Orignal Article

Clinical Interaction Effect of Azithromycin and Citalopram in New 2-[2- - (4,5-Di Methoxy Carboxy Phenyl) Azo]-Imidazole (DMCPAI) - Metal Complexes Against Nikethamide Induce Lethal Convulsion

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ABSTRACT

Objectives: The ligand of heterocyclic azo dye 2- [2-- (4,5- dimethoxy carboxy phenyl) azo]- Imidazole, (DMCPAI) was achieve by coupling between a diazonium chloride solution of 2- amino - 4,5- dimethoxy benzoic acid with imidazole in alkaline ethanol solution.

Setting: Two complexes with Co(III), Ni(II)and Cu(II) ions were achieved and qualified using existing techniques such as Mass spectral and 1H- NMR.

Methods: Thirty male mice were randomly divided into six groups for determination of analgesic activity of new compounds. Animal primary randomly divided into six groups for Interaction study between new Azo imidazole derivatives by using inducer drug for CYP450 as Citalopram and inhibitors drugs for enzyme as azithromycin for 7 days given orally and efficacy of interaction to protect nikethamide lethality. Experimental design of study listed as group one still control, group 2 given Nikethamide high dose 200 mg/kg.I.P alone for induced over stimulation and tonic clonic convulsion, animals in group 3,4,5,6 after given 7 day orally daily citalopram and azithromycin for inducer and inhibitor of CYP450,at eight day pretreatment ([Co(L)2].Cl) or [Cu(L)2] with dose 3mg/kg and post treatment with nikethamide group treated with [Cu(L)2].

The results showed protective effect against convulsion of nikethamide via delay onset of action nikethamide, minimize number of convulsion with simple partial type of seizures as compared with nikethamide reveal status epilepticus convulsion persistent, in **conclusion**, the present study concluded that azithromycin potent inducer for CYP450 and has the ability to increase the activity of Cu(L)2 compound to decrease toxicity of nikethamide.

Keywords: Interaction, Nikethamide, citalopram, azithromycin, cyp450.

Introduction

Azo imidazole compounds have been an interesting origin for researchers for more than a century. The imidazole ring is an ingredient of many important natural products, including purine, histamine, histidine, and nucleic acid.[1] Azo imidazole derivatives ligands have important roles in assortment chemistry because of the formation of stable complexes

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with most transition metals.[2,3] Azo imidazole derivatives are a very influential category of chemical agents receiving interest in scientific research.[4] Azo to include agents are used in dyeing processes cotton, cosmetics and biological activities including antibacterial, retardation the growth of microorganism.[5-7] Azo imidazole derivatives dyes are known as excellent analytical reagents in spectrophotometric determination of most transition metal ions.[8] Nikethamide (N, N-diethylnicotinamide) has been widely utilized in the clinical inspection as: first, applied stimulation of respiratory centrally, it can excite respiratory center selectively in patients suffering from a cardiopulmonary disease with respiratory failure and encephalopathy.[9] Nikethamide has actions likely to those of doxapram It was formerly used as a respiratory stimulant, antidote for a barbiturate, benzodiazepine overdose but has been largely eliminated because of risk of convulsions and toxicity.

Material and Methods

All chemicals and solvents were of the highest purity obtained from multiple companies such as Fluk, B.D.H, Merck, J.T.Baker, Sigma and Alderich. Analysis of elemental azo dye ligands and complexes of their metals were done by means of a micro analytical unit of EA 300 C.H.N element analyzer (Isfahan University, Iran).

A-Synthesis of Azo Dye Ligand (DMCPAI)

A diazonium solution was prepared by dissolving 2-amino - 4,5- dimethoxy benzoic acid 1.9 gm (0.01 mmol) in 30 ml distilled water containing 3ml concentrated hydrochloric acid. The filtered solution was cooled to 0°C. To this mixture, a solution of 0.75 gm (0.01 mol) of sodium nitrate in 25ml distilled water was added dropwise at (0-5) °C and stirred for 30 min. This diazonium chloride solution was added dropwise into a 500 ml beaker containing 0.68gm (0.01 mol) of imidazole was dissolved in 150 ml of ethanol and 20 ml of 7% sodium hydroxide with cooling and stirring continuously for one hour at (0-5) °C in ice- bath and allowed to stand overnight and acidified with dilute hydrochloric acid to PH= 6.0. The precipitate was filtered off and washed several times with cold distilled water and recrystallized twice from hot ethanol and then dried in the oven at 60 °C for several hours and stored in a desiccator over anhydrous calcium chloride. The yield was 83% of red crystals and the melting point found to be 152 °C. The purity was confirmed by the elemental analysis and TLC techniques. The structure of the ligand (DMCPAI) is shown below in (Scheme 1)::

B-Synthesis of Metal Complexes:

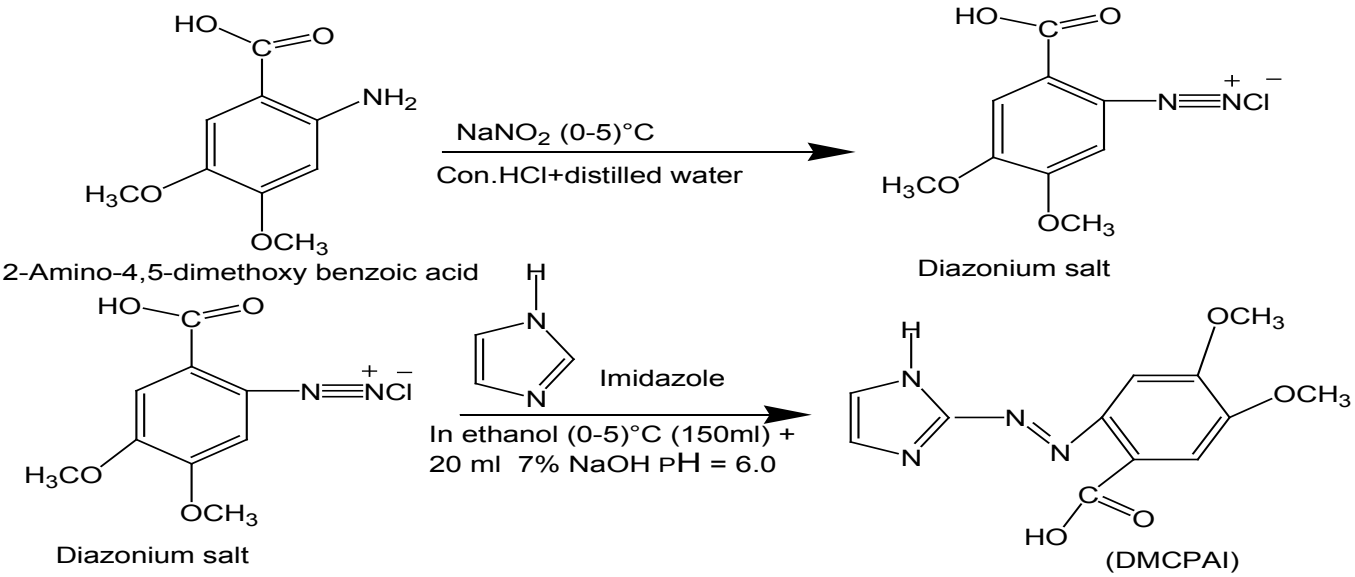
The metal complexes were prepared according to [10], and Analytical and physical data of ligand (DMCPAI) and its metal complexes.

C-Animals

Animals primary randomly divided into six groups for Interaction study between new Azo imidazole derivatives by using inducer drug for CYP450 as Citalopram and inhibitors drugs for enzyme as azithromycin for 7 days given orally and efficacy of interaction to protect nikethamide lethality.

D- Experimental design

Study listed as group one still controls, group 2 given nikethamide high dose 200mg/kg.bw. alone for induced over stimulation and tonic clonic convulsion, group 3 after given 7 day 0.2 mg/kg.bw orally daily and at eight day pretreatment ([Co(Ly)2].Cl) with dose 3mg/kg and post treatment .Group 4 after given 7 day 0.2 mg/kg.bw orally daily and at eight day pretreatment [Cu(L)2] at dose 3mg/kg and post treatment. Group 5 after given 7 day 5 mg/kg.bw orally Azithromycin and given at eight day pretreatment ([Co(L)2].Cl) 3mg/kg and



2-[2- - (4,5-Di methoxy carboxy phenyl) azo]-Imidazole, (DMCPAI)

Scheme 1: Synthesis of hetrocyclic azo dye ligand (DMCPAI)

post treatment .group 6 after given 7 day 5 mg/kg.bw orally Azithromycin daily for 7 day and at eight day pretreatment [Cu(L)2] 3mg/kg and post treatment .

E-Treatment protocol to evaluate adverse effect of nikethamide

Five groups of rats were applied in this research. The animals were subjected to pre-treatment (Co(L)2]. Cl or [Cu(L) 2] and pretreatment (nikethamide) according to [11]. Pretreatment was carried out 30 min prior to treatment. In pretreatment 0.2 mL of each new compound tested, then exposed to potent excitement nikethamide in a dose of 200 mg/kg body weight was given to all mice of all groups then mice were observed, and the time where rats died was recorded.[11]

Results

The data of referring onset action of nikethamide at point beginning was fast in the animal has taken nikethamide alone and that administrated NK-5 while NK-7- treated animal exhibit delay in appearance of exciting action, that due to mechanism of action on Benzodiazepines like activity bind to the γ-aminobutyric acid type alpha receptor (GABA- alpha) at the alpha-subunit and support GABA activity, thereby enhancement transmission of the chloride channel that tends the activity of cell become negative, so that encourages sedation, analgesic, anticonvulsant, activity. The result showed that control animal record high degree of analgesia compares with other treated groups highly excited by due to nikethamide activity, while NK-7-AZ reveal the degree of analgesia due to efficacy of compound and inhibitory effect of azithromycin to CYP 450. Nikethamide high dose 200 mg/kg.bw. alone for induced over stimulation, the Cu(L)2 new compound-related imidazole derivatives become clear high significant differences at $p< 0.05$ preferable to protect against the lethal effect of nikethamide to reach 57.6±5.02 min compared [Co(L)2]. Cl) 2 that still within 42.2±3.61.

Table 1: Analytical and physical data of ligand (DMCPAI) and its metal complexes

Compound	*Color*	*m.p °C*	*Yield %*	*Molecular Formula (Mol.wt)*	*Found (calc.)% C*	*H*	*N*	*M*
$[Co(L)_2].Cl$	Dark purple	185	61	$C_{24}H_{22}O_8N_8CoCl$	(44.92	(3.55	(17.48	(9.32
				(644.87)	(44.70	3.44)	17.38)	9.14)
$[Cu(L)_2]$	Purple	210	83	$C_{24}H_{22}O_8N_8Cu$	(47.18	(3.72	(18.38	(10.54
				(614.03)	(46.95	3.61)	18.25)	10.35)

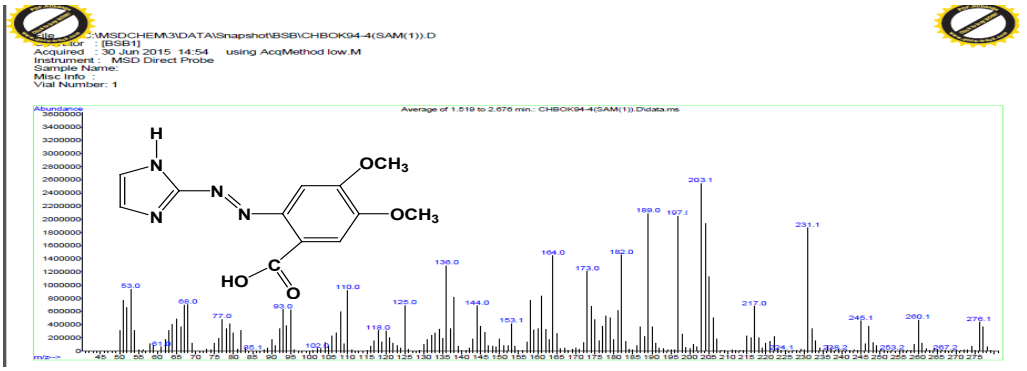


Figure (A): Mass spectrum of azo dye ligand (DMCPAI)

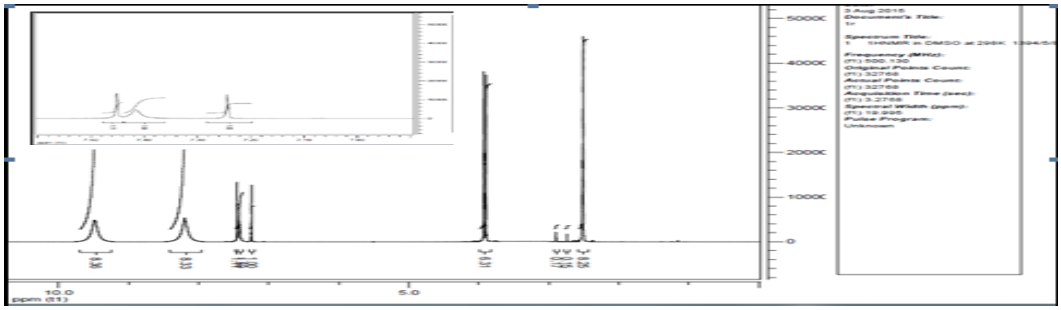


Figure (B): ^{1}H-NMR spectrum of azo dye ligand (DMCPAI)

Table 2: Onset of Action, Latency of Death in Minute, Analgesic Effect of New Imidazole Derivatives Drugs Against Nikethamide Induce Convulsion –Epileptics –Status at Min

Groups	*Onset of action*	*Latency of death*	*Analgesic effect*
C	0	0	28.8 ± 2.59^{a}
NK	9.6 ± 0.92^{a}	30 ± 3.53^{a}	10 ± 0.89^{b}
NK-7-Ci	17.6 ± 2.61^{b}	45.8 ± 4.75^{bd}	13.6 ± 0.92^{b}
NK-7-AZ	18 ± 2.54^{b}	57.6 ± 5.02^{d}	20.8 ± 2.74^{c}
NK-5-Ci	10.8 ± 2.08^{a}	39.4 ± 3.62^{ab}	10.4 ± 1.16^{b}
NK-5-AZ	14 ± 1.22^{ab}	42.2 ± 3.61^{ab}	11.2 ± 1.15^{b}
$LSD_{0.05}$	6.5	13.572	5.748

Similar letters : non-significant differences at $p<0.05$

Different letters : significant differences at $p< 0.05$.

C: control NK : nikethamide 7: number referCu(L)2 new compound related imidazole derivatives 5: number refer [Co(L)2].Cl) 2 new compound related imidazole derivatives Ci: abbreviation of citalopram, AZ: abbreviation of azithromycin.

Discussion

In our study, the compound 2-[2- - (4,5-Di methoxy carboxy phenyl) azo]-Imidazole, (DMCPAI) and its metal complexes (Co(L)2].Cl), ([Zn(L)2]) contain compound have activity similar to benzodiazepine activity due to possessing diazonium chloride in their chemical structure so that pharmacological activity was tested to evaluate to determine this activity to modulate nikethamide that considers a potent conversant agent. Clonazepam Undergo Metabolic Pathway Nitroreduction Acetylation via enzyme CYP3A4, CYP2C19, and NAT2 to metabolism into 7-amino-clonazepam, on the other hand, CYP2C19 and CYP3A4 are the main players in the metabolism of benzodiazepines that undergo phase I metabolism into Desmethyldiazepam, Temazepam, and Oxazepam [12]. Cytochrome P450 3A4 (CYP3A4) is the most common of all Cytochromes P450 (CYPs) enzymes. It is available in great extent quantities in the hepatic and in the GIT and involved in the metabolism of hundreds of therapeutic compounds including antidepressants, calcium channel blockers, steroids and opiate analgesics antipsychotics, mood stabilizers, hypnotics, and antianxiety drugs .[13]

Our result was concord with this research via that azithromycin-treated group for 7 days at 5 mg/kg.bw orally indicate a significant increase in data of analgesia, the latency of death in minutes and reduction in time and persistence of convulsion. In 2000 Westphal confirmed coadministration of clarithromycin, azithromycin, and dirithromycin with benzodiazepines should be avoided, or the dose of the benzodiazepine should be substantially reduced.[14] on other aspects data of this paper showed the mice treated for 7day with 0.2 mg/kg.bw orally daily to determine inducer activity of SSRI drugs representing by citalopram reveal result significantly less than azithromycin groups, although that result of [Cu(L)2] drug more preferable than ([Co(L)2]. Cl) related agent. The serum concentration of Ketazolam can be increased when it is combined with Azithromycin may be due to some interaction substrate for CYP450 2D6, CYP450 3A4, and YP450 2C9 and Inhibitor for CYP450 2C9, CYP450 2D6, CYP450 2C19, and CYP450 3A4 [15, 16]. The present study concluded that azithromycin is a potent inducer for CYP450 and has the ability to increase the activity of Cu(L)2 compounds to decrease the toxicity of nikethamide.

Conclusions

The present study concluded that azithromycin is a potent inducer for CYP450 and has the ability to increase the activity of Cu(L)2 compound to decrease the toxicity of nikethamide. The present aimed to evaluate the interaction between citalopram and azithromycin on nikethamide metabolism in mice.

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