Synthesis, Complexation, and Biological Activity Studies of 4-Aminomethyl-7,8-dihydroxy Coumarines and Their Crown Ether Derivatives

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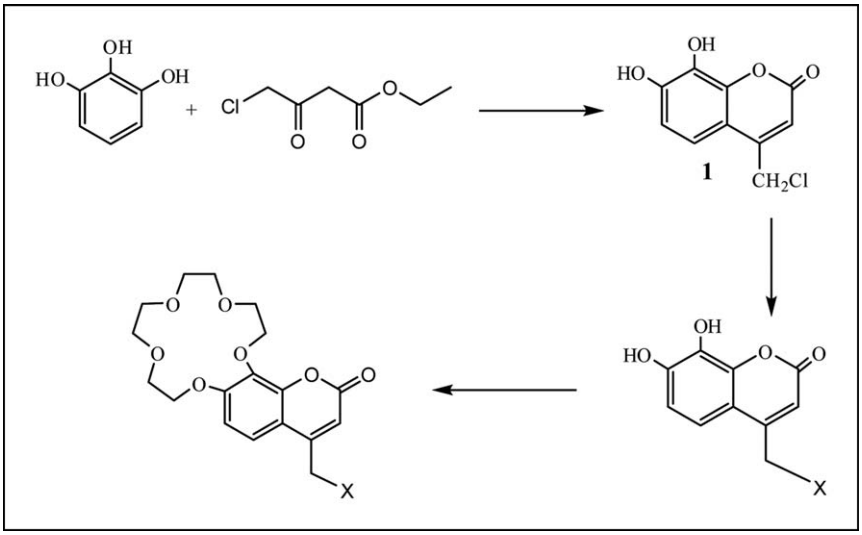

The article focuses synthesis of novel 4-aminomethyl-7,8-dihydroxy coumarins and their crown ether derivatives. The purified novel coumarins and their crown ether derivatives were identified by ^{1}H NMR, ^{13}C NMR, mass spectrometry and elemental analysis. The 1:1 binding constants of Ca^{2+}, Mg^{2+}, Fe^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+}, Co^{2+}, and Mn^{2+} ions at 25°C ± 0.1 with the 4-aminomethyl-7,8-dihydroxy coumarins and their crown ether derivatives estimated using extraction procedure with Inductively Coupled Plasma-Atomic Emission Spectroscopy in dichloromethane/water membrane systems. Synthesized compounds were investigated for complexation and biological activity properties. Best results in biological activity studies were observed for antioxidant activity.

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INTRODUCTION

Coumarins are members of the class of compounds called benzopyrones and display a variety of pharmacological properties [1] depending on their substitution pattern. They are also known to possess antifungal and antibacterial properties [2,3], natural [4], and synthetic origin [5]. The diverse biological activity of chromenone derivatives as anticoagulants and antithrombotics is well known [6].

3-Alkyl and 4-alkylcoumarins are well known [7] for their anthelmintic, hypnotic, insecticidal, antifungal activities, and anticoagulant effect on blood and diuretic properties. Extensive work has been done on the synthesis [8] of these classes of compounds. Methylene halide that is attached at the 4th position is a very highly reactive group in coumarins. Some coumarine compounds obtained from reaction of coumarin-4-methylene halide group with various amino compounds have been investigated for complexation [9], biological [10], photopysical [11,12], and spectroscopic [13–15] properties.

Macrocyclic molecules have attracted much attention because of their potential use in a variety of chemical processes, complexation ability, selective complexing

agents for metal ions, and photoinduced electron transfer since its discovery by Pedersen [16,17]. The complexation selectivity of crown ethers has often been explained in terms of the size-fit concept that the crown ether forms a more stable complex with the cation which is more suitable in size for the crown ether cavity. From many investigations of crown ether and cation interactions it was determined that crown ethers can form variable complexes with cations that are too large to fit into the macrocyclic cavity [18–23]. This led to the synthesis of new crown ethers derivatives which are attachment of functional side arm.

In ion-pair extractive separation of metal cations using a neutral chelation reagent and a counter anion, selection of the chelation reagent is one of the most important factors to realize preferable separation. Especially, investigation of effect of steric structure around electron donor atoms in the reagent on the separation ability is very important for the development of novel reagents having high separation performance. The determination of complexation constants of organic ligands-metal ion complexes in water can be examined with different methods by following the extraction of metals to the varied organic solvents with organic ligands [24–29]. The solvent extraction of metal cations which contains macrocyclic ligands is preferred to use for its easy determination by spectrophotometric methods [23,30,31].

We report in this study the synthesis, complexation, and biological activity studies of novel 4-aminomethyl-7,8-dihydroxy coumarins and their crown ether derivatives.

RESULTS AND DISCUSSION

Synthesis of compounds. 4-chloromethyl 7,8-dihydroxy coumarin compound (**2**) was synthesized by reaction of 1,2,3-trihydroxybenzene with ethyl-4-chloroacetoacetate. The 4-aminomethyl coumarins (**2–9**) were synthesized at room temperature by stirring and then refluxing the reaction mixture of amino compound and triethylamine with 4-chloromethyl coumarin in dry acetone. (Scheme 1). Next stage, the substituted 4-aminomethyl coumarin crown ether derivatives (**10–17**) were synthesized by refluxing 4-aminomethyl coumarins, tetraethyleneglycole ditosylate and Na_2CO_3 in CH_3CN for 3–4 days.

Complexation studies. K_{ex} is extraction equilibrium constant; $[M^{+m}]$ and $[MLA_m]$ are the concentrations of metal cation in aqueous phase and organic phase, respectively. $K_{D,L}$ denotes a distribution constant of ligand between organic solvent and water [25,32,33]. Extraction values, complexation, and distribution constants of synthesized ligands collected in Table 1.

$$M^{+m}_{(aq)} + L_{(org)} + mA_{(aq)} \overset{K_{ex}}{\rightleftharpoons} (MLA_m)_{(org)}, \quad (1.1)$$

$$K_{ex} = \frac{[MLA_m]_{(org)}}{[M^{+m}]_{(aq)}[L]_{(org)}[A]^m_{(aq)}}, \quad (1.2)$$

$$K_{D,L} = [L]_{(org)}/[L]_{(L)}. \quad (1.3)$$

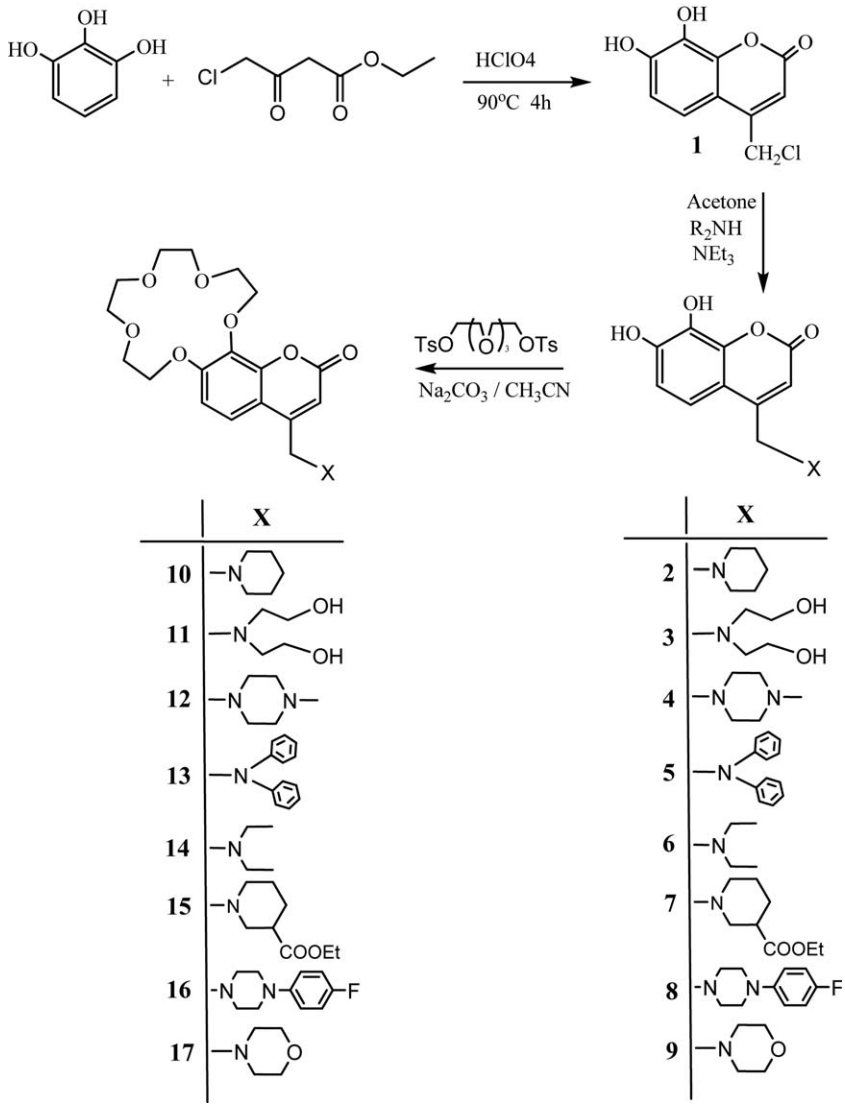
The complexation of metal cations Ca^{2+}, Mg^{2+}, Fe^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+}, Co^{2+}, and Mn^{2+} have been examined with the synthesized ligands and extracting solvent. Association constants based on 1:1 stoichiometry of various metal complexes of these ligands were calculated from eqs. (1.1)–(1.3) (see Table 1) as the interactions of the ligands with different metal ions in dichloromethane solvent media. Assuming that formation of 1:1 complexes of the metal cations have been extracted at the natural pH of the aqueous metal salt solutions. The results are interesting, namely, the binding order of hydroxycoumarin compounds observed for only **3**,**7**,**8**, and **9** as ($Ni^{2+} > Co^{2+} > Mn^{2+} > Fe^{2+} > Ca^{2+} > Zn^{2+} > Cd^{2+} > Mg^{2+}$), ($Cd^{2+} > Ca^{2+} > Co^{2+} > Fe^{2+} > Ni^{2+} > Zn^{2+} > Mn^{2+} > Mg^{2+}$), ($Co^{2+} > Ni^{2+} > Cd^{2+} > Mn^{2+} > Fe^{2+} > Ca^{2+} > Zn^{2+} > Mg^{2+}$), ($Co^{2+} > Cd^{2+} > Fe^{2+} > Ca^{2+} > Zn^{2+} >>> Ni^{2+} = Mn^{2+} = Mg^{2+}$), respectively. It is found that the values of stability constants of coumarine crown derivatives were nearly in the same range for most examined metal cations except for Cd^{2+} and Co^{2+} cations. Cd^{2+} is found to be the best extracted metal cation for the coumarine crowns. It followed by Co^{2+} for all synthesized coumarine crown derivatives. One of the most important findings was the increase in Mg^{2+} selectivity for only **8** and **11** among the all examined ligands. However, the five hydroxycoumarins (**1**,**2**,**4**,**5**, and **6**) and two coumarin crown compounds (**10** and **13**) did not show any complexation towards metal cations.

The synthesized compounds were tested for antibacterial (Table 2), antituberculosis (Table 2), antifungal (Table 2), and antioxidant (Table 3) activity. High values were obtained in antimicrobial activity studies especially for some hydroxycoumarin compounds. Best results in biological activity studies were observed for antioxidant activity. In comparison with the control substance, extremely high values have been obtained for **2**,**3**,**4**,**6**, and **8** ligands.

EXPERIMENTAL

General. The starting chemicals were purchased from Aldrich or Merck unless otherwise cited. $CaCl_2$, $MgCl_2$, $ZnCl_2$, $CoCl_2$, $FeSO_4$, $MnCl_2$, $NiCl_2$, and $CdCl_2$ were analytical grade reagents from Fluka dried over P_2O_5 for 48 h at 0.1

Scheme 1. Synthesized compounds.



torr. The CH_2Cl_2 used was of analytical reagent grade. FT-IR spectra have taken as a KBr pellet with a Perkin Elmer Spectrum spectrometer, model BX-II, High resolution EI mass spectra have been obtained with Agilent 1100 LC/MSD, NMR spectra have been obtained with a Bruker-Specrospin AvanceDPX-400 Ultra-Shield ^{1}H: 400 MHz ^{13}C: 100 MHz. CPX and TMS was the initial standard. All melting points reported are uncorrected. The concentrations of metal ions in the aqueous phases have been determined spectroscopically: ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES): Perkin Elmer Optima 3100 XL).

Organic synthesis. ***4-Chloromethyl-7,8-dihydroxy-2H-chromen-2-one(1).*** A mixture of pyrogallol (0.03 mol), ethyl 4-chloroacetoacetate (0.06 mol) and $HClO_4$ (10 mL, 70%) was heated to 90°C for 4 h. The resulting mixture was cooled, diluted with water and the precipitates were collected by filtration. The dried crude product was purified by recrystallisation from ethanol. Yield (brown):48%. mp; 105–107°C. ^{1}H NMR (400 MHz, Acetone/TMS) δ(ppm): 7.5 (s), 7.2 (d), 6.9 (d), 6.6 (s), 6.4 (s), 4.9 (s).

General synthesis of 4-aminomethyl 7,8-dihydroxy coumarins. A mixture of 4-chloromethyl-7,8-dihydroxy coumarin (0.010 mol), amino compound (0.010 mol) and acetone (300 mL) was stirred to room temparature under N_2 for 24 h. Triethyl amine (0.010 mol) added and stirred for 1 h and then the mixture was refluxed for 3 h. After the removal of solution by evaporation, the resulting mixture was triturated with water, and the precipitates were collected by filtration. The crude product was dried under vacuum.

7,8-Dihydroxy-4-piperidine-1-ylmethyl-2H-chromen-2-one(2). Yield (brown); 71%. mp; 65–68°C. ^{1}H NMR (400 MHz, Acetone/TMS) δ(ppm): 7.73 (s), 7.28 (dd), 6.85 (dd), 6.38 (s), 6.33 (s), 4.72 (s), 2.65 (m), 1.7 (m), 1.36 (m).

4-{(Bis-(2-hydroxy-ethyl)-amino)-methyl}-7,8-dihydroxy-2H-chromen-2-one (3). Yield (darkgreen); 67%. mp; 155–159°C. ^{1}H NMR (400 MHz, Acetone/TMS) δ(ppm): 7.78 (s),

Table 1

$K_{D,L}$, % Ext, and Log K_{ex} values for extraction of synthesized compounds in CH_2Cl_2 with Ca^{2+}, Mg^{2+}, Fe^{2+}, Zn^{2+}, Ni^{2+}, Cd^{2+}, Co^{2+}, and Mn^{2+} ions at 25°C ± 0.1.[a]

		Cations							
Ligand	Value	Zn^{2+}	Cd^{2+}	Ni^{2+}	Ca^{2+}	Mn^{2+}	Co^{2+}	Fe^{2+}	Mg^{2+}
1	$K_{D,L}$	–	–	–	–	–	–	–	–
	% Ext	–	–	–	–	–	–	–	–
	Log K_{ex}	–	–	–	–	–	–	–	–
2	$K_{D,L}$	–	–	–	–	–	–	–	–
	% Ext	–	–	–	–	–	–	–	–
	Log K_{ex}	–	–	–	–	–	–	–	–
3	$K_{D,L}$	0.26	0.14	2.65	0.68	1.19	1.78	0.77	–
	% Ext	20.49	12.30	72.57	40.52	54.37	64.09	43.46	–
	Log K_{ex}	8.41	8.06	10.34	9.08	9.55	9.94	9.18	–
4	$K_{D,L}$	–	–	–	–	–	–	–	–
	% Ext	–	–	–	–	–	–	–	–
	Log K_{ex}	–	–	–	–	–	–	–	–
5	$K_{D,L}$	–	–	–	–	–	–	–	–
	% Ext	–	–	–	–	–	–	–	–
	Log K_{ex}	–	–	–	–	–	–	–	–
6	$K_{D,L}$	–	–	–	–	–	–	–	–
	% Ext	–	–	–	–	–	–	–	–
	Log K_{ex}	–	–	–	–	–	–	–	–
7	$K_{D,L}$	0.63	1.98	1.41	1.86	0.31	1.80	1.64	–
	% Ext	38.69	66.39	58.56	65.02	23.86	64.35	62.10	–
	Log K_{ex}	9.02	10.04	9.71	9.98	8.53	9.95	9.85	–
8	$K_{D,L}$	0.70	1.34	2.33	0.86	0.95	3.63	0.88	0.09
	% Ext	41.02	57.23	69.93	46.13	48.72	78.40	46.91	8.23
	Log K_{ex}	9.10	9.66	10.21	9.27	9.35	10.69	9.29	7.82
9	$K_{D,L}$	11.22	1.88	–	1.41	–	3.01	1.50	–
	% Ext	85.21	65.24	–	58.48	–	75.08	59.99	–
	Log K_{ex}	5.76	9.99	–	9.71	–	10.48	9.77	–
10	$K_{D,L}$	–	–	–	–	–	–	–	–
	% Ext	–	–	–	–	–	–	–	–
	Log K_{ex}	–	–	–	–	–	–	–	–
11	$K_{D,L}$	1.27	2.49	2.19	0.48	0.90	2.46	1.04	0.04
	% Ext	55.96	71.31	68.61	32.23	47.27	71.14	50.99	4.12
	Log K_{ex}	9.61	10.28	10.14	8.81	9.30	10.27	9.43	7.47
12	$K_{D,L}$	1.03	2.51	1.35	0.85	0.56	1.95	1.10	–
	% Ext	50.84	71.49	57.41	45.95	35.97	66.09	52.28	–
	Log K_{ex}	9.43	10.28	9.67	9.26	8.93	10.02	9.48	–
13	$K_{D,L}$	–	–	–	–	–	–	–	–
	% Ext	–	–	–	–	–	–	–	–
	Log K_{ex}	–	–	–	–	–	–	–	–
14	$K_{D,L}$	1.72	2.73	1.71	1.06	0.67	2.28	0.95	–
	% Ext	63.23	73.22	63.03	51.56	40.21	69.52	48.79	–
	Log K_{ex}	9.90	10.38	9.89	9.45	9.07	10.19	9.36	–
15	$K_{D,L}$	1.32	3.12	1.55	1.24	0.72	1.80	1.72	–
	% Ext	56.92	75.71	60.82	55.30	41.71	64.26	63.22	–
	Log K_{ex}	9.65	10.52	9.80	9.59	9.12	9.94	9.90	–
16	$K_{D,L}$	1.46	3.23	1.88	0.63	0.66	2.49	0.85	–
	% Ext	59.40	76.33	65.29	38.59	39.71	71.31	45.92	–
	Log K_{ex}	9.74	10.56	9.99	9.02	9.05	10.28	9.26	–
17	$K_{D,L}$	1.68	2.72	1.64	0.86	0.97	2.32	0.91	–
	% Ext	62.69	73.13	62.18	46.26	49.32	69.86	47.76	–
	Log K_{ex}	9.88	10.37	9.86	9.27	9.37	10.20	9.32	–

[a] Corr. coefficient 0.999.

7.24 (d), 6.9 (d), 6.71 (s), 6.38 (s), 4.89 (s), 3.15 (m), 2.82 (br).

Bis-(4-piperazinyl methyl-7,8-dihydroxy)-2H-chromen-2-one(4). Yield (darkgreen); 63%. mp; 82–84°C. ^{1}H NMR (400 MHz, Acetone/TMS) δ(ppm): 7.38 (d), 6.93 (d), 6.86 (d), 6.41 (s), 6.3 (s), 4.91 (s), 3.84 (t).

4-((N,N-diphenylamino)-methyl)-7,8-dihydroxy-2H-chromen-2-one(5). Yield (brown); 77%. mp; 60–62°C. ^{1}H NMR (400

Table 2

Antibacterial, antituberculosis, antifungal, and antimicrobial activities results of synthesized compounds.

	Inhibiton zone (mm)								
Ligands	*M. tuberculosis*[a]	*M. simiae*	*M. kansasii*	*M. terrae*	*M. szulgai*	*E. coli*	*S. aureus*	*C. albicans*	*M. smegmatis*
1	NT	NT	NT	NT	NT	NA	NA	NA	NA
2	NT	NT	NT	NT	NT	7	NA	NA	NA
3	NA	NA	9	NA	9	5	7	NA	7
4	NA	NA	NA	NA	6	NA	NA	6	7
5	NT	NT	NT	NT	NT	NA	NA	NA	NA
6	NT	NT	NT	NT	NT	7	NA	NA	NA
7	NA	NA	6	NA	5	6	NA	7	7
8	NA	NA	NA	3	5	5	NT	6	6
9	NT	NT	NT	NT	NT	NA	NA	7	NA
10	NA	NA	NA	NA	9	7	NA	NA	8
11	NT	NT	NT	NT	NT	NA	NA	8	NA
12	NT	NA	NA	NA	NA	NA	NA	NA	13
13	NT	NT	NT	NT	NT	NA	NA	NA	NA
14	NT	NT	NT	NT	NT	NA	NA	6	NA
15	NA	NA	NA	NA	NA	6	NA	NA	12
16	NT	NT	NT	NT	NT	NA	NA	6	NA
17	NA	NA	NA	NA	NA	NA	NA	NA	7
Rifampicin	NT	NT	NT	NT	NT	NT	NT	NT	35

NA: not active, NT: not tested.
[a] MIC values (μg/mL).

MHz, Acetone/TMS) δ(ppm): 7.41 (s), 7.22 (dd), 7.11 (d), 6.91 (d), 6.84 (dd), 6.38 (s), 4.88 (s).

4-(N,N-diethylamino)methyl-7,8-dihydroxy-2H-chromen-2-one(6). Yield (yellow); 82%. mp; 79–82°C. ^{1}H NMR (400 MHz, Acetone/TMS) δ(ppm): 7.56 (s), 7.25 (d), 6.93 (d), 6.65 (s), 6.4 (s), 4.91 (s), 3.1 (m), 2.12 (t).

1-(7,8-Dihydroxy-2-oxo-coumarin-4-ylmethyl)-piperidine-3-carboxylic acid ethyl ester (7). Yield (brown); 71%. mp; 101–103°C. ^{1}H NMR (400 MHz, acetone/TMS) δ(ppm): 7.65 (s). 7.03 (s), 6.84 (s), 6.65 (s), 6.33 (s), 4.53 (s), 3.4 (m), 2.52 (m), 1.68 (m), 1.50 (t).

4-(4-(4-Fluoro-phenyl)-piperazin-1-ylmethyl)-7,8-dihydroxy-2H-chromen-2-one (8). Yield (brown); 70%. mp; 94–96°C. ^{1}H NMR (400 MHz, Acetone/TMS) δ(ppm): 7.36 (d), 6.99 (m), 6.85 (d), 6.32 (s), 3.73 (s), 3.18 (m), 2.72 (m).

7,8-dihydroxy-4-(morpholinomethyl)-2H-chromen-2-one(9). Yield (yellow); 78%. mp; 199–202°C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ(ppm): 7.8 (d), 7.2 (d), 6.7 (s), 4.7 (s), 3.2 (t), 2.4 (t).

General synthesis of 4-aminomethyl 7,8-coumarin crown ethers. A mixture of 4-alkylamino substitued 7,8-dihydroxy coumarin (0.005 mol), tetraethylenglycole ditosylate (0.005 mol), Na_2CO_3 (0.01 mol) and 700 mL CH_3CN was refluxed for 3–4 days under N_2. After the removal of solution by evaporation, the residue was extracted with $CHCl_3$, the organic layer washed with water and dried on $MgSO_4$. After the evaporation of $CHCl_3$, the residue was purified by column chromatography on silicagel (chloroform–methanol).

20-Piperidine-1-ylmethyl-2,5,8,11,14,17-hexaoxa-tricyclo(13.8.0.0^{16,21})tricosa-1(23),15,19,21-tetraen-18-on (10). Yield (yellow solid); 33%, mp; >300°C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ(ppm): 7.7 (d), 7.15 (d), 6.3 (s), 4.78 (s), 4.03 (t), 3.93 (t), 3.6 (m),1.87 (m), 1.69 (m). ^{13}C NMR (100 MHz) δ(ppm): 158, 149, 143.5, 139.34, 128.64, 125.95, 122, 112.88, 103, 71.88, 71.00, 70.9, 70.48, 70.24, 68.6, 69.1, 65, 60.22, 60.02, 42, 21.9. EI-MS (*m/z*). M^+ :434(8), 351(100), 219(47). Elemental Analysis: Anal. Calcd. for C, 63.73; H, 7.21; N, 3.23; O, 25.84; Found: C, 63.69; H, 7.18; N, 3.28; O, 25.85.

20-{(Bis-(2-hydroxy-ethyl)-amino)-methyl}-2,5,8,11,14,17-hexaoxa-tricyclo(13.8.0.0^{16,21})tricosa-1(2),15,19,21-tetraen-18-on (11). Yield (yellow); 19%, mp; 217–220°C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ(ppm): 7.4 (d), 7.08 (d), 6.79 (s), 4.72 (s), 3.35 (m), 2.47 (t), 4.2 (m), 3.6 (m), 2.64 (t). ^{13}C NMR (100 MHz) δ(ppm): 166.78, 153.43, 144.4, 132, 131.2, 129,

Table 3

Antioxidant activity results.

Compounds	TEAC CUPRAC
1	NA
2	1.28
3	2.40
4	3.64
5	NA
6	2.26
7	NA
8	0.89
9	NA
10	NA
11	NA
12	NA
13	NA
14	NA
15	NA
16	NA
17	NA
Askorbik acid	0.93/0.96[a]
Tocopherol	0.97/1.01[a]

[a] Values in literature.

110, 108.65, 102.73, 71.65, 71.19, 70.88, 70.32, 69.73, 68.69, 68.16, 67.66, 53, 49.8, 39.65. EI-MS (*m/z*). M^+: 453(7), 391(57), 358(100), 351(75). Elemental Analysis: Anal. Calcd. for C, 58.27; H, 6.89; N, 3.09; O, 31.75; Found: C, 58.20; H, 6.99; N, 3.00; O, 31.81.

16,16′-(piperazin-1,4-düilbis(methylen))bis(5,6,8,9,11,12-hexahidro-2H-(1,4,7,10,13) pentaoxa cyclopentadeca (2,3-h)chromen-18(3H)-one) (12). In this procedure tetraethylenglycole ditosylate and Na_2CO_3 was used twofold. Yield (yellow); 19%, mp; 285–290°C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ(ppm): 7.75 (d), 7.11 (d), 6.4 (s), 4.4 (s), 3.9 (m), 3.7 (m), 2.6 (m). ^{13}C NMR (100 MHz) δ(ppm): 163.55, 142, 140.4, 138.55, 130.23, 128.6, 126, 113.45, 101.11, 70.77, 70.44, 70.29, 69.88, 69.08, 68.73, 68.11, 67.24, 53, 43, 39.87. EI-MS (*m/z*). M^+: 783(100), 611.5(74). Elemental Analysis: Anal. Calcd. for C, 61.37; H, 6.44; N, 3.58; O, 28.61; Found: C, 61.27; H, 6.53; N, 3.58; O, 28.62.

20-((Diphenylamino)-methyl)-2,5,8,11,14,17-hexaoxa-tricyclo(13.8.0.0^{16,21})tricosa-1(23),15,19,21-tetraen-18-on (13). Yield (yellow); 29%, mp; 270–275°C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ(ppm): 7.45 (d), 7.09 (d), 6.9 (m), 6.2 (s), 4.65 (s), 4.1 (m), 3.32 (m). ^{13}C NMR (100 MHz) δ(ppm): 161, 147.65, 145.23, 142.95, 127, 123.2, 118.2, 116.56, 110.43, 109.33, 107.46, 107.33, 101, 71.39, 71.28, 71.05, 70.8, 70.46, 68.77, 68.39, 68.04, 36.55. EI-MS (*m/z*). M^+: 517(17), 439(21), 416(48), 384(100), 300(52). Elemental Analysis: Anal. Calcd. for C, 69.62; H, 6.04; N, 2.71; O, 21.64; Found: C, 69.56; H, 6.01; N, 2.78; O, 21.65.

20-Diethylaminomethyl-2,5,8,11,14,17-hexaoxa-tricyclo (13.8.0.0^{16,21}) tricosa-1(23),15,19,21-tetraen-18-on (14). Yield (yellow); 23%, mp; 278–280. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ(ppm): 7.49 (d), 7.1 (d), 6.87 (s), 6.3 (s), 4.7 (s), 4.1 (m), 3.5 (t), 2.48 (q), 2.26 (t). ^{13}C NMR (100 MHz) δ(ppm): 161, 145.4, 142.2, 138.55, 132.22, 128.61, 125, 101, 107, 71.37, 71.06, 70.65, 70.29, 70, 69.64, 68.91, 68.21, 56, 41.21, 36.55. EI-MS (*m/z*). M^+: 422.5(100), 407(32), 253(45), 219(47). Elemental Analysis: Anal. Calcd. for C, 62.69; H, 7.41; N, 3.32; O, 26.57; Found: C, 62.75; H, 7.43; N, 3.35; O, 26.47.

1-(18-Oxo-2,5,8,11,14,17-hexaoxa-tricyclo(13.8.0.0^{16,21})tricosa-1(23),15,19,21-tetraen-20-ylmethyl)-piperidine-3-carboxylic acid ethyl ester (15). Yield (brown); 35%, mp; >310. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ(ppm): 7.75 (d), 7.13 (d), 6.27 (s), 4.67 (s), 4.1 (m), 3.6 (m), 2.32 (m), 1.25 (m). ^{13}C NMR (100 MHz) δ(ppm): 172, 161, 145, 142.65, 140.11, 128.65, 125.94, 113, 112, 109, 71.85, 71.22, 70.75, 70.50, 70.32, 69.86, 68.56, 68.24, 59.60, 58, 57.7, 56.5, 55.29, 40, 36.35. EI-MS (*m/z*). M^+: 506(17), 489(52), 475(17), 316(45), 302(100). Elemental Analysis: Anal. Calcd. for C, 61.77; H, 6.98; N, 2.77; O, 28.48; Found: C, 61.70; H, 7.02; N, 2.71; O, 28.57.

20-(4-(4-Fluoro-phenyl)-piperazin-1-ylmethyl)-2,5,8,11,14,17-hexaoxa-tricyclo (13.8.0.0^{16,21}) tricosa-1(23),15,19,21-tetraen-18-on (16). Yield (brown); 37%, mp; 276–280°C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS): 7.47 (d), 7.1 (d), 7.0 (m), 4.7 (s), 3.7 (m), 3.6 (m), 2.5 (m). ^{13}C NMR (100 MHz): 167, 140, 139, 133, 131.55, 130.46, 128.9, 128.34, 126.65, 124.98, 116, 115.89, 111.76, 70.79, 70.68, 70.43, 70.12, 69.69, 69.17, 68.49, 68.04, 54, 50.88, 39. EI-MS (*m/z*). M^+: 529(100). Elemental Analysis: Anal. Calcd. for C, 63.62; H, 6.29; F, 3.59; N, 5.30; O, 21.19; Found: C, 63.57; H, 6.27; F, 3.61; N, 5.33; O, 21.22.

16-(morpholinomethyl)-5,6,8,9,11,12-hexahidro-2H-(1,4,7,10,13) pentaoxacyclopentadeca (2,3-H) chromen-18(3H)-on (17). Yield (yellow); 27%, mp; 255–260°C. ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ(ppm): 7.5 (d), 7.1 (d), 7.0 (s), 4.5 (s), 3.5 (m), 2.4 (t). ^{13}C NMR (100 MHz) δ(ppm): 161, 150.22, 146.26, 137.98, 133, 128.59, 110, 108.9, 103, 71.84, 71.12, 70.95, 70.42, 69.75, 69.4, 69, 68.78, 67.66, 56.63, 44.3. EI-MS (*m/z*). M^+: 436(100). Elemental Analysis: Anal. Calcd. for C, 60.68; H, 6.71; N, 3.22; O, 29.39; Found: C, 60.61; H, 6.75; N, 3.27; O, 29.37.

EXTRACTION PROCEDURE

The extraction measurements were done in 100 mL glass thermostated cell compartment with a mechanical stirrer where a solution 10 mL (1×10^{-5} *M*) of an aqueous salt and ligand in CH_2Cl_2 organic solvent in appropriate concentration were placed and stirred for 120 min at 25 ± 0.1°C and subsequently allowed to stand for 60 min to complete the phase separation. The optimum concentrations of the ligands were determined by extracting the alkali salts with 10 mL aliquot of various concentrations of the ligands (1×10^{-5} *M*).

After extraction, the metal concentrations in the aqueous phase were determined using ICP-AES. Each value was the average of three subsequent measurements. Complexation and distribution constants summarized in Table 1.

BIOLOGICAL ASSESSMENTS

Antibacterial and antifungal activities studies; Disc diffusion method was used [34]. The compounds were tested against standard bacterial strains; *E. coli*, *S. aureus*, *M. smegmatis*, *M. tuberculosis*, *M. simiae*, *M. kansasii*, *M. terrae*, *M. szulgai*, and a fungi *C. albicans*. Disc diffusion method was applied for the determination of antimicrobial activities of the samples. Compounds were dissolved in dichloromethane (CH_2Cl_2) and then filter-sterilized using a 0.20-μm membrane filter. A suspension of the tested microorganism (0.1 mL of 10^8 cells/mL) was spread over the surface of agar plates (MHA and SDA). Filter papers having a diameter of 6 mm, soaked with 10 μL of samples and 10 μg compound in solution were placed on the inoculated agar plates. Before incubation all petri dishes were kept in the refrigerator (4°C) for 2 h. Then they were incubated at 37°C for 24 h for bacteria and at 30°C for 48 h for the yeasts. The diameters of the inhibition zones were measured in millimeters. The biological activity results of synthesized compounds are displayed in Table 2.

ANTIOXIDANT ACTIVITY STUDIES

Antioxidant activity studies were measured by using the cuprac method in the literature [35]. Antioxidant activity studies results are showed in Table 3.

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