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Allosteric inhibitors of hepatitis C virus NS5B polymerase thumb domain site II: Structure-based design and synthesis of new templates

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ABSTRACT

Chronic hepatitis C virus (HCV) infections are a significant medical problem worldwide. The NS5B Polymerase of HCV plays a central role in virus replication and is a prime target for the discovery of new treatment options. We recently disclosed 1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-diones as allosteric inhibitors of NS5B Polymerase. Structural and SAR information guided us in the modification of the core structure leading to new templates with improved activity and toxicity/activity window.

1. Introduction

The hepatitis C virus (HCV) is the principal etiological agent of chronic hepatitis C infection,[1] affecting 170 million people worldwide. If untreated, more than 60% of these individuals will develop chronic liver disease that leads to chronic hepatitis, liver cirrhosis and hepatocellular carcinoma.[2] The current therapy, pegylated α-interferon (IFN) alone or in combination with ribavirin, is poorly tolerated. Additionally, it is of limited efficacy in patients infected with HCV genotype 1, which accounts for about 70% of the infections in the western world.[3] In response to these limitations, more efficacious and better tolerated agents are needed.

HCV is a linear single-stranded enveloped RNA virus classified in the hepacivirus genus of the flaviviridae family.[4] Similarly to other single-stranded RNA viruses, HCV lacks the ability to proofread and correct errors during replication. This accounts for the highly heterogeneous viral genome classified in six major genotypes (1–6) and a variety of subtypes. The expected onset of resistance upon treatment with a single agent targeting a viral protein and the possibility of pre-resistance in patients that harbor multiple quasi-species, suggests the need for multiple drugs to efficaciously treat the viral infection.[5] The NS5B RNA-dependent RNA polymerase plays a central role in virus replication and is considered an attractive target for drug discovery efforts because it has no counterpart in mammalian cells and so its inhibition is not expected to cause target-related side effects.[6] The three-dimensional structure of NS5B reveals the characteristic right hand architecture for this class of enzymes with finger, thumb and palm domains that fully encircle the active site cavity.[7] Several small molecules acting as allosteric inhibitors of NS5B have been disclosed during the past years reflecting the major effort in this area from pharmaceutical industries and academia.[8]

Recently, we disclosed a series of 1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-diones as allosteric inhibitors of NS5B polymerase[9] (Table 1). The lead compound **1a** showed nanomolar activity on the main NS5B polymerase genotypes (1b, 2b), while activity was in the micromolar range in the cell based assays (Table 1). X-ray analysis of **1a** bound to both DC55_1b and DC21_2b enzymes revealed that the inhibitor binds to the thumb site II. The tricyclic core interacts with a flat lipophilic surface, the bromophenyl substituent makes extensive Van der Waals and hydrophobic interactions, whereas the imide oxygens and heteroatoms of the ethanolamine side chain are involved in direct or water mediated H-bonds.[9]

The bromine present in **1a** can be replaced with a chlorine as in **1b** without changing the activity profile both on the enzyme and in the cell based assay. The structural information gained with X-ray guided the SAR studies that culminated in the identification of the more active compound **2** in which the ethanolamine side chain had been substituted by a benzylpiperidine moiety. Comparing **2** with the lead compounds **1a** or **1b**, it showed a significant improvement in enzymatic activity for both genotypes. Unfortunately, the improvement of the enzymatic activity corresponded to a poor cellular response with slight separation (<3-fold) between EC_{50} and CC_{50}. In this article we report the work which has been done in our laboratories to overcome the limitations of this series of HCV polymerase inhibitors via modification of the core scaffold.

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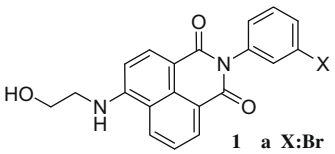
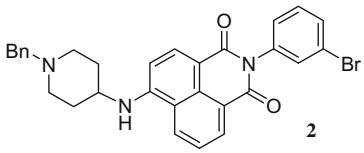
Table 1
1*H*-Benzo[*de*]isoquinoline-1,3-diones as HCV polymerase inhibitors: activity profile of **1a**,**b** and **2**





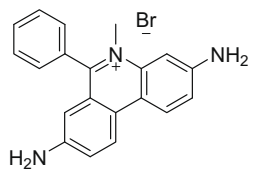
Compd	ΔC21-1b IC_{50}[a] (μM)	ΔC21-2b IC_{50}[a] (μM)	HBI10A EC_{50}[a] (μM)	H2B2 EC_{50}[a] (μM)	HBI10A CC_{50}[a] (μM)	SI[b]
1a	0.020	0.18	7.1	18	68	9.6
1b	0.039	0.21	8.4	35	57	6.8
2	<0.014	0.043	2.2	1.7	5.2	2.4

[a] Values are means of two or more experiments with standard deviations <30% of the mean value.
[b] SI = CC_{50} (HBI10A)/EC_{50} (HBI10A).

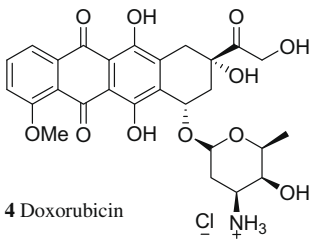
2. Results and discussion

2.1. Rationale for core structures modifications

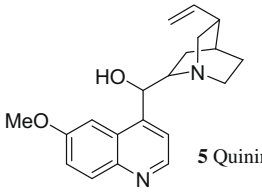
We hypothesized that the observed cytotoxicity of 1*H*-benzo[*de*]isoquinoline-1,3-(2*H*)-diones could be due to DNA intercalation properties of the scaffolds.[10] Generally DNA intercalators are characterized by typical features like a flat bi- or tricyclic fragment acting as the intercalating part and the presence of a positive charge (Fig. 1). The positive charge can be located on the ring itself as for Ethidium bromide **3**, giving interactions with the negative π-electron density of the base pairs, or it can be located on a side chain of the intercalating fragment, establishing interactions with the sugar-phosphate backbone as for Doxorubicin **4** and Quinine **5**. The intercalation induces local structural changes to DNA such as lengthening of the DNA strand or twisting of the base pairs, thus interfering with replication and leading to adverse biological effects like mutagenesis and cytotoxicity.[11] Nevertheless, if the side effects are tolerated and inferior to the benefit coming from the treatment, molecules acting as DNA-intercalators are used in therapies, like Quinine **5** as an antimalarial or Doxorubicin **4** used in cancer therapy.[12] We evaluated these known intercalators in our assays and compared them with our inhibitors **1a** and **2** (Table 2). The parameters considered were IC_{50}, EC_{50} and CC_{50} for genotype 1b. We also included a very specific mutation M423K that abolishes the activity of this class of compounds because it is located in the pocket were the 3-bromophenyl substituent is deeply buried. Compound **5** was inactive in all assays, proving that the ability to intercalate DNA alone is not sufficient to cause toxicity in the HCV cell based assay. Compounds **3** and **4** were both active in the enzymatic assay, moreover they were active on the mutant M423K. These results meant that the mechanism of action in the enzyme assays is different for the two classes of compounds (**1a**, **2** vs **3**, **4**). In the cell based assay **3** and **4** were active in the low micromolar range and they did not show a window between cytotoxicity and activity suggesting that their intercalation properties are responsible for both activity and toxicity. In contrast the existence of a window between CC_{50} and EC_{50} in our compounds, could offer the possibility to dial out the toxicity. For the reasons mentioned above going from **1a** to **2** the observed increase in cytotoxicity could be related to the introduction of the basic residue. In order to improve the activity-toxicity window we designed templates able to maintain the key interactions of **1** with HCV Polymerases: the general strategy was to reduce the lipophilic contact surface, the aromatic character to have less flat systems, and introduce more polar fragments (Scheme 1). In order to identify an inhibitor with a broad spectrum of applicability we monitored the activities for genotypes 1b and 2b both in enzymes and cellular assays. The cytotoxicity was measured for genotype 1b and the Selectivity Index was calculated as the ratio between CC_{50} and EC_{50}. This parameter was used to evaluate the activity-toxicity window: when SI < 3 the compound is considered toxic.[13]



3 Ethidium Bromide



4 Doxorubicin



5 Quinine

Figure 1. Known DNA-intercalators.

2.2. Chemistry

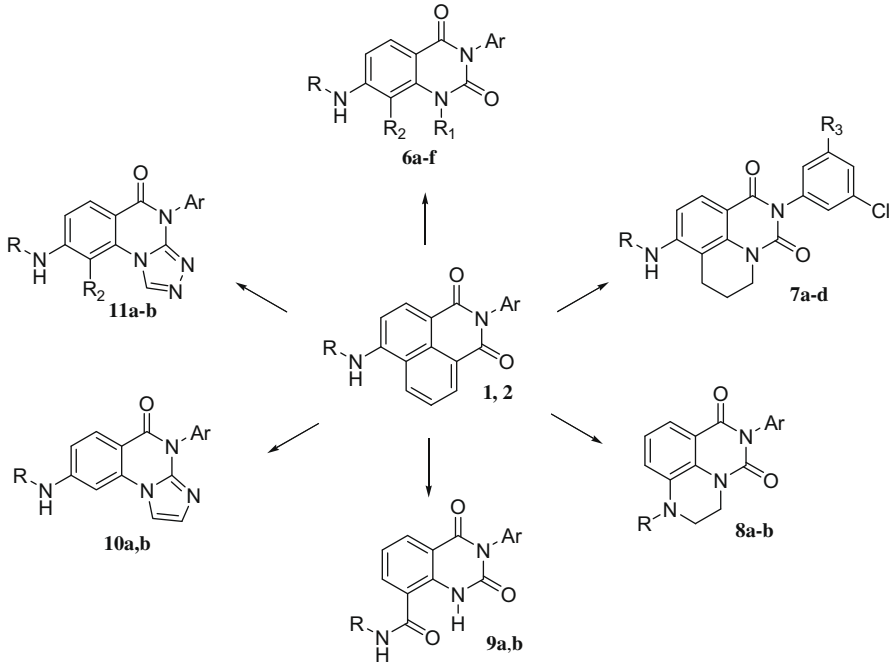
Quinazolinediones[14] **6a–e** were synthesized according to the route reported in Scheme 2 from the commercially available anthranilic acid **12a**. This was first reacted with phenyl chloroformate followed by amide bond formation with 3-chloroaniline. Ring closure to **13a** was achieved in DMF under microwave irradiation in good yield.[15] After standard alkylation at N-1 (base/R_1X) to furnish **14a**, the side chain was introduced by aromatic nucleophilic substitution with amines under microwave irradiation. This methodology allowed the synthesis of a number of analogs in moderate to good yields. For the synthesis of **6f** 2-amino-4-fluoro-3-methylbenzoic acid[16] **12b** was first converted to the methyl ester by treatment with $SOCl_2$ in MeOH and then reacted with 3-chlorophenylisocyanate. The resulting intermediate was cyclised in basic media to afford **13b**.[17] Methylation at N-1 and introduction of the ethanolamine side chain as previously described gave **6f**.

Pyridoquinazolinedione **7a** was prepared starting from 5-fluoroquinoline-8-carboxylic acid[18] **15** by reduction with H_2/PtO_2 in acetic acid (Scheme 3). Unfortunately dehalogenation and decomposition were prevalent side reactions that drastically reduced the yield of the desired product. Attempts to optimize the reaction conditions varying the solvent, the acid source and the catalyst, did not improve the yield. In the next step esterification of the acid gave **16**. Ring formation to **17** was obtained by reaction with 3-chlorophenylisocyanate followed by treatment with sodium hydroxide.[17] The ethanolamine side chain was introduced via

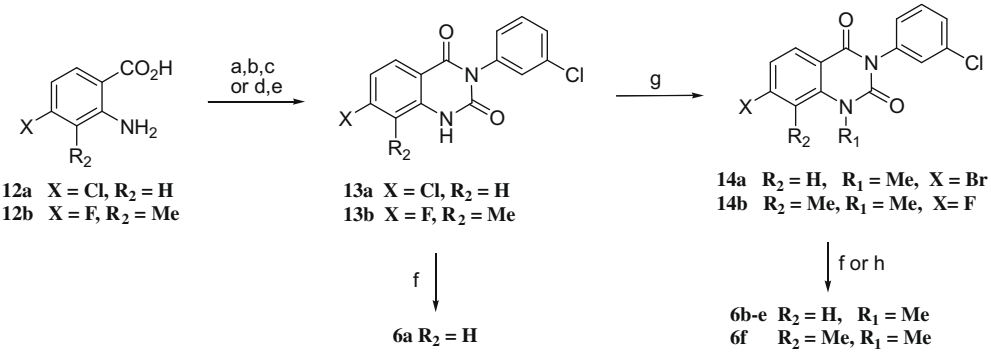
Table 2
Profiling of Known DNA-intercalators in HCV assays

Compd	ΔC21-1b IC_{50}[a] (μM)	ΔC21 M423K IC_{50}[a] (μM)	HBI10A EC_{50}[a] (μM)	HBI10A CC_{50}[a] (μM)	SI[b]
1a	0.020	NA at 2 μM	7.1	68	9.6
2	<0.014	ND	2.2	5.2	2.4
3	0.81	3.8	0.6	0.7	0.85
4	2.6	12	<0.3	<0.3	<1.0
5	NA[c] up to 50 μM	NA[c] up to 50 μM	NA[c] up to 20 μM	NA[c] up to 20 μM	–

[a] Values are means of two or more experiments with standard deviations <30% of the mean value.
[b] SI = CC_{50} (HBI10A)/EC_{50} (HBI10A).
[c] NA = Not active.



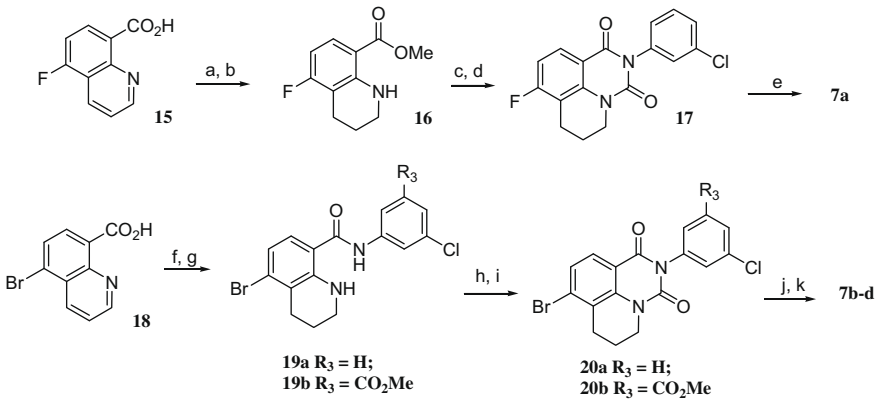
Scheme 1. Core structure modifications.



Scheme 2. Synthesis of compounds **6a–f**. Reagents and conditions: (a) Phenylchloroformate, Py, THF, 0 °C to rt; (b) 3-chloroaniline, HOBt, DCC, NMM, THF; (c) DMF, μW 150 °C, 15 min; (d) $SOCl_2$, MeOH, rt; (e) 3-chlorophenylisocyanate, Et_2O, then NaOH, EtOH, rt; (f) RNH_2, NMP, μW 170–200 °C; (g) MeI, DBU, DMF, rt; (h) RNH_2, DBU, NMP, μW 200 °C, 30 min.

fluorine displacement under microwave irradiation to afford **7a**. An alternative approach was explored to improve the low yielding reduction step. 5-Bromoquinoline-8-carboxylic acid **18** was first coupled with 3-chloroaniline, the amide was then reduced to **19a**,**b** in the presence of sodium cyanoborohydride and $BF_3{\cdot}Et_2O$ in high yield.[19] A two step sequence furnished **20a**,**b**. The introduction of the side chains by aromatic nucleophilic substitution gave unsatisfactory results. Alternatively the side chain were introduced using the more general palladium catalyzed amination in moderate-low yield.

2,3-Dihydro-1*H*,5*H*-pyrazino[3,2,1-*ij*]quinazoline-5,7(6*H*)-diones **8a**,**b** were prepared starting from quinoxaline-5-carboxylic acid[20] **21**, which was first coupled with 3-chloroaniline to furnish the corresponding amide and then partial ring reduction ($NaBH_4$/AcOH) gave **22**.[21] The side chains were introduced at this stage by reductive amination. The tricyclic core was established as usual



Scheme 3. Synthesis of compounds **7a–d**. Reagents and conditions: (a) H_2/PtO_2, AcOH; (b) $SOCl_2$, MeOH, reflux; (c) 3-chorophenylisocyanate, toluene, 90 °C; (d) NaOH, EtOH, rt; (e) RNH_2, NMP, μW 120 °C, 2 h; (f) 3-chloroaniline, HATU, DIPEA, DCM, 16 h; (g) $NaCNBH_3$, $BF_3{\cdot}Et_2O$, 80 °C; (h) phenylchloroformate, DCE, 80 °C; (i) TEA, MeOH; (j) RNH_2, NaO^tBu, BINAP, $Pd_2(dba)_3$, toluene, 80 °C, 16 h; (k) NaOH, dioxane, 40 °C.

upon treatment with phenylchloroformate and ring closure in basic media (Scheme 4).

Substituted anthranilic acids[22] **23a**,**b** were converted to intermediates **24a**,**b** according to the chemistry previously described. Side chains were introduced via transamidation of the ester **24a**, or upon methyl ester hydrolysis followed by amine coupling to give **9a**,**b** (Scheme 5).

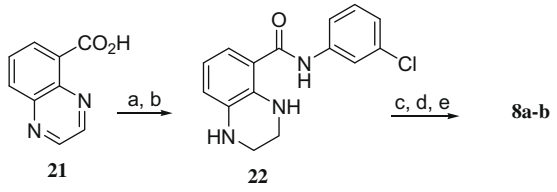
In Scheme 6 is reported the synthesis we followed to prepare compounds **10a**,**b** and **11a**,**b**. Substituted benzoic acids[16] **25a**,**b** were first coupled with 3-chloroaniline to give the amide followed by nitro-reduction for **25b** to furnish **26a**,**b**. Ring closure with carbon disulfide gave the mercaptoquinazolones **27a**,**b**. Compound **27a**, upon treatment with $SOCl_2$ followed by displacement of the chlorine intermediate with dimethoxyethylamine and ring closure in acid media, afforded **28** and then the desired side chains were introduced (**10a**,**b**). Compound **27b** was reacted with hydrazine followed by ring closure upon heating in formic acid to give **29**; introduction of the side chains produced **11a**,**b**.

2.3. Biological results

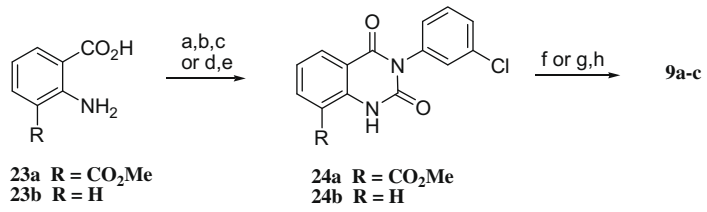
Quinazolinediones **6a–f** (Table 3) were designed to have a reduced lipophilic surface area compared to the tricyclic lead **1a**. Modeling studies (data not shown) pointed out that **6b** and **1b** adopt the same conformation and are engaged in the same interactions. The main difference between the two compounds is the reduced lipophilic contact surface area. By calculation for genotype 1b, the total contact surface area of **6b** is reduced by 16 $Å^2$ with respect to **1b**.

In the SAR study compounds **6a–e** maintain the meta-chlorophenyl substitution, while substitution at N-1 as well as the side chains were varied. The N-1 unsubstituted **6a** showed modest enzymatic activity only on genotype 1b, whereas the *N*-methyl analog **6b** showed enzymatic activity in the micromolar range against both genotypes. In the cell based assay **6b** showed marginal activity only for genotype 1b. Other changes to the ethanolamine side chain or substitutions on N-1 with ethyl, benzyl, and branched alkyl did not improve the activity (data not shown). Keeping the methyl group at N-1, the introduction of the benzylpiperidine moiety, derived from the SAR on the lead **1**, gave **6c** with a significant improvement in enzymatic activity showing nanomolar activity for genotype 1b and low micromolar on genotype 2b. In contrast the cellular assays gave a toxic response. Since the increased toxicity could be related to the introduction of the basic amino group, in compounds **6d** and **6e** the basic nitrogen was removed and the lipophilicity reduced by the truncation of the substituent. Compared with **6c** both compounds showed the expected improvement in the selectivity index (more than fourfold for **6e**) but they lost in the enzymatic activity. These results showed that the drastic reduction of the lipophilic surface area is responsible for the loss in potency. We postulated that introduction of a methyl substituent in the 8-position of the quinazoline ring as in **6f** could restore part of the surface area, and consequently restore the activity on the enzymes. Moreover steric strain between the two methyl groups (at N-1 and C-8) should twist the molecule reducing the planarity as evidenced in the superimposition of **1a** and **6f** (Fig. 2). Unfortunately the designed molecule **6f** exhibit an activity profile similar to **6b**.

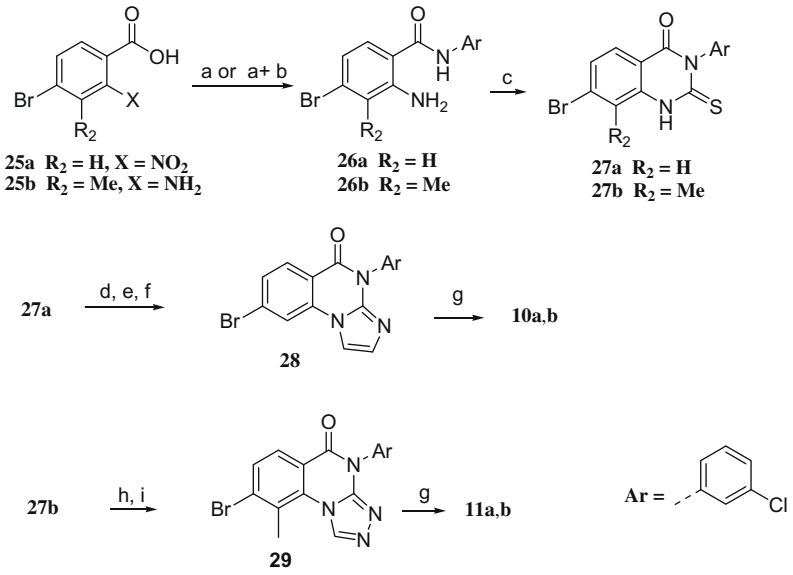
The two methyl substituents of **6f** were then constrained into a saturated ring as outlined in compounds **7a–d**: this modification was made to increase the lipophilic contact surface area respect to **6f** and reduce the aromatic character of the original tricyclic core scaffold **6b**. Compound **7a** with an ethanolamine side chain resulted 12-fold more active than the analog **6f** in the enzymatic assay for genotype 1b and eightfold for genotype 2b. Unfortunately, this significant improvement in enzymatic potency for **7a** did not translate into an improved cellular response. In fact, this relatively minor structural change led to an unexpected increase in cellular toxicity. When the benzylpiperidine side chain was introduced on this scaffold (**7b**), as expected we gained an excellent improvement of the enzymatic activities comparing with **7a**. More interestingly the compound is in the low micromolar range in the cellular assays and the selectivity index around six, meaning that in this case we were able to separate the activity from cytotoxicity. To further improve the window between EC_{50} and CC_{50}, in compound **7c** the basic amine was removed from the side chain and the benzyl moiety replaced by a carboxylic group.[9] While **7c** showed modest potency, loosing both in enzymatic and cells assays and mainly with respect to genotype 1b, it was less cytotoxic (SI = 8). The beneficial effect of the carboxylic acid presence was not general since



Scheme 4. Synthesis of compounds **8a**,**b**. Reagents and conditions: (a) 3-Chloroaniline, HATU, DIPEA, DCM; (b) $NaBH_4$, AcOH; (c) RCHO, $NaCNBH_3$, MeOH, AcOH; (d) phenylchloroformate, DCE, 80 °C; (e) TEA, MeOH.



Scheme 5. Synthesis of compounds **9a–d**. Reagents and conditions: (a) 3-Chloroaniline, Et_2O, rt; (b) phenylchloroformate, DCE, 80 °C, 2 h; (c) TEA, MeOH; (d) 3-chlorophenylisocyanate, PE, reflux; (e) HCl (concd), EtOH, reflux; (f) RNH_2, NMP, μW 170–200 °C; (g) NaOH, MeOH; (h) RNH_2, HATU, DIPEA, DCM.



Scheme 6. Synthesis of compounds **10a**,**b** and **11a**,**b**. Reagents and conditions: (a) 3-Chloroaniline, HATU, DIPEA, DCM; (b) Fe, AcOH, EtOH/H_2O; (c) CS_2, DBU, DMF; 45 °C; (d) $SOCl_2$, $CHCl_3$, 80 °C; (e) 2,2-dimethoxyethylamine, DMF, 80 °C; (f) HCl, DMF; (g) RNH_2, NMP, μW 170 °C or RNH_2, XANTPHOS, Cs_2CO_3, $Pd_2(dba)_3$, toluene, 80 °C; (h) NH_2NH_2/THF, EtOH, 80 °C; (i) HCO_2H, 120 °C.

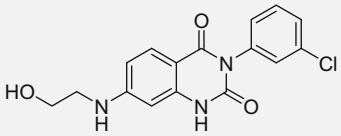
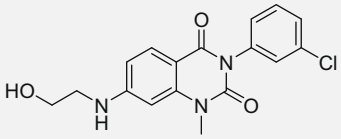
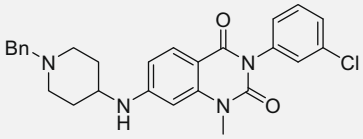
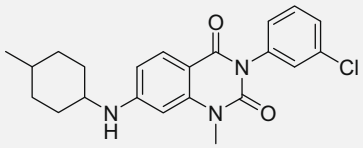
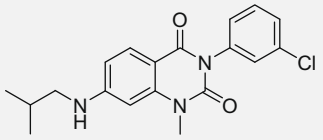
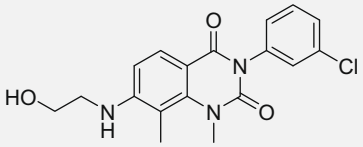
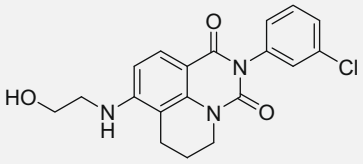
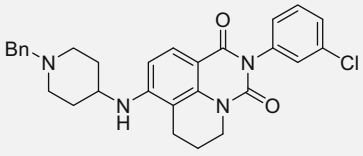
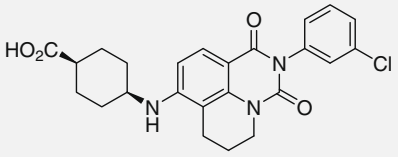
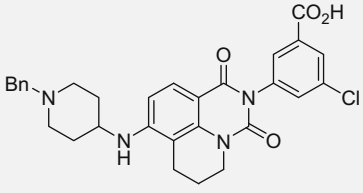
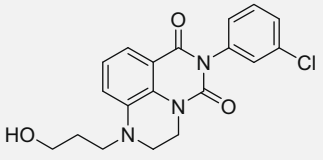
other analogs in the series exhibited a lower selectivity index (data not shown). The introduction of the carboxylic acid substituent on the phenyl ring in the right portion of the molecule (**7d**) gave a beneficial effect regarding the toxicity issue. Compared to **7b**, compound **7d** was less potent in enzyme (20-fold for genotype 1b and fivefold for 2b) and cellular assays (twofold for genotype 1b and fourfold for 2b), but more importantly the window between toxicity and activity was improved (SI = 10).

In a different design of the core structure, the nitrogen of the side chain was incorporated into the saturated ring and the side chain attached to it (compounds **8a–b**). The first compound in this class, **8a** having a propanol side chain to maintain the same chain length of the lead, showed modest affinity for both enzymes but it was essentially inactive in the cellular assays. Other analogs such as **8b** were prepared but biological results were disappointing and the scaffold did not show any advantages over the original lead.

Another designed template was based on the possibility that an internal H-bond between the carbonyl moiety of an amide and the N^1–H of the quinazolinedione could mimic the second aromatic ring of the original tricyclic scaffold. 2,4-Dioxo-1,2,3,4-tetrahydroquinazoline-8-carboxamides **9a**,**b** were considered as hybrid structures between **6f** and **1b**. More specifically, the hypothesis was that the amide in position 8 could form an intramolecular H-bond with the N^1–H of the quinazoline core ring, directing as a consequence the amide side chain in the same direction as the ethanolamine substituent does in **1a**. This hypothesis was confirmed by evaluation of the temperature coefficient ($\Delta\delta/\Delta T$) of N^1–H.[23] In spite of the fact that compounds **9a**,**b** have a similar conformation compared to the lead **1a**, the first analog **9a**, which bears the classical ethanolamine side chain attached to the amide nitrogen was essentially inactive in all assays. Also for this series the benzylpiperazine analogue **9b** showed a restored activity in enzymes, but the compound was toxic.

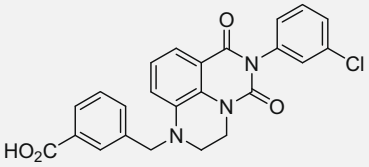
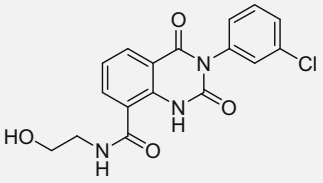
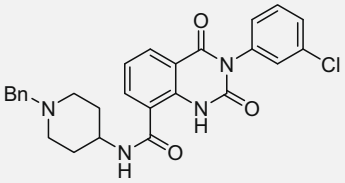
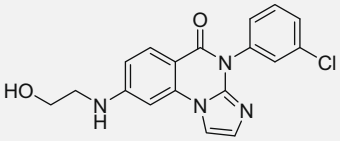
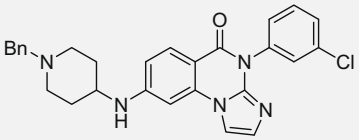
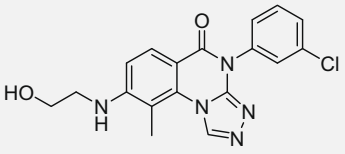
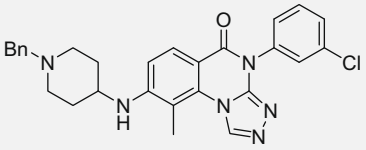
All compounds discussed so far maintained the two carbonyl groups of the imide portion of the lead. From the X-ray analysis of **1a** we knew that they are involved in H-bonds. We evaluated structures like **10a**,**b** and **11a**,**b** in which an H-bond accepting carbonyl oxygen was replaced by a nitrogen still capable of forming H-bonds but incorporated into a five membered ring. The modeling of **10a** (Fig. 3) suggested that two possible superimpositions with compound **1a** were possible. The red lines indicate unfavorable interactions. In one orientation one of the two water molecules can not be bound by the carbonyl O-atom and in the other orientation the imidazole C-H comes close to the Lys (501) side chain. Indeed compounds **10a**,**b** showed weak inhibitory activity: probably this could be explained by the unfavorable interaction we have postulated. To overcome this problem we investigated structures like **11a**,**b** in which the unfavorable interaction of the C–H of the imidazole with the Lys residue has been suppressed by replacing the imidazole with a triazole and a methyl substituent at C-8 has been introduced to increase the lipophilic contact surface with the protein. In fact **11a** showed an increased enzymatic activity respect to **10a** for both genotypes. The compound is moderate active in the cellular assay only for genotype 1b. The benzylpiperidine analog **11b** showed a peculiar profile in the enzymatic assays showing a inverted selectivity between genotype 1b and 2b respect to the compounds described in this article. **11b** gave a toxic response in cellular assays.

Table 3
Biological evaluation of the new scaffolds **6a–f**, **7a–d**, **8a–b**, **9a–b**, **10a–b** and **11a–b**

	Compound	ΔC21-1b IC_{50}[a] (μM)	ΔC21-2b IC_{50}[a] (μM)	HBI10A EC_{50}[a] (μM)	H2B2 EC_{50}[a] (μM)	HBI10A CC_{50}[a] (μM)	SI[b]
6a		5.5	>50	>100	>100	>100	–
6b		2.0	8.2	40	>100	>100	–
6c		0.075	1.3	11	>25	9.9	0.9
6d		5.2	2.3	22	16	36	1.6
6e		3.0	5.0	24	>100	>100	>4
6f		2.4	5.3	40	>100	>100	>2.5
7a		0.20	0.67	15	26	12	0.8
7b		0.008	0.14	1.7	4.9	10	5.9
7c		1.1	0.49	13	17	>100	7.8
7d		0.16	0.72	4.1	20	42	10
8a		5.2	7.7	<100	>100	NA[c]	–

(*continued on next page*)

Table 3 (*continued*)

	Compound	ΔC21-1b IC_{50}[a] (μM)	ΔC21-2b IC_{50}[a] (μM)	HBI10A EC_{50}[a] (μM)	H2B2 EC_{50}[a] (μM)	HBI10A CC_{50}[a] (μM)	SI[b]
8b		0.13	2.0	27	>100	>100	>3.7
9a		>10	>10	<100	NA[c]	NA[c]	–
9b		0.076	0.73	4.0	6.6	7	1.8
10a		>10	>10	>100	NA[c]	NA[c]	–
10b		0.84	>5.0	3.7	2.6	3.2	0.9
11a		0.38	5.3	14	NA[c]	NA[c]	–
11b		6.6	0.40	3.0	<100	9.0	3

[a] Values are means of two or more experiments with standard deviations <30% of the mean value.
[b] SI = CC_{50} (HBI10A)/EC_{50} (HBI10A).
[c] NA = Not active.

3. Conclusions

In conclusion we have illustrated the work done to overcome the limitations of 1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-diones as allosteric inhibitors of NS5B polymerase. The goals we wanted to achieve were the increase of the activities and the dialing out the toxicity related to the series. Several core scaffolds were synthesized and evaluated. As observed for the original series the highest affinity for the enzyme is conferred by the benzylpiperidinyl fragment giving inhibitors in the nanomolar range (**6c**, **7b**, **9b**) on genotype 1b. The activities for genotype 2b are generally lower (exception **11b**) than for 1b and the most active compound **7b** is threefold more active than the reference compound **2**. The same trend was observed for the activities in the cell based assays for both genotypes. The structural changes we introduced did not improved the potency in cell based assays: **7b** is equipotent with **2**, but an improvement in the activity-toxicity window has been achieved. A beneficial effect on the selectivity index is observed when a carboxylic acid is introduced (**7c** and **7d**) in the right or left hand side of the molecules. The encouraging results obtained could be used to further develop this class of compounds.

4. Experimental

4.1. Chemistry

4.1.1. General

Solvents and reagents were obtained from commercial sources and were used without further purification. With the exception of routine deprotection and coupling steps, reactions were carried out under an atmosphere of nitrogen in oven dried (110 °C) glassware. Organic extracts were dried over sodium sulfate, and were concentrated (after filtration of the drying agent) on rotatory

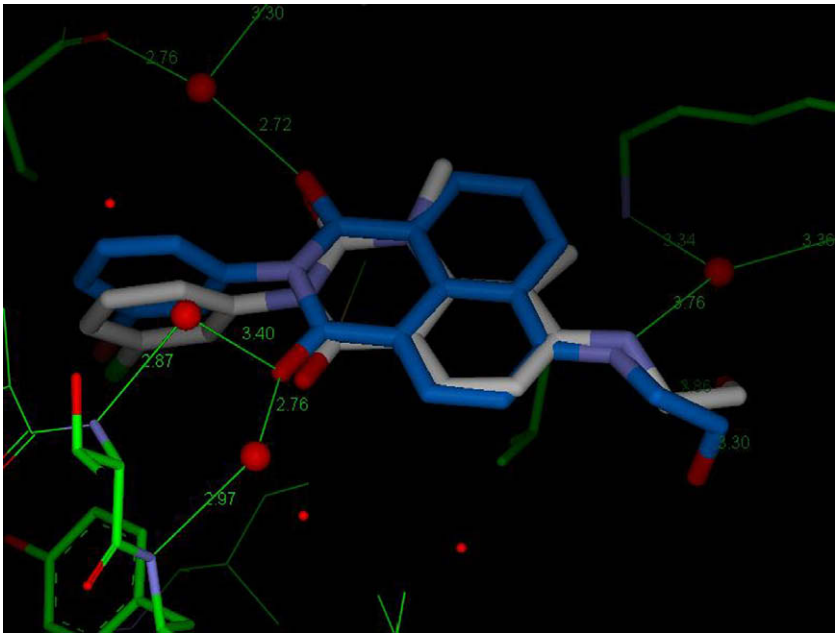


Figure 2. Superimposition of compounds **1a** and **6f**.

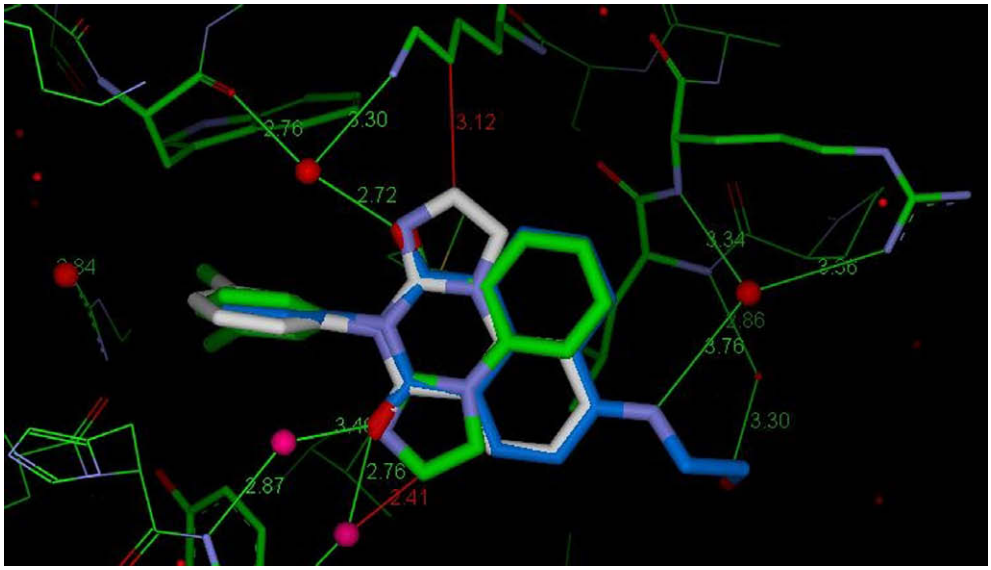


Figure 3. Superimpositions of compounds **1a** and **10a**.

evaporators operating under reduced pressure. Flash chromatography was carried out on silica gel or on commercial flash chromatography systems (Biotage corporation and Jones Flashmaster) utilising pre-packed columns. Microwave irradiation was performed in a Personal Chemistry (now Biotage) Optimizer, Model Emrys. ^{1}H NMR spectra were recorded on Bruker Avance series spectrometers operating at 300 K and at (reported) frequencies between 300 and 600 MHz. Chemical shifts (δ) for signals corresponding to non-exchangeable protons (and exchangeable protons where visible) are referenced to the residual proton signal of the deuterated solvent ($CDCl_3$ at 7.26 ppm; DMSO-d_6 at 2.50 ppm; CD_3CN at 1.94 ppm). Signals are tabulated in the order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad, and combinations thereof); coupling constant(s) in Hertz; number of protons. Mass spectral (MS) data were obtained on a Perkin Elmer API 100 operating in negative (ES^-) or positive (ES^+) ionization mode and results are reported as the ratio of mass over charge (m/z) for the parent ion only. Detection of High-Resolution Mass (HR-MS) was carried out using a SYNAPT High Definition Mass Spectrometer equipped with an electrospray interface (ESI). Source temperature was set at 120 °C. Desolvation gas flow was set at 600 L/h. Identical source conditions were used for all analytes. Detection of the ions was performed in the TOF MS mode with a scan time of 100 ms. UPLC components were a Waters binary pump, an Acquity UPLC Sample Manager, a PDA Detector and an Acquity UPLC Sample Organizer kept refrigerated at 4 °C during analysis, (Waters Corporation, USA). Both UPLC pump, detector and autosampler were controlled by MassLynx version 4.1. The same software was interfaced for subsequent data acquisition and data analysis.

Automated (mass-triggered) RP-HPLC purifications were performed using a Waters Micromass system incorporating a 2525 pump module, a Micromass ZMD detector, and a 2767 collection module, operating under Fraction Lynx software. The stationary phases used were Waters Symmetry C_{18} 5 μm, 19 × 100 mM

(Column A); Waters XTerra 5 μm, 19 mm × 100 mm (Column B). The mobile phase comprised a linear gradient of binary mixtures of MeCN (containing 0.1% TFA) (solvent A) and H_2O (containing 0.1% TFA) (solvent B). The conditions used were as follows: 20% solvent A (2 min) to 80% solvent A over 12 min, solvent A to 100% over 2 min, Column A (method 1); 40% solvent A (2 min) to 90% solvent A over 12 min, solvent A to 100% over 2 min, Column A (method 2); 20% solvent A (2 min) to 80% solvent A over 12 min, solvent A to 100% over 2 min, Column B (method 3); 40% solvent A (2 min) to 90% solvent A over 12 min, solvent A to 100% over 2 min, Column B (method 4). HPLC-MS were performed on a Waters Alliance 2795 apparatus, equipped with a diode array and a ZQ mass spectrometer using an X-Terra C_{18} column (5μ, 4.6 × 50 mm). The mobile phase comprised a linear gradient of binary mixtures of H_2O containing 0.1% formic acid (solvent A) and MeCN containing 0.1% formic acid (solvent B).

Purity of the compounds was determined by analytical RP-HPLC, data were obtained by two methods on an Acquity Waters UPLC using a flow rate of 0.5 mL/min, an Acuity UPLC BEH C18 1.7 μm, 2.1 mm × 50 mm column as the stationary phase, and a mobile phase comprising MeCN + 0.1% HCO_2H (solvent A) and H_2O + 0.1% HCO_2H (solvent B). Method 1: 30% solvent A to 100% solvent A over 2.6 min, then 100% solvent A for 0.3 min. Method 2: 0% solvent A to 100% over 2.6 min and then 100% solvent A for 0.3 min. All the compounds described in this article showed purities higher than 95% in both analytical methods.

4.1.2. General procedure for the synthesis of compounds 6a–f

4.1.2.1. 7-Chloro-3-(3-chlorophenyl)quinazoline-2,4(1*H*,3*H*)-dione (13a). 2-Amino-4-chlorobenzoic acid (2.0 g, 11.7 mmol) was dissolved in dry THF (40 mL) and cooled to 0 °C. Pyridine (1.3 mL, 16.3 mmol) and phenyl chloroformate (1.8 mL, 14.0 mmol) were added dropwise. The reaction mixture was stirred at 0 °C for 2 h. After dilution with EtOAc the organic phase was filtered and washed with 1 N HCl, brine and evaporated. The resulting crude was dissolved in THF (40 mL) and cooled to 0 °C. The solution was treated with 3-chloroaniline (1.5 g, 11.9 mmol), HOBt (3.6 g, 23.8 mmol) and NMM (1.3 mL, 11.9 mmol). After 30 min of stirring, DCC (2.5 g, 11.9 mmol) was added and the reaction mixture was stirred at 60 °C for 4 h. After cooling down, the reaction was diluted with water and the resulting precipitate was filtered off and washed with Et_2O, affording phenyl (5-chloro-2-{[(3-chlorophenyl)amino]carbonyl}-phenyl)carbamate as a pale yellow powder (1.65 g, 35%); MS (ES^+) *m/z* 401, 403 $(M+H)^+$. This crude (0.4 g, 1.0 mmol) was dissolved in DMF (5 mL) and irradiated in a microwave apparatus at 150 °C for 15 min. Water was added and the resulting precipitate was isolated by filtration affording the title compound (250 mg, 80%) as an off-white powder. ^1H NMR (300 MHz, DMSO-d_6) δ 11.71 (s, 1H), 7.93 (d, *J* = 8.4 Hz, 1H), 7.52–7.50 (m, 3H), 7.35–7.25 (m, 3H); MS (ES^+) *m/z* 307, 309 $(M+H)^+$.

4.1.2.2. 3-(3-Chlorophenyl)-7-[(2-hydroxyethyl)amino]quinazoline-2,4(1*H*,3*H*)-dione (6a). Compound **13a** (50 mg, 0.16 mmol) and 2-aminoethanol (29 μL, 0.5 mmol) in NMP (0.5 mL) were irradiated in a microwave apparatus at 200 °C for 30 min. The reaction was diluted with MeCN, followed by purification by RP-HPLC (method 1) affording the title compound (3 mg, 5%). ^1H NMR (400 MHz, DMSO-d_6) δ 11.16 (s, 1H), 7.59 (d, *J* = 8.8 Hz, 1H), 7.50–7.42 (m, 3H), 7.28–7.24 (m, 1H), 6.81 (bt, *J* = 5.4 Hz, 1H), 6.50 (dd, *J* = 8.8, 1.8 Hz, 1H), 6.21 (d, *J* = 1.8 Hz, 1H), 4.79 (br s, 1H), 3.61–3.56 (m, 2H), 3.18–3.13 (m, 2H); HRMS (ES^+) *m/z* calcd for $C_{16}H_{15}ClN_3O_3$ $(M+H)^+$: 332.0802; found: 332.0809.

4.1.2.3. 7-Chloro-3-(3-chlorophenyl)-1-methylquinazoline-2,4-(1*H*,3*H*)-dione (14a). A solution of **13a** (0.38 g, 1.2 mmol) in DMF (5.5 mL) was treated with DBU (0.22 mL, 1.5 mmol) and MeI (92 μL, 1.5 mmol). The reaction mixture was stirred at rt for 1 h. After dilution with water, the resulting precipitate was filtered off affording the title compound (350 mg, 90%) as a white solid. ^1H NMR (400 MHz, DMSO-d_6) δ 8.04 (d, *J* = 8.4 Hz, 1H), 7.65 (d, *J* = 1.5 Hz, 1H), 7.53–7.49 (m, 3H), 7.39 (dd, *J* = 8.4, 1.5 Hz, 1H) 7.34–7.30 (m, 1H), 3.53 (s, 3H); MS (ES^+) *m/z* 365, 367 $(M+H)^+$.

4.1.2.4. 3-(3-Chlorophenyl)-7-[(2-hydroxyethyl)amino]-1-methylquinazoline-2,4(1*H*,3*H*)-dione (6b). Compound **14a** (80 mg, 0.25 mmol) and 2-aminoethanol (0.15 mL, 2.55 mmol) in NMP (0.8 mL) were irradiated in a microwave apparatus at 200 °C for 30 min. The reaction was diluted with MeCN, followed by purification by RP-HPLC (method 1) affording the title compound (15 mg, 18%). ^1H NMR (400 MHz, DMSO-d_6) δ 7.71 (d, *J* = 8.8 Hz, 1H), 7.51–7.45 (m, 2H), 7.43–7.41 (m, 1H), 7.27–7.24 (m, 1H), 6.88 (bt, *J* = 5.5 Hz, 1H), 6.58 (dd, *J* = 8.8, 1.9 Hz, 1H), 6.39 (d, *J* = 1.9 Hz, 1H), 4.80 (t, *J* = 5.3 Hz, 1H), 3.61 (td, $J_1 = J_2 = 5.7$ Hz, $J_3 = 5.2$ Hz, 2H), 3.46 (s, 3H), 3.30–3.25 (m, 2H); HRMS (ES^+) *m/z* calcd for $C_{17}H_{17}ClN_3O_3$ $(M+H)^+$: 346.0958; found: 346.0955.

4.1.2.5. 7-[(1-Benzylpiperidin-4-yl)amino]-3-(3-chlorophenyl)-1-methylquinazoline-2,4(1*H*,3*H*)-dione (6c). Prepared as described for **6b** from **14a** (50 mg, 0.16 mmol), purification by RP-HPLC (method 3) affording the title compound (8 mg, 11%) as a solid. ^1H NMR (400 MHz, DMSO-d_6 + TFA) δ 9.84 (br s, 1H), 7.93 (d, *J* = 8.7 Hz, 1H), 7.76–7.64 (m, 10H), 7.49–7.41 (m, 1H), 6.78 (bd, *J* = 8.7 Hz, 1H), 6.58 (s, 1H), 4.60–4.47 (m, 2H), 3.92 (m, 1H), 3.74–3.61 (m, 5H), 3.38–3.22 (m, 2H), 2.45–2.37 (m, 2H), 1.94–1.80 (m, 2H); HRMS (ES^+) *m/z* calcd for $C_{27}H_{28}ClN_4O_2$ $(M+H)^+$: 475.1901; found: 475.1898.

4.1.2.6. 3-(3-Chlorophenyl)-1-methyl-7-[(4-methylcyclohexyl)-amino]quinazoline-2,4(1*H*,3*H*)-dione (6d). Compound **14a** (70 mg, 0.22 mmol), 4-methylcyclohexanamine (0.23 mL, 1.74 mmol) and DBU (0.039 mL, 0.26 mmol) in NMP (0.8 mL) were irradiated in a microwave apparatus at 200 °C for 30 min. The reaction was diluted with MeCN and was purified by RP-HPLC (method 2) affording the title compound (26 mg, 30%) as a solid in a mixture cis/trans (*c*/*t*) = 60/40. ^1H-NMR (600 MHz, DMSO-d_6) δ 7.70 (d, *J* = 8.7 Hz, 0.6H, *c*), 7.68 (d, *J* = 8.7 Hz, 0.4H, *t*), 7.50–7.46 (m, 2H), 7.42–7.40 (m, 1H), 7.26–7.24 (m, 1H), 6.77–6.71 (br s, 1H), 6.61 (dd, *J* = 8.7, 1.7 Hz, 0.6H, *c*), 6.53 (dd, *J* = 8.7, 1.7 Hz, 0.4H, *t*), 6.43 (d, *J* = 1.7 Hz, 0.6H, *c*), 6.34 (d, *J* = 1.7 Hz, 0.4H, *t*), 3.69 (m, 0.6H, *c*), 3.44 (s, 3H), 3.46 (m, 0.4H, *t*), 2.00–1.96 (m, 0.8H, *t*), 1.74–1.66 (m, 2H), 1.65–1.59 (m, 1.2H, *c*), 1.58–1.49 (m, 1.8H, *c*), 1.40–1.31 (m, 1.6H), 1.24–1.18 (m, 0.8H, *t*), 1.13–1.07 (m, 0.8H, *t*), 0.93 (d, *J* = 6.6 Hz, 1.8H, *c*), 0.09 (d, *J* = 6.6 Hz, 1.2H); HRMS (ES^+) *m/z* calcd for $C_{22}H_{25}ClN_3O_2$ $(M+H)^+$: 398.1635; found: 398.1637.

4.1.2.7. 3-(3-Chlorophenyl)-7-(*iso*butylamino)-1-methylquinazoline-2,4(1*H*,3*H*)-dione (6e). Prepared as described for **6d** from **14a** (50 mg, 0.16 mmol); purification by RP-HPLC (method 2) affording the title compound (12 mg, 22%) as a solid. ^1H NMR (300 MHz, DMSO-d_6) δ 7.76 (d, *J* = 8.7 Hz, 1H), 7.58–7.51 (m, 2H), 7.50–7.46 (m, 1H), 7.33–7.29 (m, 1H), 6.97 (bt, *J* = 5.8 Hz, 1H), 6.63 (dd, *J* = 8.7, 1.8 Hz, 1H), 6.41 (d, *J* = 1.8 Hz, 1H), 3.51 (s, 3H), 3.07 (bt, *J* = 5.8 Hz, 2H), 1.96 (m, 1H), 1.03 (d, *J* = 6.7 Hz, 6H); HRMS (ES^+) *m/z* calcd for $C_{19}H_{21}ClN_3O_2$ $(M+H)^+$: 358.1322; found: 358.1323.

4.1.2.8. 3-(3-Chlorophenyl)-7-fluoro-8-methylquinazoline-2,4-(1*H*,3*H*)-dione (13b). 2-Amino-4-fluoro-3-methylbenzoic acid[15] **12b** (130 mg, 0.77 mmol) was dissolved in MeOH (8 mL) and treated with $SOCl_2$ (0.084 mL, 1.15 mmol). The reaction was stirred at RT overnight, then the solvent was partially evaporated and the

residue partitioned between $NaHCO_3$ (saturated solution) and EtOAc. Separated organic layer was washed with $NaHCO_3$ (saturated solution), brine, dried and evaporated to afford the intermediate methyl 2-amino-4-fluoro-3-methylbenzoate (109 mg, 77%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (dd, J = 8.7, 8.2 Hz, 1H), 6.34 (t, J = 8.7 Hz, 1H), 6.71 (br s, 2H), 3.83 (s, 3H), 2.02 (s, 3H). The latter compound (54 mg, 0.29 mmol) was dissolved in Et_2O (1.0 mL) and 3-chlorophenylisocyanate was added dropwise. The reaction mixture was stirred 18 h at rt. Evaporation of the solvent gave a residue that was disolved in EtOH (1.0 mL) and treated with 1 M NaOH (0.5 mL). After stirring for 2 h at RT, the reaction was diluted with water, acidified with 6 N HCl and the precipitate isolated by filtration affording the title compound (85 mg, 94%) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.06 (br s, 1H), 7.87 (dd, J = 8.8, 6.6 Hz, 1H), 7.54–7.49 (m, 3H), 7.36–7.32 (m, 1H), 7.08 (t, J = 8.8 Hz, 1H), 2.30 (s, 3H); MS (ES$^+$) m/z 304, 306 (M+H)$^+$.

4.1.2.9. 3-(3-Chlorophenyl)-7-fluoro-1,8-dimethylquinazoline-2,4-(1*H*,3*H*)-dione (14b). Prepared as described for **14a** from **13b** (82 mg, 0.27 mmol) to afford the title compound (80 mg, 93%) as a solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.95 (dd, J = 8.7, 6.6 Hz, 1H), 7.53–7.47 (m, 3H), 7.33–7.28 (m, 1H), 7.18 (t, J = 8.7 Hz, 1H), 3.58 (s, 3H), 2.46 (s, 3H); MS (ES$^+$) m/z 318, 320 (M+H)$^+$.

4.1.2.10. 3-(3-Chlorophenyl)-7-[(2-hydroxyethyl)amino]-1,8-dimethylquinazoline-2,4(1*H*,3*H*)-dione (6f). Prepared as described for **6b** from **14b** (86 mg, 0.27 mmol), purification by RP-HPLC (method 1) afforded the title compound (4 mg, 4%) as a solid. ^{1}H NMR (600 MHz, DMSO-d_6 + TFA) δ 7.70 (d, J = 8.7 Hz, 1H), 7.49–7.41 (m, 3H), 7.28–7.25 (m, 1H), 6.65 (d, J = 8.7 Hz, 1H), 3.60 (t, J = 6.0 Hz, 2H), 3.45 (s, 3H), 3.31 (t, J = 6.0 Hz, 2H), 2.20 (s, 3H); HRMS (ES$^+$) m/z calcd for $C_{18}H_{19}ClN_3O_3$ (M+H)$^+$: 360.1115; found: 360.112.

4.1.3. Synthesis of 7a

4.1.3.1. Methyl 5-fluoro-1,2,3,4-tetrahydroquinoline-8-carboxylate (16). 5-Fluoroquinoline-8-carboxylic acid[17] **15** (612 mg, 3.22 mmol) in acetic acid (13 mL) was treated with PtO_2 (219 mg, 0.97 mmol) and stirred under hydrogen at atmospheric pressure for 3 h. The catalyst was filtered off and the solution was concentrated to dryness under reduced pressure to give a residue that after purification by RP-HPLC (method 3) afforded 5-fluoro-1,2,3,4-tetrahydroquinoline-8-carboxylic acid (253 mg, 40%) as a white solid; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.34 (br s, 1H), 8.06 (br s, 1H), 7.62 (dd, J = 8.7, 7.1 Hz, 1H), 6.26 (t, J = 8.9 Hz, 1H), 3.31–3.36 (m, 2H), 2.61–2.69 (m, 2H), 1.71–1.85 (m, 2H); MS (ES$^+$) m/z 196 (M+H)$^+$. The latter compound (137 mg, 0.7 mmol) was dissolved in MeOH (18 mL), treated with $SOCl_2$ (1.4 mL, 19.6 mmol) and heated to reflux for 24 h. Volatiles were evaporated under reduced pressure and the residue partitioned between CH_2Cl_2 and aqueous $NaHCO_3$ (saturated solution). The organic layer was separated and dried. Evaporation of the solvent afforded the title compound (118 mg, 80%) as a solid; MS (ES$^+$) m/z 210 (M+H)$^+$.

4.1.3.2. 2-(3-Chlorophenyl)-8-fluoro-6,7-dihydro-1*H*,5*H*-pyrido-[3,2,1-*ij*]quinazoline-1,3(2*H*)-dione (17). A solution of **16** (118 mg, 0.56 mmol) in toluene (2 mL) was treated with 1-chloro-3-isocyanatobenzene (206 μL, 1.68 mmol) and heated at 90 °C for 20 h. Solvent was evaporated under reduced pressure to give a residue that was dissolved in ethanol/1 N NaOH (2:1, 3 mL). The resulting solution was stirred at rt for 3 h, and concentrated to dryness under reduced pressure to afford a residue which was purified by RP-HPLC (method 2) affording the title compound (65 mg, 35%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.94 (dd, J = 8.9 Hz, 6.4, 1H), 7.46–7.56 (m, 3H), 1.97–2.08 (m, 2H), 7.28–7.36 (m, 1H), 7.14 (t, J = 8.9 Hz, 1H), 3.90–4.00 (m, 2H), 2.82–2.91 (m, 2H); MS (ES$^+$) m/z 331, 333 (M+H)$^+$.

4.1.3.3. 2-(3-Chlorophenyl)-8-[(2-hydroxyethyl)amino]-6,7-dihydro-1*H*,5*H*-pyrido[3,2,1-*ij*]quinazoline-1,3(2*H*)-dione (7a). Intermediate **17** (30 mg, 0.09 mmol) was dissolved in NMP (1 mL) and treated with ethanolamine (33 μL, 0.54 mmol). The reaction mixture was irradiated in a microwave apparatus at 200 °C for 2 h. The solution was purified by RP-HPLC (method 3) affording the title compound (17 mg, 35%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.72 (d, J = 8.8 Hz, 1H), 7.51–7.46 (m, 2H), 7.44–7.41 (m, 1H), 7.29–7.22 (m, 1H), 6.62 (d, J = 8.8 Hz, 1H), 5.90 (br s, 1H), 4.79 (br s, 1H), 3.92–3.87 (m, 2H), 3.59 (t, J = 5.9 Hz, 2H), 3.39 (t, J = 5.9 Hz, 2H), 2.54–2.52 (m, 2H), 2.05–1.96 (m, 2H); HRMS (ES$^+$) m/z calcd for $C_{19}H_{19}ClN_3O_3$ (M+H)$^+$: 372.1115; found: 372.1121.

4.1.4. General procedure for the synthesis of compounds 7b–d

4.1.4.1. 5-Bromoquinoline-8-carboxylic acid (18). A solution of 8-methylquinoline (4.0 g, 28.0 mmol) in H_2SO_4 (80 mL) was treated with Ag_2SO_4 (13.1 g, 42 mmol) then bromine (4.5 g, 28 mmol) was added. The reaction mixture was stirred at rt for 5 h and then poured into ice and filtered. The resulting filtrate was basified with aqueous Na_2CO_3 (saturated solution) and the aqueous phase was extracted with EtOAc. The combined organic layers were evaporated affording 5-bromo-8-methylquinoline (5.0 g, 71%) as a solid, MS (ES$^+$) m/z 222, 224 (M+H)$^+$. The latter was dissolved in H_2SO_4/H_2O (2:3, 200 mL) and treated with CrO_3 (22.4 g, 22 mmol) at 90 °C. The reaction mixture was heated at 90 °C for 2 h. Then, the mixture was poured into ice and the precipitate was filtered affording the title compound (3.54 g, 62%) as an orange solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.25–9.13 (m, 1H), 8.82 (d, J = 7.7 Hz, 1H), 8.47–8.37 (m, 1H), 8.23 (d, J = 7.7 Hz, 1H), 8.01–7.91 (m, 1H); MS (ES$^+$) m/z 252, 254 (M+H)$^+$.

4.1.4.2. 5-Bromo-*N*-(3-chlorophenyl)-1,2,3,4-tetrahydroquinoline-8-carboxamide (19a). Compound **18** (1.0 g, 3.94 mmol) in CH_2Cl_2 (40 mL) was treated with DIPEA (1.5 mL, 8.7 mmol), 3-chloroaniline (0.13 mL, 4.33 mmol), and HATU (1.6 g, 4.33 mmol). The reaction mixture was stirred at rt for 16 h. Then, it was treated with aqueous $NaHCO_3$ (saturated solution) and the organic phase separated. The organic phase was washed with 1 N HCl, aqueous $NaHCO_3$ (saturated solution) and dried. Evaporation of the solvent gave a crude that was treated with Et_2O. The solid obtained (1 g, 2.75 mmol) was filtered and dissolved in THF (30 mL). The resulting solution was treated with $NaCNBH_3$ (0.52 g, 8.26 mmol) and $BF_3{\cdot}Et_2O$ (1.05 mL, 8.26 mmol). The reaction mixture was heated at 80 °C for 1 h, left to cool down and then treated with NH_3 and Et_2O. The aqueous phase was extracted with Et_2O. The combined organic layers were dried and evaporated to give a crude which was resubmitted to the same reaction conditions to afford after workup the title compound (1.00 g, 70%) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.18 (s, 1H), 7.90 (t, J = 2.1 Hz, 1H), 7.80 (br s, 1H), 7.61 (dd, J = 8.2, 2.1 Hz, 1H), 7.43 (d, J = 8.5 Hz, 1H), 7.36 (t, J = 8.3 Hz, 1H), 7.14 (dd, J = 8.2, 2.1 Hz, 1H), 6.78 (d, J = 8.5 Hz, 1H), 3.30 (bt, J = 6.8 Hz, 2H), 2.74 (t, J = 6.4 Hz, 2H), 1.86–1.78 (m, 2H); MS (ES$^+$) m/z 365, 367 (M+H)$^+$.

4.1.4.3. 8-Bromo-2-(3-chlorophenyl)-6,7-dihydro-1*H*,5*H*-pyrido-[3,2,1-*ij*]quinazoline-1,3(2*H*)-dione (20a). To a solution of **19a** (1.00 g, 2.72 mmol) in 1,2-dicloroethane (25 mL) was added phenyl chloroformate (0.34 mL, 2.72 mmol) and the reaction mixture was stirred at 80 °C. After 1 h the solvent was evaporated and the crude was dissolved in MeOH (25 mL). The resulting solution was treated with Et_3N (7.6 mL, 54.4 mmol) and heated at 80 °C for 30 min. The solvent was evaporated and the crude washed with Et_2O and

filtered off affording the title compound (300 mg, 28%) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.81 (d, J = 8.5 Hz, 1H), 7.58–7.48 (m, 5H), 3.96 (dd, J = 7.5, 5.9 Hz, 2H), 2.93 (t, J = 6.7 Hz, 2H), 2.10–2.01 (m, 2H); MS (ES$^+$) m/z 391, 393 (M+H)$^+$.

4.1.4.4. 8-[(1-Benzylpiperidin-4-yl)amino]-2-(3-chlorophenyl)-6,7-dihydro-1*H*,5*H*-pyrido[3,2,1-*ij*]quinazoline-1,3(2*H*)-dione (7b). To a solution of **20a** (20 mg, 0.05 mmol) in toluene (1 mL), 4-amino-benzyl piperidine (0.012 mL, 0.06 mmol), NaO^{t}Bu (6.7 mg, 0.07 mmol), BINAP (0.93 mg, 0.0015 mmol) and $Pd_2(dba)_3$ (0.92 mg, 0.001 mmol) were added. The reaction mixture was heated at 80 °C for 16 h. The solvent was evaporated and the residue purified by RP-HPLC (method 3) affording the title compound (5.1 mg, 20%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.54 (br s, 1H), 7.71 (d, J = 8.8 Hz, 1H), 7.55–7.47 (m, 6H), 7.44–7.40 (m, 1H), 7.28–7.23 (m, 2H), 6.71 (d, J = 8.8 Hz, 1H), 5.77 (bd, J = 8.1 Hz, 1 H), 4.33 (d, J = 4.2 Hz, 2H), 3.92–3.88 (m, 2H), 3.71 (m, 1H), 3.46 (bd, J = 13.2 Hz, 2H), 3.14–3.06 (m, 2H), 2.53–2.51 (m, 2H), 2.12 (bd, J = 13.1 Hz, 2H), 2.04–1.96 (m, 2H), 1.83–1.73 (m, 2H); HRMS (ES$^+$) m/z calcd for $C_{29}H_{30}ClN_4O_2$ (M+H)$^+$: 501.2057; found: 501.2064.

4.1.4.5. *cis*-4-{[2-(3-Chlorophenyl)-1,3-dioxo-2,3,6,7-tetrahydro-1*H*,5*H*-pyrido[3,2,1-*ij*]quinazolin-8-yl]amino}cyclohexanecarboxylic acid (7c). Prepared as described for **7b** from intermediate **20a** (100 mg, 0.25 mmol), affording after RP-HPLC purification (method 3) the title compound (14 mg, 12%) as a solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.10 (s, 1H), 7.70 (d, J = 8.7 Hz, 1H), 7.50–7.47 (m, 2H), 7.43–7.41 (m, 1H), 7.26–7.24 (m, 1H), 6.67 (d, J = 8.7 Hz, 1H), 5.60 (d, J = 8.3 Hz, 1H), 3.90–3.88 (m, 2H), 3.54 (m, 1H), 2.53–2.51 (m, 2H), 2.42 (tt, $J_1 = J_2$ = 12.2 Hz, $J_3 = J_4$ = 3.3 Hz, 1H), 2.11 (bd, J = 12.7 Hz, 1H), 2.01–1.97 (m, 2H), 1.92–1.88 (m, 2H), 1.79 (dt, J_1 = 13.5 Hz, $J_2 = J_3$ = 3.3 Hz, 1H), 1.45–1.39 (m, 2H), 1.35–1.19 (m, 2H); HRMS (ES$^+$) m/z calcd for $C_{24}H_{25}ClN_3O_4$ (M+H)$^+$: 454.1534; found: 454.1547.

4.1.4.6. 3-[8-[(1-Benzylpiperidin-4-yl)amino]-1,3-dioxo-6,7-dihydro-1*H*,5*H*-pyrido[3,2,1-*ij*]quinazolin-2(3*H*)-yl]-5-chlorobenzoic acid (7d). Prepared according to the procedure for **7b**, from **20b** (52 mg, 0.115 mmol) to afford methyl 3-(8-(1-benzylpiperidin-4-ylamino)-1,3-dioxo-6,7-dihydropyrido[3,2,1-*ij*]quinazolin-2(1*H*,3*H*,5*H*)-yl)-5-chlorobenzoate. This was dissolved in dioxane (1 mL) and treated with 2 M NaOH (0.2 mL, 0.4 mmol). After stirring for 8 h at 40 °C, solvents were evaporated and the resulting crude was purified by RP-HPLC (method 3) affording the title compound (15 mg, 24%) as a solid. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 13.40 (br s, 1H), 7.95 (s, 1H), 7.80 (s, 1H), 7.74 (s, 1H), 7.71 (d, J = 8.9 Hz, 1H), 7.55–7.48 (m, 5H), 6.72 (d, J = 8.9 Hz, 1H), 5.79 (br s, 1H), 4.43 (s, 2H), 3.96–3.87 (m, 2H), 3.72 (m, 1H), 3.50–3.41 (m, 2H), 3.16–3.03 (m, 2H), 2.12 (bd, J = 13.4 Hz, 2H), 2.06–1.97 (m, 2H), 1.86–1.71 (m, 2H); HRMS (ES$^+$) m/z calcd for $C_{30}H_{30}ClN_4O_4$ (M+H)$^+$: 545.1956; found: 545.1965.

4.1.5. General procedure of compounds 8a–b

4.1.5.1. *N*-(3-Chlorophenyl)-1,2,3,4-tetrahydroquinoxaline-5-carboxamide (22). A solution of quinoxaline-5-carboxylic acid[19] **21** (500 mg, 2.87 mmol) in CH_2Cl_2 (30 mL) was treated with DIPEA (1.1 mL, 6.32 mmol), 3-chloroaniline (0.33 mL, 3.16 mmol) and HATU (1.20 g, 3.16 mmol). The reaction mixture was stirred at rt overnight. The solution was diluted with EtOAc and the organic phase was washed with aqueous $NaHCO_3$ (saturated solution) and dried. Evaporation of the solvent gave a crude that was washed with MeCN and filtered off affording *N*-(3-chlorophenyl)quinoxaline-5-carboxamide (775 mg, 93%), MS (ES$^+$) m/z 284, 286 (M+H)$^+$. The latter was dissolved in AcOH (30 mL) and treated with $NaBH_4$ (201 mg, 5.46 mmol). The reaction mixture was stirred at rt for 2 h, then the mixture was diluted with EtOAc and H_2O. The aqueous phase was separated and the organic phase was washed with brine and dried. Evaporation of the solvent afforded the title compound (613 mg, 78%) as an orange solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.32 (s, 1H), 7.95 (s, 1H), 7.64 (d, J = 8.1 Hz, 1H), 7.44 (d, J = 8.1 Hz, 1H), 7.38 (t, J = 8.1 Hz, 1H), 7.16 (d, J = 7.8 Hz, 1H), 7.07 (d, J = 7.8 Hz, 1H), 6.71 (t, J = 7.8 Hz, 1H), 3.50–3.43 (m, 2H), 3.41–3.36 (m, 2H); MS (ES$^+$) m/z 287, 289 (M+H)$^+$.

4.1.5.2. 6-(3-Chlorophenyl)-1-(3-hydroxypropyl)-2,3-dihydro-1*H*,-5*H*-pyrazino[3,2,1-*ij*]quinazoline-5,7(6*H*)-dione (8a). A solution of **22** (72 mg, 0.25 mmol) in MeOH (5 mL) was treated with 3-{[*tert*-butyl(dimethyl)silyl]oxy}propanal (0.052 mL, 0.25 mmol), $NaBH_3CN$ (15.7 mg, 0.25 mmol) and catalytic amount of AcOH. The reaction mixture was stirred at rt overnight. Then, aqueous $NaHCO_3$ (saturated solution) was added and the aqueous phase extracted with EtOAc. The combined organic layers were dried and evaporated to give a solid which was purified to give *N*-(3-chlorophenyl)-1-(3-hydroxypropyl)-1,2,3,4-tetrahydroquinoxaline-5-carboxamide (MS (ES$^+$) m/z 346,348 (M+H)$^+$). The latter (20 mg, 0.058 mmol) was dissolved in 1,2-dichloroethane (5 mL). The resulting solution was treated with phenyl chloroformate (0.008 mL, 0.058 mmol). The reaction mixture was stirred at 80 °C for 2 h. Evaporation of the solvent gave a crude which was dissolved in MeOH (5 mL) and treated with Et_3N (0.08 mL, 0.58 mmol). The reaction mixture was heated at 80 °C for 30 min. The solvent was evaporated and the residue purified by RP-HPLC (method 3) affording the title compound (8 mg, 20%) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.55–7.46 (m, 3H), 7.34–7.30 (m, 2H), 7.14 (t, J = 7.9 Hz, 1H), 7.08 (dd, J = 8.4, 1.2 Hz, 1H), 4.58 (br s, 1H), 4.02 (bt, J = 5.4 Hz, 2H), 3.51 (t, J = 6.1 Hz, 2H), 3.46–3.42 (m, 4H), 1.76–1.69 (m, 2H); HRMS (ES$^+$) m/z calcd for $C_{19}H_{19}ClN_3O_3$ (M+H)$^+$: 372.1115; found: 372.112.

4.1.5.3. 3-{[6-(3-Chlorophenyl)-5,7-dioxo-2,3,6,7-tetrahydro-1*H*,-5*H*-pyrazino[3,2,1-*ij*]quinazolin-1-yl]methyl}benzoic acid (8b). Prepared according to the procedure described for **8a** from intermediate **22** (100 mg, 0.35 mmol) and methyl-3-formylbenzoate (57 mg, 0.35 mmol) to obtain the intermediate methyl 3-{[6-(3-chlorophenyl)-5,7-dioxo-2,3,6,7-tetrahydro-1*H*,5*H*-pyrazino[3,-2,1-*ij*]quinazolin-1-yl]methyl}benzoate MS (ES$^+$) m/z 462, 465 (M+H)$^+$. This was dissolved in dioxane (3 mL) and treated with 2 M NaOH (0.2 mL, 0.4 mmol). After stirring for 8 h at rt, solvents were evaporated and the resulting crude was purified by RP-HPLC (method 3) affording the title compound (11 mg, 20%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.98 (br s, 1H), 7.93 (s, 1H), 7.85 (d, J = 7.8 Hz, 1H), 7.64–7.43 (m, 5H), 7.40–7.26 (m, 2H), 7.11–7.01 (m, 2H), 4.69 (s, 2H), 4.15–4.05 (m, 2H), 3.62–3.52 (m, 2H); HRMS (ES$^+$) m/z calcd for $C_{24}H_{19}ClN_3O_4$ (M+H)$^+$: 448.1064; found: 448.1077.

4.1.6. General procedure for the synthesis of compounds 9a,b

4.1.6.1. Methyl 3-(3-chlorophenyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-8-carboxylate (24a). A solution of 2-amino-3-(methoxycabonyl)benzoic acid[21] **23a** (2.00 g, 10.2 mmol) in CH_2Cl_2 (60 mL) was treated with DIPEA (2.5 mL, 14.28 mmol), 3-chloroaniline (1.5 mL, 14.28 mmol), and HATU (4.26 g, 11.2 mmol). The reaction mixture was stirred at rt for 16 h. Then, it was treated with aqueous $NaHCO_3$ (saturated solution) and the organic phase separated. The organic phase was washed with 1 N HCl, aqueous $NaHCO_3$ (saturated solution) and dried. Evaporation of the solvent gave a crude that was treated with PE-CH_2Cl_2. The precipitate was filtered to give methyl 2-amino-3-{[(3-chlorophenyl)amino]carbonyl}benzoate (2.45 g, 77%); MS (ES$^+$) m/z 305, 307 (M+H)$^+$. This intermediate (2.45 g, 8 mmol) was dissolved in 1,2-dicloroethane (50 mL) and phenylchloroformate (1 mL, 8 mmol) was added. The

reaction mixture was stirred at 80 °C. After 2 h the solvent was evaporated and the crude was dissolved in MeOH (50 mL). The resulting solution was treated with Et_3N (11 mL, 80 mmol). The reaction mixture was heated at 80 °C for 30 min. The solvent was evaporated and the crude washed with Et_2O-PE and filtered off affording the title compound (2.95 g, 100%) as a solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.81 (s, 1H), 8.35 (dd, J = 7.8, 1.3 Hz, 1H), 8.28 (dd, J = 7.9, 1.4 Hz, 1H), 7.58–7.53 (m, 3H), 7.42–7.36 (m, 2H), 3.97 (s, 3H); MS (ES$^+$) m/z 332, 334 (M+H)$^+$.

4.1.6.2. 3-(3-Chlorophenyl)-*N*-(2-hydroxyethyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-8-carboxamide (9a). A solution of **24a** (50 mg, 0.15 mmol) in NMP (3 mL) was treated with 2-aminoethanol (92.4 mg, 1.51 mmol). The reaction mixture was irradiated in a microwave apparatus at 170 °C for 300 s. The solution was purified by RP-HPLC (method 1) affording of the title compound (6 mg, 11%) as a solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.85 (s, 1H), 9.02 (t, J = 5.8 Hz, 1H), 8.27 (d, J = 7.8 Hz, 1H), 8.14 (d, J = 7.8 Hz, 1H), 7.56–7.50 (m, 3H), 7.39–7.32 (m, 2H), 4.82 (br s, 1H), 3.56 (t, J = 5.8 Hz, 2H), 3.39 (q, J = 5.8 Hz, 2H); HRMS (ES$^+$) m/z calcd for $C_{17}H_{15}ClN_3O_4$ (M+H)$^+$: 360.0751; found: 360.0754.

4.1.6.3. *N*-(1-Benzylpiperidin-4-yl)-3-(3-chlorophenyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-8-carboxamide (9b). To a solution of **24a** (55 mg, 0.17 mmol) in MeOH (3 mL) was added 2 N NaOH (0.17 mL, 0.34 mmol). The reaction mixture was stirred at rt for 6 h. After evaporation of the solvent the residue was dissolved in EtOAc and the resulting solution was washed with 1 N HCl, dried. Evaporation of the solvent gave 3-(3-chlorophenyl)-2,4-dioxo-1,2,3,4-tetrahydroquinazoline-8-carboxylic acid, MS (ES$^+$) m/z 317, 319 (M+H)$^+$. This intermediate (50 mg, 0.16 mmol) was dissolved in CH_2Cl_2 (5 mL) and treated with 4-amino benzylpiperidine (0.039 mL, 0.19 mmol), DIPEA (0.033 mL, 0.19 mmol) and HATU (66.2 mg, 0.17 mmol). The reaction mixture was stirred at rt for 48 h. After dilution with CH_2Cl_2, the resulting solution was washed with water and dried. Evaporation of the solvent gave a crude that was purified by RP-HPLC (method 1) affording the title compound (33 mg, 35%) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.60 (s, 1H), 9.54 (br s, 1H), 9.01 (d, J = 7.6 Hz, 1H), 8.24 (dd, J = 7.8, 1.3 Hz, 1H), 8.17 (d, J = 7.8 Hz, 1H), 7.56–7.49 (m, 8H), 7.39–7.33 (m, 2H), 4.32 (s, 2H), 4.07 (m, 1H), 3.44 (bd, J = 12.2 Hz, 2H), 3.20–3.10 (m, 2H), 2.13 (bd, J = 12.3 Hz, 2H), 1.87–1.76 (m, 2H); HRMS (ES$^+$) m/z calcd for $C_{27}H_{26}ClN_4O_3$ (M+H)$^+$: 489.1693; found: 489.1702.

4.1.6.4. 3-(3-Chlorophenyl)quinazoline-2,4(1*H*,3*H*)-dione (24b). Methyl 2-aminobenzoate (9.05 g, 66.2 mmol) were dissolved in PE (50 mL) at rt and 1-chloro-3-isocyanatobenzene (15.1 g, 99.3 mmol) was added dropwise. The mixture was then stirred overnight at reflux. The reaction was cooled down and the solid filtered off. The residue was triturated with clean PE and dried overnight under vacuum affording methyl 2-({[(3-chlorophenyl)amino]carbonyl}-amino)benzoate (19.0 g, 95%); MS (ES$^+$) m/z 305, 307 (M+H)$^+$. The latter was dispersed in absolute EtOH (90 mL) and concentrated HCl (60 mL) were added dropwise at rt. The mixture was stirred at reflux for 6 h, a clear solution was obtained. The mixture was cooled down and a solid started to precipitate. The precipitate was filtered off, washed extensively with Et_2O and dried at vacuum overnight, affording the title compound (13.8 g, 77%) as off-white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.59 (s, 1H), 7.94 (d, J = 8.1 Hz, 1H), 7.71 (t, J = 8.1 Hz, 1H), 7.53–7.49 (m, 3H), 7.35–7.32 (m, 1H), 7.25–7.21 (m, 2H); MS (ES$^+$) m/z 273, 275 (M+H)$^+$).

4.1.7. General procedure for the synthesis of compounds 10a,b and 11a,b

4.1.7.1. 2-Amino-4-bromo-*N*-(3-chlorophenyl)benzamide (26a). To a solution of 4-bromo-2-nitrobenzoic acid **25a** (2.10 g, 8.5 mmol) in CH_2Cl_2 (40 mL), 3-chloroaniline (0.99 mL, 9.39 mmol), DIPEA (3.25 mL, 18.7 mmol) and HATU (3.60 g, 9.39 mmol) were added. The reaction mixture was stirred at rt for 16 h and then treated with aqueous $NaHCO_3$ (saturated solution). The organic phase was separated, washed with 1 N HCl and dried. Evaporation of the solvent gave a solid that was washed with Et_2O affording 4-bromo-*N*-(3-chlorophenyl)-2-nitrobenzamide (2.00 g, 67%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.89 (s, 1H), 8.40 (s, 1H), 8.13 (d, J = 8.1 Hz, 1H), 7.85 (s, 1H), 7.77 (d, J = 8.1 Hz, 1H), 7.51 (d, J = 8.2 Hz, 1H), 7.41 (t, J = 8.2 Hz, 1H), 7.21 (d, J = 8.2 Hz, 1H); MS (ES$^+$) m/z 355, 357, 359 (M+H)$^+$. The latter (2.00 g, 5.6 mmol) was dissolved in EtOH/H_2O/AcOH (12:1:0.008) (130 mL). Iron (6.25 g, 112 mmol) was added and the resulting suspension was heated to 66 °C for 5 h. The resulting precipitated was filtered and the filtrated concentrated. The residue was dissolved in EtOAc and the resulting organic phase was washed with aqueous $NaHCO_3$ (saturated solution), brine and dried over Na_2SO_4. Evaporation of the solvent gave the title compound (1.37 g, 76%) as a solid; MS (ES$^+$) m/z 325, 327, 329 (M+H)$^+$.

4.1.7.2. 7-Bromo-3-(3-chlorophenyl)-2-thioxo-2,3-dihydroquinazolin-4(1*H*)-one (27a). Compound **26a** (1.37 g, 4.21 mmol) was dissolved in DMF (4 mL) and added slowly to a solution of CS_2 (6 mL) and DBU (626 mL, 4.2 mmol) in DMF (9 mL) at rt. The resulting suspension was heated at 45 °C for 30 min and then at rt for 16 h. The reaction mixture was poured in 1 N HCl and the resulting precipitated was collected and washed with EtOH and PE affording of the title compound (920 mg, 60%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.11 (s, 1H), 7.87 (d, J = 8.3 Hz, 1H), 7.62 (s, 1H), 7.53–7.47 (m, 4H), 7.30 (d, J = 6.8 Hz, 1H); MS (ES$^+$) m/z 367, 369, 371 (M+H)$^+$.

4.1.7.3. 8-Bromo-4-(3-chlorophenyl)imidazo[1,2-*a*]quinazolin-5(4*H*)-one (28). To a solution of **27a** (320 mg, 0.87 mmol) in $CHCl_3$ (5 mL) at rt, SO_2Cl_2 (0.035 mL, 0.43 mmol) was added and the resulting solution was heated at 80 °C. After 3 h the solution was poured into ice, CH_2Cl_2 was added and the organic phase was separated, washed with water and dried over Na_2SO_4. Evaporation of the solvent gave 7-bromo-2-chloro-3-(3-chlorophenyl)quinazolin-4(3*H*)-one (150 mg, 46%), MS (ES$^+$) m/z 369, 371, 373 (M+H)$^+$. The latter was dissolved in EtOH (2 mL). The resulting solution was treated with 2,2-dimethoxyethanamine (0.26 mL, 2.4 mmol) and Et_3N (0.33 mL, 2.4 mmol). The reaction mixture was heated at 80 °C. After 30 min the solvent was evaporated, water was added and the aqueous phase extracted with EtOAc. The combined organic phase was dried and evaporated to give a residue which was disolved in DMF/HCl_{conc} (2:1) (3 mL). The resulting solution was heated at 80 °C for 1 h. After cooling down it was purified by RP-HPLC (method 3) affording the title compound (50 mg, 33%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.52 (br s, 1H), 8.22 (br s, 1H), 8.12 (d, J = 8.6 Hz, 1H), 7.73 (d, J = 8.6 Hz, 1H), 7.64 (s, 1H), 7.60–7.40 (m, 3H), 7.03 (s, 1H); MS (ES$^+$) m/z 374, 376, 378 (M+H)$^+$.

4.1.7.4. 4-(3-Chlorophenyl)-8-[(2-hydroxyethyl)amino]imidazo-[1,2-*a*]quinazolin-5(4*H*)-one (10a). A solution of **28** (15 mg, 0.04 mmol) in NMP (0.5 mL) was treated with 2-aminoethanol (24.4 mg, 0.4 mmol). The reaction mixture was irradiated in a microwave apparatus at 170 °C 70 min. The solution was purified by RP-HPLC (method 3) affording the title compound (2 mg, 14%)

as a white solid. ^{1}H NMR (400 MHz, CD_3CN) δ 7.97 (d, J = 8.7 Hz, 1H), 7.70 (d, J = 1.5 Hz, 1H), 7.58–7.48 (m, 3H), 7.42–7.39 (m, 1H), 7.01 (d, J = 1.5 Hz, 1H), 6.82 (d, J = 1.9 Hz, 1H), 6.77 (dd, J = 8.7, 1.9 Hz, 1H), 3.78–3.72 (m, 3H), 3.42 (t, J = 5.4 Hz, 1H), 3.07–3.05 (m, 2H); HRMS (ES^+) m/z calcd for $C_{18}H_{16}ClN_4O_2$ $(M+H)^+$: 355.0962; found: 355.0965.

4.1.7.5. 8-[(1-Benzylpiperidin-4-yl)amino]-4-(3-chlorophenyl)-imidazo[1,2-*a*]quinazolin-5(4*H*)-one (10b). To a solution of **28** (35 mg, 0.089 mmol) in toluene (1 mL) were added 4-amino-benzylpiperidine (0.022 mL, 0.107 mmol), NaO^tBu (12 mg, 0.125 mmol), XANTPHOS (15.5 mg, 0.027 mmol) and $Pd_2(dba)_3$ (16.3 mg, 0.018 mmol). The reaction mixture was heated at 80 °C for 2 h. After evaporation of the solvent the residue was purified by RP-HPLC (method 3) affording the title compound (30 mg, 70%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.71 (br s, 1H), 8.04 (br s, 1H), 7.86 (d, J = 8.8 Hz, 1H), 7.61–7.49 (m, 8H), 7.44–7.41 (m, 1H), 7.14 (bd, J = 7.1 Hz, 1H), 7.04 (br s, 1H), 6.91 (d, J = 1.8 Hz, 1H), 6.76 (dd, J = 8.8, 1.8 Hz, 1H), 4.38 (s, 2H), 3.70 (m, 1H), 3.53 (bd, J = 12.1 Hz, 2H), 3.10–3.01 (m, 2H), 2.23 (bd, J = 13.2 Hz, 2H), 1.72–1.62 (m, 2H); HRMS (ES^+) m/z calcd for $C_{28}H_{27}ClN_5O$ $(M+H)^+$: 484.1904; found: 484.1908.

4.1.7.6. 7-Bromo-3-(3-chlorophenyl)-8-methyl-2-thioxo-2,3-dihydroquinazolin-4(1*H*)-one (27b). Prepared according to the procedure reported for **27a** from 2-amino-4-bromo-3-methylbenzoic acid[15] **25b** (800 mg, 3.48 mmol) affording the title compound (600 mg, 67%) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.75 (d, J = 8.6 Hz, 1H), 7.62 (d, J = 8.4 Hz, 1H), 7.56–7.46 (m, 3H), 7.32–7.28 (m, 1H), 2.61 (s, 3H); MS (ES^+) m/z 381, 383, 385 $(M+H)^+$.

4.1.7.7. 7-Bromo-3-(3-chlorophenyl)-8-methyl-2-thioxo-2,3-dihydroquinazolin-4(1*H*)-one (29). To a solution of **27b** (600 mg, 1.57 mmol) in EtOH (15 mL), hydrazine (1 M solution in THF (31 mL, 31.3 mmol) was added at rt. The reaction mixture was heated at 80 °C for 16 h. After evaporation of the solvent, the resulting crude was washed with PE (465 mg, 78%). The latter was disolved in formic acid (5 mL) and heated at 120 °C for 2 h. After cooling, H_2O was added and the precipitate washed with $NaHCO_3$ (saturated solution), H_2O and Et_2O, affording the title compound 355 mg, 74%). MS (ES^+) m/z 389, 391, 393 $(M+H)^+$.

4.1.7.8. 4-(3-Chlorophenyl)-8-[(2-hydroxyethyl)amino]-9-methyl-[1,2,4]triazolo[4,3-*a*]quinazolin-5(4*H*)-one (11a). To a solution **29** (60 mg, 0.15 mmol) in NMP (2 mL) at rt, ethanolamine (100 mg, 1.53 mmol) was added. The reaction mixture was irradiated in a microwave apparatus at 170 °C for 40 min. After cooling down the reaction it was purified by RP-HPLC (method 3) affording the title compound (4 mg, 7%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.36 (s, 1H), 7.95 (d, J = 8.9 Hz, 1H), 7.55–7.63 (m, 1H), 7.60–7.57 (m, 2H), 7.51–7.47 (m, 1H), 6.89 (d, J = 8.9 Hz, 1H), 6.35 (t, J = 5.5 Hz, 1H), 4.84 (t, J = 5.6 Hz, 1H), 3.66–3.61 (m, 2H), 3.38–3.35 (m, 2H), 2.48 (s, 3H); HRMS (ES^+) m/z calcd for $C_{18}H_{17}ClN_5O_2$ $(M+H)^+$: 370.1071; found: 370.1063.

4.1.7.9. 8-[(1-Benzylpiperidin-4-yl)amino]-4-(3-chlorophenyl)-9-methyl[1,2,4]triazolo[4,3-*a*]quinazolin-5(4*H*)-one (11b). Prepared as described for **11a** from **29** (7 mg, 15%). ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.48 (s, 1H), 7.94 (d, J = 9.0 Hz, 1H), 7.65–7.46 (m, 9H), 6.99 (d, J = 9.0 Hz, 1H), 4.33 (s, 2H), 3.76 (bt, 1H), 3.49–3.47 (m, 2H), 3.14–3.08 (m, 2H), 2.50 (s, 3H; overlapped by residual protonic DMSO signal), 2.19–2.14 (m, 2H), 1.86–1.79 (m, 2H); HRMS (ES^+) m/z calcd for $C_{28}H_{28}ClN_6O$ $(M+H)^+$: 499.2013; found: 499.2006.

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Supplementary data

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