Bioorganic & Medicinal Chemistry Letters 19 (2009) 5539–5542

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

A direct synthesis of 5,6-dihydroindolo[2,1-*a*]isoquinolines that exhibit immunosuppressive activity

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ARTICLE INFO

Article history:
Received 21 May 2009
Revised 13 August 2009
Accepted 14 August 2009
Available online 20 August 2009

Keywords:
Immunosuppressive
Indoloisoquinoline
Phosphazine
Condensation

ABSTRACT

Dihydroindolo[2,1-*a*]isoquinolines were synthesized from tetrahydroisoquinolines and α-fluoroaldehydes by a novel two-step procedure. These compounds exhibited significant immunosuppressive activity against IL-2, IL-10 and IFN-γ.

The indolo[2,1-*a*]isoquinolines **1** represent a growing class of natural and synthetic compounds with useful biological activity. A sub-class whose members contain a quaternary ammonium salt is represented by mangochinine (**2a**),[1] cryptaustoline (**2b**),[2] and *O*-methylcryptaustoline (**2c**)[3] and is shown in Figure 1. Certain indolo[2,1-*a*]isoquinolines have been reported to inhibit the growth of human mammary carcinoma cells,[4] to treat multiple sclerosis,[5] and to exhibit antiviral activity.[6] Compound **3** strongly inhibited tubulin polymerization.[7] To the best of our knowledge, there have been no reports that indolo[2,1-*a*]isoquinolines exhibit immunosuppressive activity. We report herein that compounds synthesized by our novel two-step procedure exhibit immunosuppressive activity against IL-2, IL-10 and IFNγ.

Scheme 1 depicts four versatile methods for the synthesis of indolo[2,1-*a*]isoquinolines that have been reported. Orito reported the cyclization of 1-bromobenzyl-5,6-dihydroisoquinolines **4** by the nucleophilic addition of the dihydroisoquinoline nitrogen atom to the bromobenzyl moiety.[8] He constructed several analogs with different patterns of oxygenation. Lautens and co-workers reported an innovative palladium-catalyzed tandem reaction sequence starting from a *N*-(2-bromoethyl)indole **5** and an aryl iodide.[9] Importantly, this sequence can accommodate both electron-withdrawing and electron-donating groups on the aromatic ring. Saa and co-workers reported the synthesis of **1** from 3,4-dihydroisoquinolines **6** and benzyne.[10] Although this pathway is a direct one, the yields were modest. Kametani reported the synthesis of an indolo[2,1-*a*]isoquinoline via an intramolecular benzyne reaction.[11] Several groups reported intramolecular radical cyclizations onto indoles **7** to form the indolo[2,1-*a*]isoquinoline ring system.[12,13] The radicals were generated using either trialkyltin hydrides or trialkylgermanium hydrides. This pathway is flexible with regard to substitution on either the indole or the bromobenzene ring.

Our approach to the synthesis of indolo[2,1-*a*]isoquinolines is depicted in Scheme 2. This approach involves the preparation of aldehyde **8** by the coupling of **9** and **10** followed by a base-induced cyclization to generate the indolo[2,1-*a*]isoquinoline system. Since tetrahydroisoquinolines are readily available[14] and several 2-fluorobenzaldehydes are commercially available, this approach has the potential to be a very flexible one. This synthetic strategy is distinctly different from the four general synthetic routes to indolo[2,1-*a*]isoquinolines described above. It may also be noted that recently, De Koning reported the deprotonation and cyclization of *N*-benzyl pyrroles using *t*-BuOK to form related heterocyclic systems.[15]

In order to test the concept, we treated 2-fluorobenzaldehyde (**10a**) with anhydrous potassium carbonate and tetrahydroisoquinoline in DMF to generate **8a** in 48% yield.[16] Cyclization of aldehyde **8a** was attempted using *t*-BuOK, LDA, Li-TMP, KH, and P_4-*t*-Bu. Only P_4-*t*-Bu (Scheme 3), a sterically hindered phosphazine base developed by Schwesinger,[17] generated the desired tetracyclic product **1a**. When aldehyde **8a** was treated with P_4-*t*-Bu at 80 °C for 2 h, indolo[2,1-*a*]isoquinoline **1a** was produced in 35% isolated yield.[18]

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doi:10.1016/j.bmcl.2009.08.057

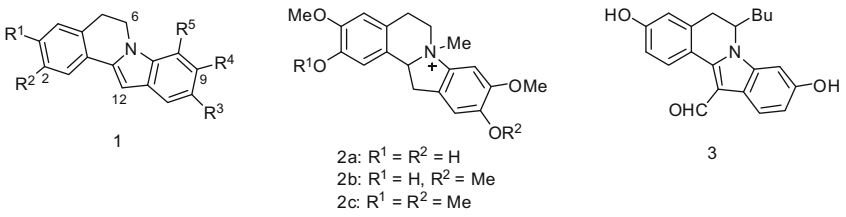
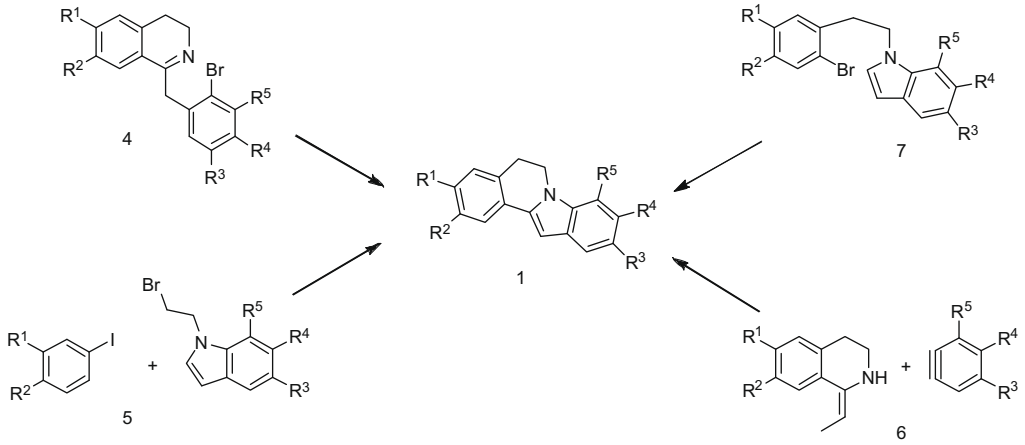
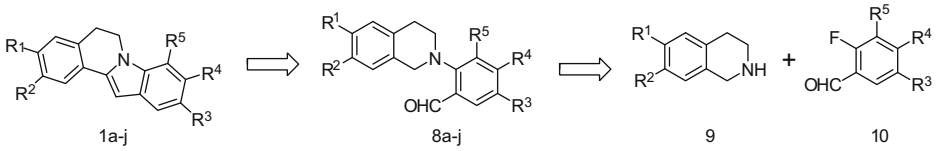


Figure 1.



Scheme 1.

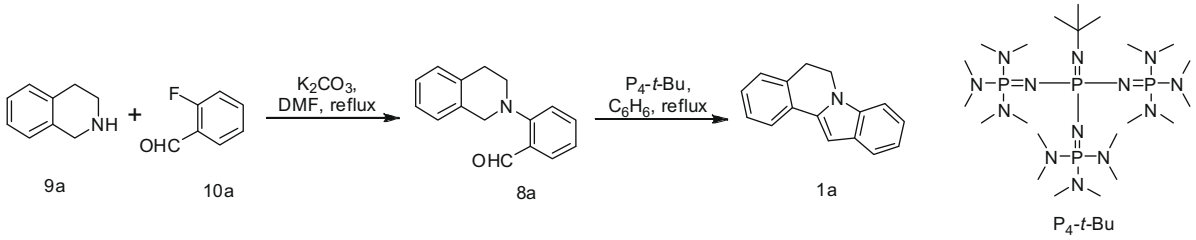


Scheme 2.

With a successful two-step synthesis of dihydroindolo[2,1-*a*]isoquinolines, we generated a number of related compounds from commercially available tetrahydroisoquinolines and 2-fluorobenzaldehydes. The results of this effort are shown in Table 1.

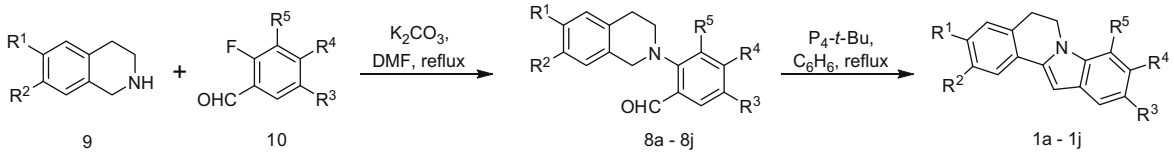
Compound **1f** could be used in a direct synthesis of *O*-methylcryptaustoline **3c** as shown in Scheme 4. The reduction of compound **1f** using sodium cyanoborohydride in acetic acid at ambient temperature afforded the tetrahydro compound **11**[1] which on treating with methanol containing excess methyl iodide over 48 h afforded **2c** in 73% overall yield from **16**. The NMR and melting point of our synthetic compound were identical to that of the literature[19] compound.

We next evaluated compounds **1a–j** for their ability to modulate immune response. Spleen cells obtained from influenza virus-infected mice were cultured in vitro with compounds **1a–j** and influenza virus. Addition of virus to cultures significantly increases cytokine production. The virus-induced change was observed in the comparison between vehicle treatment and vehicle + virus treatment in Figures 2a–c. With respect to the cytokine interleukin-10 (IL-10), viral infection may induce secretion from T lymphocytes as well as monocytes and macrophages. IL-10 often acts to limit inflammation. Each compound (**1a–j**) suppressed the production of IL-10 (Fig. 2a). In Figure 2b, influenza virus-induced Interferon-γ (IFNγ) results are shown. IFNγ is typi-

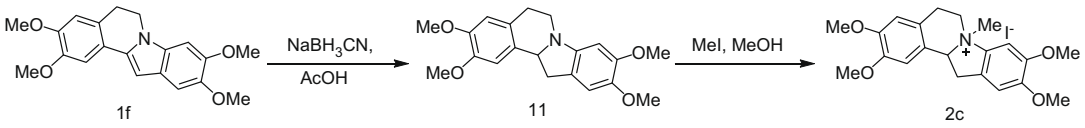


Scheme 3.

Table 1
Synthesis of 5,6-dihydroindolo[2,1-*a*]isoquinolines



S.No	R^1	R^2	R^3	R^4	R^5	Yield of **8a–j** (%)	Yield of **1a–j** (%)
a	H	H	H	H	H	48	35
b	H	H	H	H	OMe	42	52
c	H	H	OMe	OMe	H	48	38
d	OMe	OMe	H	H	H	48	25
e	OMe	OMe	H	H	OMe	37	32
f	OMe	OMe	OMe	OMe	H	40	39
g	H	H	CF_3	H	H	60	44
h	OMe	OMe	CF_3	H	H	66	35
i	H	H	H	CF_3	H	52	42
j	OMe	OMe	H	CF_3	H	53	39



Scheme 4.

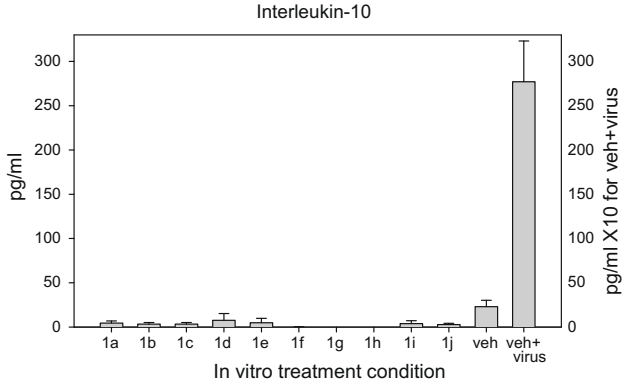


Figure 2a. Suppression of IL-10 by **1a–j**.

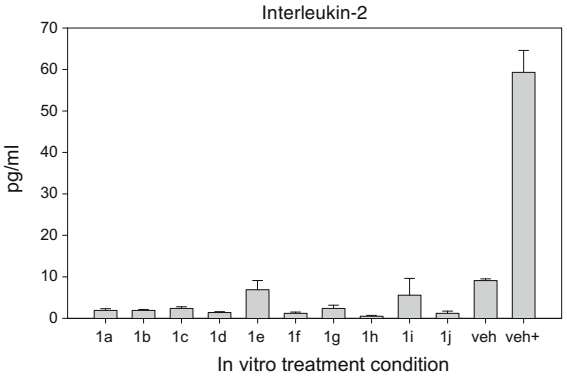


Figure 2c. Suppression of IL-2 by **1a–j**.

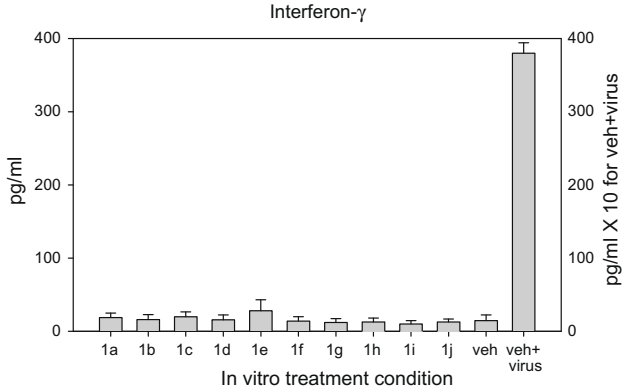


Figure 2b. Suppresion of IFNγ by **1a–j**.

cally secreted by cytotoxic T lymphocytes, and exhibits anti-viral activity, but prolonged release may result in immunopathology. Again, every compound (**1a–j**) inhibited production of IFNγ. Interleukin-2 (IL-2) is produced primarily by T-helper cells during influenza infection and plays an important role in activating T lymphocytes. Compounds **1a–j** also significantly reduced IL-2 production.

All compounds were then evaluated for their impact on cell viability, as reduced cell viability may limit cytokine production. Spleen cells typically do not proliferate in culture without stimulus, and in these experiments, no stimulus was added. Therefore, these results likely reflect cell viability rather than proliferation. Each of the compounds was incubated with spleen cells obtained from non-infected mice for 24, 48 and 72 h. Compounds **1a–d**, **1f**, and **1h** significantly reduced viability at all three times points as compared to the vehicle control (Fig. 3). Compounds **1i** and **1j** reduced viability at 24 h, but not after 48 or 72 h, however, there

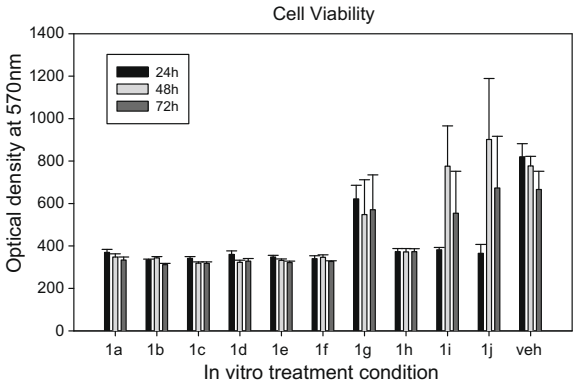


Figure 3. Cell viability data of compounds **1a–j**.

was a large degree of variability in the response with spleen cells from some mice showing a large increase in viability whereas spleen cells from other mice showed a decrease in viability. Compound **1g** did not significantly reduce viability as compared to vehicle at any time point. In Figure 2a, it is apparent that adding virus induces production of each cytokine as compared to the control wells without virus [vehicle compared to vehicle (DMSO) + virus]. Each chemical compound (**1a–j**) when added to the well with virus resulted in a significant (p <0.05) decline of cytokine produced (Fig. 2a, IL-10; Fig. 2b, IFNγ, and Fig. 2c, IL-2). In contrast to the cytokine production, not all compounds reduced viability. Instead, compounds **1a–f**, and **1h** reduced viability as compared to vehicle control whereas **1g**, **1i**, and **1j** were not different from vehicle control. However, it is important to note that the effects of compounds **1g**, **1i**, and **1j** were variable. In some mice these compounds reduced viability whereas in other mice these compounds actually increased viability as assessed with Cell Titer reagent.

In conclusion, this methodology constitutes a novel and direct route to dihydroindolo[2,1-*a*]isoquinolines. The route is flexible with respect to functionality and can be scaled up to prepare gram quantities of dihydroindolo[2,1-*a*]isoquinolines. These compounds exhibited significant immunosuppressive activity against IL-2, IL-10 and IFN-γ, and the majority of the compounds reduced cell viability with the exception of **1g**, **1i**, and **1j**.[20]

Acknowledgment

We thank the Department of Chemistry at Iowa State University for partial support of Vinayak Gupta.

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16. *Representative procedure A*: for the preparation of 2-[3,4-dihydroisoquinolin-2(1*H*)-yl]benzaldehyde **8a**: To a solution of 1,2,3,4-tetrahydroisoquinoline (0.56 g, 4.2 mmol) in dry DMF (6 mL), dry K_2CO_3 (0.58 g, 4.2 mmol) was added followed by solution of 2-fluorobenzaldehyde (0.50 g, 4.0 mmol) in DMF at rt. Reaction mixture was heated to reflux for 20 h. After the completion of reaction, reaction mixture was cooled to rt, diluted with water and extracted with ethyl acetate (three times). Organic layer was then washed with water, brine and dried over $MgSO_4$. Excess solvent was evaporated in vacuo to obtain crude product. Crude product was subjected to column purification using 5% ethyl acetate: petroleum ether to obtain pure product as yellow liquid (48% yield).^{1}H NMR (400 MHz, $CDCl_3$) δ 3.07 (t, J = 5.6 Hz, 2H), 3.46 (t, J = 5.6 Hz, 2H), 4.34 (s, 2H), 7.10–7.13 (m, 2H), 7.19–7.22 (m, 4H), 7.54 (dt, J = 7.8 Hz, J = 2 Hz, 1H), 7.86 (dd, J = 7.6 Hz, J = 1.6 Hz, 1H), 10.34 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 29.1, 53.6, 54.8, 119.0, 122.3, 126.1, 126.4, 126.6, 128.6, 129.0, 130.0, 134.1, 134.2, 134.9, 155.2, 191.3; MS (*m*/*z*): 237, 149, 125, 123, 95, 83, 69, 55; HRMS: calcd for $C_{16}H_{15}NO$: 237.1154, found 237.1156.
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18. *Representative procedure B*: for the preparation of 5,6-dihydroindolo[2,1-*a*]isoquinoline **1a**: to a solution of aldehyde (0.10 g, 0.42 mmol) in freshly distilled dry benzene (25 mL), P_4-*t*-Bu solution (0.46 mL, 0.46 mmol) was added at rt and reaction mixture was heated to reflux with monitoring (2 h). After the completion of reaction, benzene was partially evaporated and reaction mixture was purified by column purification using 3% ethyl acetate: petroleum ether to obtain pure product as Pale green solid (yield 35%).mp: 165–167 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.21 (t, J = 6.8 Hz, 2H), 4.27 (t, J = 6.4 Hz, 2H), 6.89 (s, 1H), 7.12 (t, J = 7.2 Hz, 1H), 7.20–7.36 (m, 5H), 7.65 (d, J = 8.0 Hz, 1H), 7.78 (d, J = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 29.4, 40.3, 96.6, 109.1, 115.5, 120.0, 120.9, 121.8, 124.5, 127.4, 128.6, 128.5, 128.9, 132.3, 135.8, 143.7; MS (*m*/*z*): 219, 109, 108; HRMS: calcd for $C_{16}H_{13}N$: 219.1048, found 219.1052.
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20. *Procedure to evaluate immunomodulation*: spleen cells were obtained from mice infected with a mouse-adapted influenza virus (A/PR/8/34-H1N1) seven days prior to cell collection. Mice were infected with a dose of virus resulting in <5% mortality. Single cell suspensions were prepared from the spleens and red blood cells were lysed with ammonium chloride. After washing with Hanks Balanced Salt Solution to remove remaining ammonium chloride, the cells were then adjusted to a concentration of 5×10^6 cell/ml in AIM-V cell-culture medium (Invitrogen, Carlsbad CA). Cells were plated in 24 well plates. Control wells contained either no influenza virus (no immune stimulation), vehicle with no virus, virus (immune stimulation with influenza A/PR/8/34 virus conc. of 10 HAU/ml), or vehicle + virus. Test wells contained one each of the chemical compounds (**1a–j**) at a final concentration of 0.5 mg/ml performed in triplicate. Cells were incubated at 37 °C, 5% CO_2, in a humidified atmosphere, and cell supernatant were collected 48–96 h later. Cell supernatants were collected at 48 h for the cytokines interleukin-2 and interferon-γ, and at 96 h for the cytokine interleukin-10. ELISA kits were used to analyze cytokines (BD Biosciences). Briefly, immunosorbent 96 wells plates were coated with a monoclonal capture antibody specific for each cytokine and incubated overnight at 4 °C. Assay diluent (PBS) containing 10% fetal bovine serum was used to block plates. After washing, appropriate cell culture samples and cytokine standards were added to the plate, followed by 2 h incubation at room temperature. Plates were again washed, and a second biotinylated detection antibody as well as avidin-horseradish peroxidase conjugate (enzyme reagent) was added. After another 1 h incubation at room temperature, plates were washed again and TMB substrate containing 3,3′,5,5′-tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) was added to each well. Following 30 min incubation, the absorbance was read on a Fluostar microplate reader. A standard curve was created from the known standards and the cytokine protein concentration was calculated by software using a four parameter curve fit.
A cell viability study of these compounds was also tested in spleen cells from non-infected mice. Briefly, mouse spleen cells at a concentration of 5×10^6 cell/ml in AIM-V medium were cultured in 96-well plates. Each well contained a volume of 250 μl (1.25×10^5 cells/well). The cells were incubated with each of the compounds for 24, 48 or 72 h at 37 °C, 5% CO_2, in a humidified atmosphere. At the appropriate time point (24, 48 or 72 h), 20 μl volume of cell titer reagent [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt; MTS] plus phenazine ethosulfate) (Promega, Madison, WI) was added to each well. Plates were then returned to the incubator for 3 h. After this time, the plates were read on a Fluostar microplate reader at an absorbance of 490 nm.