Fused Pyrrolo[2,3-*c*]carbazol-6-ones: Novel Immunostimulants That Enhance Human Interferon-γ Activity

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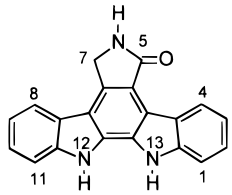
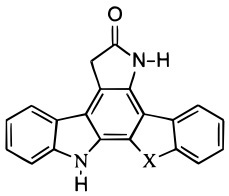
The clinical need for therapeutic agents which restore or enhance an immune response in immunocompromised patients such as occurs in viral infections, cancer, autoimmune diseases, and acquired immune deficiency syndrome (AIDS) has led to the search for novel immunostimulants.[1] Interferon-γ (IFN-γ) is a potent activator of the immune system and has been used to successfully treat tumors and virus infections in humans. However, dose-related side effects limit its effectiveness. Small molecule enhancers of the activity of IFN-γ might permit lower doses of IFN-γ to be administered while achieving an equal therapeutic effect.

A proposed mechanism for the antiviral and antitumor effect of IFN-γ results from IFN-γ-induced activation of cells of the immune system which then function to destroy the virus-infected or tumor cell.[2] One major response of the immune system to IFN-γ is the induction of class I and class II antigens of the major histocompatability complex (MHC-I and MHC-II) on the surface of B-cells and T-cells.[3] The induction of MHC-II antigen is limited to IFN-γ, and moreover IFN-α and IFN-β antagonize the IFN-γ dependent induction of this pathway.[4] We used the induction of MHC class II antigens by IFN-γ in a human monocyte-derived cell line to screen compounds for enhancement of the biological activity of IFN-γ. We report here a series of fused pyrrolo[2,3-*c*]carbazol-6-ones which potentiate the IFN-γ induction of MHC class II molecules. These compounds are structurally distinct from the indolocarbazole natural products such as K-252c (**1**), K-252a (**2a**), and, staurosporine (**2b**), and as opposed to the indolocarbazoles, do not display protein kinase C (PKC) inhibitory activity.

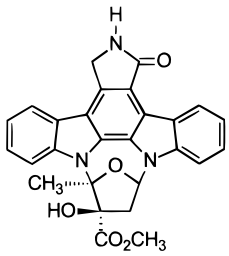
The compounds were prepared by a novel tandem Michael acid-catalyzed condensation sequence.[5] The reaction of the 2-arylindoles **3**−**6** with maleimide (method A, Scheme 1) or ethyl *cis*-β-cyanoacrylate (method B, Scheme 2) gives the corresponding carbazole derivatives **7**−**10** in a one-pot reaction. The indole analog **9** was prepared by method A (maleimide route) using toluene as a solvent and TFA as a catalyst. Examples **8**−**10** in method A were prepared using TFA as a solvent. The proposed mechanism for the synthesis of indolocarbazole **9** by the maleimide and cyanoacrylate approaches has been previously described.[5] Indole analog **9** (Scheme 3) was formed in 27% yield from **5**, while indene **10** was prepared in 12% yield. The 2-arylindole intermediates, 2-(2-furyl)indole (**3**), 2-(benzothienyl)indole (**4**), and 2-(2-indolyl)indole (**5**), were prepared by coupling the corresponding 2-bromoheteroaryl with 1-carboxy-2-(tributylstannyl)indole.[6] 2-(2-Indenyl)indole (**6**) was prepared from 2-bromoindene and 1-carboxy-2-(tributylstannyl)-indole or by alkylation of lithio 2-lithioindole-1-carboxylate[7] (**13**) with 2-indanone to give alcohol **14** followed by dehydration (2 N HCl, acetone) to give **6** in 35% overall yield (Scheme 3).



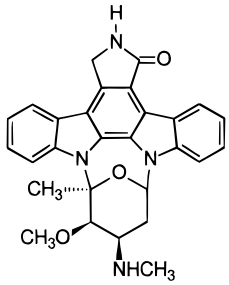
1 K-252c



Pyrrolo[2,3-c]carbazole-6-ones
X = NH, O, S, CH_2



2a K-252a



2b Staurosporine

The pyrrolo[2,3-*c*]carbazol-6-ones potentiate the activity of human IFN-γ in inducing the expression of MHC-II molecules. The MHC-II complex is made up of three protein heterodimers consisting of two peptide chains in each heterodimer. The three protein heterodimers in the human MHC-II complex are designated HLA-DP, HLA-DQ, and HLA-DR. All three heterodimers in the human MHC-II complex are induced by IFN-γ.[3] HLA-DR was selected for measurement in THP-1 cells, a human monocyte cell line. Induction of HLA-DR was performed using standard literature procedures.[2,8]

The four heterocyclic analogs were screened at 2 μM for potentiation of IFN-γ-induced expression of HLA-DR above that of IFN-γ alone (Table 1). The enhancement of HLA-DR by IFN-γ (100 units/mL) is designated as 100%. HLA-DR is not expressed in the absence of IFN-γ, and there is no induction of HLA-DR by the compounds alone. The more effective compounds which enhance IFN-γ-induced expression were the indenyl (**10**) and benzothienyl (**8**) analogs which showed 55% and 60% enhancement above IFN-γ alone, respectively. The indole analog **9** showed a weaker IFN-γ potentiation (30%), while the benzofuran derivative showed a minimal effect on enhancement of HLA-DR above IFN-γ alone. The enhanced activity of the methylene analog **10** (X = CH_2) and sulfur analog **8** (X = S) compared to the oxygen derivative **7** (X = O) and nitrogen derivative **9** (X = N) may result from the electronic effects induced by the more electronegative oxygen and nitrogen atoms.

A number of substances antagonize the induction of MHC class II expression by IFN-γ. These include glucocorticoids or IFN-α/β,[4,9a] and agents that induce the intracellular accumulation of cAMP such as prostaglandins[9b] and catecholamines.[9c] While low concentrations of adrenergic agents antagonize the induction of MHC class II expression by IFN-γ, very high concentrations of isoproterenol (>100 μM) reportedly induce MHC class II expression on bovine brain capillary endothelial

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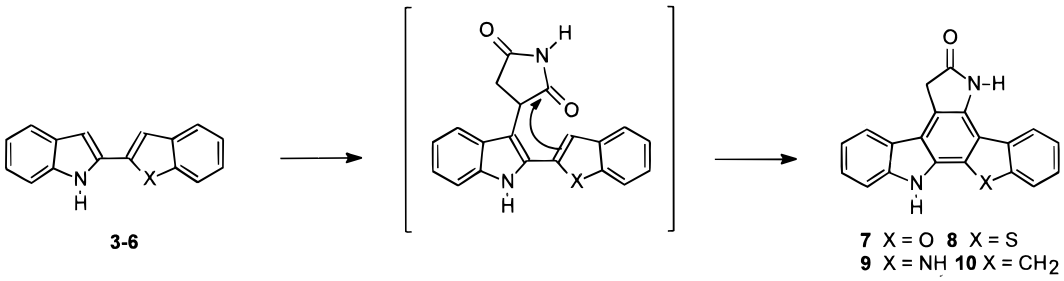
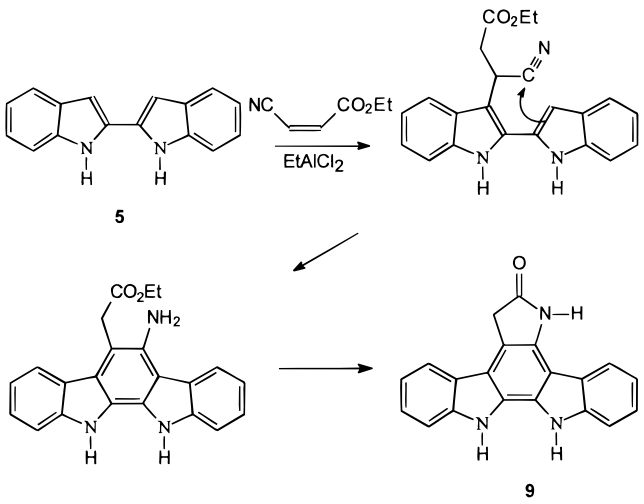
Scheme 1



Scheme 2



Scheme 3

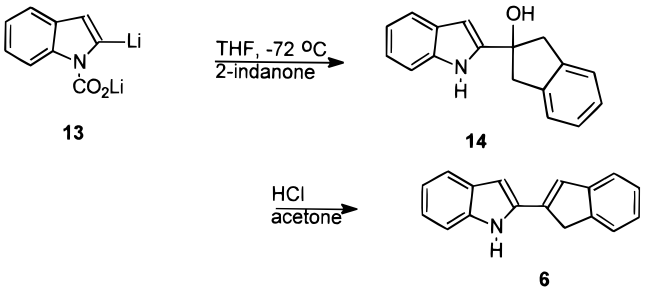


Table 1. Effect of Pyrrolo[2,3-*c*]carbazol-6-one Derivatives on Enhancement of IFN-γ Induction of HLA-DR

compd (X)	concn, μM	IFN-γ, units/mL	% (±SD) enhancement of HLA-DR
7 (X = O)	0	0	0
	2	0	0
	0	100	100 ± 1
	2	100	108 ± 1
8 (X = S)	0	0	0
	2	0	0
	0	100	100 ± 1
	2	100	153 ± 1
9 (X = NH)	0	0	0
	2	0	0
	2	0	0
	0	100	100 ± 1
	2	100	130 ± 5
10 (X = CH_2)	0	0	0
	2	0	0
	0	100	100 ± 1
	2	100	160 ± 2

cells.[10] A variety of hormones have been reported to enhance the production of HLA-DR induced by IFN-γ in various cell types. Thyrotropin (TSH)[11a] and TNF-α[11b] enhance the induction of HLA-DR by IFN-γ in thyroid cells, while 1,25-dihydroxyvitamin D_3 potentiates the induction of IFN-γ in osteoblasts.[11c] The thyroid hormone, L-thyroxine (T_4), and 3,3′,5-L-triiodothyronine (T_3) potentiate induction by IFN-γ of HLA-DR antigen expression in HeLa cells in addition to the indolocarbazole natural product staurosporine (**2a**).[11d] However, staurosporine inhibits the T_4 expression of HLA-DR by IFN-γ.[11d] The mechanism of staurosporine-induced expression of IFN-γ is postulated to be due to its potent PKC inhibitory activity. KT-5720, a K-252a analog which lacks PKC activity, was inactive in this assay.[11d] In our assay K-252a and K-252c (both potent PKC inhibitors) were not active in inducing expression of HLA-DR by IFN-γ. PKC activation has been proposed as a pharmacological requirement for induction of MHC expression by IFN-γ in macrophage activation.[12] The PKC inhibitors H7 and staurosporine inhibit IFN-γ-induced FcγR and IFN-γ Ia Ag expression. The fused pyrrolo[2,3-*c*]carbazol-6-one derivatives were evaluated for PKC inhibition in a mixed PKC isozyme rat brain preparation[13] and found to display IC_{50} values > 10 μM (Table 2). The lactam amide of staurosporine (and other indolo[2,3-*a*]pyrrolo[3,4-*c*]carbazoles such as K-252a and K-252c) is proposed to be an important hydrogen bond donor−acceptor system for binding to the kinase ATP site.[14] In the pyrrolo[2,3-*c*]carbazol-6-one series the amide group is reversed and therefore lacks the critical hydrogen-bonding orientation for PKC inhibition. The mechanism of IFN-γ induction by the fused pyrrolo[2,3-*c*]carbazol-6-ones is under investigation.

Table 2. PKC Activity of the Fused Pyrrolo[2,3-*c*]-carbazol-6-ones

compd	X	PKC (IC_{50}, μM)
7	O	>10
8	S	>10
9	NH	>10
10	CH_2	>10
K-252a		0.250 ± 0.025
staurosporine		0.003 ± 0.0008

Enhancement of MHC class II expression has not previously been a therapeutic target for indications such as viral infections and cancer. In contrast, antagonists of MHC class II are being developed for the suppression of autoimmune disease and transplant rejection[15] despite the potential that such agents may compromise the host defense against pathogens. The research reported here demonstrates that the fused pyrrolo[2,3-*c*]carbazol-6-one derivatives enhance IFN-γ induction of HLA-DR and do not inhibit PKC activity. The most active ring systems were the indenyl (**10**) and benzothienyl (**8**) derivatives. This series may provide an approach for restoring or enhancing the effectiveness

of the immune system, showing therapeutic benefit for inhibiting virus and tumor growth.

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Supporting Information Available: Experimental details and spectral and analytical data for the preparation of compounds **7**−**10** and methods for assay of HLA-DR by flow cytometry for evaluation of expression of MHC class II (5 pages). Ordering information is given on any current masthead page.

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