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Natural Product Synthesis

Total Synthesis and Biological Evaluation of Jerantinine E**

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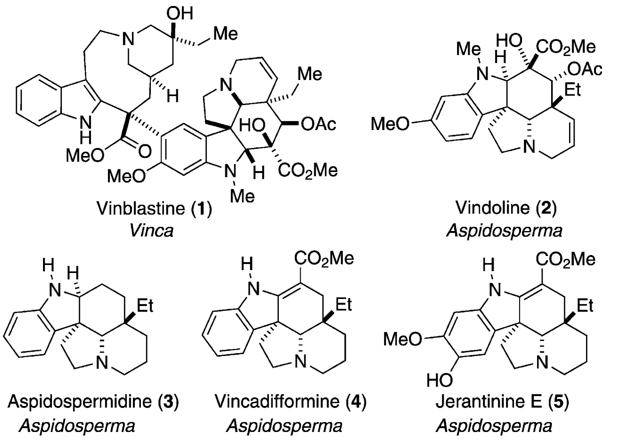
Indole alkaloids have attracted the attention of synthetic chemists because of their intriguing structural features and remarkable bioactivities. This interest is perhaps best represented by the *Vinca* alkaloid vinblastine (**1**; Scheme 1), isolated from *Catharanthus roseus*, which is currently among the foremost drugs used to combat cancer.[1] However, de novo synthesis of novel vinblastine analogues remains a daunting task owing to its highly complex structure. Consequently, the discovery of simpler bioactive *Aspidosperma* alkaloids is of high interest to facilitate the discovery of new anticancer agents. Not surprisingly, intensive synthetic efforts have been directed towards the synthesis of vindoline (**2**),[2] or simpler *Aspidosperma* alkaloids such as aspidospermidine (**3**)[3] and vincadifformine (**4**).[4]



Scheme 1. *Vinca* and *Aspidosperma* alkaloids.

In 2008, Kam and co-workers reported the isolation of seven new *Aspidosperma* indole alkaloids, jerantinine A–G, from a leaf extract of the Malayan plant *Tabernaemontana corymbosa*.[5] Among them was jerantinine E (**5**), which is structurally related to vincadifformine (**4**) but has a more oxidized indole core. The jerantinines displayed significant cytotoxic activity (half-maximal inhibitory concentration; $IC_{50} = 0.27–0.96\ \mu g\,L^{-1}$ ($0.70–2.50\ \mu\text{M}$)) against human KB cells, which is rare among simple *Aspidosperma* alkaloids. Nevertheless, the mode of action for the cytotoxicity of the jerantinines is currently unknown. Furthermore, the highly oxygenated core of the jerantinines renders them challenging synthetic targets, and a total synthesis of jerantinines A-G has not yet been reported.

Herein, we describe the first total synthesis of (±)-jerantinine E (**5**) in 17 steps and 16% overall yield from δ-valerolactam (**10**). The availability of a significant amount of the natural product permitted the separation of the enantiomers, in-depth biological evaluation of the cytotoxic activity in various human cancer cell lines, as well as a first investigation on the origin of the observed cytotoxicity.

As shown in our retrosynthesis (Scheme 2, **A**), we envisaged that the free hydroxy group of jerantinine E (**5**) could be generated by selective demethylation during the last step of the total synthesis. This strategy would simplify the synthesis by avoiding a multistep hydroxy group protecting/deprotecting sequence. We expected this selective methyl group removal to proceed under oxidative conditions owing to the presence of the electron-rich nitrogen in the *para* position.[6] As such, the indole core could be oxidized to the corresponding iminoquinone, followed by in situ reduction to give **5**. We next planned to build-up the e-ring system by a bis(alkylation) and to install the methyl ester group on the c-ring by acylation using Mander's reagent. This ring formation/acylation sequence was developed during the synthesis of *Aspidosperma* alkaloids by Rawal and co-workers, albeit with a less oxygenated indole core.[3d] The requisite starting material for this sequence could be generated through *N*-protecting group removal and deoxygenation of intermediate **6**. Tetracyclic product **6** was envisaged to be accessed by a selective cyclization (formal homo-Nazarov reaction) of aminocyclopropane **7**, previously developed in our group,[7] forming the central ring system c. Cyclization precursor **7** would finally be obtained by adding an organometallic reagent derived from commercially available indole **9** onto Weinreb amide **8**.

Our synthesis commenced with the coupling of Weinreb amide **8**, obtained in seven steps and 51% overall yield from

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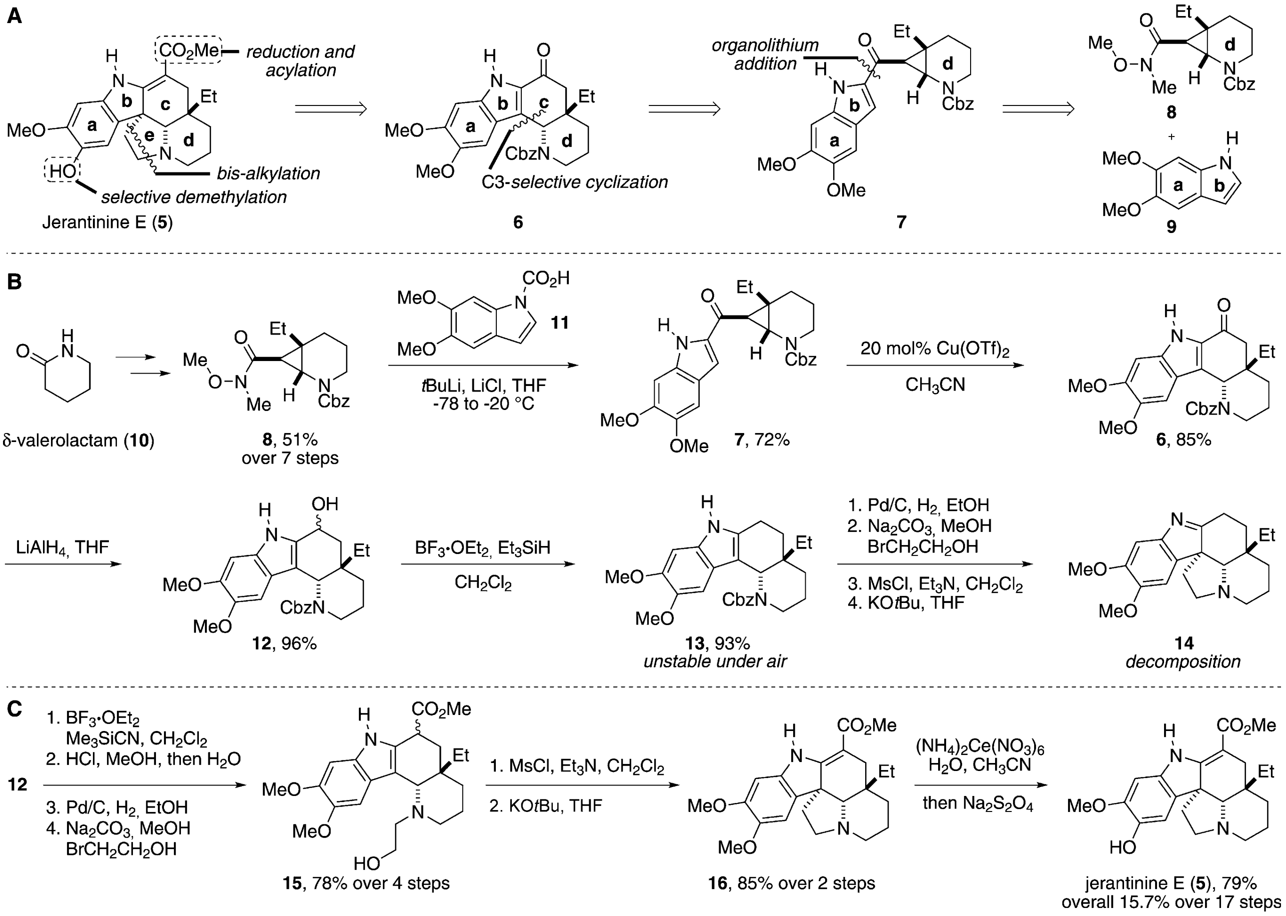

Scheme 2. A) Retrosynthetic analysis, B) initial approach, and C) final synthetic route for the total synthesis of jerantinine E (**5**). Ms = mesyl.

commercially available δ-valerolactam (**10**),[7] and the doubly lithiated organolithium reagent derived from *N*-carboxy indole **11** (Scheme 2, **B**). Selecting the carboxylate as *N*-indole protecting group had a dual purpose, as it directed the lithiation to the C2 indole position and was readily removed during the aqueous work-up. The desired aminocyclopropane **7** was obtained in 72 % yield. The formal homo-Nazarov cyclization of **7** afforded the *cis*-diastereoisomer of **6** exclusively in 85 % yield with high C3 regioselectivity.[8]

At this point of the synthesis, four of the five ring systems of jerantinine E (**5**) were installed, and we turned our attention to the formation of the e-ring. Deoxygenation of the ketone was unexpectedly challenging however, and the reported one-step reduction by Wenkert and co-workers led only to decomposition of the starting material.[9] Thus, a two-step procedure was devised to remove the ketone functionality. Alcohol **12** was obtained in 96 % yield employing lithium aluminum hydride under mild conditions. We reasoned that an ionic reduction could be ideal at this stage to remove the hydroxy group, as a facile carbocation formation can be envisaged owing to the highly electron-rich adjacent indole ring system. Indeed, treatment of alcohol **12** with boron trifluoride etherate at low temperature led to carbocation formation, which upon triethylsilane-mediated hydride transfer gave intermediate **13** in 93 % yield. Compound **13** was highly unstable under air, and the subsequent transformations, including Cbz-removal and Rawal's three-step procedure[3d] had to be completed in one pot while carefully using Schlenk techniques. Although liquid chromatography–mass spectrometry analysis of the reaction progress indicated that Cbz deprotection, alkylation, mesylation, and base-mediated ring closure were working, we were unable to isolate the desired pentacyclic compound **14** as it immediately decomposed into several unidentifiable products during workup.

At this point, it became clear that a new approach avoiding highly electron-rich intermediates and reactive imines, such as **14**, had to be designed to gain access to jerantinine E (**5**). We speculated that these objectives could be realized by introducing the electron-withdrawing ester group present on the natural product prior to the installment of the last ring system (Scheme 2, **C**). Thus, the presumably reactive imine double bond would migrate into conjugation with the ester group upon closure of the fifth ring system.

To test this hypothesis, we made use of the facile carbocation formation through the treatment of alcohol **12** with boron trifluoride etherate. The carbocation intermediate was reacted with trimethylsilylcyanide and the resulting nitrile product was transformed into ester **15** by Pinner methanolysis, Cbz-deprotection, and alkylation with 2-bromoethanol. Gratifyingly, intermediate **15** successfully underwent the ring-closing procedure, generating pentacyclic product **16** in 85 % yield. As postulated, the presence of the ester group facilitated double-bond migration into conjugation, producing a perfectly stable compound. Finally, selective demethylation of **16** was possible by cerium ammonium nitrate mediated oxidation followed by in situ reduction with sodium dithionite to give jerantinine E (**5**) in 79 % yield. Thus, the first total synthesis of jerantinine E (**5**) was accomplished in 17 steps and a 16 % overall yield starting from δ-valerolactam (**10**).

With significant amounts of jerantinine E (**5**) in hand, we focused our attention on exploring its potential as an anticancer agent. Considering that breast and lung cancers are among the most frequent and fatal types of this disease,[10] two human-derived breast- and lung-cancer cell lines were selected to investigate the cytotoxic activity of jerantinine E (**5**). A first moderately invasive cell line (MCF-7) was selected from breast ductal carcinoma in conjunction with a second highly invasive breast cancer cell line (MDA-MB-231).[11] The human adenocarcinoma cell line (A549) derived from alveolar epithelial cells and adenosquamous carcinoma cell line (HTB-178)[12] were chosen to serve as lung cancer model cell lines. The two enantiomers of jerantinine E (**5**) were first separated by chiral chromatography and their cytotoxic activity examined separately. The cellular assays showed that only naturally occurring (−)-jerantinine E (**5**) was significantly bio-active (Figure 1).[13]

Both breast cancer cell lines gave comparable inhibition values for cell growth after 24 and 72 h (Figure 1 A and B) having IC_{50} values between 1.7 and 2.3 $\mu g\,mL^{-1}$ (4.4 to 6.0 μM). The screening assay in the two lung cancer cell lines revealed that (−)-jerantinine E (**5**) exhibits greater cytotoxicity against the adenocarcinoma A549 cell line compared to adenosquamous HTB-178 cells at both 24 and 72 h (Figure 1 C and D). In the four tested human cancer cell lines, (−)-jerantinine E (**5**) displayed the highest cytotoxic effect in the A549 cell line with an IC_{50} after 24 and 72 h of exposure of 1.6 and 0.4 $\mu g\,mL^{-1}$ (4.2 and 1.0 μM), respectively (Figure 1 C and D).

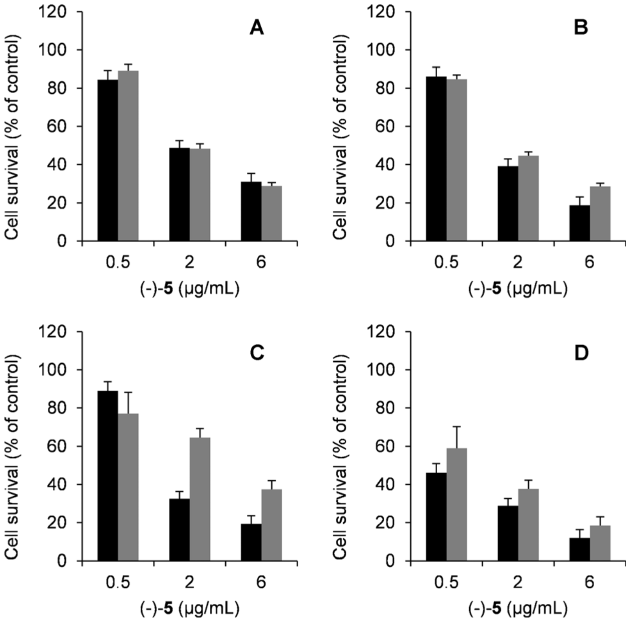

Figure 1. Effect of (−)-jerantinine E (**5**) on the survival of breast cancer cells (A, 24 h exposure and B, 72 h exposure; black bar: MDA-MB-231, gray bar: MCF-7) and lung cancer cells (C, 24 h exposure and D, 72 h exposure; black bar: A549, gray bar: HTB-178). For additional experimental details, see the Supporting Information.

After studying the cytotoxic activity profile for jerantinine E (**5**), we set out to elucidate possible mechanisms for the observed cell growth inhibition. First, a cell migratory assay was carried out with the above described human-derived cancer cell lines. Inhibition of cell migration ranging between 50 and 90 % was observed in all four cancer cell lines after 15 h of exposure to 2 and 6 $\mu g\,mL^{-1}$ of jerantinine E (**5**).[14] Second, an impedance study of jerantinine E (**5**) against the L-929 (mouse fibroblast) cell line was carried out. In this assay, cells are placed on a gold electrode plate and treated with the test compound at their IC_{90} concentration. During a five day time period, the change in impedance, owing to changes in the morphological state of the cells upon compound exposure, is measured. Through cluster analysis, the obtained data can then be matched to the data of a reference compound with a known target.[15] Using this approach, we found that jerantinine E (**5**) displayed an impedance profile in accordance with reference compounds, such as colchicine, which affects the microtubule network. An immunofluorescent staining assay was then carried out (Figure 2) with PtK2 kidney cells to confirm this possible mode of action. As shown in Figure 2 B, multi-lobed nuclei were observed after exposing PtK2 kidney cells to 2 $\mu g\,mL^{-1}$ jerantinine E (**5**). Furthermore, disruption of the microtubule network already became apparent at this stage and is nearly complete at higher concentration (Figure 2 C and D). Such observations further suggested microtubule disruption as a plausible mode of action. To confirm this hypothesis, the ability of both enantiomers and racemic jerantinine E (**5**) to

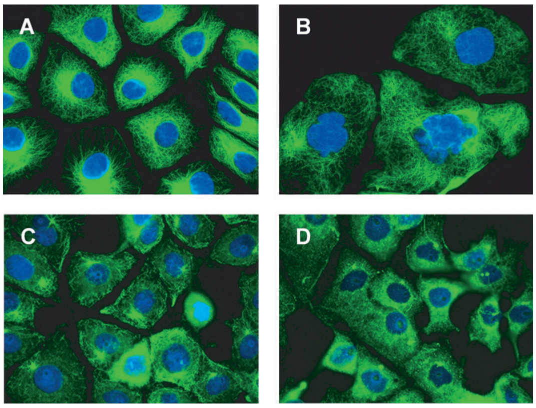

Figure 2. Immunofluorescent staining of A) untreated PtK2 kidney cells and cells exposed to B) 2 $\mu g\,mL^{-1}$, C) 6 $\mu g\,mL^{-1}$, and D) 10 $\mu g\,mL^{-1}$ jerantinine E (**5**), showing the effect on microtubules (green) and the cell nucleus (blue). For additional experimental details, see the Supporting Information.

inhibit tubulin polymerization was studied on the purified tubulin. The assay showed (−)-jerantinine E (**5**) to be a potent inhibitor of tubulin polymerization with an IC$_{50}$ value of 0.17 µg mL^{-1} (0.45 µM).[14] It is noteworthy that (−)-jerantinine E (**5**) was even slightly more active than the known microtubule skeleton disruptor colchicine.

In conclusion, we have described the first total synthesis of the indole alkaloid jerantinine E (**5**) in 17 steps and 16% overall yield starting from δ-valerolactam (**10**). An efficient and highly selective formal homo-Nazarov cyclization of an aminocyclopropane was used to give access to four of the five rings contained in the complex polycyclic core of the natural product. A novel strategy to install the ester group onto the highly sensitive oxygenated indole alkaloid core of the molecule was devised. Lastly, a late-stage selective demethylation led to an efficient synthetic sequence. The total synthesis enabled us to investigate the biological profile of jerantinine E (**5**). The cytotoxic activity of the natural product was determined in several human-derived breast and lung cancer cell lines, displaying for instance an IC$_{50}$ value of 0.4 µg mL^{-1} (1.0 µM) in the A549 lung cancer cell line. Investigations into the mode of action suggested that jerantinine E (**5**) acts via disruption of the microtubule network, as indicated by its potent inhibitory activity displayed in tubulin polymerization. Future work will focus on the synthesis of analogues for a detailed structure–activity relationship study with the goal of finding compounds with improved activity and potency. Additional experiments to elucidate the exact origin of the observed microtubule disruption will also be considered to further understand the source of the observed cytotoxicity.

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[1] a) S. E. Malawista, H. Sato, K. G. Bensch, *Science* **1968**, *160*, 770; b) B. Gigant, C. G. Wang, R. B. G. Ravelli, F. Roussi, M. O. Steinmetz, P. A. Curmi, A. Sobel, M. Knossow, *Nature* **2005**, *435*, 519; c) F. Gueritte, J. Fahy, *The vinca Alkaloids, Anticancer agents from natural products* (Eds.: D. J. C. Newman, G. M. Kingston), Taylor & Francis group, New York, **2005**.

[2] Selected examples: a) M. Ando, G. Buchi, T. Ohnuma, *J. Am. Chem. Soc.* **1975**, *97*, 6880; b) J. P. Kutney, U. Bunzlitrepp, K. K. Chan, J. P. D. Souza, Y. Fujise, T. Honda, J. Katsube, F. K. Klein, A. Leutwiler, S. Morehead, M. Rohr, B. R. Worth, *J. Am. Chem. Soc.* **1978**, *100*, 4220; c) P. L. Feldman, H. Rapoport, *J. Am. Chem. Soc.* **1987**, *109*, 1603; d) H. Ishikawa, G. I. Elliott, J. Velcicky, Y. Choi, D. L. Boger, *J. Am. Chem. Soc.* **2006**, *128*, 10596; e) D. Kato, Y. Sasaki, D. L. Boger, *J. Am. Chem. Soc.* **2010**, *132*, 3685.

[3] Selected examples: a) G. Stork, J. E. Dolfini, *J. Am. Chem. Soc.* **1963**, *85*, 2872; b) J. P. Kutney, N. Abdurahm, P. Lequesne, E. Piers, I. Vlattas, *J. Am. Chem. Soc.* **1966**, *88*, 3656; c) T. Gallagher, P. Magnus, J. C. Huffman, *J. Am. Chem. Soc.* **1982**, *104*, 1140; d) S. A. Kozmin, T. Iwama, Y. Huang, V. H. Rawal, *J. Am. Chem. Soc.* **2002**, *124*, 4628; e) J. P. Marino, M. B. Rubio, G. F. Cao, A. de Dios, *J. Am. Chem. Soc.* **2002**, *124*, 13398; f) I. Coldham, A. J. M. Burrell, L. E. White, H. Adams, N. Oram, *Angew. Chem.* **2007**, *119*, 6271; *Angew. Chem. Int. Ed.* **2007**, *46*, 6159; g) C. Sabot, K. C. Guerard, S. Canesi, *Chem. Commun.* **2009**, 2941; h) L. McMurray, E. M. Beck, M. J. Gaunt, *Angew. Chem.* **2012**, *124*, 9422; *Angew. Chem. Int. Ed.* **2012**, *51*, 9288; i) L. Jiao, E. Herdtweck, T. Bach, *J. Am. Chem. Soc.* **2012**, *134*, 14563; j) M. Kawano, T. Kiuchi, S. Negishi, H. Tanaka, T. Hoshikawa, J. Matsuo, H. Ishibashi, *Angew. Chem.* **2013**, *125*, 940; *Angew. Chem. Int. Ed.* **2013**, *52*, 906.

[4] Selected examples: a) J. P. Kutney, R. T. Brown, E. Piers, *J. Am. Chem. Soc.* **1964**, *86*, 2286; b) S. Kobayashi, G. Peng, T. Fukuyama, *Tetrahedron Lett.* **1999**, *40*, 1519.

[5] K. H. Lim, O. Hiraku, K. Komiyama, T. S. Kam, *J. Nat. Prod.* **2008**, *71*, 1591.

[6] a) G. Meunier, B. Meunier, *J. Am. Chem. Soc.* **1985**, *107*, 2558; b) J. D. White, K. M. Yager, T. Yakura, *J. Am. Chem. Soc.* **1994**, *116*, 1831; c) M. Alvarez, M. A. Bros, G. Gras, W. Ajana, J. A. Joule, *Eur. J. Org. Chem.* **1999**, 1173; d) M. B. Andrus, E. L. Meredith, E. J. Hicken, B. L. Simmons, R. R. Glancey, W. Ma, *J. Org. Chem.* **2003**, *68*, 8162.

[7] a) F. De Simone, J. Gertsch, J. Waser, *Angew. Chem.* **2010**, *122*, 5903; *Angew. Chem. Int. Ed.* **2010**, *49*, 5767; b) F. De Simone, J. Waser, *Chimia* **2012**, *66*, 233.

[8] A 12:1 regioselectivity was observed for C3 vs N1 cyclization. For a further discussion of the selectivity of the cyclization reaction of aminocyclopropanes on indoles, see: a) F. De Simone, J. Waser, *Synlett* **2011**, 589; b) F. De Simone, T. Saget, F. Benfatti, S. Almeida, J. Waser, *Chem. Eur. J.* **2011**, *17*, 14527.

[9] E. Wenkert, T. Hudlicky, *J. Org. Chem.* **1988**, *53*, 1953.

[10] J. Ferlay, D. M. Parkin, E. Steliarova-Foucher, *Eur. J. Cancer* **2010**, *46*, 765.

[11] D. Staedler, E. Idrizi, B. H. Kenzaoui, L. Juillerat-Jeanneret, *Cancer Chemother. Pharmacol.* **2011**, *68*, 1161.

[12] D. Staedler, T. Magouroux, R. Hadji, C. Joulaud, J. Extermann, S. Schwung, S. Passemard, C. Kasparian, G. Clarke, M. Gerrmann, Le R. Dantec, Y. Mugnier, D. Rytz, D. Ciepielewski, C. Galez, S. Gerber-Lemaire, L. Juillerat-Jeanneret, L. Bonacina, J. P. Wolf, *ACS Nano* **2012**, *6*, 2542.

[13] Please see the Supporting Information for exact IC$_{50}$-values, including error range, for both enantiomers and racemic jerantinine E (**5**) in the four described cancer cell lines.

[14] Please see the Supporting Information for more details.

[15] a) K. Solly, X. Wang, X. Xu, B. Strulovici, W. Zheng, *Assay Drug Dev. Technol.* **2004**, *2*, 363; b) J. M. Atienza, N. Yu, S. L. Kirstein, B. Xi, X. Wang, X. Xu, Y. A. Abassi, *Assay Drug Dev. Technol.* **2006**, *4*, 597; c) Y. A. Abassi, B. Xi, W. Zhang, P. Ye, S. L. Kirstein, M. R. Gaylord, S. C. Feinstein, X. Wang, X. Xu, *Chem. Biol.* **2009**, *16*, 712; d) W. N. Venables, B. D. Ripley, *Modern Applied Statistics with S*, 4th ed., Springer, New York, **2002**; e) G. R. Warnes gplots: Various R programming tools for plotting data. **2010**. Retrieved from http://cran.rproject.org/package = gplots; f) N. Ke, B. Xi, P. Ye, W. Xu, M. Zheng, L. Mao, M. J. Wu, J. Zhu, J. Wu, W. Zhang, J. Zhang, J. Irelan, X. Wang, X. Xu, Y. A. Abassi, *Anal. Chem.* **2010**, *82*, 6495.