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The Discovery of RFI-641 as a Potent and Selective Inhibitor of the Respiratory Syncytial Virus

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Abstract—The design and synthesis of a new potent and selective inhibitor of the respiratory syncytial virus are described. This compound, RFI-641, emerged from analysis of the structure–activity relationship in a series of biphenyl triazine anionic compounds possessing specific anti-RSV activity. The key synthetic step involves coupling of diaminobiphenyl **11** with two equivalents of chlorotriazine **10** under microwave conditions. RFI-641 inhibited RSV in vitro and in vivo models.

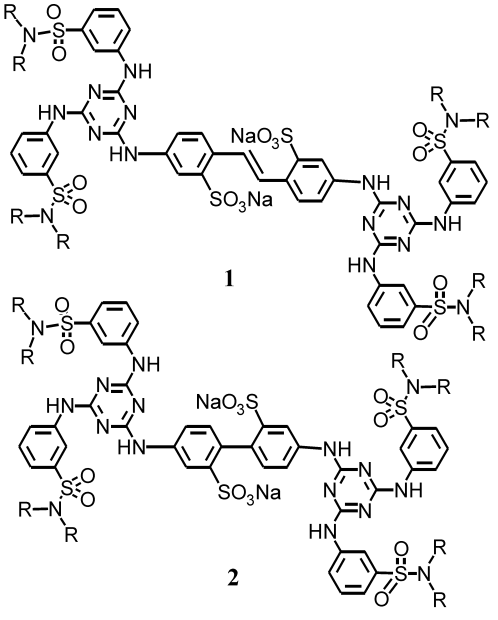
Respiratory syncytial virus (RSV) is a cause of respiratory infection that occurs seasonally and has a worldwide distribution. It is the most important cause of respiratory infection in infants, which is associated with a high morbidity and mortality.[1] RSV is considered as a possible cause of exacerbations of asthma, necrosis, and cystic fibrosis. It causes fever and pneumonia in transplant and cancer patients (mortality ca. 50%).[2] RSV is an important cause of respiratory infection in healthy school-age children and adults.[1] Investigations among elderly people (over 65 years) showed that RSV is the cause of 14% pneumonia and 10% of cardiopulmonary cases; 10% mortality from these diseases is considered being related to RSV.[3]

As the significance of RSV infection has been acknowledged, serious efforts are put into finding an effective treatment for the disease. Back in 1945, an oxygen tent was the only remedy against RSV infection.[3] Nowadays, there are several groups of anti-RSV agents, different by their structure and mechanism of action. In prophylaxis there is active immunization and passive administration of anti-RSV antibodies by various routes.[4]

Ribavirin, the only drug licensed for the therapy of RSV, has limited clinical utility. It works as an indirect inhibitor of RNA transcription and is characterized by a broad spectrum of antiviral activity, potential toxicity, and relatively high cost.

Other structural groups of potential anti-RSV agents, such as antisense oligonucleotides designed to inhibit viral replication,[5,6] heparin-like inhibitors of viral attachment,[7] compounds of various structure that prevent viral penetration into the host cell,[8–10] are at various stages of investigation, but none of them has advanced to the clinical stage.

Recently, a screening program, initiated at Wyeth-Ayerst to look for inhibitors of RSV, identified a compound **1**, specifically active against RSV in vitro ($IC_{50} = 0.15\ \mu M$).



1

2

R = $CH_2CH_2CONH_2$

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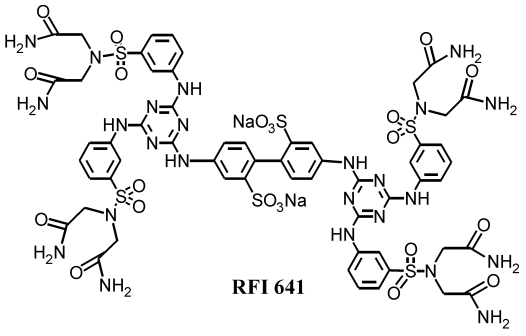
Further investigations showed that the biphenyl analogue **2** is even more active against RSV ($IC_{50} = 0.05\,\mu M$). These compounds have a very interesting structure, unusual for a drug: the charged core, four substituted sulfonamides in the periphery, and two triazine linkers.

The basic structure–activity relationships determined on the various series of analogues have been reported.[4,11,12] It has been determined by fluorescence spectroscopy technique that these compounds function as fusion protein inhibitors.[11] Further efforts were directed to the identification of inhibitors with greater potency and higher specificity than **2** against RSV. Herein, we describe the culmination of our work, which led to the identification of RFI-641 as a promising anti-RSV agent. Among the synthesized compounds of this general structure, RFI-641 is the most potent and selective RSV inhibitor, currently going through Phase I of clinical trials.

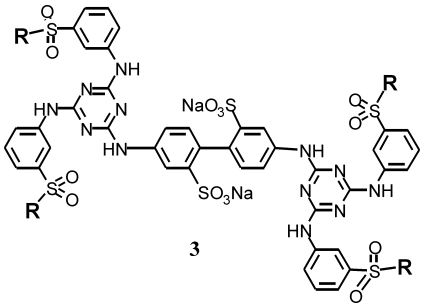


Previous data led us to focus on negatively charged biphenyl as the core and triazines as linkers with phenyl sulfonamides as peripheral arms. This has resulted in replacing the stilbene core group with the biphenyl disulfonic acid.

Subsequently, our efforts were concentrated on exploring the peripheral substituent R in the general structure **3**, a region that seemed to provide numerous opportunities for modifications and at the same time was very sensitive to changes. Among these variations were extension of the length of carbon chains in R, bearing peripheral functional groups.

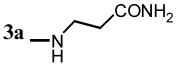
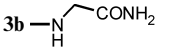
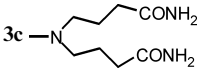
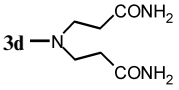
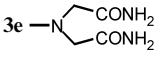
Table 1 summarizes the RSV inhibition data and selectivity as measured against human cytomegalovirus and herpes simplex virus type 1 in cell cultures.[13]

The results showed that both for the monosubstituted analogues **3a**,**3b** and for disubstituted analogues **3c**,**3d** shorter chain analogues **3b** and **3d** exhibited enhanced inhibition of RSV in vitro in comparison to **3a** and **3c**, respectively. Monosubstituted nitrogen analogue **3a** was less active than its disubstituted analogue **3d**. With that in mind, we synthesized **3e**, which carries disubstituted glycylamide peripheral arms instead of the propylamide substituents presented in **3d**. To our satisfaction, it was found that **3e** emerged as the most potent and selective inhibitor of RSV in vitro (Table 1). Therefore, in this case the combination of disubstitution and shorter carbon chain led to a cumulative increase of anti-RSV activity.



3

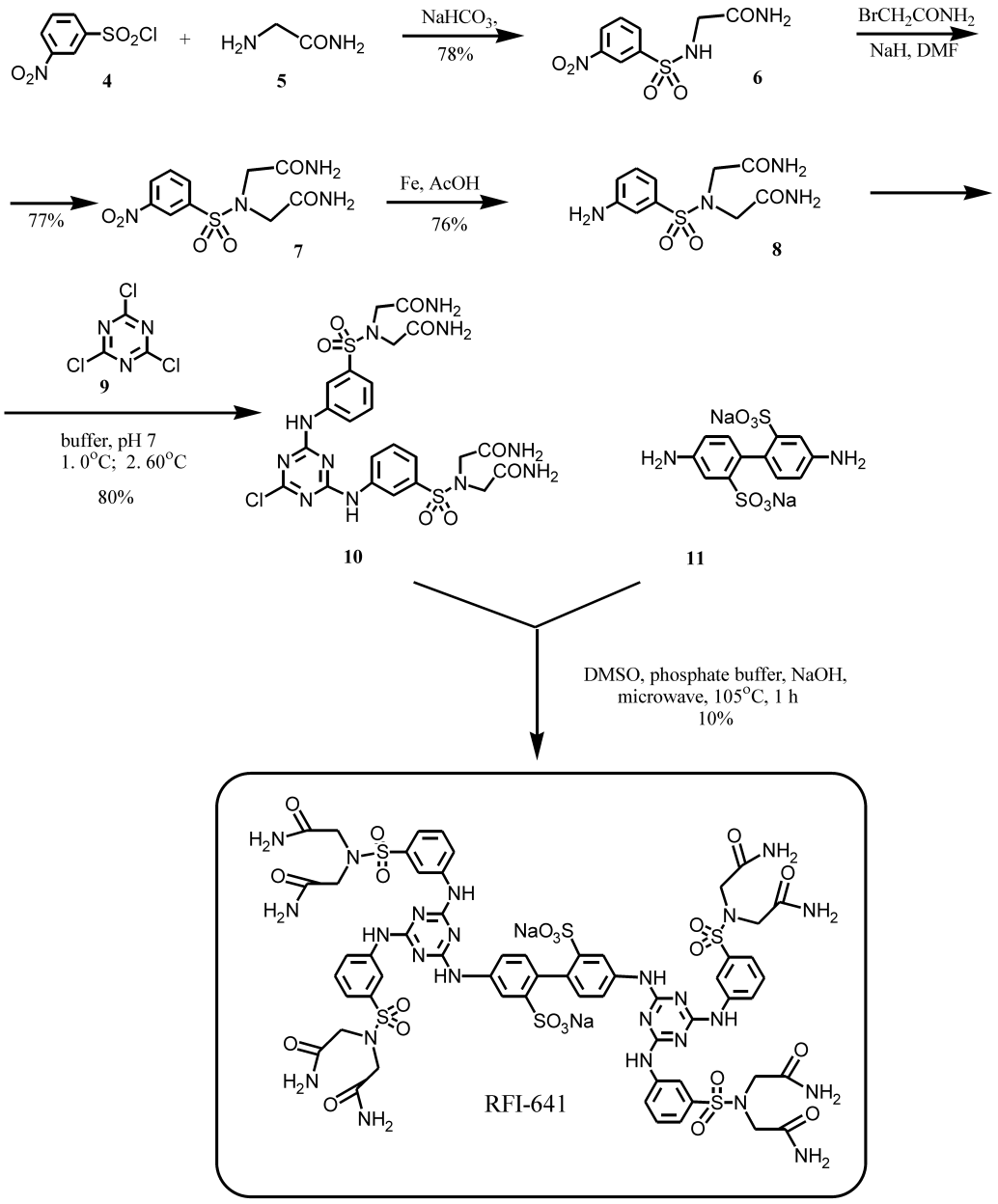
Table 1. Effect of varying the side-chain length on activity and specificity of RSV inhibitors **3a–e**

	IC_{50} (μM)[a]		
R	HRSV	HCMV	HSV
3a 	0.66	2.64	23.2
3b 	0.27	1.37	>25
3c 	0.1	5.8	8.9
3d 	0.08	13.9	19.5
3e 	0.05	2.55	7.72

[a]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.

First, the peripheral fragment, 3-aminophenyl-*N*,*N*-bis-carbamoylmethyl-sulfonylimine **8**, was synthesized as outlined in Scheme 1. 3-Nitrobenzenesulfonyl chloride **4** was converted to the correspondent amide **6** by reaction with glycylamide **5** in Shotten–Baumann conditions. The second glycynamido chain was added by reaction with sodium hydride and bromoacetamide in DMF. The nitro group of **7** was reduced to the amino group by treatment with iron powder in water/acetic acid to give the amino compound **8**. Next, nucleophilic addition of **8** to triazine **9** under controlled conditions led us to the disubstituted monochlorotriazine **10** in good yield.

In order to minimize the side reactions, we decided to apply microwave for the last substitution. In our previous experience, the microwave conditions allowed to reduce reaction time from 20 h to less than 1 h. We heated the disubstituted monochlorotriazine **10** with the core part **11** in the microwave[14] at 105 °C for 1 h, and obtained RFI-641 as a major component of the reaction mixture.



Scheme 1.

RFI-641 inhibits laboratory (six viruses) and clinical isolates (18 viruses) of RSV subtypes A and B in the range of 0.008–0.11 μM (0.013–0.18 μg/mL).[15] The lack of cytotoxicity on growing HF cells gives RFI-641 a selectivity index with a range of $>417->2500$-fold, based on the range of IC_{50} for all 24 virus. In three animal models of RSV infection (mice, cotton rats and African green monkeys), prophylactic administration of RFI-641 significantly reduced in viral titers in nasal washes (measured at the peak of virus infection). In addition, in the African green monkey model the drop in viral titers after therapeutic intranasal application of RFI-641 was 1.7 logs.[16] These data, together with the in vitro results, support the development of RFI-641 for the prevention and treatment of RSV disease.

Thus, chemical optimization has led to RFI-641, a candidate molecule for the prevention and treatment of RSV disease.

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13. 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 11.
14. For these experiments, the SYNTHEWAVE 402 unit from PROLABO company, equipped with a monomode focused system and continuous infra-red temperature feedback was used. The reactions were carried out in an open 50 mL quartz vessel reactor with the following concentrations: **10**, 500 mg, 0.73 mmol; **11**, 100 mg, 0.29 mmol in 2.5 mL of DMSO, 2.5 mL of the phosphate buffer (1 N, PH 7) and 1.0 mL of 1 N NaOH.
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