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Synthetic Analogues of the Microtubule-Stabilizing Sponge Alkaloid Ceratamine A Are More Active than the Natural Product

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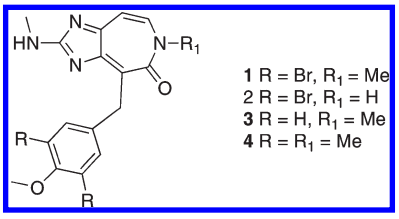
Desbromoceratamine A (**3**) exhibits significantly less potent activity than the natural product ceratamine A (**1**) in a cell-based assay for antimitotic activity. Synthesis of the ceratamine A analogue **4** has shown that replacing the bromine atoms in the natural product with methyl groups generates an analogue that is more active than natural ceratamine A (**1**). Further enhancement of the antimitotic activity of the ceratamine pharmacophore has been achieved in the synthetic analogue **33**, which has both bromine atoms replaced with methyl groups and an additional methyl substituent on the amino nitrogen at C-2. An efficient synthetic route has been developed to **33** that should enable the first in vivo evaluation of the new ceratamine microtubule-stabilizing pharmacophore and has provided several additional analogues for structure−activity relationship evaluation.

Introduction

Ceratamines A (**1**) and B (**2**) are antimitotic alkaloids isolated from extracts of the marine sponge *Pseudoceratina* sp. collected in Papua New Guinea.[1] The imidazo[4,5-*d*]-azepine core heterocycle of the ceratamines has not been encountered at any oxidation state in other natural products.[2] Ceratamines stabilize microtubules, but they do not bind to the same site as paclitaxel, and they generate an unusual mitotic arrest phenotype that is characterized by the formation of pillarlike structures of tubulin.[3] The recent approval of the epothilone analogue ixabepilone for clinical use highlights the continued interest in microtubule-stabilizing agents as anticancer drugs.[4] Ceratamines are attractive lead compounds for anticancer drug development because they have novel, but nevertheless relatively simple, chemical structures as compared with other natural product microtubule-stabilizing agents, they are achiral, they do not bind to the paclitaxcel site on microtubules, and they generate an unprecedented mitotic arrest phenotype.



The original isolation of ceratamines A (**1**) and B (**2**) from the *Pseudoceratina* sp. generated only small amounts of material that was not adequate to support in vivo evaluation in mouse models of cancer.[1] To provide the material required for further evaluation of the new ceratamine microtubule-stabilizing pharmacophore, we embarked on a synthetic program aimed at making ceratamine A (**1**) and simpler analogues. These efforts resulted in the synthesis of several desbromo analogues of ceratamine A (**1**) that confirmed the structure proposed for the natural product and the microtubule-stabilizing activity of the new pharmacophore.[5,6] However, the reduced potency and efficacy of synthetic desbromo ceratamine A (**3**) compared with the activity of natural ceratamine A (**1**) revealed that the bromine atoms were necessary for the full biological activity of the natural product.

Intrigued by the important role that the bromine atoms play in the antimitotic activity of the ceratamines, we set out to see if other substituents might provide the same benefit. The initial hypothesis was that the bromine atoms were simply adding steric bulk to the aromatic ring that either helped to fill a binding pocket or predefined a preferred binding conformation. Therefore, we decided to replace the bromine atoms in ceratamine A (**1**) with methyl groups, reasoning that since their van der Waals radii were nearly equal (Br, 1.86 Å; Me, 1.80 Å),[7] they would fill roughly comparable volumes of space, and the new target became the analogue **4**.

Results and Discussion

The synthesis of **4** (R = Me) followed the route used in our earlier synthesis of desbromoceratamine A (**3**) (R = H) as outlined in Scheme 1.[6] Vinyl bromide **7** was prepared from 3,5-dimethylanisaldehyde via a Cr(II)-mediated condensation with tribromomethylacetate[8] followed by amide formation as previously described for the synthesis of **6**.[6] Stille coupling between stannane **5** and the vinyl bromide **7** gave **9** in high yield. *Z*-Vinyl bromide **11** was synthesized by reaction of the aldehyde **9** with (bromomethyl)triphenylphosphoniumbromide

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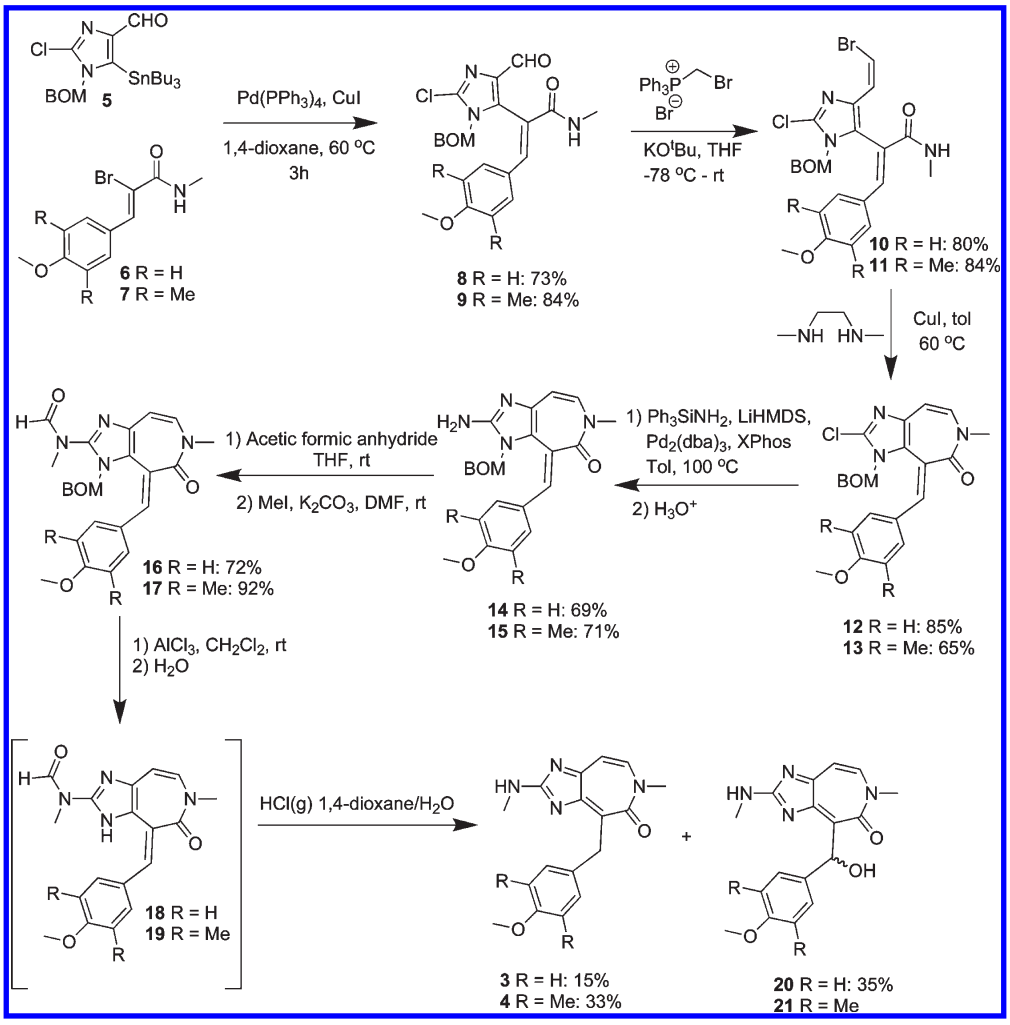
Scheme 1



and potassium *tert*-butoxide (KOtBu).[a9] Copper(I)-catalyzed ring closure of **11** to form the enamide **13** followed a procedure developed by Buchwald.[10] A dilute tetrahydrofuran (THF) solution of **11** was treated with CuI, Cs_2CO_3, and *N*,*N*′-dimethylethylenediamine at elevated temperatures to yield the 5,7-bicyclic system **13** in excellent yield. Heating chloroimidazole **13**, triphenylsilylamine, and lithium hexamethyldisilazide (LiHMDS) in the presence of tris(dibenzylidenacetone)dipalladium(0) [$Pd_2(dba)_3$] and the Buchwald ligand 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (XPhos) in toluene (Tol) followed by acid hydrolysis of the triphenylsilyl group gave the primary amine **15**.[11] Reaction of **15** with acetic formic anhydride in THF for 24 h followed by alkylation with methyl iodide (MeI) and K_2CO_3 in dimethylformamide (DMF) gave *N*-methyl formamide **17** in high yield. Removal of the benzyloxymethyl (BOM) group from **17** with $AlCl_3$ gave formamide **19**. The desired dimethylceratamine A analogue **4** and the corresponding C-11 hydroxy analogue **21** were formed when a stream of dry HCl(g) was bubbled through a solution of formamide **19** in 50%1,4-dioxane and water.

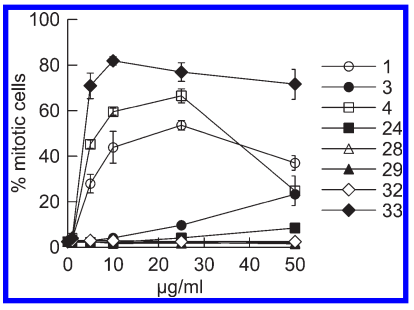
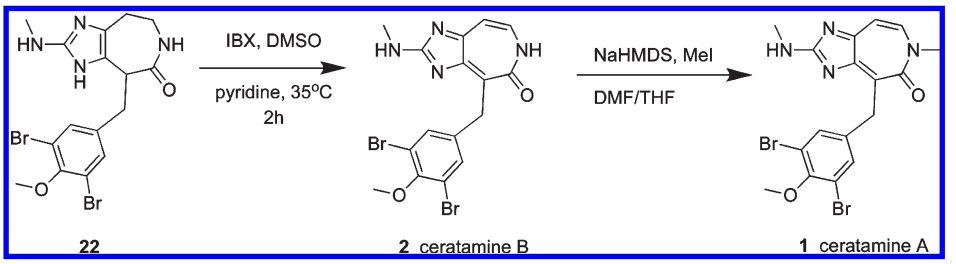


Figure 1. Dose−response curves for natural and synthetic ceratamines in a cell-based assay for mitotic arrest.

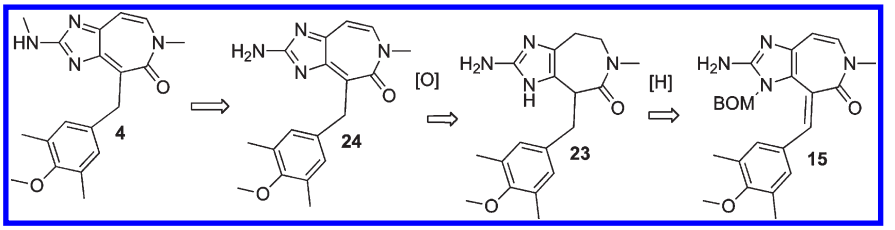
As we had hoped, biological evaluation showed that replacement of the bromine atoms in ceratamine A (**1**) with methyl groups to give **4** produced an analogue that was slightly more potent ($IC_{50} \approx 3.5$ μg/mL) and significantly more efficacious (67% cells arrested in mitosis at optimal concentration) than the natural product ($IC_{50} \approx 5$ μg/mL; 54% cells arrested in mitosis at optimal concentration) as shown in Figure 1, making it a strong candidate for testing the ceratamine antimitotic pharmacophore in mouse models of cancer. However, the formation of the C-11 hydroxyl byproduct **21** hampered the

[a] Abbreviations: BOM, benzyloxymethyl; DMAP, 4-dimethylaminopyridine; DMF, dimethylformamide; DMSO, dimethylsulfoxide; HRESIMS, high-resolution electrospray ionization mass spectrometry; IBX, 2-iodobenzoic acid; IC_{50}, concentration giving 50% of full biological response; KOtBu, postassium tertiary butoxide; LiHMDS, lithium hexamethyldisilazide; MeI, methyl iodide; NMR, nuclear magnetic resonance; $Pd_2(dba)_3$, tris(dibenzylidenacetone)dipalladium(0); RP-HPLC, reversed-phase high-performance liquid chromatography; SAR, structure−activity relationship; THF, tetrahydrofuran; TLC, thin-layer chromatography; Tol, toluene; XPhos, 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl.

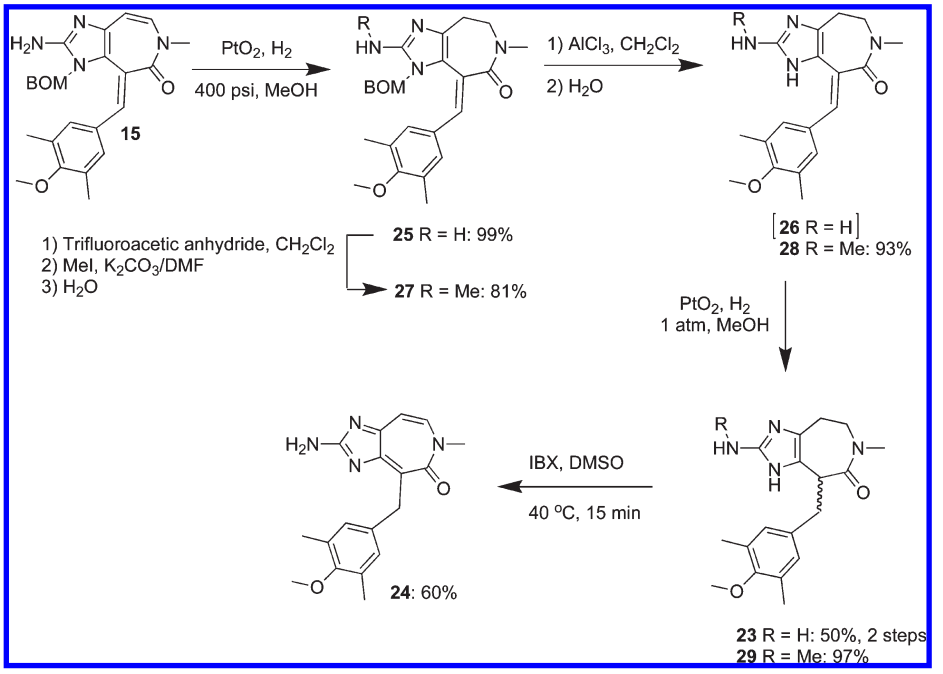
Scheme 2



Scheme 3



Scheme 4



efficiency of the synthesis of **4**, and we set out to circumvent this limitation.

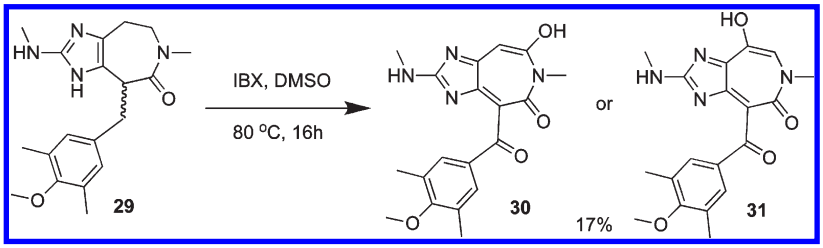
While our work was in progress, Coleman's group reported the first synthesis of the natural products **1** and **2**.[12] The end game of their synthesis featured an elegant 2-iodobenzoic acid (IBX)-mediated double dehydrogenation to create the aromatic imidazo[4,5-*d*]azepine heterocycle (Scheme 2, **22** to **2**), an approach that had earlier failed in our hands.[6]

Coleman's report led us to consider a hybrid synthesis of **4** shown retrosynthetically in Scheme 3, whereby heterocycle **15** would be assembled at the ceratamine oxidation state as before, reduced to **23**, and then reoxidized to the all-aromatic tautomer **24** using Coleman's IBX methodology, presumably without the formation of the undesirable C-11 oxidized side product **21**. Mono *N*-methylation of **24** would then yield the desired analogue **4**.

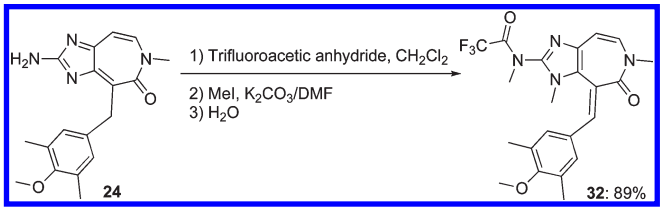
Scheme 4 shows the realization of the hybrid route to ceratamines. The enamide functionality in BOM-protected primary amine **15** could be reduced in excellent yield to give **25** using PtO_2-catalyzed hydrogenation at 400 psi. Removal of the BOM group in **25** with $AlCl_3$ cleanly gave intermediate **26**, which could be further hydrogenated at atmospheric pressure, again using PtO_2 as a catalyst, to give the tetrahydro ceratamine analogue **23** in high yield. IBX oxidation of **23** gave the ceratamine **24** without any detectable formation of the corresponding C-11 oxidized analogues. As shown in Figure 1, compound **24** had almost no antimitotic activity in the cell-based assay, demonstrating the critical requirement for *N*-2 methylation in the ceratamine antimitotic pharmacophore.

Our first attempt to incorporate *N*-2 methylation into the hybrid synthetic route started with methylation of the partially reduced intermediate **25** as shown in Scheme 4. Conversion of the primary amine **25** into the corresponding trifluoroacetamide followed by alkylation with methyliodide and K_2CO_3 and subsequent aqueous workup gave the *N*-2 methylated analogue **27**. Removal of the BOM protecting group in

Scheme 5



Scheme 6



Scheme 7

27 with $AlCl_3$ followed by atmospheric pressure hydrogenation with PtO_2 catalysis gave **29**. Unexpectedly, attempted IBX oxidation of **29**, using the conditions that readily converted **23** to **24** (Scheme 4) and **22** to **2** (Scheme 2), gave no detectable transformation of starting material to product. Heating the reaction to 80 °C resulted in conversion of the starting material **29** into overoxidized products. Although these oxygenated compounds were not completely purified or characterized because they were not the desired products, the MS and nuclear magnetic resonance (NMR) data obtained for the impure mixture were consistent with structures **30** or **31** (Scheme 5). We were unable to find any conditions that would transform **29** into the desired *N*-2 methylated ceratamine analogue **4**.

Next, we turned our attention to direct *N*-2 methylation of the ceratamine analogue **24** using the conditions that had cleanly converted the primary amine **25** to the *N*-2 monomethyl analogue **27** (Scheme 4). As shown in Scheme 6, reaction of the *N*-2 primary amine **24** with trifluoracetic anhydride followed by methylation with MeI/K_2CO_3 and aqueous workup does indeed accomplish monomethylation at *N*-2 as desired, but it also leads to tautomerization to give **32**. This unanticipated dearomatization turns out to be consistent with all of the previous experience that we have had with the imidazo[4,5-*d*]azepine ring system in the ceratamines. When the C-2 substituent is hydrogen, chlorine, thiomethyl, formylamide, or trifluoracetamide, the ceratamines prefer the nonaromatic tautomer observed in **32**.[6] The only ceratamine analogues that we have made to date that prefer the aromatic tautomer present in **24** are compounds (**1**, **2**, **3**, **4**, **24**, etc.) having a strongly electron-donating primary amine or *N*-methyl amine substituent at C-2. It is interesting that reducing the electron-donating ability of an amino subsitutent at C-2 by acylating the amine is sufficient to drive the tautomeric equilibrium away from aromaticity in the imidazo[4,5-*d*]azepine ring system.

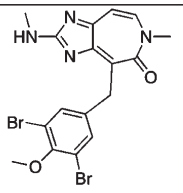
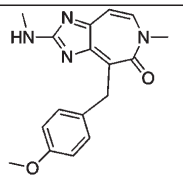
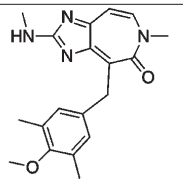
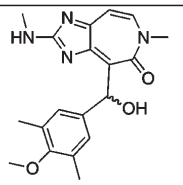
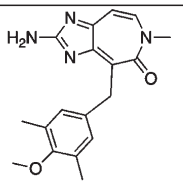
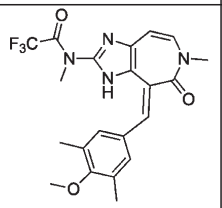
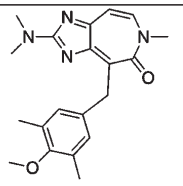
Because methylation of the acylated *N*-2 amine in **24** had failed to produce the desired result, we next attempted direct stiochiometric alkylation. Treatment of **24** with NaH and 1 equiv of MeI converts it to the *N*-2 dimethyl amine **33** (Scheme 7). It was not possible to stop the *N*-2 alkylation at the momomethyl product **4** nor was it possible to generate the *N*-2 trimethylated product. Using excess MeI produces the dimethyl compound **33** in nearly quantitative yield. Although **33** was not the desired monomethyl product **4**, we nevertheless tested it in the cell-based antimitotic assay. As shown in Figure 1, the dimethyl analogue **33** had even better antimitotic activity (**33**: $IC_{50} \approx 3.0\ \mu g/mL$; 82% cells arrested in mitosis at optimal concentration) than the monomethyl analogue **4** ($IC_{50} \approx 3.5\ \mu g/mL$; 67% cells arrested in mitosis at optimal concentration), and it is easier to make.

Our original analysis of the ceratamine microtubule-stabilizing pharmacophore focused on the imidazo[4,5-*d*]azepine core heterocycle as the primary structural requirement for biological activity. This was based in part on the observation that the 3,5-dibromo-4-methoxyphenyl substructure is commonly encountered in sponge metabolites, but none of the other natural products containing this moiety are known to target tubulin.[13] As a result, our initial analogue synthesis program was designed to make compounds that did not contain the bromine atoms found in the natural product, and our finding that desbromo ceratamine A was significantly less active than the natural products was unexpected.[5,6] The current study has shown that replacing the bromine atoms in ceratamine A (**1**) with methyl groups to give the analogue **4** recaptures and even exceeds the antimitotic activity of the natural product (Figure 1 and Table 1). We assume that since bromine atoms and methyl groups have roughly comparable van der Walls radii,[7] the effect is steric and the two subsituents are simply filling the same space.

The current study has also revealed an additional structure−activity relationship (SAR) for the ceratamine pharmacophore. Compound **21**, which is the C-11 hydroxy analogue of **4**, is completely inactive at the concentrations tested (Table 1). Similarly, compound **32**, which differs from **4** by being acylated at *N*-2 with a trifluoracetyl group and existing in the nonaromatic tautomeric form, is inactive. The primary amine **24**, which just lacks the *N*-2 methyl substituent in **4**, is essentially inactive, while compound **33**, which is dimethylated on *N*-2, is significantly more active than **1** or **4**.

The above results show that the structural requirements for activity in the ceratamine antimitotic pharmacophore are quite tightly constrained. There is a strict requirement for bulky substituents at C-14 and C-16 on the phenyl ring. This steric bulk is supplied by the bromine atoms in the natural products **1** and **2**. As described above, we have found that methyl groups at these positions in the synthetic analogues **4** and **33** produce

Table 1. Antimitotic Activity of Ceratamines in a Cell-Based Assay for Antimitotic Activity

		IC_{50} (μg/mL)	% cells arrested at mitosis at optimal concentration
1		5	54
3		>50	23
4		3.5	67
21		Inactive at 50 μg/mL	
24		Inactive at 50 μg/mL	
32		Inactive at 50 μg/mL	
33		3	82

enhanced antimitotic activity. Oxygen substituents, either hydroxyl or ketone functionalities, at C-11 strongly attenuate the activity. Methyl substituents are required on *N*-2. If there is no methyl substituent on *N*-2, the compounds are inactive, and dimethylation on *N*-2 enhances activity relative to monomethylation. All compounds tested to date that exist in the nonaromatic tautomeric form of the imidazo[4,5-*d*]azepine heterocycle are inactive.[5,6] However, all of these compounds are also missing a nonacylated methyl amine substituent at C-2. Because these two structural changes are thus far always interrelated, it is not possible to tell if it is just the *N*-2 methyl amine susbtituent or both the *N*-2 methyl amine and the aromatic tautomeric form that is required for antimitotic activity.

The new ceratamine analogues **4** and **33** show roughly the same potency as ceratamine A, suggesting that they bind microtubules with comparable affinity. However, **4** and **33** arrest a higher proportion of cells at mitosis (67 and 82%, respectively) than ceratamine A (54%). It is increasingly recognized that cells do not remain arrested in mitosis indefinitely in the presence of microtubule-targeting agents.[14] After a period of arrest typically lasting several hours, cells may

enter interphase without having undergone cell division. This process, termed mitotic slippage, can lead to the formation of abnormal multiploid cells. We speculate that **4** and **33** may induce a more robust mitotic arrest than ceratamine A, resulting is a lower rate of slippage and perhaps enhanced antitumor activity.

Conclusions

In summary, an efficient new synthetic route to the highly methylated ceratamine analogue **33** has been developed. Compound **33**, in which the C-14 and C-16 bromine substituents in the natural product ceratamine A (**1**) have been replaced by methyl groups and the C-2 monomethylamino substituent in the natural product has been replaced by a dimethylamino substituent, is significantly more active than the inspirational natural product. Evaluation of this highly methylated ceratamine **33** in mouse models of cancer is ongoing, and these animal experiments will provide the first in vivo data on the microtubule-stabilizing ceratamine pharmacophore. The results of these in vivo experiments will be reported elsewhere.

Experimental Section

General Methods. All nonaqueous reactions were carried out in flame-dried glassware and under an Ar atmosphere unless otherwise noted. Air- and moisture-sensitive liquid reagents were manipulated via a dry syringe. Anhydrous THF was obtained from distillation over sodium. Fresh IBX was prepared according to a literature procedure.[15] All other solvents and reagents were used as obtained from commercial sources without further purification. ^{1}H and ^{13}C spectra were obtained on Bruker Avance 400 direct or Bruker Avance 600 cryoprobe spectrometers. Flash chromatography was performed using Silicycle Ultra Pure silica gel (230−400 mesh). The purity of compounds was evaluated via reversed-phase high-performance liquid chromatography (RP-HPLC) on a C18 analytical column eluting with MeOH/H_2O mixtures. Samples of all compounds tested for antimitotic activity in the cell-based assay were >95% pure, as were all other synthetic intermediates unless otherwise noted.

(*Z*)-2-Bromo-3-(4-methoxy-3,5-dimethylphenyl)-*N*-methylacrylamide (7). Vinylbromide **7** was prepared as described using a Cr(II)-mediated olefination of 4-methoxy-3,5-dimethyl-benzaldehyde with tribromomethyl acetate, followed by functional group interchange.[6] ^{1}H NMR (CD_2Cl_2, 400 MHz): δ 2.32 (s, 6H), 2.94 (d, J = 4.6 Hz, 3H), 3.75 (s, 3H), 6.95 (s, broad, 1H), 7.49 (s, 2H), 8.17 (s, 1H). ^{13}C NMR (CD_2Cl_2, 100 MHz): δ 16.1, 27.3, 59.7, 114.0, 129.6, 130.9, 131.1, 136.8, 158.4, 163.0. High-resolution electrospray ionization mass spectrometry (HRESIMS) calcd for $C_{13}H_{17}NO_2{}^{79}Br$ ([M + H]$^+$), 298.0443; found, 298.0445.

(*E*)-2-(1-((Benzyloxy)methyl)-2-chloro-4-formyl-1*H*-imidazol-5-yl)-3-(4-methoxy-3,5-dimethylphenyl)-*N*-methylacrylamide (9). Stannane **5** (1.83 g, 3.39 mmol), bromide **7** (1.01 g, 3.39 mmol), $Pd(PPh_3)_4$ (392 mg, 0.339 mmol), and CuI (646 mg, 3.39 mmol) were combined in 12 mL of dry 1,4-dioxane. The reaction mixture was heated to 60 °C for 2 h, cooled, filtered through a Celite pad, and concentrated to dryness. The crude product was purified by column chromatography to yield **9** (1.33 g, 2.84 mmol, 84%) as a pale yellow solid. ^{1}H NMR (CD_2Cl_2, 400 MHz): δ 2.13 (s, 6H), 2.80 (d, J = 4.8 Hz, 3H), 3.65 (s, 3H), 4.49 (s, 2H), 5.02 (d, J = 10.7 Hz, 1H), 5.20 (d, J = 10.7 Hz, 1H), 6.00 (d, br, J = 4.8 Hz, 1H), 6.64 (s, 2H), 7.18 (m, 2H), 7.31 (m, 3H), 7.97 (s,1H), 9.72 (s, 1H). ^{13}C NMR (CD_2Cl_2, 100 MHz): δ 16.1, 26.9, 59.6, 71.5, 73.6, 119.0, 127.6, 128.3, 128.6, 128.9, 130.8, 131.8, 136.0, 136.3, 136.7, 138.2, 143.7, 159.1, 165.2, 183.9. HRESIMS calcd for $C_{25}H_{26}N_3O_4{}^{35}ClNa$ ([M + Na]$^+$), 490.1510; found, 490.1504.

(*E*)-2-(1-((Benzyloxy)methyl)-4-((*Z*)-2-bromovinyl)-2-chloro-1*H*-imidazol-5-yl)-3-(4-methoxy-3,5-dimethylphenyl)-*N*-methylacrylamide (11). (Bromomethyl)triphenylphosphonium bromide (2.5 g, 5.68 mmol) was slurried in 25 mL of dry THF. Potassium *tert*-butoxide (638 mg, 5.68 mmol) was added in one portion, and the resulting bright yellow solution was stirred at room temperature for 30 min. The reaction mixture was then cooled to −78 °C, and a solution of **9** (1.33 g, 2.84 mmol) in 10 mL of dry THF was added over a period of 10 min. The solution was stirred at −78 °C for 1.5 h, allowed to warm to room temperature, and stirred for an additional 30 min. The reaction was quenched by the addition of water, and the reaction mixture was extracted with 2 × EtOAc. The organic phase was dried over Na_2SO_4, filtered, and concentrated to dryness. The crude product was purified by column chromatography to yield **11** (1.30 g, 2.39 mmol, 84%) as a pale yellow foam. ^{1}H NMR (CD_2Cl_2, 400 MHz): δ 2.15 (s, 6H), 2.75 (d, J = 4.9 Hz, 3H), 3.66 (s, 3H), 4.45 (s, 2H), 4.99 (d, J = 10.8 Hz, 1H), 5.15 (d, J = 10.8 Hz, 1H), 5.79 (s, br, 1H), 6.32 (d, J = 8.2 Hz, 1H), 6.73 (m, 3H), 7.16 (m, 2H), 7.30 (m, 3H), 8.00 (s, 1H). ^{13}C NMR (CD_2Cl_2, 100 MHz): δ 16.0, 26.8, 59.6, 71.1, 73.4, 106.6, 118.9, 122.1, 127.5, 128.1, 128.5, 128.6, 129.2, 130.9, 131.6, 134.4, 136.3, 136.6, 143.8, 158.9, 165.5. HRESIMS calcd for $C_{26}H_{27}N_3O_3{}^{35}Cl^{79}BrNa$ ([M + Na]$^+$), 566.0822; found, 566.0815.

(*E*)-3-((Benzyloxy)methyl)-2-chloro-4-(4-methoxy-3,5-dimethylbenzylidene)-6-methyl-4,6-dihydroimidazo[4,5-*d*]azepin-5(3*H*)-one (13). Vinyl bromide **11** (62.3 mg, 0.114 mmol), CuI (4.4 mg, 0.23 mmol), and powdered K_3PO_4 (49 mg, 0.23 mmol) were combined in 1.5 mL of Tol. *N*,*N*′-Dimethylethylenediamine (5.0 μL, 0.46 mmol) was then added, and the reaction mixture was heated to 55 °C for 16 h. At this point, another portion of CuI (4.4 mg, 0.23 mmol) and *N*,*N*′-dimethylethylenediamine (5 mL, 0.46 mmol) were added, and the reaction mixture was stirred at 55 °C for another 5 h. The reaction mixture was cooled to room temperature, filtered through a Celite pad, and concentrated to dryness. The crude product was purified by column chromatography to yield **13** (34.4 mg, 0.074 mmol, 65%) as a pale yellow solid. ^{1}H NMR (CD_2Cl_2, 400 MHz): δ 2.16 (s, 6H), 3.29 (s, 3H), 3.68 (s, 3H), 4.36 (s, 2H), 4.52 (d, J = 11.4 Hz, 1H), 4.85 (d, J = 11.4 Hz, 1H), 6.14 (d, J = 9.2 Hz, 1H), 6.20 (d, J = 9.2 Hz, 1H), 6.60 (s, 2H), 7.27 (m, 6H). ^{13}C NMR (CD_2Cl_2, 100 MHz): δ 15.9, 38.2, 59.7, 70.9, 73.2, 107.9, 122.4, 123.4, 127.8, 127.9, 128.4, 128.9, 129.5, 129.9, 131.6, 134.8, 136.1, 136.3, 136.9, 158.0, 169.2. HRESIMS calcd for $C_{26}H_{26}N_3O_3{}^{35}ClNa$ ([M + Na]$^+$), 486.1560; found, 486.1552.

(*E*)-2-Amino-3-((benzyloxy)methyl)-4-(4-methoxy-3,5-dimethylbenzylidene)-6-methyl-4,6-dihydroimidazo[4,5-*d*]azepin-5(3*H*)-one (15). Chloride **13** (273.1 mg, 0.59 mmol), triphenylsilylamine (196 mg, 0.71 mmol), $Pd_2(dba)_3$ (54 mg, 0.59 mmol), and XPhos (67 mg, 0.14 mol) were combined in 6.5 mL of dry Tol. LiHMDS (1.53 mmol, 1.0 M in Tol, 1.53 mmol) was added, and the reaction mixture was heated to 90 °C for 1 h. The reaction mixture was cooled to room temperature and diluted with EtOAc. A 1 M concentration of HCl was added, and the biphasic mixture was stirred rapidly for 5 min. The aqueous layer was basified by the addition of saturated $NaHCO_3$, and the crude reaction mixture was extracted with 3 × EtOAc. The organic phase was dried over Na_2SO_4, filtered, and concentrated to dryness. The crude product was purified by column chromatography to yield **15** (185 mg, 0.42 mmol, 71%) as a bright yellow solid. ^{1}H NMR (CD_2Cl_2, 400 MHz): δ 2.17 (s, 6H), 3.28 (s, 3H), 3.67 (s, 3H), 4.25 (d, J = 3.9 Hz, 2H), 4.51 (d, J = 3.3 Hz, 2H), 4.80 (s, br, 2H), 6.04 (d, J = 9.1 Hz, 1H), 6.10 (d, 9.1 Hz, 1H), 6.68 (s, 2H), 7.12 (s, 1H), 7.22 (m, 2H), 7.30 (m, 3H). ^{13}C NMR (CD_2Cl_2, 100 MHz): δ 15.9, 38.2, 59.7, 70.4, 72.2, 108.7, 122.9, 127.3, 128.1, 128.3, 128.5, 129.6, 130.6, 131.2, 131.3, 136.8, 157.5, 169.4. HRESIMS calcd for $C_{26}H_{29}N_4O_3$ ([M + H]$^+$), 445.2240; found, 445.2245.

(*E*)-*N*-(1-((Benzyloxy)methyl)-8-(4-methoxy-3,5-dimethylbenzylidene)-6-methyl-7-oxo-1,6,7,8-tetrahydroimidazo[4,5-*d*]azepin-2-yl)-*N*-methylformamide (17). To a slurry of sodium formate

(215 mg, 3.16 mmol) in 3 mL of dry THF was added acetyl chloride (150 μL, 2.1 mmol). The slurry was then heated to 45 °C for 4 h. The slurry was cooled to room temperature, and a solution of **15** (44 mg, 0.1 mmol) in 1 mL of THF was added, followed by a catalytic amount of 4-dimethylaminopyridine (DMAP). The solution was then stirred at room temperature for 24 h. A 1 M concentration of HCl was added, and the solution was then stirred for 10 min. The solution was basified with the addition of saturated $NaHCO_3$, and the crude reaction mixture was extracted into EtOAc. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude product was then dissolved in 0.5 mL of DMF, and K_2CO_3 (33 mg, 0.24 mmol) was added to the solution, followed by MeI (50 mL, 0.15 mmol), and the reaction mixture was stirred at room temperature for 24 h. The solution was then extracted into EtOAc and washed with 3 × H_2O. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography to yield **17** (45 mg, 0.092 mmol, 92% yield) as a dull yellow solid. ^{1}H NMR (CD_2Cl_2, 400 MHz): δ 2.15 (s, 6H), 3.19 (s, 3H), 3.31 (s, 3H), 3.68 (s, 3H), 4.32 (s, 2H), 4.50 (d, J = 11.3 Hz, 1H), 4.58 (d, *J* = 11.3 Hz, 1H), 6.22 (m, 2H), 6.58 (s, 2H), 7.21 (m, 2H), 7.25 (s, 1H), 7.32 (m, 3H), 8.32 (s, 1H). ^{13}C NMR (CD_2Cl_2, 100 MHz): δ 16.0, 32.6, 38.2, 59.7, 71.0, 72.6, 108.1, 122.6, 127.9, 128.1, 128.5, 129.2, 129.5, 129.8, 130.0, 131.4, 134.6, 136.6, 158.0, 162.2, 169.2. HRESIMS calcd for $C_{28}H_{30}N_4O_4Na$ ([M + Na]$^+$), 509.2165; found, 509.2169.

4-(4-Methoxy-3,5-dimethylbenzyl)-6-methyl-2-(methylamino)-imidazo[4,5-*d*]azepin-5(6*H*)-one (4). Compound **15** (45 mg, 0.09 mmol) was dissolved in 3 mL of CH_2Cl_2. Anhydrous $AlCl_3$ (120 mg, 0.9 mmol) was added, and the resulting blood red slurry was stirred at room temperature for 10 min. The reaction mixture was then cooled to 0 °C and quenched with the careful addition of H_2O. EtOAc was added, and the aqueous layer was basified by the addition of saturated $NaHCO_3$. The biphasic mixture was extracted with 3 × EtOAc, the combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The crude residue was dissolved in CH_2Cl_2, passed through a silica plug, and concentrated again to yield crude **19** as a pale yellow solid. Without further purification, this product was dissolved in 5 mL of 1,4-dioxane. Five milliliters of deionized H_2O was added, and the resulting solution was sparged with nitrogen with sonication for 10 min. The solution was then cooled to 0 °C, and a stream of HCl (g) was passed through the solution for 5 min. The solution was then basified by addition of $NaHCO_3$ and extracted with 3 × EtOAc to give a bright yellow solution. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography to yield **4** (10 mg, 0.030 mmol, 33%) as a bright yellow solid. Compound **21** was also isolated from the reaction mixture but not fully purified or characterized. The NMR spectra of **4** in dimethylsulfoxide (DMSO)-d_6 showed a 3:1 ratio of two rotamers. Only the major isomer is described. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.09 (s, 6H), 3.04 (d, *J* = 4.7 Hz, 3H), 3.51 (s, 3H), 3.54 (s, 3H), 4.19 (s, 2H), 6.38 (d, *J* = 9.8 Hz, 1H), 6.97 (s, 2H), 7.69 (d, *J* = 9.8 Hz, 1H), 8.50 (d, *J* = 4.7 Hz, 1H). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 29.2, 35.5, 43.7, 59.1, 100.3, 123.4, 129.2, 135.8, 142.5, 154.6, 160.7, 164.1, 169.6, 175.4. HRESIMS calcd for $C_{19}H_{23}N_4O_2$ ([M + H]$^+$), 339.1821; found, 339.1832.

(*E*)-2-Amino-3-((benzyloxy)methyl)-4-(4-methoxy-3,5-dimethylbenzylidene)-6-methyl-4,6,7,8-tetrahydroimidazo[4,5-*d*]azepin-5(3*H*)-one (25). Primary amine **15** (56.8 mg, 0.13 mmol) was dissolved in 1.0 mL of MeOH. PtO_2 (20 mg) was added, and the resulting slurry was hydrogenated at 400 psi for 16 h. The slurry was then filtered through a 0.4 mm membrane, and the filtrate was washed with 3 × MeOH. The resulting solution was concentrated to dryness to yield **25** (58 mg, 0.13 mmol, quant yield) as a pale yellow solid. ^{1}H NMR (CD_2Cl_2, 400 MHz): δ 2.19 (s, 6H), 2.72 (d, br, *J* = 17.4 Hz, 1H), 2.87 (td, *J* = 13.3, 3.2 Hz, 1H), 3.07 (s, 3H), 3.34 (dt, *J* = 14.8, 2.7 Hz, 1H), 3.67 (s, 3H), 4.04 (t, br, *J* = 13.3 Hz, 1H), 4.22 (d, *J* = 2.7 Hz, 2H), 4.55 (d, *J* = 11.3 Hz, 1H), 4.62 (d, *J* = 11.3 Hz, 1H), 5.04 (s, br, 1H), 6.84 (s, 1H), 6.88 (s, 2H), 7.80 (m, 5H). ^{13}C NMR (CD_2Cl_2, 100 MHz): δ 15.9, 27.5, 33.6, 47.9, 59.7, 70.2, 72.3, 116.6, 125.9, 127.9, 128.3, 128.4, 129.6, 130.7, 131.0, 131.2, 135.3, 137.2, 151.5, 157.3, 172.3. HRESIMS calcd for $C_{26}H_{31}N_4O_3$ ([M + H]$^+$), 447.2396; found, 447.2405.

(*E*)-3-((Benzyloxy)methyl)-4-(4-methoxy-3,5-dimethylbenzylidene)-6-methyl-2-(methylamino)-4,6,7,8-tetrahydroimidazo[4,5-*d*]-azepin-5(3*H*)-one (27). Primary amine **25** (42 mg, 0.094 mmol) was dissolved in 1 mL of CH_2Cl_2 and cooled to 0 °C. Trifluoroacetic anhydride (130 μL, 0.94 mmol) was added neat, and the solution was stirred at 0 °C for 15 min. The solution was then concentrated to dryness, redissolved in Tol, and concentrated to dryness again. The resulting residue was dissolved in 1 mL of DMF, and K_2CO_3 (26 mg, 0.19 mmol) was added, followed by MeI (60 μL, 0.94 mmol). This slurry was stirred rapidly at room temperature for 1 h, then H_2O (15 mL) was added, and the solution was stirred for another 5 min. The crude product was extracted into EtOAc and washed with 2 × H_2O. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography to yield **27** (35 mg, 0.076 mmol, 81%) as a pale yellow solid. ^{1}H NMR (CD_2Cl_2, 400 MHz): δ 2.21 (s, 6H), 2.74 (m, 2H), 3.11 (s, 3H), 3.34 (s, 3H), 3.45 (m, 1H), 3.71 (s, 3H), 4.05 (m, 1H), 4.37 (s, 2H), 4.50 (d, *J* = 11.3 Hz, 1H), 5.08 (d, *J* = 11.3 Hz, 1H), 6.88 (s, 2H), 7.08 (s, 1H), 7.26 (m, 5H). ^{13}C NMR (CD_2Cl_2, 100 MHz): δ 15.9, 24.1, 30.8, 33.9, 46.2, 59.8, 71.7, 74.1, 118.4, 122.3, 125.6, 127.6, 127.9, 128.0, 129.5, 129.7, 132.0, 136.6, 137.2, 151.9, 154.6, 158.6, 171.3. HRESIMS calcd for $C_{27}H_{33}N_4O_3$ ([M + H]$^+$), 461.2542; found, 461.2546.

(*E*)-4-(4-Methoxy-3,5-dimethylbenzylidene)-6-methyl-2-(methylamino)-4,6,7,8-tetrahydroimidazo[4,5-*d*]azepin-5(3*H*)-one (28). Secondary amine **27** (62 mg, 0.13 mmol) was dissolved in 10 mL of CH_2Cl_2. Anhydrous $AlCl_3$ (173 mg, 1.4 mmol) was added, and the slurry was stirred rapidly for 10 min. The slurry was cooled to 0 °C and quenched carefully with H_2O. The aqueous phase was basified by addition of saturated $NaHCO_3$, and the product was extracted with 3 × EtOAc. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography to yield **28** (46 mg, 0.13 mmol, 93%) as a pale yellow solid. ^{1}H NMR (CD_2Cl_2, 400 MHz): δ 2.24 (s, 6H), 2.83 (t, *J* = 5.2 Hz, 2H), 3.10 (s, 3H), 3.50 (s, 3H), 3.74 (s, 3H), 3.78 (t, *J* = 4.5 Hz, 2H), 7.06 (s, 2H), 7.34 (s, 1H), 10.9 (s, br, 1H). ^{13}C NMR (CD_2Cl_2, 100 MHz): δ 15.9, 24.2, 28.9, 34.6, 46.7, 59.6, 115.7, 123.2, 125.6, 129.1, 129.9, 131.8, 137.1, 149.5, 158.1, 164.3, 169.9. HRESIMS calcd for $C_{19}H_{25}N_4O_2$ ([M + H]$^+$), 341.1978; found, 341.1985.

4-(4-Methoxy-3,5-dimethylbenzyl)-6-methyl-2-(methylamino)-4,6,7,8-tetrahydroimidazo[4,5-*d*]azepin-5(3*H*)-one (29). Secondary amine **28** (33.6 mg, 0.099 mmol) was dissolved in 2 mL of EtOH. PtO_2 (10 mg) was added, and the resulting slurry was hydrogenated under balloon pressure for 4 h, until thin-layer chromatography (TLC) analysis (5% HOAc/CH_2Cl_2) showed the reaction to be complete. The slurry was filtered through a 0.4 mm membrane, and the filtrate was washed with 3 × MeOH. The resulting solution was concentrated to dryness to yield **29** (33 mg, 0.096 mmol, 97%) as a colorless solid. This product was used without further purification. The NMR spectra of this compound showed the presence of two isomers. HRESIMS calcd for $C_{19}H_{27}N_4O_2$ ([M + H]$^+$), 343.2134; found, 343.2140.

2-Amino-4-(4-methoxy-3,5-dimethylbenzyl)-6-methyl-4,6,7,8-tetrahydroimidazo[4,5-*d*]azepin-5(3*H*)-one (23). Primary amine **25** (40 mg, 0.090 mmol) was dissolved in 2 mL of CH_2Cl_2. Anydrous $AlCl_3$ (120 mg, 0.9 mmol) was added, and the slurry was stirred at room temperature for 15 min. The slurry was cooled to 0 °C and quenched carefully with H_2O. The aqueous layer was basified by the addition of $NaHCO_3$, and the crude product was extracted with 5 × EtOAc. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated to yield **26**, which was used without further purification. HRESIMS calcd

for $C_{18}H_{23}N_4O_2$ ($[M + H]^+$), 327.1821; found, 327.1825. Crude **26** was slurried in 5 mL of 1:1 MeOH/EtOH. PtO_2 (10 mg) was added, and the solution was hydrogenated at 400 psi for 18 h. The slurry was then filtered through a 0.4 mm membrane, and the filtrate was washed with 2 × MeOH and 2 × EtOAc. The resulting solution was concentrated to dryness to yield **23** (15 mg, 0.045 mmol, 50%) as a colorless solid. This material was used without further purification. ^{1}H NMR (CD_2Cl_2, 400 MHz): δ 2.20 (s, 6H), 2.63 (m, 2H), 2.95 (s, 3H), 3.11 (m, 2H), 3.53 (m, 1H), 3.66 (s, 3H), 3.86 (m, 1H), 4.10 (m, 1H), 6.87 (s, 2H). ^{13}C NMR (CD_2Cl_2, 100 MHz): δ 15.9, 24.9, 29.9, 34.8, 38.9, 47.2, 59.6, 122.3, 128.3, 129.1, 129.4, 130.5, 135.4, 148.0, 155.4, 173.2. HRESIMS calcd for $C_{18}H_{25}N_4O_2$ ($[M + H]^+$), 329.1978; found, 329.1973.

7- or 8-Hydroxy-4-(4-methoxy-3,5-dimethylbenzoyl)-6-methyl-2-(methylamino)imidazo[4,5-*d*]azepin-5(6*H*)-one (30/31). Compound **29** (10.3 mg, 0.03 mmol) was dissolved in 0.5 mL of DMSO. IBX (33.7 mg, 0.12 mmol) was added, and the solution was heated to 80 °C for 16 h. The yellow reaction mixture was cooled to room temperature, diluted with saturated $NaHCO_3$, and extracted with EtOAc. The organic phase was dried over Na_2SO_4, filtered, and concentrated to yield a dull yellow residue. This mixture was purified by column chromatography to yield **30/31** (2 mg, 0.005 mmol, 17%) as the only isolable product. ^{1}H NMR (CD_2Cl_2, 600 MHz): δ 2.27 (s, 6H), 3.34 (s, 3H), 3.37 (s, 3H), 3.74 (s, 3H), 6.22 (s, 1H), 7.51 (s, 2H). ^{13}C NMR (CD_2Cl_2, 150 MHz): δ 15.7, 28.7, 31.9, 59.4, 105.1, 129.8, 131.6, 132.2, 162.7, 163.9, 192.6. HRESIMS calcd for $C_{19}H_{20}N_4O_4Na$ ($[M + Na]^+$), 391.1382; found, 391.1378.

2-Amino-4-(4-methoxy-3,5-dimethylbenzyl)-6-methylimidazo-[4,5-*d*]azepin-5(6*H*)-one (24). Reduced ceratamine **23** (15 mg, 0.045 mmol) was dissolved in 0.5 mL of DMSO. IBX (50 mg, 0.18 mmol) was added, and the slurry was heated to 40 °C for 15 min, after which the solution turned homogeneous. The resulting dark yellow solution was diluted with saturated $NaHCO_3$ and extracted with 3 × EtOAc. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography to yield **24** (8.7 mg, 0.027 mmol, 60%) as a bright yellow solid. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.08 (s, 6H), 3.52 (s, 3H), 3.54 (s, 3H), 4.16 (s, 2H), 6.43 (d, J = 9.8 Hz, 1H), 6.88 (s, 2H), 7.74 (d, J = 9.8 Hz, 1H), 8.04 (s, 1H), 8.18 (s, 1H). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 15.8, 35.7, 43.8, 59.1, 100.3, 123.2, 129.0, 129.3, 135.6, 142.6, 154.5, 160.8, 164.0, 170.1, 176.3. HRESIMS calcd for $C_{18}H_{21}N_4O_2$ ($[M + H]^+$), 325.1665; found, 325.1670.

(*E*)-2,2,2-Trifluoro-*N*-(8-(4-methoxy-3,5-dimethylbenzylidene)-1,6-dimethyl-7-oxo-1,6,7,8-tetrahydroimidazo[4,5-*d*]azepin-2-yl)-*N*-methylacetamide (32). Amine **24** (3 mg, 0.009 mmol) was dissolved in 0.3 mL of CH_2Cl_2. Trifluoroacetic anhydride (13 μL, 0.09 mmol) was added, and the solution was stirred at room temperature for 30 min. The reaction mixture was then concentrated to dryness, redissolved in Tol, and dried again. The residue was dissolved in 0.2 mL of DMF, and K_2CO_3 (5 mg, 0.036 mmol) was added, followed by MeI (5.7 μL, 0.09 mmol). This reaction mixture was stirred at room temperature for 1 h, followed by addition of H_2O and an additional 15 min of stirring. The reaction mixture was extracted with EtOAc, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography to yield **32** (3.5 mg, 0.008 mmol, 89%) as a pale yellow solid. ^{1}H NMR (CD_2Cl_2, 600 MHz): δ 2.21 (s, 6H), 2.83 (s, 3H), 3.26 (s, 3H), 3.53 (s, 3H), 3.71 (s, 3H), 6.00 (d, J = 9.1 Hz, 1H), 6.29 (d, J = 9.1 Hz, 1H), 6.83 (s, 2H), 7.42 (s, 1H). ^{13}C NMR (CD_2Cl_2, 150 MHz): δ 15.6, 31.5, 31.0, 37.9, 59.8, 99.4, 130.0, 132.1, 138.6. HRESIMS calcd for $C_{22}H_{23}N_4O_3F_3Na$ ($[M + Na]^+$), 471.1620; found, 471.1610.

2-(Dimethylamino)-4-(4-methoxy-3,5-dimethylbenzyl)-6-methyl-imidazo[4,5-*d*]azepin-5(6*H*)-one (33). Amine **24** (10 mg, 0.031 mmol) was dissolved in 1 mL of DMF. NaH (12 mg, 60% in oil, 0.31 mmol) was added, and the resulting dark red solution was stirred at room temperature for 10 min. MeI (20 μL, 0.31 mmol) was then added, and the reaction mixture was stirred at room temperature for 1 h. After this time, the reaction was quenched by the careful addition of H_2O, and the product was extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography to yield **33** (10 mg, 0.028 mmol, 90%) as a bright yellow solid. ^{1}H NMR (DMSO-d_6, 600 MHz): δ 2.11 (s, 6H), 3.34 (s, 6H), 3.55 (s, 3H), 3.56 (s, 3H), 4.20 (s, 2H), 6.48 (d, J = 9.9 Hz, 1H), 6.99 (s, 2H), 7.78 (d, J = 9.9 Hz, 1H). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 15.9, 35.6, 37.7, 43.9, 59.1, 100.5, 123.3, 129.3, 135.8, 143.1, 154.6, 160.3, 164.1, 169.9, 175.1. HRESIMS calcd for $C_{20}H_{25}N_4O_2$ ($[M + H]^+$), 353.1978; found, 353.1982.

Cell-Based Assay for Compounds Causing Mitotic Arrest. We used a modification of a literature protocol.[16] MCF-7mp53 cells were seeded in 96-well plates (PerkinElmer Viewplate) and treated in triplicate with compounds at 1−50 μg/mL for 20 h at 37 °C. The plates were centrifuged at 50*g* for 2 min to increase adherence of mitotic cells. The cells were then fixed and permeabilized with 3.7% formaldehyde and 0.1% Triton X-100 in TBS (10 mM Tris-HCl, pH 7.4, 150 mM NaCl) for 15 min. Protein-binding sites were blocked using 1% bovine serum albumin in TBS for 30 min. The cells were then incubated with 1:50 TG-3 antibody[17,18] for 1 h, washed in TBS, and incubated with 1:500 goat antimouse AlexaFluor 568 (Invitrogen) for 1 h at room temperature. The wells were rinsed once with TBS, and nuclei were stained with 500 ng/mL Hoechst 33342 (Invitrogen) in TBS for 10 min and rinsed three times with TBS. The plates were analyzed with a Cellomics ArrayScan VTI using the Target Activation protocol. The cells were identified by their nuclear staining in the first channel, and the number of cells in each field with the strong TG-3 staining indicative of a mitotic cell was quantified in the second red channel, allowing calculation of the percentage of cells in mitosis.

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Supporting Information Available: NMR spectra for compounds **4**, **7**, **9**, **11**, **13**, **15**, **17**, **23**−**25**, and **27**−**33**. This material is available free of charge via the Internet at http://pubs.acs.org.

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