Synthesis and Antioxidant Activities of [5-fluoro *N, N′*-bis (salicylidene) ethylenediamine] and [3, 5-fluoro *N, N′*-bis (salicylidene) ethylenediamine] Manganese (III) Complexes

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ABSTRACT: *Antioxidants act as free radical scavengers in the oxidation processes. Thus, they will certainly play diverse roles in the biological systems and the therapy of a wide variety of diseases. Regarding this fact, in the present study, we synthesized two new salen ligand compounds by the condensation of ethylendiamine and salicylaldehyde derivatives in excellent yields. The structures of these ligands were confirmed by IR, ^{1}H NMR and mass spectroscopy techniques. Furthermore, we evaluated the relative dismutase, catalase and peroxidase activities of the newly synthesized complexes named as EUKs 131, 132, 141 and 142 relative to EUKs 108 and 8, as the reference compounds. The results demonstrated that all Mn-salen complexes (EUKs) illustrated significant dismutase, catalase and peroxidase activities. EUKs 131 and 8 showed the most catalase and peroxidase activities while their dismutase activities were almost the same as the other compounds. In addition, our data indicated that the biological activities of the EUKs are modulated by manganese element as well as the types and the positions of substituents on the ligand.*

KEY WORDS: *Mn-salen complexes, Synthesis, Antioxidant, Dismutase, Catalase, Peroxidase.*

INTRODUCTION

Antioxidants are compounds capable of inhibiting the process of oxidation [1]. They act as free radical scavengers, convert radicals to less reactive species and therefore, protect the cell components against the destructive effects of endogenous free radicals [2-3]. The biological systems normally balance the endogenous free radical content through their own enzymatic and non-enzymatic antioxidant defense systems. The enzymatic antioxidant components are superoxide dismutase, catalase and glutathione peroxidase. The non-enzymatic antioxidant group consists of small organic molecules such as vitamins C and E. The defense antioxidants can effectively interact with free radicals and terminate their chain reactions before damage to vital molecules [1, 4-5].

The level higher than normal level of ROS, which is toxic to biological systems, is resulted due to the imbalance between the extent of ROS production and consumption. This situation will lead to a wide array of human diseases [6-8]. Based on this fact, appropriate consumption of antioxidants would have beneficial

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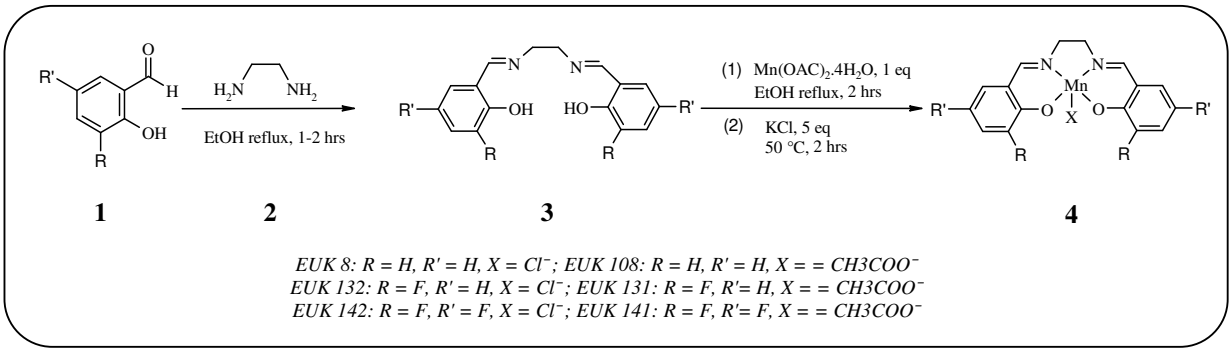

Fig. 1: The synthetic pathway of the new Mn-salen complexes used in this study.

effects on the onset and progression of oxidative stress-associated diseases. In addition, the role of antioxidants in augmentation of immune defense system has also been reported [1, 3, 9-14].

It is well established that natural and / or synthetic antioxidants including vitamins, phenols, flavonoids, carotenoids are able to scavenge radicals and attenuate oxidant content to the normal level [14]. However, antioxidant enzymes due to their short circulating half life, antigenisity and the cost have limited application as therapeutic agents. Therefore, worldwide attention has been toward synthetic and/or natural products acting as enzymes. In that line, global attention has been devoted to the SOD and CAT mimetics such as salen complexes [14, 16-22]. Salens are chelating agents used in coordination chemistry and homogeneous catalysis. They form various complexes with most transition metal ions such as Mn^{2+}, Cr^{2+}, Fe^{2+}, Ru^{2+}, Co^{2+}, V^{2+} and Ti^{2+} [23]. Mn-salen complexes with SOD/CAT mimetic properties have been reported which protect cells against structural and functional injuries induced by high levels of ROS [16]. Because of this SOD/CAT mimicry, some of them have been investigated as possible therapeutic agents in disorders resulting from oxidative stress. It has been shown that the central metal ion in these complexes, along with the ligand structure, modulates the antioxidant activities of salen derivatives [24]. Herein we describe some initial results including the synthesis and antioxidant activities of 5-fluoro N, N' bis (salicylaldehyd) ethylenediamine and 3, 5-difluoro *N, N*'-bis (salicylaldehyd) ethylenediamine manganese (III) acetate and chloride complexes. The general method, which is used for the preparation of Mn-salen complexes, is illustrated in Fig. 1 [16, 25].

EXPERIMENTAL SECTION

Melting points were measured on an Electrothermal Buchi 535 apparatus. The IR spectra were recorded using a Shimadzu IR-470 spectrometer. The ^{1}H-NMR spectra were done on a Bruker DRX-500 Avance instrument with $CDCl_3$ as the solvent at 500 and 75 MHz. The mass spectra were recorded on an Agilent Technology (HP) 5973 mass spectrometer operating at an ionization potential of 70 eV.

Chemicals

Nitro Blue Tetrazolium (NBT), Nicotinamide Adenine Dinucleotide reduced form (NADH), Phenazine MethoSulfate (PMS), hydrogen peroxide (H_2O_2), 3-Fluorosalicyl aldehyde (97%), 5-fluorosalicyl aldehyde (97%), 3, 5-difluorosalicyl aldehyde (97%), manganese acetate and potassium chloride were purchased from Merck company (Darmstadt, Germany). 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) was prepared from Sigma company (Sigma-Aldrich, Sternheim, Germany). Salicyl aldehyde and ethylene diamine were purchased from Fluka company (Buchs, Switzerland). All other chemicals used were analytical grade and prepared from either Sigma-Aldrich or Merck company (Germany). All chemicals were used without further purification.

General procedure for the synthesis of [5-fluoro N, N'-bis (salicylidene) ethylenediamine] and [3, 5-fluoro N, N'-bis (salicylidene) ethylenediamine] ligands

EUKs 108 and 8 were prepared according to modified method of *Ruch et al.* [16]. To produce EUKs 131, 132, 141 and 142, 1 equiv of ethylenediamine in absolute ethanol was added to a solution of 2 equiv of

Table 1: The synthesized compounds in this study.

Compound	Products	R	R'	X	Yield (%)
3a	EUKs108/8 ligand	H	H	_	88
3b	EUKs131/132 ligand	5-F	5-F	_	92
3c	EUKs141/142 ligand	3,5F	3,5F	_	93
4a	EUK-108	H	H	OAC	73
4b	EUK-131	5-F	5-F	OAC	79
4c	EUK-141	3,5F	3,5F	OAC	94
4d	EUK- 8	H	H	Cl	95
4e	EUK-132	5-F	5-F	Cl	55
4f	EUK-142	3,5F	3,5F	Cl	35

the substituted aldehyde in absolute ethanol (0.05-0.2 M solution). The mixture was refluxed for 2 hours, cooled and kept in a refrigerator for overnight to allow more precipitate to be formed. The yellow precipitate was then filtered, washed with ethanol, and air-dried to give the desired ligand in 92-93% yield (Table 1). Structures of the ligands were confirmed by IR, ^{1}H NMR and mass spectroscopy.

General procedure for the synthesis of [5-fluoro N, N'-bis (salicylidene) ethylenediamine] and [3, 5-fluoro N, N'-bis (salicylidene) ethylenediamine] manganese (III) complexes

One equiv of solid manganese (II) acetate tetrahydrate was added to a stirred suspension of 1 equiv of the ligand in 95% ethanol (0.025-0.03 M) and the reaction was refluxed for 2 hours. The dark brown solutions were then evaporated to dryness by a rotary evaporator. The crude product, a dark brown solid, was washed with acetone, filtered, and air-dried. Each product was obtained as hydrates in 79-94% yield (Table 1). The acetate complexes were converted to the corresponding chlorides by treating an aqueous solution (0.03-0.06 M) of the acetate, warmed to 50 °C, with 5 equiv of KCl dissolved in distilled water. A dark brown precipitate formed. The suspension was cooled in an ice/water bath and then filtered. After solvent removal, the solid was washed with water and acetone. The products were obtained as hydrates in 35-55% yield (Table 1).

Superoxide dismutase activity assay

SOD activity was measured based on the inhibition extent of amino blue tetrazolium formazan formation in the mixture of Nicotinamide Adenine Dinucleotide, Phenazine MethoSulphate and Nitroblue Tetrazolium (NADH–PMS–NBT) system, according to the method of *Kakkar et al.* [26]. The reaction mixture contained 100 µg/mL of the Mn-salen complexes, 1.2 mL of sodium pyrophosphate buffer (pH 8.3; 52 mM), 0.1 mL of PMS (0.2 mM), 0.3 mL of NBT (0.3 mM) and 0.2 mL of NADH (0.75 mM). Reaction was started by addition of NADH. After incubation at 30 °C for 90 sec, the reaction was stopped by addition of 0.1mL of glacial acetic acid. Reaction mixture was stirred vigorously with 4.0 mL of *n*-butanol. All measurements were carried out at 25.0 ± 0.2 ºC. Colour intensity of the chromogen in the butanol was measured against the corresponding blank solutions spectrophotometrically at 560 nm. One unit of enzyme activity was defined as that amount of enzyme which caused 50% inhibition of NBT reduction.

Catalase activity assay

CAT activity was assayed by the method of *Aebi* [27]. Each Mn-salen complex, in the range of 20-100 µM, was added to a cuvette containing 1.995 mL of 50 mM phosphate buffer (pH 7.4). Reaction was started by addition of 1.0 ml of freshly prepared 30 mM H_2O_2 at 25.0 ± 0.1 ºC. Control compounds were tested

at the appropriate equivalent concentrations, that is, 2 equiv for salicyl aldehydes and 1 equiv for manganese salts, ligand, and ethylenediamine. Stock solutions of complexes and control compounds were prepared in water or methanol, depending on solubility. At final concentration, methanol did not exceed 5% and did not affect assay activities. After initiation of the reaction, the rate of decomposition of H_2O_2 was measured at 15sec intervals spectrophotometrically at 240 nm. Activity of CAT was expressed as $\times 10^{-1}$ k, where k represents the rate constant of the first order reaction of CAT.

Peroxidase activity assay

The peroxidase activity of each Mn-salen complex was assayed by monitoring the hydrogen peroxide-dependent oxidation of ABTS (2, 2′-azino-bis-(3-ethylbenzothiazoline-6- sulfonic acid)) spectrophotometrically [16]. The assay mixture consisted of 50 mM sodium phosphate (pH 8.1), 0.9% sodium chloride, 0.5 mM ABTS, 0.2 mM hydrogen peroxide, and different concentrations of each Mn-salen complex (10-100 µM). Assays were conducted at 25 ºC. The ABTS oxidation was monitored at 740 nm ($\epsilon_{740} = 20\ 300\ M^{-1}\ cm^{-1}$) to eliminate interferences from the absorption of metal complexes. There was no detectable ABTS oxidation in the absence of each Mn-salen complexes or hydrogen peroxide under the assay condition.

RESULTS AND DISCUSSION

Synthesis

The reaction of ethylendiamine **1** with salicyl aldehydes **2** in absolute ethanol afforded salen ligands **3** in good yields. The synthesis of each complex consists of three steps: (i) condensation of ethylendiamine **1** with salicylaldehyde derivative **2** in absolute ethanol to form the salen ligand; (ii) chelating manganese element to the tetradonating ligand to produce the acetate complex **4**; (iii) converting the acetate to chloride complex by treating with a chloride anion solution [16, 25]. The structures were deduced by melting point determination, ^{1}H NMR, IR, UV-Vis and mass spectrometric data:

EUK-131/132 ligand (3b)

Yield: 0.79 g (92%); yellow crystals, mp 205.8–206.1ºC. IR (KBr) (υ_{max}/cm^{-1}): 2945 and 2935 (C-H stretching), 1629 (C=N), 1495 (C-O), 1268 and 1245 (C-O stretching), 1136 (C-N). ^{1}H NMR ($CDCl_3$, ppm): δ 12.90 (bs, 2H), 8.35 (s, 2H), 6.93 to 7.09 (m, 6H), 4.01 (s, 2H). MS (EI) m/z: 304 (M^+).

EUK-141/142 ligand (3c)

Yield: 0.47 g (93%); yellow crystals, mp 183.2–184.3ºC. IR (KBr) (υ_{max}/cm^{-1}): 3055 and 2955 (C-H stretching), 1648 (C=N), 1540 (C-O), 1269 and 1214 (C-O stretching), 1121 (C-N). ^{1}H NMR ($CDCl_3$, ppm): δ 13.16 (bs, 2H), 8.36 (s, 2H), 6.82 to 7.31 (m, 4H), 4.05 (s, 2H). MS (EI) m/z: 340 (M^+).

^{1}H NMR analysis clearly indicated the formation of the corresponding salen ligands. The ^{1}H NMR spectrum of **3b** exhibited two sharp signals for methylene (δ 4.01 ppm), and methine (δ 8.35 ppm) protons. The aromatic moiety appeared at δ 6.93 to 7.09 ppm (Fig. 2a). The ^{1}H-NMR spectrum of **3c** is similar to that of **3a** (Fig. 2b). The structural assignments of **3b-c** were made on the basis of their NMR and IR spectra. The IR spectrum of **3b-c** showed an absorption at 1629 cm^{-1} (EUK-131/132 ligand) and 1648 cm^{-1} (EUK-141/142 ligand) indicating the presence of an imine bond. The mass spectrum of 3b-c displayed the molecular ion (M^+) peak at m/z = 304 and 340, which was consistent with the 1:2 adduct of ethylenediamine and salicyl aldehydes with loss of two H_2O molecules (Fig. 3). The UV–Vis spectra are not significantly different from those of corresponding reference compounds (EUKs 108 and 8), in the same environment (Fig. 4).

Antioxidant activity elevation

The SOD-like activities of **4a–f** complexes were determined indirectly using NBT as an electron acceptor [16]. At 100 µg/mL, SOD-like activities were 1.93, 1.89, 1.88, 1.86, 1.85, and 1.84 for EUKs 141, 108, 131, 142, 132 and 8, respectively (Fig. 5). All complexes have significant superoxide dismutase activity, although acetate complexes, EUKs 108, 131 and 141 were slightly more potent SOD mimetic than chloride complexes (EUKs 8, 132 and 142). Our observations were parallel to the literature values [16]. Thus, it might be concluded that fluoride substituents on the ligand did not modulate the SOD activity to a significant degree.

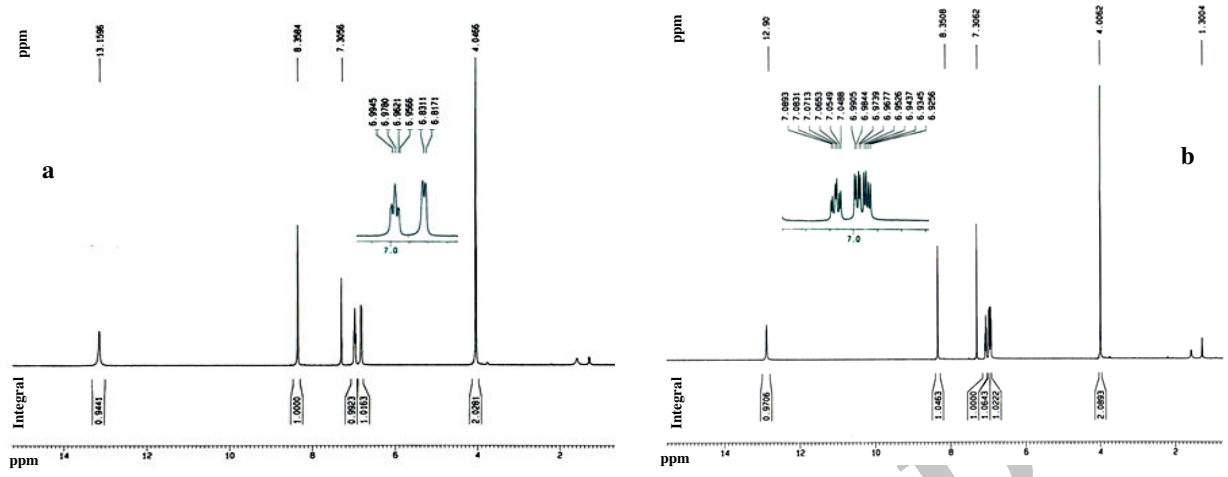

Fig. 2: ^{1}H NMR spectra ($CDCl_3$, 500 MHz) of 3b (a) and 3c (b) compounds.

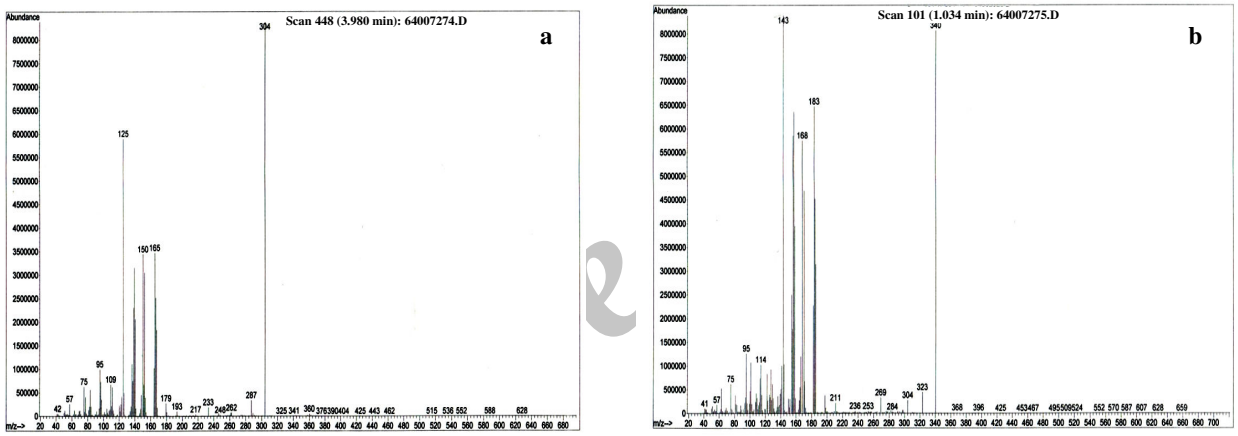

Fig. 3: Mass Spectra of 3b (a) and 3c (b) compounds.

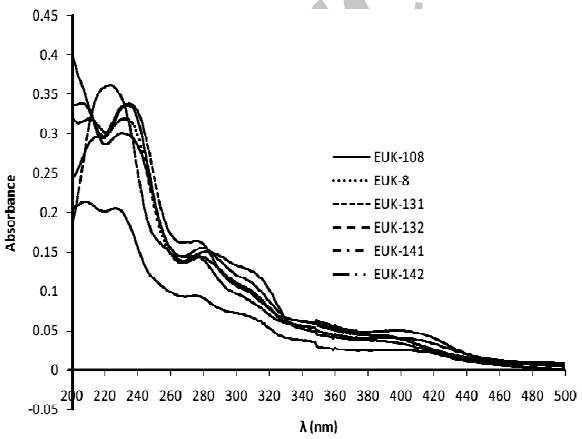

Fig. 4: UV–Vis spectra of Mn-salen complexes at 10 μM in H_2O.

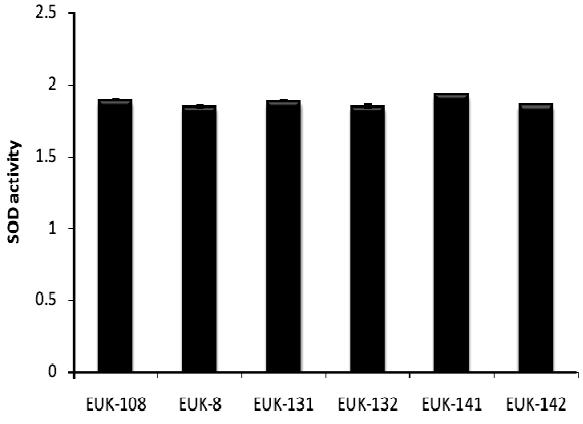

Fig. 5: The OD-like activities of EUKs 108, 8, 131, 132, 141, and 142 at 100 μM.

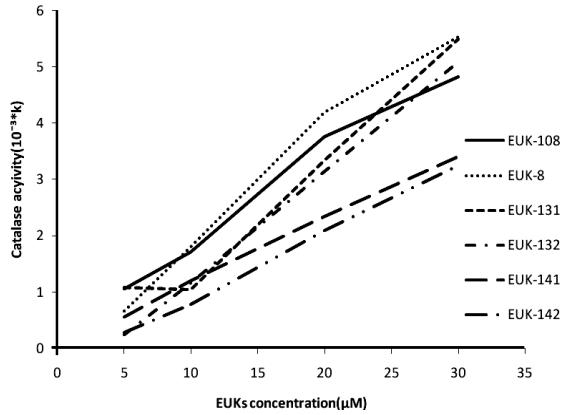

Fig. 6: The catalase-like activities of EUKs 108, 8, 131, 132, 141, and 142 at the concentration range of 5-30 μM. Concentration of substrate = 10 mM.

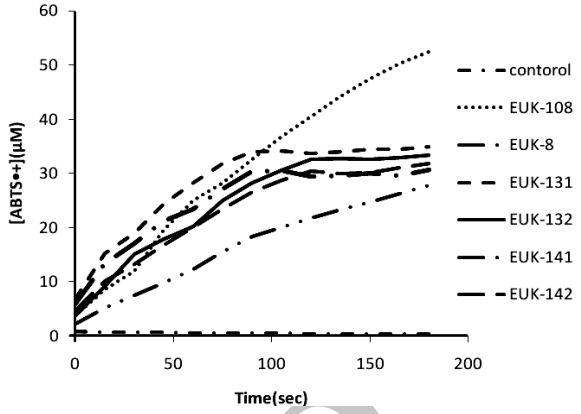

Fig. 7: Time-dependent formation of the $ABTS^{\bullet+}$ in the presence of 10 μM of each evaluated EUK..

The catalase activities, in term of H_2O_2 decomposition, were found to be 5.54, 5.50, 5.08, 4.82, 3.41 and 3.24 $k\times10^{-3}$/min for EUKs 8, 131, 132, 108, 141 and 142, respectively. Based on Fig. 6, at lower concentrations (5-10 μM), no difference between the CAT-like activities of evaluated salen compounds in acetate and/or chloride from was observed. However, this difference became significant at higher concentrations (20-30 μM). Regarding CAT-like activity at 30 μM, the following rank order was obtained for EUKs: 131 (*para* F) > 108 > 141 (*para* and *ortho* F) in acetate as well as 8 > 132 (*para* F) > 142 (*para* and *ortho* F) in chloride forms. Based on our data, it could clearly be stated that the fluoride substituents affect the CAT enzymatic behavior of the newly synthesized Mn-salen derivatives. These results confirmed those of the literature. The evaluation of the CAT activity as a consequence of the similar substitutions on the salen ring has been formerly explained [16-18, 29-30]. On the basis of the data reported in the literature [16], it has been specified that salen ring substitution influences catalase activity in Mn-salen complexes [16]. According to these data, the following rank order was observated for Mn-salen complexes: 113 (*ortho* OMe) > 121 (*ortho* F) > 189 (*ortho* OEt) > 108 > 123 (*pare* OMe) > 114(*meta* OMe) in acetate as well as 134 (*ortho* OMe) > 122 (*ortho* F) > 15 (*para* OMe) > 8 > 115 (*meta* OMe) in chloride forms. According to these ranking, fluoride substitution at the *ortho* position acted as the electron donor alkoxy group at the *ortho* position and showed higher catalase activity than EUKs 108 and 8 [16]. However, our results indicated that the newly synthesized compounds with fluoride substitution at the *ortho* and *para* positions had CAT-like activity similar to slightly lower than EUKs 108 and 8.

Mn-salen complexes also exhibited peroxidase activity using a wide range of substrates. Among them, 2, 2'-azino-bis (3-ethylbenz-thiazoline- 6-sulfonic acid) (ABTS) acts as the specific substrate [16, 28]. Fig. 7 illustrates the extent of $ABTS^{\bullet+}$ formation in the presence of EUKs 131, 132, 141and 142 in comparison to EUKs 108 and 8, versus time. As it is evident from this Fig, at 10 μM, all EUK derivatives showed pronounced peroxidase activities. The peroxidase activities of the EUKs 108, 8, 131, 141, 132 and 142 were found to be 21.17, 10.14, 21.40, 16.43, 17.54 and 15.23 μM $ABTS^{\bullet+}$/min, respectively. These results show that the peroxidase activities of the newly synthesized complexes are slightly higher than those of the reference compounds, EUKs 108 and 8. EUK 131 is the most potent compound in this series for peroxidase activity.

It has previously been shown that EUK-121, as an acetate Mn-salen complex with a fluoride group at the *ortho* position on the salen ring, is a weaker antioxidant than the unsubstituted compound of EUK-108 [16]. Based on our results, although EUK-141 (with *para* and *ortho* F groups) had lower activity than EUK-108, EUK-131 (with a *para* F group) showed a slightly higher peroxidase activity relative to EUK-108. In addition, our results indicated that EUK-132 (with a *para* F group)

and EUK-141 (with *para* and *ortho* F groups), both in chloride form, had high peroxidase activity than unsubstituted reference compound, EUK-108. These observations are parallel to that reported for EUK-122, with *an ortho* F group in chloride form [16].

Overall, our data indicated that substituents on the salen ring can strengthen and/or weaken the free radical scavenging activity of the salen derivatives. This is most probably due to modulation of electron density on the ring through a combination of resonance and inductive effects. The electron donating groups such as alkoxys, at the *para* and *ortho* positions usually act through resonance effect [16]. However, halogen substituents have dual inductive electron withdrawing effect and resonance electron donating activity [31]. Fluorine is the smallest halogen and the most electronegative element. Its high electronegativity causes σ electrons to be withdrawn away from the ring. Its small size increases the distance between the energetic levels of fluoride HOMO and salen ring LUMO. Thus, the inductive effect of fluoride substituents predominate the resonance effect and thus, it could modulate the antioxidative activities of the Mn-salen complexes.

CONCLUSIONS

Regarding the increasing interest in Mn-salen complexes as potential drugs against oxidative stress related diseases, new salen derivatives with fluoride substituents were synthesized using *N, N′* bis (salicylaldehyd) ethylenediamine. All Mn-salen complexes described in this study, exhibited catalase, SOD and peroxidase activities using in vitro models. The fluoride substituents, based on our data, did not affect SOD activities. However, the CAT and peroxidase activities were positively modulated by fluoride substituents. These findings support the concept that the newly synthesized Mn-salen Complexes are SOD/CAT mimetics and thus, they might have potential biological applications in the antioxidant therapy of oxidative stress-related diseases.

Abbreviations

ABTS	2, 2'-Azino-bis, 3-ethylbenzthiazoline-6-sulfonic acid
CAT	Catalase
EUK-131	Manganese 5-fluoro *N,N′*-bis(salicylidene) ethylenediamine acetate
EUK-132	Manganese 5-fluoro *N,N′*-bis(salicylidene) ethylenediamine chloride
EUK-141	Manganese 3, 5-fluoro *N,N′*-bis(salicylidene) ethylenediamine acetate
EUK-142	Manganese 3, 5-fluoro *N,N′*-bis(salicylidene) ethylenediamine chloride
Equiv	Equivalent
GPX	Glutathione peroxidase
H_2O_2	Hydrogen peroxide
HOMO	High occupied molecular orbital
IR	Infrared
LUMO	Low occupied molecular orbital
Mn-salens	Mn-salen complexes
MS	Mass
NADH	Nicotinamide adenine dinucleotide
NBT	Nitro blue tetrazolium
NMR	Nuclear magnetic resonance
PD	Parkinson's disease
PMS	Phenazine methosulfate
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
Sec	Second
SOD	Superoxide dismutase

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REFERENCES

[1] Ames B.N., Shigenega M.K., Hagen T.M., Oxidants and the Degenerative Diseases of Ageing, *Proc Nati Acad Sci*, **90**, p. 7915 (1993).

[2] Mandal S., Yadav S., Yadav S., Nema R. K., Antioxidants, *Journal of Chemical and Pharmaceutical Research*, **1**, p. 102 (2009).

[3] Badarinath A.V., Rao K.M., Madhusudhana C.C., Ramkanth S., Rajan T.V.S, Gnanaprakash K., A Review on In-vitro Antioxidant Methods: Comparisons, Correlations and Considerations, *International Journal of PharmTech Research*, **2**, p. 1276 (2010).

[4] Ardestani A., Yazdanparast R., Antioxidant and Free Radical Scavenging Potential of Achillea Santolina Extract, *Food Chem.*, **104**, p. 21 (2007).

[5] Mammadov R., Makasçı - Afacan A., Uysal - Demir D.; Görk, Ç., Determination of Antioxidant Activities of Different Urginea Maritima (L.) Baker Plant Extracts, *Iran. J. Chem. Chem. Eng.,* **29**(3), p. 47 (2010).

[6] Riley D.P., Functional Mimics of Superoxide Dismutase Enzymes as Therapeutic Agents, *Chem. Rev.*, **99**, p. 2573 (1999).

[7] Autzen S., Korth H.G., Boese R., Groot H., Sustmann R., Studies of Pyridinyl-containing 14-membered Macrocyclic Copper (II) Complexes, *Eur. J. Inorg. Chem.*, **7**, p. 1401 (2003).

[8] Stanely M.P.P., Menon V.P., Antioxidant Activity of Tinospora Cordifolia Roots in Experimental Diabetes, *J. Ethnopharmacol.*, **65**, p. 277 (1999).

[9] Cheeseman K.H., Slater T.F., Free Radicals in Medicine, *British Med. Bull.*, **49**, p. 479 (1993).

[10] Blokhina O., Virolainen E., Fagerstedt K.V., Antioxidants, Oxidative Damage and Oxygen Deprivation Stress, *Annals of Botany*, **91**, p. 179, (2003).

[11] Bjelakovic G., Nikolova D., Gluud L.L., Simonetti RG, Gluud C. Antioxidant Supplements for Prevention of Mortality in Healthy Participants and Patients with Various Diseases. *Cochrane Database of Systematic Reviews*, CD 007176 (2008).

[12] Jung, C., Rong, Y., Doctrow, S., Baudry, M., Malfroy, B., Xu, Z., Synthetic Superoxide Dismutase/Catalase Mimetics Reduce Oxidative Stress and Prolong Survival in a Mouse Amyotrophic Lateral Sclerosis Model, *Neurosci. Lett.*, **304**, p. 157 (2001).

[13] Samai, M., Sharpe, M. A.. Gard, P. R., Chatterjee, P. K., Comparison of the Effects of the Superoxide Dismutase Mimetics EUK-134 and Tempol on Paraquat-induced Nephrotoxicity, *Free Radical Biol. Med.*, **43**, p. 528 (2007).

[14] Sharpe M.A., Ollosson R., Stewart V.C., Clark J.B., Oxidation of Nitric Oxide by Oxomanganese–Salen Complexes: A New Mechanism for Cellular Protection by Superoxide Dismutase/Catalase Mimetics, *Biochem. J.,* **366**, p. 97 (2002).

[15] Gutteridge J. M. C., Halliwell B., Free Radicals and Antioxidants in the Year 2000. A Historical Look to the Future, *Ann. N. Y. Acad. Sci.*, **899**, p. 136 (2000).

[16] Doctrow S.R., Huffman K., Marcus C.B., Tocco G., Malfroy E., Adinolfi C.A., Kruk H., Baker K., Lazarowych N., Mascarenhas J., Malfroy B., Salen-Manganese Complexes as Catalytic Scavengers of Hydrogen Peroxide and Cytoprotective Agents: Structure-Activity Relationship Studies, *J. Med. Chem.*, **45**, p. 4549 (2002).

[17] Lanza V., Vecchio G., New Conjugates of Superoxide Dismutase/Catalase Mimetics with Cyclodestrins, *Journal of Inorganic Biochemistry*, **103**, p. 381 (2009).

[18] Park W., Lim D., Effect of the oligo(ethylene glycol) Group on the Antioxidant Activity of Manganese Salen Complexes, *Bioorganic & Medicinal Chemistry Letters*, **19**, p. 614 (2009).

[19] Giblin G. M., Box P. C., Campbell I. B., Hancock A. P., Roomans S., Mills G. I., Molloy C., Tranter G. E., Walker A. L., Doctrow S. R., Huffman K., Malfroy B., 6, 6'-Bis(2-hydroxyphenyl)-2, 2'-bipyridine Manganese(III) Complexes: A Novel Series of Superoxide Dismutase and Catalase Mimetics, *Bioorganic & Medicinal Chemistry Letters*, **11**, p. 1367 (2001).

[20] Melov, S., Doctrow, S. R., Schneider, J. A., Haberson, J., Patel, M., Coskun, P. E., Huffman, K., Wallace, D. C. & Malfroy, B., Lifespan Extension and Rescue of Spongiform Encephalopathy in Superoxide Dismutase 2 Nullizygous Mice Treated with Superoxide Dismutase-Catalase Mimetics. *J. Neurosci.,* **21,** p. 8348 (2001).

[21] Rong, Y., Doctrow, S. R., Tocco, G. & Baudry, M. EUK-134, a Synthetic Superoxide Dismutase and Catalase Mimetic, Prevents Oxidative Stress and Attenuates Kainate-Induced Neuropathology, *Proc. Natl. Acad. Sci. USA*, p. 9897 (1999).

[22] Baudry M., Etienne S., Bruce A., Palucki M., Jacobsen E., Malfroy B., Salen-Manganese Complexes are Superoxide Dismutase-Mimics. *Biochem. Biophys. Res. Commun.*, **96**, p. 964 (1993).

[23] Venkataramanan N.S., Kuppuraj G., Rajagopal S. Metal–Salen Complexes as Efficient Catalysts for the Oxygenation of Heteroatom Containing Organic Compounds-Synthetic and Mechanistic Aspects., *Coord Chem Rev.*, **249**, p. 1249 (2005).

[24] Patel M., Day B. J., Metalloporphyrin Class of Therapeutic Catalytic Antioxidants,Trends *Pharmacol Sci.*, **20**, p. 359 (1999).

[25] Boucher, L. J. Manganese Schiff's base complexes II: Synthesis and Spectroscopy of Chloro- Complexes of Some Derivatives of (salicylaldehydeethylenediimato) Manganese (III). *J. Inorg. Nucl. Chem.*, ***36***, p. 531 (1974).

[26] Kakkar P., Das B., Viswanathan P.N., A Modified Spectrophotometric Assay of Superoxide Dismutase, *Ind. J. Biochem. Biophys*, **21**, p. 130 (1984).

[27] Aebi H., Catalase in Vitro, *Methods Enzymol*, **105**, p. 121 (1984).

[28] Childs R. E., Bardsley W. G., The Steady-State Kinetics of Peroxidase with 2, 2'-Azino-di- (3-ethylbenzthiazoline-6-sulphonic acid) as Chromogen, *Biochem. J.*, **145**, p. 93 (1975).

[29] Gonzalez P. K., Zhuang J., Doctrow S. R., Malfroy B., Benson P. F., Menconi M. J., Fink M. P., EUK-8, A Synthetic Superoxide Dismutase and Catalase Mimetic, Ameliorates Acute Lung Injury in Endotoxemic Swine. *J. Pharmacol. Exp. Ther.*, **275**, p. 798 (1995).

[30] Doctrow S. R., Huffman K., Marcus C. B., Musleh W., Bruce A., Baudry M., Malfroy B., Salen-Manganese Complexes: Combined Superoxide Dismutase/Catalase Mimics with Broad Pharmacological Efficacy, *Adv Pharmacol*., **38**, p. 247 (1997).

[31] Graham Solomons T. W., "Organic Chemistry", sixth edition, John Wiley and Sons, PP. 675-676; 691-693 (1996).