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Substituted tetrahydro-β-carbolines as potential agents for the treatment of human papillomavirus infection

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ABSTRACT

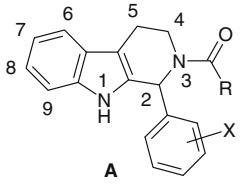
The identification and optimization of a series of substituted tetrahydro-β-carbolines with potent activity against human papillomavirus is described. Structure–activity studies focused on the substitution pattern and chirality of the β-carboline ring system are discussed. Optimization of these parameters led to compounds with antiviral activities in the low nanomolar range.

Human papillomaviruses (HPVs) comprise a family of small double-stranded DNA viruses that are implicated as the causal agents in a variety of benign and pre-malignant epithelial tumors. Several HPV variants are known to infect the genital mucosa and HPV infection is now known to be the most common sexually transmitted disease worldwide.[1] HPVs associated with genital infections are classified as either low or high risk depending on their particular disease associations.[2] Low risk HPVs (i.e., HPV 6 and 11) cause genital warts whereas the high variants (HPV 16 and 18) are clearly linked with genital cancers including cervical carcinoma. The majority of HPV infections are transient, however, a high proportion of persistent infections with high risk types are known to progress to cervical dysplasia, a precursor to cervical cancer.

Currently employed treatments for HPV-associated genital lesions include various ablative and cytodestructive therapies as well as topical treatment with the immunostimulator imiquimod (Aldara®). Two anti-HPV vaccines (Cervarix® and Gardasil®) were recently approved, however, the long term effect of these agents on cancer incidence remains to be seen.[3] There are currently no small molecule oral agents available for the treatment of HPV, therefore this constitutes a significant unmet medical need.

Researchers from our laboratories recently reported the lead optimization of a series of substituted tetrahydrocarbazoles that originated from a high throughput screen.[4] The screen employed a human cervical keratinocyte cell line (W12-20850) isolated from a low grade cervical lesion containing HPV 16 episomal DNA.[5] In addition to the tetrahydrocarbazole hits, we also identified several substituted tetrahydro-β-carbolines with single digit micromolar potencies. These compounds, which possessed aryl and acyl substituents at the 2- and 3-positions respectively (Fig. 1), show a close structural resemblance to the previously reported tetrahydrocarbazoles. Herein we report our efforts toward the optimization of the β-carboline series.

In our initial structure–activity studies we chose to hold the 2-substituent constant as *p*-tolyl and examine the effect of varying the substituent on the nitrogen atom at the 3-position. These compounds were prepared as shown in Scheme 1. Tryptamine **1** was



A

Figure 1. Tetrahydro-β-carboline lead series (**A**).

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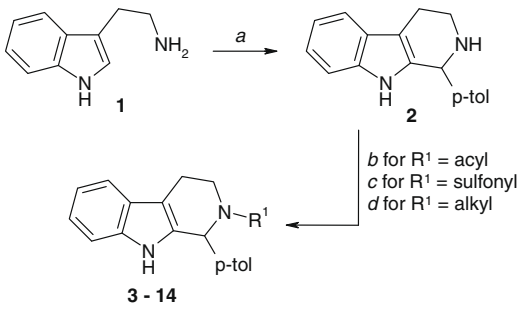


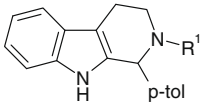
Scheme 1. Reagents and conditions (a) *p*-tolualdehyde, glacial AcOH, 80 °C (86%); (b) RCOCl, DMAP (1.5 equiv), CH_2Cl_2 (73% for **4**) or RCOOH, EDCI, HOBt, DMF (85% for **5**); (c) RSO_2Cl, DMAP (1.5 equiv), CH_2Cl_2 (63% for **11**); (d) RCHO, $NaBH(OAc)_3$, 1,2-dichloroethane (83% for **13**).

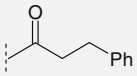
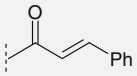
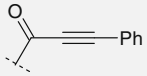
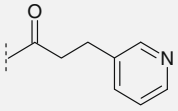
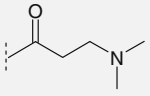
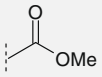
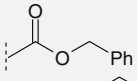
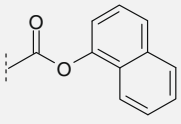
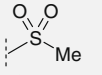
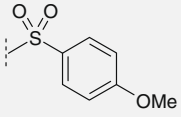
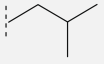
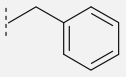
subjected to Pictet-Spengler cyclization with *p*-tolualdehyde to give intermediate **2**.[6] Access to amides **3**–**7** and carbamates **8**–**10** was provided by acylation with acid chlorides/chloroformates or by coupling with carboxylic acids under standard carbodiimide coupling conditions. Similarly, sulfonamide derivatives **11** and **12** were prepared by reaction with the appropriate sulfonyl chloride. Finally, amine derivatives **13** and **14** were prepared by reductive alkylation of **2** with isobutyraldehyde and benzaldehyde, respectively.

Table 1 illustrates HPV activity and cytotoxicity data for the 3-position analogs **3**–**14**. The phenylpropionyl derivative **3** provided a 1.3 μM IC_{50} in the W12 assay and an appreciable 100-fold cytotoxicity window. Unsaturation of the 3-substituent sidechain to afford cinnamoyl derivative **4** led to little change in activity whereas the analogous linear acetylene derivative **5** displayed a 10-fold loss of activity. The basic sidechain analogs **6** and **7** showed modest activities in the 15 μM range accompanied by marked erosion of the cytotoxicity windows, indicating that polar and/or ionizable groups are not well tolerated. Carbamate derivatives **8** and **9** provided low micromolar activities while maintaining good cytotoxicity windows whereas the sterically more demanding naphthyl carbamate **10** showed a 20-fold reduction in potency. The sulfonamide derivatives **11** and **12** showed relatively weak activities with some preference for the smaller methyl analog **11**. The isobutyl and benzyl amine analogs **13** and **14** showed 25 and 5.8 μM activities respectively. It is interesting that compound **14** maintains the low micromolar potency characteristic of the generally more active acyl derivatives even though it possesses a different hybridization geometry (sp^3 vs sp^2) at the ring attachment point.

Having explored the 3-position SAR in the β-carboline series, we turned our attention to the 2-position. In this exercise we held the 3-substituent constant as phenylpropionyl and varied the 2-substituent. Our synthetic approach is illustrated in Scheme 2. Compounds **15**–**19** were prepared as described earlier by Pictet-Spengler cyclization of **1** with the appropriate aldehydes followed by acylation with phenylpropionyl chloride. Catalytic hydrogenolysis of **19** afforded alcohol **20** which was subjected to Mitsunobu reaction with phenol to give phenyl ether **21**. Alternatively, **20** was converted to the PNP active carbonate by treatment with *p*-nitrophenyl chloroformate followed by reaction with pyrrolidine to give carbamate **22**. Compounds **24** and **25** were synthesized from ester intermediate **23** which itself was obtained via Pictet-Spengler reaction of **1** with glyoxylic acid followed by Fisher esterification and then acylation with phenylpropionyl chloride.[7] Saponification of **23** with LiOH followed by EDCI coupling of the resulting carboxylic acid with pyrrolidine afforded amide derivative **24**. DIBAL reduction of **23** gave an aldehyde intermediate which was subjected to reductive amination with pyrrolidine to give **25**.

Table 1
Effect of the 3-position substituent on HPV activity and cytotoxicity

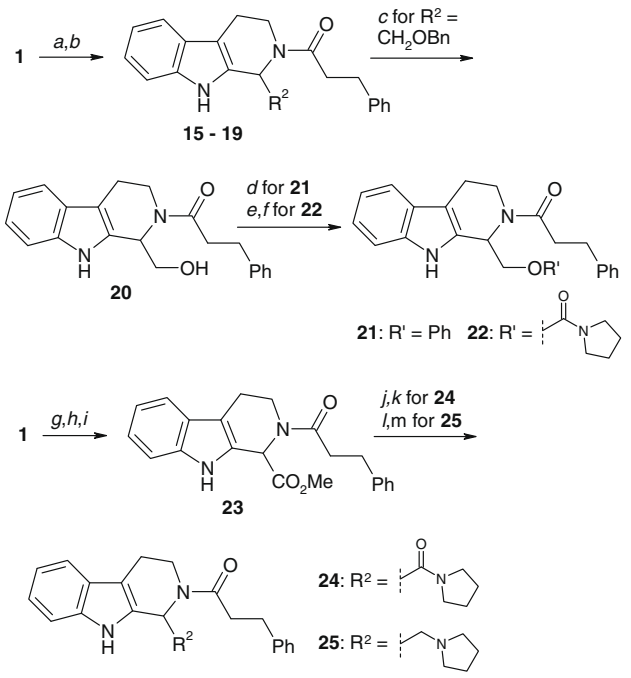


Compound	R^1	IC_{50}[a] (μM)	CC_{50}[b] (μM)
3		1.3	>100
4		2.3	>100
5		20	>100
6		18	94
7		15	40
8		1.7	31
9		0.9	>100
10		23	>100
11		23	>100
12		66	>100
13		25	50
14		5.8	94

[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).

Table 2 shows SAR data for the 2-position analogs **15**–**25**. Phenyl derivative **15** was equipotent with the *p*-tolyl analog **3** whereas insertion of a nitrogen atom into the aromatic ring to give pyridine **16** led to a complete loss of activity. The phenethyl derivative **17** illustrates that chain extension of the aromatic unit maintains HPV activity. Insertion of an oxygen atom into the ethylene tether of **17** affords benzyl ether **19** which shows a modest loss of activity. However, replacement of one of the methylene units in **17** with an oxygen atom leads to a nearly complete loss of potency as illustrated by phenyl ether **21**. Interestingly, the isobutyl derivative **18** was the most potent analog in the entire set showing a 330 nM IC_{50} while maintaining a reasonable cytotoxicity window. Methyl carbamate **23** showed a relatively weak 20 μM IC_{50} while



Scheme 2. Reagents and conditions: (a) RCHO, glacial AcOH, 80 °C or TFA, CH_2Cl_2, rt (80% for R = Ph); (b) phenylpropionyl chloride, $(i\text{-Pr})_2EtN$, CH_2Cl_2 (86% for **16**); (c) H_2, $Pd(OH)_2$, MeOH (62%); (d) PhOH, di-*t*-butyl azodicarboxylate, Ph_3P, CH_2Cl_2 (30%); (e) 4-nitrophenyl chloroformate, pyridine, CH_2Cl_2 (91%); (f) pyrrolidine, $(i\text{-Pr})_2EtN$, CH_2Cl_2 (83%); (g) glyoxylic acid, aqueous HCl (88%); (h) HCl, MeOH, reflux, 97%; (i) phenylpropionyl chloride, $(i\text{-Pr})_2EtN$, CH_2Cl_2 (66%); (j) LiOH, THF, H_2O (95%); (k) pyrrolidine, EDCI, HOBt, DMF (78%); (l) DIBAL, ether, −78 °C; (m) pyrrolidine, $NaBH(OAc)_3$, 1,2-dichloroethane, (25% over two steps).

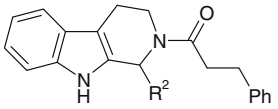
the carbamate and amide derivatives **22** and **24** lost all activity. The basic pyrrolidine analog **25** showed 8.5 μM activity in the HPV assay and little separation between antiviral activity and cytotoxicity.

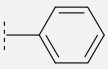
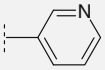
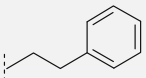
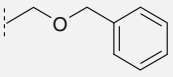
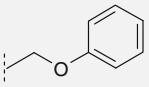
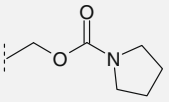
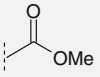
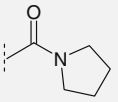
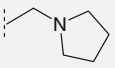
The next structural feature we were interested in exploring was substitution on the left-hand benzo ring of the β-carboline ring system. The syntheses in this case were very straightforward involving Pictet-Spengler cyclization of commercially available substituted tryptamines with *p*-tolualdehyde followed by acylation with phenylpropionyl chloride (Scheme 3).

Table 3 shows the SAR data for the benzo substituted analogs **27**–**33**. Collectively, the benzo ring substitutions are well tolerated with several conferring substantial increases in HPV activity relative to the unsubstituted parent compound **3**. In addition, all of the analogs in this set maintain >100-fold cytotoxicity windows. Of particular interest is the 7-chloro analog **30** which shows a 62 nM anti-HPV IC_{50}. This activity trend is consistent with observations in the previously discussed, structurally related tetrahydrocarbazole series.[4]

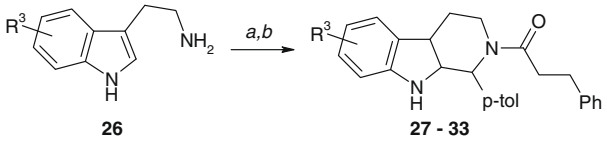
The final structural feature that we were interested in exploring was the absolute configuration at the 2-position of the β-carboline ring system. Therefore, the racemic 7-chloro analog **30** was subjected to enantiomeric separation via chiral supercritical fluid chromatography.[8] The absolute stereochemistries were then assigned using Ab Initio Vibrational Circular Dichroism (VCD) Spectroscopy.[9] In addition, we combined the optimal 2-position isobutyl substituent with the 7-chloro feature by synthesizing racemic **34** which was also subjected to chiral separation and VCD-based absolute stereochemical assignment.[8,9] The SAR data for these four compounds is shown in Table 4. A clear trend emerged with the *S* absolute configuration being preferred affording 23 nM and 18 nM activities for compounds **30b** and **34b**, respectively.

Table 2
Effect of the 2-position substituent on HPV activity and cytotoxicity



Compound	R^2	IC_{50}[a] (μM)	CC_{50}[b] (μM)
15		1.2	>100
16		>100	>100
17		2.7	>100
18		0.33	22
19		7.3	>100
21		72	>100
22		>100	>100
23		20	>100
24		>100	>100
25		8.5	16

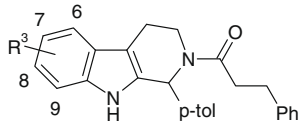
[a,b] See footnotes a and b in Table 1.



Scheme 3. Reagents and conditions: (a) *p*-tolualdehyde; glacial AcOH, 80 °C for compounds **27**–**32**; TFA, $CHCl_3$, reflux for compound **33** (73% for **30**); (b) phenylpropionyl chloride, DMAP (1.5 equiv), CH_2Cl_2 (91% for **30**).

In order to assess their viability as potential drug candidates, compounds **30b** and **34b** were subjected to additional biological profiling including rat pharmacokinetic analysis. Following IV dosing of 1 mg/kg in rats, compound **30b** showed a 6.2 h $t_{1/2}$, and a 3.6 mL/min/kg clearance value while **34b** showed a 0.8 h $t_{1/2}$, and a 17.3 mL/min/kg clearance value. Unfortunately, both compounds showed negligible oral bioavailability in rat when dosed at 10 mg/kg. However, **30b** showed a 73% oral bioavailability in mouse following a 5 mg/kg dose. Screening of **30b** against a panel of enzymes and receptors (PanLabs) showed little risk of undesirable off-target activity. In addition, **30b** was found to be devoid of deleterious cytochrome-P450 activity following screening against a CYP panel. Thus, compound **30b** has been identified

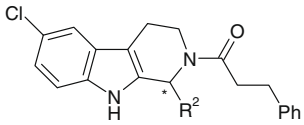
Table 3
Effect of the benzo ring substituent on HPV activity and cytotoxicity



Compound	R^3	IC_{50}[a] (μM)	CC_{50}[b] (μM)
27	7-Me	0.35	>100
28	7-MeO	0.57	>100
29	7-F	0.44	>100
30	7-Cl	0.062	>100
31	8-MeO	5.8	>100
32	8-F	1.7	>100
33	9-Me	2.2	>100

[a,b] See footnotes a and b in Table 1.

Table 4
Effect of chirality in the 7-chloro series on HPV activity and cytotoxicity



Compound	R^2	Chirality	IC_{50}[a] (μM)	CC_{50}[b] (μM)
30a	*p*-Tol	*R*	7.7	>100
30b	*p*-Tol	*S*	0.023	>100
34a	*i*-Butyl	*R*	0.88	>100
34b	*i*-Butyl	*S*	0.018	>100

[a,b] See footnotes a and b in Table 1.

as a potential new agent for the treatment of human papillomavirus infection.

Acknowledgments

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References and notes

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