Efficient synthesis of DHA transition metal chelates as potent antioxidants, enzyme inhibitor and antimicrobial agents.

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**Abstract:** A large in vitro biological screening and an efficient with easy access to a family of transition metal complexes of dehydroacetic acid (DHA) are reported. The obtained complexes (1-4) with some transition metal of interest: Ni (II), Co (II), Zn (II), Mn (II) respectively, were fully characterized by MP, UV-Vis and FT-IR spectroscopy; several in vitro biological tests were performed on this series of compounds to explore its therapeutically potential in order to continue further investigations and exploring it as new target drugs. In this case, enzymatic activity: as urease inhibitors and antioxidant activities: ABTS scavenging activity, β-carotène linoleic acid bleaching activity, Ferrous ions binding effect, Copper (CCA) and ferrous chelating activity, gave good values of IC50 for all studied complexes 1-4 in range of 8,20 ±0,39-10,62 ±0,01 μg/mL for urease inhibiting test better than DHA and used standard Thiourea (IC50=11,57±0,68 μg/mL), interesting results are also obtained for compound 2 in ability of chelating ferrous ions with an IC50=14,53±0,92 μg/mL, comparing with tested standard EDTA (CI50=8,80±0,47 μg/mL), for all cited applications complex 4 is mostly a hit, while antimicrobial activity gave better results with free ligand DHA, discussion on molecular structure and predicted SAR will be given.

**Keywords:** DHA, Transition metal complexes, Biological activities.
DHA or 3-acetyl-4-hydroxy-6-methyl-2-pyrone

Identified structure in 1924 by Rassweiler et Adams.

Several active centers:

- Position C3.
- Positions C2, C4 et C6.
- Position C5.
Bloc «d».

| Metal      | Electrons de valence de la couche « d » |
|------------|----------------------------------------|
| Nickel     | 8 électrons                            |
| Cobalt     | 7 électrons                            |
| Zinc       | 10 électrons                           |
| Manganèse  | 5 électrons                            |
Biological activities of DHA and derivatives

M = Ni, Co, Mn, Zn

Maiti, (1998)
Ullah et al., (2012)
Malik, M-A et al., (2018)

DHA Amines
Synthesis of DHA chelates

M = Ni, Zn, Co, Mn

\[ \text{MC} \text{Cl}_2 \times \text{Aceton} + \text{CH}_3\text{COONa} \rightarrow \text{EtOH} \]

| Chelates          | Color | Yield% |
|-------------------|-------|--------|
| Co(DHA)\text{2H}_2\text{O} | Rose  | 81     |
| Ni (DHA)\text{2H}_2\text{O} | Vert  | 72     |
| Zn(DHA)\text{2H}_2\text{O}  | Blanc | 91     |
| Mn(DHA)\text{2H}_2\text{O}  | Jaune | 43     |
Sythesis pathways
Spectroscopic identification
### Melting Point

| Chelates               | MP    |
|------------------------|-------|
| Co(DHA)$_2$.2H$_2$O    | 260   |
| Ni (DHA)$_2$.2H$_2$O   | 243   |
| Zn(DHA)$_2$.2H$_2$O    | 168   |
| Mn(DHA)$_2$.2H$_2$O    | > 260 |
IR spectroscopy

DHA-Zn

DHA-Ni

DHA-Mn

DHA-Co
Commun band in the range of 251-267 nm

Bathochrom effect

In the range of 285-313 nm

$\lambda = 480$ nm

Transition d-d

Mn (DHA)
In vitro Biological screening
Antioxidant activity

- Scavenging test of ABTS radical
- Fe chelating capacity (activité de chélation des ions ferreux)
- Copper chelating ability
- Fe²⁺ chelating ability by UV-VIS
- β-carotène blanching activity
- Antioxydante capacity by copper reducing
- Scavenging test of hydroxyle radical
- Scavenging test of l’hydrogène peroxyde

Enzymatic activity

- Scavenging test of superoxyde (pyrogallol) radical
- Metal chelating activity (phénontroline)
- Superoxyde DMSO alcalin test
- Scavenging test of DPPH radical
- Scavenging test of galvinoxyle radical
- Antioxydante capacity by fe reducing

- Uréase
- Acetylcholine estérase
- Alpha amylase
- Butyrylcholine estérase
Scavenging test of ABTS radical

Antioxidant agent

K\textsubscript{2}C\textsubscript{2}O\textsubscript{8}

Back to non radical form

\[ \lambda = 734 \text{ nm} \]
- BHT
- BHA
- DHA
- DHA-Co
- DHA-Zn
- DHA-Mn

**Inhibition (%)**

![Graph showing inhibition percentage vs concentration](image)

**Concentration µg/mL**

- 12.5
- 25
- 50
- 100
- 200
- 400
- 800

**Activity starts at 50 µg**

**Best reactivity**

- DHA-Mn

**CI50**

- 258.36 µg/mL

**IC50 en µg/mL**

- 1.29
- 1.81
- 258.36

**DHA-Mn**
β-carotène blunching test

Oxydation

Dans ce test, la capacité antioxydante est déterminée en présence d'un antioxydant. 

λ = 470 nm
**β-carotène blunching test**

**Chelates + DHA cinetic**
- Slow

**DHA-Mn chelate**
- Better activity

**CI50**
- > BHT and BHA

**IC50 en µg/ml**
- BHT: 0.91
- BHA: 1.05
- DHA: 77.07
- DHA-Ni: 220.87
- DHA-Zn: 185.72
- DHA-Mn: 22.69

**Concentrations en µg/mL**
- BHT
- BHA
- DHA
- DHA-Ni
- DHA-Zn
- DHA-Mn
Metal chelating activity

Fe\(^{2+}\) (Cu\(^{2+}\)) + H\(_2\)O\(_2\) → Fe\(^{3+}\) (Cu\(^{3+}\)) + OH• + OH

Fe chelating ability by UV-Vis

Chelates binding to Fe

Abs ± intense and > to free chelates

Co-Fe\(^{2+}\)

Weakest absorbance

DHA and chelates have a potent Fe chelating ability

Absorbance at 420 nm

DHA

DHA-Ni

DHA-Co

DHA-Zn

DHA-Mn

DHA-R

DHA-Ni-R

DHA-Co-R

DHA-Zn-R

DHA-Mn-R

Fe\(^{2+}\)
**Fe chelating test**

Fe\(^{2+}\) (Cu\(^{2+}\)) + H\(_2\)O\(_2\) $\rightarrow$ Fe\(^{3+}\) (Cu\(^{3+}\)) + OH• + OH

**Metal chelating activity**

Ferrozine-Fe\(^{2+}\)

Chelating agent

Cloudy chelates
Fe chelating activity

**Concentration μg/mL**

| Concentration (μg/mL) | EDTA | DHA-Ni |
|-----------------------|------|--------|
| 0                     | 0    | 0      |
| 25                    |      |        |
| 50                    |      |        |
| 100                   |      |        |
| 200                   |      |        |
| 400                   |      |        |
| 800                   |      |        |

**Inhibition (%)**

- **Cl50**
  - EDTA: 12.5 µg/mL
  - DHA-Ni: 14.53 µg/mL

**IC50 en μg/mL**

- EDTA: 8.8 µg/mL
- DHA-Ni: 14.53 µg/mL

**Best activity**

DHA-Ni
Copper chelating activity

\[
\text{Cu}^{2+} + \text{OH}_2 \rightarrow \text{Cu}^{2+}-\text{PV}
\]

Fe\(^{2+}\) (Cu\(^{2+}\)) + H\(_2\)O\(_2\) \rightarrow Fe\(^{3+}\) (Cu\(^{3+}\)) + OH\(^-\) + OH

Chelating agent

λ = 632 nm

Cloudy chelate
Metal chelating activity

DHA and chelates → Copper chelating agents

DHA-Mn → Best activity

CI50 → 106.36 μg/mL
Uréase inhibiting activity

**Principe**

- DHA-Ni, DHA-Co, DHA-Zn et DHA-Mn

**Activity**

- 6,25 µg
- 3,125 µg

Free DHA

**Activity < Chelates and thioura**

CI50 > 200 µg/mL

**Graph**

- Inhibition (%)
- Thiourée, DHA, DHA-Ni, DHA-Co, DHA-Zn, DHA-Mn

**Bar Chart**

- Thiourea, DHA-Ni, DHA-Co, DHA-Zn, DHA-Mn
- CI50 in µg/mL:
  - Thiourea: 11.57
  - DHA-Ni: 10.38
  - DHA-Co: 10.62
  - DHA-Zn: 9.42
  - DHA-Mn: 8.2
Uréase inhibiting activity

Amtul. Z et al, (2002)

Zerner and al model

Uréase synthetic inhibitors

Ni atoms inactivation

Enzyme activity inhibition
Conclusion

Dehydroacetic acid and its transition metal complexes (1-4) were efficiently synthesized, characterized and fully screened for over than 20 *in vitro* biological activities, which exhibit a high urease inhibiting capacity for all chelates, Mn chelate as a hit for antioxidant activity and DHA free ligand as better antimicrobial agent. Discussion on molecular structures and comparison with observed effect helped to explain the structure activity relationship that may or not improve observed therapeutically effect of Dehydroacetic acid by chelating in comparison with DHA free ligand, and suppose that tested compounds adopt different mechanism of action depending on biological application. In regards of these promising results, kinetic studies, pharmacomodulation of tested oraganometallic complexes to increase medicinal effect and *in vivo* preclinical tests, are recommended as future investigations.
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