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Deccan J. Medicinal Chem and Analysis 6(1): Jan-feb 2015

DECCAN PHARMA JOURNAL SERIES

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(Research Article)

Received:Dec 2014 accepted: Feb 2015

SYNTHESIS AND ANALGESIC ACTIVITY OF DIFFERENT HALOGENATED DERIVATIVES OF 1,4- NAPHTHOQUINONES & THEIR METAL CHELATES

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Keywords:
analgesic activity, tail flick, Eddy's hot plate.

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ABSTRACT

Various halogenated derivatives of 1,4-Naphthoquinones and their Zinc chelates were synthesized. All the synthesized compounds have been screened for their analgesic activity by tail flick and Eddy's hot plate method. Among the compounds tested 2-Ethylcarboxamido-3-chloro-1,4-naphthoquinone and it's Zinc chelate shows better analgesic activity as compared with standard Diclofenac sodium.

INTRODUCTION

Nowadays life of human being is in danger because of he is suffering from many upsetting diseases like, tuberculosis, arthritis, cancer, AIDS, and the diseases caused by several bacterial infections.In market some medicines are available to overcome these diseases, but there is need to synthesize a new chemical entity[NCE] to overcome the adverse effect and resistance of the current drug.

Literature survey reveals that, Lapachol, a yellow coloring matter obtained from *Tabebuia avellanedae* have the structure of an amylene hydroxynaphthoquinone used as an analgesic, anti-inflammatory, antineoplastic and diuretic[1] by the local people in Brazil.

Metal chelates of Lawsone oxime found to posses considerable anti-microbial activity[2].One of the hydroxyl derivative of 1,4-Naphthoquinone, i.e. Lawsone (2-Hydoxy-l,4-naphthoquinone, III) is a monohydroxy naphthoquinone pigment of henna**3** plant possessing various biological properties, including antitumor ativity.It is also an effective chelator of divalent and trivalant metal ions due to its juxtaposed phenolic hydroxyl and keto groups. Some hydroxynaphthoquinone metal complexes shows good analgesic and antitumour activity.

The above detailed literature survey, encourage us to synthesize Zn, & Cd metal chelates of 2-Hydroxy-3-bromo-1,4-naphthoquinone, 2-Methoxy-3-bromo-1,4-Naphthoquinone, 2-Hydroxy-3-chloro-1,4-naphthoquinone and 2-Ethylcarboxamido-3-chloro1,4-Naphthoquinone to evaluate their analgesic activities by Tail flick & Eddy's Hot Plate method on albino mice. The experimental results shows a good analgesic activity as compared to standard Diclofenac sodium.

MATERIALS AND METHODS

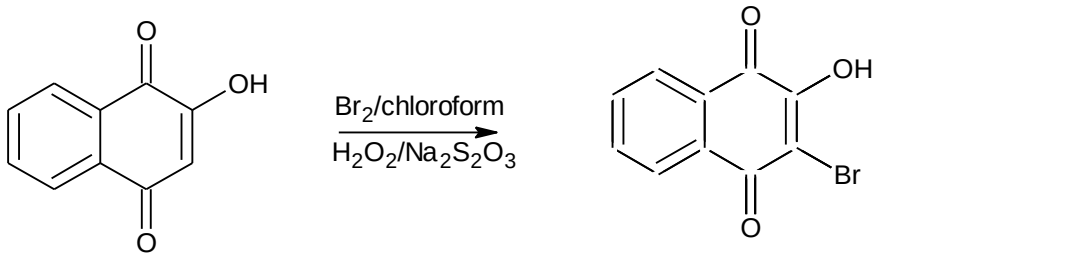
Experimental:

Scheme of Synthesis:-

Synthesis of Halogenated derivatives of 1.4-Naphthoquinone derivative.

1.Synthesis of 2-hydroxy-3-bromo-1,4-naphthoquinone from lawsone:

Synthesis of 2-Hydroxy- 3-bromo-1, 4-Naphthoquinone

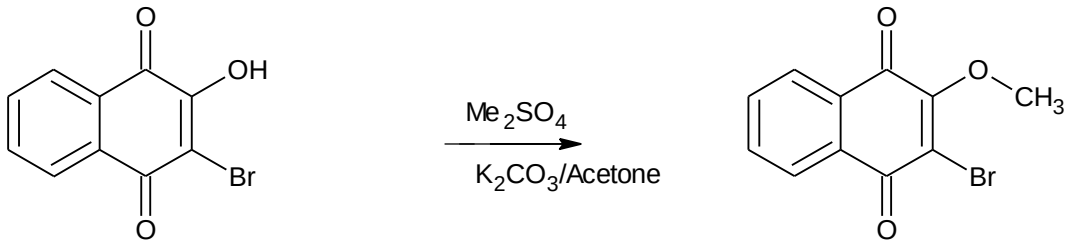


Lawsone

2-Hydroxy- 3-bromo-1, 4-Naphthoquinone

2.Synthesis of 2-methoxy-3-bromo-1,4-naphthoquinone:

Synthesis of 2-Methoxy- 3-bromo-1, 4-Naphthoquinone

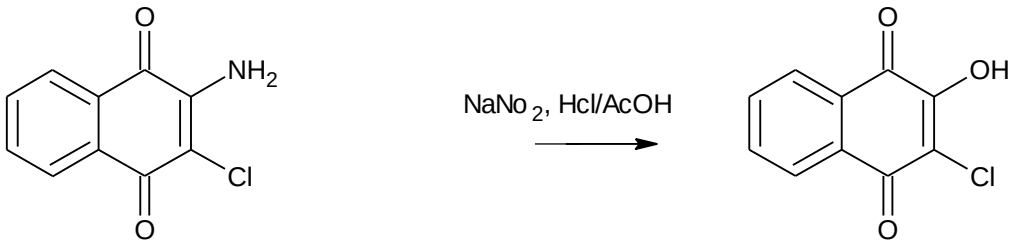


2-Hydroxy- 3-bromo-1, 4-Naphthoquinone

2-Methoxy- 3-bromo-1, 4-Naphthoquinone

3.Synthesis of 2-hydroxy-3-chloro-1,4-naphthoquinone:

Synthesis of 2-Hydroxy - 3-chloro-1, 4-Naphthoquinone

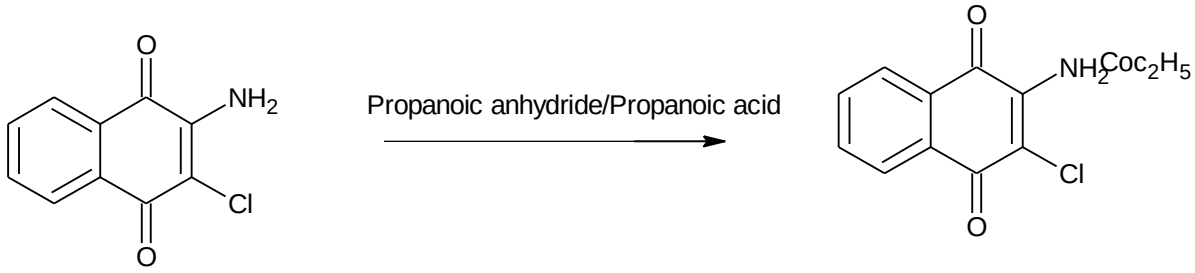


2-Amino- 3-chloro-1, 4-Naphthoquinone

2-Hydroxy - 3-chloro-1, 4-Naphthoquinone

***4.*Synthesis of 2-ethylcarboxamido-3-chloro-1,4-naphthoquinone:**

Synthesis of 2-Ethylcarboxamide- 3-chloro-1, 4-Naphthoquinone



Experimental Procedure:

1. ***Synthesis of 2-hydroxy-3-bromo-1,4-naphthoquinone from lawsone:***

A stirred solution of lawsone in chloroform was cooled to 20^0Cand then 2N H_2SO_4 was added. The mixture was then slowly added bromineand 0.7ml of solution H_2O_2 30%. The reaction was monitored by TLC until complete consumption of lawsone. The reaction was allowed to warm to room temperature and then a solution of $Na_2S_2O_3$ 10% was added to eliminate any bromine remaining. The reaction mixture was extracted with chloroform. The combined organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure.

2.Synthesis of 2-methoxy-3-bromo-1,4-naphthoquinone:

To a solution of 2-hydroxy-3-bromo-1,4-naphthoquinone in acetone was slowly added solid K_2CO_3 and dimethylsulfate. The mixture was refluxed in 6 hours and then was filtered to eliminate the solid K_2CO_3. The solution was then concentrated under reduced pressure.

3.Synthesis of 2-hydroxy-3-chloro-1,4-naphthoquinone:

Dissolve 2-amino-3-chloro-1,4-napthoquinone in 20 ml mixture of glacial acetic

acid – water – HCl (7:2:1). To above solution cooling at 10 - 15oC, a solution of $NaNO_2$

1% was slowly added. The solution color turned form red to orange yellow. The mixture was warmed to 50 oC in 30 minutes to hydrolyze the diazonium salt and then extracted with dichloromethane (3 x 20mL). The combined organic phase was dried over

anhydrous Na2SO4 and then concentrated under reduced pressure to obtain an orange solid product.

4. ***Synthesis of 2-Ethylcarboxamido-3-chloro-1,4-naphthoquinone:***

To a suspension of 2-amino-3-chloro-1,4-naphthoquinone in propionic acid (10mL), 6.7g of anhydride propionic was added. Then one drop of concentrated sulfuric acid was added. The stirred mixture was refluxed at 70^0C in 6 hours.The reaction was cooled to 0^0C in 2 hours to form a pale yellow precipitate. The solid material was collected by vacuum filtration.

Preparation of Metal Chelates of Halogenated derivatives of 1.4-Naphthoquinone derivative.

Preparation of Ligand Solutions:-

The ligands were weighed appropriately to obtain their accurate $2x10^{-3}$ M solutions. The weighed ligands were dissolved in sufficient amount of methanol to dissolve the ligand completely. In case of each experiment, clear solution of the ligand was obtained.

Preparation of the Metal salt Solution:

Aquous solution of the metal ions like Zn[II] &Cd[II] corresponding to $1x10^{-3}$M were prepared by dissolving the appropriate amounts of metal acetates in distilled water. Aquous Ammonia solution [10%] was prepared in distilled water and was used to adjust the pH of reaction mixture.

Preparation of the Metal Chelates:

All the chelates were prepared by following a general procedure as described below:

The ligand solution $2x10^{-3}$ M was taken in a 3 neck-flask. The metal ion solution $1x10^{-3}$M was added drop by drop from a dropping funnel into the ligand solution, with constant stirring. After the addition of the metal solution aq. Ammonia solution[10%] was added dropwise to adjust pH 5-6. The flask containing the reaction mixture was then kept overnight in refrigerator, after stirring the reaction mixture for about one hour. The products were then filtered under vacuum at room temperature, thoroughly washed with distilled water and then with little methanol. Finally they were dried in vacuum desicator.

PHARMACOLOGICAL STUDIES.:-

Material and methods:

Materials:-

Different Synthesized compounds, Diclofenac Sodium.& carboxy methyl cellulose were used as received.

Acute Toxicity Studies:

The acute toxicity study, before analgesic activity was carried out by Karber's method[4], and the LD_{50} value of synthesized compounds were found to by 200mg/kg body weight of albino mice. Hence the ED_{50} was selected as 20mg/kg body weight of albimino mice for the present study.

Study of Analgesic activity:-

1. **Tail Flicking Method/ Heat Conduction Method[5]:**

Swiss albino mice weighing 20-30gm of either sex were maintained under controlled condition of light (12 hr) and temperature 25 ± 1^0c. in the animal house. These animals were grouped into ten[10] groups of six animals each. Group-I received distilled water, which served as control group. Group-II received Sodium Diclofenac (10 mg/kg. i.p) and served as standard group. Group-III-X received oral 20mg/kg body weight of synthesized compounds A, A_1,B,B_1,C,C_1,D&D_1. respectively. Sixty minutes after oral administration of synthesized compounds and 30 minutes after i.p. injection of Sodium Diclofenac, the tail tip of individual animals was dipped up to 5cm into hot water (maintained at 58^0c) and the response/reaction time was noted as sudden withdrawl of the tail from the hot water. The cut off period of 12 seconds was maintained.

2. **Eddy's Hot Plate Method[6]:**

The animals were grouped into ten groups of six animals each. Group-I received distilled water, which served as control group. Group-II received Sodium Diclofenac (10 mg/kg. i.p) and served as standard group. Group-III-X received oral 20mg/kg body weight of synthesized compounds A, A_1,B,B_1,C,C_1,D&D_1 respectively. Sixty minutes after oral administration of synthesized compounds and 30 minutes after i.p. injection of Sodium Diclofenac, animals were individually placed on the Hot plate(maintained at 55^0C) and the responses such as paw licking or jump response, whichever appeared first were noted out. The cut off period of 15 sec. was maintained.

Statistical analysis: The data were statistically analyzed using one-way ANOVA followed by Dunnett's test for individual comparision of groups with control . "p" values below 0.001 were considered as singnificant. All values of statistical analysis are expressed as mean ± SEM.

RESULTS AND DISCUSSION

Table No.1 :

Physicochemical data for Halogenated derivatives of 1.4-Naphthoquinone derivative and their metal chelates.

Sr.No.	Compd. Name.	Mol.formula	% Yield	Melting Point(0^{o}c)
1	2-Hydroxy-3-bromo-1,4-Naphthoquinone[A]	$C_{10}H_5O_3Br$	92%	192-194^{0}C.
2	Zn chelate of 2-Hydroxy-3-bromo-1,4-Naphthoquinone [A_1]	$C_{10}H_5O_3Br.Zn$	85%	210-212^{0}C.
3	2-Methoxy-3-bromo-1,4-Naphthoquinone [B]	$C_{11}H_7O_3Br$	80%	186-188^{0}C.
4	Zn chelate of 2-Methoxy-3-bromo-1,4-Naphthoquinone [B_1]	$C_{11}H_7O_3Br.Zn$	75%	198-200^{0}C.
5	2-Hydroxy-3-Chloro-1,4-Naphthoquinone [C]	$C_{10}H_5O_3Cl$	62%	299-300^{0}C.
6	Zn chelate of 2-Hydroxy-3-chloro-1,4-Naphthoquinone [C_1]	$C_{10}H_5O_3Cl.Zn$	60%	310-312^{0}C.
7	2-Ethylcarboxamido-3-chloro-1,4-Naphthoquinone.[D]	$C_{13}H_{11}O_3Cl.N.$	45%	205-207^{0}C.
8	Zn chelate of 2-Ethylcarboxamido-3-chloro-1,4-Naphthoquinone [D_1]	$C_{13}H_{11}O_3Cl.N.Zn$	50%	216-218^{0}C.

*All melting points are uncorrected.

Table-2 :
Analgesic activity of Halogenated derivatives of 1.4-Naphthoquinone derivative and their metal chelates. By Tail Flick Method.

Sr. No.	Groups	Dose [mg/kg]	Reaction Time (seconds)
1	Group-I (Control)	Distilled water	2.6±0.254
2	Group-II(Standard)	10	11.2±0.123
3	Group-III (Compd.A)	20	6.2±0.243
4	Group-IV(Compd.A_1)	20	6.4±0.321
5	Group-V(Compd.B)	20	6.5±0.316
6	Group-VI(Compd.B_1)	20	7.2±0.421
7	Group-VII(Compd.C)	20	6.8±0.324
8	Group-VIII(Compd.C_1)	20	7.1±0.381
9	Group-IX(Compd.D)	20	8.1±0.354
10	Group-X (Compd. D_1)	20	8.5±0.215

Values are expressed as mean ± SEM; n=6; $p<0.001$

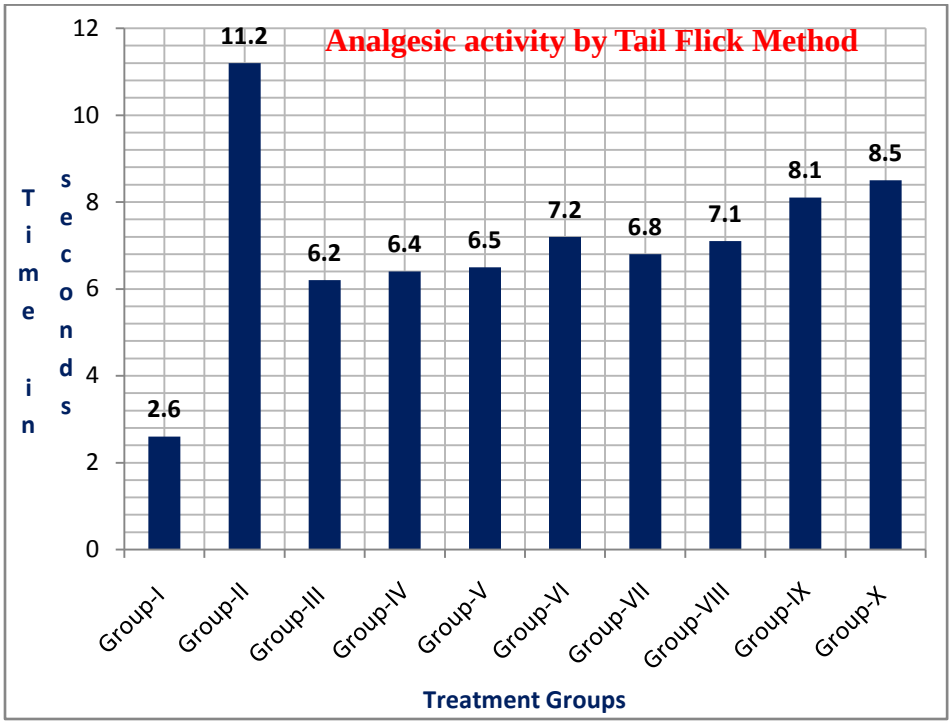
Table-3

Analgesic activity of Halogenated derivatives of 1.4-Naphthoquinone derivative and their metal chelates. By Eddy's Hot Plate Method.

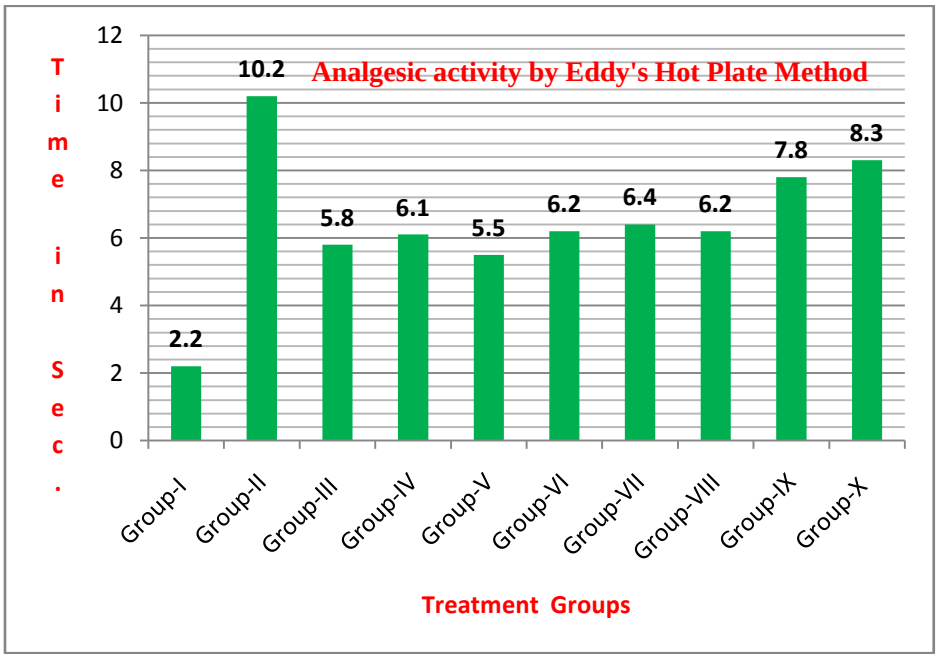
Sr. No.	Groups	Dose [mg/kg]	Reaction Time (seconds)
1	Group-I (Control)	Distilled water	2.2±0.142
2	Group-II(Na.Diclofenac)	10	10.2±0.223
3	Group-III (Compd.A)	20	5.8±0.143
4	Group-IV(Compd.A_1)	20	6.1±0.224
5	Group-V(Compd.B)	20	5.5±0.413
6	Group-VI(Compd.B_1)	20	6.2±0.323
7	Group-VII(Compd.C)	20	6.4±0.124
8	Group-VIII(Compd.C_1)	20	6.2±0.152
9	Group-IX(Compd.D)	20	7.8±0.254
10	Group-X (Compd. D_1)	20	8.3±0.114

Values are expressed as mean ± SEM; n=6; $p<0.001$

Histogram-1 Analgesic activity by Tail Flick Method.



Histogram-2 Analgesic activity by Eddy's Hot Plate Method.



Conclusion:-

Different halogenated derivatives of 1,4-Naphthoquinones and their Zinc and Cadmium metal chelates were synthesized. The synthesized compounds Percentage yield and Physical constants were obtained and shown in table 1. Further the synthesized compounds and ther metal chelates were screened for their Analgesic activities and shown in Table-3 and Table-4 and represented in Graph-I and Graph-II and found to possess moderately good Analgesic activity as compared to standard Sodium Diclofenac.

From this study it can be concluded that, the newly synthesized halogenated derivatives of 1,4-Naphthoquinone and their metal chelates are stable and can be subustituted for exsiting analgesic drugs to increase efficiency with less toxicity and side effects.

ACKNOWLEDGEMENTS:

The authors are thankful to Rajgad Dnyanpeeth's College of Pharmavy Bhor, for providing necessary facilities to carry out this research work.

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