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Identification of novel allosteric modulators for the G-protein coupled US28 receptor of human cytomegalovirus

Ana Kralj [a], Alexander Wetzel [b], Shohreh Mahmoudian [c], Thomas Stamminger [c], Nuska Tschammer [a,*], Markus R. Heinrich [a,*]

[a] Department of Chemistry and Pharmacy, Emil Fischer Center, Friedrich-Alexander-Universität Erlangen-Nürnberg, Schuhstraße 19, D-91052 Erlangen, Germany
[b] Department of Chemistry and Biochemistry, Technische Universität München, Lichtenbergstraße 4, D-85747 Garching b. München, Germany
[c] Institute for Clinical and Molecular Virology, University Hospital Erlangen, Schlossgarten 4, D-91054 Erlangen, Germany

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ABSTRACT

The highly constitutively active G-protein coupled receptor US28 of human cytomegalovirus (HCMV) is an interesting pharmacological target because of its implication on viral dissemination, cardiovascular diseases and tumorigenesis. We found that dihydroisoquinolinone and tetrahydroisoquinoline scaffolds may be promising lead structures for novel US28 allosteric inverse agonists. These scaffolds were rapidly synthesized by radical carboamination reactions followed by non-radical transformations. Our novel US28 allosteric modulators provide valuable scaffolds for further ligand optimization and may be helpful chemical tools to investigate molecular mechanisms of US28 constitutive signaling and its role in pathogenesis.

Human herpesviruses are widespread pathogens involved in acute and chronic diseases.[1] The omnipresent human cytomegalovirus (HCMV) causes a life-long latent infection in healthy hosts.[1,2] In patients with immature or suppressed immune systems (e.g., neonates, AIDS [Acquired Immune Deficiency Syndrome], cancer patients and transplant patients), HCMV can lead to severe and life-threatening disease. HCMV infection is also associated with a number of chronic diseases, including atherosclerosis, cancer and transplant rejection.[1,2] Viruses like HCMV encode viral G-protein coupled receptors (vGPCRs) that are often highly homologous with the host's chemokine receptors; these vGPCRs couple very efficiently to signaling networks of the host.[3] Most vGPCRs signal independently from a ligand. Constitutive signaling of vGPCRs augments virus survival, host invasion and, in some cases, oncogenesis or cardiovascular disease, by exploiting preferred signaling cascades.[4] HCMV encodes US28, a highly constitutively active viral GPCR receptor, which has the ability to bind different human CC-chemokines (e.g., CCL5/Rantes) as well as the CX_3C-chemokine CX_3CL1/fractalkine.[1,2] Because of the potential role of US28 in viral dissemination and persistence as well as in cardiovascular disease and tumorigenesis, US28 remains an interesting pharmacological target.[2,5,6] The search for a potent inverse agonist that would reverse the constitutive activity of US28 is an ongoing challenge.

VUF2274 [5-(4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl)-2,2,-diphenylpentanenitrile], initially discovered as the CCR1 receptor antagonist,[7] has also been described as the first US28 inverse agonist.[8,9] VUF2274 and its derivatives behave as inverse agonists in the PLCβ pathway (measured as an accumulation of inositol triphosphate). Despite significant efforts and detailed structure–activity relationship (SAR) studies, the potency of this class of compounds and some non-VUF2274-like compounds (e.g., methiothepin) could not be improved beyond micromolar range.[8,10,11]

With the intention to identify novel molecular scaffolds that would serve as a template for the development of US28 inverse agonists, we dedicated our attention to the known chemokine CXCR3 receptor ligands. The human CXCR3 receptor possesses 28% sequence homology with US28. As a comparison, US28 has a 30% amino acid sequence homology with the human chemokine CCR1 receptor,[12] the primary target of VUF2274. Inspired by the structure of the CXCR3 receptor antagonist, VUF5834,[13] with no reported activity at US28, we identified tetrahydroisoquinolines and dihydroisoquinolinones as promising lead scaffolds for further structural optimization. We applied radical carboamination reactions starting from simple precursors to rapidly and efficiently access the pharmaceutically important class of β-arylamines, which were subjected to non-radical transformations to afford dihydroisoquinolinone and tetrahydroisoquinoline deriva-

* Corresponding authors. Tel.: +49 9131 852 3931; fax: +49 9131 852 2585.
E-mail addresses: nuska.tschammer@medchem.uni-erlangen.de (N. Tschammer), markus.heinrich@medchem.uni-erlangen.de (M.R. Heinrich).

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tives. Radical carboamination reactions starting from such simple precursors as aryldiazonium salts and olefins offer fast and efficient access to the pharmaceutically important class of β-arylamines.[14–16] In combination with other non-radical transformations, a wide range of further products, especially heterocycles, becomes available in only a few steps. Compared to organometallic methods for carboamination, which currently have to be conducted in a partially intramolecular fashion (amine equivalent usually attached to olefinic substrate), radical reactions are not limited in this way.[17,18] Herein, we present the first application of this powerful synthetic strategy in the field of medicinal chemistry. The HPLC purity of investigated compounds was >95%.

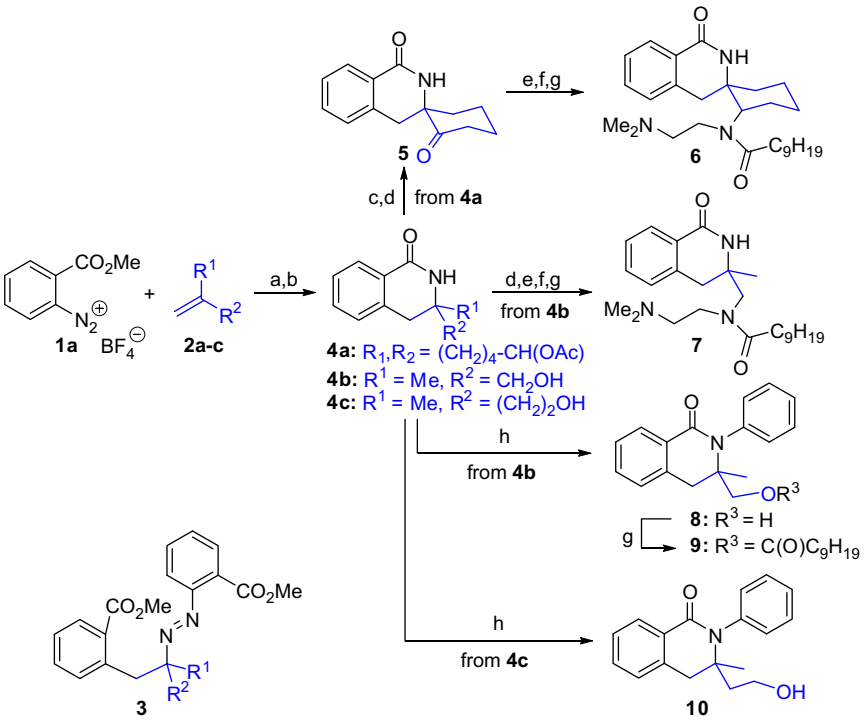
Dihydroisoquinolinones are usually obtained when the two-step carboamination sequence is carried out with aryldiazonium salts bearing a carboxylic ester functionality in the *ortho* position to the diazonium group. Initially (step a), azo compounds of type **3** are prepared by the iron(II)-mediated reaction of diazonium salt **1a** and olefin of type **2** (Scheme 1). In the next step (b), which is commonly carried out without isolation of azo compound **3**, the reductive cleavage of the N–N double bond is accompanied by lactamization to give the desired target molecules **4**. According to this pathway, we prepared three dihydroisoquinolinones **4a**–**c**, which served as key intermediates for further transformations. The spirocyclic ketone **5** was obtained from acetate **4a** by hydrolysis (step c) and Swern oxidation (step d). Reductive amination of ketone **5** with *N*,*N*-dimethyl ethylenediamine (steps e and f) and subsequent coupling to decanoyl chloride provided the spirocyclic test compound **6**. Compound **4a** was obtained as a 2:1 mixture of diastereoisomers (*trans*:*cis* of –NH- and –OAc on the cyclohexane ring). Compound **6** was obtained as a single isomer. Its relative stereochemistry could not be determined due to an overlap of signals in the NMR spectrum. Comparable reagents and conditions (steps d–g) were employed to convert dihydroisoquinolinone **4b** into test compound **7**.

Because the structural designs of compounds **6** and **7** were inspired by VUF5834,[13] we conducted several further experiments to introduce a phenyl substituent at the nitrogen atom of the isoquinolinone. These attempts to prepare compounds even more closely related to the known CXCR3 ligands failed. To get access to the N-phenylated dihydroisoquinolinones **8** and **10**, the key intermediates **4b** and **4c** were submitted to a copper-catalyzed arylation protocol (step h).[19] Esterification of **8** with decanoyl chloride gave test compound **9** (step g).

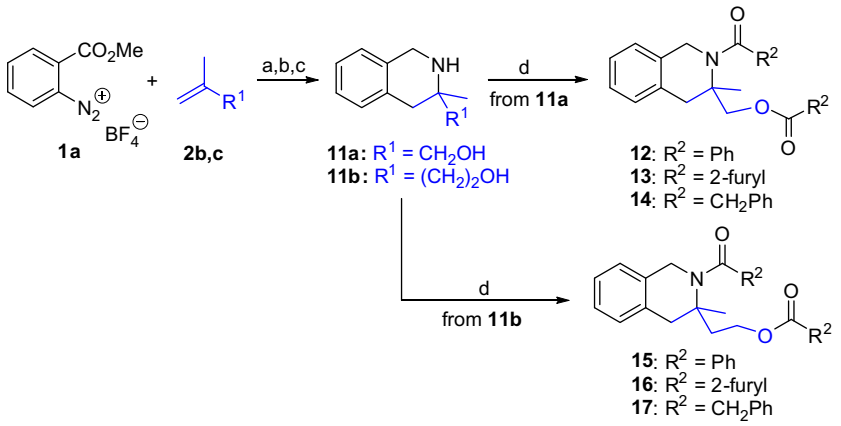
For the preparation of tetrahydroisoquinolines **11a** and **11b** the two-step carboamination sequence shown in Scheme 2 was modified by a further reductive step with lithium aluminum hydride (Scheme 2, steps a–c). Each of the two intermediates **11a** and **11b** was then reacted with benzoyl chloride, 2-furoyl chloride and 2-phenacetyl chloride under reaction conditions leading to double acylation at the nitrogen atom and the oxygen atom. In this way, the six tetrahydroisoquinoline-derived test compounds **12**–**17** were available.

Without a carboxylic ester functionality on the aromatic core of the arenediazonium salt, β-arylamines of type **18** are obtained as products from the carboamination sequence (Scheme 3, steps a and b) instead of the isoquinolinones shown above (Scheme 1). By applying a protocol reported by Ohwada[20], we were able to convert seven β-arylamines **18a**–**g** into 1-phenyltetrahydroisoquinolines **19**–**25**. For this purpose, the amines **18a**–**g** were condensed with benzaldehyde to yield imines (step c), which cyclized at elevated temperatures when treated with trifluoromethanesulfonic acid (step d). The reaction conditions for the cyclization step led to a strong predominance of the *cis*-configured products with a selectivity usually higher than 10:1 (*cis*:*trans*). In a few cases we were also able to isolate the minor *trans* isomers, such as compounds **20′** and **23′**.

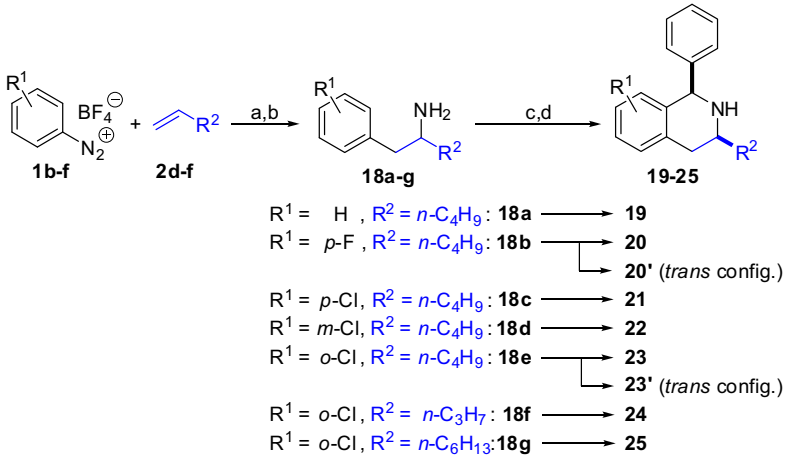
For the characterization of novel allosteric modulators of US28, we used the luciferase based PathDetect Elk1 gene reporter assay. Functional assays have the capability to detect ligands that



Scheme 1. Reagents and conditions: (a) $FeSO_4$, H_2O–DMSO, rt; (b) Raney-Ni/H_2 (50 bar), MeOH or EtOH, rt or Zn, HCl, rt; (c) NaOH, MeOH; (d) $(COCl)_2$, NEt_3, DMSO, CH_2Cl_2, −78 °C; (e) *N*,*N*-dimethyl ethylenediamine, $CHCl_3$, 90 °C ; (f) $NaBH_4$, MeOH, rt; (g) $H_{19}C_9COCl$, NEt_3, 1,2-$C_2H_4Cl_2$, 50 °C; (h) Ph–I, Cu, DMF, 150 °C.



Scheme 2. Reagents and conditions: (a) $FeSO_4$, H_2O–DMSO, rt; (b) Zn, HCl, 50 °C; (c) $LiAlH_4$, THF, reflux; (d) PhCOCl, furan-2-carboxylic acid chloride or $PhCH_2COCl$, NEt_3, 1,2-$C_2H_4Cl_2$, 50 °C.



Scheme 3. Reagents and conditions: (a) $FeSO_4$, H_2O–DMSO, rt; (b) Zn, HCl, MeOH, 50 °C; (c) PhCHO, $MgSO_4$, Et_2O, rt; (d) CF_3SO_3H, 120 °C.

allosterically alter the affinity and/or efficacy for any species that interacts with the receptor, including cytosolic signaling proteins.[21,22] The US28 receptor that we used was cloned from the clinically relevant and highly endotheliotropic HCMV strain TB40/E (Genbank Accession No. ABV71518.1, Supplementary data).[23] Constitutive activity of US28 mediates the activation of transcription factors through p42/p44 mitogen-activated protein kinase (MAPK) and p38 MAPK-dependent pathways.[2,3] The transcription factor Elk-1 is phosphorylated and activated by p42/p44 MAPK.[24] The activation of Elk-1 results in an increased expression of the reporter protein luciferase. For the quantitation of the luciferase expression, BrightGlo reagent was used and luminescence was measured with a microplate reader. The increase in the luminescence was directly proportional to the activation of Elk-1 transcription factor. The constitutive activity of US28 increased the basal luminescence by 20-fold compared to mock transfected HEK cells (Supplementary data). The degree of inverse agonism (maximal effect) was entirely dependent on the level of constitutive activity of the system (i.e., the difference between the constitutive and non-constitutive basal responses).[25]

Antipsychotic methiothepin that has been described as an agonist on US28,[26] barely showed any agonist effect on the US28 wild type receptor in our test system. VUF2274 is a weak inverse agonist on US28 (EC_{50} = 4.5 μM, efficacy = −22%). VUF2274 was reported as full inverse agonist in the PLCβ pathway (measured as an accumulation of inositol triphosphate).[9] The observed difference in the maximal efficacy of VUF2274 can be attributed to different assay conditions. In the reporter gene assay, the reporter gene is transcribed and accumulated in the cell as soon as the constitutive active US28 is expressed. Unless compounds are added immediately after transfection, the efficacy of the inverse agonists is likely underestimated.[9]

The substituted dihydroisoquinolinone **7** and its spirocyclic derivative **6** demonstrated an inverse agonism on US28, with the EC_{50} values of 1.50 and 4.80 μM and efficacies of −20 and −21%, respectively (Table 1, Fig. 1). The N-phenylated dihydroisoquinolinone derivative **8** behaved as an agonist on US28. The exchange of the hydroxymethyl group of **8** for hydroxyethyl group of **10** yielded the inverse agonist with EC_{50} value 1.00 μM, and the efficacy of −14 (Table 1, Fig. 1).

Further modifications of the dihydroisoquinolinone scaffold resulted in a series of strong US28 inverse agonists. The best compounds in the series were benzoyl substituted tetrahydroisoquinoline **12** with the EC_{50} value of 8.50 μM and the efficacy −62% and phenacetyl substituted tetrahydroisoquinoline **14** with the EC_{50} value of 3.40 μM and the efficacy −37% (Table 1, Fig. 1). The exact position of ester acyl oxygen as a potential hydrogen bond acceptor seems to be important for the binding affinity of the inverse agonists we examined. The exchange of hydroxymethyl with hydroxyethyl linker as in **15**, **16** and **17** resulted in a three to four fold loss of affinity. To confirm that inverse agonism of the described compounds does not originate from their cytotoxicity, the

Table 1
Functional characterization of compounds **6**–**25** employing the US28 receptor[a]

Compound	EC_{50} (μM)	pEC_{50} ± SEM	Efficacy (% over basal)
VUF2274	4.50	5.34 ± 0.46	−22 ± 8
Methiothepin	0.35	6.45 ± 0.45	10 ± 3
6	4.80	5.32 ± 0.27	−22 ± 4
7	1.50	5.82 ± 0.21	−20 ± 3
8	4.40	5.36 ± 0.18	41 ± 7
9	n.d.	n.d.	n.d.
10	1.00	5.99 ± 0.40	−14 ± 3
12	8.50	5.07 ± 0.02	−62 ± 2
13	7.40	5.13 ± 0.09	−18 ± 2
14	3.40	5.46 ± 0.22	−37 ± 6
15	13.50	4.87 ± 0.07	−70 ± 5
16	n.d.	n.d.	n.d.
17	7.50	5.12 ± 0.08	−51 ± 6
19	0.28	6.56 ± 0.32	22 ± 3
20	1.80	5.75 ± 0.26	15 ± 3
20'	2.00	5.70 ± 0.51	15 ± 5
21	7.80	5.11 ± 0.49	12 ± 3
22	n.d.	n.d.	n.d.
23	0.13	6.89 ± 0.21	21 ± 2
23'	1.40	5.85 ± 0.36	16 ± 3
24	1.50	5.81 ± 0.26	27 ± 5
25	2.30	5.64 ± 0.39	21 ± 5

[a] Functional data were obtained on transfected HEK cells that transiently expressed US28 as shown by the PathDetect trans Elk-1 reporter gene assay. Dose response curves of 4–8 experiments performed in triplicates have been normalized and pooled to get a mean curve from which the EC_{50} value and the maximum intrinsic activity of each compound was obtained. n.d.—not detectable.

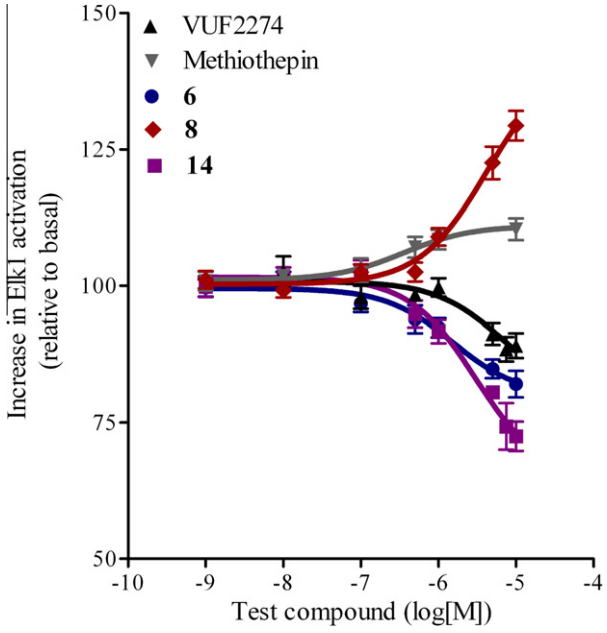


Figure 1. Functional characterization of reference compounds VUF2274 and methiothepin, and representative novel compounds **7**, **8** and **14**. HEK cells were transiently transfected with US28 and components of PathDetect Elk-1. Normalized curves from 3 to 6 experiments, each performed in triplicate, are shown. The error bars represent the SEM.

cell viability was investigated. The compounds with the highest rate of inverse agonism (compounds **12**, **15** and **17**) had minor but significant cytotoxicity, which could account for the pronounced inverse agonist effect (Supplementary data). The other novel inverse agonists (**7**, **10**, **13** and **14**) had no cytotoxicity. The observed inverse agonism was thus mediated by US28.

Structural modifications of the dihydroisoquinolinone core resulted in a series of 1-phenyltetrahydroisoquinolines (**19**–**25**) which behaved as weak allosteric agonists on the US28 wild type (Table 1). Unfortunately, this group of compounds demonstrated visible affinity and efficacy on the D_{2L} receptor (Supplementary data), indicating that this molecular scaffold might be used for the development of biogenic amine receptor ligands. The unfavorable D_{2L} receptor affinity (2 μM) was reported also for the US28 inverse agonist VUF2274.[7] Methiothepin displayed agonist activity on US28 and has an antipsychotic action on serotonin and dopamine receptors.[27] The cross-reactivity of small-molecular-weight chemokine ligands with biogenic amine receptors is one of the main issues regarding the design of highly selective chemokine ligands.[28]

Our attempts to design a novel inverse agonist for US28 resulted in a series of allosteric modulators with the potency and efficacy comparable or superior to the reference VUF2274. These novel US28 allosteric modulators provide valuable chemical tools to investigate molecular mechanisms of US28 constitutive signaling and its role in the pathogenesis of viral infection, tumorigenesis and development of cardiovascular disease. The further development of novel US28 allosteric inverse agonists by synthesis procedures that will yield enantiopure compounds and the determination of preferred receptor ligand interactions, which depend on the absolute stereochemistry of the enantiomers, are in progress.

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Supplementary data

Supplementary data (experimental procedures, sequence alignment, results of the cytotoxicity assay, the reporter gene assay performed on mock transfected HEK cells, and synthesis procedures) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.06.120.

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