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# NOVEL INDOLOCARBAZOLE PROTEIN KINASE C INHIBITORS WITH IMPROVED BIOCHEMICAL AND PHYSICOCHEMICAL PROPERTIES

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**Abstract:** Novel lactam and imide indolocarbazoles were synthesized and characterized with respect to inhibition of protein kinase C and other protein kinases. In both series potent and selective PKC inhibitors could be identified. Structure activity relationships are discussed.

Protein kinase C (PKC) has initially been described as a $Ca^{2+}$/phospholipid-activated protein kinase.[1] Meanwhile 10 PKC isoenzymes have been identified that differ in their cofactor requirement.[2]

This serine/threonine kinase is believed to play a key role in intracellular signal transduction related to cell growth and differentiation.[3] Consequently, inhibitors of PKC might provide a novel class of therapeutics for cancer treatment.[4]

Most of the potent and selective PKC inhibitors described in the literature are related to the structure of staurosporine, an alkaloid isolated from *Streptomyces staurosporeus*.[5] Although there was early evidence that staurosporine and structurally related PKC inhibitors interfere with the ATP binding site of the PKC molecule[6], a domain which is highly homologous in most protein kinases, rather selective PKC inhibitors either of the indolocarbazole[7] or the bisindolylmaleimide[8] substructure have been developed by others and our own group.[9]

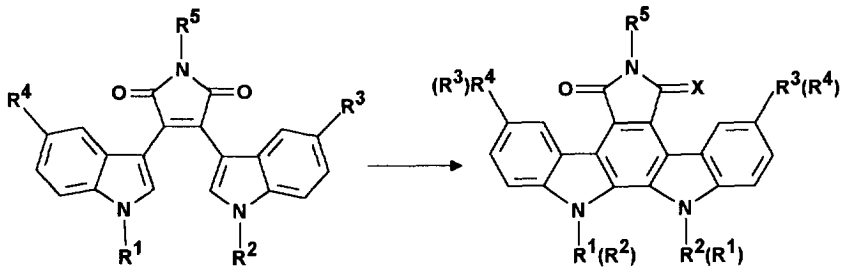
From a series of non-glycosidic/non-aminoalkyl-substituted indolocarbazole lactams compound **3a** (**Gö 6976**) emerged as a potent and selective inhibitor.[10] This compound, however, exhibited low solubility and bioavailability, which appeared to be an obstacle for therapeutic application. In search for novel potent and selective PKC inhibitors with improved solubility and/or bioavailability, we studied the effects of aminoalkyl substitution at the indolyl nitrogens and of ring substitution in non-glycosidic indolocarbazole lactams (**3**, X=H,H) as well as in corresponding imides (**3**, X=O). Results are shown in Table 1.

We found potent and selective PKC inhibitors not only in the lactam series (**3i, Gö 7852**), but also in the imide series (**3m, Gö 7612**; **3q, Gö 7874**). Furthermore, due to the aminoalkyl function of compounds **3i** (**Gö 7852**) and **3q** (**Gö 7874**) water-soluble hydrochloride salts of these substances could be prepared.

**Synthesis of Compounds**

The preparation of indolocarbazole **3a** and related compounds by appropriate substitution reactions of the aglycone of staurosporine **1** has been described.[10] Compounds **3b** and **3d** were synthesized in a manner analogous to that described earlier[10] (the 3,9-dimethoxy derivative of staurosporine aglycone was prepared by Clemmensen reduction[11] of the corresponding imide[12]). Compound **3c** was prepared from **3b** by $BBr_3$ cleavage of both methoxy groups.

Key step of the synthesis of compounds **3e - 3q** was the oxidative cyclisation of substituted bisindolylmaleimides **2** to indolocarbazoles **3** (DDQ, p-TsOH, toluene or chlorobenzene)[13], followed by a Clemmensen reduction (Zn/Hg, HCl, EtOH)[11] in the cases of the lactams **3e - 3j** (mixture of regioisomers):




**2** **3**

**$R^5$=H: 3a-3q** (see Table 1)

Synthetic routes to bisindolylmaleimides **2** were adapted from literature.[14,15]

Compounds **3g** and **3k - 3o** were prepared by the oxidative cyclisation of bisindolylmaleimides **2**, already bearing the final substitution pattern, followed by a Clemmensen reduction in the case of **3g**. The corresponding bisindolylmaleimides **2** were prepared from appropriately substituted indoles and 1-methyl-3-indolylacetic acids.[15]

Lactam **3h** (1:1 mixture of regioisomers) was prepared from **3k** by Raney nickel catalyzed hydrogenation of the 2-cyanoethyl group, followed by Clemmensen reduction of one of the carbonyl groups.

For the synthesis of **3e** and **3f** 2,3-bis(5-methoxy-3-indolyl)-N-methylmaleimide **2** ($R^1$=$R^2$=H, $R^3$=$R^4$=OMe, $R^5$=Me), prepared from 2,3-dibromo-N-methylmaleimide and 5-methoxyindolylmagnesiumbromide[14], was oxidatively cyclised in the usual manner. The intermediate indolocarbazole **3** (X=O) was alkylated at one indole nitrogen atom using 3-dimethylaminopropyl chloride and NaH in DMF. Subsequently the N-methylimide was converted to the anhydride and finally to the N-unsubstituted imide by a reaction sequence already described for bisindolylmaleimides[14], followed

Table 1. Inhibition of Protein Kinases by Indolocarbazole Derivatives in *in vitro* Enzyme Assays (Mean Values of $IC_{50}$; n=2-3)

| Compound[18] | $R^1(R^2)$# | $R^2(R^1)$ | $R^3(R^4)$ | $R^4(R^3)$ | X | Ratio of Regioisomers | $IC_{50}$ (μM) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | PKC | cAPK | cGPK | MLCK | TPK |
| 1 | | Staurosporine | | | | | 0.009 | 0.040 | 0.018 | 0.010 | 0.400 |
| 3a* | Me | $-(CH_2)_2CN$ | H | H | H,H | single isomer | 0.020 | >100 | 6.2 | 5.8 | >10 |
| 3b | Me | $-(CH_2)_2CN$ | OMe | OMe | H,H | single isomer | 0.056 | 45 | 2.0 | 1.6 | >10 |
| 3c | Me | $-(CH_2)_2CN$ | OH | OH | H,H | single isomer | 0.009 | n.d. | n.d. | 0.030 | n.d. |
| 3d | H | $-(CH_2)_3NMe_2$ | H | H | H,H | 6:1 | 0.230 | >10 | 0.420 | 0.390 | n.d. |
| 3e | H | $-(CH_2)_3NMe_2$ | OMe | OMe | H,H | 6:5 | 0.550 | n.d. | n.d. | 0.220 | n.d. |
| 3f | H | $-(CH_2)_3NMe_2$ | OH | OH | H,H | 1:1 | 0.016 | n.d. | n.d. | 0.031 | n.d. |
| 3g | Me | $-(CH_2)_3NMe_2$ | H | H | H,H | 1:1 | 0.045 | 9.9 | 0.870 | 0.250 | n.d. |
| 3h | Me | $-(CH_2)_3NH_2$ | H | H | H,H | 1:1 | 0.032 | 0.750 | 0.220 | 0.018 | n.d |
| 3i** | Me | $-CH_2-CHOH-CH_2NMe_2$ | H | H | H,H | 1:1 | 0.030 | 24 | 9.4 | 5.5 | >100 |
| 3j | Me | $-CH_2-CHOH-CH_2NMe_2$ | OMe | H | H,H | 1:1 | 0.086 | 15 | 23 | 9.2 | >10 |
| 3k | Me | $-(CH_2)_2CN$ | H | H | O | - | 0.038 | >30 | 7.2 | 0.055 | n.d. |
| 3l | Me | $-(CH_2)_2CN$ | H | OMe | O | - | 0.016 | n.d. | n.d. | 0.049 | n.d. |
| 3m*** | Me | $-(CH_2)_2CN$ | OMe | H | O | - | 0.002 | 0.400 | >3 | 0.200 | >10 |
| 3n | Me | $-(CH_2)_2CN$ | OMe | OMe | O | - | 0.006 | n.d | n.d. | 0.180 | n.d. |
| 3o | Me | $-(CH_2)_3CN$ | OMe | H | O | - | 0.005 | 0.200 | 1.0 | 0.032 | n.d. |
| 3p | Me | $-CH_2-CHOH-CH_2NMe_2$ | H | H | O | - | 0.021 | 6.3 | 2.1 | 0.120 | n.d. |
| 3q**** | Me | $-CH_2-CHOH-CH_2NMe_2$ | OMe | H | O | - | 0.004 | 0.510 | 4.8 | 0.120 | n.d. |

* Gö 6976 ** Gö 7852 *** Gö 7612 **** Gö 7874 n.d.: not determined

# The residues $R^1$ - $R^4$ given in brackets denote the structure of the respective isomer in regioisomeric mixtures 3d - 3j and refer either to the minor (3d, 3e) or the second isomer in equal mixtures (3f - 3j).[18]

**by cleavage of** both methoxy groups ($BBr_3$, $CH_2Cl_2$) in the case of **3f**. The resulting imides were reduced by Clemmensen reduction to the corresponding lactams **3e** and **3f**.

For the preparation of compounds **3p** and **3q** bisindolylmaleimides **2** ($R^1=R^5=Me$, $R^2=R^4=H$, $R^3=H$ or OMe), monomethylated at the indole nitrogen atoms, were alkylated by epichlorohydrin (NaH, DMF) followed by oxidative cyclisation to indolocarbazoles **3**. Ring-opening of the epoxides with dimethylamine in refluxing ethanol occured in a regioselective manner. Subsequently the N-methylimides were converted to the N-unsubstituted imides **3p** and **3q** as described above.

Compounds **3i** and **3j** resulted from Clemmensen reduction of **3p** and **3q**.

## Structure Activity Relationships

From a study of the aminoalkyl substitution effect in the lactam series, it was found by comparison of the close analogs **3d**, **3g** and **3i** that a methyl and a dimethylaminohydroxypropyl group at the indolyl nitrogen positions are most beneficial for potency and selectivity (**3i, Gö 7852**).[9]

The positive effect of hydroxyl in position 2 of the aminopropyl group on selectivity is mainly caused by decreasing potency for MLCK inhibition. In contrast, N-unsubstituted aminoalkyl groups (e.g. **3h**) decrease selectivity by increasing potency for MLCK inhibition. Aryl ring substitution in the lactam series by methoxy groups in positions $R^3/R^4$ (**3b**, **3e**, **3j**) has no significant effect. Phenolic hydroxy groups in the same positions (**3c**, **3f**) enhance potency for both PKC and MLCK inhibition. From a limited series of indolocarbazoles, comprising derivatives either unsubstituted at the indolyl nitrogens or substituted with simple alkyl groups, it appeared that the lactam moiety might be essential for high activity and selectivity.[9] Now , however, we have found potent inhibitors also in the imide series using appropriate substitution patterns at the indolyl nitrogens, such as methyl at $R^1$ and cyanoalkyl or dimethylaminohydroxypropyl at $R^2$. Potency and selectivity of these compounds (**3k, 3p**) could be further increased by introducing a ring methoxy group in position $R^3$ (**3m**, **3q**).

## Testing of Protein Kinases

All test compounds were dissolved in 100% DMSO at a concentration of 20 mM. Stock solutions were further prediluted with 10% DMSO as required, and finally 10-fold diluted by addition to the assay mixture. The final DMSO concentration in the assay was 1% for all experiments. The assays for the various protein kinases (**PKC**: protein kinase C from rat brain; **cAPK**: catalytic subunit of cAMP-dependent protein kinase from bovine heart; **cGPK**: cGMP-dependent protein kinase from bovine lung; **MLCK**: myosin light-chain kinase from chicken gizzard; **TPK**: generell tyrosine-specific protein kinase activity from murine B cell lysate) were performed as described.[10]

Briefly, the assay conditions for the protein kinase C enzyme assay were as follows. PKC was

purified from rat brain according to the procedure described by Inagaki et al.[16] This resulted in a homogenous, mixed preparation of the 4 $Ca^{2+}$-dependent PKC isozymes as shown by immunoblotting with isozyme-specific polyclonal antibodies. Enzyme activity was determined in a reaction cocktail of 200 µl which contained 50 mM HEPES-NaOH (pH 7.5), 5 mM $MgCl_2$, 1 mM EDTA, 1.25 mM EGTA, 1.32 mM $CaCl_2$, 1 mM DTT, 1 µg phosphatidylserine, 0.2 µg 1,2-diolein, 40 µg histone III-S (Sigma), 10 µM ATP (0.3 µCi [$\gamma$-$^{32}$P]ATP), 10 units (pmol $P_i$/min) of enzyme and test compound. Incubation was started by the addition of enzyme and performed for 5 min at 30° C. Reaction was stopped by addition of 2 ml of 8.5% $H_3PO_4$ and filtration through nitrocellulose filters (0.45 µm). Incorporation of $^{32}P_i$ was determined by liquid scintillation counting.

### Mode of PKC Inhibition

For the indolocarbazole lactam compound **3a (Gö 6976)** it has been shown by kinetic analysis that this compound interferes with the ATP binding site of the PKC molecule.[17] This behaviour could be confirmed for the lactam **3i (Gö 7852;** data not shown). In order to evaluate the mode of PKC inhibition by indolocarbazole imides kinetic studies were performed with **3m (Gö 7612)**. Results from a representive experiment are shown in Fig. 1. Increasing concentrations of the inhibitor caused a shift of the $K_m$ for ATP to higher concentrations, whereas $V_{max}$ was nearly unaffected implying competitive inhibition. The $K_i$ calculated from a secondary plot (Fig. 1a, inset) was 1.1 ± 0.1 nM (mean ± SD; n = 3). In contrast, a clear decrease of $V_{max}$ was observed with increasing concentrations of the inhibitor in the presence of various amounts of histone, suggesting non-competitive inhibition with respect to this substrate site. In this case a $K_i$ value of 9.7 ± 1.5 nM (mean ± SD; n = 3) could be determined by means of secondary plots (Fig. 1b, inset).

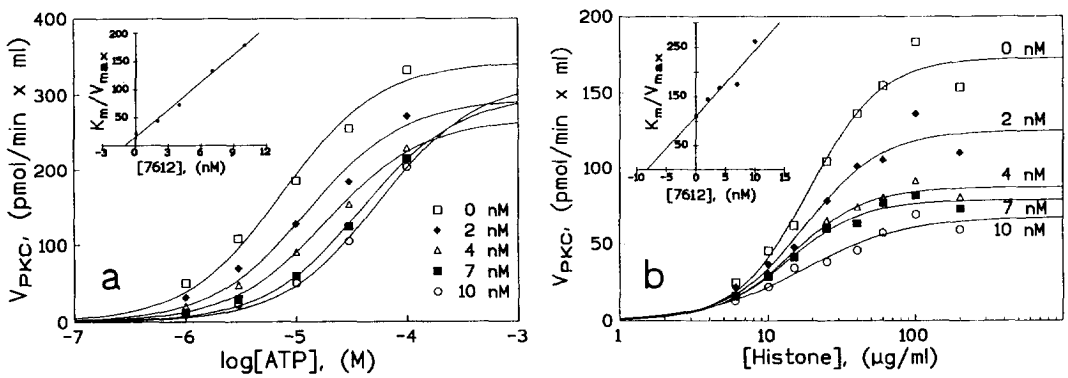



**Fig. 1. Kinetic analysis of PKC inhibition by Gö 7612.** A curve fitting computer program (GraphPad InPlot, GraphPad Software, San Diego) was used to calculate $K_m$, $V_{max}$ (nonlinear regression) and $K_i$ (linear regression).

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18. All new compounds gave IR, 1H NMR, MS and elemental analytical data consistent with their structures. The structural assignments of regioisomers in isomeric mixtures of monoalkylated compounds **3d - 3f** were derived from 1H NMR according to ref. 10 (note 21). In the same manner structural assignments of the single isomers **3a - 3c** are based on the 1H NMR of precursors with $R^1$ = H. Due to the presence of small amounts of the second regioisomer ($R^2$ = H) in the crude reaction mixture clear-cut structural assignment for the single isomers **3a - 3c** could be made. Regioisomers of the 1:1 isomeric mixtures **3g - 3j** were unambiguously assigned from 1H NMR for **3j** only. Due to a large downfield shift in 1H NMR caused by an anisotropic effect of the lactam carbonyl group one of the aromatic protons in positions 4 and 8 can be easily distinguished on the basis of the ortho coupling pattern in the case of **3j**.


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