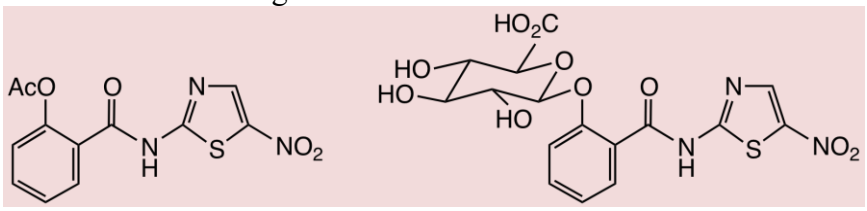
The collaborative project between Romark Laboratories and the University of Liverpool

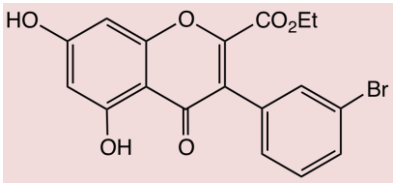
A personal recollection by Dr. Andrew Stachulski

It was in 1998, while I was Research Manager at Ultrafine Chemicals in Manchester, that I first became aware of Romark Laboratories and their interest in medicinal chemistry. At that time Romark's lead compound, nitazoxanide **1**, was being marketed as an antiparasitic and anthelmintic agent. During human metabolism, **1** is metabolized to the *O*-aryl glucuronide **2**, and we were asked to synthesise a reference standard of this metabolite. I found an efficient four-step synthesis, which we published, and later repeated, as well as using the method for other related glucuronides.



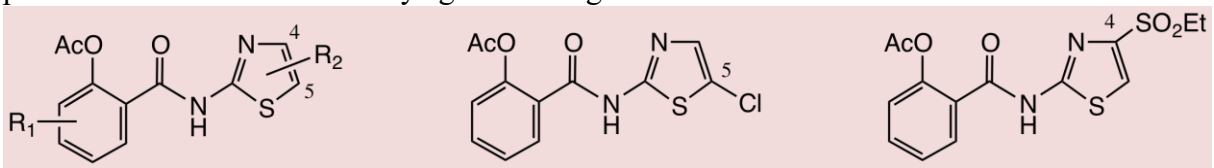
1 **2**

At that time I was invited to meet Dr. Rossignol, who had first synthesised **1** in the 1970s, and it became clear that his company wanted to begin a drug discovery programme. We were not able to begin this immediately, but after I took up a lectureship at Liverpool in 2001 we met again and began a collaboration which has continued ever since.



3

We initially made a series of isoflavones typified by compound **3**, which were promising antiparasitic agents, but it was the discovery in 2003 that nitazoxanide possessed good broad-spectrum antiviral activity that gave us the real breakthrough. From then we carried out an extensive synthetic programme, eventually preparing around 150 analogues of the thiazolides, as we christened them, of general structure **4**. A number of analogues were also made by Kalexsyn, in the USA, around 2008-2009. We have published SAR papers on the activities of these compounds against hepatitis B and C viruses: many of the analogues possess low micromolar activity against a range of viruses.

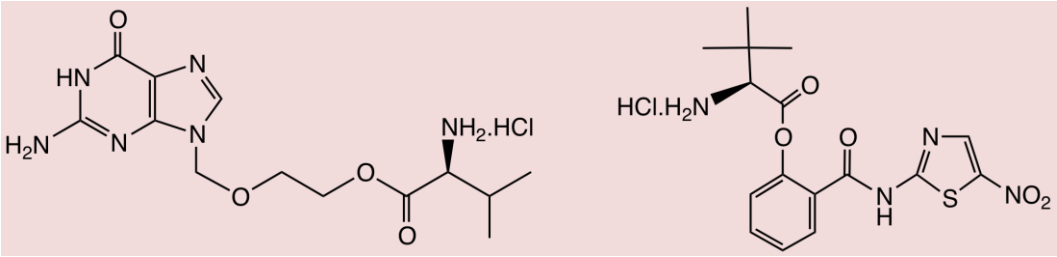


4 **5** **6**

Of the 'second generation' derivatives, the 5-chloro analogue **5** is still one of the most promising and is ready for phase 1 clinical trials. The activity of the 4-substituted series is generally more restricted, but sulfones such as **6** are highly active against influenza A virus, IC_{50} = 0.14 μmolar. There is undoubtedly still scope for new analogues, and future work can be tied into our growing knowledge of the mode of action of these compounds.

Nevertheless, the pharmacokinetic parameters of thiazolides **1** and **5** are far from ideal. They are passively absorbed following oral administration, but once the emphasis switched to anti-influenza compounds it was clearly highly desirable to seek derivatives-

prodrugs- that might be more effectively absorbed by the oral route. Taking our cue from the nucleoside analogue antiviral agent valacyclovir **7**, which greatly improves the oral bioavailability of acyclovir, we designed the L-*tert*-leucyl amino-acid ester **8**, which has a good absolute bioavailability of 20%. This should permit the use of a much smaller dose. The phenolic acetate in thiazolides such as **1** and **5** is itself a simple prodrug for the free phenol, which is the active form of the drug *in vivo*.Very recently, nitazoxanide **1** has successfully completed phase 3 trials against acute uncomplicated influenza and its market launch is expected soon.



8 **9**

The thiazolides have beguilingly simple structures, but their mechanism of action is still not known in detail. However, they are not directly acting antiviral agents. In the case of influenza A, it has been shown that they interfere with the maturation of the viral glycoprotein, and hence with viral excretion from the host cell. Further investigation of the biological mechanism is ongoing.

In summary, the thiazolides are a significant group of drug molecules whose full properties are still being discovered. The synthesis of new active analogues, further efficient prodrugs and more investigation of the mode of action are important ongoing tasks. We hope that this collaboration will continue to offer research opportunities at Liverpool for years to come.

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