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Accelerated Discovery of Potent Fusion Inhibitors for Respiratory Syncytial Virus

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[‖]Author Contributions

N.P. and T.M.K. contributed equally to this study.

The authors declare no competing financial interest.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsinfecdis.9b00524.

Complete description of the experimental conditions and characterization data of all of the compounds described in the manuscript; detailed description of the *in vitro* biological assessments (PDF)

Detailed data of the compounds (TXT)

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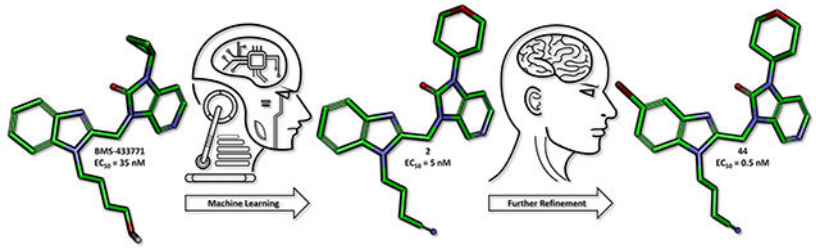
Abstract

A series of five benzimidazole-based compounds were identified using a machine learning algorithm as potential inhibitors of the respiratory syncytial virus (RSV) fusion protein. These compounds were synthesized, and compound **2** in particular exhibited excellent *in vitro* potency with an EC_{50} value of 5 nM. This new scaffold was then further refined leading to the identification of compound **44**, which exhibited a 10-fold improvement in activity with an EC_{50} value of 0.5 nM.

Graphical Abstract



Keywords

RSV; fusion inhibitor; benzimidazole; machine learning

Respiratory syncytial virus (RSV), an orthopneumovirus, has remained the global leading cause of lower respiratory tract infections in vulnerable patient populations since its discovery in 1956.[1,2] It accounts for 68% of acute respiratory infections in infants during their first viral season and infects nearly all children by 2–3 years of age,[3] 40% of whom will develop a secondary respiratory infection.[4] In 2015 alone, RSV-related acute lower respiratory infections resulted in the hospitalization of ~3.2 million children worldwide and 66 000 deaths.[5,6] RSV infection of the small airways of the lungs (bronchiolitis) in children has been associated with a higher risk of developing asthma, but a direct causal link has yet to be established.[7] RSV infection is also prevalent among the elderly population, where it is often misdiagnosed as influenza, and among immunocompromised patients[8] as well those with cardiopulmonary diseases.[9,10] Emerging epidemiological evidence has suggested that RSV has a morbidity burden in older adults similar to that of nonpandemic influenza.[11,12] Unfortunately, despite its widespread occurrence, there are currently no direct-acting therapeutics for RSV and treatment is often limited to palliative care.[13] A prophylactic agent, palivizumab (Synagis), is available for high-risk infants, but its use is typically limited to the developed world due to its cost.[14–16] There is therefore a major unmet medical need

for safe and effective therapeutics, both for treating active RSV infections and for prophylaxis.

In recent years, attempts to combat RSV infection have mostly focused on the inhibition of viral polymerase activity or membrane fusion. A number of anti-RSV agents targeting the F protein in particular, the protein responsible for mediating membrane fusion,[17] have been reported in the literature, some of which have advanced into clinical trials (Figure 1).[3,16,18–22] This provided us with a promising opportunity to employ our machine learning algorithm in the form of Naive Bayes Networks (NBNs) to scan the known structure activity relationship (SAR) information and design new, potent RSV fusion protein inhibitors, an approach that has served us well in the past.[23–25]

METHODS

Our machine learning approach focused on a series of RSV fusion inhibitors developed by Bristol-Myers Squibb (BMS) that resulted in the orally bioavailable clinical candidate BMS-433771 with nanomolar potency (Figure 1).[18,26] The development of BMS-433771 was halted due to a change in the strategic focus of the company, which left this series available for further exploration.[18]

With the chemical space and biological target selected, we created a machine learning algorithm designed to predict highly active compounds. We employed the ChEMBL database as our source of information[27] as well as the patent literature.[28] We selected an NBN approach, as this algorithm is highly tolerant of any incidental noise in the data, is extremely fast to generate, and in our experience is good at predicting potencies.[29] Additionally, we used PipelinePilot as our platform to perform both our informatics processing and machine learning. Having obtained a set of known RSV fusion inhibitors with associated EC_{50} values, we split the data into an inactive class ($EC_{50} > 30$ nM) and an active class ($EC_{50} \leq 30$ nM). For our independent variable, we employed extended connectivity fingerprints, which are quick to compute and are often utilized for machine learning.[23,24] We used a 70–30 split to perform training and testing and used the leave-one-out cross validation method as a training control. Using the 30% hidden data as a test of our classification algorithm, we found that a test set receiver operating characteristics area under the curve (ROC AUC) of 0.929 and the algorithm had a perfect enriching capability at the top 5% of predicted values (all of the top 5% structures were active).

With a highly predictive algorithm in hand, we set out to explore the chemical space of several families of fusion inhibitors known in the literature. These structurally diverse core scaffolds (Figure 2), which had all been advanced to late stage development, were enumerated in PipelinePilot using the ZINC15 database of commercially available building blocks.[30] This data set was then prioritized by our NBN and subsequently screened using several cheminformatics filters to generate a prioritized virtual library (>115 000 analogs). These filters removed all non-novel structures and structures containing reactive functional groups. It also included a Lipinski Rule of 5 filter and a cLogD < 1.8 filter (the evaluation of the BMS series showed a decrease in metabolic stability in human liver microsomes for

compounds with cLogD > 1.8).[23,24,26,31,32] The resulting top 50 list of compounds was further refined on the basis of their synthetic tractability.

Using this workflow, we identified molecules **1–3** (Figure 3), which met all the criteria as candidates for synthesis. To validate the utility of the cLogD <1.8 filter, we also identified compounds **4** and **5**, which were predicted to be potent but were removed by the cLogD filter.

The synthesis of compounds **1–3** is shown in Scheme 1. Th nucleophilic aromatic substitution (S_NAr) reaction of commercially available 4-chloro-3-nitropyridine or 4-methoxy-3-nitropyridine (**6**) with the requisite amines produced substituted pyridines **7–9**. The reduction of nitro groups of each using classical hydrogenation conditions led to diaminopyridines **10–12**, which were subsequently cyclized with carbonyl diimidazole (CDI)to yield the desired substituted imidazopyridine-2-ones **13–15**. Each substituted imidazopyridin-2-one was then alkylated with the requisite chloromethyl benzimidazole **20** and **21** (*vide infra*) to afford the desired targets, **1** and **2**, as well as precursor **16**. Subsequent removal of the *tert*-butyldimethylsilyl (TBS) protecting group of **16** with tetrabutylammonium flouride (TBAF) afforded target compound **3**.

The synthesis of **4** and **5** commenced with the generation of the benzimidazole-2-methanol intermediates **18** and **19** (Scheme 2) from commercially available 1*H*-benzimidazole-2-methanol **17**. Subsequent chlorination resulted in the production of multigram quantities of **20** and **21**, which were then individually coupled with commercially available spirocyclopropyl pyrrolopyridinone **11** to produce **4** and **22**, respectively. The latter was subsequently deprotected to yield **5**.

Once in hand, target compounds **1–5** were evaluated in a whole-cell (Hep-2 or BEAS-2B) RSV replication assay using recombinant RSV strain A2-L19F expressing renilla luciferase or nano luciferase as additional transcription units, respectively.[33] In parallel, all candidates were tested against recombinant RSV-A2-L19F$_{D489E}$, carrying a previously described point mutation in the F protein that provides pan-resistance to all current RSV entry inhibitor classes.[33] The resulting antiviral activities are listed in Tables 1 and S1. Most candidates identified by our machine learning algorithm were found to exhibit antiviral activities that were comparable to BMS-433771. Our best compound **2** exhibited an EC_{50} value of 5 nM, an approximately 7-fold improvement in potency compared to BMS-433771. Moreover, all our compounds possessed CC_{50} values greater than 300 μM, indicating good to excellent therapeutic indices for all. When the compounds were incubated with human liver microsomes (HLMs), they all exhibited $t_{1/2}$ > 30 min (see Table 1). Compounds **1** and **3** exhibited the greatest stability with $t_{1/2}$ values of >2 h, while BMS-433771 had a $t_{1/2}$ of 64 min. Our most potent compound **2** exhibited a $t_{1/2}$ of 84 min. We note that compounds **4** and **5** had poorer metabolic stability than compounds **1–3**, consistent with our hypothesis that compounds with cLogD > 1.8 produce unfavorable metabolic stability.

These results successfully demonstrated the utility of employing machine learning to efficiently identify a series of novel and potent inhibitors of RSV fusion.

While we were performing our studies, Roymans et al. disclosed the structures and binding modes of a series of RSV fusion inhibitors with structural similarity to BMS-433771 (JNJ-53718678 and JNJ-49153390, shown in Figure 1) but with significantly improved antiviral activities.[34] In this work, strategic incorporation of a chlorine or bromine atom facilitated a halogen bonding interaction with the backbone of Thr397 and is the likely reason for the improved potency.[34] We envisaged that the binding affinity of our compounds for the F protein could similarly be improved with the introduction of a halogen at the 5-position on the benzimidazole portion of our compounds. To validate this, we generated another small library of analogues based on compound **2**, now featuring a halogen at the 5-position on the benzimidazole core.

The synthesis of these compounds started with the alkylation of commercially available 4-bromo-, 4-chloro-, or 4-fluoro-2-nitroanilines **23–25** to give intermediates **26–31** (Scheme 3). This was followed by the reduction of the nitro group in the presence of iron with ultrasonic irradiation or hydrogenation using Pt/C to give **32–37** and a ring-closing reaction with 2-chloro-1,1,1-trimethoxy-ethane to yield the desired benzimidazole precursors **38–43**. These were subsequently coupled with substituted imidazopyridine-2-ones **14** or **15** (the synthesis of which is described in Scheme 1) to give the desired products **44–49** and precursor **50**. A final TBS deprotection reaction of **50** using HF-pyridine produced compound **51**.

The antiviral activities of compounds **44–49** and **51** (Table 2) confirmed that the installation of a bromine (**44**) or a chlorine (**45**) at the 5-position on the benzimidazole core resulted in a 10-fold gain in potency relative to compound **2** (EC_{50} = 0.5 and 0.7 nM, respectively) with no change in toxicity (CC_{50} > 20 μM). These compounds were ~70-fold more potent than BMS-433771 and approximately equipotent with JNJ-53718678. On the other hand, the installation of a fluorine (**46**) resulted in a significant loss in potency (EC_{50} > 250 nM). These compounds were also analyzed for their stability in HLMs. The introduction of a halogen reduced the $t_{1/2}$ of compounds **44** and **45** about 5-fold relative to compound **2**, likely due to an increase in cLogD.

Of the 5 compounds described in Table 1, compounds **1** and **3**, featuring a thietane-1,1-dioxide in place of the tetrahydropyran of compound **2**, exhibited greater stability in HLMs. With this in mind, we synthesized compound **47** featuring a tetrahydro-2*H*-thiopyran-1,1-dioxide. While this did not result in the same subnanomolar potencies seen with compounds **44** and **45**, compound **47** exhibited a significantly improved $t_{1/2}$ of 151 min.

Finally, compounds **48**, **49**, and **51** were synthesized to assess the importance of the nitrile group for antiviral activity. Compound **48** features the methyl sulfone thought to be responsible for the protein–ligand water-mediated hydrogen bonding interactions, as described for JNJ-5371876.[34] The introduction of the methyl sulfone on our series of compounds, however, resulted in a slight loss in potency relative to compounds **44** and **45**. The introduction of the trifluoromethyl group (**49**) and the hydroxyl group (**51**) had no significant impact on potency, with EC_{50} values of 3 and 1 nM, respectively. Compounds **48**, **49**, and **51** all exhibited $t_{1/2}$ values of <30 min.

In this assay, our compounds, while extremely potent against wild-type RSV, lose significant potency against the viral escape mutant, D489E.[33] Although its clinical impact remains unclear, this mutation, as well as other resistance hot spots that are observed clinically,[33,34] reminds us of the important lesson learned in treating viral diseases over the past three decades; i.e., success is rarely achieved using only monotherapy. Moreover, while the clinical utility of RSV fusion inhibitors remains unclear (e.g., a recently completed phase 2 clinical trial with presatovir in adult hematopoietic cell transplant recipients has revealed no therapeutic benefit),[35] several other fusion inhibitors are currently undergoing clinical evaluations, and the results of these studies will likely tell the tale of the potential of this class of drugs.

By employing a machine learning algorithm, we were able to rapidly identify a series of novel compounds as highly potent inhibitors of the RSV F protein. In this series, compound **2** was found to be 7-fold more potent than BMS-433771. Subsequently, we could further improve the potency by introducing a halogen onto the benzimidazole portion of our compounds to exploit a halogen bonding interaction with Thr397. This approach proved to be successful, and potency improved by an additional 10-fold. Our work provides important proof-of-concept for the use of a machine learning algorithm-based strategy to achieve chemotype diversification and lead optimization.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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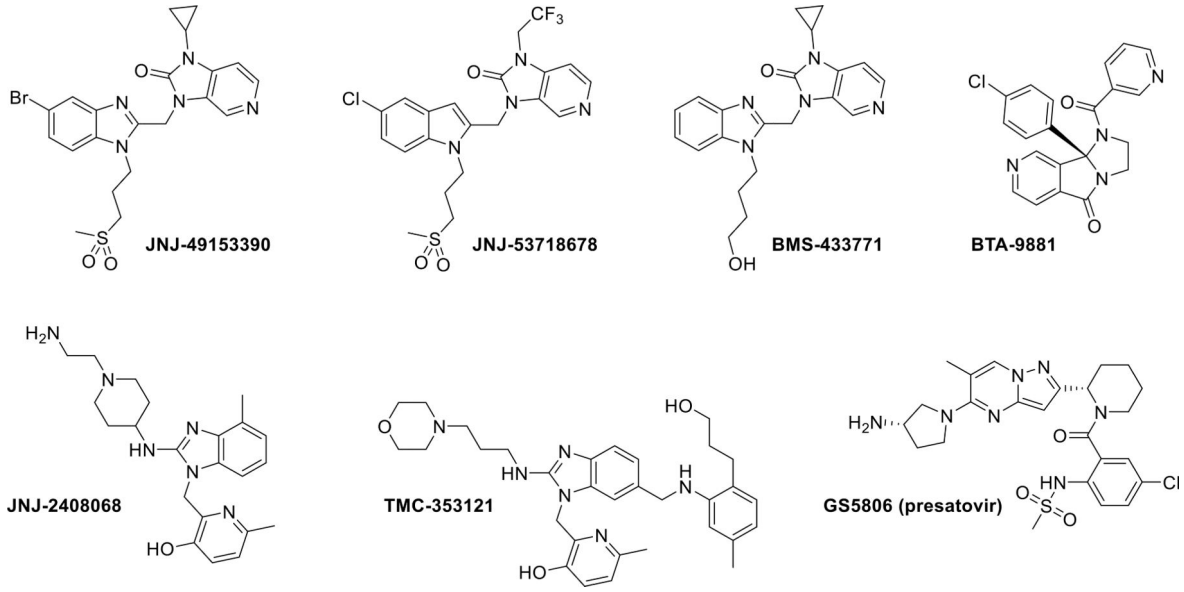


Figure 1.
Examples of known RSV fusion inhibitors.

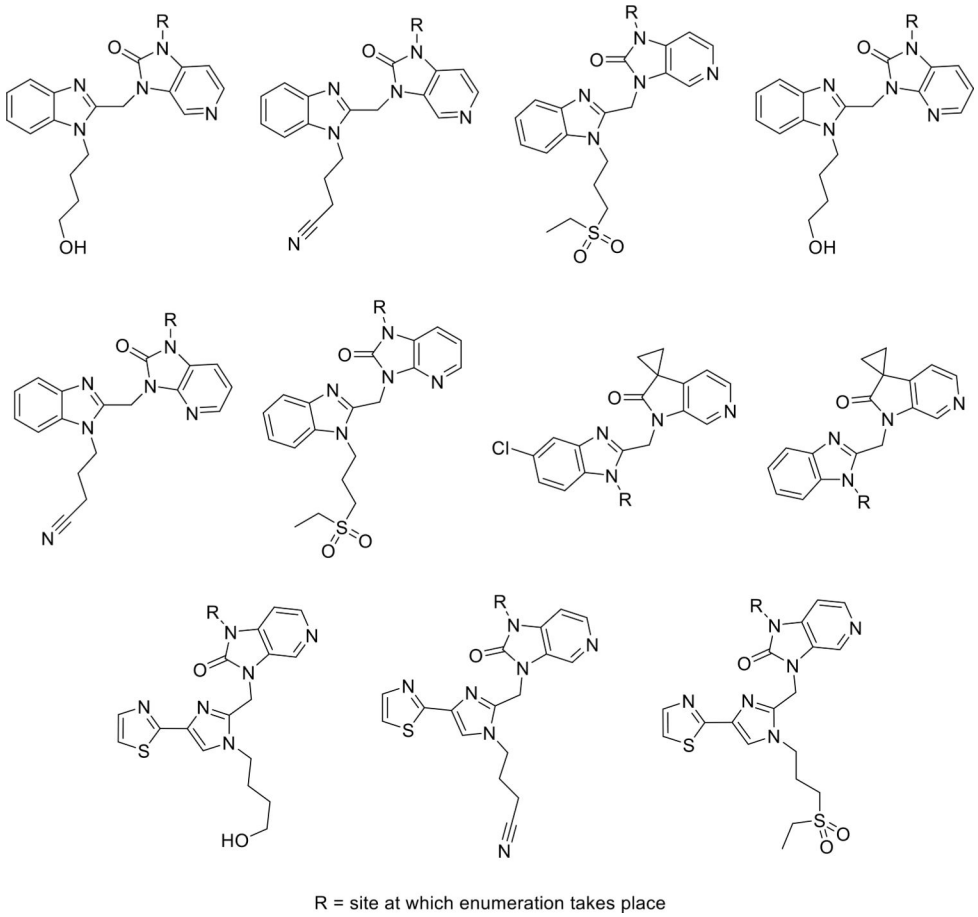


Figure 2.
Examples of core scaffolds identified and sites of enumeration.

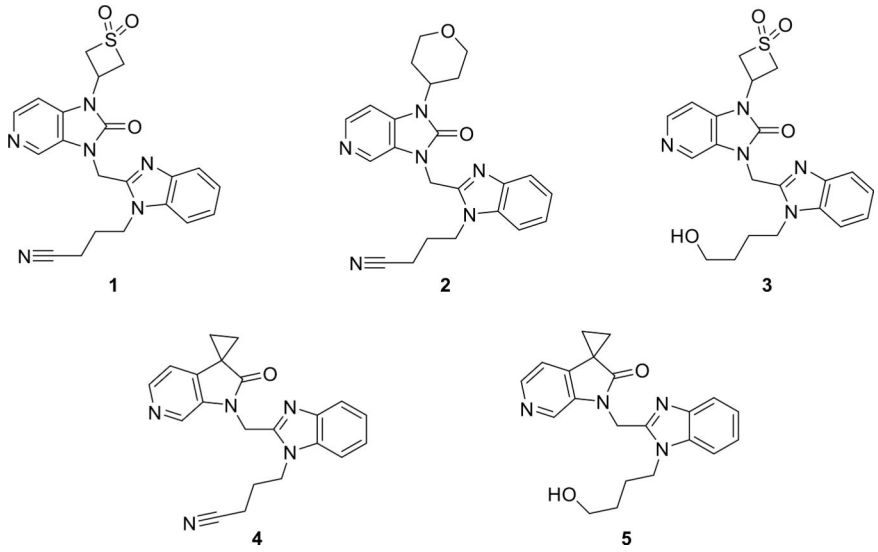
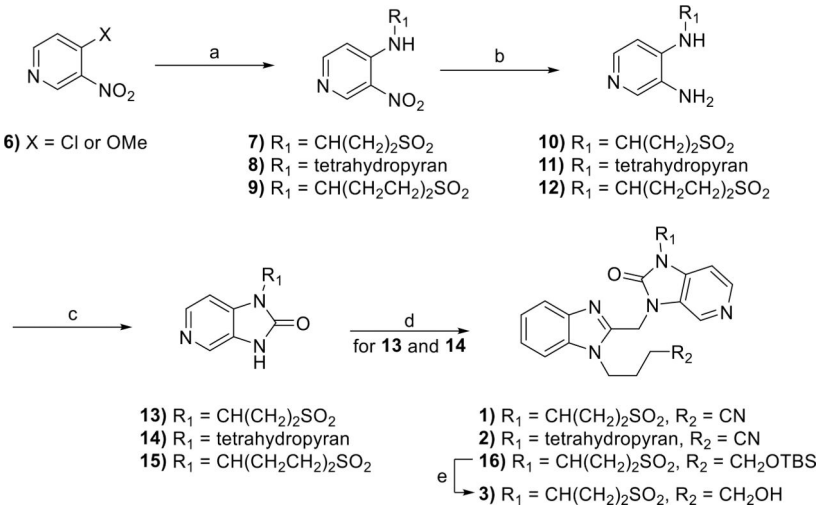
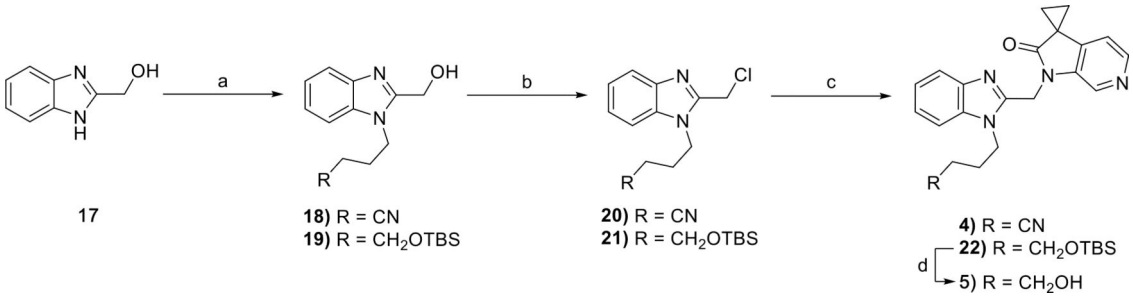


Figure 3.
Candidates for synthesis identified by machine learning.



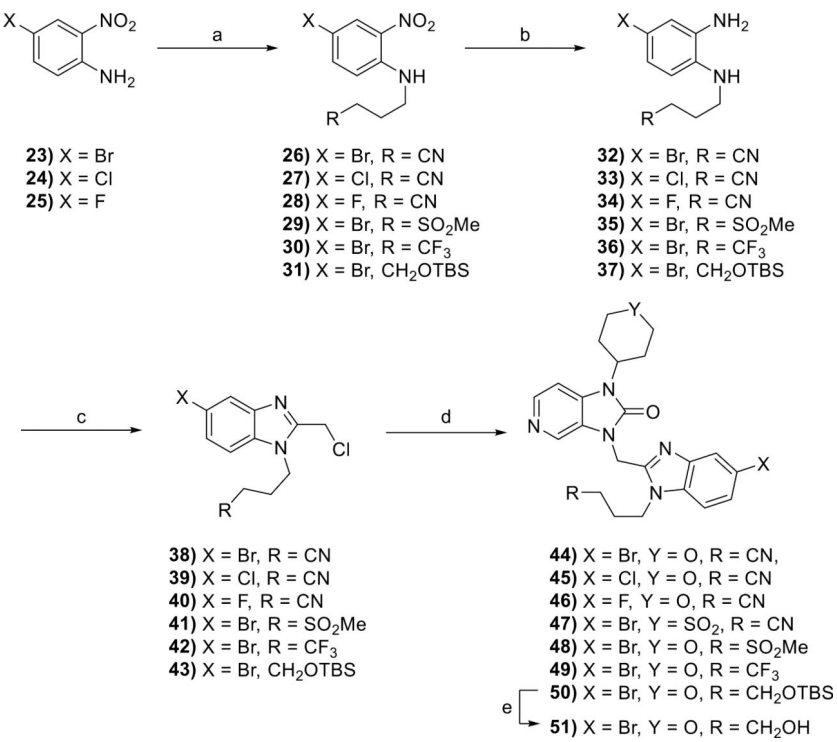
Scheme 1.

Reagents and conditions: (a) tetrahydro-2*H*-pyran-4-amine, 3-aminothietane 1,1-dioxide hydrochloride, or 1,1-dioxothian-4-amine, Hünig's base, EtOH, 90 °C, 18 h, 30–61%; (b) Pd/C, H_2, MeOH, rt, 40 min to 3 h, 100%; (c) CDI, MeCN, 3–12 h, 21–77%; (d) Cs_2CO_3, MeCN, 70 °C, 18 h, 33–43% or Cs_2CO_3, KI, DMF, 80 °C, 8 h, 31%; (e) TBAF, THF, rt, 32 h, 65%.



Scheme 2.

Reagents and conditions: (a) alkyl halide, NaH or K_2CO_3, DMF, 0 °C to rt, 18 h, 76–94%; (b) $SOCl_2$, Et_3N, DCM, rt, 3 h, 80–93%; (c) Cs_2CO_3, MeCN, rt, 4–18 h, 39–75%; (d) TBAF, THF, rt, 16 h, 84%.

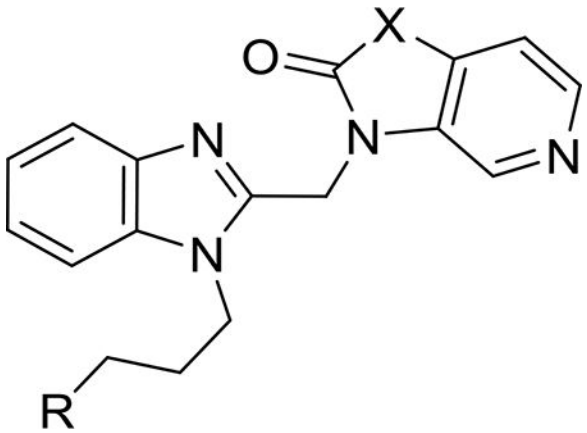


Scheme 3.

Reagents and conditions: (a) alkyl halide, NaH, DMF, 0 °C to rt, 15–51%; (b) $Fe_{(s)}$, EtOH, AcOH, H_2O, rt, 2 h, 48–67% or Pt/C, H_2, EtOAc, rt, 18 h, 70%; (c) 2-chloro-1,1,1-trimethoxy-ethane, p-TsOH·H_2O, DCM, rt, 4 h, 26–87%; (d) 14 or 15, K_2CO_3, DMF, 50 °C, 18 h, 16–86%; (e) HF-pyridine, THF, rt, 3 h, 58%.

Table 1.

Antiviral Activities and Stability Data of Compounds Identified by Our Machine Learning Algorithm



compd	R	X	EC_{50} (nM)	EC_{90} (nM)	CC_{50} (nM)	therapeutic index	HLM ($t_{1/2}$) (min)
1	CN	$NCH(CH_2)_2SO_2$	11	1180	>300 000	>27 272	1380
2	CN	N-THP	5	470	>300 000	>60 000	84.4
3	CH_2OH	$NCH(CH_2)_2SO_2$	13	1010	>300 000	>23 076	433
4	CH_2OH	$C(CH_2)_2$	1940	>2000	>300 000	>154	27.8
5	CN	$C(CH_2)_2$	22	1530	>300 000	>13 636	30.1
BMS-433771	CH_2OH	N-*c*Pr	34.5	>2000	>300 000	>8695	64.2

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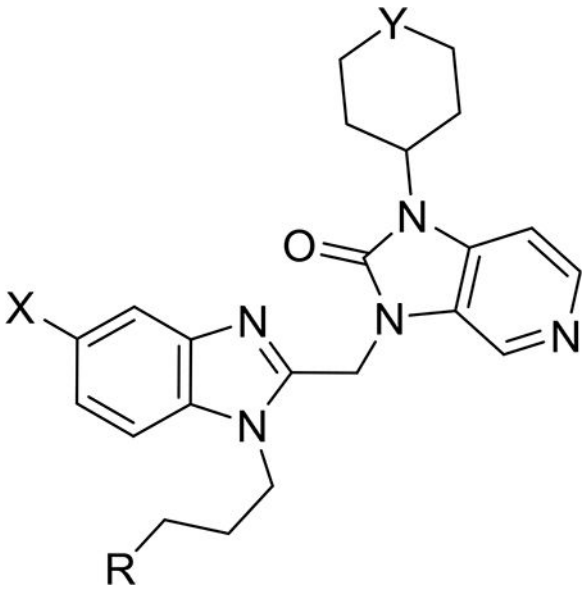
Table 2.

Antiviral Activities and Stability Data of Compounds Featuring a Halogen on the Benzimidazole



compd	R	X	Y	EC_{50} (nM)	EC_{90} (nM)	CC_{50} (nM)	therapeutic index	HLM ($t_{1/2}$) (min)
2	CN	H	O	5	470	>300 000	>60 000	84.4
44	CN	Br	O	0.5	2.0	>20 000	>40 000	15
45	CN	Cl	O	0.7	1.3	>20 000	>28 571	16.8
46	CN	F	O	>250	>250	15 710	>62	37.9
47	CN	Br	SO_2	3.1	10.4	>20 000	>6451	150.7
48	SO_2Me	Br	O	8.3	18.2	>20 000	>2409	22.4
49	CF3	Br	O	2.5	15.3	>20 000	>8000	3.6
51	CH_2OH	Br	O	1.3	52.2	>20 000	>15 385	19.2
BMS-433771				34.5	>2000	>300 000	>8695	64.2
JNJ-53718678				0.9	1.1	16 056	17 840	52.9