Pyrrolocarbazole Analogs of Aromatic Skeleton of Indolocarbazole Natural Products

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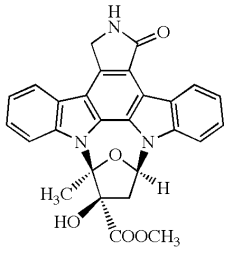
Received October 13, 2004

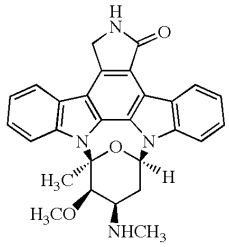
The synthesis of seven pyrrolo[2,3-*a*]carbazoles derivatives using Fischer indole cyclization conditions is described. Polyphosphoric acid trimethylsilyl ester was used as a mild catalyst for the cyclization step of intermediate arylhydrazones which were prepared from ethyl 7-oxo-4,5,6,7-tetrahydroindole-2-carboxylate and used as such without further purification. In all cases a mixture of two products was obtained, the dihydro and the corresponding dehydrogenated one. The completion of the dehydrogenation was achieved by treatment of this resultant mixture with dichlorodicyanoquinone.

J. Heterocyclic Chem., **41**, 349 (2004).

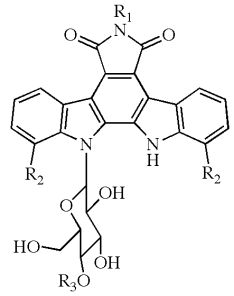
Indolocarbazoles represent an important class of natural products with interesting and diverse biological activities. Among them (Figure 1), the antibiotic staurosporine, isolated from *Streptomyces staurosporeus* [1], and the furanosylated congener K252a, isolated from *Norcadiopsis* species [2], which bear a sugar moiety linked to both indole nitrogen atoms, have been proved potent, nonselective protein kinase C (PKC) inhibitors [3-5]. Staurosporine has also shown interesting biological properties [6-8]. Furthermore, the structurally related antibiotic rebeccamycin, isolated from *Saccharotrix aerocolonigenes* [9,10] and the semisynthetic derivatives ED-110 and NB-506 also bearing a carbohydrate residue attached only to one nitrogen atom of the indolocarbazole skeleton, have been shown to possess antitumor properties mainly *via* topoisomerase I inhibition [11-14]. On the other hand, aglycon indolocarbazoles such as arcyriaflavin A and its synthetic analogs have been reported to be potent, selective



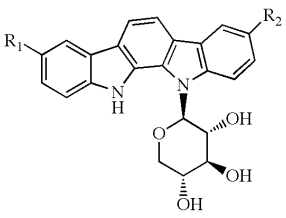
K - 252a



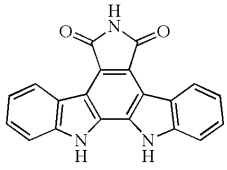
Staurosporine



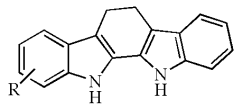
Rebeccamycin R_1=H, R_2=Cl, R_3=CH_3
ED - 110 R_1=R_3=H, R_2=OH
NB - 506 R_1=NHCHO, R_2=OH, R_3=H



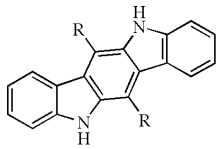
Tjipanazole F1 R_1=Cl, R_2=H
Tjipanazole F2 R_1=H, R_2=Cl



Arcyriaflavin A



5,6,11,12-tetrahydroindolo[2,3-*a*]carbazoles
R=H, CH_3, Cl, Br, $COOCH_3$, OCH_3



5,11-dihydroindolo[3,2-*b*]carbazoles
R=H, CH_3, CHO

Figure 1

inhibitors of human cytomegalovirus (HCMV) replication [15,16]. Several 5,11-dihydroindolo[3,2-*b*]carbazoles [17,18] have been demonstrated to be extremely efficient bioassay ligands for the arylhydrocarbon (Ah) receptor [19] or TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) receptor, as it is alternatively referred to in literature. Tjipanazoles, microbial agents isolated from the blue-green alga *Tolypothrix tjipanasensis*, are desamido analogs of rebeccamycin, except for tjipanazole J and exhibit antifungal as well as antitumor activity [20]. Finally, a series of 5,6,11,12-tetrahydroindolo[2,3-*a*]carbazole derivatives have been synthesized and screened for their *in vitro* antibacterial, antifungal and tuberculostatic activity with moderate and promising results [21].

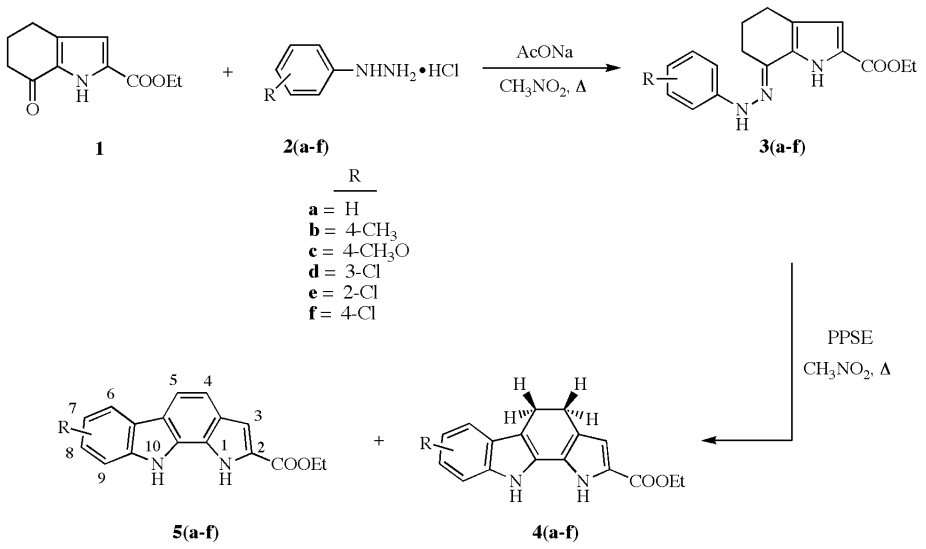
Based on the above considerations and consulting the great range of biological activities of the indolocarbazole core contained compounds, we decided to synthesize a series of derivatives modified in the basic aromatic skeleton. Specifically by replacing one indolic moiety of the indolocarbazole skeleton with a pyrrolic one, results in a tetracyclic pyrrolo-carbazole skeleton [22].

At the outset of our investigation we had to develop a versatile process that would enable us to easily introduce various functional groups to the pyrrolocarbazole skeleton under construction. To this end, the Fischer cyclization process was investigated by using as a building block a functionalized pyrrole derivative namely the ethyl 7-oxo-4,5,6,7-tetrahydroindole-2-carboxylate [23] **1**. It was envisioned that such a cyclization process would provide a simple approach to pyrrolocarbazole derivatives. Accordingly this reaction was attempted by using arylhydrazine derivatives to prepare the corresponding arylhydrazones of **1**. However, the produced arylhydrazones proved to be relatively fragile except the one prepared from the *p*-nitrophenylhydrazine. They were not stable to chromatography undergoing significant decomposition. Thus, they were used in the next cyclization step without isolation as a one pot approach to the tetracyclic skeleton. The use though of conventional catalysts in the Fischer cyclization step (acetic acid/*p*-toluenesulfonic acid, ethanol-hydrochloric acid, ethanol-trifluoroacetic acid, polyphosphoric acid, polyphosphoric acid -toluene, *etc*) was found to give complex mixtures from which the products were isolated with difficulty with the usual methods albeit in low yields (<10%).

After some experimentation, it was found that catalysts that were essentially aprotic such as the polyphosphoric acid trimethylsilyl ester (PPSE), gave satisfactory results. This catalyst in combination with nitromethane as a solvent was employed through out this work [24]. The reaction of ketone **1** with the arylhydrazines **2(a-f)** in the presence of anhydrous sodium acetate in dry nitromethane afforded the corresponding arylhydrazones **3(a-f)** (Scheme 1). Then, the crude reaction mixture of arylhydrazone was added to a freshly prepared solution of PPSE in dry nitromethane (or *vise versa*) and the whole reaction mixture was refluxed for 6-8 hours under a nitrogen atmosphere. Thus, the mixture of the products **4(a-f)** and **5(a-f)** was increased to 30-38%.

In all cases a mixture of two desirable products was obtained, the dihydro and the corresponding dehydrogenated one, as confirmed by the ^{1}H nmr spectra of the reaction mixture. Two triplets at 2.8-3.0 δ are characteristic of the four hydrogen atoms at C-ring of the dihydro compounds.

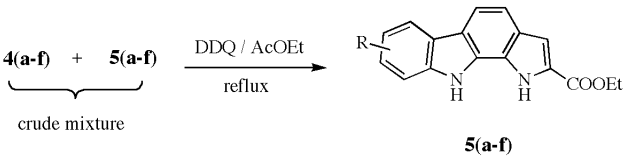
Scheme 1



1 **2(a-f)** **3(a-f)** **5(a-f)** **4(a-f)**

The chromatographic separation of the mixture of dihydro and fully aromatic product was difficult even when high performance liquid chromatography (hplc) was used. In order to avoid these difficult separations, the crude reaction mixture was subjected to dehydrogenation conditions as such. It was found that the use of palladium on powdered charcoal (10%)-dipentene in refluxing xylenes or refluxing diglyme caused partial destruction of the materials involved. Particularly the compounds carrying chlorine or methoxy groups are prone to significant decomposition. The best results were obtained by treating the reaction mixture with dichlorodicyanoquinone (DDQ) in refluxing ethyl acetate (Scheme 2).

Scheme 2



During the preparation of the **5d** analog, a second product was isolated. Not surprisingly, this one was characterized as the regioisomeric ethyl 6-chloro-1*H*-pyrrolo-[2,3-*a*]carbazole-2-carboxylate $\mathbf{5d_2}$ (Scheme 3).

It should be pointed out that the isolated *p*-nitrophenylhydrazone of **1** resisted cyclization under the conditions employed in this work. Presumably, the electron-withdrawing character of the nitro group deactivates the aromatic ring involved sufficiently to inhibit cyclization.

The use [25] of an HPLC-ESI MS instrument, applied in the negative mode, for the mass spectral analysis of pyrrolocarbazole derivatives synthesized in this work, proved to be very practical and sensitive.

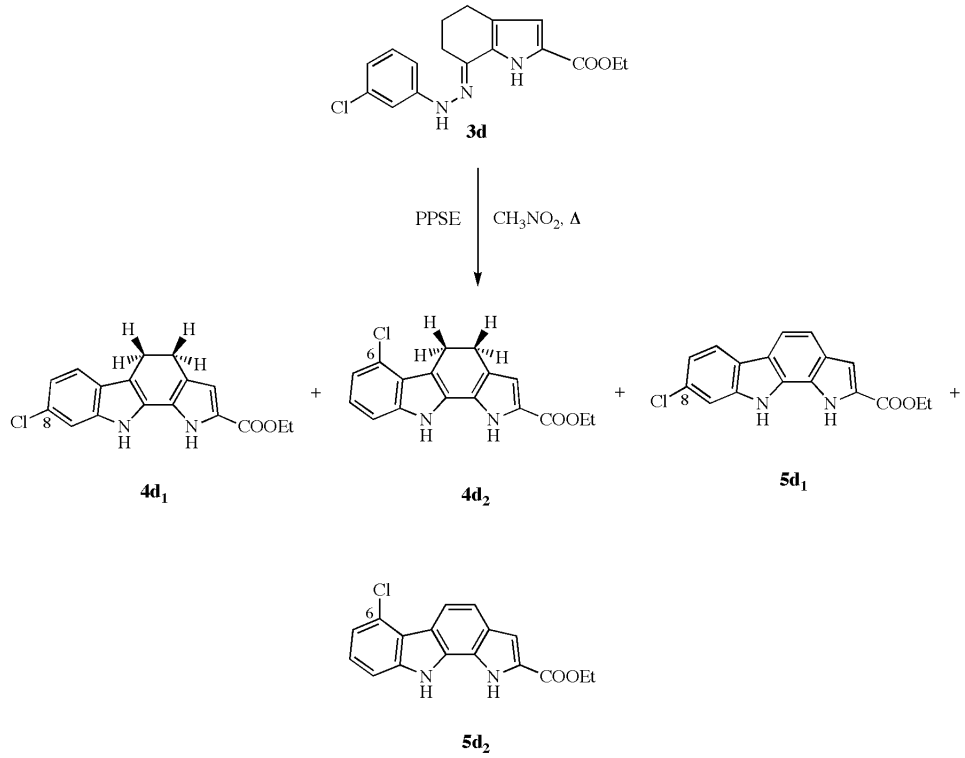
Thus, 1 μg of compound was diluted in 40% ethyl acetate in methanol and injected in the ESI source at 100 μL/min using 50% methanol in water as mobile phase. Hot nitrogen gas (Dominic-Hunter UHPLCMS-10) was used for desolvation. In the electrospray source the spray needle was grounded. Voltages of -4,5, -3,5 and –3,0 kV were applied to the capillary, plate and cylindrical electrodes respectively.

The mass spectra obtained are characterized by the deprotonated $[M-H]^-$ molecular ion peak.

In conclusion, the Fischer cyclization process which has maintained its prominent role as a route to indoles and hence to the construction of numerous carbazoles and certain indolocarbazoles, has been extended to the construction of pyrrolo[2,3-*a*]carbazoles.

The annelation on this tetracyclic pyrrolo[2,3-*a*]carbazole with a pyrrolo[3,4-*c*] ring and the introduction of glycosidic substituents to the heterocyclic core are under investigation.

Scheme 3



EXPERIMENTAL

Melting points were measured on a Kallenkamp melting point apparatus in capillary tubes and are uncorrected. The ir spectra were recorded on a FT-IR Jasco spectrophotometer and the ^{1}H nmr spectra were obtained in deuteriochloroform and dimethyl sulfoxide-d_6 solutions with a Brucker 400 spectrophotometer. Mass spectra were acquired in an AQA Navigator, Finnigan, LC-MS Spectrometer.

Thin layer-chromatography (tlc) was performed on E. Merck precoated silica gel plates (Kieselgel 60 F_{254}). Visualization was achieved by exposure to iodide vapours or/and under uv light (254 and 365 nm). Column chromatography was conducted with silica gel (E. Merck, 70-230 mesh). Analytical samples of the compounds were obtained after hplc purification of crude samples on a Waters model 501 system equipped with a Lambda-Max 481 LC spectophotometer (visualization at 256 nm) by using a Lichrosorb Si60 (5 μm, 250x10 mm) column and hexane/ethyl acetate mixtures as mobile phases.

Preparation of Arylhydrazones.

General Procedure.

A mixture of the cyclic ketone **1** (1 mmole, 207 mg), the appropriate arylhydrazine hydrochloride (1.4 mmoles) and anhydrous sodium acetate (2 mmoles, 164 mg) in dry nitromethane (10 ml) was refluxed at 100° for 2-4 hours under a nitrogen atmosphere. After the formation of the corresponding arylhydrazone (tlc monitoring) the reaction mixture was cooled in room temperature and kept in refrigerator until it was used.

Preparation of PPSE [26-29].

A solution of phosphorus pentoxide (1 mmole, 142 mg) and hexamethyldisiloxane (1.6 mmoles, 0.34 ml) in dry dichloromethane (5 ml) was refluxed for 30 minutes under a nitrogen atmosphere. The solvent and low boiling reaction products were removed by gradually increasing the temperature and the clear residue was heated to 180° for 5-10 minutes. The resulting pale yellow oil was cooled and used immediately.

Preparation of Pyrrolo[2,3-*a*]carbazole Derivatives.

General Procedure.

The freshly prepared crude reaction mixture of arylhydrazone **3** (1 mmole) was added in one portion at 100° to freshly prepared PPSE (3.5 mmoles based on phosphorus pentoxide) in dry nitromethane (5 ml) and the resulting solution was heated to reflux for 6-8 hours under a nitrogen atmosphere. Then, the reaction mixture was poured onto ice-water and extracted with ethyl acetate three times. The combined extracts were washed with water and a saturated aqueous solution of sodium chloride, dried over magnesium sulfate and concentrated under reduced pressure. Flash chromatography of the residue on silica gel using toluene/ethyl acetate gradient (from 90:10% to 60:40% correspondingly) gave a mixture containing the fully aromatized and the dihydro products.

Dehydrogenation of the 4,5-Dihydro-pyrrolo[2,3-*a*]carbazoles (**4a-f**).

General Procedure.

The mixture of the dihydro and the fully aromatized product was dissolved in ethyl acetate (~15 ml) and dichloro-dicyanoquinone (1 mmole, 227 mg) was added. The mixture was stirred at 70° for 4-6 hours under a nitrogen atmosphere. The solvent was evaporated under reduced pressure and the residue was chromatographed by flash chromatography on silica gel using toluene/ethylacetate gradient (from 100:0 to 80:20 respectively). The fully aromatized product was isolated almost quantitatively.

Ethyl 1*H*-Pyrrolo[2,3-*a*]carbazole-2-carboxylate (**5a**).

This compound was obtained in 52% yield as a white solid, mp 325-326° (from ethyl acetate) (lit [22] mp 302-303°); ir (potassium bromide): 3362, 1672, 1204, 733 cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 11.40 (s, 1H, NH), 10.44 (s, 1H, NH), 8.17 (d, 1H, J = 7.6 Hz), 7.91 (d, 1H, J = 8.4 Hz), 7.63 (d, 1H, J = 8 Hz), 7.55 (d, 1H, J = 8.4 Hz), 7.47 (t, 1H), 7.43 (s, 1H), 7.33 (t, 1H), 4.53 (q, 2H), 1.55 (t, 3H,); ms: m/z 277.3 [M-H]$^-$.

Anal. Calcd. for $C_{17}H_{14}N_2O_2$: C, 73.37; H, 5.07; N, 10.07. Found: C, 73.53; H, 5.09; N, 10.13.

Ethyl 7-Methyl-1*H*-pyrrolo[2,3-*a*]carbazole-2-carboxylate (**5b**).

This compound was obtained in 42% yield as a white solid, mp 311-313° (from ethyl acetate); ir (potassium bromide): 3360, 1667, 1208, 740 cm^{-1}; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 11.67 (s, 1H, NH), 10.61 (s, 1H, NH), 7.76 (s, 1H), 7.66 (d, 1H, J=8.1 Hz), 7.45 (d, 1H, J=8.6 Hz), 7.27 (d, 1H, J=8.5 Hz), 7.18 (s, 1H), 7.07 (d, 1H, J=8.6 Hz), 4.27 (q, 2H), 2.36 (s, 1H), 1.26 (t, 3H); ms: m/z 291.3 [M-H]$^-$.

Anal. Calcd. for $C_{18}H_{16}N_2O_2$: C, 73.95; H, 5.52; N, 9.58. Found: C, 73.85 ; H, 5.53; N, 9.63.

Ethyl 7-Methoxy-1*H*-pyrrolo[2,3-*a*]carbazole-2-carboxylate (**5c**).

This compound was obtained in 41% yield as a white solid, mp 297-298° (from ethyl acetate); ir (potassium bromide): 3364, 3335, 1672, 1208, 752 cm^{-1}; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 11.81 (s, 1H, NH), 10.71 (s, 1H, NH), 7.78 (d, 1H, J=8.3 Hz), 7.61 (d, 1H, J=2.5 Hz), 7.56 (d, 1H, J=8.8 Hz), 7.35 (d, 1H, J=8.6 Hz), 7.27 (s, 1H), 6.97-6.95 (dd, 1H), 4.36 (q, 2H), 3.83 (s, 3H), 1.35 (t, 3H); ms: m/z 307.4 [M-H]$^-$.

Anal. Calcd. for $C_{18}H_{16}N_2O_3$: C, 70.12; H, 5.23; N, 9.09. Found: C, 70.14; H, 5.25; N, 9.12.

Ethyl 8-Chloro-1*H*-pyrrolo[2,3-*a*]carbazole-2-carboxylate (**5d$_1$**).

This compound was obtained in 24% yield as a white solid, mp 327-329° (from ethyl acetate); ir (potassium bromide): 3358, 1676, 1206, 737 cm^{-1}; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 11.89 (s, 1H, NH), 11.0 (s, 1H, NH), 8.08 (d, 1H, J=8.3 Hz), 7.83-7.79 (m, 2H), 7.43 (d, 1H, J=8.6 Hz), 7.29 (s, 1H), 7.18 (d, 1H, J=8.3 Hz), 4.36 (q, 2H), 1.35 (t, 3H); ms: m/z 311.2 [M-H]$^-$.

Anal. Calcd. for $C_{17}H_{13}ClN_2O_2$: C, 65.29; H, 4.19; Cl, 11.34; N, 8.96. Found: C, 65.49; H, 4.20; N, 8.90.

Ethyl 6-Chloro-1*H*-pyrrolo[2,3-*a*]carbazole-2-carboxylate (**5d$_2$**).

This compound was obtained in 21% yield as a white solid, mp 317-318° (from ethyl acetate); ir (potassium bromide): 3351, 1671, 1208, 727 cm^{-1}; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 11.89 (s, 1H, NH), 11.25 (s, 1H, NH), 8.15 (d, 1H, J=9.2 Hz), 7.69 (d, 1H, J=8.1 Hz), 7.48 (d, 1H, J=9.1 Hz), 7.36-7.32 (m, 2H), 7.22 (d, 1H, J=7.7 Hz), 4.38 (q, 2H), 1.36 (t, 3H); ms: m/z 311.2 [M-H]$^-$.

Anal. Calcd. for $C_{17}H_{13}ClN_2O_2$: C, 65.29; H, 4.19; Cl, 11.34; N, 8.96. Found: C, 65.50; H, 4.20; N, 9.02.

Ethyl 9-Chloro-1*H*-pyrrolo[2,3-*a*]carbazole-2-carboxylate (**5e**).

This compound was obtained in 47% yield as a white solid, mp 285-286° (from ethyl acetate); ir (potassium bromide): 3427, 3288, 1682, 1206, 748 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 11.70 (s, 1H, NH), 11.30 (s, 1H, NH), 8.10 (d, 1H, J=7.8 Hz), 7.85 (d, 1H, J=8.4 Hz), 7.50-7.46 (m, 2H), 7.34 (s, 1H), 7.22 (t, 1H), 4.40 (q, 2H), 1.39 (t, 3H); ms: m/z 311.2 $[M-H]^-$.

Anal. Calcd. for $C_{17}H_{13}ClN_2O_2$: C, 65.29; H, 4.19; Cl, 11.34; N, 8.96. Found: C, 65.42; H, 4.18; N, 8.99.

Ethyl 7-Chloro-1*H*-pyrrolo[2,3-*a*]carbazole-2-carboxylate (**5f**).

This compound was obtained in 39% yield as a white solid, mp 336-337° (from ethyl acetate); ir (potassium bromide): 3350, 1672, 1204, 733 cm^{-1}; ^1H nmr (dimethyl sulfoxide-d_6): δ 11.93 (s, 1H, NH), 11.06 (s, 1H, NH), 8.22 (s, 1H), 7.88 (d, 1H, J=8.6 Hz), 7.75 (d, 1H, J=8.6 Hz), 7.46 (d, 1H, J=8.5 Hz), 7.38 (d, 1H, J=8.4 Hz), 7.34 (s, 1H), 4.41 (q, 2H), 1.4 (t, 3H) ms: m/z 311.2 $[M-H]^-$.

Anal. Calcd. for $C_{17}H_{13}ClN_2O_2$: C, 65.29; H, 4.19; Cl, 11.34; N, 8.96. Found: C, 65.53; H, 4.18; N, 9.05.

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