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Substituted tetrahydrocarbazoles with potent activity against human papillomaviruses

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ABSTRACT

The synthesis and SAR of a series of substituted 1-aminotetrahydrocarbazoles with potent activity against human papillomaviruses are described. Synthetic approaches allowing for variation of the substitution pattern of the tetrahydrocarbazole are outlined and resulting changes in antiviral activity are highlighted. Several compounds with in vitro antiviral activity (W12 antiviral assay) in the low nanomolar range were identified and (1*R*)-6-bromo-*N*-[(1*R*)-1-phenylethyl]-2,3,4,9-tetrahydro-1*H*-carbazole-1-amine was selected for further evaluation.

Human papillomaviruses (HPVs) are small nonenveloped DNA viruses that cause a wide variety of benign and pre-malignant epithelial tumors. Several HPVs infect the genital mucosa. HPV infection is the most common sexually transmitted disease throughout the world, with an incidence roughly twice that of herpes simplex infection.[1] There are over 5.5 million new cases of sexually transmitted HPV infection that occur in the US each year, with at least 20 million people currently infected.[2]

Papillomaviruses that cause genital infections are classified as either low risk or high risk HPVs.[3] The low risk HPVs (such as HPV 6 and 11) cause genital warts, but the high risk HPVs (such as HPV 16 and 18) can cause genital cancers including cervical carcinoma. The role of HPV as the principal agent in the etiology of cervical cancer has been clearly established[4] with a lifetime risk of invasive cancer in the range of 5–10% for untreated infections. While most HPV infections are transient, lasting less than 1 year, a high proportion of persistent infections with high risk types progress to cervical dysplasia, the precursor to cervical cancer.

Currently available treatments for genital warts and cervical dysplasia involve surgical removal or chemical destruction of the infected tissue. The only drug available is the immunomodulator imiquimod (Aldara®), which is only approved for the treatment of external genital warts. None of the present treatments directly target the viral infection. Recently, vaccines (Cervarix® and Gardasil®) have been developed to prevent some types of HPV infection, but the impact of these vaccines on cancer incidence remains to be seen.[5]

A human cervical keratinocyte cell line, W12-20850, isolated from a low grade cervical lesion and containing HPV-16 episomal DNA, served as a primary assay for anti-HPV activity.[6] This cell-based HPV screen (W12 assay) was used to screen in excess of 500 K compounds, resulting in identification of several 5–10 μM inhibitors that contained a 1-aminotetrahydrocarbazole core,[7] substituted at the C6 and 1-amine positions (Fig. 1).

We became interested in exploring the SAR of these 1-aminotetrahydrocarbazoles and herein we describe optimization of the substitution pattern of the tetrahydrocarbazole core. Substituted 2,3,4,9-tetrahydro-1*H*-carbazol-1-ones (**E**) were prepared from anilines (**A**) and 2-(hydroxymethylene)cyclohexanone (**C**),[8] via cyclohexane-1,2-dione(phenyl)hydrazone (**D**) as outlined in

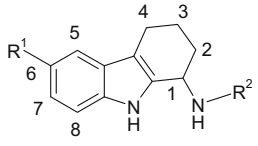

Figure 1. Aminotetrahydrocarbazole, where R^1 and R^2 are hydrophobic groups.

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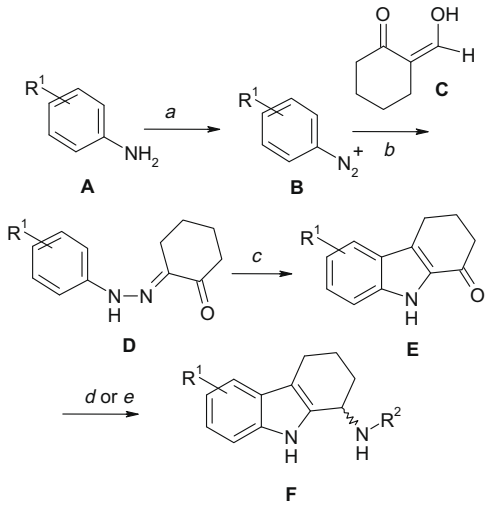
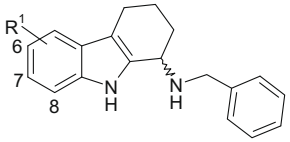


Scheme 1. Reagents and conditions: (a) $NaNO_2$, aq HCl, 20 °C, 15 h; (b) NaOAc, aq MeOH, reflux, 2 h (49% from **A** for R^1 = Br); (c) concd HCl in AcOH; 120 °C for 20 min (88% for R^1 = Br); (d) R^2NH_2, *p*TsOH, toluene then BH_3 in THF; (e) R^2NH_2, $NaBH(OAc)_3$, AcOH, DCE (31% for R^1 = Br, R^2 = Bn). (Compound **F** is racemic at 1-amine position.)

Scheme 1. The reaction, known as the Borsche synthesis, is a special case of the Fischer indole synthesis.[9] Desired amines **F** were prepared from tetrahydrocarbazolones (**E**) by either a two step (imine formation followed by reduction) or one pot reductive amination.

The activity of tetrahydrocarbazoles where the C6 substituent is varied is shown in Table 1 (compounds **1**–**9**). A lipophilic electron withdrawing substituent appeared to be preferred at C6, with the bromo derivative **9** showing the best activity. To investigate if the substitution pattern was optimal we next explored the effect of moving the bromine to the C7 and C8 positions. The 7-bromo derivative **10** was synthesized from 3-bromoaniline in a similar fashion as outlined in Scheme 1. To access the 8-bromo derivative a different methodology was employed (Scheme 2).

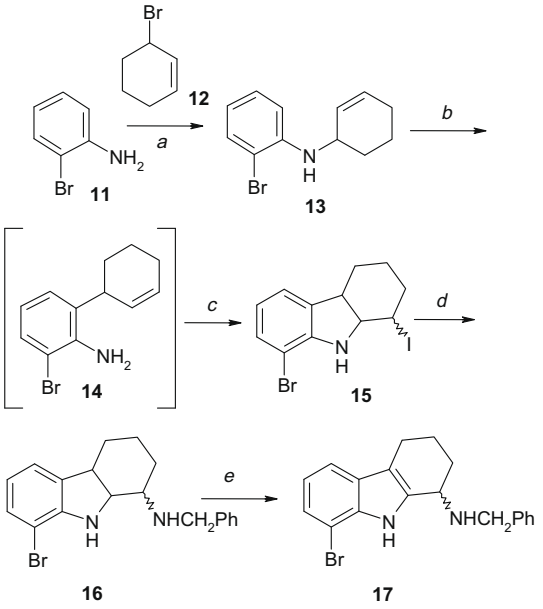
Table 1
HPV activity and cytotoxicity of tetrahydrocarbazoles



Compd	R^1	IC_{50}[a] (μM)	CC_{50}[b] (μM)
1	H	6.7	40.4
2	6-CO_2CH_3	19.7	44.5
3	6-OCH_3	7.6	57.9
4	6-CH_3	1.9	21.3
5	6-CF_3	1.0	>100
6	6-NO_2	0.23	20.4
7	6-F	1.6	35.9
8	6-Cl	0.33	27.9
9	6-Br	0.15	18.8
10	7-Br	11.1	>40
17	8-Br	11.4	>40

[a] HPV activity measured in W12-20850 cells containing episomal HPV-16 DNA. IC_{50} is the concentration at which 50% efficacy in the W12 assay is observed using a hybrid capture method.

[b] CC_{50} is the concentration at which 50% cytotoxicity is observed in human foreskin fibroblasts (HFF cells).



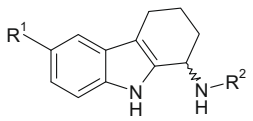
Scheme 2. Reagents and conditions: (a) K_2CO_3, DMF, 75 °C for 16 h (73%); (b) $ZnCl_2$, xylenes, reflux, 6 h (23%); (c) I_2 (4 equiv), $NaHCO_3$ (8 equiv), CH_2Cl_2, 20 °C for 3 h (49%); (d) $NaHCO_3$, benzylamine (neat), 150 °C in SmithCreator microwave for 10 min (54%); (e) DDQ (1.5 equiv), CH_2Cl_2, 16 h at rt (26%).

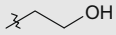
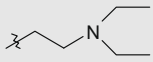
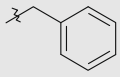
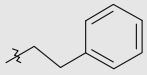
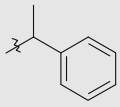
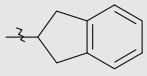
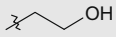
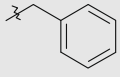
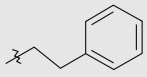
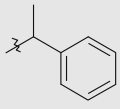
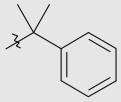
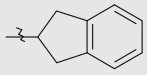
Condensation of aniline **11** and 3-bromocyclohexene **12** gave compound **13**. Lewis acid catalyzed aza-Claisen rearrangement of **13** gave **14**. Treatment of **14** with iodine under basic conditions[10] affected ring closure via an iodonium intermediate and resulted in formation of hexahydrocarbazole **15**. Nucleophilic substitution of iodine with benzylamine in a microwave gave **16**. Oxidation of **16** by DDQ gave the desired tetrahydrocarbazole **17**. The effect of moving the bromine from the C6-position (**9**) to C7 or C8-position (**10** and **17**, respectively) resulted in reduced anti-HPV activity of nearly two orders of magnitude.[11] Next we investigated optimizing the amine substituent of the 1-aminotetrahydrocarbazole. We chose the 6-bromo and the 6-methyl as the C6 substituents for these studies. The *N*-benzyl-6-bromotetrahydrocarbazoleamine **9** showed the best anti-HPV activity of the C6 substituted compounds, while the *N*-benzyl-6-methyltetrahydrocarbazole **4** was about 10-fold less active than **9**. We wanted to see if this difference in activity between the 6-bromo and 6-methyl substituents was maintained when the 1-amine was changed from benzylamine (Table 2 shows activity of several of those amines). In general, compounds with small alkylamine substituents (**18**) or polar amine substituents (**19** and **20**) showed limited activity. More lipophilic alkylaryls, such as benzylamine (**4**) or phenethylamine (**21**), were more potent. The α-methylbenzylamine (**22**) and the indaneamine (**23**) showed still better activity, indicating that α-substitution of the alkylaryl was optimal.

For the 6-bromo substituted compounds the activity trends were the same. Notable is the very good anti-HPV potency of the α-methylbenzylamine **26** in that it is 5-fold more active than the benzylamine **9** and over 10-fold more potent than the dimethylbenzylamine compound **27**. For all analogs, the C6 bromo substituted compounds showed about 10-fold better activity than the C6 methyl substituted compounds. The promising activity of the α-methylbenzylamine derivatives **22** and **26** prompted us to synthesize their stereoisomers from the chiral (*R*)- and (*S*)-α-methylbenzylamines, respectively.

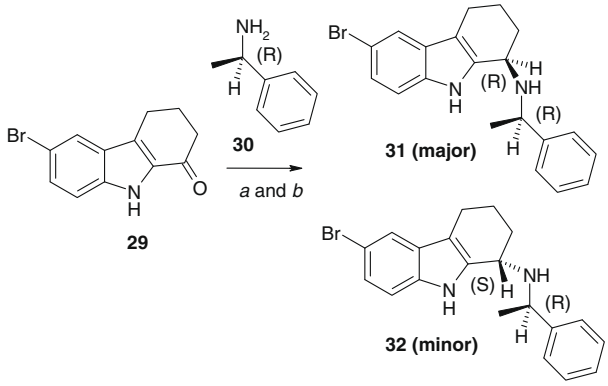
Use of chiral α-methylbenzylamines resulted in high diastereofacial selectivity in the reductive amination of the tetrahydrocarbazolones. Thus, reductive amination of 6-

Table 2
HPV activity and cytotoxicity of tetrahydrocarbazoles with different amine substitutions



Compd	R^1	R^2	IC_{50}[a] (μM)	CC_{50}[b] (μM)
18	Me		>20	>20
19	Me		>30	>30
20	Me		>30	>30
4	Me		1.9	21.3
21	Me		2.7	13.5
22	Me		0.54	40
23	Me		0.47	40
24	Br		6.83	20.1
9	Br		0.15	18.8
25	Br		0.41	11.4
26	Br		0.03	22.9
27	Br		0.6	>40
28	Br		0.12	30.8

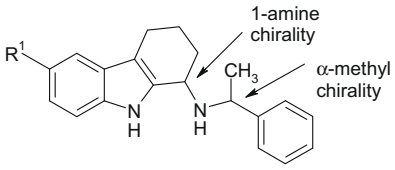
[a] HPV activity measured in W12-20850 cells containing episomal HPV-16 DNA. IC_{50} is the concentration at which 50% efficacy in the W12 assay is observed using a hybrid capture method.
[b] CC_{50} is the concentration at which 50% cytotoxicity is observed in human foreskin fibroblasts (HFF cells).



Scheme 3. Reagents and conditions: (a) $NaBH(OAc)_3$, AcOH, DCE, rt 12 h (52%, 90:10 ratio of *R*,*R*-diastereomer **31** vs *S*,*R*-diastereomer **32**); (b) separation of diastereomers via Supercritical Fluid Chromatography using a Berger Amine column from Chiral Technologies, 10% MeOH (with 2% diethylamine, 10% $CHCl_3$) as mobile phase at 1500 psi, 50 °C, 2 mL/min. Retention time for *R*,*R*-diastereomer **31** 17.5 min, for *S*,*R*-diastereomer **32** 19.8 min. HCl salt of **31** obtained by dissolving **31** in Et_2O and dropwise addition of 1 M HCl in Et_2O.

bromotetrahydrocarbazolone **29** with (*R*)-α-methylbenzylamine (**30**) gave the *R*,*R*-diastereomer **31** in 9:1 excess over the *S*,*R*-diastereomer **32** (Scheme 3). These diastereomers could be separated using supercritical fluid chromatography. Chirality of the 1-amine chiral center was initially assigned by Ab Initio Vibrational Circular Dichroism (VCD) Spectroscopy[12] and later confirmed by X-ray crystallography for **31**. The *R*,*S*-diastereomer (**33**) as well as the *S*,*S*-diastereomer (**34**) were also prepared from (*S*)-α-methylbenzylamine, in this case the *S*,*S*-isomer **34** was the major diastereomer (**34**:**33** in 9:1 ratio). Finally, all diastereomers of the 6-methyltetrahydrocarbazoles were prepared and their anti-HPV activity tested (Table 3). Antiviral testing of the diastereomers revealed that while the chirality of the α-methyl group had little influence on the antiviral activity, the stereochemistry of the 1-amine was very important. Thus diastereomers with *R*-stereochemistry at the 1-amine showed good potency (**31**, **33**, **35** and **37**), while the corresponding 1-amino-*S*-isomers (**32**, **34**, **36** and **38**) were virtually inactive. Again the 6-bromo compounds showed better potency than the corresponding 6-methyl derivatives.

Table 3
HPV activity and cytotoxicity of chiral tetrahydrocarbazoles



Compd	R^1	Chirality (1-amine)	Chirality (α-methyl)	IC_{50}[a] (μM)	CC_{50}[b] (μM)
31	Br	*R*	*R*	0.03	9.0
32	Br	*S*	*R*	>0.5	>30
33	Br	*R*	*S*	0.02	15
34	Br	*S*	*S*	2.85	28.5
35	Me	*R*	*R*	0.3	29
36	Me	*S*	*R*	7.1	41
37	Me	*R*	*S*	0.2	29
38	Me	*S*	*S*	6.7	36

[a] HPV activity measured in W12-20850 cells containing episomal HPV-16 DNA. IC_{50} is the concentration at which 50% efficacy in the W12 assay is observed using a hybrid capture method.
[b] CC_{50} is the concentration at which 50% cytotoxicity is observed in human foreskin fibroblasts (HFF cells).

Compound **31** was selected for further studies based on potency and ease of synthesis.[13] Cytotoxicity testing in additional cell lines (human keratinocytes, Vero cells) showed good separation between the demonstrated anti-HPV activity, as measured by the W12 assay and cytotoxicity (SI >300 in human keratinocyte cells, SI >500 in Vero cells). When the compound was dosed (10 mg/kg) as a suspension (0.5% HPMC:0.1% Tween 80) to female rats it showed good pharmacokinetics ($t_{1/2}$ 4.4 h; V_d 1.9 L/kg; F 47%; Cl 13.4 mL/min/kg), indicating it might be suitable for oral dosing. Furthermore, screening against a panel of enzymes and receptors (PanLab) showed little risk of unwanted enzyme or receptor inhibition. Given the very potent anti-HPV activity in the W12 assay, low cytotoxicity and suitable pharmacokinetic profile, compound **31** was progressed for testing in animal models.

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11. Due to the poor activity of C-7 (and C-8) substituted compounds, C-5 and other alternative substitution patterns were not pursued.
12. Experimental VCD spectra acquired in $CDCl_3$ using Bomem Chiral RTM VCD spectrometer operating between 2000 and 800 cm^{-1}. The GAUSSIAN 98 Suite of computational programs was used to calculate model VCD spectra. Stereochemical assignments made by comparing calculated and measured spectra. For reference: (a) Chesseman, J. R.; Frisch, M. J.; Devlin, F. J.; Stephens, P. J. *Chem. Phys. Lett.* **1996**, *252*, 211; (b) Stephens, P. J.; Devlin, F. J. *Chirality* **2000**, *12*, 172.
13. While compound **33** shows similar potency and selectivity as **31** it is only obtained as a minor isomer from the reductive amination of 6-bromotetrahydrocarbazolone and (*S*)-α-methylbenzylamine.