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2-Phenylquinolones as Inhibitors of the HIV-1 Tat–TAR Interaction

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Tat (transactivator of transcription) is a regulatory protein essential for viral gene expression, replication, and pathogenesis and represents an attractive target in anti-HIV-1 drug development.[1] Tat interacts with the RNA structure TAR (transactivation responsive region), a short stem-loop located in the long terminal repeat (LTR) region at the 5′ end of all nascent HIV-1 transcripts.[2] The Tat–TAR interaction results in an exponential increase of viral transcript production and expression of all proteins necessary to complete the HIV life cycle.[3,4]

Small molecules able to interfere with TAR and compete for Tat binding have been reported (see references [1] and [5] for recent reviews). We recently designed the first series of 2-phenylquinolones (2-PQs, Figure 1) on the basis of the structural

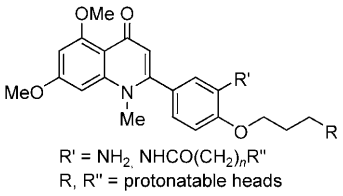

Figure 1. General structure of the first series of 2-phenylquinolones (2-PQs).

requirements that are known to ensure efficient Tat–TAR recognition.[5–7] In these compounds, a quinolone ring lacking the C3 carboxyl group serves as a stacking moiety between base pairs of the nucleic acid and as a scaffold for a phenyl ring bearing one or two protonatable side chains intended to electrostatically bind the phosphate backbone of TAR.[8]

By using a fluorescence quenching assay (FQA), we show that these novel quinolones lacking the 4-keto-3-carboxyl functionality can efficiently interfere with the Tat–TAR complex.[8] Moreover, structure–activity relationship (SAR) studies of the phenyl ring requirements have evidenced that, in sharp contrast to previously reported biaryl-heterocyclic derivatives directly related to our compounds,[6] 2-PQs require only a single cationic residue for efficient Tat–TAR inhibition, thus decreasing the overall charge density and molecular mass of the molecule. From these indications, we have expanded our analysis by synthesizing and testing an enlarged series of analogues (compounds **3**–**14**, Table 1) with the aim of improving Tat–TAR complex inhibition, delineating the SAR, and increasing cellular penetration.

Lead compounds for the expanded series were the piperidinyl (**1**) and piperazinyl (**2**) derivatives (Table 1), whose K_i values in the Tat–TAR competition assay were in the low micromolar range.[8] The newest 2-PQs are characterized by a monoalkyl side chain, and further structural modifications involved either the quinolone nucleus or the protonatable side chain. Modifications of the quinolone nucleus were carried out to verify whether variation of the stacking moiety could affect inhibition of the TAR complex with Tat. An initial modification, the deletion of the *N*1-alkyl substituent, yielded **3** and **4**. In these analogues, tautomeric equilibria allow formation of a 4-hydroxy group which is potentially involved in a hydrogen bond with the vicinal 5-methoxy group, thus affecting lipophilicity, cellular permeation, and activity of the derivatives. Other modifications involved the 5,7-dimethoxy substituents, while retaining a methyl group at the N1 position. For **5** and **6,** both C5 and C7 are unsubstituted, while replacement of the C5 methoxy group with a hydroxy group yielded **7** and **8**. The 5,7-dihydroxy analogues were also planned as part of the extended SAR study but could not be obtained due to instability.

In regards to the protonatable side chain, various head groups were explored by the addition of pyrrolidine, thiomorpholine, and morpholine, as in compounds **11**, **12**, and **13**. To determine the optimal length of the *O*-propyl side chain for Tat–TAR recognition, the spacing *n*-propyl chain was lengthened to obtain the *n*-butyl (**9**) and *n*-pentyl (**10**) congeners, while maintaining the piperidinyl moiety as the cationic head. Finally, for the purpose of bioisosteric substitution, the oxygen atom bridging the 2-phenyl ring to the protonatable side chain was replaced by a sulfur atom (compound **14**).

The synthesis of target compounds **3**–**6** (Scheme 1) included the preparation of amide intermediates **16** and **17**. Condensation of 3,5-dimethoxyaniline with 4-chloro-3-nitrobenzoyl chloride yielded **15,** which, upon Friedel–Crafts acylation using $SnCl_4$ as a catalyst, afforded the desired regioisomer, **16**. In contrast, **17** was prepared directly by condensation of 1-[2-(methylamino)phenyl]ethanone with 4-chloro-3-nitrobenzoyl chloride. The subsequent basic cyclization of **16** and **17** with

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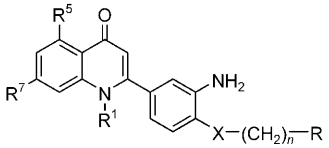
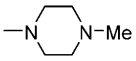
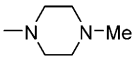
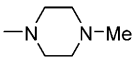
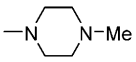
Table 1. Structure and biological evaluation of 2-phenylquinolones.



Compd	R^1	R^5	R^7	X	*n*	R	K_i [μM][a]	Uptake [%][b]
1	Me	OMe	OMe	O	3		0.81 ± 0.15	0.9[8]
2	Me	OMe	OMe	O	3		0.80 ± 0.10	0.8[8]
3	H	OMe	OMe	O	3		NA[c]	19.6
4	H	OMe	OMe	O	3		NA[c]	13.4
5	Me	H	H	O	3		0.96 ± 0.02	11.1
6	Me	H	H	O	3		0.99 ± 0.16	10.3
7	Me	OH	OMe	O	3		ND[d]	ND[d]
8	Me	OH	OMe	O	3		ND[d]	ND[d]
9	Me	OMe	OMe	O	4		0.77 ± 0.13	2.1
10	Me	OMe	OMe	O	5		0.57 ± 0.05	4.1
11	Me	OMe	OMe	O	3		1.09 ± 0.16	0.4
12	Me	OMe	OMe	O	3		0.68 ± 0.11	7.9
13	Me	OMe	OMe	O	3		0.88 ± 0.14	0.5
14	Me	OMe	OMe	S	3		0.76 ± 0.16	0.5

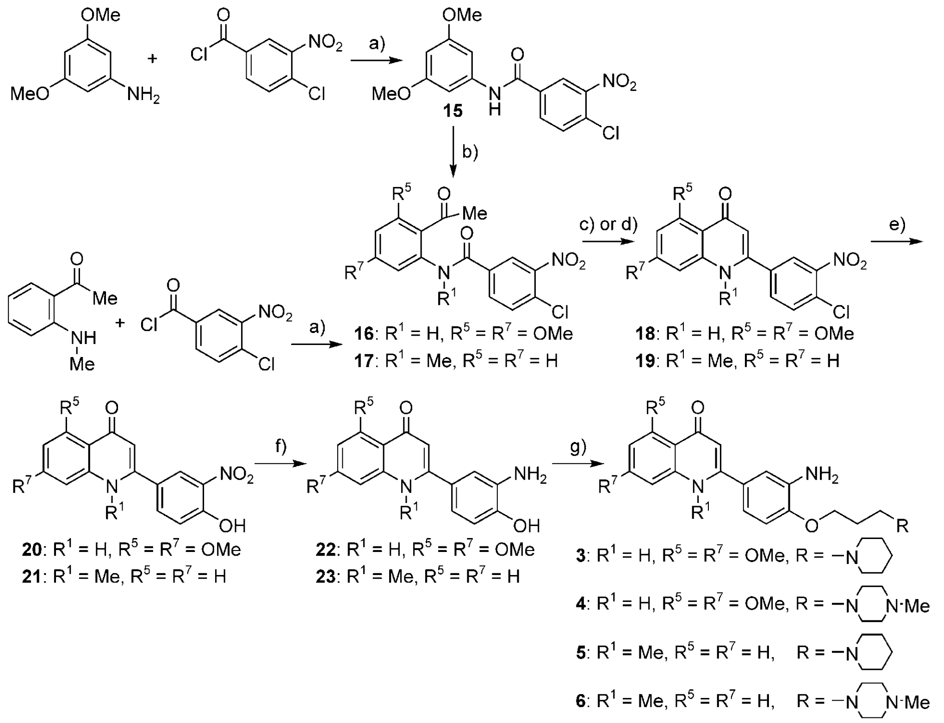
[a] From FQA experiments performed with 10 nM fluorescein–Tat (sequence 37–72) in 50 mM Tris-HCl pH 7.5, 80 mM KCl, 0.1% DMSO, 0.01% Triton X-100, 5 μg mL^{-1} BSA, and 20 nM dsTAR competitor. [b] Uptake experiments were performed after incubation for 3 h at 37 °C in Jurkat cells as described.[8] [c] Not active. [d] Not determined.

NaH and *t*BuOK, respectively, yielded **18** and **19,** which in turn afforded the 2-phenylquinolone scaffolds **22** and **23** after treatment with KOH at 90 °C, followed by catalytic reduction of the nitro group.

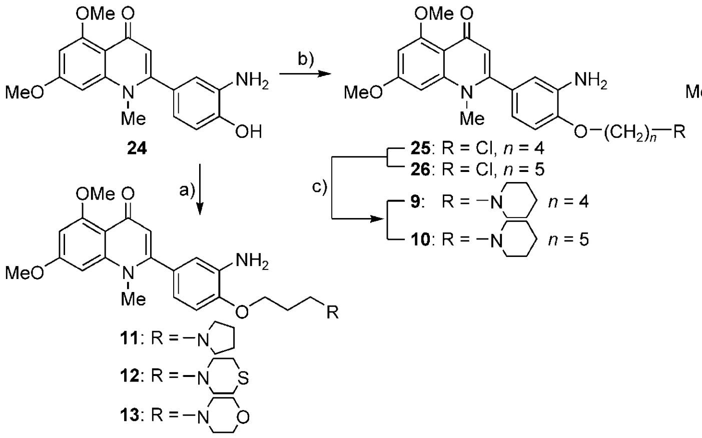
*N*1-hydrogen-*O*-alkyl derivatives **3**, **4** and *N*1-methyl-*O*-alkyl derivatives **5**, **6** were prepared by reacting synthons **22** and **23** with the protonatable side chains 1-(3-chloropropyl)piperidine[9] and 1-(3-chloropropyl)-4-methylpiperazine[10] in the presence of Cs_2CO_3. Analogously, the reaction of **24**[8] (Scheme 2) with 1-(3-chloropropyl)pyrrolidine,[11] 4-(3-chloropropyl)thiomorpholine,[12] and 4-(3-chloropropyl)morpholine[10] yielded 2-phenylquinolones **11**–**13**. It was not possible to obtain the *O*-butyl derivative **9** and the *O*-pentyl derivative **10** directly by reaction of **24**[8] with the appropriate chloroalkylpiperidines. Thus, the synthetic route first entailed the reaction of **24**[8] with 1-bromo-5-chlorobutane and 1-bromo-5-chloropentane to afford **25** and **26**, which were then subjected to nucleophilic displacement with piperidine, furnishing the corresponding target compounds, **9** and **10**.

The 5-hydroxy derivatives **7** and **8** were obtained as shown in Scheme 3. Selective de-O-methylation of the corresponding 5,7-dimethoxy derivatives **27**[8] and **28**[8] using LiCl in DMF at reflux permitted the mono-*O*-methyl derivatives to be obtained, although in low yield. Compound **14** (Scheme 4) was synthesized by combining **29**[8] with Na_2S in DMSO to yield **30**, which was successively treated with 1-(3-chloropropyl)piperidine[9] and finally reduced using iron powder. See the Supporting Information for a detailed description of synthetic procedures.

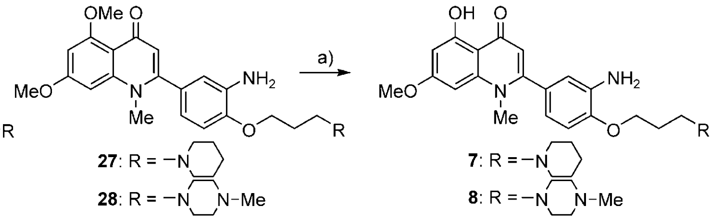
To establish the effect of these novel compounds on the Tat–TAR complex by FQA, titrations of the fluoresceinated Tat peptide with increasing quantities of dabcyl-TAR ($K_d = 6.85 \pm 1.21$ nM) were carried out in the presence of fixed concentrations of quinolones, allowing the evaluation of the inhibition



Scheme 1. Reagents and conditions: a) Et_3N, THF; b) MeCOCl, $SnCl_4$, CH_2Cl_2, 0 °C; c) NaH, THF, 50 °C; d) *t*BuOK, *t*BuOH, 30 °C; e) KOH/H_2O, DMSO, 90 °C; f) H_2, Raney Ni, DMF/2-methoxyethanol; g) $Cl(CH_2)_3R$, Cs_2CO_3, DMF, 60 °C.



Scheme 2. Reagents and conditions: a) $Cl(CH_2)_3R$, Cs_2CO_3, DMF, 60 °C; b) $Br(CH_2)_nCl$, Cs_2CO_3, DMF, 60 °C; c) piperidine, K_2CO_3, DMF, 50 °C.

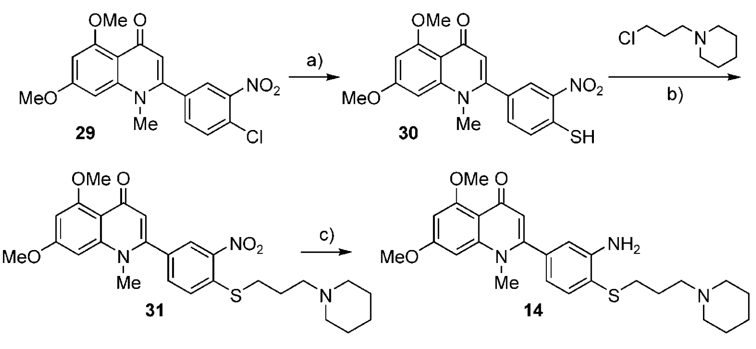


Scheme 3. Reagents and conditions: a) LiCl, DMF, reflux.

constants (K_i) for each compound (Table 1). Competition experiments for the analogues were performed essentially as described,[8] employing the 36 amino acid Tat peptide (CFTTKALGISYGRKKRRQRRRPPQGS-QTHQVSLSKQ), whose specificity for TAR resembles that of the full-length protein.[13] Lead compounds **1** and **2** were also analyzed using these more stringent conditions, exhibiting excellent in vitro activity and further validating our previous results.[8]

As clearly shown in Table 1, modifications made to the protonatable side chains led to derivatives with excellent Tat–TAR inhibition properties; an increase in in vitro activity was achieved by increasing the length of the *O*-propyl side chain. In fact, while the butyl derivative **9** has similar activity to its direct analogue **1**, a clear decrease in K_i was observed for the pentyl derivative **10**, the best of this new series, demonstrating that optimal electrostatic interaction with TAR can be achieved by adjusting the length of the protonatable side chain. The piperidine or piperazine protonatable heads (**1** and **2**, respectively), can be suitably replaced by the morpholino- (compound **13**) and thiomorpholino- (compound **12**) heterocyclic ring analogues, which exhibit similar activity. In contrast, reduction to a 5-membered ring size, as in pyrrolidine derivative **11**, is detrimental to activity. These studies also show that exchanging the ether for a thioether side chain does not substantially affect activity (compound **14**).

Contrasting results were obtained in modifications of the quinolone nucleus. In particular, the *N*1-desmethyl analogues **3** and **4** were not active in the in vitro assay ($K_i > 10$ μM), indicat-



Scheme 4. Reagents and conditions: a) $Na_2S{\cdot}9H_2O$, DMF, 0 °C; b) Cs_2CO_3, DMF, 60 °C; c) Fe, 8 N HCl, MeOH.

ing that the presence of an alkyl substituent at N1 is essential for the in vitro activity of 2-PQs. However, compounds characterized by a lack of substituents on the quinolone nucleus (compounds **5** and **6**) are still active, although they exhibit less activity than the 5,7-dimethoxy analogues. Phenols **7** and **8** yielded non-reproducible results, linked to evident spectral modifications of the stock solutions with time, and were not considered further in our studies.

Cellular uptake studies, conducted with this new series of analogues as previously described by us[8] gave divergent results: modifications at the quinolone nucleus resulted in an increase in the percentage of quinolones internalized into cells, as shown in Table 1. However, while for **3** and **4** a marked increase in uptake was made useless by loss of Tat–TAR inhibition, structural modifications as in **5** and **6** were encouraging, as they led to an increase in uptake coupled with favorable in vitro activity. As for modifications at the protonatable side chain, the increase in Tat–TAR activity was paralleled by a modest increase in uptake for only **10** and **12**.

In conclusion, we have synthesized an extended series of 2-PQs, tested their activity on the Tat–TAR complex, and obtained novel compounds that exhibit higher activity while expanding our structure–activity analysis study for this new class of inhibitors. Our results demonstrate that the achievement of in vitro activity is often not compatible with an improvement in cellular permeation parameters, in agreement with what has been reported by other authors developing small molecules directed at TAR recognition.[5,6]

Finally, to confirm our hypothesis that the development of antiviral agents can be achieved by preliminary screening of activity toward isolated targets coupled with uptake analysis, the anti-HIV-1 activity and cytotoxicity for these newly synthesized compounds were studied in the Jurkat lymphoblastoid T cell line. As expected, the compounds exhibiting null or very marginal uptake do not show any cytotoxicity or activity in de novo infected cells, similar to what was found for lead compounds **1** and **2**.[8] Modifications at the quinolone ring, as in **3** and **4**, are incompatible with Tat–TAR inhibition, although they lead to a distinct increase in uptake and result in appreciable cytotoxicity (EC_{50} = 51.3 and 64.3 μM, respectively). Derivatives **5** and **6**, bearing no substituents at the 5- and 7-positions of the quinolone nucleus, gave encouraging results, exhibiting a residual dose-dependent anti-HIV-1 activity (IC_{50} = 75 and 85 μM, respectively) coupled with lower or null cytotoxicity (CC_{50} = 90 and > 100 μM, respectively). For **10**, the compromise between good activity in vitro and marginal increase in uptake relative to the reference compound **1** led to an observed dose-dependent antiviral activity. Unfortunately, an increase in cytotoxicity was observed at similar concentrations. Clearly, uptake must be further improved, perhaps by rendering the test drugs more hydrophobic, although extension of the side chain beyond five rotatable bonds is not advisable, according to Lipinski's rule of five. Additional studies are warranted to address the issue of activity versus toxicity in order to find a viable therapeutic window, as seen with WM5, a previously investigated and effective aminoquinolone targeted at Tat–TAR and endowed with remarkable antiviral activity.[14]

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Keywords: 2-phenylquinolones · antiviral agents · fluorescence quenching assay · medicinal chemistry · Tat–TAR inhibitors

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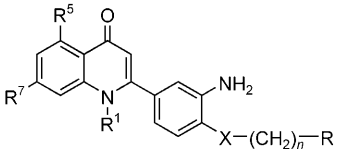
COMMUNICATIONS

Novel 2-phenylquinolones aimed at the Tat–TAR complex were synthesized and tested. Derivatives characterized by precise modifications of the quinolone nucleus and to the side chain of the 2-phenyl ring allowed a thorough structure–activity study, confirming 2-phenylquinolone as a suitable scaffold for inhibition of the Tat–TAR interaction.



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