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Tetrahydrocarbazole amides with potent activity against human papillomaviruses

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ABSTRACT

Synthesis of a series of tetrahydrocarbazole amides with potent activity against human papillomaviruses is described. Synthetic approaches allowing for variation of the substitution pattern of the tetrahydrocarbazole and the amide are outlined and resulting changes in antiviral activity and certain developability parameters are highlighted. Several compounds with in vitro antiviral activity (W12 antiviral assay) in the single digit nanomolar range were identified and *N*-[(1*R*)-6-chloro-2,3,4,9-tetrahydro-1*H*-carbazol-1-yl]-2-pyridinecarboxamide 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 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 while 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 treatment 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, and indeed due to the simplicity of the virus it provides few unique viral targets for drug development. 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 optimization of anti-HPV activity.[6] Previously we described optimization of the 1-aminotetrahydrocarbazole scaffold, where we showed that C6-substitution of the tetrahydrocarbazole core with a lipophilic halogen (Cl or Br), and α-methylbenzylamine substitution with (*R*)-stereochemistry at the 1-amine, were optimal for anti-HPV activity (compound **1**).[7]

Compound **1** (Fig. 1) showed good antiviral activity (IC_{50} 30 nM) and selectivity (SI >100 in multiple cell lines). However, upon further studies we found it to have potential cytochrome P450 inhibition (against CYP2D6, IC_{50} 0.16 μM) as well as modest hERG potassium channel inhibition (IC_{50} 1.7 μM). Inhibition of both CYP2D6[8] as well as the hERG channel[9] have been linked to compounds with charged amines and a heterocyclic (aromatic) moiety. We thus became interested in changing the basicity of the 1-amine and investigating how this would affect the anti-HPV activity.

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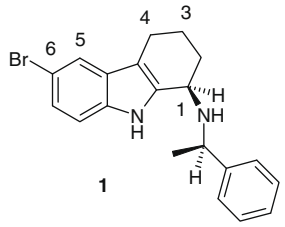
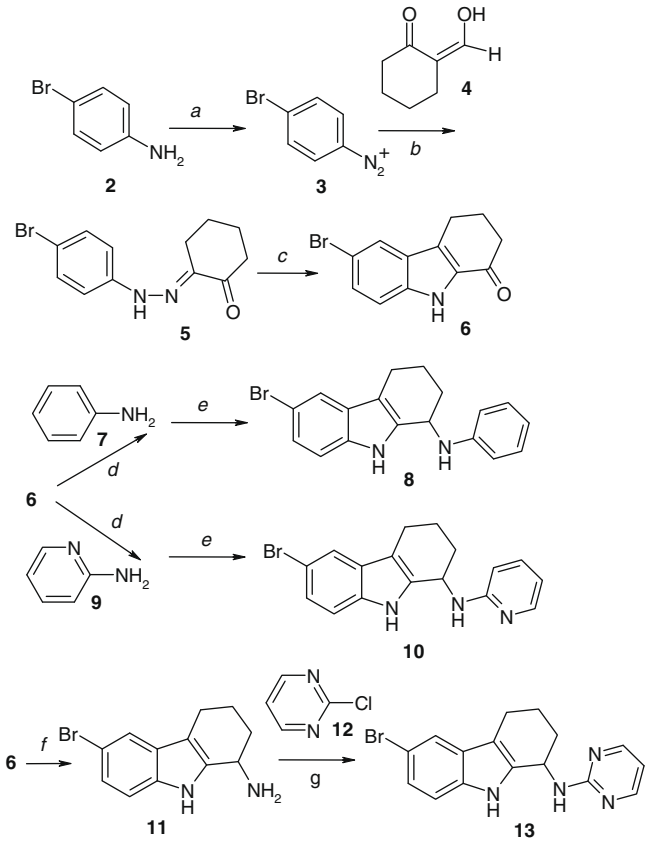


Figure 1. (1*R*)-6-Bromo-*N*-[(1*R*)-1-phenylethyl]-2,3,4,9-tetrahydro-1*H*-carbazole-1-amine (**1**).

6-Bromo-2,3,4,9-tetrahydro-1*H*-carbazol-1-one (**6**) was prepared from *p*-bromoaniline (**2**) and 2-(hydroxymethylene)cyclohexanone (**4**),[10] via cyclohexane-1,2-dione(*p*-bromophenyl)hydrazone (**5**) as outlined in Scheme 1. The reaction, known as the Borsche synthesis, is a special case of the Fischer indole synthesis.[11] Tetrahydrocarbazolone **6** was used to make aniline derivative **8** and aminopyridine derivative **10** via two step reductive amination as shown in Scheme 1.

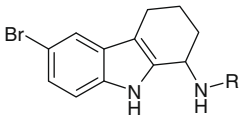
Tetrahydrocarbazole-1-amine **11** was prepared by treatment of **6** with NH_4OAc and sodium cyanoborohydride in methanol. Condensation of 2-chloropyrimidine (**12**) and **11** in a microwave at 150 °C was used to prepare pyrimidine derivative **13**.

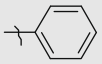
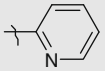
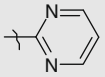
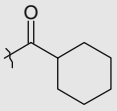
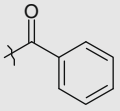
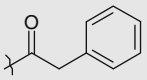
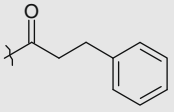
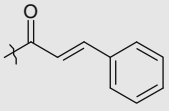
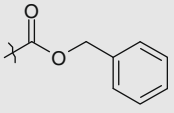
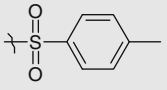
Anti-HPV activity for compounds **8**, **10** and **13** is shown in Table 1. These compounds showed potent anti-HPV activity as well as little CYP2D6 (32 μM for **8**) and hERG (>30 μM for **8**) inhibition. This demonstrated that by changing the basicity of the 1-amine we



Scheme 1. Reagents and conditions: (a) $NaNO_2$, aq HCl, 20 °C, 15 h; (b) NaOAc, aq MeOH, reflux, 2 h, (49% from **2**); (c) concd HCl in AcOH; 120 °C for 20 min, (88%); (d) *p*-TsOH (cat), toluene (Dean Stark), reflux, 16 h; (e) $NaBH_4$, MeOH, 30 min (yellow solid, 51% for **8**); (f) NH_4OAc, $NaCNBH_3$, MeOH, 60 °C, 15 h (52%); (g) DMF, Microwave (Smith Synthesizer), 150 °C, 15 min (26%).

Table 1
HPV activity and cytotoxicity of tetrahydrocarbazole amides and sulfonamides



Compd	R	IC_{50}[a] (μM)	CC_{50}[b] (μM)
8		0.01	50.8
10		0.04	20.7
13		0.09	89.5
14		12.9	>50
15		0.4	50
16		0.03	>20
17		0.58	>50
18		1.2	>50
19		0.32	>40
20		0.045	>20
21		2.26	>50
22		0.024	>20

[a] HPV activity measured in W12-20850 cells containing episomal HPV-16 DNA. IC_{50} is the concentration at which 50% efficacy in the W-12 assay is observed using a hybrid capture method.

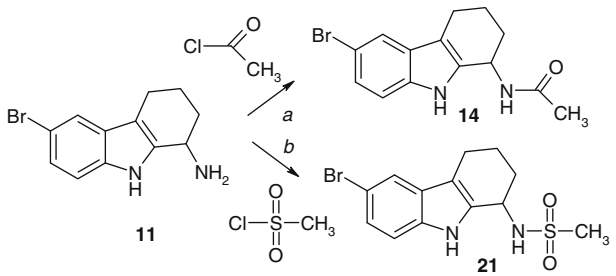
[b] CC_{50} is the concentration at which 50% cytotoxicity is observed in human foreskin fibroblasts (HFF cells).[12]

could modulate CYP2D6 and hERG inhibition. However modulation of the basicity of the 1-amine reduced solubility and resulted in poor bioavailability in rats (bioavailability <5% from a solution formulation of **8**).

We thus turned our attention to synthesis of amides and sulfonamides. Amides could be conveniently prepared from amine **11** and acid chlorides as outlined in Scheme 2. While simple alkylamides, like acetamide **14**, showed little activity, more lipophilic amides like cyclohexanecarboxamide **15** and especially benzamide **16**, showed much better anti-HPV activity. The benzamide **16** was about 2 orders of magnitude more potent than the acetamide **14**. This prompted us to change the distance between the amide and the phenyl moiety by synthesis of the phenylacetamide **17** and phenylpropaneamide **18**, as well as phenylpropenamide **19** and finally the benzyl carbamate **20**. Moving the amide and the phenyl moiety 1 or 2 'carbons' apart did not improve activity. In fact these derivatives were, with the exception of the benzyl carbamate **20**, about an order of magnitude less potent than **16**. We then synthesized a couple of sulfonamide derivatives by condensing amine **11** with sulphonyl chlorides under basic conditions as outlined in Scheme 2. Sulfonamides **21** and **22** showed similar activity trends to the amides, thus the simple methylsulfonamide **21** showed poor activity while toluyl sulfonamide **22** was equally potent to the benzamide **16**. For further optimization we chose to look at exploring the substitution pattern of the benzamide moiety of **16**, as the molecular weight of the amides is lower than the weight of the sulfonamides, and more benzoyl chlorides are commercially available than sulphonyl chlorides.

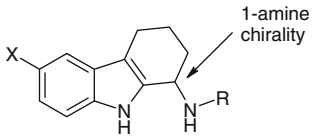
For a systematic look at the optimum substitution of the benzamide, we synthesized benzamides substituted with methyl, methoxy and fluorine in the *ortho* (**23**–**25**), *meta* (**27**–2**9**) and *para* (**31**–**33**) positions, as well as 2, 3 and 4-pyridinylamides (**26**, **30** and **34**, respectively) (Table 2). The 2-fluorosubstituted benzamide **25** and the 2-pyridinylamide **26** showed the most potent anti-HPV activity, while the *ortho*-methyl derivative **23** and the *ortho*-methoxy derivative **24** showed the worst activity. In contrast *meta* and *para* substituents had less dramatic effect on anti-HPV activity. Derivatives **25** and **26**, being the most potent compounds, were optimized further.

As mentioned previously for compound **1** the (*R*)-enantiomer at the 1-amine showed potent activity, while the *S*-isomers had limited activity. As the amides described were synthesized as racemates we became interested in separating the enantiomers and testing them independently. For benzamide **16**, the *R*- and *S*-enantiomers were separated using supercritical fluid chromatography[13] and stereochemistry was assigned by Ab Initio Vibrational Circular Dichroism (VCD) Spectroscopy.[14] Again, the *R*-enantiomer **35** was found to be very active, while the *S*-enantiomer **36** showed virtually no activity.



Scheme 2. Reagents and conditions: (a) CH_3COCl added to solution of **11** and $(i\text{-Pr})_2EtN$ in CH_2Cl_2 at 0 °C, then stirred at rt for 16 h (white solid, 52%); (b) CH_3SO_2Cl added to solution of **11** and $(i\text{-Pr})_2EtN$ in CH_2Cl_2 at 0 °C, then stirred at rt for 5 h (tan solid, 57%).

Table 2
HPV activity and cytotoxicity of tetrahydrocarbazoles benzamides



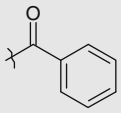
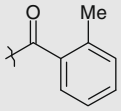
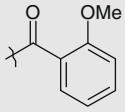
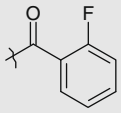
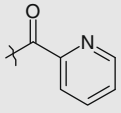
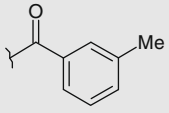
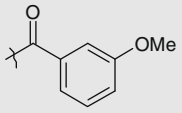
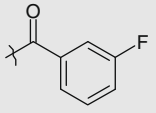
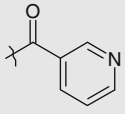
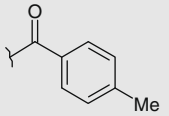
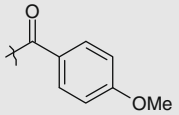
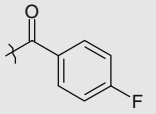
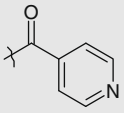
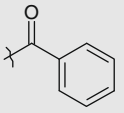
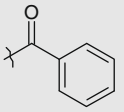
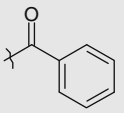
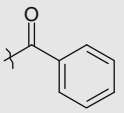
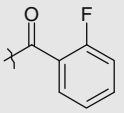
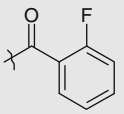
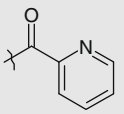
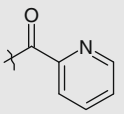
Compd	X	R	Chirality	IC_{50}[a] (μM)	CC_{50}[b] (μM)
16	Br		Racemic	0.03	>20
23	Br		Racemic	0.5	40
24	Br		Racemic	21	100
25	Br		Racemic	0.008	49
26	Br		Racemic	0.005	72
27	Br		Racemic	0.024	41
28	Br		Racemic	0.021	26
29	Br		Racemic	0.018	65
30	Br		Racemic	0.19	100
31	Br		Racemic	0.010	87
32	Br		Racemic	0.017	88
33	Br		Racemic	0.044	47

Table 2 (*continued*)

Compd	X	R	Chirality	IC_{50}[a] (μM)	CC_{50}[b] (μM)
34	Br		Racemic	0.2	100
35	Br		(*R*)	0.008	39
36	Br		(*S*)	5.7	52
37	Cl		(*R*)	0.020	26
38	Cl		(*S*)	11.3	55
39	Cl		(*R*)	0.008	24
40	Cl		(*S*)	2.1	36
41	Cl		(*R*)	0.005	16
42	Cl		(*S*)	9.0	61

[a] HPV activity measured in W12-20850 cells containing episomal HPV-16 DNA. IC_{50} is the concentration at which 50% efficacy in the W-12 assay is observed using a hybrid capture method.

[b] CC_{50} is the concentration at which 50% cytotoxicity is observed in HFF cells.

As the 6-bromosubstituted tetrahydrocarbazole compounds showed very good activity we next looked at the corresponding 6-chloro analogs in an attempt to reduce the molecular weight of the compounds and potentially improve pharmacokinetics. The *R*- and *S*-enantiomers were obtained via synthesis of racemates followed by separation by supercritical fluid chromatography.[15] The 6-chloro compounds, especially the (*R*)-enantiomers of the 2-fluorosubstituted benzamide **39** and the 2-pyridinylamide **41**,[16] again demonstrated very impressive anti-HPV activity. Because of their lower molecular weight and otherwise similar activity profile to the corresponding 6-bromo derivatives, these 6-chloro derivatives (**39** and **41**) were selected for further progression. Both **39** and **41** showed suitable cytochrome P450 (CYP2D6 >20 μM for both compounds) and hERG (IC_{50} >20 μM for both compounds) profile for further development, thus solving the potential problems associated with earlier generations of the tetrahydrocarbazoles (such as compound **1**). Head to head comparison of rat pharmacokinetics on solution formulations of **39** and **41** revealed that **41** had significantly better bioavailability in rats than **39** (56% compared with 21% from a 10 mg/kg dose in a solution formulation), hence **41** was progressed. Cytotoxicity testing in a number of additional cell lines (human keratinocytes, Vero cells) showed good separation between anti-HPV activity and cytotoxicity (SI >1000 in human keratinocytes, SI >1000 in Vero cells). Pharmacokinetic behavior of **41** was studied in three species and is outlined in Table 3.

Table 3
Plasma pharmacokinetics of **41**

Parameter	Rat	Dog	Monkey
Cl (mL/min/kg)	13.1	5	1.1
$V_d$ss (L/kg)	1.6	1.7	0.13
$T_{1/2}$ (h)	4.1	9.5	3.6
F (%, solution)	56	35	56

Clearance (Cl) and volume of distribution (V_d ss) calculated following a 2 mg/kg iv dose. Half life ($T_{1/2}$) and oral bioavailability (*F*) calculated following solution doses of 10 mg/kg (rat) and 5 mg/kg (dog and monkey).

Pharmacokinetic behavior of **41** indicates that this compound might be suitable for oral administration. Further screening against a panel of enzymes and receptors (PanLab) showed little risk of unwanted enzyme or receptor inhibition at concentrations close to those demonstrating anti-HPV activities. Because of the extremely promising anti-HPV potency and suitable pharmacokinetic profile, compound **41** was progressed for further testing. This testing showed that **41** had a unique profile, showing not only potent anti-HPV activity but also activity against some other viruses,[6c] while being selective (**41** did not impact the growth of several primary cell lines studied). Preliminary mechanism studies indicate that **41** most likely targets a host cell process which results in the induction of a subset of interferon-stimulated genes.[6c] This would explain its activity in some virally infected and immortalized cells as well as lack of toxicity in primary cell lines. The intriguing profile of **41** is being further evaluated and a suitable route to prepare kilogram quantities of **41** has been developed.[17]

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12. Decision to compare activity (IC_{50}) in W-12 (keratinocytes) with cytotoxicity (CC_{50}) in HFF cells (fibroblasts) was based on the fact that a screening of over 50 tetrahydrocarbazole showed CC_{50} values to be virtually identical in keratinocytes and fibroblasts. HFF cells (fibroblasts) were amenable to high throughput screening, whereas keratinocytes are not.
13. Separated on a Berger analytical SFC with HP1100 diode array detector. Eluted with 30% MeOH in CO_2; flow rate 2 mL/min at 2250 psi, 50 °C on Diacel AD-H column (Chiral Technologies), 4.6 × 250 mm, 5 μm; *R*-isomer retention time 12.37 min; *S*-isomer retention time 16.11 min.
14. 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. Assignments were later confirmed by X-ray crystallography of 41.
15. Separated on a Berger analytical SFC with HP1100 diode array detector. Eluted with 30% MeOH in CO_2; flow rate 2 mL/min at 1500 psi, 40 °C on Diacel AS-H column (Chiral Technologies), 4.6 × 250 mm, 5 μm; For 41 retention time 5.08 min, for 42 retention time 7.45 min.
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