OnLine Journal of Biological Sciences 2 (3): 145-150, 2002

Synthesis and Evaluation of Biological and Pharmacological Activities of a Novel Acetyl-Derivative and Copper Complexes Tranexamic Acid

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Abstract: The objective of this study was to synthesize, characterize and evaluate biological and pharmacological activities of acetyl-substitutive derivative of Tranexamic acid (T.A) and their copper (II) complexes. N-Acetyltranexamic acid (A-2), Di-Tranexamate Diaquo Copper (II) (C-1) and Di-N-Acetyltranexamate Diaquo Copper (II) (C-3) were synthesized, using novel and reproducible procedures. These compounds were characterized using techniques like FTIR, Mass Spectroscopy and FT ^{1}H NMR. Different methods, reported in literature, have successfully been applied for qualitative and structural characterization of these compounds. C-1 showed unidentate bonding of carboxylic group to copper (II) while C-3 indicated bidentate bonding of carboxylate group to copper (II). Bioactivities of the compounds were carried out and the compounds exhibited excellent anti-tumor, significant analgesic and antifungal activities, leading to the conclusion that the derivatives and the complexes are more effective than their parent drug.

Key word: Tranexamic acid, acetyltranexamate derivative, copper complexes, synthesis, bio-activity evaluation

Introduction

Tranexamic acid (Trans-4-aminomethylcyclohexane carboxylic acid-C_8 H_{15} NO_2) is the derivative of amino acid lysine. This drug inhibits the proteolytic activity of plasmin and the conversion of plasminogen to plasmin by plasminogen activators. It is used for its antiplasminic, hemostatic, antiallergic and anti-inflammatory activities (Anthony *et al.*, 1988; Nakanishi, 1999).

A review of the literature revealed that the substitutive derivatives of some drugs show different and in most of the cases, more effective activities than their parent compounds (Daidone *et al.*, 1989; Ahmad *et al.*, 2000; Pang *et al.*, 1988). In another study it was found that the O-heterocyclic substituted salicylamides are more effective than salicylamide and in some cases less toxic (Fahmy and El-Eraki, 2001). Moreover, in our previous work we have found that copper complexes of nonsteroidal anti-inflammatory drugs (NSAID) are more effective than their parent drugs (Khan *et al.*, 1997; 1997a).

In view of the above findings, interest was emphasized in the present investigation to synthesize some new and novel phthaloyl and acetyl derivatives of Tranexamic acid. Moreover, copper (II) complexes of the drug and the derivatives were also synthesized. In order to ascertain if the newly synthesized derivatives and/or the copper complexes would offer some better and different activities than their parent compound, comparative evaluation of their biological and pharmacological activities were performed.

Materials and Methods

Materials: Sodium hydroxide and copper sulfate (Merck-Germany) were used as such without further purification. Tranexamic acid was a gratis supply from Tabros & Organon Pharmaceuticals (Pvt.) Ltd. Methanol, ethanol, chloroform, acetone, methyl acetate and acetic anhydride, etc. were of analytical grade. Pyrex glasswares were used to carryout all experiments. They were properly washed using detergent washing powder, chromic mixture, distilled water and organic solvents, etc. After washing, all the apparatus were dried at 110 °C for two hours in the oven (Memmert-Germany).

The experimental work was carried out in the Faculty of Pharmacy, Gomal University, D. I. Khan. Infrared absorption spectra were recorded on Fourier Transform IR (FTIR) spectrophotometer (Mediac Corporation prospect-IR U.S.A) at Ferozsons Labs., Nowshera. ^{1}HNMR spectra were recorded on Multinuclear FT NMR 400 MHZ (Brucker Co, model AM-400) and Mass spectra on model MAT 112 & 113, Doubled Focusing Mass Spectrophotometer (Finnigan) connected to IBM – at compatible PC based system, at H.E.J. International Research Institute of Chemistry, Karachi. Various bio-activity tests were also performed at this Institute. Melting points were determined on Reichert Thermo-var (F.G. Bode Co., Austria) by taking crystals of the samples on a cover slip. Digital pH/MV meter, model Nova–210 °C (Nova Scientific Co. Ltd., Korea) was used for pH measurement of the samples.

Chemical studies

Synthesis of derivative (A-2) and copper complexes (C-1 and C-3): Tranexamic acid (T.A) was made to react with acetic anhydride and the derivative (N-Acetyltranexamic Acid (A-2)) was synthesized according to the reported procedure (Daniel *et al.*, 1975). Similarly the copper complexes, Di-Tranexamate Diaquo Copper (II) (C-1) and Di-N-Acetyltranexamate Diaquo Copper (II) (C-3) were synthesized following an established method (Khan *et al.*, 1997; 1997a)

Qualitative metal analysis of the complexes: To check whether, the metal cations are free or complexed in nature, elemental identification tests were carried out on the metal complexes with or without decomposition. Without decomposition tests failed, indicating the complexed nature of metal cations. To make metal cations, free metal complexes were decomposed. For this purpose, small amount of complex was taken in a china dish. A few drops of concentrated HNO_3 and HCl were added into it and the mixture was heated up to dryness by evaporation. Into the dry residue, a few more drops of concentrated HCl were added and evaporated again to dryness. The dry residue was dissolved in distilled water and the resultant aqueous solution was used to perform the identification tests for copper.

Biological studies: The derivatives and the complexes were checked against various organisms. The procedures used for measuring their activities are described as follows:

***In vitro* bactericidal bioassay:** The agar well diffusion method (Kazmi *et al.*, 1991) was used for carrying out the bactericidal activity test for the derivatives and the complexes. The human pathogens were studied for this purpose. The bacterial zone of inhibition (mm) was measured. The agar medium with standard drugs and the complexes, including the pathogens, was incubated for 18 hours at 37 °C. Tetracycline was used as a reference drug.

***In vitro* fungicidal bioassay:** The fungicidal activity test for the derivatives and the complexes was performed using the agar tube dilution method (Kazmi *et al.*, 1991). Human, animal and plant pathogens were studied for this purpose. The % linear growth inhibition (mm) was estimated, taking 400 μg of the samples in one ml of the media. The samples were incubated at 37 °C for a period of 7 days. Miconazole, Ketoconazole, Amphotericin-B, Flucytosine, Benlate and Nabane were used as standard drugs.

Anti-yeast bioassay: The anti-yeast activity test for the derivatives and the complexes was carried out through detection of DNA damaging effects of the agents. The mutant and wild type strains of *Saccharomyces cerevisiae* were studied for the purpose. The yeast zone of inhibition (mm) was measured using Streptonigrin as a reference drug.

Pharmacological studies

Effect on blood pressure and heart rate: The effects of the derivative on blood pressure and heart rate were studied in normotensive anaesthetized male Sprague-Dawely Wistar rats (230-260 g) (Gilani, 1991). The animals were anaesthetized with thiopental sodium (60-80 mg/kg body weight, i.p). The arterial blood pressure was recorded from the carotid artery via the arterial cannula connected to a pressure transducer (Statham P_{23}) coupled with a grass model 79 polygraph. Drugs were injected via the cannula

inserted in the jugular vein. Mean blood pressure was calculated as the diastolic blood pressure plus one-third of pulse width. The normal response of the animal was checked through the standard compounds, acetylcholine (hypotensive) and noradrenaline (hypertensive). Acetylcholine (1μg /kg) was used as a positive control which produced 59.5 $\pm$ 8.3% fall (mean $\pm$ SEM, n = 3) in the mean blood pressure (Gilani, 1991).

Analgesic activity: Male albino mice (25-30 g) were randomly divided into several groups and injected intraperitoneally with 1 % acetic acid in a volume of 0.1mL/10g body weight. The symptoms of the acetic acid induced abdominal writhing were similar to those described by Emele (Emele and Shanaman, 1963). Five minutes after the injection of acetic acid, the mice were observed for 10 minutes and the number of writhing responses for each mouse was recorded. Aspirin (acetyl salicylic acid of chemical grade) was used as a reference drug.

***In vitro* phyto-toxic (Anti-Tumor) bioassay:** The anti-tumor activity test (potato disc assay) for derivative and the copper (II) complexes was performed, using 10 plants of Lemna acquinoctialis welv for the purpose. The highest doses 500 μg per ml of the derivatives and the copper (II) complexes were employed. The sample was incubated at a temperature of 28 $\pm$1 ^{0}C (Rehman, 1999).

Results and Discussions

Synthesis: The synthesis of A-2 took place through a chemical reaction between T.A and acetic anhydride in 1:1 molar ratios and the yield was 83.2 % (Eq. 1).

It is reported (Rao *et al.*, 1988) that copper (II) reacts with carboxylic acids to form copper (II) complexes with a molar ratio of 2:1.

The synthesis of C-1 took place through the chemical reaction between T.A and copper sulfate in 2:1 molar ratio of the two constituents and the yield was 82% (Eq. 2).

C-3 was synthesized through a chemical reaction carried out in 2:1 molar ratio of A-2 and copper sulfate and the yield was 78% (Eq. 3).

Qualitative metal analysis of complexes

Tests for Copper: A dark-blue color was developed upon addition of excess NH_4OH to the aqueous solution obtained through the qualitative metal analysis of the complexes, described above. This confirms the presence of copper in the complexes.

Addition of a few drops of potassium ferrocyanide to the aqueous solution gave red color, which further confirms the presence of copper.

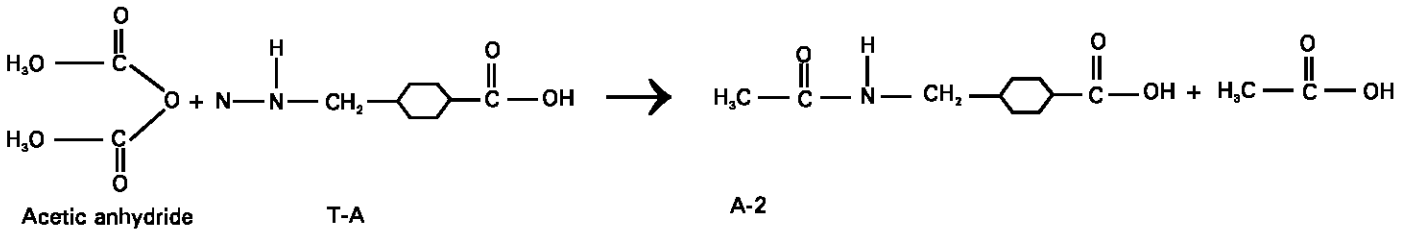
Characterization Techniques

Physical nature: The observed physical properties such as melting points and solubility of the derivative and the copper (II) complexes are given in Table 1. The melting points of A-1, C-1, and C-3 are totally different than that of the T.A (286-290 ^{0}C). This may indicate the confirmation about the synthesis of the respective derivatives and complexes.

I.R. spectroscopic studies: The I.R. data and vibrational behavior of the derivatives and the copper (II) complexes are given in Table 2. The I.R. spectra of A-2 showed stretching for NH_2 at 3300 cm^{-1}, acetyl (C=O) at 1690 cm^{-1} and for carbonyl (COO) at 1630 cm^{-1} and 1430 cm^{-1}, that confirms the A-2 synthesis.

The I.R. spectra of C-1 showed that the broad peak between 3100 cm^{-1} to 2400 cm^{-1} in the spectra for T.A, due to carboxylic (COOH) group, has been disappeared. This shows the linkage of copper with the ligand (T.A) through the hydroxyl bond of carboxylic group. Furthermore, the bands associated with ν_{asym} and ν_{sym} (COO) mode were stretched at range 1625 cm^{-1} and 1350 cm^{-1} respectively. Comparing the ν_{asym} and $_{sym}$ vibration values of the complex (C-1) with that of the ligand, it was found that ν_{asym} values were raised and ν_{sym} values were lowered for the complex. This indicates the unidentate or asymmetric bonding of carboxylate group to the Copper (II). In case of C-3, the I.R. spectra showed that the medium peak of A-2 at 3110

Equation No. 1



Equation No. 2

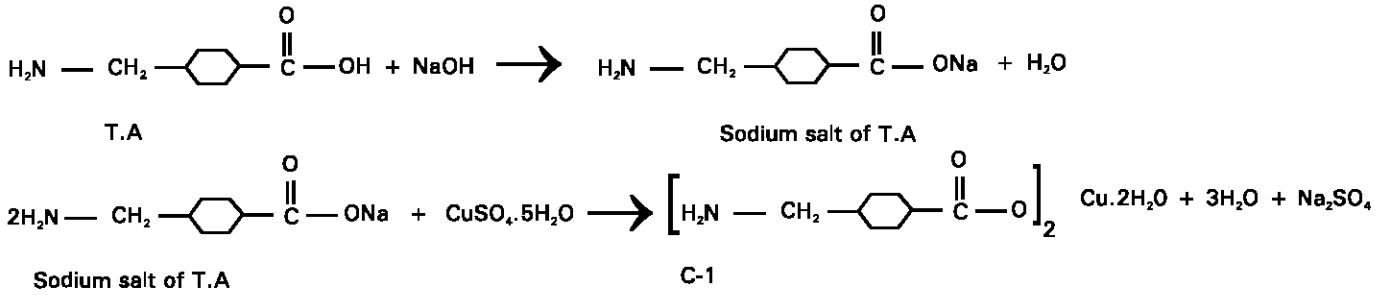
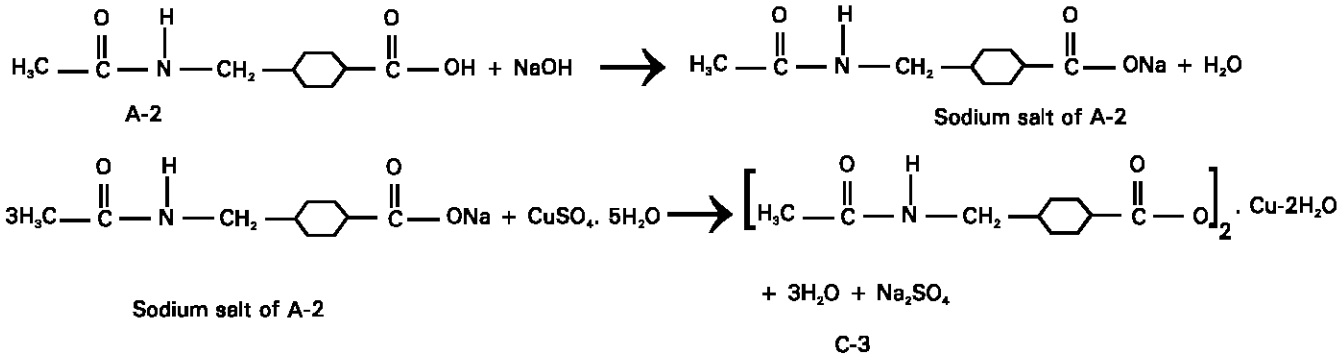


Table 1: Physical properties of the derivative and copper (II)complexes

Comp. No.	Mol. formula	Odour, color and physical state	Meltiing points	Solubilities							Methyl Acetate
				H_2O	CH_3OH	C_2H_5OH	$CHCl_3$	CCl_4	0.IN HCl	0.1N HCl	
A-2	$C_{10}H_{17}NO_3$	Odourless, white crystalline	247-250°C	P.sol	F.sol	F.sol	Insol.	Insol.	------	-------	-------
C-1	$(C_8H_{14}NO_2)_2.Cu.2H_2O$	Odourless, blue amorphos (solid)	220°C (completely black)	Insol.	Insol.	Insol.	Insol.	Insol.	Sol.	Insol.	Sparingly soluble
C-3	$(C_{10}H_{16}NO_3)_2.Cu.2H_2O$	Odourless, ligth green, amorphos (solid)	280°C (completely black)	Insol.	Insol.	Insol.	Insol.	Insol.	Sol.	Insol.	Sparingly soluble

A-2= N-Acetyltranexamic acid, C-1= Copper complex of Tranexamic acid, C-3= Copper complex of N-Acetyltranexamic acid. Sol= soluble, Insol= Insoluble, F. Sol. = Freely soludle, P. Sol. = Partially soluble. Comp. = Compound, Mol. = Molecular

Equation No. 3



Equation No. 4

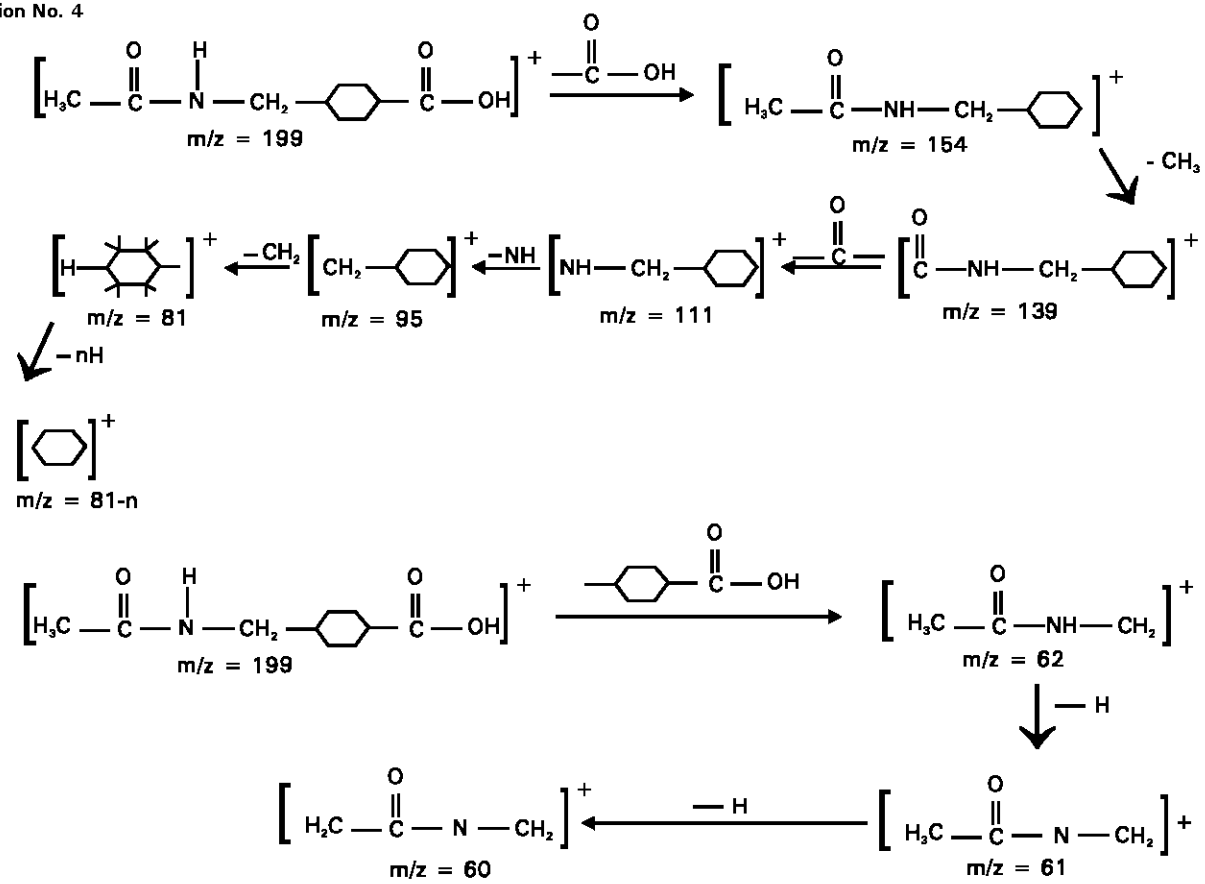


Table 2: I. R. Data of the derivative and copper (11) complexes

Comp. No.	Carbonyl (COO)		Acetyl(C=O)	COOH	NH_2	Cu-O(Copper-oxygen)
	Asym.	Sym.				
T.A.	1600-1500cm^{-1} (s.b)	1390cm^{-1} (s)	–	3100-2400cm^{-1} (b)	3550-3320cm^{-1} (b)	–
A-2	1630cm^{-1} (s.sp)	1430cm^{-1} (w)	1690cm^{-1} (s.sp)	3110cm^{-1} (m)	3300cm^{-1}	–
C-1	1625cm^{-1} (w)	1330cm^{-1} (sp)	–	–	3550-3150cm^{-1} (s.b)	815cm^{-1} (w)
C-3	1530cm^{-1} (w)	4560cm^{-1} (w)	1640cm^{-1}	–	–	815cm^{-1} (m)

T.A.= N-Acetyltranexamic acid, C-1= Copper complex of Tranexamic acid, C-3= Copper complex of N-Acetyltranexamic acid
S=strong , b= broad, sp= sharp, w=weak, m=medium. Asym=Asymmrtric, sym=symmetric.

Table 3: ^{1}H NMR data of N-Acetyltranexamic acid

Proton No.	A-2 (δppm)
2	2.10-2.17 (m)
3,7	1.32-1.51 (m)
4,6	---------
5	2.17-2.21 (m)
8	2.99-3.03 (m)
9	7.80-7.82 (m)
10	1.93 (S)
11,12	---------

A-2 = N-Acetyltranexamic acid, M = multiplet, d = doublet, S = singlet

Table 4: *In vitro* bactericidal bioassay

Name of bacteria	Clinical Implication	Activity			Activity of reference durg (Doxyycyline)
		A-2	A-1	A-3	
Human pathogens					
Bacillus subtilis	Food poisoning	–	–	–	+ + +
Corynebacterium diptheriae	Dipthera, infecton of ear, nose, throat and skin. Toximia: Cardio-respiratory failure	–	–	–	+ + + +
Escherchia coli ETEC	Infections of wounds and urinary tract. Inflammation of Peritoneum and GIT, dysentry, septicemia, neonatal meningitis	–	–	–	+ + +
Klebslella penumonia	Infections of respiratory and urinary tract. Supporetive infection in sinuses and Middle ear etc, septicemia	–	–	–	+ + +
Proteus vulgaris	Infections of urinary tract and wounds, septicemia	–	–	–	+ + +
Pseudomonas aeroginosa	Infections of wounds, uruinay tract and eyes. Septicemia	–	–	–	+ +
Salmonella typhi	Typhoid fever, salmonella food poisoning. Localized infection: pyelonephatis, endocarditis, salpingitis, chronic osteomyelitis	–	–	–	+ + + +
Shigella dysentry	Inflammation of GIT, bacterial dysentery	–	– (+)	–	+ + + +
Staphylococcus aureus	Food poisoining, scalded skin syndrome, toxic shock syndrme. Infections of upper respiratory tract and wounds Abacesses, endocarditis	–	–	–	+ + +
Streptococcus pyrogenes	Acute rheumatic fever, scarlet fever, sore throat, orysipelas, aeptic wounds, impetigo, inflammations of post glomerulonephrone (kidney), tonsils and middle ear pueperal sepsis, erythema nodosum	–	–	–	+ +

Key: A-2 = N-acetyltranexamic acid, C-1 = Copper complex of Tranexamicd acid, C-3 = Copper complex of N-Acetyltranexamic acid, + + + + = Very high activity + + + = High activity + + = Optimum activity + = Low activity - = No activity

Table 5: *In vitro* fungicidal bioassay

Name of fungi	Clinical implication	Activities			Standared drugs	Activity
		A-2	C-1	C-3		
Human pathogens	***Cutaneous mycoses***					
Epidermophyton floccosum	Ring worm of groins, arms and torso	+ +	+ +	+ +	Miconazole, Ketoconazole	+ + + + +,+ + + + +
Trichophyton schoenleinii	Scaring of the scalp, permanent alopecia	+ + + +	+ + + +	+ +	Miconazole, Ketoconazole	+ + + + +,+ + + + +
Pseudallescheria boydii	***Subcutaneous mycoses*** Infection of skin sbcutaneous tissue, nasalsinuses, mycetoma and brains abscess	+ +	+ + +	+ + +	Miconazole, Ketoconazole	+ + + +, + + + +
Candida albicans	***Opportunistic mycoses*** Candidosis, infection of lungs, vagina, ear, bones, heart and thrush	–	–	+	Miconazole, Ketoconazole	+ + + + +,+ + + + +
Aspergillus niger	Infection of lungs, eyes and CNS. Hypersensitivity and fungal ball	+	+ +	+ + + +	Amphotericin-B Flucytosine	+ + + + +,+ + + + +
Animal pathogens	***Cutaneous mycoses***					
Microsporum canis	Ringworm infection of hair and skin in dogs and cats.	+ + + +	+ + + +	+ +	Miconazole, Ketoconazole	+ + + + +,+ + + + +
Trichophyton mentagrophytes	Ringworm of feet, nails, fore arms and groins in rodents	+ +	+ +	+ +	Miconazole, Ketoconazole	+ + + + +,+ + + + +
Trichophyton sirnii	Sever combined inflammatory and hypersensitivity Reaction "kerion" in monkeys, rare (India).	+ + + +	+ + +	+ + + +	Miconazole, Ketoconazole	+ + + + +,+ + + + +
Plant pathogens	**Seed borne pathogens**					
Fusariumum solani var. *Lycopersici* (tomato)	Root rot, stemcankers associated with wounds, damping off seedling, destruction of spawn inbeds of cultivated mushrooms and pea crop	+	+ + + +	+	Benlate, Nabam	+ + + + +,+ + + + +
Macrophomina phaseolina	Seed rot, wilt, Root rot (Charcoal rot)	+	+ +	+ +	Benlate, Nabam	+ + + + +,+ + + + +
Rhizoctonia solani	Root rot (necrosis), wilt.	+ +	+ + + +	-	Benlate	+ + + + +

Key: A-2 = N-acetyltranexamic acid, C-1 = Copper complex of Tranexamicd acid, C-3 = Copper complex of N-Acetyltranexamic acid, + + + + + = Very high activity + + + + = High activity + + + = Optimum activity + + = Low activity + = Very low activity – = No activity

Table 6: Anit-yeast bioassay

Name of yeast	Activities of copper (II) complexes			Activity of Streptonigrin (standard drug)
	A-2	C-1	C-3	
Saccharomyces cerevisiae (m) RS322Y (RAD52)	+	+	–	+ + +
Saccharomyces cerevisiae (w) LF 15(RAD+)	–	–	–	+ +

A-1 = N-Acetyltranexamic acid, C-1 = Copper complex of Tranexamic acid, C-3 = Copper complex of N-Acetyltranexamic acid. + + + = Very high activity, + + = High activity, + = Low activity, – = no activity, m = mutant strain, w = wild type strain.

Table 7: Analgesis activity of ligands

Compound	Dose (mg/kg)	Acetic acid induced writhing response		Remarks
		No. of writhes	% inhibition	
Control	-	48	-	This derivative well inhibited the acetic acid induced writhes and showed the excellent analgesic activities
Aspirin	150	16	67	
A-2	10	24	50	

Animal used = Male Albino mice (25-30gm)NMRI straing, Route of administration = Intraperitioneal (I.P), Reference standard used = Aspirin (Acetyl salicylic acid of chemical grade)

Table 8: *In vitro* Phytotoxic (Anti-Tumor) bioassay

Name of Plant	Concentration (μg/ml)	No. of fronds		% Growth regultion	Reference inhibitor	FI 50* (μg/ml)
A-2 Control						
Lemna	500	10	21	100	Praqual	0.000
Acquinoctialis	50	17	19	100	100 %	0.125
Walv	05	19	20	85		
C-1 Control						
Lemna	500	00	11	100	Praqual	0.000
acquinoctialis	50	00	09	100	100%	0.125
Waly	05	07	09	22		
C-3 Control						
Lemna	500	00	11	100	Praqual	0.000
Acquinoctialis	50	00	9	100	100 %	0.125
Walv	05	2	9	17		

A-2 = N-Acetyltranexamic acid, C-1 = Copper complex of Tranexamic acid, C-3 = Copper complex of N-Acetyltranexamic acid. FI50* = Concentration necessary to inhibit and promrote 50% of found proliferation

cm^{-1}, which was due to the presence of carboxylic acid (COOH), has been totally disappeared. This shows the linkage of Copper (II) with its ligand (A-2) through the carboxylate COOH group. Moreover, the acetyl (C=O) band was seen at 1640 cm^{-1}. The bands associated with ν_{asym} and ν_{sym} (COO) modes were stretched at range 1530 Cm^{-1} and 1450Cm^{-1}, respectively. The ν_{asym} and ν_{sym} vibration values of the C-3 were compared with the values of its ligand (A-2). The ν_{asym} values were lowered and the ν_{sym} values were raised for the complex, which indicated the bidentate or symmetric bonding of carboxylate group to the Copper.

Mass spectroscopic studies: A-2 ($C_{10}H_{17}NO_3$) produced intense molecular ion peaks. The most important fragmentation pathways involved the loss of COOH to form $C_9H_{16}No^+$ion (m/z =154), followed in sequence, by loss of CH_3 to form $C_8H_{13}NO^+$ion (m/z 139), loss of CO to form $C_7H_{13}NO^+$ion (m/z =111), loss of NH to form $C_7H_{12}^+$ ion (m/z = 95), and loss of CH_2 to form $C_6H_{10}^+$ ion (m/z =81). The fragmentation pathways are given in (Eqe. 4).

^{1}H-NMR Spectroscopic Studies: The results regarding the chemical shift of protons of A-2, as revealed from its ^{1}HNMR spectrum are given in Table 3. The proton No. 2 was seen in range 2.17-2.10 PPM values and it was not properly resolved. The protons No. 3 and 7 were seen in the range 1.51-1.32 PPM values. They were not properly resolute since they were overlapped with each other (Pavia *et al.*, 1979). The proton No.8 was seen in range 3.03-2.99 PPM values and was seen as multiplet (Thorntonton and Collet, 1979). The proton No.9 was seen in range 7.82-7.80 PPM values and was seen as broad doublet peak. The proton No. 10 was seen in range 1.93PPM value and it appeared as a singlet peak.

Biological Activities

***In vitro* bactericidal bioassay:** The derivative as well as the complexes showed little activity towards the pathogens (Table 4). The only exception being that of the C-1, which showed low activity against *Shigella dyscentry* (causative organism of GIT inflammation-bacterial dysentery). It may be due to the presence of copper in the complex. Doxycycline was used as a reference drug.

***In vitro* fungicidal bioassay:** The results of the fungicidal activity tests for the derivative and complexes against various fungi are given in Table 5. As seen in the table, the derivative as well as the Copper (II) Complexes proved themselves to be good fungicides, by inhibiting the growth of various fungi, harmful to human beings, animals and plants.

Anti-yeast bioassay: The anti-yeast activities of the derivative and the complexes are shown in Table 6. A-2 and C-1 showed low anti-yeast activities, while C-3 was found to have no anti-yeast activity against mutant strain {RS 322 Y (RAD52)} and wild type strain {LF15 (RAD_+)} of *Saccharomyces cerevisiae*. Streptonigrin was used as a reference drug.

Effect on blood pressure and heart rate: A-2 at the doses of 10, 25 and 40 mg/kg body weight showed no effect on blood pressure and heart rate of the male Sprague-Dawely rats (230-260g). The normal response of animal was checked through standard compounds such as acetylcholine (Hypotensive) and noradrenaline (Hypertensive).

Analgesic activity: The results of the tests performed for knowing the analgesic activities of A-2 are shown in Table 7. It was found that A-2 has excellent analgesic activities. Aspirin was used as a standard drug.

***In vitro* Phyto-toxic (Anti-Tumor) activity:** The results of the *in vitro* anti-tumor activity test (potato disc assay) for the derivative and the complexes are shown in Table 8. It was found that A-2 showed low anti-tumor activity at the dose of 500μg/ml; while the same dose of C-1 and C-3 exhibited 100% anti-tumor activities.

A-2 was synthesized from T.A and its synthesis was confirmed by physical methods and through various spectroscopic techniques.

In the second stage, C-1 and C-3 were synthesized from their respective ligands i.e. T-A and A-2, respectively. Their synthesis was confirmed by physical method and through spectroscopic techniques. C-1 indicated unidentate bonding of carboxylate group to copper (II). While C-3 indicated bidentate bonding of carboxylate group to copper (II).

A-2 showed excellent analgesic activity, significant anti-fungal and low anti-tumor (phytotoxic) activities. However, it showed no anti-bacterial and anti-yeast activities. Also, it exhibited no effect on blood pressure and heart rate of anesthetized rats.

C-1 exhibited high anti-tumor (phytotoxic) and significant anti-fungal activities. It showed low anti-yeast activity. However, it showed no anti-bacterial activity. C-3 showed high anti-tumor (phytotoxic) and significant anti-fungal activities. However it exhibited no anti-yeast and no anti-bacterial activity.

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