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Diversity Oriented Synthesis of a Vinblastine-Templated Library of 7-Aryl-Octahydroazonino[5,4-*b*]indoles via a Three-Component Reaction

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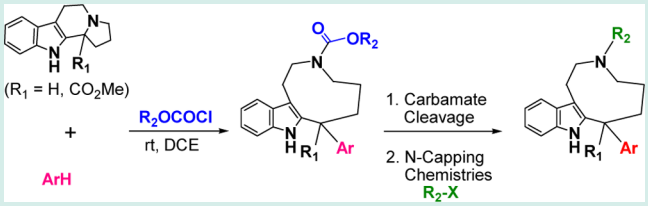
Supporting Information

ABSTRACT: A vinblastine-templated library of 7-aryl-octahydroazonino[5,4-*b*]indoles was prepared by a three-component reaction from indolizino[8,7-*b*]indoles, chloroformates, and activated arenes via a chloroformate mediated fragmentation of the indolizinoindole nucleus followed by insertion of an activated arene. In addition to N3-carbamoyl-7-aryl-octahydroazonino-[5,4-*b*]indoles prepared in one step, a wide range of N3-substituted substrates were synthesized in one pot via the derivatization of a versatile N3−H-azonino[5,4-*b*]indole intermediate generated in situ by application of the same strategy. A subset of 308 compounds out of a virtual library of 3216, representing 13 different chemotypes, was prepared by high throughput solution-phase synthesis and subsequently purified by mass-triggered high performance liquid chromatography (HPLC). A total of 188 compounds with a minimum purity of 80% by $UV_{214\ nm}$ and 85% by evaporative light scattering detection (ELSD) was isolated for primary screening.



KEYWORDS: *7-aryl-octahydroazonino[5,4-b]indole, indolizino[8,7-b]indole, chloroformate, arene, three-component reaction*

INTRODUCTION

The design and synthesis of screening libraries for the generation of new leads constitutes an integral part of the drug discovery process since the advent of combinatorial chemistry.[1] Natural products have achieved an unrivaled success rate in generating lead structures for drug discovery because of their inherent complexity and high density of functional and pharmacophoric groups.[2−4] They cover diversity and chemistry space not yet readily available from synthetic drug-like libraries and continue to inspire the design of novel natural product-like libraries by diversity oriented synthesis (DOS) strategies.[5−9] Natural product-like molecules could populate chemical space occupied by bioactive natural products and lead to the discovery of novel modulators of protein−protein interactions as well as chemical probes for dissecting dynamic signaling pathways.[10,11]

In the search for natural product-like screening libraries, we turned our attention to the design of structurally diverse azonino-[5,4-*b*]indoles as new simplified vinblastine analogues. Vinblastine and vincristine were among the earliest clinically active antimitotic agents identified to inhibit tubulin polymerization by binding *β*-tubulin at the Vinca alkaloid site.[12,13] They are widely used as antitumor drugs, and research toward the discovery of new analogues of vinblastine and vincristine is ongoing.[14−17] Inspired by early model studies on vinblastine's hypothetical mechanism of action,[18] we surmised that a series of octahydro-azonino[5,4-*b*]indoles (**I**) with an activated aryl group at the C7 carbon center might exhibit some of vinblastine's structural features required for cytotoxic activity and lead to a series of new truncated vinblastine analogues (Figure 1). Access to a diverse set of the target compounds would involve the intermediacy of

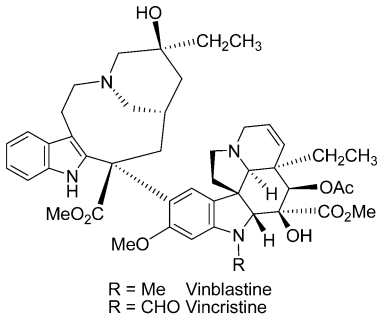


Figure 1. 7-Aryl-octahydroazonino[5,4-*b*]indoles from *β*-THC scaffolds.

7-aryl-octahydroazonino[5,4-*b*]indole **II**, containing an activated aryl group at the C7 carbon center as well as a secondary N3 nitrogen center. Compound **II** would in turn result from the fragmentation of tertiary amine **III**.

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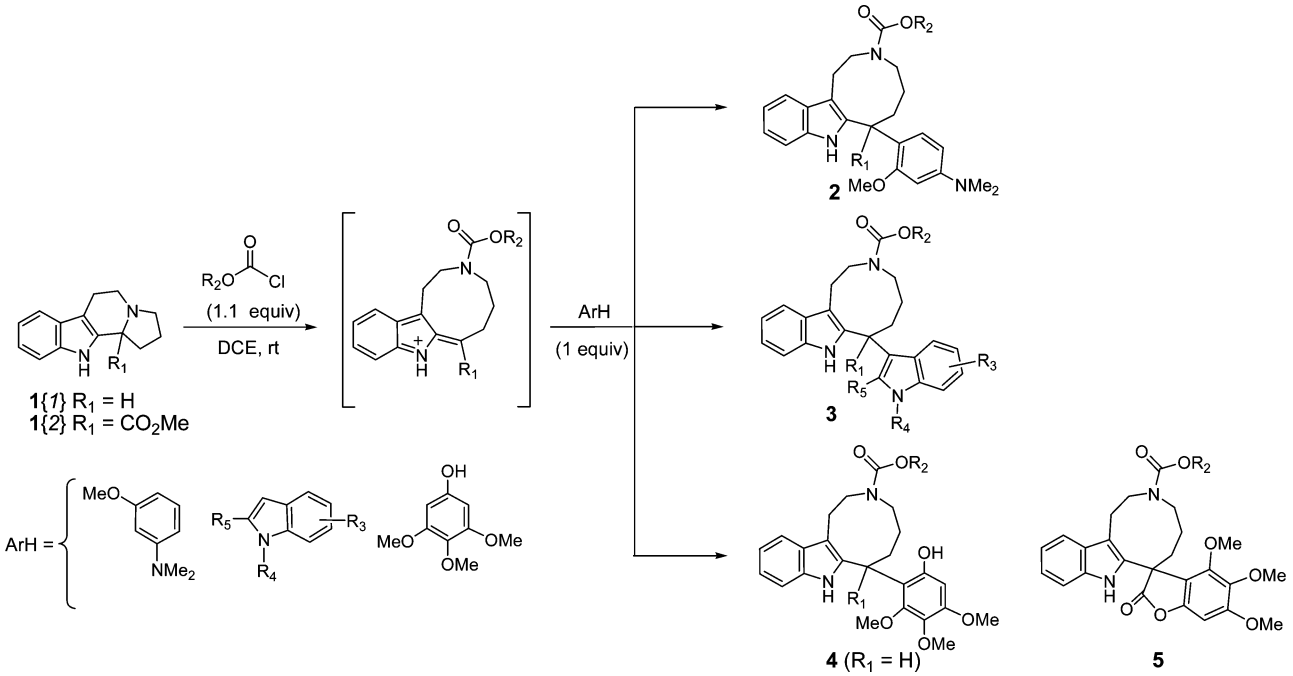
Scheme 1. Synthesis of 7-Aryl-octahydroazonino[5,4-*b*]indoles via a Three-Component Reaction



Aiming to develop a strategy amenable to high throughput solution-phase synthesis of the target compounds, we turned our attention to the chloroformate mediated fragmentation[19−21] of indolizino[8,7-*b*]indole **III** (R_1 = H, CO_2Me). Herein, we wish to report the results of our studies pertaining to the solution-phase synthesis of a diverse library of 7-aryl-octahydroazonino[5,4-*b*]indoles with general structure **I**, resulting from the chloroformate mediated fragmentation of indolizinoindole **III** (R_1 = H, CO_2Me) accompanied by insertion of an activated arene to the newly generated azonino nucleus.

RESULTS AND DISCUSSION

Library Design. On the basis of prior studies,[22,23] we realized that a series of structurally diverse N3-carbamoyl-7-aryloctahydroazonino[5,4-*b*]indoles with general structures **2**−**4** could result in one step from indolizino[8,7-*b*]indole **1**{*1,2*}, chloroformates and the corresponding activated arenes, that is, *m*-dimethylaminoanisole, indoles, and 3,4,5-trimethoxyphenol (Scheme 1). Treatment of indolizinoindole **1**{*1,2*} with a chloroformate would induce cleavage of the C_1-N_2 bond in the *β*-THC nucleus, providing a transient iminium ion which could then be trapped by an activated arene to produce the corresponding azoninoindole.

The participation of activated anilines in the chloroformate mediated fragmentation of indolizinoindoles has been described in the past.[24,25] Thus, combination of **1**{*1,2*} with *m*-dimethylaminoanisole and a chloroformate in dichloroethane (DCE), upon stirring at room temperature, resulted in the formation of N-carbamoyl-azonino[5,4-*b*]indole **2**.[26] Activated arenes such as indoles and oxygenated aromatics were also found to participate in this fragmentation/insertion chemistry.[23] Indolizinoindoles **1**{*1*}[27] and **1**{*2*}[28] underwent a chloroformate induced facile ring expansion with indoles in DCE to produce the corresponding bis-indole alkaloids with general structure **3**. A number of indoles with various substitution patterns were shown to be reactive, with the alkylation occurring exclusively at the *β*-indolic carbon. Of a series of oxygenated aromatics tested, 3,4,5-trimethoxylphenol was that found to insert to the azonino nucleus during the fragmentation of **1**{*1*} and **1**{*2*}. Indeed, treatment of **1**{*1*} with a chloroformate, in the presence of 3,4,5-trimethoxylphenol, gave phenol adduct **4**. However, spirolactone **5**, instead of azonine ester **4** (R_1 = CO_2Me), was formed from scaffold **1**{*2*} under the same reaction conditions.[23] Attempts to generate compound **4** (R_1 = CO_2Me) were not fruitful and spirolactone **5** was obtained as the sole product, presumably via an intramolecular lactonization reaction between the C7 carboxymethyl group and the neighboring phenolic hydroxyl.[29] Both aromatic and aliphatic chloroformates induced azonine ring formation equally well.

Given the limited number of available *m*-dialkylaminoanisoles, the use of N-substituted-4-(3-methoxyphenyl)piperazines seemed a viable alternative since they could exhibit similar reactivity and furthermore introduce additional diversity based on the substitution pattern of the secondary piperazine nitrogen. However, the limited reactivity of N-alkyl substrates,[30] coupled with the limited availability of N-acyl and N-sulfonyl-4-(3-methoxyphenyl)-piperazines, led us to explore the use of N-Boc-4-(3-methoxyphenyl)piperazine instead (Scheme 2). The presence of the N-Boc carbamate group would reduce the basicity of the piperazine nitrogen and thus prevent its competitive side reaction with the chloroformate reagent during the fragmentation of the indolizinoindole nucleus. In addition, subsequent cleavage of the N-Boc carbamate group would enable the introduction of additional diversity at the secondary piperazine nitrogen through a wide range of capping reagents.

Indeed, indolizinoindoles **1**{*1*} and **1**{*2*} underwent a similar insertion reaction with N-Boc-4-(3-methoxyphenyl)piperazine[31] to afford octahydroazonino[5,4-*b*]indole **6** (Scheme 2). Subsequent cleavage of the N-Boc group with HCl in dioxane resulted in piperazine **7**, a versatile intermediate for the synthesis of several chemotypes. For example, treatment of crude piperazine **7** with acid chlorides, in the presence of an excess of Hünig's base at room temperature, afforded a set of amides **8**. Similarly, reaction of **7** with sulfonyl chlorides under the same reaction conditions gave sulfonamides **9**. Also, piperazine **7** underwent a reductive amination with aldehydes in the presence of $NMe_4BH(OAc)_3$ to produce N-alkylated piperazines **10**.[32]

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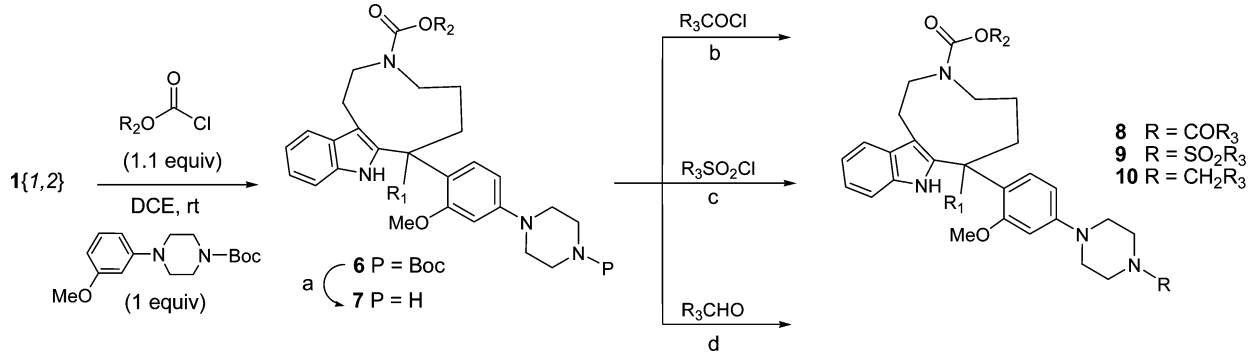
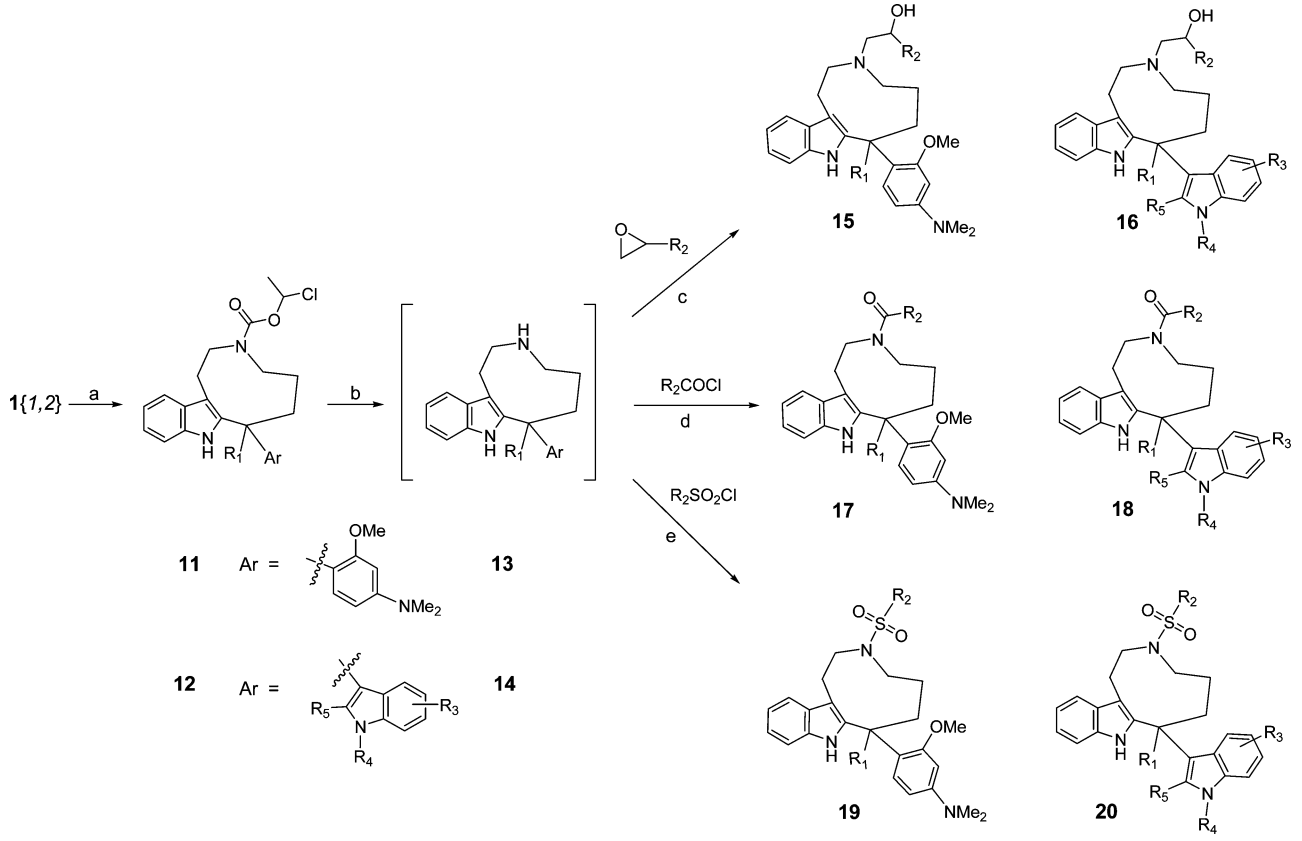
Scheme 2. Entry to Azonino[5,4-*b*]indoles Containing a 4-(3-Methoxyphenyl)piperazine Moiety at C7[a]



[a]***Reagents and conditions:*** (a) 4 N HCl in dioxane, DCE, rt, 3 h; (b) R_3COCl (1 equiv), N*i*Pr_2Et (9 equiv), CH_3CN, rt, 16 h; (c) R_3SO_2Cl (1 equiv), N*i*Pr_2Et (7.5 equiv), CH_3CN, rt, 16 h; (d) R_3CHO (1 equiv), $NMe_4BH(OAc)_3$ (2.5 equiv), DCE, rt, 15 h.

Scheme 3. Access to a Diverse Set of N3-Substituted-7-aryl-octahydroazonino[5,4-*b*]indoles via the in Situ Derivatization of a Secondary Azonine[a]



[a]***Reagents and conditions:*** (a) ACE-Cl (1.1 equiv), ArH (1 equiv), DCE, rt, 12 h; (b) MeOH, 50 °C, 3 h; (c) Epoxide (1 equiv), N*i*Pr_2Et (5 equiv), MeOH, 55 °C, 8 h; (d) R_2COCl (1 equiv), N*i*Pr_2Et (7.5 equiv), CH_3CN, rt, 16 h; (e) R_2SO_2Cl (1 equiv), N*i*Pr_2Et (7.5 equiv), CH_3CN, rt, 16 h.

The strategies described in Schemes 1 and 2 provide access to a diverse set of N3-carbamoyl-7-aryl-octahydroazonino[5,4-*b*]-indoles in one pot. Although enrichment of diversity at the C7-carbon center is achieved through the insertion of different activated arenes, the diversity at the newly generated azonine N3-center is limited to the carbamate group. Therefore, access to a structurally diverse set of octahydroazonino[5,4-*b*]indoles should be attained via the derivatization of the secondary azonine nitrogen and should require the intermediacy of the corresponding N3−H-azonino[5,4-*b*]indole. Considering the rather harsh conditions required for the cleavage of nonactivated alkyl or aryl carbamates, access was necessary to a carbamate intermediate which could be cleaved to the requisite secondary azonine in a high throughput synthesis platform under mild conditions. Among several activated carbamate groups which could be readily cleaved, *α*-chloroethylcarbamate[33] seemed to constitute an attractive candidate since it could be cleaved by heating in MeOH. Indolizinoindoles **1**{*1*} and **1**{*2*} underwent a facile ring expansion as well with *α*-chloroethylchloroformate (ACE-Cl), in the presence of *m*-dimethylaminoanisole and indoles, to produce the corresponding *α*-chloroethylcarbamates **11** and **12** respectively (Scheme 3). Subsequent cleavage of the labile carbamates **11** and

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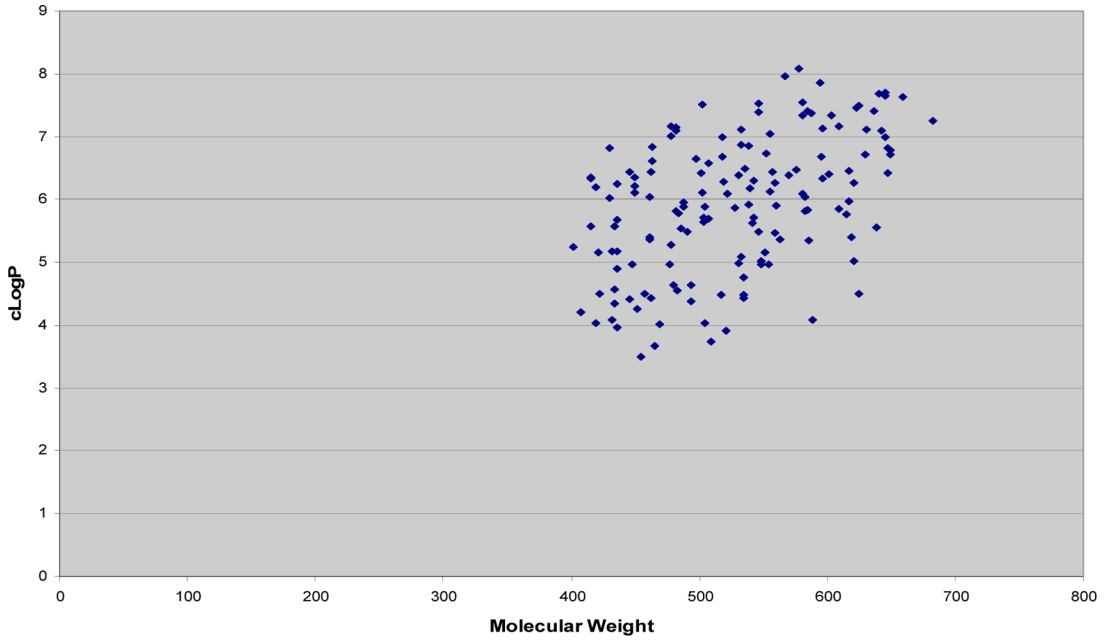

Figure 2. cLogP plot versus MW profile of a set of 160 compounds selected for synthesis.

12 under heating in MeOH at 50 °C for 3 h, produced the requisite N3−H-azonino[5,4-*b*]indoles **13** and **14**, respectively.[34] Azonines **13** and **14** could subsequently be derivatized in situ, without having to be isolated, and result in a diverse set of N3-substituted-7-aryl-octahydroazonino[5,4-*b*]indoles. Thus, treatment of crude **13** with epoxides in MeOH and an excess of Hünig's base, produced aminoalcohols **15**. Reaction of **13** with a series of acid chlorides as well as sulfonyl chlorides in the presence of an excess of Hünig's base, produced amides **17** and sulfonamides **19**, respectively.[35] Likewise, elaboration of azonine **14** under the same reaction conditions afforded azonino[5,4-*b*]indoles **16**, **18**, and **20**.

Indolylazonines with general structure **14** (R_1 = H) were found to be unstable, and they gradually decomposed at room temperature. They were acid sensitive and decomposed to indolizinoindole **1**{*1*}.[23] Decomposition was observed even when samples were dissolved in $CDCl_3$, presumably by traces of HCl present in the solvent. Therefore, immediate derivatization of azonine **14** was necessary.

Library Synthesis. On the basis of the chemistry depicted in Schemes 1, 2, and 3, the synthesis of a diverse library of 7-aryl-octahydroazonino[5,4-*b*]indoles, consisting of 13 different chemotypes, utilized indolizinoindoles **1**{*1,2*}, *m*-dimethylaminoanisole, 3,4,5-trimethoxyphenol, N-Boc-4-(3-methoxyphenyl)-piperazine, and a set of commercially available indoles, chloroformates, acid chlorides, sulfonyl chlorides, aldehydes, and epoxides. Given that the design of these octahydroazonino[5,4-*b*]indoles is based on a large natural product, it was expected that the overall library profile would not comply with Lipinski's[36] rule of five criteria. Indeed, reagent selection according to the rule of five criteria was rather limited because of prevalent molecular weight and clogP violations observed in a large portion of the library (Figure 2). Therefore, chemsets of 10 chloroformates, 23 indoles, 25 acid chlorides, 25 sulfonyl chlorides, 18 aldehydes, and 12 epoxides were selected from a larger pool of commercially available reagents, based on diversity criteria, using an integrated ArQule library design tool (Figure 3).[37]

Library synthesis was performed on 100 μmol scale with a small set of compounds being produced for each chemotype, mainly by cherry-picking[38] of the virtual library. A subset of 308 compounds was selected for synthesis from a potential virtual library of 3216 octahydroazoninoindoles (Table 1). These compounds were then purified on an in-house high throughput purification platform by reverse phase high performance liquid chromatography (HPLC) with mass-triggered fraction collection,[39,40] quantified by weight, and characterized by LC/MS to establish the purity and identity of each collected compound. The overall passing rate of the library was 61%, and 188 good compounds were isolated for primary screening assays according to our purity and quantity criteria (quantity ≥5 μmol, purity: $UV_{214\ nm}$ ≥80% and ELSD ≥ 85%). Compounds that did not fulfill the purity and quantity criteria were immediately culled. Representative compounds from the library are illustrated in Figure 4. Numbers in parentheses indicate the isolated yield in μmol, the $UV_{214\ nm}$ and the evaporative light scattering detection (ELSD) purity of samples isolated after high throughput HPLC purification, respectively. More details regarding the purity and quantity of all compounds synthesized, as well as the synthesis layout of the chemotypes described, can be found in the Supporting Information section. A few samples were randomly selected from each chemotype subset for evaluation by ^{1}H NMR and ^{13}C NMR to confirm their structures and purities.[41]

A passing rate >50% was observed for the majority of chemotypes synthesized, as shown in Table 1, except for azoninoindoles **16**, **18**, and **20** which were produced with a passing rate <40%. The low passing rate for these chemotypes is not surprising and may be attributed to the instability of the intermediate azonine **14** (R_1 = H), as it was observed in the early library design process. Although a high passing rate was observed for the synthesis of chemotypes **9** and **10**, their average recovery was rather low presumably because of incomplete derivatization of the intermediate piperazine **7**.

CONCLUSIONS

We have designed and prepared by solution-phase synthesis a vinblastine-templated library of 7-aryl-octahydroazino[5,4-*b*]-indoles, constisting of 13 different chemotypes. The chemistry

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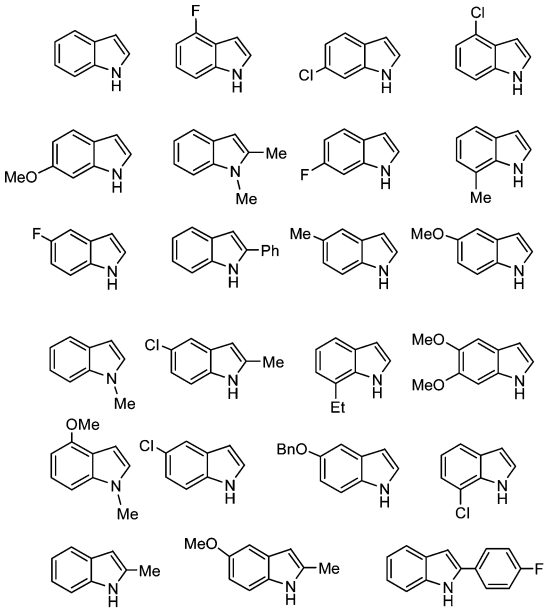
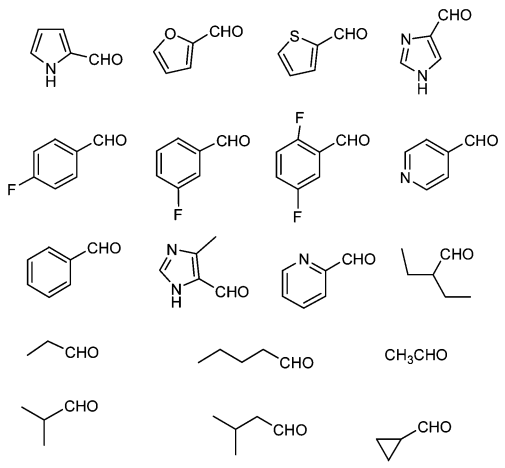
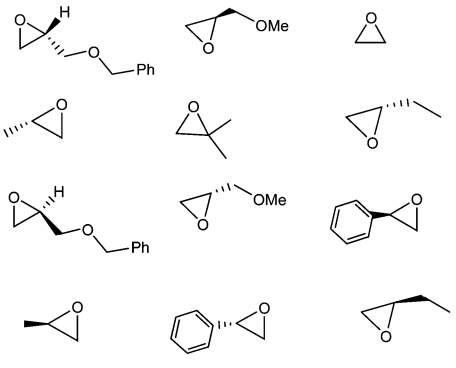


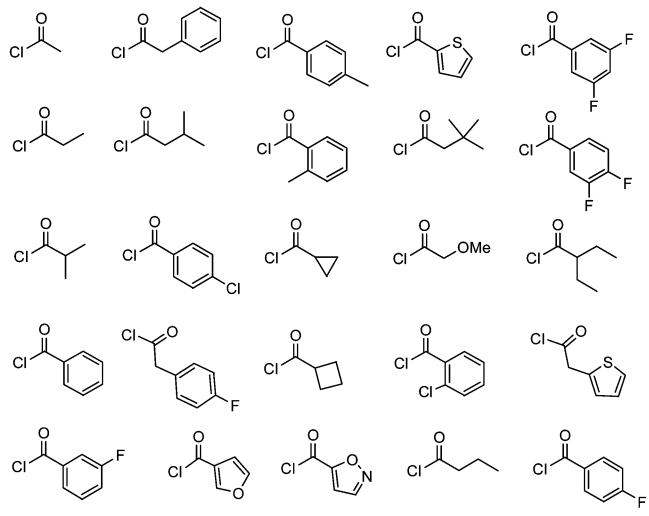
Indoles{*1-23*}



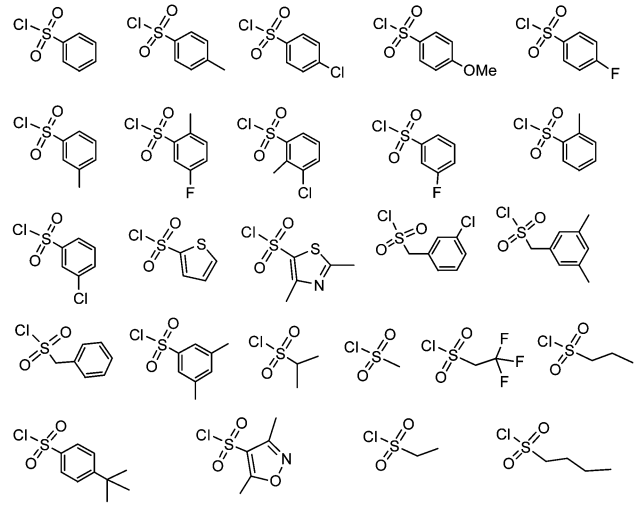
Aldehydes{*1-18*}



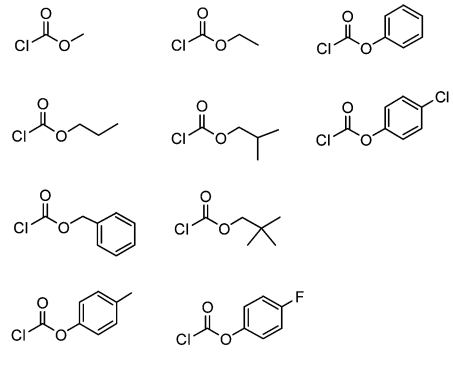
Epoxides{*1-12*}



Acid chlorides{*1-25*}



Sulfonyl chlorides{*1-25*}



Chloroformates{*1-10*}

Figure 3. Chemsets of commercially available monomers used in library synthesis.

described here contains all the attributes of a multicomponent reaction[42−44] resulting in diverse natural product-like structures in an efficient and convergent manner. In addition, it illustrates an example of diversity oriented synthesis of natural product-like molecules encompassing elements of appendage and functional diversity.[45,46] Furthermore, entry to spirolactone chemotype **5**

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Table 1. Library Results Per Azonino[5,4-*b*]indole Chemotype Synthesis

chemotype	virtual library size	produced	isolated[a]	quantity[b] (μmol)	purity[c] UV_{214}/ELSD (%)	passing rate[d] (%)
2	20	20	17	50.3	95/100	85
3	460	24	17	36.4	97/100	71
4	10	10	6	36.9	99/100	60
7	20	20	14	27.2	92/98	70
8	500	43	23	27	95/99	54
9	250	23	16	9.8	96/100	67
10	180	24	19	8.9	98/100	79
15[e]	24	24	17	36.7	95/100	71
16[e]	552	24	9	23.9	94/100	38
17	25	24	18	36	92/100	75
18	575	24	4	27.6	90/100	17
19	25	24	19	27.3	99/100	79
20	575	24	9	15.4	93/100	38
total	**3216**	**308**	**188**	**27.9**[f]	**95/99.8**[f]	**61**[f]

[a]Isolated compounds after HPLC purification meeting the purity/quantity criteria. [b]Average recovery per chemotype set isolated based on 100 μmol scale reactions. [c]Average purity per chemotype set isolated. [d]Percentage of the library that passed the purity/quantity culling criteria. [e]Aminoalcohol derivatives from optically active epoxides were isolated as a mixture of diastereomers. [f]Overall library recovery, purity, and passing rate.

represents an example of skeletal diversity[47] where a new scaffold can be generated within the same library. Results of our studies, including biological data, will be reported in due course.

EXPERIMENTAL PROCEDURES

General Experimental Procedure for the Synthesis of N3-Carbamoyl Azonino[5,4-*b*]indoles (2, 3, 4, 6, 11, 12). Stock solutions of indolizinoindoles **1**{*1,2*} (0.1 M), chloroformates (0.5 M), and activated arenes (0.5 M) were prepared in anhydrous DCE. Solutions of **1**{*1,2*} (1000 μL, 100 μmol), arene (200 μL, 100 μmol) and chloroformate (220 μL, 110 μmol) were subsequently dispensed to a round-bottom two-drum vial in a 24 well plate. The reaction mixture was shaken at room temperature overnight and then concentrated under vacuum to generate the crude product. The samples of chemotypes **2**, **3**, and **4** were dissolved in 500 μL of DMSO and subsequently purified by mass-triggered reverse phase prep-HPLC.

*Compound **2**{11}*. 300 MHz ^{1}H NMR ($CDCl_3$, as a mixture of rotamers) δ 7.45 (d, 1 H, *J* = 7.6 Hz), 7.29 (m, 1 H), 7.15−7.06 (m, 3 H), 6.32 (br s, 2 H), 4.02 (m, 2 H), 3.75 (d, 3 H), 3.63 (s, 3 H), 3.32−2.60 (m, 8 H), 2.92 (s, 6 H), 1.62 (q, 4 H, *J* = 6.7, 7.0 Hz), 0.88 (q, 3 H, *J* = 6.4, 7.3 Hz). 75.4 MHz ^{13}C NMR ($CDCl_3$, as a mixture of rotamers) δ 157.1, 157.0, 156.3, 150.9, 134.5, 133.0, 129.0, 128.2, 125.3, 121.6, 118.8, 117.9, 117.8, 110.5, 104.3, 96.8, 66.8, 66.7, 55.4, 52.3, 47.6, 47.3, 46.7, 45.9, 41.0, 26.3, 25.8, 23.1, 22.4, 10.6, 10.5. MS (ES^+) *m*/*z* for $C_{29}H_{37}N_3O_5$: calcd, 507.27; found, 508.39 (M+H).

*Compound **3**{7}*. 300 MHz ^{1}H NMR ($CDCl_3$, as a mixture of rotamers) δ 8.20 (s, 1 H), 7.65 (s, 1 H), 7.60 (d, 1 H, *J* = 6.4 Hz), 7.36−6.88 (m, 11 H), 6.98 (d, 1 H, *J* = 7.9 Hz), 6.91 (t, 1 H, *J* = 7.9 Hz), 4.83 (m, 1 H), 4.32 (m, 2 H), 3.54 (m, 1 H), 3.32−3.12 (dddd, 1 H, *J* = 4.7, 15.8 Hz), 3.06−2.83 (m, 2 H), 2.60−2.07 (m, 2 H), 1.48 (d, 1 H, *J* = 13.2 Hz), 1.21 (m, 1 H). 75.4 MHz ^{13}C NMR ($CDCl_3$, as a mixture of rotamers) δ 156.0, 155.0, 151.7, 151.6, 137.7, 137.3, 136.8, 135.8, 129.5, 129.4, 129.3, 128.7, 128.6, 128.4, 127.3, 127.2, 125.53, 125.5, 125.4, 122.7, 122.6, 122.2, 122.1, 121.3, 120.8, 120.6, 120.0, 119.9, 119.8, 119.5, 119.3, 118.6, 118.5, 117.9, 117.7, 112.6, 111.8, 111.3, 111.1, 110.9, 110.8, 51.4, 50.5, 49.9, 49.8, 33.6, 32.8, 32.7, 24.6, 24.3, 24.2, 22.4, 21.7. MS (ES^+) *m*/*z* for $C_{29}H_{27}N_3O_2$: calcd, 449.21; found, 450.29 (M+H).

*Compound **3**{17}*. 300 MHz ^{1}H NMR ($CDCl_3$) δ 7.50 (d, 1 H, *J* = 7.6 Hz), 7.40−6.99 (m, 9 H), 4.05 (t, 1 H, *J* = 6.1 Hz), 3.96 (m, 1 H), 3.76 (s, 3 H), 3.72 (s, 3 H), 3.1 (br s, 5 H), 2.62 (br s, 1 H), 1.62 (m, 6 H), 0.94 (t, 3 H, *J* = 7.3 Hz). MS (ES^+) *m*/*z* for $C_{29}H_{33}N_3O_4$: calcd, 487.25; found, 488.31 (M+H).

*Compound **4**{4}*. 300 MHz ^{1}H NMR ($CDCl_3$, as a mixture of rotamers) δ 7.75 (br s, 1 H), 7.48 (d, 1 H, *J* = 7.0 Hz), 7.26−7.06 (m, 3 H), 6.39 (s, 0.7 H), 6.30 (s, 0.3 H), 5.20 (dd, 0.7 H, *J* = 5.3, 13.2 Hz), 5.12 (dd, 0.3 H, *J* = 4.4, 13.1 Hz), 4.18−4.0 (m, 2 H), 3.96 (s, 2 H), 3.88 (d, 3 H), 3.79 (d, 6 H), 3.73 (s, 1 H), 3.39 (t, 0.7 H, *J* = 12, 14.9 Hz), 3.24 (t, 0.3 H, *J* = 11.7, 14.1 Hz), 3.04 (m, 1 H), 2.81−2.60 (m, 2 H), 1.85−1.62 (m, 2 H), 1.35 (m, 1 H), 1.05 (q, 1 H, *J* = 12, 13.5 Hz). 75.4 MHz ^{13}C NMR ($CDCl_3$, as a mixture of rotamers) δ 158.1, 157.3, 152.7, 152.0, 150.4, 149.9, 138.4, 138.3, 135.4, 129.0, 128.2, 127.8, 125.3, 120.8, 118.6, 117.5, 117.4, 116.1, 115.6, 112.2, 111.7, 110.6, 97.5, 97.0, 61.4, 60.9, 55.9, 52.8, 52.7, 51.0, 50.1, 49.6, 49.2, 31.9, 31.5, 31.3, 24.7, 23.7, 22.4, 21.4. MS (ES^+) *m*/*z* for $C_{25}H_{30}N_2O_6$: calcd, 454.21; found, 455.25 (M+H).

Synthesis of Azonino[5,4-*b*]indoles 7. A solution of crude carbamate **6** (∼100 μmol) in DCE, prepared according to the general method, was treated with 500 μL (2 mmol) of a solution of 4 N HCl in dioxane. The reaction mixture was shaken at room temperature for 3 h and then concentrated to afford the crude product. The samples were dissolved in 500 μL of DMSO and subsequently purified by mass-triggered reverse phase prep-HPLC.

*Compound **7**{11}*. 300 MHz ^{1}H NMR ($CDCl_3$, as a mixture of rotamers) δ 9.40 (br s, 1 H), 7.45 (d, 1 H, *J* = 7.0 Hz), 7.28−7.06 (m, 4 H), 6.43 (t, 2 H, *J* = 6.4 Hz), 4.60−4.30 (br s, 1 H), 4.03 (m, 2 H), 3.75 (m, 6 H), 3.16 (s, 6 H), 3.04 (s, 6 H), 2.55 (s, 4 H), 1.62 (q, 4 H, *J* = 7.0 Hz), 0.93 (q, 3 H, *J* = 6.7 Hz). 100.6 MHz ^{13}C NMR ($CDCl_3$, as a mixture of rotamers) δ 157.0, 151.7, 134.5, 129.0, 128.2, 125.3, 121.8, 119.0, 118.0, 117.9, 110.7, 107.7, 100.6, 66.9, 66.7, 55.5, 52.4, 49.3, 49.2, 47.6, 47.2, 46.7, 45.9, 45.4, 26.3, 25.8, 23.1, 22.4, 10.6, 10.5. MS (ES^+) *m*/*z* for $C_{31}H_{40}N_4O_5$: calcd, 548.30; found, 549.50 (M+H).

Synthesis of Azonino[5,4-*b*]indoles 8 and 9. Crude piperazine **7**, resulting from the deprotection of crude carbamate **6** prepared on 100 μmol scale, was dissolved in CH_3CN (1 mL) and in 1 mL (0.75 mmol) of a 0.75 M solution of Hunig's base in CH_3CN. Addition of 440 μL (110 μmol) of a 0.25 M solution of acid chloride or sulfonyl chloride in CH_3CN followed, and the

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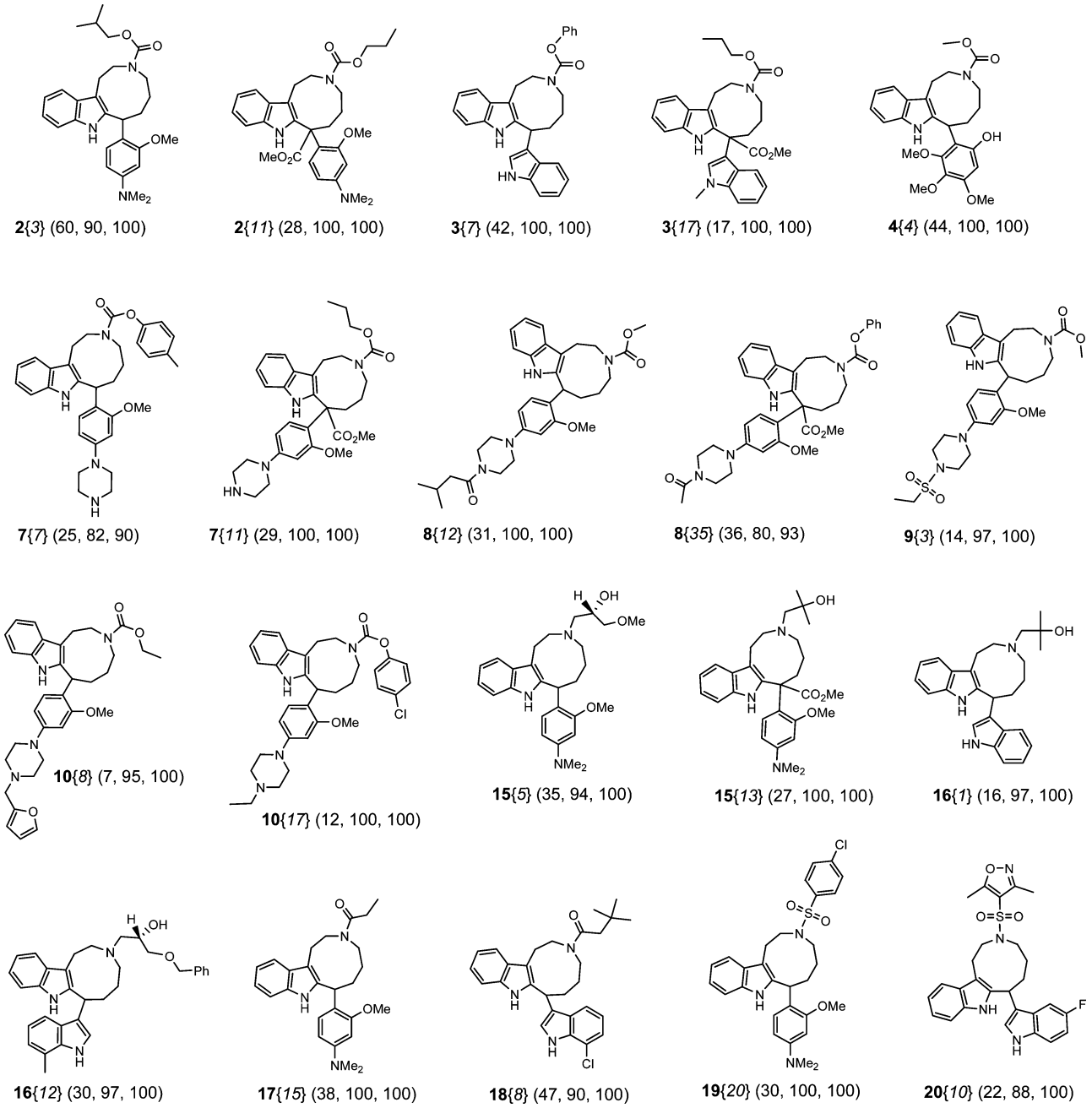

Figure 4. Representative library members isolated after HPLC purification.

reaction mixture was then shaken at room temperature overnight. The solvent was removed under vacuum, and the resulting residue was dissolved in 2 mL of DCE, followed by a liquid−liquid extraction workup on an automated station with 2 mL of H_2O. The organic layer was transferred to clean collection vials and then concentrated to afford the crude product. The samples were dissolved in 500 μL of DMSO and subsequently purified by mass-triggered reverse phase prep-HPLC.

Compound ***8****{12}*. 300 MHz ^{1}H NMR ($CDCl_3$, as a mixture of rotamers) δ 8.10 (s, 0.5 H), 7.92 (s, 0.5 H), 7.50 (d, 1 H, *J* = 3.5 Hz), 7.26−7.17 (m, 2 H), 7.08 (m, 2 H), 6.52 (dd, 1 H, *J* = 1.8, 8.2 Hz), 6.45 (d, 1 H, *J* = 10.5 Hz), 4.64 (m, 1 H), 3.97 (m, 2 H), 3.83 (s, 1 H), 3.81 (s, 2 H), 3.78 (s, 2 H), 3.73 (s, 1 H), 3.64 (s, 3 H), 3.40−2.80 (m, 10 H), 2.43−2.05 (m, 2 H), 2.0−1.77 (m, 2 H), 1.42−1.25 (m, 2 H), 1.0 (d, 6 H). 75.4 MHz ^{13}C NMR ($CDCl_3$, as a mixture of rotamers) δ 171.0, 157.9, 157.5, 156.9, 151.2, 151.1, 137.6, 137.3, 135.4, 129.0, 128.3, 128.2, 128.0, 127.3, 125.3, 124.1, 124.0, 120.8, 118.9, 117.6, 117.5, 112.2, 111.6, 110.4, 108.5, 101.2, 101.1, 55.7, 55.6, 52.5, 50.3, 50.2, 50.0, 49.8, 49.5, 49.0, 48.5, 45.7, 42.0, 41.4, 41.0, 36.6, 35.8, 31.3, 31.2, 25.8, 25.0, 24.5, 23.8, 22.7, 21.4. MS (ES^+) *m*/*z* for $C_{32}H_{42}N_4O_4$: calcd, 546.32; found, 547.36 (M+H).

Synthesis of Azonino[5,4-*b*]indoles 10. 500 μL (125 μmol) of a 0.25 M solution of aldehyde in DCE-DMF (4:1) and 1200 μL (300 μmol) of a 0.25 M solution of $Me_4NBH(OAc)_3$ in DCE were added to a suspension of crude piperazine **7** (∼100 μmol) in 0.5 mL of DCE. The reaction mixture was shaken at room temperature overnight and a liquid−liquid extraction workup was conducted on an automated station with 2.5 mL of 10% aqueous NH_4OH. The organic layer was transferred to a clean collection vials and then concentrated to afford the crude product. The resulting residues

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were dissolved in 500 μL of dimethylsulfoxide (DMSO) and subsequently purified by mass-triggered reverse phase prep-HPLC.

Synthesis of N3-hydroxyalkylazonines 15 and 16. The crude α-chloroethylcarbamates **11** and **12** (~100 μmol), prepared according to the general method, were dissolved in 1 mL of MeOH and heated at 50 °C for 3 h. The methanol solution was cooled down at room temperature and then treated with 100 μL (0.57 mmol) of neat Hunig's base and 700 μL of a 0.15 M solution of epoxide in MeOH (105 μmol). The reaction mixture was heated at 55 °C for 8 h and then concentrated. The resulting residue was subsequently dissolved in 2 mL of DCE followed by a liquid−liquid extraction workup on an automated station with 2 mL of saturated aqueous NH_4Cl. The organic layer was transferred to clean collection vials and then concentrated to afford the crude product. The samples were dissolved in 500 μL of DMSO and subsequently purified by mass-triggered reverse phase prep-HPLC.

*Compound **15**{13}*. 300 MHz ^{1}H NMR ($CDCl_3$) δ 8.85−8.45 (br s, 1 H), 7.45 (d, 1 H, J = 7.3 Hz), 7.26−7.05 (m, 4 H), 6.23 (s, 2 H), 3.79 (s, 3 H), 3.69 (s, 3 H), 3.30−3.0 (m, 6 H), 2.94 (s, 6 H), 2.80−2.37 (m, 6 H), 1.15 (s, 6 H). MS (ES^+) m/z for $C_{29}H_{39}N_3O_4$: calcd, 493.29; found, 494.23 (M+H).

*Compound **16**{1}*. 300 MHz ^{1}H NMR ($CDCl_3$) δ 8.12 (s, 1 H), 7.54 (s, 1 H), 7.52 (s, 1 H), 7.34 (d, 1 H, J = 8.2 Hz), 7.27−7.02 (m, 6 H), 6.93 (t, 1 H, J = 7.9 Hz), 5.46 (t, 1 H, J = 8.2 Hz), 3.20−2.94 (m, 3 H), 2.88−2.72 (m, 4 H), 2.60 (d, 1 H, J = 13.8 Hz), 2.27 (m, 2 H), 1.39 (m, 2 H), 1.24 (s, 3 H), 1.20 (s, 3 H). 100.6 MHz ^{13}C NMR ($CDCl_3$) δ 137.6, 136.6, 135.4, 129.0, 128.7, 128.2, 127.1, 122.4, 120.8, 120.5, 119.7, 119.6, 118.8, 117.6, 110.9, 110.5, 73.0, 70.7, 59.9, 34.3, 33.5, 28.6, 28.4, 26.3, 24.9. MS (ES^+) m/z for $C_{26}H_{31}N_3O$: calcd, 401.25; found, 402.19 (M+H).

*Compound **16**{12}*. 300 MHz ^{1}H NMR ($CDCl_3$, as a mixture of diastereomers) δ 8.11 (s, 1 H), 7.56 (s, 1 H), 7.52 (d, 1 H, J = 7.3 Hz), 7.31−6.80 (m, 12 H), 5.38 (br s, 1 H), 4.45 (s, 2 H), 4.0 (br s, 1 H), 3.59−3.42 (m, 2 H), 3.35−2.95 (m, 4 H), 2.72 (s, 4 H), 2.47 (s, 3 H), 2.20 (s, 2 H), 1.36 (m, 2 H). MS (ES^+) m/z for $C_{33}H_{37}N_3O_2$: calcd, 507.29; found, 508.12 (M+H).

Synthesis of Azonino[5,4-*b*]indoles 17−18 and 19−20. The crude α-chloroethylcarbamates **11** and **12** (~100 μmol), prepared according to the general method, were dissolved in 1 mL of MeOH and heated at 50 °C for 3 h. The solvent was concentrated, and the resulting residue was dissolved in 1 mL (0.75 mmol) of a 0.75 M solution of Hunig's base in CH_3CN, followed by the addition of 500 μL (125 μmol) of a 0.25 M solution of acid chloride or sulfonyl chloride in CH_3CN. The reaction mixture was stirred at room temperature overnight and then concentrated under vacuum. The resulting residue was dissolved in 2 mL of DCE followed by a liquid−liquid extraction workup on an automated station with 2 mL of H_2O. The organic layer was transferred to clean collection vials and then concentrated to afford the crude product. The samples were dissolved in 500 μL of DMSO and subsequently purified by mass-triggered reverse phase prep-HPLC.

*Compound **17**{15}*. 300 MHz ^{1}H NMR ($CDCl_3$, as a mixture of rotamers) δ 8.10 (s, 0.7 H), 7.87 (s, 0.3 H), 7.52 (m, 1 H), 7.22 (m, 2 H), 7.10 (s, 2 H), 6.73 (s, 0.3 H), 6.40 (t, 2 H, J = 7.3, 8.2 Hz), 4.62 (dd, 0.7 H, J = 4.4, 13.8 Hz), 4.53 (dd, 0.3 H, J = 3.8, 12.9 Hz), 4.28 (dd, 0.7 H, J = 5.3, 13.2 Hz), 4.05 (m, 0.3 H), 3.80 (d, 3 H), 3.72 (t, 1 H, J = 12.0 Hz), 3.42−3.12 (m, 2 H), 3.05 (m, 1 H), 2.90 (s, 6 H), 2.75 (t, 1 H, J = 12.0 Hz), 2.55 (m, 3 H), 1.85 (m, 1 H), 1.52 (m, 1 H), 1.25 (m, 1 H), 1.15 (t, 3 H, J = 7.3 Hz). MS (ES^+) m/z for $C_{26}H_{33}N_3O_2$: calcd, 419.26; found, 420.31 (M+H).

*Compound **18**{8}*. 300 MHz ^{1}H NMR ($CDCl_3$, as a mixture of rotamers) δ 8.45 (s, 1 H), 8.26 (s, 0.5 H), 7.64−7.41 (m, 2 H), 7.27 (m, 1 H), 7.20−6.93 (m, 4 H), 6.82 (m, 1 H), 4.62 (m, 1 H), 4.45 (m, 1 H), 4.15 (m, 0.3 H), 3.90 (t, 0.7 H, J = 13.2, 14.6 Hz), 3.55−3.10 (m, 2 H), 3.0 (m, 1 H), 2.70 (t, 1 H, J = 10.8 Hz), 2.55 (m, 1 H), 2.32−2.05 (m, 2 H), 1.80 (dd, 1 H, J = 2.3, 7.0 Hz), 1.50 (br d, 1 H, J = 13.5 Hz), 1.25 (br s, 1 H), 1.06 (d, 3 H), 1.03 (d, 6 H). MS (ES^+) m/z for $C_{28}H_{32}ClN_3O$: calcd, 461.22; found, 462.24 (M+H).

*Compound **19**{20}*. 300 MHz ^{1}H NMR ($CDCl_3$, as a mixture of rotamers) δ 8.26 (s, 0.7 H), 8.14 (s, 0.3 H), 7.78 (d, 2 H, J = 8.5 Hz), 7.50 (d, 2 H, J = 8.5 Hz), 7.40 (d, 1 H, J = 7.6 Hz), 7.30−7.12 (m, 2 H), 7.05 (m, 2 H), 6.78 (s, 0.3 H), 6.73 (d, 0.3 H, J = 8.2 Hz), 6.34 (d, 0.7 H, J = 8.5 Hz), 6.30 (s, 0.7 H), 5.54 (dd, 0.3 H, J = 3.8, 12.9 Hz), 5.14 (dd, 0.7 H, J = 4.4, 12.9 Hz), 3.86 (s, 2 H), 3.80 (s, 1 H), 3.60 (m, 2 H), 3.40 (m, 1 H), 3.24 (m, 1 H), 2.94 (s, 6 H), 2.87 (m, 2 H), 2.60 (m, 1 H), 1.98 (m, 1 H), 1.43−1.25 (m, 2 H). MS (ES^+) m/z for $C_{29}H_{32}ClN_3O_3S$: calcd, 537.19; found 537.97 (M+H).

■ ASSOCIATED CONTENT

Supporting Information

General methods and purification conditions. Copies of ^{1}H and ^{13}C NMR spectra (PDF) of compounds **2**{*11*}, **2**{*16*}, **2**{*18*}, **3**{*5*}, **4**{*1*}, **4**{*8*}, **3**{*7*}, **3**{*17*}, **4**{*4*}, **7**{*11*}, **8**{*12*}, **8**{*32*}, **8**{*33*}, **8**{*39*}, **13** (R_1 = H), **13** (R_1 = CO_2Me), **15**{*13*}, **15**{*23*}, **16**{*1*}, **16**{*12*}, **17**{*15*}, **18**{*8*}, **19**{*9*}, **19**{*12*}, and **19**{*20*}. Copies of ^{1}H NMR spectra (PDF) of compounds **2**{*11*}, **3**{*7*}, **4**{*4*}, **7**{*11*}, **8**{*12*}, and **19**{*20*} recorded at 55 °C. Tables reporting the purity and quantity of all 308 compounds synthesized as well as the synthesis layout of each chemotype, are also included. Copies of LC/MS chromatograms (PDF) of the aforementioned compounds and other library members are also provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(26) LC/MS on crude reaction mixture as well as postpurification samples of chemotype **2** (R = H) resulting from scaffold **1**{*1*}, indicated the presence of a small amount (5−10%) of a regioisomeric product which might presumably be generated from the alkylation of *m*-dimethylaminoanisole *para* to the OMe group.

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(30) N-benzyl-4-(3-methoxyphenyl)piperazine failed to promote the expected ring expansion, presumably because of a competitive reaction of the chloroformate with the basic tertiary piperazine nitrogen. However, reaction of N-acetyl- and N-benzoyl-4-(3-methoxyphenyl) piperazine with **1**{*1*} and **1**{*2*} afforded the corresponding azoninoindoles under the same reaction conditions.

(31) Prepared by acylation of the corresponding N-H piperazine with Boc_2O (1.1 equiv) and NEt_3 (2 equiv) in DCE at room temperature.

(32) The same trend as with *m*-dimethylaminoanisole was observed in the reaction of scaffold **1**{*1*} with N-Boc-4-(3-methoxyphenyl)

piperazine. Similarly, LC/MS on crude reaction mixture as well as postpurification samples of chemotypes **7**−**10** (R = H), indicated the formation of a small amount (5−10%) of a regioisomeric product which might presumably result from the alkylation of N-Boc-4-(3-methoxyphenyl)piperazine *para* to the OMe group.

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(34) For the synthesis, isolation, and characterization of azonines **14** (R_1 = H, CO_2Me), see reference 23. Azonines **13** were prepared accordingly and isolated in 61% (R_1 = H) and 53% (R_1 = CO_2Me) yield, respectively. **Compound 13** (R_1 = H): ^{1}H NMR (300 MHz, CD_3OD): δ 7.51 (d, 1 H, J = 7.6 Hz), 7.34 (s, 1 H), 7.32−7.18 (m, 3 H), 7.04 (m, 2 H), 4.73 (dd, 1 H, J = 12. 6, 3.2 Hz), 3.98 (s, 3 H), 3.62−2.90 (m, 6 H), 3.25 (s, 6 H), 2.56 (q, 1 H, J = 12.3 Hz), 2.18 (m, 1 H), 2.12−1.84 (m, 2 H). ^{13}C NMR (75.4 MHz, CD_3OD): δ 157.9, 142.5, 136.5, 136.0, 134.4, 129.4, 129.3, 127.8, 121.7, 119.2, 117.2, 110.8, 107.8, 103.6, 55.7, 47.4, 46.0, 45.0, 38.7, 29.2, 25.9, 21.1. MS (ES^+) m/z for $C_{23}H_{29}N_3O$: calcd, 363.23; found, 364.22 (M+H). **Compound 13** (R_1 = CO_2Me): ^{1}H NMR (300 MHz, CD_3OD): δ 7.89 (s, 1 H), 7.51 (d, 1 H, J = 7.6 Hz), 7.40 (s, 1 H,), 7.37 (s, 1 H), 7.24−7.05 (m, 3 H), 3.94 (s, 3 H), 3.70 (s, 3 H), 3.56−3.34 (m, 3 H), 3.29 (s, 6 H), 3.26−3.05 (m, 4 H), 2.64 (br d, 1 H, J = 14.6 Hz), 1.83 (br s, 2 H). ^{13}C NMR (75.4 MHz, CD_3OD):δ 172.2, 156.5, 142.0, 134.6, 131.2, 129.6, 128.8, 126.5, 121.0, 118.0, 115.9, 109.9, 108.5, 103.2, 76.8, 54.4, 53.8, 50.6, 44.4, 43.9, 28.5, 21.4, 20.3. MS (ES^+) m/z for $C_{25}H_{31}N_3O_3$: calcd, 421.23; found, 422.17 (M+H).

(35) LC/MS on crude reaction mixture as well as postpurification samples showed that chemotypes **15**, **17**, and **19** (R = H), resulting from scaffold **1**{*1*}, contain a small amount (5−10%) of a regioisomeric product which might presumably be generated from the alkylation of *m*-dimethylaminoanisole *para* to the OMe group.

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