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Cytotoxic, antiglycation and carbonic anhydrase inhibition studies of chromium(III)-aroylhydrazine complexes

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ABSTRACT

In order to further reveal the chemistry and biochemistry of chromium(III) complexes, the present work illuminates the formation of chromium(III) complexes with aroylhydrazine ligands with their physical, chemical and spectral studies. Another significant contribution of this study is the evaluation of the cytotoxic activity, antiglycation property and carbonic anhydrase inhibition study of synthesized chromium(III)-aroylhydrazine complexes. Synthesis and structural investigation of aroylhydrazine ligands (1-7) and their chromium(III) complexes (1a-7a) were carried out by using elemental analysis (C, H, N), physical (conductivity measurements) and spectral (EI-Mass, ESI-Mass, FTIR and UV-Visible) methods. These physical, analytical and spectral data supports that all chromium(III)-aroylhydrazine complexes exhibit an octahedral geometry in which ligand exhibits as a bidentate coordination and two water molecules coordinated at equatorial positions with general formula $[Cr(L)_2(H_2O)_2]Cl_3$. Cytotoxic investigations shows that synthesized chromium(III)-aroylhydrazine complexes were not found to be toxic against normal cells so these compounds were further studied for other biological activities. Moreover, aroylhydrazine ligands and their chromium(III) complexes were examined for their antiglycation activity in which ligands were found inactive whereas chromium(III)-aroylhydrazine complexes showed significant inhibition of the process of protein glycation. Similarly, in carbonic anhydrase inhibition studies all aroylhydrazine ligands were observed inactive while some of chromium(III)-aroylhydrazine complexes showed potential in carbonic anhydrase inhibition.

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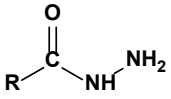
1. Introduction

Chromium(III) is an important mineral that shows a significant part in metabolism of glucose and it is required as a supplement in a control of diabetes mellitus [1]. Literature suggests that chromium(III) complexes have a number of biological activities [2-5] but some are cytotoxic in nature [6]. So it is necessary to investigate new non-toxic chromium(III) complexes. Moreover, ligands such as aroylhydrazines have also an enormous biologically importance and their activities are known to be more enhanced after complexation with certain metal ions [7-15].

Chromium(III) and chromium(VI) are the common forms of chromium in which chromium(VI) compound have reported as more toxic, harmful and carcinogenic as compared to compounds of chromium(III) that has its own beneficial biological importance [16]. In biological tissues, chromium is usually occur in trivalent form that regulates the normal metabolism of glucose, proteins and fats [17,18]. If chromium(III) ingestion is thousands of µg/day, it affects the human body dangerously. Another important form of chromium is chromium(VI) that is highly toxic to humans as well as animals due to its oxidizing capability, mutagenic and carcinogenic nature [17]. Strong evidences have been found on mutagenicity of chromium(VI) complexes in bacterial also in mammalian cells besides the chromium(III) complexes non-mutagenicity [19]. Chromium(III) exists inside the cells and it cannot pass through the membrane, so found to be non-carcinogenic due to critical binding of DNA inside the cells [20,21]. The beneficial and harmful effects of chromium supplements are due to these two oxidation states of chromium in biological systems. Hence several biological studies have discussed the toxicity of chromium(III) compounds. But very few of the research work have been done on the safety and non-cytotoxic behavior of chromium(III) complexes [3].

Chromium(III) assists the interaction of insulin through its receptor and the cell surface [22,23]. Chromium(III) is known to increases binding of insulin to cells, numerous insulin



1 - 12

1	R = 3-F-C_6H_4	7	R = 3-Br-C_6H_4
2	R = 3-OCH_3-C_6H_4	8	R = C_6H_5
3	R = 3-NH_2-C_6H_4	9	R = 2-F-C_6H_4
4	R = 4-NH_2-C_6H_4	10	R = 2-OCH_3-C_6H_4
5	R = 3-I-C_6H_4	11	R = 2-NH_2-C_6H_4
6	R = 4-I-C_6H_4	12	R = NH-C_6H_5

Figure 1. Structures of aroylhydrazine ligands (**1-12**).

receptors, which cause to make active insulin receptor kinase leading to improved insulin sensitivity [1]. The increase in diabetic issues and failure of existing antidiabetic drugs is important aspect to motivate researchers in the investigation of antiglycation agents for inhibition of protein that might be responsible for glycation. Diabetic problems such as retinopathy, neuropathy, cataract and atherosclerosis have straight relevance with advanced glycation end products (AGEs) [24]. Hence, agents with antiglycation and antioxidant properties may impede the route of AGEs formation by inhibiting further oxidation of Amadori products. The formation of AGEs is a multipart process comprising a variety of chemical reactions mediated without the support of any enzyme. Production of Schiff bases is the starting stage of glycation and in middle the Amadori products form due to readjustment of Schiff bases. The Amadori products go over further rearrangement, dehydration, condensation and addition reactions with other proteins [25]. It has been reported that multifunctional agents such as metal ion chelation, carbonyl scavenging and antioxidant activities within the similar molecule may also successfully inhibit the glycation reaction [25]. In fact, the analysis of compounds with both antioxidative and AGEs inhibition properties may act as anticipatory agents against diabetic problems [24]. Metal complexes of Co(II), Ni(II), Mn(II), Cu(II) and Zn(II) with isatinhydrazone have a prominent antiglycation as well as antioxidant activity [26]. Isatinthiosemicarbazone with zinc, nickel and cobalt also show a good antiglycation activity due to existence of thiourea moiety, chelation of metals with different substituents which contribute in the direction of their protein antiglycation activity [27].

Carbonic anhydrases (CAs) are highly active group of enzymes that are involved in different pathological processes and they have a major role in the growth and virulence of pathogens [28]. CAs initially catalyzes the physiological conversion of CO_2 into bicarbonate and proton. This reversible reaction is associated with a number of pathological and physiological processes like transportation and respiration of CO_2 between lungs and tissues, pH regulation, electrolyte secretion, homeostasis, biosynthetic processes (gluconeogenesis, ureagenesis and lipogenesis), bone resorption and calcification [29-33]. The most active CAs are CA II and CA IX that catalyses CO_2 into bicarbonates [34].

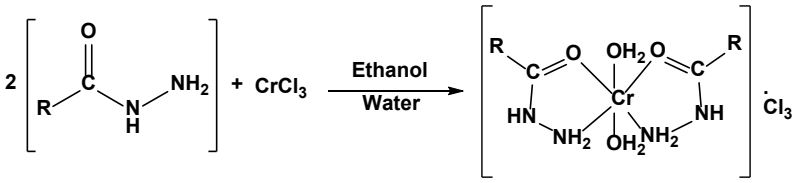
Carbonic anhydrase inhibitors (CAIs) establish their role as effective antiglaucoma, diuretics, antiobesity as well as anti-infective agents. Recently, it was found that CAIs have a great potential to act as anticancer and anti-infective drugs [28]. The acetazolamide, methazolamide, ethoxozolamide and dorazolamide are the standard CAIs, they have an important CA inhibitory properties [35-38]. Thiadiazole sulphonamides (such as benzolamide, acetazolamide and methazolamide) with multiple metals in which Zn(II), Fe(II), Hg(II), Cd(II), Co(II), V(IV), Cu(II), Cr(III), Ni(II) and lanthanides(III) have been examined by CA inhibition strengths against isozymes for their prospective pharmacological applications [39-46]. These complexes show very powerful action against CA I and CA II as linked to their sulphonamides [43].

In literature, very small records of non-toxic chromium compounds were found with enzyme inhibition potential and antiglycation properties. However, this work illustrates the studies of non-toxic nature of chromium(III)-aroylhydrazine complexes with antiglycation, CA-II enzyme inhibition potential that could be beneficial in the identification of new compounds for the control of damaging effects of cancer and other physiological disorders.

2. Experimental

2.1. Chemistry

All the chemicals were of reagent grade obtained from Merck, Sigma Aldrich or BDH which were utilized without additional purification. Structures of aroylhydrazine ligands are depicted in Figure 1 and synthesis of chromium(III)-aroylhydrazine complexes are presented in Scheme 1. CHN contents were analyzed on a CHN/S elemental analyzer Perkin Elmer 2400. Chromium content was determined in digested sample of chromium(III) complex in the form of lead chromate by gravimetric analysis using lead nitrate as a precipitating agent [47]. Non-coordinated chloride in digested sample of complex was estimated using cation exchange chromatography [47]. Molar conductance of chromium(III)-aroylhydrazine complexes were determined by conductivity meter of HANNA (HI-8633). Infrared (IR) spectra of all aroylhydrazines and their chromium(III) complexes were observed on a IR spectrophotometer (Shimadzu-460) at wavelength region 4000-400cm^{-1} on KBr disks. ^1H NMR spectroscopic analysis of aroylhydrazines were performed on Bruker spectrometer at 400 MHz using TMS as internal standard at room temperature. Chemical shifts were defined in δ (ppm) as well as coupling constants were specified in Hz. EI-MS spectroscopic analysis of aroylhydrazines were done on Finnigan-MAT-311-A apparatus and ESI-Mass spectroscopic analysis of chromium(III) complexes were performed on Qstar XL MS/MS system company applied biosystem. UV-Visible spectroscopy was done on a Shimadzu UV-1800 spectrophotometer by UV Probe software starting 200 to 800 nm. UV-Visible study was performed in two parts: Fresh solutions of aroylhydrazines and their corresponding chromium(III) complexes in DMSO with concentration 1.0×10^{-4} M were recorded in UV region from 200-350 nm. Then, in visible region from 350-800 nm solutions of chromium(III)-aroylhydrazine complexes with 7.5×10^{-3} M concentration and their spectra were recorded immediately after complete dissolution.



1a	**R = 3-F-C_6H_4**	**5a**	**R = 3-I-C_6H_4**
2a	**R = 3-OCH_3-C_6H_4**	**6a**	**R = 4-I-C_6H_4**
3a	**R = 3-NH_2-C_6H_4**	**7a**	**R = 3-Br-C_6H_4**
4a	**R = 4-NH_2-C_6H_4**		

Scheme 1. Synthesis of chromium(III) complexes of aroylhydrazines.

Chemicals required for antiglycation activity were procured from different chemical companies such as BSA (Research Organics), Rutin (Carl Roth GmbH & Co), sodium azide (Scharlau Chemie), methylglyoxyl 40% aqueous solution with sodium dihydrogen phosphate (Sigma Aldrich), DMSO (Fischer Scientific) and disodium hydrogen phosphate (Merck). Carbonic anhydrase (CA-II) was obtained from Sigma and Aldrich with 99% pure, HEPES buffer from DOJINDO Mol. Tech. Inc. (Rockville, MD USA), 4-nitro-phenyl acetate (NPA) from MP Biomedicals (Solon, Ohio, USA) and reagent grade *tris*-(hydroxymethyl)-amino methane was obtained from Scharlau. In cytotoxic activity, 3-[4,5-dimethylthiazol-2yl]-2,5-diphenyl-tetrazolium bromide (MTT) and penicillin and streptomycin were from MP Biomedicals (Solon, Ohio, USA). Delbecco's modified Eagle's medium (DMEM), Fetal Bovine Serum (FBS) purchased from Gibco (Gaithersburg, MD, USA). 3T3 normal cell lines were generously provided by Molecular Immunology Laboratory, Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan. The cells were grown in DMEM medium supplemented with 10% (*v*:*v*) fetal bovine serum (FBS). 1% (*v*:*v*) L-glutamine, 100 U penicillin and 100 µg/mL streptomycin at 37 °C in a humidified atmosphere with 5% CO_2.

2.1.1. Synthesis of aroylhydrazine ligands

All of the aroylhydrazine ligands (Figure 1) have been synthesized and characterized previously. The physical and analytical data of these synthesized ligands have been described [7-15]. Aroylhydrazine ligands (**1**-**7**) were re synthesized here from previous method [13].

3-Fluorobenzoylhydrazine (**1**): Color: Colorless solid. Yield: 74%. M.p.: 94-96 °C. FT-IR (KBr, ν, cm^{-1}): 3298(NH), 3219, 3032 (NH_2 stretch), 1666 (C=O), 1564 (NH bend.), 1620, 1483 (C=C), 1348 (C-N). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.85 (s, 1H, NH), 7.67 (d, 1H, *J* = 8.1 Hz, H-6), 7.60 (dd, 1H, *J* = 10.4 Hz, *J* = 2.0 Hz, H-2), 7.49 (dd, 1H, *J* = 6.1 Hz, *J* = 2.1 Hz, H-5), 7.34 (dt, 1H, *J* = 8.9 Hz, *J* = 2.8 Hz, H-4), 4.52 (s, 2H, NH_2). MS (EI, *m/z* (%)): 154 (M^+, 47), 124 (15), 123 (100), 95 (95), 75 (47), 57 (4), 51 (13). Anal. calcd. for $C_7H_7N_2OF$: C, 54.54; H, 4.54; N, 18.18. Found: C, 54.58; H, 4.56; N, 18.15%. UV/Vis (DMSO, λ_{max}, nm, (ε)): 269 (4691).

3-Methoxybenzoylhydrazine (**2**): Color: Colorless solid. Yield: 79%. M.p.: 229-231 °C. FT-IR (KBr, ν, cm^{-1}): 3298 (NH), 3207, 3072 (NH_2 stretch), 1640 (C=O), 1530 (NH bend.), 1548, 1479 (C=C), 1326 (C-N). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.73 (s, 1H, NH), 7.4 (d, 1H, *J* = 7.6 Hz, H-6), 7.5 (d, 1H, *J* = 6.4 Hz, H-5), 7.3 (s, 1H, H-2), 7.0 (d, 1H, *J* = 7.5 Hz, H-4), 4.47 (s, 2H, NH_2), 3.79 (s, 3H, OCH_3). MS (EI, *m/z* (%)): 166 (M^+, 19), 150 (20), 135 (100), 134 (18), 107 (30), 92 (40), 77(51), 64 (33), 50 (37). Anal. calcd. for $C_8H_{10}N_2O_2$: C, 57.81; H, 6.01; N, 16.89. Found: C, 57.85; H, 6.02; N, 16.86%. UV/Vis (DMSO, λ_{max}, nm, (ε)): 235 (279).

3-Aminobenzoylhydrazine (**3**): Color: Colorless solid. Yield: 75%. M.p.: 125-127 °C. FT-IR (KBr, ν, cm^{-1}): 3377 (NH), 3329, 3291 (NH_2 stretch), 1621 (C=O), 1522 (NH bend.), 1600, 1486 (C=C), 1354 (C-N). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.83 (1H, s, NH), 7.0 (t, 1H, *J* = 7.8 Hz, H-5), 6.9 (s, 1H, H-2), 6.8 (d, 1H, *J* = 7.7 Hz, H-6), 6.6 (d, 1H, *J* = 7.9 Hz, H-4), 6.31 (*s*, 2H, Ar-NH_2), 4.56 (2H, s, NH_2). MS (EI, *m/z* (%)):151 (40), 136 (100), 121 (50), 120 (100), 106 (9), 92 (100), 82 (35), 77 (50), 54 (32). Anal. calcd. for $C_7H_9N_3O$: C, 55.62; H, 5.96; N, 27.81. Found: C, 56.30; H, 6.25; N, 28.22%. UV/Vis (DMSO, λ_{max}, nm, (ε)): 263 (3970), 318 (1649).

4-Aminobenzoylhydrazine (**4**): Color: Colorless solid. Yield: 72%. M.p.: 79-81 °C. FT-IR (KBr, ν, cm^{-1}): 3429 (NH), 3344, 3234 (NH_2 stretch), 1650 (C=O), 1547 (NH bend.), 1606, 1502 (C=C), 1313 (C-N). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.69 (s, 1H, NH), 7.5 (d, 2H, *J* = 8.6 Hz, H-2/H-6), 6.7 (d, 2H, *J* = 8.6Hz, H-3/H-5), 6.30 (*s*, 2H, Ar-NH_2), 4.49 (2H, s, NH_2). MS (EI, *m/z* (%)): 151 (33), 137 (87), 136 (100), 121 (81), 120 (100), 107 (29), 92 (100), 83 (55), 65 (100), 54 (38). Anal. calcd. for $C_7H_9N_3O$: C, 55.62; H, 5.96; N, 27.81. Found: C, 56.30; H, 6.25; N, 28.22%. UV/Vis (DMSO, λ_{max}, nm, (ε)): 306 (7612).

3-Iodobenzoylhydrazine (**5**): Color: Colorless solid. Yield: 78%. M.p.: 138-140 °C. FT-IR (KBr, ν, cm^{-1}): 3312 (NH), 3180, 3035 (NH_2 stretch), 1651 (C=O), 1553 (NH bend.), 1622, 1464 (C=C), 1342 (C-N). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.80 (1H, s, NH), 8.1 (s, 1H, H-2), 7.9 (d, 1H, *J* = 7.9 Hz, H-4), 7.8 (d, 1H, *J* = 7.9 Hz, H-6), 7.2 (t, 1H, *J* = 7.7 Hz, H-5), 4.50 (2H, s, NH_2). MS (EI, *m/z* (%)): 262 (M^+, 42), 231 (100), 203 (91), 104 (9), 76 (59), 50 (20). Anal. calcd. for $C_7H_7N_2OI$: C, 32.06; H, 2.67; N, 10.68. Found: C, 32.05; H, 2.65; N, 10.70%. UV/Vis (DMSO, λ_{max}, nm, (ε)): 280 (6135).

4-Iodobenzoylhydrazine (**6**): Color: Colorless solid. Yield: 84%. M.p.: 141-143 °C. FT-IR (KBr, ν, cm^{-1}): 3209 (NH), 3123, 3056 (NH_2 stretch), 1626 (C=O), 1536 (NH bend.), 1591, 1477 (C=C), 1340 (C-N). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.91 (s, 1H, NH), 7.5 (d, 2H, *J* = 8.5 Hz, H-2/H-6), 7.8 (d, 2H, *J* = 8.6 Hz, H-3/H-5), 4.47 (s, 2H, NH_2). MS (EI, *m/z* (%)): 262 (421, M^+), 231 (100), 203 (39), 104 (9), 76 (25). Anal. calcd. for $C_7H_7N_2OI$: C, 32.06; H, 2.67; N, 10.68. Found: C, 32.04; H, 2.68; N, 10.67%. UV/Vis (DMSO, λ_{max}, nm, (ε)): 306 (7612).

3-Bromobenzoylhydrazine (**7**): Color: Colorless solid. Yield: 87%. M.p.: 170-172 °C. FT-IR (KBr, ν, cm^{-1}): 3302 (NH), 3224, 3037 (NH_2 stretch), 1662 (C=O), 1554 (NH bend.), 1618, 1467 (C=C), 1338 (C-N). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.81 (1H, s, NH), 8.0 (s, 1H, H-2), 7.74 (d, 1H, *J* = 7.7 Hz, H-6), 7.68 (d, 1H, *J* = 8.4 Hz, H-4), 7.37 (t, 1H, *J* = 7.9 Hz, H-5), 4.49 (s, 2H, NH_2). MS (EI, *m/z* (%)): 216 (18, M^{2+}), 214 (19), 185 (95), 183 (100), 155 (40), 76 (15), 50 (9). Anal. calcd. for $C_7H_7N_2OBr$: C,

39.09; H, 3.24; N, 12.96. Found: C, 38.92; H, 3.23; N, 12.95%. UV/Vis (DMSO, λ_{max}, nm, (ε)): 215 (508), 263 (3502).

2.1.2. Synthesis of chromium(III) complexes of aroylhydrazines

Chromium(III)-aroylhydrazine complexes were synthesized using Scheme 1, 5 mmol solution of $CrCl_3.6H_2O$ was dissolved in ethanol (5 mL), in the same way 10 mmol solution of suitable aroylhydrazine ligand was also dissolved in ethanol (10 mL) [13]. At room temperature mixing was done and the resulting mixture was refluxed. Solid product was precipitated out after 3-4 hrs which was then cooled, filtered and washed with distilled ethanol and then dried in evaporating dish in air. All ligands were dissolved at room temperature in ethanol except compound **2** and **4** that were dissolved on heating. Analytical and physical data of chromium(III) complexes (**8a**-**12a**) were discussed previously [13] while chromium (III) complexes (**1a**-**7a**) are presented in this study.

Diaquabis(3-fluorobenzoylhydrazine)chromium(III)chloride (**1a**): Color: Grayish. Yield: 63%. FT-IR (KBr, ν, cm^{-1}): 3500-2400 (br, NH, NH_2 stretch), 1629 (C=O), 1539 (NH bend.), 1567, 1482 (C=C), 1369(C-N). ESI-Mass (*m/z*) calcd. for $CrC_{14}H_{18}N_4O_4F_2Cl_3$, 502.6858; found 502.3654; $CrC_{14}H_{12}N_4O_2F_2$, 358.2635; found 358.0121. Anal. calcd. for $CrC_{14}H_{18}N_4O_4F_2Cl_3$: C, 33.43; H, 3.50; N, 11.11, Cr^{3+}, 10.34. Found: C, 33.63; H, 3.60; N, 11.32, Cr^{3+}, 10.37%. UV/Vis (DMSO, λ_{max}, nm, (ε)): 270 (18990), 320 (9914), 410 (98), 610 (44). Δ° (cm^{-1}): 16194.60, β: 0.71. Λ_m (Ω^{-1} cm^2 mol^{-1}): 110.12.

Diaquabis(3-methoxybenzoylhydrazine)chromium(III)chloride (**2a**): Color: Violet. Yield: 65%. FT-IR (KBr, ν, cm^{-1}): 3600-2800 (br, NH, NH_2 stretch), 1628 (C=O), 1525 (NH bend.), 1600, 1486 (C=C), 1354 (C-N). ESI-Mass (*m/z*) calcd. for $CrC_{16}H_{24}N_4O_6Cl_3$, 526.7601; found 526.3657; $CrC_{16}H_{18}N_4O_4$, 382.3346; found 382.1342. Anal. calcd. for $CrC_{16}H_{24}N_4O_6Cl_3$: C, 36.46; H, 4.55; N, 10.60, Cr^{3+}, 9.87. Found: C, 36.31; H, 4.45; N, 10.50, Cr^{3+}, 9.91%. UV/Vis (DMSO, λ_{max}, nm, (ε)): 265 (6793), 287 (7206), 400 (117), 550 (70). Δ° (cm^{-1}): 18751.40, β: 0.54. Λ_m (Ω^{-1} cm^2 mol^{-1}): 121.90.

Diaquabis(3-aminobenzoylhydrazine)chromium(III)chloride (**3a**): Color: Grayish. Yield: 50%. FT-IR (KBr, ν, cm^{-1}): 3600-2900 (br, NH, NH_2 stretch), 1628 (C=O), 1538 (NH bend.), 1600, 1486 (C=C), 1354 (C-N). ESI-Mass (*m/z*) calcd. for $CrC_{14}H_{22}N_6O_4Cl_3$, 496.7144; found 496.3571; $CrC_{14}H_{16}N_6O_2$, 352.3510; found 352.0701. Anal. calcd. for $CrC_{14}H_{22}N_6O_4Cl_3$: C, 33.83; H, 4.43; N, 16.90, Cr^{3+}, 10.47. Found: C, 34.40; H, 4.54; N, 16.27, Cr^{3+}, 10.89%. UV/Vis (DMSO, λ_{max}, nm, (ε)): 265 (8310), 325 (4957), 380 (332), 560 (42). Δ° (cm^{-1}): 15572.31, β: 0.63. Λ_m (Ω^{-1} cm^2 mol^{-1}): 111.90.

Diaquabis(4-aminobenzoylhydrazine)chromium(III)chloride (**4a**): Color: Brown. Yield: 60%. FT-IR (KBr, ν, cm^{-1}): 3400-2300 (br, NH, NH_2 stretch), 1610 (C=O), 1498 (NH bend.), 1552, 1438 (C=C), 1332 (C-N). ESI-Mass (*m/z*) calcd. for $CrC_{14}H_{22}N_6O_4Cl_3$, 496.7144; found 496.4015; $CrC_{14}H_{16}N_6O_2$, 352.3118; found 352.0573. Anal. calcd. for $CrC_{14}H_{22}N_6O_4Cl_3$: C, 33.83; H, 4.43; N, 16.90, Cr^{3+}, 10.47. Found: C, 34.10; H, 4.48; N, 16.91, Cr^{3+}, 10.77%. UV/Vis (DMSO, λ_{max}, nm, (ε)): 300 (36557), 360 (371), 410 (347), 560 (124). Δ° (cm^{-1}): 18150.61, β: 0.51. Λ_m (Ω^{-1} cm^2 mol^{-1}): 112.30.

Diaquabis(3-iodobenzoylhydrazine)chromium(III)chloride (**5a**): Color: Violet. Yield: 64%. FT-IR (KBr, ν, cm^{-1}): 3900-2900 (br, NH, NH_2 stretch), 1631 (C=O), 1526 (NH bend.), 1582, 1471 (C=C), 1327(C-N). ESI-Mass (*m/z*) calcd. for $CrC_{14}H_{18}N_4O_4I_2Cl_3$, 718.4778; found 718.0153; $CrC_{14}H_{12}N_4O_2I_2$, 572.9948; found 573.8369. Anal. calcd. for $CrC_{14}H_{18}N_4O_4I_2Cl_3$: C, 23.40; H, 2.52; N, 7.79, Cr^{3+}, 7.23. Found: C, 22.90; H, 2.50; N, 7.88, Cr^{3+}, 7.45%. UV/Vis (DMSO, λ_{max}, nm, (ε)): 260 (9677), 410 (96), 570 (43). Δ° (cm^{-1}): 17986.87, β: 0.54. Λ_m (Ω^{-1} cm^2 mol^{-1}): 127.48.

Diaquabis(4-iodobenzoylhydrazine)chromium(III) chloride (**6a**): Color: Violet. Yield: 70%. FT-IR (KBr, ν, cm^{-1}): 3200-2600 (br, NH, NH_2 stretch), 1671 (C=O), 1586 (NH bend.), 1637, 1527 (C=C), 1389 (C-N). ESI-Mass (*m/z*) calcd. for $CrC_{14}H_{18}N_4O_4I_2Cl_3$, 718.4778; found 718.3105; $CrC_{14}H_{12}N_4O_2I_2$, 572.9948; found 573.8326. Anal. calcd. for $CrC_{14}H_{18}N_4O_4I_2Cl_3$: C, 23.40; H, 2.52; N, 7.79, Cr^{3+}, 7.23%. Found: C, 23.43; H, 2.49; N, 7.78, Cr^{3+}, 7.88%. UV/Vis (DMSO, λ_{max}, nm, (ε)): 260 (26683), 390 (87), 560 (33). Δ° (cm^{-1}): 18880.66, β: 0.65. Λ_m (Ω^{-1} cm^2 mol^{-1}): 123.40.

Diaquabis(3-bromobenzoylhydrazine)chromium(III) chloride (**7a**): Color: Grayish. Yield: 57%. FT-IR (KBr, ν, cm^{-1}): 3600-2700 (br, NH, NH_2 stretch), 1624 (C=O), 1540 (NH bend.), 1584, 1470 (C=C), 1357 (C-N). ESI-Mass (*m/z*) calcd. for $CrC_{14}H_{18}N_4O_4Br_2Cl_3$, 624.4770; found 624.3107; $CrC_{14}H_{12}N_4O_2Br_2$, 480.2010; found 480.0201. Anal. calcd. for $CrC_{14}H_{18}N_4O_4Br_2Cl_3$: C, 26.93; H, 3.04; N, 8.97, Cr^{3+}, 8.32. Found: C, 26.90; H, 3.01; N, 8.91, Cr^{3+}, 8.75%. UV/Vis (DMSO, λ_{max}, nm, (ε)): 270 (9469), 360 (288), 530 (48). Δ° (cm^{-1}): 19209.13, β: 0.77. Λ_m (Ω^{-1} cm^2 mol^{-1}): 125.60.

2.2. Cytotoxicity assay

Standard MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl -tetrazolium bromide) colorimetric assay was utilized for the determination of cytotoxic activity of compounds and they were evaluated in 96-well flat-bottomed micro plates [48,49]. In Dulbecco's Modified Eagle medium, 3T3 cells (mouse fibroblast) were cultured and it was supplemented with fetal bovine serum (FBS) 5%, streptomycin (100 µg/mL) and penicillin (100 IU/mL). These all were kept in flask of 75 cm^3 at 37 °C with 5% CO_2 incubator. Then these growing cells were harvested, counted by haemocytometer and a particular medium was used for dilution. 5×10^4 cells/mL was the concentration that used for the preparation of cell culture and then 100 µL/well was introduced into 96-well plates. Medium was removed after overnight incubation and then fresh medium (200 µL) was added by variable concentration of compounds (1-100 µM), 200 µL MTT (0.5 mg/mL) was added following 48 hrs to each well. At 540 nm absorbance was noted and calculated with in cells the reduction of MTT to formazan using a microplate reader (spectra Max plus, Molecular Devices, CA, USA). Concentration causing 50% growth inhibition (IC_{50}) for 3T3 cells was recorded as the cytotoxicity. The following formula was used for the calculation of the percent inhibition, where O.D represents the optical density.

Inhibition (%) = 100 – (average of O.D of test compound – average of O.D of negative control)/average of O.D of positive control – average of O.D of negative control)* 100) (1)

Percent inhibition was evaluated using Soft- Max pro software (Molecular Device, USA).

2.3. Antiglycation activity assay (in vitro)

The antiglycation activity was done using the testified method [25,50] with the amendments mentioned in below process. This assay based on testing of inhibition of methylglyoxyl (MGO) mediated glycation of bovine serum albumin (BSA) by fluorometry. 0.1 M Na_2HPO_4 and NaH_2PO_4 were used for the preparation of pH = 7.4 phosphate buffer that also comprising 30 mM sodium azide (NaN_3) to inhibit growth of bacteria. Triplicate sets of solution, each sample comprises of 50 µL BSA 10 mg/mL in buffer, 0.1 M of pH = 7.4 phosphate buffer containing NaN_3 (30 mM), 50 µL of 14 mM

Table 1. Calculation of energy ratios (E_2/E_1) using (Cr^{+3}) d^3 system Tanabe-Sugano diagram.

Δ°/B	^4A_2g (F) to ^4T_1g (F)	^4A_2g (F) to ^4T_2g (F)	Energy ratios (E_2/E_1)
10	16	9	1.77
20	29	19	1.52
30	41	29	1.41
40	51	39	1.30

Table 2. Calculations of ligand field parameters Δ°, B and *β*.

Comp.	v_2 (nm)	v_1 (nm)	v_2 (cm^{-1})	v_1 (cm^{-1})	v_2/v_1	Δ°/B	E/BV_2	E/BV_1	B V_2	B V_1	B AV	Δ°	β = B/B'
$CrCl_3$	480	670	20833	14925	1.39	31	42	31	496.03	481.46	488.74	15517.74	0.47
1a	410	620	24390	16129	1.51	22	33	22	739.09	733.13	736.11	16194.60	0.71
2a	400	550	25000	18181	1.37	33	43	34	581.39	534.75	558.07	18751.40	0.54
3a	380	560	26315	17857	1.47	23	38	28	690.70	617.89	654.29	15572.31	0.63
4a	410	560	24390	17857	1.36	34	45	35	542.00	510.20	526.10	18150.61	0.51
5a	410	570	24390	17543	1.39	32	41	33	582.10	531.63	556.86	17986.87	0.54
6a	390	560	25641	17857	1.43	28	38	26	674.76	673.85	674.30	18880.66	0.65
7a	360	530	27777	18867	1.47	24	34	24	814.59	786.16	800.38	19209.13	0.77

MGO and 20 µL of test sample prepared in DMSO were incubated in sterilized settings at 37 °C for 9 days. After incubation, all samples were observed for the increase of particular fluorescence (excitation on 330 nm and emission on 440 nm) going on a microtitre plate reader (Spectra Max, Molecular Devices, USA) spectrophotometer against blank solution. A positive control Rutin was used that have an IC_{50} = 294±1.50 µM. In the test sample the percent inhibition of AGE formation was calculated against control for every compound with the formula:

Percent inhibition = (1- fluorescence of sample solution/ fluorescence of the control solution) × 100 (2)

Concentration of test compound that inhibits 50% the MGO mediated glyoxidation of protein BSA is represented as IC_{50} value of test compound. EZ-Fit Enzyme kinetics program (Perrella Scientific Inc., Amherst, USA) was used for the estimation of IC_{50} values of test compounds.

2.4. Carbonic anhydrase inhibition assay

Esterase method [51] was used in carbonic anhydrase inhibition. Colorless substrate 4-nitrophenyl acetate (4-NPA) hydrolyzes by carbonic anhydrases and converted into CO_2 and 4-nitrophenoxide ion (yellow product). This inhibition process is measured by decrease in absorbance at 400 nm (λ_{max} of 4-nitrophenoxide ion) in the presence of inhibitor [52].

Spectrophotometric analysis [53,54] was utilized to evaluate carbonic anhydrase inhibition potential of sample *in vitro*. This assay was performed at 25 °C in HEPES- *tris* buffer of 20 mM with pH = 7.4. In each sample tube, 140 µL of tris buffer solution, 20 µL of fresh enzyme solution (0.1 mg in 1 mL deionized water) of purified bovine erythrocyte CA-II and 20 µL of test compound in DMSO at different concentrations were taken. This inhibitor and enzyme in a solution were mixed and pre incubated at room temperature for 15 min to allow enzyme inhibition complex formation. Substrate reaction was initiated by adding 20 µL of 4-NPA (0.7 mM) ethanolic solution. This reaction was continuously measured with 1 min interval for 30 min at 400 nm during the formation of product in 96 well plate reader, by ELISA Reader SPECTRA-Max 340 spectrophotometer (USA). In above mentioned process, 100% activity of control was taken in the absence of inhibitor.

IC_{50} signifies the test compound's concentration producing a 50 % reduction of CA-catalyzed hydrolysis of substrate, 4-NPA. The IC_{50} values of all compounds were calculated through enzyme kinetics software EZ-Fit (Perrella Scientific Inc. Amherst, USA), by means of % activity against inhibitor concentration plots.

3. Results and discussions

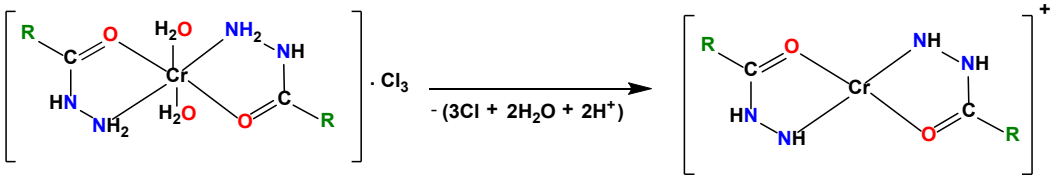
3.1. Synthesis and physicochemical properties

Synthesis and structural studies of aroylhydrazine ligands (**1**-**12**) have reported previously [7-15]. Chromium(III)-aroyl-hydrazine complexes (**8a**-**12a**) were discussed in reference [13] while remaining chromium(III) complexes (**1a**-**7a**) with aroylhydrazine ligands (**1**-**7**) are specified in Figure 1 and Scheme 1. In the synthesis of chromium(III)-aroyl-hydrazine complexes (**1a**-**7a**), a mixture of chromium (III) chloride using distilled ethanol were refluxed in 1:2 mole ratio with a given aroylhydrazine ligands. The physical, analytical and spectral studies of aroylhydrazine ligands and their chromium(III) complexes such as ^{1}H NMR, EI-Mass, IR, ESI mass fragmentation and UV-Visible spectroscopy are mentioned in experimental section. In UV-Visible study, different ligand field parameters were also calculated and they were listed in Tables 1 and 2. Characterization of chromium(III) complexes were performed using different methods and techniques like metal content (Cr^{+3}) were determined in the form of lead chromate ($PbCrO_4$) by gravimetric analysis using lead nitrate ($PbNO_3$) as a precipitating agent [47]. Other elemental analysis (C, H, N), shifts in position of peaks in Infrared spectroscopy and conductivity values indicates the coordination of Cr centre with aroylhydrazine ligand in 1:2 molar ratio. In conductivity measurements, fresh solution of complex in DMSO shows conductance in the range 110.12-127.48 $\Omega^{-1}.cm^2.mol^{-1}$ signifying the presence of counter ions making it outer sphere complex [55]. The counter ion was determined to be Cl^- as a white precipitate was formed upon addition of $AgNO_3$. Moreover, the percentage of chromium(III) obtained in complex supports the suggested structure of complex. Hence, consider on above studies, the structures of chromium(III)-aroylhydrazine complexes (**1a**-**7a**) are assigned to be an octahedral in which chromium(III) centres acquire an octahedral arrangements, form coordination bond with an aroylhydrazine ligand in a bidentate fashion and have two water molecule at the equatorial position (Scheme 1) [56]. Broad peaks in ^{1}H NMR spectra of chromium(III) complexes (**1a**-**7a**) indicate the paramagnetic nature of metal centre in complexes.

3.2. Spectroscopy

3.2.1. ^{1}H NMR spectroscopy

Proton NMR spectra of aroylhydrazine ligands showed the protons corresponding to the benzene ring with appropriate splitting pattern.



Scheme 2. ESI-Mass fragmentation of complexes (**1a**-**7a**).

3.2.2. Fourier-transform infrared spectroscopy

The Infrared spectroscopic data of chromium(III)-aroylhydrazine complexes(**1a**-**7a**) are described in characterization data of experimental. Strong carbonyl stretching absorptions exhibited in all the ligands around 1654±34 cm^{-1} were shifted by 8-45 cm^{-1} in the spectra of their respective complexes indicated that chromium is coordinated through carbonyl oxygen of aroylhydrazine [57,58].

The coordination of amino nitrogen of aroylhydrazine ligand to the metal center is supported by a shift in the position of NH bending vibrations from 1561±55 cm^{-1} in the free ligands to 1547±33 cm^{-1} in the complexes. Hence, it can be concluded that aroylhydrazine act as bidentate ligand coordinated with carbonyl oxygen and amine N atoms of the ligand [13].

All the ligands displayed intense N-H stretching vibrations in the range of 3032-3441 cm^{-1}. These stretching vibrations were cautiously assigned to amino and imino-NH groups present in aroylhydrazine ligands. The narrow absorption peaks in this range indicate hydrogen bonding among -NH protons. The spectrum of all chromium(III) complexes show broad peaks at ~3250 cm^{-1} (3900-2300 cm^{-1}). These broad absorption bands containing NH and OH stretching vibrational modes derived from aroylhydrazine and coordinated water molecule, respectively that usually give absorption in the similar region. Existence of broad band illustrates existence of non-hydrogen bonded moieties. Nevertheless, the probability of the existence of both non hydrogen and hydrogen bonded groups may not be ruled out as broad band may eclipse the sharp peaks.

3.2.3. ESI-Mass fragmentation spectroscopy

ESI-Mass spectroscopy has shown a characteristic molecular ion peak and a base peak (i.e., the most intense peak representing a stable fragment) along with other fragments at suitable *m/z* positions corresponding to each chromium(III)-aroylhydrazine complex. The electrospray mass fragmentation data of the chromium(III)-aroylhydrazine complexes (**1a**-**7a**) as defined in experimental section. A mass spectrum was obtained in 1:1 mixture of acetonitrile and THF. Peaks in the ESI mass spectra were identified by using the most abundant *m/z* value in the isotopic mass distribution. It is noteworthy that all complexes produce fragments which are assigned to the removal of three chlorides (counter ion) and two water molecule coordinated at equatorial positions. Charge balance indicates that each aroylhydrazine molecule is coordinated with chromium(III) as neutral ligand. Removal of proton from aroylhydrazine ligand makes it anionic in nature with -1 charge, which results in change of charge on coordination sphere from +3 to +1 [13]. Fragmentation of chromium(III)-aroylhydrazine complexes (**1a**-**7a**) is shown in general Scheme 2.

3.2.4. UV-Visible spectroscopy

UV-Visible spectra of freshly prepared solution of aroylhydrazines (**1**-**7**) and their chromium(III) complexes (**1a**-**7a**) were collected in DMSO and observed electronic transitions are mentioned in experimental section. For comparative purpose electronic transitions of ligands were also listed. All the ligands have carbonyl group attached with benzene ring with different substituents along with NH-NH_2 described in Figure 1. The paramagnetic chromium(III) chloride in DMSO have a d^3 system with ^4A_2g ground state and three spin allowed transitions with ground state ^4A_2g (F) to ^4T_2g (F), ^4T_1g (F) and ^4T_1g (P) at 670, 480 and 280 nm, respectively. Molar absorptivity values suggest these transition as Laporte forbidden, spin allowed transitions. The presence of these bands proves the octahedral geometry of complexes [59,60]. All chromium(III)-aroylhydrazine complexes showed these transitions with low molar absorptivity values (33-347 $M^{-1}.cm^{-1}$) except ^4A_2g (F) → ^4T_1g (P) showing higher molar absorptivity value (6793-36557 $M^{-1}.cm^{-1}$). It may be due to overlapping of this band with ligand's transitions in the same range. All ligands showed π-π* transitions in UV region which originate from the π bonds of the aroylhydrazine ligands.

3.2.4.1. Calculations of Δ°, B and β using Tanabe-Sugano diagram

Chromium(III)-aroylhydrazine complexes have a d^3 electronic configuration so first the TS diagram of d^3 system was selected. λ_{max} for spin-allowed and spin forbidden transitions were identified, then these wave length (λ_{max}) were converted into wavenumbers (ν) and energy ratios (E_2/E_1) were calculated (Table 1). These energy ratios were plotted and graphically Δ°/B was calculated (Figure 2). These Δ°/B values were used on the printed TS diagram of d^3 system and E/B ratios on ν_2 and ν_1 were determined. These ratios were used in calculation of B ν_2 and B ν_1 and the average of these were B_{avg} (Racha parameter). Finally naphalauxetic ratio (β) was also calculated by dividing the B of complex with the B of Cr(III) metal ion as mentioned in Table 2. β values are in the range of 0.47-0.77. It shows 30-56 % reduction in the Racha parameter indicating appreciable covalent character due to strong naphalauxetic effect [61]. All the ligands generate stronger ligand field than DMSO. Δ° values suggest that compound **7a** is strongest (Δ° = 19209 cm^{-1}) among all tested complexes. Figure 3 displays the shifting of chromium(III) bands toward lower wavelength upon coordination with different ligands. Experimental data reveals the strength of ligands in the order, **7** > **6** > **2** > **4** > **5** > **1** > **3**.

3.3. Cytotoxic activity

Several studies have been declared on the toxicity of chromium(III) compounds. Instead of these a few of the research have been done on the safety and non-cytotoxic behavior of chromium(III) complexes [3]. Genotoxicity of chromium(III) in cellular system have been detected in which HaCaT human keratinocytes [62-64], dermal fibroblasts and bacterial cells [19] were used to investigate the cytotoxicity and genotoxicity of chromium(III) complexes.

Table 3. Percent inhibition and IC_{50} values of cytotoxicity of chromium(III)-aroylhydrazine complexes at 100 (µM) concentration *.

Compound	% Inhibition	IC_{50} (µM) ±SEM
1a	15.32	>500
2a	1.26	>1000
3a	21.40	>500
4a	1.13	>1000
5a	6.20	>1000
6a	30.63	>500
7a	17.96	>500
8a	13.12	>500
9a	7.21	>1000
10a	34.12	>500
11a	1.69	>1000
12a	14.70	>500
$CrCl_3.6H_2O$	21.68	>500
Cyclohexamide	86.00	0.3±0.2

* NA = Not active; Cyclohexamide: Standard inhibitor of cytotoxic activity; SEM: Standard error of the mean.

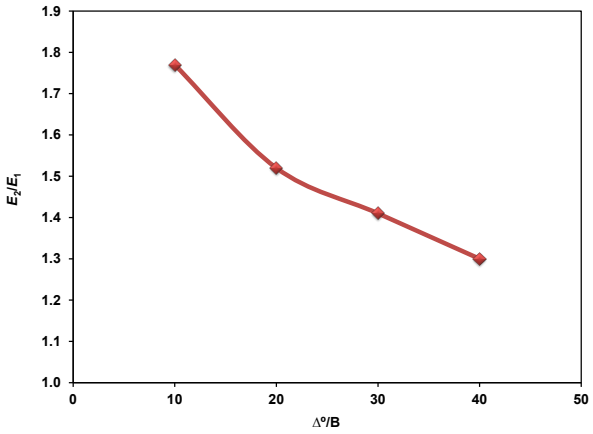


Figure 2. Plot of energy ratios and by graph calculated Δ°/B.

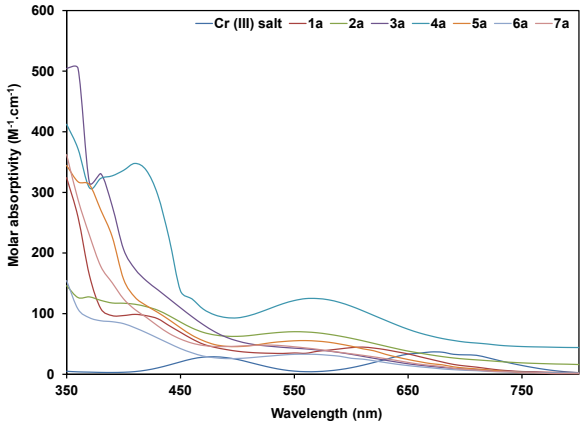


Figure 3. UV-Visible spectrum of $CrCl_3$ and chromium(III)-aroylhydrazine complexes (**1a**-**7a**).

The cytotoxic activity of chromium(III) chloride and its complexes were calculated by using MTT assay [10,48,49]. All of the chromium(III)-aroylydrazine complexes and their metal salt chromium(III) chloride at 100 µM concentration exhibited a very low inhibition potential against 3T3 normal cell line as compared to cyclohexamide, a standard inhibitor of cytotoxic activity that showed 86 percent inhibition on same concentration (Table 3). Hence, no cytotoxic activity was observed at 100 µM proved that chromium(III) chloride and their aroylhydrazine complexes were not found toxic. Moreover, below 100 µM is the concentration of chromium(III) compound that is mainly found in nutritional supplements [3]. In addition, all of chromium(III)-aroylydrazine complexes acquired useful antioxidant effects which were discussed in previous [13]. So it is worth stating here that this study also supports a lack of chromium(III) toxicity.

3.4. Antiglycation activity

Aroylhydrazine ligands and their chromium(III) complexes (Table 4) were screened for their antiglycation potential**.** Ligands and metal salt itself found to be inactive but the chromium(III)-aroylhydrazine complexes have found IC_{50} values are in the range of (368-892 µM). Compound **1a**, **2a**, **5a**-**7a**, **9a** and **10a** were exhibited moderate antiglycation activity which can be comparable with standard Rutin (IC_{50} = 294.5 µM) used in antiglycation activity.

Table 4. IC_{50} values of antiglycation activity of aroylhydrazines (**1-12**) and their chromium(III)-aroylhydrazine complexes (**1a-12a**) *.

Compound	IC_{50} (μM) ± SEM
1a	428.66±1.5
2a	429.01±4.4
3a	NA
4a	NA
5a	389.39±1.8
6a	368.39±3.8
7a	378.75±3.5
8a	892.40±4.5
9a	428.62±4.7
10a	394.30±4.6
11a	NA
12a	NA
$CrCl_3.6H_2O$	NA
Rutin	294.5±1.5

* NA = Not active; All aroylhydrazines are not active; Rutin: Standard inhibitors of anti-glycation activity; SEM: Standard error of the mean.

Chromium(III) chloride salt and free aroylhydrazine ligands both are inactive to the glycation inhibition but the combination of ligand with chromium(III) salt acquiring an antiglycation potential in chromium(III)-aroylhydrazine complexes are based on this fact that an apparent understanding about the structure activity relationship could be developed. Between the different chromium(III) compounds (**1a-12a**) a varying degree of inhibition were found with IC_{50} value ranging from 368 to 892 μM compared with standard Rutin (IC_{50} = 294.5±1.5). Compound **5a** (IC_{50} = 389.39±1.8), **6a** (IC_{50} = 368.39±3.8), **7a** (IC_{50} = 378.75±3.5) and **10a** (IC_{50} = 394.30±4.6) showed active antiglycation activity but the compounds **3a**, **4a**, **11a**, **12a** and all aroylhydrazine ligands are inactive against glycation inhibition, that means complexation might take part in a major role in reducing the toxicity of chromium(III) ion and increasing their antiglycation potential.

The carbonyl and amino groups in a compound are highly critical in the inhibition of glycation process. The process of glycation starts with the reaction of free amino group of proteins and carbonyl group of reducing sugar [65,66]. Similarly, rutin (a standard inhibitor) can trap amino groups of protein by inhibiting protein glycation [67,68]. Concluding the above discussion aroylhydrazines should have a strong antiglycation potential because of presence of amino and carbonyl groups but these aroylhydrazines are inactive because it may be relative affinity among carbonyl group and amino group in hydrazine molecule to amino group of protein and carbonyl group of methylglyoxyl. Furthermore, due to a small aroylhydrazine molecule, a small distance between carbonyl and amino groups unable to form *bis*-Schiff base. Hence free aroylhydrazine ligands have not found antiglycation potential.

To detect the effect of different substituents antiglycation activity of compounds (**1a**, **2a**, **5a-10a**) have been compared. Compound **8a** in which no substituent are on phenyl ring and directly attached to the aroylhydrazine carbonyl group that attained a very less inhibition potential with IC_{50} value of 892 μM. All of the compounds have same metal chromium but possess different substituents on benzoylhydrazine. Compound **5a**, **6a** and **7a** have strong antiglycation potential in which iodo and bromo groups are present on *meta* and *para* positions respectively as well as compounds **1a** and **9a** contain *ortho*-fluoro group and *meta*-fluoro groups have also a significant glycation inhibition potential. Methoxy containing substituents (**2a**, **10a**) also have a valuable antiglycation activity. It means the substitution by halo or oxygen containing groups might increases hydrophilicity plus hydrogen bonding properties, which can support the contact of a compound with protein. Moreover, the halo and oxygen comprising substituents may reduce the electron density on carbon atom of adjacent carbonyl group through negative inductive effect that creates carbonyl group more labile for nucleophilic attack by amino groups of proteins. The consequential possible Schiff base adducts formation among protein and chromium(III)-aroylydrazine complex inhibits the methyl glyoxyl mediated glycation of protein. From now it was assumed that the substitution on phenyl ring of aroylhydrazine may improve the antiglycation efficiency. In distinction, the NH moiety in between carbonyl group and phenyl ring of aroylhydrazine (as in complex **12a**) is accountable for the inactivity in antiglycation activity. The NH moiety is proposed to be convoluted in intramolecular H-bonding with adjacent carbonyl group which may prevent carbonyl to relate effectively with protein producing less inhibition potential. The intramolecular H-bonding has likewise previously been specified as possible source of low antiglycation activity in hydroxyl compounds [69].

A new interesting feature is the number of nitrogen atoms existing in the complex which is adversely related with antiglycation ability for majority of the compounds. As well the presence of NH_2 group at *ortho, meta* and *para* position in compounds **3a**, **4a** and **11a** displays inactivity in antiglycation activity.

In previous studies it was found that the different group substitution results in varying mark of antiglycation activity [70-72]. The outcome of present study clearly indicates that the alteration in the structure of a compound could be used to enhance the antiglycation activity of chromium(III)-aroylhydrazine complexes. The active inhibition of protein glycation is an important tool to control diabetic problems [73].

From these results it can be concluded that halo and methoxy substitution group containing chromium(III)-aroylhydrazine complexes is decisive to slow down the process of protein glycation more efficiently. Besides, in vitro antiglycation potential of chromium(III)-aroylhydrazine complexes is affected by various factors such as metal-ligand complexation, binding pattern of ligand in complex, presence of nitrogen and also a nature of ligand. This study provides the opportunity for future researchers to work in this area in order to support the hypothesis and mechanism of action of antiglycation and to catch more chromium(III) based antiglycating agents in order to control diabetes.

3.5. Carbonic anhydrase inhibition

In vitro carbonic anhydrase prospective of the aroylhydrazine ligands and their chromium(III) complexes was determined by decrease in absorbance of 4-nitrophenol at 400 nm. 4-nitrophenol was formed by hydrolytic reaction of carbonic anhydrase with 4-nitrophenylacetate (substrate) [51]. All of the aroylhydrazine ligands (**1-12**) have no inhibition potential against carbonic anhydrase however, the chromium(III)-aroylhydrazine complexes having a contrast degree of IC_{50} values represent an excellent, moderate and weak inhibition of CA II (Table 5).

Table 5. IC_{50} values of Carbonic anhydrase II inhibition activity of aroylhydrazines (**1-12**) and chromium(III)-aroylhydrazine complexes (**1a-12a**) *.

Compound	IC_{50} (μM) ± SEM
1a	20.01±0.03
2a	91.50±0.04
3a	150.22±0.04
4a	>500
5a	105.34±0.96
6a	144.24±0.07
7a	117.31±0.07
8a	185.31±0.09
9a	258.45±0.59
10a	200.23±0.32
11a	134.23±0.60
12a	34.05±0.16
$CrCl_3.6H_2O$	>500
Acetazolamide	0.13±0.06

* SEM: Standard error mean of thee results; all aroylhydrazines are not inhibit carbonic anhydrase II enzyme; acetazolamide is positive control.

All the aroylhydrazine ligands are unable to inhibit CA II but complexes of Cr(III) originate some excellent Cr(III)-based carbonic anhydrase inhibitors. The IC_{50} values of Cr(III) complexes ranges from 20.01 to 258.45 μM. These outcomes were related with earlier studies unfolding CA inhibition due to various metal containing compounds such as V(IV), Cr(III), Fe(II), Co(II) and Ni(II) complexes of 5-chloroacetamido-1,3,4-thiadiazole-2-sulfonamide [44]. Similarly, the sulfanilamide derivatives of Schiff bases with Co(II), Cu(II) and Ni(II) have been reported a strong inhibition beside CA I, II and IV isozymes but their ligands are inactive against carbonic anhydrase [74].

Chromium(III) complex of 3-flourobenzoylhydrazine (**1**) and 4-phenyl semicarbazide (**12**) enhances inhibitory potential as compared to $CrCl_3.6H_2O$. These carbonic anhydrase inhibitors, compounds **1a** and **12a** show IC_{50} values of 20.01 μM and 34.05 μM, respectively. These two complexes can be comparable to acetazolamide, a standard inhibitor of CA which has an IC_{50} value 0.13 μM. In chromium(III)-aroylhydrazine complexes, the presence of 3-methoxy (**2a**), 3-amino (**3a**), 3-iodo (**5a**), 4-iodo (**6a**), 3-bromo (**7a**) and 2-amino (**11a**) rate in moderate inhibition of CA-II. The compounds **2a**, **3a**, **5a**, **6a**, **7a** and **11a** have IC_{50} values 91.50, 134.23, 150.22, 105.34, 144.24 and 117.31 μM, respectively. In two of chromium(III) complexes absence of substituent group on phenyl ring hydrazide (**8a**) and 2-methoxy group (**10a**) exhibited weak carbonic anhydrase (II) inhibition have IC_{50} values 185.31 and 200.23 μM, respectively. Chromium(III) complex (**4a**) in which 4-amino group is present and metal salt of chromium(III) shows a poor activity against carbonic anhydrase was found to have IC_{50} values above 500 μM.

The results evaluate that the existence of amino group (**3a** and **11a**) and also an iodo group (**5a** and **6a**) substituents play a strong role in expressing carbonic anhydrase inhibition potential to chromium(III) complexes. The polarizability of chromium(III)-aroylhydrazine complexes in which halo groups are present can interact with hydrophilic portion at the entrance of CA-II site [39]. Other important fact is that the substitution group on *meta* position (**2a**, **3a** and **5a**) in complexes have good effects as compared to the *ortho* (**9a** and **10a**) and *para* (**4a**) position of substituents. The chromium (III) complexes which have substitution group on *meta* position relate more with enzyme due to orientation of interactive sites of enzymes. Hence above study prove that substitution on meta position provide an excellent carbonic anhydrase inhibition.

4. Conclusions

Herein, we report a sequence of chromium(III)-aroylhydrazine complexes were synthesized and characterized. These chromium(III) containing complexes were characterized using different instrumental analysis such as IR, ESI-Mass and UV-Visible spectroscopy. All of chromium(III) complexes exhibited an octahedral geometry with 1:2 metal to ligand ratio in solid state. UV-Visible study were further used for the calculation of ligand field parameters to elaborates the strength of ligands in chromium(III)-aroylhydrazine complexes. In cytotoxic activity, all of the chromium(III)-aroylhydrazine complexes and their metal salt were found to be non-toxic against 3T3 normal cell line as compared to standard cyclohexamide, so these compounds were further scanned in different activities such as antiglycation and carbonic anhydrase inhibition. Moreover, Antiglycation activity justifies that halo and methoxy containing chromium(III)-aroylhydrazine complexes is decisive to inhibit the process of protein glycation more efficiently as compared to amino groups complexes. In carbonic anhydrase inhibition activity, amino and iodo groups substituents play a strong role in expressing carbonic anhydrase inhibition potential to chromium(III) complexes. Other interesting conclusion is that *meta* position of substituents interacts with enzyme more effectively. Hence, this study assess that cytotoxic, antiglycation and CA II inhibition studies of these complexes are dependent upon various factors such as metal-ligand complexation, binding pattern of ligands in the complexes, presence of nitrogen and nature of the ligands.

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Disclosure statement

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