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STUDIES ON THE ARILDONE CLASS OF ANTIVIRAL AGENTS

BY

BALJIT BETTADAPUR

A thesis
submitted to the Faculty of Graduate Studies and Research
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of Master of Science
in
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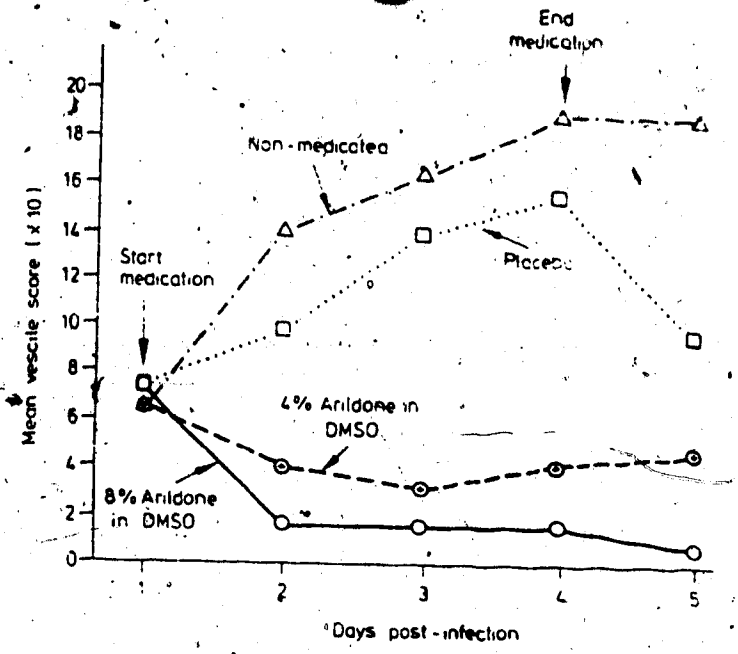

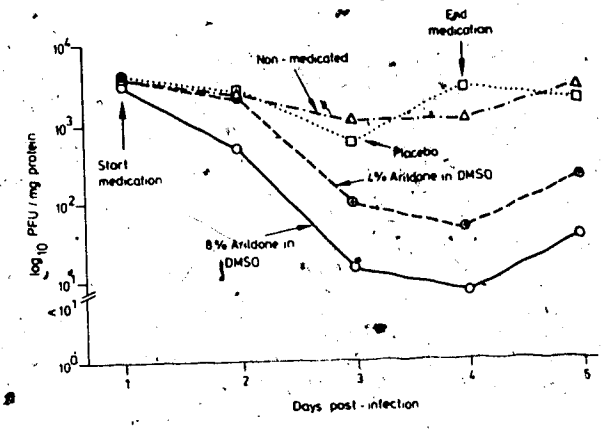

Fig. 4. The effect of 8% and 4% arildone in 90% DMSO on the virus content of herpetic lesions in guinea pigs. Animals were infected and medication applied as described in Fig. 3. The infected site was scraped into balanced salt solution with a sterile scalpel, virus was released by sonication, and large debris was removed by low speed centrifugation. The virus in the supernatant was quantitated by plaque formation and protein analysis.

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ABSTRACT

Antiviral chemotherapy has presented a major challenge to medical science. Numerous antiviral compounds have been reported in the literature, but only a few of them have been studied clinically and very few are available on the market. These drugs are effective only against specific viruses. The search for effective antiviral agents which have a broad spectrum of activity with low toxicity is continuing.

A novel class of antiviral agents(the aryloxyalkyl diketones), was recently discovered and found to possess broad spectrum antiviral activity. Further studies on this series have resulted in chemotherapeutic agents such as 4-[6-(2-chloro-4-methoxyphenoxy)hexyl]-3,5-diethyl-1H-pyrazole methanesulfonate, 6 (WIN 41258-3), 5-[7-(2-chloro-4-methoxyphenoxyheptyl]-3-methylisoxazole, 9 (WIN 49321) and 5-[7-[4-(4,5-dihydro-2-oxazolyl)phenoxy]heptyl]-3-methylisoxazole, 15 (WIN 51711), with activity against rhinovirus-2, poliovirus-2 and herpes simplex viruses. Further investigation, based on viral receptor studies, on the modification of 15 (WIN 51711) has resulted in the compound 15a (WIN 52084) with a $-CH_3$ substituent at the 4-position of the oxazoline ring. This compound 15a was found to possess the best activity against human rhinovirus-2 and -14.

This research project involves a systematic structure-activity study of compounds such as WIN 41258-3, WIN 49321 and WIN 51711, in which I determined the effect of changes in structural components of the molecule on activity. The effect of changes in the heterocyclic

system, the aryl system and in the connecting (spacer) carbon chain on activity were studied. The compounds prepared were tested in vitro by a plaque reduction assay method against herpes simplex virus 1 in Vero cells. Our studies indicate that changes in the heterocyclic moiety such as the use of isoxazoles, pyrazoles, isothiazoles, triazoles and imidazoles result in active compounds. Compounds with the -O-aryl system are also active but the activity decreases when the -O-aryl is replaced by -Br and a heteroaryl group. Compounds with -O-heteroaryl systems are also active. Connecting carbon chain lengths of 6 and 7 in the compounds result in comparable activity and produce the most active compounds. The inclusion of sulfoxide or sulfone groups in the molecule enhances the activity.

I believe that our work has led to the development of several new compounds which have antiviral activity particularly against herpesvirus.

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LIST OF ABBREVIATIONS

Ar	Aromatic
ATCC	American type culture collection
bp	Boiling point
BSC-1 cells	A line of African green monkey kidney cells
C	Centigrade
CPE	Cytopathic effect
d	Doublet
δ	Delta
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DS	Double strand
g	Gram
HeLa cells	A line of human epitheloid carcinoma cells
HSV	Herpes simplex virus
Hz	Hertz
IR	Infrared
L-cells	A line of mouse fibroblasts cells
m	Multiplet
MEM	Minimum essential medium
MIC_{50}	Minimal inhibitory concentration required to reduce the number of plaques formed by a given virus preparation by 50%
ml	Milliliter
mp	Melting point
mRNA	Messenger ribonucleic acid

n_{20}	Refractive index
NMR	Nuclear magnetic resonance
Pfu	Plaque forming unit
RNA	Ribonucleic acid
RV	Rhinovirus
s	Singlet
SS	Single strand
t	Triplet
TK	Thymidine Kinase
ug	Microgram
uM	Micromolar
Vero Cells	A line of African green monkey kidney cells
VP1	Virion protein 1
	Five-membered heteroaromatic ring

Chapter 1

INTRODUCTION

1.1. Viruses as infectious agents

Microorganisms such as bacteria, fungi and protozoa consist of living cells which possess sufficient biochemical machinery for their replication. They synthesize their own structural and functional macromolecules; their genetic information is stored, transmitted, and modified in the form of double-stranded DNA, and they reproduce without losing the fundamental integrity of their basic cellular unit. Viruses constitute a unique class of infectious agents. They are acellular infectious particles which are incapable of metabolic activity outside the living cells. Their genetic information is stored either in the form of DNA or RNA, and can only be expressed within a living cell, at the expense of that cell's own energy and metabolic machinery.

Viruses infect virtually all living organisms. Many different viruses are known which infect human beings. It is estimated that viruses cause more than 60% of the infectious diseases, including the common cold, influenza, bronchitis, hepatitis, herpes genitalis and labialis, poliomyelitis, gastroenteritis, and rabies, as well as many common childhood diseases like chicken pox, measles and mumps. One of the most dreadful diseases recently encountered is Acquired Immune Deficiency Syndrome (AIDS), is also caused by a virus.

1.2. Treatment of viral infections

1.2.1. Problems in antiviral therapy

The development of compounds useful for the prophylaxis and therapy of viral diseases has presented more difficult problems than those encountered in the search for chemotherapeutic agents effective in disorders caused by other microorganisms. This is because, in contrast to most other infectious agents, viruses are obligate intracellular parasites that require the active participation of the metabolic processes of the invaded cell.

Structure of a virion

A virion, a mature virus particle, contains one type of nucleic acid, either RNA or DNA in single-stranded or double-stranded form. Most DNA-containing viruses have double-stranded (DS) DNA, while most of the RNA viruses have single-stranded (SS) RNA. The viral nucleic acid is surrounded by a protein coat, called the capsid (Figure 1.1). The nucleic acid and the capsid together constitute the nucleocapsid. The capsid is made up of a large number of capsomers held together by noncovalent bonds. Each capsomer consists of one or more polypeptide chains. The capsid may be naked or enclosed within a lipoprotein envelope. Some virions have virus specific enzymes such as transcriptases or polymerases.

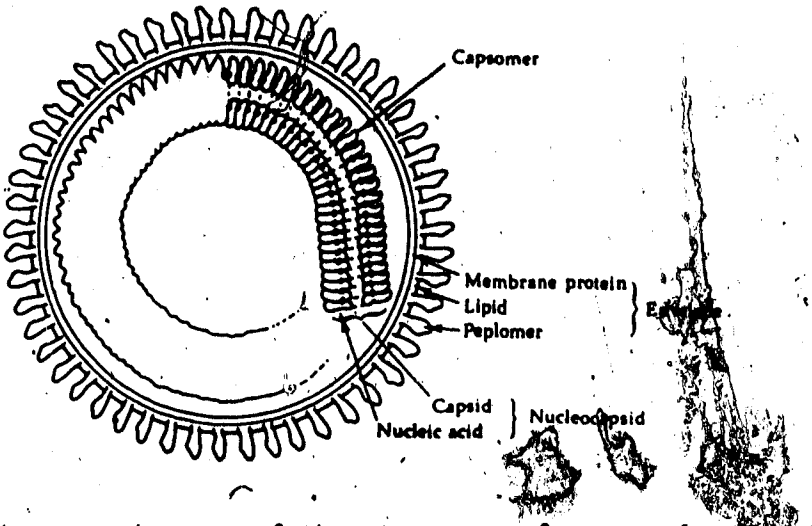


Figure 1.1 Schematic diagram of the structure of an enveloped virion (taken from Ref. 1 with permission)

Viruses have a unique mode of reproduction as they multiply only within the host cells and are totally dependent on them for replication. With some viruses, for example, herpes simplex virus (HSV), various structural components are synthesized in different compartments of the host cell and are finally assembled into whole virions.

The major problem in chemotherapy is one of selective toxicity: How to inhibit or interrupt the cycle of viral replication without interfering with the macromolecules of the host cell? Viral growth can be inhibited by compounds which interfere with RNA or DNA synthesis, but these substances often affect both virus and host cells. The absence of selective toxicity limits the possible therapeutic use of many potential antiviral agents.

Another problem in antiviral chemotherapy is that most of the

available antiviral agents need to be administered early in the treatment of viral infections. Thus an agent may not be effective in treating a viral infection with a long incubation period because the virus may be well disseminated by the time symptoms of the disease develop.

Drug resistance has also become an important consideration as some viruses susceptible to antiviral agents have become resistant to the drug.

1.2.2. Requirements for an effective antiviral agent

Considering the above problems (toxicity, early administration and drug resistance), we need to design effective antiviral drugs, with broad spectrum activity, that can satisfy the following requirements. The antiviral agent should

(a) be sufficiently potent to completely inhibit viral replication because incomplete inhibition may only prolong the disease.

(b) have favorable pharmacokinetic properties so that drug can reach the target organ where viral infection may be localized.

(c) lack immunosuppressive activity.

(d) not be susceptible to the development of resistant viral variants.

(e) have selective toxicity; should not be toxic to the host cell.

1.2.3. Antiviral treatment presently available

A. Interferons and their inducers

In response to a variety of inducing stimuli, vertebrate cells

synthesize proteins known as interferons, which confer an antiviral state on host cells through mechanisms that require further cellular RNA and protein synthesis. The production of these protein interferons is the main defense mechanism of the body during the acute phase of a viral infection. The three types of interferons, Alpha, Beta and Gamma, are produced by leukocytes, fibroblasts and T-lymphocytes respectively.

In most cases, these interferons inhibit viral replication by preventing the translation of viral mRNA[2]. Interferons can be produced in vitro by inducing their synthesis in lymphocytes with inactivated viruses, bacterial endotoxins and dsRNA[3,4,5]. Interferons have been cloned and expressed in bacteria[6,7]. Since topical interferon is rapidly cleared from the nasal passage[8], purified and recombinant interferon can be used for the treatment of respiratory viral infections[9].

In clinical situations, the effects of interferons have been studied[10]. They may be useful in life-threatening infections[11] such as hemorrhagic fevers, and herpesvirus caused encephalitis, and may also eliminate persistent[11,12] infections caused by hepatitis B virus, herpes zoster virus, papilloma (wart) viruses, and cytomegalovirus.

Unfortunately, it appears that the interferons, in therapeutic doses, may cause some undesirable side effects[13] such as fever, hypotension, and impaired liver function. Interferon may still be potentially the best broad-spectrum antiviral, but it has not yet been

shown to have sufficient efficacy to be generally useful to the clinician. It does not satisfy the need for an effective broad spectrum antiviral drug which has minimum side effects.

B. Vaccines

The most successful approach to the prevention of viral infection is the use of a potent virus vaccine. This can be produced either by inactivation of an infectious virus or by development of live attenuated virus strains. A number of live attenuated vaccines[14] are available, for example, yellow fever, rubeola, rubella, mumps and poliomyelitis. Inactivated vaccines[14] are also available, for example, prevention of influenza, poliomyelitis and rabies. A new vaccine approved for hepatitis-B[15], produced from its surface antigens (HB_5Ag) found in human blood. An exciting development is the preparation of synthetic vaccine as in the case of the poliovirus[16].

Unfortunately, vaccines are not available for all viruses. The rhinoviruses consist of over 100 different serotypes and vaccination would be the most unlikely procedure for prevention[17] because of the specificity of the immune reaction of the vaccines. The influenza-A virus presents another problem as it continuously changes its antigenic composition[17]. Recombinant herpesvirus can be used as genital herpes vaccines[18,19], free of those genes responsible for oncogenicity.

In the absence of an effective vaccine for interaction with the target virus, we depend on the development of antiviral drugs.

C. Antiviral Chemotherapy

Extensive study has led to the discovery of many antiviral agents with selective activity. These can inhibit a particular event of viral replication. Different events in the replication of viruses and the compounds known to inhibit these events will be discussed in the following sections.

I. Early events of replication

Virus Adsorption

The process of infection begins with the interaction of virus and specific receptors on the surface of cells.

Virus Penetration

Adsorbed virions can penetrate by several mechanisms —

(a) Endocytosis: This results in the delivery of the virion to endocytic vesicles where viral envelopes fuse with the vesicle membrane, resulting in the release of nucleocapsids into the cytoplasm[20]. This is the most common mechanism.

(b) Fusion of the virion envelope with plasma membrane which enables the nucleocapsid to enter cytoplasm[21].

(c) Direct penetration of the virion into the cytoplasm[21]. This mechanism is quite uncertain, but may be necessary to account for penetration of non-enveloped virions.

Virus Uncoating

Following penetration, the viral protein coat opens to release

the nucleic acid contents into the cytoplasm. With some enveloped virions, uncoating occurs at the time of penetration while with others, uncoating takes place near nuclear pores[22].

A number of compounds are known which inhibit these early events of the viral replicative cycle:

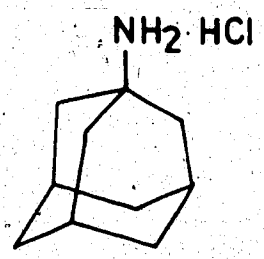
HEPARIN (an acid mucopolysaccharide)

Heparin forms a complex with HSV and prevents its adsorption to the host cell. Therefore, it is no longer effective if added after the adsorption of intact virions to cells[23].

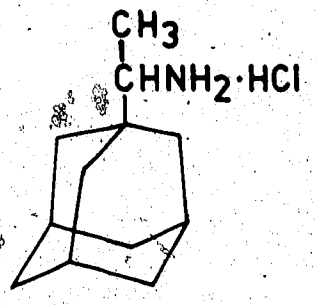
OLIGOPEPTIDES

Oligopeptides that mimic the N-terminal region of the paramyxovirus F_1 polypeptide are specific inhibitors of paramyxovirus and oligopeptides that mimic the N-terminal region of the orthomyxovirus HA_2 polypeptide inhibit influenza viruses[24,25]. Different viruses are inhibited by different oligopeptides. The heptapeptides Z-D-Phe-L-Phe-Gly-D-Ala-D-Val-D-Ile-Gly is the most potent inhibitor of measles virus and the tetrapeptides Z-Gly-L-Leu-L-Phe-Gly and Z-Gly-L-Phe-L-Phe-Gly are the most important inhibitors of influenza A and B virus respectively[25]. These oligopeptides inhibit the penetration of the virus into the cell which occurs by fusion of the viral membrane with the cell membrane[26].

AMANTADINE AND RIMANTADINE



AMANTADINE



RIMANTADINE

Both amantadine and rimantadine inhibit the replication of type A influenza viruses without serious side effects[27,28,29]. Although the exact mechanisms of action are not known, it has been shown that both affect a virus-specific process that takes place between uncoating and primary transcription[30,31]. Studies on the effect of amantadine on rhabdovirus has indicated that the drug inhibits viral uncoating and the later steps, but not the viral attachment and penetration[32].

ARILDONE

Arildone is active against many RNA and DNA viruses. It interacts directly with the poliovirus capsid and inhibits the uncoating of the virus. It is expected that arildone interacts in a similar manner with herpesvirus[33] because capsid is the common structure in both the viruses.

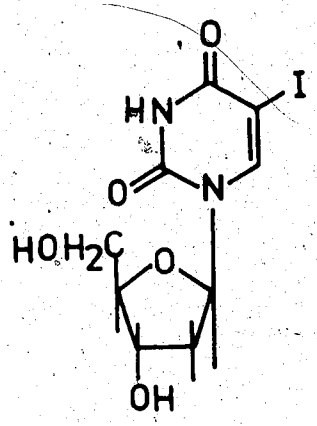
II. Synthetic Events of replication

After viral uncoating, the transcription of early mRNA from viral nucleic acid starts. Sometimes, virions may need their own

transcriptases or reverse transcriptases. These early mRNAs translate into proteins on cytoplasmic polyribosomes. Once these enzymes are synthesized, the replication of the viral nucleic acid starts, followed by the transcription of late mRNAs, which translate to structural and virus-coded proteins.

Agents inhibiting these events are:

5-IODO-2'-DEOXYURIDINE (IDOXURIDINE) IdUrd



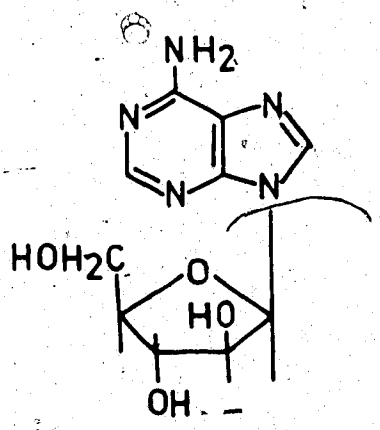
IdUrd was synthesized by Prusoff[34,35] as an anticancer agent and found to be active against HSV and vaccinia virus in vitro, and herpes simplex keratitis in rabbits[36] and in monkeys[37]. In humans, it was found to be active against ocular herpesvirus infections. Idoxuridine in DMSO has shown some benefits on local symptoms in herpes-zoster[38].

Mechanism of action

IdUrd replaces thymidine in newly synthesized viral DNA[39] and its metabolites (mono-, di- and tri-phosphates) can also act as substrates

for many enzyme systems, for example, deoxythymidine kinase, deoxycytidylate deaminase, cytidine diphosphate reductase and DNA polymerase[40]. IdUrd is phosphorylated in both virus-infected and uninfected cells by both virus-induced and cellular deoxythymidine kinase[41]. The incorporation of IdUrd into the DNA of normal cells causes inhibition of cellular DNA synthesis, which is responsible for the toxicity[39,42].

9-β-D-ARABINOFURANOSYLADENINE (VIDARABINE) Ara-A



Vidarabine was synthesized as an anticancer agent[43] and found to be active against herpesviruses. In humans, it has been shown to be highly effective in herpes simplex keratitis[44], herpes simplex encephalitis[45] and varicella-zoster virus[46,47]. Recent studies have indicated that Ara-AMP[48] can be used for the treatment of chronic type B hepatitis and Ara-A[49] can be used for the treatment of herpes-zoster in cancer patients.

Mechanism of action

In herpesvirus infected cells, Ara-A inhibits DNA synthesis[50]. The metabolism of this nucleoside analogue to its 5'-triphosphate

(Ara-ATP) is first required, and then DNA synthesis may be inhibited by several possible mechanisms:

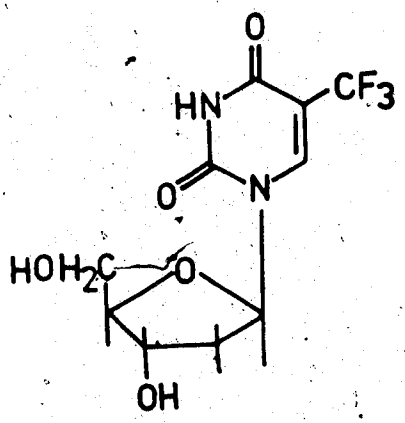
(a) Inhibition of the virus-specific DNA polymerase[50,51]

(b) Inhibition of the virus-specific ribonucleotide reductase[52]

(c) Incorporation of Ara-AMP into viral DNA[53]

Decreased methylation of macromolecules results from the inhibitory effect of vidarabine on the transmethylases, and this in turn results in cytotoxicity[54].

5-TRIFLUOROMETHYL-2'-DEOXYURIDINE (TRIFLURIDINE) F_3dThd



Trifluridine was first synthesized by Heidelberger et. al.[55]. This compound has shown marked efficacy in the treatment of HSV eye infections in rabbits and humans[56,57,58]. It has shown some activity against cytomegalovirus[59] also. It has 10-fold greater antiviral potency and 10-fold greater solubility in aqueous solution than idoxuridine[39].

Mechanism of action

Trifluridine incorporates into viral DNA and thus inhibits late virus-specific mRNA synthesis. It is readily phosphorylated in

eukaryotic cells by thymidine kinase to mono-, di- and tri-phosphates. The parent compound and its metabolites can affect DNA synthesis of both virus and cell at four different biochemical sites[60].

(a) Inhibition of deoxythymidine kinase by F_3dThd

(b) Inhibition of deoxythymidylate synthetase by F_3dTMP

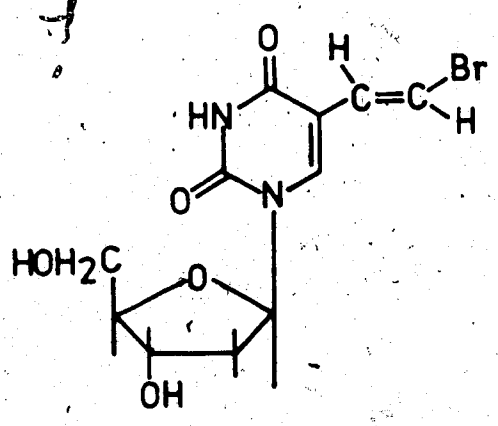
(c) Inhibition of DNA polymerases by F_3dTTP

(d) DNA strand breakage by incorporation of this thymidine analogue into DNA.

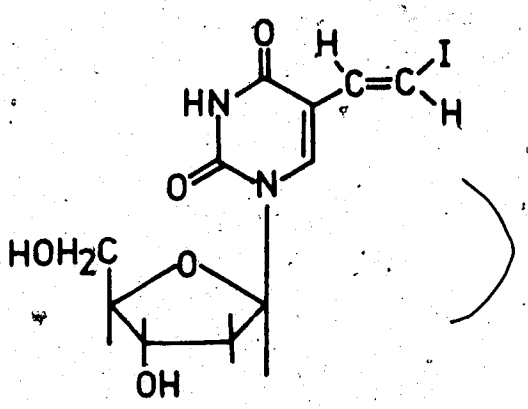
Toxicity is observed because the drug incorporates into the DNA and causes sister chromatid exchange in human cells[61].

(E)-5-(2-BROMOVINYL)-2'-DEOXYURIDINE (BVdU) and

(E)-5-(2-IODOVINYL)-2'-DEOXYURIDINE (IVdU)



BVdU



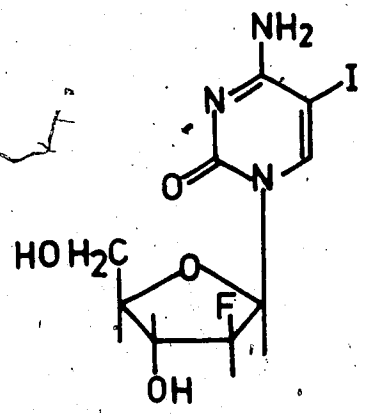
IVdU

Both BVdU and IVdU have been found to be selective antiherpes agents[62,63] and are specifically phosphorylated in infected cells by HSV-1 and varicella-zoster virus-induced deoxythymidine (dThd) kinase[64,65,66]. Recent studies have indicated that BVdU can be used orally for the treatment of varicella and zoster in children with

cancer[67] and for severe herpes-zoster in cancer patients[68]. The HSV-1 induced dThd-dTMP kinase converts BVdU to BVdUMP and then to its 5'-diphosphate (BVdUDP), whereas the HSV-2 induced dThd-dTMP kinase phosphorylates the BVdU to only the 5'-monophosphate stage. Therefore, the activity of BVdU and IVDU were observed only against HSV-1, when studied in mice[69].

BVdUDP is converted to the triphosphate by cellular kinase, and this triphosphate inhibits henpesvirus-induced DNA polymerase to a greater extent than cellular DNA polymerases[70].

1-(2'-DEOXY-2'-FLUORO-β-D-ARABINOFURANOSYL)-5-IODOCYTOSINE (FIAC)

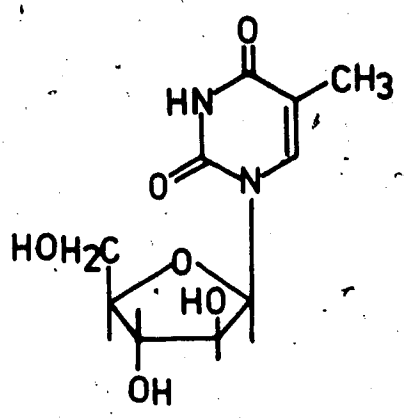


FIAC is active against HSV-1, HSV-2, herpes-zoster virus, and human cytomegalovirus[71]. This selective activity is due to inhibition of the virus coded DNA polymerase[72].

Several other compounds in the series, such as 1-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-5-iodouracil (FIAU) and its corresponding

5-methyl analogue (FMAU) and 5-ethyl analogue (FEAU) are active against herpesviruses[73]. Comparative efficacy and selectivity of these nucleoside analogues against Epstein-Barr virus have indicated[74] that relative potential of these drugs is FIAC = FIAU > FMAU.

1-β-D-ARABINOFURANOSYLTHYMINE (Ara-T)



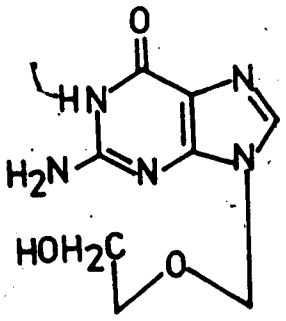
Activity has been reported with Ara-T in vivo against herpes simplex keratitis in rabbits[75] and HSV encephalitis in mice[76].

Ara-T is phosphorylated to Ara-TTP by the herpesvirus-induced deoxypyrimidine kinase in infected cells[77]. Mutant strains of HSV that are incapable of inducing this virus-specific enzyme are not susceptible to inhibition by Ara-T[78]. Toxicity results from the incorporation of Ara-TMP into cellular DNA[79].

9-(2-HYDROXYETHOXYMETHYL)GUANINE (ACYCLOVIR)

Acyclovir is active against herpesviruses, especially herpes zoster virus, HSV-1 and HSV-2[80] but inactive against vaccinia virus, adenovirus, cytomegalovirus and RNA viruses[81]. Recent studies

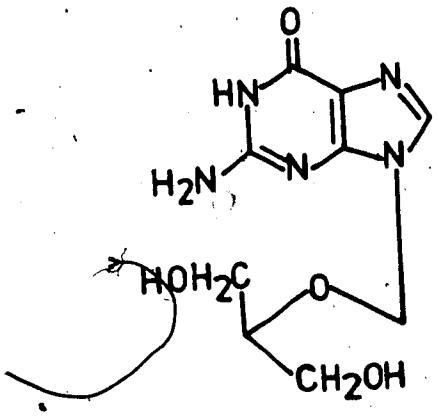
indicate[82] the effective use of acyclovir for the prophylaxis of herpes infections in renal transplant recipient. It can be used for varicella-zoster infection[83,85] and genital HS infection[84]. Inhibition of human cytomegalovirus (HCMV)[86] by combined acyclovir and vidarabine was found to be synergistic for three of four HCMV clinical isolates studied and additive for one HCMV isolate. Similar effect was observed when acyclovir and recombinant human alpha interferon were administered for HSV-1 infection in mice[87]. Acyclovir is converted to its monophosphate by a viral-specified thymidine kinase[88,89], further phosphorylated to the diphosphate by host cell guanosine monophosphate kinase[90] and to the triphosphate by unidentified cellular enzymes[91]. This triphosphate inhibits HSV-1 viral DNA polymerase[92] but does not affect cellularα-DNA polymerase at the same concentration[93]. Mutant strains are resistant to acyclovir[94].



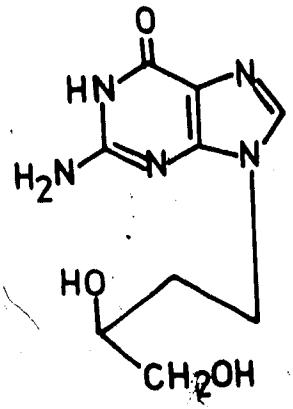
Two other guanosine analogues are

9-(1,3-DIHYDROXY-2-PROPOXYMETHYL)GUANINE (DHPG)

9-(3,4-DIHYROXYBUTYL)GUANINE (DHBG)



DHPG

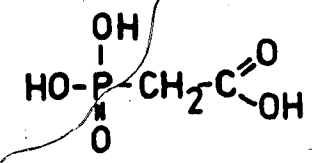


DHBG

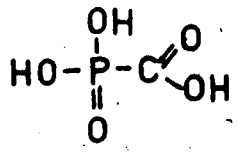
Like acyclovir, RS-DHBG[95] and DHPG[96] are phosphorylated by herpesvirus TK. The phosphorylation to mono-, di-, and tri-phosphate is more efficient for DHPG than for acyclovir[97,98], because DHPG has a hundred times higher affinity than acyclovir towards viral TK. DHPG is also significantly more active than acyclovir against human, monkey and rodent cytomegalovirus[99]. DHBG and DHPG are similar to acyclovir in activity and mode of action.

PHOSPHONOACETIC ACID (PAA)

PHOSPHONOFORMIC ACID (PFA, FOSCARNET)



PAA

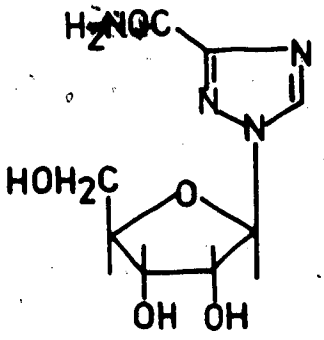


PFA

PAA has been shown to selectively inhibit the replication of herpesviruses[100,101]. It exerts its antiviral activity by phosphorylation with HSV TK and preferential inhibition of the herpesvirus-specified DNA-polymerases[102]. It has been reported[103] that partially purified HeLa cell DNA polymerase is also affected by PAA. The related compound PFA selectively blocks the replication of herpesviruses as effectively as PAA[104,105,106] and these two agents have similar mechanisms of action.

1-β-D-RIBOFURANOSYL-1,2,4-TRIAZOLE-3-CARBOXAMIDE (RIBAVIRIN)

Ribavirin possesses broad spectrum antiviral activity against both RNA and DNA viruses[107,108]. The drug has been effective in the treatment of influenza A and B infections[109,110]. For antiviral



action, it is converted to its 5'-mono-, di- and triphosphates by cellular enzymes[111] and these nucleotides act upon several different enzymes. Ribavirin-5'-monophosphate inhibits the synthesis of guanosine 5'-monophosphate (GMP), resulting in the lowering of GTP levels in the cell and inhibition of nucleic acid synthesis[112].

Ribavirin inhibits guanine deaminase and therefore the formation

of dTTP[113]. In HSV, DNA synthesis is inhibited by the inhibition of the virus-induced DNA polymerase by ribavirin 5'-triphosphate[114], which also selectively inhibits the influenza virus-associated RNA polymerase and also inhibits virus-specific protein synthesis[115]. Ribavirin 5'-triphosphate also inhibits vaccinia virus-associated mRNA guanyl transferase activity and blocks the capping of virus-specific mRNA[116]. Ribavirin inhibits tobacco mosaic virus replication[117] by preventing an early function that is necessary to initiate viral RNA synthesis. Resistance against ribavirin has not developed probably because of its multiple sites of antiviral action.

III. Late events of replication

Assembly and Release[118]

There are two general mechanisms involved in assembly and release. In one case, the newly synthesized viral nucleic acids and structural proteins come together intracellularly, either in the cytoplasm or the nucleus. The virions are released as the infected cells disintegrate. In the other strategy, employed by enveloped viruses, viral proteins are inserted into cell membranes, the nucleocapsid is attached on the cytoplasmic side of the membrane and the virus buds into the extracellular environment.

Compounds inhibiting these events are:

2-DEOXY-D-GLUCOSE AND MODIFIED POLYSACCHARIDES

2-Deoxy-D-glucose is effective against enveloped viruses[119]. It

impairs or alters the glycosylation of virus specific polypeptides[120]. Glucosamine also inhibits the multiplication of enveloped viruses by interference with the synthesis of viral envelope glycoprotein[121,122].

1.2.4. Limitations of currently available antiviral agents

As described in the previous sections, there are many antiviral agents known that interfere with virus-specific functions or interact with virus specified enzymes and thus inhibit particular phases of viral replication. However, very few of them have been approved by U.S. Food and Drug Administration and are available on the market. The problem is usually toxicity of the agent to the host cells. Antiviral drugs currently available[123] on the market are listed in Table 1.1.

Drug	Trade Name	Indication(s)
Acyclovir	Zovirax	Mucocutaneous herpes simplex infection in compromised hosts, Initial genital herpes simplex infections
Amantadine	Symmetrel	Prophylaxis and treatment of influenza A
Idoxuridine	Stoxil	Herpes simplex keratitis
Trifluridine	Viroptic	Herpes simplex keratitis
Vidarabine	Vira-A	Herpes simplex keratitis, Herpes simplex encephalitis, Neonatal herpes simplex CNS and disseminated infection

Table 1.1. Currently available antiviral agents

Even these drugs are limited in their usefulness as antiviral agents. Each has a narrow spectrum of activity, e.g. idoxuridine is useful only for herpes simplex keratitis and amantadine only for

respiratory infections caused by influenza A virus. In addition, it has been found that even some susceptible viruses become resistant to these drugs. A recent report[94] describes the drug resistance patterns of HSV isolated from patients treated with acyclovir. This type of resistance to thymidine kinase (TK)-dependent drugs may be due to the appearance of strains with TK deficienty, altered TK-substrate specificities or changes in viral DNA polymerase[124]. Since most of the available drugs are nucleoside analogues, it is believed that a fresh approach might circumvent the difficulties encountered with this class of compounds. Therefore, a new class arildone is being studied.

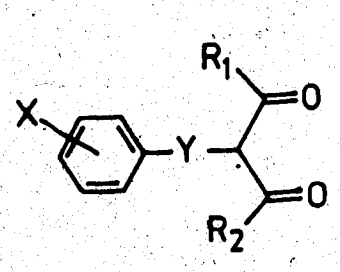
1.3. Arildone: A β-Diketone

1.3.1. Introduction

A new class of antiviral compounds, the aryloxyalkyl-β-diketones, is recently discovered. The most promising compound, arildone, has been extensively studied by Sterling-Winthrop Research Institute and is active against a wide variety of RNA and DNA viruses. Arildone and its metabolites[125] are well tolerated in animal toxicity studies. Currently, arildone is undergoing clinical trials for herpesvirus infection in humans.

1.3.2. Research leading to the discovery of arildone

During the course of routine screening of compounds for antiviral activity, it was discovered at the Sterling-Winthrop Research Institute that several aryl diketones[126] of the general structure(1) were active against equine rhinovirus in vitro.



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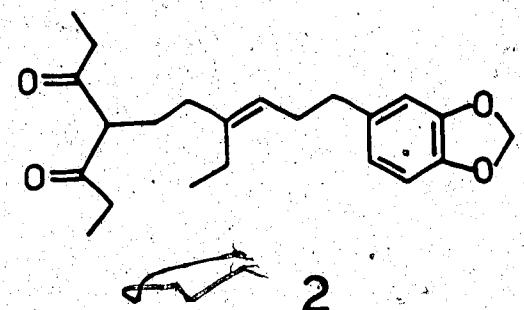

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The methylenedioxy diketone(2) was first prepared. Several analogues were synthesized to examine the following factors as they affect antiviral activity towards equine rhinovirus and HSV-2[126]:

(a) The size of the diketone moiety

(b) The necessity of the ethyl side chain in carbon spacer

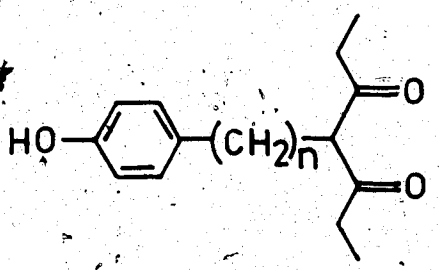
(c) The necessity of the double bond in the carbon spacer

(d) The effect of various substituents on the ring

(e) The size of the carbon spacer

No significant difference in activity against equine rhinovirus was observed between the corresponding heptanedione, hexanedione and pentanedione. Reducing the ethyl side chain in carbon spacer to methyl and finally to hydrogen caused a slight increase in activity(MIC_{50} from 6 to 3 ug/ml) Reduction of the double bond of (2) did not result in any loss of antiviral acitivity. A series of compounds was prepared by maintaining both hepatanedione and the hexamethylene spacer and varying the substituents on the phenyl ring[126]. The 4-hydroxy homologue was found to be active against equine rhinovirus (MIC_{50} from 3 to 6 ug/ml). It is believed that the role of the substituents on the phenyl ring is mainly to contribute to the lipophilicity of the

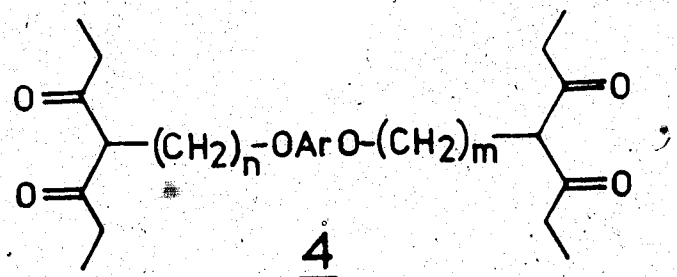
molecule. Further study[126] was done to examine the effect of the bridge between the diketone and aryl portion of the molecule. The peak activity was attained against equine rhinovirus with n=6 (3a, MIC_{50} =3 to 6 ug/ml) and was maintained through n=8 (3b). In the case of HSV-2, maximum activity was exhibited with 3b (MIC_{50} = 3 to 6 ug/ml) but not with 3a (MIC_{50} = 12 ug/ml).



n = 6, 3a

n = 8, 3b

In an attempt to prepare β-diketones with potentially broader spectrum antiviral activity, a series of aryl bis(β-diketones) with the general structure (4) was prepared[127]. The most promising compound with hexyl bridge (m=n=6, 4a) was found to have good antiherpetic activity. At 4 ug/ml, this compound gave a 89% and 93% reduction in plaque count, when tested against HSV-1 and -2 respectively[127].



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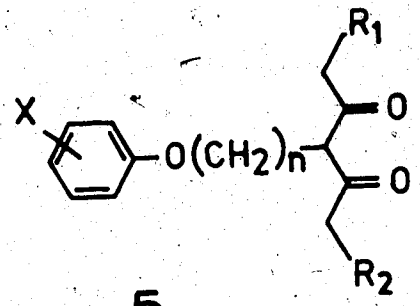
m = n = 6 4a

As an extension of the work, a related series of compounds, the aryloxyalkyl diketones (5) was synthesized to find compounds with improved antiherpetic activity[128].



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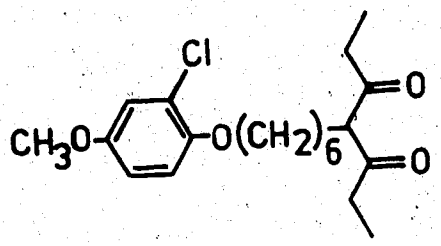


5a

Variations in the aromatic substituent pattern and chain length were investigated[128]. The most promising compound (5a) was found to possess a 2-chloro-4-methoxyphenyl moiety and n=6 (MIC_{50} = 6 ug/ml for HSV-1 and HSV-2, MIC_{50} = 1.5 ug/ml for equine rhinovirus).

This compound (5a) is known as arildone and is composed of a β-diketone unit separated from a substituted benzene ring by an alkyl chain of six carbons. Substituents on the benzene ring contribute to lipophilicity. Compounds with an alkyl chain of six to eight carbon atoms have antiviral activity[128].

1.3.3. Antiviral activity of arildone

A. In vitro studies

Inhibition of cytopathic effects in cell culture:

Kim and coworkers showed that the MIC_{50} values of arildone ranged

from 0.8uM for poliovirus to 16.2uM for HSV-1 and HSV-2 (Table 1.2). Higher concentrations caused intracellular granulation[129].

Plaque reduction tests:

Table 1.2 (data from Ref.129) represents a list of viruses tested by plaque reduction assays. Picornaviruses and herpesviruses were very sensitive to the drug, whereas other viruses were less sensitive.

Virus	CPE(MIC_{50} in uM)	Plaque reduction(MIC_{50} in uM)
Poliovirus-2	0.8	< 0.27
HSV-1 (Sheely strain)	16.2	< 1.35
HSV-2 (Curtis strain)	16.2	< 5.4
Varicella-zoster virus	ND	< 2.7
Vaccinia virus	8.1	> 13.5
Adeno virus	ND	> 27

Table 1.2. Effect of arildone on virus infectivity (In vitro studies)

ND: Not Done

Effect on virus yield:

Cells were infected at multiplicity of infection (m.o.i.) between 0.1 and 1 plaque forming unit/cell (pfu/cell) and arildone was present from the time of addition of virus throughout the entire growth cycle. Virus yield was determined by plaque assay 24 hours after infection except for murine cytomegalovirus in which it was determined after 72 hours.(Table 1.3, data from Ref. 129)

Virus	MIC_{50} (uM)
Poliovirus-2	< 2.7
HSV-1	2.7
HSV-2	< 2.7
Murine cytomegalovirus	< 8.1

Table 1.3 Effect of arildone on virus replication (In vitro studies)

These studies had indicated that arildone, at low concentrations, inhibited the replication of certain DNA and RNA viruses in vitro. As in the plaque reduction tests, the yields of herpesviruses and picornaviruses were sensitive to arildone.

B. In vivo studies

Effect on the development of lesions

In vivo studies have demonstrated that arildone is effective topically in a guinea pig skin infection caused by HSV-1 and HSV-2[130,131]. In guinea pigs the application of 8% arildone in cream formulation or 8% arildone in 90% DMSO five times daily starting twenty four hours post infection suppressed the formation and progression of herpetic vesicles and significantly reduced virus titers in the lesion sites (Figure 1.2). During the next twenty four hour period of therapy, drying and crusting began in all arildone-treated sites, while in placebo DMSO-treated animals drying of the skin around the vesicles was the only change observed. In untreated animals, vesicles were moist and continued to increase in size[130].

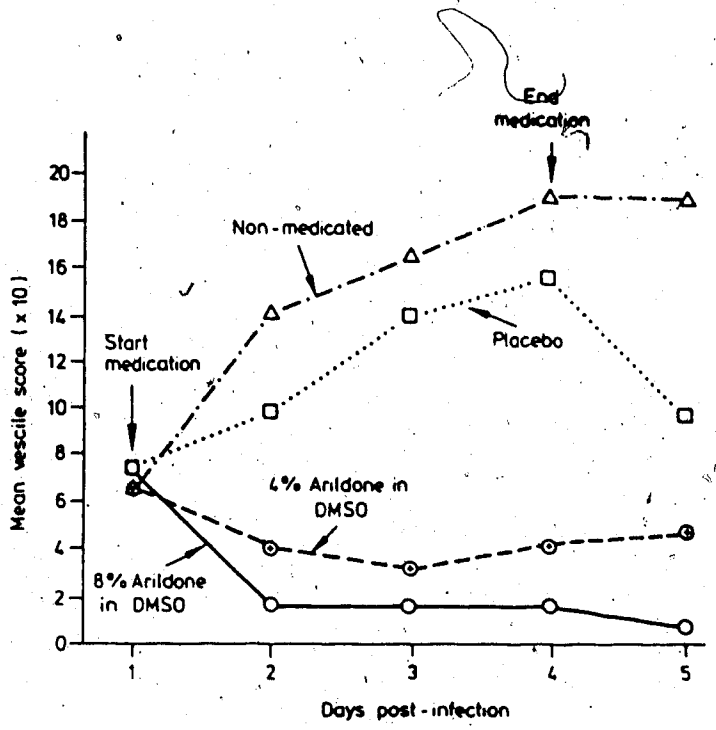


Figure 1.2 Effect of arildone on the development of lesions produced by HSV in guinea pigs(taken from Ref.130 with permission)

Effect on virus growth

The drug concentrations of 4% and 8% arildone in 90% DMSO, applied to the infected animals, reduced the virus content in the lesions compared with virus content in placebo-treated and nonmedicated infected animals, Figure 1.3.

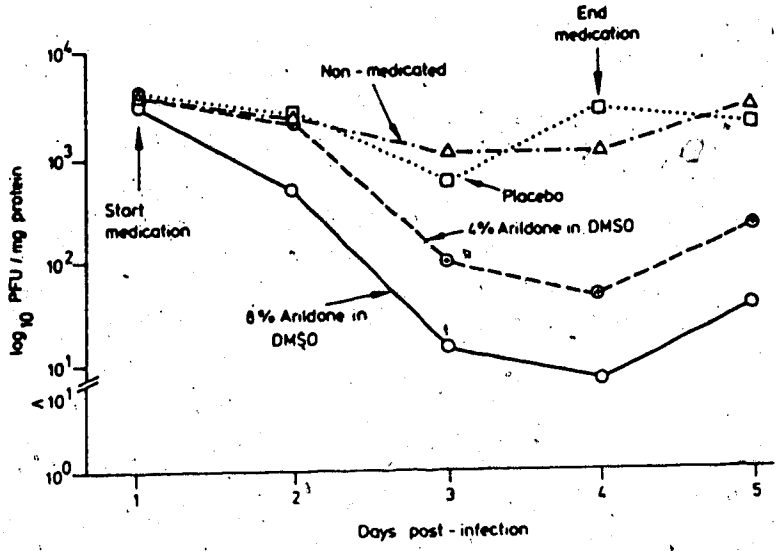


Figure 1.3 Effect of arildone on virus content in lesions produced by HSV in guinea pigs (taken from Ref.130 with permission)

Arildone in cream

The therapeutic effect was not as rapid and marked as that produced by arildone prepared in DMSO. The superior activity of arildone in a DMSO preparation is probably due to deeper and more effective penetration of the drug[130].

C. Mode of action

Arildone inhibits replication of HSV-2 in infected BSC-1 cells by interfering with a sensitive event prior to six hours post infection and possibly after four hours post infection. Neither viral DNA nor

viral proteins are synthesized in the presence of arildone. This indicates that arildone interferes with early events of viral replication[132]. Similar results are obtained with HSV-1 in the same cell line[33].

In the case of poliovirus-2, the mode of action was studied by adding arildone at different times[133]. A three-fold increase in the MIC_{50} was obtained when the drug was present only in the agar overlay medium after the adsorption period. This increase may be due to initiation of virus replication in some cells before the drug was added. A ten-fold increase in the MIC_{50} was obtained when arildone was present only during the adsorption period and washed away before the agar overlay medium was added. This increase may be due to the initiation of replication by cell associated virus, which was able to uncoat the viral nucleic acid in the absence of the drug. These results also suggest the partial reversibility of the antiviral activity of the drug and the failure of the drug to affect adsorption or integrity of the poliovirus[133].

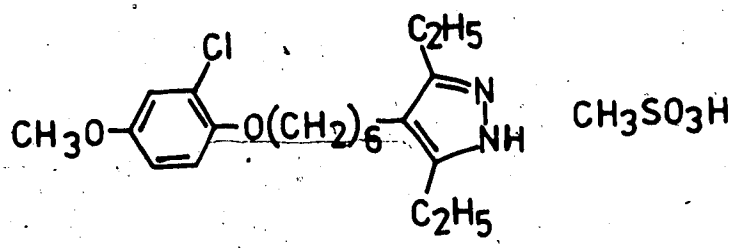
Thus arildone inhibits simple, nonenveloped, RNA containing viruses, as well as large, enveloped, DNA containing viruses. If inhibition of uncoating of the viral nucleic acid is the common mode of action of arildone, it suggests that arildone may interact with the icosahedral protein capsid, a structure common to both poliovirions and herpes simplex virions[133]. Direct, but reversible interaction of arildone with the icosahedral capsid of these virions may prevent the conformational alterations required to uncoat and release the viral genome into the cell[133]. Studies on purified poliovirus have suggested

that arildone prevents uncoating of the virion by stabilizing the protein-protein interaction in the capsid[134].

1.3.4. Analogues of arildone

Further study was done in the Sterling-Winthrop Research Institute by modifying the acyclic β- diketone moiety to cyclic systems such as pyrazoles and isoxazoles.

A. Pyrazole analogue



6

This analogue 6 (WIN 41258-3) can be easily prepared from arildone by heating with hydrazine hydrochloride[135]. The water soluble methanesulfonate salt was prepared, and it was found to be active against HSV-1 and HSV-2 *in vitro* and *in vivo*. Results shown in Table 1.4 (data from Ref. 136) indicate that the compound prevented plaque formation by HSV-1 and HSV-2 in BSC-1 cell monolayers.

Virus	Drug concentration (ug/ml)	% Inhibition
HSV-1	2	33
	3	100
HSV-2	2	72
	3	100

Table 1.4 Effect of pyrazole analogue on virus infectivity (In vitro study)

The compound was effective in mouse genital herpes infection after intravaginal administration. The compound was administered to mice at four hours post infection with solutions containing 1.25, 2.5, 5.0 or 10% on saturated tampons. Therapy resulted in a high survival rate (80 to 100%) of treated animals versus 20 to 30% of placebo-treated controls. The compound was also effective in the treatment of guinea pig skin infections produced by HSV-1. Solutions of 2.5, 5 and 10% of the compound, applied to the skin starting twenty four hours post infection, resulted in rapid suppression of development of herpetic vesicles and significant reduction of the virus titers in the lesion sites[136].

The data obtained in Sterling-Winthrop Research Institute is summarized in Table 1.5.

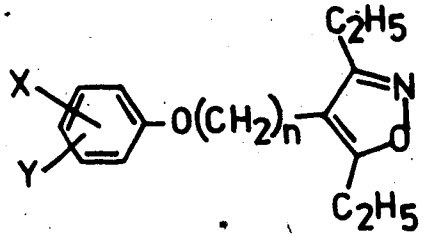
Compound	MIC_{50} (ug/ml)		
	HSV-1	HSV-2	equine rhinovirus
3a	ND	12	3 to 6
4a	3	3	ND
5a	6	6	1.5
6	>2	<2	ND

Table 1.5 Improved antiviral activity of β-diketones related compounds (In vitro studies)

ND: Not Done

B. Isoxazole analogues

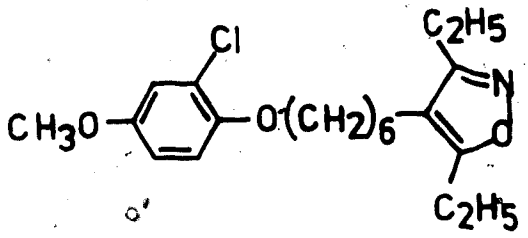
In view of the lack of chemotherapeutic agents available for the treatment of picornavirus infections, a program directed towards the discovery of compounds active against this class of viruses was initiated at Sterling-Winthrop Research Institute. As a result of the screening of compounds related to arildone, it was found that the isoxazole analogues (7) possessed in vitro activity against both rhinovirus-2 (RV-2) and poliovirus-2[137].



7

These analogues can be synthesized from arildone by heating with

hydoxylamine hydrochloride[137]. Consequently, a variety of related 3,4,5-trisubstituted as well as some 3,5-disubstituted isoxazoles were prepared. First, trisubstituted isoxazoles were evaluated against RV-2 *in vitro* by the plaque reduction method. The initial compound(7a) exhibited an MIC_{50} of 0.8 ug/ml[137].



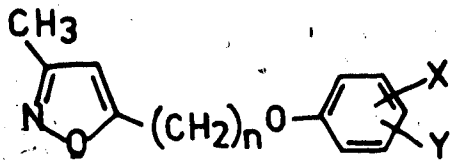
7a

A series of 3,5-disubstituted isoxazoles, 8, was also tested against RV-2 and polio-2 *in vitro* by the plaque reduction method[137] (Table 1.6). Compounds 9 and 10 were found to be active against RV-2. However, compound 9 (WIN 49321) was significantly more active than 10 against polio-2.

When 10 was administered orally to poliovirus-infected mice, it did not show any effect. This may be due to metabolic hydrolysis of the compound to the inactive acid. Compound 9 was tested in mice which were infected intracerebrally with polio-2. The lowest dose that resulted in significantly increased survival was 31mg/Kg bid.

Several modifications of the ester group were made without obtaining any improvement in systemic antiviral activity. The oxazoline 15 (WIN 51711) was prepared[138] as a cyclic variation of the

ethyl ester with similar space-filling characteristics. This modification necessitated replacing one of the oxygens with a nitrogen atom and potentially minimized the metabolic hydrolysis to an inactive acid. Indeed, this compound (15, WIN 51711) showed significantly improved antiviral activity against polio-2 virus (Table 1.6), and reduced plaque formation by 9 enteroviruses and 33 rhinoviruses with MIC_{50}s of 0.004 to 0.17 and 0.004 to 6.2ug/ml respectively[139].



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Compound Number	X	Y	n	MIC_{50} (ug/ml)	
				RV-2	Polio-2
9	2-Cl	4-CH_3O	7	0.04	0.05
10	H	4-$COOC_2H_5$	6	0.01	>0.80
11	H	4-$COOCH_3$	7	3.00	0.40
12	H	4-CH_3CO	7	1.60	0.01
13	H	4-C_2H_5CO	7	1.70	0.40
14	H	4-$CH(CH_3)_2$	7	1.00	0.04
15	H	4 	7	0.10	0.004

Table 1.6 In vitro antipicornavirus activity of 3,5-disubstituted isoxazoles

A number of compounds related to WIN 51711 has been synthesized and tested against several different picornaviruses. Recent X-ray crystallographic studies[140] by a group at Purdue University and Sterling-Winthrop Research Institute have shown clearly the site of attachment of two WIN antiviral compounds in human rhinovirus 14 (HRV-14) that prevents the pH-mediated uncoating of the viral RNA. This consists of a 3-methylisoxazole group that inserts itself into the hydrophobic interior of the VP1 protein. The 4-oxazolinylphenoxy group covers the entrance to an ion channel into the virion and the 6 (or 7) membered aliphatic chain provides an appropriate spacer (Fig. 1.4). Thus uncoating of the viral RNA may be inhibited by preventing the collapse of the VP1 hydrophobic pocket or by blocking the flow of ions into the virus inte

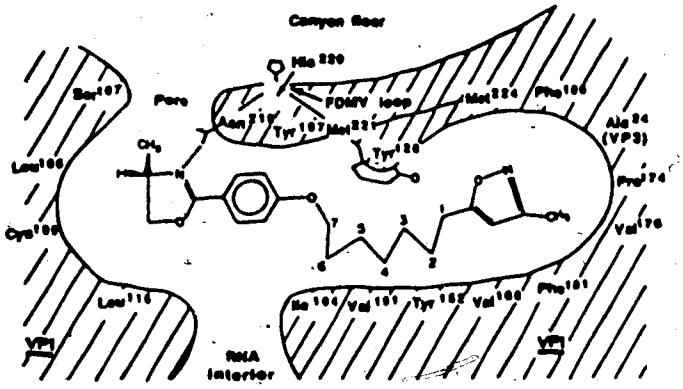
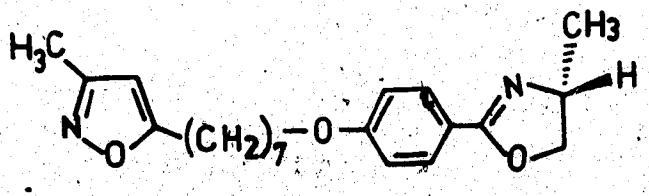


Figure 1.4 Diagrammatic representation of WIN 52084 binding site in HRV-14 capsid (taken from Ref. 140 with permission).

The most active compound against HRV-14 is the chiral compound 15a with MIC_{50} of 0.06 uM, which has a methyl group at position 4 on

the oxazoline ring, producing an asymmetric carbon atom (15a, WIN 52084).



15a

The S-isomer was found to be ten times more active than the R-isomer against HRV-14 and fifteen times more active against HRV-2. This may be due to the improved hydrophobic interactions of the S-methyl group with a hydrophobic pocket formed in HRV-14. The R-methyl group would be expected to have no positive effect on such interactions and hence has similar MIC_{50} values (0.4 uM) to the desmethyl compound (WIN 51711)[140]. It is expected that these WIN antiviral compounds prevent uncoating by stabilizing the virus.

1.4. Objective of research

In designing antiviral drugs, among the factors to be considered are their cost, ease of production, route of administration, safety and broad spectrum of antiviral activity. A few drugs are presently available on the market. These are effective for the early treatment of certain acute virus infections, but have many limitations such as a narrow spectrum of antiviral activity, side effects and development of viral resistance. New or improved antiviral drugs are needed.

Most of the available antiviral drugs on the market are nucleoside analogues and with antiherpetic activity. The action of several nucleoside analogues on cellular as well as viral enzymes leads to toxicity and limits their use. Efforts are being made to develop an antiviral agent with no toxicity and with broad spectrum of antiviral activity. Extensive studies in the Sterling-Winthrop Research Institute led to the discovery of arildone. It is not a nucleoside analogue, and is active against a wide variety of RNA and DNA viruses. Compound 6 possesses antiherpetic activity, whereas compound 9 possesses antipicornavirus activity. The objective of the present research in our laboratory was to chemically synthesize an arildone type of compound with broad spectrum antiviral activity against both picornavirus and herpesvirus.

A series of compounds related to WIN 49321 (9) was prepared to study the effect of the following factors on antiviral activity.

(a) The effect of change in carbon chain length on antiviral activity

(b) The effect of changes in -O-aryl substitution on antiviral activity

(c) The effect of changes in heteroaromatic moiety on antiviral activity

(d) The effect of changes in -O-aryl moiety on antiviral activity

(e) The effect of substitution of α-CH_2 of carbon chain on antiviral activity.

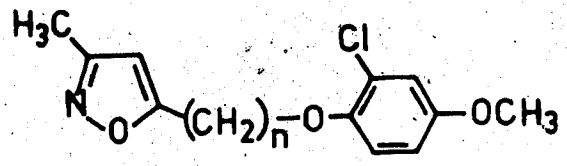
Chapter 2

CHEMICAL SYNTHESIS

2.1. Outline of chemical synthesis of compounds

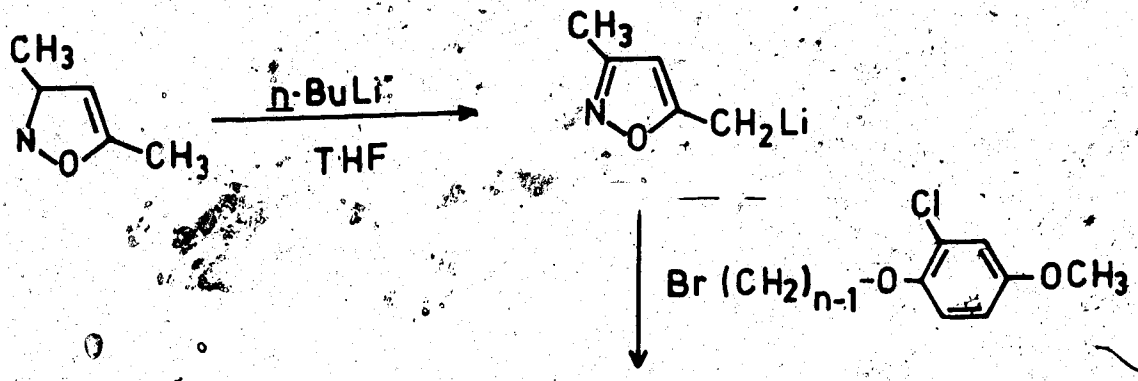
A series of compounds was synthesized by the routes summarized in the following schemes, to study the structure-activity relationships. WIN 49321 (9) was considered as reference for this study.

(a) The effect of change in carbon chain length on the antiviral activity was studied by preparing the compounds (general formula 16), with different values of n (n=5, 6, 7, 8, 9).



16

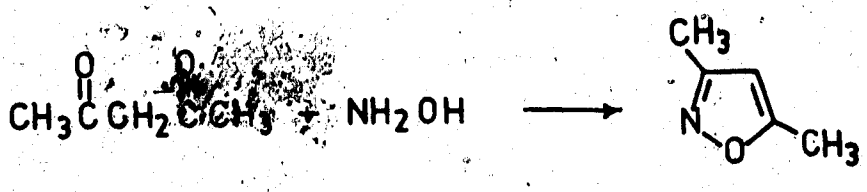
The synthesis of these compounds involved the lithiation of 3,5-dimethylisoxazole by using n-butyllithium at -75°C to get lithium salt. Further alkylation of lithium salt with appropriate bromoalkylaryl ether gave the desired product (Scheme I).



16

SCHEME I

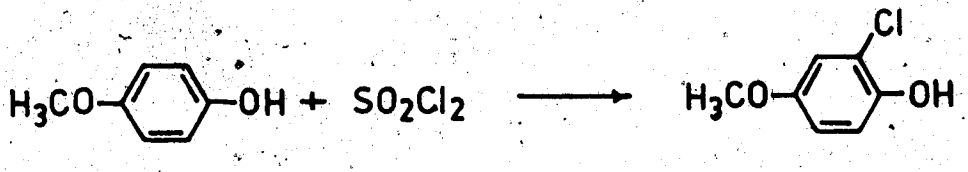
3,5-Dimethylisoxazole (17) can be prepared[141] by refluxing the mixture of acetylacetone (0.10 mole) and hydroxylamine hydrochloride (0.10 mole) for one hour, using ethanol as the solvent (Scheme II).



17

SCHEME II

2-Chloro-4-methoxyphenol (18) can be prepared[148] by refluxing 4-methoxyphenol (0.10 mole) with sulfuryl chloride (0.10 mole) in chloroform for two hours (Scheme III).

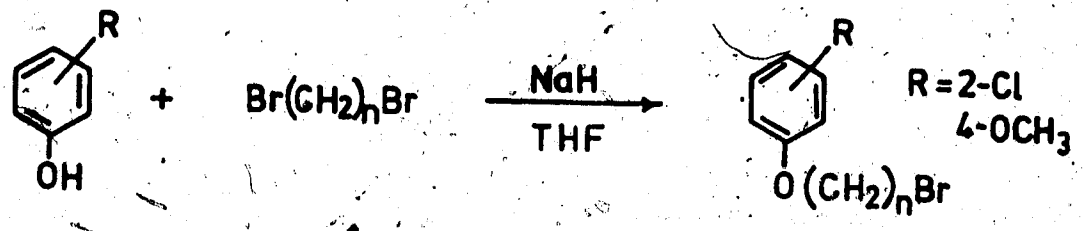


18

SCHEME III

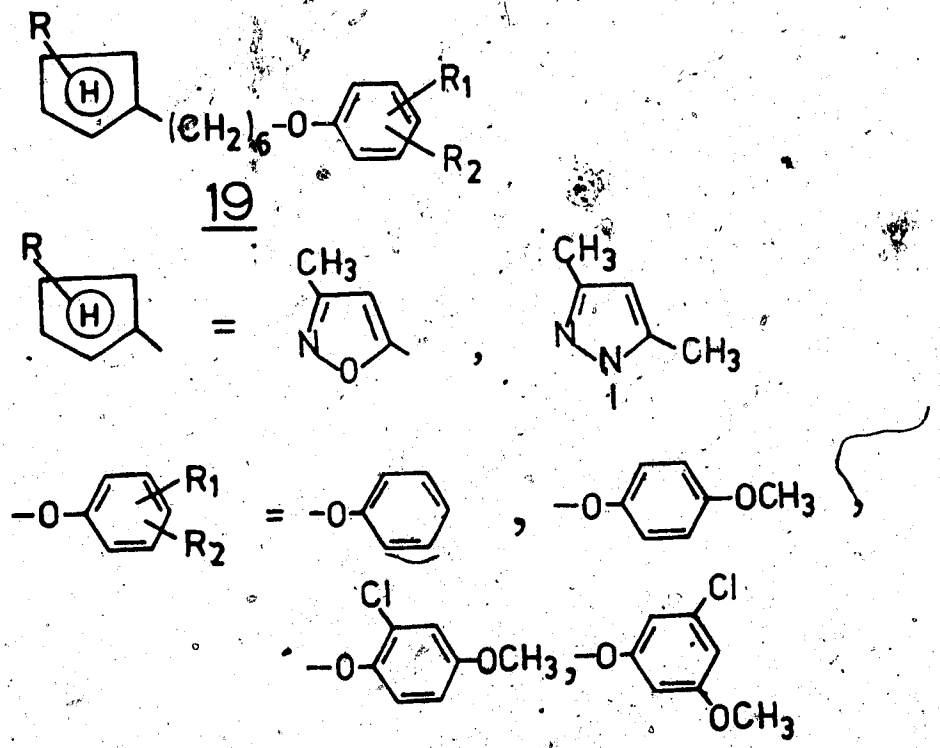
Bromoalkylaryl ethers preparation involved the treatment of 2-chloro-4-methoxyphenol (0.10 mole) with sodium hydride (0.11 mole) in THF at room temperature, and then refluxing the reaction mixture with

dibromoalkane (0.15 mole) (Scheme IV).

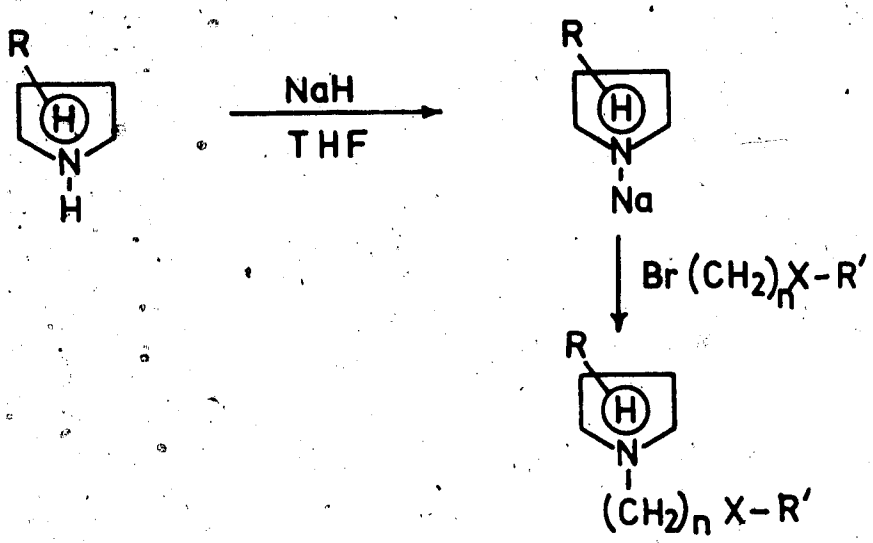


SCHEME IV

(b) A series of compounds (general formula 19), was prepared to study the effect of different substituents in -O-aryl moiety on antiviral activity.

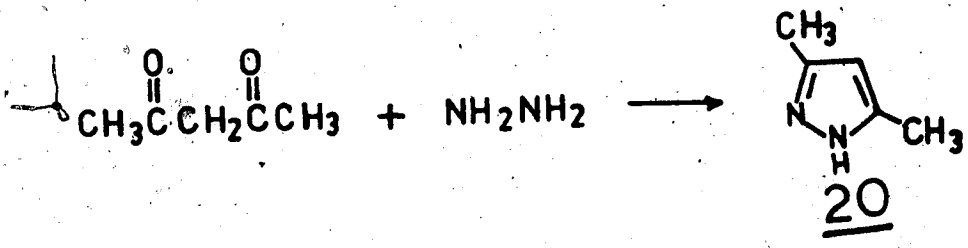


The compounds in isoxazole series were synthesized by the route summarized in scheme I. In pyrazole series, the compounds were prepared by the metallation of 3,5-dimethylpyrazole (0.10 mole) with sodium hydride (0.10 mole), followed by alkylation with the appropriate bromoalkylaryl ether (0.10 mole) (Scheme V).



SCHEME V

3,5-Dimethylpyrazole (20) can be prepared[142] by refluxing the mixture of acetylacetone (0.10 mole) and hydrazine hydrate (0.10 mole) in ethanol for thirty minutes (Scheme VI).

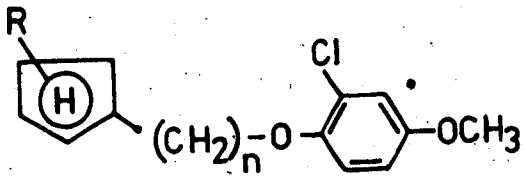


SCHEME VI

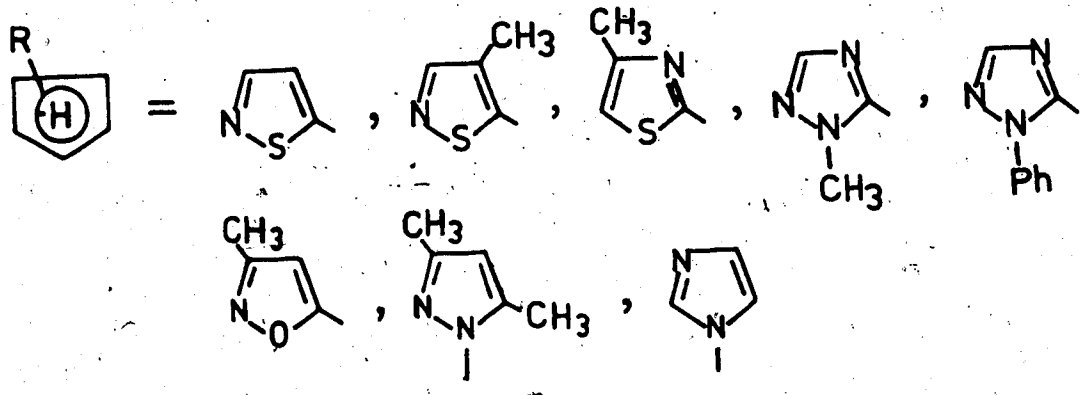
Bromoalkylaryl ethers were synthesized by the route summarized in Scheme IV.

3-Chloro-5-methoxyphenol was bought from Aldrich Chemical Company.

(c) The effect of changes in heteroaromatic moiety on antiviral activity was studied by preparing the compounds (general formula 21) by the route summarized in Scheme I, except in pyrazoles and imidazoles, where Scheme V was followed.

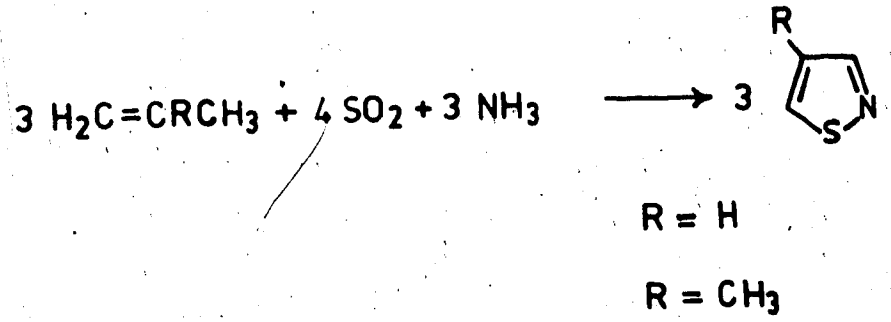


21 n = 6 and 7



Isothiazole [143] can be prepared by passing the gaseous mixture of propylene, ammonia and sulfur dioxide over catalyst (activated

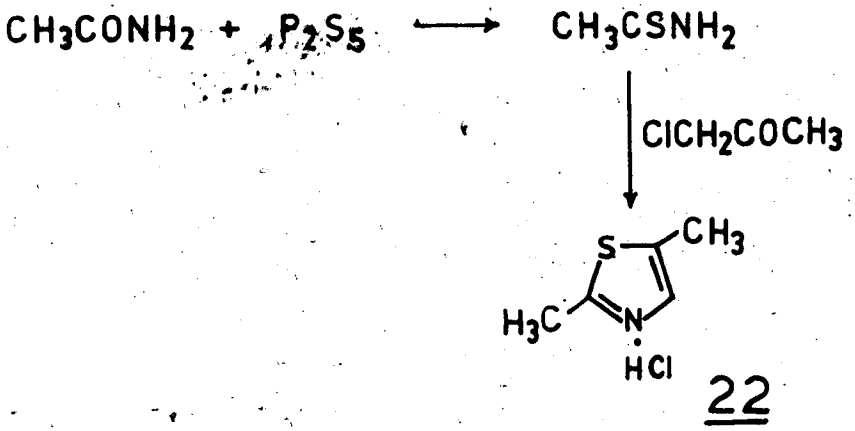
aluminum oxide) at temperature above 250°C (Scheme VII).



SCHEME VII

Similarly, isobutylene furnishes 4-methylisothiazole[143].

2,4-Dimethylthiazoles (22) can be prepared[144] by refluxing the mixture of thioacetamide (0.10 mole) and chloroacetone (0.10 mole) for thirty minutes, using benzene as the solvent (Scheme VIII).

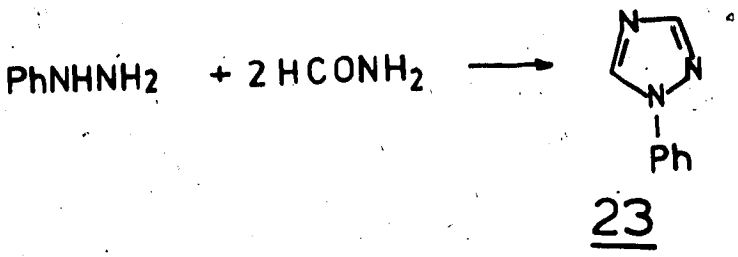


22

SCHEME VIII

1-Phenyl-1,2,4-triazole (23) can be obtained[145] by refluxing the mixture of phenylhydrazine (0.10 mole) and formamide (0.30 mole) for

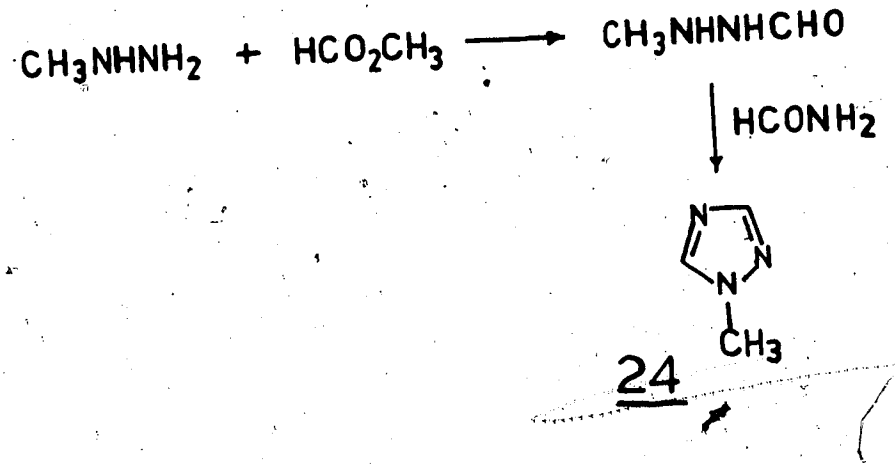
thirty hours at 135-140°C (Scheme IX).



23

SCHEME IX

Similarly, 1-methyl-1,2,4-triazole (24) is obtained[146] by stirring the mixture of methylhydrazine (0.50 mole) and methyl formate (1.10 mole) for two hours at room temperature, followed by refluxing with formamide (1.10 mole) for ten hours at 135-145°C (Scheme X).



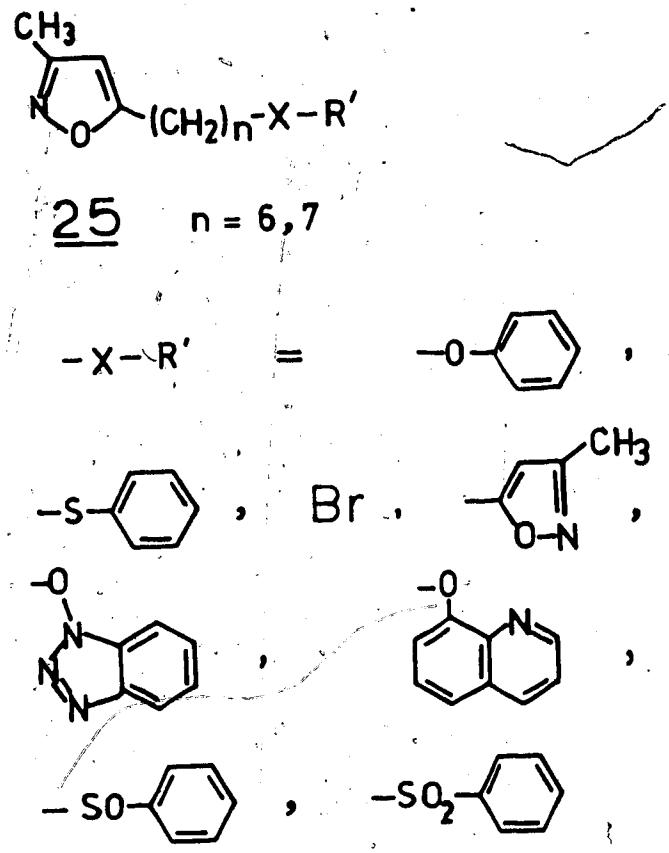
24

SCHEME X

Imidazole was bought from Aldrich Chemical Company.

Bromoalkylaryl ethers can be prepared by the route summarized in Scheme IV.

(d) The effect of changes in -O-aryl moiety on antiviral activity was studied by preparing the compounds of general formula 25. These compounds were prepared by the route shown in Scheme I.



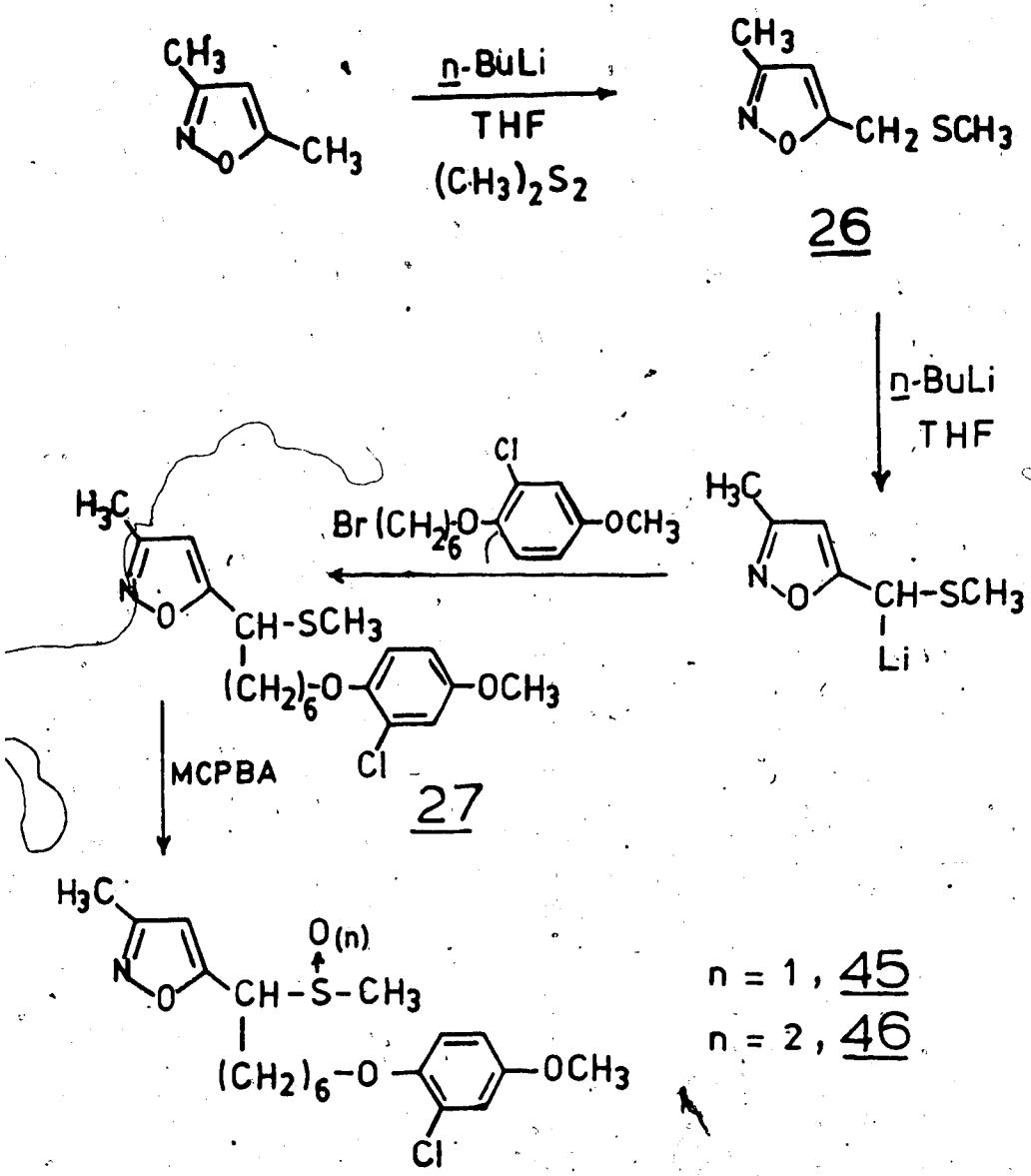
25 n = 6,7

-X-R' = -O- , -S- , Br , , , , -SO- , -SO2-

Bromoalkylaryl thioethers can be prepared by the same route as Scheme IV, using thiophenol.

The oxidation of thioethers with m-chloroperbenzoic acid(MCPBA) gives the sulfoxide or sulfone depending on the stoichiometry.

(e) The effect of substitution on α-CH_2 of carbon chain on antiviral activity was studied by preparing 27, 45 and 46, by the route shown in Scheme XI.



SCHEME XI

Preparation of 3-methyl-5-methylthiomethylisoxazole[147] 26 involved the lithiation of 3,5-dimethylisoxazole (0.10 mole) with n-butyllithium (0.11 mole) and further alkylation of the resulted lithium salt with dimethyl disulfide (0.12 mole). Compound 27 was prepared by lateral lithiation of the C-5 methylene group of 26, followed by the alkylation with 1-bromo-6-(2-chloro-4-methoxyphenoxy)-hexane. Further oxidation of S in 27 with m-chloroperbenzoic acid (MCPBA) gave sulfoxide 45 or sulfone 46 depending on stoichiometry (Scheme XI).

2.2. Experiments

Melting points were measured on a Thomas Hoover "Unimelt" capillary melting point apparatus and are uncorrected. Refractive indices were determined using an Abe Refractometer model Carl Zeiss 13657.

Infrared spectra were recorded on a Unicam SP 1000 or Nicolet FT-IR spectrophotometer. Proton nuclear magnetic resonance spectra were taken in deuteriochloroform unless otherwise stated, using tetramethylsilane as internal standard, on Varian EM-360A, EM-390 or Brucker AM-300 NMR spectrometers. Microanalysis were obtained on a Perkin-Elmer 2408 analyzer and the results were within the acceptable range (±0.5%).

The reactions were monitored by thin layer chromatography. Column chromatography was carried out utilizing silica gel 60 (E.Merck). Tetrahydrofuran, used as a solvent in most of the reactions, was dried by refluxing with sodium metal and benzophenone and stored over 4-A molecular sieves.

2.2.1 Methods of preparation for starting materials

Preparation of 3,5-Dimethylisoxazole (17)

A solution of acetylacetone (10g, 0.10 mole) in ethanol (10 ml) was added to a solution of hydroxylamine hydrochloride (7g, 0.10 mole) in water (15 ml). The mixture was heated under reflux for one hour and then was poured onto cold water (60 ml). The aqueous mixture was extracted with ether (2 x 30 ml). The combined extracts were dried ($MgSO_4$), filtered and the filtrate was concentrated under reduced pressure to give a brown oil. Distillation gave 6.0g(62%) of a colorless oil, bp 140-42°C, (lit.[141] 140-42°C).

Preparation of 3,5-Dimethylpyrazole (20)

A solution of acetylacetone (10g, 0.10 mole) and hydrazine hydrate (5 ml, 0.10 mole) in ethanol was stirred at room temperature for 10 minutes and then refluxed for 30 minutes. This mixture was cooled and poured into saturated brine (100 ml). The oily yellow product was extracted with ether (2 x 20 ml). The combined extracts were dried ($MgSO_4$), filtered and concentrated to give a yellow residue as crude product. Crystallization from petroleum ether gave 7.5g (78%) of white plates, mp 105°C (lit.[142] 106°C).

Preparation of 2,4-Dimethylthiazole (22)

A solution of chloroacetone (200 ml, 2.48 mole) in benzene (50 ml) was added slowly to a stirred mixture of acetamide (150g, 2.54 mole) and powdered phosphorus pentasulfide (100g, 0.45 mole) in benzene (100 ml), in a flask fitted with a reflux condenser, with initial heating

on a water bath. As soon as an exothermic reaction started the reaction flask was removed from the bath and the addition continued through the reflux condenser. After the addition was completed, the reaction mixture was heated under reflux for 30 minutes and then cooled. To this cooled mixture, water (400 ml) was added and the reddish upper layer containing benzene with some impurities was discarded. The lower layer was made alkaline by the addition of 5N sodium hydroxide and the crude thiazole was extracted with ether. The combined ethereal extracts were dried ($MgSO_4$), filtered, concentrated under reduced pressure. Distillation gave 132.2g (44.5%) of a colorless oil, bp 140-42°C (lit.[144] 143-45°C).

Preparation of 1-Phenyl-1,2,4-triazole (23)

A mixture of phenylhydrazine (25.9g, 0.19 mole) and formamide (25.9g, 0.57 mole) was stirred and heated under reflux in a nitrogen atmosphere in an oil bath at 135-40°C for 30 hours. The reaction mixture was cooled, dissolved in dichloromethane (75 ml), washed with water (2 x 60 ml), dried ($MgSO_4$), filtered and concentrated to give a brown low melting solid. Distillation under reduced pressure gave 18.3g (67%) of the product bp 88-90°C/0.4 mm, as a clear oil which crystallized on cooling, mp 46-47°C (lit.[145] 46-47°C).

Preparation of 1-Methyl-1,2,4-triazole (24)

Methyl formate (66g, 1.10 mole) was added dropwise to methylhydrazine(23g, 0.50 mole) at room temperature with stirring and the mixture stirred for two hours. The excess of methyl formate was

removed by evaporation under reduced pressure. Formamide (45g, 1.00 mole) was added to this residue and the solution heated under reflux for ten hours (135-145°C). The reaction mixture was cooled, extracted with dichloromethane (2 x 100 ml) and the combined dichloromethane layers washed with water (2 x 25 ml), dried ($MgSO_4$), filtered and concentrated to give 36g of a colorless oil. Distillation of this oil gave 17.3g(41.1%) of the desired product, bp 71-74°C/13 mm (lit.[146] 175-7°C).

Preparation of 3-Methyl-5-methylthiomethylisoxazole (26)

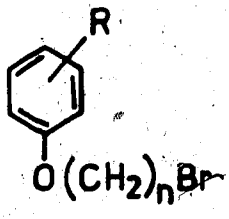
n-Butyllithium (625 ml; 1.6M in hexane, 1 mole) was added slowly to a stirred, cold (-70°C) solution of 3,5-dimethylisoxazole (97.1g, 1 mole) in dry THF (900 ml) in a nitrogen atmosphere. The clear yellow solution was stirred at -75°C for one hour. This solution was added slowly under nitrogen atmosphere to a stirred, cold (-75°C) solution of dimethyl disulfide (110 ml, 1.2 mole) in dry THF (900 ml) in nitrogen atmosphere over a 30 minute period maintaining the temperature of the reaction mixture below -65°C, stirred an additional 15 minutes at -75°C and then allowed to reach room temperature. At about -25°C a solid separated out of the solution. The reaction mixture was concentrated and extracted the residue with ether. The ether extract was washed with water (2 x 100 ml), dried ($MgSO_4$) and concentrated to give 146.5g of a yellow oil. The crude product was distilled at 100-102°C/9 mm to give 124.5g (87%) of desired product[147].

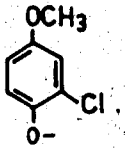
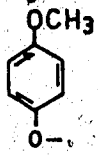
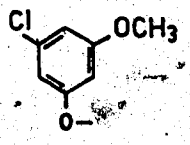
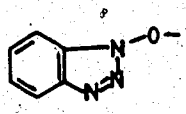
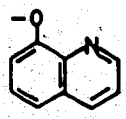
Preparation of 2-Chloro-4-methoxyphenol (18)

p-Methoxyphenol (37.2g, 0.3 mole) in chloroform (100 ml) was treated at room temperature with sulfuryl chloride (41.5g, 0.3 mole) dissolved in chloroform (25 ml). The mixture was heated under reflux for two hours and distilled to remove excess sulfuryl chloride. The residue was purified by silica gel column chromatography using 66% dichloromethane and 33% hexane as eluant and gave 30.5g (63.5%) of the pure product as a white solid, mp 44-45°C (lit.[148] 44-45°C).

2.2.2 General method of preparation of Bromoalkylaryl ether (28-33) used for alkylation of heterocyclic compounds

Sodium hydride (0.11 mole) was washed with hexane (3 times) and suspended in THF. A solution of substituted phenol or 1-hydroxybenzotriazole or 8-hydroxyquinoline (0.10 mole) in THF was added dropwise to the suspension, resulting in the evolution of hydrogen gas. After the addition, the reaction mixture was stirred at room temperature for 45 minutes. This mixture was added dropwise to a solution of the dibromoalkane (0.20 mole) in THF and the reaction mixture heated under reflux overnight. The resulting mixture was concentrated and the residue taken up in ether. The ethereal solution was washed with water, dried ($MgSO_4$), filtered and concentrated under reduced pressure to give an oil which was distilled to remove excess of dibromoalkane. the residue was purified by silica gel column chromatography.



	n	Compound Number
	6	28
	5	29
	5	30
	5	31
	6	32
	6	33

Preparation of 1-Bromo-6-(2-chloro-4-methoxyphenoxy)hexane(28)

The crude product was purified by column chromatography using 90% hexane and 10% ethyl acetate as eluant to give an oil (80.6%), bp 120-22°C/0.1 mm.

IR (neat): 3066, 2934, 2877, 1467, 1208, 1170, 1051 cm^{-1}.

NMR: δ 7.00-6.75(m, 3H, Ar-H); 3.95(t, 2H, J=7Hz, -O-CH_2-); 3.80(S, 3H, -OCH_3); 3.39(t, 2H, J=7Hz, -CH_2-Br); 2.09-1.26(m, 8H, -$(CH_2)_4$-).

Preparation of 1-Bromo-5-phenoxypentane (29)

The purification of crude product by column chromatography using hexane-ethyl acetate mixture (85:15) as eluant gave a colorless oil (61.6%), bp 84-86°C/0.1 mm.

IR(neat): 3066, 2943, 2869, 1239, 1167, 1038 cm^{-1}.

NMR: δ 7.46-6.82(m, 5H, Ar-H); 4.00(t, 2H, J=6.0Hz, -O-CH_2-); 3.46(t, 2H, J=7.5Hz, -CH_2-Br); 2.23-1.42(m, 6H, -$(CH_2)_3$).

Preparation of 1-Bromo-5-(4-methoxyphenoxy)pentane (30)

The column chromatography of the crude product using hexane-ethyl acetate mixture (9:1) as eluant gave a colorless oil (73.7%), bp 118-20°C/0.1 mm.

IR(nujol): 1236, 1170, 1050 cm^{-1}

NMR: δ 6.79(s, 4H, Ar-H); 3.85(m, 5H, -O-CH_2-, -OCH_3); 3.36(t, 2H, J=6.0Hz, -CH_2-Br); 2.00-1.16(m, 6H, -$(CH_2)_3$-)

Preparation of 1-Bromo-5-(3-chloro-5-methoxyphenoxy)pentane (31)

Hexane:Ethyl acetate solvent mixture(9:1) was used as eluant in column chromatography to purify the crude product to give a colorless

solid (80.0%), mp 35-6°C.

IR(nujol): 1611, 1061 cm^{-1}

NMR: δ 6.52-6.33(m, 3H, Ar-H); 3.92(m, 5H, $-OCH_2-$, $-O-CH_3$); 3.46(t, 2H J=6Hz, $-CH_2-Br$); 2.19-1.42(m, 6H, $-(CH_2)_3-$)

Preparation of 1-Bromo-6-(benzotriazol-1-yloxy)hexane (32)

The crude product was purified by column chromatography using 60% hexane and 40% ethyl acetate as eluant to give a thick oil (63.5%), n_{20} 1.5385.

IR(neat): 3060, 2951, 2860, 1234, 1160, 1091 cm^{-1}

NMR: δ 8.06(d, 1H, J=7.5Hz, 7'-H); 7.66-7.33(m, 3H, Ar-H); 4.56(t, 2H, J=7.5Hz, $-O-CH_2-$); 3.46(t, 2H, J=7.5Hz, $-CH_2-Br$); 2.06-1.46(m, 8H, $-(CH_2)_4-$).

Preparation of 1-Bromo-6-quinolin-8-yloxy)hexane (33)

The crude product was purified by column chromatography using 40% hexane and 60% ethyl acetate as eluant to give a thick oil (49.7%), n_{20} 1.5800.

IR(neat): 3066, 2941, 2867, 1251, 1176, 1091 cm^{-1}

NMR: δ 9.00(d, 1H, J=6Hz, 2'-H); 8.09(d, 1H, J=7.5Hz, 4'-H); 7.52-7.00 (m, 4H, Ar-H); 4.19(t, 2H, J=6Hz, $-O-CH_2-$); 3.36(t, 2H, J=6Hz, $-CH_2-Br$) 2.16-1.33(m, 8H, $-(CH_2)_4-$).

2.2.3 Preparation of 5-Thiophenoxypentyl bromide

A solution of thiophenol (11g, 0.10 mole) in THF (50 ml) was added dropwise to a solution of sodium hydride (2.64, 0.11 mole) in THF (25 ml). After the addition the reaction mixture was stirred at

room temperature for two hours. This reaction mixture was added dropwise to a solution of dibromopentane (41.3g, 0.18 mole) in THF (25 ml) and the mixture heated under reflux overnight. The mixture was concentrated and the residue taken up in ether. The ether extract was washed with water, dried ($MgSO_4$), filtered and the filtrate concentrated at reduced pressure to give an oil which was purified by column chromatography using 85% hexane and 15% ethyl acetate as eluant to give the pure product (22.8g, 88.7%) as a yellow oil, bp 110-12°C/0.1 mm.

IR(neat): 3077, 2932, 2860, 1229, 1170, 1070 cm^{-1}

NMR: δ 7.36-6.95(m, 5H, Ar-H); 3.33(t, 2H, J=7.5Hz, $-CH_2Br$); 2.85(t, 2H, J=7.5Hz, $-S-CH_2-$); 2.06-1.29(m, 6H, $-(CH_2)_3-$).

2.2.4. General methods for the alkylation of heterocyclic compounds to obtain desired products

Method A:

n-Butyllithium in hexane (0.1 mole) was added dropwise to a stirred solution of the heteroaromatic compound (0.10 mole) in THF under nitrogen at -70°C. After the addition was complete the mixture was stirred for 30 minutes to two hours at -70°C and the solution of bromoalkylaryl ether (0.10 mole) in THF was added dropwise. During the addition the temperature was maintained at -65°C. The reaction mixture was stirred for an additional one hour at this temperature. The reaction mixture was gradually allowed to warm up to room temperature and stirred overnight after which time the mixture was concentrated and the residue extracted with ether. The ether layer was

washed sequentially with 2N HCl , water and brine and then dried ($MgSO_4$). Removal of solvent gave an oil which was purified by silica gel column chromatography.

Method B:

A solution of the heterocyclic compound (0.10 mole) in THF was added to a stirred solution of sodium hydride (0.10 mole) in THF. The mixture was stirred at room temperature for one hour and a solution of the bromoalkylaryl ether (0.10 mole) in THF was added slowly. The reaction mixture was heated under reflux overnight (10 hours), concentrated and the resulting solution was extracted with ether (3 times). The combined ether extracts were washed with water and dried ($MgSO_4$). Removal of solvent gave the crude compound which was purified by silica gel column chromatography.

2.2.5. General method for the oxidation of sulfur in thio compounds

m-Chloroperbenzoic acid (0.10 mole) was added in batches to a stirred solution of thioether (0.10 mole) in dichloromethane, to maintain temperature (-5° to 0°C). After the addition was completed, the reaction mixture was allowed to stir at 0°C for 30 minutes. This mixture was washed with sodium bicarbonate solution and water, dried ($MgSO_4$) and filtered. The filtrate was concentrated under reduced pressure to get crude product which was purified by column chromatography to give sulfoxide.

A similar experiment was carried out to prepare sulfone by us

two equivalents of MCPBA and stirring the reaction mixture at room temperature for about two hours.

2.2.6 Preparation of the desired products

Preparation of 5-[5-(2-Chloro-4-methoxyphenoxy)pentyl]-3-methyl-isoxazole (34)

Method of preparation: A

Eluant for column chromatography: 55% hexane and 45% ethyl acetate

% Yield: 63.9; mp 42-44°C.

IR(KBr): 3074, 2934, 2877, 1605, 1507, 1270, 1056 cm^{-1}

NMR: δ 7.03-6.80(m, 3H, Ar-H); 5.86(s, 1H, -N=C-CH-); 4.00(t, 2H, J=6Hz, -O-CH_2-); 3.80(s, 3H, -O-CH_3); 2.77(t, 2H, J=7Hz, -CH_2-C=C-); 2.30(s, 3H, -CH_3); 2.15-1.15(m, 6H, -$(CH_2)_3$-).

Anal. Calcd. for $C_{16}H_{20}NO_3Cl$: C, 62.03; H, 6.46; N, 4.52. Found: C, 62.42; H, 6.49; N, 4.69.

Preparation of 5-[6-(2-Chloro-4-methoxyphenoxy)hexyl]-3-methyl-isoxazole (35).

Method of preparation: A

Eluant for column chromatography: 50% hexane and 50% ethyl acetate

% Yield: 55.0; mp 26-27°C (lit.[137] as oil)

IR(neat): 3074, 2943, 2860, 1605, 1500, 1210, 1052 cm^{-1}

NMR: δ 6.93-6.66(m, 3H, Ar-H); 5.76(s, 1H, -N=C-CH-); 3.90(t, 2H, -CH_2-O-); 3.71(s, 3H, -OCH_3); 2.66(t, 2H, J=6Hz, -CH_2-C=C); 2.23(s, 3H, -CH_3); 2.03-1.16(m, 8H, -$(CH_2)_4$-).

Preparation of 5-[7-(2-Chloro-4-methoxyphenoxy)heptyl]-3-methyl-isoxazole (9)

Method of preparation: A

Eluant for column chromatography: 60% hexane and 40% ethyl acetate

% Yield: 60.5; mp 44-6°C.(lit.[137] 45-6°C)

IR(KBr): 2951, 2852, 1602, 1495, 1211, 1050 cm^{-1}

NMR: δ 6.95-6.73(m, 3H, Ar-H); 5.80(s, 1H, -N=C-CH); 3.94(t, 2H, J=5Hz, $-CH_2-O-$); 3.76(s, 3H, $-O-CH_3$); 2.68(t, 2H, J=7Hz, $-CH_2-C=C-$); 2.26(s, 3H, $-CH_3$); 2.03-1.16(m, 10H, $-(CH_2)_5-$)

Preparation of 5-[8-(2-Chloro-4-methoxyphenoxy)octyl]-3-methyl-isoxazole (36)

Method of preparation: A

Eluant for column chromatography: 50% hexane and 50% ethyl acetate

% Yield: 68.2; mp 34-35°C

IR(KBr): 3076, 2926, 2869, 1608, 1502, 1212, 1049 cm^{-1}

NMR: δ 7.00-6.80(m, 3H, Ar-H); 5.83(s, 1H, -O-C=CH-); 3.94(t, 2H, J=6Hz, $-CH_2-O-$); 3.73(s, 3H, $-OCH_3$); 2.69(t, 2H, J=7.5Hz, $-CH_2-C=C-$); 2.30(s, 3H, $-CH_3$); 2.05-1.15(m, 12H, $-(CH_2)_6-$)

Anal. Calcd. for $C_{19}H_{26}NO_3Cl$: C, 64.86; H, 7.39; N, 3.98. Found: C, 65.08; H, 7.40; N, 4.07

Preparation of 5-[9-(2-Chloro-4-methoxyphenoxy)nonyl]-3-methyl-isoxazole (37)

Method of preparation: A

Eluant for column chromatography: 50% hexane and 50% ethyl acetate

% Yield: 71.5; mp 49-51°C

IR(KBr): 3074, 2934, 2860, 1605, 1505, 1219, 1045 cm^{-1}

NMR: δ 7.00-6.80(m, 3H, Ar-H); 5.83(s, 1H, -N=C-CH-); 3.96(t, 2H, J=6Hz, $-CH_2-O-$); 3.75(s, 3H, $-OCH_3$); 2.69(t, 2H, J=7Hz, $-CH_2-C=C-$); 2.25(s, 3H, $-CH_3$); 2.00-1.05(m, 14H, $-(CH_2)_7-$)

Anal. Calcd. for $C_{20}H_{28}NO_3Cl$: C, 66.02; H, 7.70; N, 3.85. Found: C, 65.88; H, 7.77; N, 3.77

Preparation of 5-[6-(3-Chloro-5-methoxyphenoxy)hexyl]-3-methyl-isoxazole (38)

Method of preparation: A

Eluant for column chromatography: 60% hexane and 40% ethyl acetate

% Yield: 60.2; mp 49-50°C

IR(KBr): 3074, 2943, 2869, 1605, 1499, 1223, 1028 cm^{-1}

NMR: δ 6.53-6.33(m, 3H, Ar-H); 5.80(s, 1H, -N=C-CH-); 4.03-3.76(m, 5H, $-CH_2-O$, $-OCH_3$); 2.71(t, 2H, J=7.5Hz, $-CH_2-C=C$); 2.26(s, 3H, $-CH_3$); 2.03-1.16(m, 8H, $-(CH_2)_4-$)

Anal. Calcd. for $C_{17}H_{22}NO_3Cl$: C, 63.06; H, 6.80; N, 4.32. Found: C, 63.28; H, 6.74; N, 4.39

Preparation of 3-Methyl-5-(6-phenoxyhexyl)isoxazole (39)

Method of preparation: A

Eluant for column chromatography: 50% hexane and 50% ethyl acetate

% Yield: 79.5; n_{20} 1.5171

IR(Neat): 3074, 2943, 2860, 1597, 1499, 1236, 1031 cm^{-1}

NMR: δ 7.46-6.80(m, 5H, Ar-H); 5.80(s, 1H, -N=C-CH); 3.96(t, 2H,

J=6Hz, $-C\underline{H}_2-O$); 2.71(t, 2H, J=7Hz, $-C\underline{H}_2-C=C$); 2.23(s, 3H, $-C\underline{H}_3$); 2.06-1.10(m, 8H, $-(C\underline{H}_2)_4$)

Anal. Calcd. for $C_{16}H_{21}NO_2$: C, 74.13; H, 8.10; N, 5.40. Found: C, 74.20; H, 8.17; N, 5.24.

Preparation of 5-[6-(4-Methoxyphenoxy)hexyl]-3-methylisoxazole (40)

Method of preparation: A

Eluant for column chromatography: 80% hexane and 20% ethyl acetate

% Yield: 66.8; mp 29-31°C.

IR(KBr): 3074, 2934, 2850, 1607, 1508, 1228, 1036 cm^{-1}

NMR: δ 6.83(s,4H, Ar-H); 5.83(s, 1H, $-N=C-C\underline{H}-$); 4.01-3.76(m, 5H, $-C\underline{H}_2-O-$, $-OC\underline{H}_3$); 2.71(t, 2H, J=7Hz, $-C\underline{H}_2-C=C-$); 2.26(s, 3H, $-C\underline{H}_3$); 2.10-1.20(m, 8H, $-(C\underline{H}_2)_4-$)

Anal. Calcd. for $C_{17}H_{23}NO_3$: C, 70.58; H, 7.95; N, 4.84. Found: C, 70.13; H, 8.08; N, 4.71.

Preparation of 5-(6-Bromohexane)-3-methylisoxazole (41)

Method of preparation: A

Eluant for column chromatography: 90% hexane and 10% ethyl acetate

% Yield: 43.5; n_{20} 1.5005

IR(neat): 2934, 2869, 1605, 1167 cm^{-1}

NMR: δ 5.80(s, 1H, $-O-C=C\underline{H}-$); 3.40(t, 2H, J=6Hz, $-C\underline{H}_2-Br$); 2.74(t, 2H, J=7Hz, $-C\underline{H}_2-C=C-$); 2.23(s, 3H, $-C\underline{H}_3$); 2.15-1.25(m, 8H, $-(C\underline{H}_2)_4-$)

Anal. Calcd. for $C_{10}H_{16}NOBr$: C, 48.90; H, 6.53; N, 5.71. Found: C, 48.56; H, 6.56; N, 5.96.

Preparation of 1,7-Di-(3-methylisoxazol-5-yl)heptane (42)

Method of preparation: A

Eluant for column chromatography: 75% hexane and 25% ethyl acetate

% Yield: 40.0; bp 132-34°C/0.2 mm

IR(neat): 2934, 2852, 1597, 1500 cm^{-1}

NMR: δ 5.82(s, 2H, Ar-H); 2.70(t, 4H, J=7Hz, 2X-CH_2-C=C-); 2.23(s, 6H, 2X-CH_3); 2.00-1.30(m, 10H, -$(CH_2)_5$-)

Anal. Calcd. for $C_{15}H_{22}N_2O_2$: C, 68.70; H, 8.39; N, 10.68. Found: C, 68.53; H, 8.21; N, 10.26.

Preparation of 5-[7-(Benzotriazol-1'-yloxy)heptyl]-3-methyl-isoxazole (43)

Method of preparation: A

Eluant for column chromatography: 30% hexane and 70% ethyl acetate

% Yield: 52.8; n_{20} 1.5345

IR(neat): 3074, 2943, 2860, 1608, 1095 cm^{-1}

NMR: δ 8.03(d, 1H, J=7.5Hz, 7'-H); 7.79-7.29(m, 3H, Ar-H); 5.86(s, 1H, -N=C-CH); 4.56(t, 2H, J=6Hz, -CH_2-O-); 2.72(t, 2H, J=6Hz, -CH_2-C=C-); 2.23(s, 3H, -CH_3); 2.00-1.23(m, 10H, -$(CH_2)_5$-)

Anal. Calcd. for $C_{17}H_{22}N_4O_2$: C, 64.90; H, 7.00; N, 17.83. Found: C, 64.99; H, 7.16; N, 17.56.

Preparation of 3-Methyl-5-[7-(quinolin-8-yloxy)heptyl]isoxazole (44)

Method of preparation: A

Eluant for column chromatography: 20% hexane and 80% ethyl acetate

% Yield: 49.38; mp 90-92°C

IR(KBr): 3074, 2934, 2860, 1605, 1474, 1260, 1040 cm^{-1}

NMR: δ 9.00(d, 1H, J=6Hz, 2'-H); 8.17(d, 1H, J=7.5Hz, 4'-H); 7.59-7.06(m, 4H, Ar-H); 5.82(s, 1H, -N=C-CH); 4.23(t, 2H, J=7.5Hz, $-CH_2-O$); 2.81(t, 2H, J=7.5Hz, $-CH_2-C=C-$); 2.23(s, 3H, $-CH_3$); 2.00-1.24(m, 10H, $-(CH_2)_5-$)

Anal. Calcd. for $C_{20}H_{24}N_2O_2$: C, 74.00; H, 7.40; N, 8.64. Found: C, 73.91; H, 7.43; N, 8.91.

Preparation of 5-[7-(2-Chloro-4-methoxyphenoxy)-1-methylthioheptyl]-3-methylisoxazole (27)

Method of preparation: A

Eluant for column chromatography: 75% hexane and 25% ethyl acetate

% Yield: 85.0; mp 45-47°C

IR(KBr): 3075, 2943, 2860, 1600, 1498, 1209, 1052 cm^{-1}

NMR: δ 6.96-6.73(m, 3H, Ar-H); 5.93(s, 1H, -N=C-CH-); 4.03-3.73(m, 6H, -CH-S-, $-CH_2-O-$, $-O-CH_3$); 2.23(s, 3H, $-CH_3$); 2.00(s, 3H, $-SCH_3$); 2.03-1.10(m, 10H, $-(CH_2)_5-$)

Anal. calcd. for $C_{19}H_{26}NO_3SCl$: C, 59.45; H, 6.77; N, 3.65. Found: C, 59.05; H, 6.88; N, 3.52.

Preparation of 5-[7-(2-Chloro-4-methoxyphenoxy)-1-methylsulfoxyheptyl]-3-methylisoxazole (45)

Method of preparation: Oxidation of Sulfur in 27 with 1 eq. of MCPBA

Eluant for column chromatography: 33% hexane and 66% ethyl acetate

% Yield: 75.3; mp 43-45°C

IR(neat): 3076, 2943, 2860, 1607, 1496, 1224, 1050 cm^{-1}

NMR: δ 6.96-6.76(m, 3H, Ar-H); 6.19(d, 1H, J=6Hz, -N=C-CH-); 4.03-3.76 (m, 6H, -CH-S=O, $-CH_2-O-$, $-OCH_3$); 2.40(s, 3H, $O=S-CH_3$); 2.30(s, 3H, $-CH_3$); 2.00-1.00(m, 10H, $-(CH_2)_5$)

Anal. calcd. for $C_{19}H_{26}NO_4SCl$: C, 57.07; H, 6.15; N, 3.50. Found: C, 57.31; H, 6.46; N, 3.49.

Preparation of 5-[7-(2-Chloro-4-methoxyphenoxy)-1-methylsulfonyl-heptyl]-3-methylisoxazole (46)

Method of preparation: Oxidation of Sulfur in 27 with 2 eq. MCPBA

Eluant for column chromatography: 60% hexane and 40% ethyl acetate

% Yield: 80.5; mp 73-75°C

IR(KBr): 3078, 2934, 2860, 1605, 1507, 1302, 1220, 1050 cm^{-1}

NMR: δ 6.96-6.76(m, 3H, Ar-H); 6.33(s, 1H, -N=C-CH-); 4.33-4.16(m, 1H, $-CH-SO_2-$); 4.00-3.66(m, 5H, $-CH_2-O-$, $-OCH_3$); 2.76(s, 3H, $-SO_2-CH_3$); 2.30(s, 3H, $-CH_3$); 2.00-1.00(m, 10H, $-(CH_2)_5-$)

Anal. calcd. for $C_{19}H_{26}NO_5SCl$: C, 55.80; H, 6.25; N, 3.36. Found: C, 55.99; H, 6.35; N, 3.26.

Preparation of 3-Methyl-5-(6-phenylthiohexyl)isoxazole (47)

Method of preparation: A

Eluant for column chromatography: 80% hexane and 20% ethyl acetate

% Yield: 85.5; bp 160-62°C/0.1 mm

IR(neat): 3070, 2943, 2860, 1605, 1491 cm^{-1}

NMR: δ 7.40-7.13(m, 5H, Ar-H); 5.80(s, 1H, -N=C-CH-); 3.03-2.56(m, 4H, $-CH_2-C=C-$, $-CH_2-S-$); 2.23(s, 3H, $-CH_3$); 2.00-1.10(m, 8H, $-(CH_2)_4-$)

Anal. calcd. for $C_{16}H_{21}NOS$: C, 69.80; H, 7.63; N, 5.09. Found: C, 69.62; H, 7.29; N, 4.92.

Preparation of 3-Methyl-5-(6-phenylsulfoxyhexyl)isoxazole (48)

Method of preparation: Oxidation of Sulfur in 47 with one eq. MCPBA

Eluant for column chromatography: 60% hexane and 40% ethyl acetate

% Yield: 74.8; n_{20} 1.5466

IR(neat): 3076, 2934, 2860, 1614, 1039 cm^{-1}

NMR: δ 7.69-7.49(m, 5H, Ar-H); 5.82(s, 1H, -N=C-CH-); 3.00-2.59(m, 4H, $-CH_2-C=C-$, $-CH_2-SO-$); 2.26(s, 3H, $-CH_3$); 2.00-1.39(m, 8H, $-(CH_2)_4-$)

Anal. calcd. for $C_{16}H_{21}NO_2S$: C, 65.90; H, 7.21; N, 4.81. Found: C, 65.60; H, 7.03; N, 4.61.

Preparation of 3-Methyl-5-(6-phenylsulfonylhexyl)isoxazole (49)

Method of preparation: Oxidation of Sulfur in 47 with 2 eq. MCPBA

Eluant for column chromatography: 30% hexane and 70% ethyl acetate

% Yield: 79.9; mp 50-52°C

IR(KBr): 3074, 2943, 2852, 1605, 1294, 1146 cm^{-1}

NMR: δ 8.13-8.06(m, 2H, Ar-H); 7.81-7.37(m, 3H, Ar-H); 5.93(s, 1H, -N=C-CH-); 3.21(t, 2H, J=7.5Hz, $-CH_2-SO_2-$); 2.79(t, 2H, J=7.5Hz, $-CH_2-C=C-$); 2.36(s, 3H, $-CH_3$); 2.10-1.15(m, 8H, $-(CH_2)_4-$)

Anal. calcd. for $C_{16}H_{21}NO_3S$: C, 62.50; H, 6.84; N, 4.56. Found: C, 62.61; H, 6.92; N, 4.59.

Preparation of 1-[6-(2-Chloro-4-methoxyphenoxy)hexyl]-3,5-dimethylpyrazole (50)

Method of preparation: B

Eluant for column chromatography: 40% hexane and 60% ethyl acetate

% Yield: 72.0; mp 27-28°C

IR(KBr): 2943, 2869, 1502, 1212, 1052 cm^{-1}

NMR: δ 6.93-6.70(m, 3H, Ar-H); 5.70(s, 1H, -N-C=CH); 3.90(t, 4H, J=6Hz, $-CH_2-O-$, $-CH_2-N-$); 3.70(s, 3H, $-OCH_3$); 2.20(s, 6H, 2 X-CH_3); 2.01-1.13(m, 8H, $-(CH_2)_4-$)

Anal. calcd. for $C_{18}H_{25}N_2O_2Cl$: C, 64.19; H, 7.42; N, 8.30. Found: C, 63.78; H, 7.48; N, 7.96.

Preparation of 1-[7-(2-Chloro-4-methoxyphenoxy)heptyl]-3,5-dimethylpyrazole (51)

Method of preparation: B

Eluant for column chromatography: 50% hexane and 50% ethyl acetate

% Yield: 70.12; mp 35-37°C

IR(KBr): 3074, 2943, 2860, 1502, 1212, 1049 cm^{-1}

NMR: δ 6.96-6.70(m, 3H, Ar-H); 5.73(s, 1H, -N-C=CH); 3.91(t, 4H, J=6Hz, $-CH_2-O-$, $-CH_2-N-$); 3.73(s, 3H, $-O-CH_3$); 2.20(s, 6H, 2 X-CH_3); 2.03-1.13(m, 10H, $-(CH_2)_5-$)

Anal. calcd. for $C_{19}H_{27}N_2O_2Cl$: C, 65.00; H, 7.70; N, 7.69. Found: C, 64.55; H, 7.77; N, 7.64.

Preparation of 1-[6-(3-Chloro-5-methoxyphenoxy)hexyl]-3,5-dimethylpyrazole (52)

Method of preparation: B

Eluant for column chromatography: 80% hexane and 20% ethyl acetate

% Yield: 83.8; mp 48-49°C

IR(KBr): 3074, 2934, 2860, 1589, 1203, 1056 cm^{-1}

NMR: δ 6.96-6.73(m, 3H, Ar-H); 5.76(s, 1H, -N-C=CH-); 3.96(t, 4H, J=6Hz, $-CH_2-O-$, $-CH_2-N-$); 3.76(s, 3H, $-OCH_3$); 2.23(s, 6H, 2 X-CH_3); 2.00-1.10(m, 8H, $-(CH_2)_4$)

Anal. calcd. for $C_{18}H_{25}N_2O_2Cl$: C, 64.19; H, 7.42; N, 8.32. Found: C, 64.50; H, 7.47; N, 8.20.

Preparation of 5-[6-(2-Chloro-4-methoxyphenoxy)hexyl]-4-methyl-isoxazole (53)

Method of preparation: A

Eluant for column chromatography: 85% hexane and 15% ethyl acetate

% Yield: 59.6; mp 29-31°C

IR(nujol): 1499, 1210, 1048 cm^{-1}

NMR: δ 8.20(s, 1H, -N=CH-); 6.93-6.73(m, 3H, Ar-H); 3.96(t, 2H, J=6Hz, $-O-CH_2-$); 3.43(s, 3H, $-OCH_3$); 2.85(t, 2H, J=7Hz, $-CH_2-C=C-$); 2.20(s, 3H; $-CH_3$); 2.03-1.10(m, 8H, $-(CH_2)_4-$)

Anal. calcd. for $C_{17}H_{22}NO_2SCl$: C, 60.08; H, 6.48; N, 4.12. Found: C, 60.34; H, 6.52; N, 3.97.

Preparation of 5-[7-(2-Chloro-4-methoxyphenoxy)heptyl]-4-methyl-isothiazole (54)

Method of preparation: A

Eluant for column chromatography: 85% hexane and 15% ethyl acetate

% Yield: 52.3; bp 182-8[illegible]/0.1 mm

IR(neat): 3074, 2934, 2869, 1500, 1215, 1058 cm^{-1}

NMR: δ 8.26(s, 1H, -N=CH-); 6.96-6.76(m, 3H, Ar-H); 3.94(t, 2H, J=6Hz,

$-CH_2-O-$); 3.76(s, 3H, $-OCH_3$); 2.76(t, 2H, J=7Hz, $-CH_2-C=C-$); 2.16(s, 3H, $-CH_3$); 2.01-1.15(m, 10H, $-(CH_2)_5-$)

Anal. calcd. for $C_{18}H_{24}NO_2SCl$: C, 61.10; H, 6.78; N, 3.96. Found: C, 61.35; H, 7.04; N, 4.18.

Preparation of 5-[6-(2-Chloro-4-methoxyphenoxy)hexyl]isothiazole (55)

Method of preparation: A

Eluant for column chromatography: 75% hexane and 25% ethyl acetate

% Yield: 49.0; mp 40-42°C

IR(KBr): 3074, 2931, 2843, 1605, 1508 cm^{-1}

NMR: δ 8.36(s, 1H, -N=CH-); 7.00-6.73(m, 4H, -S-C=CH-, Ar-H); 3.93(t, 2H, J=6Hz, $-O-CH_2-$); 3.70(s, 3H, $-OCH_3$); 2.93(t, 2H, J=7.5Hz, $CH_2-C=C-$); 2.00-1.10(m, 8H, $-(CH_2)_4-$)

Anal. calcd. for $C_{16}H_{20}NO_2SCl$: C, 58.99; H, 6.14; N, 4.30. Found: C, 59.22; H, 6.14; N, 4.39.

Preparation of 5-[7-(2-Chloro-4-methoxyphenoxy)heptyl]isothiazole (56)

Method of preparation: A

Eluant for cholumn chromatography: 80% hexane and 20% ethyl acetate

% Yield: 55.9; bp 178-80°C/0.1 mm

IR(neat): 3074, 2934, 2852, 1502, 1212, 1058 cm^{-1}

NMR: δ 8.36(s, 1H, -N=CH-); 7.00-6.73(m, 4H, -S-C=CH, Ar-H); 3.93(t, 2H, J=6Hz, $-CH_2-O-$); 3.73(s, 3H, $-OCH_3$); 2.90(t, 2H, J=7.5Hz, $CH_2-C=C-$); 2.03-1.10(m, 10H, $-(CH_2)_5-$)

Anal. calcd. for $C_{17}H_{22}NO_2SCl$: C, 60.08; H, 6.48; N, 4.12. Found: C,

60.39; H, 6.67; N, 3.77.

Preparation of 2-[6-(2-Chloro-4-methoxyphenoxy)hexyl]-4-methyl-thiazole (57)

Method of preparation: A

Eluant for column chromatography: 25% hexane and 75% ethyl acetate

% Yield: 69.3; bp 170-72°C/0.1 mm

IR(neat): 3074, 2943, 2852, 1495, 1217, [illegible]54 cm^{-1}

NMR: δ 6.96-6.66(m, 4H, -S-C<u>H</u>=C-, Ar-<u>H</u>); 3.96(t, 2H, J=6Hz, -C<u>H</u>$_2$-O-); 3.76(s, 3H, -OC<u>H</u>$_3$); 2.99(t, 2H, J=7.5Hz, -C<u>H</u>$_2$-C=N-); 2.46(s, 3H, -C<u>H</u>$_3$); 2.10-1.15(m, 8H, -(C<u>H</u>$_2$)$_4$-)

Anal. calcd. for $C_{17}H_{22}NO_2SCl$: C, 60.00; H, 6.48; N, 4.12. Found: C, 59.94; H, 6.48; N, 4.06.

Preparation of 2-[7-(2-Chloro-4-methoxyphenoxy)heptyl]-4-methyl-thiazole (58)

Method of preparation: A

Eluant for column chromatography: 66% hexane and 33% ethyl acetate

% Yield: 68.5; mp 30-32°C

IR(nujol): 1503, 1213, 1050 cm^{-1}

NMR: δ 6.96-6.66(m, 4H, -S-C<u>H</u>=C-, Ar-<u>H</u>); 3.93(t, 2H, J=6Hz, -C<u>H</u>$_2$-O-); 3.73(s, 3H, -C<u>H</u>$_3$); 2.96(t, 2H, J=7.5Hz, -C<u>H</u>$_2$-C=N-); 2.40(s, 3H, -C<u>H</u>$_3$); 2.10-1.10(m, 10H, -(C<u>H</u>$_2$)$_5$-)

Anal. calcd. for $C_{18}H_{24}NO_2SCl$: C, 61.10; H, 6.79; N, 3.96. Found: C, 61.37; H, 6.82; N, 3.83.

Preparation of 1-[7-(2-Chloro-4-methoxyphenoxy)heptyl]imidazole (59)

Method of preparation: B

Eluant for column chromatography: 10% hexane and 90% ethyl acetate

% Yield: 80.2; bp 196-98°C/0.1 mm

IR(neat): 3088, 2959, 2852, 1605, 1501, 1210, 1030 cm^{-1}

NMR: δ 7.43(s, 1H, -N-C<u>H</u>=N-); 7.06-6.59(m, 5H, Ar-<u>H</u>); 4.00-3.62(m, 7H, -N-C<u>H</u>$_2$-, -O-C<u>H</u>$_2$-, -OC<u>H</u>$_3$); 1.85-1.23(m, 10H, -(C<u>H</u>$_2$)$_5$-)

Anal. calcd. for $C_{17}H_{23}N_2O_2Cl$: C, 63.25; H, 7.18; N, 8.60. Found: C, 62.96; H, 7.03; N, 8.15.

Preparation of 5-[6-(2-Chloro-4-methoxyphenoxy)hexyl]-1-methyl-1,2,4-triazole (60)

Method of preparation: A

Eluant for column chromatography: 20% hexane and 80% ethyl acetate

% Yield: 50.3; n_{20} 1.5400

IR(neat): 2934, 2860, 1497, 1216, 1046 cm^{-1}

NMR: δ 7.80(s, 1H, -N=C<u>H</u>-); 7.00-6.80(m, 3H, Ar-<u>H</u>); 3.99(t, 2H, J=6Hz, -C<u>H</u>$_2$-O-); 3.83(s, 3H, -N-C<u>H</u>$_3$); 3.76(s, 3H, -OC<u>H</u>$_3$); 2.78(t, 2H, J=7.5Hz, -C<u>H</u>$_2$-C=N-); 2.00-1.25(m, 8H, -(C<u>H</u>$_2$)$_4$-)

Anal. calcd. for $C_{16}H_{22}N_3O_2Cl$: C, 59.35; H, 6.80; N, 12.98. Found: C, 59.62; H, 6.76; N, 12.95.

Preparation of 5-[7-(2-Chloro-4-methoxyphenoxy)heptyl]-1-methyl-1,2,4-triazole (61)

Method of preparation: A

Eluant for column chromatography: 20% hexane and 80% ethyl acetate

% Yield: 48.0; mp 42-43°C

IR(KBr): 3074, 2943, 2864, 1497, 1222, 1041 cm^{-1}

NMR:δ7.80(s, 1H, -N=C<u>H</u>-N-); 6.96-6.76(m, 3H, Ar-<u>H</u>); 3.96(t, 2H, J=6Hz, -C<u>H</u>$_2$-O-); 3.80(s, 3H, -N-C<u>H</u>$_3$); 3.73(s, 3H, -OC<u>H</u>$_3$); 2.71(t, 2H, J=7.5Hz, -C<u>H</u>$_2$-C=N-); 2.10-1.10(m, 10H, -(C<u>H</u>$_2$)$_5$-)

Anal. calcd. for $C_{17}H_{24}N_3O_2Cl$: C, 60.44; H, 7.11; N, 12.44. Found: C, 60.29; H, 7.18, N; 12.78.

Preparation of 5-[7-(2-Chloro-4-methoxyphenoxy)heptyl]-1-phenyl-1,2,4-triazole (<u>62</u>)

Method of preparation: A

Eluant for column chromatography: 30% hexane and 70% ethyl acetate

% Yield: 50.5; n_{20} 1.5740

IR(neat): 3070, 2934, 2860, 1599, 1504, 1212, 1051 cm^{-1}

NMR: δ 8.64(s, 1H, -N-C<u>H</u>=N-); 7.82-6.79(m, 8H, Ar-<u>H</u>); 3.99(t, 2H, J=6Hz, -C<u>H</u>$_2$-O-); 3.72(s, 3H, -OC<u>H</u>$_3$); 2.77(t, 2H, J=6Hz, -C<u>H</u>$_2$-C=N-); 2.20-1.15(m, 10H, -(C<u>H</u>$_2$)$_5$-)

Anal. calcd. for $C_{22}H_{26}N_3O_2Cl$: C, 66.08; H, 6.50; N, 10.51. Found: C, 66.38; H, 6.13; N, 10.62.

Chapter 3

ANTIVIRAL TESTING EXPERIMENTS

In vitro antiviral activity experiments were performed by a plaque reduction assay method. Vero cells were infected with HSV-1 to calculate percentage inhibition (drug concentration of 5 ug/ml) and MIC_{50} values of the synthesized compounds.

3.1 Percentage inhibition of plaque formation by HSV-1 in Vero cells by compounds at 5 ug/ml

Materials:

Vero cells (originally from ATCC) were infected with HSV-1 of JLJ strain, isolated from a patient with herpes encephalitis. The minimum essential medium (MEM), eagle (modified) was bought from Flow Laboratories, Inc. and was supplemented with 100 iu/ml pencillin G, 100 ug/ml streptomycin and 4% calf serum. The compounds were dissolved in DMSO to get concentration of 10 mg/ml and then further diluted by MEM to 5 ug/ml.

Procedure:

Confluent monolayers of Vero cells were prepared in MEM in 60 mm tissue culture plates and maintained at 37°C in a humidified 5% CO_2 incubator. For plaque assays, the medium was removed from the cells and replaced with 0.2 ml/plate of MEM containing HSV-1 with or without (control) the drug to be tested. Virus was allowed to adsorb for one hour in the incubator before the addition of 4 ml/plate overlay (1% agarose in MEM) containing the drug at the same concentration as in the virus preparation. After two or three days in the incubator at 37°C, at a time when plaques were visible, the cells were stained with

0.02% neutral red in MEM for at least four hours before the plaques were counted. Each test was done in duplicate and the average of the two plaque counts used in the calculations.

Tables 3.1 to 3.5 show the inhibition of plaque formation by HSV-1 in Vero cells by compounds at 5 ug/ml.

Compound Number	Pfu/ml x 10^6 *	% Inhibition** at 5 ug/ml
Control	2.4	0
34	0.4	83.4
35	0	100
9	0	100
36	1.0	58.5
37	0.8	66.7

Table 3.1. Inhibition by compounds with different carbon chain lengths—isoxazole series.

* $$\text{Pfu/ml} = \frac{\text{number of plaques counted}}{\text{vol. of (virus and/or drug) solution added} \times \text{dilution factor}}$$

** $$\%\ \text{Inhibition} = 100 - \frac{\text{Pfu/ml measured in the presence of drug}}{\text{Pfu/ml measured in the absence of drug}} \times 100$$

Compound Number	Pfu/ml x 10^8	% Inhibition at 5 µg/ml
Control	1.9	0
39	0	100
40	0	100
35	0	100
38	0.1	94.8
50	0	100
52	0.9	52.7

Table 3.2: Inhibition by compounds with different 4-O-aryl substitution—isoxazole and pyrazole series.

Compound Number	Pfu/ml x 10^8	% Inhibition at 5 ug/ml
Control	3.6	0
35	0	100
9	0	100
50	0	100
51	0	100
53	0	100
54	0	100
55	0	100
56	0	100
57	*	
58	*	
60	0	100
61	0	100
62	0	100
59	0	100

Table 3.8: Inhibition by compounds with different heteroaromatic moieties.

* There were too many small sized plaques to count

ND: Not Done

Compound Number	Pfu/ml x 10^8	% Inhibition at 5 ug/ml
Control	3.3	0
9	0	100
41	2.6	21.3
42	1.3	60.7
39	0	100
47	0.4	87.9
48	0	100
49	0	100
44	0	100
43	0	100

Table 3.4: Inhibition by compounds with different -O-aryl moieties.

Compound Number	Pfu/ml x 10^6	% Inhibition at 5 ug/ml
Control	2.4	0
9	0	100
27	0.5	79.2
45	0	100
46	0	100

Table 3.5: Inhibition by compounds with substitution on α-CH_2 of carbon chain—isoxazole series.

3.2 Determination of MIC_{50} values

Similar experiments (Section 3.1) were carried out to determine the MIC_{50} values of the compounds, showing 100% inhibition, by varying the concentration of the drugs (10.0 to 0.1 ug/ml). For each compound, a graph of pfu/ml versus drug concentration was plotted. An example is shown for the reference compound 9 (Figure 3.1).

The following tables (3.6 to 3.24) show the minimum drug concentration required to reduce the plaque formation, by HSV-1 in Vero cells, by 50%.

Compound Number	Drug Conc. ug/ml	Pfu/ml x 10^8	MIC_{50} ug/ml
Control	0	5.1	
9	10	0	1.3
	5	0	
	1	2.6	
	0.5	3.6	
	0.1	4.0	

Table 3.6

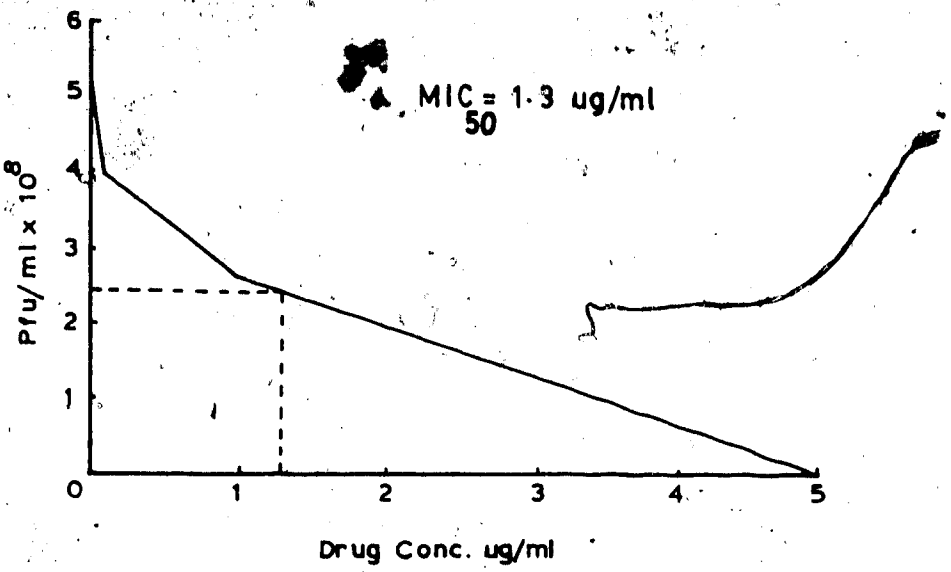


Figure 3.1: Graph of Pfu vs Drug concentration for WIN 49321

Tables showing the effect of the concentrations of different compounds on pfu/ml of HSV-1 preparation in Vero cells.

Compound Number	Drug Conc. ug/ml	Pfu/ml x 10^6	MIC_{50} ug/ml
Control	0	12.3	
35	5	0	1.4
	2	3.6	
	1	6.8	
	0.5	7.6	
	0.1	9.6	

Table 3.7

Compound Number	Drug Conc. ug/ml	Pfu/ml x 10^8	MIC_{50} ug/ml
Control	0	5.1	
39	10	0	1.5
	5	0	
	1	2.8	
	0.5	3.2	
	0.1	3.9	

Table 3.8

Compound Number	Drug Conc. ug/ml	Pfu/ml x 10^6	MIC_{50} ug/ml
Control	0	12.3	
40	5	0	1.0
	2	3.2	
	1	6.0	
	0.5	7.2	
	0.1	8.6	

Table 3.9

Compound Number	Drug Conc. ug/ml	Pfu/ml x 10^8	MIC_{50} ug/ml
Control	0	5.1	
43	10	0	1.8
	5	0	
	1	3.1	
	0.5	3.8	
	0.1	4.2	

Table 3.10

Compound Number	Drug Conc. ug/ml	Pfu/ml x 10^8	MIC_{50} ug/ml
Control	0	5.1	
44	10	0	1.7
	5	0	
	1	3.0	
	0.5	3.7	
	0.1	4.4	

Table 3.11

Compound Number	Drug Conc. ug/ml	Pfu/ml x 10^6	MIC_{50} ug/ml
Control	0	2.4	
27	5	0.5	3.4
	2	1.8	
	1	2.0	
	0.5	2.2	
	0.1	2.3	

Table 3.12

Compound Number	Drug Conc. ug/ml	Pfu/ml x 10^6	MIC_{50} ug/ml
Control	0	2.4	
45	5	0	1.3
	2	0	
	1	1.8	
	0.5	2.0	
	0.1	2.2	

Table 3.13

Compound Number	Drug Conc. ug/ml	Pfu/ml x 10^6	MIC_{50} ug/ml
Control	0	2.4	
46	5	0	1.2
	2	0.2	
	1	1.5	
	0.5	2.1	
	0.1	2.2	

Table 3.14

Compound Number	Drug Conc. ug/ml	Pfu/ml x 10^6	MIC_{50} ug/ml
Control	0	7.5	
47	5	1.8	1.6
	2	3.4	
	1	4.1	
	0.5	4.6	
	0.1	6.3	

Table 3.15

Compound Number	Drug Conc. ug/ml	Pfu/ml x 10^6	MIC_{50} ug/ml
Control	0	7.5	
48	5	0	0.4
	2	0.7	
	1	2.6	
	0.5	3.4	
	0.1	5.1	

Table 3.16

Compound Number	Drug Conc. ug/ml	Pfu/ml x 10^6	MIC_{50} ug/ml
Control	0	7.5	
49	5	0	0.5
	2	0.9	
	1	2.3	
	0.5	3.9	
	0.1	5.0	

Table 3.17

Compound Number	Drug Conc. ug/ml	Pfu/ml x 10^6	MIC_{50} ug/ml
Control	0	5.3	
50	10	0	0.7
	5	0	
	1	2.2	
	0.5	2.9	
	0.1	3.3	

Table 3.18

Compound Number	Drug Conc. ug/ml	Pfu/ml x 10^6	MIC_{50} ug/ml
Control	0	2.5	
51	5	0	1.3
	2	0.6	
	1	1.5	
	0.5	2.0	
	0.1	2.0	

Table 3.19

Compound Number	Drug Conc. ug/ml	Pfu/ml x 10^6	MIC_{50} ug/ml
Control	0	2.5	
54	5	0	0.7
	2	0.5	
	1	1.0	
	0.5	1.3	
	0.1	1.9	

Table 3.20

Compound Number	Drug Conc. ug/ml	Pfu/ml x 10^6	MIC_{50} ug/ml
Control	0	2.5	
56	5	0	1.3
	2	0.6	
	1	1.5	
	0.5	1.8	
	0.1	2.0	

Table 3.21

Compound Number	Drug Conc. ug/ml	Pfu/ml x 10^6	MIC_{50} ug/ml
Control	0	7.5	
59	5	0	0.5
	2	0.1	
	1	1.6	
	0.5	4.0	
	0.1	4.3	

Table 3.22

Compound Number	Drug Conc. ug/ml	Pfu/ml x 10^6	MIC_{50} ug/ml
Control	0	12.3	
61	5	0	1.0
	2	2.5	
	1	6.1	
	0.5	7.2	
	0.1	9.8	

Table 3.23

Compound Number	Drug Conc. ug/ml	Pfu/ml x 10^6	MIC_{50} ug/ml
Control	0	12.3	
62	5	0	0.8
	2	1.3	
	1	5.7	
	0.5	7.1	
	0.1	9.0	

Table 3.24

3.3 Preliminary study of mode of action

Recent studies indicate that two WIN compounds (15, 15a) inhibit the replication of HRV-2 and -14 by inhibiting the uncoating of the viral RNA[140]. In our laboratory three experimental variations, using WIN 49321 (9), were carried out with HSV-1 to obtain preliminary data on the mechanism of action of these synthesized compounds on HSV-1 virion.

Experiment 1: Cells + virus + drug in overlay

Experiment 2: Cells + virus, drug + drug in overlay

Experiment 3: Cells + virus, drug + overlay

In the first experiment, cells were infected with virus, kept in the incubator for one hour and then overlay (1% agarose) with or without drug (5 ug/ml) was added to each plate. The number of pfu/ml in the presence of the drug was found to be very close to the number obtained in the absence of drug (Table 3.25), showing that WIN 49321 did not inhibit the viral replication, after the virus was bound and entered the cell.

In the second experiment, the solution of drug (5 ug/ml) with virus was added to cells which were kept in the incubator for one hour. Overlay (1% agarose) with drug solution was added to the plate which were kept for two to three days in the incubator, at which time plaques were counted. In this experiment, WIN 49321 showed 100% inhibition (Table 3.25).

In the third experiment, the solution of drug (5 ug/ml) with virus was added to cells which were kept in the incubator for one

hour. Overlay (1% agarose) was added to the plates which were kept for two to three days in the incubator, at which time plaques were counted. In this experiment, the plaque formation was not inhibited completely by the compound (Table 3.25).

Expt.	Compound Number	Pfu/ml x 10^8	% Inhibition @ 5 ug/ml
1	Control	3.6	0
	WIN 49321	2.9	19.5
2	Control	3.8	0
	WIN 49321	0	100
3	Control	2.9	0
	WIN 49321	2.2	24.2

Table 3.25 Effect of WIN 49321 on HSV-1 replication when drug was added at different times.

The experiments suggest that the active compounds inhibit one of the early events of viral replication and should be present throughout the viral replication cycle. The mode of action of these compounds against HSV-1 is probably similar to that recently described for HRV-14.

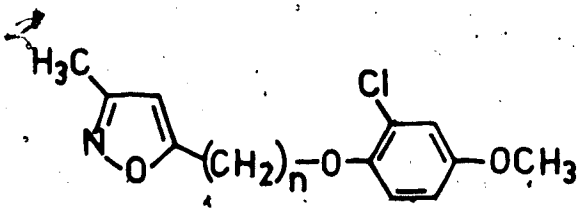
Chapter 4

RESULTS AND DISCUSSION OF ANTIVIRAL EXPERIMENTS

4.1 Results of antiviral experiments

All the synthesized compounds were tested *in vitro* to establish the structure-activity relationships by a plaque reduction assay method. Vero cells were infected with HSV-1 for these experiments. MIC_{50} Values were calculated for the compounds with 100% inhibition at 5 ug/ml.

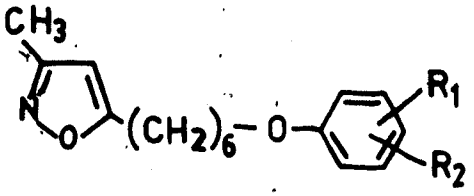
(a) The effect of change in carbon chain length on antiviral activity. In the isoxazole series (Table 4.1), the compounds with the best activity against HSV-1 are those in which the connecting carbon chain is 6 or 7.



Compound number	n	%Inhibition at 5 ug/ml	MIC_{50} (ug/ml)
34	5	83.4	ND
35	6	100	1.4
9	7	100	1.3
36	8	58.5	ND
37	9	66.7	ND

Table 4.1 Isoxazole series - The effect of change in carbon chain length on antiviral activity
ND: Not Done

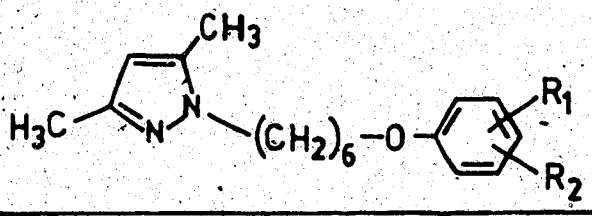
(b) The effect of changes in -O-aryl substitution on antiviral activity.



Compound number	-O-(R1, R2)	% Inhibition at 5 ug/ml	MIC_{50} (ug/ml)
39	-O-	100	1.5
40	-O-OCH3	100	1.0
35	Cl, -O-OCH3	100	1.4
38	Cl, -O-OCH3	94.8	ND

Table 4.2a Isoxazole series--The effect ofchanges in -O-aryl substitution on antiviral activity.
ND: Not Done

In vitro antiviral testing experiments against HSV-1 (Tables 4.2a and 4.2b) have indicated that the substitution pattern of the -O-aryl group has a profound effect on the activity of the compound. Among the isoxazole series (39, 40, 35 and 38), the 2-chloro-4-methoxyphenyl isomer 35 is more active than the 3-chloro-5-methoxy-phenyl isomer 38 and has comparable activity to the phenyl 39, and the 4-methoxyphenyl 40 compounds (Table 4.2a). The same conclusion can be drawn in the pyrazole derivatives 50 and 52 (Table 4.2b).



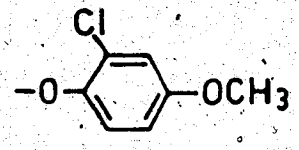
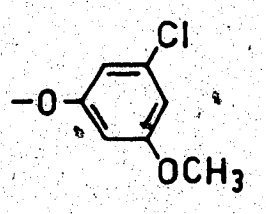
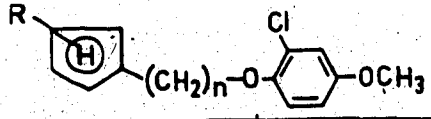
Compound Number	-O-(R1, R2)	% Inhibition (at 5ug/ml)	MIC_{50} (ug/ml)
50		100	0.7
52		52.7	ND

Table 4.2b Pyrazole series—The effect of changes in -O-aryl substitution on antiviral activity.
ND: Not Done



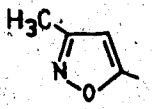
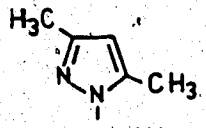
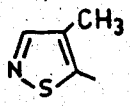
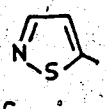
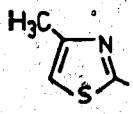
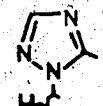
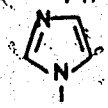
Compound Number	R	n	% Inhibition (at 5 ug mL)	MIC_{50} (ug/ml)
35		6	100	
9		7	100	1.3
50		6	100	
51		7	100	1.3
53		6	100	
54		7	100	0.7
55		6	100	
56		7	100	1.3
57		6	*	ND
58		7	*	
60		6	100	
61		7	100	1.0
62		7	100	0.8
59		7	100	0.5

Table 4.3 The effect of changes in heteroaromatic moiety on antiviral activity.

* There were too many small sized plaques to count.

ND: Not Done

(c) The effect of changes in heteroaromatic moiety on antiviral activity.

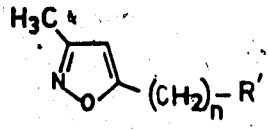
In vitro antiviral activity studies against HSV-1 by plaque reduction assay method have indicated that the isoxazole or pyrazole rings can be replaced by other five-membered heteroaromatic moieties with retention of activity (Table 4.3). Isothiazoles 53-56, 1,2,4-triazoles 60, 61 and 62 and imidazole 59 are equipotent with the isoxazoles 35 and 9 or pyrazoles 50 and 51. Also, in the case of the 1,2,4-triazoles, the 1-phenyl 62 is as active as the 1-methyl 61, indicating that the steric requirement of this part of the molecule may not be critical for activity. In thiazoles 57 and 58, anomalous results (too many small sized plaques to count) are obtained indicating that the compounds are not as active as the reference.

(d) The effect of changes in -O-aryl moiety on antiviral activity

Table 4.4 considers the effect of variations in the -O-aryl group on antiherpetic activity. When this function is replaced by -Br as in 41 or by 3-methylisoxazole as in 42, the activity decreases. The -O- of -O-phenyl 39 can be replaced by -S- or -SO- or $-SO_2-$ as in 47, 48 and 49 respectively, with retention of activity. The -O-phenyl group can also be replaced by an -O-N-heteroaryl group 43 or -O-heteroaryl group 44 without loss of antiviral activity.

(e) The effect of substitution on α-CH_2 of carbon chain on antiviral activity.

Antiviral activity experiments against HSV-1 have indicated that the substitution of the α-CH_2 of the connecting carbon chain with $-SCH_3$ in 27, $-SOCH_3$ in 45 and $-SO_2CH_3$ in 46 (Table 4.5) also results in



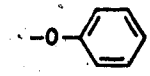
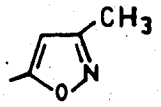
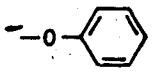
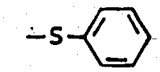
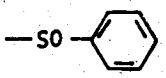
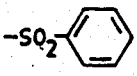
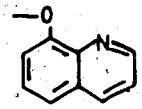
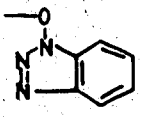
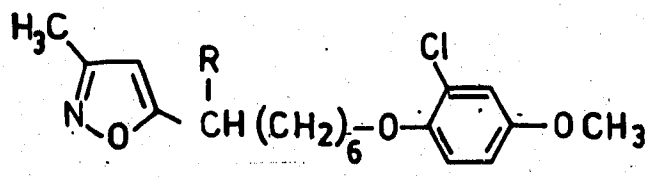
Compound Number	R′	n	% Inhibition (at 5 ug ml)	MIC_{50} (ug/ml)
9		7	100	1·3
41	— Br	6	21.3	ND
42		7	60·7	ND
39		6	100	1·5
47		6	87·9	1·6
48		6	100	0·4
49		6	100	0·5
44		7	100	1·7
43		7	100	1·8

Table 4.4 The effect of changes in –O–aryl moiety on antiviral activity.

ND: Not Done

retention of activity similar to thio compounds, 47, 48 and 49. The sulfoxide 45 and sulfone 46 show significantly better activity than the sulfide 27.



Compound Number	R	% Inhibition (at 5 ug/ml)	MIC_{50} (ug/ml)
9	-H	100	1·3
27	$-SCH_3$	79·2	3·4
45	$-SOCH_3$	100	1·3
46	$-SO_2CH_3$	100	1·2

Table 4.5 The effect of substitution on α-CH_2 of carbon chain on antiviral activity.

4.2. Discussion on antiviral experiments results

For antiviral experiments, Vero cells were used because plaques formed in these cells by HSV-1 were easily countable compared to other cells such as Hela and L-cells.

A preliminary data (Table 3.25) on the mechanism of action of WIN 49321 on HSV-1 indicates that active compounds inhibit one of the early events of viral replication and the compound should be present throughout the viral replication cycle because the attachment of the compound to the virus is a reversible step. An assumption can be made that these compounds inhibit viral uncoating step similar to the mode of action of arildone in poliovirus and HSV. This particular step can be inhibited by stabilizing the viral capsid. The active compounds need interaction with the capsid, for example, π-bonding, H-bonding or hydrophobic interactions (as found in 15a), to inhibit viral uncoating.

A recent study on HRV in Sterling-Winthrop Research Institute and Purdue University has indicated that in 15a, isoxazole portion of the molecule fits into the hydrophobic portion of VP1 and oxazoline group blocks the ion channel, whereas carbon chain (n=7) acts as the proper spacer.

Similarly, in our studies, the compounds with the best activity against HSV-1 are those in which the length of the connecting carbon chain is 6 or 7 (Table 4.1).

The study on the effect of changes in -O-aryl substitution on antiviral activity (Tables 4.2a,b) has indicated that the positions of -Cl and $-OCH_3$ on phenoxy group are important for activity, but no

conclusion can be drawn here.

The effect of changes in heteroaromatic moiety on antiviral activity was studied by the compounds in Table 4.3. All 1,2 azoles are more active compared to 1,3 azoles because some plaques are found in thiazoles 57 and 58 but not in isothiazoles 53-56. On the other hand, imidazole analogue 59 (1,3 azole) shows even better activity compared to reference. This may be due to the attachment of the carbon chain to N in imidazole and to C in thiazole.

Experiments for the study of the effect of changes in -O-aryl moiety have indicated that this portion (-O-aryl) of the compound is important for antiviral activity (Table 4.4). Replacement of -O-aryl with Br or isoxazole decreases the activity. This may be because of inefficient π-bonding with the viral capsid. The inclusion of sulfoxide 48 and sulfone 49 enhances the activity because of better interaction (H-bonding) with the viral capsid. Similar conclusions can be drawn from the study on the effect of substitution on α-CH_2 of carbon chain on antiviral activity, where sulfoxide and sulfone show better activity compared to sulfide (Table 4.5).

The plaque reduction assay method for in vitro antiviral testing has following advantages:

(i) It is simple, easy to handle and rapid assay.

(ii) The screening of the rapid assay compounds for antiviral activity can be done easily.

(iii) It is an inexpensive method as it does not involve expensive instruments.

However, there are some limitations:

(i) The number of virions produced per cell cannot be calculated.

(ii) It is difficult to obtain reproducible results because of different cell type, virus type and growth environment.

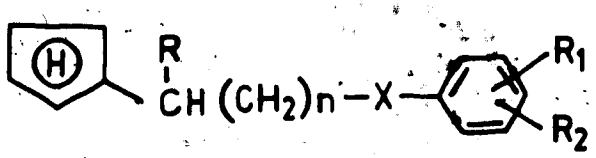
(iii) This assay cannot determine the exact mode of action so further extensive molecular studies should be done.

(iv) Formulation, bioavailability, metabolism and other pharmacokinetic properties of the drugs cannot be studied.

Chapter 5

GENERAL SUMMARY AND CONCLUSIONS

Based on preliminary publications from Sterling-Winthrop Research Institute on a new class of antiviral agent—the arildone class--which appears to have broad spectrum antiviral activity, a systematic study on this class of compounds was undertaken. The general structure of this class of compounds is given by 63.



63

A series of compounds was synthesized by varying the following:

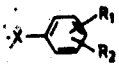
(a)  can be five-membered heteroaromatic rings

(b) R can be H, SR^3, SOR^3 or SO_2R^3

(c) n can have values from 4 to 8

(d) X can be O, S, SO, SO_2 and CH_2

(e)  can be a substituted phenyl group or may also represent a heteroaromatic group such as quinoline or benzotriazole.

(f)  can also be replaced by Br or isoxazole.

All these compounds were tested for their activity against HSV-1 in vitro by a plaque reduction assay method using Vero cells.

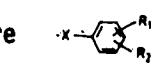
The following general conclusions have been made based on our results:

1. Compounds in which the heteroaromatic group is isoxazole, pyrazole, isothiazole, imidazole and 1,2,4-triazole show comparable activities against HSV-1. Thiazoles give anomalous results since small sized plaques are produced which are difficult to count, showing that the compounds are not as active as reference.

2. In isoxazole series, where R=H, the best results are obtained with compounds where n is 5 and 6.

3. The compounds where R is $SOCH_3$ and SO_2CH_3 are comparable in antiherpetic activity with compounds where R=H and better than those with R=SCH_3.

4. In isoxazole series, the compounds with X=O, SO and SO_2 are equipotent and better than compounds in which X=S or CH_2.

5. The compounds where  is replaced by Br or isoxazole are inactive compared to reference.

6. In phenyl substituted compounds, the substituents R_1 and R_2 and their positions of attachment to the ring are important in determining the antiherpetic activity. Compounds in which the aryl group is replaced by heteroaryl group, such as quinoline and benzotriazole, show high antiherpetic activity.

This work shows that there is a considerable difference in the antiherpetic activity of members of this class of compounds with changes in various moieties of the molecule. We have now identified some modified compounds which have comparable or superior activity to

reference compound WIN 49321 against HSV-1. Compounds 48, 49, 50, 54, 59 and 62 can be used for further testing because these compounds have two to three folds more antiherpetic activity (in vitro studies) than reference compound(WIN 49321)9. Because of the lipophilic nature of these compounds, the drug solutions can be prepared by dissolving in suitable solvent. These drugs in solution may penetrate the skin for the treatment of skin infection caused by HSV. The sulfoxides and sulfones are also expected to show better activity in vivo because of efficient bonding (H-bonding) to viral capsid and higher lipophilicity compared to the reference compound(WIN 49321).

These compounds should also be tested against rhinovirus, in vitro and in vivo to identify compounds with broad spectrum antiviral activity.

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