Bioorganic & Medicinal Chemistry Letters 25 (2015) 976–981

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

1,2,3,9*b*-Tetrahydro-5*H*-imidazo[2,1-*a*]isoindol-5-ones as a new class of respiratory syncytial virus (RSV) fusion inhibitors. Part 2: Identification of BTA9881 as a preclinical candidate

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ARTICLE INFO

Article history:
Received 24 September 2014
Revised 5 November 2014
Accepted 6 November 2014
Available online 14 November 2014

Keywords:
Respiratory syncytial virus
Antiviral
Synthesis

ABSTRACT

Respiratory syncytial virus (RSV) is a major cause of respiratory tract infections in infants, young children and adults. 1,2,3,9*b*-Tetrahydro-5*H*-imidazo[2,1-*a*]isoindol-5-ones with general structure **1** were previously identified as promising inhibitors of RSV targeting the fusion glycoprotein. In particular, the introduction of a nitrogen at the 8-position of the tricyclic core yielded lead compounds **2** and **3**. Extensive exploration of the R^2 group established that certain heterocyclic amides conferred potent RSV A&B activity and a good balance of physicochemical and pharmacokinetic properties. The antiviral activity was found to reside in a single enantiomer and compound **33a**, (9b*S*)-9b-(4-chlorophenyl)-1-(pyridin-3-ylcarbonyl)-1,2,3,9*b*-tetrahydro-5*H*-imidazo[1′,2′:1,2]pyrrolo[3,4-*c*]pyridin-5-one (known as **BTA9881**), was identified as a candidate for preclinical development.

Respiratory syncytial virus (RSV) is the predominant cause of acute lower respiratory tract infection in young children and results in substantive hospital admissions. Global mortality rates in children under five years of age have been estimated at 200,000 annually.[1] RSV is known primarily as a pediatric pathogen; however it can infect individuals of all ages, particularly those with underlying disease. Symptoms range from severe pneumonia and bronchiolitis to much milder symptoms similar to the common cold.[2] *Palivizumab*, a humanized monoclonal antibody targeting the fusion glycoprotein of RSV, is registered for the prevention of serious lower respiratory tract disease caused by RSV in high-risk infants (less than two years old).[3] Prophylactic use of *palivizumab* is limited due to its cost and ribavirin, the only agent approved for treatment of RSV, provides marginal benefit and is potentially toxic.[4] Several small molecule inhibitors have been discovered[5] and there are two RSV fusion inhibitors currently in Phase 2 studies (MDT-637, formerly VP-14637,[6] and GS-5806[7]).

In a previous publication,[8] we described the discovery of 1,2,3,9*b*-tetrahydro-5*H*-imidazo[2,1-*a*]isoindol-5-ones (**1**) as a new class of potent respiratory syncytial virus (RSV) fusion inhibitors and identified compounds **2** and **3** bearing a nitrogen at the 8-position of the tricyclic core as candidates for further optimisation (Fig. 1). Here, we report the results of these optimisation efforts and the subsequent nomination of BTA9881 (**33a**) as a preclinical candidate for the treatment of RSV.

Our previous investigations into the SAR of the 1,2,3,9*b*-tetrahydro-5*H*-imidazo[2,1-*a*]isoindol-5-one series revealed that the 4-chlorophenyl group was preferred at R^1 and identified the R^2 group as a promising starting point for further optimisation efforts. Compounds containing the 1,2,3,9*b*-tetrahydro-5*H*-imidazo[1′,2′:1,2]pyrrolo[3,4-*c*]pyridin-5-one core were initially synthesised according to the three-step, racemic procedure outlined in Scheme 1.[9] Friedel–Crafts acylation of chlorobenzene (**5**) with 3,4-pyridine anhydride (**4**) gave a 1:1 mixture of keto acids **6** and **7**. Separation of the desired regioisomer **6** was achieved through careful manipulation of the pH during work-up.[9] Condensation with ethylenediamine in refluxing xylenes yielded the core **8**, which was then reacted with the appropriate acid chlorides or isocyanates to give final compounds **9**–**34**.

Further development work led to a selective and scalable synthesis of 3-(4-chloro-benzoyl)-isonicotinic acid, **6**, based on published methodology showing that isonicotinanilide **39** can be efficiently *ortho*-lithiated[10] (Scheme 2). Briefly, amide **39** was readily prepared and formed a stable dianion at −45 °C that reacted with electrophiles such as methyl 4-chlorobenzoate (**40**). Aqueous

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http://dx.doi.org/10.1016/j.bmcl.2014.11.024
0960-894X/

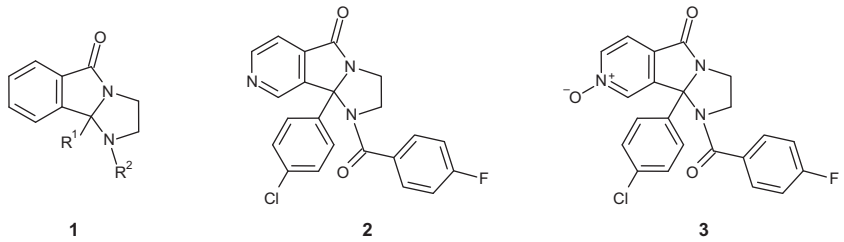
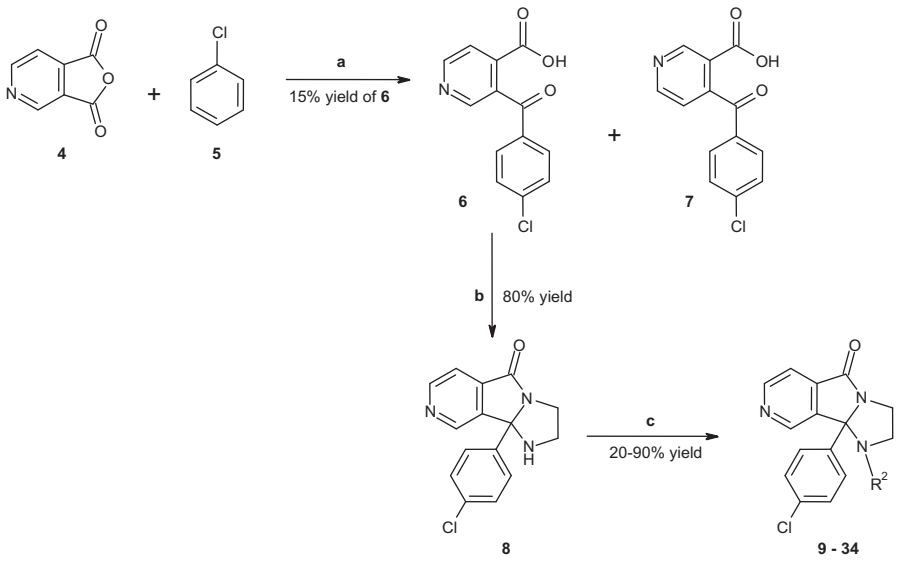
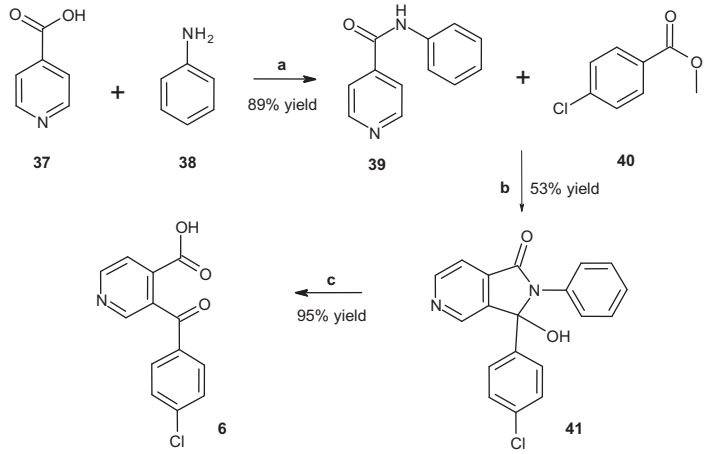


Figure 1. General structure of 1,2,3,9*b*-tetrahydro-5*H*-imidazo[2,1-*a*]isoindol-5-ones (**1**) and structures of compounds **2** and **3**.



Scheme 1. Reagents and conditions: (a) $AlCl_3$ (2.2 equiv), 110 °C, 5 h; (b) ethylenediamine (5 equiv), xylenes, reflux, 4 h; (c) acid chloride or isocyanate (2 to 5 equiv), pyridine, 0 °C to rt.



Scheme 2. Reagents and conditions: (a) 1,1′-Carbonyldiimidazole (1 equiv), DMF, 40 °C (b) **39**, nBuLi (2 equiv), *N*,*N*,*N*′,*N*′-Tetramethylethylenediamine (2 equiv), −45 °C, then **40** (2 equiv) (c) 15% KOH aq, 60–90 °C.

workup resulted in ring closure to the hydroxy lactam **41** which was readily hydrolysed under basic conditions to desired ketoacid, **6**.[11]

Compounds were tested for activity against RSV in a cytopathic effect (CPE) assay[12] (see Table 1). In addition to optimising compound potency, variation at the R^2 position also offered an opportunity to 'tune' the drug-like properties of the molecule. With this in mind, compounds with promising activity against RSV were also assayed in the presence of alpha-1-acid glycoprotein (AAG) in order to assess the influence of binding to a human serum protein (no activity shift was observed for this class of compounds in the presence of human serum albumin).[13]

Our previous work on the 1,2,3,9*b*-tetrahydro-5*H*-imidazo[2,1-*a*]isoindol-5-one series identified ureas and 5- and 6-member heterocyclic amides as promising avenues for further investigation for variation at R^2. A small set of ureas was synthesised (compounds **9**–**11**), but while all showed some level of activity against RSV (see Table 1), none were as potent as compound **2**. Our

Table 1
Antiviral activity, protein binding properties and cytotoxicity of compounds **2** and **9**–**34**

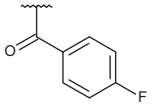
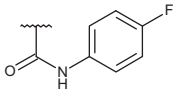
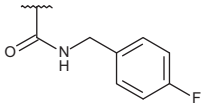
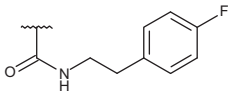
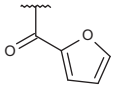
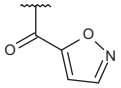
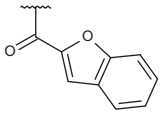
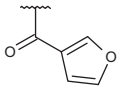
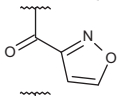
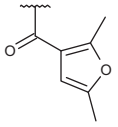
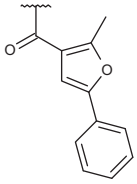
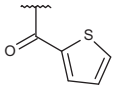
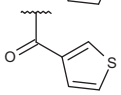
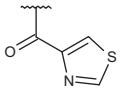
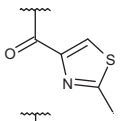
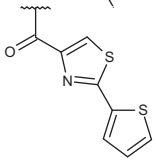
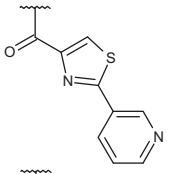
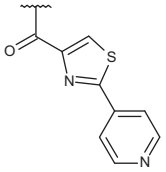
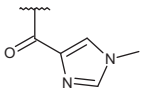
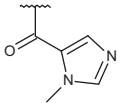
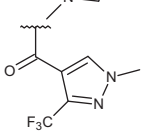
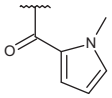
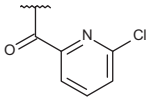
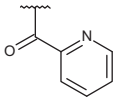
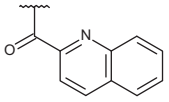
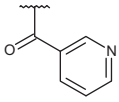
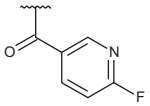
Compd	R^2	RSV A2 EC_{50} (μM) CPE assay[a]	EC_{50} fold shift RSV A2 + AAG[b]	Cytotoxicity, CC_{50} (μM)
2		0.23 ± 0.07 (n = 40)	7.2	>49
9		1.0	ND	>20
10		0.93	ND	>20
11		0.62	ND	>20
12		0.12	3.6	>19
13		0.37	ND	>19
14		6.4	ND	>17
15		0.096	2.4	>19
16		0.18	11	>20
17		0.12	14	>18
18		1.6	ND	>15
19		0.13	12	>18
20		0.17	27	>18
21		0.18	16	>18
22		0.073	5.7	>18
23		0.035, 0.027	16	>15

Table 1 (*continued*)

Compd	R^2	RSV A2 EC_{50} (μM) CPE assay[a]	EC_{50} fold shift RSV A2 + AAG[b]	Cytotoxicity, CC_{50} (μM)
24		0.025 ± 0.007 (n = 4)	10	>15
25		2.3	ND	>15
26		0.75	ND	>20
27		0.73	ND	>20
28		0.031 ± 0.007 (n = 3)	4.3	>16
29		0.020	91	>20
30		0.11	27	>17
31		0.77	ND	>19
32		8.5	ND	>17
33		0.22, 0.21	2.8	>51
34		0.14, 0.093	1.8	>48

ND = not determined.
[a] Standard deviations are quoted where n > 2.
[b] Alpha-1-acid glycoprotein present at a concentration of 1.5 mg/mL.

attention therefore concentrated on investigating heterocyclic amides as the most promising sub-class.

The 2-furoyl group, which had previously shown promising activity in the hit series, also showed good activity on the pyridine tricyclic core (see compound **12**), giving an almost 2-fold improvement in potency compared to compound **2**. The corresponding 5-isoxazolyl (**13**) showed a loss of potency and the addition of a fused phenyl ring (benzofuran **14**) was poorly tolerated. Replacing the 2-furoyl group with the 3-furoyl isomer (compound **15**) gave a comparably active compound. In this case, modification to an isoxazole (**16**) also resulted in a reduction in potency, but to a lesser extent. Substitution of the 3-furan ring with two methyl groups (compound **17**) was tolerated without improving activity, but

increasing the size of the 5-substituent to phenyl (compound **18**) resulted in a loss of potency. Replacing the furan ring with a thiophene did not have a significant impact on activity, with similar levels of potency seen for the 2-isomer (compare compounds **19** and **12**) and a less than two-fold reduction observed for the 3-isomer (compare compounds **20** and **15**), but the level of protein binding displayed by these compounds increased. The synthesis of a small set of thiazole analogues (compounds **21**–**25**) yielded a number of active compounds, with analogues **23** and **24** in particular displaying excellent activity against RSV. Unfortunately, both compounds also suffered from high binding to alpha-1-acid glycoprotein. Interestingly, compound activity was highly sensitive to the 2-substituent on the thiazole ring, with the 4-pyridyl isomer **25** proving to be 10-fold less active than its 3-pyridyl counterpart **24**. A small set of nitrogen-based 5-membered heterocyclic amides was also investigated. Imidazoles **26** and **27** were both less active than compound **2**, but pyrazole **28** showed excellent anti-RSV activity, together with good protein binding properties. Pyrrole **29** also demonstrated excellent potency, but was highly protein-bound.

Our starting point for investigating 6-membered heterocyclic amides was the 3-chloro-2-pyridine-carbonyl group, which had previously shown encouraging potency.[8] Compound **30** displayed improved activity over **2**, but with high protein binding. Removal of the chloro-substituent (see compound **31**) resulted in a loss of potency, and, as was seen for the furoyl analogue, the addition of a fused phenyl ring (quinolone **32**) was not tolerated. However, the replacement of the 2-pyridine ring with its 3-pyridine analogue (compound **33**) yielded a compound with improved activity against RSV and good protein binding properties. The addition of a 4-fluoro substituent (compound **34**) resulted in a further improvement in potency whilst maintaining the compound's favourable protein binding characteristics.

Separation of a selection of racemic compounds into their enantiomers using chiral chromatography, followed by subsequent testing against RSV in the CPE assay indicated that the antiviral activity appeared to reside in a single enantiomeric series (Table 2). Single crystal X-ray crystallography performed on compounds **15a** and **33a** revealed the configuration at the chiral centre of the active enantiomers to be (*S*) (Fig. 2). A highly efficient chiral resolution of core **8** was subsequently developed utilising (*R*)-(−)-1,1′-binaphthyl-2,2′-diyl hydrogenphosphate ((*R*)-(−)-BNPPA) as a resolving agent.[14] The binary diastereomeric salt was readily formed when racemic core **8** was heated in ethanol (or an ethanol/methanol mixture) with ((*R*)-(−)-BNPPA (0.5–1.0 equiv) and allowed to cool. The resolved core was recovered in high chiral purity (>99% ee) following a single resolution and liberation of the free base (aqueous K_2CO_3).

A set of compounds combining potent activity against RSV with good protein binding properties were assessed for RSV cross-strain activity, lipophilicity, solubility and rat pharmacokinetics (see Tables 3 and 4).

All compounds profiled showed good activity against the three strains of RSV tested, together with moderate to high solubility and low lipophilicity. The rat PK profiles for furoyl compounds **12a** and **15a** demonstrated high plasma clearance and low exposure. Pyrazole **28** showed good bioavailability but had a long terminal half-life, presumably due to low plasma clearance and a moderate volume of distribution. Pyridine **33a** had a very good PK profile, demonstrating high exposure (the highest C_{max} of any analogue tested), excellent bioavailability, and a moderate half-life. Its 4-fluoro-substituted analogue **34a** also displayed high exposure and bioavailability, but with a longer elimination half-life.

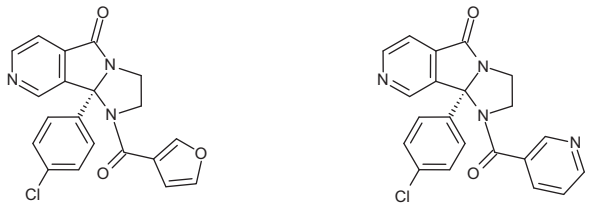


Figure 2. Structures of active enantiomers **15a** and **33a**.

N-oxide **3** had previously demonstrated a significantly shorter half-life than parent compound **2**.[8] In addition, the pyridine N-oxides of compounds **28** and **34** (**35a** and **36** respectively, Fig. 3) were detected in rat plasma and urine following intravenous and oral dosing. It appears that these active metabolites are formed rapidly in vivo and were synthesised (according to the method of Sharpless)[17] in order to independently assess their pharmacokinetic properties.

The bioavailability of N-oxide **35a** improved and plasma clearance was reduced (see Table 5) compared to the parent pyridyl compound **28**. However, the PK profile still demonstrated an elongated terminal half-life. Similarly, N-oxide **36** retained high exposure and good bioavailability but the half-life following oral exposure was not lower than that of pyridyl compound **34a**. Measurable plasma concentrations of **28** were detected for the duration of the post-dose sampling period after IV and oral dosing of **35a**, indicating that the N-oxide was subject to reductive metabolism in vivo.

Further virology studies were conducted to characterise the most promising compound, **33a**, as a direct and selective inhibitor of RSV fusion. Briefly, **33a** displayed potent antiviral activity against RSV in cytopathic effect, plaque reduction and yield reduction assays. A time of addition experiment demonstrated that the compound acted early in the virus replication cycle consistent with inhibition of attachment or fusion of the virus to the host cell. In vitro resistance selection studies were conducted with **33a** in RSV-A Long and RSV-B B1 strains. Mutations were identified in the F-gene leading to single amino acid substitutions; Q494L (Long) and L141F (B1). These mutations were associated with reduced susceptibility to **33a** and to other known small molecule fusion inhibitors (Table 6). Cross-resistance is a common feature

Table 2
Antiviral activity for racemates and separated enantiomers of selected compounds

Racemate	RSV A2 EC_{50} (μM) CPE assay[a]	Active enantiomer	Chiral purity (% ee)	RSV A2 EC_{50} (μM) CPE assay[a]	Inactive enantiomer	Chiral purity (% ee)	RSV A2 EC_{50} (μM) CPE assay[a]
2	0.23 ± 0.07 (*n* = 40)	**2a**	>95	0.11	**2b**	>95	14
3	0.25 ± 0.10 (*n* = 3)	**3a**	92	0.21, 0.17	**3b**	95	11, 8.6
15	0.096	**15a**	>99.5	0.010 ± 0.003 (*n* = 3)	**15b**	98	0.67, 1.0
24	0.025 ± 0.007 (*n* = 4)	**24a**	97	0.013	**24b**	98	0.92 ± 0.020 (*n* = 3)
33	0.22, 0.21	**33a**	>98	0.048 ± 0.027 (*n* = 3)	**33b**	96	>1.2
34	0.14, 0.093	**34a**	99	0.075	**34b**	99	7.8

[a] Standard deviations are quoted where *n* > 2.

Table 3
Antiviral activity and physicochemical properties of selected compounds

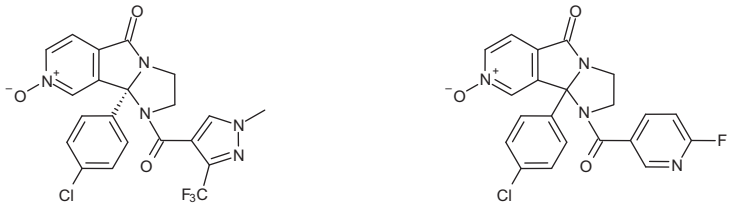
Compd	EC_{50} (μM) CPE assay[a]			log*D*[b]	Solubility pH 2[c] (μg/mL)	Solubility pH 6.5[c] (μg/mL)
	RSV A2	RSV Long	RSV B1			
2	0.23 ± 0.07 (*n* = 40)	0.22 ± 0.10 (*n* = 12)	0.68 ± 0.34 (*n* = 12)	2.8	25–50	12.5–25
3	0.25 ± 0.10 (*n* = 3)	0.35, 0.12	0.91	2.3	50–100	25–50
12	0.12	0.37	0.17	2.8	50–100	>100
15a	0.010 ± 0.003 (*n* = 3)	0.032 ± 0.022 (*n* = 3)	0.085 ± 0.057 (*n* = 4)	2.5[d]	50–100[d]	>100[d]
28a	0.0072, 0.0078	0.0064	0.10 ± 0.05 (*n* = 3)[d]	3.0	>100	25–50
33a	0.048 ± 0.027 (*n* = 3)	0.059 ± 0.024 (*n* = 3)	0.16 ± 0.09 (*n* = 4)	2.1[d]	>100[d]	50–100[d]
34a	0.075	0.13[d]	0.29	2.7	>100	25–50

[a] Standard deviations are quoted where *n* > 2.
[b] Effective logD values were measured using a chromatographic method employing a SUPELCOSIL LC-ABZ column using an octanol saturated mobile phase at pH 7.4.[15]
[c] Kinetic solubility measured by nephelometry.[16]
[d] Data collected on the racemate.

Table 4
Intravenous and oral PK properties of selected compounds in male Sprague Dawley rat

Compd	Rat PK IV (*n* = 2)				Rat PK Oral (*n* = 2)			
	Dose (mg/kg)	$T_{1/2}$ (h)	CLplasma (mL/min/kg)	V_z (L/kg)	Dose (mg/kg)	$T_{1/2}$ (h)	C_{max} (μM)	*F* (%)
2	2.0[a]	>24	2.7	6.0	19[b]	>24	6.3	28
3	2.0[c]	6.5	2.4	1.3	20[d]	5.6	23	89
12a	1.1[e]	2.4	79	8.7	20[d]	2.8	0.44	21
15a	2.0[e]	2.4	55	11	19[d]	3.1	0.77	35
28	0.9[e]	5.6	4	1.9	19[d]	7.1	3.6	38
33a	8.8[f]	4.5	4.5	1.7	19[d]	4.5	23	~100
34a	5.1[e]	11	4.3	3.8	19[g]	9.3	8.8	75

[a] Dosed as a solution in 0.1 M Captisol and 10–12.5% DMSO.
[b] Dosed as a suspension in hydroxypropylmethylcellulose.
[c] Dosed as a solution in 5% glucose and 10% DMSO.
[d] Dosed as a suspension in carboxymethylcellulose.
[e] Dosed as a solution in 35–40% propylene glycol and 10% ethanol in water.
[f] Dosed as a solution in 20 mM citrate (pH 2.6) with 5% glucose and 10% DMSO.
[g] Dosed as a suspension in 0.5% hydroxypropylmethylcellulose, 0.5% benzyl alcohol and 0.4% Tween80.



35a
RSV A2 EC_{50}: 0.012, 0.010μM
Cytotoxicity, CC_{50}: >5.0μM

36
RSV A2 EC_{50}: 0.15μM
Cytotoxicity, CC_{50}: >17μM

Figure 3. Structures and antiviral activities of N-oxides **35a** and **36**.

Table 5
Intravenous and oral PK properties of compounds **35a** and **36** in male Sprague Dawley rat

Compd	Rat PK IV				Rat PK Oral (*n* = 2)			
	Dose (mg/kg)	$T_{1/2}$ (h)	CLtotal (mL/min/kg)	V_z (L/kg)	Dose (mg/kg)	C_{max} (μM)	$T_{1/2}$ (h)	*F* (%)
35a	3.1[a] (*n* = 1)	11	2.2	2.1	18[b]	9.3	11	57
36	2.1[c] (*n* = 2)	1.7	7.9	1.2	21[b]	2.5	20	85

[a] Dosed as a solution in 40% propylene glycol, 10% ethanol and 0.4% Tween80 in water.
[b] Dosed as a suspension with carboxymethylcellulose.
[c] Dosed as a solution in 20 mM citrate (pH 2.6) with 5% glucose and 10% DMSO.

for this class of fusion inhibitors which appear to have a similar mechanism of action.[6,18,19]

In summary, further optimisation of the acyl substituent of RSV fusion inhibitor **2** resulted in the discovery of a number of compounds combining potent cross-strain RSV activity with good drug-like properties. As a result of this work, compound **33a** (BTA9881), which provided the best overall combination of potency, physicochemical properties, cross-species pharmacokinetics

Table 6
Cross resistance profiles of **33a**, MDT-637 (VP-14637), BMS-433771 and TMC353121

Mutation (F protein)	Mutation raised against	RSV Strain	EC_{50} fold shift			
			33a	VP-14637[6]	BMS-433771[18]	TMC353121[19]
L141F	**33a**	B-1	>100	ND	>345	>3333
Q494L	**33a**	Long	40	ND	10	11.5
D489Y	VP-14637	Long	>1800	>2174	>20,000	>10,000

ND = not determined.

(including approximately 100% oral bioavailability in the dog with a slow elimination phase) and preliminary safety pharmacology was nominated as a preclinical candidate for the treatment of RSV infections. Further details of the virology, mechanism of action and in vivo efficacy of BTA9881 will be described in a separate publication.

Acknowledgments

Physicochemical, metabolic and pharmacokinetic experiments were conducted at the Centre for Drug Candidate Optimisation, Monash University (Melbourne, Australia). We acknowledge the financial assistance of a START Grant from the Australian Government.

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9. Synthesis of **8** (Scheme 1): $AlCl_3$ (2.88 mol) was added to a stirred suspension of anhydride **4** (1.31 mol) in chlorobenzene (1.2 L) and heated to 110 °C for 5 hours. The cooled mixture was treated with water (2 L), heated to reflux for 1 hour and filtered. The collected solids were suspended in water (3.5 L) and 10% NaOH (aq) (350 mL), filtered and acidified to pH 3.1 with 2 M HCl. The precipitate was recovered by filtration and washed in boiling EtOH (2 L) to give a white solid (67 g). This material was dissolved in 10% NaOH (400 mL), acidified to pH 6.3 with 2 M HCl and filtered to yield **6** as a white solid (53 g). A solution of **6** (53 g) and ethylenediamine (68 mL) were heated at reflux in xylenes (1.8 L) for 4 h. The hot solution was filtered, the filtrate was evaporated and the residue was recrystallised from ethanol to give **8** as a white solid (46.4 g).
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11. Synthesis of **6** (Scheme 2): A solution of 1,1′-carbonyldiimidazole (130 g, 0.81 mol), isonicotinic acid (100 g, 0.81 mol) and DMF (300 mL) was heated at 40 °C for 2.5 h before aniline (100 g, 0.81 mol) was added in a single portion. After 20 h, water (1.2 L) was added. The resulting suspension was filtered and the solids dried under vacuum to afford isonicotinanilide (**39**) (142.6 g, 89%). In an acetone/dry ice bath, a solution of isonicotinanilide (**39**) (150 g, 0.76 mol) and TMEDA (228 mL, 1.51 mol) in THF (2.25 L) was maintained at −45 °C while it was treated with 2.37 M *n*-BuLi in hexane (637 mL, 1.51 mol), stirred for 1 hour, treated with a solution of methyl 4-chlorobenzoate (129.1 g, 1.51 mol) in THF (260 mL) and finally stirred for a further 1.5 h. Water (750 mL) was slowly added and the biphasic mixture allowed to warm to room temperature with stirring for 18 h. While cooling in ice/water, the aqueous layer was adjusted to pH 7 by addition of HCl (conc., 300 mL). The organic layer was washed with water (650 mL), concentrated to 1 L and filtered. The solid was dried under vacuum to afford **41** (62.9 g, 25%). Further concentration of the filtrate and washing with isopropanol (150 mL) yielded additional product (70 g, 28%). Lactam **41** (130 g, 0.386 mol) was added to a solution of KOH (182 g) in water (1.2 L). The resulting suspension was stirred at 60–90 °C for 6 h. The pH was adjusted to 4 by addition of HCl (conc., 268 mL) while cooling in an ice bath and the resulting suspension was stirred for 3 h. The solid was isolated by filtration, washed with water (2 × 100 mL), isopropanol (2 × 70 mL) and dried under vacuum to afford 3-(4-chlorobenzoyl)-isonicotinic acid (**6**) as an off-white solid (96.2 g, 95%).
12. Cytopathic effect (CPE) assays were performed essentially as described in the literature (Watanabe, W.; Konno, K.; Ijichi, K.; Inoue, H.; Yokota, T.; Shigeta, S. *J. Virol. Methods* **1994**, *48*, 257). Serial dilutions of the test compounds were made in 96 well plates. HEp2 cells (1.0×10^4 cells/well) were infected with RSV at a low multiplicity of infection (e.g., RSV A2 at an moi of 0.01) and added to plates to assess antiviral activity. Uninfected HEp2 cells were used to assess compound cytotoxicity. Assays were incubated for 5 days at 37 °C in a 5% CO_2 atmosphere. The extent of CPE was determined via metabolism of the vital dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). MTT (1 mg/ml) was added to each well and plates incubated for 2 h incubation at 37 °C. Wells were aspirated, isopropanol (200 µL) was added and absorbance values read at 540 nm. Compound concentrations that inhibited CPE by 50% (EC_{50}) and developed cytotoxicity (CC_{50}) were calculated using non-linear regression analysis.
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