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Research Article

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Synthesis of 3-aryl amido/imido-methyl-4-hydroxy-phenyl-5'-phenyl-3'-oxazolyl acetophenone azines as potential antiviral agents

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ABSTRACT

3-Aryl-amido/imido-methyl-4-hydroxy-phenyl-acetophenones (I) were prepared in excellent yields by condensing equilmolar amounts or p-hydroxy acetophenones and aryl-amido/imido-alcohols in the presence of conc. H_2SO_4. Reaction of (I) with semicarbazide, hydro-chloride in ethanol afforded 3-Aryl-amido/imido-methyl-4-hydroxy-phenyl-acetophenone-semicarbazones (II) in the yields ranging from 60-70%. Reaction of (II) with acetophenone and iodine in glacial acetic acid afforded 3-Aryl-amido/imido-methyl-4-hydroxy-phenyl-5'-phenyl-3'-oxazolyl-acetophenone azines (III) in the yields ranging from 65-70%. Some of the compounds showed promising antiviral activity against three viruses viz., JEV, HSV-I and Influenza A viruses respectively. The present paper describes the synthesis and biological activity screening procedures.

Keywords: Thiosemicarbazones, Methisazone, biological screening

INTRODUCTION

Thiosemicarbazones have been claimed to possess very promising antibacterial as well as antiviral activities amongst various thiosemicarbazones, Methisazone (N-Methylisatin--thiosemicarbazone) has been found most effective. It interferes with the translation of RNA messages into protein-synthesis on the cell ribosome. Ultimately, it produces a defect in protein incorporation into virus. Although viral-DNA increases and host cell are damaged, infectious virus is not produced. It is active against pox-viruses, including variola and vaccinia[1]. Some RNA-viruses such as rhino-viruses, echo-viruses, reo-viruses, influenza, Para-influenza and polio-viruses are also inhibited. It has also been used as a prophylactic agent against small-pox. Historically, methisazone was one of the first antiviral compound(s) used in chemical practice. It is orally absorbed with nausea and vomiting as the principal side effects. The drug is also used in vaccinia gangrenosa and disseminated vaccinia infections[2]. A potential side-effect of methisazone is a suppression of host immune-system[3]. Complexes of methisazone and copper have been shown to interact with nucleic-acids[4], RNA-tumors viruses, herpes viruses etc. are inactivated by direct contact with methisazone which may be a functions of the chelating ability of the drug[5]. Incorporation of the thiosemicarbazone group into a cyclic-system appears to increase the spectrum of antiviral activity of these compounds[6-8]. These valid observation led the author to synthesize the cyclic derivatives of semicarbazone for studying their effects against three human viruses.

EXPERIMENTAL SECTION

Melting points were determined on electrically heated apparatus and the values are uncorrected. Purity of all the compounds was checked by TLC. I.R. spectrum of one compound was recorded on a Perkin Elmer 157 infrared spectrophotometer (wave length $_{max}$ in Cm^{-1}) and ^{1}H-NMR spectrum of the same compound was recorded on a spectrometer (chemical shifts in s-scale) using TMS as internal reference.

3-Aryl-amido/imido-methyl-4-hydroxy-phenyl-acetophenone (I)
A mixture or p-hydroxyacetophenone (0.2 mole) and an amidoalcohol (0.2 mole) was dissolved in conc. H_2SO_4 (30 ml.) by stirring. While dissolving, the contents were cooled. The resultant solution was stirred for half-an-hour at room temperature and kept in an ice-chest over night. It was poured into ice-cold water (100 ml.).A reddish-brown solid separated out which was allowed to settle for 1h. It was filtered off and washed repeatedly with water. The C-amidoalkylated product thus obtained, was dried in a vacuum desiccators and crystallised from dilute ethanol. The compounds of this category are recorded in Table (I).

3-Aryl-amido/imido-methyl-4-hydroxy-phenyl-acetophenone semicarbazones (II)
3-Aryl-amido/imido-methyl-4-hydroxy-phenyl-acetophenone (I) (0.1 mole) , semicarbazide, hydrochloride (0.15 mole) and fused sodium acetate (1 gm) in ethanol (50 ml.) were heated under reflux on a water bath for 2 h. Subsequently, the solvent was distilled off. The solid thus isolated was washed with water to remove the inorganic materials. It was dried in a vacuum desiccator and crystallised from ethanol. Semicarbazones thus synthesized, are presented in Table (II).

3-Aryl-amido/imido-methyl-4-hydroxy-phenyl-5'-phenyl-3'-oxazolyl-aceto-phenone azines (III)
A mixture of 3-Aryl-amido/imido-methyl-4-hydroxy-phenyl-acetophenone semicarbazones (II) (0.01 mole), acetophenone (0.01 mole) and iodine (0.1 gm) in glacial acetic acid (50 ml.) was refluxed for 8 h. on a sand bath. The reaction mixture was cooled and the separated solid was filtered off. It was repeatedly washed with water and dried in a vacuum desiccator. The crude azines thus synthesized were crystallized from methanol. The compounds of this category are recorded in Table (III) along with their characterization data.

Biological Activity
All the four compounds were screened for their antiviral activity against three viruses viz., Influenza A (H_3N_2), Japanese encephalitis virus (P 20778) and Herpes simplex virus (753166).

Maintenance of Virus
Japanese Encephalitis Virus (JEV):
It was maintained by intracranial inoculation in 1-3 days old suckling albino swiss mice. The brains of the infected mice with specific paralytic symptoms were triturated and 10% homogenate (W/V) was made in phosphate buffered saline (PBS) pH 7.2. Virus 'was titrated by two-fold serial dilutions and LD_{50} was calculated.[9]

Herpes Simplex Virus Type I (HSV)
Virus was maintained in 5-6 gms albino swiss mice by the same route as JEV. The brains of infected mice with peculiar paralytic symptoms were collectively triturated with MEM and 10% homogenate (W/V) was made. The virus was titrated for its final concentration by calculating the LD_{50}.

Influenza Virus
Virus was maintained in 10 days old embryonated hen's eggs by inoculating it in allantoic cavity. The fluid was harvested after 48 hours. The virus titre was established by haemagglutination method with fowl RBC[10].

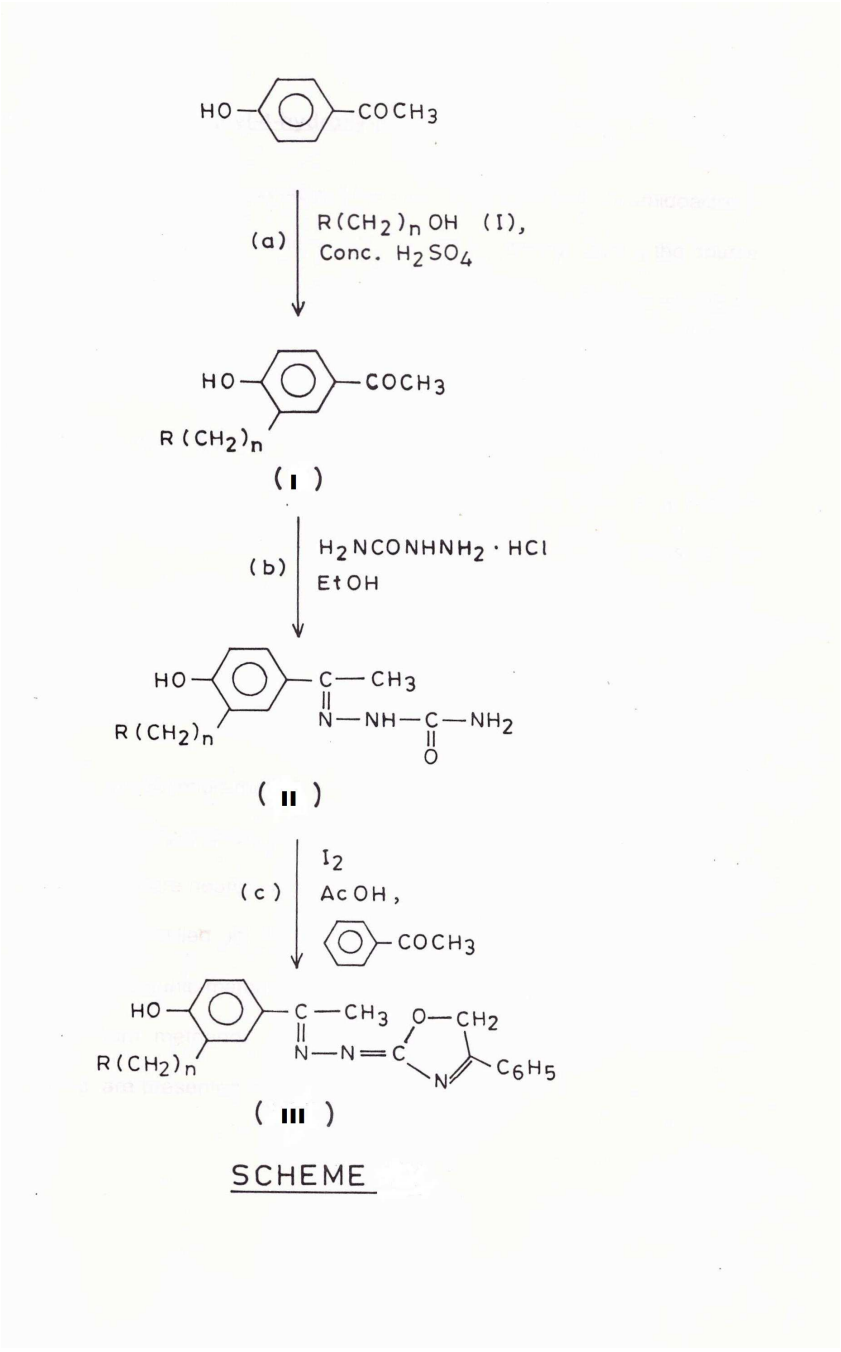
Maintenance of cells
Vero cells were maintained with MEM supplemented with 10% foetal bovine serum, 100 units of penicillin G, 100 micro gram of streptomycin and 40 micro gram of gentamycin/ml.

Antiviral Assay (*in vitro*)
The antiviral assay was done according to sidewell and Hauffmann[11]. Vero cells were seeded in 96 well micro titre plates and incubated at 37^0C for 24 hours. The confluent monolayers were inoculated with 100 $TCID_{50}$ concentration of the test virus and incubated for 90 minutes at 37^0C for adsorption of the virus. The plate was washed with MEM and treated with test compound(s) in two-fold serial dilutions. The test plate were incubated for 48-50 hours and stained with crystal violet. The plates were now observed for cytopathic effects like swelling, distortion and sloughing of cells. The toxicity of the test compound(s) was established by treating the cells with two-fold serial dilutions of the compound(s) and observing the state of cells after 18 hrs.

***In Vivo* (in mice)**
HSV I - The test compound (s) were administered in 15-16 gm swiss mice at the concentration of 0.5 mg/dose (6 mice/compound). After 18 hrs. the animals were challenged with 5-10 LD_{50} of the virus followed by two doses of the test materials at 24 h. intervals. The animals were observed for viremia, paralysis, mortality and increase in average survival time (AST) as compared to untreated control as detailer, Kant, et al, 1997[12].

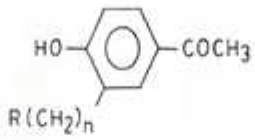
Influenza *(in vivo)*

For in ovo screening, the test compounds at the concentration of 0.5 mg/embryo were administered in the allantoic cabity of the 10 days old embryonated eggs (6 embryos/compound). These embryos were then challenged with 0.064 HA units of virus form the same route i.e. allantoic cavity, simultaneously after virus infection. These eggs were incubated at 37^0C for 48-72 hrs. The mortality of the embryos were observed and the allantoic fluid was haevested from each batch of the eggs. The haemagglutination was don with the fowl RBC and the presence of virus was calculated[13].



SCHEME

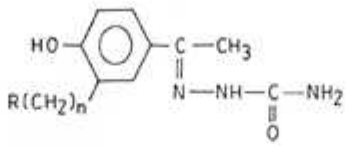
TABLE – I



3-Aryl-amido/imido-methyl-4-hydroxy-phenyl acetophenone (I)

Compound No.	R	m.p. (°C)	Molecular Formula	Nitrogen analysis (%)	
				Calcd.	Found
1.	Nicotinamido-	124	$C_{15}H_{14}N_2O_3$	10.37	10.02
2.	Benzamido-	198	$C_{16}H_{15}NO_3$	5.21	4.94
3.	Salicylamido-	86	$C_{22}H_{19}NO_4$	3.87	3.48
4.	Phthalimido-	114	$C_{17}H_{13}NO_4$	4.75	4.37

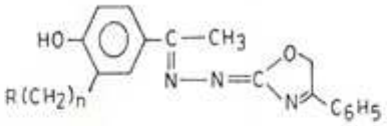
TABLE - II



3-Aryl-amido/imido-methyl-4-hydroxy-phenyl-acetophenone-semicarbazones (II)

Compound No.	R	m.p. (°C)	Molecular Formula	Nitrogen analysis (%)	
				Calcd.	Found
1.	Nicotinamido-	226	$C_{16}H_{17}N_5O_3$	21.41	21.04
2.	Benzamido-	130	$C_{17}H_{18}N_4O_3$	17.17	16.85
3.	Salicylamido-	146	$C_{23}H_{22}N_4O_4$	13.39	13.12
4.	Phthalimido-	132	$C_{18}H_{16}N_4O_4$	15.91	15.56

TABLE -III



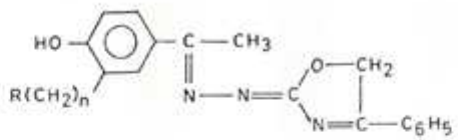
3-Aryl-amido/imido-methyl-4-hydroxy-phenyl-5'-phenyl-3'-oxazolyl-acetophenone azines (III)

Compound No.	R	m.p. (°C)	Molecular Formula	Nitrogen analysis (%)	
				Calcd.	Found
III*a.	Nicotinamido-	240	$C_{34}H_{21}N_5O_3$	16.34	16.08
IIIb.	Benzamido-	semi solid	$C_{35}H_{22}N_4O_3$	13.14	12.86
IIIc.	Salicylamido-	155	$C_{31}H_{20}N_4O_4$	10.81	10.42
IIId.	Phthalimido-	158	$C_{30}H_{20}N_4O_4$	12.38	12.13

*IR (KBr): 1620 (C=N), 1680 (amide CO), 3264 and 3148 (N-H Str.) and 3730 (ArOH) cm^{-1}.

*^{1}H-NMR ($CDCl_3$): δ 6.1-7.7 (m,12H ArH), δ 1.2 (S,3H,CH_3), δ 3.2 (d,2H,CH_2NH), δ 4.1 (S,2H,CH_2O) and δ8.7 (brs, 1H,NH).

TABLE -IV



3-Aryl-amido/imido-methyl-4-hydroxy-phenyl-5'-phenyl-3'-oxazolyl-acetophenone-azines

Antiviral activity against JEV in vitro

Compound No.	R	Dose (μg mL^{-1})	Maximum 50% cytotoxic concentration	Effective Concentration	Therapeutic index	Protection of Cells
III^a	Nicotinamido-	500-4	125.0	31.5	4.0	50.0
III^b	Benzamido-	500-4	31.2	----	None	None
III^c	Salicylamido-	500-4	62.5	----	None	None
III^d	Phthalimido-	500-4	125.0	31.5	4.0	75.0

TABLE - V

Follow-up study of in vitro active compounds against JEV in-vivo

Compound No.	Dose mg/mouse/day	Percent Protection	Increase in average survival time (days) as compared to control
III^a	0.5	None	3.2
III^d	0.5	40.0	9.0

TABLE - VI

In-vivo Antiviral activity against Influenza A, HSV-I

Compound No.	R	Influenza A % Inhibition	HSV-I % Protection
III^a	Nicotinamido-	60	None
III^b	Benzamido-	40	25
III^c	Salicylamido-	None	None
III^d	Phthalimido-	None	None

RESULTS AND DISCUSSION

The antiviral activity data recorded in Table (IV), (V) and (VI) show that out of four compounds which were screened for their antiviral activity against three viruses, the compound No. III^a was found to exhibit virus inhibitory activity to the ectent of 50% against JEV and 60% against Influenza A. However, this compound was completely inactive against Herpes Simplex virus I. The compound No. III^d was found to exhibit virus inhibitory activity to the extent of 75% against Japanese encephalitis virus, in vitro and 40 % in vivo. It is very interesting to observe that the same compound (III^d) showed virus inhibitory property to the extent of 40% against influenza A and 25% against HSV-I.

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REFERENCES

[1] do Valle, L.A., et al. , *Lancet*, **1965**, 2, 976.
[2] Thompson, R.L., et al., J. Immunol, **1956**, 70, 229.
[3] Levy, J.A., Levy, S.B. and Levinson, W., *Virology*, **1976**, 74, 426.
[4] Mikelens, P.E., Woodson, B.A. and Levinson, W.E., *Bio-chem. Pharmacol*., **1976**, 25, 821.
[5] Levinson, W.E., Faras, A., Morris, R. Mikelems, P., Ringold, G., Kass, S., Levinson, B., and Jackson, J., in Virus research (ICN-UCLA Symposium), Fox, F., and Robinson, W., Eds., Academic Press, New York, 1973, 403.
[6] Schauer, P., Likar, M., Karbavcic, A. and Pollak, A., *Pathol-Microb*., **1965**, 28, 382.
[7] Schauer, P., Karbavcic, A., Tisler, M., and Likar, M., *Experimentia*, **1966**, 22, 304.
[8] Schauer, P., Likar, M., and Klement-vebek, S., *Ann. N.Y. Acad. Sci*., **1970**, 173, 603.
[9] Reed, L.J., and Muench, H.A., *Amer. J. Hyg*., **1938**, 27, 493-497.
[10] Babbar, O.P., Joshi, M.N., Madan, A.R., *Indian. J. Med. Res*., **1982**, 76 (Suppl.) 54-65.
[11] Sidwell, R.A., and Hoffman, T.H., *Appl. Microbiol*., **1971**, 22 (5), 797-801.
[12] Kant, R., Chandra, K., Gupta, B.M., Mathur, K.B., Kishor, K. and Dhar, M.M., *Indian J. Med. Res*. ,**1977**, 3, 314-319.