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The anti-ulcer activity of the Schiff base: 1H-indole-3-ethylenesalicyldamine and its zinc (II), nickel (II) and copper (II) complexes

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ABSTRACT: The anti-ulcer effect of the Schiff base 1H-indole-3-ethylenesalicyldamine and its zinc (II), nickel (II) and copper (II) complexes have been reported. The compounds were tested against ethanol-induced gastric lesions in rats in high (60 mg/kg) and low (30 mg/kg) doses. They show high efficacy as anti-ulcer agents than Cimetidine control. Research finding laid suitable backgrounds for further studies and investigations for promising alternatives to traditional anti-ulcer drugs.

Keywords: Schiff bases; metal complexes; Indole; anti-ulcer; cimetidine.

INTRODUCTION: Peptic ulcer is still an annoying disease. Many people, in today's world, suffer from gastric ulcer specially those prone to alcoholic drinks, or use nonsteroidal anti-inflammatory drugs for pain-killing such as Aspirin[1].

No single cause has been found for ulcers. However, it is now clear that an ulcer is the end result of an imbalance between digestive fluids in the stomach and duodenum. Most ulcers are caused by an infection with a type of bacteria called Helicobacter pylori (H. pylori)[2 & 3].

Schiff bases and their metal complexes were reported to possess a broad spectrum of biological activities among them anti-ulcer activity[4-6]. Salicylaldehyde Schiff bases have been shown to have anti-inflammatory and anti ulcer activities[6 & 7]. The compound salicylidene anthranilic acid was found to possess antiflammatory and antiulcer activity, while its copper complexes showed an increased antiulcer activity. Similar study was carried out by K. D. Rainsford and co-workers on a number of Cu(II) complexes [7]. Results show that Cu(II) preparations which are highly irritating outside the stomach, caused no ulceration when applied intra gastric, but always elicited a copious mucus effusion into the stomach. This non ulcerant effect was confirmed by light microscopic observations of formaline-fixed tissues stained with periodic acid Schiff reagent, Alcian blue (pH 2.5) or the haematoxylin and eosin stains.

Zinc is an essential element that plays an important role in cell-mediated immune functions. Zinc is vital in the proper functioning of mucosal cells to suppress the advancement of gastrointestinal disease by free radical scavenging and interruption of the inflammatory process[8 & 9].

Of course there are several treatments that are in use today to cure or relief ulcer formation, but several disadvantages related to general toxicity, side effects, allergy and drug resistance phenomenon limit the use of many existing ones. This encourages us to search for new treatments that can be used as suitable replacements with minimal side effects.

Some information regarding the synthesis and characterization of the Schiff base: 1H-indole-3-ethylenesalicyldamine and its derivatives have been published[10]. Yet no data was recorded regarding the biological activity of zinc, nickel and copper complexes of this ligand. We aim to synthesize zinc, nickel and copper complexes of the ligand and to examine their anti-ulcerogenic activity in view to complete the picture for their biological applications.

MATERIAL AND METHODS:

Materials: Chemicals including tryptamine, salicylaldehyde, zinc (II) acetate dihydrate, copper (II) acetate monohydrate, nickel (II) acetate tetrahydrate, triethyl amine, and other organic solvents were purchased from Sigma-Aldrich and used without further purification. Ethanol was redistilled before use. Dry dimethylsulfoxide and dimethylformamide were utilized for recrystallization purposes.

Physical measurements: IR spectra were recorded with a Perkin-Elmer FT-IR spectrophotometer model Spectrum 2000 using KBr pellets as support in the range 4000-370 cm^{-1}. ^1H NMR spectra were recorded at room temperature on a JEOL ECA-400 spectrometer, operating with a frequency of 400 MHz, using DMSO-d^6 as solvent. Electronic spectra, in DMSO solution, were obtained using a Varian 50 Conc UV-

visible spectrophotometer over the wavelength range 200-800 nm.

Preparation of the ligand 1H-Indole-3-ethylene salicylaldimine (TS): The ligand 1H-Indole-3-ethylenesalicylaldimine (TS) is prepared according to the literature method[10].

Preparation of Complexes: Cu (II) and Ni (II) complexes were also prepared according to the literature method[10]. Zn (II) complex was prepared as follows: To a solution of zinc acetate dihydrate in absolute ethanol was added a solution of tryptamine in the same solvent in a molar ration of 1:2 (Metal: ligand) followed by a few drops of triethylamine. The mixture was heated under reflux for 5 hours. The product that had formed was filtered off, washed with absolute ethanol and dried over anhydrous silica gel, and finally recrystallized from DMF. Percentage yield was 82%. m.p 275 °C, Anal. Calc. for [$C_{34}H_{30}N_4O_2Zn$; FW: 592.02]: C, 68.89; H, 5.06; N, 9.45%. Found: C, 68.97; H, 4.96; N, 9.62%. Selected FTIR data (KBr, cm^{-1}): 3411 (m, N-H Indolic), 3170 (m, C-H Aliphatic), 1623 (s, C=N), 1302 (s, C-O), 596 (w, M-O), 489 (w, M-N).

Anti-ulcer experiment: Adult male Sprague-Dawley rats weighted between 200 - 220g were obtained from the animal house, Faculty of Medicine, University Malaya and treated according to the criteria outlined in the "Guide for the Care and Use of laboratory Animals" prepared by the National Academy of Sciences and published by the U.S. National Institutes of Health. The rats were fastened for 24h before experiment but allowed to access water. They are distributed into 4 groups, each group contains 6 rats.

Gastric ulcer was induced according to a modified method reported by Robert et al[11] for all rats.

Group 1 rats were negative controls that received 5 mls of 10% Tween-20 orally by orogastric intubations; whereas Group 2 rats received oral doses of 50 mg/kg (rat body weight) Cimetidine (in 5 ml volume) as positive controls. Group 3 and 4 rats received oral high dose (60 mg/kg) and low dose (30 mg/kg) of TS and its zinc, copper and nickel complexes respectively. The rats were euthanized 60 minutes later[12] by overdoses of diethyl ether and their stomachs were immediately excised. Each stomach was opened along the greater curvature; gastric juice was collected from each stomach, and then stomachs were washed with distilled water and fixed in 10% buffered formalin for 15 minutes. Surface areas (mm^2) were measured individually for each stomach and the sum of the lesion parts was estimated by the aid of microscope. Percentage surface area affected was obtained according to the formula:

$$\text{US } (mm^2) = \frac{\text{Total area covered by ulcers}}{\text{Total corpus mucosal surface}} \times 100\%$$

and the percentage inhibition (I %) was determined as [(UI in control – UI in test group) ÷ UI in control group] ×100[13].

The mucus content was weighed and expressed in terms of grams[14] and the pH of the stomach juice was also recorded.

RESULTS AND DISCUSSION:

The physical properties of the ligands and their complexes were listed in Table 1.

Table 1: Physical properties of the ligand and its complexes.

S. No.	Name of the compound	Molecular formula	Molecular weight	Color	Melting point (ºC)
1.	TS	$C_{17}H_{16}N_2O$	264.32	Yellow	100
2.	$(TS)_2Zn$	$C_{34}H_{30}N_4O_2Zn$	592.02	White	275 (decomposition)
3.	$(TS)_2Ni$	$C_{34}H_{30}N_4O_2Ni$	585.33	Green	222 (decomposition)
4.	$(TS)_2Cu$	$C_{34}H_{30}N_4O_2Cu$	590.18	Brown	214 (decomposition)

Elemental analyses for the complexes confirmed 1:2 metal to ligand stoichiometry. The compounds were very stable at room temperature in the solid state. The ligand is soluble in ethanol, methanol, acetone, and high boiling point solvents, whereas the complexes dissolved only in DMSO and DMF and not soluble in either ethanol or methanol.

Infra red spectra: The IR spectra of the newly prepared zinc complex are closely related to their reported copper (II) and nickel (II) congeners[10]. It is evident that for this complex the hydroxyl band at 3049 cm^{-1} of the ligand was disappeared indicating deprotonation of the ligand, and confirming coordination of the phenolic oxygen atom to the metal[15]. This fact can also be supported further by the shift of the C-O stretching band with respect to the same band in the free ligand. The azomethine stretching band in the free ligand shifts from 1630 cm^{-1} to 1623 cm^{-1} in the complex. This supports the participation of the azomethine nitrogen atom in coordinate bond to the metal center.

New bands at 596 cm^{-1} and 489 cm^{-1} could be assigned to vibrations associated with Zn-O, and Zn-N bonds respectively[16]. All IR data were recorded in Table 2.

Proton NMR spectra: The ^1H NMR spectra of the ligand TS was recorded in d^6-DMSO with chemical shifts expressed in ppm using tetramethylsilane (TMS) as internal standard was given in Table 3.

Table 2: IR frequencies of the ligand and complexes.

Compound	N-H	O-H	C-H aliphatic	C=N	C-O	C-H Out of plane Ar	M-O	M-N
TS	3420	3049-2857 broad	2936	1630	1281	746	-	-
$(TS)_2Zn$	3411	-	3170-2924	1623	1302	738	596	489
$(TS)_2Ni$	3447	-	2926	1612	1332	740	597	421
$(TS)_2Cu$	3424	-	2901	1623	1326	743	579	427

Table 3: 1H-NMR spectra of the ligand and complexes.

Compound	H_a	H_b	H_c	H_d	H_e	$H_{aromatic}$
TS	10.79(s)	3.03(t)	3.85(t)	8.43(s)	-	6.81-7.32(m)
$(TS)_2Zn$	10.76(s)	2.88(t)	3.71(t)	8.39(s)	-	6.56-7.30(m)
$(TS)_2Ni$	No clear spectra					
$(TS)_2Cu$	No clear spectra					

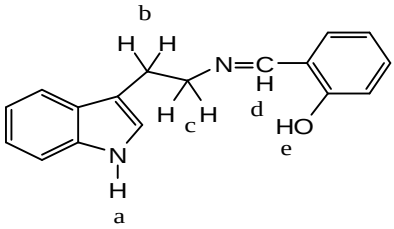

Figure 1: ligand TS.

The spectrum of zinc complex is somewhat similar to the ligand indicating a symmetrical arrangement of the ligand around the metal ion. The O-H proton signal was disappeared as a result of proton substitution by a cation. H_b protons go to slightly lower chemical shift value 2.88 ppm and so H_c protons to 3.71 ppm. The aldehydic proton appears at 8.39 ppm as a singlet and the aromatic protons in the usual range 6.56 – 7.30 ppm as a multiplet.

No ^1H NMR spectra was detected for Ni and Cu complexes of the ligand because they are paramagnetic in solution.

UV-Vis spectra: The electronic spectra of the ligand TS and its zinc with their tentative assignments is given in Table 4. $(TS)_2Zn$ complex exhibits two absorption maxima. The band at 364 nm is due to charge transfer probably from metal to ligand. The intensity and position of this band seems to indicate the presence of a tetrahedral environment around the zinc ion[17]. The bands at 275 nm and 244 nm could be attributed to the ligand.

Anti-ulcer activity: The anti-ulcer results of TS and its Zn(II), Ni(II) and Cu(II) complexes were shown in table 5, and presented diagrammatically in Figures 2 and 3.

Figure 2 shows the ulcer area (mm^2) in rat stomachs for the ligand and its complexes together with the positive and negative controls. It is clearly evident that TS and its complexes significantly reduced the ulcer area compared to Cimetidine when applied as high or low dose. Complexation of zinc metal to the ligand doesn't manifest any difference in lesions inhibition compared to the uncomplexed ligand, but still can be considered as potent anti-ulcer drug. Reduction of ulcer areas in the case of complexes probably have been achieved by stimulating the production of more mucus as it was shown in Table 1 (Mucus weight column).

It could be also concluded from Figures 1 and 2 that, among the series, TS and its zinc complex reveal the greatest inhibition percentage and the oral administration of the ligand or its zinc complex before ethanol administration significantly prevent ulceration up to 100%. Copper complex, when applied as a high dose, appears to have stronger effect in reducing the ulcer area than the nickel complex. Since copper complexes seem to have superoxide dismutating activity, it is reasonable to suggest that the inactivation of superoxide anions protects the stomach wall. The radical scavenging property of the copper complex together with an increase of prostaglandins may help to explain the gastroprotective effect of the compound[18].

Table 4: UV-Vis spectra of ligand and complexes.

Compound	Conc (mol/L)	ε ($mol^{-1}Lcm^{-1}$)	λ max (nm)	Assignment
TS	2.84×10^{-4}	5.32×10^{3}	314	n - π^*
		8.75×10^{3}	291	$\pi - \pi^*$ chelate ring
		9.13×10^{3}	283	$\pi - \pi^*$ chelate ring
		9.41×10^{3}	267	$\pi - \pi^*$ indole group
$(TS)_2Zn$	1.26×10^{-4}	8.67×10^{3}	364.5	CT
		1.77×10^{4}	275	$\pi - \pi^*$ ligand
		2.50×10^{3}	244	$\pi - \pi^*$ ligand

Table 5: Anti-ulcer data for ligand and complexes in comparison to negative control Tween-20 and positive control Cimetidine.

Compound	Total ulcer area(mm^2) (Mean ± S.E.M)	Mucus weight (g)	pH	% inhibition
Tween-20 (-ve control)	1438 ± 58^a	0.58	7.00	-
Cimetidine (+ve control)	168 ± 5^b	0.61	7.00	88
TS (HD)	Zero^c	0.88	3.61	100
TS (LD)	Zero^c	0.71	5.28	100
$(TS)_2Zn$ (HD)	Zero^c	1.31	5.00	100
$(TS)_2Zn$ (LD)	Zero^c	0.58	3.44	100
$(TS)_2Ni$ (HD)	9.6 ± 0.2^c	1.50	6.25	99
$(TS)_2Ni$ (LD)	20.4 ± 0.2^c	0.86	4.77	98
$(TS)_2Cu$ (HD)	2.4 ± 0.1^c	1.43	3.54	100
$(TS)_2Cu$ (LD)	52.8 ± 0.2^c	1.33	4.54	96

All values are expressed as mean ± standard error mean. Means with different superscripts are significantly different. The mean difference is significant at the p<0.05 level. HD (High dose: 60 mg/kg), LD (Low dose: 30 mg/kg)

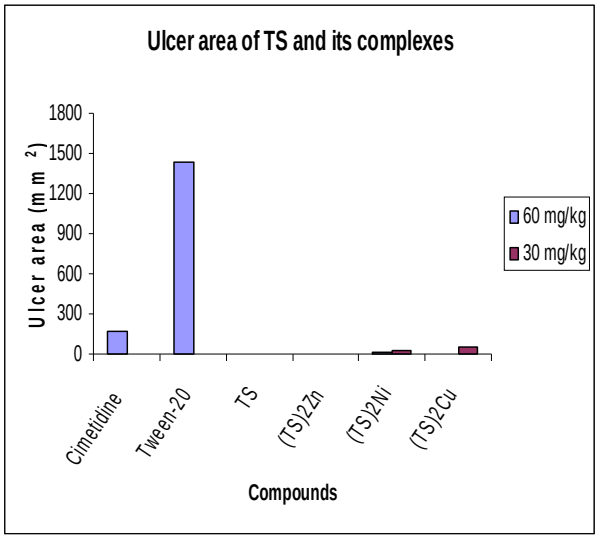

Figure 2: Ulcer areas encountered in rat stomachs after administration of TS and its complexes in comparison to positive and negative controls.

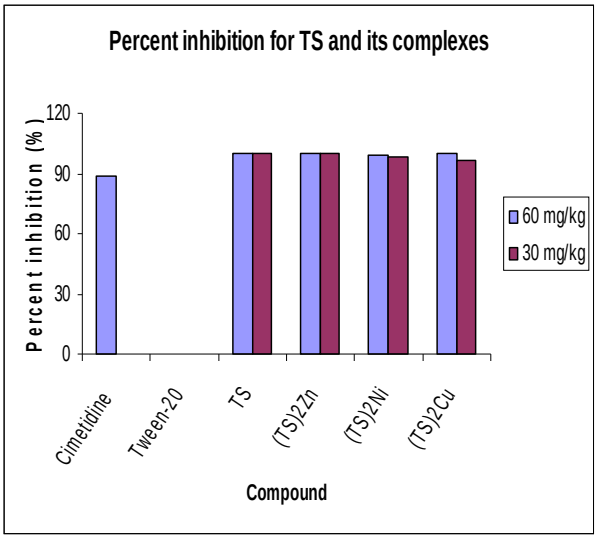

Figure 3: Percent inhibition for TS and its Zn(II), Ni(II) and Cu(II) complexes.

CONCLUSION:

The anti-ulcer effect of the Schiff base: 1H-indole-3-ethylenesalicyldamine and its zinc (II), copper (II) and nickel (II) was studied. The Schiff base ant its complexes exhibit potent anti-ulcer activity when applied as high (60 mg/kg) or low (30 mg/kg) doses compared to Cimetidine drug.

More efforts are recommended to expand the study to discover the other biological factors that may seem important for these promising compounds.

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REFERENCES:

1. F. Mary, C. Brucker, M. Ann (1997) Pharmacologic management of common gastrointestinal health problems in women, *J. Nurse-Midwifery,* 42, 145–162.

2. E. S. de Souza Almeida, V. C. Filho, R. Niero, B. K. Clasen, S. O. Balogun, D. T. O. Martins (2011) Pharmacological mechanisms underlying the anti-ulcer activity of methanol extract and canthin-6-one of Simabaferruginea A. St-Hil in animal models, *J. Ethnopharmacol*., 134, 630–636.

3. M. Saleh Salga, H. Mohd Ali, M. Ameen Abdulla, S. Ibrahim Abdelwahab (2012) Gastroprotective activity and mechanism of novel dichloridozinc(II)-4-(2-(5-methoxybenzylideneamino)ethyl) piperazin-1-iumphenolate complex on ethanol-induced gastric ulceration, *Chem. boil. Interact*., 195, 144–153.

4. I. Mohamed Mustafa, M. A. Hapipah, M. Ameen Abdulla, T. Robinson Ward (2009) Synthesis, structural characterization, and anti-ulcerogenic activity of Schiff base ligands derived from tryptamine and 5-chloro, 5-nitro, 3,5-ditertiarybutyl salicylaldehyde and their nickel(II), copper(II), and zinc(II) complexes, *Polyhedron*, 28, 3993–3998.

5. I. Mohamed Mustafa, M. A. Hapipah, M. Ameen Abdulla, P. Hassandarvish (2012) Acute Toxicity and Gastroprotective Effect of the Schiff Base Ligand 1*H*-Indole-3-ethylene-5-nitrosalicylaldimine and Its Nickel (II) Complex on Ethanol Induced Gastric Lesions in Rats, *Molecules*, 17, 12449–12459.

6. R. K. Parashar, R. C. Sharma, G. Mohan (1990) Biological Activity of Some Schiff Bases and Their Metal Complexes, *Bio. Trace element Res.*, 23, 145-149.

7. K. D. Rainsford and M. W. (1976) Whitehouse; Experientia, 32/9 1172.

8. W. P. John (2007) Rapid Review Biochemistry, second ed., Mosby, Inc. Elsevier.

9. X. Mei, X. Luo, S. Xu, D. Xu, Y. Zheng, J. Lv (2009) Gastroprotective effects of a new zinc (II)–curcumin complex against pylorus-ligature-induced gastric ulcer in rats, *Chem. Biol. Interact*., 181, 316–321.

10. M. G. Martin Reyes, P. Gili, P. Martin Zarza, A. Medina and M. C. Diaz (1986) Complexes of Cu(II), Ni(II) and Co(II) with the Schiff Base: 1 H-Indole-3-ethylensalicylaldimine as Ligand, *Inorg Chim. Acta.*, 116, 153-156.

11. A. Robert, J. E. Nezamis, C. Lancaster and A. J. Hanchar (1979) *Gastroenterology,* 77, 433-443.

12. L. A. F. Paiva, V. S. N. Rao, N. V. Gramosa and F. R. Silveira (1998) *J. Ethnopharmacology,* 62, 73-78.

13. V. C. O. Njar, J. K. Adesanwo and J. Raji (1995) *Planta Medica*, 61, 91-92.

14. H. Varley, A. H. Gowenlock, M. Bell (1980) Practical Clinical Biochemistry, 535-595, The Whitefrairs Press, London.

15. G. C. Peroy (1975) *J. Inorg. Nucl. Chem.*, 37, 2071.

16. D. M. Adams (1967) Metal-ligand and related vibrations, Edward Arnold, London, 248 & 284.

17. D. Todor, and Carmy Lim (2000) *J. Am. Chem Soc.*, 122, 11146.

18. L. Franco and D. Doria (1997) *Pharm. Res.*, 36/5 395-399.