbonyls, which absorb in the region of 2,050–1,900 cm^{-1} from the bridged carbonyls absorbing below 1,900 cm^{-1} [6]. The most recent fairly comprehensive review of the vibrational spectra of transition metal carbonyls is contained in the book by Braterman [7].

The success of amide group ligands and its derivatives as biological active agents leads to stimulate the synthesis development of maleanilic acid derivatives and its compounds [8,9,10,11,12]. Maleanilic acid and its derivatives are amino acid compounds which display a variety of biological activities. They can be used as potential inhibitors [13] or antitubercular agents [14]. They can be used to prepare the maleimides which are an important class of substrates for preparing chemical probes for protein structure [10]. They also can be used as a protective and curative fungicide [15]. They are used as essential materials in polymer chemistry (i.e., as photoinitiators for free-radical polymerization and monomers in polymaleimides and copolymers synthesis) [16,17].

In view of these reports, we are interested in synthesis of p-chlorophenyl maleanilic acid ligand and its corresponding metal chelates of the general formula $[M(CO)_4L]$, where M = Cr, Mo or W. The newly synthesized products were characterized and the free ligand and its chromium complex are screened for their antitumor activities against cell lines of HCT-116 (human colon carcinoma), hepG-2 (human hepatocellular carcinoma), and MCF-7 (human breast carcinoma), which showed inhibition activities with good IC_{50} values.

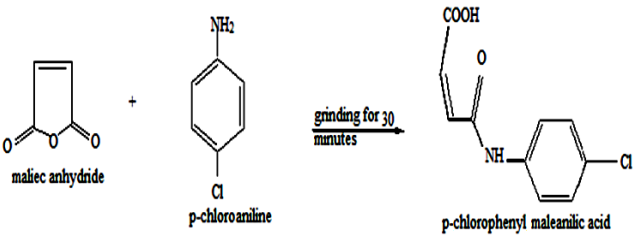
2. Experimental

2.1. Materials and reagents

All the chemicals used in this study are of the analytical grade and of highest purity available; they included 4-chloroaniline, maleic anhydride, $Cr(CO)_6$, $Mo(CO)_6$, and $W(CO)_6$. They are purchased from Sigma Aldrich (Germany) and used without further purification. The solvents such as absolute ethanol alcohol, DMF, and DMSO are purchased from Alpha Aesar.

2.2. Instrumentation

Melting points were determined in a capillary tube using (Gallen Camp) electrothermal melting point instrument (fine



Scheme 1: Preparation of p-chlorophenyl maleanilic acid.

controlled, measured temperature up to 370 °C) and elemental analysis data were recorded on the microanalytical center at Cairo University. ^{1}H-NMR studies were recorded on Bruker DPX 400 spectrometer (300.068787 MHZ) and DMSO was used as the internal reference solvent. The electron impact (EI) mass spectra (MS) at 70 eV of the tested compounds had been done using MS-5988 GS-MS Hewlett-Packard instrument. Thermal analyses (TGA and DTG) were carried out in dynamic nitrogen atmosphere (20 mL min^{-1}) with a heating rate of 10 °C min^{-1} using Shimadzu TGA Q500 V20.10 Build 36 instrument.

2.3. Methods

2.3.1. Preparation of free ligand

p-Chlorophenyl maleanilic acid free ligand was prepared according to the following procedures [18]: p-chloroaniline (12.75 g, 0.1 mol) was mixed and grinding at room temperature with maleic anhydride (9.8 g, 0.1 mol) in an agate mortar. During the grinding, a nice yellowish-white product appeared. The crude product was recrystallized from absolute ethanol and dried under vacuum over P_2O_5. The yield was 90% and the melting point was measured. The preparation procedure for the given novel ligand is presented in Scheme 1.

2.3.2. Synthesis of metacomplexes

Chelates of general formula $[M(CO)_4L]$, where L is p-chlorophenyl maleanilic acid and M = Cr, Mo, or W, were synthesized by adding the DMF solution of p-chlorophenyl maleanilic acid ligand (0.5 mmol, 0.11 g) to $M(CO)_6$ (0.5 mmol) in DMF solvent with constant stirring at (90 °C–100 °C) continued for 60 min under reflux in inert nitrogen atmosphere. The reaction mixture was heated with stirring to evaporate all the solvents to endure the resulted precipitate. The precipitate was cooled, filtered off, washed thoroughly with absolute ethanol several times, purified and recrystallized from DMF/absolute ethanol mixture solvent. Finally, it was dried in desiccator containing drier P_2O_5. All the above steps were repeated for all the selected transition metal chelates. The suggested procedures used for preparation of all chelates are shown in Scheme 2 according to the modification of Cooper et al. methods [19,20].

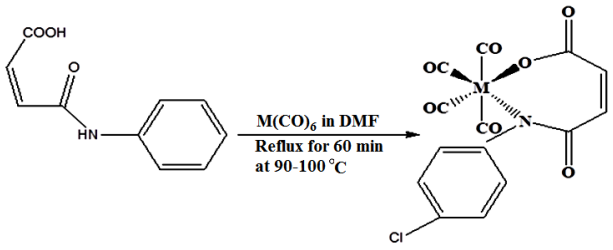
Table 1: Properties of p-chlorophenyl maleanilic acid ligand (L) and its new complexes of molar ratio (1:1) of transition metal cation (M = Cr, Mo or W metal carbonyls).

Compound	Mol. formula	Mol. wt	Color	M.P °C	%C calc. (found)	%H calc. (found)	%N calc. (found)
L(p-ClMA)	$C_{10}H_7O_3NCl$	224.62	Yellowish white	200–203	53.47 (52.35)	3.14 (3.71)	6.2 (6.47)
$Cr(CO)_4$-L complex	$C_{17}H_{14}O_8N_2ClCr$	461.76	Oily green	179–182	44.21 (43.92)	3.03 (3.7)	6.06 (6.8)
$Mo(CO)_4$-L complex	$C_{17}H_{14}O_8N_2ClMo$	505.7	Yellow	157–160	40.37 (39.88)	2.77 (3.04)	5.54 (6.1)
$W(CO)_4$-L complex	$C_{17}H_{14}O_8N_2ClW$	594.63	Yellow	152–154	34.34 (35.33)	2.35 (2.71)	4.71 (5.4)

Table 2: Infrared spectrum data of p-chlorophenyl maleanilic acid ligand and its metal complexes (band maxima in cm^{-1}).

Compound	υ(C=O) COOH	υ(C=O) amide(I) (N−C=O)	υ(O−H)	υ(N−H) amide	υ(C=O) carbonyl	υ(M−O)	υ(C−Cl)
L	1,702	1,630	3,282	3,205	—	—	1,092
$Cr(CO)_4L$	1,709 s	1,629 m	—	3,273 w	1,971 m	502	1,089
$Mo(CO)_4L$	1,706 s	1,630 m	—	3,480 w	1,971 m	508	1,089
$W(CO)_4L$	1,705 s	1,654 m	—	3,423 w	2,057 m	506	1,090

Band property: s = strong, m = medium, w = weak.



Scheme 2: Preparation of $[M(CO)_4L]$ complexes, where M = Cr, Mo or W and L is p-chlorophenyl maleanilic acid.

2.4. Biological activity

2.4.1. Materials and reagents

MCF-7 cells (human breast cancer cell line), HepG-2 cells (human Hepatocellular carcinoma), and HCT-116 (colon carcinoma) were obtained from VACSERA Tissue Culture Unit, dimethyl sulfoxide (DMSO), and crystal violet and trypan blue dye were purchased from Sigma (St. Louis, Mo., USA). Fetal bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25% Trypsin-EDTA were purchased from Lonza, and crystal violet stains (1%).

2.4.2. Cytotoxicity evaluation using viability assay

The cells were propagated in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50 μg mL^{-1} gentamycin. All cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2 and were subcultured two times a week. The cells were seeded in 96-well plate at a cell concentration of 1×10^4 cells per well in 100 μL of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell mono layers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37 °C in a humidified incubator with 5% CO_2 for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO.

The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells at 37 °C, various concentrations of sample were added, and the incubation was continued for 24 h and viable cells yield was determined by a colorimetric method [21,22].

3. Results and discussion

p-Chlorophenyl maleanilic acid free ligand was prepared by the condensation reaction of maleic anhydride and p-chloroaniline. Then it was complexed with metal ions by (1:1) molar ratio to yield $[M(CO)_4L]$ complex [M= Cr, Mo or W]. Both of the free ligand and its corresponding metal complexes were isolated, purified by recrystallization, and characterized.

3.1. Elemental analysis and physical properties

The elemental analysis data of the free ligand and its corresponding transition metal chelates agreed with the theoretical values within the limit of experimental error; as shown in Table 1.

These analytical data confirm the proposed general formulae of the prepared compounds.

3.2. FT-IR spectroscopy

The FT-IR data of the free ligand and its corresponding metal chelates are examined and the results are presented in Table 2.

The FT-IR spectra of p-chlorophenyl maleanilic acid free ligand (L) show that a broad band at 3,282 cm^{-1} may be revealed to υ(OH) of carboxylic group; this band was disappeared in the spectra of its chelates [5,6,7,8]. The band

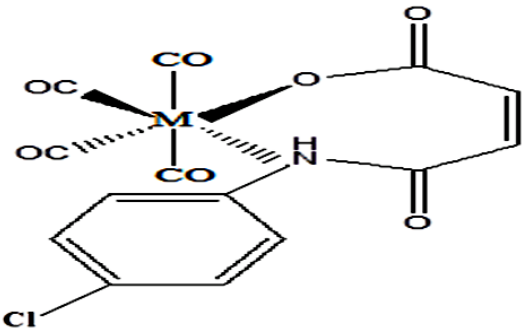


Figure 1: The proposed structure of the prepared organometallic chelates (M = Cr, Mo or W).

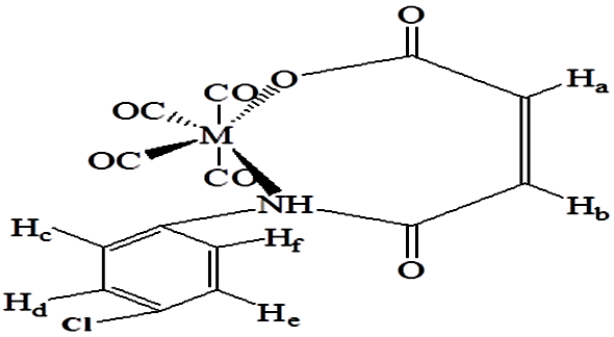


Figure 2: The proposed protonated structure of metal chelates (M = Cr, Mo or W).

Table 3: Chemical shifts δ (ppm) relative to DMSO.

Compound	δO−H	δN−H	δH^a	δH^b	δH^c	δH^d	δH^e	δH^f
L	12.96	10.44	6.44	6.26	7.66	7.39	7.39	7.66
			6.29	6.28	7.64	7.38	7.38	7.64
$Mo(CO)_4L$	—	10.62	6.27	6.31	7.36	7.39	7.39	7.36
			6.42	6.46	7.63	7.66	7.66	7.63
$W(CO)_4L$	—	10.61	6.24	6.28	7.56	7.59	7.59	7.56
			6.64	6.68	7.65	7.68	7.68	7.65

resonated at 1,702 cm^{-1} in the spectra of free ligand may be attributed to υ(C=O) of carboxylic group. This band was slightly shifted to 1,709, 1,706, and 1,705 cm^{-1} in the spectra of its chelates. The weak υ(N−H) amide stretching band observed at 3,205 cm^{-1} in the spectra of free ligand (L) was shifted to higher frequency at 3,273, 3,480, and 3,423 cm^{-1} in the spectra of [$Cr(CO)_4L$], [$Mo(CO)_4L$], and [$W(CO)_4L$] complexes, respectively. This conclusion indicates the coordination of p-chlorophenyl maleanilic acid ligand to the metal ions through the nitrogen of amide group [15]. The coordination is also evident from the FT-IR spectra of the complexes which show stretching bands observed at 502–508 cm^{-1}; these bands may be assigned to υ(M−O) [23].

The comparative studies of FT-IR for the free ligand and its corresponding complexes prove the proposed structure of the complexes which is shown in Figure 1.

3.3. ^{1}H-NMR measurements

The ^{1}H-NMR data of p-chlorophenyl maleanilic acid free ligand (L) and its corresponding metal chelates are studied. The results are tabulated in Table 3.

A singlet at δ 12.96 ppm may be assigned to the carboxylic OH proton in free ligand (L) spectrum. This band is disappeared in the spectra of its metal chelates indicating the sharing of free ligand carboxylic group in the chelate form. The signal resonated at δ 10.44 ppm of H-NCO proton of L free ligand is slightly shifted to δ 10.62 and δ 10.61 ppm in the spectra of $Mo(CO)_4L$ and $W(CO)_4L$ complexes, respectively. This indicates the possibility of sharing the free ligand amide group in the coordination process. A doublet of doublets at δ 7.39–7.38 ppm of relative intensity 1H of the aromatic protons *ortho* to chloro group (H^e, H^d) of free ligand (Figure 2) is slightly shifted to δ 6.39–7.66 ppm and δ 6.59–7.68 ppm for $Mo(CO)_4L$ and $W(CO)_4L$, respectively. The second doublet of doublets at δ 7.66–7.64 ppm of the aromatic protons, which are *ortho* to amide group (H^c, H^f), is slightly shifted to δ 7.36–7.63 ppm and δ 7.56–7.65 ppm for $Mo(CO)_4L$ and $W(CO)_4L$, respectively. A doublet of doublets which are observed at δ 6.44–6.29 ppm of free ligand vinylic proton H^a is slightly shifted to δ 6.27–6.42 ppm and δ 6.24–6.64 ppm for Mo and W metal chelates, respectively. The other vinylic proton H^b, which resonated at 6.26–6.28 ppm, is slightly shifted to δ 6.31–6.46 ppm and δ 6.28–6.68 ppm for $Mo(CO)_4L$ and $W(CO)_4L$, respectively [24]. These shifts are confirming the chelation possibility of L through carboxylic and amide groups to the Mo and W metal cations (Figure 2).

3.4. Mass spectra of p-chlorophenyl maleanilic acid metal chelates

The electron impact mass spectra (EI-MS) of the newly prepared complexes are recorded at 70 eV and investigated. The electron ionization (EI-MS) mass spectrum for $Cr(CO)_4L$ complex at 70 eV was recorded and investigated; see Figure 3.

The mass spectrum of $Cr(CO)_4L$ shows a signal at $m/z = 388$ with RI = 48%; this signal may be attributed to the main molecular ion. This fragment is broken through three parallel pathways which are presented in Scheme 3.

Pathway I shows fragment ion at $m/z = 164$ (mole mass = 164, RI = 25%) due to the rupture of p-chlorophenyl maleanilic acid. The signal at $m/z = 109$ (mole mass = 108; RI = 21%) is due to the rupture of two CO gas molecules. Pathway II shows fragment ions at $m/z = 225$ and 113 (RI = 63% and 40%, resp.); these fragments may be attributed to the loss of $Cr(CO)_4$ followed by the loss of 4-chlorobenzene. The third pathway shows signal at $m/z = 332$ (mole mass = 332, RI = 35%), which may be referred to the loss of two molecules of CO gas.

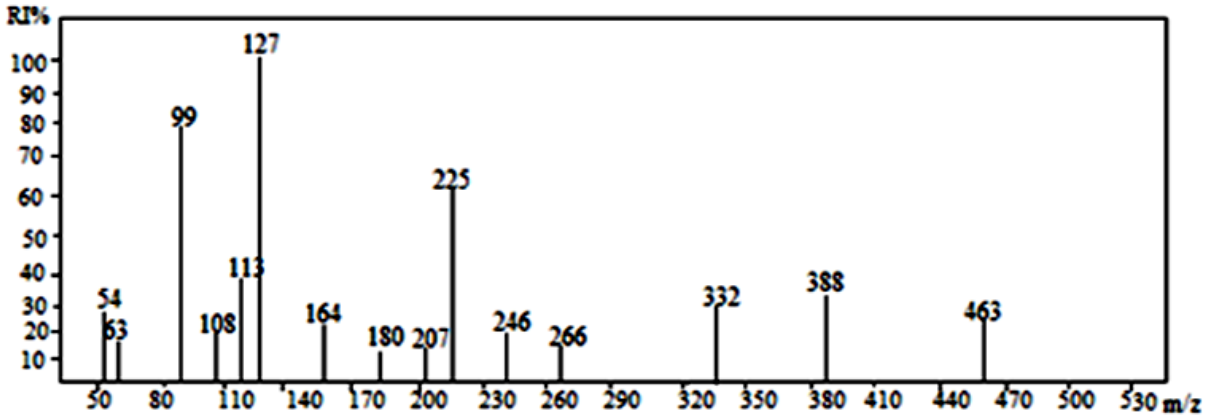
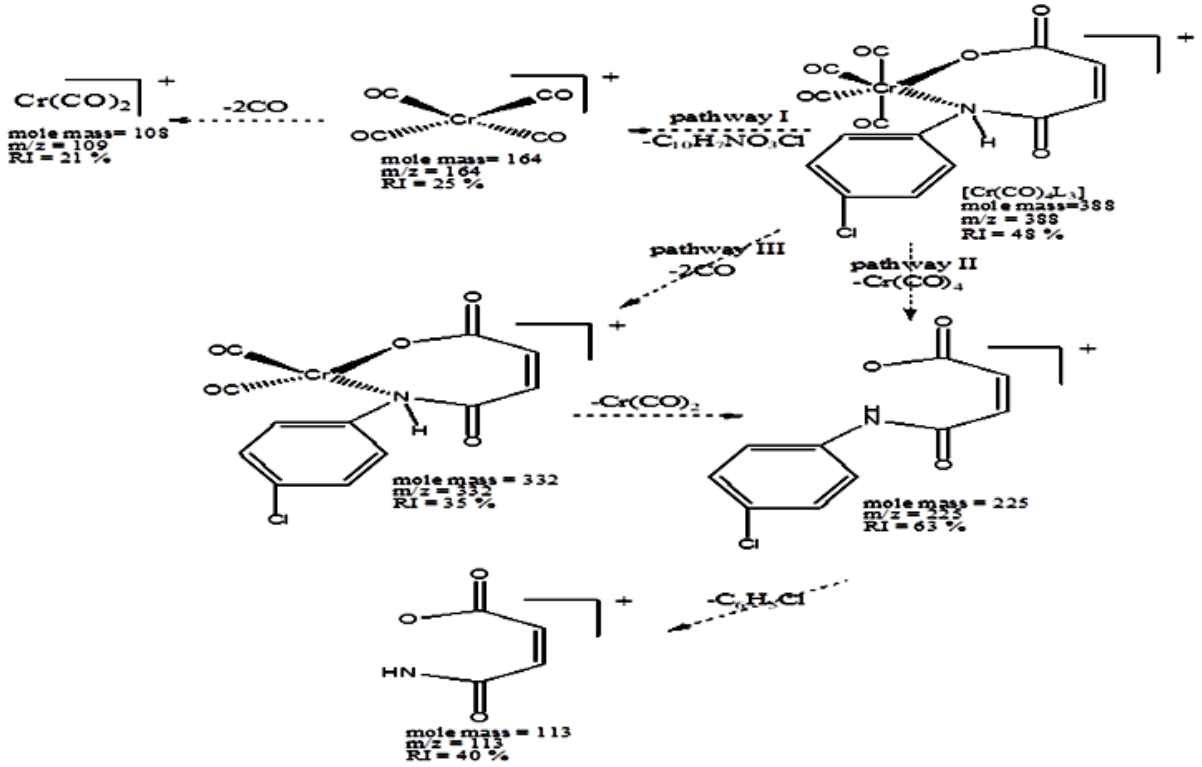


Figure 3: Mass spectra of $[Cr(CO)_4L]$, where L = p-chlorophenyl maleanilic acid.



Scheme 3: The mass fragmentation pathways of the principle fragment ions of $[Cr(CO)_4L]$ chelate.

3.4.1. Mass spectra of [Mo(CO)$_4$L]

The electron ionization mass spectrum for $Mo(CO)_4L$ is measured at 70 eV and investigated. The signals are presented in Figure 4.

The spectrum of $Mo(CO)_4L$ at 70 eV is characterized by many competitive and consecutive pathways, thus forming many intense fragment ions which are presented in Scheme 4.

The mass fragmentation of $Mo(CO)_4L$ after ionization of neutral molecule at 70 eV consists of three principal pathways as rationalized in Scheme 4. The signal that appears at $m/z = 434$ (RI = 35%) may be referred to the appearance of the main molecular ion. This molecular ion is due to the loss of 4-chlorobenzene. The appearance of the signal at $m/z = 322$ (mole mass = 322, RI = 48%) is followed by signal at $m/z = 207$ (mole mass = 207, RI = 65%), which may be attributed to the loss of $C_4H_5O_3N$. Pathway II shows a signal at $m/z = 226$ (mole mass = 226, RI = 40%) due to the loss of $Mo(CO)_4$ from the molecular ion. The signal at $m/z = 115$ (mole mass = 115, RI = 29%) may be referred to the loss of 4-chlorobenzene, followed by the elimination of CO_2 gas with a signal at $m/z = 71$ (mole mass = 71,

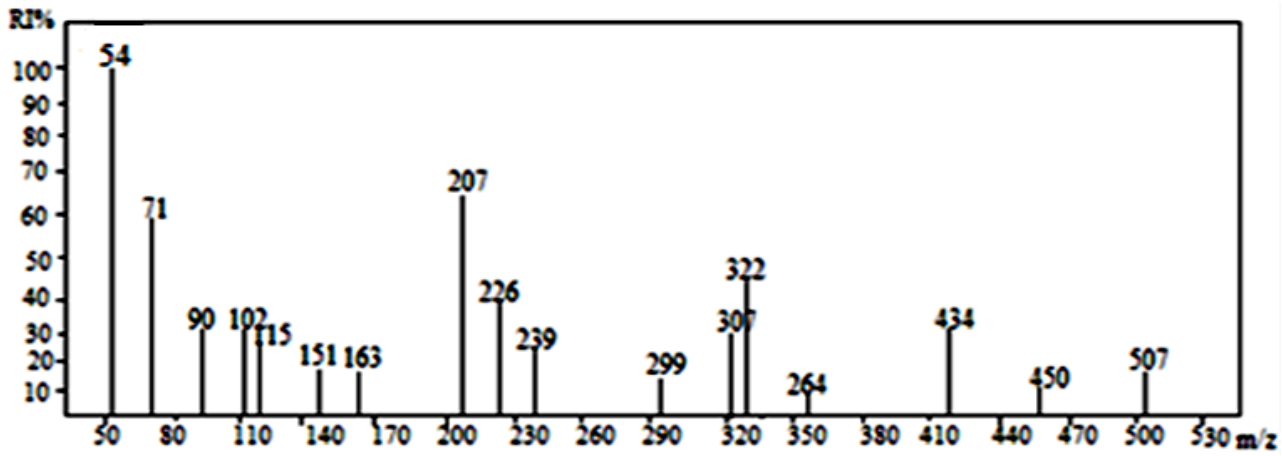
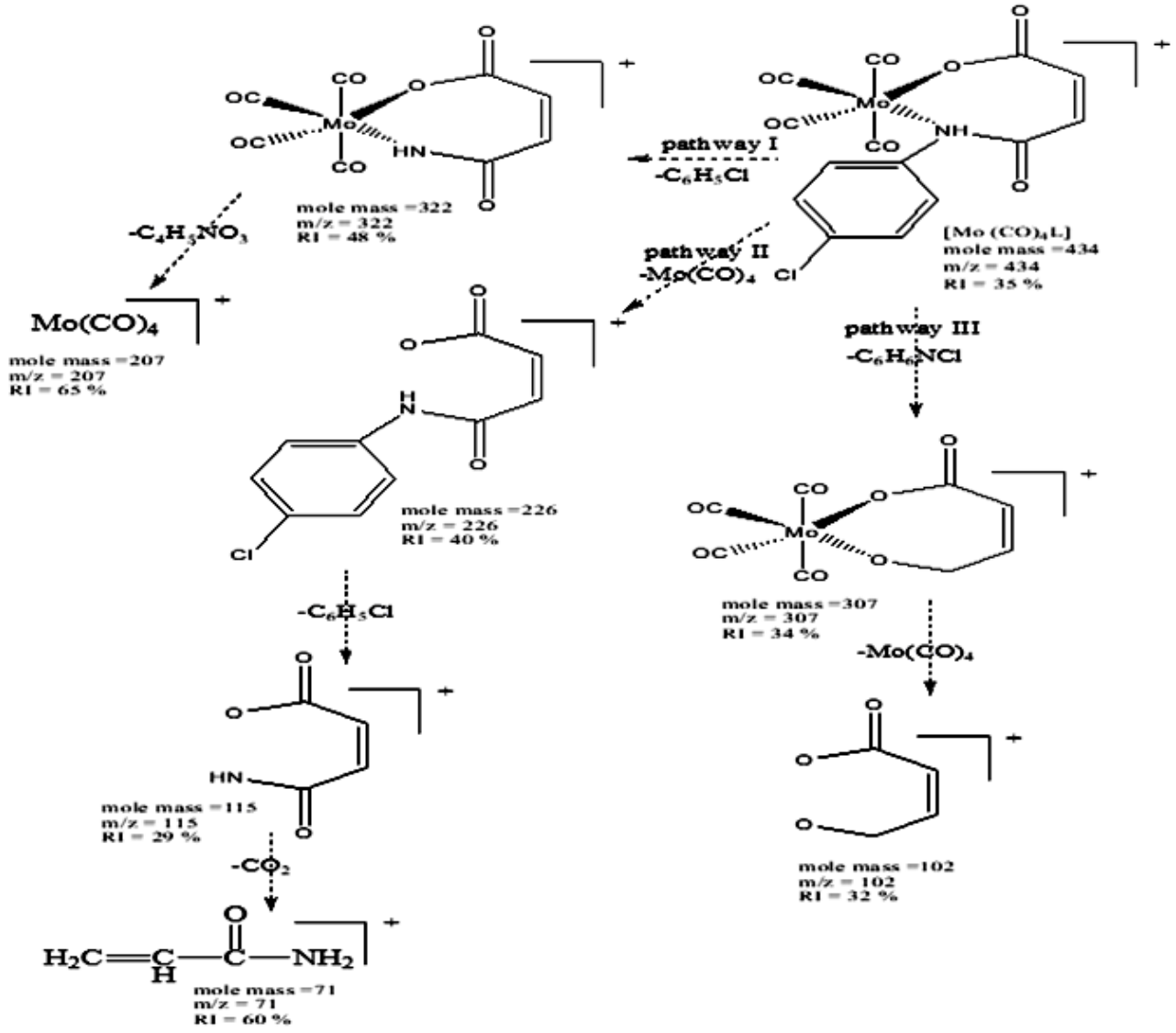


Figure 4: Mass spectra of $Mo(CO)_4L$, where L = p-chlorophenyl maleanilic acid.



Scheme 4: The mass fragmentation pathways of the principle fragment ions of $Mo(CO)_4L$.

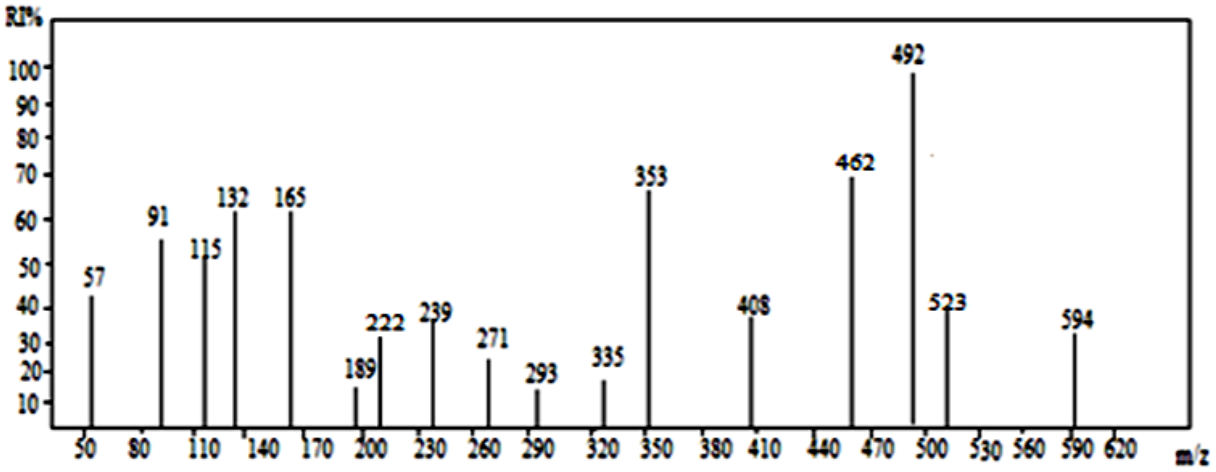


Figure 5: Mass spectra of $W(CO)_4L$, where L = p-chlorophenyl maleanilic acid.

RI = 60%). The final pathway shows two consecutive fragments at $m/z = 307$ and 102 (mole masses = 307 and 102, RI = 34% and 32%); these fragments may be referred to the loss of p-chloroaniline and $Mo(CO)_4$, respectively.

3.4.2. Mass spectra of [W(CO)$_4$L]

The electron ionization mass spectrum for $W(CO)_4L$ at 70 eV is recorded in Figure 5.

The spectrum of $W(CO)_4L$ is characterized by many competitive and consecutive pathways and forming many intense fragmentations, which are presented in Scheme 5.

The mass fragmentation of $W(CO)_4L$ chelate consists of four principal pathways. The signal at $m/z = 523$ (mole mass = 523, RI = 42.41%) refers to the main molecular ion and its high RI value refers to the stability of this metal chelate. In Scheme 5 pathway I, the fragment at $m/z = 408$ (mole mass = 408, RI = 37.17%) refers to the loss of chlorobenzene from the main molecular ion. This step is followed by the loss of maleic acid with $m/z = 293$ (mole mass = 293, RI = 38.46%), then the remainder product loosed 2CO groups with $m/z = 239$ (mole mass = 239, RI = 38.22%). Pathway II shows different signals at $m/z =$ 393 and 97 (RI = 59.69% and 40.84%, resp.), which are due to the loss of fragment ions of mole masses = 393 and 100, respectively. The signals at $m/z = 462$ and 339 in Scheme 5, pathway III (RI = 46.6% and 32.46%, resp.) are attributed to the loss of fragment ions of mole masses = 463 and 339, respectively. Most of these fragment ions are related the rupture of two molecules of CO gas followed by the loss of 4-chlorobenzene respectively from the main molecular ion.

3.5. Thermal analyses

The TGA and DTG thermal analyses data of the synthesized metal chelates are tabulated in Table 4.

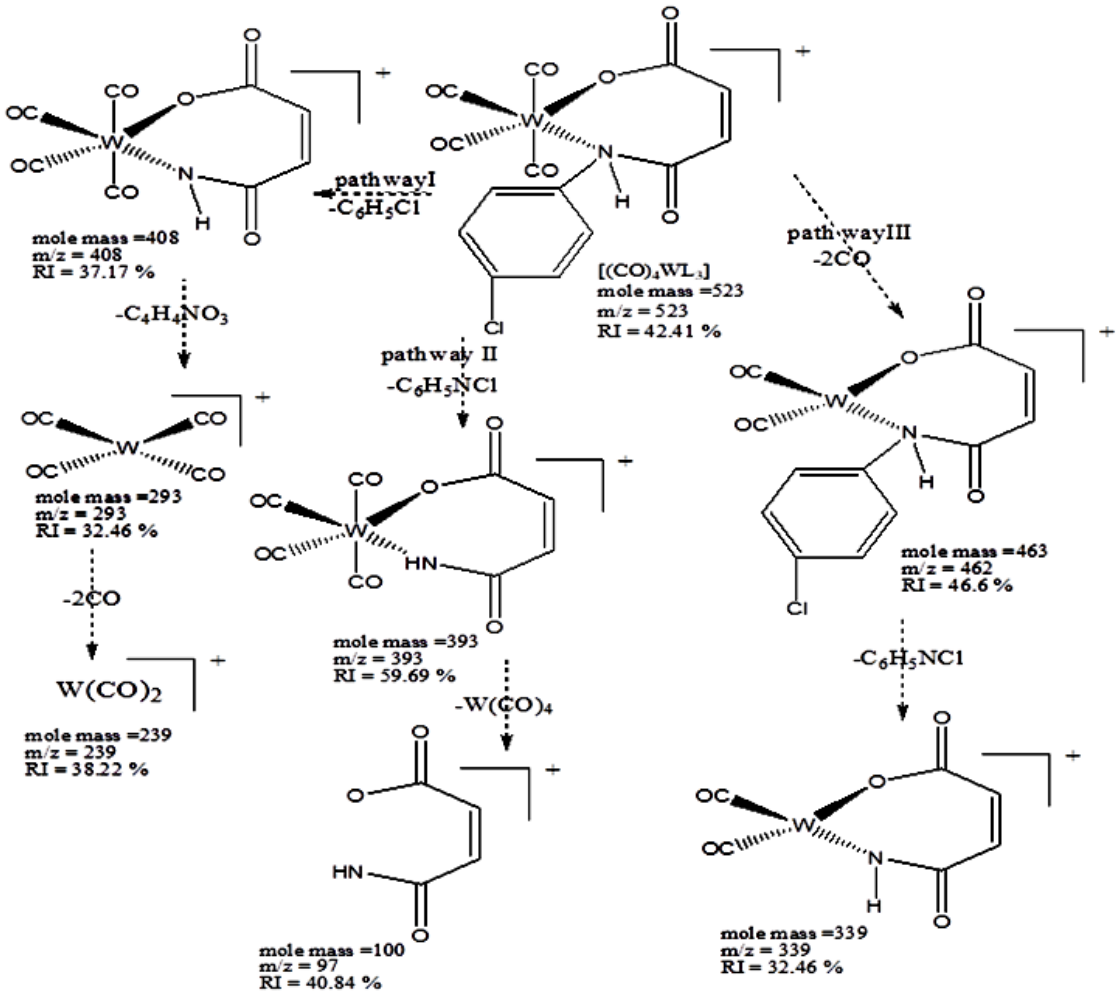
The thermal decomposition of $Cr(CO)_4L$ metal chelate as an example occurs through two steps. The first step occurs at temperature 110 °C–184 °C and exact DTG peak temperature of 134 °C with mass loss of 48.64% (calcd. 49.33%). This step may be assigned to the separation of p-chlorophenyl maleanilic acid ligand from the metal chelate. The second step occurs at temperature range of 184 °C–278 °C with DTG peak at 273.5 °C; this range may correspond to the removal of two CO groups with observed mass loss of 12.13% (calcd. 13.18%). The total practical mass loss may be 60.77% (calcd. 62.51%). The remainder product may be $Cr(CO)_2$ with practical mass 39.23% (calcd. 37.49%).

The second metal chelate $Mo(CO)_4L$ decomposed through one step occurs at range 110 °C–271 °C and DTG peak at 190 °C with mass loss of 44.42% (calcd. 43.02%). This mass loss may be attributed to the removal of p-chlorophenyl maleanilic acid ligand from the entity of the metal chelate leaving $Mo(CO)_4$ as remainder product with practical mass 55.58% (calcd. 57.98%).

Three decomposition steps appear in the thermal analysis of $W(CO)_4L$ complex. The first one may correspond to the loss of p-chlorophenyl maleanilic acid ligand with mass loss of 34.6% (calcd. 37.17%). The second occurs at 280 °C–350 °C with a mass loss of 4.33% (calcd. 4.71%), which may be attributed to the loss of CO gas. The third step of decomposition (350 °C–589 °C) may be assigned to the loss of another molecule of CO gas leaving $W(CO)_2$ as a remainder product with practical mass 56.43% (calcd. 53.41%).

3.6. Biological activity

The cytotoxic activity of p-chlorophenyl maleanilic acid ligand and its chromium chelate against cell lines of HCT-116 (human colon carcinoma), hepG-2 (human hepatocellular carcinoma), and MCF-7 (human breast carcinoma) was evaluated by viability assay [25]. The results obtained are shown in Figures 6(a) and 6(b). The concentrations of them ranged from 3.9 μg mL^{-1} to 500 μg mL^{-1}.



Scheme 5: The mass fragmentation pathways of the principle fragment ions of $W(CO)_4L$.

Table 4: Thermal analyses data of the newly synthesized chelates.

Compound	TGA temp. range (°C)	DTG temp (°C)	Mass loss calcd. %	TGA description	Residue
$C_{14}H_7O_7NClCr$	110–184	134	49.33 (48.64)	– The loss of p-chlorophenyl maleanilic acid ligand	$Cr(CO)_2$
$Cr(CO)_4L$	185–278	273.5	13.18 (12.13)	– The loss of 2CO groups	37.49 (39.23)
$C_{14}H_7O_7NClMo$	110–271	190	43.02 (44.42)	– The loss of p-chlorophenyl maleanilic acid ligand	$Mo(CO)_4$
$Mo(CO)_4L$					57 (55.6)
$C_{14}H_7O_7NClW$	131–280	169.69	34.6 (37.17)	– The loss of p-chlorophenyl maleanilic acid ligand	$W(CO)_2$
$W(CO)_4L$	280–350	252.52	4.33 (4.71)	– The loss of CO group	46.09 (44.57)
	350–589	391.92	4.64 (4.71)	– The loss of CO group	

The toxicity of ligand and its chromium chelate are found to be concentration dependent. The cell viability decreased with increasing the concentration of both ligand and chelate against the tested cancer cell lines. Evaluation of the efficacy of p-chlorophenyl maleanilic acid free ligand (L) as inhibitor revealed a moderate potency against [(HepG-2), (MCF-7), and (HCT-116)] human cancer cell lines, as shown in Figure 6(a) with IC_{50} values about 123 μg mL^{-1}, 95.2 μg mL^{-1}, and 60.4 μg mL^{-1} for [(HepG-2), (MCF-7), and (HCT-116)] human cancer cell lines, respectively. The IC_{50} values are estimated from the respective dose-response curve and are summarized in Table 5. The data obtained from the cytotoxic activity assay of $Cr(CO)_4L$ chelate illustrated that the inhibitory potency of the free ligand was obviously weakened when complexed with $Cr(CO)_6$ except for HepG-2 cell line. $Cr(CO)_4L$ chelate shows higher potency to HepG-2 cell line than the free ligand.

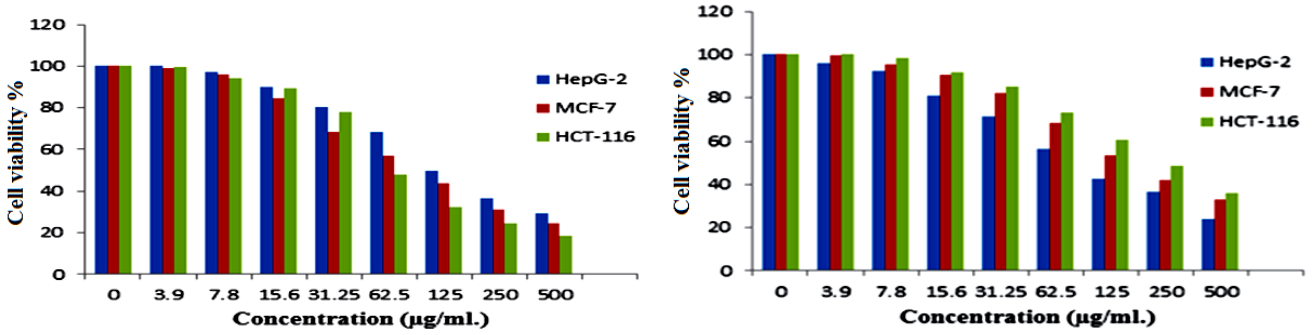


Figure 6: (a) Cell viability of (L) against the three tested cell lines. (b) Cell viability of $[Cr(CO)_4L]$ complex against the three tested cell lines.

Table 5: Influence of the ligand and chromium chelate on the viability of MCF-7, HepG-2, and HCT-116 cell lines.

Compound	In vitro cytotoxicity IC_{50} ($\mu g\,mL^{-1}$)		
	HepG-2	MCF-7	HCT-116
p-Chlorophenyl maleanilic acid (L)	123	95.2	60.4
$Cr(CO)_4L$ chelate	91.2	163	236

4. Conclusions

A novel p-chlorophenyl maleanilic acid ligand was prepared and structurally identified. The isolated p-chlorophenyl maleanilic acid organometallic W, Mo, and Cr chelates during the present study are proved to have stoichiometric ratio of 1:1 ($L:M(CO)_4$) and found to be mononuclear compounds. The structures of this novel ligand and its isolated metal chelates are proved by elemental analyses and applying spectroscopic measurements (FT-IR, H-NMR, and mass spectra) and confirmed by thermal analyses. The anticancer activity of the synthesized novel p-chlorophenyl maleanilic acid free ligand and its corresponding chromium chelate are investigated. The free ligand shows inhibitory activity higher than those of its chelate against MCF-7 and HCT-116 cell lines and it has lower inhibitory activity than the chelate against HepG-2 cell line.

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Conflict of interest The authors declare that they have no conflict of interest.

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