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ORIGINAL ARTICLE

Protein Kinase Inhibitory Potential and Anti-Fungal Activities of Metal Complexes of Anti-Viral Drug Ribavirin

Samar Akhtar[1], Hammad Ahmad[1], Hashaam Akhtar[1], Rehman Zafar[1,]*, Wajeeha Waseem[2], Maryam Khan Sherwani[1]

[1] Yusra Institute of Pharmaceutical Sciences, Yusra Medical and Dental Collage, Zaraj Housing Society, Opposite DHA Phase 2 Gate III, Main G.T. Road, Islamabad, 44000, Pakistan

[2] Riphah Institute of Pharmaceutical Sciences, G-7/4, Islamabad, 44000, Pakistan

Authors' Contributions
1 Conception & Study Design, Data Collection, Data Analysis.
2 Conception & Study Design, Data Collection.
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****Address of Correspondence Author:***
rehmanzafar016@gmail.com

ABSTRACT

Background: Ribavirin, a known antiviral drug, potentially recognised for a number of pathological conditions like hepatitis C, Lassa fever and Hanta virus infection. It predominantly works by inhibiting viral RNA synthesis that in turn stop viral multiplication. The best prosperous derivative of this drug is Taribavirin (3-carboxamidine derivative).

Objective: In this research, we aim to synthesize the metal complexes of already marketed drug ribavirin, because the complexation of such approved marketed drug will save time and currency that may spend in relation to clinical development of new drug molecule.

Methods: Eleven derivatives (S-01-S-11) of ribavirin were synthesized in combination with di/tri organotin, Zn, Cu and Fe. Characterization was done through IR and H^1 NMR spectrophotometer and these compounds were analysed for *in vitro* biological activities including anti-microbial, anti-fungal and protein kinase inhibition assay.

Results: Research revealed that addition of trace metals to approved marketed drug have enhanced the biological properties of unbound drug.

Conclusion: Outcomes of this research clearly indicates the enhancement of biological activities of free drug through immersion of metals which could be further analysed for toxicity studies.

Keywords: Ribavirin, trace metals, protein kinase inhibition.

INTRODUCTION

Circumstantial

Ribavirin proves to be imminent antiviral product in pharmaceutical industry [1]. It is potentially prescribed in contradiction of many viral species including Congo virus [2-5]. Hepatitis C, other viral infections and HIV management can be done with its interference to interferon [6, 7]. Research has shown that ribavirin can be used to treat rabies when combined with other drugs [8]. They have freshly been identified for their events in contradiction of severe viral infections [9, 10].

In 1970, ribavirin was recognized and further in 1972 described to have antiviral potential [11]. It works by obstruction with duplication of the viral hereditary material as it takes after structure squares of RNA particle. Fundamentally ribavirin consists of a ribose sugar with purine like nitrogenous base. Its component of activity is as yet not surely known [12]. Organometallic compounds were utilized from the beginning of time to treat numerous sorts of sickness including bacterial, contagious, viral, leishmanial diseases and different issue [13]. The field of organometallic therapeutic science picked up its blast later revelation of anti-cancer medication cisplastin. They proved to have ability of having anti-tumour and anti-microbial operators [14]. Further tin ethyl etiopurpurin were accounted for to be utilized for restoring growth related macular deterioration [15].

Iron, copper and zinc are basic follow reserves needed to perform typical working of our body. Deficiency of the over 3 elements in a person prompts bizarre development, diminished in susceptibility, and particularly life becomes hard to vision without iron because it has the capacity to deliver O_2 to all cells [16]. Other metals have their own importance in normal working of body [17, 18].

Meanwhile hundreds of years, antimony was utilized to treat dermal diseases. During the 20th century, metals mixes stayed utilized for leishmaniasis as 1st strategy treatment [19, 20].

METHODOLOGY

Substantial

Chemicals with the various synthetic mixtures that involves metal salts were purchased then used moving forward deprived of any more change.

Methods

Medication ribavirin was taken from local pharmaceutical manufacturer. Brisk fitting china stood utilized with dissolving focuses were verified to have Gallen light device. IR spectrophotometer was utilized to describe functional groups. Combination then hostile to bacterial inhibition activity was done in research lab of Riphah University Pharmacy Department. The complexation of metals with free drug ribavirin was carried in the way as shown in Figure **1**.

Common Way for Combination of Compounds 1-6 (S01-S06)

Ribavirin and di/tri organotin stayed blended according to the previously reported procedure [21]. With triethylamine Et_3N (1 mmol) ribavirin (2 mmol) was gone to reflux condenser for 3 hours. with steady blending in dry toluene. It remained permitted for chilling and 2 mmol of organotin which was included after reflux and the blend was allowed to mix for 360 min. At that point, result precious stones of Et_3NHCl_2 were sifted off and the organotin edifices were filtered from the remainder by means of dissipation [22].

Compound 7 Zinc Derivative (S-07)

Zinc chloride, ribavirin and methanol in their respective quantities was added and refluxed for 3 hours. They were blended through consistent mixing with warmed and afterward chilled off. In the wake of cooling, they were elucidated by vaporization of product after filtration and washed with chloroform and methanol [23].

Compound 8 Copper Derivative (S-08)

Marketed drug and Cu(II) acetic acid were broken down in 15 ml methanol independently afterward combined. The environment was kept up at pH 7. Mixture was stirred at 37°C for 240 min. What's more, cooling down medium-term. At that point item was filtered and sprinkled with addition of methanol and vacuum dried [24].

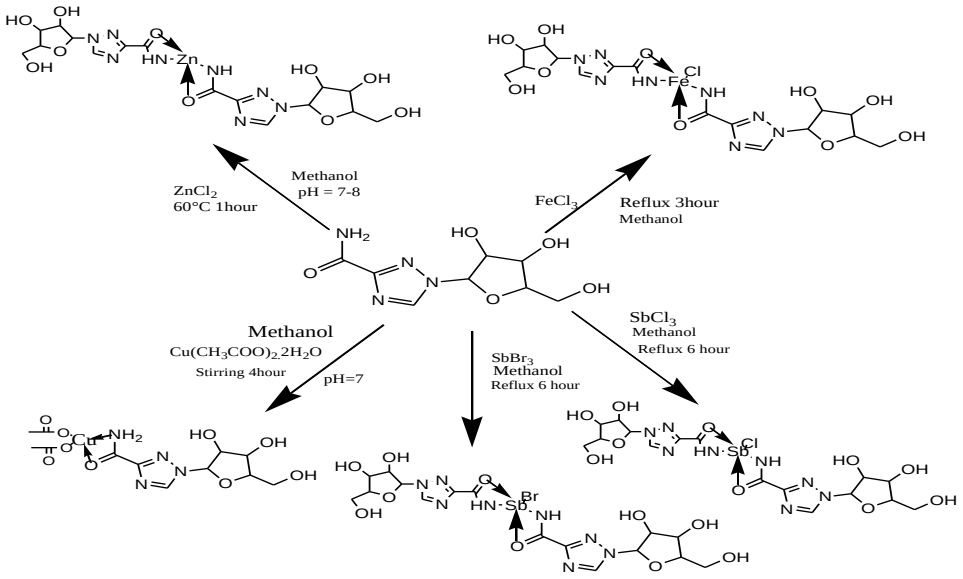

Figure 1. Showing synthesis Ribavirin with trace metal**.**

Compound 9 Iron Derivative (S-09)

$FeCl_3$ (1 mmol) with methanol and 1 mmol of ribavirin were mixed. Then they were allowed to reflux on oil bath for 3 hours to avoid direct heating of compounds [25]. In wake of cooling the arrangement was illuminated and coming about hastens were methanol mixed and got dehydrated out with gel as shown in Figure **1**.

Compound 10-11 Organotin Derivative (S10-S11)

1 mmol of 25 ml of dry methanol ribavirin arrangement was broken down gradually with consistent mixing to naturally arranged arrangement of antimony halide (1 mmol) at 37°C in 10 ml methanol with reflux for 3 hours. At that point, subsequent collection was placed in murkiness. The item remained gotten as hastens afterward percolation. This accelerates stood clear with ethanol with purged by recrystallization strategy [26].

Anti-Microbial Activity

Anti-bacterial examine of coordinated mixers against the bacterial stains and the paternal sedate were done of microscopic organisms utilizing dispersion strategy [27, 28]. Supplement nutrient agar remained utilized. The microbial species comprised of *S. aureus* and *E. coli*. DMSO and third generation cephalosporin's 20 µg/plate were utilized as a negative control and positive control separately. The results were analysed as triplicate and their arithmetic mean was considered.

Anti-Fungal Assay

Ribavirin and integrated edifices got analysed for 6 irresistible strains of growths to be specific Mucor sp., *A. flavous*, *A. fumigatus*, *A. niger*, and *S. fusarium*. DMSO was taken as deleterious control and Terbinafine was taken as optimistic control. Cylinder dissemination strategy [29, 30] followed utilizing dextrose agar. Readings were taken as triplicate.

% restraint potential = 100 – (Lined development in test/Lined development of control) x 100

Enzyme Peptide Kinase Inhibitory Potential Assay

Chemicals particularly protein kinases undergo ascended in critical lethal concentrations as threatening development treatment as protein phosphorylation lessens by catalysts which had turned out to be one of the primary authoritative frameworks in natural strategies including cell death, cell increase along with assimilation. Peptide kinase activity was performed on all compounds. Experimental 2 zones of inhibition were a straightforward limiting zone speaking to toxicity of cells with an uncovered restraint zone indicating positive peptide hindrance capability all the synthesized compounds. 9 mm of hindrance zone was dynamic considered.

RESULTS AND DISCUSSION

The complexation of marketed drug ribavirin with metals was done in a streamline manner. The products were obtained in handsome amount. Review of IR spectrum uncovered trademark possibilities of natural surface of the edifices that enabled us for confirmation of their binding complexes. Recurrence about 3299-3199 cm^{-1} might be described as v(N-H) that is available in allowed medication however missing through metal buildings. Among all edifices, metal coordination was supported with moving of v(C-N) and v(C-O) which relates to non-bounded medication inside spectrum of IR. Buildings displaying presence v(M-N) *i.e*. metal nitrogen in scope of 449-559 cm^{-1}. The tin edifices demonstrated very week and width groups allotted to v(Sn-O) at district of 399-489 for each cm supporting Sn-O security arrangement. Auxiliary explanation of edifices was finished with ^{1}H and ^{13}C atomic attractive reverberation spectrum. It was critical move of signs in ^{1}H NMR of edifices and unbound medication ribavirin. Chelation of ribavirin with metal particles brought about lower filed moves of sugar proton flags, because of bringing down negative ion on hydrogen iota. In ^{13}C NM resonance, distinction along with concoction diversion were peaceful striking of carbon particles that are nearness to mind boggling focus that is agreeable to intricate arrangement. Spectroscopic information demonstrates effectiveness blend of edifices.

Anti-Microbial Activity

Organic exercises of elements mixes remained confirmed in contradiction of accessible species of microorganisms with their growths. Unbound medication ribavirin that additionally tried however demonstrated for all intents and purposes no action in double tries yet the integrated buildings indicated amazing results when tested on bacterial species. Microbe against activity consequences just tin based

compounds buildings remained calculable. Calculations for anti-microbial and anti-fungal activity are given in Table **1**, Table **2** and graphically displayed in Figure **2**, Figure **3**, respectively [31, 32].

Table 1. Synthesized compounds anti-microbial activity.

Compounds	Inhibitory Region (2)	
	S. aureus	***E. coli***
S	000	000
S-01	010	008
S-02	000	008
S-03	000	010
S-04	013	012
S-05	011	013
S-06	000	011
S-07	015	014
S-08	006	000
S-09	015	014
S-10	015	016
S-11	000	008
Cephalosporin	021	022
Dimethylsulfoxide	000	000

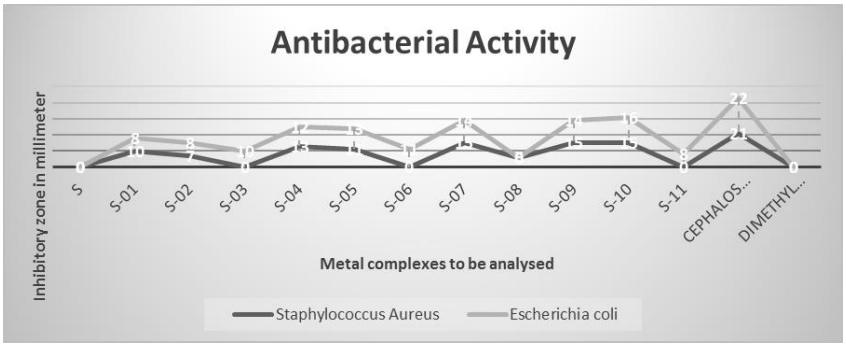

Figure 2. Anti-bacterial activity.

Table 2. Synthesized compounds activity against fungus.

S. No.	Compounds	Inhibitory Area (2)				
		A. flavous	***A. niger***	***F. solani***	***A. fumigatus***	**Mucor sp.**
1	S	0	0	0	0	0
2	S-01	14	17	13	16	18
3	S-02	18	17	14	15	16
4	S-03	0	0	0	0	0
5	S-04	0	8	0	0	0
6	S-05	28	25	18	29	18
7	S-06	28	0	0	0	0
8	S-07	11	10	14	13	12
9	S-08	0	0	0	0	0
10	S-09	0	0	0	0	0
11	S-10	0	0	0	0	0
12	S-11	0	0	0	0	0
13	Terbinafine	15	32	34	30	32
14	DMSO	0	0	0	0	0

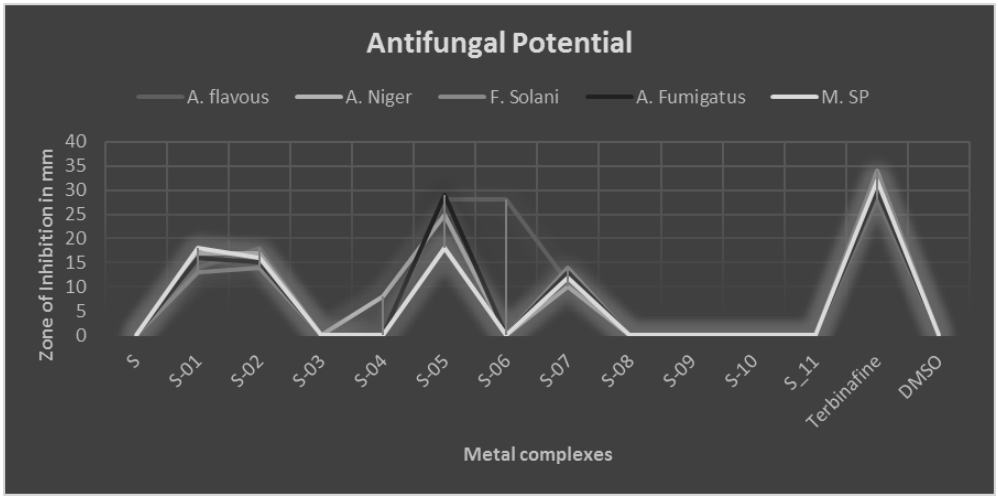

Figure 3. Anti-fungal potential shown.

Table 3. Inhibition of protein kinase results.

S. No.	Compounds	Inhibitory Calculated (2) Conc.	
		Transparent Area (2)	Colour Area (2)
1	S	00	00
2	S-01	02	27
3	S-02	03	22
4	S-03	00	10
5	S-04	00	25
6	S-05	00	25
7	S-06	00	18
8	S-07	00	07
9	S-08	00	09
10	S-09	00	00
11	S-10	00	00
12	S-11	00	00

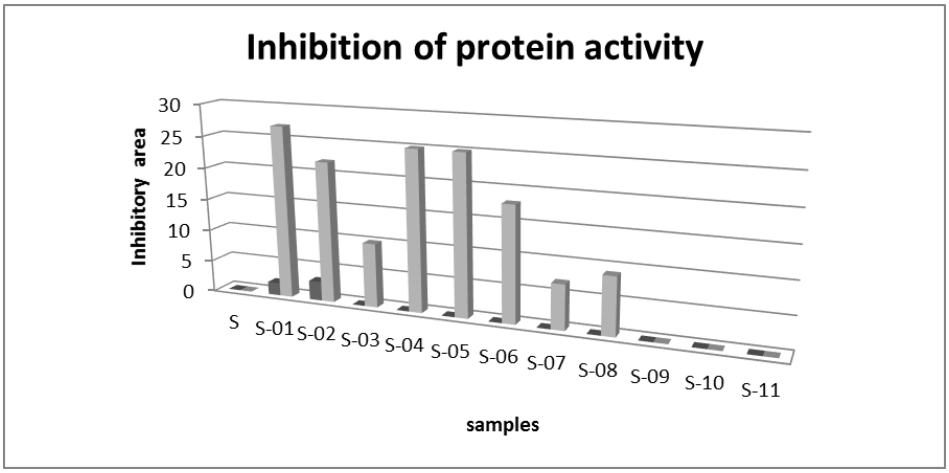

Figure 4. Representation of protein inhibition activity.

Inhibition of Protein Kinase

Anti-viral drug along with integrated buildings were examined for peptide restraint test. Character 6 shows graphical portrayal of protein kinase measure of ribavirin and its buildings. The drug and its integrated element buildings demonstrated extremely encouraging outcomes. Test S-1 indicated most noteworthy zone of hindrance pursued by S-04, S-05, S-06, S-08, S-07 and S-03 individually. While samples S 09-11 demonstrated not at all unmistakable area of restraint by any stretch of the imagination. Calculated values for protein kinase inhibitory potential are given in Table **3**. These calculations are drawn graphically in Figure **4**.

CONCLUSION

The trial information exhibited in this paper gives proof of effective development of normally dynamic organotin, Zn(II), Cu(II), Sb(III) and Fe(III) ribavirin edifices. The examination uncovered that the metal complexation has influenced and upgraded the natural impacts of the free medication. Further examinations to divulge other pharmacological properties of the integrated edifices are expected to investigate in future research.

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