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Respiratory syncytial virus fusion inhibitors. Part 7: Structure–activity relationships associated with a series of isatin oximes that demonstrate antiviral activity in vivo

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ABSTRACT

A series of bezimidazole-isatin oximes were prepared and profiled as inhibitors of respiratory syncytial virus (RSV) replication in cell culture. Structure–activity relationship studies were directed toward optimization of antiviral activity, cell permeability and metabolic stability in human liver microsomes (HLM). Parallel combinatorial synthetic chemistry was employed to functionalize isatin oximes via O-alkylation which quickly identified a subset of small, lipophilic substituents that established good potency for the series. Further optimization of the isatin oxime derivatives focused on introduction of nitrogen atoms to the isatin phenyl ring to provide a series of aza-isatin oximes with significantly improved PK properties. Several aza-isatin oximes analogs displayed targeted metabolic stability in HLM and permeability across a confluent monolayer of CaCo-2 cells. These studies identified several compounds, including **18i, 18j** and **18n** that demonstrated antiviral activity in the BALB/c mouse model of RSV infection following oral dosing.

Respiratory syncytial virus (RSV) is an enveloped, negative strand RNA virus that infects the respiratory tract of both children and adults.[1–3] Most children are infected with RSV before 2 years of age, re-infection is a common occurrence and morbidity due to complications is high among premature infants and those with underlying cardiopulmonary problems.[4] Moreover, RSV infections have been associated with increased prevalence of asthma in later childhood.[5] Adults are also susceptible to morbidity as the result of an RSV infection, especially elderly populations and patients that are immunocompromised.[6,7] The current standard of care for an RSV infection is limited to aerosol administration of ribavirin as a therapeutic agent while a series of intramuscular injections of the humanized monoclonal antibody palivizumab (Synagis™), an RSV immune globulin, provides prophylactic protection for children at risk.[8] However, ribavirin is not a specific antiviral agent and is teratogenic while prophylaxis with Synagis™ is expensive and requires a series of monthly injections in advance of the winter season when RSV is more prevalent.

In an effort to address the unmet need for an orally active and effective inhibitor of RSV, we have examined a series of 1,2-disubstituted benzimidazole derivatives that are potent inhibitors of RSV fusion and for which the basic structure–activity relationships (SAR) have been defined.[9–15] These inhibitors have been shown to bind to a conserved pocket in the RSV F protein amino terminus heptad repeat that assembles during the initial stages of the-fusion process and are postulated to interfere with the formation and/or stability of the F protein 6-helix bundle that is observed in the post-fusion state.[15] Optimization of this series of antiviral agents using in vitro assays to assess cell permeability and metabolic stability resulted in the identification of several analogs that displayed acceptable metabolic stability in human liver microsomes and adequate CaCo-2 cell permeability while maintaining excellent antiviral potency. Emerging from that work, BMS-433771 (**1**) was identified as a potential development compound that demonstrated antiviral activity in both the BALB/c and cotton rat models of RSV infection following oral dosing.[16,17] As part of the overall

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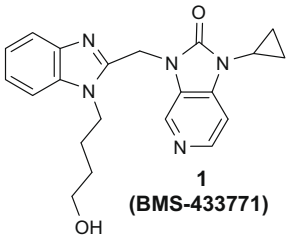
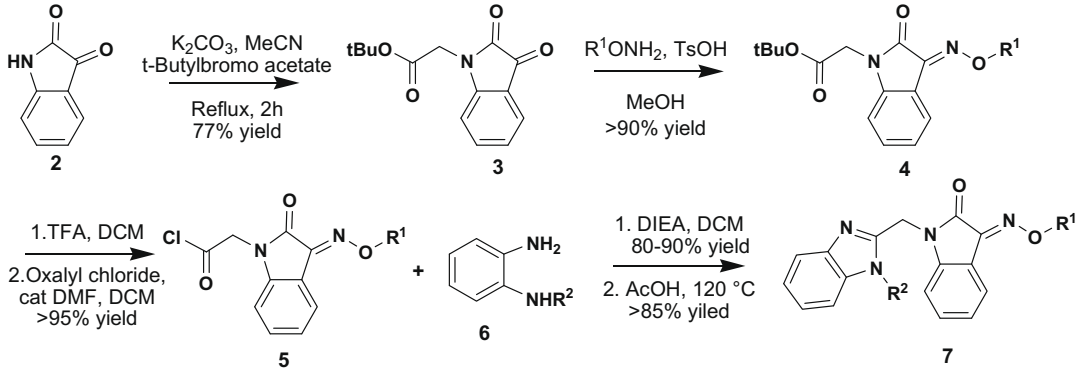
survey of this class of RSV fusion inhibitor, we examined a series of isatin oxime derivatives that were envisioned as bioisosteric substitutes for the bezimidazol-2-one moiety found in BMS-433771 (**1**).[12] In this communication, we describe the structure–activity relationships associated with this template in which the oxime moiety offers a convenient structural moiety for probing an element of the pharmacophore which had proven to be of considerable importance in the benzimidazol-2-one series.

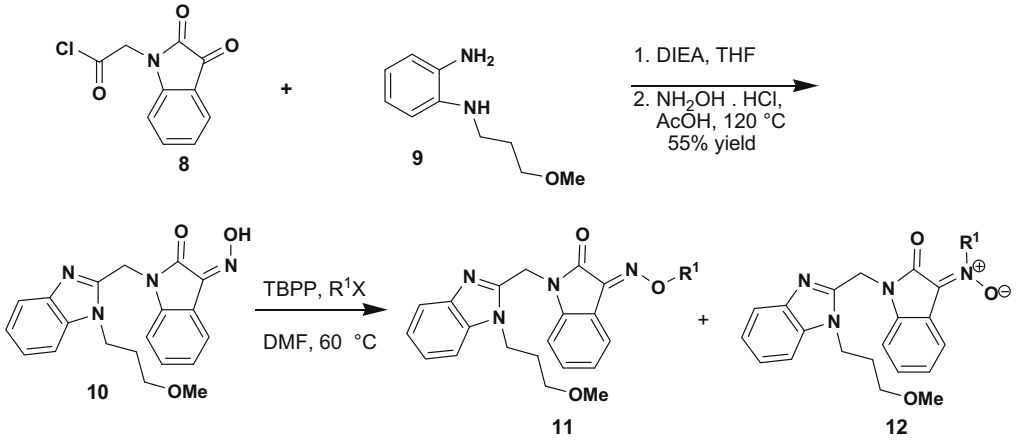


The preparation of benzimidazole isatin oxime derivatives was accomplished according to the methods outlined in Schemes 1 and 2. These two synthetic routes were developed in order to take advantage of the versatility and convenience of introducing side chains to either the benzimidazole or the isatin oxime moiety. Both methods employed *tert*-butyl 2-(2,3-dioxoindolin-1-yl)acetate (**3**) as the common intermediate, obtained in 77% yield from isatin **2** by alkylation with *tert*-butyl bromoacetate in the present of K_2CO_3, as summarized in Scheme 1. Reaction of **3** with an-O-substituted hydroxyl amine in the presence of *p*-toluenesulfonic acid in MeOH provided oximes **4** typically in >90% yield. Acid-catalyzed removal of the *tert*-butyl ester of **4** followed by reaction of the carboxylic acid with oxalyl chloride gave acid chlorides **5** in good yield. Coupling of **5** to substituted phenylenediamines **6** followed by heating in acetic acid at reflux yielded the benzimidazole isatin oximes **7**.

Following the identification of 3-methoxypropane as a preferred benzimidiazole side chain, an alternative synthetic approach that relies upon alkylation of the isatin oxime **10** as a convenient penultimate intermediate was examined as a means of introducing substituents under a parallel synthesis protocol (Scheme 2). Coupling of acid chloride **8**, obtained from **3** following the same protocol used for the preparation of acid chlorides **5**, with the substituted phenylenediamine **9** followed by cyclization of the amide in acetic acid in the presence of hydroxylamine gave **10** in 55% overall yield. Alkylation of the sodium salt derived from oxime **10** is potentially a complex process that can afford a mixture of products depending upon whether alkylation occurs at oxygen or nitrogen.[18–20] In the course of preparing a library of O-alkylated oximes, it was found that alkylation of **10** with alkyl halides led primarily to the formation of the desired oxime ether **11,** with only small amounts of nitrone, which were not isolated during purification of the oxime targets. However, in three individual cases where



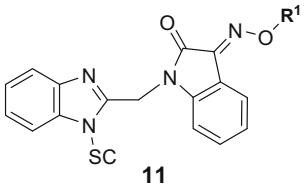
Scheme 1.



Scheme 2.

simple secondary alkyl halides were used as the electrophiles, it was found that both the O- and N-alkylated products were formed in approximately equal quantity and the two products were readily separated by column chromatography. The isatin oxime ethers **11** and nitrones **12** that were prepared as part of this survey are compiled in Tables 1 and 2, respectively.

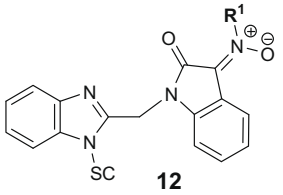
Table 1
Structure, RSV inhibitory activity and cytotoxicity associated with isatin-oxime ether derivatives **11**



11

#	Side chain (SC)	R^1	EC_{50} (μM)	CC_{50} (μM)	Profiling data
11a	$-(CH_2)_2CH(Me)_2$	H	0.010 (0.014, 0.006)	7.46 (12.7, 2.20)	
11b	$-(CH_2)_2CH(Me)_2$	Me	0.087 (0.108, 0.065)	20.6 (11.0, 30.1)	
11c	$-(CH_2)_4OH$	Me	0.010 (0.010, 0.010)	190.8 (145.5, 236.1)	
11d	$-(CH_2)_4OAc$	Me	0.030 (0.019, 0.042)	124.5 (119.9, 129.1)	HLM $T_{1/2}$ = 1.5 min
11e	$-(CH_2)_3OMe$	Me	0.030 (0.030, 0.030)	34.8 (23.1, 46.5)	HLM $T_{1/2}$ = 16 min Caco-2 Pc = 188 nm/s
11f	$-(CH_2)_3OMe$	Et	0.059 (0.030, 0.088)	21.9 (2.2, 41.4)	
11g	$-(CH_2)_3OMe$	$-CH_2CH_2F$	0.071 (0.050, 0.091)	10.7 (10.2, 11.6)	
11h	$-(CH_2)_3OMe$	$-CH_2CF_3$	2.74 (5.17, 0.309)	133.6 (224.0, 43.3)	HLM $T_{1/2}$ = 3.2 min Caco-2 Pc = 652 nm/s
11i	$-(CH_2)_3CN$	$-CH_2CF_3$	0.066 (0.091, 0.040)	126.6 (226.5, 46.6)	HLM $T_{1/2}$ = 18 min
11j	$-(CH_2)_3OMe$	*i*-Pr	0.257 (0.182, 0.331)	56.9 (28.9, 84.9)	
11k	$-(CH_2)_4OH$	*t*Bu	1.130 (1.330, 0.921)	237.8 (237.8, 237.8)	
11l	$-(CH_2)_3OMe$	$-CH(CH_2CH_3)_2$	0.935 (0.933, 0.936)	20.3 (12.8, 26.8)	
11m	$-(CH_2)_3OMe$	$-CH_2cPr$	0.096 (0.116, 0.076)	61.8 (100.0, 23.5)	
11n	$-(CH_2)_3OMe$	$-CH_2cBu$	0.246 (0.387, 0.098)	29.2 (23.6, 34.8)	
11o	$-(CH_2)_3OMe$	–4-Tetrahydro-2*H*-pyran	0.206 (0.149, 0.264)	100.0 (100.0, 100.0)	
11p	$-(CH_2)_3OMe$	–Cyclo-hexyl	0.652 (0.512, 0.792)	71.9 (66.1, 77.6)	
11q	$-(CH_2)_3OMe$	$-CH_2$-1-tetrahydrofuran	0.152 (0.128, 0.175)	35.0 (34.5, 35.4)	
11r	$-(CH_2)_3OMe$	$-(CH_2)_2cHex$	0.384 (0.200, 0.567)	40.5 (26.8, 54.2)	
11s	$-(CH_2)_3OMe$	*n*-Pr	0.147 (0.110, 0.183)	29.9 (29.8, 30.1)	
11t	$-(CH_2)_3OMe$	$-CH_2CH_2{\cdot}CH_2F$	0.125 (0.118, 0.153)	70.6 (43.2, 98.1)	
11u	$-(CH_2)_3OMe$	*n*-Bu	0.261 (0.120, 0.391)	29.5 (24.3, 34.6)	
11v	$-(CH_2)_3OMe$	*n*-Pentyl	0.188 (0.166, 0.029)	54.0 (20.5, 87.4)	
11w	$-(CH_2)_3OMe$	$-CH_2CH{=}CH_2$	0.428 (0.039, 0.466)	100.0 (100.0, 100.0)	
11x	$-(CH_2)_3OMe$	$-(CH_2)_2CH{=}CH_2$	0.260 (0.167, 0.353)	25.5 (17.2, 33.9)	
11y	$-(CH_2)_3OMe$	$-(CH_2)_3CH{=}CH_2$	0.192 (0.116, 0.267)	45.8 (41.7, 49.9)	
11z	$-(CH_2)_3OMe$	$-(CH_2)_3C{\equiv}CH$	0.064 (0.048, 0.080)	67.1 (34.1, 100.0)	
11aa	$-(CH_2)_3OMe$	$-(CH_2)_3CN$	0.079 (0.068, 0.090)	100.0 (100.0, 100.0)	
11ab	$-(CH_2)_3OMe$	$-CH_2CH(OH)CH_2CN$	0.065 (0.036, 0.094)	66.0 (44.2, 87.9)	
11ac	$-(CH_2)_3OMe$	$-(CH_2)_4OAc$	0.062 (0.033, 0.090)	57.3 (48.2, 66.5)	
11ad	$-(CH_2)_3OMe$	$-CH_2CONEt_2$	0.026 (0.021, 0.030)	40.7 (29.8, 51.6)	
11ae	$-(CH_2)_3OMe$	$-CH_2CONH_2$	0.154 (0.123, 0.185)	15.6 (28.2, 2.9)	HLM $T_{1/2}$ = 21 min Caco-2 Pc = 15 nm/s
11af	$-(CH_2)_3CN$	$-CH_2CONH_2$	0.045 (0.024, 0.068)	126.9 (194.3, 79.5)	Caco-2 Pc = 30 nm/s
11ag	$-(CH_2)_3OMe$	$-(CH_2)_2NMe_2$	0.370 (0.347, 0.393)	74.0 (57.5, 90.5)	
11ah	$-(CH_2)_3OMe$	$-(CH_2)_3NMe_2$	0.240 (0.159, 0.321)	29.8 (28.7, 30.9)	
11ai	$-(CH_2)_3OMe$	$-(CH_2)_2$piperidine	0.682 (1.078, 0.286)	28.2 (21.0, 35.4)	
11aj	$-(CH_2)_3OMe$	$-(CH_2)_3$piperidine	0.290 (0.275, 0.305)	17.0 (29.4, 4.6)	
11ak	$-(CH_2)_3OMe$	$-CH_2$-2-pyridine	0.238 (0.168, 0.308)	100.0 (100.0, 100.0)	
11al	$-(CH_2)_3OMe$	$-CH_2$-3-pyridine	0.141 (0.198, 0.085)	74.2 (52.7, 95.7)	
11am	$-(CH_2)_3OMe$	$-CH_2$-4-pyridine	0.242 ± 0.145 (*n* = 3)	23.9 ± 25.8 (*n* = 3)	HLM $T_{1/2}$ = 0.6 min Caco-2 Pc = 214 nm/s
11ao	$-(CH_2)_3OMe$	$-CH_2CO_2H$	0.295 (0.173, 0.418)	100.0 (100.0, 100.0)	
11an	$-(CH_2)_3OMe$	$-(CH_2)SO_3H$	0.046 (0.016, 0.076)	84.1 (68.3, 100.0)	
11ap	$-(CH_2)_3OMe$	$-CH(CO_2H)CH_2CO_2H$	0.019 (0.017, 0.020)	25.0 (21.4, 28.5)	
11aq	$-(CH_2)_2CHMe_2$	Ph	0.375 (0.346, 0.404)	32.9 (19.2, 46.7)	
11ar	$-(CH_2)_2CHMe_2$	4′-Ph-Br	0.705 (1.075, 0.335)	9.6 (15.6, 3.7)	
11as	$-(CH_2)_2CHMe_2$	$-CH_2$-Ph	0.202 (0.195, 0.211)	27.1 (26.3, 27.9)	
11at	$-(CH_2)_2CHMe_2$	$-CH_2$-Ph-4-CO_2H	0.016 (0.018, 0.013)	137.8 (201.4, 74.2)	
11au	$-(CH_2)_2NMe_2$	$-CH_2$-4′-Ph-CO_2H	0.0165 (0.014, 0.019)	36.2 (31.4, 41.0)	
11av	$-(CH_2)_3OMe$	$-CH_2$-4′-Ph-CO_2Me	0.050 (0.027, 0.072)	21.3 (38.3, 4.3)	
11aw	$-(CH_2)_2CHMe_2$	$-CH_2$-4′-Ph-CO_2Me	0.023 (0.014, 0.032)	15.4 (11.7, 19.2)	HLM $T_{1/2}$ = 0.6 min
11ax	$-(CH_2)_2NMe_2$	$-CH_2$-4′-Ph-CO_2Me	0.023 ± 0.012 (*n* = 3)	29.1 ± 17.1 (*n* = 3)	HLM $T_{1/2}$ = 0.6 min
11ay	$-(CH_2)_2NSO_2Me$	$-CH_2$-4′-Ph-CO_2Me	0.051 (0.023, 0.079)	34.6 (22.5, 46.7)	
11az	$-(CH_2)_3OMe$	$-CH_2$-4′-Ph-$CONMe_2$	0.030 (0.012, 0.047)	53.6 (31.4, 75.8)	Caco-2 Pc = 237 nm/s
11ba	$-(CH_2)_2CHMe_2$	$-CH_2$-4′-Ph-$CONMe_2$	0.009 (0.008, 0.009)	16.4 (26.3, 6.4)	
11bc	$-(CH_2)_4OH$	$-CH_2$-4′-Ph-SO_2Me	0.012 (0.013, 0.010)	8.1 (1.6, 14.4)	HLM $T_{1/2}$ = 13 min Caco-2 Pc = 152 nm/s
11bb	$-(CH_2)_4OAc$	$-CH_2$-4′-Ph-SO_2Me	0.005 (0.003, 0.007)	115.6 (57.1, 174.0)	

Table 2
Structure, RSV inhibitory activity and cytotoxicity associated with nitrone derivatives **12**



#	Side chain (SC)	R^1	EC_{50} (μM)	CC_{50} (μM)
12j	$-(CH_2)_3OMe$	*i*-Pr	0.230 (0.287, 0.172)	68.7 (100.0, 37.4)
12l	$-(CH_2)_3OMe$	$-CH(CH_2CH_3)_2$	0.898 (0.305, 1.49)	105.7 (100.0, 111.4)
12p	$-(CH_2)_3OMe$	–cHex	0.285 (0.168, 0.403)	100.0 (100.0, 100.0)

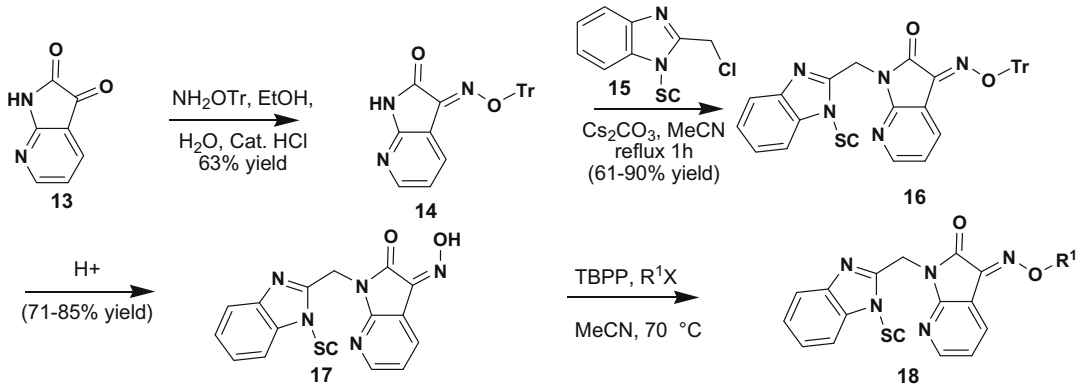
In analogy with the earlier work, a series aza-isatin oxime analogs **18** were synthesized following the route outlined Scheme 3, an approach that represents a variation of the procedures used to prepare the benzimidazol-2-one series that led to the identification of BMS-433771.[12] Acid-catalyzed condensation of *O*-trityl hydroxylamine with 7-aza-isatin (**13**) in aqueous EtOH afforded the oxime **14** in 63% yield.[21] Alkylation of **14** with a 2-chloromethy-benzimidazole derivative **15** incorporating the appropriate side chain provided the trityl-protected oximes **16** in 61–90% yield. The trityl protecting group was removed under acidic conditions to provide oximes **17** which were subjected to solution phase alkylation using a solid support-bound phosphazene base in CH_3CN at 70 °C in a parallel synthesis mode, to provide the series of O-alkylated oximes **18**. The compounds that constitute this facet of the study are compiled in Table 3.

In the present study, we selected the isatin core as a potential bioisostere of the benzimidazol-2-one heterocycle that had proven to be a suitable vehicle for drug discovery in the earlier series.[10–13] The isatin oxime ethers **11** and **18** present a silhouette similar to that of the N-alkylated benzimidazol-2-ones derivatives, the oxime ether substituent exploring a similar vector to the benzimidazolone N-substituent but extending planarity from the heterocyclic nucleus.[10–13] The initial SAR survey within this isatin oxime ether series was directed toward optimization of antiviral activity, cell permeability and metabolic stability in HLM. By selecting benzimidaozle side chains known to be optimal from the earlier work, effort was most effectively focused initially on optimization of the oxime substituent.[9–15]

The SAR evident from the data presented in Table 1 correlate quite well with that established for the benzimidazol-2-one series although the isatin oximes **11** generally exhibit slightly improved potency. Small oxime substituents such as methyl, ethyl, and fluoroethyl, as found in **11b–11g**, are preferred for potent antiviral activity, with these three compounds demonstrating EC_{50}s of less than 100 nM. Although the parent oxime **11a** reveals that substitution of the oxime oxygen is not essential for potent antiviral activity, it is critical for good cell permeability (vide infra), analogous to findings with the earlier series.[9–15] Compounds **11j–11s** demonstrate that branching at the α or β-position of the side chain in the context of simple alkyl or cycloalkyl substituents generally confers reduced potency with the exception of the cyclopropylmethyl analogue **11m**. Extension of the simple alkyl chain up to five carbon atoms resulted in 2–7-fold reduced potency compared to **11f**, as exemplified by examples **11t–11y**. Compounds **11z** and **11aa**, which examine the effect of higher levels of unsaturation at the chain termini, show improved antiviral activity with cell culture potency EC_{50}s below 100 nM. Polarity in the oxime ether substituent is quite well tolerated, as exemplified by **11ab** which incorporates a polar hydroxyl moiety as a branching element at the β-position in conjunction with a nitrile capping moiety and demonstrates reasonable potency, EC_{50} = 65 nM. This aspect of the SAR was probed further by evaluating the series of amides, esters, amines and sulfones represented by **11ac–11bb**. Polarity can be incorporated at the terminus of the side chain providing that these moieties are either neutral or acidic since the overtly and mildly basic amines **11ag–11am** are associated with reduced antiviral activity. Amongst the neutral polar groups, acetate (**11ac**) and amide (**11ad–11af**) offered good potency with the exception of the primary amide **11ae**. However, this result appears to depend on the identity of the benzimidazole side chain since the butyronitrile analogue **11af** is threefold more potent. Acidic ether chain termini generally afforded potent antiviral agents, reflecting the SAR established in the benzimidazol-2-one series. The introduction of a phenyl spacer between the oxime and the carboxylic acid moiety generally affords potent antiviral agents, exemplified by **11at–au**, improving the potency of the underlying benzyl element **11as**. This extends to other polar, neutral substituents incorporated at the 4-position of the aryl ring, represented by compounds **11av–bb**, which demonstrate excellent in vitro antiviral properties.

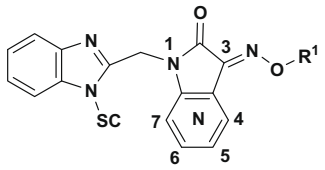
The three nitrones **12j**, **12l** and **12p** that were evaluated exhibit modest antiviral activity that is comparable to the corresponding oximes, as summarized in Table 2. However, the nitrone **12p** is an exception, being almost fourfold more active than its oxime counterpart **11p**.

The criteria established to identify compounds suitable for further study included a half life ($T_{1/2}$) in human liver microsomes of >30 min, predictive of moderate clearance in humans, and permeability across a confluent layer of Caco-2 cells of >100 nm/s, indicative of good intestinal absorption.[12] A select series of compounds



Scheme 3.

Table 3
Structure, RSV inhibitory activity and cytotoxicity associated and PK data of aza-isatin oxime derivatives **18**



18

#	Aza position	SC	R^1	EC_{50} (μM)	CC_{50} (μM)	HLM $T_{1/2}$ (min)	CaCo-2 Pc (nm/s)
18a	6	$-(CH_2)_4-F$	–H	0.0179 (0.0298, 0.0060)	97.8 (140.4, 55.1)	100	27
18b	7	$-(CH_2)_3-CN$	–H	0.0227 (0.0303, 0.0151)	159.0 (187.8, 120.3)	100	42
18c	7	$-(CH_2)_4-OAc$	–H	0.0497 (0.0267, 0.0727)	245.4 (245.4, 245.4)	1.3	12
18d	6	$-(CH_2)_4-F$	$-CH_3$	0.106 (0.1726, 0.0397)	16.4 (15.1, 17.7)	26	128
18e	6	$-(CH_2)_4-OH$	$-CH_3$	0.0309 (0.0321, 0.0297)	223.4 (240.5, 206.3)	84	98
18f	7	$-(CH_2)_3-CN$	$-CH_3$	0.0928 (0.0408, 0.1449)	172.0 (204.7, 129.2)	28	160
18g	7	$-(CH_2)_4-OH$	$-CH_3$	0.0312 (0.0248, 0.0377)	100.2 (117.7, 82.8)	31	141
18h	6	$-(CH_2)_4-F$	$-CH_2CH_2F$	0.0619 (0.0612, 0.0624)	12.4 (12.0, 12.7)	38	158
18i	6	$-(CH_2)_4-OH$	$-CH_2CH_2F$	0.0542 (0.0240, 0.0844)	65.0 (63.7, 66.3)	100	53
18j	7	$-(CH_2)_4-OH$	$-CH_2CH_2F$	0.0431 (0.0334, 0.0528)	123.5 (176.3, 70.64)	44	115
18k	7	$-(CH_2)_4-OAc$	$-CH_2CH_2F$	0.0546 (0.0533, 0.0558)	89.4 (44.1, 89.4)	ND	ND
18l	7	$-(CH_2)_3-CN$	$-CH_2CH_2F$	0.0573 (0.0349, 0.0797)	165.1 (150.0, 180.2)	55	155
18m	6	$-(CH_2)_3-SO_2Me$	$-CH_2CH_2F$	0.0125 (0.0176, 0.0074)	217.4 (217.6, 217.4)	>100	10
18n	7	$-(CH_2)_3-SO_2Me$	$-CH_2CH_2F$	0.0292 (0.0230, 0.0354)	125.3 (217.6, 52.9)	>100	182
18o	7	$-(CH_2)_4-OH$	$-CH_2CF_3$	0.0798 (0.0568, 0.1028)	156.6 (178.1, 125.06)	65	154
18p	7	$-(CH_2)_3-CN$	$-CH_2CF_3$	0.112 (0.0537, 0.1719)	96.8 (197.7, 12.8)	48	180
18q	6	$-(CH_2)_3-SO_2Me$	$-CH_2CH{=}CH_2$	0.0124 (0.0117, 0.0120)	217.4 (220.5, 214.4)	ND[a]	ND[a]
18r	6	$-(CH_2)_3-SO_2Me$	$-CH_2$-2-pyridine	0.0290 (0.0188, 0.0392)	103.8 (151.0, 56.6)	21	16
18s	7	$-(CH_2)_4-OH$	$-CH_2$-4′-Ph-SO_2CH_3	0.0073 (0.0112, 0.0033)	85.9 (121.8, 50.1)	5.3	71
18t	5	$-(CH_2)_3-SO_2Me$	$-CH_2$-4′-Ph-SO_2CH_3	10.596 (1.192, 20.000)	34.4 (26.9, 41.9)	200	ND
18u	5	$-(CH_2)_3-CN$	$-CH_2$-4′-Ph-SO_2CH_3	10.182 (1.543, 18.822)	126.8 (64.5, 189.2)	29	ND

[a] ND = Not determined.

presented in Table 1 that met the targeted antiviral activity of an EC_{50} of <100 nM were screened in one or both of these in vitro assays as a prelude to selecting candidates for pharmacokinetic profiling in the rat. As can be appreciated from an analysis of the data presented in Table 1, those analogs incorporating lipophilic side chains demonstrated good permeability across Caco-2 cells. For example, the permeability coefficients (Pc) for compounds **11c**, **11h**, **11am**, **11az** and **11bc** range from 152 to 652 nm/s. As might be anticipated and in line with earlier studies, analogs with polar side chains, exemplified by **11ae** and **11af,** showed much reduced Caco-2 cell permeability, with Pc = 15 and 30 nm/s, respectively. However, the metabolic stability determined for compounds **11d**, **11e**, **11h**, **11i**, **11ae**, **11am**, **11aw**, **11ax** and **11bc** in the HLM preparation precluded advancement of these compounds into rat pharmacokinetic studies. Most of these compounds were consumed within minutes, with only **11i** and **11ac** approaching the targeted half life, with $T_{1/2}$ = 18 and 21 min, respectively.

Consequently, attention was focused on the series of azaisatin oximes presented in Table 3, designed after the success of this tactical approach in the benzimidazol-2-one series. This structural modification presented as an opportunity to address the metabolic liability that could arise from ring hydroxylation of the isatin phenyl ring system by CYP 450 enzymes whilst concomitantly improving the water solubility of these RSV inhibitors. In the benzimidazol-2-one series studied earlier, this approach provided increased HLM stability, improving half life from a few minutes with benzimidazol-2-ones to greater than 30 min in the aza homologues.[12] Since that work established that the 6 and 7-aza benzimidazol-2-one series was associated with significantly greater antiviral potency than the 4 and 5-aza isomers, this topology became the focus of aza-isatin analogues.[12] The five bendazimidazole side chains employed in this survey, 4-fluorobutane, 4-butyronitrile, 4-acetoxy butane, 4-butanol, and 4-(methylsulfonyl)propane, were selected based on the properties established in the previous study.[12] The unsubstituted oximes **18a–c** combine potent antiviral activity, EC_{50}s ranging from 18 to 50 nM, with excellent HLM stability, particularly for **18a** and **18b** with $T_{1/2}$ = 100 min. However, all 3 compounds suffer from low Caco-2 cell permeability, ranging from 12 to 42 nm/s, presumably a function of the polarity associated with the presence of the oxime OH. Masking this moiety with a methyl group provided a series of compounds **18d–g** that exhibit good to acceptable antiviral potency, EC_{50}s = 31–106 nM, whilst affording excellent cell permeability and preserving targeted HLM stability. Compounds **18h–q** incorporate more lipophilic fluoroethyl, trifluoroethyl, and propene side chains, respectively, and all compounds in this series, with the exception of **18p**, show acceptable antiviral activity with EC_{50}s ranging from 13 to 80 nM. These derivates also demonstrate enhanced half lives in HLM and excellent Caco-2 cell permeability, although the sulfone **18m** is an outlier in the latter assay, presumably a function of the polarity of this structural element in conjunction with the polarity of the 6-azaisatin.[22] The two isomeric azaisatins **18m** and **18n** provide for an interesting comparison with both compounds demonstrating potent antiviral activity and excellent metabolic stability. However, whilst the 7-aza compound **18n** demonstrates good CaCo-2 cell permeability, Pc = 182 nm/s, the 6-aza isomer **18m** is essentially impermeable, with a Pc of 10 nm/s. This trend extends to the other matched pairs represented by the 7-aza-isatin oximes **18g** and **18j** and the 6-aza-isatin oxime isomers **18e** and **18i**, respectively, although it is less severe. This phenomenon is presumably a function of the polarity differences between the 6-aza- and 7-aza-isatin series, attributable to the more exposed ring N atom in the 6-aza isomer.

Table 4
Biological data comparison of **18n** and BMS-433771

Biological data		Compound **18n**	BMS-433771
Virology	EC_{50} (nM)	30	10
	CC_{50} (nM)	135	>218
In vitro profiling	HLM (min)	100	34
	CaCo-2 (nm/sec)	182	143
	CYP 450 (μM)	CYP 2C9 = 20, 2C19 = 18; All other >40	All CYP >28
Rat in vivo PK profile (IV 1 mg/kg; PO 5 mg/kg)	%F	14	13
	Oral Cmax (ng/mL)	14	187
	CLtot (mL/min/kg)	130	61
	Vss (L/kg)	1.8	0.57
	Terminal $T_{1/2}$ (min)	12	18
In vivo antiviral activity in the mouse, 50 mg/kg PO	Δ TCID50 (log viral titer drop)	1.05	1.0
	RT-PCR (fold reduction)	45	7.5

Three compounds containing the fluoroethyl oxime substituent, the alcohols **18i** and **18j** and sulfone **18n**, emerged as optimally combining potent antiviral activity with good CaCo-2 cell permeability and metabolic stability in HLM that are predictive of good pharmacokinetic properties in vivo. Consequently, these compounds were selected for evaluation in the BALB/c mouse model of RSV infection. After oral administration of single doses of 50 mg/kg 1 h prior to inoculation with virus a dosing regimen established previously,[17] compounds **18i**, **18j** and **18n** reduced viral titers in the lungs of these mice by 0.68, 0.62 and 1.05 $\log_{10}$, respectively. Additional profiling data, summarized in Table 4, revealed that **18n** exhibited pharmacokinetic properties in the rat comparable to BMS-433771 (**1**) although **18n** is a slightly inferior antiviral.

In summary we have explored a series of isatin oxime derivatives as an isostere of the benzimidazol-2-one moiety found in a family of potent inhibitors of RSV active in cell culture assays. These isatin oxime derivatives represent an evolution of the class of small molecule RSV fusion inhibitors represented by BMS-433771 (**1**), a compound with oral bioavailability in 4 species and antiviral activity in 2 animal models of RSV infection. The strategy of balancing lipophilicity and polarity in the oxime substituent and isatin heterocycle with that of the benizimidazole side chain produced a series of compounds with potent antiviral activity in cell culture that combined good metabolic stability in vitro with high cell membrane permeability. This investigation led to the identification the azaisatin oxime **18n** as a compound demonstrating antiviral activity in the BALB/c mouse model of RSV infection after oral dosing.

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