Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 5199–5203

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

Aiming Sun,[a,*] Nizal Chandrakumar,[a] Jeong-Joong Yoon,[b] Richard K. Plemper[b] and James P. Snyder[a]

[a]*Department of Chemistry, 1515 Dickey Drive, Emory University, Atlanta, GA 30322, USA*
[b]*Department of Pediatrics, Division of Pediatric Infectious Diseases, Emory Children's Center, 2015 Uppergate Drive, Emory University School of Medicine, Atlanta, GA 30322, USA*

Received 3 May 2007; revised 25 June 2007; accepted 28 June 2007
Available online 4 July 2007

Abstract—High-throughput screening has identified 1-methyl-3-(trifluoromethyl)-*N*-[4-(pyrrolidinylsulfonyl)phenyl]-1*H*-pyrazole-5-carboxamide **16677** as a novel and potent (IC_{50} = 35–145 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. Published by Elsevier Ltd.

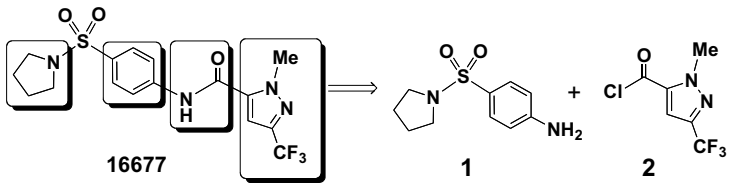
Paramyxoviruses are negative stranded RNA viruses, most of which are highly contagious airborne pathogens that spread via the respiratory route. Members of this viral family include major human and animal pathogens such as measles virus (MV), human parainfluenza viruses (HPIV), mumps virus, respiratory 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} = 0.6–3.0 μM) 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. Toward this goal, we have developed and implemented a novel cell-based assay for high-throughput screening (HTS) identification of MV inhibitors.[12] This approach has yielded several confirmed hit candidates, the most potent of which, compound **16677** (IC_{50} = 250 nM against MV Edmonston strain), was found to act as a well-behaved, 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) activity,[12] was prepared by coupling of 4-amino-prolidinyl 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 circum-

Keywords: Measles virus; HTS (high-through screening) hits; Pyrazoles.
* Corresponding author. Tel.: +1 404 727 6689; fax: +1 404 727 6689; e-mail: asun2@emory.edu

0960-894X/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2007.06.084



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

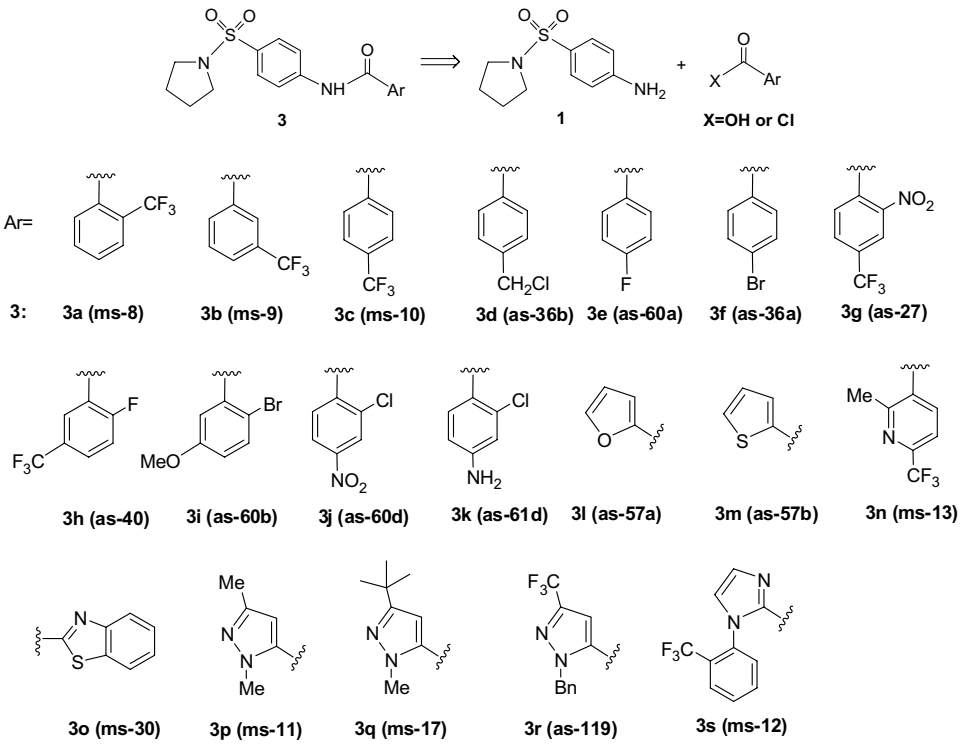


Figure 1. *N*-[4-(Pyrrolidinylsulfonyl)phenyl]-1*H*-amide analogs **3**.

Table 1. MV antiviral IC_{50}s and CC_{50}s for *N*-[4-(pyrrolidinylsulfonyl)phenyl]-1*H*-phenyl amides **3**

ID	Compound	EC_{50} (μM)[a] (MV-Alaska)		CC_{50} (μM)[b] (Vero cells)	
3a	MS-8	CPE inhibit	>75	MTT cytotox	>300
3b	MS-9	CPE inhibit	>75	MTT cytotox	>300
3c	MS-10	CPE inhibit	48 ± 6.1	MTT cytotox	>300
3d	AS-36b	CPE inhibit	ND[c]	MTT cytotox	11 ± 1.3
3e	AS-60a	CPE inhibit	>75	MTT cytotox	>300
3f	AS-36a	CPE inhibit	ND[c]	MTT cytotox	59 ± 4
3g	AS-27	CPE inhibit	>75	MTT cytotox	>300
3h	AS-40	CPE inhibit	⩾75	MTT cytotox	76 ± 3
3i	AS-60b	CPE inhibit	>75	MTT cytotox	>300
3j	AS-60d	CPE inhibit	>75	MTT cytotox	>300
3k	AS-61d	CPE inhibit	>75	MTT cytotox	>300
3l	AS-57a	CPE inhibit	>75	MTT cytotox	>300
3m	AS-57b	CPE inhibit	>75	MTT cytotox	>300
3n	MS-13	CPE inhibit	>75	MTT cytotox	>300
3o	MS-30	CPE inhibit	>75	MTT cytotox	>300
3p	MS-11	CPE inhibit	>75	MTT cytotox	>300
3q	MS-17	CPE inhibit	25	MTT cytotox	140
3r	AS-119	CPE inhibit	>75	MTT cytotox	37 ± 1.5
3s	MS-12	CPE inhibit	65	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] EC_{50} not determined (ND) when $CC_{50} \leqslant 75$ μM.

scribed 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 (Fig. 1 and Table 1).

Phyrazole ring replacement. *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.

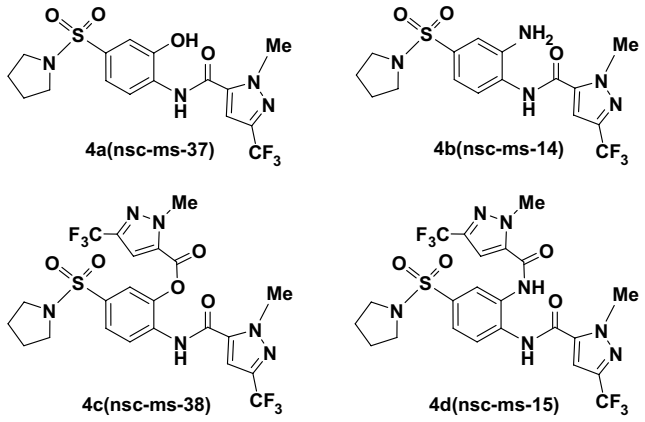


Figure 2. Substituted phenyl derivatives.

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.

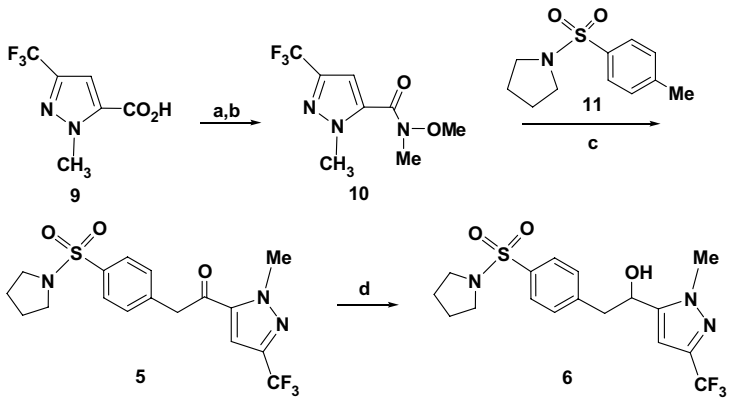
Middle phenyl ring substitution. Preliminary modification of this phenyl ring was accomplished by installing a limited set of substituents as indicated by **4a**–**d** (Fig. 2). Polar moieties *ortho* to the amide nitrogen (**4a** and **4b**) eliminate activity altogether, while ester **4c** and amide **4d** show fairly high toxicities. The CC_{50}s of both compounds were determined as 13–14 μM (Table 2). While a more thorough-going exploration of substitution at the central phenyl is called for, it is noteworthy that the critical CF_3-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** (Schemes 2 and 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

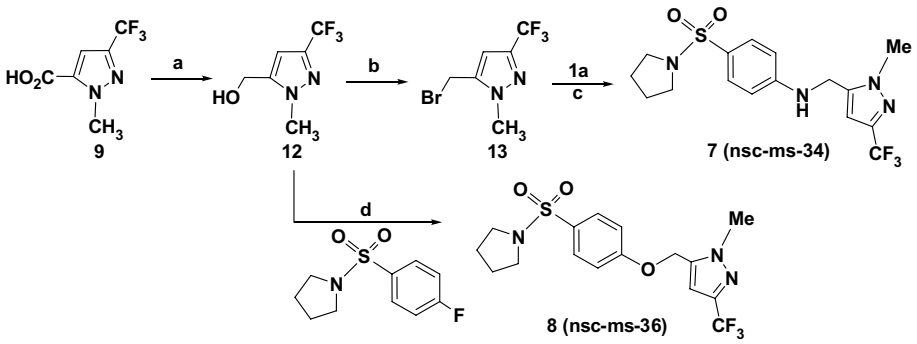
Table 2. MV antiviral IC_{50}s and CC_{50}s of substituted phenyl and different linker compounds

ID	Compound	EC_{50} (μM)[a] (MV-Alaska)		CC_{50} (μM)[b] (Vero cells)	
4a	MS-37	CPE inhibit	>75	MTT cytotox	288 ± 13
4b	MS-14	CPE inhibit	⩾75	MTT cytotox	>300
4c	MS-38	CPE inhibit	ND[c]	MTT cytotox	14 ± 0.5
4d	MS-15	CPE inhibit	ND[c]	MTT cytotox	13 ± 0.3
5	MS-26	CPE inhibit	15.5 ± 2.4	MTT cytotox	>300
6	MS-27	CPE inhibit	41 ± 39	MTT cytotox	>300
7	MS-34	CPE inhibit	>75	MTT cytotox	>300
8	MS 36	CPE inhibit	>75	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] EC_{50} not determined (ND) when $CC_{50} \leqslant 75$ μM.



Scheme 2. Synthesis of ketone and hydroxyl analogs of compound **16677**. Reagents: (a) oxalyl chloride; (b) *N*,*O*-dimethlyhydroxylamine HCl, DIPEA, DMF; (c) *n*-BuLi, THF; (d) $NaBH_4$.



Scheme 3. Synthesis of amine analog **7** and ether analog **8**. Reagents and conditions: (a) $LiAlH_4$, THF; (b) PBr_3, THF, −50 °C–rt; (c) $CsCO_3$, DMF, 80 °C, 16 h; (d) $CsCO_3$, DMF, 80°C.

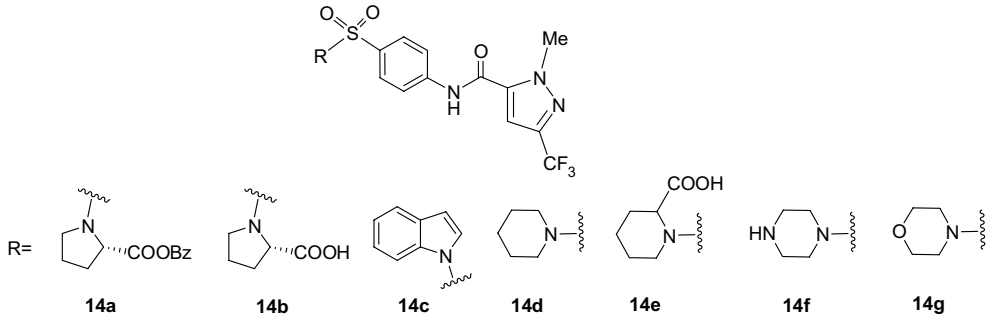


Figure 3. 1-Methyl-3-(trifluoromethyl)-*N*-[4-(pyrrolidinylsulfonyl)-phenyl]-1*H*-heterocyclic ring-5-carboxamide derivatives.

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.

Analogs **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 PBr_3. 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, while 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 (Fig. 3). The most active piperidine derivative **14d**, when subjected to a secondary virus titer reduction assay, revealed activity against live MV (0.012 ± 0.017 μM, 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 ± 27μM, resulting in a selectivity index (CC_{50}/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**

Table 3. MV antiviral IC_{50}s and CC_{50}s of 1-methyl-3-(trifluoromethyl)-*N*-[4-(pyrrolidinylsulfonyl)-phenyl]-1*H*-heterocyclic ring-5-carboxamides

ID	Compound	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] EC_{50} not determined (ND) when $CC_{50} \leqslant 75$ μM.

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.

Acknowledgments

This work was supported by a research grant from the American Lung Association, Public Health Service Grants AI056179 and AI071002 from NIH/NIAID (to R.K.P.), and by the National Institutes of Health 1 U54 HG003918-02 (to J.P.S.).

References and notes

1. Lamb, R. A.; Kolakosky, D. Paramyxoviruses: the viruses and their replication. In *Fundamental Virology*; Fields, B. N., Knipe, D. M., Howley, P. M., Eds.; Lippencott-Raven: Philadelphia, PA, 1996; p 577.
2. Hilleman, M. R. *Vaccine* **2001**, *20*, 651.
3. Shigeta, S.; Mori, S.; Baba, M.; Ito, M.; Honzumi, K.; Nakamura, K.; Oshitani, H.; Numazaki, Y.; Matsuda, A., et al. *Antimicrob. Agents Chemother.* **1992**, *36*, 435.
4. Gabrielsen, B.; Phelan, M. J.; Barthel-Rosa, L.; See, C.; Huggins, J. W.; Kefauver, D. F.; Monath, T. P.; Ussery, M. A.; Chmurny, G. N., et al. *J. Med. Chem.* **1992**, *35*, 3231.
5. Barnard, D. L. *Antivir. Chem. Chemother.* **2004**, *15*, 111.
6. Pokrovskii, A. G.; Il'icheva, T. N.; Kotovskaya, S. K.; Romanova, S. A.; Charushin, V. N.; Chupakhin, O. N. *Biochem. Biophys.* **2004**, *398*, 285.
7. Plemper, R. K.; Erlandson, K. J.; Lakdawala, A. S.; Sun, A.; Prussia, A.; Boonsombat, J.; Aki-Sener, E.; Yalcin, I.; Yildiz, I.; Temiz-Arpaci, O.; Tekiner, B.; Liotta, D. C.; Snyder, J. P.; Compans, R. W. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 5628.
8. Plemper, R. K.; Lakdawala, A. S.; Gernert, K. M.; Snyder, J. P.; Compans, R. W. *Biochemistry* **2003**, *42*, 6645.
9. Plemper, R. K.; Doyle, J.; Sun, A.; Prussia, A.; Cheng, L. T.; Rota, P. A.; Liotta, D. C.; Snyder, J. P.; Compans, R. W. *Antimicrob. Agents Chemother.* **2005**, *49*, 3755.
10. Sun, A.; Prussia, A.; Zhan, W.; Murray, E. E.; Doyle, J.; Cheng, L. T.; Yoon, J. J.; Radchenko, E. V.; Palyulin, V. A.; Compans, R. W.; Liotta, D. C.; Plemper, R. K.; Snyder, J. P. *J. Med. Chem.* **2006**, *49*, 5080.
11. Doyle, J.; Prussia, A.; White, L. K.; Sun, A.; Liotta, D. C.; Snyder, J. P.; Compans, R. W.; Plemper, R. K. *J. Virol.* **2006**, *80*, 1524.
12. White, L. K.; Yoon, J.-J.; Lee, Jin K.; Sun, A.; Du, Y.; Fu, H.; Snyder, J. P.; Plemper, R. K. *Antimicrob. Agents Chemother.* **2007**, *51*, 2293.
13. Schlosser, M.; Volle, J.; Leroux, F.; Schenk, K. *Eur. J. Org. Chem.* **2002**, 2913.