Selection of a Respiratory Syncytial Virus Fusion Inhibitor Clinical Candidate. 2. Discovery of a Morpholinopropylaminobenzimidazole Derivative (TMC353121)

Jean-François Bonfanti,*[,†] Christophe Meyer,[†] Frédéric Doublet,[†] Jérôme Fortin,[†] Philippe Muller,[†] Laurence Queguiner,[†] Tom Gevers,[‡] Peggy Janssens,[‡] Heidi Szel,[‡] Rudy Willebrords,[‡] Philip Timmerman,[§] Koen Wuyts,[§] Pieter van Remoortere,[#] Frans Janssens,[⊥] Piet Wigerinck,[#] and Koen Andries[‡]

Medicinal Chemistry Department, Johnson & Johnson Pharmaceutical Research and Development, Campus de Maigremont, B.P. 615, F-27106 Val de Reuil, France, Antimicrobial Research Department, ADME-Tox & Bioanalysis Department, and Medicinal Chemistry Department, Johnson & Johnson Pharmaceutical Research and Development, Turnhoutseweg 30, B-2340 Beerse, Belgium, and Tibotec BVBA, Generaal de Wittelaan L 11B 3, B-2800 Mechelen, Belgium

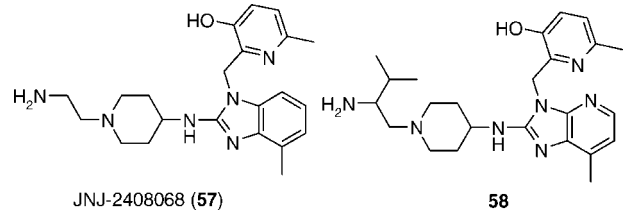
Received October 11, 2007

A preceding paper (Bonfanti et al. *J. Med Chem.* **2007**, *50*, 4572−4584) reported the optimization of the pharmacokinetic profile of substituted benzimidazoles by reducing their tissue retention. However, the modifications that were necessary to achieve this goal also led to a significant drop in anti-RSV activity. This paper describes a molecular modeling study followed by a lead optimization program that led to the recovery of the initial potent antiviral activity and the selection of TMC353121 as a clinical candidate.

Introduction

Human respiratory syncytial virus (RSV[a]) is a negative-sense enveloped RNA virus, a member of the Paramyxoviridae family, subfamily Pneumovirinae. Human RSV causes a spectrum of respiratory tract diseases in people of all ages throughout the world. In healthy adults, a relatively mild infection results in symptoms similar to those of the common cold. Among more susceptible populations, particularly infants under the age of two and immunosuppressed and elderly people, RSV infection can induce severe bronchiolitis or pneumonia, resulting in significant morbidity and mortality. RSV is highly contagious, and the infection in young children can cause lung damage that persists for years, contributing to chronic lung diseases in later life (chronic wheezing, asthma).

There are no vaccines available to prevent RSV infection.[2,3] Only three drugs have been approved so far. The only chemotherapeutic on the market is ribavirin (Virazole).[4] This aerosol treatment is used in high-risk or severely ill hospitalized infants. The route of administration, the toxicity (risk of teratogenicity), the cost, and the highly variable efficacy limit its use. Respigam, replaced now by palivizumab (Synagys), respectively polyclonal and monoclonal antibodies, were licensed to prevent RSV infection in high-risk infants.



JNJ-2408068 (**57**) **58**

Figure 1. Chemical structures of compounds JNJ-2408068 (**57**) and **58**.

Thus, there is a clear need for a new drug to prevent and treat RSV infection. Our RSV inhibition program first led to the discovery of JNJ-2408068 (**57**) (Figure 1).[5] This derivative demonstrated picomolar anti-RSV activity, but its long elimination half-life from several tissues ($t_{1/2}$ in lung = 153 h) created cause for concern. Initial attempts to improve the pharmacokinetic profile were focused on the identification of critical substructures responsible for the long tissue retention. We learned that reducing the basicity of the molecule by replacing the aminoethylpiperidine moiety successfully reduced the tissue half-life, but the resulting compounds were significantly less active against RSV.[1] We then initiated a molecular modeling study in combination with a lead optimization program aiming at recovering the original RSV inhibitory activity.

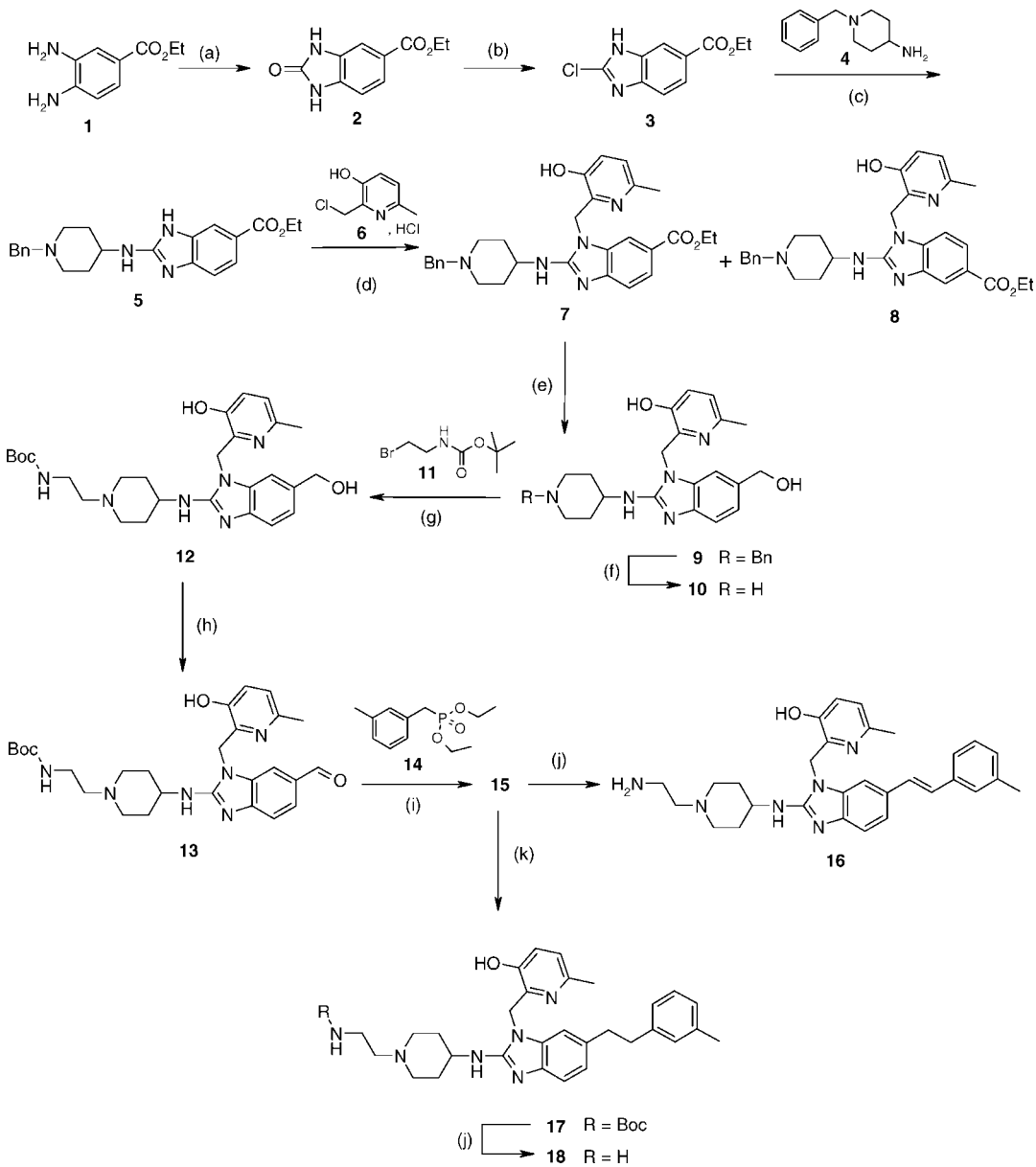
On the basis of the discovery of resistance mutations in the fusion protein of the virus, substituted benzimidazole compounds were assumed to inhibit the RSV fusion process.[5] More precisely, we assumed that these inhibitors act through direct binding to a putative site located in the core domain of the RSV fusion protein (F protein). This hypothesis was supported by time of addition studies demonstrating that **57** inhibits the fusion of the virus to uninfected cells early in the infection cycle as well as the fusion of infected cells to uninfected cells at the end of the replication cycle.[5] Single-point mutations were identified in the fusion protein of several compound **57**-resistant variants of RSV. We,[5] and others,[6] have described two sets of mutations on the core and the head domains respectively. Evidence for the presumed mechanism of action came from

* Author to whom correspondence should be addressed (telephone, +33 2 32 61 74 72; fax, +33 2 32 61 72 98; e-mail, jbonfant@prdfr.jnj.com).
† Johnson & Johnson Pharmaceutical Research and Development, Val de Reuil.
‡ Antimicrobial Research Department, Johnson & Johnson Pharmaceutical Research and Development, Beerse.
§ ADME-Tox & Bioanalysis Department, Johnson & Johnson Pharmaceutical Research and Development, Beerse.
# Tibotec BVBA.
⊥ Medicinal Chemistry Department, Johnson & Johnson Pharmaceutical Research and Development, Beerse.

[a] Abbreviations: RSV, respiratory syncytial virus; RNA, ribonucleic acid; F protein, fusion protein; Boc, *tert*-butoxycarbonyl; PK, pharmacokinetic; LC; liquid chromatography; MS, mass spectroscopy; SAR, structure–property relationship; rt, room temperature; EDTA, ethylenediaminetetraacetic acid; DIPE, diisopropyl ether; HOBT, 1-hydroxybenzotriazole; EDCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; AUCinf, area under concentration × time curve (total systemic exposure to test compound); CL, plasma clearance; C_{max}, maximum plasma concentration; Fabs, absolute bioavailability; $t_{1/2}$, half-life; V_{dz}, apparent volume of distribution during the terminal phase; po, oral; iv, intravenous.

10.1021/jm701284j CCC: $40.75
Published on Web 02/07/2008

Scheme 1[a]



[a] Reagents and conditions: (a) urea, xylene, 140 °C, 12 h; (b) $POCl_3$, HCl, 120 °C, 6 h; (c) 160 °C, 2 h; (d) K_2CO_3, CH_3CN, reflux, 12 h; (e) $LiAlH_4$, THF, rt, 12 h; (f) H_2, Pd/C 10%, CH_3OH, 8 bar, 40 °C 12 h; (g) NEt_3, DMF, 80 °C, 12 h; (h) MnO_2, CH_2Cl_2, rt, 12 h; (i) NaH, THF, rt, 12 h; (j) HCl/2-propanol 5 N, 2-propanol, 60 °C, 4 h; (k) H_2, Pd/C 10%, CH_3OH/THF, 2 bar, 4 h.

mutations D486N/E/V, E487D, F488I/L, and D489Y, which cluster on the core domain over a putative binding site described as a potential target for small-molecule inhibitors.[7] RSV fusion inhibitors are thought to be able to disrupt the formation of a six-helix bundle that is essential to start the fusion between the viral and the cellular membrane.[8]

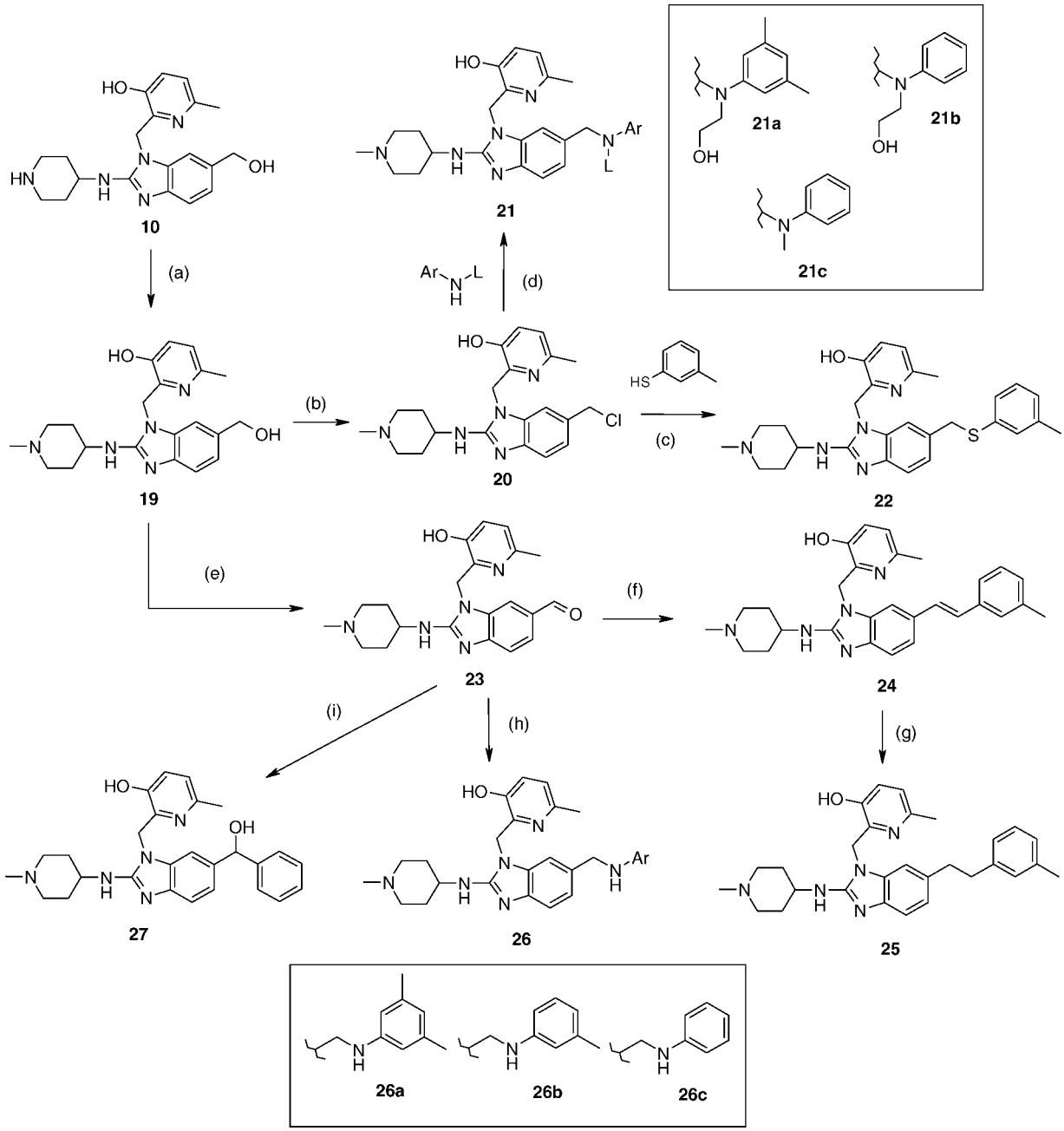
A common feature shared by class 1 fusion proteins is a conformational rearrangement that those proteins undergo upon fusion[9] to adopt a stable postfusion form. An X-ray structure of the RSV F protein core domain in the postfusion state[7] revealed the details of the intramolecular interactions between two heptad repeats, named HR-N and HR-C. The HR-C peptides insert in an antiparallel way into hydrophobic grooves located at the surface of a central HR-N coiled coil. Of particular interest is a cavity where most of the contacts between the two partners occur and which accommodates the side chains of two aromatic residues F483 and F488 of the HR-C peptides. Although disruption of protein–protein interaction by small molecules is traditionally considered to be a difficult task in drug design, this cavity has been described as a potential target for such small-molecule inhibitors.[8,10]

The above considerations allowed a structure-based design strategy: the most potent compound of the series (**58**, pEC_{50} = 10) (Figure 1) and the lead compound (**57**, pEC_{50} = 9.6) were submitted to a conformational analysis to identify their most stable conformation. Both structures were then docked in the putative binding site both manually and automatically. The best docking solutions were selected and analyzed for potential areas of chemical modification. A subsequent lead optimization program led to the discovery of new compounds with picomolar anti-RSV activity and devoid of accumulation issues.

Chemistry

The common building block **10** was obtained in six steps starting from 3,4-diaminobenzoic acid ethyl ester **1** (Scheme

Scheme 2[a]



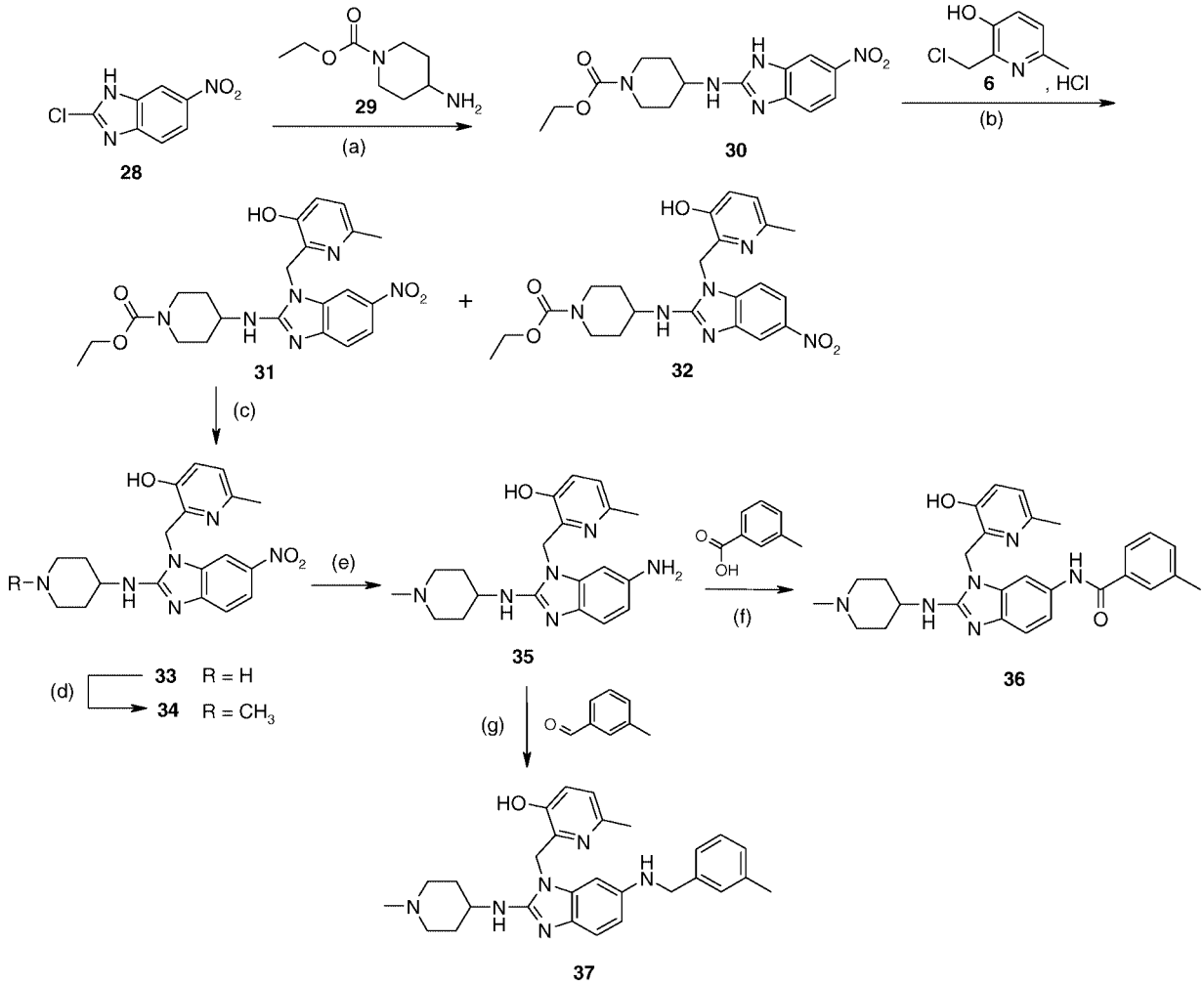
[a] Reagents and conditions: (a) CHOH 37% in water, $NaBH_3CN$, CH_3CO_2H, CH_3CN, rt, 12 h; (b) $SOCl_2$, CH_2Cl_2, 5 °C to rt, 12 h; (c) K_2CO_3, CH_3CN, 50 °C 12 h; (d) K_2CO_3, DMF, 80 °C, 4 h; (e) MnO_2, CH_2Cl_2, rt, 12 h; (f) (3-methylbenzyl)phosphonic acid diethyl ester, NaH, THF, 5 °C to rt, 12 h; (g) H_2, Pd/C 10%, CH_3OH/THF, 2 bar, rt, 4 h; (h) $ArNH_2$, BH_3CN- on solid support or $NaBH_3CN$, AcOH, CH_3OH, rt, 24 h; (i) bromobenzene, Mg, THF, rt, 3 h.

1). Cyclization with urea in xylene at high temperature gave the corresponding benzimidazolone **2**. $POCl_3$ mediated chlorination in acidic medium followed by a melting reaction with *N*-benzyl-4-aminopiperidine **4** afforded intermediate **5**. The introduction of the hydroxypyridine moiety was not selective and led to the formation of two isomers, **7** and **8**. The two new analogues were separated by column chromatography over silica gel. Reduction using $LiAlH_4$ in THF was performed on isomer **7**. The resulting alcohol analogue **9** was finally debenzylated, affording building block **10**.

Building block **10** was used to synthesize analogues of JNJ-2408068 bearing an extra phenethyl chain on the benzimidazole group (Scheme 1). N-Alkylation reaction with Boc-protected bromoethylamine **11** in the presence of NEt_3 gave intermediate **12**. The aldehyde group necessary for a Wittig–Horner reaction was formed via oxidation of the methyl−alcohol group with MnO_2. 3-Methylbenzyl phosphonate, deprotonated with NaH, reacted on the aldehyde to afford the methylstyrene derivative **15**. Part of intermediate **15** was used to reduce the double bond with hydrogen and palladium 10% on charcoal. Acid-mediated deprotection of the primary amino group in intermediates **15** and **17** led to the final expected compounds **16** and **18**.

The selective methylation of the piperidine moiety was performed by reductive amination using *p*-formaldehyde and sodium borohydride (Scheme 2). A two-step process, involving $SOCl_2$-mediated chlorination and coupling reaction in the presence of K_2CO_3, allowed us to introduce different thio- and aminoaryl groups. The aldehyde building block **23** gave access

Scheme 3[a]



[a] Reagents and conditions (a) 120 °C, 10 h; (b) K_2CO_3, DMF, 70 °C, 24 h; (c) HBr 48% in water, 70 °C, 12 h; (d) HCHO 37% in water, $NaBH_3CN$, AcOH, CH_3CN, rt, 12 h; (e) Ni (Ra), H_2, CH_3OH, 3 bar, rt, 1 h; (f) EDCI, HOBT, CH_2Cl_2, rt, 12 h; (g) BH_3CN^- on solid support, AcOH, CH_3OH, rt, 24 h.

to the styrene (**24**) and phenethyl (**25**) analogues, to different aniline derivatives (**26a**, **26b**, **26c**), and to a shorter chain with a secondary hydroxyl group after a Grignard reaction (**27**).

To continue the modulation of the spacer group between the benzimidazole core heterocycle and the phenyl ring, we synthesized building block **35** bearing an amino functional group (Scheme 3).

This building block was obtained using the synthetic pathway already described in Scheme 1, starting from the nitrochlorobenzimidazole **28**. A carboethoxy replaced the benzyl as protecting group for the piperidine moiety. The final reduction was performed in the presence of Raney nickel under a pressure of hydrogen to yield compound **35**. This new starting material was employed in a coupling reaction to afford the amide derivative **36** and in a reductive amination reaction to afford the *N*-*m*-methylbenzyl targeted analogue **37**.

Bromoacetic acid ethyl ester was used to introduce the hydroxyethyl chain on the piperidine group (Scheme 4). The synthetic route leading to the aniline derivatives remained as described earlier in Scheme 2. Final compounds **41a** and **41b** were finally obtained after reduction of the carboxylic ester into alcohol.

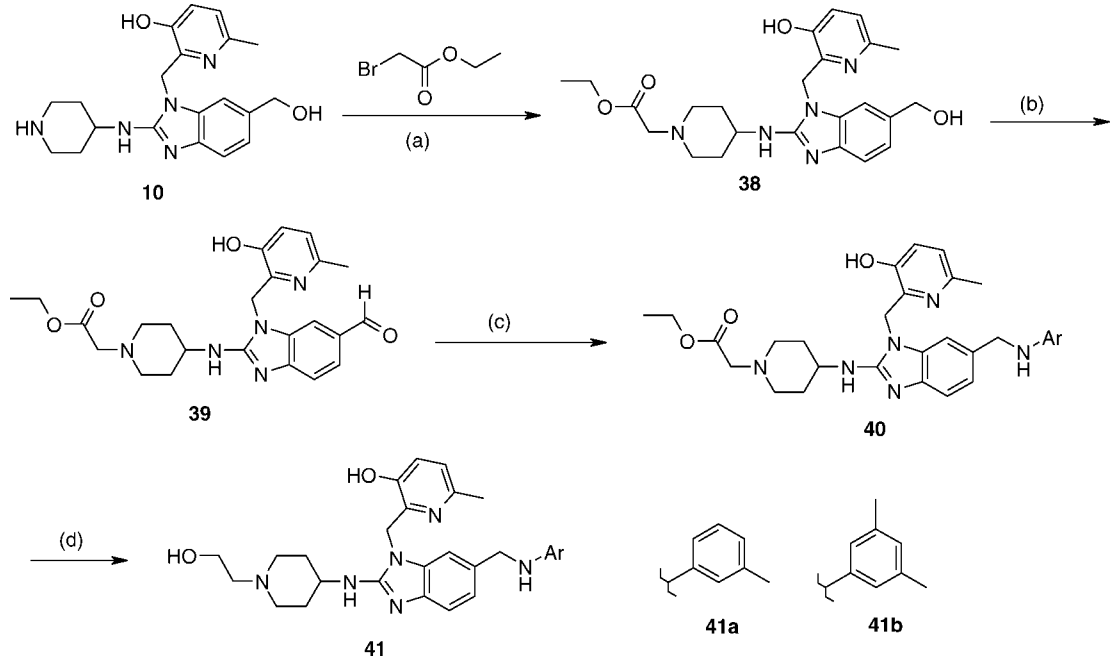
In the series covering the modulation of the entire aminoethylpiperidine moiety, we introduced the various chains at the beginning of the synthesis scheme by melting with chlorobenzimidazole **3** (Scheme 5). As before, the introduction of the hydroxypyridine "head part" led to the formation of two isomers. After separation by column chromatography over silica gel, isomer **45** was smoothly oxidized and the resulting aldehyde reacted with 3,5-dimethylaniline to yield final compounds **48a**−**48e**. **48f** was obtained after deprotection of the diol function in **48b**.

The morpholinopropyl series was explored extensively, and an optimized synthetic pathway was used to produce the two requested building blocks **53** and **54** (Scheme 6). Chlorobenzimidazole ester **3** was first reduced into alcohol in the presence of $LiAlH_4$. This reduction prevented a possible side reaction of the ester group with the amino moiety of the next-step reactant and allowed us to improve significantly the yield of the melting reaction (from 47 to 82%). The reaction with the lutidine derivative **6** afforded again two isomers in the same proportion and with a yield comparable to what was achieved with the ester starting material **42**. Secondary and primary anilines were involved in reactions with the two building blocks **53** and **54** utilizing already described nucleophilic substitution and reductive amination conditions.

Molecular Modeling

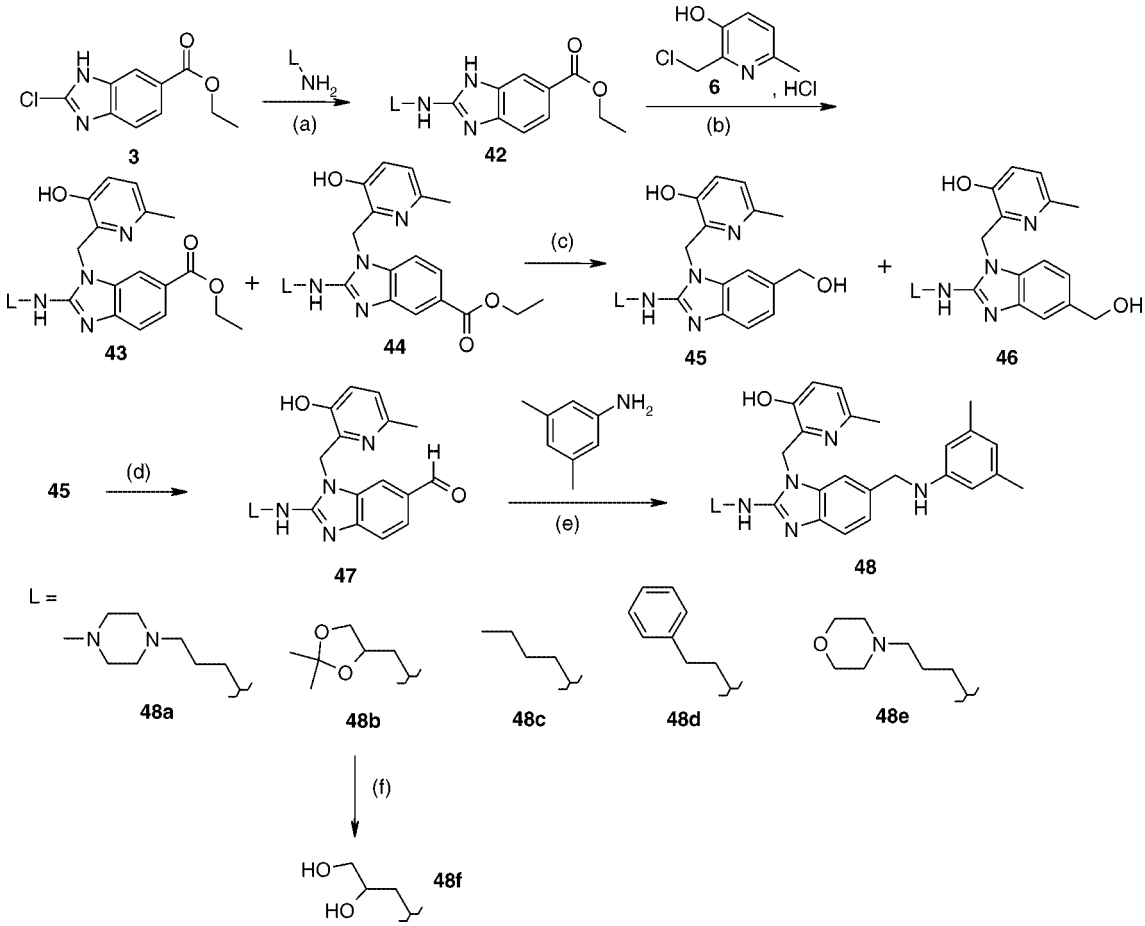
The conformational analysis of compounds **58** and **57** yielded 1244 and 735 conformers, respectively, within the energy window of 8 kcal/mol above the global minimum. Respectively,

Scheme 4[a]



[a] Reagents and conditions: (a) NEt_3, DMF, 50 °C, 1 h; (b) MnO_2, CH_2Cl_2, rt, 12 h; (c) $ArNH_2$, $NaBH_3CN$, CH_3CN, rt, 6 h; (d) $LiAlH_4$, THF, 5 °C to rt, 5 h.

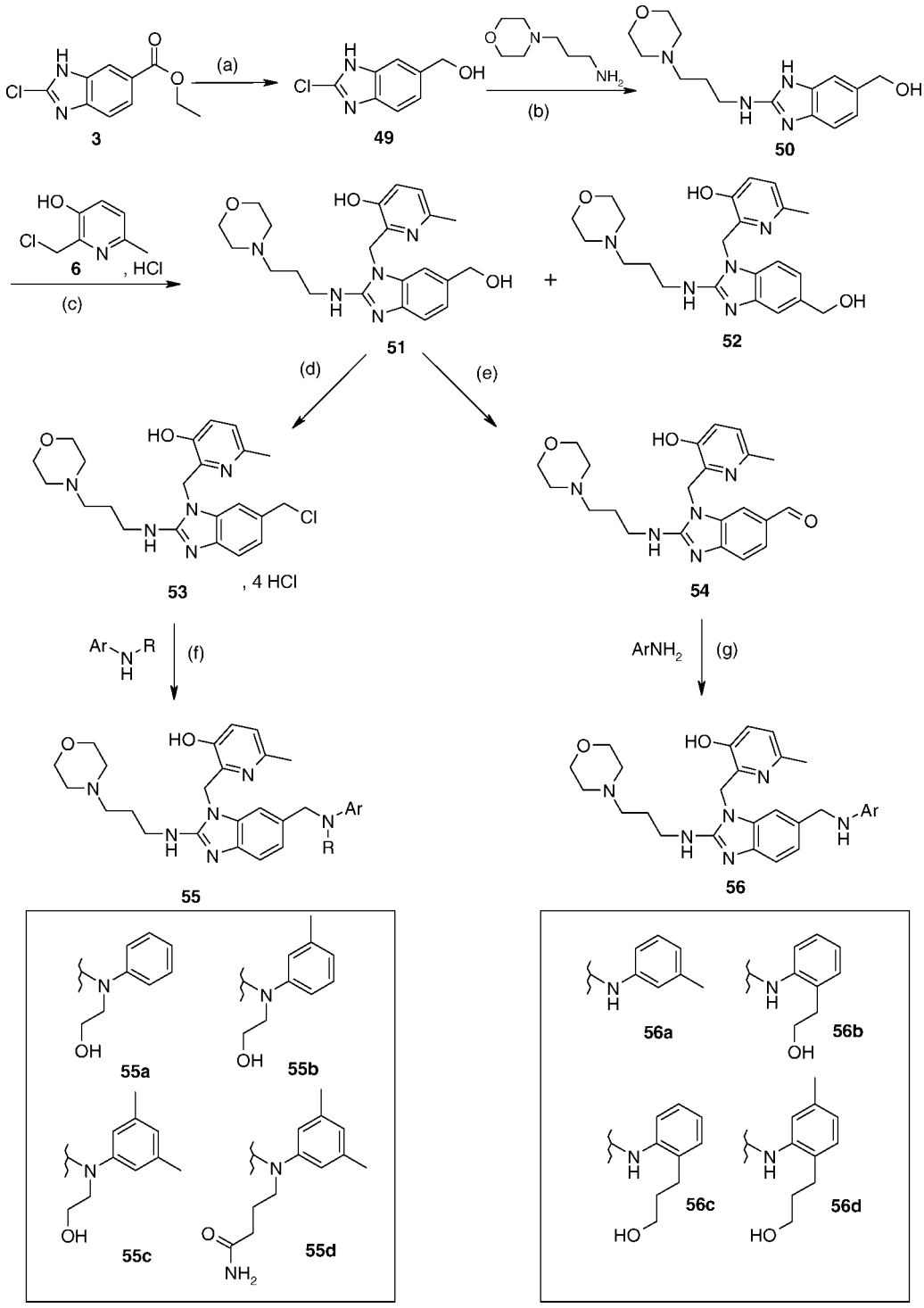
Scheme 5[a]



[a] Reagents and conditions: (a) 140 °C, 3 h; (b) K_2CO_3, DMF, rt, 24 h; (c) $LiAlH_4$, THF, 5 °C to rt, 13 h; (d) MnO_2, CH_2Cl_2, rt, 12 h; (e) AcOH, $NaBH_3CN$, CH_3CN, rt, 12 h; (f) HCl 3N, THF, rt, 6 h.

84 and 91% of the conformers were found at least twice, meaning that the conformational space was properly sampled. Most notably, the lowest energy conformer of both compounds (64.4 and 57.2 kcal/mol, respectively) showed an intramolecular

Scheme 6[a]



[a] Reagents and conditions (a) $LiAlH_4$, THF, 5 °C, 3 h; (b) 125 °C, 4 h; (c) K_2CO_3, DMF, rt, 12 h; (d) $SOCl_2$, CH_2Cl_2, 5 °C to rt, 14 h; (e) MnO_2, CH_2Cl_2, rt, 12 h; (f) K_2CO_3 or Cs_2CO_3, DMF, 80 °C, 1−4 h; (g) $NaBH_3CN$− on solid support or $NaBH_3CN$, AcOH, CH_3OH, or CH_3CN, rt, 12 to 24 h.

H-bond between the planar NH group and the pyridyl nitrogen. However, the N−H···N angles (128.8 and 131.9°, respectively) deviated significantly from the ideal 180° geometry such that it was not expected that this weak H-bond strongly influences the ligand conformation upon protein binding. Both lowest energy conformers were selected as the starting point of the docking study.

Automated and manual docking was performed on both compounds. It has to be stressed that docking tools perform better on well-delineated globular cavities where directional protein–ligand H-bonds help the algorithm to converge to a solution. Because the shape of the putative binding site is a hydrophobic groove and the HR-C/HR-N interaction takes place at the surface of the HR-N coiled coil, it is not surprising that only a few solutions were obtained with FlexX. Additional solutions were obtained by rigid, manual docking of the lowest energy conformer of both compounds. All complexes were subsequently optimized using the MMFF94s force field.

The most interesting solutions are given in Figure 2. **58** (left) binds in two (P1 and P2) of the three subpockets forming the hydrophobic binding site. In addition to the ligand's isopropyl group making favorable steric and hydro-

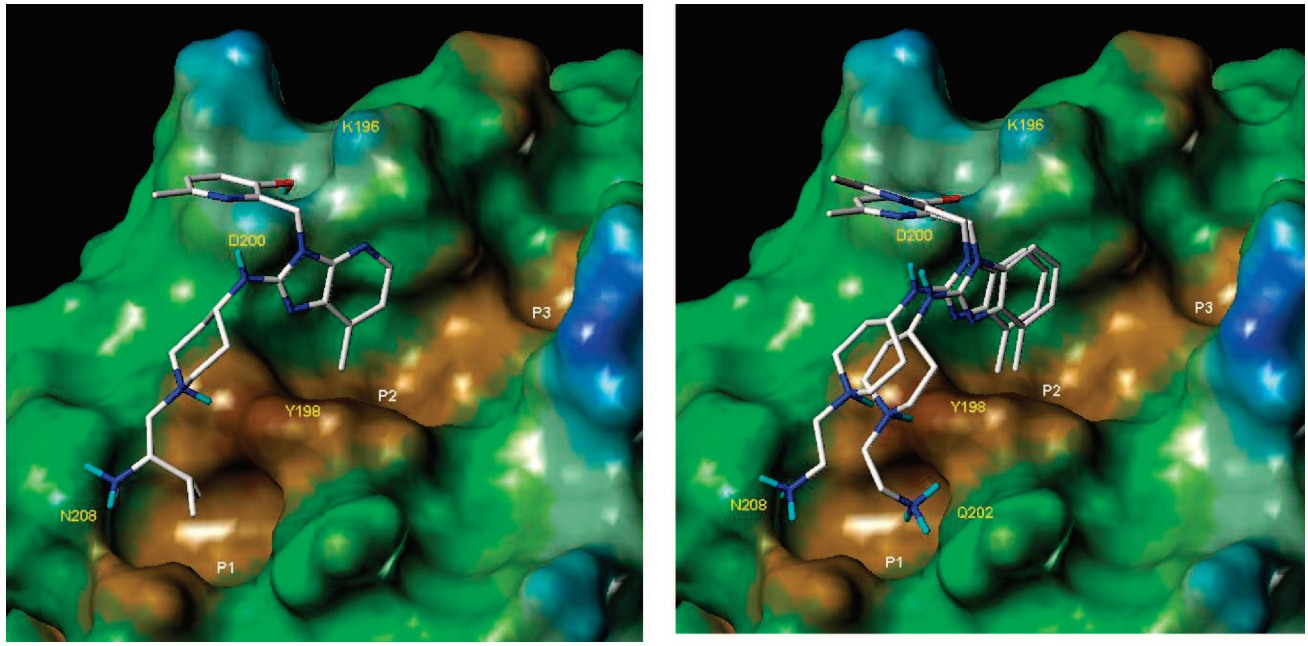


Figure 2. Automated docking solutions for compounds **58** (left) and **57** (right). Similar binding modes have been obtained; both compounds occupy subpockets P1 and P2, thus leaving subpocket P3 unexploited. Major interactions can be observed with residues N208, Q202, and Y198 and the salt bridge D200−K196. (Left) Best docking solution obtained for compound **58**. The isopropyl group perfectly mimics the interactions made by the side chain of HR-C residue L481. (Right) Two best docking solutions obtained for compound **57**. The differences lie in the orientation of the flexible amino chain able to interact with asparagine N208 or glutamine Q202.

phobic interactions in P1, three major interactions with surrounding residues can be observed: the ammonium substituent is performing electrostatic interactions with the side chain of polar residue N208; the hydroxyl group located on the pyridyl ring makes a H-bond with the salt bridge D200−K196; and the methyl substituent at position 4 of the benzimidazole scaffold is engaged in an alkyl-pi stacking with the aromatic ring of Y198. **57** (right) shares a similar binding mode with **58**. However, the absence of the isopropyl group confers a greater flexibility to the amino chain, which enables the ammonium group to span a wider conformational space. Two docking solutions were obtained that differ only by the region of interaction of the ammonium group either in the vicinity of N208 or close to Q202. Neither the consensus scoring nor the potential energy difference between the minimized complexes allowed us to favor one solution over the other.

Evidence in favor of our docking solutions comes from the comparison with the F protein core domain X-ray structure. The "natural substrate" HR-C peptide and the docked inhibitor **58** show remarkable similarities in their interaction scheme with the F protein (Figure 3): both molecules have an isopropyl chain interacting in subcavity P1; their respective hydroxyl group is located in the same region in space and makes a H-bond with the D200−K196 salt bridge. The two molecules also have an aromatic moiety lying in the same subpocket P2.

Another observation that adds confidence to our model comes from the resistant RSV mutants that were raised through exposure to **57**.[5] They were shown to have single-point mutations in the F-gene that affect residues at position 486−489 of the HR-C peptide. Those residues cover subpocket P3 and a part of subpocket P2 with residue D486 being in direct contact with the docked inhibitors (Figure 3). It was questioned why those mutations occur on the HR-C peptide, whereas they would have been expected to affect residues of the HR-N coiled coil. Consistent with a scenario

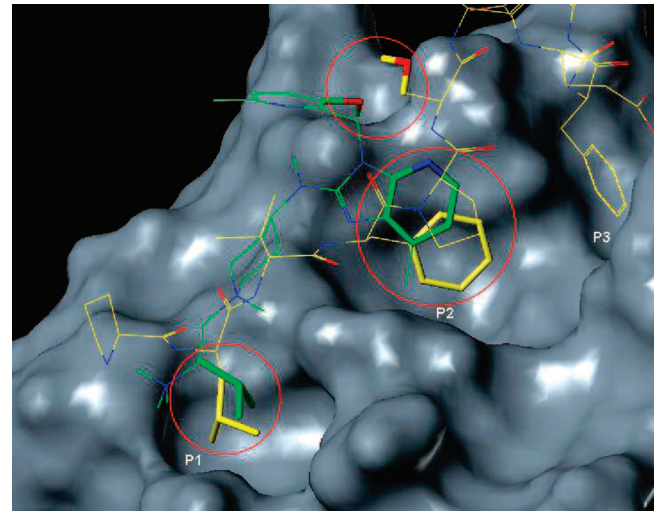
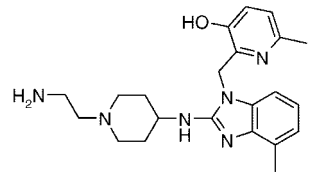
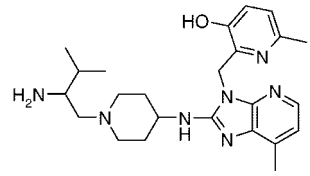
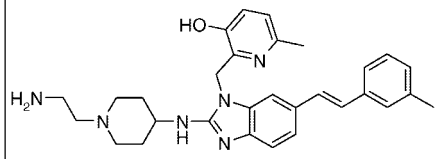
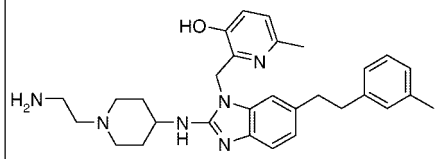


Figure 3. Interaction matches between HR-C (yellow) and **58** (green). **58** in its docked orientation has been superimposed to the X-ray structure of the F-protein core domain. The red circles indicate a match between equivalent groups in both substrates.

proposed recently,[11] one explanation is the establishment of a ternary HR-N/inhibitor/HR-C complex upon binding of the inhibitor. Due to its presence in the hydrophobic groove, the competitive inhibitor would prevent zipping of the HR-C/HR-N peptides. Starting in the mutation region, the remaining HR-C sequence of residues would unzip from the "hydrophobic rail". Local stabilization would be achieved by establishment of new favorable interactions between affected HR-C residues and the inhibitor. Mutations clearly indicate that residues D486, E487, F488, and D489 could be actively involved in a ternary complex formation and might influence inhibitor binding. As a consequence, docking studies involving only residues from the HR-N binding site and the inhibitor

Table 1. In Vitro RSV Inhibitory Activities of Compounds Synthesized To Validate the Molecular Modeling Model

Compound no	Structure	pEC_{50}	pCC_{50}
57		9.6 ± 0.4	< 4
58		10.0 ± 0.2	4.3
16		8.5 ± 0.2	5.1
18		9.0 ± 0.4	4.9

should be analyzed with caution and within the context of a possible ternary complex.

Results and Discussion

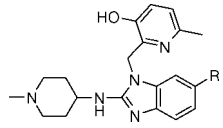
We have previously outlined the importance of the aminoethylpiperidine moiety of compound **57** regarding RSV inhibitory activity and tissue retention propensity.[1] Its replacement led to an improvement of the pharmacokinetic profile at the expense of a large drop in antiviral activity. This drop is consistent with the assumption that the suppression of the terminal ammonium group would lead to a large increase in binding energy (~18 kcal/mol). Analysis of the docking model clearly indicated that compounds **57** and **58** bind in subpockets P1 and P2, thereby leaving subpocket P3 free. It was hypothesized that the increase in free binding energy obtained upon removal of the aminoethylpiperidine moiety might be compensated by new favorable interactions made in subpocket P3. This pocket is known to be a key subsite for HR-C binding, where numerous contacts occur with residue F488. Ideally, a new substituent should be introduced that mimics the aromatic side chain of HR-C residue F488. If successful, this would in turn translate into some activity recovery while preserving the enhanced pharmacokinetic profile.

A modeling design showed that, to reach subpocket P3, new substituents should be anchored at position 6 of the benzimidazole scaffold and should extend by three bonds along the hydrophobic groove. An aromatic 6-membered ring was deemed to be optimal to mimic a phenylalanine side chain. Finally, introduction of a methyl substituent in the meta position of the phenyl ring was meant to optimize steric and hydrophobic interactions. To validate these results, we synthesized the corresponding **57** derivatives **16** and **18** (Table 1). Because of chemical feasibility considerations, the methyl substituent at position 4 of the benzimidazole scaffold was not introduced. If properly designed, these compounds were supposed to retain an activity level close to that of their parent compound. This was indeed the case with compound **18**, showing nanomolar activity against RSV. The corresponding unsaturated analogue **16** was slightly less potent, presumably as a consequence of inherent rigidity, which could prevent the substituent to fit optimally in the hydrophobic subpocket.

Lead Optimization. The strategy was to introduce substituents in position 6 of the new compounds with improved pharmacokinetic properties,[1] in an attempt to increase their in vitro RSV inhibitory activity.

The results obtained with compounds **16** and **18** suggested that introduction of bulky hydrophobic substituents in position 6 was allowed, and we tried to further optimize the first substituents. *N*-Methylpiperidine building blocks were the most accessible, and we decided to perform the optimization in this series (Table 2). As expected, the strict analogues of compounds

Table 2. Structure–Activity Relationship in the *N*-Methylpiperidine Series



Compound no	R	pEC_{50}	pCC_{50}
21a		9.4 ± 0.2	4.4
21b		8.5 ± 0.4	< 4
21c		7.4 ± 0.2	5.2
22		6.6 ± 0.3	4.4
24		6.3 ± 0.2	5.6
25		7.7 ± 0.3	5.9
26a		8.6 ± 0.1	4.3
26b		8.3 ± 0.3	4.7
26c		7.8 ± 0.2	4.5
27		6.8 ± 0.3	< 4
36		7.2 ± 0.1	4.3
37		7.8 ± 0.4	4.8

16 and **18** were found to be less potent (compound **24**, pEC_{50} = 6.3; compound **25**, pEC_{50} = 7.7). Despite this 2 log reduction in activity, they could be used as a basis for a further optimization. Replacement of the methylene linked to the phenyl group with a heteroatom led to mixed results: although the introduction of a sulfur atom resulted in somewhat reduced potency (compound **22**, pEC_{50} = 6.6), the gain in activity for the aniline derivative was substantial (compound **26b**, pEC_{50} = 8.3). The importance of the methyl substituent on the phenyl ring was well demonstrated by the reduction in activity when removed (compound **26c**, pEC_{50} = 7.8). Reduction of the chain length alongside addition of a hydroxyl group to mimic the NH of compound **26c** (compound **27**, pEC_{50} = 6.8) afforded a less potent compound. Moving the NH one carbon atom away from the ring reduced activity (compound **37**, pEC_{50} = 7.8). An even larger decrease in potency was seen upon replacement of the methylene with a carbonyl in this inhibitor (compound **36**, pEC_{50} = 7.2). The addition of a second methyl group in the meta-position of the aniline moiety led to a very potent molecule (compound **26a**, pEC_{50} = 8.6). We finally investigated substitution of the aniline nitrogen. The molecule with a simple methyl substituent was less effective (compound **21c**, pEC_{50} = 7.4). Most promisingly, incorporation of an ethanol chain resulted in a further increase in activity (compound **21b**, pEC_{50} = 8.5). Combination with the di-*m*-methyl substitution of the aniline moiety afforded the most potent inhibitor (compound **21a**, pEC_{50} = 9.4). This last compound was nearly equipotent to JNJ-2408068.

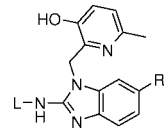
We combined the substitution of compounds **26a** and **26b** with different new chains L reported in our previous paper[1] (Table 3). For the hydroxyethylpiperidine chain (compounds **41a** and **41b**), the additional substituent led to a 1 log increase in pEC_{50} compare to the original compound (from pEC_{50} = 7.7 to pEC_{50} = 8.8). No difference was observed between the monomethyl and dimethyl substitution of the phenyl ring. A comparable improvement in potency was obtained for the *N*-methylpropylpiperazine analogue (compound **48a**, pEC_{50} = 8.4 instead of 7.6 for the original derivative). The addition of the new R-group did not result in any improvement to the inhibitory activity of poorly active compounds with pEC_{50} values below 4 (compounds **48c** and **48d**). The same modification led to a disappointing outcome for the dihydroxypropyl analogue (compound **48f**, pEC_{50} = 7.5, equipotent to the original compound). Finally, the best results were obtained for the morpholinopropyl derivatives. A 1.7 log increase was observed with the mono-*m*-methyl substituent (compound **56a**, pEC_{50} = 8.6). Incorporation of a second methyl in the meta-position resulted in a further increase in RSV inhibitory activity (compound **48e**, pEC_{50} = 9.0).

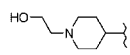
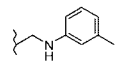
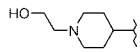
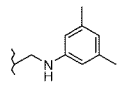
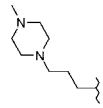
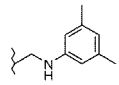
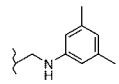
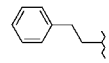
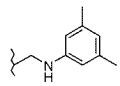
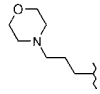
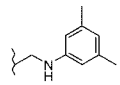
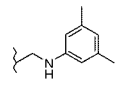
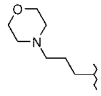
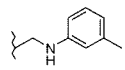
Three molecules underwent in vivo pharmacokinetics (PK) experiments to study tissue retention. Combination of the new substitution with the hydroxyethylpiperidine chain proved to have an unfavorable effect on PK profile in terms of tissue retention. Whereas the original compound gave a $t_{1/2}$ in lung of 29 h, the new analogues led to much longer half-lives, comparable for the two derivatives (67.7 h for compound **41a** and 84.9 h for compound **41b**). Most promisingly, the good trend was confirmed for the propylmorpholine analogue (compound **48e**, $t_{1/2}$ = 13.7 h).

In an attempt to further study the phenomenon of potent RSV inhibitors with improved PK profile, we tested additional morpholinopropyl analogues (Table 4). Substantial gains in potency were seen with the introduction of polar chains on the aniline part of the molecules. A hydroxyethyl chain on the nitrogen of compound **56a** led to a 0.7 log increase in RSV inhibitory activity (compound **55b**, pEC_{50} = 9.3). The addition of a second methyl group in the meta-position resulted in an antiviral of equivalent potency (compound **55c**, pEC_{50} = 9.3). In agreement with what we saw in the previous series, the unsubstituted analogue still showed an interesting antiviral potency, although 1 order of magnitude lower (compound **55a**, pEC_{50} = 8.5). The hydroxyethyl chain was favorably replaced with an amide-propyl chain (compound **55d**, pEC_{50} = 9.6). We discovered that dislocation of the alcohol chain to the ortho-position of the phenyl ring was the optimal choice (compound **56b**, pEC_{50} = 9.1). Increasing the length of the linear alkyl chain from two to three methylene groups led to a very potent molecule (compound **56c**, pEC_{50} = 9.6). Finally, combination with the *meta*-methyl substitution afforded the most potent analogue (compound **56d**, pEC_{50} = 9.9).

Subsequently, four new morpholinopropyl derivatives were selected for in vivo half-life determination. We confirmed that this series of very potent RSV inhibitors was devoid of unfavorable tissue retention (10.4 h $\leq t_{1/2} \leq$ 25.1 h).

Table 3. Transfer of the SAR in the Series Covering the Modulation of the Aminoethylpiperidine Moiety



Compound no	L	R	pEC_{50}	pCC_{50}	$T_{1/2\ (24\text{-}96\ h)}$ in lung (h)
41a			8.8 ± 0.2	4.4	67.7
41b			8.8 ± 0.3	4.5	84.9
48a			8.4 ± 0.3	5	nd*
48c			< 4	5.1	nd
48d			< 4	4.4	nd
48e			9.0 ± 0.2	5	13.7
48f			7.5 ± 0.3	4.3	nd
56a			8.6 ± 0.2	4.9	nd

* nd: not determined

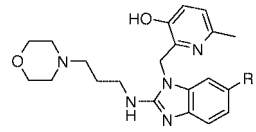
Several compounds with improved PK properties were identified for preclinical PK and safety assessment, leading to the selection of compound **56d** (TMC353121) for clinical evaluation. **56d** is further evaluated in clinical trials.

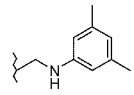
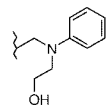
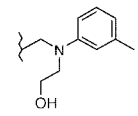
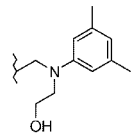
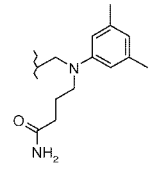
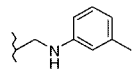
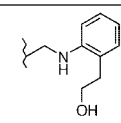
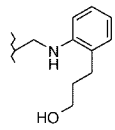
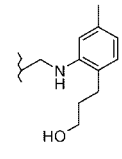
Antiviral Spectrum and Mechanism of Action of 56d. Antiviral activity was measured against group A and group B respiratory syncytial viruses and clinical isolates with equal potency (Table 5). The extent of resistance and/or cross-resistance of **56d** on mutants raised against **57**[1] (R1LO, R2LO in Table 5) or palizivumab (R6LO in Table 5), where all mutations are located on the fusion protein, was assessed using the antiviral assay. The activity profile of **56d** was found to be identical to the profile of JNJ-2408068. Also, the R1LO and R2LO RSV mutants were resistant against two demonstrated binders of the fusion protein, VP-146373[11] and BMS4337714.[8] Moreover, it has recently been demonstrated that JNJ-2408068 competes with VP-14637 for the same binding site.[11] These observations all confirm that **56d** interferes with the entry mechanism by binding to the fusion protein and, hence, inhibiting virus−cell fusion and cell−cell fusion (syncytia formation).

Pharmacokinetic Profile of 56d in Plasma. After iv administration, **56d** was rapidly eliminated with a plasma clearance of 5.4 L/h/kg (Table 6). The plasma concentrations dropped quickly within the first hour after dosing and then much more slowly. The plasma half-life determined between 4 and 24 h (last blood sampling time point) was about 10 h. The volume of distribution was 79 L. After oral administration of a 10 mg/kg dose, the compound was slowly absorbed and the absolute bioavailability was moderate (about 14%). Maximum plasma concentrations of about 47 ng/mL were observed at 4 h postdose.

56d in the Cotton Rat Model. The cotton rat model was used to demonstrate the in vivo efficacy of **56d**. The cotton rat is semipermissive for RSV infection; that is, the amount of virus recovered from the lungs is directly proportional to the amount

Table 4. Structure–Activity Relationship in the Morpholinopropyl Series



Compound no	R	pEC_{50}	pCC_{50}	$T_{1/2\ (24\text{-}96\ h)}$ in lung (h)
48e		9.0 ± 0.2	5	13.7
55a		8.5 ± 0.3	4.3	nd*
55b		9.3 ± 0.4	5	nd
55c		9.3 ± 0.2	4.4	19.6
55d		9.6 ± 0.3	4.7	10.4
56a		8.6 ± 0.2	4.9	nd
56b		9.1 ± 0.4	4.7	20.1
56c		9.6 ± 0.2	4.9	nd
56d		9.9 ± 0.3	5.1	25.1

* nd: not determined

of challenge virus, suggesting that there is only one round of replication in the cotton rat.[12]

The results of the different experiments are summarized in Table 7.

56d was shown to be active by intravenous, oral, and aerosol administration in the cotton rat model at doses of 10 mg/kg, 40 mg/kg, and 5 mg/mL, respectively.

The results of the studies in which **56d** (10 mg/kg) was administered intravenously at different time points before and after RSV infection suggest that the compound has to be administered shortly before the virus challenge (i.e., 5 min) to achieve a maximal antiviral effect. Only minor reductions were measured when the compound was administered 24 h before or 3 h after virus challenge. This is due to the fact that RSV replication in the cotton

rat model results in only one replication cycle and that **56d** has to be present before viral fusion occurs at the beginning of that replication cycle. RSV infection in humans involves several replication cycles, and it can therefore not be excluded that **56d** will have a therapeutic effect if administered after the onset of symptoms.

Conclusion

A molecular modeling study allowed us to identify a possible modulation to improve the binding of our RSV inhibitors with the target protein and recover the initial nanomolar activity. A subsequent lead optimization program led to the discovery of new morpholinopropyl-containing substituted benzimidazoles. These new compounds not only were very potent RSV inhibitors but were also devoid of any tissue retention.

Among these new antiviral compounds, **56d** was selected for clinical development. **56d** showed activity against groups A and B RSV and against a panel of clinical isolates with equal potency. In vivo activity in the cotton rat model by different routes of administration was demonstrated. **56d** is a potent novel RSV fusion inhibitor further evaluated in clinical trials.

Experimental Section

In Vitro Antiviral Assay. Our multicycle replication assay was developed on the basis of the HeLaM cell using a low amount of virus as the inoculum. This ensures that compounds interfering with any of the many molecular targets that are important for virus replication in vitro will be detected. RSV was added to HeLaM cells, an epithelial-like cell line, in the presence and absence of various compound concentrations. The viability of treated and untreated cells was assessed by the addition of Thiazolyl blue (MTT) after an incubation period of 1 week. pEC_{50} values were calculated from the optical density (OD) values, measured spectrophotometrically. Virus controls, cell controls, and cytotoxicity controls were included in each plate. Reference compounds were included in each test. Tests in which the virus/cell/compound controls did not meet the objectives were not approved.

The in vitro model was validated using ribavirin and palivizumab as controls. Both substances produced EC_{50} values in the range of published data. Ribavirin and JNJ-2408068 were included as positive control in each test.

Cotton Rat Model. Cotton rat experiments were carried out using prophylactic and therapeutic drug administration schedules. Efficacy was measured at 4 days after virus challenge, at a time when RSV pulmonary titers reach their maximum level (i.e., $\sim 10^6$ infectious viruses/mL of BALF) in untreated control animals. For sample collection, lungs were flushed with physiological water and the virus concentration in these broncho-alveolar lavage fluids (BALF) was determined by plaque titration. Compounds were administered via inhalation (INH) with a SATURN (small animal therapeutic ultrasonic nebulizer) aerosol-generating device, intravenously (iv) or orally by gavage (po).

A rough calculation of the dosage per kilogram of body weight of drug delivered to the cotton rats by aerosol (inhalation) was made using the following formula: estimated dose = aerosol concentration of drug × minutes of treatment × respiratory volume per minute/kg for cotton rats × pulmonary retention fraction.[13] Because **56d** was added to the aerosol delivery reservoir at a concentration of 5 mg/mL, it is estimated that under the conditions utilized, a 75 g cotton rat administered a single aerosol for 60 min would have received an estimated dose of 1.25 mg/kg.

Pharmacokinetic Determination. Male Sprague–Dawley rats (250–300 g, Charles River) were used (three animals per time group).

Each individual compound was dissolved in a 10% hydroxypropyl-β-cyclodextrin solution at an individual concentration of 1 mg/mL. For some compounds, HCl was added to obtain their total dissolution. An equal volume of each individual solution was added together with an equal volume of demineralized water to make a formulation with a final concentration of 0.25 mg of compound/mL. The osmolarity was measured and brought to 283 mosmol/kg with NaCl. The final pH of the formulation was 5.35. Before dosing, the formulation was stored at room temperature and protected from light. Immediately after dosage, the formulation was frozen and stored at ≤ -18 °C until analysis.

Table 5. Median in Vitro Results (HeLaM Cells) for **56d**

RSV subtype	strain	EC_{50} (ng/mL)	pEC_{50}	pEC_{90}	CC_{50} (ng/mL)	SI (CC_{50}/EC_{50})
A	LO	0.07	9.9	9.6	4436	63371
A	CI11	0.03	10.3	10.0	4436	147867
A	CI57	0.04	10.1	9.8	4436	110900
B	CI41	0.02	10.5	10.1	4436	221800
B	CI42	0.02	10.5	10.1	4436	221800
A	R1LO	7.03	7.9	7.5	4436	631
A	R2LO	279.91	6.3	<4	4436	16
A	R6LO	0.04	10.1	<6.7	4436	110900

For the tissue retention studies, all animals were dosed by a 10 min infusion in the tail vein, to provide a final dose of 1 mg of compound/kg of body weight. The exact infusion speed was calculated by taking the exact body weight of the individual animals into account. For the bioavailability study, a dose of 2 mg/kg of compound **56d** was given intravenously in a saphenous vein, and a 10 mg/kg dose was given orally by gastric intubation.

For the tissue retention studies, at 4, 24, 96, and 168 h after dose administration, three individual animals per time group were sacrificed by decapitation for blood collection and dissection of lung, liver, and kidney. For the bioavailability study, the sample time points were at 7 and 20 min and 1, 2, 4, and 8 h (after intravenous administration) and at 30 min and 1, 2, 4, 8, and 24 h (after oral administration). Blood was collected on EDTA K3 in 10 mL BD Vacutainer tubes. Plasma was obtained following centrifugation at 4 °C. Individual tissue samples were dissected, blotted on filter paper, and weighed immediately. Individual tissue homogenates were prepared in demineralized water (1:9 w/v). Plasma and tissue homogenate samples were stored at ≤ -18 °C prior to analysis.

Individual plasma and tissue samples were analyzed using a qualified research bioanalytical method (LC-MS/MS), that is, a bioanalytical method that takes into account all scientific features of a method stipulated in the FDA guidelines with respect to stability, accuracy, and precision, but not fully validated.

Chemistry. All analytically pure compounds were dried under vacuum in a drying pistol using a Buchi Glass Oven B-580 apparatus. Melting points were determined using a Leica VMHB apparatus and are uncorrected. TLC analyses were run on silica gel 60 F_{254} plates (Merck) using a variety of solvent systems and a fluorescent indicator for visualization. Spots were visualized under 254 nm UV illumination. Column chromatography was performed with silica gel 60 (Merck) (0.015–0.040 mm) or Kromasyl (Akzo Nobel) (0.010 mm). Proton NMR spectra were recorded on a Bruker Avance 300 (300 MHz) and a Bruker Avance 400 (400 MHz) spectrometer using internal deuterium lock. Chemical shifts are reported relative to internal DMSO (δ 2.54) in parts per million and coupling constants (J) in hertz. Exact mass spectra (TOF) were recorded with a Micromass LCT instrument. Elemental analyses were performed with a Thermo Electron Corp. instrument EA 1110 or EA 1108 for C, H, and N, and the results were within ±0.4% of the theoretical values. Chemicals and solvents were purchased from either Acros Co. or Aldrich Chemical Co. Yields refer to purified products and are not optimized.

2-Hydroxy-3*H*-benzoimidazole-5-carboxylic Acid Ethyl Ester (Intermediate 2). A mixture of **1** (0.166 mol) and urea (0.199 mol) in xylene (300 mL) was stirred under reflux for 12 h. The reaction was cooled to room temperature. The precipitate was filtered off, rinsed with xylene and DIPE, and then dried (32 g, 93%, melting point > 260 °C): ^{1}H NMR (DMSO-d_6) δ 1.30 (t, 3 H, J = 7.0

Table 6. Pharmacokinetic Profile in Plasma

iv					po			
dose (mg/kg)	AUC_{inf} (ng·h/mL)	CL (L/h/kg)	V_{dz} (L/kg)	$t_{1/2(4–24\ h)}$ (h)	dose (mg/kg)	C_{max} (ng/mL)	AUC_{inf} (ng·h/mL)	F_{abs} (%)
2	354	5.4	79.3	10.3	10	47.3	246	14

Table 7. Results in Cotton Rat Model

log reduction[a]	route[b]	timing[c]	dose[d]
0.6	INH	−1 h	0.25 mg/mL
1	INH	−1 h	1.25 mg/mL
1.5	INH	−1 h	5 mg/mL
1.6	INH	−1 h	20 mg/mL
0.4	iv	−5 min	2 mpk
0.2	iv	−96 h	10 mpk
0.4	iv	−72 h	10 mpk
0.3	iv	−48 h	10 mpk
0.5	iv	−24 h	10 mpk
0.9	iv	−4 h	10 mpk
0.9	iv	−1 h	10 mpk
1.2	iv	−5 min	10 mpk
0.5	iv	+3 h	10 mpk
0.2	iv	+24 h	10 mpk
0.4	po	−2 h	10 mpk
0.8	po	−2 h	40 mpk

[a] Log reduction in BALF of cotton rats was calculated versus control animals. [b] All single doses (duration inhalation treatment = 1 h). [c] Indicates when the compound was administered before (minus) or after (plus) virus challenge. [d] Inhalation doses are expressed in mg/mL, i.e., the concentration of the solution in the reservoir of the nebulizer.

Hz), 4.26 (qd, 2 H, J = 7.0 Hz), 7.02 (d, 1 H, J = 7.7 Hz), 7.48 (s, 1 H), 7.62 (d, 1 H, J = 7.7 Hz), 10.93 (br s, 2 H).

2-Chloro-3*H*-benzoimidazole-5-carboxylic Acid Ethyl Ester (Intermediate 3). A mixture of **2**(0.073 mol) in $POCl_3$ (150 mL) was stirred at 100 °C. Concentrated HCl (around 1.5 mL) was added dropwise, very carefully, until dissolution of **2**. Next, the mixture was stirred at 120 °C for 6 h. The solvent was evaporated until dryness. The residue was taken up in H_2O/ice, basified with K_2CO_3 (powder), and extracted with EtOAc + 10% CH_3OH. The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent was evaporated until dryness (13.5 g, 83%, melting point 178 °C).

2-(1-Benzylpiperidin-4-ylamino)-3*H*-benzoimidazole-5-carboxylic Acid Ethyl Ester (Intermediate 5). A mixture of **3** (0.051 mmol) and **4** (0.056 mol) was stirred at 160 °C for 2 h. The residue was taken up in CH_2Cl_2/H_2O and basified with a 10% solution of K_2CO_3 in water. The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent was evaporated until dryness. The residue was purified by column chromatography over silica gel (eluent, $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.5). The pure fractions were collected, and the solvent was evaporated (15.3 g, 79%): ^{1}H NMR (DMSO-d_6) δ 1.30 (t, 3 H, J = 7.2 Hz), 1.52 (qd, 2 H, J = 11.1 Hz), 1.93 (d, 2 H, J = 11.1 Hz), 2.07 (t, 2 H, J = 11.1 Hz), 2.79 (d, 2 H, J = 11.1 Hz), 3.48 (s, 2 H), 3.55–3.65 (m, 1 H), 4.27 (qd, 2 H, J = 7.2 Hz), 7.04 (d, 1 H, J = 7.7 Hz), 7.13–7.19 (m, 1 H), 7.20–7.35 (m, 5 H), 7.59 (d, 1 H, J = 7.7 Hz), 7.72 (s, 1 H), 10.28 (s, 1 H).

2-(1-Benzylpiperidin-4-ylamino)-3-(3-hydroxy-6-methylpyridin-2-ylmethyl)-3*H*-benzoimidazole-5-carboxylic Acid Ethyl Ester (Intermediate 7) and 2-(1-Benzylpiperidin-4-ylamino)-1-(3-hydroxy-6-methylpyridin-2-ylmethyl)-1*H*-benzoimidazole-5-carboxylic Acid Ethyl Ester (Intermediate 8). A mixture of **5** (0.0396 mol), **6** (0.059 mol), and K_2CO_3 (0.1584 mol) in CH_3CN (180 mL) was stirred and refluxed for 12 h. The solvent was evaporated until dryness. The residue was taken up in CH_2Cl_2. The organic layer was washed with H_2O, dried (over $MgSO_4$), and filtered, and the solvent was evaporated until dryness. The residue (20 g) was purified by column chromatography over silica gel (eluent, toluene/2-propanol/NH_4OH 85:15:1). Two fractions were collected, and the solvent was evaporated [5.3 g of fraction 1 (27%) and 6.3 g of fraction 2 (32%)]. Fraction 1 was crystallized twice in 2-propanone/CH_3CN/DIPE. The precipitate was filtered off and dried (4.9 g of intermediate 7, 25%, melting point 179 °C): ^{1}H NMR (DMSO-d_6) δ 1.32 (t, 3 H, J = 7.2 Hz), 1.57 (qd, 2 H, J = 11.1 Hz), 2.02 (d, 2 H, J = 11.1 Hz), 2.13 (t, 2 H, J = 11.1 Hz), 2.36 (s, 3 H), 2.81 (d, 2 H, J = 11.1 Hz), 3.50 (s, 2 H), 3.73–3.85 (m, 1 H), 4.28 (qd, 2 H, J = 7.2 Hz), 5.19 (s, 2 H), 7.03–7.08 (m, 2 H), 7.15 (d, 1 H, J = 7.7 Hz), 7.20 (d, 1 H, J = 7.7 Hz), 7.22–7.28 (m, 1 H), 7.29–7.38 (m, 4 H), 7.62 (dd, 1 H, J = 2.0, 7.7 Hz), 7.89 (d, 1 H, J = 2.0 Hz), 10.34 (br s, 1 H).

Fraction 2 was crystallized from 2-propanone/CH_3CN/DIPE. The precipitate was filtered off and dried (5.5 g of intermediate**8**, 28%, melting point 238 °C): ^{1}H NMR (DMSO-d_6) δ 1.30 (t, 3 H, J = 7.2 Hz), 1.55 (qd, 2 H, J = 11.1 Hz), 2.02 (d, 2 H, J = 11.1 Hz), 2.13 (t, 2 H, J = 11.1 Hz), 2.34 (s, 3 H), 2.80 (d, 2 H, J = 11.1 Hz), 3.50 (s, 2 H), 3.70–3.81 (m, 1 H), 4.26 (qd, 2 H, J = 7.2 Hz), 5.19 (s, 2 H), 6.92–7.00 (m, 1 H), 7.06 (d, 1 H, J = 7.7 Hz), 7.15 (d, 1 H, J = 7.7 Hz), 7.22–7.28 (m, 1 H), 7.29–7.38 (m, 5 H), 7.59 (d, 1 H, J = 7.7 Hz), 7.75 (s, 1 H), 10.34 (br s, 1 H).

2-[2-(1-Benzylpiperidin-4-ylamino)-6-hydroxymethylbenzoimidazol-1-ylmethyl]-6-methylpyridin-3-ol (Intermediate 9). $LiAlH_4$ (0.009 mol) was added portionwise to a mixture of **7** (0.003 mol) in THF (60 mL) at 5 °C under N_2 flow. The reaction was stirred at 5 °C for 1 h and then at room temperature for 12 h. AcOEt and H_2O were added carefully, and the aqueous layer was saturated with K_2CO_3 (powder). The organic layer was separated, dried (over $MgSO_4$), and then filtered over Celite. The filtrate was evaporated until dryness (1.3 g, 97%): ^{1}H NMR (DMSO-d_6) δ 1.54 (qd, 2 H, J = 11.1 Hz), 2.04 (d, 2 H, J = 11.1 Hz), 2.13 (t, 2 H, J = 11.1 Hz), 2.36 (s, 3 H), 2.80 (d, 2 H, J = 11.1 Hz), 3.50 (s, 2 H), 3.68–3.78 (m, 1 H), 4.48 (d, 2 H, J = 4.6 Hz), 5.01 (t, 1 H, J = 4.6 Hz), 5.10 (s, 2 H), 6.78 (d, 1 H, J = 7.2 Hz), 6.88 (d, 1 H, J = 7.7 Hz), 7.06 (d, 1 H, J = 7.7 Hz), 7.11 (d, 1 H, J = 7.7 Hz), 7.15 (d, 1 H, J = 7.7 Hz), 7.22–7.28 (m, 2 H), 7.30–7.38 (m, 4 H), 10.28 (br s, 1 H).

2-[6-Hydroxymethyl-2-(piperidin-4-ylamino)benzoimidazol-1-ylmethyl]-6-methylpyridin-3-ol (Intermediate 10). A mixture of **9** (0.0028 mol) and Pd/C 10% (2.5 g) in CH_3OH (40 mL) was hydrogenated at 40 °C for 12 h under an 8 bar pressure and then filtered over Celite. The Celite was rinsed with a solution of CH_3OH/THF (50:50). The filtrate was concentrated under reduced pressure (1.8 g, 95%, melting point 260 °C): MS ($C_{20}H_{25}N_5O_2$), m/z 368 $(M + H)^+$.

(2-{4-[6-Hydroxymethyl-1-(3-hydroxy-6-methylpyridin-2-ylmethyl)-1*H*-benzoimidazol-2-ylamino]piperidin-1-yl}ethyl)carbamic Acid *tert*-Butyl Ester (Intermediate 12). A mixture of **10** (0.0079 mol), **11** (0.0095 mol), and NEt_3 (0.0118 mol) in DMF (60 mL) was stirred at 80 °C for 12 h. The solvent was evaporated until dryness. The residue was taken up in CH_2Cl_2/H_2O. The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent was evaporated. The residue (7 g) was purified by column chromatography over silica gel (eluent, $CH_2Cl_2/CH_3OH/NH_4OH$ 90:10:0.5). The pure fractions were collected, and the solvent was evaporated (1.2 g, 30%): MS ($C_{27}H_{38}N_6O_4$), m/z 511 $(M + H)^+$.

(2-{4-[6-Formyl-1-(3-hydroxy-6-methylpyridin-2-ylmethyl)-1*H*-benzoimidazol-2-ylamino]piperidin-1-yl}ethyl)carbamic Acid *tert*-Butyl Ester (Intermediate 13). A mixture of **12** (0.0023 mol) and MnO_2 (2.4 g) in CH_2Cl_2 (80 mL) was stirred at room temperature for 12 h and then filtered over Celite. The Celite was washed with H_2O. The filtrate was concentrated under reduced pressure. The residue (1.2 g) was purified by column chromatography over silica gel (eluent, $CH_2Cl_2/CH_3OH/NH_4OH$ 95:5:0.1). The pure fractions were collected, and the solvent was evaporated (0.8 g, 67%): MS ($C_{27}H_{36}N_6O_4$), m/z 509 $(M + H)^+$.

(2-{4-[1-(3-Hydroxy-6-methylpyridin-2-ylmethyl)-6-(2-*m*-tolylvinyl)-1*H*-benzoimidazol-2-ylamino]piperidin-1-yl}ethyl)carbamic Acid *tert*-Butyl Ester (Intermediate 15). Diethyl 3-methylbenzylphosphonate **14** (0.0023 mol) was added at 5 °C to a mixture of NaH in oil (0.0047 mol) in THF (20 mL) under N_2 flow. The mixture was stirred at 5 °C for 30 min. A solution of **13** (0.0007 mol) in THF (10 mL) was added. The mixture was stirred at 5 °C for 1 h and then stirred at room temperature for 12 h. H_2O was added. The mixture was extracted with CH_2Cl_2. The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent was evaporated. The residue was taken up in 2-propanone. The precipitate was filtered off and dried (0.32 g, 68%): ^{1}H NMR (DMSO-d_6) δ 1.38 (s, 9 H), 1.53 (qd, 2 H, J = 11.1 Hz), 2.01 (d, 2 H, J = 11.1 Hz), 2.11 (t, 1 H, J = 11.1 Hz), 2.30–2.39 (m, 8 H), 2.83 (d, 2 H, J = 11.1 Hz), 3.05 (qd, 2 H, J = 6.1 Hz), 3.65–3.75 (m, 1 H), 5.17 (s, 2 H), 6.65 (t, 1 H,J = 5.1 Hz), 6.75–6.85 (m, 1 H), 7.00 (d, 1 H, J = 16.2 Hz), 7.02–7.09 (m, 2 H), 7.13–7.20 (m, 3 H), 7.20–7.27 (m, 2 H), 7.35 (d, 1 H, J = 7.7 Hz), 7.39 (s, 1 H), 7.54 (s, 1 H), 10.38 (br s, 1 H).

2-[2-[1-(2-Aminoethyl)piperidin-4-ylamino]-6-(2-*m*-tolylvinyl) benzoimidazol-1-ylmethyl]-6-methylpyridin-3-ol (Compound 16). A mixture of **15** (0.0001 mol) in HCl 5 N/2-propanol (0.6 mL) and 2-propanol (6 mL) was stirred at 60 °C for 4 h and then cooled to room temperature. The precipitate was filtered off, rinsed with 2-propanol and then with DIPE, and dried (0.057 g, HCl, salt, 71%, melting point 211 °C): ^{1}H NMR (DMSO-d_6) δ 2.10–2.30 (m, 4 H), 2.33 (s, 3 H), 2.37 (s, 3 H), 3.13 (qd, 2 H, J = 11.1 Hz), 3.30–3.45 (m, 4 H), 3.70–3.79 (m, 2 H), 4.15–4.27 (m, 1 H), 5.62 (s, 2 H), 7.09 (s, 1 H, J = 7.7 Hz), 7.19–7.30 (m, 4 H), 7.35 (d, 1 H, J = 7.7 Hz), 7.40 (s, 1 H), 7.43–7.50 (m, 3 H), 7.75 (s, 1 H), 11.33 (br s, 1 H); HRMS (ESI), calcd for $C_{30}H_{37}N_6O$ 497.3029, found $[MH]^+$ 497.3017.

(2-{4-[1-(3-Hydroxy-6-methylpyridin-2-ylmethyl)-6-(2-*m*-tolylethyl)-1*H*-benzoimidazol-2-ylamino]piperidin-1-yl}ethyl)carbamic Acid *tert*-Butyl Ester (Intermediate 17). A mixture of **15** (0.0002 mol) and Pd/C 10% (0.03 g) in CH_3OH (5 mL) and THF (5 mL) was hydrogenated at room temperature for 4 h under a 2 bar pressure and then filtered over Celite. The organic layer was washed with H_2O, dried (over $MgSO_4$), and filtered, and the solvent was evaporated until dryness (0.15 g, 100%). The crude compound was used directly in the next reaction step.

2-[2-[1-(2-Aminoethyl)piperidin-4-ylamino]-6-(2-*m*-tolylethyl)benzoimidazol-1-ylmethyl]-6-methylpyridin-3-ol (Compound 18). A mixture of **17** (0.0002 mol) in HCl 5 N/2-propanol (1.5 mL) and 2-propanol (15 mL) was stirred at 60 °C for 4 h and then cooled to room temperature. The precipitate was filtered off, rinsed with 2-propanol/DIPE, and dried (0.128 g, HCl salt, 76%, melting point 188 °C): ^{1}H NMR (DMSO-d_6) δ 2.03–2.20 (m, 4 H), 2.22 (s, 3 H), 2.33 (s, 3 H), 2.72–2.82 (m, 4 H), 2.83–2.93 (m, 4 H), 3.05–3.20 (m, 2 H), 3.30–3.45 (m, 4 H), 3.70–3.80 (m, 2 H), 4.10–4.22 (m, 1H), 5.50 (s, 2 H), 6.88–6.95 (m, 2 H), 6.99 (s, 1 H), 7.07(d, 1 H, J = 7.7 Hz), 7.12 (d, 1 H, J = 7.7 Hz), 7.19 (d, 1 H, J = 7.7 Hz), 7.29 (s, 1 H), 7.32–7.40 (m, 2 H), 9.00 (br s, 1 H), 11.30 (br s, 1 H); HRMS (ESI), calcd for $C_{30}H_{39}N_6O$ 499.3185, found $[MH]^+$ 499.3197.

2-[6-Hydroxymethyl-2-(1-methylpiperidin-4-ylamino)benzoimidazol-1-ylmethyl]-6-methylpyridin-3-ol (Intermediate 19). HCHO 37% in water (0.0098 mol), $NaBH_3CN$ (0.0059 mol), and then CH_3CO_2H (2 mL) were added at room temperature to a mixture of **10** (0.0049 mol) in CH_3CN (50 mL). The mixture was stirred at room temperature for 12 h. The solvent was evaporated until dryness. The residue was taken up in EtOH (30 mL), and a 5 N solution of HCl in 2-propanol (4 mL) was added. The mixture was stirred at 80 °C for 8 h. The solvent was evaporated until dryness. The residue was taken up in CH_2Cl_2/K_2CO_3 10%. The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent was evaporated under reduced pressure. The residue was crystallized from CH_3OH/2-propanone/CH_3CN. The precipitate was filtered off and dried (1.65 g, 88%). Part of this fraction (0.15 g) was crystallized from CH_3OH/2-propanone. The precipitate was filtered off and dried (melting point 165 °C): ^{1}H NMR (DMSO-d_6) δ 1.56 (qd, 2 H, J = 11.1 Hz), 2.04 (d, 2 H, J = 11.1 Hz), 2.12–2.30 (m, 5 H), 2.38 (s, 3 H), 2.85 (d, 2 H, J = 11.1 Hz), 3.67–3.78 (m, 1 H), 4.48 (s, 2 H), 4.95–5.05 (m, 1 H), 5.10 (s, 2 H), 6.81 (d, 1 H, J = 5.0 Hz), 6.88 (d, 1 H, J = 7.7 Hz), 7.05 (d, 1 H, J = 7.7 Hz), 7.12 (d, 1 H, J = 7.7 Hz), 7.16 (d, 1 H, J = 7.7 Hz), 7.25 (s, 1 H), 10.28 (br s, 1 H).

2-[6-Chloromethyl-2-(1-methylpiperidin-4-ylamino)benzoimidazol-1-ylmethyl]-6-methylpyridin-3-ol (Intermediate 20). $SOCl_2$ (2.1 mL) was added dropwise to a mixture of **19** (0.0018 mol) in CH_2Cl_2 (20 mL) at 5 °C. The mixture was stirred at 5 °C for 1 h and then at room temperature for 12 h. The solvent was evaporated until dryness (0.93 g, 100%). The crude product was used directly in the next reaction step.

2-[6-{[(3,5-Dimethylphenyl)-(2-hydroxyethyl)amino]methyl}-2-(1-methylpiperidin-4-ylamino)benzoimidazol-1-ylmethyl]-6-methylpyridin-3-ol (Compound 21a). A mixture of 20 (0.0003 mol), 2-(3,5-dimethylphenylamino)ethanol (0.0005 mol), and K_2CO_3 (0.0019 mol) in DMF (30 mL) was stirred at 80 °C for 4 h and poured into H_2O. The aqueous layer was saturated with K_2CO_3 and extracted with CH_2Cl_2/CH_3OH. The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent was evaporated until dryness. The residue (0.25 g) was purified by column chromatography over silica gel (eluent, CH_2Cl_2/CH_3OH/NH_4OH 90:10:1). The pure fractions were collected, and the solvent was evaporated. The residue (0.05 g, 24%) was crystallized from 2-propanone/DIPE. The precipitate was filtered off and dried (0.042 g, 20%, melting point 176 °C): ^{1}H NMR (DMSO-d_6) δ 1.53 (qd, 2 H, J = 11.1 Hz), 1.97–2.10 (m, 4 H), 2.13 (s, 6 H), 2.20 (s, 3 H), 2.33 (s, 3 H), 2.75 (d, 2 H, J = 11.1 Hz), 3.44 (t, 2 H, J = 6.4 Hz), 3.55 (t, 2 H, J = 6.4 Hz), 3.60–3.70 (m, 1 H), 4.53 (s, 3H), 5.05 (s, 2 H), 6.20 (s, 1 H), 6.33 (s, 2 H), 6.75 (br s, 1 H), 6.79 (s, 1 H, J = 7.7 Hz), 7.03 (d, 1 H, J = 7.7 Hz), 7.10 (d, 1 H, J = 7.7 Hz), 7.12–7.18 (m, 2 H), 11.30 (br s, 1 H); HRMS (ESI), calcd for $C_{31}H_{41}N_6O_2$ 529.3291, found $[MH]^+$ 529.3305. Anal. ($C_{31}H_{40}N_6O_2 \cdot 0.4H_2O$) C, H, N.

2-[6-{[(2-Hydroxyethyl)phenylamino]methyl}-2-(1-methylpiperidin-4-ylamino)-benzoimidazol-1-ylmethyl]-6-methylpyridin-3-ol (Compound 21b). 21b was synthesized from 2-phenylaminoethanol with the procedure described for compound **21a** (28.2%, melting point 229 °C): ^{1}H NMR (DMSO-d_6) δ 1.52 (qd, 2 H, J = 11.1 Hz), 1.95–2.08 (m, 4 H), 2.20 (s, 3 H), 2.33 (s, 3 H), 2.33 (s, 3 H), 2.75 (d, 2 H, J = 11.1 Hz), 3.49 (t, 2 H, J = 6.4 Hz), 3.58 (t, 2 H, J = 6.4 Hz), 3.62–3.72 (m, 1 H), 4.55 (s, 2 H), 4.68 br s, 1H), 5.05 (s, 2 H), 6.53 (t, 1 H, J = 7.7 Hz), 6.65–6.75 (m, 3 H), 6.80 (d, 1 H, J = 7.7 Hz), 7.01–7.12 (m, 4 H), 7.13–7.18 (m, 2 H), 10.25 (br s, 1 H); HRMS (ESI), calcd for $C_{29}H_{37}N_6O_2$ 501.2978, found $[MH]^+$ 501.2981. Anal. ($C_{29}H_{36}N_6O_2 \cdot 0.3H_2O$) C, H, N.

6-Methyl-2-[6-[(methylphenylamino)methyl]-2-(1-methylpiperidin-4-ylamino)benzoimidazol-1-ylmethyl]pyridin-3-ol (Compound 21c). 21c was synthesized from methylphenylamine with the procedure described for compound **21a** (30%, melting point 189 °C): ^{1}H NMR (DMSO-d_6) δ 1.53 (qd, 2 H, J = 11.1 Hz), 1.95–2.09 (m, 4 H), 2.18 (s, 3 H), 2.33 (s, 3 H), 2.73 (d, 2 H, J = 11.1 Hz), 2.97 (s, 3H), 3.58–3.70 (m, 1 H), 4.52 (s, 2H), 5.05 (s, 2 H), 6.58 (t, 1 H, J = 7.7 Hz), 6.70–6.78 (m, 3 H), 6.80 (d, 1 H, J = 7.7 Hz), 7.04 (d, 1 H, J = 7.7 Hz), 7.07–7.20 (m, 5 H), 10.30 (br s, 1 H); HRMS (ESI), calcd for $C_{28}H_{35}N_6O$ 471.2872, found $[MH]^+$ 471.2875. Anal. ($C_{28}H_{34}N_6O$) C, H, N.

6-Methyl-2-[2-(1-methylpiperidin-4-ylamino)-6-*m*-tolylsulfanylmethylbenzoimidazol-1-ylmethyl]pyridin-3-ol (Compound 22). A mixture of **20** (0.0018 mol), 3-methylthiophenol (0.002 mol), and K_2CO_3 (0.0077 mol) in CH_3CN (70 mL) was stirred at 50 °C for 12 h. The solvent was evaporated under reduced pressure. The residue was taken up in H_2O. The mixture was extracted with CH_2Cl_2. The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent was evaporated until dryness. The residue (0.55 g) was purified by column chromatography over silica gel (eluent, CH_2Cl_2/CH_3OH/NH_4OH 88:12:2). The pure fractions were collected, and the solvent was evaporated. The residue (0.35 g, 39%) was crystallized from CH_3CN/DIPE. The precipitate was filtered off and dried (0.32 g, melting point 202 °C): ^{1}H NMR (DMSO-d_6)

δ 1.53 (qd, 2 H, J = 11.1 Hz), 1.95–2.09 (m, 4 H), 2.18 (s, 3 H), 2.24 (s, 3 H), 2.35 (s, 3 H), 2.75 (d, 2 H, J = 11.1 Hz), 3.60–3.72 (m, 1 H), 4.20 (s, 1 H), 5.08 (s, 2 H), 6.80 (s, 1 H), 6.90 (d, 1 H, J = 7.7 Hz), 7.03–7.10 (m, 4 H), 7.17 (d, 1 H, J = 7.7 Hz), 7.23 (d, 2 H, J = 7.7 Hz), 7.28 (s, 1 H), 10.35 (br s, 1 H); HRMS (ESI), calcd for $C_{28}H_{34}N_5OS$ 488.2484, found $[MH]^+$ 488.2469. Anal. ($C_{28}H_{33}N_5OS$) C, H, N.

3-(3-Hydroxy-6-methylpyridin-2-ylmethyl)-2-(1-methylpiperidin-4-ylamino)-3*H*-benzoimidazole-5-carbaldehyde (Intermediate 23). A mixture of **19** (0.0028 mol) and MnO_2 (2.5 g) in CH_2Cl_2 (40 mL) was stirred at room temperature for 12 h and then filtered over Celite. Celite was rinsed with CH_2Cl_2. The filtrate was concentrated under reduced pressure. The residue was taken up in 2-propanone. The precipitate was filtered off and dried (0.75 g, 69%, melting point 250 °C): ^{1}H NMR (DMSO-d_6) δ 1.56 (qd, 2 H, J = 11.1 Hz), 1.95–2.08 (m, 4 H), 2.20 (s, 3 H), 2.34 (s, 3 H), 2.75 (d, 2 H, J = 11.1 Hz), 3.72–3.82 (m, 1 H), 5.22 (s, 2 H), 7.05 (d, 1 H, J = 7.7 Hz), 7.15 (d, 1 H, J = 7.7 Hz), 7.18–7.28 (m, 1 H), 7.30 (d, 1 H, J = 7.7 Hz), 7.54 (dd, 1 H, J = 2.0, 7.7 Hz), 7.73 (d, 1 H, J = 2.0 Hz), 9.85 (s, 1 H).

6-Methyl-2-[2-(1-methylpiperidin-4-ylamino)-6-(2-*m*-tolylvinyl)benzoimidazol-1-ylmethyl]-pyridin-3-ol (Compound 24). 3-Methylbenzylphosphonic acid diethyl ester **14** (0.0027 mol) was added at 5 °C to a mixture of NaH (0.0027 mol) in THF (15 mL) under N_2 flow. The mixture was stirred at 5 °C for 30 min. A solution of **23** (0.0009 mol) in THF (5 mL) was added. The mixture was stirred at 5 °C for 1 h and then stirred at room temperature for 12 h. H_2O was added. The mixture was extracted with CH_2Cl_2. The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent was evaporated until dryness. The residue was purified by column chromatography over silica gel (eluent, CH_2Cl_2/CH_3OH/NH_4OH 85:14:1). The pure fractions were collected, and the solvent was evaporated. The residue was crystallized from 2-propanone. The precipitate was filtered off and dried (0.12 g, 28%, melting point 244 °C): ^{1}H NMR (DMSO-d_6) δ 1.54 (qd, 2 H, J = 11.1 Hz), 1.95–2.09 (m, 4 H), 2.19 (s, 3 H), 2.33 (s, 3 H), 2.37 (s, 3 H), 2.75 (d, 2 H, J = 11.1 Hz), 3.64–3.75 (m, 1 H), 5.17 (s, 2 H), 6.84 (s, 1 H), 6.99 (d, 1 H, J = 16.2 Hz), 7.02–7.10 (m, 2 H), 7.13–7.30 (m, 5 H), 7.36 (d, 2 H, J = 7.7 Hz), 7.39 (s, 1 H), 7.53 (s, 3 H), 10.35 (br s, 1 H); HRMS (ESI), calcd for $C_{29}H_{34}N_5O$ 468.2763, found $[MH]^+$ 468.2751. Anal. ($C_{29}H_{33}N_5O \cdot 0.3H_2O$) C, H, N.

6-Methyl-2-[2-(1-methylpiperidin-4-ylamino)-6-(2-*m*-tolylethyl)benzoimidazol-1-ylmethyl]-pyridin-3-ol (Compound 25). A mixture of **24** (0.0001 mol) and Pd/C 10% (0.015 g) in CH_3OH (3 mL) and THF (3 mL) was hydrogenated at room temperature for 4 h under a 2 bar pressure and then filtered over Celite. The Celite was washed with H_2O. The filtrate was concentrated under reduced pressure. The residue was crystallized from CH_3OH/2-propanone. The precipitate was filtered off and dried (0.04 g, 80%, melting point 246 °C): ^{1}H NMR (DMSO-d_6) δ 1.53 (qd, 2 H, J = 11.1 Hz), 1.95–2.09 (m, 4 H), 2.18 (s, 3 H), 2.27 (s, 3 H), 2.35 (s, 3 H), 2.70–2.90 (m, 6 H), 3.60–3.72 (m, 1 H), 5.09 (s, 2 H), 6.65–6.75 (m, 1 H), 6.79 (d, 2 H, J = 5.5 Hz), 6.95–7.08 (m, 5 H), 7.12–7.22 (m, 3 H), 10.35 (br s, 1 H); HRMS (ESI), calcd for $C_{29}H_{36}N_5O$ 470.2920, found $[MH]^+$ 470.2911. Anal. ($C_{29}H_{35}N_5O \cdot 0.3H_2O$) C, H, N.

2-[6-[(3,5-Dimethylphenylamino)methyl]-2-(1-methylpiperidin-4-ylamino)benzoimidazol-1-ylmethyl]-6-methylpyridin-3-ol (Compound 26a). A mixture of **23** (0.0004 mol), 3,5-dimethylaniline (0.00047 mol), BH_3CN- on solid support (0.00047 mol), and CH_3CO_2H (4 drops) in CH_3OH (8 mL) was stirred at room temperature for 24 h. The solution was filtered. The filtrate was concentrated under reduced pressure. The residue was purified by column chromatography over silica gel (eluent, CH_2Cl_2/CH_3OH/NH_4OH 87:12:1.5). The pure fractions were collected, and the solvent was evaporated (0.12 g). The compound was crystallized in CH_3CN/DIPE (0.08 g, 42%, melting point 235 °C): ^{1}H NMR (DMSO-d_6) δ 1.47–1.60 (m, 2 H), 1.97–2.08 (m, 4 H), 2.10 (s, 6 H), 2.20 (s, 3 H), 2.33 (s, 3 H), 2.72–2.80 (m, 2 H), 3.65–3.75 (m, 1 H), 4.20 (d, 2 H, J = 5.5 Hz), 5.10 (s, 2 H), 5.80 (t, 1 H, J = 5.5 Hz), 6.15 (s, 1 H), 6.22 (s, 2H), 6.70–6.75 (m, 1 H), 6.95 (d, 1 H, J = 7.7 Hz), 7.05 (d, 1 H, J = 7.7 Hz), 7.12 (d, 1 H, J = 7.7 Hz), 7.17 (d, 1 H, J = 7.7 Hz), 7.28 (s, 1H), 10.25 (br s, 1 H); HRMS (ESI), calcd for $C_{29}H_{37}N_6O$ 485.3029, found $[MH]^+$ 485.3028.

6-Methyl-2-[2-(1-methylpiperidin-4-ylamino)-6-(*m*-tolylaminomethyl)benzoimidazol-1-ylmethyl]pyridin-3-ol (Compound 26b). 23 (0.0005 mol), $NaBH_3CN$ (0.0006 mol), and then CH_3CO_2H (0.2 mL) were added at room temperature to a mixture of 3-methylaniline (0.0006 mol) in CH_3CN (20 mL). The mixture was stirred at room temperature for 12 h. H_2O was added. The mixture was saturated with K_2CO_3 (powder) and extracted with CH_2Cl_2/CH_3OH. The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent was evaporated until dryness. The residue (0.3 g) was purified by column chromatography over silica gel (eluent, CH_2Cl_2/CH_3OH/NEt_3 90:10:0.1). The pure fractions were collected, and the solvent was evaporated. The residue (0.17 g, 68%) was crystallized from CH_3OH/2-propanone/DIPE. The precipitate was filtered off and dried (0.13 g, 52%, melting point 141 °C): ^{1}H NMR (DMSO-d_6) δ 1.53 (qd, 2 H, J = 11.1 Hz), 1.97–2.10 (m, 4 H), 2.15 (s, 3 H), 2.20 (s, 3 H), 2.33 (s, 3 H), 2.75 (d, 2 H, J = 11.1 Hz), 3.63–3.72 (m, 1 H), 4.20 (d, 2 H, J = 5.5 Hz), 5.10 (s, 2 H), 5.94 (t, 1 H, J = 5.5 Hz), 6.31 (d, 1 H, J = 7.7 Hz), 6.38 (d, 1 H, J = 7.7 Hz), 6.42 (s, 1 H), 6.77 (br s, 1H), 6.91 (t, 1 H, J = 7.7 Hz), 7.95 (d, 1 H, J = 7.7 Hz), 7.04 (d, 1 H, J = 7.7 Hz), 7.10–7.19 (m, 2 H), 7.29 (s, 1H), 10.30 (br s, 1 H); HRMS (ESI), calcd for $C_{28}H_{35}N_6O$ 471.2872, found $[MH]^+$ 471.2898. Anal. ($C_{28}H_{34}N_6O \cdot 0.5H_2O$) C, H, N.

6-Methyl-2-[2-(1-methylpiperidin-4-ylamino)-6-phenylaminomethylbenzoimidazol-1-ylmethyl]pyridin-3-ol (Compound 26c). 26c was synthesized from phenylamine with the procedure described for compound **26b** (51.4%, melting point 206 °C): ^{1}H NMR (DMSO-d_6) δ 1.53 (qd, 2 H, J = 11.1 Hz), 1.95–2.08 (m, 4 H), 2.19 (s, 3 H), 2.33 (s, 3 H), 2.75 (d, 2 H, J = 11.1 Hz), 3.63–3.72 (m, 1H), 4.23 (d, 1 H, J = 5.5 Hz), 5.09 (s, 2 H), 6.03 (t, 1 H, J = 5.5 Hz), 6.48 (t, 1 H, J = 7.7 Hz), 6.58 (d, 2 H, J = 7.7 Hz), 6.78 (br s, 1 H), 6.95 (d, 1 H, J = 7.7 Hz), 6.97–7.07 (m, 3 H), 7.10–7.18 (m, 2 H), 7.30 (s, 1 H), 10.30 (br s, 1 H); HRMS (ESI), calcd for $C_{27}H_{33}N_6O$ 457.2716, found $[MH]^+$ 457.2731. Anal. ($C_{27}H_{32}N_6O \cdot 0.4H_2O$) C, H, N.

2-[6-(Hydroxyphenylmethyl)-2-(1-methylpiperidin-4-ylamino)benzoimidazol-1-ylmethyl]-6-methylpyridin-3-ol (Compound 27). A solution of bromobenzene (0.002 mol) in THF was added to a mixture of magnesium (0.002 mol) in a minimum of THF under N_2 flow. The mixture was stirred at a temperature above 30 °C and then cooled to room temperature. A solution of **23** (0.0003 mol) in THF (7 mL) was added dropwise. The mixture was stirred at room temperature for 3 h. H_2O was added. Then, CH_2Cl_2 was added. The mixture was filtered over Celite. The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent was evaporated until dryness. The residue was purified by column chromatography over silica gel (eluent, CH_2Cl_2/CH_3OH/NH_4OH 84:14:1). The pure fractions were collected, and the solvent was evaporated. The residue (0.1 g, 55%) was crystallized from DIPE. The precipitate was filtered off and dried (0.092 g, 51%, melting point 144 °C): ^{1}H NMR (DMSO-d_6) δ 1.52 (qd, 2 H, J = 11.1 Hz), 1.95–2.09 (m, 4 H), 2.18 (s, 3 H), 2.33 (s, 3 H), 2.70–2.80 (m, 2 H), 3.60–3.70 (m, 1 H), 5.08 (s, 2 H), 5.68 (s, 1 H), 5.73 (s, 1 H), 6.75 (s, 1 H), 6.88 (d, 1 H, J = 7.7 Hz), 7.05 (t, 2 H, J = 7.7 Hz), 7.03–7.19 (m, 2 H), 7.25 (t, 2 H, J = 7.7 Hz), 7.32–7.40 (m, 3 H), 10.35 (br s, 1 H); HRMS (ESI), calcd for $C_{27}H_{32}N_5O_2$ 458.2556, found $[MH]^+$ 458.2564. Anal. ($C_{27}H_{31}N_5O_2 \cdot 1H_2O$) C, H, N.

4-(6-Nitro-1*H*-benzoimidazol-2-ylamino)piperidine-1-carboxylic Acid Ethyl Ester (Intermediate 30). A mixture of **28** (0.0524 mol) and **29** (0.1048 mol) was stirred at 120 °C in a Parr pressure vessel for 10 h, then taken up into H_2O, and extracted with AcOEt. The concentrated organic layer was purified by short open column chromatography over silica gel (eluent, CH_2Cl_2/CH_3OH 96:4). The product fractions were collected, and the solvent was evaporated until dryness (7.7 g, 44%): ^{1}H NMR (DMSO-d_6) δ 1.19 (t, 3H, J = 6.9 Hz), 1.40 (qd, 2 H, J = 11.1 Hz), 1.95 (d,

2 H, J = 11.1 Hz), 2.90–3.05 (m, 2 H), 3.80–3.90 (m, 1 H), 3.95 (d, 2 H, J = 11.1 Hz), 4.05 (qd, 2 H, J = 6.9 Hz), 7.24 (d, 1 H, J = 7.7 Hz), 7.40–7.55 (m, 1 H), 7.88 (d, 1 H, J = 7.7 Hz), 7.95 (s, 1 H), 11.25 (br s, 1 H).

4-[1-(3-Hydroxy-6-methylpyridin-2-ylmethyl)-6-nitro-1*H*-benzoimidazol-2-ylamino]piperidine-1-carboxylic Acid Ethyl Ester and 4-[1-(3-Hydroxy-6-methylpyridin-2-ylmethyl)-5-nitro-1*H*-benzoimidazol-2-ylamino]piperidine-1-carboxylic Acid Ethyl Ester (Intermediates 31 and 32). A mixture of **30** (0.0312 mol), **6** (0.0343 mol), and K_2CO_3 (0.1092 mol) in DMF (100 mL) was stirred at 70 °C for 24 h. H_2O was then added. The mixture was extracted with CH_2Cl_2. The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent was evaporated until dryness. The residue (12.2 g) was purified by column chromatography over silica gel (eluent, toluene/iPrOH/NH_4OH 90:10:0.5). Two fractions were collected, and the solvent was evaporated. Four grams of intermediate **31** (28%) were obtained: ^{1}H NMR (DMSO-d_6) δ 1.20 (t, 3H, J = 6.9 Hz), 1.48 (qd, 2 H, J = 11.1 Hz), 2.00 (d, 2 H, J = 11.1 Hz), 2.30 (s, 3, H), 3.00–3.10 (m, 2 H), 3.94 (d, 2 H, J = 11.1 Hz), 4.05 (qd, 2 H, J = 6.9 Hz), 5.28 (s, 2 H), 7.05 (d, 1 H, J = 7.7 Hz), 7.15 (d, 1 H, J = 7.7 Hz), 7.28 (d, 1 H, J = 7.7 Hz), 7.34 (d, 1 H, J = 7.7 Hz), 7.94 (dd, 1 H, J = 2.5, 7.7 Hz), 8.13 (d, 1 H, J = 2.5 Hz), 10.32 (br s, 1 H). Intermediate **32** as obtained in a yield of 5.4 g (38%): ^{1}H NMR (DMSO-d_6) δ 1.19 (t, 3 H, J = 7.1 Hz), 1.39–1.51 (m, 2 H), 1.98–2.05 (m, 2 H), 2.28 (s, 3 H), 3.00–3.10 (m, 2 H), 3.88–3.96 (m, 3 H), 4.06 (qd, 2 H, J = 7.1 Hz), 5.28 (s, 2 H), 7.05 (d, 1 H, J = 7.7 Hz), 7.12–7.20 (m, 3H), 7.24 (d, 1 H, J = 7.7 Hz), 7.89 (dd, 1 H, J = 2.0, 7.7 Hz), 8.00 (d, 1 H, J = 2.0 Hz), 10.28 (br s, 1H).

6-Methyl-2-[6-nitro-2-(piperidin-4-ylamino)benzoimidazol-1-ylmethyl]pyridin-3-ol (Intermediate 33). Intermediate **31** (0.0088 mol) was added portionwise to a 48% solution of HBr in water (40 mL). The mixture was brought slowly to 70 °C and then stirred for 12 h. The precipitate was filtered, washed with CH_3CN, and dried. The residue (4.6 g, 80%) was taken up in H_2O and basified with K_2CO_3 (powder). The precipitate was filtered and then rinsed with EtOH. The filtrate was concentrated under reduced pressure (3 g, 52%).

6-Methyl-2-[2-(1-methylpiperidin-4-ylamino)-6-nitrobenzoimidazol-1-ylmethyl]pyridin-3-ol (Intermediate 34). HCHO 37% in H_2O (0.0152 mol) and then $NaBH_3CN$ (0.0091 mol) were added at room temperature to a mixture of **33** (0.0075 mol) in CH_3CN (100 mL). Acetic acid (3.5 mL) was added slowly at room temperature. The mixture was stirred at room temperature for 12 h and poured into H_2O. The aqueous layer was saturated with K_2CO_3 (powder). The mixture was extracted with EtOAc/CH_3OH. The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent was evaporated until dryness (2.6 g, 87%).

2-[6-Amino-2-(1-methylpiperidin-4-ylamino)benzoimidazol-1-ylmethyl]-6-methylpyridin-3-ol (Intermediate 35). A mixture of **34** (0.0065 mol) and Raney nickel (2.6 g) in CH_3OH (100 mL) was hydrogenated at room temperature for 1 h under a 3 bar pressure and then filtered over Celite. The Celite was washed with CH_3OH. The filtrate was concentrated under reduced pressure. The residue (2.2 g) was purified by column chromatography over silica gel (eluent, CH_2Cl_2/CH_3OH/NH_4OH 85:14:1). The pure fractions were collected, and the solvent was evaporated (0.85 g, 35%): ^{1}H NMR (DMSO-d_6) δ 1.50 (qd, 2 H, J = 11.1 Hz), 1.97–2.10 (m, 4 H), 2.20 (s, 3 H), 2.35 (s, 3 H), 2.70–2.80 (m, 2 H), 3.55–3.65 (m, 1 H), 4.60 (br s, 2 H), 4.95 (s, 2 H), 6.25 (dd, 1 H, J = 2.1, 7.7 Hz), 6.55 (d, 1 H, J = 2.1 Hz), 6.60 (br s, 1 H), 6.86 (d, 1 H, J = 7.7 Hz), 7.05 (d, 1 H, J = 7.7 Hz), 7.15 (d, 1 H, J = 7.7 Hz), 10.20 (br s, 1 H).

***N*-[3-(3-Hydroxy-6-methylpyridin-2-ylmethyl)-2-(1-methylpiperidin-4-ylamino)-3*H*-benzoimidazol-5-yl]-3-methylbenzamide (Compound 36).** A mixture of **35** (0.000125 mol) and 3-methylbenzoic acid (0.00025 mol) in CH_2Cl_2 (4 mL) was stirred at room temperature. HOBT (0.00025 mol) and EDCI (0.00025 mol) were added. The reaction was stirred at room temperature for 12 h. The solution was concentrated under reduced pressure, and a 10% solution of $NaHCO_3$ in water (2 mL) and CH_3OH (2 mL) were added. The mixture was stirred and refluxed for 4 h. CH_3OH was then removed under reduced pressure, and the resulting solution extracted with CH_2Cl_2. The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent was evaporated until dryness. The residue was purified by column chromatography over silica gel (eluent, CH_2Cl_2/CH_3OH/NH_4OH 90:10:0.5). The pure fractions were collected, and the solvent was evaporated (0.04 g, 60%): HRMS (ESI), calcd for $C_{28}H_{33}N_6O_2$ 485.2665, found $[MH]^+$ 485.2660.

6-Methyl-2-[6-(3-methylbenzylamino)-2-(1-methylpiperidin-4-ylamino)benzoimidazol-1-ylmethyl]pyridin-3-ol (Compound 37). A mixture of **35** (0.0005 mol), 3-methylbenzaldehyde (0.0006 mol), BH_3CN- on solid support (0.0006 mol), and acetic acid (8 drops) in CH_3OH (10 mL) was stirred at room temperature for 24 h. The solid support was filtered off and rinsed with CH_3OH. The filtrate was concentrated under reduced pressure. The residue (0.53 g) was purified by column chromatography over silica gel (eluent, CH_2Cl_2/CH_3OH/NH_4OH 92:8:0.5 to 89:10:1). The pure fractions were collected, and the solvent was evaporated. The residue (0.11 g) was crystallized from CH_3OH/DIPE. The precipitate was filtered off and dried (0.072 g, 28%, melting point 240 °C): ^{1}H NMR (DMSO-d_6) δ 1.490 (qd, 2 H, J = 11.1 Hz), 1.95–2.07 (m, 4 H), 2.17 (s, 3 H), 2.27 (s, 3 H), 2.35 (s, 3 H), 2.75 (d, 2 H, J = 11.1 Hz), 3.52–3.72 (m, 1 H), 4.15 (d, 2 H, J = 5.5 Hz), 4.95 (s, 2 H), 5.67 (t, 1 H, J = 5.5 Hz), 6.28 (d, 1 H, J = 7.7 Hz), 6.53 (br s, 1 H), 6.63 (s, 1 H), 6.90 (d, 1 H, J = 7.7 Hz), 6.99–7.08 (m, 2H), 7.14–7.23 (m, 4 H), 10.33 (br s, 1 H); HRMS (ESI), calcd for $C_{28}H_{35}N_6O$ 471.2872, found $[MH]^+$ 471.2853. Anal. ($C_{28}H_{34}N_6O$) C, H, N.

{4-[6-Hydroxymethyl-1-(3-hydroxy-6-methylpyridin-2-ylmethyl)-1*H*-benzoimidazol-2-ylamino]piperidin-1-yl}acetic Acid Ethyl Ester (Intermediate 38). A mixture of **10** (0.0027 mol), ethyl bromoacetate (0.0032 mol), and NEt_3 (0.004 mol) in DMF (40 mL) was stirred at 50 °C for 1 h, poured into ice–water, and extracted three times with CH_2Cl_2. The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent was evaporated until dryness. The residue was taken up in 2-propanone/DIPE. The precipitate was filtered off, rinsed with H_2O, and dried (1 g, 82%, melting point 206 °C): ^{1}H NMR (DMSO-d_6) δ 1.20 (t, 3H, J = 6.9 Hz), 1.54 (qd, 2 H, J = 11.1 Hz), 2.03 (d, 2 H, J = 11.1 Hz), 2.28–2.40 (m, 5 H), 2.85 (d, 2 H, J = 11.1 Hz), 3.25 (s, 2 H), 3.65–3.75 (m, 1 H), 4.09 (qd, 2 H, J = 6.9 Hz), 4.48 (d, 1 H, J = 5.0 Hz), 5.03 (t, 1 H, J = 5.0 Hz), 5.12 (s, 2 H), 6.80–6.85 (m, 1 H), 6.88 (d, 1 H, J = 7.7 Hz), 7.05 (d, 1 H, J = 7.7 Hz), 7.12 (d, 1 H, J = 7.7 Hz), 7.15 (d, 1 H, J = 7.7 Hz), 7.25 (s, 1 H), 10.28 (s, 1 H).

{4-[6-Formyl-1-(3-hydroxy-6-methylpyridin-2-ylmethyl)-1*H*-benzoimidazol-2-ylamino]piperidin-1-yl}acetic Acid Ethyl Ester (Intermediate 39). A mixture of **38** (0.0044 mol) and MnO_2 (4 g) in CH_2Cl_2 (60 mL) was stirred at room temperature for 12 h then filtered over Celite. The Celite was rinsed with H_2O. The filtrate was concentrated under reduced pressure. The residue was taken up in CH_3CN. The precipitate was filtered off and dried (1.8 g, 91%): ^{1}H NMR (DMSO-d_6) δ 1.20 (t, 3H, J = 6.9 Hz), 1.58 (qd, 2 H, J = 11.1 Hz), 2.00 (d, 2 H, J = 11.1 Hz), 2.30–2.40 (m, 5 H), 2.86 (d, 2 H, J = 11.1 Hz), 3.25 (s, 2 H), 3.75–3.85 (m, 1 H), 4.09 (qd, 2 H, J = 6.9 Hz), 5.23 (s, 2 H), 7.05 (d, 1 H, J = 7.7 Hz), 7.15 (d, 1 H, J = 7.7 Hz), 7.20 (d, 1 H, J = 7.7 Hz), 7.31 (d, 1 H, J = 7.7 Hz), 7.54 (d, 1 H, J = 7.7 Hz), 7.73 (s, 1 H), 9.85 (s, 1 H), 10.32 (s, 1 H).

{4-[1-(3-Hydroxy-6-methylpyridin-2-ylmethyl)-6-(*m*-tolylaminomethyl)-1*H*-benzoimidazol-2-ylamino]piperidin-1-yl}acetic Acid Ethyl Ester (Intermediate 40a). CH_3CO_2H (0.2 mL) was added at room temperature to a mixture of **39** (0.0004 mol), 3-methylaniline (0.0005 mol), and $NaBH_3CN$ (0.0005 mol) in CH_3CN (20 mL). The mixture was stirred at room temperature for 6 h. CH_3CO_2H (0.2 mL) was added. The mixture was stirred at room temperature for 12 h. The solvent was evaporated until dryness. The residue was taken up in CH_2Cl_2/K_2CO_3 10%. The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent

was evaporated until dryness (0.22 g, 100%). This product was used directly in the next reaction step.

{4-[6-[(3,5-Dimethylphenylamino)methyl]-1-(3-hydroxy-6-methylpyridin-2-ylmethyl)-1*H*-benzoimidazol-2-ylamino]piperidin-1-yl}acetic Acid Ethyl Ester (Intermediate 40b). 40b was synthesized from 3,5-dimethylphenylamine with the procedure described for intermediate **40a** (100%).

2-[2-[1-(2-Hydroxyethyl)piperidin-4-ylamino]-6-(*m*-tolylaminomethyl)benzoimidazol-1-ylmethyl]-6-methylpyridin-3-ol (Compound 41a). $LiAlH_4$ (0.0008 mol) was added to a mixture of **40a** (0.0004 mol) in THF (20 mL) at 5 °C under N_2 flow. The mixture was stirred at 5 °C for 1 h, then brought to room temperature, and stirred for 4 h. A minimum of H_2O and then CH_2Cl_2 were added. The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent was evaporated until dryness. The residue (0.22 g) was purified by column chromatography over silica gel (eluent, $CH_2Cl_2/CH_3OH/NH_4OH$ 85:15:1). The pure fractions were collected, and the solvent was evaporated. The residue (0.1 g, 50%) was crystallized from CH_3OH/CH_3CN/DIPE. The precipitate was filtered off and dried (0.08 g, 40%, melting point 137 °C): ^{1}H NMR (DMSO-d_6) δ 1.50–1.65 (m, 2 H), 2.05 (d, 2H, J = 11.1 Hz), 2.13 (s, 3 H), 2.25–2.38 (m, 5 H), 2.45–2.60 (m, 2 H), 2.92–3.04 (m, 2 H), 3.52–3.60 (m, 2 H), 3.68–3.80 (m, 1 H), 4.20 (d, 2 H, J = 5.2 Hz), 5.10 (s, 2 H), 5.95 (t, 1 H, J = 5.2 Hz), 6.30 (d, 1 H, J = 7.7 Hz), 6.37 (d, 1 H, J = 7.7 Hz), 6.42 (s, 1 H), 6.77 (s, 1 H), 6.89 (t, 1 H, J = 7.7 Hz), 6.95 (d, 1 H, J = 7.7 Hz), 7.04 (d, 1 H, J = 7.7 Hz), 7.12 (d, 1 H, J = 7.7 Hz), 7.17 (d, 1 H, J = 7.7 Hz), 7.28 (s, 1 H), 10.30 (br s, 1 H); HRMS (ESI), calcd for $C_{29}H_{37}N_6O_2$ 501.2978, found $[MH]^+$ 501.2970. Anal. ($C_{29}H_{36}N_6O_2 \cdot 2.8H_2O$) C, H, N.

2-{6-[(3,5-Dimethylphenylamino)methyl]-2-[1-(2-hydroxyethyl)piperidin-4-ylamino]benzoimidazol-1-ylmethyl}-6-methylpyridin-3-ol (Compound 41b). 41b was synthesized from intermediate **40b** with the procedure described for compound **41a** (0.153 g, 66%, melting point 128 °C): ^{1}H NMR (DMSO-d_6) δ 1.57 (qd, 2H, J = 11.1 Hz), 2.05 (d, 2H, J = 11.1 Hz), 2.14 (s, 6 H), 2.23 (t, 2H, J = 11.1 Hz), 2.37 (s, 3 H), 2.46 (t, 2H, J = 6.1 Hz), 2.86 (d, 2H, J = 11.1 Hz), 3.53 (t, 2H, J = 6.1 Hz), 3.70–3.80 (m, 1 H), 4.24 (d, 2H, J = 5.1 Hz), 5.11 (s, 2 H), 5.42 (s, 1 H), 6.19 (s, 1 H), 6.27 (s, 2 H), 6.52 (br s, 1 H), 6.95 (d, 1 H, J = 7.7 Hz), 7.04 (d, 1 H, J = 7.7 Hz), 7.13 (d, 1 H, J = 7.7 Hz), 7.17 (d, 1 H, J = 7.7 Hz), 7.33 (s, 1 H), 10.35 (br s, 1 H); HRMS (ESI), calcd for $C_{30}H_{39}N_6O_2$ 515.3134, found $[MH]^+$ 515.3119.

2-[3-(4-Methylpiperazin-1-yl)propylamino]-3*H*-benzoimidazole-5-carboxylic Acid Ethyl Ester (Intermediate 42a). A mixture of **3** (0.0222 mol) and 1-(3-aminopropyl)-4-methylpiperazine (0.0267 mol) was stirred at 140 °C for 1 h and then taken up in CH_2Cl_2/CH_3OH. The organic layer was washed with K_2CO_3 10%, dried (over $MgSO_4$), and filtered, and the solvent was evaporated until dryness. The residue was purified by column chromatography over silica gel (eluent, $CH_2Cl_2/CH_3OH/NH_4OH$ 88:12:1). The pure fractions were collected, and the solvent was evaporated (1 g, 13%): ^{1}H NMR (DMSO-d_6) δ 1.30 (t, 3 H, J = 6.8 Hz), 1.72 (qt, 2 H, J = 7.0 Hz), 2.15 (s, 3 H), 2.20–2.45 (m, 10 H), 3.30 (qd, 2 H, J = 7.0 Hz), 4.25 (qd, 2 H, J = 6.8 Hz), 6.93–7.05 (m, 1 H), 7.15 (d, 1 H,J = 7.7 Hz), 7.57 (d, 1 H, J = 7.7 Hz), 7.70 (s, 1 H), 11.00 (br s, 1 H).

3-(3-Hydroxy-6-methylpyridin-2-ylmethyl)-2-[3-(4-methylpiperazin-1-yl)propylamino]-3*H*-benzoimidazole-5-carboxylic Acid Ethyl Ester (Intermediate 43a) and 1-(3-Hydroxy-6-methylpyridin-2-ylmethyl)-2-[3-(4-methylpiperazin-1-yl)propylamino]-1*H*-benzoimidazole-5-carboxylic Acid Ethyl Ester (Intermediate 44a). A mixture of **42a** (0.0055 mol), **6** (0.006 mol), and K_2CO_3 (0.0165 mol) in CH_3CN (25 mL) was stirred at room temperature for 24 h, poured into ice–water, and extracted with CH_2Cl_2. The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent was evaporated until dryness. The residue was purified by column chromatography over silica gel (eluent, $CH_2Cl_2/CH_3OH/NH_4OH$ 84:15:1). The pure fractions were collected, and the solvent was evaporated [1.4 g of the mixture of intermediates **43a** + **44a** (60:40), 54.7%]; ^{1}H NMR (DMSO-d_6) δ 1.31 (t, 3 H, J = 6.8 Hz), 1.78 (qt, 2 H, J = 7.0 Hz), 2.14 (s, 3 H), 2.20–2.40 (m, 11 H), 3.40–3.50 (m, 2 H), 4.25 (qd, 2 H, J = 7.0 Hz), 5.18 (s, 2 H), 7.03 (d, 1 H, J = 7.7 Hz), 7.12–7.25 (m, 3 H), 7.55–7.64 (m, 1 Hm), 7.74–7.82 (m, 1 H).

2-{6-Hydroxymethyl-2-[3-(4-methylpiperazin-1-yl)propylamino]benzoimidazol-1-ylmethyl}-6-methylpyridin-3-ol (Intermediate 45a) and 2-{5-Hydroxymethyl-2-[3-(4-methylpiperazin-1-yl)-propylamino]benzoimidazol-1-ylmethyl}-6-methylpyridin-3-ol (Intermediate 46a). $LiAlH_4$ (0.009 mol) was added slowly at 5 °C to a mixture of **43a** + **44a** (0.0015 mol) in THF (50 mL) under N_2 flow. The mixture was stirred at 5 °C for 1 h and then stirred at room temperature for 12 h. A minimum of H_2O was added. Next, CH_2Cl_2/CH_3OH was added. The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent was evaporated until dryness. The residue (1.2 g) was purified by column chromatography over silica gel (eluent, $CH_2Cl_2/CH_3OH/NH_4OH$ 80:20:2). Two fractions were collected, and the solvent was evaporated (0.37 g of F1 and 0.53 g of F2). F1 was crystallized from 2-propanone/CH_3CN/DIPE. The precipitate was filtered off and dried (0.34 g of intermediate **45a**, 25.2%; melting point 230 °C): ^{1}H NMR (DMSO-d_6) δ 1.78 (qt, 2 H, J = 6.4 Hz), 2.12 (s, 3 H), 2.20–2.42 (m, 13 H), 3.39 (t, 2 H, J = 6.4 Hz), 4.46 (s, 2 H), 4.98 (br s, 1 H), 5.10 (s, 2 H), 6.79 (br s, 1 H), 6.88 (d, 1 H, J = 7.7 Hz), 7.03 (d, 1 H, J = 7.7 Hz), 7.09 (d, 1 H, J = 7.7 Hz), 7.13 (d, 1 H, J = 7.7 Hz), 7.18 (s, 1 H), 10.12 (br s, 1 H).

F2 was crystallized from 2-propanone/CH_3CN/DIPE. The precipitate was filtered off and dried (0.5 g of intermediate **46a**, 39.4%; melting point 154 °C): ^{1}H NMR (DMSO-d_6) δ 1.77 (qt, 2 H, J = 6.8 Hz), 2.12 (s, 3 H), 2.16–2.44 (m, 13 H), 3.39 (t, 2 H, J = 6.8 Hz), 4.45 (s, 2 H), 4.93 (s, 1 H), 5.10 (s, 2 H), 6.80–6.85 (m, 2 H), 7.02 (d, 1 H, J = 7.7 Hz), 7.07–7.17 (m, 3 H), 10.09 (br s, 1 H).

3-(3-Hydroxy-6-methylpyridin-2-ylmethyl)-2-[3-(4-methylpiperazin-1-yl)propylamino]-3*H*-benzoimidazole-5-carbaldehyde (Intermediate 47a). A mixture of **45a** (0.0006 mol) and MnO_2 (0.6 g) in CH_2Cl_2 (60 mL) was stirred at room temperature for 12 h and then filtered over Celite. The Celite was rinsed with H_2O. The filtrate was concentrated under reduced pressure (0.28 g, 100%).

2-{6-[(3,5-Dimethylphenylamino)methyl]-2-[3-(4-methylpiperazin-1-yl)propylamino]benzoimidazol-1-ylmethyl}-6-methylpyridin-3-ol (Compound 48a). Acetic acid (0.3 mL) was added at room temperature to a mixture of **47a** (0.0006 mol), 3,5-dimethylaniline hydrochloride (0.0007 mol), and $NaBH_3CN$ (0.0007 mol) in CH_3CN (30 mL). The mixture was stirred at room temperature for 30 min. Acetic acid (0.3 mL) was added, and the mixture was stirred at room temperature for 12 h. Then, the solvent was evaporated until dryness, the residue was taken up in EtOH/2-propanol/saturated HCl, and the mixture was stirred at 80 °C for 12 h. Next, the solvent was evaporated until dryness. The mixture was extracted with CH_2Cl_2/K_2CO_3 10%. The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent was evaporated until dryness. The residue (0.43 g) was purified by column chromatography over silica gel (eluent, $CH_2Cl_2/CH_3OH/NH_4OH$ 88:12:0.5). The pure fractions were collected, and the solvent was evaporated. The residue (0.14 g, 40%) was crystallized from 2-propanone/DIPE. The precipitate was filtered off and dried (0.107 g, 30.6%; melting point 150 °C): ^{1}H NMR (DMSO-d_6) δ 1.78 (qt, 2 H, J = 6.4 Hz), 2.08 (s, 6 H), 2.12 (s, 3 H), 2.20–2.40 (m, 13 H), 3.39 (t, 2 H, J = 6.4 Hz), 4.17 (d, 2 H, J = 6.4 Hz), 5.10 (s, 2 H), 5.82 (t, 1 H, J = 5.1 Hz), 6.14 (s, 1 H), 6.20 (s, 2 H), 6.93 (d, 1 H, J = 7.7 Hz), 7.02 (d, 1 H, J = 7.7 Hz), 7.10 (d, 1 H, J = 7.7 Hz), 7.14 (d, 1H, J = 7.7 Hz), 7.20 (s, 1 H), 10.20 (brs, 2 H); HRMS (ESI), calcd for $C_{31}H_{42}N_7O$ 528.3451, found $[MH]^+$ 528.3434. Anal. ($C_{31}H_{41}N_7O \cdot 0.45H_2O$) C, H, N.

2-[(2,2-Dimethyl-[1,3]dioxolan-4-ylmethyl)amino]-3*H*-benzoimidazole-5-carboxylic Acid Ethyl Ester (Intermediate 42b). 42b was synthesized from (2,2-dimethyl-[1,3]dioxolan-4-yl)methylamine with the procedure described for compound **42a** (43.7%): ^{1}H NMR (DMSO-d_6) δ 1.25 (s, 3 H), 1.32 (t, 3 H, J = 6.8 Hz), 1.37 (s, 3 H), 3.37–3.50 (m, 2 H), 3.72 (t, 1 H, J = 7.7 Hz), 4.02

(t, 1 H, J = 7.7 Hz), 4.20–4.30 (m, 3 H), 6.90–7.00 (m, 1 H), 7.10–7.20 (m, 1 H), 7.55–7.65 (m, 1 H), 7.72 (s, 1 H), 10.90 (br s, 1H).

2-[(2,2-Dimethyl-[1,3]dioxolan-4-ylmethyl)amino]-3-(3-hydroxy-6-methylpyridin-2-ylmethyl)-3*H*-benzoimidazole-5-carboxylic Acid Ethyl Ester (43b) and 2-[(2,2-Dimethyl-[1,3]dioxolan-4-ylmethyl)amino]-1-(3-hydroxy-6-methylpyridin-2-ylmethyl)-1*H*-benzoimidazole-5-carboxylic Acid Ethyl Ester (44b). 43b and **44b** were synthesized from **42b** with the procedure described for compounds **43a** + **44a** (40:60 mixture, 78.9%): ^{1}H NMR (DMSO-d_6) δ 1.27 (s, 3 H), 1.31 (t, 3 H, J = 6.8 Hz), 1.37 (s, 3 H), 2.30–2.35 (m, 3 H), 3.50–3.60 (m, 2 H), 3.75 (t, 1 H, J = 7.7 Hz), 4.03 (t, 1 H, J = 7.7 Hz), 4.25 (qd, 3 H, J = 6.8 Hz), 4.35 (qt, 1 H, J = 5.7 Hz), 5.15–5.25 (m, 2 H), 7.05 (d, 1 H, J = 7.7 Hz), 7.12–7.35 (m, 3 H), 7.56–7.65 (m, 1 H), 7.75 (s, 1 H), 10.23 (br s, 1 H).

2-{2-[(2,2-Dimethyl-[1,3]dioxolan-4-ylmethyl)amino]-6-hydroxymethylbenzoimidazol-1-ylmethyl}-6-methylpyridin-3-ol (45b) and 2-{2-[(2,2-Dimethyl-[1,3]dioxolan-4-ylmethyl)amino]-5-hydroxymethylbenzoimidazol-1-ylmethyl}-6-methylpyridin-3-ol (46b). 45b and **46b** were synthesized from the mixture **43b** + **44b** with the procedure described for **45a** and **46a**. 22% of **45b**: ^{1}H NMR (DMSO-d_6) δ 1.28 (s, 3 H), 1.38 (s, 3 H), 2.34 (s, 3 H), 3.45–3.55 (m, 2 H), 3.75 (t, 1 H, J = 7.7 Hz), 4.04 (t, 1 H, J = 7.7 Hz), 4.35 (qt, 1 H, J = 6.4 Hz), 4.47 (d, 2 H, J = 5.1 Hz), 5.0 (t, 1 H, J = 6.4 Hz), 5.05–5.20 (m, 2 H), 6.89 (d, 1 H, J = 7.7 Hz), 6.95–7.07 (m, 2 H), 7.13 (d, 1 H, J = 7.7 Hz), 7.15 (d, 1 H, J = 7.7 Hz), 7.23 (s, 1 H), 10.22 (br s, 1 H). 6.2% of **46b**: ^{1}H NMR (DMSO-d_6) δ 1.28 (s, 3 H), 1.37 (s, 3 H), 2.34 (s, 3 H), 3.45–3.65 (m, 2 H), 3.75 (t, 1 H, J = 7.7 Hz), 4.04 (t, 1 H, J = 7.7 Hz), 4.30–4.40 (m, 1 H), 4.45 (d, 2 H, J = 5.1 Hz), 4.95 (t, 1 H, J = 5.1 Hz), 5.05–5.20 (m, 2 H), 6.85 (d, 1 H, J = 7.7 Hz), 6.95–7.02 (m, 1 H), 7.03 (d, 1 H, J = 7.7 Hz), 7.10–7.18 (m, 3 H), 10.20 (br s, 1 H).

2-[(2,2-Dimethyl-[1,3]dioxolan-4-ylmethyl)amino]-3-(3-hydroxy-6-methylpyridin-2-ylmethyl)-3*H*-benzoimidazole-5-carbaldehyde (47b). 47b was synthesized from **45b** with the procedure described for **47a** (90%).

2-{2-[(2,2-Dimethyl-[1,3]dioxolan-4-ylmethyl)amino]-6-[(3,5-dimethylphenylamino)methyl]benzoimidazol-1-ylmethyl}-6-methylpyridin-3-ol (48b). 48b was synthesized from **47b** with the procedure described for **48a** (45.5%, melting point 212 °C): ^{1}H NMR (DMSO-d_6) δ 1.28 (s, 3 H), 1.37 (s, 3 H), 2.08 (s, 6H), 2.32 (s, 3 H), 3.42–3.58 (m, 2 H), 3.74 (t, 1 H, J = 7.3 Hz), 4.02 (t, 1 H, J = 7.3 Hz), 4.18 (d, 2 H, J = 5.6 Hz), 4.36 (qt, 1 H, J = 7.3 Hz), 5.11 (dd, 2 H, J = 3.6, 11 Hz), 5.84 (t, 1 H, J = 5.6 Hz), 6.14 (s, 1 H), 6.20 (s, 2 H), 6.95 (d, 1 H, J = 8.1 Hz), 6.97–7.05 (m, 2 H), 7.13 (t, 1 H, J = 8.1 Hz), 7.24 (s, 1 H), 10.21 (br s, 1 H).

3-[6-[(3,5-Dimethylphenylamino)methyl]-1-(3-hydroxy-6-methylpyridin-2-ylmethyl)-1*H*-benzoimidazol-2-ylamino]propane-1,2-diol (48f). A mixture of **48b** (0.0004 mol) in a 3 N solution of HCl in water (20 mL) and THF (20 mL) was stirred at room temperature for 6 h, basified with K_2CO_3 (powder), and extracted with CH_2Cl_2. The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent was evaporated until dryness. The residue (0.25 g) was purified by column chromatography over silica gel (eluent, CH_2Cl_2/CH_3OH/NH_4OH 92:8:0.5). The pure fractions were collected, and the solvent was evaporated. The residue (0.17 g, 92%) was crystallized from CH_3CN/DIPE. The precipitate was filtered off and dried (0.127 g, 69%, melting point 128 °C): ^{1}H NMR (DMSO-d_6) δ 2.08 (s, 6 H), 2.32 (s, 3 H), 3.30–3.42 (m, 3 H), 3.47–3.56 (m, 1 H), 3.70 (qt, 1 H, J = 4.6 Hz), 4.18 (d, 2 H, J = 5.1 Hz), 5.05–5.18 (m, 4 H), 5.84 (t, 1 H, J = 5.1 Hz), 6.15 (s, 1 H), 6.20 (s, 2 H), 6.92–7.04 (m, 3 H), 7.10 (d, 1 H, J = 7.7 Hz), 7.13 (d, 1 H, J = 8.2 Hz), 7.25 (s, 1 H), 10.20 (brs, 2 H); HRMS (ESI), calcd for $C_{26}H_{32}N_5O_3$ 462.2505, found $[MH]^+$ 462.2513. Anal. ($C_{26}H_{31}N_5O_3 \cdot 0.35H_2O$) C, H, N.

2-Butylamino-3*H*-benzoimidazole-5-carboxylic Acid Ethyl Ester (42c). 42c was synthesized from butylamine with the procedure described for **42a** (26%): ^{1}H NMR (DMSO-d_6) δ 0.92 (t, 3 H, J = 7.7 Hz), 1.27–1.40 (m, 5 H), 1.50–1.60 (m, 2 H), 3.29 (qd, 2 H, J = 7.0 Hz), 4.25 (qd, 2 H, J = 6.8 Hz), 7.01 (t, 1 H, J = 7.0 Hz), 7.15 (d, 1 H, J = 7.7 Hz), 7.57 (dd, 1 H, J = 2.0, 7.7 Hz), 7.70 (d, 1 H, J = 2.0 Hz), 11.05 (br s, 1 H).

2-Butylamino-3-(3-hydroxy-6-methylpyridin-2-ylmethyl)-3*H*-benzoimidazole-5-carboxylic Acid Ethyl Ester (43c) and 2-Butylamino-1-(3-hydroxy-6-methylpyridin-2-ylmethyl)-1*H*-benzoimidazole-5-carboxylic Acid Ethyl Ester (44c). 43c and **44c** were synthesized from **42c** with the procedure described for **43a** + **44a** (16.4% of **43c** and 23.7% of **44c**): ^{1}H NMR (DMSO-d_6) δ 0.93 (t, 3 H, J = 7.7 Hz), 1.30 (t, 3 H, J = 6.8 Hz), 1.45 (sx, 2 H, J = 7.7 Hz), 1.62 (qt, 2 H, J = 7.7 Hz), 2.30 (s, 3 H), 3.41 (qd, 2 H, J = 5.1 Hz), 4.25 (qd, 2 H, J = 6.8 Hz), 5.17 (s, 2 H), 6.94 (t, 1H, J = 5.1 Hz), 7.04 (d, 1 H, J = 7.7 Hz), 7.15 (d, 1 H, J = 7.7 Hz), 7.25 (d, 1 H, J = 7.7 Hz), 7.55 (d, 1 H, J = 7.7 Hz), 7.74 (s, 1 H), 10.24 (br s, 1 H)).

2-(2-Butylamino-6-hydroxymethylbenzoimidazol-1-ylmethyl)-6-methylpyridin-3-ol (45c). 45c was synthesized from **43c** with the procedure described for **45a** (100%).

2-Butylamino-3-(3-hydroxy-6-methylpyridin-2-ylmethyl)-3*H*-benzoimidazole-5-carbaldehyde (47c). 47c was synthesized from **45c** with the procedure described for **47a** (94%).

2-{2-Butylamino-6-[(3,5-dimethylphenylamino)methyl]benzoimidazol-1-ylmethyl}-6-methylpyridin-3-ol (48c). 48c was synthesized from **47c** with the procedure described for **48a** (14.5%, melting point 181 °C): ^{1}H NMR (DMSO-d_6) δ 0.92 (t, 2 H, J = 7.2 Hz), 1.39 (sx, 2 H, J = 7.5 Hz), 1.61 (qt, 2 H, J = 7.5 Hz), 2.08 (s, 6 H), 2.30 (s, 3 H), 3.35–3.45 (m, 2 H), 4.17 (d, 2 H, J = 5.1 Hz), 5.10 (s, 2 H), 5.82 (t, 1 H, J = 5.1 Hz), 6.13 (s, 1 H), 6.20 (s, 2 H), 6.70 (br s, 1 H), 6.93 (d, 1 H, J = 7.7 Hz), 7.02 (d, 1 H, J = 7.7 Hz), 7.09 (d, 1 H, J = 7.7 Hz), 7.14 (d, 1H, J = 7.7 Hz), 7.23 (s, 1 H), 10.25 (br s, 2 H); HRMS (ESI), calcd for $C_{27}H_{34}N_5O$ 444.2763, found $[MH]^+$ 444.2760. Anal. ($C_{27}H_{33}N_5O$) C, H, N.

2-Phenethylamino-3H-benzoimidazole-5-carboxylic Acid Ethyl Ester (42d). 42d was synthesized from phenethylamine with the procedure described for **42a** (48.4%, melting point 153 °C): ^{1}H NMR (DMSO-d_6) δ 1.30 (t, 3 H, J = 6.8 Hz), 2.90 (t, 2 H, J = 7.1 Hz), 3.55 (qd, 2 H, J = 7.1 Hz), 4.27 (qd, 2 H, J = 6.8 Hz), 7.00–7.13 (m, 1 H), 7.15–7.25 (m, 2 H), 7.25–7.35 (m, 4 H), 7.58 (d, 1 H, J = 7.7 Hz), 7.73 (s, 1H), 11.00 (br s, 1H).

3-(3-Hydroxy-6-methylpyridin-2-ylmethyl)-2-phenethylamino-3*H*-benzoimidazole-5-carboxylic Acid Ethyl Ester (43d) and 1-(3-Hydroxy-6-methylpyridin-2-ylmethyl)-2-phenethylamino-1*H*-benzoimidazole-5-carboxylic Acid Ethyl Ester (44d). 43d and **44d** were synthesized from **42d** with the procedure described for **43a** + **44a**. 19.8% of **43d**: ^{1}H NMR (DMSO-d_6) δ 1.32 (t, 3 H, J = 6.8 Hz), 2.25 (s, 3 H), 2.95 (t, 2 H, J = 7.1 Hz), 3.65 (t, 2 H, J = 7.1 Hz), 4.25 (qd, 2 H, J = 6.8 Hz), 5.18 (s, 2 H), 7.03 (d, 1 H, J = 7.7 Hz), 7.13 (d, 1 H, J = 7.7 Hz), 7.18–7.33 (m, 7 H), 7.64 (dd, 1 H, J = 2.0, 7.7 Hz), 7.81 (d, 1 H, J = 2.0 Hz), 10.25 (br s, 1H). 49.5% of **44d**: ^{1}H NMR (DMSO-d_6) δ 1.32 (t, 3 H, J = 6.8 Hz), 2.24 (s, 3 H), 2.95 (t, 2 H, J = 7.1 Hz), 3.64 (t, 2 H, J = 7.1 Hz), 4.27 (qd, 2 H, J = 6.8 Hz), 5.18 (s, 2 H), 7.03 (d, 1 H, J = 7.7 Hz), 7.10–7.17 (m, 2 H), 7.18–7.32 (m, 6 H), 7.58 (dd, 1 H, J = 2.0, 7.7 Hz), 7.78 (d, 1 H, J = 2.0 Hz), 10.25 (br s, 1H).

2-(6-Hydroxymethyl-2-phenethylaminobenzoimidazol-1-ylmethyl)-6-methylpyridin-3-ol (45d). 45d was synthesized from **43d** with the procedure described for **45a** (70%, melting point 185 °C): ^{1}H NMR (DMSO-d_6) δ 2.25 (s, 3 H), 2.96 (t, 2 H, J = 7.1 Hz), 3.61 (t, 2 H, J = 7.1 Hz), 4.47 (s, 2 H), 4.99 (br s, 1H), 5.10 (s, 2 H), 6.74–7.4 (m, 11H), 10.23 (br s, 1 H).

3-(3-Hydroxy-6-methylpyridin-2-ylmethyl)-2-phenethylamino-3*H*-benzoimidazole-5-carbaldehyde (47d). 47d was synthesized from **45d** with the procedure described for **47a** (100%).

2-{6-[(3,5-Dimethylphenylamino)methyl]-2-phenethylaminobenzoimidazol-1-ylmethyl}-6-methylpyridin-3-ol (48d). 48d was synthesized from **47d** with the procedure described for **48a** (75.4%, melting point 190 °C): ^{1}H NMR (DMSO-d_6) δ 2.08 (s, 6 H), 2.25 (s, 3 H), 2.95 (t, 2 H, J = 7.2 Hz), 3.45–3.55 (m, 2 H), 4.18 (d, 2 H, J = 5.1 Hz), 5.10 (s, 2 H), 5.82 (t, 1 H, J = 5.1 Hz), 6.14 (s, 1 H), 6.20 (s, 2 H), 6.88 (br s, 1 H), 6.94 (d, 1 H, J = 7.7 Hz), 7.00 (d, 1 H, J = 7.7 Hz), 7.13 (d, 2 H, J = 7.7 Hz), 7.18–7.24

(m, 2 H), 7.25–7.33 (m, 4 H), 10.20 (br s, 2 H); HRMS (ESI), calcd for $C_{31}H_{34}N_5O$ 492.2763, found $[MH]^+$ 492.2788. Anal. ($C_{31}H_{33}N_5O$) C, H, N.

2-(3-Morpholin-4-yl-propylamino)-3*H*-benzoimidazole-5-carboxylic Acid Ethyl Ester (42e). 42e was synthesized from 3-morpholin-4-yl-propylamine with the procedure described for **42a** (47%): ^{1}H NMR (DMSO-d_6) δ 1.30 (t, 3 H, J = 7.1 Hz), 1.62 (qt, 2 H, J = 6.9 Hz), 2.30–2.40 (m, 6 H), 3.30–3.38 (m, 2 H), 3.57 (t, 4 H, J = 4.0 Hz), 4.25 (qd, 2 H, J = 7.1 Hz), 7.00–7.08 (m, 1 H), 7.15 (d, 1 H, J = 7.7 Hz), 7.53–7.63 (m, 1 H), 7.70 (s, 1 H), 11.10 (s, 1 H).

3-(3-Hydroxy-6-methylpyridin-2-ylmethyl)-2-(3-morpholin-4-ylpropylamino)-3*H*-benzoimidazole-5-carboxylic Acid Ethyl Ester (43e) and 1-(3-Hydroxy-6-methylpyridin-2-ylmethyl)-2-(3-morpholin-4-ylpropylamino)-1*H*-benzoimidazole-5-carboxylic Acid Ethyl Ester (44e). 43e and **44e** were synthesized from **42e** with the procedure described for **43a** + **44a**. 20%, melting point 184 °C for **43e**: ^{1}H NMR (DMSO-d_6) δ 1.31 (t, 3 H, J = 7.4 Hz), 1.79 (qt, 2 H, J = 6.6 Hz), 2.26–2.42 (m, 9 H), 3.45 (t, 2 H, J = 6.6 Hz), 3.55 (t, 4 H, J = 5.6 Hz), 4.25 (qd, 2 H, J = 7.4 Hz), 5.18 (s, 2 H), 7.05 (d, 1 H, J = 7.7 Hz), 7.10–7.18 (m, 2 H), 7.19 (d, 1 H, J = 7.7 Hz), 7.62 (d, 1 H, J = 7.7 Hz), 7.80 (s, 1 H), 10.25 (br s, 1 H). 34%, melting point 176 °C for **44e**: ^{1}H NMR (DMSO-d_6) δ 1.30 (t, 3 H, J = 7.4 Hz), 1.79 (qt, 2 H, J = 6.6 Hz), 2.30 (s, 3 H), 2.31–2.40 (m, 6 H), 3.45 (t, 2 H, J = 6.6 Hz), 3.55 (t, 4 H, J = 5.6 Hz), 4.25 (qd, 2 H, J = 7.4 Hz), 5.18 (s, 2 H), 6.92–7.02 (m, 1 H), 7.05 (d, 1 H, J = 7.7 Hz), 7.15 (d, 1 H, J = 7.7 Hz), 7.23 (d, 1 H, J = 7.7 Hz), 7.55 (d, 1 H, J = 7.7 Hz), 7.75 (s, 1 H), 10.25 (br s, 1 H).

2-[6-Hydroxymethyl-2-(3-morpholin-4-ylpropylamino)benzoimidazol-1-ylmethyl]-6-methyl-pyridin-3-ol (45e). 45e was synthesized from **43e** with the procedure described for **45a** (83%, melting point 192 °C): ^{1}H NMR (DMSO-d_6) δ 1.79 (qt, 2 H, J = 6.4 Hz), 2.31 (s, 3 H), 2.33–2.45 (m, 6 H), 3.41 (t, 2 H, J = 6.4 Hz), 3.54 (t, 4 H, J = 5.1 Hz), 4.47 (s, 2 H), 4.99 (br s, 1 H), 5.11 (s, 2 H), 6.80 (br s, 1 H), 6.88 (d, 1 H, J = 7.7 Hz), 7.03 (d, 1 H, J = 7.7 Hz), 7.09 (d, 1 H, J = 7.7 Hz), 7.15 (d, 1 H, J = 7.7 Hz), 7.19 (s, 1 H), 10.26 (br s, 1 H).

3-(3-Hydroxy-6-methylpyridin-2-ylmethyl)-2-(3-morpholin-4-ylpropylamino)-3*H*-benzoimidazole-5-carbaldehyde (47e). 47e was synthesized from **45e** with the procedure described for **47a** (90%, melting point 206 °C): ^{1}H NMR (DMSO-d_6) δ 1.80 (qt, 2 H, J = 6.6 Hz), 2.30 (s, 3 H), 2.31–2.40 (m, 6 H), 3.48 (t, 2 H, J = 6.6 Hz), 3.55 (t, 4 H, J = 5.6 Hz), 5.22 (s, 2 H), 7.03 (d, 1 H, J = 7.7 Hz), 7.14 (d, 1 H, J = 7.7 Hz), 7.22–7.32 (m, 2 H), 7.53 (d, 1 H, J = 7.7 Hz), 7.65 (s, 1 H), 9.81 (s, 1 H).

2-[6-[(3,5-Dimethylphenylamino)methyl]-2-(3-morpholin-4-yl-propylamino)benzoimidazol-1-ylmethyl]-6-methylpyridin-3-ol (48e). 48e was synthesized from **47e** with the procedure described for **48a** (48%, melting point 199 °C): ^{1}H NMR (DMSO-d_6) δ 1.78 (qt, 2 H, J = 7.4 Hz), 2.08 (s, 6 H), 2.30 (s, 3 H), 2.31–2.42 (m, 6 H), 3.40 (t, 2 H, J = 6.4 Hz), 3.55 (t, 4 H, J = 4.0 Hz), 4.18 (t, 2 H, J = 5.1 Hz), 5.10 (s, 2 H), 5.82 (t, 1 H, J = 5.1 Hz), 6.14 (s, 1 H), 6.20 (s, 2 H), 6.77 (br s, 1 H), 6.93 (d, 1 H, J = 7.7 Hz), 7.02 (d, 1 H, J = 7.7 Hz), 7.09 (d, 1 H, J = 7.7 Hz), 7.14 (d, 1 H, J = 7.7 Hz), 7.20 (s, 1 H), 10.24 (br s, 1 H); HRMS (ESI), calcd for $C_{30}H_{39}N_6O_2$ 515.3134, found $[MH]^+$ 515.3144. Anal. ($C_{30}H_{38}N_6O_2$) C, H, N.

(2-Chloro-3*H*-benzoimidazol-5-yl)methanol (49). $LiAlH_4$ (0.146 mol) was added portionwise to a solution of tetrahydrofuran (200 mL) at 5 °C under N_2 flow. A solution of **3** (0.073 mol) in tetrahydrofuran (200 mL) was then added dropwise. The mixture was stirred at 5 °C for 3 h. A minimum of H_2O was then added, followed by a solution of CH_2Cl_2/CH_3OH (90:10). The resulting mixture was dried (over $MgSO_4$) and filtered, and the solvent was evaporated until dryness (12.6 g, 95%, melting point 179 °C): ^{1}H NMR (DMSO-d_6) δ 4.58 (s, 2 H), 5.20 (br s, 1 H), 7.18 (d, 1 H, J = 7.7 Hz), 7.40–7.49 (m, 2 H), 13.14 (br s, 1 H).

[2-(3-Morpholin-4-ylpropylamino)-3*H*-benzoimidazol-5-yl]-methanol (50). A mixture of **49** (0.069 mol) and 3-morpholin-4-ylpropylamine (0.207 mol) was stirred at 125 °C for 4 h and then taken up in CH_2Cl_2/CH_3OH. The organic layer was washed with a 10% solution of K_2CO_3 in water, dried (over $MgSO_4$), and filtered and the solvent was evaporated until dryness. The residue (37 g) was purified by column chromatography over silica gel (eluent, CH_2Cl_2/CH_3OH/NH_4OH 90:10:0.5). The pure fractions were collected, and the solvent was evaporated (16.5 g, 82%): ^{1}H NMR (DMSO-d_6) δ 1.72 (qt, 2 H, J = 6.4 Hz), 2.28–2.42 (m, 6 H), 3.29 (qd, 2 H, J = 6.4 Hz), 3.57 (t, 4 H, J = 5.1 Hz), 4.46 (s, 2 H), 5.07 (br s, 1 H), 6.54 (t, 1 H, J = 6.4 Hz), 6.81 (d, 1 H, J = 7.7 Hz), 7.04 (d, 1 H, J = 7.7 Hz), 7.08 (s, 1 H), 10.71 (br s, 1 H).

2-[6-Hydroxymethyl-2-(3-morpholin-4-ylpropylamino)benzoimidazol-1-ylmethyl]-6-methylpyridin-3-ol (51) and 2-[5-Hydroxymethyl-2-(3-morpholin-4-ylpropylamino)benzoimidazol-1-ylmethyl]-6-methylpyridin-3-ol (52). A mixture of **50** (0.0396 mol), **6** (0.0475 mol), and K_2CO_3 (0.1188 mol) in dimethylformamide (110 mL) was stirred at room temperature for 12 h. The reaction was poured into ice/water. The aqueous layer was saturated with K_2CO_3 (powder) and extracted with a solution of CH_2Cl_2/CH_3OH (95:5). The residue was purified by chromatography over silica gel (eluent, CH_2Cl_2/CH_3OH/NH_4OH 90:10:1). The pure fractions were collected, and the solvent was evaporated. 5.4 g of **51** (33%, melting point 192 °C): ^{1}H NMR (DMSO-d_6) δ 1.79 (qt, 2 H, J = 6.4 Hz), 2.31 (s, 3 H), 2.33–2.45 (m, 6 H), 3.41 (t, 2 H, J = 6.4 Hz), 3.54 (t, 4 H, J = 5.1 Hz), 4.47 (s, 2 H), 4.99 (br s, 1 H), 5.11 (s, 2 H), 6.80 (br s, 1 H), 6.88 (d, 1 H, J = 7.7 Hz), 7.03 (d, 1 H, J = 7.7 Hz), 7.09 (d, 1 H, J = 7.7 Hz), 7.15 (d, 1 H, J = 7.7 Hz), 7.19 (s, 1 H), 10.26 (br s, 1 H). 5 g of **52** (31%, melting point 134 °C): ^{1}H NMR (DMSO-d_6) δ 1.79 (qt, 2 H, J = 6.4 Hz), 2.31 (s, 3 H), 2.33–2.43 (m, 6 H), 3.41 (t, 2 H, J = 6.4 Hz), 3.56 (t, 4 H, J = 5.1 Hz), 4.45 (s, 2 H), 4.94 (br s, 1 H), 5.11 (s, 2 H), 6.76 (br s, 1 H), 6.82 (d, 1 H, J = 7.7 Hz), 7.02 (d, 1 H, J = 7.7 Hz), 7.06–7.17 (m, 3 H), 10.26 (br s, 1 H).

2-[6-Chloromethyl-2-(3-morpholin-4-ylpropylamino)benzoimidazol-1-ylmethyl]-6-methylpyridin-3-ol (53). $SOCl_2$ (0.81 mL) was added dropwise to a mixture of **51** (0.0006 mol) in CH_2Cl_2 (10 mL) at 5 °C. The mixture was stirred at 5 °C for 2 h, then brought to room temperature, and stirred for 12 h. The solvent was evaporated until dryness (0.42 g, 100%, 4 HCl). The crude compound was used directly in the next reaction step.

3-(3-Hydroxy-6-methylpyridin-2-ylmethyl)-2-(3-morpholin-4-ylpropylamino)-3*H*-benzoimidazole-5-carbaldehyde (54). 54 is the same compound as **47e**.

2-[6-{[(2-Hydroxyethyl)phenylamino]methyl}-2-(3-morpholin-4-ylpropylamino)benzoimidazol-1-ylmethyl]-6-methylpyridin-3-ol (55a). A mixture of **53** (0.0009 mol), 2-(phenylamino)ethanol (0.0004 mol), and K_2CO_3 (0.0019 mol) in DMF (30 mL) was stirred at 80 °C for 1 h. H_2O was added, and the mixture was extracted with CH_2Cl_2. The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent was evaporated. The residue (0.16 g) was crystallized from CH_3CN/DIPE. The precipitate was filtered off, rinsed with DIPE, and dried (0.051 g, 25%, melting point 190 °C): ^{1}H NMR (DMSO-d_6) δ 1.78 (qt, 2 H, J = 7.4 Hz), 2.32 (s, 3 H), 2.33–2.42 (m, 6 H), 3.40 (t, 2 H, J = 6.4 Hz), 3.47 (t, 2 H, J = 6.4 Hz), 3.53–3.63 (m, 6 H), 4.55 (s, 2 H), 5.05 (s, 2 H), 6.53 (t, 1 H, J = 7.7 Hz), 6.68 (d, 2 H, J = 7.7 Hz), 6.78 (d, 1 H, J = 7.7 Hz), 6.98–7.15 (m, 6 H), 10.20 (br s, 1 H); HRMS (ESI), calcd for $C_{30}H_{39}N_6O_3$ 531.3084, found $[MH]^+$ 531.3088. Anal. ($C_{30}H_{38}N_6O_3 \cdot 0.5H_2O$) C, H, N.

2-[6-{[(2-Hydroxyethyl)-*m*-tolylamino]methyl}-2-(3-morpholin-4-ylpropylamino)benzoimidazol-1-ylmethyl]-6-methylpyridin-3-ol (55b). A mixture of **53** (0.0005 mol), 2-[(3-methylphenyl)amino]ethanol (0.0006 mol), and K_2CO_3 (0.0025 mol) in DMF (30 mL) was stirred at 80 °C for 4 h and then poured into H_2O. The aqueous layer was saturated with K_2CO_3 (powder) and extracted with CH_2Cl_2. The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent was evaporated. The residue (0.7 g) was purified by column chromatography over silica gel (eluent, CH_2Cl_2/CH_3OH/NH_4OH 93:7:0.5). The pure fractions were collected, and the solvent was evaporated. The residue (0.12 g) was crystallized from 2-propanone/DIPE. The precipitate was filtered off, rinsed with DIPE, and dried (0.1 g, 36%, melting point 205

°C): ^{1}H NMR (DMSO-d_6) δ 1.78 (qt, 2 H, J = 7.4 Hz), 2.32 (s, 3 H), 2.33–2.42 (m, 6 H), 3.40 (t, 2 H, J = 6.4 Hz), 3.47 (t, 2 H, J = 6.4 Hz), 3.53–3.63 (m, 6 H), 4.55 (s, 2 H), 5.05 (s, 2 H), 6.53 (t, 1 H, J = 7.7 Hz), 6.68 (d, 2 H, J = 7.7 Hz), 6.78 (d, 1 H, J = 7.7 Hz), 6.98–7.15 (m, 6 H), 10.20 (br s, 1 H); HRMS (ESI), calcd for $C_{31}H_{41}N_6O_3$ 545.3240, found $[MH]^+$ 545.3254. Anal. ($C_{31}H_{40}N_6O_3 \cdot 0.3H_2O$) C, H, N.

2-[6-{[(3,5-Dimethylphenyl)-(2-hydroxyethyl)amino]methyl}-2-(3-morpholin-4-ylpropylamino)benzoimidazol-1-ylmethyl]-6-methylpyridin-3-ol (55c). A mixture of 3,5-dimethylaniline (0.04 mol), 2-bromoethanol (0.033 mol), and K_2CO_3 (0.033 mol) in CH_3CN (50 mL) was stirred at 80 °C for 12 h. The reaction was cooled to room temperature, and the solvent was evaporated. The residue was taken up in CH_2Cl_2/CH_3OH (95:5) and washed with a saturated solution of K_2CO_3 in water. The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent was evaporated until dryness. The residue was purified by column chromatography over silica gel (eluent, CH_2Cl_2/CH_3OH/ NH_4OH 98:2:0.1). The pure fractions were collected, and the solvent was evaporated [1.9 g of 2-(3,5-dimethylphenylamino)ethanol (29%)]: ^{1}H NMR (DMSO-d_6) δ 2.12 (s, 6 H), 3.05 (qd, 2 H, J = 6.1 Hz), 3.51 (qd, 2 H, J = 6.1 Hz), 4.63 (t, 1 H, J = 6.1 Hz), 5.25 (t, 1 H, J = 6.1 Hz), 6.12–6.23 (m, 3 H).

A mixture of **53** (0.000695 mol), 2-(3,5-dimethylphenylamino) ethanol (0.0009 mol), and K_2CO_3 (0.0035 mol) in dimethylformamide (40 mL) was stirred at 80 °C for 4 h. H_2O was added. The solution was saturated with K_2CO_3 (powder) and extracted with CH_2Cl_2/CH_3OH (95:5). The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent was evaporated. The residue (0.5 g) was purified by column chromatography over silica gel (eluent, CH_2Cl_2/CH_3OH/NH_4OH 93:7:0.5). The pure fractions were collected, and the solvent was evaporated (0.120 g, 31%). The residue was crystallized from CH_3CN/DIPE. The precipitate was filtered off, rinsed with DIPE, and dried (0.1 g, 26%, melting point 180 °C): ^{1}H NMR (DMSO-d_6) δ 1.79 (qt, 2 H, J = 7.4 Hz), 2.13 (s, 6 H), 2.30 (s, 3 H), 2.32–2.41 (m, 6 H), 3.40 (qd, 4 H, J = 6.8 Hz), 3.51–3.66 (m, 6 H), 4.52 (s, 2 H), 4.65 (br s, 1 H), 5.07 (s, 2 H), 6.20 (s, 1 H), 6.32 (s, 2 H), 6.74 (br s, 1H), 6.78 (d, 1 H, J = 7.7 Hz), 7.01 (t, 1 H, J = 7.7 Hz), 7.05–7.15 (m, 3 H), 10.20 (br s, 1 H); HRMS (ESI), calcd for $C_{32}H_{43}N_6O_3$ 559.3397, found $[MH]^+$ 559.3395. Anal. ($C_{32}H_{42}N_6O_3$) C, H, N.

4-{(3,5-Dimethylphenyl)-[3-(3-hydroxy-6-methylpyridin-2-ylmethyl)-2-(3-morpholin-4-ylpropylamino)-3*H*-benzoimidazol-5-ylmethyl]amino}butyramide (55d). 3-(3,5-Dimethylphenylamino)butyric acid ethyl ester was prepared analogously to 2-(3,5-dimethylphenylamino)ethanol starting from 3,5-dimethylaniline and 3-bromopropionic acid ethyl ester: ^{1}H NMR (DMSO-d_6) δ 1.18 (t, 3 H, J = 7.1 Hz), 1.75 (qt, 2 H, J = 6.9 Hz), 2.13 (s, 6 H), 2.38 (t, 2 H, J = 6.9 Hz), 2.98 (qd, 2 H, J = 6.9 Hz), 4.05 (qd, 2 H, J = 7.1 Hz), 5.40 (t, 1 H, J = 6.9 Hz), 6.15 (s, 3 H).

3-(3,5-Dimethylphenylamino)butyric acid ethyl ester (0.0026 mol) in a 7 N solution of NH_3 in CH_3OH was stirred at 80 °C in a sealed vessel. The reaction was cooled to room temperature, and the solvent was evaporated until dryness [0.5 g of 3-(3,5-dimethylphenylamino)butyramide (100%)]: ^{1}H NMR (DMSO-d_6) δ 1.72 (qt, 2 H, J = 7.1 Hz), 2.10–2.18 (m, 8 H), 2.95 (qd, 2H, J = 7.1 Hz), 5.35 (t, 1 H, J = 7.1 Hz), 6.15 (s, 3 H), 6.74 (s, 1 H), 7.25 (s, 1 H).

A mixture of **53** (0.0125 mol), 3-(3,5-dimethylphenylamino)butyramide (0.0145 mol), and Cs_2CO_3 (0.0605 mol) in DMF (300 mL) was stirred at 80 °C for 4 h, poured into ice–water, and extracted with CH_2Cl_2. The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent was evaporated. The residue (11.3 g) was purified by column chromatography over silica gel (eluent, CH_2Cl_2/CH_3OH/NH_4OH 93:7:0.5). The pure fractions were collected, and the solvent was evaporated (2.6 g, 35%). This fraction was crystallized from 2-propanone/CH_3OH/DIPE. The precipitate was filtered off and dried (2.17 g, 29%, melting point 170 °C): ^{1}H NMR (DMSO-d_6) δ 1.70–1.85 (m, 4 H), 2.04–2.12 (m, 2 H), 2.13 (s, 6 H), 2.30 (s, 3 H), 2.31–2.41 (m, 6 H), 3.30 (t, 2 H, J = 6.8 Hz), 3.40 (t, 2 H, J = 6.8 Hz), 3.55 (t, 4 H, J = 3.8 Hz), 4.47 (s, 2 H), 5.06 (s, 2 H), 6.20 (s, 1 H), 6.32 (s, 2 H), 6.73 (br s, 1H), 6.77 (d, 1 H, J = 7.7 Hz), 6.83 (s, 1 H), 7.01 (d, 1 H, J = 7.7 Hz), 7.05–7.15 (m, 3 H), 7.33 (s, 1 H), 10.23 (br s, 1 H); HRMS (ESI), calcd for $C_{34}H_{46}N_7O_3$ 600.3662, found $[MH]^+$ 600.3704. Anal. ($C_{34}H_{45}N_7O_3$) C, H, N.

6-Methyl-2-[2-(3-morpholin-4-ylpropylamino)-6-(*m*-tolylaminomethyl)benzoimidazol-1-ylmethyl]pyridin-3-ol (56a). Acetic acid (0.2 mL) was added at room temperature to a mixture of **54** (0.0004 mol), 3-methylaniline (0.0005 mol), and $NaBH_3CN$ (0.0005 mol) in CH_3CN (25 mL). The mixture was stirred at room temperature for 30 min. Acetic acid (0.2 mL) was added, and the mixture was stirred at room temperature for 12 h. The solvent was evaporated until dryness. The residue was taken up in CH_2Cl_2/K_2CO_3 10%. The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent was evaporated until dryness. The residue (0.22 g) was purified by column chromatography over silica gel (eluent, CH_2Cl_2/CH_3OH/NH_4OH 94:6:0.5). The pure fractions were collected, and the solvent was evaporated. The residue (0.16 g, 65%) was crystallized from 2-propanone/DIPE. The precipitate was filtered off and dried (0.114 g, 46.5%, melting point 195 °C): ^{1}H NMR (DMSO-d_6) δ 1.78 (qt, 2 H, J = 7.4 Hz), 2.12 (s, 3 H), 2.30 (s, 3 H), 2.31–2.40 (m, 6 H), 3.40 (t, 2 H, J = 6.4 Hz), 3.55 (t, 4 H, J = 4.0 Hz), 4.18 (t, 2 H, J = 5.1 Hz), 5.10 (s, 2 H), 5.94 (t, 1 H, J = 5.1 Hz), 6.30 (d, 1 H, J = 7.7 Hz), 6.37 (d, 1 H, J = 7.7 Hz), 6.40 (s, 1 H), 6.77 (br s, 1 H), 6.88 (t, 1 H, J = 7.7 Hz), 6.94 (d, 1 H, J = 7.7 Hz), 7.03 (d, 1 H, J = 7.7 Hz), 7.10 (d, 1 H, J = 7.7 Hz), 7.14 (d, 1 H, J = 7.7 Hz), 7.20 (s, 1 H), 10.24 (br s, 1 H); HRMS (ESI), calcd for $C_{29}H_{37}N_6O_2$ 501.2978, found $[MH]^+$ 501.2991. Anal. ($C_{29}H_{36}N_6O_2$) C, H, N.

2-[6-{[2-(2-Hydroxyethyl)phenylamino]methyl}-2-(3-morpholin-4-ylpropylamino)benzoimidazol-1-ylmethyl]-6-methylpyridin-3-ol (56b). A mixture of **54** (0.0002 mol), 2-(2-aminophenyl)ethanol (0.0002 mol), and BH_3CN- (polymer support) (0.0003 mol) in CH_3OH (10 mL) and acetic acid (0.1 mL) was stirred at room temperature for 16 h and then filtered. The filtrate was concentrated under reduced pressure. The residue was taken up in K_2CO_3 10%, saturated with K_2CO_3 (powder), and extracted with CH_2Cl_2/CH_3OH. The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent was evaporated until dryness. The residue was crystallized from 2-propanone/DIPE. The precipitate was filtered off and dried (0.12 g, 93%, melting point 140 °C): ^{1}H NMR (DMSO-d_6) δ 1.78 (qt, 2 H, J = 7.4 Hz), 2.30 (s, 3 H), 2.32–2.40 (m, 6 H), 2.67 (t, 2 H, J = 6.4 Hz), 3.40 (t, 2 H, J = 6.4 Hz), 3.55 (t, 2 H, J = 3.9 Hz), 3.63 (t, 2 H, J = 6.4 Hz), 4.25 (d, 2 H, J = 5.1 Hz), 5.10 (s, 2 H), 5.57 (t, 1 H, J = 5.1 Hz), 6.40–6.50 (m, 2 H), 6.77 (br s, 1 H), 6.88 (t, 1 H, J = 7.7 Hz), 6.90–6.98 (m, 2 H), 7.03 (d, 1 H, J = 7.7 Hz), 7.09 (d, 1 H, J = 7.7 Hz), 7.15 (d, 1 H, J = 7.7 Hz), 7.24 (s, 1 H), 10.20 (br s, 1 H); HRMS (ESI), calcd for $C_{30}H_{39}N_6O_3$ 531.3084, found $[MH]^+$ 531.3097. Anal. ($C_{30}H_{38}N_6O_3 \cdot 1.35H_2O$) C, H, N.

2-[6-{[2-(3-Hydroxypropyl)phenylamino]methyl}-2-(3-morpholin-4-ylpropylamino)benzoimidazol-1-ylmethyl]-6-methylpyridin-3-ol (56c). A mixture of **54** (0.0004 mol), 3-(2-aminophenyl)propan-1-ol (0.0005 mol), and BH_3CN- (polymer support) (0.0007 mol) in CH_3OH (20 mL) and acetic acid (0.2 mL) was stirred at room temperature for 24 h. The polymer was filtered off. The filtrate was concentrated under reduced pressure. The residue was taken up in CH_2Cl_2. The organic layer was washed with K_2CO_3 10%, dried (over $MgSO_4$), and filtered, and the solvent was evaporated until dryness. The residue was crystallized from CH_3OH/DIPE. The precipitate was filtered off and dried (0.14 g, 66%, melting point 178 °C): ^{1}H NMR (DMSO-d_6) δ 1.72 (qt, 2 H, J = 7.4 Hz), 1.79 (qt, 2 H, J = 7.4 Hz), 2.30 (s, 3 H), 2.30–2.40 (m, 6 H), 2.50–2.58 (m, 2 H), 3.40 (t, 2 H, J = 6.4 Hz), 3.47 (t, 2 H, J = 6.4 Hz), 3.55 (t, 4 H, J = 3.9 Hz), 4.30 (d, 2 H, J = 5.1 Hz), 5.10 (s, 2 H), 5.48 (t, 1 H, J = 5.1 Hz), 6.40–6.50 (m, 2 H), 6.80 (br s, 1 H), 6.85 (t, 1 H, J = 7.7 Hz), 6.90–7.00 (m, 2 H), 7.04 (d, 1 H, J = 7.7 Hz), 7.09 (d, 1 H, J = 7.7 Hz), 7.15 (d, 1 H, J = 7.7 Hz), 7.24 (s, 1 H), 10.20 (br s, 1 H); HRMS (ESI), calcd for $C_{31}H_{41}N_6O_3$ 545.3240, found $[MH]^+$ 545.3230. Anal. ($C_{31}H_{40}N_6O_3$) C, H, N.

2-[6-{[2-(3-Hydroxypropyl)-5-methylphenylamino]methyl}-2-(3-morpholin-4-ylpropylamino)benzoimidazol-1-ylmethyl]-6-methylpyridin-3-ol (56d). A mixture of 1-iodo-4-methyl-2-nitrobenzene (0.019 mol), acrylic acid ethyl ester (0.0238 mol), NEt_3 (0.019 mol), and $Pd(OAc)_2$ in CH_3CN was stirred at 100 °C for 2.5 h and then cooled to room temperature. The mixture was filtered through a pad of Celite. The Celite was rinsed with CH_2Cl_2. The filtrate was concentrated under reduced pressure. The residue was taken up in DIPE with a few drops of acetone. The precipitate was filtered off and dried [4.47 g of 3-(4-methyl-2-nitrophenyl)acrylic acid ethyl ester, 100%, melting point 67 °C]: ^{1}H NMR (DMSO-d_6) δ 1.28 (t, 3 H, J = 7.1 Hz), 2.45 (s, 3 H), 4.22 (qd, 2 H, J = 7.1 Hz), 6.62 (d, 1 H, J = 16.2 Hz), 7.60 (d, 1H, J = 7.7 Hz), 7.85–7.93 (m, 3H).

Dibal-H 20% in toluene (0.0255 mol) was added at −35 °C to a mixture of 3-(4-methyl-2-nitrophenyl)acrylic acid ethyl ester (0.0085 mol) in THF (80 mL) under N_2 flow. The mixture was stirred at −35 °C for 15 min. H_2O (20 mL) was added dropwise at −35 °C under N_2 flow. The mixture was half-evaporated, and CH_2Cl_2 was added. The mixture was filtered over Celite. The Celite was washed with CH_2Cl_2. The filtrate was washed with H_2O. The organic layer was separated, dried ($MgSO_4$), and filtered, and the solvent was evaporated [2 g of 3-(4-methyl-2-nitrophenyl)prop-2-en-1-ol, 100%]: ^{1}H NMR (DMSO-d_6) δ 2.40 (s, 3 H), 4.15 (t, 2 H, J = 4.9 Hz), 5.03 (t, 1 H, J = 4.9 Hz), 6.47 (dt, 1 H, J = 4.3, 16.2 Hz), 6.80 (d, 1 H, J = 16.2 Hz), 7.50 (d, 1 H, J = 7.7 Hz), 7.70 (d, 1H, J = 7.7 Hz), 7.75 (s, 1 H).

A mixture of 3-(4-methyl-2-nitrophenyl)prop-2-en-1-ol (0.0085 mol) and Raney nickel (1.6 g) in CH_3OH (30 mL) was hydrogenated at room temperature for 2 h under a 3 bar pressure and then filtered over Celite. The Celite was rinsed with CH_3OH. The filtrate was concentrated under reduced pressure [1.7 g of 3-(2-amino-4-methylphenyl)propan-1-ol, 86%, melting point 65 °C]: ^{1}H NMR (DMSO-d_6) δ 1.62 (qt, 2 H, J = 7.1 Hz), 2.12 (s, 3 H), 2.40 (t, 2 H, J = 7.1 Hz), 3.40 (qd, 2 H, J = 6.0 Hz), 4.45 (t, 1 H, J = 5.1 Hz), 4.70 (s, 2 H), 6.30 (d, 1 H, J = 7.7 Hz), 6.42 (s, 1 H), 6.77 (d, 1 H, J = 7.7 Hz).

Acetic acid (0.2 mL) was added at room temperature to a mixture of **54** (0.0004 mol), 3-(2-amino-4-methylphenyl)propan-1-ol (0.0005 mol) and BH_3CN^- on solid support (0.0007 mol) in CH_3OH (20 mL). The mixture was stirred at room temperature for 12 h. The solid support was filtered off and rinsed with CH_3OH, and the filtrate was concentrated. The residue was taken up in a 10% solution of K_2CO_3 in water and extracted with CH_2Cl_2/CH_3OH (95:5). The organic layer was separated, dried (over $MgSO_4$), and filtered, and the solvent was evaporated until dryness. The residue was purified by column chromatography over silica gel (eluent, CH_2Cl_2/CH_3OH/NH_4OH 92:8:1). The pure fractions were collected, and the solvent was evaporated. The residue was crystallized from 2-propanone/DIPE. The precipitate was filtered off and dried (0.223 g, 82%, melting point 208 °C): ^{1}H NMR (DMSO-d_6) δ 1.69 (qt, 2 H, J = 7.4 Hz), 1.79 (qt, 2 H, J = 7.4 Hz), 2.05 (s, 3 H), 2.30 (s, 3 H), 2.32–2.52 (m, 8 H), 3.40 (t, 2 H, J = 6.4 Hz), 3.45 (t, 2 H, J = 6.4 Hz), 3.54 (t, 4 H, J = 3.9 Hz), 4.28 (d, 2 H, J = 5.1 Hz), 5.10 (s, 2 H), 5.32 (t, 1 H, J = 5.1 Hz), 6.25–6.31 (m, 2 H), 6.80 (d, 1 H, J = 7.7 Hz), 6.81 (br s, 1 H), 6.95 (d, 1 H, J = 7.7 Hz), 7.02 (d, 1 H, J = 7.7 Hz), 7.10 (d, 1 H, J = 7.7 Hz), 7.13 (d, 1 H, J = 7.7 Hz), 7.24 (s, 1 H), 10.10 (br s, 1 H); HRMS (ESI), calcd for $C_{32}H_{43}N_6O_3$ 559.3397, found $[MH]^+$ 559.3403. Anal. ($C_{32}H_{42}N_6O_3$) C, H, N.

Conformational Analysis. The initial structures of compounds **58** and **57** (Figure 1) were constructed using the sketcher of SYBYL[14] and further optimized using the MMFF94S[15] force field and charge set to yield a suitable starting point for the searching algorithm.

The Random Search tool available in SYBYL was used to perform a random torsional search of each compound. All rotatable bonds were searched except those of the terminal groups. The Random Search parameters were set to default values except for the following: max cycle 20000, E cutoff 8 kcal/mol, and chirality check turned on (for **58** only). A maximum of 1200 iterations was allowed for the minimization step with the Powell method and the MMFF94S force field and charge set. A dielectric constant of 80 with a distance-dependent attenuation function was used to simulate solvent effects. All calculations were performed on a Silicon Graphics O2 workstation [400 MHz MIPS R12000 (IP35) processor].

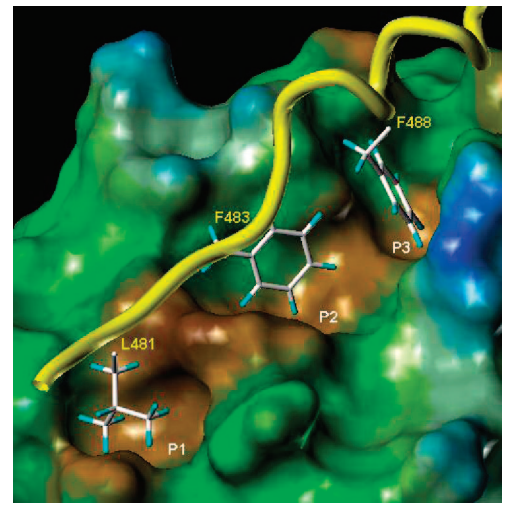


Figure 4. Molcad representation of the binding site used for molecular docking. The molecular lipophilic potential has been mapped on the Connolly surface (brown, lipophilic regions; blue, polar regions). The binding site encompasses subcavities P1, P2, and P3 that accommodate the hydrophobic side chains of the three HR-C residues L481, F483, and F488, respectively (yellow tube, HR-C backbone; white-capped sticks, the three residue side chains).

Docking. The initial protein coordinates were retrieved from the Protein Data Bank (code 1g2c) and processed to yield a model appropriate for molecular docking. According to the indications given by the X-ray structure authors,[7] the inhibitor-binding region was identified on a hydrophobic groove located on the HR-N coiled coil structure. The C-terminal end of the groove features a cavity where the majority of contacts occur between six cavity-lining HR-N residues and two aromatic HR-C residues, F483 and F488. To define the binding site required for docking, we extended this region by including one adjacent cavity in which the hydrophobic side chain of the HR-C residue L481 is deeply buried. The three binding site subcavities have been denoted P1, P2, and P3 as shown in Figure 4.

Compounds **58** and **57** were docked both manually and automatically within the putative binding site. We used FlexX, as implemented in the SYBYL 6.7.2 package, for automatic docking with the genetic algorithm parameters set to their default value. Consensus scoring was applied and used in the decision-making process. All protein–ligand complexes were optimized using the MMFF94s force field. Within the binding site region all residues and ligand atoms were allowed to move. Residues outside this region were kept rigid during the calculation.

Acknowledgement. We gratefully acknowledge Line Harmier for analysis and Cyril Henry for purification of compounds. We are also grateful to Luc Geeraert for proofreading the manuscript.

Supporting Information Available: Elemental analyses of the target compounds. This material is available free of charge via the Internet at http://pubs.acs.org/.

References

(1) Bonfanti, J.-F.; Doublet, F.; Fortin, J.; Lacrampe, J.; Guillemont, J.; Muller, P.; Queguiner, L.; Arnoult, E.; Gevers, T.; Janssens, P.; Szel, H.; Willebrords, R.; Timmerman, P.; Wuyts, K.; Janssens; F.; Sommen, C.; Wigerinck, P.; Andries, K. Selection of a respiratory syncytial virus inhibitor clinical candidate, part 1: Improving the pharmacokinetic

profile using the structure−property relationship. *J. Med. Chem.* **2007**, *50*, 4572–4584.
(2) Hall, C. B. Prospects for a respiratory syncytial virus vaccine. *Science* **1994**, *265*, 1393–1394.
(3) Meijas, A.; Ramilo, O. New approaches to reduce the burden of RSV infection. *Drug Discovery Today: Ther. Strategies/Infect. Dis.* **2006**, *3*, 173–181.
(4) Broughton, S.; Greenough, A. Drugs for the management of respiratory syncytial virus infection. *Curr. Opin. Invest. Drugs* **2004**, *5*, 862–865.
(5) Andries, K.; Moeremans, M.; Gevers, T.; Willebrords, R.; Sommen, C.; Lacrampe, J.; Janssens, F.; Wyde, P. R. Substituted benzimidazoles with nanomolar activity against respiratory syncytial virus. *Antiviral Res.* **2003**, *60*, 209–219.
(6) Morton, C. J.; Cameron, R.; Lawrence, L. J.; Lin, B.; Lowe, M.; Luttick, A.; Mason, A.; McKimm-Breschkin, J.; Parker, M. W.; Ryan, J.; Smout, M.; Sullivan, J.; Tucker, S. P.; Young, P. R. Structural characterization of respiratory syncytial virus fusion inhibitor escape mutants: homology model of the F protein and a syncytium formation assay. *Virology* **2003**, *311*, 275–288.
(7) Zhao, X.; Singh, M.; Malashkevich, V. N.; Kim, P. S. Structural characterization of the human respiratory syncytial virus fusion protein core. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 14172–14177.
(8) Cianci, C.; Langley, D. R.; Dischino, D. D.; Sun, Y.; Yu, K.-L.; Stanley, A.; Roach, J.; Li, Z.; Dalterio, R.; Colonno, R.; Meanwell, N. A.; Krystal, M. Targeting a binding pocket within the trimer-of-hairpins: Small-molecule inhibition of viral fusion. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 15046–15051.
(9) Debnath, A. K. Prospects and strategies for the discovery and development of small-molecule inhibitors of six-helix bundle formation in class 1 viral fusion proteins. *Curr. Opin. Invest. Drugs* **2006**, *7* (2), 118–127.
(10) Cianci, C.; Meanwell, N.; Krystal, M. Antiviral activity and molecular mechanism of an orally active respiratory syncytial virus fusion inhibitor. *J. Antimicrob. Chemother.* **2005**, *55*, 289–292.
(11) Douglas, J. L.; Panis, M. L.; Ho, E.; Lin, K-Y.; Krawczyk, S. H.; Grant, D. H.; Cai, R.; Swaminathan, S.; Chen, X.; Cihlar, T. Small molecules VP-14637 and JNJ-2408068 inhibit respiratory syncytial virus fusion by similar mechanisms. *Antimicrob. Agents Chemother.* **2005**, *49*, 2460–2466.
(12) Prince, G. A.; Jenson, A. B.; Horswood, R. L.; Camargo, E.; Chanock, R. M. The pathogenesis of respiratory syncytial virus infection in cotton rats. *Am. J. Pathol.* **1978**, *93*, 771–791.
(13) Wyde, P. R.; Wilson, L. Z.; Petrella, R.; Gilbert, B. E. Efficacy of high dose-short duration ribavirin aerosol in the treatment of respiratory syncytial virus infected cotton rats and influenza B virus infected mice. *Antiviral Res.* **1987**, *7*, 211–220.
(14) SYBYL 6.7.2, Tripos Inc., St. Louis, MO.
(15) Halgren, T. A. MMFF VI. MMFF94s option for energy minimization studies. *J. Comput. Chem.* **1999**, *20*, 720–729.

JM701284J