Total Synthesis and Biological Evaluation of the Nakijiquinones

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Abstract: The Her-2/Neu receptor tyrosine kinase is vastly overexpressed in about 30% of primary breast, ovary, and gastric carcinomas. The nakijiquinones are the only naturally occurring inhibitors of this important oncogene, and structural analogues of the nakijiquinones may display inhibitory properties toward other receptor tyrosine kinases involved in cell signaling and proliferation. Here, we describe the first enantioselective synthesis of the nakijiquinones. Key elements of the synthesis are (i) the reductive alkylation of a Wieland−Miescher-type enone with a tetramethoxyaryl bromide, (ii) the oxidative conversion of the aryl ring into a *p*-quinoid system, (iii) the regioselective saponification of one of the two vinylogous esters incorporated therein, and (iv) the selective introduction of different amino acids via nucleophilic conversion of the remaining vinylogous ester into the corresponding vinylogous amide. The correct stereochemistry and substitution patterns are completed by conversion of two keto groups into a methyl group and an endocyclic olefin via olefination/reduction and olefination/isomerization sequences, respectively. This synthesis route also gave access to analogues of nakijiquinone C with inverted configuration at C-2 or with an exocyclic instead of an endocyclic double bond. Investigation of the kinase-inhibiting properties of the synthesized derivatives revealed that the C-2 epimer **30** of nakijiquinone C is a potent and selective inhibitor of the KDR receptor, a receptor tyrosine kinase involved in tumor angiogenesis. Molecular modeling studies based on the crystal structure of KDR and a model of the ATP binding site built from a crystal structure of FGF-R revealed an insight into the structural basis for the difference in activity between the natural product nakijiquinone C and the C-2 epimer **30**.

Introduction

Natural products embodying a Decalin-type core structure and a quinoid or related aromatic side chain often are characterized by pronounced and manifold biological properties. For instance, the marine sesquiterpene quinones nakijiquinone A−D (**1a**−**d**),[1] ilimaquinone (**2**),[2] avarol (**3**),[3] smenospongine (**4**), smenospongidine (**5**), smenospongiarine (**6**),[4] and mamanuthaquinone (**7**)[5] (Figure 1) display antimicrobial, antiviral, and cytotoxic activities. Therefore, they offer promising opportunities for the development of new compounds which may be employed efficiently for the elucidation of intracellular events and/or the development of new drugs. A prime example which clearly shows the possible impact of this natural product-based approach to study biological phenomena is the synthesis of ilimaquinone and its use for the study of intracellular vesicular trafficking by Snapper et al.[6]

Among this class of natural products, the nakijiquinones (**1**) are of particular relevance. These marine sesquiterpene quinones display pronounced cytotoxicity against L 1210 murine leukemia cells and KB human epidermoid carcinoma cells and, most notably, were identified as the first naturally occurring inhibitors of the Her-2/Neu (also called erbB-2) receptor tyrosine kinase (RTK).[1] Her-2/Neu is vastly overexpressed in about 30% of primary breast, ovary, and gastric carcinomas.[7,8] Amplification is closely correlated with the clinical behavior of these neoplasms, such that tumors with Her-2 amplification are more aggressive and are associated with reduced patient survival.

Receptor tyrosine kinases are crucial for proliferation, survival, and differentiation. They have been implicated in several pathologies such as tumor growth and tumor angiogenesis. Compounds that can selectively block RTK activity are of paramount importance for the development of new anticancer drugs.[8]

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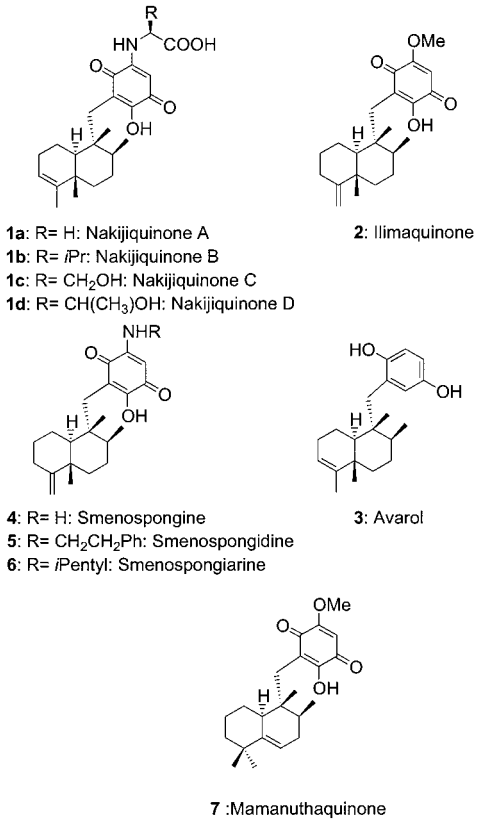


Figure 1. Structures of the nakijiquinones and related natural products.

During the last years, many small molecule inhibitors of RTKs have been discovered, and some of them have already entered clinical trails (see in a following section).

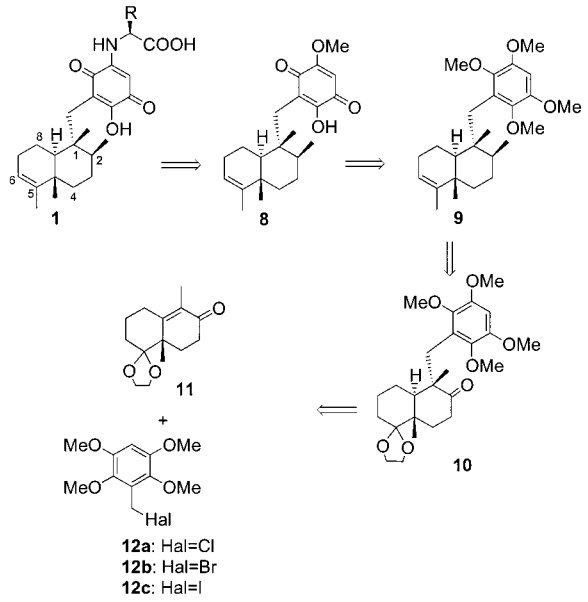
In addition to the opportunities for the treatment of cancer and other diseases opened up by RTK inhibitors, such drugs are also likely to be useful in dissecting signaling pathways.[9−11]

In this paper, we describe the first enantioselective total synthesis of the nakijiquinones and closely related analogues of these natural products and a first biological evaluation aimed at the determination of their potency and their selectivity against different tyrosine kinases.[12]

Results and Discussion

Retrosynthetic Considerations. The nakijiquinones embody three basic structural elements, an amino acid, a central *p*-quinoid unit, and a diterpenoid system. To reach a high degree of convergency, the nakijiquinones were first dissected in a retrosynthetic sense into isospongiaquinone (**8**), an interesting natural product in its own right (vide infra), and an amino acid that can be introduced in the last step by conjugate addition to the vinylogous methyl ester present[1] (Scheme 1). It was further

Scheme 1. Retrosynthetic Analysis of the Nakijiquinones



planned to generate the selectively functionalized quinoid system by oxidation of tetramethoxy-substituted aromatic precursor **9** to a 1,4-dicarbonyl compound and subsequent selective saponification of one of the two vinylogous methyl esters generated thereby. Tracing the quinoid system back to a stable aromatic compound was necessary, because we resorted to the reductive alkylation of α,β-unsaturated Wieland−Miescher-type enone **11** with a benzyl halide **12** for the synthesis of the diterpene structure and its coupling with the alkoxy-substituted aromatic compound. This strategy had already proven to be useful in the construction of related natural products.[13] This synthesis plan called for an intermediary keto group at C-2 which later on would be converted to the required methyl group by an olefination and subsequent isomerization. Finally, we envisaged generating the endocyclic 5,6-alkene by olefination of a ketone at C-5 and subsequent isomerization of the generated exocyclic double bond. Thus, compound **10** was aimed at as an intermediate.

Synthesis of the Nakijiquinones. Benzyl halides (**12**) were synthesized from catechol (**13**) as shown in Scheme 2; attempts to synthesize these compounds from *p*-benzoquinone failed. First, aromatic diol **13** was oxidized to the corresponding *ortho*-quinone, which readily added sodium methoxide. Subsequent reoxidation led to *ortho*-quinone (**14**), which upon treatment with sulfuric acid in methanol rearranged to the corresponding *para*-quinoid compound (**15**).[14] Reduction of the carbonyl groups proved unexpectedly difficult; that is, the use of $CrCl_2$, $NaHSO_3$, B_2H_6, ascorbic acid, and $TiCl_3$ failed. However, upon treatment with $NaBH_4$ in ethanol, the carbonyl groups were cleanly reduced. The resulting diol (**16**) was unstable and, therefore, directly converted into tetramethoxybenzene (**17**).[15]

To vary size and quality of the leaving group in the planned reductive alkylation, benzyl halides **12a**−**c** were synthesized. Chloromethylation was achieved in 71% by treatment with 1-chloro-4-(chloromethoxy)butane in the presence of $SnCl_4$[16] in THF/cyclohexane; in neat cyclohexane, the reaction did not

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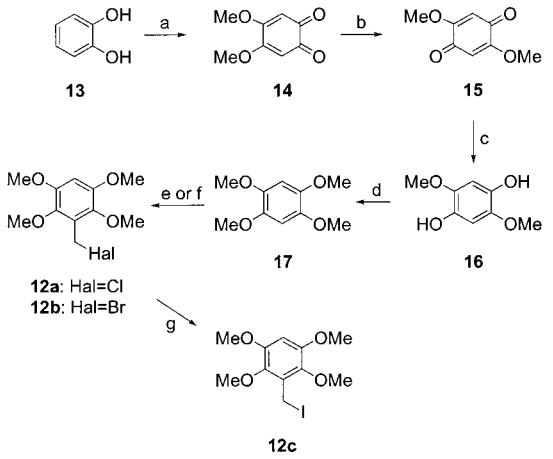
Scheme 2. Synthesis of Benzyl Halides **12a**−**c**



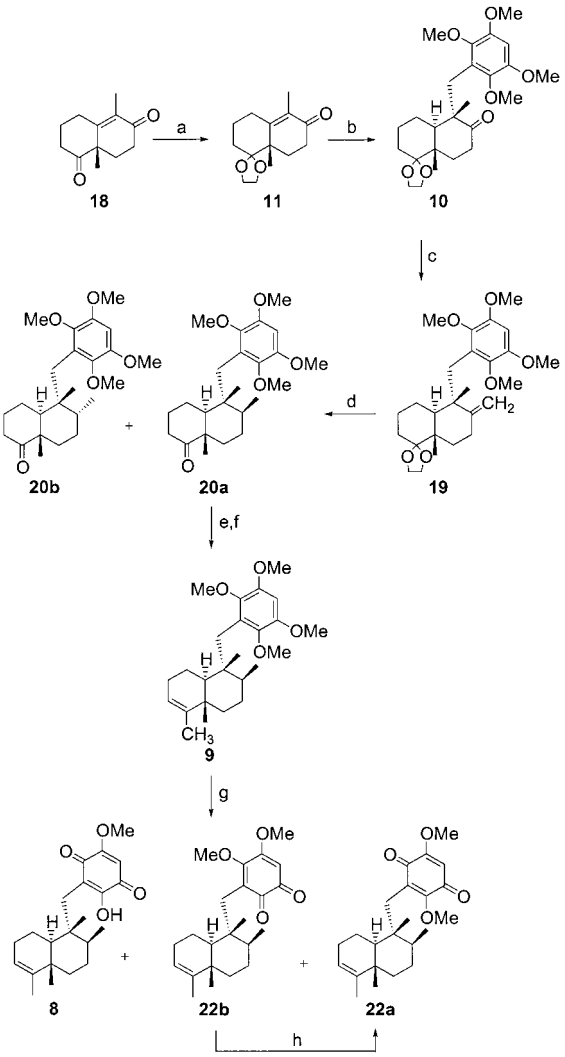
[a] PbO_2, NaOMe, MeOH, 20−22 °C, 65%. [b] H_2SO_4, MeOH, 96%. [c] $NaBH_4$, EtOH, 0 °C. [d] $(CH_3O)_2SO_2$, NaOH, $NaHSO_3$, EtOH, reflux, 75% (2 steps). [e] $Cl(CH_2)_4OCH_2Cl$, $SnCl_4$, THF, cyclohexene, 71% **12a**. [f] $(HCHO)_n$, HBr/CH_3COOH, 74% **12b**. [g] NaI, acetone, 94%.

occur. Bromomethyl derivative **12b** was obtained by treatment with 1.1 equiv HBr in acetic acid in the presence of 2 equiv of paraformaldehyde.[17] Benzyl iodide **12c** was accessible from either chloride **12a** or bromide **12b** via Finkelstein reaction. α,β-Unsaturated ketone **18** was synthesized as described via Robinson annelation employing L-phenylalanine as source of chirality.[18] After recrystallization from *n*-hexane/ethyl acetate, the diketone was obtained in 98% ee (the ee value was determined by means of NMR spectroscopy after reduction of the keto group at C-5 with $NaBH_4$ and esterification of the resulting alcohol with (*R*)-Mosher acid).

Selective monoprotection of the C-5 keto group was achieved in high yield by transacetalization with ethyl-2-methyl-1,3-dioxolane.[19] Under these conditions, only the more electrophilic keto group was attacked, whereas direct ketalization with ethylene glycol led to protection of both carbonyl groups.

The decisive reductive coupling between benzyl halides **12** and enone **11** turned out to be particularly challenging. Such couplings employing less-substituted benzylic halides have been described in the literature[13a,b,d] and proved to be reproducible in our hands. However, the reported reaction conditions could not be employed successfully to the synthesis of compound **10** (Scheme 3). In initial experiments, none of the benzyl halides **12** reacted with the radical anion generated from enone **11** by treatment with lithium in ammonia at −78 °C. Alternatively, we tried to effect the coupling by synthesis of the silylenol ether via treatment with methyllithium[20] or via direct generation of the enolate by treatment of the α,β-unsaturated ketone with L-selectride.[20] However, only addition of MeLi to the carbonyl group or reduction of the ketone was observed. Because of the failure of these attempts, we carefully reexamined the conditions for the originally considered reductive alkylation procedure, and finally, conditions were found under which the desired coupling product was obtained in 76% yield. The reaction is run best in

Scheme 3. Synthesis of Advanced Intermediate **22a**



[a] MED, ethylene glycol, *p*-TsOH, CH_2Cl_2, 84%. [b] Li, NH_3, THF, H_2O −78 °C; −33 °C, 45 min; −78 °C **12b**, 2h then reflux, 76%. [c] KO*t*Bu, CH_3PPH_3Br, toluene, reflux, 98% **19**. [d] 5% HCl, THF, rt, quant, then 10%Pd/C, H_2, Et_3N, MeOH, 12h, rt, 92% (97:3 (**20a:20b**)). [e] KO*t*Bu, CH_3PPH_3Br, toluene, reflux, 98% **21**. [f] $RhCl_3$, $CHCl_3$/EtOH, 2 d, reflux, 95% **9**. [g] AgO, HNO_3, dioxane, rt, 22% **22a**, 60% **22b**, 9.5% **8**. [h] H_2SO_4, MeOH, 96%.

such a way that 30 equiv of lithium dust are first added to liquid ammonia at −78 °C. Then, a solution of enone **11** in THF containing traces of water (1:0.018) is added slowly over 30 min. The resulting solution is refluxed at −33 °C for 45 min. It is important that the solution remains blue during this time, indicating the formation and existence of the enolate. The solution is then cooled to −78 °C, and THF is added to guarantee that the enolate remains in solution (final ratio NH_3/THF = 1.0:0.7). Then, immediately, a solution of 12 equiv of benzyl bromide **12b** in THF is added, and the solution is refluxed for 2 h (final ratio THF/NH_3/H_2O = 1:1.5:0.005). To achieve a high yield, it is important to ensure that the solution does not contain excess solvated electrons after addition of the benzyl halide is complete, because they would induce dimerization of the benzyl bromide. If required, surplus electrons can be trapped by addition of isoprene. If the benzyl halide is added to the reaction mixture at −33 °C, the amount of byproducts formed increases.

With an efficient process for the synthesis of α,α-dialkylated ketone **10** developed, the generation of the stereocenter at C-2

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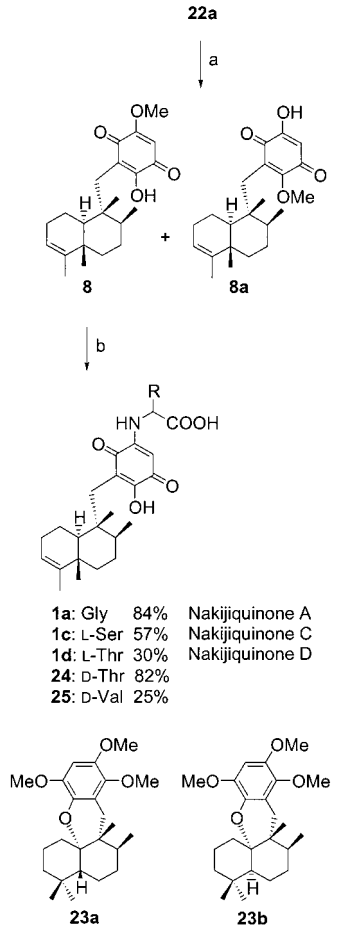
was approached. The conversion of the keto group present in **10** into the corresponding *exo*-methylene compound **19** was unexpectedly difficult. Several established methylenation reagents such as the Tebbe reagent[21] and Cp_2TiMe_2[22] failed completely, and under various conditions, for example, employing BuLi in dioxane or DME or NaH in DMSO, the Wittig reaction either yielded no product at all or only an unsatisfactory low amount of the desired olefin. Also, attempts to add MeLi to the keto group followed by deoxygenation of the tertiary alcohol in a Barton protocol failed. Finally, the use of 9 equiv of H_3CPPh_3Br together with 9 equiv of KO*t*Bu in refluxing toluene[23] turned out to be the method of choice, yielding exocyclic olefin **19** in nearly quantitative yield. Disappointingly, reduction of olefin **19** with PtO_2 proceeded without recordable stereoselectivity. We felt that this might be due to an unfavorable conformation of the bicyclic system and envisaged that removal of the acetal protecting group might improve the situation. This turned out to be the case. After cleavage of the acetal, the stereoselectivity of the reduction with PtO_2 in CH_2Cl_2 was raised to 80:20, and use of Pd/C as catalyst in NEt_3 as solvent yielded ketone **3** in 92% as a 97:3 mixture of epimers at C-2 (Scheme 3). Diastereomers **20a** and **20b** are readily separated by chromatography. As planned, the ketone at C-5 was converted in very high overall yield to endocyclic alkene **9** by olefination (for which the conditions described previously once more proved to be very efficient) and subsequent rhodium-catalyzed isomerization of the exocyclic to the endocyclic double bond.

The next two steps in the synthetic sequence, that is, the oxidation of the tetramethoxyphenyl ring to the *para*-quinoid system and the subsequent selective removal of the correct *O*-methyl group, posed major problems. Oxidation of the aromatic system was initially attempted by applying CrO_3 or cerium ammonium nitrate which had proven to be useful reagents in related studies.[6,13c,24] However, oxidized intermediate **22a** was formed only in low yield or not at all. After substantial variation of the reaction conditions, the use of AgO and catalytic amounts of HNO_3 at room temperature in dioxane as solvent finally evolved as the method of choice.[25] Under these conditions, *p*-diketone **22a** and regioisomeric *o*-diketone **22b** were obtained in a total yield of 82% as an isomeric mixture. The undesired *o*-diketone **22b** was readily rearranged to **22a** by treatment with acid.

Initial attempts directed toward the regioselective removal of the correct *O*-methyl group from **22a** concentrated on the use of different Lewis acids. From the synthesis of maesanin and related compounds, it is known that such quinoid systems can be regioselectively deprotected by means of $HClO_4$ or BCl_3.[26] However, application of this method to **22a** resulted in decomposition of the starting material. If milder methods such as the use of TMSI or $AlCl_3$ were used, the starting material was reisolated. Also, application of alternative techniques for the cleavage of methyl ethers (e.g., LiI in pyridine, or $BF_3 \cdot Et_2O$ together with EtSH or NaSEt) only resulted in reisolation or decomposition of the starting material. Furthermore, attempts to cleave a methyl ether from tetramethoxyaryl intermediate **20a**, that is, prior to oxidation to the quinoid system, were frustrated by Lewis acid mediated rearrangement of the terpene framework and led to the formation of undesired compounds **23a** and **23b** (Scheme 4).

Scheme 4. Synthesis of Nakijiquinone **1c**



[a] KOH, MeOH, H_2O, rt, 71% **8**, 11% **8a**. [b] D- or L-Amino acid, $NaHCO_3$, EtOH, 30 °C or 40 °C, 24 h.

Finally, the problem could be solved by following a different train of thought. Compound **22a** can not only be regarded as a double methyl ether but also as a double vinylogous methyl ester which should be cleavable under basic conditions. Gratifyingly, this reasoning proved to be correct, and treatment of **22a** with 1 N KOH resulted in the formation of selectively deprotected vinylogous acid **8** in 71% yield. In addition, regioisomeric saponification product **8a** was formed in 11% yield. Presumably, the reaction proceeds via conjugate addition of hydroxide to one of the vinylogous esters and elimination of methanol. The preferred formation of the correct regioisomer under these conditions is not readily explained, in particular because variations of the reaction conditions, that is, use of pure methanol instead of H_2O/methanol or use of LiOH instead of KOH, lead to the preferred formation of undesired isomer **8a**. We assume that attack of hydroxide on both possible positions is reversible and that the two enolate intermediates are in equilibrium with each other. Obviously, the enolate leading to the desired product is the thermodynamically more stable isomer and is formed preferably under the particular reaction conditions chosen.

Compound **8** is identical to isospongiaquinone, a natural product isolated from *Stelospongia conulata*.[27] Specific rotation,

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IR, and NMR spectroscopic data of synthetic **8** were in full accord with the data reported for the natural product, thereby proving the absolute configuration of the synthesized compound.

Isospongiaquinone **8** may be employed as a central intermediate for the synthesis of all nakijiquinones. Treatment of this compound with amino acids in ethanol at 30 °C or 40 °C (see the Supporting Information) in the presence of $NaHCO_3$ results in the conversion of the remaining vinylogous ester in **8** into the corresponding vinylogous amide.[1] We have verified this finding and converted isospongiaquinone (**8**) into nakijiquinones A, C and D (**1a,c,d**) by treatment with glycine, L-serine and L-threonine, respectively (Scheme 4). In addition, D-threonine and D-valine were introduced to give nakijiquinones **24** and **25**. The IR and NMR spectroscopic data recorded for the synthetic samples matched the data published.

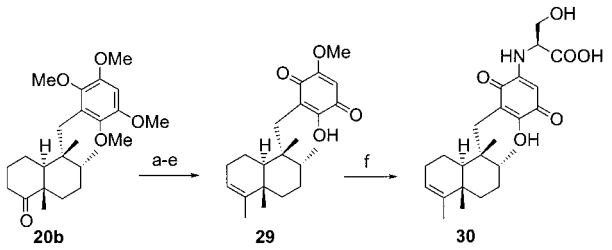
Unexpectedly, however, the values determined for the specific rotation of the synthetic nakijiquinones differed markedly from the data published for the natural products (see the Supporting Information). Most striking was the fact that in the case of nakijiquinones A, C, and D and D-threonine derivative **24** not only the value but, in particular, the direction of the specific rotation was in fact opposite to the reported data, raising the question of whether epimerization might have occurred in the last step of the synthesis.

But as described above, the spectroscopic data recorded for the synthetic nakijiquinones are in full accord with the published values for the natural nakijiquinones. In addition, for example, the diastereomer formed from isospongiaquinone and D-serine (i.e., the possible epimerization product analogous to nakijiquinone C) shows a specific rotation that markedly differs from the value for the synthetic sample.[1] Furthermore, all data (including the specific rotation) recorded for isospongiaquinone (**8**) synthesized as described here are in full accord with the values published for this natural product. Thus, if the absolute configuration of **8** is correct, nakijiquinones **1a**, **c**, **d**, **24**, and **25** must have been formed with the correct absolute configuration as well.[28]

Synthesis of Nakijiquinone Analogues. For the study of the relationship between structure and biological activity of the nakijiquinones, variation of the substitution and the stereochemistry of the trans Decaline-type core structure of the natural products is of great importance. Thus, for initial studies of this aspect, we have synthesized nakijiquinone C analogues **30** and **32** with altered configuration at C-2 and with an exocyclic instead of an endocyclic double bond, respectively. For this purpose, the reaction conditions optimized for the synthesis of the nakijiquinones could be applied directly.

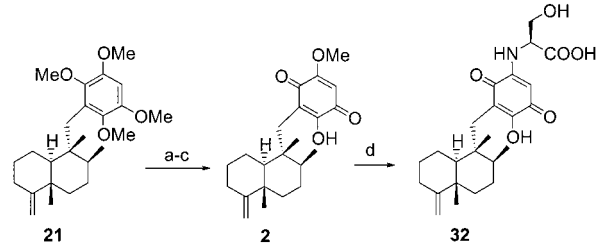
As shown in Scheme 5, C-2 epimeric nakijiquinone C analogue **30** was obtained from tetramethoxyaryl intermediate **20b** obtained as the minor diastereomer from the olefination/reduction of alkylation product **10** (see Scheme 3). To this end, ketone **20b** was converted into exocyclic olefin **26** which was isomerized to endocyclic alkene **27**. Oxidation of the aromatic ring gave *para*-quinoid intermediate **28a** and *ortho*-quinone **28b** in a nearly equal ratio as well as a substantial amount of the desired selectively unmasked C-2 epimer of isospongiaquinone (**29**). *Ortho*-quinone **28b** was rearranged to *para*-quinone **28a** by treatment with acid. Regioselective saponification of one vinylogous ester yielded desired intermediate **29**. Finally, vinylogous ester **29** was converted into the C-2 epimer of

Scheme 5. Synthesis of C2 Epimeric Nakijiquinone C Analogue **30**



[a] KO*t*Bu, CH_3PPH_3Br, toluene, reflux, 98% **26**. [b] $RhCl_3$, $CHCl_3$/EtOH, 2d, reflux, 95% **27**. [c] AgO, HNO_3, dioxane, rt, 33% **28a**, 27% **28b**, 23% **29**. [d] **28b** H_2SO_4, MeOH, 96% **28a**.tfnt[e] KOH, MeOH, H_2O, rt, 59% **29**. [f] L-Serine, $NaHCO_3$, EtOH, 40 °C, 24 h, 32%.

Scheme 6. Synthesis of Nakijiquinone C Analogue 32 Bearing an Exocyclic Double Bond



[a] AgO, HNO_3, dioxane, rt, 26% **31a**, 40% **31b**, 4% **2**. [b] **31b** H_2SO_4, MeOH, 96% **31a**. [c] KOH, MeOH, H_2O, rt, 72% **2**. [d] L-Serine, $NaHCO_3$, EtOH, 40 °C, 24 h, 30%.

nakijiquinone C (**30**). Nakijiquinone C analogue **32** was obtained accordingly from intermediate **21** formed in the synthesis of the natural product by olefination of the keto group at C-5 (see above). Thus, the tetramethoxyaryl ring embedded in **21** was oxidized to a mixture of the *para*- and the *ortho*-quinoid derivative, and the *ortho*-quinone was rearranged to the *para*-quinone under acidic conditions (Scheme 6). Regioselective saponification of one vinylogous ester yielded ilimaquinone (**2**) which finally was treated with L-serine to give nakijiquinone C analogue **32**.

Determination of the Biological Activity. To obtain a preliminary picture of the biochemical properties of the nakijiquinones and related compounds obtained in the synthesis effort detailed above, we investigated their ability to inhibit several different receptor tyrosine kinases.

To this end, apart from Her-2/Neu (vide supra), EGFR (ErbB-1), IGF1R, VEGFR2 (KDR), and VEGFR3 (flt-4) were selected to cover a broad spectrum of tyrosine kinases.

The EGFR (epidermal growth factor receptor, ErbB-1), which is closely related to Her-2/Neu, was one of the first tyrosine kinases described. It has been implicated in human tumorigenesis, for example, of glioblastoma as well as in numerous tumors of epithelial origin, including breast and esophageal tumors.[29]

The insulin-like growth factor 1 receptor (IGF1R) exerts mitogenic, cell survival, and insulin-like activities by binding its ligands IGF1 and IGF2. It is involved in postnatal growth physiology and has been shown to be connected to proliferative disorders such as breast cancer.[30]

VEGFR2 and VEGFR3 are both receptors for the vascular endothelial growth factor (VEGF) family. The VEGFRs are predominantly expressed on endothelial cells. Whereas VEGFR2

(28) The direction of the specific rotation reported for nakijiquinone C isolated from natural sources is indeed incorrect. Reexamination of the original sample yielded a value of $[\alpha]_D^{20} = +138°$ ($c = 0.1$, EtOH): Kobayashi, J. Hokkaido University, Sapporo, Japan. Personal communication.

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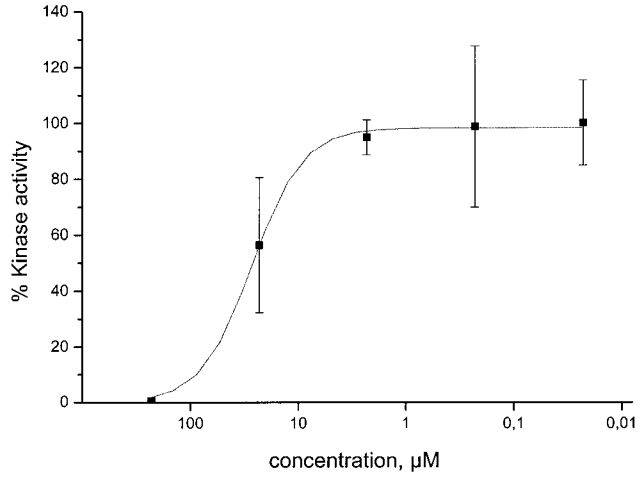

Figure 2. Dose dependent inhibition of KDR kinase activity in vitro by **30**. Each point represents the mean ± standard deviation of 4 independent reading points.

is responsible for endothelial cell proliferation and blood vessel permeability, the VEGF receptor 3 seems to be critical for lymphatic vessel development. Both receptor tyrosine kinases are essential for tumor angiogenesis and lymphangiogenesis, respectively.[31−33] The restrictive expression of VEGFR on endothelial cells predestines them as targets for selective therapeutic intervention.

Briefly, in the assay, the kinase-catalyzed phosphorylation of poly(Glu-Tyr) in the presence of varying concentrations of inhibitor was determined. The kinases were employed as fusion proteins of glutathione-*S*-transferase (GST) and the respective kinase domain. Kinase activity was determined by means of an anti-phosphotyrosine antibody conjugated to horseradish peroxidase (POD). The chemiluminescence caused by the reaction catalyzed by POD immobilized after antibody binding to phosphotyrosine residues was measured (see the Supporting Information for details).

Fourteen of the synthesized compounds were investigated as possible inhibitors for the tyrosine kinases mentioned above. This selected group included nakijiquinones **1a**, **1c**, **1d**, **24**, **25**, quinoid compounds **8**, **8a**, **22a**, and **22b** (having the same absolute configuration and the endocyclic double bond as the terpenoid nakijiquinone core), quinoid nakijiquinone analogues **2** and **32** (with an exocyclic double bond), tetramethoxyaryl intermediates **9** and **21** (with exo- and endocyclic double bond and the stereochemistry found in the natural product), and, finally, compound **30** (the C-2 epimer of nakijiquinone C).

Nakijiquinones **1a**, **1c**, **1d**, **24**, and **25** are only poor inhibitors or not inhibitors of the kinases investigated; that is, they do not display substantial inhibition of the kinases in concentrations lower that 30 μM.

The same is true for quinoid analogues **8**, **8a**, **22a**, **22b**, **2**, and **32** and tetramethoxyaryl compounds **9** and **21** which all have the same arrangement of stereocenters as the nakijiquinones.

However, 2-epi-nakijiquinone C (**30**) turned out to be a good and selective inhibitor of VEGFR2 (KDR). It inhibits this tyrosine kinase with an IC50 value of 21 μM (at a concentration of 25 μM ATP) (Figures 2 and 3) and does not display remarkable activity toward the other kinases investigated. This finding suggests that the stereochemistry of the terpenoid core structure is an important determinant of the tyrosine kinase inhibiting activity of the nakijiquinones. In particular, it appears to determine the selectivity for individual kinases, because nakijiquinone C is a selective Her-2/Neu inhibitor[1] and 2-epi-nakijiquinone C selectively targets VEGFR2.

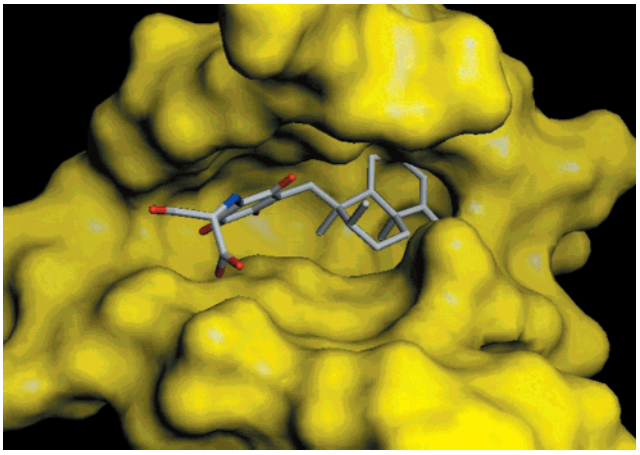

Figure 3. Proposed binding mode of **30** into the active site of KDR.

Molecular Modeling Studies. Recent molecular modeling studies performed on a homology model of the VEGFR2 (KDR) structure[34] provided valuable insights into the potential binding modes of competitive inhibitors of ATP binding to this protein. To further our understanding of the difference in activity between the natural product nakijiquinone and C-2 epimer **30**, we undertook molecular modeling studies using the recently published crystal structure of KDR[35] and a model of the ATP binding site based on a crystal structure of the FGF-R[34] kinase which has a high homology to KDR.[36] The initial docking studies performed with the GOLD software using the KDR structure provided a good indication about potential binding modes. However, these did not yield a satisfactory explanation for the observed differences in activity.[37] In contrast to the structure of the FGF-R kinase which was used to construct an ATP binding site model, the available KDR crystal structure is not a complex with an inhibitor and, thus, contains a more open active site.[38] Using the FGF-R kinase derived model, it was finally possible to determine a binding mode which is consistent with the available SAR data. The proposed binding mode of **30** is shown in Figure 3.[39]

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(39) Figures 3−5 were created using WitnotP. WitnotP is a molecular modeling program developed by and licenced from Novartis AG, Basel. For further information on WitnotP, contact A. Widmer, Novartis AG, Basel.

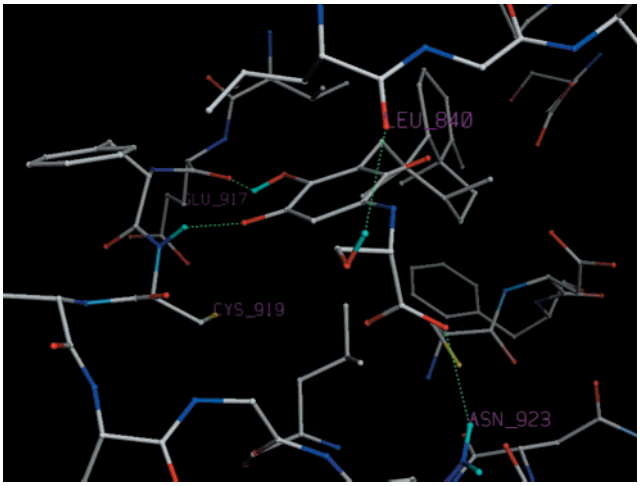

Figure 4. Compound **30** within the active site of KDR. Hydrogen bonds are indicated by green dotted lines. The hydrogen bonds to Cys 919 and Glu 917 are 2.0 and 2.7 Å long, respectively. Those hydrogen bonds possibly formed with Leu 840 and Asn 923 measure 2.7 and 2.5 Å, respectively.

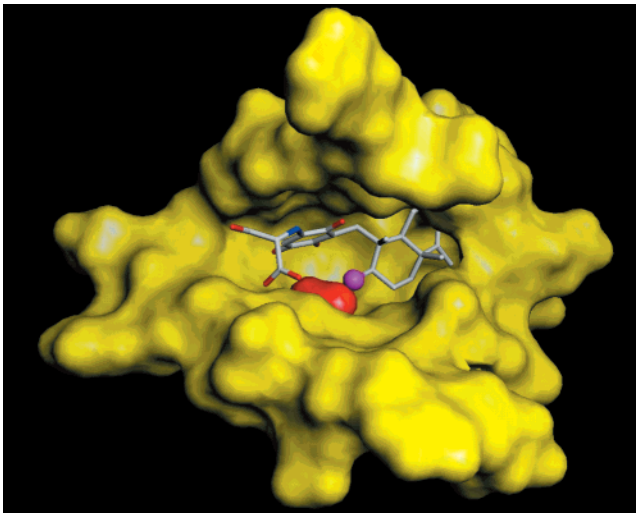

Figure 5. Nakijiquinone **1c** within the active site of KDR. The clashing groups are indicated by color: Nakijiquinone C-2 (magenta) and L1035 (red).

The Decalin subunit of **30** fits into a hydrophobic pocket formed by V916,[40] V914, V899, L889, C1045, F1047, and the hydrocarbon part of the side chain of K868 near the nucleotide binding loop of KDR. In the activated form of the kinase, K868 is thought to form a salt brigde to E885, which ensures the proper coordination for two of the phosphate oxygens of ATP. The chair−twist conformation of the Decalin moiety displayed in Figure 3 was found to be the most stable conformation in the environment of the hydrophobic pocket of our model. The L-serine moiety contributes a hydrogen bond to the backbone NH of N923. The phenolic OH and the adjacent quinone carbonyl group engage in a bidentate manner the backbone carbonyl of E917 and the backbone NH of C919 in hydrogen bonding interactions. Possibly another hydrogen bond forms between the serine NH and the backbone carbonyl group of L840, a residue belonging to the nucleotide binding loop. Considering that **30** was found to be a competitive inhibitor of ATP binding and that E917 and C919 correspond to the residues of the hinge loop that are seen to make hydrogen bonds with the purine moiety of ATP and with heterocyclic inhibitors in the crystal structure of all protein kinases determined thus far,[34,41] this binding mode appears very reasonable (see Figure 4).

While this pattern of hydrogen bonds can also be attained by nakijiquinone **1c**, the Decalin moiety of the natural product cannot be fit into the hydrophobic pocket in the way described for epimer **30**. We attribute this to a possible clash of the equatorial methyl group at C-2 with L1035 (see Figure 5).

This model also allows for an explanation for the lack of activity exhibited by compounds **1a**, **1c**, **1d**, **2**, **8**, **8a**, **9**, **21**, **22a**, **22b**, **24**, **25**, and **32**. With the exception of **1a**, **1c**, **1d**, and **32**, these compounds cannot form the hydrogen bonds described for **30** and exhibit the same stereochemistry on the Decalin moiety as **1c**. In addition, one of the methoxy groups in compounds **9** and **21** clashes with V848, which may also contribute to their inactivity toward KDR.

Conclusion

In conclusion, we have developed the first enantioselective route to the nakijiquinones, the only natural products known to selectively inhibit the Her-2/Neu protooncogene. This synthetic route gives access to various analogues of these interesting and biologically relevant kinase inhibitors. Thereby, new opportunities for the development of selective inhibitors of the Her-2/Neu and the KDR receptor tyrosine kinases and for the study of the biological phenomena influenced by these enzymes may be opened up. Compound **30** represents a promising starting point for the development of more potent inhibitors for the KDR receptor. Such a new starting point may be of particular relevance, because other KDR inhibitors have already been advanced into clinical studies. Thus, currently, a small molecule inhibitor of KDR, SU5416, is under evaluation for the treatment of different human cancers.[41] It was shown that the antitumor effect results from the inhibition of VEGF induced angiogenesis and is not caused by any cytotoxicity.[42]

Furthermore, recently, some more potent inhibitors of the FGF and KDR receptors with nanomolar affinities toward the isolated kinases have been reported. The new KDR inhibitor identified by us is significantly less potent than these compounds. However, its underlying structure is not comparable with the basic structure types of the other KDR inhibitors reported so far.

The new KDR receptor inhibitor compound may, therefore, open up the opportunity to develop a new approach for the therapy of angiogenesis-dependent diseases, such as solid and epithelial tumors, as well as for diabetic retinopathy and rheumatoid arthritis.[43,44]

(40) Residues were numbered according to the numbering proposed by McTigue et al.[35]

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(50) For further details on the construction of the VEGFR2 homology model, please contact Dr. Pascal Furet at pascal.furet@pharma.novartis.com.

Supporting Information Available: Experimental procedures and spectroscopic and analytical data for all compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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