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Synthesis of Privileged Scaffolds by Using Diversity-Oriented Synthesis

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Abstract: An elegant reagent-controlled strategy has been developed for the generation of a diverse range of biologically active scaffolds from a chiral bicyclic lactam. Reduction of the chiral lactam with LAH or alkylation with LHMDS to trigger different cyclization reactions have been shown to generate privileged scaffolds, such as pyrrolidines, indolines, and cyclotryptamines. Their amenability to substitution allows us to create various compound libraries by using these scaffolds. In silico studies were used to estimate the drug-like properties of these compounds. Selected compounds were subjected to anticancer screening by using three different cell lines. In addition, all these compounds were subjected to antibacterial screening to gauge the spectrum of biological activity that was conferred by our DOS methodology. Gratifyingly, with no additional iterative cycles, our method directly generated anticancer compounds with potency at low nanomolar concentrations, as represented by spiroindoline **14**.

Keywords: biological activity · diversity-oriented synthesis · heterocycles · scaffolds · spiro compounds

Introduction

Diversity-oriented synthesis (DOS) and biology-oriented synthesis (BIOS) are two efficient strategies that enable the generation of a diverse range of compound sets, which serve as versatile tools in chemical biology.[1] Both approaches rely on the multistep synthesis of skeletally differentiating transformations from a common precursor to generate structurally diverse scaffolds. Herein, we report the development of diastereomerically pure 2-aminophenyl bicyclic lactam **1** as a chiral platform for the generation of optically pure privileged scaffolds by using DOS.[2]

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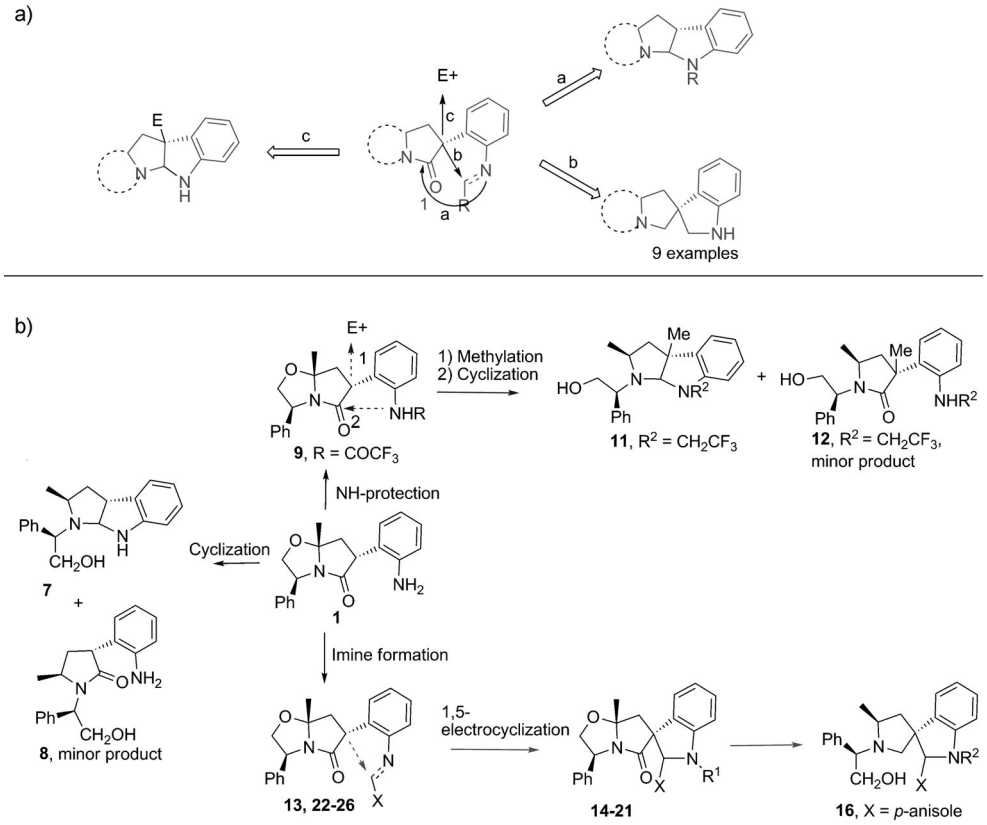
Over the past few years, we have been interested in using DOS to generate asymmetric motifs as chiral platforms to yield complex molecules. So far, this approach has been successful, as evidenced by our previous reports on the syntheses of novel scaffolds from our laboratories.[3]

Privileged scaffolds are specific molecular skeletons that significantly contribute to the biological activity of their daughter molecules. Many such scaffolds are present in natural products. Owing to their obvious utility in drug discovery, substantial synthetic efforts have been invested in the development of methods to access these scaffolds. We have been interested in developing DOS-related methodologies to access such versatile scaffolds in a synthetically efficient manner.

Diastereomerically pure bicyclic lactams have provided access to a wide range of natural and unnatural products that contained quaternary stereocenters in high enantiomeric purity.[4] They have been used extensively in enantioselective syntheses that were based on the initial substitution of the α-carbon atom to the amide carbonyl group, either by reaction with a base followed by an appropriate electrophile or by a thio-Claisen rearrangement of the corresponding thiolactam.[5] Recently, we successfully synthesized a new enantiomerically pure 2-aminophenyl bicyclic lactam (**1**). We envisaged the application of lactam **1** to access small fused tricyclic and spirocyclic fragments (Scheme 1a) through a diversity-oriented synthesis. Biologically active cyclotryptamines and spiroindoline ring systems (fused tricyclic and spirocyclic fragments) provide a good platform to demonstrate the utility of this method. Spiroxoindolines elacomine and horsfiline have been reported to have multiple biological activities (as antimalarial agents, as inhibitors of



Scheme 1. a) Summary of the conversion of bicyclic lactam **1** into cyclotryptamine and spiroindoline scaffolds. b) Our overall strategy.

p53:MDM2 receptors, and as antimicrobial agents for both plant and human pathogens).[6–9] Indolines are also known modulators of focal adhesion kinase (FAK) signaling pathways.[10] Cyclotryptamine physostigmine is a parasympathomimetic alkaloid, specifically, a reversible cholinesterase inhibitor, that occurs naturally in the Calabar bean.[11]

The application of enolate-mediated alkylation reactions of chiral lactam **1**, followed by ring closure onto a built-in electrophile has been described previously (Scheme 1b).

In an earlier communication,[3a] we reported the encouraging anticancer activity of related scaffolds that were developed in our group. Therefore, we decided to subject these molecules to screening with three different cancer cell lines. In addition, to assess the breadth of the biological activity that these scaffolds could exhibit, we chose to screen our molecules against five randomly selected bacteria pathogens (*Ralstonia eutropha*, *Bacillus sphaericus*, *Proteus vulgaris*, *Proteus mirabilis*, and *Staphylococcus aureus*). The results of the biological evaluation of our molecules are described in the following sections.

Results and Discussion

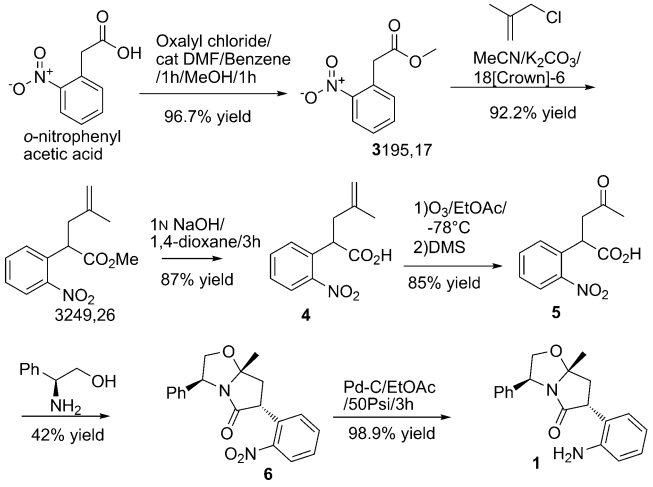
Compound libraries that are based on privileged scaffolds typically deliver a higher proportion of hits in biological assays. Facile synthetic-group transformations within privileged scaffolds induce remarkable changes in terms of potency and target affinity.[12] Consequently, substantial effort has been made towards generating libraries from these scaffolds. For example, in a recent study, Le and Kim reported the synthesis of a library of compounds around the 1,4-pyrazolodiazapin-8-one structure with the aim of using the diazepines to closely mimic the β-turn structure of a number of peptides.[13] However, there are not many diversity-oriented syntheses of privileged-scaffold-containing molecules.[14]

We have developed a unique method based on chiral bicyclic lactam **1**, which provides some of these scaffolds in an efficient manner. We aimed to keep our scaffolds as close to the "rule of three" as possible ($M_w < 300$, HBD ≤ 3 and HBA ≤ 3, clog P $= 3$, number of rotatable bonds ≤ 3, polar surface area: 60 Å^2),[15] which would allow us to further manipulate the scaffolds to generate molecules that would still generally adhere to Lipinski's guidelines.

Linear enantioselective syntheses of these scaffolds have been reported in the past.[16] However, to the best of our knowledge, this is the first reagent-based DOS method that allows access to structurally diverse privileged scaffolds through common intermediates (**1** and **2**). This method has an attractive steps-per-scaffold efficiency, whilst providing access to chemically complex end-molecules. Moreover, we believe that this method also contributes to the observed stereocontrol during the substrate assembly. Structural diversity is imparted by careful choice of substitution and cyc-

lization reactions that proceed predictably and efficiently. The ease with which the stereodirecting functionality is partially removed in the final step makes this strategy very useful in generating libraries and as also for further downstream transformations.

Bicyclic lactam **1** was synthesized as shown in Scheme 2. 2-Nitrophenylacetic acid was converted into its corresponding ester (**2**) in 96.7% yield. Allylation of compound **2** with

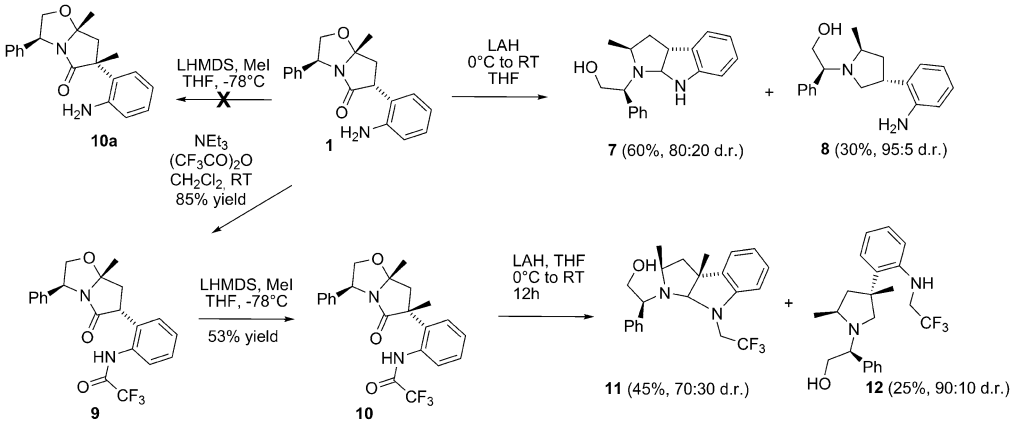


Scheme 2. Synthesis of chiral bicyclic lactam **1**.

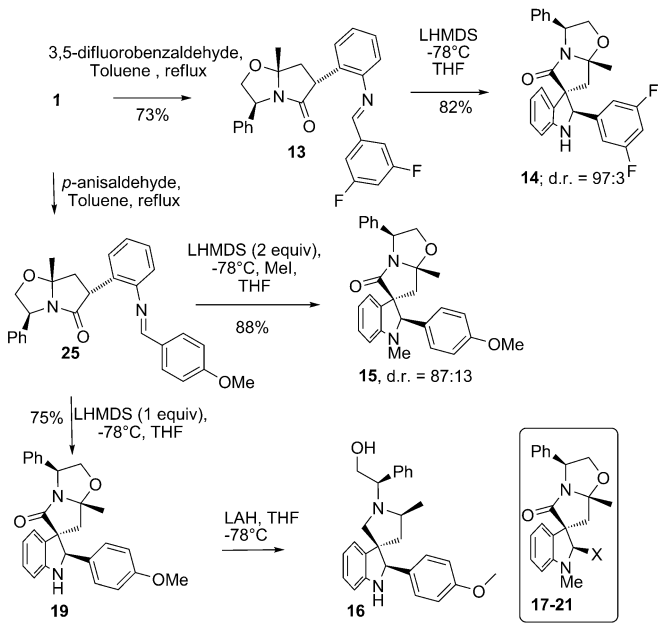
2-methylallyl chloride generated intermediate **3** in 92.2% yield. This step was followed by the hydrolysis of the ester to afford olefin **4** (87% yield), which underwent ozonolysis to generate ketone **5** (85% yield). Condensation of (*S*)-phenyl glycinol with ketone **5** afforded lactam **6** in 42% yield. Facile reduction of compound **6** with H_2 in the presence of 5% Pd/C in EtOAc under 50 psi pressure generated the desired 2-aminophenylbicyclic lactam (**1**) in about 99% % yield and 99:1 d.r. The absolute stereochemistry of compound **1** was determined by single-crystal X-ray diffraction (for the X-ray data, see the Supporting Information). Notably, the synthesis of compound **1** could be performed on a multigram scale as necessary. Next, strategies for its elaboration into various privileged scaffolds were explored.

Initial studies on the cyclization of compound **1** into cyclotryptamine scaffold **7** with diisobutyl aluminum hydride (DIBAL-H) at 0°C afforded the desired compound in low yields and poor diastereoselectivities. After a few disappointing attempts (20–30% yield and 70–75% d.r.) with various reducing agents, such as sodium bis(methoxyethoxy)aluminum hydride (Red-Al) and borane dimethylsulfide (BH_3•DMS), we were pleased to find that the treatment of compound **1** with lithium aluminum hydride (LAH) at 0°C provided a mixture of cyclotryptamine **7** (60% yield, 95:5 d.r.) and pyrrolidine **8** (30% yield, 95:5 d.r.; Scheme 3). The direct methylation of compound **1** to synthesize the α-chiral quaternary center failed to generate the desired compound. Hence, compound **1** was acylated with trifluoroacetyl chloride to generate compound **9**. Methyl-substituted cyclotryptamine **11** was synthesized from compound **9** by sequential treatment with methyl iodide in LHMDS at −78°C to give compound **10** in 53% yield and treatment with LAH at 0°C to afford compound **11** in 45% yield and 96:4 d.r. NOE experimental analysis of compound **10** confirmed the configuration of the methyl group relative to the stereocenters on the chiral auxiliary (Scheme 3). In addition, LAH reduction of compound **10** also resulted in methyl-substituted pyrrolidine **12** in 25% yield and 95:5 d.r. The concomitant partial removal of the chiral auxiliary during LAH-mediated cyclization is an added benefit of this procedure.

To further elaborate on the diversity of bicyclic lactam **1**, we envisaged the synthesis of indolines by using a 1,5-electrocyclization strategy that was inspired by the chemistry of Speckamp and co-workers (Scheme 4).[17] As a model reaction, compound **1** was heated at reflux with 3,5-difluorobenzaldehyde in toluene to generate the corresponding imine (**13**). Treatment of compound **13** with LHMDS at −78°C in THF generated the indoline scaffold (**14**) in 82% overall yield as a 97:3 mixture of diastereomers at the newly generated spirocyclic stereocenter (for the absolute configuration, see the X-ray data in the Supporting Information). We used these conditions for the synthesis of substituted spiroindo-



Scheme 3. Synthesis of cyclotryptamine and pyrrolidine scaffolds.



Scheme 4. Synthesis of spiroindolines.

lines **17**–**21** (Scheme 4) from their corresponding imines (Table 1). N-methylated spiroindoline **15** was synthesized from imine **25** by treating it with methyl iodide in the presence of LHMDS (2 equiv) at −78 °C in THF in about 60–

Table 1. Synthesis of various spiroindolines.

#	X	Imine	Indoline	Yield [%][a]	d.r.[b]
1	2-furyl	**23**	**17**	78	67:15
2	3-pyridyl	**24**	**18**	88	77:15
3	4-anisole	**25**	**19**	75	78:22
4	cyclohexyl	**26**	**20**	55	88:12
5	phenyl	**22**	**21**	67	97:3

[a] Yield of isolated product. [b] HPLC of the crude sample (the pure compound was obtained by recrystallization after column chromatography of the mixture on silica gel).

70 % yield and 80:20 d.r. The oxazoline chiral auxiliary on the indolines could be partially removed by treatment with LAH, as demonstrated on indoline **19** to obtain amino alcohol **16** (Scheme 4).

With various molecular scaffolds in hand, we evaluated the skeletal diversity that was afforded by the N-heterocycles and their related chemical descriptors by using in silico algorithms. By virtue of possessing its own set of drug-like properties, each molecule occupies a unique point in the chemical space. Hence, the larger the coverage of the chemical space by a collection of molecules, the greater its diversity. To assess the diversity of the reported compounds, we plotted them in chemical space plots that corresponded to a set of six key descriptors (that is, polar surface area (PSA), solubility, hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), log A, and log D) relative to 1011 FDA-approved drugs (light-blue dots), as reported in the GOSTAR database (Figure 1 a).[18–20]

The plots show that our molecules (Figure 1, dark-blue dots) are not "outliers" with respect to these parameters when compared to marketed drugs.

In addition, our molecules were plotted according to the normalized principal moment of inertia (PMI) formalism of Sauer and Schwartz.[21] Since molecular shape and biological activity are intricately related, the screening of molecules that have a higher degree of built-in molecular-shape diversity translates into a greater probability of discovering compounds with biological activity. The three corners of the isosceles triangle of the PMI plot are dominated by rods, spheres, and discs, respectively (the shapes are representations of the overall shape of the constituent molecules). Commercial compounds tend to occupy the left-hand edge of the PMI plot; that is, they adopt shapes that fall in between rods and discs. Natural products are distributed more evenly over the space and tend to occupy the intermediate region between disc- and sphere-like shapes. Figure 1 shows the coverage of N-heterocycles **7**–**18**. Our collection of molecules can be classified into three types of molecular cores, namely pyrrolidines (**12**), cyclotryptamines (**7** and **11**), and indolines (**16**, **17**, and **18**). These compounds tend to occupy more natural-product-like shapes and are distributed fairly centrally (between discs and spheres) in the PMI plot (Figure 1 c).[22] The bioactivity track record of natural products is well-established. We hope that the similar shapes of our molecules to those of natural products will increase the likelihood of discovering biologically relevant molecules by using this method.

In our earlier communication,[3a] we reported that spiroquinoline **27** and spiroindolone **28** inhibited the proliferation of the HeLa (human epithelial) cell line with IC_{50} values of 730 nM and 1 μM, respectively, and the MCF-7 breast cancer cell line with IC_{50} values of 890 nM and 1.7 μM, respectively. In addition, good bioactivity of spiroindolones against BSC-1, HCT116p53+/+ and p53−/−, and HeLa cells was recently reported.[23] Therefore, we decided to screen our molecules for anticancer activity. In deference to resource limitations, we screened a small selection of the spiroindolones (**14**, **15**, **17**, **18**, and **21**) against HL60 (human promyelocytic leukemia cells), U937 (human leukemic monocyte lymphoma cells), and MCF-7 (breast cancer cell cells; Table 2). Gratifyingly, three of our library compounds inhibited the growth of one, two, or all three cell lines in the range 11–248 nM. Compound **14** was the most potent, thus inhibiting cell proliferation of all the three cells with IC_{50} values of 11 nM (HL60), 24 nM (MCF-7), and 248 nM (U937). Compounds **15**/**17** inhibited the cell proliferation of HL60 and MCF-5 with IC_{50} values of 126/180 nM and 147/240 nM, respectively. To ensure that our compounds were not exerting their anticancer potency through cytotoxicity, we screened them against baby hamster kidney 21 (BHK-21), a commonly used healthy cell line. For each of the potent compounds, the dose that was required to exhibit cytotoxicity was at least 100-fold higher than their respective IC_{50} concentrations (see the Supporting Information). These anticancer "hits" at low nanomolar concentrations (**14**, **15**, and **17**) elo-

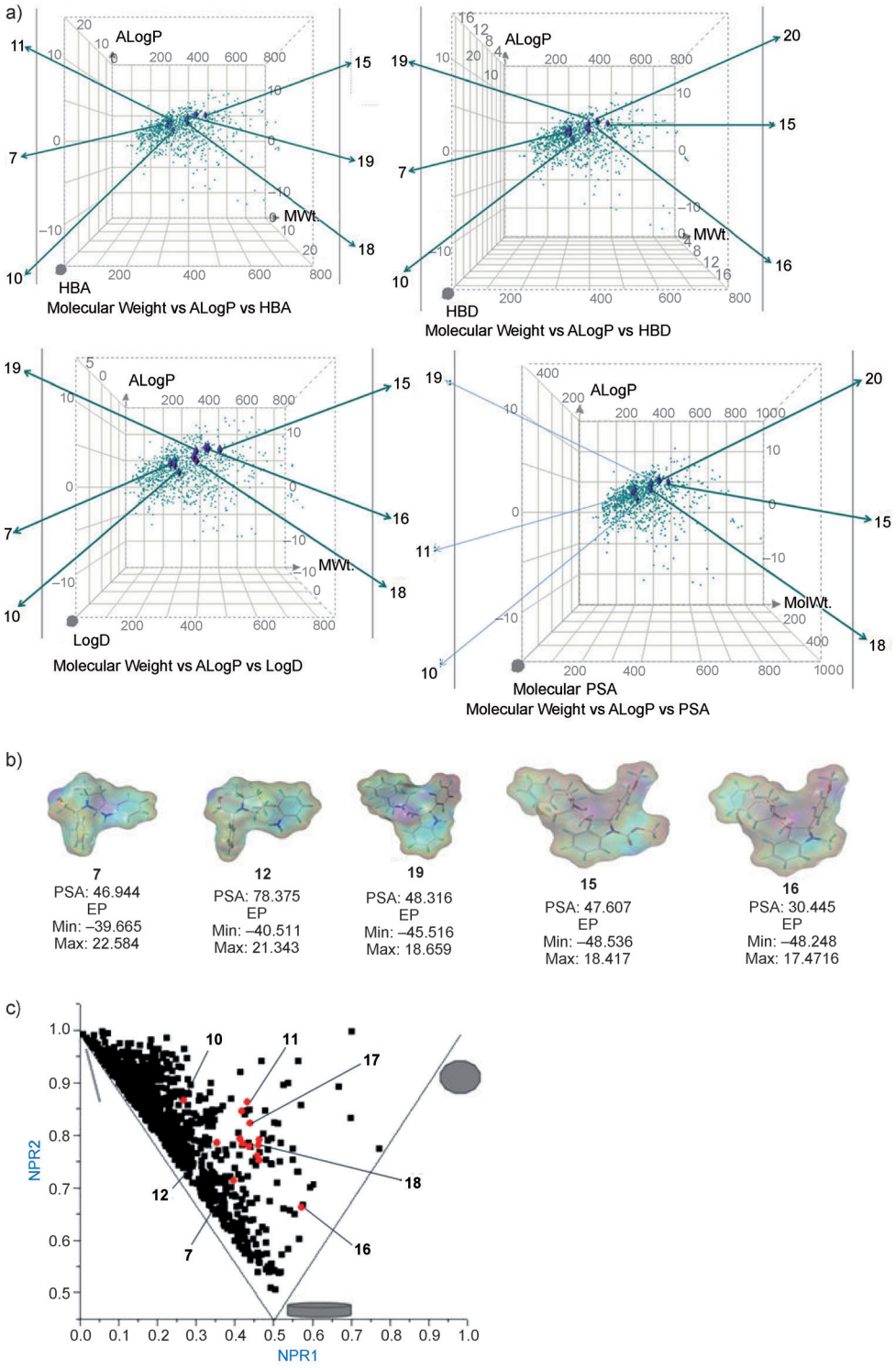
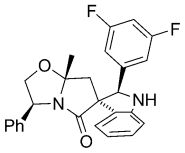
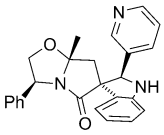
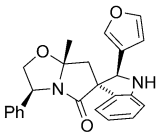
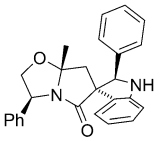
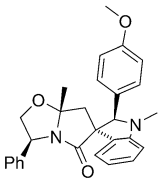
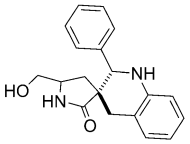
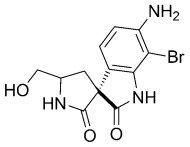


Figure 1. Cheminformatics analysis of our library of privileged scaffolds; in silico analysis, polar surface area, and polar moment of inertia (PMI) plots of a representative set of molecules are shown. a) In silico analysis of eight molecules (dark-blue spots) in a chemical-space plot that corresponds to a set of six drug-like properties (PSA, solubility, HBA, HBD, log A and log D), relative to 1011 FDA-approved drugs (light-blue dots), as reported in the GOSTAR database (GVKBioscience proprietary database). b) Surface electrostatic potential of a representative set of our compounds, that is, compounds **7**, **12**, **19**, **15**, and **16**. The surface electrostatic profiles were calculated by projecting the Gasteiger–Marsili charge distribution onto a Connolly surface that was generated by using the MOLCAD tool in SYBYL. c) PMI plot of our group of molecules relative to 1011 FDA-approved drugs. The PMI calculations involved aligning each molecule to principal moment axes by using SYBYL and the normalized PMI values were calculated by using in-house software.

quently highlight the merits of our DOS method; the usual expectations from such non-targeted, early scaffold explorations are modest (double-digit micromolar potency). Potent hits such as these also point to the "privileged" nature of the scaffolds.

To further explore the diverse biological activity of our collection of compounds, we decided to assess their antibacterial properties, prompted by the presence of cyclotryptamine, pyrrolidinone, and indoline motifs in various antibacterial natural products. We decided to screen our compounds for the inhibition of proliferation against five bacterial strains, that is, *Ralstonia eutropha* (Gram-negative), *Bacillus sphaericus* (Gram-positive), *Proteus vulgaris* (Gram-negative), *Proteus mirabilis* (Gram-negative), and *Staphylococcus aureus* (Gram-positive) by using the disc-diffusion method (see the Supporting Information).[24,25] For each microbial strain, negative controls were maintained, for which pure DMSO solvent was used, whilst, for a positive control, the standard antibiotic cefotaxime (0.2 mg disc^{-1}) was used. The minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) of the compounds were determined by using the broth-dilution technique. The lowest concentration of the drug that inhibited the growth was considered to be the MIC. Compounds with lower MIC values are those with high antimicrobial activity. The lowest concentration of the drug that caused the death of the microorganism was considered to be the MBC. We found that two of the library compounds mildly modulated bacterial growth. Pyrrolidine **11** inhibited the proliferation of *Ralsto-*

Table 2. Anticancer activities of selected spiroindolines.

Compound		HL60[a] IC_{50} [μM]	U937[b] IC_{50} [μM]	MCF-7[c] IC_{50} [μM]
	**14**	0.011(±0.008)	0.248(±0.004)	0.024(±0.002)
	**18**	0.562(±0.002)	5.670(±0.01)	1.179(±0.01)
	**17**	0.180(±0.003)	0.520(±0.015)	0.240(±0.003)
	**21**	5.828(±0.041)	14.60(±0.012)	4.733(±0.009)
	**15**	0.126(±0.004)	1.87(±0.012)	0.147(±0.002)
	**27**	–	–	8.912(±5.802)
	**20**	–	–	16.832(±2.911)
etoposide (control)		0.002	0.01	0.009

[a] Human promyelocytic leukemia cells; [b] human leukemic monocyte lymphoma cells; [c] breast cancer cell.

nia E. with an MIC of 276 μM and cyclotryptamine **12** inhibited the proliferation of *Proteus V* with an MIC of 300 μM (see the Supporting Information).

Conclusions

In conclusion, we have employed a DOS-based strategy for the generation of a library of diverse scaffolds (cyclotryptamines, pyrrolidines, and spiroindolines) from readily available starting materials. A main objective of this paper, that is, the facile diversity-oriented synthesis of privileged scaffolds, has been achieved. Cheminformatics assessments suggest that our compound library not only allows access to the regions of "chemical space" that are populated by marketed drugs, but also the space of some natural-product-like compounds, thus promising to deliver molecules with rich structural and biological diversity. Finally, we have demonstrated that selected members of this library have shown excellent activity towards cancer cell lines. Anticancer screening identified three highly potent anticancer hit compounds, that is, compounds **14**, **15**, and **17**, with potency at low nanomolar concentrations. We were also pleased to find that no cytotoxicity was observed, even at concentrations above 100 times their respective IC_{50} values. However, an antibacterial screen yielded less-encouraging results (high micromolar potencies), thus leading us to conclude that these scaffolds had much better potential in oncological applications rather than as antibacterial compounds. The varied biological activity profile of the different compounds in this collection towards the different assays underlines the fundamental concept of DOS, that is, that the architectural diversity of a DOS-mediated library is responsible for its diversity in biological activity. As part of our future plans, we intend to use these "hits" as starting points for a medicinal chemistry investigation.

Experimental Section

General procedures for the key steps in the synthesis of bicyclic lactam **1**, its intermediates (**2**–**6**), analogues **9** and **10**, cyclotryptamines **7** and **11**, pyrrolidines **8** and **12**, spiroindolines **14**–**21**, and their intermediates (**13**, **22**–**26**) are provided in the Supporting Information. Some representative procedures are given below. The syntheses of compounds **27** and **28** have been reported previously.[3a] Unless otherwise noted, all reactions were performed in flame-dried glassware under an anhydrous Ar atmosphere. For additional procedures, as well as detailed experimental and complete analytical data of all compounds, see the Supporting Information.

*Synthesis of Compound **1***

A solution of compound **6** (5 g, 14.7 mmol) in EtOAc (500 mL) was hydrogenated with Pd/C (10 wt.%, 800 mg) under hydrogenated balloon pressure (20 Psi) at RT for 16 h. Then, the catalyst was filtered through Celite under a nitrogen atmosphere and washed with EtOAc. Then, the filtrate was evaporated under reduced pressure to obtain amine **1** (4.5 g, 98.9% yield) as a white solid. ^{1}H NMR (300 MHz,$CDCl_3$): δ = 7.38–7.25 (m, 5H),7.11–7.09 (d, J = 7.8 Hz, 2H), 6.84–6.81 (d, J = 7.8 Hz, 1H), 6.78–6.75 (d, J = 7.8 Hz, 1H), 5.21–5.16 (t, J = 7.5 Hz, 1H), 4.66–4.60 (t, J = 8.4 Hz,1H),4.42–4.36 (dd, ^1J = 10.8, ^2J = 8.7 Hz, 1H), 4.19–4.14 (m, 1H), 2.82–2.74 (t, J = 12.9 Hz, 1H), 2.64–2.57 (m, 1H), 1.57 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 177.90, 145.48, 139.61, 128.68, 128.27, 127.66, 125.60, 123.21, 119.39, 117.92, 98.00, 73.06, 57.87, 46.58, 39.84, 23.96 ppm; IR (KBr): $\tilde{\nu}_{max}$ = 3338, 2931, 1706, 1087, 1013, 695 cm^{-1}; LCMS: m/z calcd: 309.15 $[M+H]^+$; found: 309.01.

General Synthesis of Cyclotryptamines and Pyrrolidines

Bicyclic Lactam **1** was dissolved in tetrahydrofuran. The resulting solution was cooled to 0 °C, LAH was added, and the mixture was stirred at that temperature until the reaction was adjudged to be complete by TLC analysis of a reaction aliquot (quenched with a saturated aqueous solution of NH_4Cl and extracted with CH_2Cl_2). The reaction mixture was further washed with water (×2) and brine and dried ($MgSO_4$). The solvent was removed by rotary evaporation. Purification by flash chromatography on silica gel (*n*-hexane/EtOAc) afforded cyclotryptamines and pyrrolidines **7**, **8**, **11**, and **12**.

*Synthesis of Compounds **7** and **8***

To an ice-cooled solution of compound **1** (120 mg, 0.389 mmol) in dry THF (3 mL) was added LAH (67 mg, 1.75 mmol) under a stream of nitrogen gas in three almost-equal portions. The ice bath was removed and the reaction mixture was stirred at RT for 1 h, by which time, complete consumption of compound **1** had occurred (TLC analysis). Then, the reaction mixture was cooled to 0°C and EtOAc (3 mL) was added to quench any unreacted LAH. The solution was filtered through a Celite plug and thoroughly washed with EtOAc. The filtrate and the washings (about 15 mL) were combined and washed with water (10 mL), and brine (10 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated to give a crude material (220 mg) that was purified by preparative HPLC (Waters 3100, mass trigger) to afford the desired compounds **7** (69 mg) and **8** (35 mg).

7: ^{1}H NMR (400 MHz,$CDCl_3$): $\delta = 8.02$ (s, 1H), 7.42–7.32 (m, 3H), 7.29–7.26 (m, 1H), 7.20–7.16 (m, 1H), 7.08–7.0 (m, 3H), 6.91 (s, 1H), 6.80–6.78 (d, $J = 7.2$ Hz, 2H), 3.88–3.85 (m, 1H), 3.65–3.58 (m, 1H), 3.31–3.26 (t, $J = 9.2$ Hz, 1H), 3.00–2.93 (m, 2H), 2.88–2.83 (m, 1H), 2.75–2.67 (m, 1H), 2.06–1.99 (m, 1H), 1.14–1.12 ppm (d, $J = 6.0$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 149.00$, 136.59, 129.65, 128.88, 128.56, 128.48, 128.43, 127.83, 124.01, 119.77, 111.67, 63.22, 60.76, 54.82, 51.39, 45.93, 29.65, 18.99 ppm; LCMS: m/z calcd: 295.17 $[M+H]^+$; found: 295.2.

8: ^{1}H NMR (400 MHz, DMSO): $\delta = 7.34$–7.22 (m, 5H), 6.84–6.80 (t, $J = 7.6$ Hz, 1H), 6.78–6.76 (d, $J = 7.6$ Hz, 1H), 6.56–6.54 (d, $J = 8.0$, 1H), 6.40–6.37 (t, $J = 7.6$ Hz, 1H), 5.19 (s, 2H), 4.40 (s, 1H), 3.78–3.72 (m, 1H), 3.72–3.68 (t, $J = 6.4$ Hz, 3H), 3.62–3.57 (m, 1H), 3.57 (m, 1H), 3.29–3.03 (t, $J = 8.8$ Hz, 1H), 2.88 (s, 1H), 1.87–1.81 (m, 1H), 1.69–1.63 (m, 1H), 1.02–1.01 ppm (d, $J = 5.6$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 144.40, 136.66, 128.90, 128.88, 128.35, 127.84, 127.79, 127.67, 127.10, 118.24, 116.06, 63.58, 62.80, 54.82, 51.49, 37.95, 37.38, 17.23 ppm; LCMS: m/z calcd: 297.19 $[M+H]^+$; found: 297.08.

General Procedure for the Synthesis of Spiroindolines

Imines **13** and **22**–**26** were dissolved in THF. The resulting mixture was cooled to −78°C, followed by the addition of lithium hexamethyldisilazide (LHMDS, 2.00 equiv). The reaction mixture was slowly warmed to RT and the mixture was stirred at that temperature until the reaction was adjudged complete by TLC analysis of a reaction aliquot (quenched with a saturated aqueous solution of NH_4Cl and extracted with CH_2Cl_2). The reaction mixture was further washed with water (×2) and brine and dried ($MgSO_4$). The solvent was removed by rotary evaporation. Purification by flash chromatography on silica gel (*n*-hexane /EtOAc) afforded spiroindolines **14** and **17**–**19**.

*Synthesis of Compound **14***

Following the general procedure with compound **13** (0.2 g, 0.462 mmol), anhydrous THF (10 mL), and LHMDS (1 M in THF, 0.154 g, 0.92 mL, 0.922 mmol). The resulting residue was purified by column chromatography on neutral alumina (petroleum ether/EtOAc, 90:10) to afford compound **14** (0.16 g, 82% yield) as a white solid. $R_f = 0.5$ (petroleum ether/EtOAc, 85:15); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.25$–7.2 (m, 3H), 7.18–7.14 (m, 3H), 7.08 (m, 1H), 6.88–6.83 (m, 2H), 6.78–6.73 (m, 3H), 5.11 (t, $J = 7.2$ Hz, 1H), 4.95 (s, 1H), 4.61 (t, $J = 8.8$ Hz, 1H), 4.2 (br s, 1H), 3.98 (m, 1H), 2.97 (d, $J = 4.4$ Hz, 1H), 2.58 (d, $J = 5.2$ Hz, 1H), 1.23 ppm (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$): $\delta = 177.24$, 164.25, 164.13, 161.77, 161.65, 150.38, 142.87, 142.79, 142.7, 140.2, 129.83, 129.18, 128.9, 128.41, 127.14, 125.19, 124.94, 123.92, 111.25, 111.18, 111.06, 110.99, 109.88, 104.11, 103.86, 103.61, 96.48, 74.73, 71.96, 63.25, 57.12, 46.84, 29.66, 24.07 ppm; LCMS: m/z calcd: 433.46 $[M+H]^+$; found: 433.03.

*Synthesis of N-Protected Spiroindoline **15***

Imine **25** was dissolved in THF. The resulting mixture was cooled to −78°C, lithium hexamethyldisilazide (2.00 equiv) was added, and the mixture was stirred for 0.5 h. MeI was added and the mixture was slowly warmed to RT and stirred at that temperature until the reaction was adjudged complete by TLC analysis of a reaction aliquot (quenched with a saturated aqueous solution of NH_4Cl and extracted with CH_2Cl_2). The reaction mixture was further washed with water (×2) and brine and dried ($MgSO_4$). The solvent was removed by rotary evaporation. Purification by flash chromatography on silica gel (*n*-hexane/EtOAc) afforded spiroindoline **15**.

Cheminformatic Analysis

In silico and PMI analysis were carried out on 1100 FDA-approved drugs (randomly taken from our GOSTAR proprietary database and our compounds. Each molecule was analyzed for a set of six physiochemical properties by using online cheminformatics tools, ChemDraw, or manual inspection. The data were collected on an Excel spreadsheet. The mean value and standard deviation of each column was calculated and the mean values were also calculated within each compound series. Analysis was performed on mean-centered normalized data by using an in-house proprietary statistical package. The results from R were used to generate PCA plots (for full details, see the Supporting Information). These results were used to generate the plot shown in Figure 1a.

The PMI calculations involved aligning each molecule to the principal moment axes in SYBYL and the normalized PMI values were calculated by using in-house software. The 2D chemical structures of the compounds were drawn in Discovery studio 3.1 (DS 3.1), whereas those of the FDA-approved drugs were collected from the GOSTAR (Gvkbio Online Structure Activity Relationship) database. These compounds were imported into DS 3.1 and converted into 3D structures. Energy minimization was performed for all of these 3D structures to identify global/local minima by applying the CHARMm force field. The resulting 3D structures were used to calculate the three principal moments of inertia; the PMI values are sorted in ascending magnitude, that is, I1, I2, and I3. Normalized PMI ratios (NPR) were calculated by dividing the two smaller PMI values (I1 and I2) by the largest PMI value (I3) and generated two characteristic values for each compound (I1/I3 and I2/I3). These values were plotted against each other and the resulting graph formed an isosceles triangle that was defined by its three corners, wherein the vector [I1/I3, I2/I3] was equal to [1,1], [0.5,0.5], and [0,1] (Figure 1c).

Biological Assay: Anticancer Assay

Cell lines and cell culture: Three cell lines, that is, HL60 (human promyelocytic leukemia cells), U937 (human leukemic monocytic lymphoma cells), and MCF-7 (human breast cancer cells), were obtained from the National Centre for Cellular Sciences (NCCS, Pune, India). The cell lines were maintained in RPMI-1640 medium (HiMedia, Mumbai, India) that was supplemented with 10% heat-inactivated fetal bovine serum (FBS; HiMedia, Mumbai, India), pencillin, and streptomycin (100 $\mu g\,mL^{-1}$ each). For the screening experiments, the cells were seeded in a 96-well microtiter culture plate (Corning Costar) with 100 μL of the medium to a final density of 2×10^4 cells $well^{-1}$. Then, the plates were incubated at 37°C under an atmosphere of 5% CO_2 and 100% relative humidity for 24 h prior to the addition of the experimental compounds.

Method: Cell proliferation and viability were measured by MTT assay (MTT = 3-(4,5-dimethylthiazol-2-yi)-2,5-diphenyltetrazolium bromide), according to the method developed by Mosmann.[26] Prior to use, all of the test compounds were dissolved in DMSO and diluted with DMSO to the desired final concentration. After 24 h of incubation, the grown cells were treated with different concentrations of the test compound (1–100 $\mu g\,mL^{-1}$), as well as the carrier solvent alone, at a final volume of 200 μL. This experiment was repeated thrice. The concentration of the DMSO carrier solvent did not exceed 0.1%, so as to be deliberately nontoxic to cells. After 48 h of incubation, MTT (10 μL, 5 $mg\,mL^{-1}$) was added to each well and the plate was subsequently incubated at 37°C in the dark for about 4 h. Later on, the supernatants were discarded and the formazan crystals were dissolved in DMSO (100 $\mu L\,well^{-1}$) by incubating at RT for 30 min. The reduction of MTT (as the rate of color development) was quantified by measuring the absorbance at 570 nm on a spectrophotometer (Model: Spectra MAX Plus; Molecular Devices; supported by SOFTmax PRO-3.0).

The inhibition effects of the test compounds on cell proliferation or viability were determined by using cells that were treated with DMSO as a control. Linear regression analysis (OriginPro 8.0) was performed on

the data and the regression lines were plotted to obtain the best straight-line fit. The IC_{50} values, that is, the inhibition of cell-viability concentrations, were determined by using the respective regression equation.

Antibacterial Assay

The antimicrobial activities of the compounds were tested by the disc-diffusion method against five microorganisms, that is, *Ralstonia eutropa, Bacillus sphaericus, Proteus vulgaris, Proteus mirabilis*, and *Staphylococcus aureus*. The test compound (15 μL, 0.2 mg disc^{-1}) was impregnated into discs of sterile filter paper (diameter: 5 mm) by soaking the discs in solutions of the test compounds in DMSO for a minimum of 2 h before being allowed to dry. Then, the impregnated discs were placed in each agar plate and the microorganisms were inoculated on the discs by using the spread-plate method. For each microbial strain, negative controls were maintained, for which pure DMSO solvent was used, because it did not possess any antimicrobial effect. For a positive control, the standard antibiotic cefotaxime (0.2 mg disc^{-1}) was used. The petri plates were incubated at 37 °C for 18–24 h and the zone of inhibition was measured (in mm).

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[1] a) G. C. Micalizio, S. L. Schreiber, *Angew. Chem.* **2002**, *114*, 3406–3410; *Angew. Chem. Int. Ed.* **2002**, *41*, 3272–3276; b) D. S. Tan, *Nat. Chem. Biol.* **2005**, *1*, 74–84; c) P. Arya, R. Joseph, Z. Gan, B. Rakic, *Chem. Biol.* **2005**, *12*, 163–180; d) A. Nören-Müller, I. Reis-Corrêa, Jr., H. Prinz, C. Rosenbaum, K. Saxena, H. J. Schwalbe, D. Vestweber, G. Cagna, S. Schunk, O. Schwarz, H. Schiewe, H. Waldmann, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 10606–10611; e) M. A. Koch, H. Waldmann, *Drug Discovery Today* **2005**, *10*, 471–483.

[2] a) M. D. Burke, S. L. Schreiber, *Angew. Chem.* **2004**, *116*, 48–60; *Angew. Chem. Int. Ed.* **2004**, *43*, 46–58; b) S. L. Schreiber, *Science* **2000**, *287*, 1964–1969.

[3] a) V. S. B. Damerla, C. Tulluri, R. Gundla, L. Naviri, U. Adepally, P. S. Iyer, Y. L. N. Murthy, N. Prabhakar, S. Sen, *Chem. Asian J.* **2012**, *7*, 2351–2360; b) S. Sen, S. R. Kamma, V. R. Potti, Y. L. N. Murthy, A. B. Chaudhury, *Tetrahedron Lett.* **2011**, *52*, 5585–5588.

[4] a) A. I. Meyers, B. A. Lefker, *J. Org. Chem.* **1986**, *51*, 1541–1544; b) A. I. Meyers, B. A. Lefker, *Tetrahedron* **1987**, *43*, 5663–5676; c) A. I. Meyers, R. Hanreich, K. T. Wanner, *J. Am. Chem. Soc.* **1985**, *107*, 7776–7778; d) A. I. Meyers, D. J. Berney, *J. Org. Chem.* **1989**, *54*, 4673–4676; e) A. I. Meyers, J. L. Romine, A. J. Robichaud, *Heterocycles* **1990**, *30*, 339–340; f) A. I. Meyers, M. Sturgess, *Tetrahedron Lett.* **1989**, *30*, 1741–1744; g) D. Romo, J. L. Romine, W. Midura, A. I. Meyers, *Tetrahedron* **1990**, *46*, 4951–4994.

[5] a) A. I. Meyers, M. A. Seefeld, B. A. Lefker, J. F. Blake, P. G. Willard, *J. Am. Chem. Soc.* **1998**, *120*, 7429–7438; b) P. Devine, A. I. Meyers, *J. Am. Chem. Soc.* **1994**, *116*, 2633–2634.

[6] a) A. Jossang, P. Jossang, H. A. Hadi, T. Sevenet, B. Bodo, *J. Org. Chem.* **1991**, *56*, 6527–6530; b) C. B. Cui, H. Kakeya, H. Osada, *J. Antibiot.* **1996**, *49*, 832–835; c) R. W. Carling, P. D. Leeson, A. M. Moseley, R. Baker, A. C. Foster, S. Gromwood, J. A. Kemp, G. R. Marshall, *J. Med. Chem.* **1992**, *35*, 1942–1953.

[7] a) P. D. Leeson, R. W. Carling, K. W. Moore, A. M. Moseley, J. D. Smith, G. Stevenson, T. Chan, R. Baker, A. C. Foster, S. Gromwood, J. A. Kemp, G. R. Marshall, K. Hoosteen, *J. Med. Chem.* **1992**, *35*, 1954–1968; b) S. M. Verbitski, C. L. Mayne, C. L. Davis, R. A. Davis, G. Cencepcion, C. M. Ireland, *J. Org. Chem.* **2002**, *67*, 7124–7130.

[8] G. Zinzalla, D. E. Thurston, *Future Med. Chem.* **2009**, ***1***, 65–72.

[9] B. K. S. Yeung, B. Zou, M. Rottmann, S. B. Lakshminarayana, S. H. Ang, S. Y. Leong, J. Tan, J. Wong, S. Keller-Maerki, C. Fischli, A. Goh, E. K. Schmitt, P. Krastel, E. Francotte, K. Kuhen, D. Plouffe, K. Henson, T. Wagner, E. A. Winzeler, F. Petersen, R. Brun, V. Dartois, T. T. Diagana, T. H. Keller, *J. Med. Chem.* **2010**, *53*, 5155–5159.

[10] R. R. Poondra, N. N. Kumar, K. Bijian, M. Prakesch, V. Campagna-Slater, A. Reayi, P. T. Reddy, A. Choudhry, M. L. Barnes, D. M. Leek, M. Daroszewska, C. Lougheed, B. Xu, M. Schapira, M. A. Alaoui-Jamali, P. Arya, *J. Comb. Chem.* **2009**, *11*, 303–309.

[11] a) B. G. Katzung, S. Masters, A. Trever, *Basic and Clinical Pharmacology*, McGraw-Hill, p. 110; b) P. L. Julian, J. Pikl, *J. Am. Chem. Soc.* **1935**, *57*, 539–544; c) P. L. Julian, J. Pikl, D. Boggess, *J. Am. Chem. Soc.* **1934**, *56*, 1797–1801.

[12] a) M. E. Welsch, S. A. Snyder, B. R. Stockwell, *Curr. Opin. Chem. Biol.* **2010**, *14*, 347–361; b) M. Feher, J. M. Schmidt, *J. Chem. Inf. Comput. Sci.* **2003**, *43*, 218–227; c) D. A. Horton, G. T. Bourne, M. L. Smythe, *Chem. Rev.* **2003**, *103*, 893–930.

[13] J.-Y. Le, Y.-C. Kim, *ChemMedChem* **2009**, *4*, 733–737.

[14] a) L. Constantino, D. Barlocco, *Curr. Med. Chem.* **2006**, *13*, 65–85; b) G. Müller, *Drug Discovery Today* **2003**, *8*, 681–691; c) J. M. dos Santos Filho, A. C. L. Leite, B. G. de Oliveria, D. R. M. Moriera, M. S. Lima, M. B. P. Soares, L. F. Leite, *Bioorg. Med. Chem.* **2009**, *17*, 6682–6691.

[15] a) D. C. Rees, M. Congreve, C. W. Murray, R. Carr, *Nat. Rev. Drug Discovery* **2004**, *3*, 660–665; b) M. Congreve, R. Carr, C. Murray, H. Jhoti, *Drug Discovery Today* **2003**, *8*, 876–879.

[16] a) T. Bui, S. Syed, C. F. Barbas III, *J. Am. Chem. Soc.* **2009**, *131*, 8758–8759; b) T. Matsuura, L. E. Overman, D. J. Poon, *J. Am. Chem. Soc.* **1998**, *120*, 6500–6503; c) K. Asakawa, N. Noguchi, S. Takashima, M. Nakada, *Tetrahedron: Asymmetry* **2008**, *19*, 2304–2309; d) B. M. Trost, Y. Zhang, *J. Am. Chem. Soc.* **2006**, *128*, 4590–4591; e) B. M. Trost, Y. Zhang, *J. Am. Chem. Soc.* **2007**, *129*, 14548–14549; f) B. K. T. Lee, G. S. Wong, *J. Org. Chem.* **1991**, *56*, 872–875; g) A. Minatti, S. L. Buchwald, *Org Lett.* **2008**, *10*, 2721–2724; h) A. S. Guram, R. A. Rennels, S. L. Buchwald, *Angew. Chem.* **1995**, *107*, 1456–1459; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1348–1350; i) J. P. Wolfe, R. A. Rennels, S. L. Buchwald, *Tetrahedron* **1996**, *52*, 7525–7546; j) S. Wagaw, R. A. Rennels, S. L. Buchwald, *J. Am. Chem. Soc.* **1997**, *119*, 8451–8458; k) B. H. Yang, S. L. Buchwald, *Org. Lett.* **1999**, *1*, 35–38; l) Y. Kitamura, A. Hashimoto, S. Yoshikawa, J.-I. Odaira, T. Furuta, T. Kan, K. Tanaka, *Synlett* **2006**, 115–117; m) T. Watanabe, S. Oishi, N. Fujii, H. Ohno, *Org. Lett.* **2008**, *10*, 1759–1762; n) A. Klapars, X. Huang, S. L. Buchwald, *J. Am. Chem. Soc.* **2002**, *124*, 7421–7423; o) K. Yamada, T. Kubo, H. Tokuyama, T. Fukuyama, *Synlett* **2002**, 0231–0234; p) H. J. Jeong, J. M. Lee, M. K. Kim, S.-G. Lee, *J. Heterocycl. Chem.* **2002**, *39*, 1019–1024; q) S. G. Lee, K. H. Park, Y.-J. Yoon, *J. Heterocycl. Chem.* **1998**, *35*, 711–715.

[17] a) R. J. Vijn, W. N. Speckamp, B. S. de Jong, H. Heimstra, *Angew. Chem.* **1984**, *96*, 165–166; *Angew. Chem. Int. Ed. Engl.* **1984**, *23*, 165–166; b) W. N. Speckamp, S. J. Veenstra, J. Dijkink, R. Fortgens, *J. Am. Chem. Soc.* **1981**, *103*, 4643–4645.

[18] a) GVKBiosciences Pvt. Ltd. proprietary database; b) stable 3D structures of all of the compounds were used to calculate their physicochemical properties, such as molecular weight, number of hydrogen-bond donors (HBD), number of hydrogen-bond acceptors (HBA), A log P, log D, and polar surface area (PSA); these values were calculated by using Discovery studio 3.1 (Accelrys. Inc.).

[19] a) P. Ertl, B. Rohde, P. Selzer, *J. Med. Chem.* **2000**, *43*, 3714–3717; b) "Polar Surface Area": P. Ertl in *Molecular Drug Properties* (Ed.: R. Mannhold), Wiley-VCH, Weinheim, **2007**, pp. 111–126.

[20] SYBYL 8.0, The Tripos Associates, St. Louis, **2008**.

[21] W. H. Sauer, M. K. Schwartz, *J. Chem. Inf. Comput. Sci.* **2003**, *43*, 987–1003.

[22] A. W. Hung, A. Ramek, Y. Wang, T. Kaya, J. A. Wilson, P. A. Clemonsa, D. W. Younga, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 6799–6804.

[23] A. P. Antonchick, C. Gerding-Reimers, M. Catarinella, M. Schürmann, H. Preut, S. Ziegler, D. Rauh, H. Waldmann, *Nat. Chem.* **2010**, *2*, 735–740.
[24] B. A. Thompson, *J. Clin. Pathol.* **1950**, *3*, 118–127.
[25] a) Anonymous, 1996. Pharmacopeia of India (The *Withania somnifera* (Ashwagandha). J. Basic Indian Pharmacopeia), 3rd ed. Govt. of India, New Microbiol, 46: 365–374. Delhi, Ministry of Health and Family Welfare; b) M. J. Pelczar, E. C. S. Chan, N. R. Krieg, *Microbiology Concepts and Applications*, McGraw-Hill, New York, **1993**.
[26] T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55–63.

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