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SYNTHESIS OF HEXAHYDROQUINOLINE-3-CARBOXAMIDE DERIVATIVES AND THEIR HIV-1 ANTIVIRAL ACTIVITY

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Abstract – Computational modelling was used to identify scaffolds with the potential to disrupt the interaction between HIV-1 integrase and lens epithelium-derived growth factor (HIV-1-IN-LEDGF/p75). Virtual screening of commercial library collections led to the identification of *N*-(4-chlorophenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide as a promising candidate. The synthesis of this compound and its derivatives involved the reaction of the corresponding carboxylic acid derivatives with aniline in the presence of coupling agent carbonyldiimidazole (CDI). This gave rise to *N*-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamides in yields of 71-85%. These compounds were found to be non-toxic in an MT4 cell line at 100 μM and were subsequently evaluated for antiviral activity in infected MT4 cells at a single dose concentration of 100 μM.

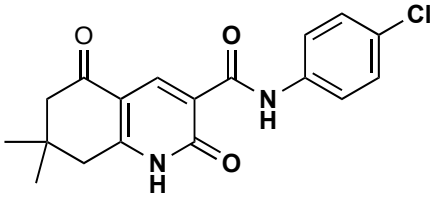
INTRODUCTION

One of the ongoing interests in our laboratories is the identification and development of small bioactive molecules able to inhibit different stages of the HIV life cycle. Of particular interest is the disruption of interactions between HIV-1-proteins and host proteins.[1-6] Computational modelling was used as a strategy to identify scaffolds as the starting point to derive small molecules that could possibly disrupt interactions between HIV-1 integrase (HIV-1-IN) and host lens epithelium-derived growth factor (LEDGF/p75) protein. Virtual screening of the ChemBridge DIVERSet library of compounds, consisting of 20,000

diverse and unique drug-like structures (ChemBridge Corporation, San Diego, CA 92121 USA) was performed using Schrödinger Maestro 11.2 software (Schrödinger Release 2017-2: Maestro version 11.2, Schrödinger, LLC, New. York, NY, 2017). Two compounds were used as references, Mut101, which is an allosteric inhibitor of the HIV-1-IN-LEDGF/p75 interaction and CX05168, an inhibitor of the HIV-1-IN-LEDGF/p75 interaction and a modulator of IN multimerization. Mut101 shows significant anti-HIV-1 activity at the integration and post-integration steps during the viral replication cycle. Mut101 binds a unique target on IN and is also capable of blocking HIV-1 replication in virus-producer cells in post-integration as an active antiretroviral agent.[7] CX05168 was designed from a series of 2-(quinolin-3-yl)acetic acid derivatives developed as inhibitors of the HIV-1-IN-LEDGF/p75 interaction. It inhibits HIV-1 replication by blocking the integration step and modulating IN multimerization.[8,9]

Virtual docking of the above-mentioned ChemBridge library led to the identification of potential active ligands based on docking scores, calculated ΔG energy values, and protein-ligand interactions. Ligands ranked according to ΔG were examined for their modelled interactions with key amino acids of the HIV-IN-dimer, and further scrutinized for their amenability to derivatization. The remaining ligands were subjected to the next selection criteria (novelty, possible chemical routes, chemical properties, other potential applications, etc.) in order to establish the best possible starting point for the synthetic work, followed by biological evaluation of the newly synthesized compounds.

From the ChemBridge library, *N*-(4-chlorophenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (Figure 1) was chosen due to its promising modelling rank, presence of functional groups amenable to protein interaction and ease of derivatization with available reagents in our laboratories.



3a

Figure 1. Selected starting point: *N*-(4-chlorophenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide **3a** (ChemBridge compound ID 9149322)

Compounds containing a quinolinone moiety are commonly evaluated in drug discovery efforts because of the wide range of pharmacological activities they exhibit. These types of compounds remain attractive as potential scaffolds for incorporation into pharmaceutical agents as they display properties such as antimicrobial[10,11] antiproliferative,[12] anticancer,[13,14] antitubercular,[15] and antioxidant[16] activities. Selected

partially saturated quinoline-carboxamides, -carboxylates and -carboxylic acids exhibiting biological activity are presented in Figure 2.

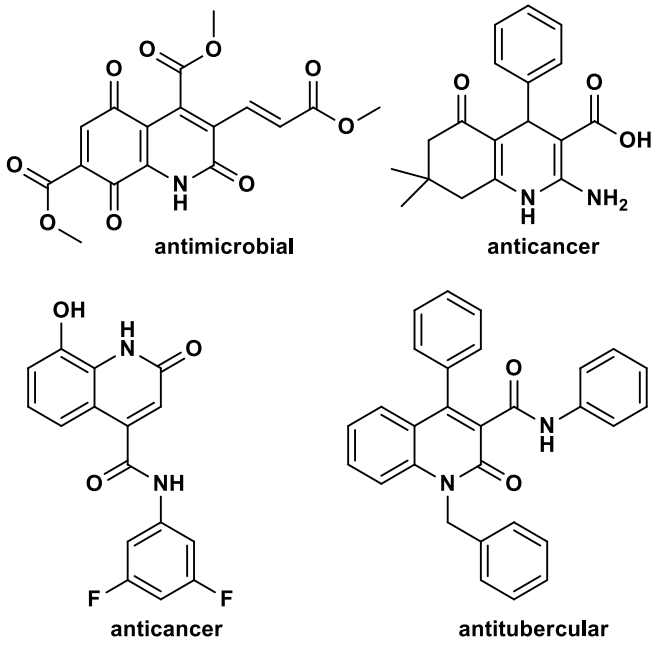
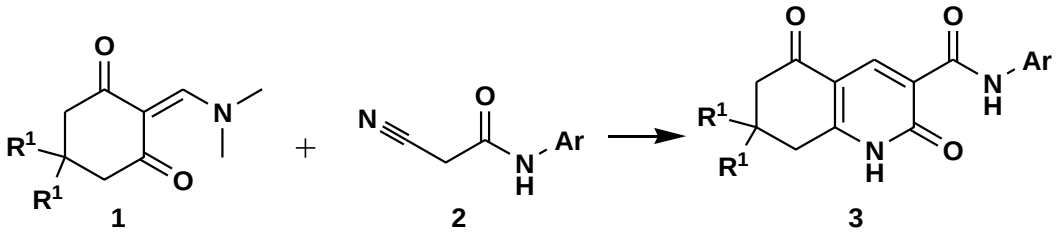


Figure 2. Quinolinone derivatives showing biological activity

The preparation of hexahydroquinoline derivatives continues to be of interest to synthetic organic chemists.[17-19] These compounds are commonly synthesized by the reaction of 1,3-cyclohexanediones, aldehydes and ethyl acetoacetate in the presence of ammonia using acetic acid or alcohol as solvent.[20] Gorobets and co-workers[21] prepared hexahydroquinoline carboxyl-derived scaffolds (Scheme 1) from the reaction of 2-((dimethylamino)methylene)cyclohexane-1,3-dione derivatives **1**, prepared from the dione and dimethylformamide dimethyl acetal (DMFDMA), and *N*1-substituted 2-cyanoacetamides **2** to form hexahydroquinoline derivatives **3**.



Scheme 1. Existing route for the synthesis of hexahydroquinolines

A similar route was reported by Dzhavakhishvili and co-workers who replaced the cyanoacetamide with a thiazole-substituted acetonitrile.[22] Fossa and co-workers, using methyl cyanoacetate in a similar reaction, synthesized and evaluated the cardiotonic effects of a family of functionalised hexahydro-dioxoquinoline carboxylates on guinea pig myocardial preparations.[23]

RESULTS AND DISCUSSION

Molecular Modelling

The HIV-1-IN-LEDGF/p75 structure used for modelling was obtained from the Protein Data Bank (PDB) as the X-ray crystal structure code 2B4J.[24] Using "Protein Preparation Wizard" from Schrödinger Maestro 11.2, chain C and D (LEDGF/p75) were removed to obtain HIV-IN (Chain A and B). Water molecules and hydrogens were removed and locations of newly-assigned hydrogens were minimized using the OPLS force field.[25] The missing residues and loop regions of chain A and B were constructed and the cap terminals were considered.[26] The Receptor Grid Generation process was followed to modify and detect the binding site for docking purposes.[27]

The molecular docking was performed to estimate the binding affinity of the compounds (ligands) with the receptor (HIV-IN) dimer. The compounds, together with reference compounds Mut101 and CX05168, were positioned in the same binding sites of the HIV-1-IN where LEDGF binds using the "ligand docking" protocol using standard precision (SP) followed by extra precision (XP) from the Schrödinger Maestro 11.2 suite.

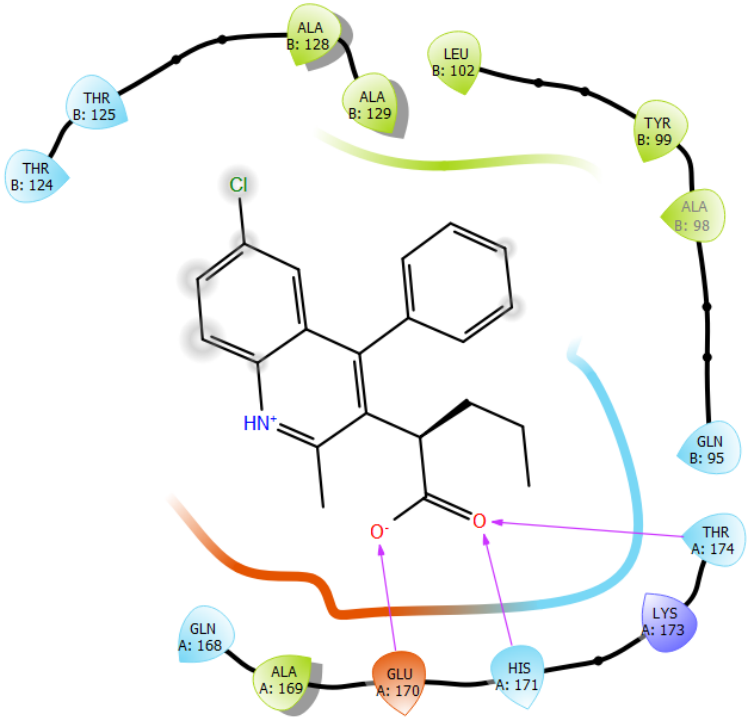


Figure 3. 2D binding mode of CX05168 in the LEDGF/p75 binding site of HIV-1-IN

During docking the program takes ligand-receptor poses as input and provides rankings or the binding affinity of the pose. These poses gave ligand docking scores, glide scores and glide emodel scores and then their binding energy was calculated using the MM-GBSA method. The HIV-IN dimer interacts with LEDGF/p75 using a hydrogen-bond network at the integrase-binding domain (IBD) from the specific

backbone conformation of amino acid residues 168-171 and the hydrophobic patch on the side chain residues of LEDGF/p75 from Ile365, Phe406, and Val408.[28] The CX05168 carboxylic acid group interacts with HIV-IN residue Glu170 through a hydrogen-bond and there are interactions of the carbonyl group oxygen atom with both His171 and Thr174 as shown in Figure 3.

The other reference compound used, Mut101, contains a carboxylic acid group that interacts with HIV-IN residues His171 and Thr174 through a hydrogen-bond, while Glu170 interacts *via* oxygen of the carbonyl group (Figure 4).

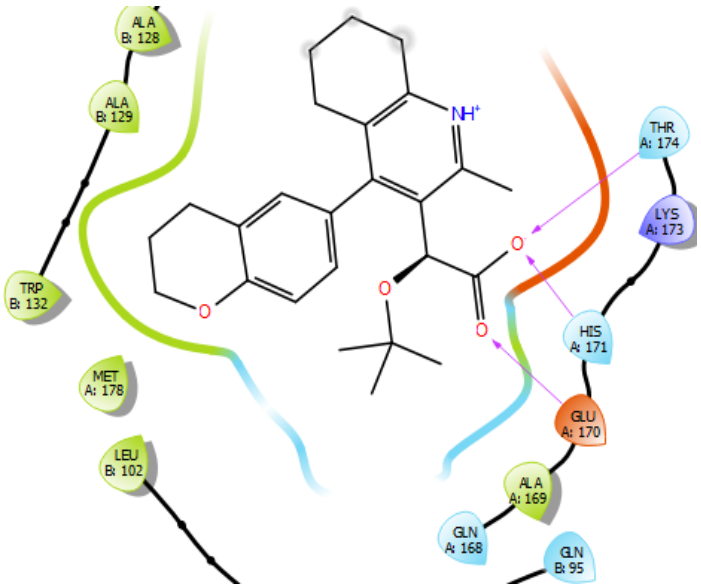


Figure 4. 2D binding mode of Mut101 in the LEDGF/p75 binding site of HIV-1-IN

Based on the virtual screening data outcomes obtained, possible facile and efficient synthetic routes and chemical reagent availability led us to choose one of the potential ChemBridge ligands containing the hexahydroquinoline scaffold. The predicted binding model at the HIV-1-IN dimer interface shows the interaction of ChemBridge ligand *N*-(4-chlorophenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide **3a** (Figure 5) interacting with amino acid residues Glu170 and Thr174 *via* oxygen from the carbonyl groups and pi-interactions with His171.

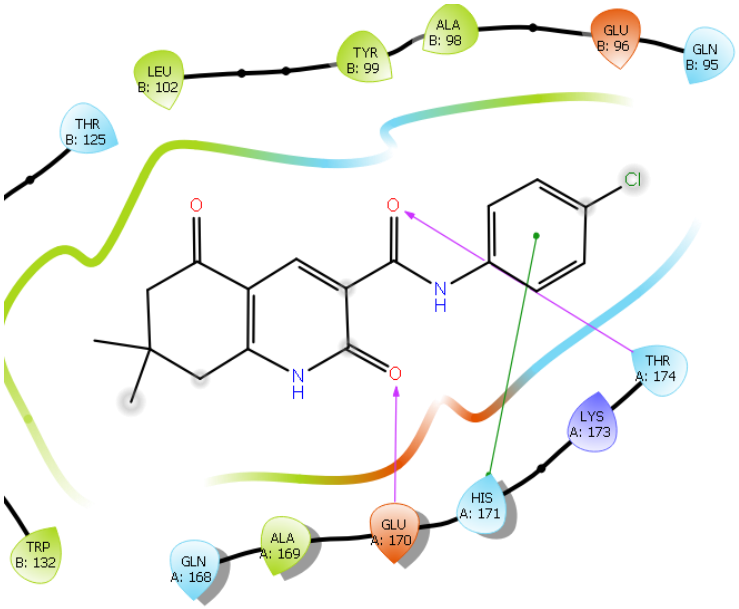


Figure 5. 2D binding mode of ChemBridge ligand **3a** in the LEDGF/p75 binding site of HIV-1-IN

The docking scores of the reference compounds were -6.176 (Mut101) and -5.329 (CX05168). The ChemBridge ligand **3a** gave a poorer docking score of -4.333. The Glide Emodel, Glide docking and MMGBSA were obtained after subjecting the ligands to the "MMGBSA" protocol. The interactions of the ChemBridge ligand **3a**, CX05168 and Mut101 with HIV-IN are represented in Table 1. The residues Glu170, His171 and Thr174 from HIV-IN were found to interact with the ChemBridge ligand, CX05168 and Mut101.

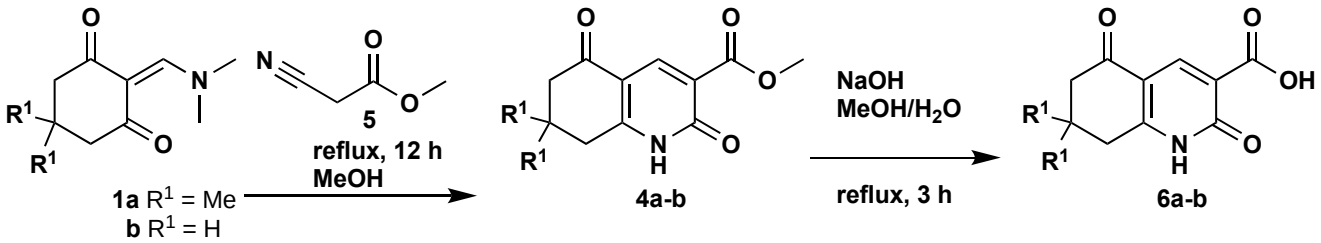
Table 1. Molecular docking results of ChemBridge ligand, CX04328 and Mut101

ENTRY	Docking score (Kcal/mol)	Glide score (Kcal/mol)	XP G score	Glide emodel (Kcal/mol))	MMGBSA (Kcal/mol)	Hydrogen bond interactions (Distance Å)	Interactions with Oxygen (Distance Å)
ChemBridge ligand (3a)	-4.333	-4.333	-4.333	-58.112	-52.523		Glu170, Thr174 (2.05, 2.17)
CX05168	-5.329	-5.339	-5.339	-52.558	-51.171	Glu170 (1.84)	His171, Thr174 (1.82, 2.14)
Mut101	-6.176	-6.621	-6.621	-63.191	-59.389	His171, Thr174 (1.87, 2.14)	Glu170 (1.87)

Chemical Synthesis

The first approach applied to the synthesis of *N*-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide derivatives was a method adopted from Gorobets and co-workers.[21] They reacted compound **1** (R^1=H) and compound **2** (Ar=Ph) in the presence of a few drops of piperidine as catalyst in *iso*-propanol as a solvent (Scheme 1) to obtain an 81% yield of the corresponding hexahydroquinoline-3-carboxamide product **3** (Scheme 1). However, in our hands this method resulted in very low to trace yields over a range of 2-cyanoacetamides **2** substituted with differently functionalised phenyl rings.

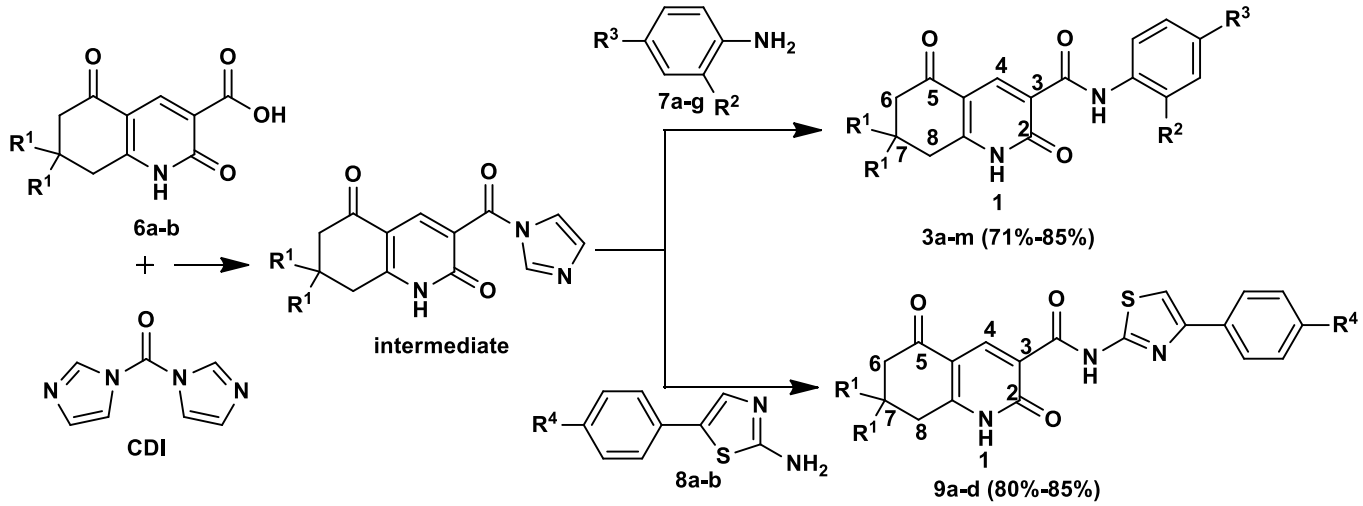
In order to execute the synthesis of hexahydroquinoline-3-carboxamides and improve the yields we used a different route by first preparing the hexahydroquinoline-3-carboxylate ester derivatives **4a-b** from diones **1a-b** and methyl cyanoacetate **5**. The ester derivatives **4a-b** were subsequently hydrolyzed to the corresponding carboxylic acid derivatives **6a-b** (Scheme 2). Reaction of 2-((dimethylamino)methylene)cyclohexane-1,3-dione derivatives **1a-b** with methyl cyanoacetate **5** under methanol reflux conditions gave rise to cyclized hexahydroquinoline-3-carboxylate derivatives **4a-b** in good yields (87-90%) after 12 h, as previously reported by Fossa and co-workers.[12] Hydrolysis of these compounds was achieved under reflux conditions using methanol and water as co-solvents (1:1) in the presence of sodium hydroxide pellets for 3 h. After cooling, 1M HCl was introduced dropwise to precipitate carboxylic acid derivatives **6a-b** in good yields of 79-85% (Scheme 2).



Scheme 2. Preparation of hexahydroquinoline-3-carboxylic acid derivatives **6a-b**

Compounds **6a-b** were then treated with appropriate anilines **7a-g** using carbonyldiimidazole (CDI) as a mediator (Scheme 3). A range of variously substituted anilines containing electron-releasing and electron-withdrawing groups was used to explore the substrate scope of the reaction and to explore the effect of different substituents on the antiviral activity. CDI remains a useful coupling reagent that promotes one-pot amide formation allowing the formation of functionalized and diverse scaffolds.[29] The key step of this reaction is the formation of an activated acyl carboxy-imidazole intermediate which reacts with the desired amine to give the amide product (Scheme 3). The reactions were carried out by heating

compound **6** with CDI under reflux conditions in acetonitrile for 2 h. After cooling to room temperature, an aniline derivative **7** was introduced and the reaction was allowed to proceed at room temperature for 24 h to give the desired products **3**. The scope of the reaction was further increased by using thiazolamine derivatives **8a-b** in reaction with **6a-b** to obtain derivatives **9a-d** (Scheme 3). Thiazole derivatives were chosen for the synthesis because of the range of biological activity displayed by this class of compounds.[30]



Scheme 3. One-pot amide preparation using CDI

Carboxamide derivatives **3** and **9** were isolated in good yields of 71-86%, with some variation being observed based on the nature of the substituent groups on the aniline **7** and 4-phenylthiazol-2-amine derivatives **8** (Table 2). Generally, using anilines **7a-g** and 4-phenylthiazol-2-amine derivatives **8a-b** as primary amine sources gave good yields of the corresponding amides. The highest yield was obtained for **3h** with a *para*-bromine substituent on the aniline, giving 86%. Compound **3i**, containing a *para*-methyl substituent on the aniline, gave the lowest yield of 71%. Products **9a-d**, derived from 4-phenylthiazol-2-amine derivatives **8a-b**, were obtained in good yields of 80-85%. Unfortunately when di-halo substituted anilines (2,4-dibromoaniline, 2,4-difluoroaniline, 2,4-dichloroaniline) and 2-nitroaniline were reacted with compound **6** using CDI mediation, the reactions were not successful. It is probable that anilines with the strongly electron-withdrawing nitro group or two electron-withdrawing halo-groups are insufficiently nucleophilic to participate in the reaction.

Table 2. Isolated yields of **3a-m** and **9a-d**

	R^1	R^2	R^3	R^4	Yield% (24 h)
3a	Me	H	Cl	-	82
3b	Me	H	Br	-	72
3c	Me	H	Me	-	76
3d	Me	Me	Me	-	78
3e	Me	OH	H	-	80
3f	Me	OMe	OMe	-	82
3g	H	H	Cl	-	80
3h	H	H	Br	-	86
3i	H	H	Me	-	71
3j	H	OH	H	-	76
3k	H	OMe	OMe	-	83
3l	Me	Me	H	-	79
3m	H	H	H	-	81
9a	Me	-	-	F	80
9b	Me	-	-	OMe	82
9c	H	-	-	F	83
9d	H	-	-	OMe	85

The structures of the synthesized compounds were confirmed by mass spectrometry, NMR spectroscopy and FTIR spectroscopy (see supplementary data). By way of example we describe the characterization of compound **3a**. The ^{1}H NMR spectrum showed broad singlets for the two NH groups at δ = 13.15 and δ = 11.70, one singlet from a CH group for H-4 at 8.71 ppm, two doublets from the aromatic ring at δ = 7.74 (J = 8.4 Hz) and δ = 7.42 (J = 8 Hz), two CH_2 singlets for H-6 and H-8 at δ = 2.85 and δ = 2.44 and one singlet corresponding to two Me groups at δ = 1.04. The ^{13}C NMR spectrum of **3a** showed 14 distinct resonances due to two methyl groups overlapping with each other and one CH_2 group not being clearly visible because of overlapping with the DMSO-d_6 residual peak (see DEPT 135 in supplementary section); similar patterns of overlap with DMSO signals were also observed for compounds **3b-f**, **3l** and **9a-b**. The mass spectrum of **3a** showed the protonated molecular ion $[M+H]^+$ peak at *m/z* 345.1012 which is consistent with the mass of the proposed product. The FTIR spectrum displayed characteristic absorption bands for carbonyl groups at 1679 cm^{-1}, 1621 cm^{-1} and 1598 cm^{-1} and for the amine group at 2971 cm^{-1} and 2864 cm^{-1}.

Cytotoxicity and Antiviral Studies

Virtual docking of the synthesized hexahydroquinoline compounds was performed and ADME-drug-likeness parameters calculated, all falling within acceptable values (See Supporting Information). Unfortunately, preliminary HIV-1 IN - LEDGF AlphaScreen^{TM} assays that are able to measure disruption of protein-protein interactions showed that none of the synthesized compounds (**3** and **9**) exhibited any significant activity as direct disruptors of the HIV-1-IN-LEDGF/p75 interaction. Nonetheless, the compounds were further subjected to screening in a cell-based anti-HIV assay. Compounds **3a-m** and **9a-d** were subjected to toxicity evaluation in the MT4 cell line at 100 μM. Auranofin was used as a cytotoxic control compound as there is documented evidence that it is cytotoxic to cancer cell lines,[31,32] and DMSO was used as a background control. All the compounds were found to cause cell reduction at CC_{50} values above 200 μM as recorded in Table 3, and were considered to be relatively non-toxic.

The hexahydroquinoline-3-carboxamide compounds **3a-m** and **9a-d** were then evaluated for their antiviral activities in infected MT4 cells. The tests were carried out in a cell-based assay and each compound was analysed at a single dose of 100 μM using FDA-approved integrase inhibitors dolutegravir and raltegravir as control compounds. The non-toxic compounds **3a-m** and **9a-d** showed antiviral activity of between 20% and 38%; except for compounds **3a** and **3h** which showed activity of less than 10% as recorded in Table 3. The highest antiviral activity (38%) was observed for **3m** which contains hydrogen at R^1, R^2 and R^3. Compound **3c**, with a methyl group at R^1 and R^3, showed antiviral activity of 36%, as did compound **3d**, with a methyl group at R^1, R^2 and R^3. The antiviral activity of compound **3i**, with hydrogen at R^1 and R^2 and methyl at R^3, was found to be 29%. Thus, compounds containing hydrogen or the activating methyl or methoxy groups at position R^3 (**3c**, **3d**, **3f**, **3i**, **3k** and **3m**) seem to show slightly better activity compared to compounds **3a**, **3b**, **3g** and **3h**, which contain deactivating groups such as chlorine and bromine. In addition, a hydroxyl group at position R^2 also seemed favourable, with compound **3e** showing 36% activity. The exception was compound **3j**, with an activating hydroxyl group at position R^2, which showed only 21% antiviral activity. When anilines were replaced by thiazolamines **8a-b** to give compounds **9a-d**, it was observed that **9a** and **9c**, both containing fluorine at position R^4, gave better antiviral activities of 33% and 35%, respectively, compared to **9b** (30%) and **9d** (22%) containing a methoxy group at position R^4. All of the hexahydroquinoline-3-carboxamide compounds **3a-m** and **9a-d** prepared showed less than 50% antiviral activity at a single dose concentration of 100 μM.

Table 3. Cytotoxicity and antiviral activity of **3a-m** and **9a-d**

Compound	Cytotoxicity CC_{50} (μM)	% Antiviral activity (infected MT4)
3a	> 200	8
3b	> 200	24
3c	> 200	36
3d	> 200	36
3e	> 200	36
3f	> 200	20
3g	> 200	25
3h	> 200	0.4
3i	> 200	29
3j	> 200	21
3k	> 200	32
3l	> 200	28
3m	> 200	38
9a	> 200	33
9b	> 200	30
9c	> 200	35
9d	> 200	22
Assay controls		
Auranofin	1.2	N.D.[a]
Dolutegravir	N.D.[b]	95
Raltegravir	N.D.[b]	97
CX05168	69.6	99
Mut101	N.D.[c]	99

CC_{50} - concentration of compounds that causes 50% reduction of cell growth

N.D. Not determined in this study; [a] Auranofin is known to be cytotoxic to the cell lines used here[31,32] and the resulting cell death would prevent any viral proliferation so auranofin was not included in the antiviral assay; [b] FDA approved anti-HIV drugs dolutegravir and raltegravir were used as controls for the antiviral assay only and were not included in the cytotoxicity study done here but their cytotoxicity in MT4 cells has been determined previously[33,34]; [c] CX05168 and

Mut101 were included due to their known inhibition of the LEDGF–HIV-IN interaction. The cytotoxicity of Mut101 has been reported previously (Mut10 CC_{50} > 50μM).[7]

CONCLUSIONS

A series of novel *N*-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide derivatives **3a-m** and **9a-d** were obtained from a CDI-mediated reaction of the corresponding carboxylic acids **6a-b** and aniline (**7a-g**) or thiazole (**8a-b**) derivatives in good yields of 71-86%. Of the reported compounds, only **3k** and **3m** have been reported previously by Gorobets and co-workers using a different synthetic method.[21] This set of compounds were found to be relatively non-toxic and were evaluated for antiviral activity against HIV-1. Apart from compounds **3a** and **3h**, which showed no activity, all other compounds (**3b-g**, **3i-m** and **9a-d**) showed antiviral inhibition percentage activities of between 20% and 38%.

EXPERIMENTAL

Chemical Synthesis

All commercially available reagents were supplied by Sigma-Aldrich and were used without further purification. Dry solvents were used directly from an LC-Tech SP-1 Solvent Purification System stored under argon. All solvents used for chromatographic purposes were supplied by Sigma-Aldrich and/or RadChem and were used without further purification.

Spectroscopic and physical data

NMR spectra were recorded at 298 K on a 400 MHz Bruker Avance instrument. Chemical shifts are reported in ppm, and are referenced internally to residual solvent resonances [7.26 ($CDCl_3$) and 2.50 (DMSO-d_6) for ^{1}H NMR; 77.16 ($CDCl_3$) 40.45 (DMSO-d_6) for ^{13}C NMR]. For mass spectrometry (LC-MS/MS) high-resolution mass spectra were obtained. Fourier infrared spectra (FTIR) were recorded neat using a PerkinElmer FT-IR Spectrometer Spectrum Two (ATR). All signals are reported on the wavenumber scale (ν/cm^{-1}).

General method for the synthesis of methyl 7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylate derivatives (4a-b)

In a 250 mL round-bottomed flask equipped with a stirrer bar, methyl cyanoacetate (**5**) (10 eq.) was added to a solution of 2-(dimethylaminomethylene)cyclohexane-1,3-dione derivative (**1**) (1 eq.) in anhydrous MeOH (60 mL). The reaction mixture was heated under reflux for 12 h while monitoring by TLC. The crude solid product was collected by filtration, washed thoroughly with Et_2O (15 mL) and recrystallized

from EtOH (15 mL) to obtain pure product.

Methyl 7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylate (4a)

2-((Dimethylamino)methylene)-5,5-dimethylcyclohexane-1,3-dione (**1a**) (10.17 g, 52 mmol) and methyl cyanoacetate (**5**) (51.53 g, 520 mmol) were reacted to give methyl 7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylate (**4a**). Physical characteristics: yellow solid powder; Yield: 11.31 g, 87%; Mp: 244-246 ºC (Lit.[12] 243-245 ºC); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.55 (1H, s, NH) 8.39 (1H, s, CH), 3.77 (3H, s, CH_3-O), 2.77 (H-6, 2H, s, CH_2), 2.39 (H-8, 2H, s, CH_2), 1.03 (6H, s, 2 × CH_3); ^{13}C NMR (101 MHz, DMSO-d_6) δ 192.9, 164.4, 160.3 (3 × C=O), 159.7 (Ar-C), 141.0 (C-4), 117.4 (Ar-C), 110.9 (Ar-C), 51.8 (O-CH_3), 49.9 (C-6, CH_2), 32.5 (C-7), 27.7 (2 × CH_3).

Methyl 2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylate (4b)

2-((Dimethylamino)methylene)cyclohexane-1,3-dione (**1b**) (14.36 g, 85.9 mmol) and methyl cyanoacetate (**5**) (85.12 g, 859 mmol) were reacted to give methyl 2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylate (**4b**); Physical characteristics: yellow solid powder; Yield: 17.21 g, 90%; Mp: 273-275 ºC (Lit.[12] 272-274 ºC); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.58 (1H, s, NH), 8.39 (1H, s, CH), 3.76 (3H, s, CH_3-O), 2.87 (H-6, 2H, t, J = 6.0 Hz, CH_2), 2.47 (H-8, 2H, t, J = 6.0 Hz, CH_2), 2.06-1.99 (H-7, 2H, m, CH_2); ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.1, 164.4, 161.9 (3 x C=O), 159.2 (Ar-C), 141.4 (C-H), 117.5 (Ar-C), 111.9 (Ar-C), 51.8 (O-CH_3), 38.9 (C-6, CH_2), 26.6 (C-8, CH_2), 20.4 (C-7, CH_2).

General method for the synthesis of 2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid derivatives (6a-b).

In a 250 mL round-bottomed flask equipped with a stirrer bar, containing a solution of methyl 2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylate derivative (**4a**) (1 eq.) in MeOH (25 mL) and water (25 mL) was added sodium hydroxide (4 eq.). The resulting reaction mixture was then heated under reflux for 3 h while monitoring by TLC. The reaction was then cooled to 0 ºC and hydrochloric acid (1 M) was slowly added until the solution became acidic. EtOAc (20 mL) and water (20 mL) were added and the aqueous layer was extracted with EtOAc (2 x 15 mL). The combined organic layers were concentrated and dried *in vacuo* to obtain the solid product.

7,7-Dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (6a).

Methyl 7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylate **4a** (10 g, 40 mmol) and sodium hydroxide (6.4 g, 160 mmol); Physical characteristics: white solid powder; Yield: 8.01 g, 85%; Mp: 298-300 ºC (Lit.[35] 299 ºC); ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.72 (1H, br s, OH), 13.49 (1H, br s, NH), 8.55 (1H, s, CH), 2.86 (2H, s, CH_2), 2.45 (2H, s, CH_2), 1.04 (6H, s, CH_3); ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.1 (C=O), 164.9 (C=O), 164.4 (C=O), 160.3 (Ar-C), 141.5 (C-H), 115.4 (Ar-C), 113.8 (Ar-C), 49.9 (C-6, CH_2), 32.6 (C-7), 27.7 (2 x CH_3).

2,5-Dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (6b).

Methyl 2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylate **4b** (10 g, 45 mmol) and sodium hydroxide (9.04 g, 226 mmol); Physical characteristics: Brown solid powder; Yield: 8.01 g, 79%; Mp: 297-299 ºC (Lit.[35] 298 ºC); ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.75 (1H, br s, OH), 13.49 (1H, br s, N-H) 8.57 (1H, s, CH), 2.97 (2H, t, J = 6.0 Hz, CH_2), 2.54 (2H, t, J = 6.4 Hz, CH_2), 2.11-2.05 (2H, m, CH_2); ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.3 (C=O), 164.5 (C=O), 164.40 (C=O), 162.0 (Ar-C), 141.7 (C-H), 115.3 (Ar-C), 114.7 (Ar-C), 36.5 (CH_2), 26.8 (CH_2), 20.3 (CH_2).

General method for the synthesis of 5-phenylthiazol-2-amine derivatives 8a-b

To a solution of THF (30 mL) and phenacyl halide (1 eq.) in a round-bottomed flask was added thiourea (1 eq.) and the mixture was stirred at room temperature for 0.5 h. The reaction mixture was filtered and washed with THF and water and the precipitate was dried to obtain a solid product which was recrystallized from EtOH.[36]

5-(4-Fluorophenyl)thiazol-2-amine (8a).

2-Chloro-4′-fluoroacetophenone (3 g, 17 mmol) and thiourea (1.3 g, 17 mmol); Physical characteristics: White crystalline solid; Yield: 2.6 g, 79%; Mp: 99-101 ºC (Lit.[37] 102-103 ºC); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.86-7.83 (2H, m, ArH), 7.35-7.31 (2H, m, ArH), 7.21 (1H, s, ArH), 4.03 (2H, br s, NH_2), ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.3 (Ar-C), 162.4 (d, J_{CF} = 246.5 Hz, Ar-C), 138.4 (Ar-C), 128.0 (d, J_{CF} = 8 Hz, Ar-CH), 125.6 (d, J_{CF} = 4.0 Hz, Ar-C), 116.0 (d, J_{CF} = 22.1 Hz, Ar-CH), 102.4 (Ar-CH).

5-(4-Methoxyphenyl)thiazol-2-amine (8b). 2-Bromo-4′-methoxyacetophenone (5.12 g, 22 mmol) and thiourea (2.02 g, 23 mmol); Physical characteristics: White solid crystals; Yield: 3.94 g, 86%; Mp: 202-204 ºC (Lit.[38] 200-203 ºC); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.66 (2H, d, J = 8.4 Hz, ArH), 7.09 (1H, s, Ar-CH), 7.04 (2H, d, J = 8.8 Hz, ArH), 3.97 (2H, br s, NH_2), 3.79 (3H, s, OCH_3); ^{13}C NMR (101 MHz, DMSO-d_6) δ 170.3 (Ar-C), 160.2 (Ar-C), 138.9 (Ar-C), 127.4(Ar-CH), 121.3 (Ar-CH), 114.5 (Ar-C), 100.8 (Ar-CH).

General method for the synthesis of 7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide derivatives using CDI as a coupling reagent (3a-m, 9a-d).

In a two-necked 100 mL round-bottomed flask equipped with a stirrer bar a solution of the appropriate 2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid derivative **6** (1 eq.) and carbonyldiimidazole (CDI, 1.3 eq.) in acetonitrile (MeCN, 25 mL) at 50 ºC were reacted for 2 h under argon. The reaction was cooled to room temperature (rt) and aniline (**7**) or thiazol-2-amine derivative (**8**) (1 eq.) was added to the solution under inert argon conditions. The resulting reaction mixture was stirred at rt for 24 h while monitoring by TLC. The precipitated solid was isolated by filtration and washed with deionised water (8 mL) and heptane (8 mL), and dried to obtain the pure product.

N-(4-Chlorophenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (3a).

7,7-Dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**6a**) (0.51 g, 2.17 mmol), CDI (0.46 g, 2.82 mmol) and 4-chloroaniline (**7a**) (0.28 g, 2.17 mmol); Physical characteristics: white solid powder; Yield: 0.61 g, 82%; Mp: 347-349 ºC; R_f: 0.7, hexane/EtOAc (1:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.15 (1H, s, N-H), 11.70 (1H, s, Ar-N-H), 8.71 (1H, s, CH), 7.73 (2H, d, *J* = 8.4 Hz, ArH), 7.41 (2H, d, *J* = 8 Hz, ArH), 2.85 (2H, s, CH_2), 2.44 (2H, s, CH_2), 1.04 (6H, s, 2 x CH_3); ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.2, 163.4, 161.1 (3 x C=O), 159.2 (Ar-C), 140.2 (Ar-CH), 137.1 (Ar-C), 128.9 (Ar-CH), 127.5 (Ar-C), 121.4 (Ar-CH), 118.1 (Ar-C), 113.0 (Ar-C), 49.9 (CH_2), 32.6 (C-7), 27.7 (2 x CH_3); FT-IR ν_{max}/cm^{-1}: 2971, 2864, 1679, 1621, 1598, 1550, 1485, 1383, 1302, 1234; HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calculated for $C_{18}H_{18}ClN_2O_3^+$ 345.1000, found 345.1012.

N-(4-Bromophenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (3b). 7,7-Dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**6a**) (0.15 g, 0.64 mmol), CDI (0.13 g, 0.83 mmol) and 4-bromoaniline (**7b**) (0.11 g, 0.64 mmol): Physical characteristics: white solid powder; Yield: 0.18 g, 72%, Mp: 234-236 ºC; R_f: 0.7, hexane/EtOAc (1:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.14 (1H, br s, N-H), 11.71 (1H, s, Ar-N-H), 8.72 (1H, s, CH), 7.68 (2H, d, *J* = 8.8 Hz, ArH), 7.54 (2H, d, *J* = 8.8 Hz, ArH), 2.85 (2H, s, CH_2), 2.44 (2H, s, CH_2), 1.04 (6H, s, 2 x CH_3); ^{13}C NMR (101 MHz, DMSO-d_6) δ 192.9, 163.3, 160.2 (3 x C=O), 159.8 (Ar-C), 140.1 (Ar-CH), 138.6 (Ar-C), 131.9 (Ar-C), 130.3 (Ar-CH), 121.5 (Ar-CH), 117.2 (Ar-CH), 111.6 (Ar-CH), 50.0 (CH_2), 32.6 (C-7), 27.7 (2 x CH_3); FT-IR ν_{max}/cm^{-1}: 3145, 2954, 1700, 1682, 1655, 1606, 1584, 1534, 1481, 1399; HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calculated for $C_{18}H_{18}BrN_2O_3^+$ 391.0495, found 391.0481.

7,7-Dimethyl-2,5-dioxo-N-(p-tolyl)-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (3c). 7,7-Dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**6a**) (0.15 g, 0.64 mmol), CDI (0.134 g, 0.83 mmol) and *p*-toluidine (**7c**) (0.110 g, 0.64 mmol; Physical characteristics: white solid powder; Yield: 0.21 g, 76%; Mp: 340-342 ºC; R_f: 0.7, hexane/EtOAc (1:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.10 (1H, br s, N-H), 11.55 (1H, s, Ar-N-H), 8.72 (1H, s, CH), 7.58 (2H, d, *J* = 7.6 Hz, ArH), 7.18 (2H, d, *J* = 7.6 Hz, Ar), 2.84 (2H, s, CH_2), 2.42 (2H, s, CH_2), 2.28 (3H, s, Ar-CH_3) 1.04 (6H, s, 2 x CH_3); ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.2, 163.4, 160.7 (3 x C=O), 158.9 (Ar-C), 140.0 (Ar-CH), 135.7 (Ar-C), 133.0 (Ar-C), 129.5 (Ar-CH), 119.7 (Ar-CH), 118.4 (Ar-C), 112.9 (Ar-C), 50.0 (CH_2), 32.7 (C-7), 27.7 (2 x CH_3), 20.5 (CH_3); FT-IR ν_{max}/cm^{-1}: 3131, 2871, 1690, 1629, 1596, 1574, 1550, 1516, 1485, 1382; HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calculated for $C_{19}H_{21}N_2O_3^+$ 325.1547, found 325.1578.

N-(2,4-Dimethylphenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (3d). 7,7-Dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**6a**) (0.25 g, 1.10 mmol), CDI (0.22 g, 1.38 mmol) and 2,4-dimethylaniline (**7d**) (0.13 g, 1.1 mmol); Physical characteristics: white solid powder; Yield: 0.28 g, 78%; Mp: 343-345 ºC; R_f: 0.7, hexane/EtOAc (1:1): ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.06 (1H, br s, N-H), 11.48 (1H, s, Ar-N-H), 8.74 (1H, s, Ar-H), 8.12 (1H, d, *J* = 8 Hz,

Ar-H), 7.06 (1H, s, Ar-H), 7.01 (1H, d, *J* = 8.4 Hz, Ar-H), 2.85 (2H, s, CH_2), 2.44 (2H, s, CH_2), 2.25 and 2.28 (6H, 2 x s, Ar-CH_3), 1.04 (6H, s, 2 x CH_3); ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 193.3, 163.6, 160.6 (3 x C=O), 158.9 (Ar-C), 140.1 ((Ar-CH), 134.2 (Ar-C), 132.9 (Ar-C), 130.9 (Ar-C), 127.3 (Ar-C), 126.8 (Ar-C), 120.9 (Ar-C), 118.5 (Ar-C), 112.9 (Ar-C), 50.0 (CH_2), 32.7 (C-7), 27.7 (2 x CH_3), 20.5 (CH_3), 17.7 (CH_3); FT-IR ν_{max}/cm^{-1}: 3131, 2942, 1674, 1621, 1593, 1543, 1483, 1458, 1426, 1389, 1303; HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calculated for $C_{20}H_{23}N_2O_3^+$ 339.1703, found 339.1711.

N-(2-Hydroxyphenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (3e). 7,7-Dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**6a**) (0.25 g, 1.10 mmol), CDI (0.22 g, 1.38 mmol) and 2-hydroxyaniline (**7e**) (0.116 g, 1.1 mmol); Physical characteristics: white solid powder; Yield: 0.28 g, 80%; Mp: 329-331 ºC; R_f: 0.6, hexane/EtOAc (1:1); ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 12.96 (1H, br s, N-H), 11.71 (1H, s, Ar-N-H), 10.03 (1H, s, O-H) 8.74 (1H, s, Ar-H), 8.38 (1H, d, *J* = 7.6 Hz, Ar-H), 6.93-6.87 (2H, m, Ar-H), 6.79 (1H, t, *J* = 7.2 Hz, Ar-H) 2.84 (2H, s, CH_2), 2.43 (2H, s, CH_2), 1.04 (6H, s, 2 x CH_3); ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 193.3, 163.1, 160.6 (3 x C=O), 158.9 (Ar-C), 146.6 (Ar-C), 140.0 (Ar-CH), 126.9 (Ar-C), 123.9 (Ar-CH), 120.2 (Ar-C), 119.1 (Ar-CH), 118.7 (Ar-C), 114.6 (Ar-CH), 112.7 (Ar-C), 50.0 (CH_2), 32.6 (C-7), 27.7 (2 x CH_3); FT-IR ν_{max}/cm^{-1}: 3038, 2961, 1670, 1623, 1602, 1575, 1537, 1499, 1442, 1417, 1391; HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calculated for $C_{18}H_{19}N_2O_4^+$ 327.1339, found 327.1335.

N-(2,4-Dimethoxyphenyl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (3f). 7,7-Dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**6a**) (0.25 g, 1.10 mmol), CDI (0.22 g, 1.38 mmol) and 2,4-dimethoxyaniline (**7f**) (0.16 g, 1.1 mmol). Physical characteristics: black solid powder; Yield: 0.32 g, 82%; Mp: 282-284 ºC; R_f: 0.6, hexane/EtOAc (1:1): ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 12.95 (1H, br s, N-H), 11.67 (1H, s, Ar-N-H), 8.72 (1H, s, CH), 8.32 (1H, d, *J* = 8.4 Hz, ArH), 6.66 (1H, s, ArH), 6.53 (1H, d, *J* = 8.4 Hz, ArH), 3.87 (3H, s, Ar-O-CH_3), 3.77 (3H, s, Ar-O-CH_3), 2.84 (2H, s, CH_2), 2.43 (2H, s, CH_2), 1.04 (6H, s, 2 x CH_3); ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 193.2, 163.1, 160.2 (3 x C=O), 158.7 (Ar-C), 156.2 (Ar-CH), 149.7 (Ar-C), 139.8 (Ar-CH), 121.2 (Ar-C), 120.7 (Ar-CH), 118.6 (Ar-C), 112.7 (Ar-C), 104.2 (Ar-C), 98.8 (Ar-CH), 56.0 (O-CH_3), 55.3 (O-CH_3), 50.0 (CH_2), 32.6 (C-8, CH_2), 30.7 (C-7), 27.7 (2 x CH_3) ; FT-IR ν_{max}/cm^{-1}: 3266, 2961, 1670, 1623, 1602, 1575, 1537, 1499, 1442, 1417, 1391; HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calculated for $C_{20}H_{23}N_2O_5^+$ 371.1600, found 371.1604.

N-(4-Chlorophenyl)-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (3g). 2,5-Dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**6b**) (0.4 g, 1.93 mmol), CDI (0.41 g, 2.51 mmol) and 4-chloroaniline (**7a**) (0.25 g, 1.93 mmol); Physical characteristics: white solid powder; Yield: 0.49 g, 80%, Mp: 200-202 ºC; R_f: 0.4, hexane/EtOAc (1:1): ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 13.11 (1H, s, N-H), 11.71 (1H, s, Ar-N-H), 8.73 (1H, s, CH), 7.69 (1H, d, *J* = 8.8 Hz, ArH), 7.55 (1H, d, *J* = 8.8 Hz,

ArH), 2.95 (2H, t, *J* = 6.0 Hz, CH_2), 2.09-2.04 (2H, m, CH_2); ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 193.3, 163.0, 161.1 (3 x C=O), 160.9 (Ar-C), 140.6 (Ar-C), 137.5 (Ar-CH), 131.8 (Ar-C), 121.7 (Ar-C), 118.0 (Ar-C), 115.5 (Ar-C), 113.9 (Ar-C), 36.6 (CH_2), 26.5 (CH_2), 20.4 (C-7, CH_2); FT-IR ν_{max}/cm^{-1}: 3157, 2954, 1700, 1683, 1654, 1606, 1584, 1481, 1381, 1380; HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calculated for $C_{16}H_{14}ClN_2O_3^+$ 317.0687, found 317.0688.

N-(4-Bromophenyl)-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (3h). 2,5-Dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**4b**) (0.4 g, 1.93 mmol), CDI (0.41 g, 2.51 mmol) and 4-bromoaniline (**7b**) (0.33 g, 1.93 mmol); Physical characteristics: brown solid powder; Yield: 0.56 g, 86%, Mp: 342-344 ºC; R_f: 0.35, hexane/EtOAc (1:1); ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 13.11 (1H, s, N-H), 11.72 (1H, s, Ar-N-H), 8.74 (1H, s, Ar-H), 7.75 (2H, d, *J* = 8.4 Hz, ArH), 7.43 (2H, d, *J* = 8.4 Hz, ArH), 2.93 (2H, t, *J* = 6.0 Hz), 2.11-2.05 (2H, m); ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 193.4, 163.0, 161.1 (3 x C=O), 160.9 (Ar-C), 140.6 (Ar-CH), 137.1 (Ar-C), 128.9 (Ar-CH), 127.5 (Ar-C), 121.4 (Ar-CH), 118.0 (Ar-C), 113.9 (Ar-C), 36.6 (CH_2), 26.6 (CH_2), 20.5 (CH_2); FT-IR ν_{max}/cm^{-1}: 3145, 2954, 1700, 1682, 1655, 1606, 1584, 1534, 1481, 1399; HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calculated for $C_{16}H_{14}BrN_2O_3^+$ 363.0182, found 363.0164.

2,5-Dioxo-N-(p-tolyl)-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (3i). 2,5-Dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**6b**) (0.4 g, 1.93 mmol), CDI (0.41 g, 2.51 mmol) and *p*-toluidine (**7c**) (0.21 g, 1.93 mmol); Physical characteristics: grey solid powder; Yield: 0.42 g, 71%, Mp: 330-332 ºC; R_f: 0.4, hexane/EtOAc (1:1) : ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 13.06 (1H, s, N-H), 11.57 (1H, s, Ar-N-H), 8.73 (1H, s, CH), 7.58 (2H, d, *J* = 8.4 Hz, ArH), 7.17 (2H, d, *J* = 7.6 Hz, ArH), 2.93 (2H, t, *J* = 6.8 Hz, CH_2), 2.27 (3H, s, Ar-CH_3), 2.08-2.03 (2H, m, CH_2); ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 193.4, 163.1, 160.7 (3 x C=O), 160.7 (Ar-C), 140.3 (Ar-CH), 135.7 (Ar-C), 132.9 (Ar-C), 129.5 (Ar-CH), 119.7 (Ar-CH), 118.3 (Ar-C), 113.9 (Ar-C), 36.6 (CH_2), 26.5 (CH_2), 20.5 (CH_3); FT-IR ν_{max}/cm^{-1}: 3131, 2871, 1679, 1629, 1596, 1574, 1550, 1516, 1485, 1382; HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calculated for $C_{17}H_{17}N_2O_3^+$ 297.1234, found 297.1240.

N-(2-Hydroxyphenyl)-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (3j). 2,5-Dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**6b**) (0.4 g, 1.93 mmol), CDI (0.41 g, 2.51 mmol) and 2-hydroxyaniline (**7e**) (0.21 g, 1.93 mmol); Physical characteristics: grey solid powder; Yield: 0.44 g, 76%, Mp: 324-326 ºC; R_f: 0.3, hexane/EtOAc (1:1): ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 12.89 (1H, br s, N-H), 11.72 (1H, s, Ar-N-H), 10.04 (1H, s, OH), 8.75 (1H, s, Ar-H), 8.37 (1H, d, *J* = 7.6 Hz, ArH), 6.90 (2H, d, *J* = 9.6 Hz, ArH), 6.79 (1H, t, *J* = 7.2 Hz, ArH), 2.92 (2H, t, *J* = 5.6 Hz, CH_2), 2.09-2.03 (2H, m, H-7), ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 193.4, 162.7, 160.7 (3 x C=O), 160.6 (Ar-C), 146.6 (Ar-C), 140.4 (Ar-CH), 126.9 (Ar-C), 123.9 (Ar-CH), 120.2 (Ar-CH), 119.1 (Ar-CH), 118.6 (Ar-C), 114.6 (Ar-CH), 113.7 (Ar-C), 36.6 (CH_2), 26.5 (CH_2), 20.5 (CH_2); FT-IR ν_{max}/cm^{-1} : 3038, 2955, 2829, 1658,

1597, 1570, 1493, 1384; HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calculated for $C_{16}H_{15}N_2O_4^+$ 299.1026, found 299.1050.

N-(2,4-Dimethoxyphenyl)-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (3k). 2,5-Dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**6b**) (0.40 g, 1.93 mmol), CDI (0.41 g, 2.51 mmol) and 2,4-dimethoxyaniline (**7f**) (0.29 g, 1.93 mmol); Physical characteristics: grey solid powder; Yield: 0.50 g, 83%, Mp: 339-341 ºC; R_f: 0.29, hexane/EtOAc (1:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.69 (1H, s, N-H), 11.56 (1H, s, Ar-N-H), 8.76 (1H, s, CH), 8.31 (1H, d, *J* = 8.4 Hz, ArH), 6.66 (1H, s, ArH), 6.54 (1H, d, *J* = 8.4 Hz, ArH), 3.88 (3H, s, Ar-O-CH_3), 3.78 (3H, s, Ar-O-CH_3), 3.06 (2H, t, *J* = 5.6 Hz, CH_2), 2.95 (2H, t, *J* = 7.6 Hz, CH_2), 2.11-2.06 (2H, m, H-7); ^{13}C NMR (101 MHz, DMSO-d_6) δ 192.7, 162.4, 159.9 (3 x C=O), 159.8 (Ar-C), 156.0 (Ar-CH), 149.7 (Ar-C), 139.8 (Ar-CH), 121.2 (Ar-C), 120.6 (Ar-CH), 118.7 (Ar-C), 113.4 (Ar-C), 104.4 (Ar-C), 98.9 (Ar-CH), 55.9 (O-CH_3), 55.1 (O-CH_3), 36.2 (C-6, CH_2), 26.2 (CH_2), 20.2 (CH_2); FT-IR ν_{max}/cm^{-1}: 3266, 3119, 2961, 1670, 1623, 1602, 1575, 1499, 1442, 1417, 1391; HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calculated for $C_{18}H_{19}N_2O_5^+$ 343.1288, found 343.1275.

7,7-Dimethyl-2,5-dioxo-N-phenyl-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (3l). 7,7-Dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**6a**) (0.25 g, 1.1 mmol), CDI (0.22 g, 1.38 mmol) and aniline hydrochloride (**7g**) (0.14 g, 1.10 mmol); Physical characteristics: white solid powder; Yield: 0.26 g, 79%; Mp: 332-334 ºC (Lit.[21] 332 ºC); R_f: 0.7, hexane/EtOAc (1:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.12 (1H, br s, N-H), 11.61 (1H, s, Ar-N-H), 8.72 (1H, s, Ar-H), 7.68 (2H, d, *J* = 7.2 Hz, Ar-H), 7.36 (2H, d, *J* = 7.6 Hz, Ar-H), 7.11 (1H, t, *J* = 6.4 Hz, Ar-H), 2.83 (2H, s, CH_2), 2.42 (2H, s, CH_2), 1.03 (6H, s, 2 x CH_3); ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.2, 163.4, 160.9 (3 x C=O), 159.0 (Ar-C), 140.1 (Ar-CH), 138.2 (Ar-C), 129.1 (Ar-C), 123.9 (Ar-C), 119.7 (Ar-C), 118.3 (Ar-C), 112.9 (Ar-C), 49.9 (CH_2), 38.9 (C-7), 27.7 (2 x CH_3); FT-IR ν_{max}/cm^{-1}: 3139, 2866, 1679, 1618, 1596, 1555, 1485, 1442, 1421, 1302; HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calculated for $C_{18}H_{19}N_2O_3^+$ 311.1390, found 311.1419.

2,5-Dioxo-N-phenyl-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (3m). 2,5-Dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**6b**) (0.40 g, 1.93 mmol), CDI (0.41 g, 2.51 mmol) and aniline hydrochloride (**7g**) (0.25 g, 1.93 mmol); Physical characteristics: grey solid powder; Yield: 0.44 g, 81%, Mp: 332-334 ºC (Lit.[21] 339 ºC); R_f: 0.4, hexane/EtOAc (1:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.11 (1H, s, N-H), 11.64 (1H, s, Ar-N-H), 8.74 (1H, s, CH), 7.69 (2H, d, *J* = 7.6 Hz, ArH), 7.36 (2H, t, *J* = 7.2 Hz, ArH), 7.12 (1H, t, *J* = 6.8 Hz, ArH), 2.94 (2H, t, *J* = 6.4 Hz, CH_2), 2.09-2.03 (2H, m, H-7); ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.4, 163.1, 160.9 (3 x C=O), 160.8 (Ar-C), 140.4 (Ar-CH), 138.2 (Ar-C), 129.1 (Ar-C), 123.9 (Ar-C), 119.7 (Ar-C), 118.2 (Ar-C), 113.9 (Ar-C), 36.6 (CH_2), 26.5 (CH_2), 20.5 (H-7, CH_2); FT-IR ν_{max}/cm^{-1}: 3026, 2957,1676, 1640, 1600, 1480, 1425; HRMS (ESI-TOF)

m/z: $[M+H]^+$ Calculated for $C_{16}H_{15}N_2O_3^+$ 283.1077, found 283.1113.

N-(4-(4-Fluorophenyl)thiazol-2-yl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (9a). 7,7-Dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**6a**) (0.25 g, 1.1 mmol), CDI (0.22 g, 1.38 mmol) and 4-(4-fluorophenyl)thiazol-2-amine (**8a**) (0.21 g, 1.10 mmol); Physical characteristics: yellow solid powder; Yield: 0.35 g, 80%; Mp: 347-349 ºC; R_f: 0.80, hexane/EtOAc (1:1); ^{1}H NMR (400 MHz,DMSO-*d*$_6$) δ 13.32 (1H, br s, N-H), 12.90 (1H, s, N-H), 8.76 (1H, s, H-4), 7.97 (2H, t, *J* = 8.0 Hz, Ar-H), 7.69 (1H, Ar-H), 7.26 (2H, t, *J* = 8.8 Hz, ArH), 2.88 (2H, s, CH_2), 2.46 (2H, s, CH_2), 1.06 (6H, s, 2 x CH_3); ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 193.0, 185.8 (C=O), 162.1 (d, J_{CF} = 248.0 Hz, Ar-C), 160.6 (C=O), 160.1 (Ar-C), 156.8 (Ar-C), 148.2 (Ar-C), 140.8 (Ar-CH), 130.6 (Ar-C), 127.8 (d, J_{CF} = 8.0 Hz, Ar-CH), 115.9 (Ar-C), 115.5 (d, J_{CF} = 21.1 Hz, Ar-CH), 113.3 (Ar-C), 108.8 (Ar-CH), 49.9 (CH_2), 32.6 (C-7), 27.6 (2 x CH_3); FT-IR ν_{max}/cm^{-1}: 3110, 2965, 2928, 1673, 1620, 1573, 1545, 1485, 1386, 1301; HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calculated for $C_{21}H_{19}FN_3O_3S^+$ 412.1126, found 412.1144.

N-(4-(4-Methoxyphenyl)thiazol-2-yl)-7,7-dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (9b). 7,7-Dimethyl-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**6a**) (0.25 g, 1.1 mmol), CDI (0.22 g, 1.38 mmol) and 4-(4-methoxyphenyl)thiazol-2-amine (**8b**) (0.22 g, 1.10 mmol); Physical characteristics: yellow solid powder; Yield: 0.36 g, 82%; Mp: 348-350 ºC; R_f: 0.7, hexane/EtOAc (1:1); ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 13.11 (1H, br s, N-H), 12.89 (1H, s, N-H), 8.75 (1H, s, Ar-H), 7.86 (2H, d, *J* = 8.4 Hz, Ar-H), 7.57 (1H, s, Ar-H), 6.99 (2H, d, *J* = 8.4 Hz, Ar-H), 3.79 (3H, s, O-CH_3), 2.87 (2H, s, CH_2), 2.49 (2H, s, CH_2), 1.05 (6H, s, 2 x CH_3); ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 193.1, 163.34, 160.8 (3 x C=O), 160.1 (Ar-C), 159.1 (Ar-C), 156.5 (Ar-C), 149.2 (Ar-C), 140.74 (Ar-CH), 127.1 (Ar-CH), 126.8 (Ar-C), 116.1 (Ar-C), 114.1 (Ar-CH), 113.3 (Ar-C), 106.9 (Ar-CH), 55.2 (O-CH_3), 49.9 (C-6, CH_2), 32.6 (C-7), 27.7 (2 x CH_3); FT-IR ν_{max}/cm^{-1}: 3119, 2930, 1624, 1573, 1540, 1486, 1453, 1418, 1303; HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calculated for $C_{22}H_{22}N_3O_4S^+$ 424.1326, found 424.1345.

N-(4-(4-Fluorophenyl)thiazol-2-yl)-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (9c). 2,5-Dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**6b**) (0.25 g, 121 mmol), CDI (0.26 g, 157 mmol) and 4-(4-fluorophenyl)thiazol-2-amine (**8a**) (0.23 g, 121 mmol); Physical characteristics: white solid powder; Yield: 0.38 g, 83%; Mp: 341-343 ºC; R_f: 0.76, hexane/EtOAc (1:1); ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 13.28 (1H, br s, N-H), 13.05 (1H, s, N-H), 8.76 (1H, s, CH), 7.97 (2H, d, *J* = 7.2 Hz, Ar-H), 7.69 (1H, s, Ar-H), 7.26 (2H, d, *J* = 8.0 Hz, Ar-H), 2.96 (2H, t, *J* = 9.2 Hz, CH_2), 2.54 (2H, t, *J* = 6.4 Hz, CH_2), 2.12-2.05 (2H, m, H-7); ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ 193.4 (C=O), 187.7 (C=O), 162.3 (d, J_{CF} = 243.4 Hz, Ar-C), 156.8 (C=O), 148.0 (Ar-C), 142.6 (Ar-C), 140.7 (Ar-CH), 133.7 (Ar-C), 130.6 (Ar-C), 127.8 (d, J_{CF} = 9.1 Hz, Ar-CH), 115.7 (Ar-C), 115.6 (d, J_{CF} = 21.1 Hz, Ar-CH), 114.3 (Ar-C),

108.7 (Ar-CH), 36.6 (CH_2), 26.9 (CH_2), 20.4 (CH_2); FT-IR ν_{max}/cm^{-1}: 3131, 2955, 2873, 1770, 1682, 1651, 1606, 1583, 1533, 1470, 1399; HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calculated for $C_{19}H_{15}FN_3O_3S^+$ 384.0813, found 384.0825.

N-(4-(4-Methoxyphenyl)thiazol-2-yl)-2,5-dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxamide (9d). 2,5-Dioxo-1,2,5,6,7,8-hexahydroquinoline-3-carboxylic acid (**6b**) (0.25 g, 121 mmol), CDI (0.26 g, 157 mmol) and 4-(4-methoxyphenyl)thiazol-2-amine (**8b**) (0.25 g, 121 mmol); Physical characteristics: yellow solid powder; Yield: 0.41g, 85%; Mp: 345-347 ºC; R_f: 0.6 hexane/EtOAc (1:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.42 (1H, br s, N-H), 13.07 (1H, br s, N-H), 8.74 (1H, s, Ar-H), 7.85 (2H, d, *J* = 8.8 Hz, Ar-H), 7.54 (1H, s, Ar-H), 6.98 (2H, d, *J* = 8.4 Hz, Ar-H), 3.79 (3H, O-CH_3), 2.94 (2H, t, *J* = 7.2 Hz, CH_2), 2.09-2.04 (2H, m, H-7); ^{13}C NMR (101 MHz, DMSO-d_6) δ 193.3, 189.9, 161.2 (3 x C=O), 159.3 (Ar-C), 156.5 (Ar-C), 149.2 (Ar-C), 141.1 (Ar-CH), 137.8 (Ar-C), 127.1 (Ar-CH), 126.9 (Ar-C), 115.7 (Ar-C), 114.3 (Ar-CH), 114.1 (Ar-C), 106.9 (Ar-CH), 55.1 (O-CH_3), 36.6 (CH_2), 27.0 (C-8, CH_2) , 20.4 (CH_2); FT-IR ν_{max}/cm^{-1}: 3142, 2954, 1700, 1682, 1652, 1606, 1584, 1469, 1399, 1381; HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calculated for $C_{20}H_{18}N_3O_4S^+$ 396.1013, found 396.1023.

Cytotoxicity Studies

The day of cytotoxicity testing, MT4 cells (obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: MT4 catalogue number 120) were counted and seeded at 5×10^4 cells per well. A total of 100 µL of cells were added to each well of a 96-well cell culture plate (TTP) for testing. The volume in the last row of wells of each plate was further increased to 197 µL of media containing MT4 cells. The plate was placed into the incubator to equilibrate to 37 ºC and 5% CO_2. Test compounds were prepared in plate format and each compound was at 25 mM stock concentration in DMSO. A total of 3.2 µL compound was added to the final row of wells containing 197 µL cells and mixed to ensure the solution was homogeneous with the cells. A 2-fold serial dilution of an 8 times series was performed and the final 100 µL was discarded. The plate was placed at 37 ºC and 5% CO_2 for 5 days. On the fifth day, the cell viability was assessed using the Cell Titre 96® Aqueous One Solution Cell Proliferation assay (Promega) as per manufacturer's instructions. Briefly, 20 µL of MTS solution was added to each well and incubated at 37 ºC for 4 h. The absorbance of the plates was then read at 490 nm on a Spectra Max spectrophotometer (Molecular Devices). The data analysis was completed on Origin 8.1 with the log value of the compound molar concentration plotted against the percentage cell viability determined by the absorbance level by sigmoidal fitting. From the curve, a half maximal cytotoxicity CC_{50} for compounds was determined. Auranofin was used as a cytotoxic control compound starting at 100 µM across a 2-fold serial dilution of an 8 dilution series. DMSO was used as background control and 2-fold serially diluted from 1.5%.

Antiviral Assays

An HIV-1 chronically infected ACH-2 clone was obtained through the NIH HIV Reagent Program, Division of AIDS, NIAID, NIH: ACH-2 Cells, ARP-349, contributed by Dr. Thomas Folks. The cells were grown and maintained in RPMI 1640 (Sigma) supplemented with 10 mM HEPES, 2 mM L-glutamine, non-essential amino acids and 10% heat inactivated fetal bovine serum at 37 ^{o}C and 5% CO_2. The ACH-2 cells were activated by 1 µM phorbol myristate acetate (PMA).[39] Supernatants were harvested at 48 h by centrifugation at 4000 x g and stored at -80 ^{o}C until use. MT4 cells were grown and maintained in RPMI (Gibco) supplemented with 2 mM L-glutamine, non-essential amino acids and 10% heat inactivated fetal bovine serum at 37 ^{o}C and 5% CO_2. Harvested supernatant containing HIV-1 was added to MT4 cells and viral absorption was allowed for 24 h similar to the method by Rosenwirth and co-workers.[40] After viral absorption, the cells were washed once with fresh media, a sample was taken, and HIV-1 infection was allowed to develop. Seven days after infection, MT4 supernatant samples were tested for the presence of p24 antigen using the bioelisa HIV-1+2 Ag/Ab kit (biokit) as per manufacturer's instructions. After HIV-1 infection was confirmed, supernatant samples were isolated by centrifugation at 4000 x g and immediately used to infect $1x10^5$ cells/mL of MT4 cells. Virus was allowed to adsorb and 24 h later the cells were washed with fresh media and 100 µL of cells, $1x10^4$ cells, were aliquoted into a round bottom 96 well plate (TPP). Uninfected cells were also prepared. A day 1 sample was taken and stored at -80 ^{o}C until final testing. Compounds were diluted in fresh media to 200 µM concentrations and 100 µL was added to each test well, the final concentration of compounds was 100 µM. Control compounds included dolutegravir and raltegravir and were used at 10 µM each, blank wells contained equal volumes of DMSO. Upper and lower asymptotes were determined by untreated MT4 cells either infected with HIV (100%) or not infected with HIV (0%). Four days after infection, p24 presence was determined using the bioelisa HIV-1+2 Ag/Ab kit as per manufacturer's instructions. The data was used to determine if the selected concentration of compounds would reduce the amount of p24 and thereby indicate a reduction in HIV infectivity.

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REFERENCES

1. T. J. Rashamuse, A. T. Harrison, S. Mosebi, S. van Vuuren, E. M. Coyanis, and M. L. Bode, *Bioorg. Med. Chem.*, 2020, **28**, 115210.

2. T. J. Rashamuse, Z. Njengele, E. M. Coyanis, Y. Sayed, S. Mosebi, and M. L. Bode, *Eur. J. Med. Chem.*, 2020, **190**, 112111.
3. R. L. Mohlala, E. M. Coyanis, M. Q. Fish, M. A. Fernandes, and M. L. Bode, *Molecules*, 2021, **26**, 5493.
4. T. J. Rashamuse, M. Q. Fish, E. M. Coyanis, and M. L. Bode, *Molecules*, 2021, **26**, 6203.
5. R. L. Mohlala, E. M. Coyanis, M. A. Fernandes, and M. L. Bode, *RSC Adv.*, 2021, **11**, 24466.
6. R. L. Mohlala, E. M. Coyanis, M. A. Fernandes, and M. L. Bode, *Tetrahedron Lett.*, 2020, **61**, 151796.
7. E. Le-Rouzic, D. Bonnard, S. Chasset, J.-M. Bruneau, F. Chevreuil, F. Le Strat, J. Nguyen, R. Beauvoir, C. Amadori, J. Brias, S. Vomscheid, S. Eiler, N. Lévy, O. Delelis, E. Deprez, A. Saïb, A. Zamborlini, S. Emiliani, M. Ruff, B. Ledoussal, F. Moreau, and R. Benarous, *Retrovirology*, 2013, **10**, 1.
8. F. Christ, A. Voet, A. Marchand, A. S. Nicolet, B. A. Desimmie, D. Marchand, D. Bardiot, N. J. Van der Veken, B. Van Remoortel, S. V. Strelkov, M. De Maeyer, P. Chaltin, and Z. Debyser, *Nat. Chem. Biol.*, 2010, **6**, 442.
9. F. Christ, S. Shaw, J. Demeulemeester, B. A. Desimmie, A. Marchand, S. Butler, W. Smets, P. Chaltin, M. Westby, Z. Debyser, and C. Pickford, *Antimicrob. Agents Chemother*., 2012, **56**, 4365.
10. S. Narwal, S. Kumar, and P. K. Verma, *Res. Chem. Intermed.*, 2017, **43**, 2765.
11. N. Fokialakis, P. Magiatis, L. Chinou, S. Mitaku, and F. Tillequin, *Chem. Pharm. Bull.*, 2002, **50**, 413.
12. P. Fossa, L. Mosti, G. Menozzi, C. Marzano, F. Baccichetti, and F. Bordin, *Bioorg. Med. Chem.*, 2002, **10**, 743.
13. M. I. Shahin, J. Roy, M. Hanafi, D. Wang, U. Luesakul, Y. Chai, N. Muangsin, D. S. Lasheen, D. A. Abou, E. Ella, K. A. Abouzid, and N. Neamati, *Eur. J. Med. Chem.*, 2018, **155**, 516.
14. M. A. Shaheena, A. A. El-Emama, and N. S. El-Goharya, *Bioorg. Chem.*, 2020, **105**, 104274.
15. G. Kumar, A. Sathe, V. S. Krishna, D. Sriram, and S. M. Jachak, *Eur. J. Med. Chem.*, 2018, **157**, 1.
16. I. Süslü, E. S. Kablan, C. Şafak, and R. Şimşek, *J. Res. Pharm.*, 2021, **25**, 52.
17. O. O. Cosmas, A. O. Mumiye, and S. Tasneem, *Appl. Sci.*, 2012, **2**, 368.
18. S.-J. Song, Z.-X. Shan, and Y. Jin, *Synth. Commun.*, 2010, **40**, 3067.
19. K.-J. Zahed and A. Maryam, *Res. Chem. Intermed.*, 2015, **41**, 6741.
20. S. Ranjbar, N. Edraki, O. Firuzi, M. Khoshneviszadeh, and R. Miri, *Mol. Divers*., 2019, **23**, 471.
21. N. Y. Gorobets, B. H. Yousefi, F. Belaj, and C. O. Kappe, *Tetrahedron*, 2004, **60**, 8633.
22. S. G. Dzhavakhishvili, N. Y. Gorobets, V. N. Chernenko, V. I. Musatov, and S. M. Desenko, *Russ. Chem. Bull., Int. Ed.*, 2008, **57**, 422.
23. P. Fossa, G. Menozzi, P. Dorigo, M. Floreani, and L. Mosti, *Bioorg. Med. Chem.*, 2003, **11**, 4749.

24. P. Cherepanov, A. L. B. Ambrosio, S. Rahman, T. Ellenberger, and A. Engelman, *Proc. Natl. Acad. Sci. USA*, 2005, **102**, 17308.
25. W. L. Jorgensen and J. Tirado-Rives, *J. Am. Chem. Soc.*, 1988, **110**, 1657.
26. M. P. Jacobson, D. L. Pincus, C. S. Rapp, T. J. F. Day, B. Honig, D. E. Shaw, and R. A. Friesner, *J. Biomol. Struct. Dyn.*, 2004, **55**, 351.
27. E. Gallicchio, N. Deng, P. He, L. Wickstrom, A. L. Perryman, D. N. Santiago, S. Forli, A. J. Olson, and R. M. Emilio, *J. Comput. Aided Mol. Des.*, 2014, **28**, 475.
28. P. Cherepanov, Z. Y. Sun, S. Rahman, G. Maertens, G. Wagner, and A. Engelman, *Nat. Struct. Mol. Biol.*, 2005, **12**, 526.
29. R. Paul and G. W. Anderson, *J. Am. Chem. Soc*., 1960, **82**, 4596.
30. M. Vinícius and N. de Souza, *J. Sulfur Chem*., 2005, **26**, 429.
31. X. Zhang, K. Selvaraju, A. A. Saei, P. D. Arcy, R. A. Zubarev, E. S. J. Arnér, and S. Linder, *Biochimie*, 2019, **162**, 46.
32. H. Luo, B. Cao, A. S. C. Chan, R. W.-Y. Sun, and T. Zou, *Angew. Chem. Int. Ed*., 2020, **59**, 11046.
33. M. Tsiang, G. S. Jones, J. Goldsmith, A. Mulato, D. Hansen, E. Kan, L. Tsai, R. A. Bam, G. Stepan, K. M. Stray, A. Niedziela-Majka, S. R. Yant, H. Yu, G. Kukolj, T. Cihlar, S. E. Lazerwith, K. L. White, and H. Jin, *Antimicrob. Agents Chemother.*, 2016, **60**, 7086.
34. A. Hombrouck, B. Van Remoortel, M. Michiels, W. Noppe, F. Christ, A. Eneroth, B. L. Sahlberg, K. Benkestock, L. Vrang, N. G. Johansson, M. L. Barreca, L. De Luca, S. Ferro, A. Chimirri, Z. Debyser, and M. Witvrouw, *Antimicrob. Agents Chemother*., 2008, **52**, 2861.
35. L. Mosti, P. Schenone, and G. Menozzi, *J. Heterocycl. Chem.*, 1985, **22**, 1503.
36. D. Mishra, A. Fatima, C. Rout, and R. Singh, *Der Chem. Sin.*, 2015, **6**, 14.
37. P. Y. Lin, R. S. Hou, H. M. Wang, I. J. Kang, and L. C. Chen, *J. Chin. Chem. Soc.*, 2009, **56**, 455.
38. P. Morales-Bonilla, A. Pérez-Cardeña, E. Quintero-Mármol, J. L. Arias-Téllez, and G. J. Mena-Rejón, *Heteroat. Chem.*, 2006, **17**, 254.
39. T. M. Folks, K. A. Clouse, J. Justement, A. Rabsonf, E. Duho, J. H. Kehrl, and A. S. Fauci, *Proc. Natl. Acad. Sci. USA*, 1989, **86**, 2365.
40. B. Rosenwirth, A. Billich, R. Datema, P. Donatsch, F. Hammerschmid, R. Harrison, P. Hiestand, H. Jaksche, P. Mayer, P. Peichl, V. Quesniaux, F. Schatz, H.-J. Schuurman, R. Traber, R. Wenger, B. Wolff, G. Zenke, and M. Zurini, *Antimicrob. Agents Chemother*., 1994, **38**, 1763.