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SUBSTITUTED *7H*-PYRIDO[4,3-*c*]CARBAZOLES WITH POTENT ANTI-HIV ACTIVITY

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Abstract: Several substituted *7H*-pyrido[4,3-*c*]carbazoles were synthesized from the natural product mukonal and tested for inhibition of HIV replication in H9 lymphocytes. 5-Methoxy-7-methyl-*7H*-pyrido[4,3-*c*]carbazole (**7**) had an EC_{50} value of 0.0054 μg/mL and the highest therapeutic index (TI = 503) in the series.
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We prepared a series of 1-*N*-alkyl-pyrido[4,3-*c*]carbazoles from mukonal, one of the chemical constituents in Rutaceous plants, for evaluation as cytotoxic agents. These compounds are related in structure to the antitumor alkaloid ellipticine[1] and its synthetic analogs,[2] and a complete report of their antitumor activity will be reported elsewhere. However, because of the continuing need for new anti-HIV agents with novel structures and mechanisms of action, we also screened the new pyridocarbazoles for anti-HIV activity. Several derivatives showed promising inhibition of HIV replication in H9 lymphocytes. We report herein the active structures, synthetic methods,[3] and anti-HIV results from this study.

Chemistry

Scheme 1 shows the synthetic route and structures of target compounds **6–11** and **13**. The amino and hydroxy groups of mukonal (**1**) were methylated using standard methodology. The resulting formylcarbazole (**2**) was refluxed with two equivalents of malonic acid to afford the unsaturated acrylic acid (**3**),[4] which was converted to the acrylic azide (**4**) with ethyl chloroformate and sodium azide.[5] Cyclization to pyrido[4,3-*c*]carbazol-1(*2H*)-one (**5**) was accomplished by reflux in *o*-dichlorobenzene and tributylamine. The carbonyl at C-1 was converted to a chlorine with $POCl_3$ giving the target 1-chloro substituted compound (**6**).

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Dechlorination of **6** using catalytic hydrogenation gave compound **7**. The 1-amino-substituted derivatives **8–11** were obtained by reacting **6** with 3-amino-1-propanol, ethylenediamine, *N,N*-dimethylethylenediamine, and 1-(2-aminoethyl)piperidine, respectively. Finally, target compound **13** was prepared from **5** by a Vilsmeier reaction and chlorination (N-methylformanilide and $POCl_3$) to give the aldehyde **12** followed by a Baeyer–Villager oxidation (conc. H_2SO_4 and H_2O_2) to give the hydroxy-substituted **13**.

Results and Discussion

The anti-HIV activities of **6–11** and **13** are shown in Table 1. Compound **7** displayed potent anti-HIV activity in acutely infected H9 lymphocytes with an EC_{50} value of 0.0054 μg/mL and a good therapeutic index (TI) of 503. Compound **6**, with a chlorine at C-1, was tenfold less cytotoxic but was also less active (EC_{50} = 0.19 μg/mL) and had a lower TI (136.8). Hydroxylation at C-10 (compound **13**) decreased activity and TI even more (EC_{50} = 1.4 μg/mL, TI = 3.6). The four compounds (**8–11**) with amino side chains at C-1 all showed similar cytotoxicity (IC_{50} = 0.15 – 0.24 μg/mL). The 2'-amino- and 2'-(dimethylamino)-ethylamine substituted compounds (**9** and **10**) were equipotent but had TI values of only 10.6 and 8.9, respectively; however, the 2-piperidinylethylamine substituted **11** had an EC_{50} of 0.005 μg/mL and was as active as **7**. Its higher cytotoxicity, however, led to a lower TI (47.9). Compound **8**, with a 3'-hydroxypropylamine group at C-1, showed the highest activity (EC_{50} = 0.001 μg/mL) in the series; however, the TI of **8** was ca. threefold less than that of **7**. Both compounds were more active than AZT (EC_{50} = 0.012 μg/mL) in this assay.

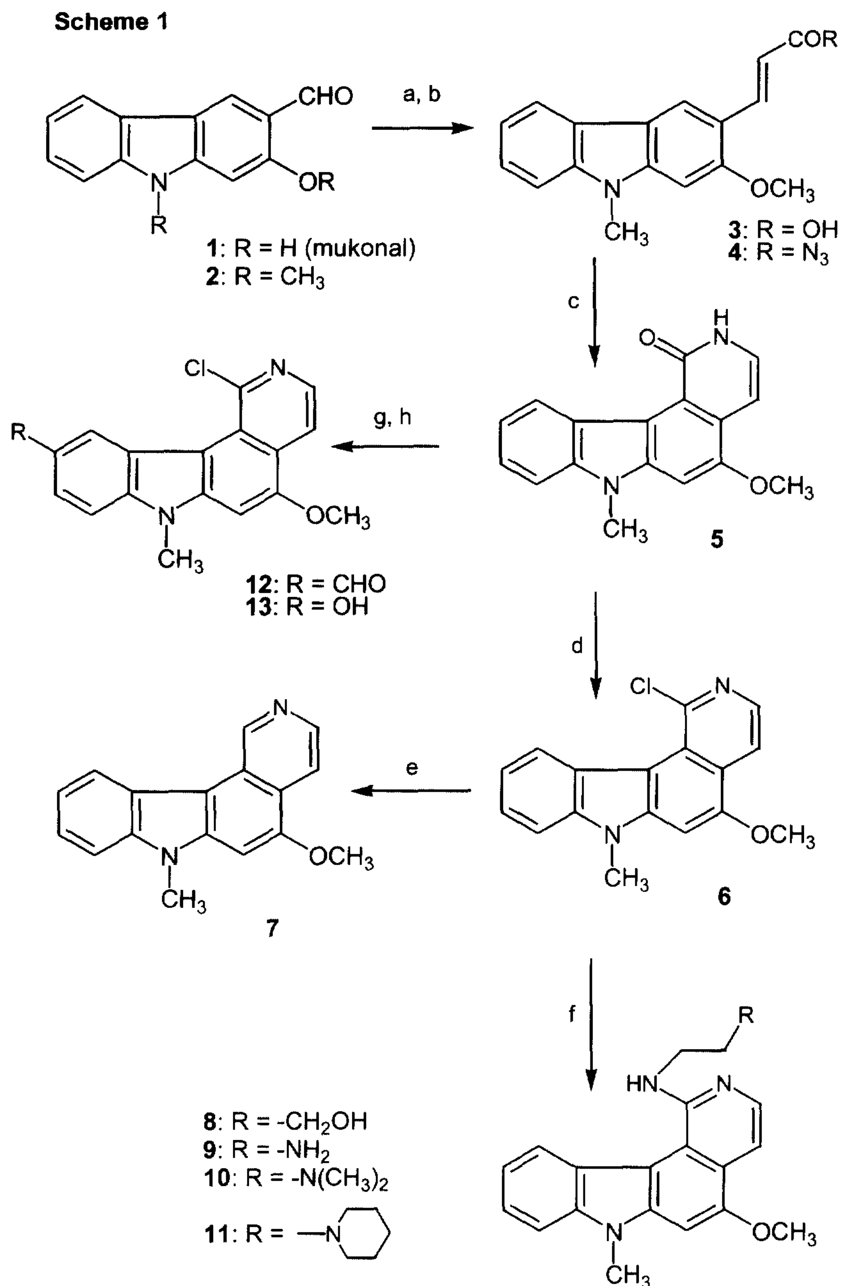
In summary, in this limited compound set, the optimal combination of activity and toxicity occurred with **7**, which has no substitution at C-1. Continuing studies are warranted with this *7H*-pyrido[4,3-*c*]carbazole class to explore additional structural modification, biological activities, and mechanism of action,. These studies will be reported in a future publication.

Table 1. Anti-HIV Activity of *7H*-pyrido[4,3-*c*]carbazoles in Acutely Infected H9 Lymphocytes[6]

Compound	IC_{50} (μg/mL)[a]	EC_{50} (μg/mL)[b]	Therapeutic index[c]
6	26	0.19 (est.)	136.8
7	2.7	0.0054	503
8	0.15	0.001	153
9	0.23	0.022	10.6
10	0.23	0.026	8.9
11	0.24	0.005	47.9
13	5	1.4	3.6
AZT	500	0.012	41,667

[a]concentration that inhibits uninfected H9 cell growth by 50%.
[b]concentration that inhibits viral replication by 50%.
[c]TI = IC_{50}/EC_{50}.

Scheme 1



(a) $CH_2(COOH)_2$ (2 equiv);(b) $ClCO_2C_2H_5$, NaN_3;(c) reflux, *o*-$C_6H_4Cl_2$, (*n*-Bu)$_3$N; (d) $POCl_3$; (e) H_2, Pd/C;(f) $H_2N(CH_2)_3OH$ (to **8**), $H_2N(CH_2)_2NH_2$ (to **9**), $(CH_3)_2N(CH_2)_2NH_2$ (to **10**), $H_2N(CH_2)_2$-piperidine (to **11**); (g) $HCON(CH_3)C_6H_5$; (h) $POCl_3$

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References and Notes

1. Goodwin, S.; Smith, A. F.; Horning, E. C. *J. Am. Chem. Soc.* **1959**, *81*, 1903.
2. Devraj, R.; Jurayj, J.; Fernandez, J. A.; Barrett, J. F.; Cushman, M. *Anti-Cancer Drug Design* **1996**, *11*, 311.
3. Bisagni, E.; Ducrocq, C.; Lhoste, J. M.; Rivalle, C.; Civier, A. *J. Chem. Soc. Perkin 1* **1979**, 1706.
4. Eloy, E.; Deryckere, A. *Helv. Chim. Acta.* **1969**, *52*, 1755.
5. Weinstock, J. *J. Org. Chem.* **1961**, *26*, 3511.
6. **HIV Growth Inhibition Assay**. The T cell line, H9, was maintained in continuous culture with complete medium (RPMI 1640 with 10% fetal calf serum [FCS] supplemented with L-glutamine at 5% CO_2 and 37 ° C. Aliquots of this cell line were only used in experiments when in log-phase of growth. Test samples were first dissolved in dimethyl sulfoxide (DMSO). The following were the final drug concentrations routinely used for screening: 100, 20, 4, and 0.8 μg/mL, but for active agents additional dilutions were prepared for subsequent testing so that an accurate EC_{50} value could be achieved. As the test samples were being prepared, an aliquot of the T cell line, H9, was infected with HIV-1 (IIIB isolate) while another aliquot was mock-infected with complete medium. The mock-infected aliquot was used for toxicity determinations (IC_{50}). The stock virus used for these studies typically had a $TCID_{50}$ value of 10^4 Infectious Units/mL. The appropriate amount of virus for a multiplicity of infection (moi) between 0.1 and 0.01 Infectious Units/cell was added to the first aliquot of H9 cells. The other aliquot of H9 cells only received culture medium and then was incubated under identical conditions as the HIV-infected H9 cells. After a 4 h incubation at 37 °C and 5% CO_2, both cell populations were washed three times with fresh medium and then added to the appropriate wells of a 24-well plate containing the various concentrations of the test drug or culture medium (positive infected control/negative drug control). In addition, AZT was also assayed during each experiment as a positive drug control. The plates were incubated at 37 °C and 5% CO_2 for 4 days. Cell-free supernatants were collected on Day 4 for use in our in-house p24 antigen ELISA assay. P24 antigen is a core protein of HIV and therefore is an indirect measure of virus present in the supernatants. Toxicity was determined by performing cell counts by a Coulter Counter on the mock-infected H9 cells that had either received culture medium (no toxicity) or test sample or AZT.