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Bioorganic & Medicinal Chemistry Letters 15 (2005) 427–430
Bioorganic & Medicinal Chemistry Letters

Pyrimidine containing RSV fusion inhibitors

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Received 3 June 2004; revised 15 October 2004; accepted 21 October 2004
Available online 6 November 2004

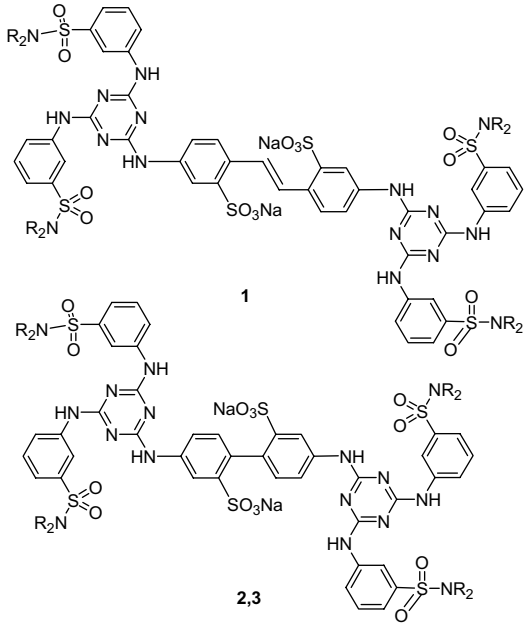
Abstract—The knowledge of SAR in a series of biphenyl anionic RSV inhibitors has been broadened by synthesis and testing of analogs with pyrimidine linkers. Generally, pyrimidine compounds were much harder to synthesize, and their anti-RSV activity was lower in comparison with triazine analogs.

1. Introduction

Human respiratory syncytial virus (RSV) is well recognized as the leading pathogen of serious upper and lower respiratory tract infection that occurs seasonally and has a worldwide distribution.[1] Severe RSV disease is associated with unacceptable morbidity and mortality in children, especially in infants.[2] Several studies suggest that RSV is an important factor in the development of asthma and possibly atopy, as well as necrosis and cystic fibrosis.[3]

More recently the pathogenicity of RSV has been demonstrated in healthy school-age children and elderly adults, institutionalized individuals, and those with compromised immune function.[4] In these populations RSV spreads with ease and frequently results in severe or fatal cardiopulmonary complications.[5] Treatment of RSV infection is primarily supportive care. The only approved agent for treating infants with severe RSV disease is Ribavirin. However, its use has been limited by the mode of administration (prolonged aerosol), cost, and low efficacy. There are currently no other effective and conveniently administered prophylactic and/or therapeutic agents for the prevention and treatment of RSV in clinical studies.[6]

Several years ago we started a program that led to novel and specific RSV inhibitors targeting virus fusion.[7–14] First in the series, compound **1**, a symmetrical triazine derivative with disulfonate stilbene core, was identified by HTS method in cell culture. It was active against RSV (IC_{50} 0.15 μM), but inactive against measles, parainfluenza, and adenovirus infection.[7,8] Further investigations showed that the biphenyl analog **2**, CL387626, is even more active against RSV in vitro (IC_{50} 0.08 μM).[7,8] Modifications of the peripheral part



Keywords: Respiratory syncytial virus; Fusion inhibition; Trichloropyrimidine; Nucleophilic substitution.
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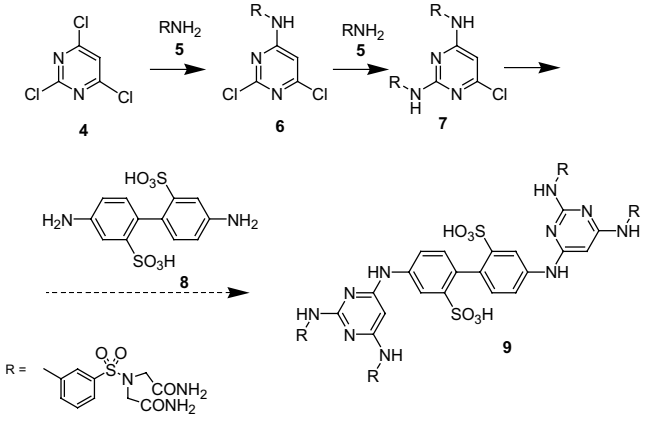
doi:10.1016/j.bmcl.2004.10.056

resulted in the most active compound in the series **3**, RFI 641 (IC_{50} 0.05 μM);[9] it has been shown that RFI 641 can significantly reduce viral titers in African green monkey, mouse, and cotton rat animal models.[10] Investigation of the mechanism of action of the dendrimer-like molecules **1–3** clearly indicated that a prerequisite for their anti-RSV activity is the inhibition of fusion and entry of the virus.[11,12]

The general structure of these compounds can be described as consisting of two parts: the charged core and four substituted sulfonamides in the peripheral area. These parts are linked together by two triazine fragments. As part of the SAR studies, numerous analogs with different charged cores and peripheral parts were synthesized, and several important SAR conclusions drawn from their biological evaluation.[13,14] Further expansion of our approach prompted us to explore the triazine linkers. We decided to replace triazine linkers with pyrimidine ones in the most active compound of the series, RFI 641.

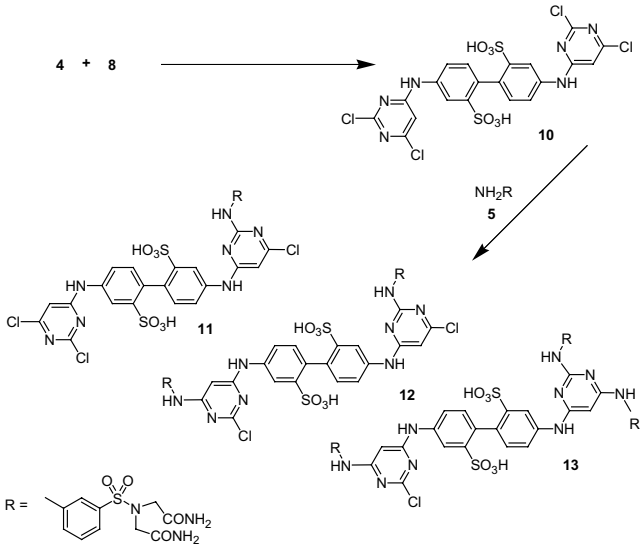
2. Synthesis

Two general methods were described for the triazine series.[7] Both methods involve stepwise assembly of the molecule from the core part, the peripheral parts, and the triazine linkers. They differ in the order of substitution of chlorine atoms in trichlorotriazine by the core part and the peripheral fragments. Both methods were applied to the synthesis of pyrimidine analogs, which turned out to be much more difficult.



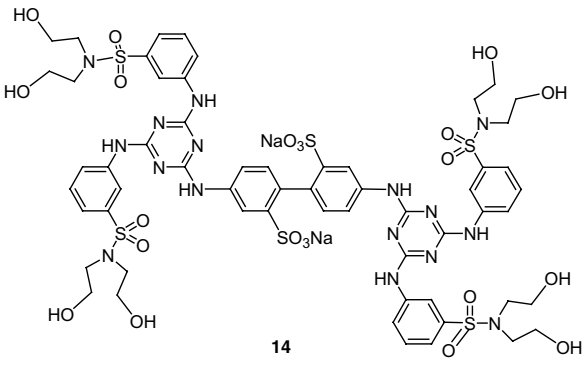
First, we tried the method that was much preferred in the triazine series due to better yields and workup conditions. It begins with sequential substitution of two chlorine atoms in trichloropyrimidine **4** with aminosulfonamide **5**. As expected, trichloropyrimidine **4** was much less reactive than trichlorotriazine. First substitution of chlorine occurred at room temperature in the phosphate buffer and took 20 h to complete. The two isomers of monosubstituted dichloropyrimidine **6** (only the major 4-substituted pyrimidine shown; ratio of 4- to 2-substituted by HPLC 7:1) was isolated, dried, and reacted with another equivalent of aminosulfonamide **5** in DMSO at 110 °C. The reaction was complete in 5 h. The product **7** (isomers unseparable by HPLC) was heated up with diaminobiphenyldisulfonate core **8** in the presence of an organic base. We tried several reaction conditions; however, no desired product **9** was observed in any of our attempts.

The next step was to try the assembly around the diaminobiphenyldisulfonate core **8**. Condensation of the core fragment **8** with the two equivalents of trichloropyrimidine **4** in 0.5 M aqueous sodium bicarbonate gave the tetrachloro-compound **10**. Unlike its triazine analog, it is stable. Analysis of compound **10** by HPLC/MS showed the 11:1 ratio of products with the same molecular mass. The major isomer was isolated by crystallization from DMF and analyzed by ^{13}C and ^{1}H NMR. The carbon spectrum had six clear signals of substituted benzene ring carbons and four signals of pyrimidine ring carbons, thus proving that the structure is symmetrical with both pyrimidines substituted at 4-position. The proton spectrum had a sharp singlet of the pyrimidine ring proton at 6.78 ppm; no signal of the second isomer was detected.

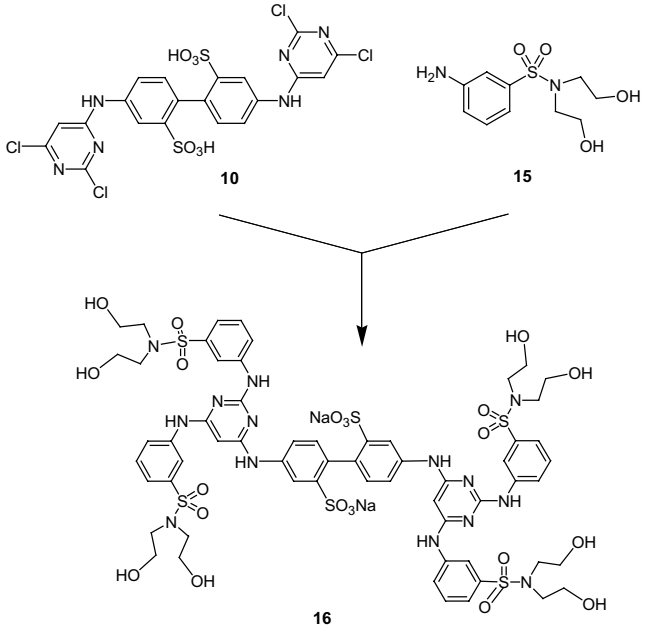


Compound **10** was then reacted with the peripheral fragment **5**. Several conditions were tried; the reaction either did not go or went too far, to thermal degradation. The best results were obtained in the microwave conditions, in DMSO, excess of the aminosulfonamide **7** working as an acid scavenger. Heating for 10 h at 130 °C produced a mixture that was separated by preparative HPLC. The major fraction, 23.5% yield, was monosubstituted trichloride **11**. As minor components, the disubstituted and trisubstituted analogs, **12** and **13**, were isolated in 7 and 4% yields, respectively.[15,16] It should be noted that these compounds, while shown as individual structures, are in fact mixtures of isomers due to the possibility of 2- or 6-substitution of the second chlorine atom in the pyrimidine linker. We decided to evaluate the antiviral activity of the mixtures prior to the separation. Our plan required a comparison of at least one fully substituted pyrimidine compound with its direct triazine analog.

As compound **9** was extremely difficult to synthesize, apparently due to the instability of the amido function in the periphery, we chose another target, related to the hydroxy analog **14**.[7,8]



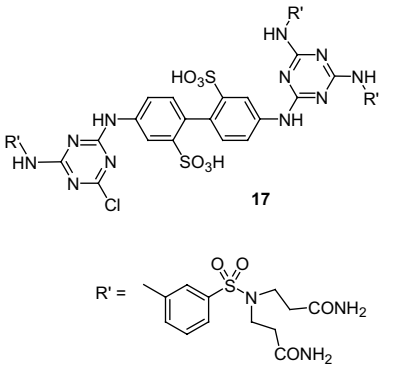
We synthesized the pyrimidine analog of compound **14** by heating a mixture of tetrachloropyrimidine **10** and excess of 3-aminophenyl-N,N-bis(3-hydroxyethyl)sulfonimine **15** neat in a 100 °C oil bath for 5.5 h. The obtained glass-like mass was pulverized in water, filtered, and the desired compound **16** isolated by preparative HPLC as amorphous solid.[15,16]



The key to the success in this case was the fusion process, with the aminosulfonamide fragment **15** playing the role of a solvent. Regrettably, these conditions were impossible to reproduce with the amido peripheral fragment **5**, a high-melting solid (mp 180–181 °C).

So, we had our pair of direct analogs for comparison. In our extensive database we found an indirect analog for compound **13**, the trisubstituted triazine **17**.[13]

The additional difference between compounds **13** and **17** is the length of amide-bearing carbon chains (glycylamide in compound **13**, propylamide in compound **17**).



The limited basis for comparison of this pair could be that, as we established before, the difference in side chain length only moderately affect the biological activity. To prove the point, we can refer to three compounds from triazine series: compounds **2** and **3**, our extensively studied leads with glycyl- and propylamido side chains, and the third analog with butylamido side chains. All three compounds have very close IC_{50} values (0.05, 0.08, and 0.1 μM, respectively).[9]

Table 1 summarized the RSV inhibition data and selectivity as measured against unrelated viruses: human cytomegalovirus and herpes simplex virus type 1 in cell cultures.[17]

A pair of compounds **14** and **16** represents the difference between pyrimidine and triazine series. The triazine compound **14** demonstrates significantly higher activity over the pyrimidine analog **16**, the difference being an order of magnitude. In a pair of compounds **13** and **17** the triazine analog **17** also has the superior anti-RSV activity over the pyrimidine compound **13**, with the similar ratio of values.

The results also show a clear correlation between the number of 'active arms' and the antiviral activity. The

Table 1. Antiviral activity of pyrimidine containing anionic compounds **11**–**13**, **16** and their triazine analogs **3**, **14**, **17**

Compound #	IC_{50} (μM)		
	HRSV	HCMV	HSV
3, RFI 641	0.05	2.55	7.72
11	16.7	24.9	47.2
12	3.5	13.8	25.4
13	1.8	7.0	18.9
14	0.09	2.3	14.7
16	0.82	4.4	4.4
17	0.25	16.8	

IC_{50}'s determined using RSV A2, HCMV AD169 and HSV-1 Patton. The RSV and HSV-1 assays are by ELISA; the HCMV assay is via an indicator gene in the virus.

trisubstituted monochloride **13** is twice as active as the disubstituted compound **12**, and almost 10 times more active then the monosubstituted trichloride **11**; the tetrachloride **10**, also tested, was completely inactive. It should be noted that the trichloride **11** and dichloride **12**, while moderately active, retain anti-RSV specificity. This confirms and expands the correlation established previously, in the triazine series, where compounds with three 'active arms' were just slightly less active than the four-substituted analogs, and compounds with two active arms were inactive.[13]

Overall, the much more difficult synthesis, isomeric composition, and lesser biological activity make the pyrimidine series more of a theoretical interest as antiviral agents.

Thus, we have investigated the last structural element of novel anti-RSV dianionic dendrimer-like compounds and found out that it is important to have an electron-rich triazine system as a linker; replacement of only one nitrogen atom by a carbon results in a 10-fold loss of the biological activity.

Acknowledgements

The authors wish to thank Dr. Boris Feld for providing the assay data; Mr. Kelvin Cruz for performing analytical HPLC; Drs. Stanley A. Lang, John Potoski, and Tarek Mansour for valuable discussions.

References and notes

1. Black, C. P. *Respiratory Care* **2003**, *48*(3), 209–231.
2. Shlaes, D. M. *Sem. Resp. Crit. Care M.* **2000**, *21*(4), 305–311.
3. Openshaw, P. J.; Dean, G. S.; Culley, F. J. *Pediatr. Infect. Dis. J.* **2003**, *22*, 58–64.
4. Hashem, M.; Hall, C. B. *J. Clin. Virol.* **2003**, *27*, 14–21.
5. Falsey, A.; Walsh, E. *Clin. Microbiol. Rev.* **2000**, *13*, 371–384.
6. Meanwell, N. A.; Krystal, M. *Drug Discov. Today* **2000**, *5*, 241–252.
7. Ding, W.-D.; Mitsner, B.; Krishnamurthy, G.; Aulabaugh, A.; Hess, C. D.; Zaccardi, J.; Cutler, M.; Feld, B.; Gazumyan, A.; Raifeld, Y.; Nikitenko, A.; Lang, S. A.; Gluzman, Y.; O'Hara, B.; Ellestad, G. A. *J. Med. Chem.* **1998**, *41*, 2671–2675.
8. LaRocque, J. P.; O'Hara, B. M.; Morin, J. E.; Ellestad, G. A.; Mitsner, B.; Ding, W.; Raifeld, Y. E.; Nikitenko, A. A. US Patent US 5, 852, 015, 1998.
9. Nikitenko, A. A.; Raifeld, Y. E.; Wang, T. Z. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1041–1044.
10. Huntley, C.; Weiss, W.; Gazumyan, A.; Buklan, A.; Feld, B.; Hu, W.; Jones, T.; Murphy, T.; Nikitenko, A.; O'Hara, B.; Prince, G.; Quartuccio, S.; Raifeld, Y.; Wyde, P.; O'Connel, J. *Antimicrob. Agents Chemother.* **2002**, *46*, 841–847.
11. Razinkov, V.; Gazumyan, A.; Nikitenko, A.; Ellestad, G. A.; Krishnamurthy, G. *Chem. Biol.* **2001**, *8*, 645–659.
12. Razinkov, V.; Huntley, C.; Ellestad, G. A.; Krishnamurthy, G. *Antiviral Res.* **2002**, *55*, 189–200.
13. Gazumyan, A.; Mitsner, B.; Ellestad, G. A. *Curr. Pharm. Design* **2000**, *6*, 525–546.
14. Aulabaugh, A.; Ding, W.-D.; Ellestad, G.; Gazumyan, A.; Hess, C. D.; Krishnamurthy, G.; Mitsner, B.; Zaccardi, J. *Drugs Future* **2000**, *25*(3), 287–294.
15. The reaction intermediates and products were analyzed by reversed-phase HPLC (column: C30-ProntoSIL, 3μ, 2×150 mm; mobile phase: A—water, B—acetonitrile, C—methanol; gradient from the mixture A:B:C 88:6:6 v/v to the mixture A:B:C 60:25:15. Preparative reversed-phase HPLC was performed on a Rainin gradient HPLC system using a YMC-Pack Polymer C18, S-10, 20×250 mm; mobile phase: A—0.05 M ammonium acetate in water (pH 4.5), B—acetonitrile; isocratic mixture A:B 59:41 v/v, then gradient to the mixture A:B 41:85 v/v in 4 min. Desalting was performed on YMC Prodigy C18 Polymer column, using acetonitrile/water 1:1 mixture.
16. Compounds **10**–**13**, **16** were characterized by mass spectroscopy. In the negative mode all spectra had half of the molecular ion ($M - 2H^2$) as the major peak in and the molecular ion as a minor peak. Particularly, compound **10** MS (ES^-): *m/z* 317.8($M - 2H^2$), 636.7($M - H^-$). Compound **11**, MS (ES^-): *m/z* 443.2($M - 2H^2$), 884.7 ($M - H^-$). Compound **12**, MS (ES^-): *m/z* 566.9($M - 2H^2$), 1132.5($M - H^-$). Compound **13**, MS (ES): *m/z* 692.3($M - 2H^2$), 714.9((M + 2Na) − $2H^-$). Compound **16**, MS (ES^-): *m/z* 765.3 ($M - 2H^2$).
17. The IC_{50} values were determined against RSV A2, HCMV AD169 and HSV Patton strains. The RSV and HSV-1 assays are ELISA based; the HCMV assay is via an indicative gene in the virus. Details of the antiviral assays are published in Ref. 7.