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Non-nucleoside inhibitors of the measles virus RNA-dependent RNA polymerase complex activity: synthesis and *in vitro* evaluation

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Abstract

High-throughput screening has identified 1-methyl-3-(trifluoromethyl)-*N*-[4-(pyrrolidinylsulfonyl)phenyl]-1H-pyrazole-5-carboxamide **16677** as a novel and potent ($IC_{50} = 35\text{--}145\text{ nM}$) inhibitor against multiple primary isolates of diverse measles virus (MV) genotypes currently circulating worldwide. The synthesis of **16677** and several analogs together with effects on MV replication is described. The most potent analog displays nanomolar inhibition against the MV and a selectivity ratio (CC_{50}/IC_{50}) of *ca.* 16,500.

Paramyxoviruses are negative stranded RNA viruses, most of which are highly contagious airborne pathogens that spread via the respiratory route. Members of this viral family include major human and animal pathogens such as measles virus (MV), human parainfluenza viruses (HPIV), mumps virus, respiratory syncytial virus and Newcastle disease virus.¹ Despite the existence of an effective live-attenuated vaccine,² MV remains a serious threat to human health globally. Ribavirin, the only drug available for the treatment of some paramyxovirus infections,^{3,4} has been used experimentally for the treatment of measles but with limited efficacy.⁵ More recently, benzimidazo-thiazole derivatives have been reported to be more potent and less cytotoxic than Ribavirin. The most active compound in the latter series demonstrated a selectivity ratio (CC_{50}/IC_{50}) of 246 compared with Ribavirin at 14.⁶

Previously, we described the development of a novel class of MV fusion inhibitors, substituted anilides, in a structure-based molecular design study.^{7, 8} The lead compound in this inhibitor class, AS-48,^{9, 10} shows activity in the low micromolar range ($IC_{50} = 0.6\text{--}3.0\text{ }\mu\text{M}$) against a panel of MV field isolates. A single Sub-Saharan isolate is resistant to inhibition by AS-48, however, and *in vitro* adaptation has resulted in the appearance of characteristic escape mutants after four to seven passages,¹¹ suggesting that resistance may emerge rapidly in the field. Identification of additional drug candidates against MV with diverse target characteristics is therefore imperative. Towards this goal, we have developed and implemented a novel cell-based assay for high-throughput screening (HTS) identification of MV inhibitors. This approach has yielded several confirmed hit candidates, the most potent of which, compound **16677** ($IC_{50} = 250\text{ nM}$ against MV Edmonston strain), was found to act as a well-behaved,

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target-specific inhibitor of MV replication with drug-like properties. The compound (1-methyl-3-(trifluoromethyl)-*N*-[4-(pyrrolidinyl-sulfonyl)phenyl]-1*H*-pyrazole-5-carboxamide) a first-in-class non-nucleoside inhibitor of the MV RNA polymerase complex (RdRp) activity¹², was prepared by coupling of 4-amino-pyrrolidinyl sulfonamide **1** with 1-methyl-3-trifluoro-pyrazole-5-acetyl chloride **2** in very good yield using pyridine as base.¹³(Scheme 1)

A structure-activity profile has begun to emerge by examination of the four molecular fragments circumscribed in Scheme 1; namely the pyrazole ring on the right, the amide linker, the central phenyl ring and the pyrrolidine ring to the left. The first stage of hit optimization focused on introducing a variety of aromatic rings as pyrazole replacements (Figure 1 and Table 1).

N-[4-(Pyrrolidinylsulfonyl)phenyl]-1*H*-amides **3a–s** were synthesized by coupling of *N*-[4-(Pyrrolidinyl sulfonyl)phenyl]-1*H*-amine with corresponding acetyl chlorides. Disappointingly, none of the compounds showed significant activity. Given the precipitous drop-off in potency, it would appear that the trifluoromethylated pyrazole ring in **16677** is essential. The pharmacophore tolerates neither substituted aromatic rings, alternative 5-membered ring heterocycles nor trifluoromethylated pyridines.

Preliminary modification of the middle phenyl ring was accomplished by installing a limited set of substituents as indicated by **4a–d** (Figure 2). Polar moieties *ortho* to the amide nitrogen (**4a** and **4b**) eliminate activity altogether, while ester **4c** and amide **4d** show fairly high toxicities. The CC₅₀s of both compounds were determined as 13–14 μ M (Table 2). While a more thorough-going exploration of substitution at the central phenyl is called for, it is noteworthy that the critical CF₃-substituted terminal pyrazole cannot be duplicated in the center of the **16677** lead molecule.

The Linker Region

Four different variations of the linker with an equivalent number of heavy chain atoms were prepared. The structures of compounds **5–8** (Scheme 2 & Scheme 3) and their corresponding activities are recorded in Table 2 (entries 5–8), illustrating a minimum 400-fold degradation in activity relative to **16677**. All the structural manipulations cause both a geometric reorganization and a variation in hydrogen bonding capacity. Thus, the planar amide in each case is replaced with a torsionally mobile surrogate. Compounds **5** and **6** eliminate NH hydrogen-bond donating capacity, while **7** and **8** deplete the C=O H-bond accepting potential. Disentangling the geometric and non-bonded effects will require additional linkers. However, it is clear that the synthetically facile amide is a powerful activity enhancing moiety.

Analogues **5** and **6** were prepared as outlined in Scheme 2. 1-Methyl-3-trifluoromethyl-5-pyrazolecarboxylic acid **9** was transformed to its acetyl chloride and coupled with *N*,*O*-dimethylhydroxylamine hydrochloride in the presence of diisopropylethyl amine in DMF to afford the Weinreb amide **10**. 4-Methyl-pyrrolidinyl sulfonamide **11** was treated with *n*-butyl lithium, followed by addition of **10** to give ketone analog **5**. Reduction of the latter's carbonyl group with sodium borohydride in methanol furnishes alcohol **6**.

Synthesis of the amine analog **7** was initiated by reduction of the carboxyl group in **9** with lithium aluminum hydride in THF to obtain alcohol **12**. Replacement of the hydroxyl group with bromide to yield **13** proceeded smoothly with PBr₃. Coupling of **13** with 4-amino-pyrrolidinyl sulfonamide **1a** in the presence of cesium carbonate in DMF provided **7**. In the meantime, alcohol **12** was likewise combined with 4-fluoro-pyrrolidinyl sulfonamide under the same conditions to form **8**. (Scheme 3)

Modification of the Pyrrolidine Ring

Considerable effort was expended to increase the potency of **16677** by modifying the central and right side of the molecule. However, as illustrated above, none of the analogs delivered increased potency, and considerable cytotoxicity was frequently encountered (i.e. compounds **4c** and **4d**). Further modification was shifted to the sulfonylated pyrrolidine ring on the left. A variety of heterocyclic rings were employed as pyrrolidine replacements while retaining the remainder of the **16677** structure (Figure 3). The most active piperidine derivative **14d**, when subjected to a secondary virus titer reduction assay, revealed activity against live MV ($0.012 \pm 0.017 \mu\text{M}$, strain Alaska) and no cytotoxicity (Promega, Table 3).

Conclusions and Prospects

In this initial optimization of the high-throughput screening MV hit **16677**, we have developed a preliminary SAR by structural manipulation within the four sectors highlighted in Scheme 1. A variety of modifications of the three sectors on the right either essentially abolished anti-MV activity or resulted in high cytotoxicity. However, a highly potent analog has been generated by replacing the pyrrolidine ring in **16677** with a piperidine to give **14d**. The compound shows activity around 10 nM and essentially no cytotoxicity when assessed in a commercially available cytotoxicity assay. Assessment of cell proliferation activity in the presence of **14d** using a trypan-blue exclusion assay has yielded a CC_{50} concentration of $199 \pm 27 \mu\text{M}$, resulting in a selectivity index ($\text{CC}_{50}/\text{IC}_{50}$) of approximately 16,500. Our previous entry inhibitor efforts uncovered substances effective in the 0.6–3 μM range,¹⁰ while one other study likewise reported anti-MV compounds in the low micromolar range without specifying the mechanistic basis for inhibition.⁶ Compounds **16677** and **14d** are the first MV inhibitors with potencies in the low nM range.¹² Future work will be devoted to expanding the class, maintaining the high selectivity ratio, extending utility to other viruses in the same family and developing analogs that are suitable for animal testing.

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Scheme 1.
Retro-synthesis of screening hit **16677**

^a Reagents: (a) oxalyl chloride; (b) N,O-dimethylhydroxylamineHCl, DIPEA, DMF; (c) *n*-BuLi, THF; (d) NaBH₄

Scheme 2.

Synthesis of ketone and hydroxyl analogs of compound **16677**.

^a Reagents: (a) LiAlH₄, THF; (b) PBr₃, THF, -50°C-rt; (c) CsCO₃, DMF, 80°C, 16h; (d) CsCO₃, DMF, 80°C.

Scheme 3.
Synthesis of amine analog **7** and ether analog **8**.

Figure 1.
N-[4-(pyrrolidinylsulfonyl)phenyl]-1H-amide analogs **3**

Figure 2.
Substituted phenyl derivatives

Figure 3.
1-Methyl-3-(trifluoromethyl)-N-[4-(pyrrolidinylsulfonyl)-phenyl]-1H-heterocyclic ring-5-carboxamide derivatives

Table 1
MV antiviral IC₅₀s and CC₅₀s for N-[4-(pyrrolidinylsulfonyl)phenyl]-1H-phenyl amides 3

ID	Comp.	EC ₅₀ (μM) ^a (MV-Alaska)	CC ₅₀ (μM) ^b (Vero cells)
3a	MS-8	CPE inhibit	MTT cytotox > 300
3b	MS-9	CPE inhibit	MTT cytotox > 300
3c	MS-10	CPE inhibit	MTT cytotox > 300
3d	AS-36b	CPE inhibit	MTT cytotox 11±1.3
3e	AS-60a	CPE inhibit	MTT cytotox > 300
3f	AS-36a	CPE inhibit	MTT cytotox 59 ± 4
3g	AS-27	CPE inhibit	MTT cytotox > 300
3h	AS-40	CPE inhibit	MTT cytotox 76 ± 3
3i	AS-60b	CPE inhibit	MTT cytotox > 300
3j	AS-60d	CPE inhibit	MTT cytotox > 300
3k	AS-61d	CPE inhibit	MTT cytotox > 300
3l	AS-57a	CPE inhibit	MTT cytotox > 300
3m	AS-57b	CPE inhibit	MTT cytotox > 300
3n	MS-13	CPE inhibit	MTT cytotox > 300
3o	MS-30	CPE inhibit	MTT cytotox > 300
3p	MS-11	CPE inhibit	MTT cytotox > 300
3q	MS-17	CPE inhibit	MTT cytotox 140
3r	AS-119	CPE inhibit	MTT cytotox 37 ± 1.5
3s	MS-12	CPE inhibit	MTT cytotox >300

^a Values represent averages of four experiments ± SD; highest concentration assessed 75 μM

^b values represent averages of two experiments ± SEM; highest concentration assessed 300 μM

^c EC₅₀ not determined (ND) when CC₅₀ ≤ 75 μM

Table 2
MV antiviral IC₅₀s and CC₅₀s of substituted phenyl and different linker compounds

ID	Comp.	EC ₅₀ (μM) ^a (MV-Alaska)	CC ₅₀ (μM) ^b (Vero cells)
4a	MS-37	CPE inhibit	MTT cytotox
4b	MS-14	CPE inhibit	MTT cytotox
4c	MS-38	CPE inhibit	MTT cytotox
4d	MS-15	CPE inhibit	MTT cytotox
5	MS-26	CPE inhibit	MTT cytotox
6	MS-27	CPE inhibit	MTT cytotox
7	MS-34	CPE inhibit	MTT cytotox
8	MS 36	CPE inhibit	MTT cytotox

^a values represent averages of four experiments ± SD; highest concentration assessed 75 μM

^b values represent averages of two experiments ± SEM; highest concentration assessed 300 μM

^c EC₅₀ not determined (ND) when CC₅₀ ≤ 75 μM

Table 3

MV antiviral IC₅₀s and CC₅₀s of 1-Methyl-3-(trifluoromethyl)-N-[4-(pyrrolidinylsulfonyl)-phenyl]-1H-heterocyclic ring-5-carboxamides.

ID	Comp.	EC ₅₀ (μM) ^a (MV-Alaska)	CC ₅₀ (μM) ^b (Vero cells)
14a	AS-85a	CPE inhibit 14 ± 2	MTT cytotox 100
14b	AS-105	CPE inhibit 23 ± 10	MTT cytotox > 300
14c	AS-103	CPE inhibit ND ^c	MTT cytotox 13 ± 0.7
14d	AS-136a	CPE inhibit <2.3	MTT cytotox >300
14e	AS-251	CPE inhibit >75	MTT cytotox >300
14f	AS-244	CPE inhibit 28 ± 9	MTT cytotox 126 ± 7
14g	AS-236	CPE inhibit 43 ± 24	MTT cytotox > 300

^a Values represent averages of four experiments ± SD; highest concentration assessed 75 μM

^b values represent averages of two experiments ± SEM; highest concentration assessed 300 μM

^c EC₅₀ not determined (ND) when CC₅₀ ≤ 75 μM