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Synthesis and SAR study of novel 3,3-diphenyl-1,3-dihydroindol-2-one derivatives as potent eIF2·GTP·Met-tRNA$_i^{Met}$ ternary complex inhibitors

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ABSTRACT

The growing recognition of inhibition of translation initiation as a new and promising paradigm for mechanism-based anti-cancer therapeutics is driving the development of potent, specific, and druggable inhibitors. The 3,3-diaryloxindoles were recently reported as potential inhibitors of the eIF2·GTP·Met-tRNA$_i^{Met}$ ternary complex assembly and 3-{5-*tert*-butyl-2-hydroxyphenyl}-3-phenyl-1,3-dihydro-2*H*-indol-2-one **#1181** was identified as the prototypic agent of this chemotype. Herein, we report our continuous effort to further develop this chemotype by exploring the structural latitude toward different polar and hydrophobic substitutions. Many of the novel compounds are more potent than the parent compound in the dual luciferase ternary complex reporter assay, activate downstream effectors of reduced ternary complex abundance, and inhibit cancer cell proliferation in the low μM range. Moreover, some of these compounds are decorated with substituents that are known to endow favorable physicochemical properties and as such are good candidates for evaluation in animal models of human cancer.

1. Introduction

Targeting translation initiation (TI) represents a new paradigm in cancer therapy [1]. Regulation of gene expression at the level of TI is essential for normal cell growth, proliferation, differentiation, and apoptosis, and as such it is under very strict control. Conversely, loss of physiological restraints on TI leads to malignant transformation *in vitro* [2–5] and plays an important role in the genesis, progression and maintenance of some cancers. Importantly, restricting TI by molecular [6] and chemical genetic approaches reverts the malignant phenotype because it preferentially reduces translation of weak mRNAs that are characterized by long and structured 5′ untranslated regions (5′UTR). These mRNAs predominantly code for oncogenic proteins, growth factors, and anti-apoptotic proteins, many of which are upregulated in and are important for the genesis and progression of cancer. Consequently, selective inhibition of weak mRNAs translation will have a detrimental effect on cancer cells with negligible impact on normal tissues.

In the TI cascade, the ternary complex (TC), which is comprised of the charged Met-tRNA$_i^{Met}$ and the GTP-coupled eukaryotic initiation factor 2 (eIF2·GTP), is an essential component of the 43S pre-initiation complex that includes 40S ribosomal subunit and other translation initiation factors such as eIF1, eIF3 and eIF4F complexes. 43S pre-initiation complex recruits mRNA to form the scanning 48S pre-initiation complex. The codon-anticodon pairing between the AUG start codon in the mRNA and the Met-tRNA$_i^{Met}$ in the ternary complex is coupled to the hydrolysis of GTP. The release of phosphate and eIF2·GDP from the pre-initiation complex is coupled to the recruitment of the 60S ribosomal subunit and to initiation of translation. Due to the much higher affinity of eIF2 to GDP than GTP, regeneration of the eIF2·GTP depends on catalysis by eIF2B, a guanine-nucleotide-exchange factor [7]. Activation of one of the eIF2α kinases by external stimuli such as starvation, hypoxia, and unfolded protein response leads to phosphorylation of S51 in the α subunit of eIF2. Phosphorylated eIF2 binds tighter with eIF2B and inhibits its guanine nucleotide exchange activity, thus preventing the regeneration of eIF2·GTP that is needed for regeneration of TC, causing reduction in its abundance and inhibition of TI.

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Experimentally, forced expression of eIF2α-S51A, a non-phosphorylatable and a constitutively active eIF2α mutant [8] or of Met-tRNA$_i^{Met}$, causes transformation of normal cells into malignant ones [9,10]. In contrast, pharmacological inhibition of TI inhibits xenograft tumor growth. Importantly, overexpression of eIF2 and inactivating mutations of eIF2α kinases have been reported in human cancers [11–14]. Both of these perturbations will increase the abundance of the TC rendering TI unrestricted.

Taken together, we hypothesize that pharmacological inhibition of TI by decreasing the availability of the eIF2·GTP·Met-tRNA$_i^{Met}$ (TC) will block preferentially the expression of oncogenes, growth regulating and anti-apoptotic proteins to which cancer cells are addicted to, and will result in the elimination of cancer cells. As such, an inhibitor of TI that targets the TC presents a novel, mechanism-based, non-toxic, and effective anti-cancer therapy. Support of this novel paradigm was offered by demonstrating that molecular probes such as clotrimazole (CLT) [15,16], troglitazone (TG) [17], and eicosapentanoic acid (EPA) [18] deplete Ca^{2+} stores and subsequently phosphorylate eIF2α, inhibit translation initiation, and reduce preferentially the expression of oncoproteins over "housekeeping" ones. Abrogation of TI accounts at least in part for the anti-proliferative activity of these agents. *In vivo*, these agents demonstrated efficacy in various mouse models [15,18,19] and inhibited the expression of oncogenes as measured in the excised tumors [18].

As an extension of our work with CLT, we have developed 3,3-diarylindolin-2-ones, which share the triarylmethyl as a common scaffold, and identified **#1181** (Fig. 1) that inhibited proliferation of a large variety of cancer cells with low micromolar potencies [20,21]. This prototypic 3,3-diarylindolin-2-one inhibits TI by reducing TC availability. Subsequently, we and others reported anti-cancer activity of this family of compounds in various mouse models [1,22–25]. Treatment of mice bearing MCF-7 human breast cancer cell derived tumors (~150 mm^3) with 140 mg/kg/bid of **#1181** sc for 3 weeks resulted in ~30% regression in tumor size [25]. This anti-tumor activity is associated with phosphorylation of eIF2α and inhibition of the expression of oncogenic proteins. These findings provide proof-of-principle that **#1181**-mediated phosphorylation of eIF2α and reduction of the TC formation are pharmacologically relevant and promising targets for anti-cancer therapy.

Many different 3,3-diarylindolin-2-one analogs have already been reported as potential drugs. As an example, 3,3-bis(4-amino-2,5-dimethoxyphenyl)-1,3-dihydroindol-2-one showed a good chemical antioxidant activity even if its antioxidative mechanism was not directly elucidated [26]. A series of N-substituted 3,3-bisaryloxindoles was identified as potent non-steroidal mineralocorticoid receptor (hMR) antagonists, displaying an excellent selectivity for hMR over human glucocorticoid, progesterone, androgen and estrogen receptors [27]. Two years beforehand, a patent had been released in which various N-substituted 3,3-bisaryloxindole analogs were identified by the same research group as nuclear receptor modulators for the treatment of congestive heart failure and other conditions [28]. In cancer therapy, 3,3-diphenyl-indol-2-one derivatives were evaluated as anti-cancer agents by Felding and coworkers [22,29,30] and lately, another research group proposed prodrugs of 3,3-diphenyl-1,3-dihydroindol-2-ones for the treatment of cancer [31]. Nevertheless, unlike our series of 3,3-diaryloxindoles, the development of the oxindole derivatives mentioned above was not targeting TI and therefore not evaluated by TI-specific bioassays.

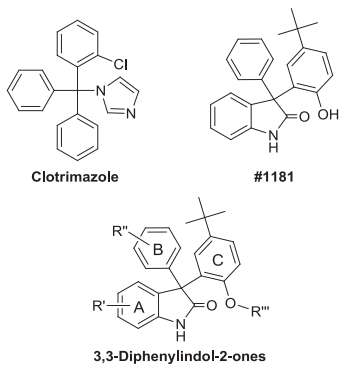


Fig. 1. Clotrimazole (CLT), **#1181** and substituted 3,3-diphenylindol-2-one scaffold.

In spite of the promising mechanism of action and the proof-of-principle achieved with **#1181**, its unfavorable physicochemical properties and insufficient potency require further optimization without compromising its *in vitro* TC inhibitory efficacy. To this end, we designed, synthesized and tested the biological activity of a focused library of structurally modified analogs of **#1181** in which we maintained the parental 3-{5-*tert*-butyl-2-hydroxyphenyl}-3-phenyl-1,3-dihydro-2*H*-indol-2-one scaffold and diversified by decorating aromatic rings **A** and **B**, and substituting the hydroxyl function on ring **C** (Fig. 1). A wide range of substituents was used, which included a variety of polar and charged groups that are commonly employed in medicinal chemistry to enhance aqueous solubility [32].

Herein, we report the development of a focused library of newly substituted 3,3-diphenyl-1,3-dihydroindol-2-ones derived from **#1181** and the characterization of their inhibition of eIF2·GTP·tRNA$_i^{Met}$ TC formation and cancer cell proliferation.

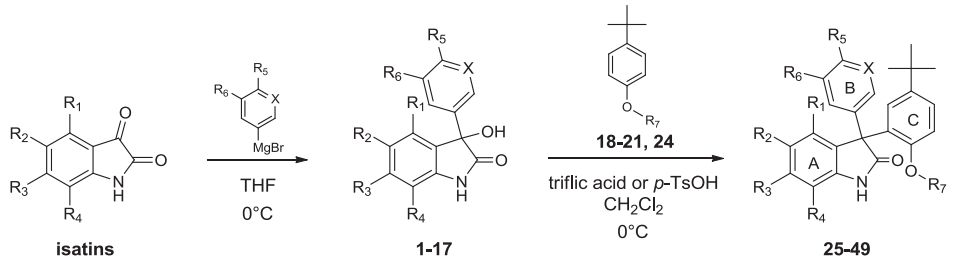
2. Results and discussion

2.1. Chemistry

The general approach to the synthesis of the library of new substituted 3,3-diphenyl-1,3-dihydroindol-2-ones is outlined in Scheme 1. The various isatins used in the condensation with the phenols were either commercially available or synthesized following the well-established Sandmeyer's methodology [33]. Accordingly, anilines such as 2,3-difluoroaniline, methylantranilate, and 6-aminobenzothiazole were condensed with hydroxylamine and chloral hydrate generating hydroxyiminoacetanilide intermediates that underwent thermal cyclization in concentrated sulfuric acid to form the corresponding isatins (Fig. 2).

Grignard reaction between an appropriately substituted phenylmagnesium bromide and isatin employing standard Grignard reaction conditions generated 3-hydroxy-3-phenyl-1,3-dihydro-2*H*-indol-2-ones **1**–**17** in moderate to good yield [34]. Yields lower than 20% were observed when one of the reactants was either 6*H*-[1,3]thiazolo[5,4,e]indol-7,8-dione or pyridin-3-ylmagnesium bromide. The second and final step of the general synthetic methodology consisted of a Friedel–Crafts acylation in which the substituted or non-substituted 4-*tert*-butylphenol reacted with the 3-hydroxy-3-phenyl-1,3-dihydro-2*H*-indol-2-ones **1**–**17** to form the 3,3-diaryloxindoles **25**–**49** [35]. The acid used in this final step was either triflic or *p*-toluene sulfonic acid depending on the reactivity of the incoming *tert*-butyl-substituted ring toward electrophilic aromatic substitution. This synthetic route resulted in racemic mixtures of the 3,3-diaryloxindoles.

In the absence of commercial sources for some of the 4-*tert*-butylphenyl ethers, we developed effective synthetic routes for their preparation. O-alkylation of 4-*tert*-butylphenol with 4-(2-hydroxyethyl)morpholine or the partially protected benzyl 4-(2-hydroxyethyl)piperazine-1-carboxylate employed a Mitsunobu reaction in the presence of triphenylphosphine and diethyl



Interm.	R_1	R_2	R_3	R_4	R_5	R_6	X	Final comp.	R_7	Modif.[a]
1	H	H	H	H	H	H	CH	**25**		C
								26		C
								27		C
								28		C
								29		C
2	H	H	H	H	OMe	H	CH	**30**	H	B
								31		B+C
3	H	H	H	H	$N(CH_3)_2$	H	CH	**32**	H	B
								33		B+C
4	H	H	H	H		H	CH	**34**	H	B
5	H	H	H	H	Cl	CH_3	CH	**35**	H	B
6	H	H	H	H	H	H	N	**36**	H	B
7	H	H	H	H	OMe	OMe	CH	**37**		B+C
8	H	H	H	H	OMe	F	CH	**38**		B+C
9	H	I	H	H	H	H	CH	**39**	H	A
10	H	OCF_3	H	H	H	H	CH	**40**	H	A
11			H	H	H	H	CH	**41**	H	A
12	H	H	F	F	OMe	H	CH	**42**	H	A+B
13	H	OMe	H	H	OMe	H	CH	**43**	H	A+B
								44		A+B+C
14	H	H	H	CO_2CH_3	OMe	H	CH	**45**	H	A+B
								46		A+B+C
15	H	I	H	H	OMe	H	CH	**47**		A+B+C
16	H	OCF_3	H	H	OMe	H	CH	**48**		A+B+C
17	H	H	F	F	$N(CH_3)_2$	H	CH	**49**		A+B+C

Scheme 1. General procedure for the synthesis of oxindoles **25**–**49**.[aa] Modifications on aryl rings **A**, **B** and/or **C** by introduction of different substituents or groups of atoms.

azodicarboxylate (DEAD) in anhydrous THF and generated the corresponding phenyl ethers **18** and **23**, respectively (Scheme 2) [36]. The latter protection strategy prevented the potential competition between the primary alcohol and the secondary amine on reacting with 4-*tert*-butylphenol. The alkylation of the same phenol with *N*,*N*-dimethylaminoethylene bromide, propargylbromide, or *tert*-butylbromoacetate was carried out in anhydrous DMF in the presence of NaH yielding the corresponding phenyl ethers **19**, **20**, and **21** respectively (Scheme 2). Both methods allowed satisfying yields (46–81%).

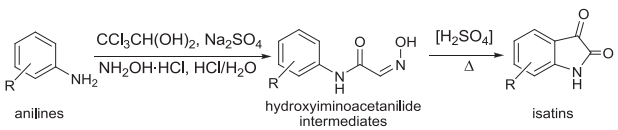
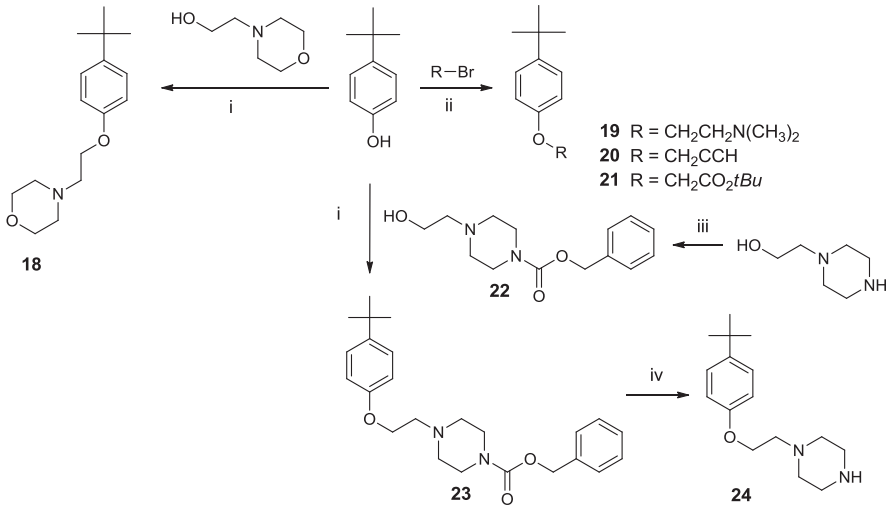


Fig. 2. Sandmeyer methodology for the synthesis of the commercially unavailable isatins from the corresponding anilines.

Following this general method of two-step synthesis we synthesized 25 new substituted 3,3-diphenyl-1,3-dihydroindol-2-ones. In some cases, post-assembly modifications such as demethylation of methoxy groups using boron tribromide methyl sulfide complex in refluxing dichloromethane [37] transformed compounds **30** and **42** into compounds **50** and **51**, respectively. Subjecting 3,3-diphenyl-1,3-dihydroindol-2-one **39** to Suzuki–Miyaura cross coupling reaction using a boronic acid pinacol ester



Scheme 2. Synthesis of intermediates **18**–**21** and **24**.[aa] Reagents and reaction conditions: (i) triphenylphosphine, DEAD, anhydrous THF, 0 °C; (ii) NaH, anhydrous DMF; (iii) benzylchloroformate, 4N NaOH, CH_3CN/H_2O 1:1; (iv) H_2, Pd–C, $AcOH_{cat.}$, MeOH, 1 atm.

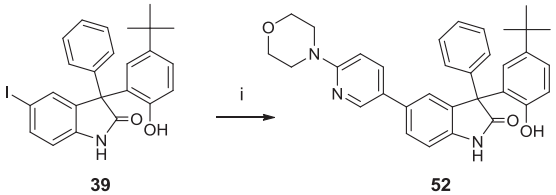
in the presence of $Pd(PPh_3)_2Cl_2$ in refluxing THF generated compound **52** in which ring **A** was decorated with the 6-(morpholin-4-yl)pyridin-3-yl substituent (Scheme 3) [38].

The only departure from the canonical scaffold comprised of a *tert*-butyl moiety at position 5 of the **C** ring was its replacement by a propan-2-yloxy group that maintained most of the bulkiness presented by *tert*-butyl but added a polar heteroatom that can act as a hydrogen bond acceptor (Scheme 4, compound **55**). Synthesis of 3-(4-methoxyphenyl)-3-{2-[2-(morpholin-4-yl)ethoxy]-5-(propan-2-yloxy)phenyl}-1,3-dihydro-2*H*-indol-2-one (**55**) included a non-discriminating, low yield (19%) double alkylation of the 1,4-hydroquinone by first 2-iodopropane and then 4-(2-chloroethyl) morpholine.

All 29 new substituted 3,3-diphenyl-1,3-dihydroindol-2-ones were purified (purity ≥95% determined by analytical reversed phase HPLC, see Table S1 in "Supporting information") either by crystallization, reverse phase (RP) flash chromatography or RP-preparative HPLC, and their structural integrity was confirmed by ^1H and ^{13}C NMR, and HR–MS.

2.2. Biological activity

The SAR study reported herein aimed at exploring the latitude for diverse decorations on the phenyl rings in the prototypic **#1181**, which has recently been reported as a potent anti-tumor agent targeting the TC and able to inhibit TI in animal models of human cancer [25]. In this study we first tested the efficacy of our new series of oxindoles to inhibit the growth of adherent murine lung squamous cell carcinoma (KLN 205) and human breast cancer and melanoma cells (CRL-2351 and CRL-2813, respectively) employing the sulforhodamine B (SRB) assay [24]. We then carried out the mechanistic TC availability assay, cell-based dual luciferase reporter gene assay [39] modified to reflect the abundance of the eIF2·GTP·Met-$tRNA_i^{Met}$ TC [24]. Subsequently, selected representatives of this oxindole series were subjected to a secondary mechanistic assay, specifically testing the effect of these compounds on the levels of C/EBP homologous protein (CHOP) mRNA, which is induced when the TC availability is reduced. We anticipated that inhibition of TI by depleting TC formation would correlate with inhibition of cancer cell proliferation.



Scheme 3. Synthesis of oxindole **52**.[aa] Reagents and reaction conditions: (i) 6-(morpholin-4-yl)pyridin-3-boronic acid pinacol ester, $Pd(PPh_3)_2Cl_2$, 2M Na_2CO_3 solution, degassed THF.

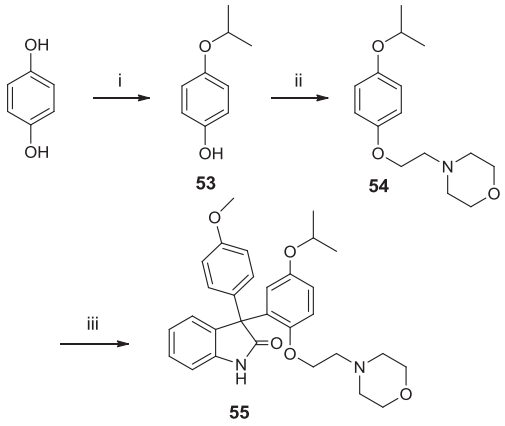
2.2.1. Inhibition of cancer cell proliferation

In an effort to modulate the physicochemical properties of **#1181** (see Table S2 in "Supporting information") we took advantage of O-alkylation of the hydroxyl in position 2 of ring **C** as a convenient site for introduction of either polar groups (analogs **25**–**27** and **29**) or a hydrophobic group (analog **28**) (Table 1). Substitutions with amines such as analogs **25**–**27** maintained the inhibitory cell proliferation activity of **#1181** while substitution with either hydrophobic or carboxyl bearing moieties (analogs **28** and **29**, respectively) led to substantial increase in the IC_{50}.

In general mono- or disubstitutions on ring **B** are well tolerated and maintain IC_{50}s in the 2–17 μM range in all 3 cell types included in this study (entries 2–5 and 13, Table 2). Likewise, mono-substitutions on ring **A** were well tolerated regardless of their chemical nature (entries 8 and 15, Table 2). In addition, simultaneous substitutions on both rings **A** and **B** resulted in compounds that had IC_{50}s in the low μM at least in one cell line except for compound **51** that was weakly potent in KLN-205 and CRL-2813 and inactive in CRL-2351 cells (entries 10–12 and 14, Table 2).

It seems that, out of the three cell lines, the murine KLN-205 is the least responsive while the human CRL-2351 is the most responsive to the parent oxindole **#1181** and its rings **B** and **C** substituted analogs. Loss of cell growth inhibitory activity ($IC_{50} > 20$ μM) was observed for analogs **36** and **45** in KLN-205 cells and for analog **51** in CRL-2351 cells (Table 2). The former, analog **36**, was associated with the introduction of 3-pyridyl ring in position **B** while analog **45** resulted in the combination of 4′-methoxy and 7-methoxycarbonyl on rings **B** and **A**, respectively. The latter was the trisubstituted analog **51** (6,7-difluoro,4′-hydroxy on rings **A** and **B**, respectively).

The most active compounds in CRL-2813 were **40** and **41** and in CRL-2351 **42** displaying IC_{50}s of ~1 μM (Table 2). Relative to the **#1181**, all three compounds are substituted on ring **A** by electron



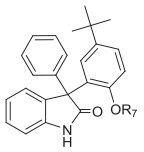
Scheme 4. Synthesis of oxindole **55**.[aa] Reagents and reaction conditions: (i) 2-iodopropane, KOH, EtOH, reflux; (ii) 4-(2-chloroethyl)morpholine hydrochloride, KOH, EtOH, reflux; (iii) intermediate **2**, triflic acid, CH_2Cl_2, 0 °C.

withdrawing substituents and an additional 4-OMe on ring **B** in compound **42**. Of interest is the remarkable difference in CRL-2351 cell growth inhibitory activity between one of the most active compounds **42** and the non-active **51**, which differ only in the substituent on ring **B** (4′-OH and 4′-OMe, respectively, Table 2). The same structural difference is presented by the two active compounds **30** and **50** but in this case it results with very similar activities in all three cell lines (Table 2).

In an attempt to merge beneficial modifications into a single structure, we chose oxindole **25** substituted by the polar 2-(morpholino-4-yl)ethoxy group on position 2 of ring **C**, which was slightly more potent than **#1181** (Table 1), as the basic scaffold and submitted it to another round of decorations (Table 3). The majority of analogs modified with either one (**31** and **33**) or two (**38**) substitutions of variable nature on ring **B** did not change the cell growth inhibitory activity in a significant way relative to the parent analog **25**. Similarly, disubstitutions on rings **A** and **B** (**44**, **47**, and **48**) did not affect significantly the IC_{50}s. A notable decrease in potency was observed with the disubstituted and trisubstituted analogs **46** and **49**, respectively, which were about 2–6-fold less potent than the parent **25**. Interestingly, these losses in potency were the result of either addition of a 7-CO_2Me to **31** or 6,7-diF to **33**. Moreover, addition of a 3′-OMe on ring **B** of **33** resulted in the inactive 3′,4′-diOMe analog **37** (Table 3).

Evidently, **#1181** tolerates a wide range of substitutions as it relates to inhibition of cancer cell growth, many of these substitutions being polar and/or charge-bearing ones.

Table 1
Inhibition of cancer cells proliferation.[a]



Entry	Comp.	R_7	IC_{50} (μM)		
			KLN	2813	2351
1	**#1181**	H	14.4 ± 1.5	4.8 ± 3.7	3.4 ± 0.66
2	**25**		6.8 ± 3.7	2.4 ± 0.3	3.35 ± 0.3
3	**26**		1.2 ± 0.3	2.4 ± 0.4	1.4 ± 0.3
4	**27**		1.2 ± 0.3	12.8 ± 0.8	0.9 ± 0.1
5	**28**		>20	>20	17.5 ± 2
6	**29**		>20	>20	>20

[a] To determine IC_{50} values on murine KLN 205 and human CRL-2813 and CRL-2351 cell lines, cell proliferation was measured by the sulforhodamine B (SRB) assay (see Experimental section for assays details). IC_{50}s are indicated as the mean values of 2 experiments, each done in triplicates.

2.2.2. Depletion of the TC

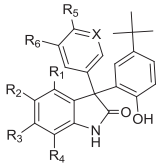
The dual luciferase TC reporter assay that measures the ability of the small molecule to reduce the availability of the eIF2·GTP·Met-$tRNA_i^{Met}$ (TC) is not only our basic high throughput screening assay but also the first line assay for gathering structure-TC-mediated TI inhibitory activity relationship. In general, reduction in TC availability will result in the inhibition of the translation of most weak mRNAs except for a small subset of mRNAs that contain multiple upstream open reading frames (uORFs) in their 5′-untranslated region (5′UTRs) such as mRNA coding for activating transcription factor 4 (ATF-4). Our dual luciferase TC reporter assay has firefly luciferase mRNA fused to the 5′UTR of ATF-4 mRNA and renilla luciferase fused to the 5′UTR that is lacking any uORFs. Validation and adaptation of this assay to HTS has been previously described [24]. Therefore, oxindoles that reduce the availability of the TC would increase firefly luciferase expression and concomitantly decrease the expression of renilla luciferase, resulting in an increased firefly/renilla luciferase ratio (F/R).

Each compound was tested at 4 different concentrations 10, 20, 40 and 80 μM. Relative to the oxindole **#1181**, the majority of the compounds in the current series were at least as potent as the parent compound in inhibiting TI (Fig. 3). Decorating **#1181** with either 4,5-[1,3]thiazolo fused to positions 4 and 5 of ring **A** (**41**), 4′-methoxy on ring **B** (**42**), or 5,4′-dimethoxy on rings **A** and **B** (**43**) led to inactive analogs. In general, substitution of ring **C** on position 2′ was more tolerated. Only one compound out of those substituted by 2′-(morpholino-4-yl)ethoxy group on position 2′ of ring **C** was inactive (**46**). In addition, substitution of the same position with 2′-piperidino-4-yl)ethoxy (**27**) or 2′-acetoxy (**29**) led to a loss of activity. Relative to the **#1181**, analogs **28**, **30**, **38**, **48**, and **49** were about 1.2–1.5-fold and analogs **31**, **33**, and **39** were about 1.5–2-fold more potent at the same concentration (40 μM). Conversely, analogs **34**, **36**, and **52** had their maximal increase of ATF-4-5′UTR-mediated expression of firefly luciferase at 80 μM (3-, 4.5-, and 4-fold, respectively). These analogs presented the same substitution pattern on ring **C** as in **#1181**, but in addition had 4′-morpholino-methyl on ring **B** (**34**), pyridyl-3-yl as ring **B** (**36**), or 5-[6-(morpholin-4-yl)pyridin-3-yl] on ring **A** (**52**). Importantly, the most potent analogs in this series displayed higher maximal TI inhibitory activity in the dual luciferase TC reporter assay but at a higher concentration compared to the maximal activity of **#1181**. It is possible that the for the compounds where the TI inhibitory activity at 80 μM is lower than at 40 μM is a result of exceeding the maximum solubility leading to lower apparent solubility.

2.2.3. Expression of CHOP mRNA

Demonstration of the impact of oxindoles as inhibitors of TI through depletion of the TC on a downstream target, such as expression of CHOP mRNA, was carried out to provide mechanistic validation of our above observations. CHOP mRNA levels are transcriptionally controlled by ATF-4, which itself is translationally upregulated when the TC availability is reduced. Therefore, reduced TC availability as demonstrated above should result in elevated CHOP mRNA levels. To this end we selected several active oxindole derivatives representing a wide range of activity in TC assay and

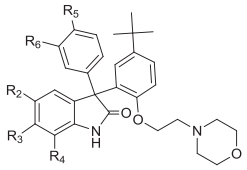
Table 2
Inhibition of cancer cells proliferation.[a]



Entry	Comp.	R_1	R_2	R_3	R_4	R_5	R_6	X	IC_{50} (μM)		
									KLN	2813	2351
1	**#1181**	H	H	H	H	H	H	CH	14.4 ± 1.5	4.8 ± 3.7	3.4 ± 0.66
2	**30**	H	H	H	H	OMe	H	CH	15.7 ± 1.1	2.75 ± 0.1	2.65 ± 0.1
3	**32**	H	H	H	H	$N(CH_3)_2$	H	CH	10.9 ± 1.3	11 ± 0.58	2.3 ± 0.3
4	**34**	H	H	H	H		H	CH	17.3 ± 2.4	7.8 ± 0.66	7.3 ± 3.6
5	**35**	H	H	H	H	Cl	CH_3	CH	4.3 ± 1	2.9 ± 0.1	2.9 ± 0. 1
6	**36**	H	H	H	H	H	H	N	>20	11.5 ± 1.8	6.4 ± 3
7	**39**	H	I	H	H	H	H	CH	14.9 ± 3.8	2.8 ± 0.2	3.5
8	**40**	H	OCF_3	H	H	H	H	CH	8 ± 1.7	1 ± 0.1	2.5 ± 0.9
9	**41**			H	H	H	H	CH	8 ± 3.3	0.95 ± 0.1	2.2 ± 1
10	**42**	H	H	F	F	OMe	H	CH	12.3 ± 2	3 ± 0.9	0.95 ± 0.08
11	**43**	H	OMe	H	H	OMe	H	CH	14.3 ± 3	11.1 ± 1.1	2.9 ± 0.3
12	**45**	H	H	H	CO_2CH_3	OMe	H	CH	>20	12.2 ± 0.5	3.4 ± 0.6
13	**50**	H	H	H	H	OH	H	CH	17.3 ± 1.8	4 ± 0.28	2 ± 0.3
14	**51**	H	H	F	F	OH	H	CH	15.3 ± 1.9	10.7 ± 3.9	>20
15	**52**	H		H	H	H	H	CH	13.3 ± 2.4	2.5 ± 1.5	1.8 ± 0.4

[a] To determine IC_{50} values on murine KLN 205 and human CRL-2813 and CRL-2351 cell lines, cell proliferation was measured by the sulforhodamine B (SRB) assay (see Experimental section for assays details). IC_{50}s are indicated as the mean values of 2 experiments, each done in triplicates.

Table 3
Inhibition of cancer cells proliferation.[a]



Entry	Comp.	R_2	R_3	R_4	R_5	R_6	IC_{50} (μM)		
							KLN	2813	2351
1	**25**	H	H	H	H	H	6.8 ± 3.7	2.4 ± 0.3	3.35 ± 0.3
2	**31**	H	H	H	OMe	H	4.3 ± 1	2.4 ± 0.9	2.65 ± 1.5
3	**33**	H	H	H	$N(CH_3)_2$	H	2.2 ± 0.6	7.5 ± 1	4.7 ± 0.3
4	**37**	H	H	H	OMe	OMe	>20	>20	>20
5	**38**	H	H	H	OMe	F	3.4 ± 1.2	2.7 ± 0.4	2.5 ± 0.6
6	**44**	OMe	H	H	OMe	H	2.75 ± 0.3	2.9 ± 0.3	4.5 ± 0.7
7	**46**	H	H	CO_2CH_3	OMe	H	17 ± 3.5	14.6 ± 2.8	16.3 ± 1.6
8	**47**	I	H	H	OMe	H	12 ± 1.2	2.6 ± 0.4	6.1 ± 1.7
9	**48**	OCF_3	H	H	OMe	H	9.3 ± 2.7	1.9 ± 0.7	2.9 ± 0.27
10	**49**	H	F	F	$N(CH_3)_2$	H	15.7 ± 1.9	11 ± 1.3	4 ± 1

[a] To determine IC_{50} values on murine KLN 205 and human CRL-2813 and CRL-2351 cell lines, cell proliferation was measured by the sulforhodamine B (SRB) assay (see Experimental section for assays details). IC_{50}s are indicated as the mean values of 2 experiments, each done in triplicates.

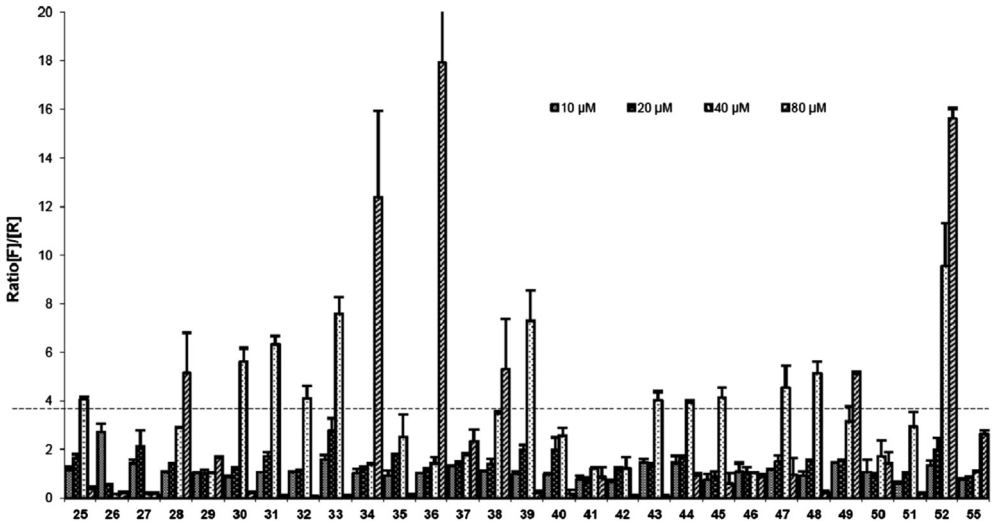


Fig. 3. Ternary complex assays on KLN-205 cell line (see Experimental section for assays details). Activity of compounds was measured by the firefly/renilla luciferase ratio (F/R) compared to vehicle treated cells, and expressed for each compound as a function of concentrations (10 μM, 20 μM, 40 μM, 80 μM). Compounds were compared to **#1181** which maximal activity (obtained at a concentration of 40 μM) is represented by the dashed line.

tested them for induction of CHOP mRNA (Table 4). We were very encouraged by the consistent and direct relationship between the compound's activity in the TC assay and the fold increase over control (DMSO) of CHOP mRNA level, both carried out in KLN-205 cells. This concurrence validates our notion that active oxindoles reduce TC availability and induce ATF-4 expression that leads to downstream increase in CHOP mRNA transcription.

Overall, these results were very encouraging. Nevertheless, it should be noted that the correlation between the inhibition of cancer cell proliferation and activity of compounds in the ternary complex assay was observed for those compounds that display significant activity (≥3-fold depletion of TC as compared to the control DMSO) in the ternary complex assay at concentrations below 40 μM. The compounds that in the ternary complex assay displayed large activity peak at 80 μM without apparent activity at the lower doses were usually devoid of activity in the cell proliferation assay. Similarly, cell growth inhibitory activity of some compounds appear to originate at least in part from the off-target effects that lead to inhibition of many other critical mechanisms required for cell growth and proliferation and have limited contribution from the depletion of TC and inhibition of translation initiation. A good example of such dissociation is displayed by the high cell inhibitory activity of **40**–**42** that are devoid of ATF-4 activity (Fig. 3). Therefore, in the future we suggest using high potency of analogs in the ternary complex assay obtained at low concentrations as the best guide during SAR aiming to improve the activity and specificity of this class of compounds.

3. Conclusion

In this study, our iterative lead optimization process has focused on the introduction of polar groups on ring **C** that could improve bioavailability (Table 1), a diverse spectrum of substituents on rings **A** and **B** that may contribute to potency enhancement (Table 2), and combination of the above to explore possible complementarity (Table 3). We are very encouraged by the structural insight gained and the realization of active compounds that display remarkable enhancement in their activities in the TC reporter assay and CHOP mRNA induction (Fig. 4). We are confident that our results will enable further improvement in bioavailability and potency that will lead to compounds of this chemotype that warrant *in vivo* evaluation.

4. Experimental section

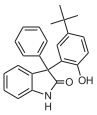
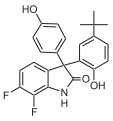
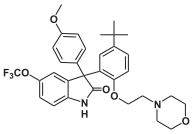
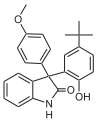
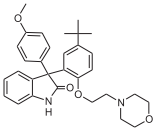
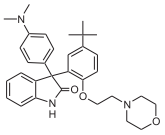
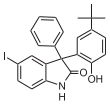
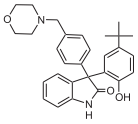
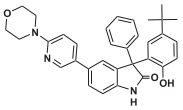
4.1. Chemistry

All reagents and solvents were purchased from commercial sources and used without further purification. All the solvents were anhydrous and maintained under nitrogen. Melting points were determined using a Mel-Temp Electrothermal apparatus and are uncorrected. Proton and carbon NMR analyses were performed on Varian 400 MHz and 500 MHz spectrometers using deuterium solvents. Chemical shifts data are reported in parts per million (ppm) relative to the residual peak of the solvent (δ 2.50 and 39.52 ppm for DMSO-d_6, and δ 3.31 and 49.00 ppm for CD_3OD) according to Gottlieb et al. [40]. All the reactions were monitored by LC-MS analysis on reverse phase (column XTerra MS C8, 3 × 100 mm, 5 μm particle size) using a ThermoFisher Finnigan/LCQ Advantage apparatus with the binary system water/acetonitrile containing 0.1% of trifluoroacetic acid (TFA) as eluent. Purifications by flash chromatography were performed on Biotage SP1 using pre-packed columns filled with silica gel and were monitored by UV detection at 254 and 280 nm. Purifications by preparative HPLC on reverse phase (column XTerra Prep MS C8, 19 × 150 mm, 5 μm particle size) were carried out on a Waters 2525 using the binary system water/acetonitrile containing 0.1% of acetic acid as eluent. The HR-MS analyses were performed by the FAS Center for Systems Biology, Harvard University. The purity of all final compounds was determined by analytical HPLC on reverse phase (column XBridge BEH130 C18, 4.6 × 100 mm, 5 μm particle size) using a Waters Alliance 2695 with the binary system water/acetonitrile containing 0.1% of acetic acid as eluent (see Table S1 in "Supporting information" for more details).

4.1.1. General procedure A for the synthesis of compounds ***1***–***17***

*4.1.1.1. 3-Hydroxy-3-phenyl-1,3-dihydro-2H-indol-2-one (**1**).* A 1 M solution of phenylmagnesium bromide in THF (20.40 mL, 20.40 mmol) was added dropwise to a stirred ice-cold solution of isatin (1 g, 6.80 mmol) in anhydrous THF (20 mL) that was kept under nitrogen. The reaction was stirred at room temperature for 2 h and then quenched with a saturated aqueous solution of NH_4Cl (20 mL). Water was then added (30 mL) and the aqueous phase was extracted twice with dichloromethane (2 × 60 mL). The organic phase was washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed in vacuum and the crude was purified by

Table 4
Expression of CHOP mRNA of active oxindole derivatives representing a wide range of increased ATF-4 activity compared to **#1181**.[a]

Comp.	Structure	ATF-4	CHOP PCR
		Max fold increase of F/R luciferase over control @ [μM]	Fold over control
#1181		3.7 ± 0.7 @ 40	3.77
51		2.9 ± 0.6 @ 40	3.09
48		5.11 ± 0.48 @ 40	3.49
30		5.6 ± 0.5 @ 40	4.09
31		6.3 ± 0.3 @ 40	7.1
33		7.15 ± 0.49 @ 40	4.15
39		7.2 ± 1.2 @ 40	4.36
34		12.4 ± 3.6 @ 80	4.68
52		15.6 ± 0.4 @ 80	20.8

[a] Ternary complex and CHOP PCR assays were carried out on murine KLN-205 cell line (see Experimental section for assays details). CHOP PCR values represent the fold increase over control (DMSO).

crystallization from EtOAc/Hexane to afford **1** (1.18 g, 77.1%) as a white powder. Mp = 215.4–216.7 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.43 (s, 1H, NH), 7.32–7.24 (m, 6H, $CH_{arom.}$), 7.12 (d, J = 7 Hz, 1H, $CH_{arom.}$), 6.98 (m, 1H, $CH_{arom.}$), 6.93 (d, J = 7.5 Hz, 1H, $CH_{arom.}$), 6.65 (s, 1H, OH). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 178.54, 141.98, 141.58, 133.79, 129.27, 128.11 (2C), 127.44, 125.46 (2C), 124.82, 122.08, 109.90, 77.37. MS (ESI) m/z 225.75 $(M + H)^+$.

*4.1.1.2. 3-Hydroxy-3-(4-methoxyphenyl)-1,3-dihydro-2H-indol-2-one (**2**).* A 0.5 M solution of 4-methoxyphenylmagnesium bromide in THF (40.80 mL, 20.40 mmol) and isatin (1 g, 6.80 mmol) were used following the **General procedure A** to afford **2** (0.90 g, 51.9%) as a white powder. Mp = 208.7–210.0 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.35 (s, 1H, NH), 7.26–7.12 (m, 4H, $CH_{arom.}$), 6.99–6.86 (m, 4H, $CH_{arom.}$), 6.55 (s, 1H, OH), 3.62 (s, 3H, CH_3). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 178.74, 158.70, 141.91, 133.78, 133.49, 129.19, 126.80 (2C), 124.78, 122.03, 113.50 (2C), 109.77, 76.96, 55.04. MS (ESI) m/z 237.96 $(M - OH)^+$.

*4.1.1.3. 3-[4-(Dimethylamino)phenyl]-3-hydroxy-1,3-dihydro-2H-indol-2-one (**3**).* A 0.5 M solution of 4-(dimethylamino)phenylmagnesium bromide in THF (20.40 mL, 10.20 mmol) and isatin (500 mg, 3.40 mmol) were used following the **General procedure A** to afford **3** (834 mg, 91.5%) as a white powder. Mp = decomp. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.28 (s, 1H, NH), 7.23 (m, 1H, $CH_{arom.}$), 7.14–7.10 (m, 3H, $CH_{arom.}$), 6.97 (m, 1H, $CH_{arom.}$), 6.87 (d,

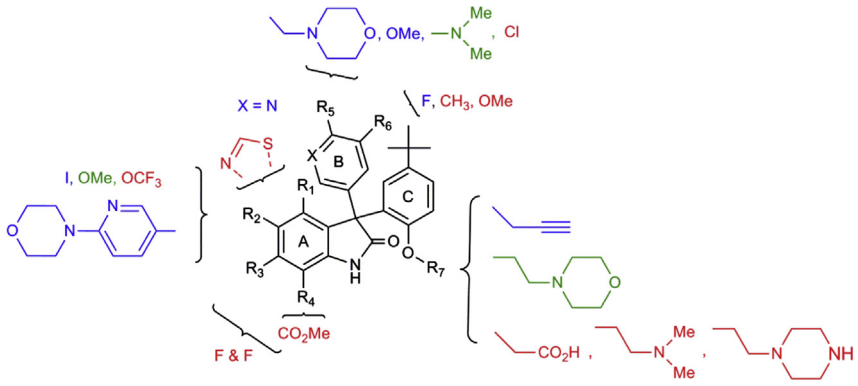


Fig. 4. Schematic summary of SAR of the series of 3,3-diphenylindol-2-ones reported herein based on the ternary complex assay (Fig. 3 and Table 4). Blue substituents are enhancing potency, green ones are most affecting the potency, and the red ones are reducing the potency relative the prototypic **#1181**. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

J = 8 Hz, 1H, $CH_{arom.}$), 6.65 (d, J = 8.8 Hz, 2H, $CH_{arom.}$), 6.39 (s, 1H, OH), 2.84 (s, 6H, CH_3). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 179.00, 149.89, 141.87, 134.01, 128.93, 128.87, 126.42 (2C), 124.83, 121.84, 111.93 (2C), 109.68, 76.96, 40.19 (2C). MS (ESI) m/z 269.17 $(M + H)^+$.

*4.1.1.4. 3-Hydroxy-3-[4-(morpholin-4-ylmethyl)phenyl]-1,3-dihydro-2H-indol-2-one (**4**).* A 0.25 M solution of 4-(morpholin-4-ylmethyl) phenylmagnesium bromide in THF (30 mL, 7.48 mmol) and isatin (550 mg, 3.74 mmol) were used following the **General procedure A**. The crude was purified by flash chromatography (0–20% of methanol in dichloromethane) to afford **4** (1.12 g, 92.4%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.50 (s, 1H, NH), 7.29 (m, 2H, $CH_{arom.}$), 7.18 (m, 2H, $CH_{arom.}$), 7.07–7.13 (m, 2H, $CH_{arom.}$), 6.92 (t, J = 7.6 Hz, 1H, $CH_{arom.}$), 6.73 (d, J = 8.0 Hz, 1H, $CH_{arom.}$), 3.56 (m, 4H, CH_2OCH_2), 3.37 (s, 2H, $CH_2\Phi$), 2.32 (m, 4H, CH_2NCH_2). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 180.81, 141.04 (2C), 139.34, 137.26, 132.97, 129.81 (2C), 125.67 (2C), 125.27, 123.48, 111.01, 78.54, 66.84 (2C), 63.02, 53.50 (2C). MS (ESI) m/z 325.10 $(M + H)^+$.

*4.1.1.5. 3-(4-Chloro-3-methylphenyl)-3-hydroxy-1,3-dihydro-2H-indol-2-one (**5**).* A 0.5 M solution of (4-chloro-3-methyl)phenylmagnesium bromide in THF (32.70 mL, 16.35 mmol) and isatin (1.20 g, 8.16 mmol) were used following the **General procedure A**. The crude was purified by flash chromatography (5–40% of ethyl acetate in cyclohexane) to afford **HW** (1.48 g, 67%) as a yellow oil. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.48 (s, 1H, NH), 7.32 (d, J = 8.0 Hz, 1H, $CH_{arom.}$), 7.22–7.25 (m, 2H, $CH_{arom.}$), 7.07 (d, J = 7.0 Hz, 1H, $CH_{arom.}$), 7.02 (dd, J = 2.5 Hz, J = 8.0 Hz, 1H, $CH_{arom.}$), 6.95 (dd, J = 2.5 Hz, J = 8.0 Hz, 1H, $CH_{arom.}$), 6.90 (d, J = 8.0 Hz, 1H, $CH_{arom.}$), 6.69 (s, 1H, OH), 2.27 (s, 3H, CH_3). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 178.75, 142.64, 141.23, 135.72, 133.99, 133.00, 130.07, 129.24, 128.84, 125.40 (2C), 122.80, 110.67, 77.53, 20.43. MS (ESI) m/z 273.85 $(M + H)^+$.

*4.1.1.6. 3-Hydroxy-3-(pyridine-3-yl)-1,3-dihydro-2H-indol-2-one (**6**).* To a suspension of magnesium (216 mg, 8.88 mmol) in anhydrous THF (5 mL) was added a few drops of 3-bromopyridine and 1,2-dibromoethane (100 μL, 1.16 mmol) under nitrogen. The reaction mixture was warmed up for 2 min to initiate the reaction and 3-bromopyridine (713 μL, 7.40 mmol) was added dropwise. After refluxing under nitrogen for 6 h, the mixture was cooled to room temperature and added dropwise to a solution of isatin (544 mg, 3.70 mmol) in anhydrous THF (20 mL) maintained at 0 °C under nitrogen. After stirring for 2 h at room temperature, the reaction was quenched with a saturated aqueous solution of NH_4Cl (20 mL). Water was then added (30 mL) and the aqueous phase was extracted twice with dichloromethane (2 × 60 mL). The organic phase was washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed in vacuum and the crude was purified by flash chromatography (10–40% of ethyl acetate in cyclohexane) to afford **6** (151 mg, 18%) as a yellow powder. Mp = 292.5–294.2 °C. ^{1}H NMR (500, MHz, DMSO-d_6): δ 8.52 (s, 1H, NH), 8.47 (s, 1H, $CH_{arom.}$), 7.84 (m, 1H, $CH_{arom.}$), 7.70 (d, J = 8.5 Hz, 1H, $CH_{arom.}$), 7.40 (m, 1H, $CH_{arom.}$), 7.35 (d, J = 8.0 Hz, 1H, $CH_{arom.}$), 7.24–7.19 (m, 2H, $CH_{arom.}$, OH), 7.08 (m, 1H, $CH_{arom.}$), 6.99 (d, J = 8.0 Hz, 1H, $CH_{arom.}$). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 179.35, 148.19, 146.53, 141.96, 134.79, 130.12, 128.62, 125.77, 124.92, 123.82, 123.12, 110.52, 84.40. MS (ESI) m/z 227.03 $(M + H)^+$.

*4.1.1.7. 3-(3,4-Dimethoxyphenyl)-3-hydroxy-1,3-dihydro-2H-indol-2-one (**7**).* A 0.5 M solution of 3,4-dimethoxyphenylmagnesium bromide in THF (20.40 mL, 10.20 mmol) and isatin (500 mg, 3.40 mmol) were used following the **General procedure A** to afford **7** (767 mg, 79.1%) as a white powder. Mp = 219.2–220.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.33 (s, 1H, NH), 7.25–7.10 (m, 3H, $CH_{arom.}$), 6.98–6.82 (m, 3H, $CH_{arom.}$), 6.56 (m, 2H, $CH_{arom.}$, OH), 3.72 (s, 3H, CH_3), 3.70 (s, 3H, CH_3). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 178.56, 148.46, 148.35, 141.89, 133.97, 133.64, 129.16, 124.83 (2C), 121.95, 117.72, 111.38 (2C), 109.78, 76.97, 55.50 (2C). MS (ESI) m/z 268.21 $(M - OH)^+$.

*4.1.1.8. 3-(3-Fluoro-4-methoxyphenyl)-3-hydroxy-1,3-dihydro-2H-indol-2-one (**8**).* A 0.5 M solution of 3-fluoro-4-methoxyphenylmagnesium bromide in THF (12.25 mL, 6.12 mmol) and isatin (300 mg, 2.04 mmol) were used following the **General procedure A** to afford **8** (443 mg, 79.5%) as a white powder. Mp = 197.8–199 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.40 (s, 1H, NH), 7.26 (m, 1H, $CH_{arom.}$), 7.19–7.13 (m, 2H, $CH_{arom.}$), 7.07 (m, 1H, $CH_{arom.}$), 6.98 (m, 1H, $CH_{arom.}$), 6.91–6.87 (m, 2H, $CH_{arom.}$), 6.67 (s, 1H, OH), 3.79 (s, 3H, CH_3). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 178.15, 152.12, 150.18, 146.50, 141.91, 134.45, 133.09, 129.42, 124.84, 122.13, 121.66, 113.39, 109.95, 76.53, 56.03. MS (ESI) m/z 256.30 $(M - OH)^+$.

*4.1.1.9. 3-Hydroxy-5-iodo-3-phenyl-1,3-dihydro-2H-indol-2-one (**9**).* A 1 M solution of phenylmagnesium bromide in THF (22 mL, 22.00 mmol) and 5-iodoisatin (2 g, 7.33 mmol) were used following the **General procedure A** to afford **9** (1.88 g, 73.1%) as a white powder. Mp = 313.6–314.3 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.56 (s, 1H, NH), 7.61 (d, J = 8.5 Hz, 1H, $CH_{arom.}$), 7.36–7.26 (m, 6H, $CH_{arom.}$), 6.79 (s, 1H, $CH_{arom.}$), 6.76 (s, 1H, OH). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 177.77, 141.70, 140.89, 137.74, 136.42, 132.88, 128.25 (2C), 127.64, 125.27 (2C), 112.51, 84.79, 77.17. MS (ESI) m/z 334.15 $(M - OH)^+$.

*4.1.1.10. 3-Hydroxy-3-phenyl-5-(trifluoromethoxy)-1,3-dihydro-2H-indol-2-one (**10**).* A 1 M solution of phenylmagnesium bromide in THF (6.50 mL, 6.50 mmol) and 5-(trifluoromethoxy)isatin (500 mg, 2.16 mmol) were used following the **General procedure A** to afford **10** (357 mg, 53.4%) as a white powder. Mp = 241.5–243.0 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.62 (s, 1H, NH), 7.44–7.26 (m, 6H, $CH_{arom.}$), 7.08 (s, 1H, $CH_{arom.}$), 6.99 (d, J = 8.5 Hz, 1H, $CH_{arom.}$), 6.83 (s, 1H, OH). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 178.35, 143.39, 141.15, 140.70, 135.33, 128.25 (2C), 127.74, 125.32 (2C), 122.55, 119.23, 118.26, 110.92, 77.42. MS (ESI) m/z 309.87 $(M + H)^+$ and 292.23 $(M - OH)^+$.

*4.1.1.11. 8-Hydroxy-8-phenyl-6,8-dihydro-7H-[1,3]thiazolo[5,4,e]indol-7-one (**11**).* A 1 M solution of phenylmagnesium bromide in THF (7.35 mL, 7.35 mmol) and 6*H*-[1,3]thiazolo[5,4,*e*]indol-7,8-dione (500 mg, 2.45 mmol) were used following the **General procedure A**. The crude was purified by flash chromatography on reverse phase (0–60% of acetonitrile in water) and the pure fractions were lyophilized to afford **11** (128 mg, 18.5%) as a white powder. Mp = decomp. ^{1}H NMR (400 MHz, CD_3OD): δ 8.99 (s, 1H, N=NH–S), 8.02 (d, J = 8 Hz, 1H, $CH_{arom.}$), 7.40–7.24 (s, 6H, $CH_{arom.}$). ^{13}C NMR (100.6 MHz, CD_3OD): δ 181.93, 155.14 (2C), 151.71, 140.99, 139.45, 130.91, 129.51 (2C), 129.27, 126.61 (2C), 125.16, 111.16, 79.74. MS (ESI) m/z 283.16 $(M + H)^+$ and 265.26 $(M - OH)^+$.

*4.1.1.12. 6,7-Difluoro-3-hydroxy-3-(4-methoxyphenyl)-1,3-dihydro-2H-indol-2-one (**12**).* A 0.5 M solution of 4-methoxy phenylmagnesium bromide in THF (9.85 mL, 4.92 mmol) and 6,7-difluoro-*1H*-indole-2,3-dione (300 mg, 1.64 mmol) were used following the **General procedure A** to afford **12** (304 mg, 63.7%) as a white powder. Mp = 169.2–170.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.14 (s, 1H, NH), 7.21 (m, 2H, $CH_{arom.}$), 6.98–6.96 (m, 2H, $CH_{arom.}$), 6.89 (m, 2H, $CH_{arom.}$), 6.73 (s, 1H, OH), 3.72 (s, 3H, CH_3). ^{13}C NMR (100.5 MHz, DMSO-d_6): δ 178.53, 158.93, 151.35, 149.40, 136.24, 134.15, 132.57, 131.46, 130.90, 126.78, 120.99, 113.62, 109.80, 76.73, 55.12. MS (ESI) m/z 273.99 $(M - OH)^+$.

*4.1.1.13. 3-Hydroxy-5-methoxy-3-(4-methoxyphenyl)-1,3-dihydro-2H-indol-2-one (**13**).* A 0.5 M solution of 4-methoxy phenylmagnesium bromide in THF (17 mL, 8.50 mmol) and 5-methoxyisatin (500 mg, 2.82 mmol) were used following the **General procedure A** to afford **13** (690 mg, 85.7%) as a white powder. Mp = 189.8–191.2 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.17 (s, 1H, NH), 7.22 (m, 2H, $CH_{arom.}$), 6.89–6.82 (m, 4H, $CH_{arom.}$), 6.73 (s, 1H, $CH_{arom.}$), 6.54 (s, 1H, OH), 3.72 (s, 3H, CH_3), 3.67 (s, 3H, CH_3). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 178.64, 158.71, 155.15, 135.07, 134.99, 133.53, 126.85 (2C), 113.91, 113.49 (2C), 111.46, 110.31, 77.38, 55.48, 55.10. MS (ESI) m/z 268.28 $(M - OH)^+$.

*4.1.1.14. Methyl 3-hydroxy-3-(4-methoxyphenyl)-2-oxindoline-7-carboxylate (**14**).* A 0.5 M solution of 4-methoxyphe nylmagnesium bromide in THF (14.65 mL, 7.32 mmol) and methyl 2,3-dioxoindoline-7-carboxylate (1 g, 4.88 mmol) were used following the **General procedure A**. The crude was purified by flash chromatography on reverse phase (30–80% of acetonitrile in water) and the pure fractions were lyophilized to afford **14** (1.06 g, 69.4%) as a white powder. Mp = 173.6–175.2 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.25 (s, 1H, NH), 7.79 (d, J = 8 Hz, 1H, $CH_{arom.}$), 7.37 (d, J = 7.5 Hz, 1H, $CH_{arom.}$), 7.20 (m, 2H, $CH_{arom.}$), 7.12 (m, 1H, $CH_{arom.}$), 6.89 (m, 2H, $CH_{arom.}$), 6.71 (s, 1H, OH), 3.89 (s, 3H, CH_3), 3.72 (s, 3H, CH_3). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 179.65, 166.48, 159.66, 143.27, 135.72, 133.05, 130.77, 130.21, 127.52 (2C), 123.24, 114.48 (2C), 112.48, 76.69, 55.88, 53.11. MS (ESI) m/z 296.20 $(M - OH)^+$.

*4.1.1.15. 3-Hydroxy-5-iodo-3-(4-methoxyphenyl)-1,3-dihydro-2H-indol-2-one (**15**).* A 0.5 M solution of 4-methoxyphenylmagnesium bromide in THF (11 mL, 5.50 mmol) and 5-iodoisatin (500 mg, 1.83 mmol) were used following the **General procedure A** to afford **15** (533 mg, 76.4%) as a white powder. Mp = 256.8–258.1 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.49 (s, 1H, NH), 7.58 (d, J = 8 Hz, 1H, $CH_{arom.}$), 7.36 (s, 1H, $CH_{arom.}$), 7.19 (m, 2H, $CH_{arom.}$), 6.88 (m, 2H, $CH_{arom.}$), 6.75 (d, J = 8 Hz, 1H, $CH_{arom.}$), 6.65 (s, 1H, OH), 3.71 (s, 3H, CH_3). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 177.95, 158.80, 141.62, 137.63, 136.47, 132.91, 132.82, 126.67 (2C), 113.63 (2C), 112.44, 84.71, 76.77, 55.10. MS (ESI) m/z 364.18 $(M - OH)^+$.

*4.1.1.16. 3-Hydroxy-3-(4-methoxyphenyl)-5-(trifluoromethoxy)-1,3-dihydro-2H-indol-2-one (**16**).* A 0.5 M solution of 4-methoxyphenylmagnesium bromide in THF (13 mL, 6.50 mmol) and 5-(trifluoromethoxy)isatin (500 mg, 2.16 mmol) were used following the **General procedure A** to afford **16** (584 mg, 79.6%) as a white powder. Mp = 226.9–228.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.55 (s, 1H, NH), 7.30–7.15 (m, 3H, $CH_{arom.}$), 7.08 (s, 1H, $CH_{arom.}$), 7.00–6.83 (m, 3H, $CH_{arom.}$), 6.73 (s, 1H, OH), 3.70 (s, 3H, CH_3). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 178.59, 158.92, 143.39, 141.10, 135.40, 132.65, 126.78 (2C), 122.50, 118.93, 118.29, 113.66 (2C), 110.88, 77.06, 55.10. MS (ESI) m/z 322.24 $(M - OH)^+$.

*4.1.1.17. 3-[4-(Dimethylamino)phenyl]-6,7-difluoro-3-hydroxy-1,3-dihydro-2H-indol-2-one (**17**).* A 0.5 M solution of 4-(dimethylamino)phenylmagnesium bromide in THF (9.85 mL, 4.92 mmol) and 6,7-difluoro-*1H*-indole-2,3-dione (300 mg, 1.64 mmol) were used following the **General procedure A** to afford **17** (387 mg, 77.6%) as a white powder. Mp = decomp. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.04 (s, 1H, NH), 7.07 (m, 2H, $CH_{arom.}$), 6.98–6.94 (m, 2H, $CH_{arom.}$), 6.65 (m, 2H, $CH_{arom.}$), 6.56 (s, 1H, OH), 2.85 (s, 6H, CH_3). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 178.78, 150.04, 136.34, 133.89, 131.69, 130.67, 127.79, 126.34 (2C), 120.90, 111.94 (2C), 109.63, 76.71, 40.15 (2C). MS (ESI) m/z 304.94 $(M + H)^+$.

*4.1.2. 4-[2-(4-tert-Butylphenoxy)ethyl]morpholine (**18**)*

A ice-cold solution of 4-*tert*-butylphenol (1 g, 6.67 mmol), 4-(2-hydroxyethyl)morpholine (0.87 g, 6.67 mmol) and triphenylphosphine (2.28 g, 8.67 mmol) in anhydrous THF (50 mL) was stirred under nitrogen for 20 min. After a dropwise addition of 40% DEAD in toluene (3.77 mL, 8.67 mmol), the reaction mixture was allowed to react for 2 more hours at room temperature. The solvent was then removed in vacuum and the residue was dissolved in dichloromethane. Addition of *n*-Hexane formed a precipitate of triphenylphosphine oxide that was filtered off. After removal of the solvents under reduced pressure, the crude was purified by flash chromatography (10–50% of ethyl acetate in cyclohexane) to afford **18** (0.96 g, 54.7%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31–7.28 (m, 2H, $CH_{arom.}$), 6.86–6.83 (m, 2H, $CH_{arom.}$), 4.09 (m, 2H, OCH_2), 3.73 (m, 4H, CH_2OCH_2), 2.79 (m, 2H, CH_2N), 2.57 (m, 4H, CH_2NCH_2), 1.29 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 156.38, 143.41, 126.16 (2C), 113.98 (2C), 66.84 (2C), 65.63, 57.65, 54.03 (2C), 34.03, 31.51 (3C). MS (ESI) m/z 264.19 $(M + H)^+$.

*4.1.3. General procedure B for the synthesis of compounds **19**–**21***

*4.1.3.1. 2-(4-tert-Butylphenoxy)-N,N-dimethylethanamine (**19**).* A solution of 4-*tert*-butylphenol (2 g, 13.33 mmol) in anhydrous DMF (25 mL) was added dropwise to a stirred mixture of NaH 60% (0.71 g, 17.75 mmol) in anhydrous DMF (50 mL) that was kept under nitrogen. After 30 min, a solution of *N,N*-dimethylaminoethyl-bromide (1.35 g, 8.89 mmol) in anhydrous DMF (25 mL) was added dropwise using a cannula and the reaction mixture was stirred under nitrogen for 16 h at 80 °C. After removal of DMF under reduced pressure, the crude was dissolved in dichloromethane

(200 ml) and the resulting solution was washed 3 times with brine and dried over anhydrous Na_2SO_4. After removal of the solvent in vacuum, the crude was purified by flash chromatography (0–10% of methanol in dichloromethane) to afford **19** (0.90 g, 45.6%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30–7.26 (m, 2H, $CH_{arom.}$), 6.87–6.84 (m, 2H, $CH_{arom.}$), 4.05 (m, 2H, OCH_2), 2.89 (m, 2H, CH_2N), 2.45 (s, 6H, $N(CH_3)_2$), 1.28 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (100.6 $CDCl_3$): δ 156.49, 143.51, 126.22 (2C), 113.99 (2C), 65.83, 57.85, 45.45 (2C), 34.08, 31.56 (2C). MS (ESI) *m/z* 222.13 $(M + H)^+$.

*4.1.3.2. 1-tert-Butyl-4-(prop-2-yn-1-yloxy)benzene (**20**).* 4-*tert*-butylphenol (1 g, 6.67 mmol), NaH 60% (0.53 g, 13.33 mmol) and propargylbromide (0.95 g, 7.98 mmol) in anhydrous DMF were used following the **General procedure B**. The crude was purified by flash chromatography (0–10% of ethyl acetate in cyclohexane) to afford **20** (0.72 g, 57.5%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.34 (m, 2H, $CH_{arom.}$), 6.94 (m, 2H, $CH_{arom.}$), 4.69 (s, 2H, CH_2), 2.53 (s, 1H, CH), 1.30 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 155.46, 144.41, 126.39 (2C), 114.45 (2C), 79.02, 75.45, 55.96, 34.26, 31.61 (3C). MS (ESI) no ionization.

*4.1.3.3. tert-Butyl-(4-tert-butylphenoxy)acetate (**21**).* 4-*tert*-butylphenol (2 g, 13.33 mmol), NaH 60% (1.07 g, 26.67 mmol) and *tert*-butylbromoacetate (1.73 g, 8.89 mmol) in anhydrous DMF were used following the **General procedure B**. The crude was purified by flash chromatography (0–10% of ethyl acetate in cyclohexane) to afford **21** (1.89 g, 80.7%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31 (m, 2H, $CH_{arom.}$), 6.84 (m, 2H, $CH_{arom.}$), 4.50 (s, 2H, OCH_2), 1.51 (s, 9H, $C(CH_3)_3$), 1.31 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 168.19, 155.69, 144.04, 126.20 (2C), 114.04 (2C), 82.05, 65.73, 34.04, 31.48 (3C), 28.01 (3C). MS (ESI) no ionization.

*4.1.4. Benzyl 4-(2-hydroxyethyl)piperazine-1-carboxylate (**22**)*

Benzylchloroformate (4.72 g, 27.69 mmol) in acetonitrile (30 mL) was added dropwise over 30 min to a solution of 1-(2-hydroxyethyl)piperazine (3 g, 23.08 mmol) in water (30 mL) via an isobar cylindrical funnel. The pH was maintained around 8–9 by addition of 4 N NaOH. The reaction was stirred overnight at room temperature. The mixture was first extracted with dichloromethane (100 mL) in order to remove the fully protected compound and then acidified with 4 N HCl. The acidic aqueous phase was extracted twice with dichloromethane (2 × 100 mL). The organic phase was washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed in vacuum and the crude was purified by flash chromatography (0–8% of methanol in dichloromethane) to afford **22** (5.41 g, 88.8%) as a colorless oil. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.37–7.29 (m, 5H, $CH_{arom.}$), 5.05 (s, 2H, $CH_2\Phi$), 4.41 (s, 1H, OH), 3.47 (m, 2H, $HOC\underline{H}_2$), 3.35 (m, 4H, $(CH_2)_2NCO$), 2.38–2.35 (m, 6H, CH_2N, CH_2NCH_2). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 155.04, 137.59, 129.09 (2C), 128.49, 128.22 (2C), 66.81, 60.82, 59.14, 53.51 (2C), 44.16 (3C). MS (ESI) *m/z* 265.02 $(M + H)^+$.

*4.1.5. Benzyl 4-[2-(4-tert-butylphenoxy)ethyl]piperazine-1-carboxylate (**23**)*

A ice-cold solution of 4-*tert*-butylphenol (0.85 g, 5.67 mmol), compound **22** (1.50 g, 5.67 mmol) and triphenylphosphine (1.93 g, 7.37 mmol) in anhydrous THF (50 mL) was stirred and kept under nitrogen for 20 min. After a dropwise addition of 40% DEAD in toluene (3.2 mL, 7.37 mmol), the reaction mixture was allowed to react for 2 more hours at room temperature. The solvent was then removed in vacuum and the residue was dissolved in dichloromethane. Addition of hexane formed a precipitate of triphenylphosphine oxide that was filtered off. After removal of the solvents under reduced pressure, the crude was purified by flash chromatography (0–50% of ethyl acetate in cyclohexane) to afford **23** (1.62 g, 72.2%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.39–7.32 (m, 7H, $CH_{arom.}$), 6.89–6.87 (m, 2H, $CH_{arom.}$), 5.18 (s, 2H, $CH_2\Phi$), 4.12 (m, 2H, OCH_2), 3.58 (m, 4H, $(CH_2)_2NCO$), 2.83 (m, 2H, CH_2N), 2.57 (m, 4H, CH_2NCH_2), 1.34 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 156.63, 155.44, 143.79, 137.00, 128.76 (2C), 128.27, 128.16 (2C), 126.49 (2C), 114.29 (2C), 67.38, 66.02, 57.52, 53.48 (2C), 44.03 (2C), 34.32, 31.82 (3C). MS (ESI) *m/z* 397.10 $(M + H)^+$.

*4.1.6. 1-[2-(4-tert-Butylphenoxy)ethyl]piperazine (**24**)*

Palladium on carbon 10% (75.7 mg, 60 mg per mmol) was carefully added to a solution of compound **23** (500 mg, 1.26 mmol) in methanol (10 mL). A catalytic amount of acetic acid was added. The reaction mixture was flushed under hydrogen at atmospheric pressure for 1.5 h and then filtered over celite. The solvent was then removed in vacuum to afford **24** (320 mg, 96.7%) as a yellow oil which was taken to the next step without further purification. MS (ESI) *m/z* 263.19 $(M + H)^+$.

*4.1.7. General procedure C for the synthesis of compounds **25**–**33**, **37**–**49** and **55***

*4.1.7.1. 3-{5-tert-Butyl-2-[2-(morpholin-4-yl)ethoxy]phenyl}-3-phenyl-1,3-dihydro-2H-indol-2-one (**25**).* Triflic acid (790 μL, 8.89 mmol) was added to a stirred ice-cold solution of compound **1** (200 mg, 0.89 mmol) and compound **18** (280 mg, 1.07 mmol) in dichloromethane (10 mL) that was kept under nitrogen. After 1.5 h, the reaction mixture was quenched over several grams of ice. The aqueous phase was then extracted twice with dichloromethane and the combined organic phases were washed with brine and dried over anhydrous Na_2SO_4. After removal of the solvent in vacuum, the crude was purified by crystallization from EtOAc/Hexane to afford **25** (250 mg, 59.8%) as a white powder. Mp = 288.8–289.4 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.39 (s, 1H, NH), 7.31–7.09 (m, 8H, $CH_{arom.}$), 6.96–6.18 (m, 4H, $CH_{arom.}$), 3.90 (m, 1H, CH_2O), 3.66 (m, 1H, CH_2O), 3.50 (m, 4H, CH_2OCH_2), 2.32–2.24 (m, 6H, CH_2N, CH_2NCH_2), 1.11 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 179.89, 154.84, 143.00, 142.74, 140.19, 133.40, 131.21, 129.03 (2C), 128.58 (2C), 127.94, 127.58 (2C), 126.27, 125.63, 121.88, 113.11, 110.17, 66.93, 66.83 (2C), 60.62, 57.02, 54.06 (2C), 34.43, 31.85 (3C). HRMS (ESI) for $C_{30}H_{34}N_2O_3$: calculated $(M + H)^+$ = 471.26422, found $(M + H)^+$ = 471.26413.

*4.1.7.2. 3-{5-tert-Butyl-2-[2-(dimethylamino)ethoxy]phenyl}-3-phenyl-1,3-dihydro-2H-indol-2-one (**26**).* Triflic acid (790 μL, 8.89 mmol), compound **1** (200 mg, 0.89 mmol) and compound **19** (236 mg, 1.07 mmol) in dichloromethane (10 mL) were used following the **General procedure C** to afford **26** (260 mg, 68.3%) as a white powder. Mp = 161.7–162.7 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.38 (s, 1H, NH), 7.40–7.32 (m, 5H, $CH_{arom.}$), 7.23–7.21 (m, 2H, $CH_{arom.}$), 7.06 (m, 1H, $CH_{arom.}$), 6.95–6.83 (m, 3H, $CH_{arom.}$), 6.80 (s, 1H, $CH_{arom.}$), 3.85 (m, 1H, OCH_2), 3.60 (m, 1H, OCH_2), 2.29 (m, 1H, CH_2N), 2.13–2.09 (m, 7H, CH_2N, $N(CH_3)_2$), 1.11 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 179.39, 154.27, 142.49, 142.18, 139.41, 132.81, 130.86, 128.53 (2C), 127.95 (3C), 127.39, 127.00, 125.51, 125.01, 121.23, 112.67, 109.51, 66.74, 59.94, 56.98, 45.44 (2C), 33.84, 31.25 (3C). HRMS (ESI) for $C_{28}H_{32}N_2O_2$: calculated $(M + H)^+$ = 429.25365, found $(M + H)^+$ = 429.25347.

*4.1.7.3. 3-{5-tert-Butyl-2-[2-(piperazin-1-yl)ethoxy]phenyl}-3-phenyl-1,3-dihydro-2H-indol-2-one (**27**).* Triflic acid (690 μL, 7.77 mmol), compound **1** (175 mg, 0.78 mmol) and compound **24** (244 mg, 0.93 mmol) in dichloromethane (10 mL) were used following the **General procedure C** to afford **27** (146 mg, 40%) as a white powder. Mp = 228.1–229.3 °C. ^{1}H NMR (500 MHz, DMSO-

d_6): δ 10.40 (s, 1H, NH), 7.30–7.21 (m, 7H, $CH_{arom.}$), 7.11 (d, J = 7 Hz, 1H, $CH_{arom.}$), 6.95–6.86 (m, 3H, $CH_{arom.}$), 6.82 (s, 1H, $CH_{arom.}$), 3.87 (m, 1H, OCH_2), 3.60 (m, 1H, OCH_2), 2.60 (m, 4H, $C\underline{H_2}NHC\underline{H_2}$), 2.28 (m, 1H, CH_2N), 2.17–2.08 (m, 5H, CH_2N, CH_2NCH_2), 1.11 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 179.81, 154.86, 143.00, 142.61, 140.21, 133.41, 131.21, 128.96 (2C), 128.52 (3C), 127.84, 127.44, 126.33, 125.58, 121.83, 113.05, 110.08, 66.82, 60.61, 57.28, 54.97 (2C), 46.21 (2C), 34.41, 31.84 (3C). HRMS (ESI) for $C_{30}H_{35}N_3O_2$: calculated $(M + Na)^+$ = 492.26215, found $(M + Na)^+$ = 492.26211.

*4.1.7.4. 3-{5-tert-Butyl-2-[(prop-2-yn-1-yl)oxy]phenyl}-3-phenyl-1,3-dihydro-2H-indol-2-one (**28**).* Triflic acid (197 μL, 2.21 mmol), compound **1** (100 mg, 0.44 mmol) and compound **20** (100 mg, 0.53 mmol) in dichloromethane (5 mL) were used following the **General procedure C**. The crude was purified by flash chromatography (10–40% of ethyl acetate in cyclohexane) to afford **28** (98 mg, 55.8%) as a white powder. Mp = 252.4–253.0 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.42 (s, 1H, NH), 7.33–7.19 (m, 7H, $CH_{arom.}$), 7.02–6.89 (m, 4H, $CH_{arom.}$), 6.83 (s, 1H, $CH_{arom.}$), 4.42 (d, J = 16 Hz, 1H, OCH_2), 4.29 (d, J = 16 Hz, 1H, OCH_2), 3.37 (s, 1H, CH), 1.11 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 179.17, 153.27, 143.04, 142.44, 139.20, 132.43, 131.26, 128.52 (2C), 128.04 (3C), 127.42, 127.08, 125.49, 124.96, 121.21, 113.26, 109.56, 78.90, 77.88, 59.81, 56.19, 33.89, 31.18 (3C). HRMS (ESI) for $C_{27}H_{25}NO_2$: calculated $(M + H)^+$ = 396.19581, found $(M + H)^+$ = 396.19537.

*4.1.7.5. [4-tert-Butyl-2-(2-oxo-3-phenyl-2,3-dihydro-1H-indol-3-yl) phenoxy]acetic acid (**29**).* Triflic acid (395 μL, 4.44 mmol), compound **1** (200 mg, 0.89 mmol) and compound **21** (282 mg, 1.07 mmol) in dichloromethane (10 mL) were used following the **General procedure C** to afford **29** (166 mg, 45%) as a white powder. Mp = 303.5–304.8 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.77 (s, 1H, OH), 10.38 (s, 1H, NH), 7.33–7.15 (m, 8H, $CH_{arom.}$), 6.96–6.83 (m, 4H, $CH_{arom.}$), 4.26 (d, J = 16 Hz, 1H, OCH_2), 4.99 (d, J = 16 Hz, 1H, OCH_2), 1.12 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 178.97, 169.82, 153.95, 143.26, 142.31, 139.54, 132.47, 131.53, 128.26–127.87 (5C), 127.19, 126.81, 125.72, 125.04, 121.17, 114.19, 109.51, 66.36, 59.89, 33.84, 31.12 (3C). HRMS (ESI) for $C_{26}H_{25}NO_4$: calculated $(M + H)^+$ = 416.18563, found $(M + H)^+$ = 416.18509.

*4.1.7.6. 3-(5-tert-Butyl-2-hydroxyphenyl)-3-(4-methoxyphenyl)-1,3-dihydro-2H-indol-2-one (**30**).* Triflic acid (348 μL, 3.92 mmol), compound **2** (200 mg, 0.78 mmol) and *tert*-butylphenol (141 mg, 0.94 mmol) in dichloromethane (10 mL) were used following the **General procedure C**. The crude was purified by flash chromatography (10–50% of ethyl acetate in cyclohexane) to afford **30** (238 mg, 78.4%) as a white powder. Mp = 132.6–134.4 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.33 (s, 1H, NH), 9.21 (s, 1H, OH), 7.20 (m, 3H, $CH_{arom.}$), 7.09 (m, 1H, $CH_{arom.}$), 6.97–6.87 (m, 5H, $CH_{arom.}$), 6.79 (m, 1H, $CH_{arom.}$), 6.60 (d, J = 8.5 Hz, 1H, $CH_{arom.}$), 3.73 (s, 3H, OCH_3), 1.10 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 180.52, 159.01, 153.46, 143.07, 140.88, 133.73, 131.88, 130.21 (2C), 129.06, 128.31, 127.39, 126.04, 125.29, 121.67, 115.58, 113.92 (2C), 119.92, 59.91, 55.74, 34.30, 31.97 (3C). HRMS (ESI) for $C_{25}H_{25}NO_3$: calculated $(M + H)^+$ = 388.19072, found $(M + H)^+$ = 388.19170.

*4.1.7.7. 3-{5-tert-Butyl-2-[2-(morpholin-4-yl)ethoxy]phenyl}-3-(4-methoxyphenyl)-1,3-dihydro-2H-indol-2-one (**31**).* Triflic acid (350 μL, 3.94 mmol) compound **18** (247 mg, 0.94 mmol) and compound **2** (200 mg, 0.78 mmol) in dichloromethane (10 mL) were used following the **General procedure C**. The crude was purified by preparative HPLC on reverse phase (20–55% of acetonitrile in water) and the pure fractions were lyophilized to afford **31** (165 mg, 42.1%) as a white powder. Mp = 222.4–223.7 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.32 (s, 1H, NH), 7.21–7.19 (m, 4H, $CH_{arom.}$), 7.00–6.86 (m, 6H, $CH_{arom.}$), 6.83 (s, 1H, $CH_{arom.}$), 3.87 (m, 1H, OCH_2), 3.73 (m, 3H, OCH_3), 3.69 (m, 1H, OCH_2), 3.49 (m, 4H, CH_2OCH_2), 2.35 (m, 1H, CH_2N), 2.28–2.24 (m, 5H, CH_2N, CH_2NCH_2), 1.12 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 179.63, 158.47, 154.15 (2C), 142.42, 142.05, 133.02, 131.02, 130.81, 129.66, 127.76, 126.98, 125.36, 124.86, 121.08, 113.33 (2C), 112.38, 109.43, 66.43, 66.18 (2C), 59.14, 56.36, 55.15, 53.45 (2C), 33.79, 31.20 (3C). HRMS (ESI) for $C_{31}H_{36}N_2O_4$: calculated $(M + H)^+$ = 501.27478, found $(M + H)^+$ = 501.27420.

*4.1.7.8. 3-[5-tert-Butyl-2hydroxyphenyl)-3-[4-(dimethylamino) phenyl]-1,3-dihydro-2H-indol-2-one (**32**).* Triflic acid (330 μL, 3.71 mmol), compound **3** (200 mg, 0.75 mmol) and *tert*-butylphenol (134 mg, 0.89 mmol) in dichloromethane (10 mL) were used following the **General procedure C**. The crude was purified by flash chromatography (2–4% of methanol in dichloromethane) to afford **32** (124 mg, 41.5%) as a white powder. Mp = 149.9–151.2 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.24 (s, 1H, NH), 9.15 (s, 1H, OH), 7.16 (m, 1H, $CH_{arom.}$), 7.09–7.05 (m, 3H, $CH_{arom.}$), 6.91–6.84 (m, 4H, $CH_{arom.}$), 6.66 (m, 2H, $CH_{arom.}$), 6.57 (d, J = 8.5 Hz, 1H, $CH_{arom.}$), 2.86 (s, 6H, $N(CH_3)_2$), 1.11 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 180.27, 152.90, 149.50, 142.51, 140.18, 133.51, 129.16 (2C), 128.68, 127.46, 127.00, 126.25, 125.33, 124.49, 120.85, 114.86, 111.79 (2C), 109.16, 59.16, 40.15 (2C), 33.71, 31.42 (3C). HRMS (ESI) for $C_{26}H_{28}N_2O_2$: calculated $(M + H)^+$ = 401.22235, found $(M + H)^+$ = 401.22205.

*4.1.7.9. 3-{5-tert-Butyl-2-[2-(morpholin-4-yl)ethoxy]phenyl}-3-[4-(dimethylamino)phenyl]-1,3-dihydro-2H-indol-2-one (**33**).* Triflic acid (200 μL, 2.24 mmol), **3** (120 mg, 0.45 mmol) and compound **18** (141 mg, 0.54 mmol) in dichloromethane (5 mL) were used following the **General procedure C**. The crude was purified by flash chromatography (2–5% of methanol in dichloromethane) to afford **33** (176 mg, 76.6%) as a white powder. Mp = 245.1–245.9 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.21 (s, 1H, NH), 7.20–7.16 (m, 3H, $CH_{arom.}$), 7.08 (m, 1H, $CH_{arom.}$), 6.90–6.84 (m, 5H, $CH_{arom.}$), 6.66 (m, 2H, $CH_{arom.}$), 3.84 (m, 1H, OCH_2), 3.68 (m, 1H, OCH_2), 3.48 (m, 4H, CH_2OCH_2), 2.86 (s, 6H, $N(CH_3)_2$), 2.36 (m, 1H, CH_2N), 2.28–2.21 (m, 5H, CH_2N, CH_2NCH_2), 1.12 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 179.95, 154.20, 149.56, 142.49, 141.99, 133.39, 131.16, 129.27, 127.48, 127.25, 125.89, 125.12, 124.63, 120.81 (2C), 112.26, 111.73 (2C), 109.24, 66.51, 66.17 (2C), 58.92, 56.36, 53.45 (2C), 40.10 (2C), 33.78, 31.27 (3C). HRMS (ESI) for $C_{32}H_{39}N_3O_3$: calculated $(M + H)^+$ = 514.30642, found $(M + H)^+$ = 514.30572.

*4.1.8. General procedure D for the synthesis of compounds **34**–**36***

*4.1.8.1. 3-(5-tert-Butyl-2-hydroxyphenyl)-3-[4-(morpholin-4-ylmethyl)phenyl]-1,3-dihydro-2H-indol-2-one (**34**).*

4-*tert*-Butylphenol (225 mg, 1.50 mmol) and compound **4** (324 mg, 1 mmol) were dissolved in 1,2-dichloroethane (10 mL). The reaction mixture was heated to 85 °C and *p*-TsOH (323 mg, 1.70 mmol) was added. After stirring at 85 °C for 6 h, the solvent was removed in vacuum and the crude was purified by flash chromatography (0–20% of ethyl acetate in cyclohexane) to afford **34** (430 mg, 94.3%) as a white powder. Mp > 314 °C (decomp). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.40 (s, 1H, NH), 9.25 (s, 1H, OH), 7.46 (m, 2H, $CH_{arom.}$), 7.19 (m, 1H, $CH_{arom.}$), 7.09 (m, 2H, $CH_{arom.}$), 7.06 (dd, J = 2.0 Hz, J = 8.0 Hz, 1H, $CH_{arom.}$), 7.00 (m, 1H, $CH_{arom.}$), 6.93 (m, 1H, $CH_{arom.}$), 6.87 (d, J = 8.0 Hz, 1H, $CH_{arom.}$), 6.67 (d, J = 2.0 Hz, 1H, $CH_{arom.}$), 6.58 (d, J = 8.4 Hz, 1H, $CH_{arom.}$), 3.56 (m, 4H, CH_2OCH_2), 3.34 (s, 2H, CH_2), 2.27 (m, 4H, CH_2NCH_2), 1.06 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 180.08, 153.51, 145.51, 143.06, 140.90, 138.89, 133.36, 130.03, 129.06 (2C), 128.91, 128.48, 127.58, 126.22 (2C), 125.45, 121.81, 115.78, 110.15, 65.82 (2C), 61.58, 60.49, 52.99 (2C), 34.90,

31.90 (3C). HRMS (ESI) for $C_{29}H_{32}N_2O_3$: calculated $(M + H)^+ = 457.24857$, found $(M + H)^+ = 457.24982$.

*4.1.8.2. 3-(5-tert-Butyl-2-hydroxyphenyl)-3-(4-chloro-3-methylphenyl)-1,3-dihydro-2H-indol-2-one (**35**).*
4-*tert*-Butylphenol (225 mg, 1.50 mmol), intermediate **5** (273 mg, 1 mmol) and *p*-TsOH (323 mg, 1.70 mmol) in 1,2-dichloroethane (10 mL) at 85 °C were used following the **General procedure D**. The crude was purified by flash chromatography (0–20% of ethyl acetate in cyclohexane) to afford **35** (300 mg, 74%) as a white powder. Mp = 140.4–141.6 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 7.23–7.20 (m, 3H, $CH_{arom.}$), 7.15 (dd, J = 2.0 Hz, J = 8.4 Hz, 1H, $CH_{arom.}$), 7.06 (m, 2H, $CH_{arom.}$), 7.00–6.95 (m, 3H, $CH_{arom.}$), 6.69 (d, J = 8.4 Hz, 1H, $CH_{arom.}$), 2.23 (s, 3H, CH_3), 1.14 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (100.6 MHz, CD_3OD): δ 182.34, 153.04, 141.86, 141.81, 139.20, 135.57, 133.26, 133.12, 130.84 (2C), 128.52, 128.22, 127.18, 126.87, 126.05, 125.57, 122.16, 116.14, 110.12, 61.24, 33.83, 30.85 (3C), 19.15. HRMS (ESI) for $C_{25}H_{24}ClNO_2$: calculated $(M + H)^+ = 406.15683$, found $(M + H)^+ = 406.15685$.

*4.1.8.3. 3-(5-tert-Butyl-2-hydroxyphenyl)-3-(pyridin-3-yl)-1,3-dihydro-2H-indol-2-one (**36**).* 4-*tert*-Butylphenol (135 mg, 0.90 mmol), intermediate **6** (140 mg, 0.62 mmol) and *p*-TsOH (190 mg, 1 mmol) in 1,2-dichloroethane (10 mL) at 85 °C were used following the **General procedure D**. The crude was purified by flash chromatography (0–20% of methanol in dichloromethane) to afford **36** (30 mg, 14%) as a white powder. Mp = 134.9–136.1 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.58 (m, 1H, $CH_{arom.}$), 8.46 (s, 1H, $CH_{arom.}$), 8.00 (m, 1H, $CH_{arom.}$), 7.64 (m, 1H, $CH_{arom.}$), 7.42 (d, J = 7.6 Hz, 1H, $CH_{arom.}$), 7.31 (dd, J = 1.6 Hz, J = 8.0 Hz, 1H, $CH_{arom.}$), 7.21 (dd, J = 2.0 Hz, J = 8.8 Hz, 1H, $CH_{arom.}$), 7.11–7.02 (m, 3H, $CH_{arom.}$), 6.73 (d, J = 8.0 Hz, 1H, $CH_{arom.}$), 1.20 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 179.85, 154.30, 153.01, 144.95, 144.46, 142.20, 141.34, 140.38 (2C), 131.56, 128.96, 126.42, 126.06 (2C), 122.70 (2C), 116.10, 110.38, 59.95, 33.80, 30.68 (3C). HRMS (ESI) for $C_{23}H_{22}N_2O_2$: calculated $(M + H)^+ = 358.16813$, found $(M + H)^+ = 359.17612$.

*4.1.9. 3-{5-tert-Butyl-2-[2-(morpholin-4-yl)ethoxy]phenyl}-3-(3,4-dimethoxyphenyl)-1,3-dihydro-2H-indol-2-one (**37**)*

Triflic acid (234 μL, 2.63 mmol), compound **7** (150 mg, 0.53 mmol) and compound **18** (166 mg, 0.63 mmol) in dichloromethane (5 mL) were used following the **General procedure C**. The crude was purified by flash chromatography (2–8% of methanol in dichloromethane) to afford **37** (187 mg, 66.9%) as a white powder. Mp = 279.1–280.0 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.29 (s, 1H, NH), 7.21–7.19 (m, 2H, $CH_{arom.}$), 7.03–6.85 (m, 7H, $CH_{arom.}$), 6.61 (s, 1H, $CH_{arom.}$), 3.87 (m, 1H, CH_2O), 3.73–3.69 (m, 4H, CH_2O, OCH_3), 3.59 (s, 3H, OCH_3), 3.48 (m, 4H, CH_2OCH_2), 2.37 (m, 1H, CH_2N), 2.30–2.23 (m, 5H, CH_2N, CH_2NCH_2), 1.12 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 180.23, 154.80, 148.95, 148.76, 143.15, 142.69, 133.47, 131.83, 131.38, 128.41, 127.88, 125.89, 125.89, 125.45, 121.62, 121.32, 113.64, 112.93, 112.01, 110.08, 67.18, 66.81 (2C), 59.89, 56.97, 56.12 (2C), 54.08 (2C), 34.42, 31.87 (3C). HRMS (ESI) for $C_{32}H_{38}N_2O_5$: calculated $(M + H)^+ = 531.28535$, found $(M + H)^+ = 531.28571$.

*4.1.10. 3-{5-tert-Butyl-2-[2-(morpholin-4-yl)ethoxy]phenyl}-3-(3-fluoro-4-methoxyphenyl)-1,3-dihydro-2H-indol-2-one (**38**)*

Triflic acid (244 μL, 2.75 mmol), compound **8** (150 mg, 0.55 mmol) and compound **18** (173 mg, 0.66 mmol) in dichloromethane (5 mL) were used following the **General procedure C** to afford **38** (98 mg, 34.4%) as a white powder. Mp = 196.9–198.2 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.45 (s, 1H, NH), 7.26–7.23 (m, 2H, $CH_{arom.}$), 7.13 (m, 1H, $CH_{arom.}$), 7.05 (m, 2H, $CH_{arom.}$), 6.98–6.89 (m, 4H, $CH_{arom.}$), 6.83 (s, 1H, $CH_{arom.}$), 3.96 (m, 1H, OCH_2), 3.82–3.77 (m, 4H, OCH_2, OCH_3), 3.55 (m, 4H, CH_2OCH_2), 3.34 (m, 2H, CH_2N), 2.35 (m, 4H, CH_2NCH_2), 1.12 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (75.47 MHz, DMSO-d_6): δ 179.14, 153.76, 152.46, 149.25, 146.40, 142.17, 132.23, 130.22, 128.07, 126.71, 125.41, 125.06, 124.45, 121.32, 115.98, 115.72, 113.17, 112.62, 109.58, 65.55 (3C), 58.91, 56.02 (2C), 52.95 (2C), 33.84, 30.99 (3C). HRMS (ESI) for $C_{31}H_{35}FN_2O_4$: calculated $(M + H)^+ = 519.26536$, found $(M + H)^+ = 519.26711$.

*4.1.11. 3-(5-tert-Butyl-2-hydroxyphenyl)-5-iodo-3-phenyl)-1,3-dihydro-2H-indol-2-one (**39**)*

Triflic acid (2.15 mL, 24.22 mmol), compound **9** (1.70 g, 4.84 mmol) and 4-*tert*-butylphenol (0.87 g, 5.81 mmol) in dichloromethane (100 mL) were used following the **General procedure C**. The crude was purified by flash chromatography (10–70% of ethyl acetate in cyclohexane) to afford **39** (1.44 g, 61.5%) as a white powder. Mp = 264.2–265.6 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.56 (s, 1H, NH), 9.38 (s, 1H, OH), 7.56 (d, J = 7.2 Hz, 1H, $CH_{arom.}$), 7.33–7.29 (m, 6H, $CH_{arom.}$), 7.12 (d, J = 7.2 Hz, 1H, $CH_{arom.}$), 6.77 (m, 2H, $CH_{arom.}$), 6.66 (d, J = 8 Hz, 1H, $CH_{arom.}$), 1.11 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 178.73, 152.76, 142.35, 140.39, 138.79, 136.42, 135.70, 133.38, 128.25 (2C), 128.05 (2C), 127.62, 127.37, 126.71, 125.17, 115.09, 111.88, 83.87, 60.04, 33.69, 31.28 (3C). HRMS (ESI) for $C_{24}H_{22}INO_2$: calculated $(M + H)^+ = 484.07680$, found $(M + H)^+ = 484.07523$.

*4.1.12. 3-(5-tert-Butyl-2-hydroxyphenyl)-3-phenyl-5-(trifluoromethoxy)-1,3-dihydro-2H-indol-2-one (**40**)*

Triflic acid (430 μL, 4.84 mmol), compound **10** (300 mg, 0.97 mmol) and 4-*tert*-butylphenol (175 mg, 1.17 mmol) in dichloromethane (10 mL) were used following the **General procedure C**. The crude was purified by flash chromatography (15–35% of ethyl acetate in cyclohexane) to afford **40** (175 mg, 40.9%) as a white powder. Mp = 278.5–279.1 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.64 (s, 1H, NH), 9.38 (s, 1H, OH), 7.36–7.31 (m, 3H, $CH_{arom.}$), 7.26–7.23 (m, 3H, $CH_{arom.}$), 7.13 (d, J = 8.5 Hz, 1H, $CH_{arom.}$), 7.09 (s, 1H, $CH_{arom.}$), 6.97 (d, J = 8.5 Hz, 1H, $CH_{arom.}$), 6.80 (s, 1H, $CH_{arom.}$), 6.64 (d, J = 8.5 Hz, 1H, $CH_{arom.}$), 1.11 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 179.27, 152.82, 142.83, 141.68, 140.43, 138.81, 134.67, 128.10 (2C), 128.08 (2C), 127.39, 127.31, 126.66, 125.19, 121.93, 121.02, 119.04, 115.14, 110.06, 60.45, 33.69, 31.25 (3C). HRMS (ESI) for $C_{25}H_{22}F_3NO_3$: calculated $(M + H)^+ = 442.16245$, found $(M + H)^+ = 442.16175$.

*4.1.13. 8-(5-tert-Butyl-2-hydroxyphenyl)-8-phenyl-6,8-dihydro-7H-[1,3]thiazolo[5,4-e]indol-7-one (**41**)*

Triflic acid (66 μL, 0.74 mmol), compound **11** (42 mg, 0.15 mmol) and 4-*tert*-butylphenol (27 mg, 0.18 mmol) in dichloromethane (5 mL) were used following the **General Procedure C**. The crude was purified by preparative HPLC on reverse phase (20–65% of acetonitrile in water) to afford **41** (21 mg, 34%) as a white powder. Mp > 300 °C (decomp). ^{1}H NMR (400 MHz, MeOD): δ 8.91 (s, 1H, N=CH–S), 8.02 (d, J = 8.8 Hz, 1H, $CH_{arom.}$), 7.32–7.30 (m, 5H, $CH_{arom.}$), 7.26 (d, J = 8.8 Hz, 1H, $CH_{arom.}$), 7.16 (dd, J = 2.8 Hz, J = 8.4 Hz, 1H, $CH_{arom.}$), 7.00 (s, 1H, $CH_{arom.}$), 6.63 (d, J = 8 Hz, 1H, $CH_{arom.}$), 1.17 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (100.6 MHz, MeOD): δ 182.65, 153.49, 153.40, 149.79, 141.46, 140.46, 137.63, 130.66, 128.59, 128.18 (2C), 127.89 (2C), 125.57–125.28 (4C), 122.65, 115.19, 109.55, 61.37, 33.77, 30.66 (3C). HRMS (ESI) for $C_{25}H_{22}N_2O_2S$: calculated $(M + H)^+ = 415.14748$, found $(M + H)^+ = 415.14738$.

*4.1.14. 3-(5-tert-Butyl-2-hydroxyphenyl)-6,7-difluoro-3-(4-methoxyphenyl)-1,3-dihydro-2H-indol-2-one (**42**)*

Triflic acid (190 μL, 2.14 mmol), compound **12** (125 mg, 0.43 mmol) and 4-*tert*-butylphenol (77 mg, 0.51 mmol) in dichloromethane (5 mL) were used following the **General procedure C** to afford **42** (86 mg, 47.3%) as a white powder. Mp = 182.8–

183.4 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.06 (s, 1H, NH), 9.35 (s, 1H, OH), 7.21 (m, 2H, $CH_{arom.}$), 7.10 (dd, J = 2 Hz, J = 8 Hz, 1H, $CH_{arom.}$), 6.94–6.92 (m, 3H, $CH_{arom.}$), 6.75 (s, 2H, $CH_{arom.}$), 6.60 (d, J = 8 Hz, 1H, $CH_{arom.}$), 3.75 (s, 3H, OCH_3), 1.10 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 179.73, 158.69, 152.69, 148.59, 140.44, 134.14, 131.51, 130.88, 129.97, 129.68, 128.00, 126.73, 125.06, 121.26, 114.82, 113.50 (2C), 108.55, 108.40, 59.06, 54.98, 33.57, 31.20 (3C). HRMS (ESI) for $C_{25}H_{23}F_2NO_3$: calculated $(M + H)^+$ = 424.17188, found $(M + H)^+$ = 424.17208.

4.1.15. 3-(5-tert-Butyl-2-hydroxyphenyl)-5-methoxy-3-(4-methoxyphenyl)-1,3-dihydro-2H-indol-2-one (**43**)

Triflic acid (310 μL, 3.49 mmol), compound **13** (200 mg, 0.70 mmol) and 4-*tert*-butylphenol (126 mg, 0.84 mmol) in dichloromethane (10 mL) were used following the **General procedure C**. The crude was purified by flash chromatography (10–45% of ethyl acetate in cyclohexane) to afford **43** (181 mg, 61.8%) as a white powder. Mp = 140.3–151.9 °C (decomp.). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.22 (s, 1H, NH), 9.23 (s, 1H, OH), 7.18 (m, 2H, $CH_{arom.}$), 7.10 (dd, J = 2.5 Hz, J = 8.5 Hz, 1H, $CH_{arom.}$), 6.90–6.79 (m, 5H, $CH_{arom.}$), 6.63–6.60 (m, 2H, $CH_{arom.}$), 3.72 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3), 1.11 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 179.63, 158.28, 154.42, 152.88, 140.26, 135.81, 134.60, 131.38, 129.35 (2C), 128.16, 126.61, 124.70, 115.16, 113.28 (2C), 112.91, 111.76, 109.47, 59.90, 55.38, 55.05, 33.66, 31.31 (3C). HRMS (ESI) for $C_{26}H_{27}NO_4$: calculated $(M + H)^+$ = 418.20128, found $(M + H)^+$ = 418.20064.

4.1.16. 3-{5-tert-Butyl-2-[2-(morpholin-4-yl)ethoxy]phenyl}-5-methoxy-3-(4-methoxyphenyl)-1,3-dihydro-2H-indol-2-one (**44**)

Triflic acid (235 μL, 2.64 mmol), compound **13** (150 mg, 0.53 mmol) and compound **18** (166 mg, 0.63 mmol) in dichloromethane (5 mL) were used following the **General procedure C**. The crude was purified by flash chromatography (0–10% of methanol in ethyl acetate) to afford **44** (108 mg, 37.2%) as a white powder. Mp = 255.8–256.9 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.17 (s, 1H, NH), 7.22–7.16 (m, 3H, $CH_{arom.}$), 6.90–6.79 (m, 6H, $CH_{arom.}$), 6.60 (s, 1H, $CH_{arom.}$), 3.88 (m, 1H, OCH_2), 3.72 (s, 3H, OCH_3), 3.70 (m, 1H, OCH_2), 3.65 (s, 3H, OCH_3), 3.32 (m, 4H, CH_2OCH_2), 2.34 (m, 1H, CH_2N), 2.27–2.22 (m, 5H, CH_2N, CH_2NCH_2), 1.12 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 179.34, 158.42, 154.47, 154.22, 142.08, 135.85, 134.47, 131.21, 130.66, 129.51 (2C), 126.91, 124.92, 113.32 (2C), 112.93, 112.51, 111.72, 109.63, 66.44, 66.13 (2C), 59.72, 56.43, 55.43, 55.12, 53.47 (2C), 33.80, 31.23 (3C). HRMS (ESI) for $C_{32}H_{38}N_2O_5$: calculated $(M + H)^+$ = 553.26729, found $(M + H)^+$ = 553.26739.

4.1.17. Methyl 3-(5-tert-butyl-2-hydroxyphenyl)-3-(4-methoxyphenyl)-2-oxoindoline-7-carboxylate (**45**)

Triflic acid (430 μL, 4.84 mmol), compound **14** (300 mg, 0.96 mmol) and 4-*tert*-butylphenol (172 mg, 1.15 mmol) in dichloromethane (10 mL) were used following the **General procedure C** to afford **45** (280 mg, 65.6%) as a white powder. Mp = 279.1–280.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.18 (s, 1H, NH), 9.32 (s, 1H, OH), 7.74 (d, J = 8 Hz, 1H, $CH_{arom.}$), 7.23–7.04 (m, 5H, $CH_{arom.}$), 6.94–6.92 (m, 2H, $CH_{arom.}$), 6.78 (s, 1H, $CH_{arom.}$), 6.59 (d, J = 8 Hz, 1H, $CH_{arom.}$), 3.86 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 1.10 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 179.74, 165.73, 158.62, 152.62, 143.50, 140.35, 134.82, 129.97–129.74 (4C), 128.45, 128.01, 126.83, 124.92, 120.96, 114.71, 113.46 (2C), 110.87, 58.09, 55.12, 52.03, 33.68, 31.31 (3C). HRMS (ESI) for $C_{27}H_{27}NO_5$: calculated $(M + H)^+$ = 446.19620, found $(M + H)^+$ = 446.19531.

4.1.18. Methyl 3-{5-tert-butyl-2-[2-(morpholin-4-yl)ethoxy]phenyl}-3-(4-methoxyphenyl)-2-oxoindoline-7-carboxylate (**46**)

Triflic acid (430 μL, 4.84 mmol), compound **14** (300 mg, 0.96 mmol) and compound **18** (302 mg, 1.15 mmol) in dichloromethane (10 mL) were used following the **General procedure C**. The crude was purified by preparative HPLC on reverse phase (50–90% of acetonitrile in water) and the pure fractions were lyophilized to afford **46** (180 mg, 33.6%) as a white powder. Mp = 207.2–208.1 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.17 (s, 1H, NH), 7.76 (d, J = 7.8 Hz, 1H, $CH_{arom.}$), 7.25–7.04 (m, 5H, $CH_{arom.}$), 6.95–6.82 (m, 4H, $CH_{arom.}$), 3.87–3.84 (m, 4H, OCH_2, OCH_3), 3.74–3.69 (m, 4H, OCH_2, OCH_3), 3.46 (m, 4H, CH_2OCH_2), 2.35–2.22 (m, 6H, CH_2N, CH_2NCH_2), 1.12 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (75.47 MHz, DMSO-d_6): δ 179.61, 165.67, 158.69, 153.88, 143.40, 142.13, 134.58, 130.07, 129.70 (2C), 129.61, 128.46, 126.96, 125.09, 120.99, 113.49 (2C), 112.07, 111.08, 66.07, 65.99 (3C), 57.95, 56.32, 55.11, 53.29 (2C), 51.95, 33.72, 31.13 (3C). HRMS (ESI) for $C_{33}H_{38}N_2O_6$: calculated $(M + H)^+$ = 559.28026, found $(M + H)^+$ = 559.27958.

4.1.19. 3-{5-tert-Butyl-2-[2-(morpholin-4-yl)ethoxy]phenyl}-5-iodo-3-(4-methoxyphenyl)-1,3-dihydro-2H-indol-2-one (**47**)

Triflic acid (175 μL, 1.97 mmol), compound **15** (150 mg, 0.39 mmol) and compound **18** (124 mg, 0.47 mmol) in dichloromethane (5 mL) were used following the **General procedure C** to afford **47** (122 mg, 49.5%) as a white powder. Mp = 274.2–275.8 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.48 (s, 1H, NH), 7.56 (d, J = 7.5 Hz, 1H, $CH_{arom.}$), 7.25–7.15 (m, 4H, $CH_{arom.}$), 6.93–6.90 (m, 3H, $CH_{arom.}$), 6.80 (s, 1H, $CH_{arom.}$), 6.75 (d, J = 7.5 Hz, 1H, $CH_{arom.}$), 3.87 (m, 1H, CH_2O), 3.77–3.63 (m, 4H, CH_2O, OCH_3), 3.50 (m, 4H, CH_2OCH_2), 2.31–2.23 (m, 6H, CH_2N, CH_2NCH_2), 1.12 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 178.96, 158.62, 154.03, 142.23, 142.22, 136.49, 135.82, 133.08, 130.23, 129.96, 129.50 (2C), 127.01, 125.25, 113.53 (2C), 112.51, 112.03, 83.76, 66.44, 66.13 (2C), 59.20, 56.36, 55.15, 53.41 (2C), 33.80, 31.20 (3C). HRMS (ESI) for $C_{31}H_{35}IN_2O_4$: calculated $(M + H)^+$ = 627.17143, found $(M + H)^+$ = 627.17127.

4.1.20. 3-{5-tert-Butyl-2-[2-(morpholin-4-yl)ethoxy]phenyl}-3-(4-methoxyphenyl)-5-(trifluoromethoxy)-1,3-dihydro-2H-indol-2-one (**48**)

Triflic acid (393 μL, 4.39 mmol), compound **16** (300 mg, 0.88 mmol) and compound **18** (279 mg, 1.06 mmol) in dichloromethane (10 mL) were used following the **General procedure C** to afford **48** (160 mg, 31%) as a white powder. Mp = decomp. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.82 (s, 1H, NH), 7.41–6.80 (m, 10H, $CH_{arom.}$), 4.20 (m, 2H, OCH_2), 3.85–3.59 (m, 7H, CH_2OCH_2, OCH_3), 3.23–2.78 (m, 6H, CH_2N, CH_2NCH_2), 1.10 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 180.63, 159.64, 153.80, 144.22, 143.64, 142.11, 135.23, 131.15–130.64 (4C), 128.09, 126.16, 122.07 (2C), 119.55, 114.33 (2C), 114.16, 111.46, 64.27, 63.82 (2C), 59.77, 55.73 (2C), 55.46, 52.13, 34.16, 31.46 (3C). HRMS (ESI) for $C_{32}H_{35}F_3N_2O_5$: calculated $(M + H)^+$ = 585.25708, found $(M + H)^+$ = 585.25694.

4.1.21. 3-{5-tert-Butyl-2-[2-(morpholin-4-yl)ethoxy]phenyl}-3-[4-(dimethylamino)phenyl]-6,7-difluoro-1,3-dihydro-2H-indol-2-one (**49**)

Triflic acid (150 μL, 1.69 mmol), compound **17** (100 mg, 0.33 mmol) and compound **18** (104 mg, 0.39 mmol) in dichloromethane (5 mL) were used following the **General procedure C.** The crude was purified by preparative HPLC on reverse phase (50–70% of acetonitrile in water) and the pure fractions were lyophilized to afford **49** (145 mg, 80.3%) as a white powder. Mp = 207.8–208.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.99 (s, 1H, NH), 7.20 (m, 1H, $CH_{arom.}$), 7.06 (m, 1H, $CH_{arom.}$), 6.92–6.94 (m, 4H, $CH_{arom.}$), 6.68–6.64 (m, 3H, $CH_{arom.}$), 3.81 (m, 2H, OCH_2), 3.47 (m, 4H, CH_2OCH_2), 2.86 (s, 6H, $N(CH_3)_2$), 2.36 (m, 2H, CH_2N), 2.22 (m, 4H, CH_2NCH_2), 1.09 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 179.87, 153.95, 149.73, 148.43, 142.08, 134.35, 132.07–131.04 (3C), 130.20 (2C), 129.18, 128.76, 127.16, 125.02, 124.43, 120.92 (2C), 111.99, 111.78, 108.30, 108.15, 66.01 (2C), 58.72, 56.34 (2C), 53.34 (2C),

33.76, 31.20 (3C). HRMS (ESI) for $C_{32}H_{37}F_2N_3O_3$: calculated $(M + H)^+ = 550.28757$, found $(M + H)^+ = 550.28718$.

4.1.22. General procedure E for the synthesis of compounds **50** and **51**

*4.1.22.1. 3-(5-tert-Butyl-2-hydroxyphenyl)-3-(4-hydroxyphenyl)-1,3-dihydro-2H-indol-2-one (**50**).* To a solution of boron tribromide methyl sulfide complex (323 mg, 1.03 mmol) in dichloroethane (10 mL) kept under nitrogen was added compound **30** (100 mg, 0.26 mmol). The reaction mixture was stirred at reflux under nitrogen overnight. When the starting material was totally consumed, the reaction mixture was cooled to room temperature, hydrolyzed by adding approx. 10 ml of water, and stirred for 20 min. The aqueous phase was extracted twice by dichloromethane (2 × 30 ml). The combined extracts were washed with 1 M $NaHCO_3$, dried over Na_2SO_4 and concentrated in vacuum. The resulting residue was purified by flash chromatography (5–55% of ethyl acetate in cyclohexane) to afford **50** (54 mg, 56% yield) as a white powder. Mp = 178.9–181.6 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.28 (s, 1H, NH), 9.39 (s, 1H, OH), 9.17 (s, 1H, OH), 7.17 (m, 1H, $CH_{arom.}$), 7.11–7.06 (m, 3H, $CH_{arom.}$), 6.93 (m, 2H, $CH_{arom.}$), 6.85 (d, J = 8 Hz, 1H, $CH_{arom.}$), 6.78 (s, 1H, $CH_{arom.}$), 6.70 (m, 2H, $CH_{arom.}$), 6.57 (d, J = 8 Hz, 1H, $CH_{arom.}$), 1.11 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 180.16, 156.53, 152.86, 142.54, 140.26, 133.33, 129.71 (2C), 129.37, 128.74, 127.62, 126.98, 125.45, 124.61, 121.00, 114.90, 114.77 (2C), 109.28, 59.22, 33.75, 31.42 (3C). HRMS (ESI) for $C_{24}H_{23}NO_3$: calculated $(M + H)^+ = 374.17507$, found $(M + H)^+ = 374.17507$.

*4.1.22.2. 3-(5-tert-Butyl-2-hydroxyphenyl)-6,7-difluoro-3-(4-hydroxyphenyl)-1,3-dihydro-2H-indol-2-one (**51**).* Boron tribromide methyl sulfide complex (250 mg, 0.80 mmol) and compound **42** (85 mg, 0.20 mmol) in dichloroethane (10 mL) were used following the **General procedure E**. The crude was purified by flash chromatography (10–30% of ethyl acetate in cyclohexane) to afford **51** (40 mg, 48.7%) as a white powder. Mp = 208.3–210.2 °C. ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.02 (s, 1H, NH), 9.52 (s, 1H, OH), 9.34 (s, 1H, OH), 7.11–7.07 (m, 3H, $CH_{arom.}$), 6.90 (m, 1H, $CH_{arom.}$), 6.75–6.68 (m, 4H, $CH_{arom.}$), 6.58 (d, J = 8.5 Hz, 1H, $CH_{arom.}$), 1.09 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 179.90, 156.80, 152.65, 148.52, 140.38, 134.11, 131.05, 131.03, 129.72, 128.23, 128.07, 126.87, 124.90, 121.18, 114.91 (2C), 114.71, 108.43, 108.29, 58.95, 33.71, 31.34 (3C). HRMS (ESI) for $C_{24}H_{21}F_2NO_3$: calculated $(M + H)^+$ = 41 0.15623, found $(M + H)^+ = 410.15582$.

4.1.23. 3-(5-tert-Butyl-2-hydroxyphenyl)-5-[6-(morpholin-4-yl) pyridin-3-yl]-3-phenyl-1,3-dihydro-2H-indol-2-one (**52**)

To a degassed solution of **39** (300 mg, 0.62 mmol), 6-(morpholin-4-yl)pyridin-3-boronic acid pinacol ester (217 mg, 0.75 mmol), and $Pd(PPh_3)_2Cl_2$ (72 mg, 0.06 mmol) in THF (6 mL) was added 2 M Na_2CO_3 solution (6.2 mL). The reaction mixture was refluxed under nitrogen for 30 min, cooled to room temperature, and concentrated in vacuum to remove most of the solvent. The formed residue was partitioned between Et_2O (30 mL) and H_2O (15 mL), and the whole was stirred for 30 min. The organic layer was separated, and the aqueous layer was extracted with Et_2O (2 × 20 mL). The combined extracts were dried over Na_2SO_4 and concentrated in vacuum. The resulting residue was purified by flash chromatography (50–60% of ethyl acetate in cyclohexane) to afford **52** (83 mg, 25.7% yield) as a white powder. Mp > 205 °C(decomp). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.53 (s, 1H, NH), 9.33 (s, 1H, OH), 8.32 (s, 1H, $CH_{arom.}$), 7.73 (d, J = 8.4 Hz, 1H, $CH_{arom.}$), 7.46 (d, J = 8 Hz, 1H, $CH_{arom.}$), 7.35–7.28 (m, 6H, $CH_{arom.}$), 7.09 (d, J = 8.4 Hz, 1H, $CH_{arom.}$), 6.96 (d, J = 8 Hz, 1H, $CH_{arom.}$), 6.86–6.84 (m, 2H, $CH_{arom.}$), 6.62 (d, J = 8.4 Hz, 1H, $CH_{arom.}$), 3.69 (m, 4H, CH_2OCH_2), 3.43 (m, 4H, CH_2NCH_2), 1.10 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ 179.42, 158.11, 152.87, 144.94, 141.52, 140.33, 139.45, 135.48, 133.91, 130.87, 128.38 (2C), 128.09, 127.99 (2C), 127.14, 126.76, 125.85, 125.53, 124.86, 123.03, 115.20, 109.83, 107.02, 65.94 (2C), 60.37, 45.24 (2C), 33.68, 31.30 (3C). HRMS (ESI) for $C_{33}H_{33}N_3O_3$: calculated $(M + H)^+ = 520.25947$, found $(M + H)^+ = 520.25935$.

4.1.24. 4-(Propan-2-yloxy)phenol (**53**)

A solution of KOH (2.64 g, 47.06 mmol) in water (40 mL) was added to a mixture of hydroquinone (2.85 g, 25.88 mmol) and 2-iodopropane (4 g, 23.53 mmol) in ethanol (50 mL). After 16 h at reflux, the dark brown reaction mixture was cooled down and filtered. After removal of ethanol in vacuum, the resulting aqueous phase was acidified with 2 N HCl and then extracted twice with ethyl acetate (2 × 60 mL). The organic phase was washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed in vacuum and the crude was purified by flash chromatography (0–20% of ethyl acetate in cyclohexane) to afford **53** (1.25 g, 35%) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.84–6.75 (m, 4H, $CH_{arom.}$), 6.60 (s, 1H, OH), 4.41 (m, 1H, $C\underline{H}(CH_3)_2$), 1.35–1.30 (m, 6H, $CH(C\underline{H}_3)_2$). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 151.32, 150.03, 118.25 (2C), 116.27 (2C), 72.00, 22.13 (2C). MS (ESI) 153.13 $(M + H)^+$.

4.1.25. 4-{2-[4-(Propan-2-yloxy)phenoxy]ethyl}morpholine (**54**)

A solution of KOH (0.74 g, 13.16 mmol) in water (10 mL) was added to a mixture of compound **53** (1 g, 6.58 mmol) and 4-(2-chloroethyl)morpholine hydrochloride (1.47 g, 7.89 mmol) in ethanol (20 mL). After 16 h at reflux, the reaction mixture was cooled down, ethanol was removed in vacuum and the resulting aqueous phase was extracted twice with ethyl acetate (2 × 30 mL). The organic phase was washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed in vacuum and the crude was purified by flash chromatography (20–80% of ethyl acetate in cyclohexane) to afford **54** (0.94 g, 54%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.77–6.75 (m, 4H, $CH_{arom.}$), 4.34 (m, 1H, $C\underline{H}(CH_3)_2$), 3.98 (t, J = 6 Hz, 2H, OCH_2), 3.66 (m, 4H, CH_2OCH_2), 2.70 (t, J = 6 Hz, 2H, CH_2N), 2.49 (m, 4H, CH_2NCH_2), 1.25–1.21 (m, 6H, $CH(C\underline{H}_3)_2$). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 152.74, 151.88, 117.18 (2C), 115.34 (2C), 70.59, 66.72 (2C), 66.10, 57.62, 53.93 (2C), 21.99 (2C). MS (ESI) 266.19 $(M + H)^+$.

4.1.26. 3-(4-Methoxyphenyl)-3-{2-[2-(morpholin-4-yl)ethoxy]-5-(propan-2-yloxy)phenyl}-1,3-dihydro-2H-indol-2-one (**55**)

Triflic acid (260 μL, 2.93 mmol), compound **2** (150 mg, 0.59 mmol) and compound **54** (187 mg, 0.71 mmol) in dichloromethane (5 mL) were used following the **General procedure C**. The crude was purified by flash chromatography on reverse phase (20–60% of acetonitrile in water) and the pure fractions were lyophilized to afford **55** (108 mg, 36.6%) as a white powder. Mp = 139.3–149.9 °C (decomp). ^{1}H NMR (500 MHz, MeOD): δ 7.27–7.24 (m, 2H, $CH_{arom.}$), 7.00–6.80 (m, 8H, $CH_{arom.}$), 6.53 (m, 1H, $CH_{arom.}$), 4.42 (m, 1H, $C\underline{H}(CH_3)_2$), 4.16 (m, 2H, OCH_2), 3.87 (m, 4H, CH_2OCH_2), 3.78 (s, 3H, OCH_3), 3.42 (m, 2H, CH_2N), 3.24 (m, 4H, CH_2NCH_2), 1.19 (d, J = 6 Hz, 3H, $CHC\underline{H}_3$), 0.60 (d, J = 6 Hz, 3H, $CHC\underline{H}_3$). ^{13}C NMR (100.6 MHz, MeOD): δ 183.32, 160.75, 152.16, 151.36, 143.92, 134.73, 131.32, 129.06 (2C), 126.57 (2C), 122.67, 119.74, 114.33–113.93 (4C), 110.81, 70.78, 65.12 (2C), 63.4ziegeler1, 60.97, 57.68, 55.73 (2C), 53.62 (2C), 21.75, 20.75. HRMS (ESI) for $C_{30}H_{34}N_2O_5$: calculated $(M + H)^+ = 503.25405$, found $(M + H)^+ = 503.25309$.

4.2. Biological evaluation

4.2.1. Cell growth assay

Adherent mouse (KLN) and human solid tumor cells (CRL-2351 and CRL-2813) were plated in 96-well plates, allowed to settle overnight, and maintained for 5 days in the presence of 0.54–

20 μM of individual compound, and cell proliferation was measured by the sulforhodamine B (SRB) assay as described [18]: briefly, cells were fixed in 10% cold trichloroacetic acid at 4 °C for 1 h, extensively washed with double-distilled H_2O and air-dried. Plates were then incubated with 0.4% SRB in 1% acetic acid for 1 h, washed with 1% acetic acid to remove the unbound dye, and air-dried. The bound dye was solubilized by addition of 10 mM Tris (pH 10), and the absorbance was determined in a Titertek Multiscan plate reader at 490 nm. The data calculations were carried out as described (the values for mean ± SD of data from replicate wells are calculated): data are expressed in terms of %T/C [(OD of treated cells/OD of control cells) × 100], as a measure of cell viability and survival in the presence of test materials. Calculations are also made for the concentration of test agents giving a T/C value of 50%, or 50% growth inhibition (IC_{50}). With the SRB assay, a measure is made of the cell population density at time 0 (the time at which drugs are added) from two extra reference plates of inoculated cells fixed with TCA at the time of drug addition to the test plates. Thus, we have three measurements: control optical density (C), test optical density (T), and optical density at time zero (T_0). The calculation is $100 \times [(T - T_0)/(C - T_0)]$. If T is less than T_0, cell killing has occurred and can be calculated from $100 \times [(T - T_0)/T_0]$. Thus, for each drug-cell line combination, a dose–response curve is generated and three levels of effect are calculated.

4.2.2. Plasmids and ternary complex assay

The dual luciferase expression vector and other plasmids used for these studies are described in Ref. [39]. The TC assay has been described elsewhere [24]. Briefly, we modified bi-directional mammalian expression vector pBI (Clontech, CA) to expand the multiple cloning sites (MCSs) and designated it thereafter as pBISA. This vector contains seven copies of the tetracycline-regulated transactivator response element (TRE), which together act as core promoter/enhancer. The TRE is flanked on both sides by minimal human cytomegalovirus (CMV) minimal promoters allowing bi-directional transcription and two MCSs. Firefly and renilla luciferases were subcloned into MCS-I and MCS-II, respectively. This plasmid, designated pBISA-DL, transcribes two mRNAs that contain the 90 nucleotide plasmid derived 5′UTR (same sequence in both mRNAs), and the ORF encoding either firefly or renilla luciferase followed by a polyadenylation sequence. This plasmid was further modified by inserting the 5′UTR of ATF-4 into MCS-I in front of the firefly luciferase mRNA. Transcription from this direction generates an mRNA that contains the firefly luciferase ORF preceded by a 5′UTR composed of 90 nucleotides derived from the plasmid and 267 nucleotides derived from the 5′UTR of ATF-4 mRNA. Transcription from the other direction generates an mRNA that contains the renilla luciferase ORF proceeded only by the 90-nucleotide plasmid-derived sequence in the 5′UTR. This expression plasmid is called pBISA-DL(ATF-4).

4.2.3. Dual luciferase assay

Cells expressing firefly and renilla luciferases were treated with compounds in 96-well plates overnight and assayed with a long-half-life glow type dual luciferase assay kit, per manufacturer's instruction (Promega Inc., Madison, WI). The data calculations were carried out as the ratio of firefly to renilla luciferase signal all corrected for the same ratio in DMSO treated wells on the same plate.

4.2.4. Real time PCR

Total RNA was extracted with TaqMan Gene Expression Cells-to-Ct Kit (Applied Biosystems) and treated with DNase I according to manufacturer's recommendations. One-step real-time PCR was performed on a Bio-Rad iCycler IQ5 system by using B-R 1-Step SYBR Green qRT-PCR Kit (Quanta BioSciences) according to manufacturer specifications. The thermal cycler conditions and the primers used are detailed under Supplementary Methods and in Ref. [41]. All PCRs were performed in triplicate in at least two independent PCR runs. Mean values of these repeated measurements were used for calculation. To calibrate the results, all the transcript quantities were normalized to 18S rRNA.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2013.08.030. These data include MOL files and InChiKeys of the most important compounds described in this article.

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