possess antiviral activity. Two active antiflogistics are not virus inhibitors. It may be supposed antiviral activity of antiflogistics in part is due to an influence at the virus penetration. It is affirmed indirectly by nonspecific antiviral activity of compounds mentioned above. 1-Methyl-4-(N-benzylcarbamido)-pyridinium iodide (amizon) is effective in treatment of herpes, influenza, and measles. Three amizon analogs are active against several viruses as well.

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Synthesis, Antiviral Activity, and Cytotoxicity of Some Novel 2-Phenyl-3-disubstituted Quinazolin-4(3H)-ones

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Quinazoline is the versatile lead molecule for designing potential antiviral agents. Its derivatives have been reported to possess broad spectrum antiviral activity. In the current study, 2-phenylbenzoxazin-4-ones was condensed with selected primary amines to form the corresponding 2,3-disubstituted quinazolin-4(3H)-ones. Their chemical structure was elucidated by means of spectral (FT-IR, ^{1}H-NMR, MS) and elemental analysis. Antiviral activity of the compounds was evaluated in plaque assays using herpes simplex virus type 1 (HSV-1), human cytomegalovirus (HMCV), vaccinia virus (VV) and cowpox virus (CPV). Cytotoxicity was determined in stationary human foreskin fibroblasts (HFF) and logarithmically growing KB cells. Compounds AA-1, AA-2, DBR-2, and MBR-2 had modest activity (IC_{50} = 20–60 μM) against VV and/or CPV, similar to cidofovir. Little cytotoxicity was observed at concentrations up to 100 μM except for MBR-2. It was cytotoxic to both HFF and KB cells at 30–40 μM thereby implying that antiviral activity was a manifestation of cytotoxicity. Similar conclusions were reached for activity of MBR-2 against HSV-1 and HCMV. The other seven compounds had modest activity against HSV-1 (25–40 μM) but with some cytotoxicity noted at 100 μM. In contrast, in an experiment using a low multiplicity of infection with HSV-1, DBR-1 and MBR-1 were active in a low micromolar range suggesting the possibility of specific antiviral activity.

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Expression of Infectious Bursal Disease Virus (IBDV) Polyprotein and VP4 Protease

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Infectious bursal disease virus consists of a bisegmented double-stranded RNA and infects the B-cells in the bursa of Fabricius of young chickens. It causes Gumboro disease, that is of considerable importance in the poultry industry. Although vaccines are available, their use is compromised by maternally derived antibodies and the emergence of very virulent strains. A promising approach is to develop anti-viral compounds that target the virus-encoded VP4 protease, which autocatalytically cleaves a 110 kDa viral polyprotein (NH_2-pVP_2-VP_4-VP_3-COOH) into pVP2, VP4 and VP3. VP2 and VP3 are necessary for the appropriate assembly of the infectious viral particles. It was initially suggested that VP4 cleaves between Arg^{458}-Arg^{459} and Lys^{722}-Arg^{723} but site-directed mutagenesis later identified Ala^{512}-Ala^{513} and Ala^{755}-Ala^{756} as the cleavage sites and VP4 (Ala^{513}-Ala^{755}) as the mature protease. We propose that this VP4 form is a product of the autocatalytic activity of the integral VP4 and that it is not the mature protease. The aim of the study was to determine the sequence required for the autocatalytic activity of VP4 and hence the sequence of the mature VP4. Thus constructs for the expression of full-length (Met^1-Glu^{1012}), truncated (Ile^{227}-Trp^{891}) polyprotein and three forms of VP4 with an alternative N- or C-terminus, namely VP4-RA (Arg^{459}-Ala^{752}), VP4-RK (Arg^{459}-Lys^{722}) and VP4-AA (Ala^{513}-Ala^{755}) were prepared. We also prepared anti-peptide antibodies against VP4-RK and VP4-AA. We report here on the expression of these various constructs in pGEX-4T-1 and pET 32a and their detection with anti-peptide antibodies towards elucidation of the sequence of the mature VP4.

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Synthesis and SAR of 9-Arylpurines as Novel Inhibitors of Enterovirus Replication

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Enteroviruses cause often mild and self-limiting infections, but may also be involved in more serious conditions, which can be life-threatening, such as pancreatitis, meningitis, encephalitis or myocarditis. There are no drugs approved for the treatment of enterovirus infections (De Palma et al., 2008). Here we report on a novel class of enterovirus inhibitors that structurally can be described as 9-arylpurines. Interestingly, scarce examples of such kind of chemical structures are reported in the literature. Two synthetic strategies were used to obtain these compounds: (i) the coupling reaction between arylboronic acids and purines catalyzed by copper salts and (ii) a two-step classical protocol based on the reaction of chloropyrimidines with anilines followed by cyclization. For this second approach, we have set up a new microwave-assisted procedure that has significantly reduced the reaction time, therefore allowing the synthesis of a considerable number of compounds in a short period of time (Aguado et al., submitted for publication). The most selective compounds in this series inhibited viral (Coxsackie B Virus 3) replication with EC_{50} values in the range 4–8 μM, and EC_{90} values around 7–10 μM. CC_{50} values were >250 μM. The most potent compounds in this series were shown to inhibit a selection of enterovirus but lacked activity against polio and rhinoviruses replication. This family of compounds is characterized by its simplicity in structure, synthetic accessibility and selective inhibitory activity against various enteroviruses replication.

References

De Palma, A.M., Vliegen, I., DeClercq, E., Neyts, 2008. J. Med. Res. Rev. 28, 823–884.

Aguado, L., Camarasa, M.J., Pérez-Pérez, M.J., J. Comb. Chem., submitted for publication.

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