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Original article

# 2-Hydroxyisoquinoline-1,3(2*H*,4*H*)-diones as inhibitors of HIV-1 integrase and reverse transcriptase RNase H domain: Influence of the alkylation of position 4

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ABSTRACT

We report herein the synthesis of a series of fifteen 2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione derivatives. Alkyl and arylalkyl groups were introduced on position 4 of the basis scaffold. All the compounds presented poor inhibitory properties against HIV-1 reverse transcriptase ribonuclease H (RNase H). Four compounds inhibited HIV-1 integrase at a low micromolar level. A docking study using the later crystallographic data available for PFV integrase allowed us to explain the slightly improved integrase inhibitory activities of 4-pentyl and 4-(3-phenylpropyl)-2-hydroxyisoquinoline-1,3(2*H*,4*H*)-diones, when compared to the basis scaffold. Physicochemical studies were consistent with 1:1 and 1:2 (metal/ligand) stoichiometries of the magnesium complexes in solution. Unfortunately all tested compounds exhibited high cellular cytotoxicity in cell culture which limited their applications as antiviral agents. However these identified integrase inhibitors provide a very good basis for the development of new hits.


## 1. Introduction

Human immunodeficiency virus type 1 (HIV-1) encodes three enzymes that are required for viral replication: reverse transcriptase, protease, and integrase (IN). Although drugs targeting reverse transcriptase and protease are in wide use and have shown effectiveness particularly when employed in combination, these highly active antiretroviral therapies (HAART) still show important limitations. These are costs, the patient's ability to adhere to the prescribed therapy, occurrence of various side effects due to drug toxicity and most importantly, the loss of drug effectiveness over time caused by development of resistance, including multidrug resistance and cross-resistance [1]. The search for new anti-HIV-1 agents remains essential and, in the last decade, two novel classes of antiretroviral agents were developed. Viral entry inhibitors were investigated, leading to Enfuvirtide, an injectable peptidic drug blocking gp-41 mediated fusion which was licensed in 2003, and to Maraviroc which was recently US FDA-approved. HIV-1 integrase (IN) has also emerged as an attractive target because it is necessary for stable infection and has no cellular human equivalent. A plethora of HIV-1 integrase inhibitors were discovered in the last decade but only one compound, Raltegravir, was US FDA-approved in October 2007 [2,3]. This is the unique currently used clinical drug targeting IN, which is administered as a new addition to HAART regimens. Raltegravir has exhibited an excellent low nanomolar and strand transfer selective in vitro IN inhibition ($IC_{50}$ value of 2.7 nM), an $IC_{95}$ value of 31 nM in the presence of normal human serum (NHS), and synergistic effects in combination with multiple current antiretroviral drugs. It is also active against various viral strains that are resistant to other classes of antiretroviral agents. Only two other compounds are in clinical development: GS-9137 (Elvitegravir) is in the late stages of clinical development [4,5] whereas S/GSK 1349572 is in phases I and II clinical trials [6,7]. Raltegravir's clinical use has already evidenced

*Abbreviations:* HAART, highly active antiretroviral therapies; HIV-1, human immunodeficiency virus type 1; IN, integrase; LDA, lithium diisopropylamide; LEDGF, lens epithelium-derived growth factor; RT, reverse transcriptase; RNase H, ribonuclease H.

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the development of viral resistances [4]. The predominant mutations of the resistant viral strains were found in the vicinity of the catalytic triad residues in the IN active site, and it is likely that they affect its catalytic activities. This evidently shows that there is a strong need to discover novel IN targeting hits with different structural scaffolds.

Second generation IN inhibitors may be provided by compounds with novel IN inhibition mechanisms like small-molecule inhibitors of the LEDGF/p75-integrase interaction [8]. An alternative may be given by multi-targeted compounds and, for example, we may take advantage of the structural similarities between the catalytic cores of HIV-1 reverse transcriptase RNase H domain [9–11] and HIV-1 IN [12–18]. They share a similar αβ-fold containing a central five-stranded mixed β-sheet surrounded by α-helices on both sides, which are in the same topological order. They also contain key acid aminoacids (D64, D116 and E152 for HIV-1 IN; D443, E478, D498 and D549 for the RNase H domain) that are able to complex two magnesium metal ions absolutely required for catalytic activity. Compounds aimed at complexing magnesium cations in both catalytic sites are likely to induce enzymatic functional impairment and inhibit HIV-1 replication [9].

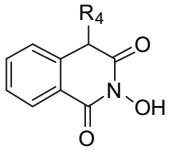
We recently focused our attention on a new scaffold, 2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione as a potential lead compound for the development of novel IN/RNase H inhibitors [19]. We elaborated a first series of derivatives variously substituted at position 7, which were mostly selective for HIV-1 IN with an $IC_{50}$ in the micromolar range. Surprisingly two new hits were discovered which displayed high selectivity for IN (vs RNase H) with submicromolar $IC_{50}$ values. These enzymatic inhibitory properties may be related to the metal-binding abilities of the compounds (1:1 stoichiometry). Unfortunately all tested compounds exhibited high cellular cytotoxicity which limited their applications as antiviral agents. Nevertheless these first results encouraged us to further investigate the pharmacomodulation of this scaffold. Our goal remains the discovery of antiviral compounds with various antienzymatic profiles.

Herein we present the elaboration of a second series of compounds substituted at position 4 by alkyl and arylalkyl chains. These synthetic modulations of the original scaffold, aimed at generating more amphiphilic molecules, were performed in order to investigate the influence of the substitution pattern on the enzymatic inhibitory and antiviral activities.

## 2. Chemistry

Novel 2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione derivatives (Table 1) were prepared from methyl homophthalate **1a** in 4 steps, according to our previously reported method, as summarized in Scheme 1 [19]. Substitution of the heterocyclic ring at position 4 was realized by alkylation of methyl homophthalate **1a** by appropriate alkyl and arylalkyl halides after LDA-promoted deprotonation of **1a** in THF, giving compounds **2a**–**k** and **2m** in 15–87% yields. Under these standard conditions and whatever the number of base equivalents, temperature and time of reaction which were applied, we were unable to isolate **2l** (where $R_4 = (CH_2)_2Ph$). We therefore supposed that 1-bromo-2-phenylethane did not react under $SN_2$ conditions which was probably due to the steric hindrance of the benzyl group. In contrast, the use of sodium amide in liquid ammonia led to **2l** with a 65% yield. Under these $SN_1$ conditions, a cyclopropyl cation could be formed with the assistance of the phenyl group and then react with the nucleophile. After saponification of the precursors **2a**–**m**, homophthalic acids **3a**–**m** were cyclized with *O*-benzylhydroxylamine in toluene at reflux in a Dean-Stark apparatus to give the corresponding 2-benzylisoquinoline-1,3(2*H*,4*H*)-dione derivatives **4a**–**m**. Final deprotection using boron tribromide (or trichloride) yielded the test compounds **5a**–**m** as mixtures of keto and enol forms with a large preference (>90% measured by $^1H$ NMR) for the keto form. Washing the crude solids with appropriate solvents allowed us to separate these two forms in some cases. The keto and enol forms were generally insoluble in dichloromethane and ethyl acetate, respectively. This was the case for **5g**/**5g′** and **5i**/**5i′**.

**Table 1**
Inhibition of HIV-1 IN and RT RNase H activities, antiviral activity and cytotoxicity of 4-substituted 2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione compounds.



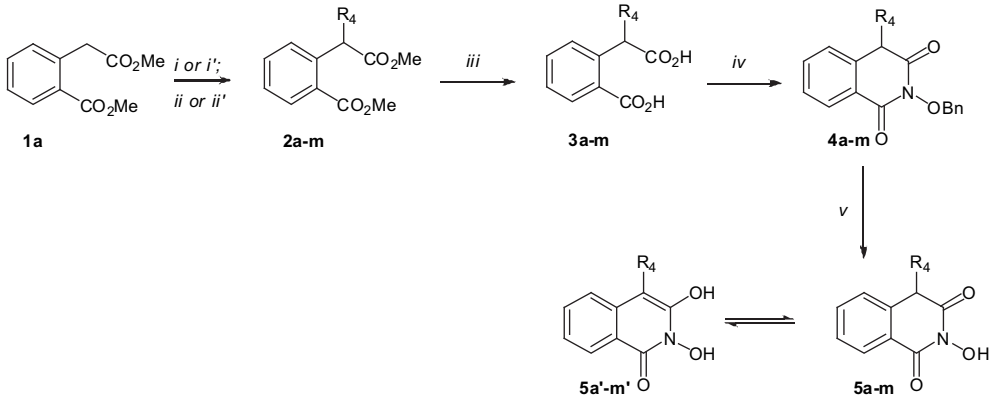

| Compd | $R_4$ | $IC_{50}$ (μM) Overall IN[a] | $IC_{50}$ (μM) RT RNase H[b] | RNase H/IN[c] | $EC_{50}$[d] (μM) | $CC_{50}$[e] (μM) |
|---|---|---|---|---|---|---|
| **5a** | $CH_3$ | 3.3 | 38.8 | 11.8 | >250 | 250 |
| **5b** | $C_2H_5$ | 15.7 | 70.0 | 4.5 | >250 | 250 |
| **5c** | $C_3H_7$ | 21.1 | 46.8 | 2.2 | >25.4 | 25.4 |
| **5d** | $CH(CH_3)_2$ | 35.5 | 18.5 | 0.52 | >122.5 | 122.5 |
| **5e′**,**5e**[i] | $C_4H_9$ | 40.2 | 13.2 | 0.3 | >25.4 | 25.4 |
| **5f** | $C_5H_{11}$ | 1.35 | 33.6 | 24.9 | >98.3 | 98.3 |
| **5g** | $C_6H_{13}$ | 11.4 | 38.0 | 3.3 | >4.4 | 4.4 |
| **5g′** | $C_6H_{13}$ | 45.4 | 7.8 | 0.2 | >128.4 | 128.4 |
| **5h′** | $C_7H_{15}$ | 113.1 | 45 | 0.4 | NT[f] | NT[f] |
| **5i** | $CH_2Ph$ | 37.7 | NA[g] | – | >95 | 95 |
| **5i′** | $CH_2Ph$ | 39.9 | 14.9 | 0.4 | >88 | 88 |
| **5j′**,**5j**[i] | $CH_2C_6H_4pCH_3$ | 3.4 | 80 | 23.5 | >5.5 | 5.5 |
| **5k** | $CH_2C_6H_4pCF_3$ | 23.2 | 42.7 | 1.8 | >6.0 | 6.0 |
| **5l** | $(CH_2)_2Ph$ | 27.8 | NA[g] | – | >30 | 30 |
| **5m′**, **5m**[i] | $(CH_2)_3Ph$ | 2.6 | 66.8 | 25.7 | >5.2 | 5.2 |
| **5n**[h] | H | 6.3 | 5.9 | 0.9 | NT[f] | NT[f] |

[a] Concentration required to inhibit by 50% the in vitro overall integrase activity. Values are means +/− standard deviations from at least three independent experiments.
[b] Concentration required to inhibit by 50% the in vitro RNase H activity.
[c] $IC_{50\ RNase\ H}/IC_{50\ HIV\text{-}1\ IN}$ ratio.
[d] Effective concentration required to reduce HIV-1-induced cytopathic effect by 50% in MT-4 cells.
[e] Cytotoxic concentration to reduce MT-4 cell viability by 50%.
[f] Non tested.
[g] Non active.
[h] Results reported from [19].
[i] Tested as mixtures of both tautomers.

## 3. Biological results

Table 1 shows enzymatic inhibitory properties for the 4-substituted derivatives (**5a**–**m**) compared to the unsusbtituted reference **5n**. As encountered for 7-substituted compounds [19], there was no improvement of RNase H inhibition amongst the series. In most cases $IC_{50}$ values ranged between 25 μM and 50 μM. Only compound **5g′** ($IC_{50}$ value of 7.8 μM) was nearly as potent as the reference unsubstituted compound **5n** ($IC_{50}$ value of 5.9 μM) whereas the other ones were 2–12-fold less inhibitory. RNase H inhibition was even completely abolished for **5i** and **5l**.

As far as integrase inhibition is concerned, the best inhibitory activity was obtained for an alkyl chain with an optimal length of five carbons. Indeed, $IC_{50}$ values progressively increased from 3.3 μM (**5a**, methyl group) to 113.1 μM (**5h′**, heptyl group), except for **5f** (pentyl group, 1.35 μM). The alkaryl sub-series gave similar results: compounds bearing substituted benzyl (**5i**, **5i′**,**5k**) and phenethyl moieties (**5l**, **5l′**) on position 4 displayed $IC_{50}$ values around 25.0–40.0 μM, whereas an optimal $IC_{50}$ value of 2.6 μM was obtained for **5m** (3-phenylpropyl group).




**Scheme 1.** Reagents and conditions: (i) 1.3 equiv LDA, −78 °C, 1 h; (i′) 1.3 equiv $NaNH_2$, −35 °C, 10 min (ii) 1.0 equiv $R_4X$, −78 °C, 1 h then room temperature, 12 h; (ii′) 1.0 equiv $\Phi(CH_2)_2Br$, −35 °C, 4 h then room temperature; (iii) 2.5 M KOH, $H_2O$/MeOH 2/1, reflux, 1 h; (iv) 1.2 equiv of $NH_2OBn$, toluene, Dean-Stark apparatus, reflux, 12 h; (v) $BBr_3$ or $BCl_3$, 4.0 equiv, room temperature, 1 h; $H_2O$, room temperature, 15 min.

The keto and enol forms of compounds bearing a hexyl group (**5g**/**5g′**) and a benzyl group (**5i**/**5i′**) were separated and tested on both enzymatic functions. Unfortunately with so few examples it is impossible to draw any conclusion concerning the influence of the tautomeric form on enzymatic inhibition.

Physicochemical studies for 7-substituted compounds using the Job method were consistent with a single 1:1 stoichiometry of the-magnesium complexes in solution and an enolization of the iso-quinoline upon magnesium cation complexation [19]. For this novel series, two metal/ligand stoichiometries (1:1 and 1:2) were evidenced. For example compound **5c** has $K_s$ values of $2.0 \pm 0.9 \times 10^5$ L mol$^{-1}$ and $4.7 \pm 0.5 \times 10^7$ L$^2$ mol$^{-2}$ for these two binding modes, respectively. It seems that the 1:2 stoichiometry, indicating a somewhat lesser ability to complex magnesium, correlates with lesser HIV-IN inhibition.

Finally, the antiviral properties were investigated by MT-4/MTT assays [19]. All novel compounds displayed important cytotoxicities, which hindered the detection of any antiviral activity in cell culture (Table 1).

## 4. Docking studies

Although RNase H inhibition was abolished in the case of this 4-substituted series, two compounds (namely **5f** and **5m**) displayed a rather encouraging low micromolar inhibition of HIV-1 integrase, with a slight 2.5–4.5-fold improvement of the $IC_{50}$ value when compared to the basis scaffold. In order to further understand the structure–activity relationship of this series and gain some insight into the binding mode of our molecules, we decided to focus our attention on integrase inhibition by carrying out molecular modeling studies.

To date, no X-ray structure of the entire HIV-1 IN in interaction with its cognate DNA with or without substrate has been solved. There are many X-ray structures of truncated integrases. But full-length integrase was used for the evaluation of the structural determinants of the strand transfer and 3′-processing reaction specificities at the DNA-binding level [23]. There are fifteen available X-ray structures of the HIV-1 IN core domain which all include one $Mg^{2+}$ ion bound by two aspartate residues (D64 and D116) in the active site [12–18]. However, there is increasing evidence in support of a two-metal ion theory. For example, X-ray structures of ASV integrase with $Cd^{2+}$or $Zn^{2+}$ showed a second binding site between D64 and E152 [20] and most docking studies now consider that IN may welcome a second $Mg^{2+}$ ion in its active site. Recently, Hare et al. solved the crystal structures of full-length prototype foamy virus (PFV) integrase–DNA complexes with various HIV-1 integrase inhibitors, exhibiting two-metal ions in the strand transfer active site ($Mg^{2+}$ or $Mn^{2+}$) [21]. This breakthrough now allows for a better understanding of the binding mode of integrase strand transfer inhibitors. In an "induced fit mechanism" theory, lipophilic side chains of the inhibitor may fit into a hydrophobic pocket created by displacement the adenine of conserved 3′-CA end dinucleotide of the viral DNA. As shown by Hare et al. [21,22], PFV integrase can be considered as a good model for the development of HIV-1 integrase strand transfer inhibitors. The secondary structures of PFV IN (PDB: 3L2T) and HIV-1 IN catalytic core domain (PDB: 1BL3) have highly conserved architectures, with a calculated RMSD of 1.04 Å (Fig. 1). Only the position of the known flexible loop (G140–G149 in HIV-1 IN) strongly varies in these structures. It has been suggested that its conformational flexibility is essential for catalytic activity, this loop adopting a fixed conformation upon DNA binding and stabilizing the 5′- end of the viral DNA [24]. On the basis of these considerations, we decided to undertake docking studies of our molecules based on the 3L2T X-ray crystallographic structure of PFV IN, according to a recently developed procedure [25]. This procedure was validated by redocking raltegravir and elvitegravir and superimposing the best obtained poses with their

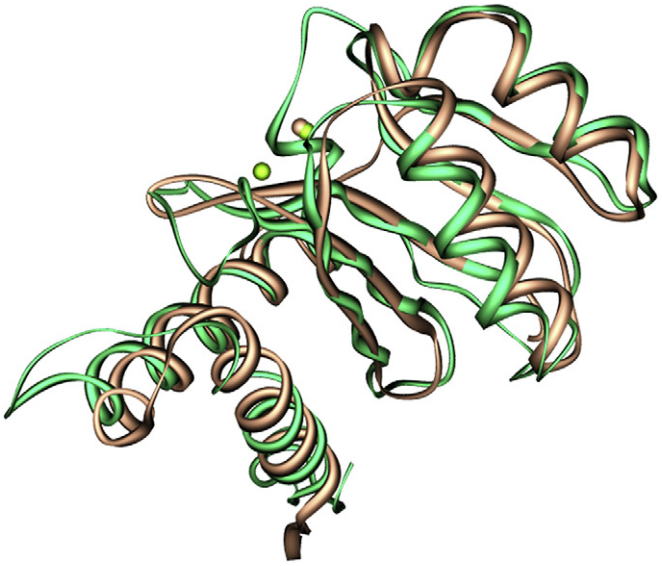


**Fig. 1.** Superimposition of integrase catalytic core domain X-Ray crystallographic structures of PFV (PDB:3L2T, green) and HIV-1 (PDB:1BL3, orange) using Pymol [42]. Calculated RMSD = 1.04 Å. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

X-Ray counterparts in the 3L2T and 3L2U structures, respectively. Using the GOLD docking suite [26], this method involves the use of the CHEMPLP fitness function [27], which is well suited to IN features as it allows for a good balance between steric complementarity, hydrogen bonding and metal binding.

In this docking model, introduction of a lipophilic alkyl side chain of at least 3 carbons in position 4 of our scaffold enables: (a) good overall burying of the ligand into the active site cavity, (b) occupancy of the hydrophobic cavity created by displacement of the 3′-end adenosine and (c) strong chelation of the 2 $Mg^{2+}$ cations. Docking calculations carried out on our series of compounds reveal a consensual mode of binding satisfying all these features (Fig. 2). The modeled optimal length of the side chain is 5–7 carbons (**5f**–**h**), as longer chains do not allow the last two interaction features to be satisfied simultaneously in this model. Indeed, docking calculations of compounds bearing a longer $C_8$, $C_9$ or $C_{10}$ alkyl chain (**5o**–**q**, see supplementary information) yield 2 main poses: in the first one, the scaffold interacts correctly with both magnesium cations, but the end of the lipophilic side chain is skewed away from the hydrophobic pocket. In the second one, it is the side chain which interacts ideally with its hydrophobic environment, but the magnesium complexation is partially lost.

Experimental results indicate an optimal length of 5 carbons (**5f**, $IC_{50} = 1.35$ μM) which correlates well with modeling results (Fig. 3), whereas a side chain of 7 carbons induces a loss of activity (**5h′**, $IC_{50} = 113.1$ μM). Docking of compounds bearing a shorter side chain ($R_4$ = H, Me, Et) does not yield poses that fit the consensual binding mode and it seems hazardous at this stage to study their interactions with the target as they are small aromatic planar scaffolds that may interact with DNA or tyrosines via π–π interactions.

Most interestingly, the modeling study of the arylalkyl series leads to similar results. Introduction of an aromatic moiety on the side chain reduces its positioning freedom, as the presence of the viral DNA cytosine can induce π-stacking that partially replaces classical hydrophobic interactions, as is the case for the *p*-fluorobenzyl group of raltegravir or elvitegravir [21]. However, in order to retain ideal magnesium complexation and gain this aromatic–aromatic interaction, a flexible arm of three carbons is required. Indeed the benzyl and phenethyl moieties (**5i** and **5l** respectively) do not provide sufficient distance between the aromatic ring and the C4 carbon to allow both interactions, thus resulting in docking poses with a partial loss of magnesium binding. The phenylpropyl side chain of **5m** however perfectly allows such a position (Fig. 4), yielding a best docking pose that fits our consensual mode of binding with: (a) π-stacking with the viral DNA cytosine, (b) overall hydrophobic interactions with the enzyme (Y212, P214, Q215, E221 of PFV IN which are all conserved in HIV-1 IN), (c) complexation of the 2 $Mg^{2+}$ cations. These results strongly correlate with biological results on HIV-1 IN, since the activity of scaffold **5n** is retained and even slightly increased for **5m** whereas it is partially lost for **5i**/**5i′** and **5l**.

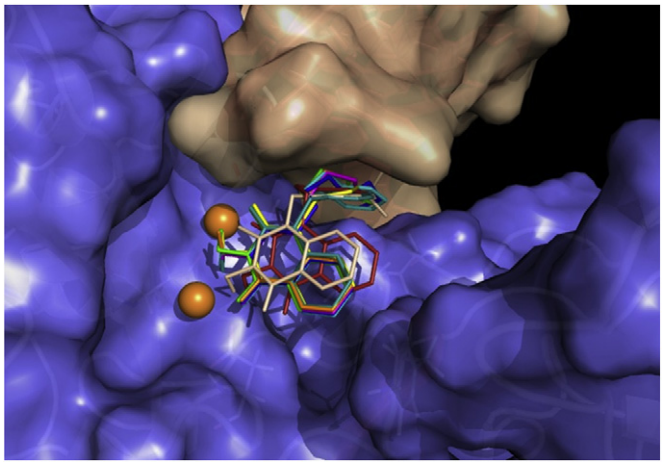


**Fig. 2.** Superimposition of the best docking pose of ligands **5c**–**m** in the active site of PFV IN using the GOLD docking suite and CHEMPLP fitness function. Conolly surfaces at a 1.4 Å radius are depicted for the enzyme (blue) and the double-stranded viral DNA (yellow). Magnesium cations are depicted in orange. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

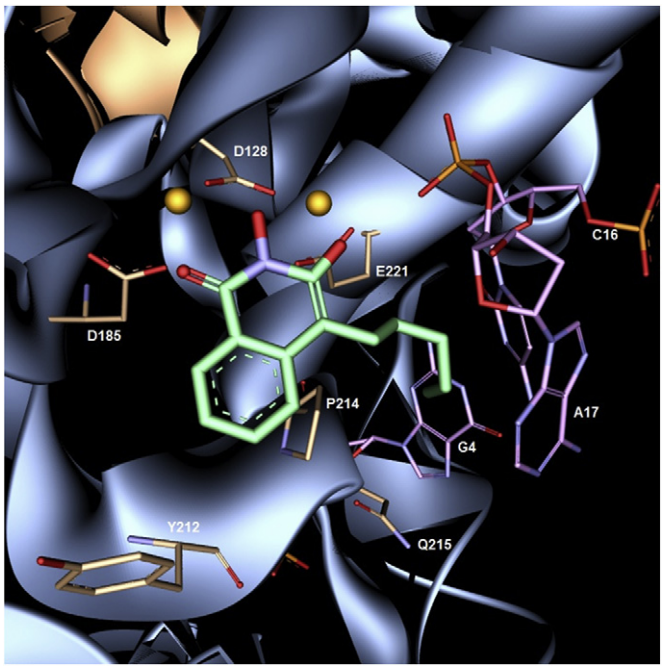



**Fig. 3.** Mode of binding of **5f**.

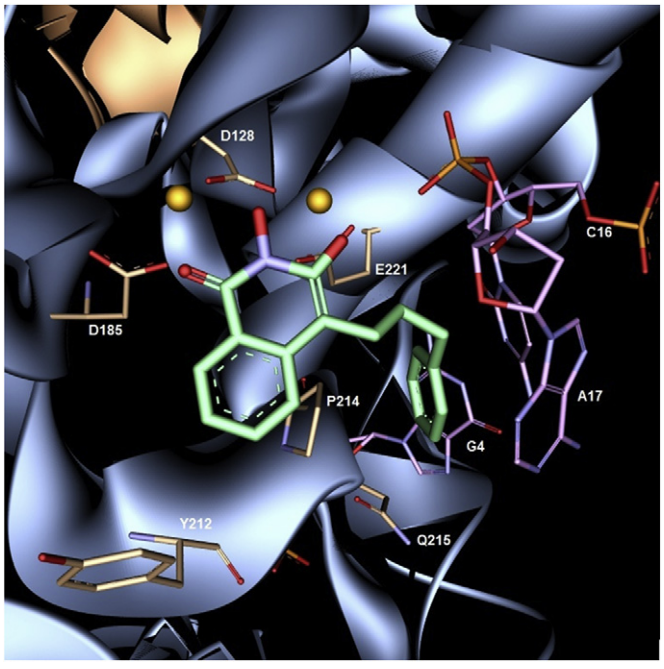



**Fig. 4.** Mode of binding of **5m**.

## 5. Conclusion

Herein we investigated the HIV-1 IN and RT RNase H inhibitory properties of a series of 4-alkylated 2-hydroxyisoquinoline-1,3 (2*H*,4*H*)-diones in order to gain further insight into the biological profile of this previously studied scaffold. Introduction of various linear alkyl chains or of alkaryl groups at position 4 led to the identification of four moderate IN inhibitors at micromolar level (**5a**, **5f**, **5j** and **5m**) with a slight 2–4.5-fold enhancement of the anti-integrase activity, when compared to the unsubstituted parent compound. These results were corroborated by docking studies using the later crystallographic data available on PFV IN including its complexes with $Mg^{2+}$ or $Mn^{2+}$ and raltegravir or elvitegravir. The best antiintegrase activities were obtained for **5f** and **5m**, in accordance with calculation results obtained from this docking model. Among tested compounds, the pentyl group of **5f** may thus be able to establish the best hydrophobic interactions inside the hydrophobic cavity created by displacement of the 3′-end adenosine, while the phenylpropyl group of **5m** is able to interact with both enzyme and viral DNA by simultaneous hydrophobic contacts and aromatic stacking. Nevertheless these interactions within the IN active site together with chelation of the 2 $Mg^{2+}$ cations are not strong enough to allow for a strong enzyme inhibition by these compounds. It is clear that alkylation of the position 4 of this scaffold is largely insufficient for its current use for the development of a new class of antiretroviral agents. However the promising results obtained for this series indicate that optimization of this scaffold may lead to a serious drug candidate. In this regard, further explorations are currently underway in our laboratories concerning the pharmacomodulation of **5f** and **5m**, in order to discover new hits amongst this family of compounds. These studies will be reported in due course and will largely benefit from our validated docking method.

## 6. Experimental

### 6.1. Chemistry

Silica gel, 200–400 mesh (Merck) was used for column chromatography. Melting points were obtained on a Reichert Thermopan melting points apparatus, equipped with a microscope. NMR spectra were obtained on a AC 300 Bruker spectrometer in the appropriate solvent with TMS as internal reference. *J* values are given in Hz. Elemental analyses were performed by CNRS laboratories (Vernaison) and were within 0.4% of the theoretical values.

#### 6.1.1. Preparation of methyl homophthalate **1a**

*6.1.1.1. Methyl 2-(2-methoxy-2-oxoethyl)benzoate* ***1a***. Homophthalic acid (5.76 g, 32.0 mmol) was dissolved in MeOH (100 mL) and thionyl chloride (5.1 mL, 70.4 mmol) was added dropwise at 0 °C. After stirring for 15 min at room temperature, the solution was heated under reflux for 2 h and concentrated *in vacuo*. The residue was dissolved in AcOEt and washed several times with 10% $NaHCO_3$. After drying over $Na_2SO_4$, the solvent was evaporated *in vacuo* to yield **1a** as a yellow oil, which crystallized on standing (95%). Crystals were carefully washed with anhydrous ether and dried at room temperature. Mp 48–49 °C (39–56 °C, [28,29]); $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta = 3.60$ (s, 3H, $OCH_3$), 3.78 (s, 3H, $OCH_3$), 4.00 (s, 2H, $CH_2$), 7.36 (m, 3H, $H_{Ar}$), 7.92 (dd, $^3J = 8.2$ Hz, $^4J = 2.0$ Hz, 1H, $H_{Ar}$); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta = 36.7$ ($CH_2$), 51.4 ($OCH_3$), 51.8 ($OCH_3$), 127.4 (CH), 129.5 (C), 130.3 (CH), 132.4 (CH), 132.6 (CH), 135.9 (C), 166.9 (CO), 172.4 (CO).

#### 6.1.2. Preparation of the alkylated methyl homophthalates **2a**–**m**

A solution of freshly distilled diisopropylamine (0.75 mL, 5.3 mmol) in 3.0 mL of dry THF under an argon atmosphere was cooled to −78 °C and 3.3 mL of 1.6 M *n*-butyllithium (5.3 mmol) in THF was added. After 30 min reaction at −78 °C, a solution of **1a** (1.00 g, 4.8 mmol) in 3.0 mL of dry THF was added dropwise. After stirring the solution for 1 h at −78 °C, a solution of alkyl or arylalkyl halide (iodide in the case of **2a** and bromide in the other cases) (9.6 mmol) in a minimum of dry THF was added dropwise and the solution was stirred for 1 h at −78 °C and then for 12 h at room temperature. The solution was quenched with 10 mL of saturated aqueous $NH_4Cl$ solution and extracted several times with ether. The organic layer was dried over $Na_2SO_4$ and concentrated *in vacuo*. After column chromatography of the residue (eluent: hexane/AcOEt, 90/10), **2a**–**k** and **2m** were obtained as colourless to yellowish oils.

*6.1.2.1. Methyl 2-(1-methoxy-1-oxopropan-2-yl)benzoate* ***2a***. Colourless oil (87%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 1.52$ (d, 3H, $CH_3$, $^3J = 7.0$ Hz), 3.64 (s, 3H, $OCH_3$), 3.88 (s, 3H, $OCH_3$), 4.62 (q, 1H, CH, $^3J = 7.0$ Hz), 7.25–7.51 (m, 3H, $H_{Ar}$), 7.90 (dd, 1H, $H_6$, $^3J = 7.6$ Hz, $^4J = 1.2$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 18.5$ ($CH_3$), 42.1 (CH), 52.0 ($OCH_3$), 52.1 ($OCH_3$), 126.9 (CH), 128.6 (CH), 129.4 ($C_{IV}$), 130.8 (CH), 132.4 (CH), 142.1 ($C_{IV}$), 167.9 (CO), 175.1 (CO); ESI–MS: $m/z = 223$ $(M + H)^+$.

*6.1.2.2. Methyl 2-(1-methoxy-1-oxobutan-2-yl)benzoate* ***2b***. Colourless oil (31%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 0.80$ (d, 3H, $CH_3$, $^3J = 7.0$ Hz), 1.70 (dq, 1H, $CH_2$, $^2J = 13.5$ Hz, $^3J = 7.3$ Hz), 2.04 (dq, 1H, $CH_2$, $^2J = 13.5$ Hz, $^3J = 6.3$ Hz), 3.50 (s, 3H, $OCH_3$), 3.76 (s, 3H, $OCH_3$), 4.45 ($t_{app}$, 1H, CH, $^3J = 7.0$ Hz), 7.13 (td, 1H, $H_{Ar}$, $^3J = 6.9$ Hz, $^4J = 1.0$ Hz), 7.33 (m, 2H, $H_{Ar}$), 7.75 (dd, 1H, $H_6$, $^3J = 6.9$ Hz, $^4J = 1.0$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 12.0$ ($CH_3$), 26.5 ($CH_2$), 48.3 (CH), 51.4 ($OCH_3$), 51.7 ($OCH_3$), 126.6 (CH), 128.4 (CH), 129.6 ($C_{IV}$), 130.4 (CH), 131.9 (CH), 140.3 ($C_{IV}$), 167.6 (CO), 174.1 (CO); ESI–MS: $m/z = 237$ $(M + H)^+$.

*6.1.2.3. Methyl 2-(1-methoxy-1-oxopentan-2-yl)benzoate* ***2c***. Colourless oil (30%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 0.84$ (t, 3H, $CH_3$, $^3J = 7.3$ Hz), 1.30 (m, 2H, $CH_2$), 1.70 (qd, 1H, $CH_2$, $^2J = 13.5$ Hz, $^3J = 7.0$ Hz), 2.07 (qd, 1H, $CH_2$, $^2J = 13.5$ Hz, $^3J = 7.0$ Hz), 3.57 (s, 3H, $OCH_3$), 3.83 (s, 3H, $OCH_3$), 4.58 (t, 1H, CH, $^3J = 7.0$ Hz), 7.23 (td, 1H, $H_{Ar}$, $^3J = 8.4$ Hz, $^4J = 2.1$ Hz), 7.40 (m, 2H, $H_{Ar}$), 7.80 (dd, 1H, $H_6$, $^3J = 8.4$ Hz, $^4J = 2.1$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 13.8$ ($CH_3$), 20.8 ($CH_2$), 35.7 ($CH_2$), 46.7 (CH), 51.7 ($OCH_3$), 52.0 ($OCH_3$), 126.7 (CH), 127.8 (CH), 129.7 ($C_{IV}$), 130.5 (CH), 132.0 (CH), 140.6 ($C_{IV}$), 167.8 (CO), 174.4 (CO); ESI–MS: $m/z = 251$ $(M + H)^+$.

*6.1.2.4. Methyl 2-(3-methyl-1-methoxy-1-oxobutan-2-yl)benzoate* ***2d***. Yellow oil (22%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 0.64$ (d, 3H, $CH_3$, $^3J = 6.7$ Hz), 1.05 (d, 3H, $CH_3$, $^3J = 6.4$ Hz), 2.30 (m, 1H), 3.57 (s, 3H, $OCH_3$), 3.85 (s, 3H, $OCH_3$), 4.45 (d, 1H, CH, $^3J = 10.2$ Hz), 7.23 (td, 1H, $H_{Ar}$, $^3J = 7.8$ Hz, $^4J = 1.6$ Hz), 7.42 (td, 1H, $H_{Ar}$, $^3J = 7.8$ Hz, $^4J = 1.6$ Hz), 7.59 (dd, 1H, $H_3$, $^3J = 7.8$ Hz, $^4J = 1.6$ Hz), 7.77 (dd, 1H, $H_6$, $^3J = 7.8$ Hz, $^4J = 1.6$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 19.8$ ($CH_3$), 21.5 ($CH_3$), 32.5 (CH), 51.7 ($OCH_3$), 52.1 ($OCH_3$), 52.9 (CH), 126.7 (CH), 128.6 (CH), 130.1 (CH), 130.9 ($C_{IV}$), 131.9 (CH), 139.5 ($C_{IV}$), 168.2 (CO), 174.5 (CO); ESI–MS: $m/z = 251$ $(M + H)^+$.

*6.1.2.5. Methyl 2-(1-methoxy-1-oxohexan-2-yl)benzoate* ***2e***. Colourless oil (15%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 0.85$ (t, 3H, $CH_3$, $^3J = 7.0$ Hz), 1.30 (m, 4H, $(CH_2)_{b,c}$), 1.73 (qd, 1H, $(CH_2)_a$, $^2J = 13.5$ Hz, $^3J = 7.0$ Hz), 2.05 (qd, 1H, $(CH_2)_a$, $^2J = 13.5$ Hz, $^3J = 7.0$ Hz), 3.62 (s, 3H, $OCH_3$), 3.88 (s, 3H, $OCH_3$), 4.59 (t, 1H, CH, $^3J = 7.0$ Hz), 7.25 (td, 1H, $H_{Ar}$, $^3J = 8.0$ Hz, $^4J = 2.4$ Hz), 7.45 (m, 2H, $H_{Ar}$), 7.85 (dd, 1H, $H_6$, $^3J = 8.0$ Hz, $^4J = 2.4$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 14.0$ ($CH_3$), 22.6 ($CH_2$), 30.0 ($CH_2$), 33.4 ($CH_2$), 47.0 (CH), 52.0 ($OCH_3$), 52.2 ($OCH_3$), 126.9 (CH), 128.7 (CH), 129.9 ($C_{IV}$), 130.7 (CH), 132.2 (CH), 140.7 ($C_{IV}$), 168.1 (CO), 174.7 (CO); ESI–MS: $m/z = 265$ $(M + H)^+$.

*6.1.2.6. Methyl 2-(1-methoxy-1-oxoheptan-2-yl)benzoate* ***2f***. Yellow oil (65%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 0.71$ (t, 3H, $CH_3$, $^3J = 7.0$ Hz), 1.14 (m, 6H, $CH_2$), 1.65 (qd, 1H, CH–C$\underline{H}_2$, $^2J = 13.5$ Hz, $^3J = 7.0$ Hz), 1.96 (qd, 1H, CH–C$\underline{H}_2$, $^2J = 13.5$ Hz, $^3J = 7.0$ Hz), 3.45 (s, 3H, $OCH_3$), 3.71 (s, 3H, $OCH_3$), 4.52 (t, 1H, CH, $^3J = 7.0$ Hz), 7.13 (td, 1H, $H_{Ar}$, $^3J = 8.0$ Hz, $^4J = 2.4$ Hz), 7.30 (m, 2H, $H_{Ar}$), 7.72 (dd, 1H, $H_6$, $^3J = 8.0$ Hz, $^4J = 2.4$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 13.6$ ($CH_3$), 22.1 ($CH_2$), 27.1 ($CH_2$), 31.3 ($CH_2$), 33.7 ($CH_2$), 46.6 (CH), 51.3 ($OCH_3$), 51.6 ($OCH_3$), 126.4 (CH), 128.3 (CH), 129.5 ($C_{IV}$), 130.3 (CH), 131.8 (CH), 140.5 ($C_{IV}$), 167.4 (CO), 174.1 (CO); ESI–MS: $m/z = 279$ $(M + H)^+$.

*6.1.2.7. Methyl 2-(1-methoxy-1-oxooctan-2-yl)benzoate* ***2g***. Orange oil (73%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 0.71$ (t, 3H, $CH_3$, $^3J = 7.0$ Hz), 1.13 (m, 8H, $CH_2$), 1.67 (qd, 1H, CH–C$\underline{H}_2$, $^2J = 13.5$ Hz, $^3J = 7.0$ Hz), 1.97 (qd, 1H, CH–C$\underline{H}_2$, $^2J = 13.5$ Hz, $^3J = 7.0$ Hz), 3.46 (s, 3H, $OCH_3$), 3.72 (s, 3H, $OCH_3$), 4.52 (t, 1H, CH, $^3J = 7.0$ Hz), 7.11 (td, 1H, $H_{Ar}$, $^3J = 8.0$ Hz, $^4J = 2.4$ Hz), 7.31 (m, 2H, $H_{Ar}$), 7.73 (dd, 1H, $H_6$, $^3J = 8.0$ Hz, $^4J = 2.4$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 13.7$ ($CH_3$), 22.3 ($CH_2$), 27.4 ($CH_2$), 28.8 ($CH_2$), 31.3 ($CH_2$), 33.4 ($CH_2$), 46.6 (CH), 51.3 ($OCH_3$), 51.6 ($OCH_3$), 126.4 (CH), 128.3 (CH), 129.5 ($C_{IV}$), 130.3 (CH), 131.8 (CH), 140.5 ($C_{IV}$), 167.4 (CO), 174.0 (CO); ESI–MS: $m/z = 293$ $(M + H)^+$.

*6.1.2.8. Methyl 2-(1-methoxy-1-oxononan-2-yl)benzoate* ***2h***. Colourless oil (67%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 0.77$ (t, 3H, $CH_3$, $^3J = 7.0$ Hz), 1.16 (m, 10H, $CH_2$), 1.60 (qd, 1H, CH–C$\underline{H}_2$, $^2J = 13.5$ Hz, $^3J = 7.0$ Hz), 2.00 (qd, 1H, CH–C$\underline{H}_2$, $^2J = 13.5$ Hz, $^3J = 7.0$ Hz), 3.52 (s, 3H, $OCH_3$), 3.78 (s, 3H, $OCH_3$), 4.56 (t, 1H, CH, $^3J = 7.0$ Hz), 7.16 (td, 1H, $H_{Ar}$, $^3J = 8.0$ Hz, $^4J = 2.1$ Hz), 7.36 (m, 2H, $H_{Ar}$), 7.78 (dd, 1H, $H_6$, $^3J = 7.6$ Hz, $^4J = 2.0$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 13.9$ ($CH_3$), 22.4 ($CH_2$), 27.6 ($CH_2$), 28.9 ($CH_2$), 29.3 ($CH_2$), 31.6 ($CH_2$), 33.5 ($CH_2$), 46.7 (CH), 51.5 ($OCH_3$), 51.8 ($OCH_3$), 126.5 (CH), 128.4 (CH), 129.6 ($C_{IV}$), 130.4 (CH), 131.9 (CH), 140.6 ($C_{IV}$), 167.6 (CO), 174.2 (CO); ESI–MS: $m/z = 307$ $(M + H)^+$.

*6.1.2.9. Methyl 2-(1-methoxy-1-oxo-3-phenylpropan-2-yl)benzoate* ***2i***. Colourless oil (57%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 3.03$ (dd, 1H, $CH_2$, $^2J = 13.5$ Hz, $^3J = 5.8$ Hz), 3.41 (dd, 1H, $CH_2$, $^2J = 13.5$ Hz, $^3J = 9.0$ Hz), 3.60 (s, 3H, $OCH_3$), 3.86 (s, 3H, $OCH_3$), 4.99 ppm (dd, 1H, CH, $^3J = 5.8$ Hz, $^3J = 9.0$ Hz), 7.14–7.35 (m, 6H, $H_{Ar}$), 7.46 (m, 2H, $H_{Ar}$), 7.86 (dd, 1H, $H_6$, $^3J = 7.6$ Hz, $^4J = 1.2$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 40.0$ ($CH_2$), 49.1 (CH), 52.1 ($OCH_3$), 52.2 ($OCH_3$), 126.3 (CH), 127.1 (CH), 128.3 (2CH), 128.9 (CH), 129.3 (2CH), 129.8 ($C_{IV}$), 130.8 (CH), 132.3 (CH), 139.3 ($C_{IV}$), 140.1 ($C_{IV}$), 167.9 (CO), 174.0 ppm (CO); ESI–MS: $m/z = 299$ $(M + H)^+$.

*6.1.2.10. Methyl 2-(1-methoxy-1-oxo-3-(4-methylphenyl)propan-2-yl)-benzoate* ***2j***. Yellow oil (74%). $^1$H NMR (300 MHz, acetone-$d_6$): $\delta = 2.23$ (s, 3H, $CH_3$), 3.01 (dd, 1H, $CH_2$, $^2J = 13.5$ Hz, $^3J = 6.2$ Hz), 3.40 (dd, 1H, $CH_2$, $^2J = 13.5$ Hz, $^3J = 8.8$ Hz), 3.50 (s, 3H, $OCH_3$), 3.83 (s, 3H, $OCH_3$), 5.05 (dd, 1H, CH, $^3J = 6.2$ Hz, $^3J = 8.8$ Hz), 7.02 (d, 2H, $H_{3'-5'}$, $^3J = 8.2$ Hz), 7.14 (d, 2H, $H_{2'-6'}$, $^3J = 8.2$ Hz), 7.30 (td, 1H, $H_{Ar}$, $^3J = 7.3$ Hz, $^4J = 2.6$ Hz), 7.48 (m, 2H, $H_{Ar}$), 7.86 (dd, 1H, $H_6$, $^3J = 7.3$ Hz, $^4J = 2.6$ Hz); $^{13}$C NMR (75 MHz, acetone-$d_6$): $\delta = 21.1$ ($CH_3$), 40.1 ($CH_2$), 49.7 (CH), 51.9 ($OCH_3$), 52.4 ($OCH_3$), 127.7 (CH), 129.3 (CH), 129.5 (2CH), 129.8 (2CH), 130.6 ($C_{IV}$), 131.3 ppm (CH), 132.9 (CH), 136.2 ($C_{IV}$), 137.1 ($C_{IV}$), 140.9 ($C_{IV}$), 168.2 (CO), 173.9 (CO); ESI–MS: $m/z = 313$ $(M + H)^+$.

*6.1.2.11. Methyl 2-(1-methoxy-1-oxo-3-(4-trifluoromethylphenyl)propan-2-yl)benzoate* ***2k***. Colourless oil (97%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 3.03$ (dd, 1H, $CH_2$, $^2J = 9.1$ Hz, $^3J = 5.1$ Hz), 3.42 (dd, 1H, $CH_2$, $^2J = 9.1$ Hz, $^3J = 5.2$ Hz), 3.54 (s, 3H, $OCH_3$), 3.77 (s, 3H, $OCH_3$), 4.90 (t, 1H, CH, $^3J = 5.1$ Hz), 7.32 (d, 2H, $H_{Ar}$, $^3J = 7.3$ Hz), 7.39 (d, 2H, $H_{Ar}$, $^3J = 7.3$ Hz), 7.53 (m, 3H, $H_{Ar}$), 7.77 (dd, 1H, $H_6$, $^3J = 7.3$ Hz, $^4J = 2.0$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 39.8$ ($CH_2$), 49.1 (CH), 52.0 ($OCH_3$), 52.2 ($OCH_3$), 124.2 (q, $CF_3$, $^1J_{C\text{-}F} = 285.0$ Hz), 125.4 (q, 2CH, $C_{3'}$, $C_{5'}$, $^3J_{C\text{-}F} = 7.0$ Hz), 125.8 (CH), 126.1 (CH), 127.1 (q, $C_{IV}$, $C_{4'}$, $^2J_{C\text{-}F} = 28.0$ Hz), 128.0 (q, 2CH, $C_{2'}$, $C_{6'}$, $^4J_{C\text{-}F} = 3.0$ Hz), 129.4 ($C_{IV}$), 130.8 (CH), 132.3 (CH), 137.3 ($C_{IV}$), 143.7 ($C_{IV}$), 167.4 (CO), 173.8 (CO); ESI–MS: $m/z = 367$ $(M + H)^+$.

*6.1.2.12. Methyl 2-(1-methoxy-1-oxo-5-phenylpentan-2-yl)benzoate* ***2m***. Yellow oil (27%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 1.62$–$1.88$ (m, 3H), 2.25 (qd, 1H, $^2J = 9.1$ Hz, $^3J = 7.0$ Hz), 2.61 (t, 2H, $^3J = 7.0$ Hz), 3.56 (s, 3H, $OCH_3$), 3.83 (s, 3H, $OCH_3$), 4.72 (t, 1H, CH, $^3J = 7.0$ Hz), 7.12–7.35 (m, 6H, HAr), 7.47 (d, 2H, HAr, $^3J = 7.5$ Hz), 7.88 (dd, 1H, $H_6$, $^3J = 8.0$ Hz, $^4J = 2.0$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 30.2$ ($CH_2$), 33.9 ($CH_2$), 36.1 ($CH_2$), 47.3 (CH), 51.9 ($OCH_3$), 52.3 ($OCH_3$), 126.4 (CH), 127.6 (CH), 129.0 (2CH), 129.1 (2CH), 129.4 (CH), 130.9 ($C_{IV}$), 131.2 (CH), 132.8 (CH), 141.3 ($C_{IV}$), 142.9 ($C_{IV}$), 168.3 (CO), 174.4 (CO); ESI–MS: $m/z = 327$ $(M + H)^+$.

*6.1.2.13. Methyl 2-(1-methoxy-1-oxo-4-phenylbutan-2-yl)benzoate* ***2l*** *[30]*. A solution of **1a** (1.00 g, 4.8 mmol) in dry ether (5.0 mL) was added dropwise to a solution of sodium amide (1.6 eq, 6.3 mmol) in ammonia (25 mL) at −35 °C [31]. Then a solution of 1-bromo-2-phenylethane (0.65 mL, 4.8 mmol) in dry ether (5.0 mL) was added and the mixture was stirred at −35 °C for four hours. After complete evaporation of ammonia, water (15 mL) was added to the mixture. After 10 min stirring at room temperature, the solution was extracted twice with 10 mL of ether. The organic layer was dried over $Na_2SO_4$ and concentrated *in vacuo*. After column chromatography of the residue (eluent: hexane/AcOEt, 80/20), **2l** was obtained as a colourless oil (65%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 2.40$–$2.70$ (m, 4H, $(CH_2)_2$), 3.64 (s, 3H, $OCH_3$), 3.82 (s, 3H, $OCH_3$), 4.67 (t, 1H, CH, $^3J = 7.3$ Hz), 7.14–7.34 (m, 6H, $H_{Ar}$), 7.47 (d, 2H, $H_{Ar}$, $^3J = 7.6$ Hz), 7.90 (d, 1H, $H_6$, $^3J = 8.0$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 33.8$ ($CH_2$), 35.1 ($CH_2$), 46.5 (CH), 51.8 ($OCH_3$), 51.9 ($OCH_3$), 125.8 (CH), 126.9 (CH), 128.2 (2CH), 128.6 (2CH), 129.8 ($C_{IV}$), 130.6 (CH), 132.1 (CH), 134.2 (CH), 140.1 ($C_{IV}$), 141.4 ($C_{IV}$), 167.7 (CO), 174.1 (CO); ESI–MS: $m/z = 313$ $(M + H)^+$.

*6.1.3. Preparation of the homophthalic acid derivatives* ***3a–m***

*6.1.3.1. 2-(1-Carboxyethyl)benzoic acid* ***3a***. Compound **2a** (0.74 g, 3.3 mmol) and KOH (1.85 g, 33.0 mmol) were dissolved in a solution of methanol (4.5 mL) and water (9.0 mL). After 1 h reflux, the solution was acidified with 3.0 M HCl and extracted several times with ether. The combined organic extracts were dried over $Na_2SO_4$ and concentrated *in vacuo* to afford **3a** as an oil, which crystallized on standing. White crystals were carefully washed with anhydrous ether and dried at room temperature. (95%). Mp 140 °C (146–161 °C, [32,33]); $^1$H NMR (300 MHz, acetone-$d_6$): $\delta = 1.45$ (d, 3H, $CH_3$, $^3J = 7.0$ Hz), 4.77 (q, 1H, CH, $^3J = 7.0$ Hz), 7.30–7.57 (m, 3H, HAr), 7.94 (dd, 1H, $H_6$, $^3J = 7.6$ Hz, $^4J = 1.2$ Hz); $^{13}$C NMR (75 MHz, acetone-$d_6$): $\delta = 18.8$ ($CH_3$), 42.0 (CH), 127.6 (CH), 129.2 (CH), 130.6 ($C_{IV}$), 131.5 (CH), 133.1 (CH), 143.5 ($C_{IV}$), 169.4 (CO), 175.8 (CO); ESI–MS: $m/z = 195$ $(M + H)^+$.

*6.1.3.2. 2-(1-Carboxypropyl)benzoic acid* ***3b***. White crystals (97%). Mp 138 °C; $^1$H NMR (300 MHz, acetone-$d_6$): $\delta = 0.87$ (t, 3H, $CH_3$, $^3J = 7.3$ Hz), 1.73 (m, 1H, CH–C$\underline{H}_2$, $^2J = 13.5$ Hz, $^3J = 7.3$ Hz), 2.06 (m, 1H, CH–C$\underline{H}_2$, $^2J = 13.5$ Hz, $^3J = 7.3$ Hz), 4.65 (t, 1H, CH, $^3J = 7.3$ Hz), 7.34 (td, 1H, $H_{Ar}$, $^3J = 8.0$ Hz, $^4J = 2.0$ Hz), 7.52 (m, 2H, $H_{Ar}$), 7.94 (dd, 1H, $H_6$, $^3J = 7.6$ Hz, $^4J = 2.0$ Hz); $^{13}$C NMR (75 MHz, acetone-$d_6$): $\delta = 12.6$ ($CH_3$), 27.3 ($CH_2$), 48.8 (CH), 127.6 (CH), 129.2 (CH), 131.2 ($C_{IV}$), 131.5 (CH), 133.0 (CH), 141.8 ($C_{IV}$), 169.6 (CO), 175.2 (CO); ESI–MS: $m/z = 209$ $(M + H)^+$.

*6.1.3.3. 2-(1-Carboxybutyl)benzoic acid* ***3c***. Green oil (97%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 0.85$ (t, 3H, $CH_3$, $^3J = 7.3$ Hz), 1.31 (m, 2H, $CH_2$), 1.72 (qd, 1H, CH–C$\underline{H}_2$, $^2J = 13.5$ Hz, $^3J = 7.0$ Hz), 2.10 (qd, 1H, CH–C$\underline{H}_2$, $^2J = 13.5$ Hz, $^3J = 7.0$ Hz), 4.58 (t, 1H, CH, $^3J = 7.0$ Hz), 7.25 (td, 1H, $H_{Ar}$, $^3J = 8.4$ Hz, $^4J = 2.4$ Hz), 7.41 (m, 2H, $H_{Ar}$), 7.79 (dd, 1H, $H_6$, $^3J = 8.4$ Hz, $^4J = 2.1$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 13.9$ ($CH_3$), 20.8 ($CH_2$), 35.0 ($CH_2$), 46.9 (CH), 127.2 (CH), 128.5 ($C_{IV}$), 129.1 (CH), 131.8 (CH), 133.3 (CH), 140.9 ($C_{IV}$), 173.4 (CO), 180.4 (CO); ESI–MS: $m/z = 223$ $(M + H)^+$.

*6.1.3.4. 2-(1-Carboxy-2-methylpropyl)benzoic acid* ***3d***. Yellow oil (99%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 0.71$ (d, 3H, $CH_3$, $^3J = 6.7$ Hz), 1.14 (d, 3H, $CH_3$, $^3J = 6.7$ Hz), 2.30 (m, 1H, CH), 4.61 (d, 1H, CH, $^3J = 10.2$ Hz), 7.34 (td, 1H, $H_{Ar}$, $^3J = 7.8$ Hz, $^4J = 1.8$ Hz), 7.54 (td, 1H, $H_{Ar}$, $^3J = 7.8$ Hz, $^4J = 1.8$ Hz), 7.61 (dd, 1H, $H_3$, $^3J = 7.8$ Hz, $^4J = 1.8$ Hz), 7.99 (dd, 1H, $H_6$, $^3J = 7.8$ Hz, $^4J = 1.8$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 19.9$ ($CH_3$), 21.7 ($CH_3$), 31.9 (CH), 53.3 (CH), 127.3 (CH), 129.0 (CH), 129.8 ($C_{IV}$), 131.5 (CH), 133.2 ($C_{IV}$), 139.8 (CH), 173.4 (CO), 179.3 (CO); ESI–MS: $m/z = 223$ $(M + H)^+$.

*6.1.3.5. 2-(1-Carboxypentyl)benzoic acid* ***3e***. White crystals (97%). Mp 124 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 0.84$ (t, 3H, $CH_3$, $^3J = 7.0$ Hz), 1.28 (m, 4H, $(CH_2)_{b,c}$), 1.78 (qd, 1H, CH–C$\underline{H}_2$, $^2J = 13.5$ Hz, $^3J = 7.0$ Hz), 2.20 (qd, 1H, CH–C$\underline{H}_2$, $^2J = 13.5$ Hz, $^3J = 7.0$ Hz), 4.60 (t, 1H, CH, $^3J = 7.0$ Hz), 7.29 (td, 1H, $H_{Ar}$, $^3J = 8.0$ Hz, $^4J = 2.1$ Hz), 7.50 (m, 2H, $H_{Ar}$), 7.98 (dd, 1H, $H_6$, $^3J = 8.0$ Hz, $^4J = 2.1$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 13.9$ ($CH_3$), 22.6 ($CH_2$), 25.6 ($CH_2$), 29.9 ($CH_2$), 47.3 (CH), 127.2 (CH), 129.0 (CH), 129.3 ($C_{IV}$), 131.5 (CH), 133.1 (CH), 140.8 ($C_{IV}$), 172.5 (CO), 178.4 ppm (CO); ESI–MS: $m/z = 237$ $(M + H)^+$.

*6.1.3.6. 2-(1-Carboxyhexyl)benzoic acid* ***3f***. Yellow oil (99%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 0.84$ (t, 3H, $CH_3$, $^3J = 7.0$ Hz), 1.24 (m, 6H, $CH_2$), 1.82 (qd, 1H, CH–C$\underline{H}_2$, $^2J = 13.5$ Hz, $^3J = 7.0$ Hz), 2.14 (qd, 1H, CH–C$\underline{H}_2$, $^2J = 13.5$ Hz, $^3J = 7.0$ Hz), 4.72 (t, 1H, CH, $^3J = 7.0$ Hz), 7.31 (td, 1H, $H_{Ar}$, $^3J = 8.0$ Hz, $^4J = 2.1$ Hz), 7.47 (m, 2H, $H_{Ar}$), 8.02 (dd, 1H, $H_6$, $^3J = 8.0$ Hz, $^4J = 2.1$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 14.0$ ($CH_3$), 22.4 ($CH_2$), 27.3 ($CH_2$), 31.7 ($CH_2$), 32.8 ($CH_2$), 47.2 (CH), 127.2 (CH), 128.9 ($C_{IV}$), 129.0 (CH), 131.6 ($C_{IV}$), 133.1 (CH), 140.8 (CH), 172.7 (CO), 179.4 ppm (CO); ESI–MS: $m/z = 251$ $(M + H)^+$.

*6.1.3.7. 2-(1-Carboxyheptyl)benzoic acid* ***3g***. Yellow oil (95%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 0.81$ (t, 3H, $CH_3$, $^3J = 7.0$ Hz), 1.19 (m, 8H, $CH_2$), 1.80 (qd, 1H, CH–C$\underline{H}_2$, $^2J = 13.5$ Hz, $^3J = 7.0$ Hz), 2.14 (qd, 1H, CH–C$\underline{H}_2$, $^2J = 13.5$ Hz, $^3J = 7.0$ Hz), 4.71 (t, 1H, CH, $^3J = 7.0$ Hz), 7.28 (td, 1H, $H_{Ar}$, $^3J = 8.0$ Hz, $^4J = 2.4$ Hz), 7.47 (m, 2H, $H_{Ar}$), 7.99 (dd, 1H, $H_6$, $^3J = 8.0$ Hz, $^4J = 2.4$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 14.0$ ($CH_3$), 22.6 ($CH_2$), 27.6 ($CH_2$), 29.1 ($CH_2$), 31.6 ($CH_2$), 33.0 ($CH_2$), 47.2 (CH), 127.1 (CH), 128.9 ($C_{IV}$), 129.2 (CH), 131.5 ($C_{IV}$), 132.9 (CH), 140.9 (CH), 172.3 (CO), 179.0 (CO); ESI–MS: $m/z = 265$ $(M + H)^+$.

*6.1.3.8. 2-(1-Carboxyoctyl)benzoic acid* ***3h***. Yellow oil (99%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 0.85$ (t, 3H, $CH_3$, $^3J = 7.0$ Hz), 1.24 (m, 10H, $5CH_2$), 1.85 (qd, 1H, $CH_2$, $^2J = 13.5$ Hz, $^3J = 7.0$ Hz), 2.15 (qd, 1H, $CH_2$, $^2J = 13.5$ Hz, $^3J = 7.0$ Hz), 4.74 (t, 1H, CH, $^3J = 7.0$ Hz), 7.32 (td, 1H, $H_{Ar}$, $^3J = 8.0$ Hz, $^4J = 2.1$ Hz), 7.51 (m, 2H, $H_{Ar}$), 8.04 (dd, 1H, $H_6$, $^3J = 8.0$ Hz, $^4J = 2.1$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 14.1$ ($CH_3$), 22.6 ($CH_2$), 27.7 ($CH_2$), 29.1 ($CH_2$), 29.5 ($CH_2$), 31.8 ($CH_2$), 32.9 ($CH_2$), 47.2 (CH), 127.2 (CH), 128.8 (CH), 129.1 ($C_{IV}$), 131.8 (CH), 133.2 ($C_{IV}$), 141.0 (CH), 173.0 (CO), 179.8 (CO); ESI–MS: $m/z = 279$ $(M + H)^+$.

*6.1.3.9. 2-(1-Carboxy-2-phenylethyl)benzoic acid* ***3i***. White crystals (97%). Mp 165 °C (154–167 °C, [34,35]); $^1$H NMR (300 MHz, acetone-$d_6$): $\delta = 3.02$ (dd, 1H, $CH_2$, $^2J = 13.5$ Hz, $^3J = 5.8$ Hz), 3.35 (dd, 1H, $CH_2$, $^2J = 13.5$ Hz, $^3J = 9.1$ Hz), 5.12 (dd, 1H, CH, $^3J = 5.8$ Hz, $^3J = 9.1$ Hz), 7.10–7.59 (m, 8H, $H_{Ar}$), 7.95 (dd, 1H, $H_6$, $^3J = 7.2$ Hz, $^4J = 1.6$ Hz); $^{13}$C NMR (75 MHz, acetone-$d_6$): $\delta = 38.6$ ($CH_2$), 48.1 (CH), 125.5 (CH), 126.4 (CH), 127.5 (2CH), 128.0 (CH), 128.5 (2CH), 129.1 ($C_{IV}$), 130.4 (CH), 131.8 (CH), 139.0 ($C_{IV}$), 139.9 ($C_{IV}$), 168.8 (CO), 173.7 ppm (CO); ESI–MS: $m/z = 271$ $(M + H)^+$.

*6.1.3.10. 2-(1-Carboxy-2-(4-methylphenyl)ethyl)benzoic acid* ***3j***. White crystals (98%). Mp 165 °C (175–176 °C, [36]); $^1$H NMR (300 MHz, acetone-$d_6$): $\delta = 2.19$ (s, 3H, $CH_3$), 2.99 (dd, 1H, $CH_2$, $^2J = 13.5$ Hz, $^3J = 5.6$ Hz), 3.31 (dd, 1H, $CH_2$, $^2J = 13.5$ Hz, $^3J = 9.1$ Hz), 5.13 (dd, 1H, CH, $^3J = 5.6$ Hz, $^3J = 9.1$ Hz), 6.99 (d, 2H, $H_{Ar}$, $^3J = 7.9$ Hz), 7.15 (d, 2H, $H_{Ar}$, $^3J = 7.9$ Hz), 7.31 (td, 1H, $H_{Ar}$, $^3J = 8.2$ Hz, $^4J = 2.0$ Hz), 7.48 (m, 2H, $H_{Ar}$), 7.96 (dd, 1H, $H_6$, $^3J = 8.2$ Hz, $^4J = 2.0$ Hz); $^{13}$C NMR (75 MHz, acetone-$d_6$): $\delta = 21.0$ ($CH_3$), 39.8 ($CH_2$), 49.4 (CH), 127.8 (CH), 129.3 (CH), 129.5 (2CH), 129.8 (2CH), 130.7 ($C_{IV}$), 131.7 (CH), 133.1 (CH), 136.1 ($C_{IV}$), 137.5 ($C_{IV}$), 141.6 ($C_{IV}$), 168.4 (CO), 174.6 (CO); ESI–MS: $m/z = 285$ $(M + H)^+$.

*6.1.3.11. 2-(1-Carboxy-2-(4-trifluoromethylphenyl)ethyl)benzoic acid* ***3k***. White crystals (97%). Mp 157 °C; $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta = 3.06$ (dd, 1H, $CH_2$, $^2J = 13.7$ Hz, $^3J = 6.1$ Hz), 3.39 (dd, 1H, $CH_2$, $^2J = 13.7$ Hz, $^3J = 8.8$ Hz), 4.99 (dd, 1H, CH, $^3J = 6.1$ Hz, $^3J = 8.8$ Hz), 7.30–7.60 (m, 6H, $H_{Ar}$), 7.69 (dd, 1H, $H_3$, $^3J = 8.0$ Hz, $^4J = 2.0$ Hz), 7.82 (dd, 1H, $H_6$, $^3J = 8.0$ Hz, $^4J = 2.0$ Hz); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta = 38.1$ ($CH_2$), 48.8 (CH), 124.7 (q, $CF_3$, $^1J_{C\text{-}F} = 285.0$ Hz), 125.2 (q, 2CH, $C_{3'}$, $C_{5'}$, $^3J_{C\text{-}F} = 7.0$ Hz), 125.9 (CH), 126.6 (CH), 127.0 (q, $C_{IV}$, $C_{4'}$, $^2J_{C\text{-}F} = 28.0$ Hz), 128.6 (q, 2CH, $C_{2'}$, $C_{6'}$, $^4J_{C\text{-}F} = 3.0$ Hz), 129.4 ($C_{IV}$), 130.8 (CH), 132.3 (CH), 134.1 ($C_{IV}$), 143.9 ($C_{IV}$), 171.5 (CO), 173.4 (CO); ESI–MS: $m/z = 339$ $(M + H)^+$.

*6.1.3.12. 2-(1-Carboxy-3-phenylpropyl)benzoic acid* ***3l***. White crystals (99%). Mp 143 °C (141 °C, [30]); $^1$H NMR (300 MHz, acetone-$d_6$): $\delta = 2.35$–$2.86$ (m, 4H, $(CH_2)_2$), 4.77 (t, 1H, CH, $^3J = 7.3$ Hz), 7.09–7.24 (m, 5H, $H_{Ar}$), 7.36 (m, 1H, $H_{Ar}$), 7.54 (m, 2H, $H_{Ar}$), 7.97 (dd, 1H, $H_6$, $^3J = 7.6$ Hz, $^4J = 2.0$ Hz); $^{13}$C NMR (75 MHz, acetone-$d_6$): $\delta = 34.6$ ($CH_2$), 36.0 ($CH_2$), 47.2 (CH), 126.6 (CH), 127.7 (CH), 129.0 (2CH), 129.1 (2CH), 129.3 (CH), 131.1 ($C_{IV}$), 131.6 (CH), 133.1 (CH), 141.6 ($C_{IV}$), 142.6 ($C_{IV}$), 169.8 (CO), 175.3 (CO); ESI–MS: $m/z = 285$ $(M + H)^+$.

*6.1.3.13. 2-(1-Carboxy-4-phenylbutyl)benzoic acid* ***3m***. Yellow crystals (98%). Mp 182 °C (184 °C, [37]); $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 1.65$–$1.92$ (m, 3H), 2.45 (qd, 1H, $^2J = 9.1$ Hz, $^3J = 7.0$ Hz), 2.66 (t, 2H, $CH_2$, $^3J = 7.0$ Hz), 4.55 (t, 1H, CH, $^3J = 7.0$ Hz), 7.10–7.38 (m, 6H, $H_{Ar}$), 7.41 (d, 2H, $H_{Ar}$), 7.97 (dd, 1H, $H_6$, $^3J = 8.0$ Hz, $^4J = 2.0$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 30.2$ ($CH_2$), 33.9 ($CH_2$), 36.1 ($CH_2$), 47.3 (CH), 126.6 (CH), 127.4 (CH), 128.2 ($C_{IV}$), 129.3 (2CH), 129.8 (2CH), 130.1 (CH), 131.2 (CH), 132.2 (CH), 138.3 ($C_{IV}$), 142.9 ($C_{IV}$), 172.3 (CO), 174.5 (CO); ESI–MS: $m/z = 299$ $(M + H)^+$.

### *6.1.4. Preparation of the 2-benzyloxyisoquinoline-1,3-diones* ***4a–m*** *[19]*

*6.1.4.1. 2-Benzyloxy-4-methylisoquinoline-1,3(2H,4H)-dione* ***4a***. After column chromatography of the residue (eluent: hexane/AcOEt, 80/20), **4a** was obtained as a yellow oil (88%). $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 1.52$ (d, 3H, $CH_3$, $^3J = 7,0$ Hz), 4.62 (q, 1H, CH, $^3J = 7.0$ Hz), 5.06 (s, 2H, $OCH_2$), 7.25–7.51 (m, 8H, $H_{Ar}$), 7.90 (dd, 1H, $H_8$, $^3J = 7.6$ Hz, $^4J = 1.2$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 18.5$ ($CH_3$), 42.1 (CH, $C_4$), 77.6 ($OCH_2$), 126.9 (CH), 128.5 (2CH), 128.6 (CH), 128.8 (CH), 129.2 (2CH), 129.4 ($C_{IV}$), 130.8 (CH), 132.4 (CH), 133.8 ($C_{IV}$), 142.1 ($C_{IV}$), 167.9 (CO), 175.1 (CO); ESI–MS: $m/z = 282$ $(M + H)^+$.

*6.1.4.2. 2-Benzyloxy-4-ethylisoquinoline-1,3(2H,4H)-dione* ***4b***. After column chromatography of the residue (eluent: hexane/AcOEt, 80/20), **4b** was obtained as a colourless oil (79%). **Keto form**: 100%. $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 0.65$ (t, 3H, $CH_3$, $^3J = 7.3$ Hz), 1.88 (m, 1H, $CH_2$, $^2J = 13.5$ Hz, $^3J = 7.3$ Hz, $^3J = 5.1$ Hz), 2.17 (m, 1H, $CH_2$,

$^2J = 13.5$ Hz, $^3J = 7.3$ Hz, $^3J = 5.1$ Hz), 3.90 (t, 1H, CH, $^3J = 5.1$ Hz), 5.09 (s, 2H, $OCH_2$), 7.16–7.39 (m, 6H, $H_{Ar}$), 7.52 (m, 2H, $H_{Ar}$), 8.17 (dd, 1H, $H_8$, $^3J = 7.6$ Hz, $^4J = 2.0$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 9.1$ ($CH_3$), 29.7 ($CH_2$), 48.1 (CH, $C_4$), 78.0 ($OCH_2$), 125.1 ($C_{IV}$), 126.8 (CH), 127.5 (CH), 127.8 ($C_{IV}$), 128.1 (2CH), 128.3 (CH), 128.8 (CH), 129.6 (2CH), 133.8 (CH), 137.9 ($C_{IV}$), 161.2 (CO), 169.2 (CO); ESI–MS: $m/z = 296$ $(M + H)^+$

*6.1.4.3. 2-Benzyloxy-4-propylisoquinoline-1,3(2H,4H)-dione* ***4c***. After column chromatography of the residue (eluent: hexane/AcOEt, 70/30), **4c** was obtained as a yellow oil (51%). **Keto form**: 100%. $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 0.79$ (t, 3H, $CH_3$, $^3J = 7.0$ Hz), 1.06–1.25 (m, 2H, $CH_2$–C$\underline{H_2}$–$CH_3$), 1.86 (qd, 1H, CH–C$\underline{H_2}$–$CH_2$, $^2J = 13.5$ Hz, $^3J = 5.3$ Hz), 2.03 (qd, 1H, CH–C$\underline{H_2}$–$CH_2$, $^2J = 13.5$ Hz, $^3J = 5.3$ Hz), 3.95 (t, 1H, CH, $^3J = 5.3$ Hz), 5.12 (s, 2H, $OCH_2$), 7.21–7.44 (m, 6H, $H_{Ar}$), 7.52 (m, 2H, $H_{Ar}$), 8.17 (dd, 1H, $H_8$, $^3J = 7.3$ Hz, $^4J = 2.0$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 13.7$ ($CH_3$), 18.4 ($CH_2$), 39.0 ($CH_2$), 47.6 (CH, $C_4$), 78.2 ($OCH_2$), 125.2 ($C_{IV}$), 127.0 (CH), 127.6 (CH), 128.3 (2CH), 128.7 (CH), 129.0 (CH), 129.9 (2CH), 133.9 (CH), 134.0 ($C_{IV}$), 138.5 ($C_{IV}$), 161.4 (CO), 169.5 (CO); ESI–MS: $m/z = 310$ $(M + H)^+$.

*6.1.4.4. 2-Benzyloxy-4-isopropylisoquinoline-1,3(2H,4H)-dione* ***4d***. After column chromatography of the residue (eluent: hexane/AcOEt, 70/30), **4d** was obtained as a yellow oil (51%). **Keto form**: 100%. $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 0.82$ (t, 3H, $CH_3$, $^3J = 7.0$ Hz), 0.99 (d, 3H, $CH_3$, $^3J = 7.0$ Hz), 2.30 (dsept, 1H, CH, $^3J = 7.0$ Hz, $^3J = 4.0$ Hz), 3.85 (d, 1H, CH, $^3J = 4.0$ Hz), 5.12 (s, 2H, $OCH_2$), 7.22–7.48 (m, 5H, $H_{Ar}$), 7.58 (m, 3H, $H_{Ar}$), 8.19 (dd, 1H, $H_8$, $^3J = 7.8$ Hz, $^4J = 1.8$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 17.9$ ($CH_3$), 19.0 ($CH_3$), 36.7 (CH), 53.6 (CH), 77.8 ($OCH_2$), 125.3 ($C_{IV}$), 127.2 (CH), 127.3 (CH), 127.8 (2CH), 128.0 (CH), 128.5 (CH), 129.3 (2CH), 132.9 (CH), 133.5 ($C_{IV}$), 136.9 ($C_{IV}$), 161.1 (CO), 168.2 (CO); ESI–MS: $m/z = 310$ $(M + H)^+$.

*6.1.4.5. 2-Benzyloxy-4-butylisoquinoline-1,3(2H,4H)-dione* ***4e***. After column chromatography of the residue (eluent: hexane/AcOEt, 80/20), **4e** was obtained as a yellow oil (98%). **Keto form**: 100%. $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 0.78$ (t, 3H, $CH_3$, $^3J = 7.0$ Hz), 1.05–1.26 (m, 4H, $(CH_2)_{b,c}$), 1.86 (qd, 1H, CH–C$\underline{H_2}$–$CH_2$, $^2J = 13.5$ Hz, $^3J = 5.3$ Hz), 2.07 (qd, 1H, CH–C$\underline{H_2}$–$CH_2$, $^2J = 13.5$ Hz, $^3J = 5.3$ Hz), 3.95 (t, 1H, CH, $^3J = 5.3$ Hz), 5.11 (s, 2H, $OCH_2$), 7.15–7.43 (m, 6H, $H_{Ar}$), 7.60 (m, 2H, $H_{Ar}$), 8.18 (dd, 1H, $H_8$, $^3J = 7.3$ Hz, $^4J = 2.0$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 13.5$ ($CH_3$), 22.1 ($CH_2$), 26.8 ($CH_2$), 36.3 ($CH_2$), 47.4 (CH, $C_4$), 77.9 ($OCH_2$), 124.9 ($C_{IV}$), 126.8 (CH), 127.4 (CH), 128.0 (2CH), 128.4 (CH), 128.8 (CH), 129.7 (2CH), 133.8 (CH), 133.9 ($C_{IV}$), 138.4 ($C_{IV}$), 161.2 (CO), 169.3 (CO); ESI–MS: $m/z = 324$ $(M + H)^+$.

*6.1.4.6. 2-Benzyloxy-4-pentylisoquinoline-1,3(2H,4H)-dione* ***4f***. After column chromatography of the residue (eluent: hexane/AcOEt, 80/20), **4f** was obtained as a yellow oil (97%). **Keto form**: 100%. $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 0.76$ (t, 3H, $CH_3$, $^3J = 7.0$ Hz), 1.00–1.27 (m, 6H, $(CH_2)_{b,c,d}$), 1.82 (qd, 1H, CH–C$\underline{H_2}$–$CH_2$, $^2J = 13.5$ Hz, $^3J = 5.3$ Hz), 2.03 (qd, 1H, CH–C$\underline{H_2}$–$CH_2$, $^2J = 13.5$ Hz, $^3J = 5.3$ Hz), 3.92 (t, 1H, CH, $^3J = 5.3$ Hz), 5.11 (s, 2H, $OCH_2$), 7.10–7.37 (m, 5H, $H_{Ar}$), 7.47–7.60 (m, 3H, $H_{Ar}$), 8.15 (dd, 1H, $H_8$, $^3J = 8.0$ Hz, $^4J = 2.0$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 13.6$ ($CH_3$), 22.1 ($CH_2$), 24.4 ($CH_2$), 31.1 ($CH_2$), 36.4 ($CH_2$), 47.3 (CH, $C_4$), 77.8 ($OCH_2$), 124.9 ($C_{IV}$), 126.8 (CH), 127.3 (CH), 128.0 (2CH), 128.4 (CH), 128.7 (CH), 129.6 (2CH), 133.6 (CH), 133.8 ($C_{IV}$), 138.3 ($C_{IV}$), 161.1 (CO), 169.3 (CO); ESI–MS: $m/z = 338$ $(M + H)^+$.

*6.1.4.7. 2-Benzyloxy-4-hexylisoquinoline-1,3(2H,4H)-dione* ***4g***. After column chromatography of the residue (eluent: hexane/AcOEt, 80/20), **4g** was obtained as a yellow oil (90%). **Keto form**: 100%. $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 0.84$ (t, 3H, $CH_3$, $^3J = 7.0$ Hz), 1.01–1.35 (m, 8H, $CH_2$), 1.84 (qd, 1H, CH–C$\underline{H_2}$–$CH_2$, $^2J = 13.5$ Hz, $^3J = 5.4$ Hz), 2.07 (qd, 1H, CH–C$\underline{H_2}$–$CH_2$, $^2J = 13.5$ Hz, $^3J = 5.4$ Hz), 3.94 (t, 1H, CH, $^3J = 5.4$ Hz), 5.14 (s, 2H, $OCH_2$), 7.10–7.40 (m, 5H, $H_{Ar}$), 7.45–7.68 (m, 3H, $H_{Ar}$), 8.18 (dd, 1H, $H_8$, $^3J = 7.8$ Hz, $^4J = 1.9$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 13.7$ ($CH_3$), 22.2 ($CH_2$), 24.7 ($CH_2$), 28.7 ($CH_2$), 31.1 ($CH_2$), 36.5 ($CH_2$), 47.3 (CH, $C_4$), 77.8 ($OCH_2$), 125.0 ($C_{IV}$), 126.8 (CH), 127.3 (CH), 128.1 (2CH), 128.4 (CH), 128.7 (CH), 129.6 (2CH), 133.6 (CH), 133.9 ($C_{IV}$), 138.4 ($C_{IV}$), 161.1 (CO), 169.2 (CO); ESI–MS: $m/z = 352$ $(M + H)^+$.

*6.1.4.8. 2-Benzyloxy-4-heptylisoquinoline-1,3(2H,4H)-dione* ***4h***. After column chromatography of the residue (eluent: hexane/AcOEt, 80/20), **4h** was obtained as a yellow oil (88%). **Keto form**: 100%. $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 0.95$ (t, 3H, $CH_3$, $^3J = 7.0$ Hz), 1.20–1.40 (m, 10H, $CH_2$), 1.98 (m, 2H, $CH_2$), 3.96 (t, 1H, CH, $^3J = 6.0$ Hz), 5.15 (s, 2H, $OCH_2$), 7.16–7.47 (m, 7H, $H_{Ar}$), 7.65 (td, 1H, $H_{Ar}$, $^3J = 7.5$ Hz, $^4J = 1.9$ Hz), 8.17 (dd, 1H, $H_8$, $^3J = 7.5$ Hz, $^4J = 1.9$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 13.7$ ($CH_3$), 21.2 ($CH_2$), 22.2 ($CH_2$), 25.6 ($CH_2$), 28.6 ($CH_2$), 31.3 ($CH_2$), 36.4 ($CH_2$), 44.9 (CH), 77.8 ($OCH_2$), 124.9 ($C_{IV}$), 126.7 (CH), 127.1 (CH), 127.4 (2CH), 127.9 (2CH), 128.6 (CH), 130.0 (CH), 134.9 (CH), 134.9 ($C_{IV}$), 138.2 ($C_{IV}$), 160.6 (CO), 167.5 (CO); ESI–MS: $m/z = 366$ $(M + H)^+$.

*6.1.4.9. 4-Benzyl-2-benzyloxy-isoquinoline-1,3(2H,4H)-dione* ***4i***. After column chromatography of the residue (eluent: hexane/AcOEt, 80/20), **4i** was obtained as a colourless oil (81%). **Keto form**: 100%. $^1$H NMR (300 MHz, acetone-$d_6$): $\delta = 3.37$ (dd, 1H, $CH_2$, $^2J = 13.0$ Hz, $^3J = 5.0$ Hz), 3.57 (dd, 1H, $CH_2$, $^2J = 13.0$ Hz, $^3J = 5.6$ Hz), 4.44 (t, 1H, CH, $^3J = 5.3$ Hz), 4.86 (d, 1H, $OCH_2$, $^2J = 9.1$ Hz), 4.99 (d, 1H, $OCH_2$, $^2J = 9.1$ Hz), 7.06–7.63 (m, 11H, $H_{Ar}$), 8.02 (m, 3H, $H_{Ar}$); $^{13}$C NMR (75 MHz, acetone-$d_6$): $\delta = 42.9$ ($CH_2$), 49.1 (CH, $C_4$), 78.3 ($OCH_2$), 125.8 (CH), 126.4 (CH), 128.0 (CH), 128.2 (CH), 128.5 (2CH), 128.7 (2CH), 128.8 (CH), 129.2 (2CH), 129.3 (2CH), 130.4 (CH), 131.8 ($C_{IV}$), 133.8 ($C_{IV}$), 139.0 ($C_{IV}$), 139.8 ($C_{IV}$), 161.2 (CO), 169.0 (CO); ESI–MS: $m/z = 358$ $(M + H)^+$.

*6.1.4.10. 2-Benzyloxy-4-(4-methylbenzyl)isoquinoline-1,3(2H,4H)-dione* ***4j***. After column chromatography of the residue (eluent: hexane/AcOEt, 80/20), **4j** was obtained as a yellow oil (98%). **Keto form**: 100%. $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 2.22$ (s, 3H, $CH_3$), 3.28 (dd, 1H, $CH_2$, $^2J = 13.0$ Hz, $^3J = 4.4$ Hz), 3.45 (dd, 1H, $CH_2$, $^2J = 13.0$ Hz, $^3J = 5.8$ Hz), 4.26 (t, 1H, CH, $^3J = 4.8$ Hz), 4.89 (d, 1H, $OCH_2$, $^2J = 8.9$ Hz), 5.02 (d, 1H, $OCH_2$, $^2J = 8.9$ Hz), 6.66 (d, 2H, $H_{Ar}$, $^3J = 7.6$ Hz), 6.92 (d, 2H, $H_{Ar}$, $^3J = 7.6$ Hz), 7.20 (m, 2H, $H_{Ar}$), 7.40 (m, 3H, $H_{Ar}$), 7.59 (m, 3H, $H_{Ar}$), 8.12 (dd, 1H, $H_8$, $^3J = 7.6$ Hz, $^4J = 2.0$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 20.7$ ($CH_3$), 42.3 ($CH_2$), 48.7 (CH), 77.9 ($OCH_2$), 125.0 ($C_{IV}$), 127.3 (CH), 127.5 (CH), 127.9 (CH), 128.1 (2CH), 128.6 (2CH), 128.7 (CH), 129.0 (2CH), 129.5 (2CH), 131.8 ($C_{IV}$), 133.3 ($C_{IV}$), 134.0 ($C_{IV}$), 136.5 (CH), 137.3 ($C_{IV}$), 160.6 (CO), 168.5 (CO); ESI–MS: $m/z = 372$ $(M + H)^+$.

*6.1.4.11. 2-Benzyloxy-4-(4-trifluoromethyl)phenylisoquinoline-1,3(2H,-4H)-dione* ***4k***. After column chromatography of the residue (eluent: hexane/AcOEt, 80/20), **4k** was obtained as a yellow oil (80%). **Keto form**: 100%. $^1$H NMR (300 MHz, acetone-$d_6$): $\delta = 3.48$ (dd, 1H, $CH_2$, $^2J = 13.5$ Hz, $^3J = 5.0$ Hz), 3.62 (dd, 1H, $CH_2$, $^2J = 13.5$ Hz, $^3J = 5.6$ Hz), 4.54 (t, 1H, CH, $^3J = 5.3$ Hz), 4.88 (d, 1H, $OCH_2$, $^2J = 9.1$ Hz), 4.99 (d, 1H, $OCH_2$, $^2J = 9.1$ Hz), 7.04 (d, 2H, $H_{Ar}$, $^3J = 7.9$ Hz), 7.30–7.70 (m, 10H, $H_{Ar}$), 8.02 (dd, 1H, $H_8$, $^3J = 7.7$ Hz, $^4J = 2.0$ Hz); $^{13}$C NMR (75 MHz, acetone-$d_6$): $\delta = 42.4$ ($CH_2$), 49.1 (CH), 78.5 ($OCH_2$), 125.7 (q, 2CH, $^3J_{C\text{-}F} = 7.6$ Hz), 127.48 ($C_{IV}$), 127.54 (CH), 128.5 (q, $CF_3$, $^1J_{C\text{-}F} = 270.0$ Hz), 128.66 (2CH), 128.74 (CH), 128.8 (q, 2CH, $^4J_{C\text{-}F} = 3.8$ Hz), 129.1 (2CH), 129.3 (q, $C_{IV}$, $^2J_{C\text{-}F} = 35.0$ Hz), 130.3 (CH), 133.3 (CH), 134.7 (CH), 135.6 ($C_{IV}$), 138.4 ($C_{IV}$), 141.7 (q, $C_{IV}$, $^5J_{C\text{-}F} = 1.1$ Hz), 161.4 (CO), 169.1 (CO); ESI–MS: $m/z = 426$ $(M + H)^+$.

*6.1.4.12. 2-Benzyloxy-4-(2-phenyl)ethylisoquinoline-1,3(2H,4H)-dione* ***4l***. After column chromatography of the residue (eluent: hexane/AcOEt, 80/20), **4l** was obtained as a yellow oil (48%). **Keto form**: 100%. $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 3.32$–$3.35$ (m, 4H, $(CH_2)_2$), 4.39 (t, 1H, CH, $^3J = 5.0$ Hz), 5.07 (s, 2H, $OCH_2$), 6.78 (m, 4H, $H_{Ar}$), 7.16–7.68 (m, 9H, $H_{Ar}$), 8.08 (dd, 1H, $H_8$, $^3J = 7.6$ Hz, $^4J = 2.0$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 31.1$ ($CH_2$), 37.8 ($CH_2$), 46.7 (CH), 77.9 ($OCH_2$), 125.1 ($C_{IV}$), 125.9 (CH), 126.8 (CH), 127.6 (CH), 128.16 (2CH), 128.22 (2CH), 128.24 (2CH), 128.6 (CH), 128.8 (CH), 129.7 (2CH), 133.8 (CH), 133.9 ($C_{IV}$), 138.1 ($C_{IV}$), 140.1 ($C_{IV}$), 161.0 (CO), 168.9 (CO); ESI–MS: $m/z = 372$ $(M + H)^+$.

*6.1.4.13. 2-Benzyloxy-4-(3-phenyl)propylisoquinoline-1,3(2H,4H)-dione* ***4m***. After column chromatography of the residue (eluent: hexane/AcOEt, 80/20), **4m** was obtained as a yellow oil (98%). **Keto form**: 100%. $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 1.54$–$1.98$ (m, 4H, $2CH_2$), 2.65 (m, 2H, $CH_2$), 4.32 (t, 1H, CH, $^3J = 5.1$ Hz), 4.98 (d, 1H, $OCH_2$, $^2J = 9.1$ Hz), 5.04 (d, 1H, $OCH_2$, $^2J = 9.1$ Hz), 7.00–7.24 (m, 5H, $H_{Ar}$), 7.35–7.54 (m, 6H, $H_{Ar}$), 7.54 (td, 1H, $H_{Ar}$, $^3J = 7.6$ Hz, $^4J = 1.6$ Hz), 7.64 (dd, 1H, $H_3$, $^3J = 7.6$ Hz, $^4J = 1.6$ Hz), 7.89 (dd, 1H, $H_8$, $^3J = 7.6$ Hz, $^4J = 1.6$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 26.4$ ($CH_2$), 35.2 ($CH_2$), 35.4 ($CH_2$), 45.9 (CH), 78.6 ($OCH_2$), 126.4 (CH), 127.7 (CH), 128.3 (CH), 128.5 (CH), 128.6 (CH), 128.7 (2CH), 128.9 (2CH), 129.3 (2CH), 129.5 ($C_{IV}$), 130.0 (2CH), 130.6 ($C_{IV}$), 133.8 (CH), 134.3 ($C_{IV}$), 137.6 ($C_{IV}$), 163.6 (CO), 166.8 (CO); ESI–MS: $m/z = 386$ $(M + H)^+$.

### *6.1.5. Preparation of the 2-hydroxisoquinoline-1,3(2H,4H)-dione derivatives* ***5a–m*** *[19]*

After boron tribromide or trichloride treatment, keto and enol forms were isolated in most cases by precipitation of the organic residues in dichloromethane and ethyl acetate, respectively. Keto and enol forms could be undoubtedly identified by $^1$H and $^{13}$C NMR measurements. The $^1$H NMR and $^{13}$C spectra of the keto forms displayed peaks around 4.0–5.0 ppm and 40–50 ppm, which could be attributed to the $H_4$ proton and $C_4$ carbon, respectively. The signal characteristic of the $H_4$ proton disappeared for the enol forms whereas the signal characteristic of the $C_4$ carbon was shifted to 80–90 ppm. IR spectroscopy corroborated the NMR data. There were two absorption bands at 1670 and 1725 $cm^{-1}$ (carbonyl functions) for the keto forms. In contrast the IR spectra of the enol forms only displayed one carbonyl vibration around 1625 $cm^{-1}$ and a new band (enol function) appeared at 1550 $cm^{-1}$.

*6.1.5.1. 2-Hydroxy-4-methylisoquinoline-1,3(2H,4H)-dione* ***5a***. Light green solid (55%). **Keto form (5a)**: 60%. $^1$H NMR (300 MHz, acetone-$d_6$): $\delta = 1.65$ (d, 3H, $CH_3$, $^3J = 7.6$ Hz), 4.12 (q, 1H, CH, $^3J = 7.6$ Hz), 7.45–7.55 (m, 2H, HAr), 7.68 (td, 1H, HAr, $^3J = 8.0$ Hz, $^4J = 2.0$ Hz), 8.09 (dd, 1H, $H_8$, $^3J = 8.0$ Hz, $^4J = 2.0$ Hz); $^{13}$C NMR (75 MHz, acetone-$d_6$): $\delta = 20.9$ ($CH_3$), 41.0 (CH, $C_4$), 124.1 ($C_{IV}$), 127.3 (CH), 127.5 (CH), 127.7 (CH), 133.7 (CH), 140.0 ($C_{IV}$), 161.3 (CO), 170.1 (CO); ESI–MS: $m/z = 192$ $(M + H)^+$; Anal. Calc. For $C_{10}H_9NO_3$: C, 62.82, H, 4.74, N, 7.33; Found: C, 62.66, H, 4.85, N, 7.42.

*6.1.5.2. 2-Hydroxy-4-ethylisoquinoline-1,3(2H,4H)-dione* ***5b***. Beige oil (75%). **Keto form (5b)**: 100%. $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 0.69$ (t, 3H, $CH_3$, $^3J = 7.3$ Hz), 2.08 (dq, 1H, $CH_2$, $^2J = 13.5$ Hz, $^3J = 4.8$ Hz), 2.35 (dq, 1H, $CH_2$, $^2J = 13.5$ Hz, $^3J = 5.4$ Hz), 4.06 ($t_{app}$, 1H, CH, $^3J = 5.2$ Hz), 7.31 (dd, 1H, $H_5$, $^3J = 7.6$ Hz, $^4J = 1.3$ Hz), 7.42 (td, 1H, $H_{Ar}$, $^3J = 7.6$ Hz, $^4J = 1.3$ Hz), 7.65 (td, 1H, $H_{Ar}$, $^3J = 7.6$ Hz, $^4J = 1.3$ Hz), 8.14 (dd, 1H, $H_8$, $^3J = 7.9$ Hz, $^4J = 1.3$ Hz), 9.38 (s, 1H, N-OH); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 9.1$ ($CH_3$), 29.6 ($CH_2$), 47.4 (CH, $C_4$), 124.6 ($C_{IV}$), 127.1 (CH), 127.9 (CH), 128.9 (CH), 134.3 (CH), 138.0 ($C_{IV}$), 160.7 (CO), 168.6 (CO); ESI–MS: $m/z = 206$ $(M + H)^+$; Anal. Calc. For $C_{11}H_{11}NO_3$: C, 64.38, H, 5.40, N, 6.83; Found: C, 64.56, H, 5.25, N, 6.62.

*6.1.5.3. 2-Hydroxy-4-propylisoquinoline-1,3(2H,4H)-dione* ***5c***. Brown oil (75%). **Keto form (5c)**: 90%. $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 0.75$ (t, 3H, $CH_3$, $^3J = 7.3$ Hz), 1.04 (m, 2H, CH–$C\underline{H_2}$–$CH_3$), 1.92 (qd, 1H, CH–$C\underline{H_2}$, $^2J = 13.5$ Hz, $^3J = 5.4$ Hz), 2.11 (qd, 1H, CH–$C\underline{H_2}$, $^2J = 13.5$ Hz, $^3J = 5.4$ Hz), 4.04 (t, 1H, CH, $^3J = 5.4$ Hz), 7.25–7.40 (m, 2H, HAr), 7.58 (td, 1H, HAr, $^3J = 7.6$ Hz, $^4J = 1.6$ Hz), 8.09 (dd, 1H, $H_8$, $^3J = 7.6$ Hz, $^4J = 1.6$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 13.7$ ($CH_3$), 18.1 ($CH_2$), 38.4 ($CH_2$), 48.6 (CH, $C_4$), 124.3 ($C_{IV}$), 127.0 (CH), 127.7 (CH), 128.6 (CH), 134.1 (CH), 138.4 ($C_{IV}$), 160.9 (CO), 169.1 (CO); ESI–MS: $m/z = 220$ $(M + H)^+$; Anal. Calc. For $C_{12}H_{13}NO_3$: C, 65.74, H, 5.98, N, 6.39; Found: C, 65.66, H, 6.08, N, 6.57.

*6.1.5.4. 2-Hydroxy-4-isopropylisoquinoline-1,3(2H,4H)-dione* ***5d***. Beige solid (47%). **Keto form (5d)**: 100%. Mp 109–111 °C; $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta = 0.76$ (t, 3H, $CH_3$, $^3J = 7.0$ Hz), 1.00 (d, 3H, $CH_3$, $^3J = 7.0$ Hz), 2.34 (dsept, 1H, CH, $^3J = 7.8$ Hz, $^3J = 7.0$ Hz), 3.97 (d, 1H, $^3J = 7.8$ Hz), 7.47 (m, 2H, $H_{Ar}$), 7.66 (td, 1H, HAr, $^3J = 7.7$ Hz, $^4J = 1.6$ Hz), 8.06 (dd, 1H, $H_8$, $^3J = 7.7$ Hz, $^4J = 1.6$ Hz); $^{13}$C NMR (75 MHz,) DMSO-$d_6$: $\delta = 18.1$ ($CH_3$), 20.0 ($CH_3$), 37.9 (CH), 53.9 (CH), 126.7 ($C_{IV}$), 128.3 (CH), 128.4 (CH), 128.9 (CH), 134.1 (CH), 138.8 ($C_{IV}$), 161.8 (CO), 168.6 (CO); ESI–MS: $m/z = 220$ $(M + H)^+$; Anal. Calc. For $C_{12}H_{13}NO_3$: C, 65.74, H, 5.98, N, 6.39; Found: C, 65.59, H, 6.13, N, 6.22.

*6.1.5.5. 2-Hydroxy-4-butylisoquinoline-1,3(2H,4H)-dione* ***5e,5e′***. Pink solid (75%). Mp 75–77 °C; **Keto form (5e)**: 90%. $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 0.72$ (t, 3H, $CH_3$, $^3J = 7.3$ Hz), 1.00–1.18 (m, 4H, $(CH_2)_{b,c}$), 2.00 (dq, 1H, $(CH_2)_a$, $^2J = 13.5$ Hz, $^3J = 5.1$ Hz), 2.17 (dq, 1H, $(CH_2)a$, $^2J = 13.5$ Hz, $^3J = 5.1$ Hz), 4.04 ($t_{app}$, 1H, CH, $^3J = 5.1$ Hz), 7.28 (dd, 1H, $H_5$, $^3J = 7.6$ Hz, $^4J = 1.3$ Hz), 7.38 (td, 1H, $H_{Ar}$, $^3J = 7.6$ Hz, $^4J = 1.6$ Hz), 7.58 (td, 1H, $H_{Ar}$, $^3J = 7.6$ Hz, $^4J = 1.6$ Hz), 8.10 (dd, 1H, $H_8$, $^3J = 7.6$ Hz, $^4J = 1.6$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 13.6$ ($CH_3$), 22.4 ($CH_2$), 26.8 ($CH_2$), 36.1 ($CH_2$), 46.6 (CH, $C_4$), 124.4 ($C_{IV}$), 127.0 (CH), 127.8 (CH), 128.6 (CH), 134.1 (CH), 138.4 ($C_{IV}$), 160.8 (CO), 169.0 (CO); **Enol form (5e′)**: 10%. $^1$H NMR (300 MHz, $CDCl_3$): $d = 1.06$ (t, 3H, $CH_3$, $^3J = 7.0$ Hz), 1.20–1.28 (m, 4H, $(CH_2)_{b,c}$), 2.83 (dq, 1H, $(CH_2)_a$, $^2J = 12.7$ Hz, $^3J = 4.5$ Hz), 2.95 (dq, 1H, $(CH_2)_a$, $^2J = 12.7$ Hz, $^2J = 4.1$ Hz), 7.63 (td, 1H, $H_{Ar}$, $^3J = 7.6$ Hz, $^4J = 1.6$ Hz), 7.81 (td, 1H, $H_{Ar}$, $^3J = 7.6$ Hz, $^4J = 1.6$ Hz), 7.96 (dd, 1H, $H_5$, $^3J = 7.6$ Hz, $^4J = 1.6$ Hz), 8.15 (dd, 1H, $H_8$, $^3J = 7.6$ Hz, $^4J = 1.6$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): d = 13.7 (CH3), 22.3 ($CH_2$), 28.1 ($CH_2$), 41.9 ($CH_2$), 76.4 ($C_{IV}$, $C_4$), 125.8 ($C_{IV}$), 127.5 (CH), 129.0 (CH), 129.7 (CH), 135.2 (CH), 138.9 ($C_{IV}$), 159.4 (CO), 161.3 (CO); ESI–MS: $m/z = 233$ $(M + H)^+$; Anal. Calc. For $C_{13}H_{15}NO_3$: C, 66.94, H, 6.48, N, 6.00; Found: C, 67.14, H, 6.32, N, 5.85.

*6.1.5.6. 2-Hydroxy-4-pentylisoquinoline-1,3(2H,4H)-dione* ***5f***. White solid (89%). **Keto form (5f)**: 90%. Mp 84–85 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 0.70$ (t, 3H, $CH_3$, $^3J = 7.0$ Hz), 1.09 (m, 6H, $CH_2$), 1.95 (qd, 1H, CH–$C\underline{H_2}$–$CH_2$, $^2J = 13.5$ Hz, $^3J = 5.1$ Hz), 2.15 (qd, 1H, CH–$C\underline{H_2}$–$CH_2$, $^2J = 13.5$ Hz, $^3J = 5.1$ Hz), 4.01 (t, 1H, CH, $^3J = 5.1$ Hz), 7.24–7.37 (m, 2H, HAr), 7.55 (td, 1H, HAr, $^3J = 7.6$ Hz, $^4J = 1.6$ Hz), 8.07 (dd, 1H, $H_8$, $^3J = 7.6$ Hz, $^4J = 1.6$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 13.7$ ($CH_3$), 22.0 ($CH_2$), 24.2 ($CH_2$), 31.2 ($CH_2$), 36.2 ($CH_2$), 46.5 (CH, $C_4$), 124.3 ($C_{IV}$), 126.8 (CH), 127.6 (CH), 128.5 (CH), 134.0 (CH), 138.3 ($C_{IV}$), 161.0 (CO), 169.2 (CO); ESI–MS: $m/z = 248$ $(M + H)^+$; Anal. Calc. For $C_{14}H_{17}NO_3$: C, 68.00, H, 6.93, N, 5.66; Found: C, 68.29, H, 6.75, N, 5.43.

*6.1.5.7. 4-Hexyl-2-hydroxy-isoquinoline-1,3(2H,4H)-dione* ***5g,5g′***. Yield (70%). **Keto form** (**5g**): 70%. Brown oil. $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 0.73$ (t, 3H, $CH_3$, $^3J = 7.3$ Hz), 1.09–1.15 (m, 8H, $CH_2$), 1.96 (qd, 1H, CH–$C\underline{H_2}$–$CH_2$, $^2J = 13.5$ Hz, $^3J = 5.1$ Hz), 2.15 (qd, 1H, CH–$C\underline{H_2}$–$CH_2$, $^2J = 13.5$ Hz, $^3J = 5.1$ Hz), 4.03 (t, 1H, CH, $^3J = 5.1$ Hz), 7.27 (dd, 1H, $H_5$, $^3J = 7.8$ Hz, $^4J = 2.0$ Hz), 7.36 (td, 1H, $H_{Ar}$, $^3J = 7.8$ Hz, $^4J = 2.0$ Hz), 7.57 (td, 1H, $H_{Ar}$, $^3J = 7.8$ Hz, $^4J = 2.0$ Hz), 8.09 (dd, 1H, $H_8$, $^3J = 7.8$ Hz, $^4J = 2.0$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 13.9$ ($CH_3$),

22.4 ($CH_2$), 24.7 ($CH_2$), 29.9 ($CH_2$), 31.3 ($CH_2$), 36.3 ($CH_2$), 46.7 (CH, $C_4$), 124.4 ($C_{IV}$), 127.0 (CH), 127.7 (CH), 128.6 (CH), 134.1 (CH), 138.4 ($C_{IV}$), 160.9 (CO), 169.1 (CO); ESI–MS: $m/z = 262$ $(M+H)^+$; Anal. Calc. For $C_{15}H_{19}NO_3$: C, 68.94, H, 7.33, N, 5.36; Found: C, 68.85, H, 7.43, N, 5.28.

**Enol form** (**5g′**): 30%. Beige solid. Mp 97–99 °C; $^1$H NMR (300 MHz, acetone-$d_6$): $\delta = 0.82$ (t, 3H, $CH_3$, $^3J = 7.0$ Hz), 1.10–1.30 (m, 6H, $CH_2$), 1.52 (m, 2H, $CH_2$), 2.68 (t, 1H, C–$CH_2$–$CH_2$, $^3J = 7.0$ Hz), 7.23 (td, 1H, HAr, $^3J = 8.0$ Hz, $^4J = 2.0$ Hz), 7.60 (m, 2H, $H_{Ar}$), 8.16 (dd, 1H, $H_8$, $^3J = 8.0$ Hz, $^4J = 2.0$ Hz); $^{13}$C NMR (75 MHz, acetone-$d_6$): $\delta = 13.9$ ($CH_3$), 22.1 ($CH_2$), 23.5 ($CH_2$), 28.5 ($CH_2$), 29.1 ($CH_2$), 31.2 ($CH_2$), 89.5 ($C_{IV}$), 121.4 (CH), 121.7 (CH), 122.0 ($C_{IV}$), 126.8 (CH), 131.0 ($C_{IV}$), 136.3 (CH), 146.9 (CO), 152.4 (CO); ESI–MS: $m/z = 262$ $(M+H)^+$; Anal. Calc. For $C_{15}H_{19}NO_3$: C, 68.94, H, 7.33, N, 5.36; Found: C, 69.12, H, 7.03, N, 5.42.

*6.1.5.8. 4-Heptyl-2-hydroxy-isoquinoline-1,3(2H,4H)-dione* ***5h′***. Beige solid (62%). **Enol form (5h′)**: 100%. Mp 121–123 °C; $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta = 0.79$ (t, 3H, $CH_3$, $^3J = 7.0$ Hz), 1.07–1.28 (m, 8H, 4 $CH_2$), 1.52 (m, 2H, $CH_2$), 2.68 (t, 2H, $CH_2$, $^3J = 6.8$ Hz), 7.24 (td, 1H, $H_{Ar}$, $^3J = 8.0$ Hz, $^4J = 2.0$ Hz), 7.60 (m, 2H, $H_{Ar}$), 8.16 (dd, 1H, $H_8$, $^3J = 8.0$ Hz, $^4J = 2.0$ Hz); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta = 13.8$ ($CH_3$), 21.9 ($CH_2$), 22.0 ($CH_2$), 28.3 ($CH_2$), 28.6 ($CH_2$), 28.8 ($CH_2$), 31.3 ($CH_2$), 89.3 ($C_{IV}$, $C_4$), 121.4 (CH), 121.6 (CH), 121.9 ($C_{IV}$) 136.3 (CH), 126.7 (CH), 131.0 ($C_{IV}$), 146.9 (CO), 152.4 (CO); ESI–MS: $m/z = 276$ $(M+H)^+$; Anal. Calc. For $C_{16}H_{21}NO_3$: C, 69.79, H, 7.69, N, 5.09; Found: C, 70.01, H, 7.48, N, 4.98.

*6.1.5.9. 4-Benzyl-2-hydroxy-isoquinoline-1,3(2H,4H)-dione* ***5i,5i′***. Yield (80%). **Keto form** (**5i**): 45%. Light yellow solid. Mp 140–142 °C; $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta = 3.36$ (dd, 1H, $CH_2$, $^2J = 8.9$ Hz, $^3J = 3.5$ Hz), 3.45 (dd, 1H, $CH_2$, $^2J = 8.9$ Hz, $^3J = 3.1$ Hz), 4.56 (t, 1H, CH, $^3J = 3.4$ Hz), 6.70 (m, 2H, $H_{Ar}$), 7.08 (m, 3H, $H_{Ar}$), 7.45 (td, 1H, $H_{Ar}$, $^3J = 7.2$ Hz, $^4J = 1.2$ Hz), 7.53 (dd, 1H, $H_5$, $^3J = 7.2$ Hz, $^4J = 1.2$ Hz), 7.68 (td, 1H, $H_{Ar}$, $^3J = 7.2$ Hz, $^4J = 1.2$ Hz), 7.86 (dd, 1H, $H_8$, $^3J = 7.2$ Hz, $^4J = 1.2$ Hz), 10.40 (s, 1H, OH); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta = 41.1$ ($CH_2$), 47.5 (CH), 125.2 ($C_{IV}$), 126.6 (CH), 127.7 (CH), 127.8 (CH), 127.9 (2CH), 129.0 (2CH), 129.6 (CH), 133.3 (CH), 135.9 ($C_{IV}$), 137.6 ($C_{IV}$), 160.9 (CO), 168.9 (CO); ESI–MS: $m/z = 268$ $(M+H)^+$; Anal. Calc. For $C_{16}H_{13}NO_3$: C, 71.90, H, 4.90, N, 5.24; Found: C, 71.78, H, 4.99, N, 5.15.

**Enol form** (**5i′**): 55%. Brown solid. Mp 135–137 °C; $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta = 3.35$ (d, 1H, $CH_2$, $^2J = 13.0$ Hz), 3.56 (d, 1H, $CH_2$, $^2J = 13.0$ Hz), 7.10–7.59 (m, 8H, $H_{Ar}$), 7.98 (dd, 1H, $H_8$, $^3J = 7.2$ Hz, $^4J = 1.2$ Hz); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta = 29.3$ ($CH_2$), 88.2 ($C_{IV}$), 121.6 ($C_{IV}$), 122.3 (CH), 122.7 (CH), 125.9 (CH), 126.7 (CH), 127.9 (2CH), 128.4 (2CH), 131.1 ($C_{IV}$), 136.3 ($C_{IV}$), 140.7 (CH), 147.7 (CO), 152.7 (CO); ESI–MS: $m/z = 268$ $(M+H)^+$; Anal. Calc. For $C_{16}H_{13}NO_3$: C, 71.90, H, 4.90, N, 5.24; Found: C, 71.75, H, 4.67, N, 5.32.

*6.1.5.10. 2-Hydroxy-4-(4-methylbenzyl)isoquinoline-1,3(2H,4H)-dione* ***5j,5j′***. Beige solid (91%). Mp 138–139 °C; **Keto form** (**5j**): 77%. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta = 2.14$ (s, 3H, $CH_3$), 3.30 (dd, 1H, $CH_2$, $^2J = 13.5$ Hz, $^3J = 5.0$ Hz), 3.42 (dd, 1H, $CH_2$, $^2J = 13.5$ Hz, $^3J = 4.6$ Hz), 4.51 (t, 1H, CH, $^3J = 4.8$ Hz), 6.57 (d, 2H, $H_{Ar}$, $^3J = 7.6$ Hz), 6.86 (d, 2H, $H_{Ar}$, $^3J = 7.6$ Hz), 7.39–7.48 (m, 2H, $H_{Ar}$), 7.67 (td, 1H, $H_{Ar}$, $^3J = 7.6$ Hz, $^4J = 1.2$ Hz), 7.86 (dd, 1H, $H_8$, $^3J = 7.6$ Hz, $^4J = 1.2$ Hz); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta = 20.5$ ($CH_3$), 40.6 ($CH_2$), 47.5 (CH), 125.1 ($C_{IV}$), 127.3 (CH), 127.5 (CH), 127.8 (CH), 128.5 (2CH), 128.8 (2CH), 132.7 ($C_{IV}$), 133.2 (CH), 135.5 ($C_{IV}$), 137.6 ($C_{IV}$), 160.9 (CO), 168.9 (CO). **Enol form** (**5j′**): 23%. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta = 2.20$ (s, 3H, $CH_3$), 4.01 (s, 2H, $CH_2$), 7.02 (d, 2H, $H_{Ar}$, $^3J = 7.6$ Hz), 7.13 (d, 2H, $H_{Ar}$, $^3J = 7.6$ Hz), 7.39–7.55 (m, 3H, $H_{Ar}$), 8.16 (dd, 1H, $H_8$, $^3J = 7.0$ Hz, $^4J = 1.2$ Hz); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta = 83.9$ ($C_{IV}$), the only detectable peak of this species; ESI–MS: $m/z = 282$ $(M+H)^+$; Anal. Calc. for $C_{17}H_{15}NO_3$: C, 72.58, H, 5.37, N, 4.98; Found: C, 72.39, H, 5.49, N, 4.76.

*6.1.5.11. 2-Hydroxy-4-(4-trifluoromethylbenzyl)isoquinoline-1,3(2H,-4H)-dione* ***5k***. Brown oil (36%). **Keto form** (**5k**): 100%. $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 3.38$ (dd, 1H, $CH_2$, $^2J = 13.4$ Hz, $^3J = 4.0$ Hz), 3.56 (dd, 1H, $CH_2$, $^2J = 13.4$ Hz, $^3J = 5.1$ Hz), 4.36 (dd, 1H, CH, $^3J = 5.1$ Hz, $^3J = 4.0$ Hz), 6.84 (d, 2H, $H_{Ar}$, $^3J = 7.6$ Hz), 7.23 (dd, 1H, $H_5$, $^3J = 7.9$ Hz, $^4J = 1.8$ Hz), 7.33 (d, 2H, $H_{Ar}$, $^3J = 7.6$ Hz), 7.43 (td, 1H, $H_{Ar}$, $^3J = 7.9$ Hz, $^4J = 1.8$ Hz), 7.58 (td, 1H, $H_{Ar}$, $^3J = 7.9$ Hz, $^4J = 1.8$ Hz), 8.02 (dd, 1H, $H_8$, $^3J = 7.9$ Hz, $^4J = 1.8$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 42.4$ ($CH_2$), 48.0 (CH), 124.5 (q, $CF_3$, $^1J_{C\text{-}F} = 280.0$ Hz), 125.4 (q, 2CH, $^3J_{C\text{-}F} = 7.0$ Hz), 125.8 ($C_{IV}$), 126.9 (CH), 127.4 (q, $C_{IV}$, $^2J_{C\text{-}F} = 27.0$ Hz), 127.7 (CH), 128.8 (CH), 129.1 (q, 2CH, $^4J_{C\text{-}F} = 3.0$ Hz), 130.2 (CH), 134.1 ($C_{IV}$), 143.8 ($C_{IV}$), 160.0 (CO), 167.2 (CO); ESI–MS: $m/z = 336$ $(M+H)^+$; Anal. Calc. For $C_{17}H_{12}F_3NO_3$: C, 60.90, H, 3.61, N, 4.18; Found: C, 61.1, H, 3.78, N, 3.99.

*6.1.5.12. 2-Hydroxy-4-(2-phenyl)ethylisoquinoline-1,3(2H,4H)-dione* ***5l***. Pink solid (70%). Mp 142–143 °C; **Keto form** (**5l**): 100%. $^1$H NMR (300 MHz, acetone-$d_6$): $\delta = 2.37$–$2.47$ (m, 4H, $(CH_2)_2$), 4.21 (t, 1H, CH, $^3J = 7.0$ Hz), 7.06–7.20 (m, 4H, $H_{Ar}$), 7.41–7.54 (m, 3H, $H_{Ar}$), 7.66 (td, 1H, $H_{Ar}$, $^3J = 7.6$ Hz, $^4J = 1.2$ Hz), 8.08 (dd, 1H, $H_8$, $^3J = 7.6$ Hz, $^4J = 1.2$ Hz); $^{13}$C NMR (75 MHz, acetone-$d_6$): $\delta = 32.0$ ($CH_2$), 38.4 ($CH_2$), 47.1 (CH), 126.8 (CH), 128.2 (CH), 128.5 (CH), 128.8 (CH), 129.1 (2CH), 129.2 (2CH), 129.5 ($C_{IV}$), 130.0 ($C_{IV}$), 134.6 (CH), 141.8 ($C_{IV}$), 161.5 (CO), 169.3 (CO); ESI–MS: $m/z = 282$ $(M+H)^+$; Anal. Calc. For $C_{17}H_{15}NO_3$: C, 72.58, H, 5.37, N, 4.98; Found: C, 72.76, H, 5.17, N, 5.08.

*6.1.5.13. 2-Hydroxy-4-(3-phenyl)propylisoquinoline-1,3(2H,4H)-dione* ***5m,5m′***. Brown oil (75%). **Keto form (5m)**: 70%. $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 1.52$–$2.13$ (m, 6H, $3CH_2$), 4.02 (t, 1H, CH, $^3J = 5.1$ Hz), 7.00–7.39 (m, 6H, $H_{Ar}$), 7.54 (m, 2H, $H_{Ar}$), 8.09 (dd, 1H, $H_8$, $^3J = 7.6$ Hz, $^4J = 1.6$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 26.2$ ($CH_2$), 35.1 ($CH_2$), 35.4 ($CH_2$), 46.2 (CH), 126.7 (CH), 127.6 (CH), 128.0 (2CH), 128.1 (2CH), 128.4 (CH), 128.8 ($C_{IV}$), 128.9 ($C_{IV}$), 136.2 ($C_{IV}$), 137.9 (CH), 141.0 (CH), 160.8 (CO), 168.8 (CO); **Enol form** (**5m′**): 30%. $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 1.52$–$2.13$ (m, 6H, $3CH_2$), 7.00–7.39 (m, 8H, $H_{Ar}$), 8.38 (dd, 1H, $H_8$, $^3J = 7.9$ Hz, $^4J = 2.0$ Hz); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 23.5$ ($CH_2$), 30.3 ($CH_2$), 33.5 ($CH_2$), 76.5 ($C_{IV}$), 121.7 ($C_{IV}$), 123.7 (CH), 124.2 (CH), 125.6 (CH), 125.7 (2CH), 126.7 (CH), 128.4 (2CH), 131.9 ($C_{IV}$), 136.2 ($C_{IV}$), 141.6 (CH), 145.2 (COH), 151.2 (CO); ESI–MS: $m/z = 296$ $(M+H)^+$; Anal. Calc. for $C_{18}H_{17}NO_3$: C, 73.20, H, 5.80, N, 4.74; Found: C, 72.99, H, 5.25, N, 5.01.

### 6.2. Docking procedure

Previous work revealed that the 2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione scaffold complexes magnesium as the enol or enolate form [19]. Preliminary calculations using the SPARC online calculator [38] indicate that such enols are deprotonated in aqueous media at physiological pH, hence we chose to model our ligands as the enolate form. All ligands were created and minimized at the B3LYP/6-31G* level using the Gaussian 03 package [39]. The study was based on the 3L2T PDB structure containing 2 magnesium cations. The co-crystallized ligand was extracted and the protein was prepared by adding hydrogens and removing water molecules and irrelevant heteroatoms in Accelrys DS Visualizer 2.5.5 [40]. Docking calculations were carried out using the CCDC GOLD docking suite [26] with the active site defined as a sphere containing all atoms within 15 Å of the X-ray ligand raltegravir. The CHEMPLP fitness function [27] was used at default settings. After manual editing of atom and bond types in *mol2* files, all ligands were submitted to 100 docking runs using a 0.75 Å RMSD clustering. The resulting docking poses were analyzed in DS Visualizer and selected poses were rendered using POV-Ray [41].

### 6.3. Biological procedures

RNase H and integrase inhibition assays, in vitro anti-HIV and drug susceptibility assays were performed according previously reported methods [19].

#### 6.3.1. Integrase inhibition

To determine the susceptibility of the HIV-1 integrase enzyme to different compounds, an enzyme-linked immunosorbent assay was used. This assay uses an oligonucleotide substrate in which one oligo (5′-ACTGCTAGAGATTTTCCACACTGACTAAAAGGGTC-3′) is labeled with biotin on the 3′ end and in which the other oligo is labeled with digoxigenin at the 5′ end. For the overall integration assay, the second 5′-digoxigenin-labeled oligo is 5′-GACCCTTT-TAGTCAGTGTGGAAAATCTCTAGCA**GT**-3′. The integrase was diluted in 750 mM NaCl, 10 mM Tris (pH 7.6), 10% glycerol, 1 mM β-mercaptoethanol, and 0.1 mg/mL bovine serum albumin. To perform the reaction, 4 μL of diluted integrase (corresponds to a concentration of WT integrase of 1.6 μM) and 4 μL of annealed oligos (7 nM) were added in a final reaction volume of 40 μL containing 10 mM $MgCl_2$, 5 mM DTT, 20 mM HEPES (pH 7.5), 5% PEG, and 15% DMSO. The reaction was carried out for 1 h at 37 °C. These reactions were followed by an immunosorbent assay on avidin-coated plates. 3′-Processing and strand transfer assays were performed according to previously reported methods.

#### 6.3.2. In vitro anti-HIV and drug susceptibility assays

The inhibitory effect of antiviral drugs on the HIV-1-induced cytopathic effect (CPE) in human lymphocyte MT-4 cell culture was determined by the MT-4/MTT assay. This assay is based on the reduction of the yellow colored 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) by mitochondrial dehydrogenase of metabolically active cells to a blue formazan derivative, which can be measured spectrophotometrically. The 50% cell culture infective dose ($CCID_{50}$) of the HIV-1 ($III_B$) strain was determined by titration of the virus stock using MT-4 cells. For the drug susceptibility assays, MT-4 cells were infected with 100–300 $CCID_{50}$ of the virus stock in the presence of 5-fold serial dilutions of the antiviral drugs. The concentration of various compounds achieving 50% protection against the CPE of the different HIV strains, which is defined as the $EC_{50}$, was determined. In parallel, the 50% cytotoxic concentration ($CC_{50}$) was determined.

#### 6.3.3. Reverse transcriptase RNase H assay

The substrate for RNase H activity was prepared as previously described. *Escherichia coli* RNA polymerase used single-stranded calf thymus DNA as a template to synthesize complementary $^3H$-labeled RNA. For RNase H activity, recombinant HIV-1 RT (4.5 pmol) was incubated with the appropriate compound for 10 min at 37 °C in 20 μL. The components of the incubation mixture were added to reach a final concentration of 50 mM Tris–HCl (pH 8.0), 10 mM dithiothreitol, 6 mM $MgCl_2$, 80 mM KCl, and the labeled nucleic acid duplex (20,000 cpm) in a final volume of 50 μL. After incubation for 10 min at 37 °C, the reaction was stopped by addition of 1 mL of cold 10% TCA containing 0.1 M sodium pyrophosphate. Samples were filtered on nitrocellulose filters and washed, and the radioactivity was determined.

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## Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2010.11.033.

## References

[1] G. Moyle, J. Gatell, C.F. Perno, W. Ratanasuwan, M. Schechter, C. Tsoukas, AIDS Patient Care STDs 22 (2008) 459–471.
[2] R.T. Steigbigel, D.A. Cooper, P.N. Kumar, J.E. Eron, M. Schechter, M. Markowitz, M.R. Loutfy, J.L. Lennox, J.M. Gatell, J.K. Rockstroh, C. Katlama, P. Yeni, A. Lazzarin, B. Clotet, J. Zhao, J. Chen, D.M. Ryan, R.R. Rhodes, J.A. Killar, L.R. Gilde, K.M. Strohmaier, A.R. Meibohm, M.D. Miller, D.J. Hazuda, M.L. Nessly, M.J. DiNubile, R.D. Isaacs, B.Y. Nguyen, H. Teppler, N. Engl. J. Med. 359 (2008) 339–354.
[3] K. Ramkumar, N. Neamati, Core Evid. 4 (2009) 131–147.
[4] L.Q. Al-Mawsawi, R.I. Al-Safi, N. Neamati, Expert. Opin. Emerg. Dr 13 (2008) 213–225.
[5] A. Zolopa, M. Mullen, D. Berger, P. Ruane, T. Hawkins, L. Zhong, S. Chuck, J. Enejosa, B. Kearney, A. Cheng, 14th Conference on Retroviruses and Opportunistic Infections. Los Angeles, USA, 143LB, 2007.
[6] S. Min, I. Song, J. Borland, S. Chen, Y. Lou, T. Fujiwara, S.C. Piscitelli, Antimicrob. Agents Chemother. 54 (2010) 254–258.
[7] M. Mascolinli, R. Kort, J. Int. AIDS Soc. 13 (Suppl. 1) (2010) S3.
[8] F. Christ, A. Voet, A. Marchand, 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, Z. Debyser, Nat. Chem. Biol. 6 (2010) 442–448.
[9] M.L. Andréola, Curr. Pharm. Des. 10 (2004) 3713–3723.
[10] W. Yang, W.A. Hendrickson, R.J. Crouch, Y. Satow, Science 249 (1990) 1398–1405.
[11] J.F. Davies II, Z. Hostomska, Z. Hostomsky, S.R. Jordan, D.A. Matthews, Science 252 (1991) 88–95.
[12] Y. Goldgur, F. Dyda, A.B. Hickman, T.M. Jenkins, R. Craigie, D.R. Davies, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 9150–9154.
[13] Y. Goldgur, R. Craigie, G.H. Cohen, T. Fujiwara, T. Yoshinaga, T. Fujishita, H. Sugimoto, T. Endo, H. Murai, D.R. Davies, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 13040–13043.
[14] J.C. Chen, J. Krucinski, L.J. Miercke, J.S. Finer-Moore, A.H. Tang, A.D. Leavitt, R.M. Stroud, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 8233–8238.
[15] G. Bujacz, J. Alexand ratos, Q. ZhouLiu, C. ClementMella, A. Wlodawer, FEBS Lett. 398 (1996) 175–178.
[16] F. Dyda, A.B. Hickman, T.M. Jenkins, A. Engelman, R. Craigie, D.R. Davies, Science 266 (1994) 1981–1986.
[17] J. Greenwald, V. Le, S.L. Butler, F.D. Bushman, S. Choe, Biochemistry 38 (1999) 8892–8898.
[18] S. Maignan, J.P. Guilloteau, Q. Zhou-Liu, C. Clément-Mella, V. Mikol, J. Mol. Biol. 282 (1998) 359–368.
[19] M. Billamboz, F. Bailly, M.L. Barreca, L. De Luca, J.F. Mouscadet, M.L. Andreola, F. Christ, Z. Debyser, M. Witvrouw, P. Cotelle, J. Med. Chem. 51 (2008) 7717–7730.
[20] G. Bujacz, J. Alexandratos, A. Wlodawer, G. Merkel, M. Andrake, R.A. Katz, A.M. Skalka, J. Biol. Chem. 272 (1997) 18161–18168.
[21] S. Hare, S.S. Gupta, E. Volkov, A. Engelman, P. Cherepanov, Nature 464 (2010) 232–236.
[22] E. Valkov, S. Gupta, S. Hare, A. Helander, P. Roversi, M. McClure, P. Cherepanov, Nucleic Acids Res. 37 (2009) 243–255.
[23] K. Carayon, H. Leh, E. Henry, F. Simon, J.F. Mouscadet, E. Deprez, Nucleic Acids Res. 38 (2010) 3692–3708.
[24] F. Dyda, A. Hickman, T. Jenkins, A. Engelman, R. Craigie, D. Davies, Science 266 (1994) 1981–1986.
[25] L. De Luca, S. De Grazia, S. Ferro, R. Gitto, F. Christ, Z. Debyser, A. Chimirri, Eur. J. Med. Chem. in press.
[26] G. Jones, P. Willett, R.C. Glen, A.R. Leach, R. Taylor, J. Mol. Biol. 267(1997) 727–748 (b) J.C. Cole, J.W.M. Nissink, R. Taylor, in: B. Shoichet, J. Alvarez (Eds.), Virtual Screening in Drug Discovery, Taylor & Francis CRC Press, Boca Raton, Florida, USA, 2005.
[27] O. Korb, T. Stützle, T. Exner, J. Chem. Inf. Model. 49 (2009) 84–96.
[28] J.C. Sheehan, R.C. O'Neill, J. Am. Chem. Soc. 72 (1950) 4614–4616.
[29] H.J.E. Loewenthal, R. Pappo, J. Am. Chem. Soc. 72 (1952) 4799–4804.
[30] J.N. Chatterjea, S.C. Bhakta, A.K. Chattopadhyay, J. Indian Chem. Soc. 51 (1974) 757–762.
[31] A. El Masmodi, P. Cotelle, D. Barbry, B. Hasiak, D. Couturier, Synthesis (1989) 327–329.

[32] J.N. Chatterjea, C. Bhakta, T.R. Vakula, J. Indian Chem. Soc. 49 (1972) 1161–1168.
[33] A. McKillop, D.P. Rao, Synthesis 11 (1977) 759–760.
[34] S. Wawzonek, G.R. Hansen, J. Org. Chem. 40 (1975) 2974–2975.
[35] M. Prashad, M. Seth, A.P. Bhaduri, R.C. Srimal, Indian J. Chem. 17B (1979) 496–498.
[36] J.N. Chatterjea, H. Mukherjee, J. Indian Chem. Soc. 37 (1960) 443–450.
[37] J.N. Chatterjea, K.D. Banerji, H. Mukherjee, J. Indian Chem. Soc. 40 (1963) 45–52.
[38] S.H. Hilal, S.W. Karickhoff, L.A. Carreira, Quant. Struc. Act. Rel. 14 (1995) 348–355.
[39] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, J.A. Jr. Montgomery, T. Vreven, K.N. Kudin, J.C. Burant, J.M. Millam, S.S. Lyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G.A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, L. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J.E. Knox, H.P. Hratchian, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmam, O. Yazyev, A.I. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, P.Y. Ayala, K. Morokuma, G.A. Voth, P. Salvador, J.J. Dannenberg, V.G. Zakrzewski, S. Dapprich, A.D. Daniels, M.C. Strain, O. Farkas, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, L.V. Ortiz, Q. Cu, A.G. Baboul, S. Clifford, L. Cioslowski, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, M. Challacombe, P.M.W. Gili, B. Johnson, W. Chen, M.W. Wong, C. Gonzalez, J.A. Popie, Gaussian 03, Revision C.02. Gaussian, Inc., Wallingford CT, 2004.
[40] D.S. Visualizer, Release 2.5.5. Accelrys Software Inc., San Diego, CA, USA, 2007.
[41] Persistence of Vision (TM) Raytracer, Release 3.6.2. Persistence of Vision Pty. Ltd., Williamstown, Victoria, Australia, 2004.
[42] The Pymol Molecular Graphics System, Version 1.3, Schrödinger, LLC.