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NOVEL INHIBITORS OF INFLUENZA SIALIDASES RELATED TO ZANAMIVIR. Heterocyclic replacements of the glycerol sidechain

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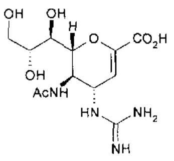
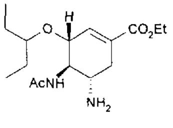
Abstract: A series of substituted 4-amino-4H-pyran-2-carboxylic acid 6-triazoles and oxadiazoles are described. The compounds are inhibitors of influenza virus sialidases which, in general, show a similar pattern of activity and selectivity to recently reported 4H-pyran-2-carboxylic acid 6-carboxamides © 1997 Elsevier Science Ltd.

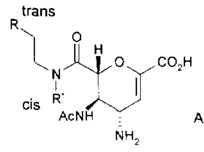
Inhibitors of influenza virus sialidases have potential for the prophylaxis and treatment of influenza infections.[1] In recent times several highly potent inhibitors of influenza sialidases have been discovered. Zanamivir **1** is a potent and selective inhibitor of both influenza A and B virus sialidases. It has excellent antiviral activity in animal models when delivered directly to the respiratory tract and is currently undergoing clinical evaluation.[2] Since the discovery of zanamivir, several groups have attempted to identify new sialidase inhibitors with modified physicochemical properties which could make them more suitable for systemic delivery. Workers from Gilead substituted the dihydropyran in zanamivir with a cyclohexene ring, and replaced the glycerol sidechain with a lipophilic ether. This led to the discovery of GS4104 **2**, a pro-drug which is orally active in mice.[3,4] In recent work, we also discovered that it is possible to replace the glycerol sidechain of zanamivir with lipophilic groups, and prepared carboxamides **3** (R = range of alkyl and arylalkyl groups, R\` = Et, propyl) with excellent inhibitory activity, particularly against influenza A sialidase.[5,6] In order to further probe the structural features important for binding within this region of the sialidase active site we have prepared a range of heterocyclic derivatives **4a-h.** Our initial molecular modelling studies suggested that the pendant alkyl or aralkyl groups (R and R\`) attached to these heterocycles would also be capable of making favourable van der Waals contacts with sialidases. Herein we describe the synthesis and biological properties of these compounds.



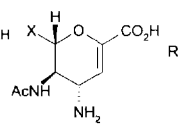
zanamivir **1**



GS4104 **2**



Carboxamides **3**



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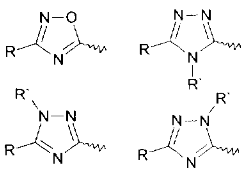

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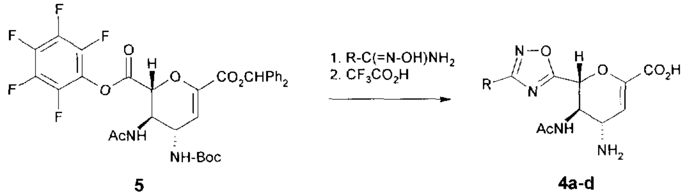
Synthesis of oxadiazoles 4a-d

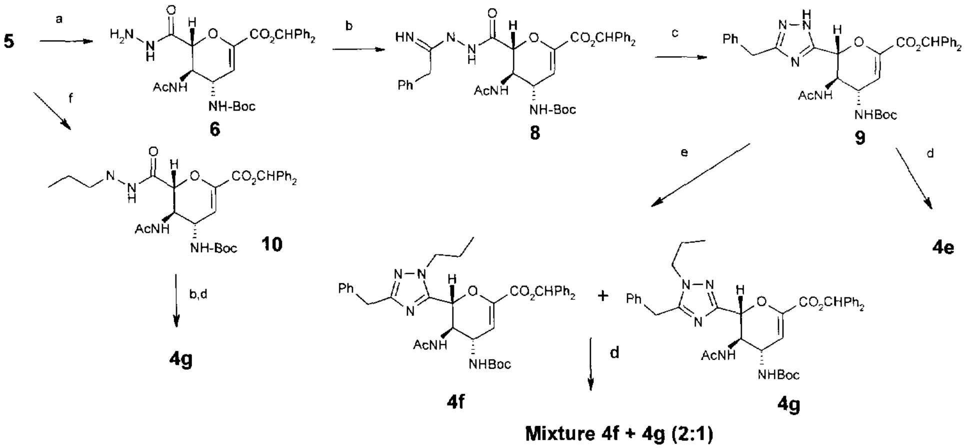
Oxadiazoles **4a-d** were prepared in two stages from the previously reported pentafluorophenyl ester **5**.[5] Thus treatment of **5** with an appropriate amidoxime in the presence of molecular sieve, and deprotection with trifluoroacetic acid:dichloromethane afforded oxadiazoles **4a-d** directly.



	R	Yield (%)
4a	Me	45
4b	Butyl	49
4c	Ph	62
4d	CH_2Ph	57

Synthesis of triazoles 4e-g

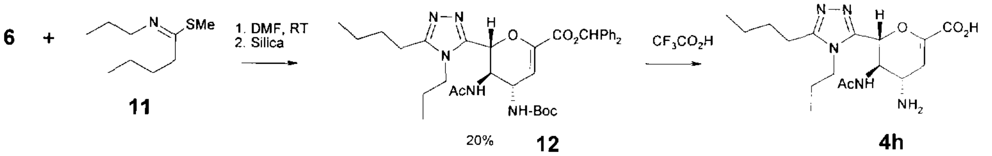
Pentafluorophenyl ester **5** was converted into the hydrazide **6** by treatment with hydrazine. Reaction of **6** with the S-methyl thioamide **7**[7] and sodium carbonate in DMF afforded the imide intermediate **8**. Cyclisation of **8** to the triazole **9** was accomplished in high yield by refluxing in a mixture of acetic acid and ethyl acetate. Deprotection of **9** with trifluoroacetic acid and a trace of anisole afforded the disubstituted triazole **4e**. Alkylation of **9** with n-propyl iodide produced a 2:1 mixture isomers which were similarly deprotected to an inseparable mixture of triazole isomers **4f** and **4g**. A pure sample of isomer **4g** was unambiguously prepared from **5** via a similar process by initial reaction of **5** with n-propyl hydrazine. This produced exclusively the hydrazide intermediate **10** which can only form **4g** when treated with **7**, cyclised and deprotected. In this case cyclisation to the triazole is spontaneous on reaction with **7**.



a) H_2NNH_2, THF b) **7**: $PhCH_2C(=NH)SMe.HI$, Na_2CO_3 c) AcOH, EtOAc, reflux d) CF_3CO_2H, anisole e) N-propyl iodide f) $CH_3(CH_2)_2NHNH_2$, DMF

Synthesis of triazole 4h

Treatment of hydrazide **6** with S-methylthioamide **11** in DMF at room temperature, followed by silica gel chromatography afforded the triazole intermediate **12** directly. Deprotection of **12** afforded exclusively triazole isomer **4h**. Thus through the appropriate choice of hydrazide and S-methyl thiourea it is possible to achieve syntheses of all possible trisubstituted triazole regioisomers

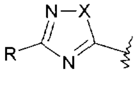


Sialidase Inhibitory Activities of 4a-h. Comparison with dihydropyran carboxamides (Table)[8-10]

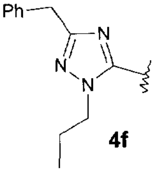
Compound	Flu A (μM)	Flu B (μM)
4a	23	43
4b	56	210
4c	14	80
4d	99	>420
4e	87	130
4f (2:1 mixture with **4g**)	0.07	56
4g	12	>360
4h	0.33	13
3a (R = Ph, R` = H)	12	67
3b (R = Ph, R` = n-Propyl)	0.002	3.6
1	0.002	0.02

The oxadiazoles **4a-d** and disubstituted triazole **4e** all show modest inhibitory activity against sialidases from both influenza A and B, similar to the activity of the secondary phenethylamide **3a** (and other secondary carboxamides in general[6]). These results are consistent with molecular modelling of **4a-e** which suggests that the heterocycle substituents in these compounds may occupy a similar position to the *trans*-phenethyl amide substituent in **3a**, and hence make only weak hydrophobic interactions with the enzyme.[11] However, introduction of the propyl group onto the triazole ring, in compounds **4f** and **4h,** results in a significant improvement in inhibitory activity against influenza A sialidase and marked selectivity over the influenza B enzyme is also observed. This is similar to the pattern of inhibitory activity previously seen with the tertiary propylamide **3b** (and other tertiary carboxamides which possess an ethyl or n-propyl *cis*-amide substituent[6]). Previous X-ray crystallographic studies with compound **3b** showed that the presence of the *cis*-propyl amide substituent induced a conformational change in sialidase upon binding, which we postulated to be more favourable for the influenza A enzyme. Salt bridge formation occurs between the sidechains of Arg 224 and Glu 276 within the glycerol binding pocket. This creates a new hydrophobic pocket in the base of the active site which is occupied by the cis-propyl group in **3b**.[6]

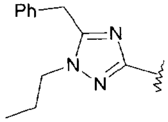
Our inference from the results described above is that a similar conformational change must be occurring upon binding of the heterocycles **4f** and **h**. Simple molecular modelling analysis of structures **4f,g** and **h** reveals that the propyl groups in compounds **4f** and **h** can easily adopt a similar orientation to the cis-propyl group in amide **3b** (below). However, the propyl group in the other trisubstituted triazole **4g** cannot occupy the same position in space. Hence this compound exhibits inhibitory activity which is more like that of the secondary amide **3a**.



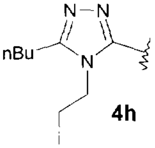
4a-e



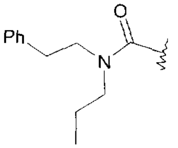
4f



4g



4h



Carboxamide 3b

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11. X-ray crystallography shows that the phenyl ring of **3a** weakly interacts with a lipophilic pocket in sialidase defined by Ala246 and Ile222.[6]

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