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Preclinical Activity of New [1,2]Oxazolo[5,4-*e*]isoindole Derivatives in Diffuse Malignant Peritoneal Mesothelioma

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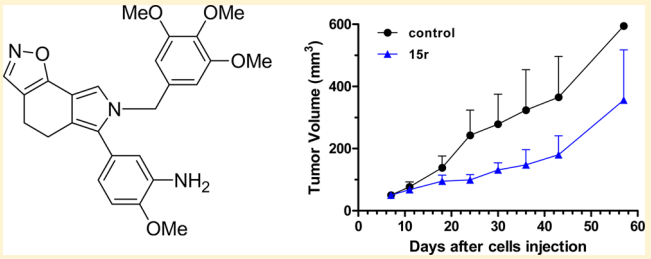
Supporting Information

ABSTRACT: A series of 22 derivatives of the [1,2]oxazolo-[5,4-*e*]isoindole system were synthesized through an efficient and versatile procedure that involves the annelation of the [1,2]oxazole moiety to the isoindole ring, producing derivatives with a wide substitution pattern. The structure−activity relationship indicates that the *N*-4-methoxybenzyl group appears crucial for potent activity. In addition, the presence of a 6-phenyl moiety is important and the best activity is reached with a 3,4,5-trimethoxy substituent. The most active compound, bearing both the structural features, was able to inhibit tumor cell proliferation at nanomolar concentrations when tested against the full NCI human tumor cell line panel. Interestingly, this compound was effective in reducing in vitro and in vivo cell growth, impairing cell cycle progression and inducing apoptosis, as a consequence of the inhibition of tubulin polymerization, in experimental models of diffuse malignant peritoneal mesothelioma (DMPM), a rapidly lethal disease, poorly responsive to conventional therapeutic strategies.



INTRODUCTION

Malignant mesothelioma is a rare tumor associated with asbestos exposure that develops from mesothelial cells of the serosal membranes, including pleura, peritoneum, tunica vaginalis, and pericardium.[1] Diffuse malignant peritoneal mesothelioma (DMPM) accounts for approximately 20% of all malignant mesotheliomas, with a yearly incidence in Europe of 1−2 cases per million.[2] DMPM prognosis is dismal. In historical case series, standard therapy with palliative surgery, systemic/intraperitoneal chemotherapy, and abdominal irradiation showed to be ineffective, with a median survival of about one year.[2] More recently, a loco-regional strategy that combines aggressive cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy (HIPEC) significantly improved median survival up to 52−92 months in selected series of patients, although half of them still experienced recurrence.[3] For these patients, and for those who are not eligible to CRS +HIPEC, the prognosis remains severe due to the lack of effective alternative treatment options, highlighting the need to develop new therapeutic strategies.

In a recent project aimed at developing new candidates for the treatment of DMPM, we identified novel molecules based on natural compound scaffolds.[4−6] Small molecules have found application in medicine, notably as cancer chemotherapeutic agents. Among these, nitrogen heterocycles are of special interest because they constitute an important class of natural and synthetic products, many of which exhibit relevant biological activities.[7−17] Isoxazoles and [1,2]oxazoles are nitrogen and oxygen containing heterocycles that belong to the azoles family, which have aroused much interest as novel anticancer agents. Among these, diaryl[1,2]oxazoles of type **1** (Chart 1) have been widely studied, showing very promising results either as HSP-90 inhibitors[18] or potent analogues of the antitubulin compound combretastatin A4 (CA4).[19,20]

In the latter series of compounds, the 3,4,5-trimethoxy group on one of the two aromatic rings was essential for the cytotoxic activity especially in the presence of a nitro or an amino group in α-position to the 4-methoxy on the other phenyl ring, reaching the best antitubulin activity.

Derivatives of the 3,5-bis(3′-indolyl)[1,2]oxazole system, recently reported by us as analogues of nortopsentins A-C, exhibited in vitro cytotoxicity in the micromolar range.[21]

3-Amino benzo[*d*]isoxazoles potently inhibited both the vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor families of receptor tyrosine kinases.[22] Other 3-amino-benzo[*d*]isoxazoles displayed potent inhibitory effects both at enzymatic and cellular levels

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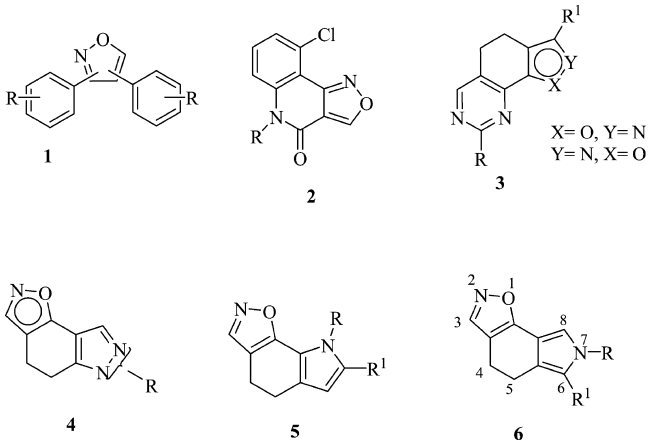
Chart 1. [1,2]Oxazole Derivatives 1−6



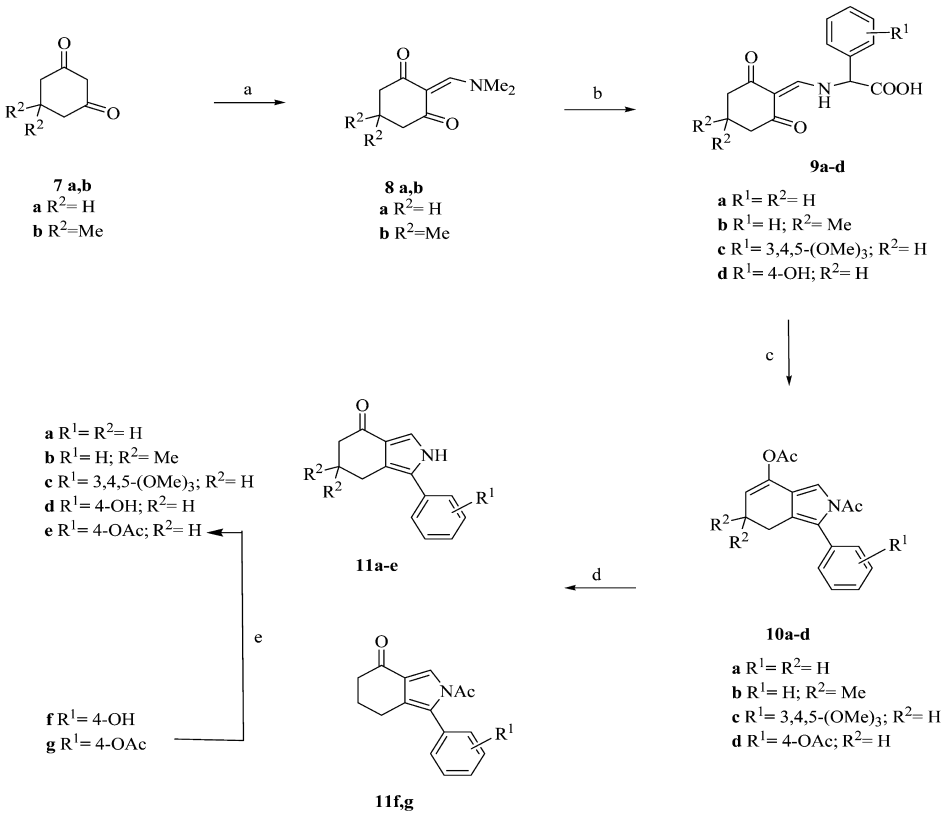
toward c-Met tyrosine kinase.[23] Tricyclic[1,2]oxazoles of type **2** have been described as selective inhibitors of the multidrug resistance protein MRP1.[24] Finally, isoxazolo-quinazolines **3** emerged as potent modulators of kinases, such as MSK1 and PERK.[25]

Drugs that interfere with tubulin dynamics (including taxanes, vinca alkaloids, and combrestatins) are commonly used in the clinic to treat a variety of cancers.[26] In particular, the vinca alkaloid vinorelbine has demonstrated clinical efficacy in malignant pleural mesothelioma, representing a reasonable alternative treatment option for the disease.[27]

The incorporation of heterocyclic rings into pharmaceutical candidates is a major strategy to enhance activity and safety profiles. As a consequence, for a long time, much of our attention has been paid to the design and synthesis of pyrrolo-fused systems.[28−32] Several examples in the literature highlighted the indole nucleus as a valuable pharmacophore for potent antimitotic agents,[33,34] but less is known about isoindole derivatives.[35−37] We have already reported the synthesis of heterocyclic systems in which the isoindole scaffold is condensed with esatomic rings such as a pyrane,[38] pyridine,[39] and pyrimidine[35,40] and with a pentatomic one such as pyrazole[41] producing compounds with interesting antitumor activity.

A literature search revealed only two reports, from the same research group, concerning the [1,2]oxazolo[5,4-*e*]isoindoles ring system. Both reports described the same synthetic approach, leading to the tricyclic system with limited yield and poor possibility of obtaining derivatives with a wide substitution pattern.[42,43] Thus, as initial rationale for our work, we decided to explore new synthetic strategies, planning the annelation of an isoxazole ring to an isoindole moiety to generate [1,2]oxazolo [5,4-*e*]isoindoles of type **6** (Chart 1). We have recently reported that tricyclic compounds incorporating the [1,2]oxazole unit fused to an indazole or an indole scaffold, namely [1,2]oxazolo[5,4-*e*]indazoles **4**[44] and [1,2]oxazolo[4,5-*g*]indoles **5**,[45] which showed promising antitumor properties (Chart 1). In particular, some derivatives of the latter class of compounds demonstrated a potent growth inhibitory effect on

Scheme 1. Synthesis of 1-Aryl Tetrahydro-4*H*-isoindol-4-ones 11a−g[a]



[a]Reagents and conditions: (a) DMFDMA, reflux, 30 min−1 h, 98−99%; (b) phenylglycine or 4-hydroxyphenylglycine or 3,4,5-trimethoxyphenylglycine, $AcONa{\cdot}3H_2O$, ethanol, reflux, 1−2 h, (90−95%); (c) Et_3N, Ac_2O, reflux, 15−30 min, (75−90%); (d) 80% acetic acid, 37% HCl, rt−60 °C, 10−55 min, 5−80%; (e) 10% K_2CO_3, ethanol, rt, 2 min, (93%).

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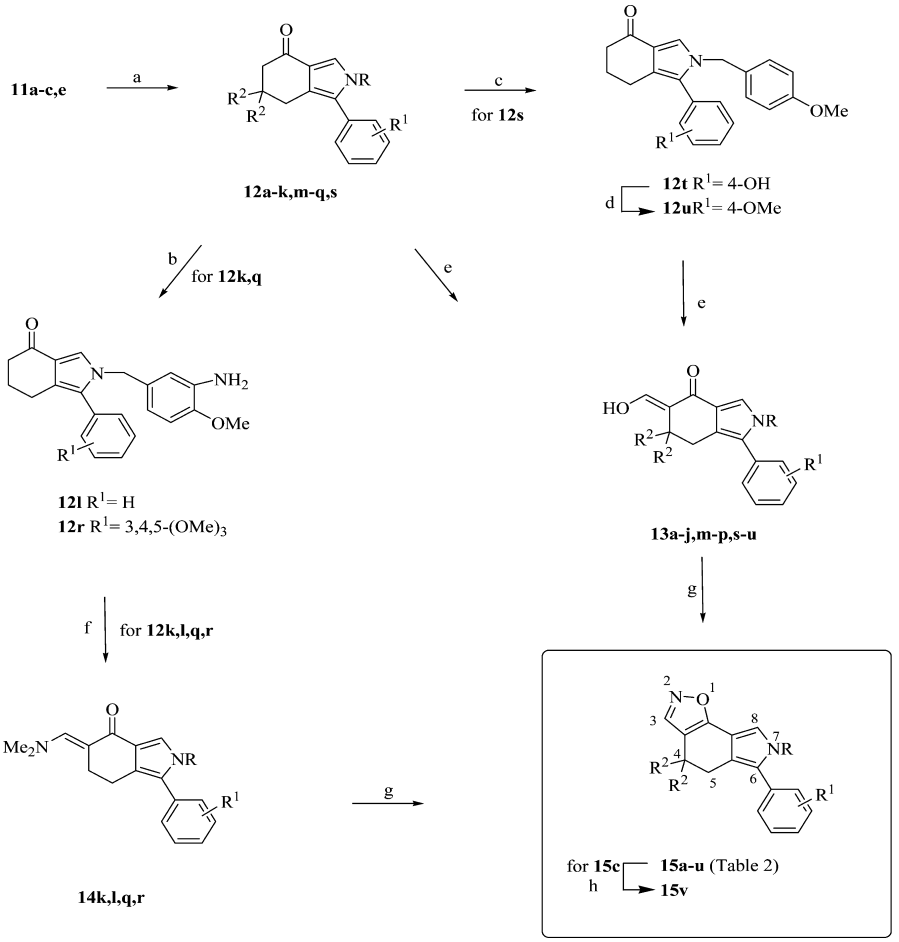
Scheme 2. Synthesis of 5,7-Dihydro-4*H*-[1,2]oxazole[5,4-*e*]isoindoles 15a−v[a]



[a]Reagents and conditions: (a) for **12a−c**,[35,40] **12d−k,n-p,r,s** NaH, DMF, 0 °C to rt, 1 h then aralkyl halide at 0 °C to rt, 1−24 h, 52−93%, for **12m**, K_2CO_3, *N*-methylpyrrolidone, N_2, rt, 1 h, then Cu(I)Br, rt 1 h, then 4-OMePhI, reflux, 48 h, 60%; (b) H_2, 10% Pd/C, ethanol, rt, 24 h, 95−98%; (c) 10% K_2CO_3, ethanol, rt, 16 h, 96%; (d) NaH, THF, 0 °C to rt, 1 h then MeI at 0 °C to rt, 20 h, 88%; (e) *t*-BuOK, toluene, 0 °C to rt, 3 h then HCOOEt, rt, 24 h, 60−88%; (f) TBDMAM, toluene, reflux, 24 h; (g) $NH_2OH{\cdot}HCl$, ethanol, reflux, 50 min, 57−84%; (h) BBr_3, dichloromethane, −78 °C to rt, 24 h, 84%.

the National Cancer Institute 60 human tumor cell line panel (NCI-60) at concentrations in the micromolar to nanomolar range. The most active compounds were particularly effective against the leukemia subpanel, and the presence of a methoxy substituted benzyl moiety at the indole nitrogen appeared crucial for conferring remarkable cytotoxic activity to the [1,2]oxazolo indole derivatives. On the basis of the above observations, the present study was designed to explore further chemical modifications of the five-membered heterocycle nucleus. Herein, we describe the synthesis and biological evaluation of new [1,2]oxazolo[5,4-*e*]isoindoles **6** as positional isomers of compounds **5** (Chart 1), bearing aryl substituted moieties including methoxy groups, in an the attempt to synthesize new active drugs against mesothelioma.

RESULTS AND DISCUSSION

Chemistry. Our approach to the synthesis of the new ring system consisted in the preparation of a number of tetrahydroisoindole-4-ones of types **11** (Scheme 1) and **12** (Scheme 2); the latter could be further functionalized in α position to the carbonyl group to have the access to key synthons of type **13** and **14** ready for the annelation leading to the [1,2]oxazole moiety. Ketones **11a** bearing the 1-phenyl moiety and **12a−c** bearing a methyl, benzyl, and 4-methoxybenzyl substituent, respectively, in position 2 were conveniently prepared by a method previously reported by us (Schemes 1 and 2).[35,40]

Several new tetrahydroisoindoles were prepared as outlined in the Scheme 1 by a multistep sequence starting from commercially available resorcine **7a** and dimedone **7b**, which were converted into their enamino derivatives **8a,b** in quantitative yields upon heating the latter under reflux in dimethylformamide dimethylacetal (DMFDMA) (99 and 98% yields, respectively). 2-[(Dimethylamino)methylidene]-cyclohexane-1,3-diones **8a,b** were subsequently reacted in refluxing ethanol with the commercially available phenylglycine, 3,4,5-trimethoxy phenylglycine, in turn prepared by a Strecker reaction from 3,4,5-trimethoxybenzaldehyde[46] or 4-hydroxy-phenylglycine to give the enamino acids **9a−d** (90−95%). Cyclization in acetic anhydride and triethylamine of **9a−c** gave the expected dihydroisoindole **10a−c** (75−90%). In the case of **9d**, the cyclization process in acetic anhydride also determined acetylation of the phenolic group, thus furnishing the 4-[2-acetyl-4-(acetyloxy)-6,7-dihydro-2*H*-isoindol-1-yl]phenyl acetate **10d** (81%). Finally, tetrahydroisoindole-2-ones **11a−c** were obtained (70−80%) by deacetylation of the correspond-

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ing derivatives **10a−c** by heating in aqueous acetic acid (80%) and HCl (37%). Hydrolysis of **10d**, performed under the same conditions, led to several byproducts, making it difficult to isolate the desired tetrahydroisoindole **11e**. A strict temperature control was crucial in order to obtain **11e** with the best yield. By using the same conditions as for its analogues (10−15 min, 60 °C), **10d** gave a mixture of two deacetylated products: the 4-acethoxyphenyl derivative **11e** (12%) and the 4-hydroxyphenyl one **11d** (32%). Prolonged heating led to **11d** (73%) as the sole product, which was not useful for our purpose. To make the deacetylation process smoother, it was conducted at room temperature.

After 90 min, four products were isolated: **11d** (17%), **11e** (19%), **11f** (9%), and **11g** (21%). Instead, after 50 min, **11d** (9%), **11e** (23%), **11f** (5%), and **11g** (51%) were isolated with the indicated yields. This finding was considered the best result considering that **11e** and **11g** were preferred for our synthetic purpose. Derivative **11g** was readily converted into **11e** (93%) in 10% K_2CO_3.

Ketones **11a−c,e**, bearing a free NH, were subjected to nucleophilic reactions with aralkyl halides to give N-substituted derivatives (Scheme 2). Thus, reaction in dimethylformamide with benzyl bromide or substituted benzyl chlorides (2-methoxybenzyl chloride, 3-methoxybenzyl chloride, 4-methoxybenzyl chloride, 2,3-dimethoxybenzyl chloride, 2,5-dimethoxybenzyl chloride, 3,4-dimethoxybenzyl chloride, 3,5-dimethoxybenzyl chloride, 3,4,5-trimethoxybenzyl chloride, 3-nitro-4-methoxybenzyl chloride) in the presence of sodium hydride gave derivatives **12a−k,m−q,s** in 62−84% yields. Instead, the 4-methoxyphenyl derivative **12m** was obtained in 60% yield by a modified Ulmann cross-coupling reaction using 4-methoxy iodoanisole as an alkylating agent in the presence of copper(I)bromide and potassium carbonate. Moreover, the 3-nitro 4-methoxybenzyl substituted derivatives **12k** and **12q** were subjected to catalytic reduction with 10% Pd on charcoal, furnishing the corresponding amino derivatives **12l** (98%) and **12r** (95%). Hydrolysis in basic media (10% K_2CO_3) of **12s** furnished the 4-hydroxyphenyl derivative **12t** (96%), which was subsequently methylated with iodomethane to give **12u** in good yield (88%).

The synthesis of [1,2]oxazolo[5,4-*e*]isoindoles **15a−v** has been accomplished as outlined in the Scheme 2. The *N*-substituted tetrahydroisoindoles **12a−s,u** were properly functionalized in α position to the carbonyl (Table 1). In particular, tetrahydroisondoles **12a−j,m−p,s,u** were subjected to formylation, using ethyl formate as the formylating agent in the presence of potassium *t*-buthoxide, to furnish the corresponding hydroxymethyl derivatives **13a−j,m−p,s,u** (60−97%). Compound **13t** was obtained as a byproduct from the formylation of **12s**, together with the expected formyl derivative **13s**.

Compounds **13m,s,t** were unstable and were used as crude products for the next step.

Only in the case of the 3-nitro 4-methoxybenzyl substituted derivatives **12k,q** and the 3-amino 4-methoxybenzyl substituted derivatives **12l,r**, the Bredereck reagent was used to convert them into the α-enaminoketons **14k,l,q,r**, which were not isolated as pure compounds, and thus used as crude products directly in the next step. Annelation of the [1,2]oxazole ring was achieved reacting the key intermediates **13** and **14** with hydroxylamine hydrochloride as 1,3 dinucleophile in refluxing ethanol.

Table 1. *N*-Substituted Tetrahydro-4*H*-isoindol-4-ones 12a−u

	R	R^1	R^2	yields[a] (%)
12a	Me	H	H	90
12b	Bn	H	H	90
12c	4-OMeBn	H	H	85
12d	3-OMeBn	H	H	71
12e	2-OMeBn	H	H	64
12f	2,3-$(OMe)_2$Bn	H	H	62
12g	2,5-$(OMe)_2$Bn	H	H	93
12h	3,4-$(OMe)_2$Bn	H	H	62
12i	3,5-$(OMe)_2$Bn	H	H	76
12j	3,4,5-$(OMe)_3$Bn	H	H	84
12k	3-NO_2,4-OMeBn	H	H	83
12l	3-NH_2,4-OMeBn	H	H	98
12m	4-OMePh	H	H	60
12n	Bn	H	Me	76
12o	4-OMeBn	H	Me	88
12p	4-OMeBn	3,4,5-$(OMe)_3$	H	88
12q	3-NO_2,4-OMeBn	3,4,5-$(OMe)_3$	H	74
12r	3-NH_2,4-OMeBn	3,4,5-$(OMe)_3$	H	95
12s	4-OMeBn	4-OAc	H	64
12t	4-OMeBn	4-OH	H	96
12u	4-OMeBn	4-OMe	H	88

[a]Figures represent the yield obtained at the final reaction step.

By this versatile route, 21 derivatives of the [1,2]oxazolo[5,4-*e*]isoindole system **15a−u** were obtained (57−84%, Table 2). Additionally, the 7-(4-methoxybenzyl)-6-phenyl-5,7-dihydro-4*H*-[1,2]oxazolo[5,4-*e*]isoindole **15c** was subjected to demethylation of the methoxy functionality with BBr_3, furnishing the 4-hydroxy analogue **15v** (84%).

Table 2. 5,7-Dihydro-4*H*-[1,2]oxazole[5,4-*e*]isoindoles 15a−v

[1,2] oxazoles	Sbt	R	R^1	R^2	yields[a] (%)
15a	**13a**	Me	H	H	62
15b	**13b**	Bn	H	H	60
15c	**13c**	4-OMeBn	H	H	67
15d	**13d**	3-OMeBn	H	H	78
15e	**13e**	2-OMeBn	H	H	80
15f	**13f**	2,3-$(OMe)_2$Bn	H	H	70
15g	**13g**	2,5-$(OMe)_2$Bn	H	H	60
15h	**13h**	3,4-$(OMe)_2$Bn	H	H	83
15i	**13i**	3,5-$(OMe)_2$Bn	H	H	69
15j	**13j**	3,4,5-$(OMe)_3$Bn	H	H	76
15k	**14k**	3-NO_2,4-OMeBn	H	H	62
15l	**14l**	3-NH_2,4-OMeBn	H	H	60
15m	**13m**	4-OMePh	H	H	76
15n	**13n**	Bn	H	Me	84
15o	**13o**	4-OMeBn	H	Me	75
15p	**13p**	4-OMeBn	3,4,5-$(OMe)_3$	H	60
15q	**14q**	3-NO_2,4-OMeBn	3,4,5-$(OMe)_3$	H	62
15r	**14r**	3-NH_2,4-OMeBn	3,4,5-$(OMe)_3$	H	58
15s	**13s**	4-OMeBn	4-OAc	H	69
15t	**13t**	4-OMeBn	4-OH	H	57
15u	**13u**	4-OMeBn	4-OMe	H	75
15v	**15c**	4-OHBn	H	H	84

[a]Figures represent the yield obtained at the final reaction step.

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Biology. *In Vitro Antiproliferative Activity of the Type 15 Compounds.* All the synthesized compounds of type **15** were submitted to the NCI and prescreened at a 10^{-5} M dose in the full panel of 60 human cell lines of different tumor types[47−49] (data not shown). Compounds **15b,c,e,g,h,l,o−r,t,u** were selected for further screenings at five concentration levels (10^{-4}−10^{-8} M), and their antiproliferative activity was defined in terms of GI_{50} value (i.e., the molar concentration that inhibits 50% net cell growth). Data analysis showed that almost all compounds exhibited growth inhibitory effect against all tested cell lines, with GI_{50} values in the micromolar to nanomolar range. In particular, the 4-methoxybenzyl substitution at the benzyl bound to pyrrole nitrogen seems to be crucial for the activity. In fact, derivatives **15c,l,p−r,t,u**, showed remarkable antiproliferative activity at submicromolar to nanomolar concentrations. The most potent derivative was **15r**, bearing a 3,4,5-trimethoxyphenyl substitution, for which nanomolar GI_{50} values were observed in 46 out of 56 cell lines (see Supporting Information, Tables S1 and S2 and Figures S1−S10).

On the basis of the remarkable cytotoxic activity observed with the **15r** derivative in the NCI panel of human tumor cell lines, including those from chemoresitant histotypes (i.e., melanoma, nonsmall cell lung cancer), the study was extended to three additional cell lines, STO, MP4, and MP8 which were established from clinical specimens of DMPM, a tumor type highly resistant to conventional treatments. The antiproliferative activity of **15r** was assessed by MTS assay following 72 h exposure to increasing concentrations of the compound. A dose- and time-dependent cell growth inhibition was consistently observed in all cell models (Figure 1). STO cells

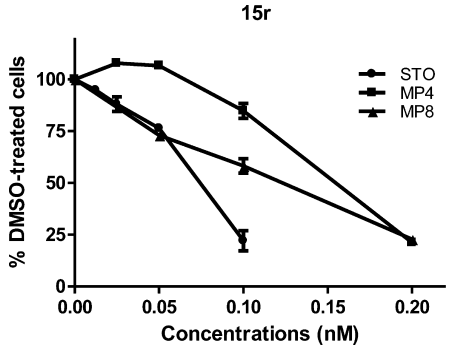

Figure 1. 15r derivative impairs DMPM cell growth. DMPM cells were cultured for 72 h in the presence of increasing concentrations of the compound and the cytotoxic activity was assessed by MTS assay. Data are expressed as percentage values with respect to DMSO-treated cells and represent the mean values ± SD (standard deviation) of three independent experiments.

were highly responsive to the compound, as indicated by the concentration required to inhibit growth by 50% (IC_{50}) of 0.07 ± 0.02 μM, whereas a slightly less pronounced and comparable sensitivity to **15r** was displayed by MP4 and MP8, with IC_{50} values of 0.16 ± 0.05 and 0.13 ± 0.04 μM, respectively. Interestingly, **15r** did not alter the growth of WI38 normal human lung fibroblasts (IC_{50} > 100 μM).

Effects of 15r Derivative on Tubulin Polymerization. On the basis of the evidence that several derivatives structurally related to our [1,2]oxazolo[5,4-*e*]isoindole were previously reported to interfere with tubulin polymerization,[19,20] we investigated whether **15r** was able to affect tubulin dynamics. Western blot results showed that treatment with **15r** derivative markedly inhibited tubulin polymerization in STO and MP4 cells, as evidenced by the reduction in the polymerized compared to soluble fraction of tubulin (Figure 2A). The

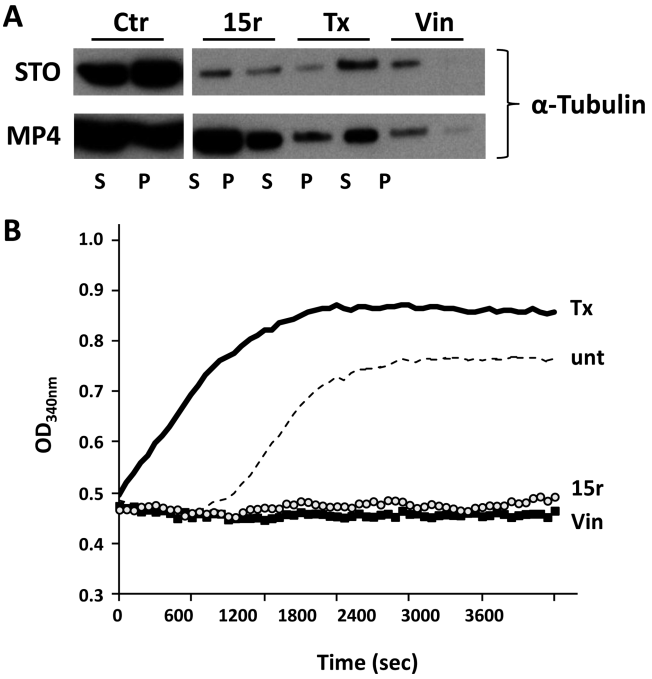

Figure 2. 15r derivative interferes with tubulin polymerization. (A) Representative Western blot showing the soluble (S) or polymerized (P) tubulin fraction in STO and MP4 cells after 24 h of exposure to **15r** at the concentrations corresponding to the IC_{50} at 72 h. Vinorelbine (Vin; 2 and 10 nM for STO and MP4, respectively) and taxol (Tx; 1 and 20 nM for STO and MP4, respectively) were selected as reference drugs due to their opposite mechanism of action on tubulin polymerization. (B) In vitro assessment of tubulin polymerization in the absence (unt) or presence of **15r**, vinorelbine (Vin) and taxol (Tx), used at 10 μM. A representative experiment of two is shown.

direct interference of **15r** with tubulin polymerization was further demonstrated by monitoring in vitro changes in the GTP-induced assembly of purified tubulin monomers. Specifically, results indicated that tubulin polymerization was markedly inhibited in samples treated with **15r** with respect to untreated controls (Figure 2B). Our findings also suggested that the compound acts in a vinca alkaloid-like manner because its effect on tubulin polymerization was superimposable to that observed following exposure to vinorelbine but opposite to that induced by taxol (Figure 2).

15r Derivative Promotes Cell-Cycle Arrest and Induces Apoptosis. The biological effects consequent to **15r**-induced tubulin polymerization impairment and responsible for the observed antiproliferative activity of the compound were further investigated. Exposure to **15r** induced a marked perturbation in the distribution of the cells in the different cell cycle phases (Figure 3A), as assessed by flow cytometry. Specifically, drug treatment (IC_{50} and IC_{80}) of asynchronously growing STO and MP4 cells resulted in a time-dependent accumulation of cells in the G2/M phase, with a concomitant reduction in the percentage of cells in G1 and S compartments (Figure 3A). The data are comparable to those obtained in the same cell lines following exposure to drug interfering with

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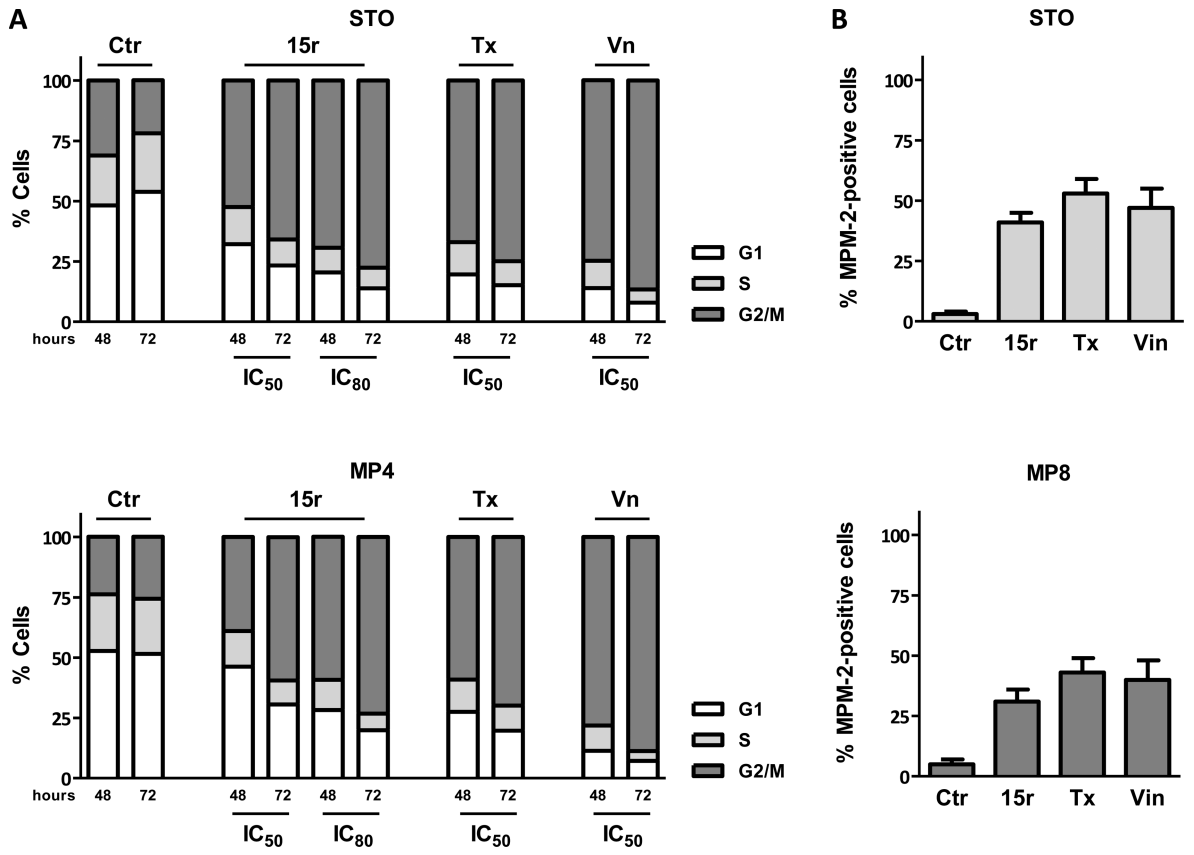

Figure 3. 15r derivative causes mitotic arrest in DMPM cells. (A) DMPM cells were exposed to 1% (v/v) DMSO (control cells; Ctr), derivative **15r** (IC_{50} and IC_{80}), or taxol (Tx) and vinorelbine (Vin) (IC_{50}) for 48 and 72 h, and the effect on the cell cycle distribution was assessed as described in the Experimental Section. Data are reported as the percentage of cells in G1, S, and G2/M phases and represent the mean values of three independent experiments; SDs were always within 5%. (B) Percentage of mitotic cells upon the exposure of DMPM cells to equitoxic concentrations (IC_{50}) of derivative **15r**, vinorelbine (Vin; 2 and 13 nM for STO and MP8, respectively), and taxol (Tx; 1 and 18 nM for STO and MP8, respectively). Data are expressed as mean values ± SD of three independent experiments.

tubulin dynamics, such as taxol and vinorelbine (Figure 3A). In addition, fluorescence microscopy analysis of DMPM cells stained with the Mitotic Protein Monoclonal no. 2 (MPM-2) antibody, which recognizes the phosphorylated epitope in specific phosphoproteins which are phosphorylated in the onset of mitosis, showed the presence of 30−50% mitotic cells within the overall cell population following 72 h exposure of STO and MP8 cells to equitoxic (IC_{50}) concentrations of **15r**, vinorelbine, and taxol (Figure 3B). Treatment of DMPM cells with **15r** also resulted in a marked and time-dependent apoptotic response, as indicated by a significant increase in caspase-3 catalytic activity, determined in vitro by the hydrolysis of the specific fluorogenic substrate. Specifically, in STO cells exposed for 72 h to derivative **15r** (IC_{80}), caspase-3 catalytic activity was 38.5-fold higher than that observed in control samples (Figure 4). Consistently, a 35-fold increase in the enzyme activity was observed in MP4 cells under the same experimental conditions (Figure 4).

*Antitumor Activity of **15r** Derivative in STO Xenograft Model.* In the last step of the study, the in vivo antitumor activity of **15r** was evaluated in STO cells following subcutaneous xenotransplantation into athymic nude mice. As shown in Figure 5A, intraperitoneal administration of the compound resulted in a statistical significant ($P < 0.05$) tumor growth delay compared to control mice, with a maximum tumor volume inhibition of 59%. Consistently, Ki67 immunohistochemical staining of tumor xenografts after the last treatment showed a marked **15r**-induced decline of cell proliferation (Figure 5B). The compound was well tolerated with minimal weight loss and no toxic deaths.

CONCLUSION

We have reported a versatile method for the synthesis of 5,7-dihydro-4*H*-[1,2]oxazole[5,4-*e*]isoindoles involving the annelation of the [1,2]oxazole moiety to the isoindole ring. Several derivatives with a wide substitution pattern were efficiently obtained and evaluated for their anticancer activity. The structure−activity relationship indicated that the *N*-4-methoxybenzyl group appears crucial for the antiproliferative activity of the compounds, with the most potent derivative being **15r**, bearing a 3,4,5-trimetoxy substituent. Indeed, in vitro studies indicated that this compound induced significant antiproliferative effects in a large panel of human cancer cell lines and did not appreciably interfere with the growth of human normal fibroblasts, suggesting a preferential activity against malignant cells. It is worth noting that the compound markedly impaired the growth of three experimental models of DMPM, a rapidly lethal malignancy, poorly responsive to chemotherapy.

The antiproliferative activity of **15r** was found to rely on the ability to impair microtubule assembly during mitosis, in a vinca alkaloid-like manner, with a consequent cell cycle arrest at the G2/M compartment and the induction of caspase-dependent apoptosis. Most importantly, our results documented a significant in vivo antitumor activity of **15r** derivative at well-

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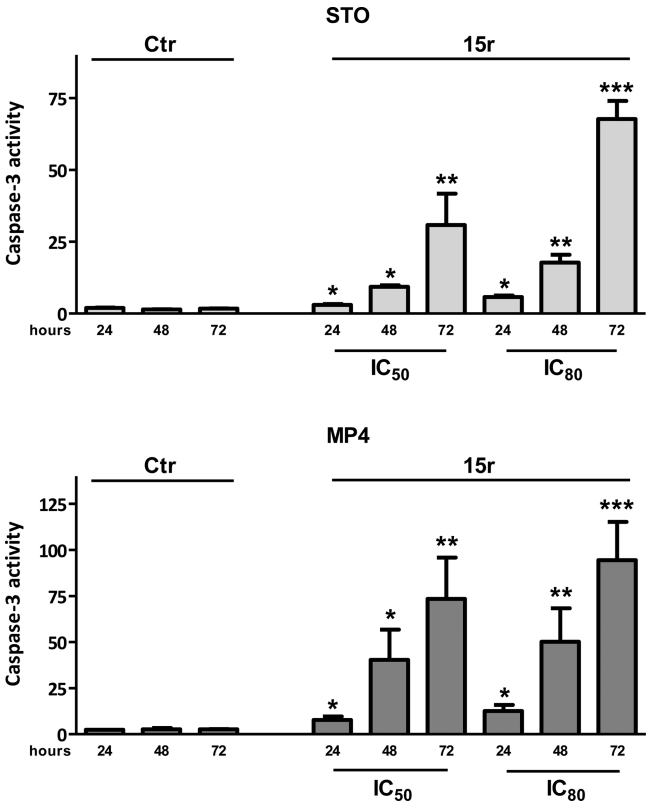

Figure 4. **15r** derivative induces apoptosis in DMPM cells. The catalytic activity of caspase-3 was assessed by the in vitro hydrolysis of the fluorogenic substrates (DEVD-pNA) after exposure of STO and MP4 cells to 1% (v/v) DMSO (control cells; Ctr) or to **15r** derivative. Data are expressed as mean values ± SD of three independent experiments. ***$P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

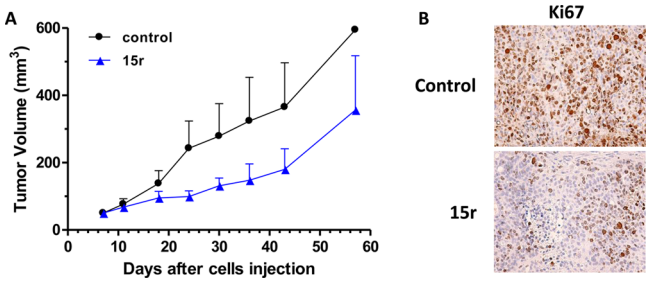

Figure 5. Efficacy of **15r** derivative against DMPM xenografts. (A) Tumor growth curves of STO cells subcutaneously injected into right flank of nude mice. Mice (eight mice/group) were randomly grouped to receive vehicle (Control) or **15r** (5 mg/kg, q7dx4). The treatment started 10 days after cell inoculum. Data are expressed as mean values ± SE. (B) Representative Ki67 staining performed in FFPE sections of tumors from the experimental groups reported in A. Images for one representative mouse per group are shown. Original magnification: ×20.

tolerated doses in a DMPM xenograft model, suggesting a possible therapeutic potential of the compound for the management of the disease.

EXPERIMENTAL SECTION

Chemistry. *General Methods.* All melting points were taken on a Büchi melting point M-560 apparatus. IR spectra were determined in bromoform with Shimadzu FT/IR 8400S spectrophotometer. ^{1}H and ^{13}C NMR spectra were measured at 200 and 50.0 MHz, respectively, in DMSO-d_6 or $CDCl_3$ solution using a Bruker Avance II series 200 MHz spectrometer. Column chromatography was performed with Merck silica gel (230−400 mesh ASTM) or a Büchi Sepacor chromatography module (prepacked cartridge system). Elemental analyses (C, H, N) were within ±0.4% of theoretical values and were performed with a VARIO EL III elemental analyzer. The purity of all the tested compounds was >95%, determined by HPLC (Agilent 1100 series). In particular, $(NH_4)_2HPO_4$ 0.4 M/acetonitrile 68:32 v/v was used as eluent, with a flow rate of 1 mL/min, filtered on a cellulose regenerated filter, cut off 0.2 μm, and samples injected in a C18 Gemini HPLC column. The purity was calculated by using a calibration curve obtained for serially diluted concentrations of compounds (K_{max} = 254 nm) in the eluent and expressed as the amount of intact molecules per unit mass.

Compounds **8a**, **9a**, **10a**, **11a**, **12a**−**c**, and **13a**−**c** were conveniently prepared by a method previously reported by us.[35,40]

*Synthesis of 2-[(Dimethylamino)methylidene]ciclohexane-1,3-dione (**8b**).* A solution of **7b** (71 mmol) in *N*,*N*-dimethylformamide dimethyl acetal (20 mL) was heated under reflux up to completeness (30 min, TLC). After cooling, the solvent was evaporated at reduced pressure. The solid obtained was used in the next step without further purification. Brown solid; yield 98%; mp 86−87 °C. IR (cm^{-1}) 1718 (CO), 1662 (CO). ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.96 (s, 6H, 2 × CH_3), 2.22 (s, 4H, 2 × CH_2), 3.04 (s, 3H, CH_3), 3.38 (s, 3H, CH_3), 7.95 (s, 1H, CH). ^{13}C NMR (50 MHz, DMSO-d_6) δ 28.1 (2 × q), 44.2 (q), 47.7 (q), 51.7 (2*t*), 99.8 (s), 107.1 (s), 193.3 (s), 193.4 (s). Anal. Calcd for $C_{11}H_{17}NO_2$: C, 67.66; H, 8.78; N, 7.17. Found: C, 67.79; H, 8.45; N, 7.45.

*General Procedure for the Synthesis of {[(2,6-Dioxocyclohexylidene)methyl]amino}arylacetic Acid (**9b**−**d**).* To a solution of **8a**,**b** (18 mmol) in ethanol (42 mL), a solution of the suitable phenylglycine (21.6 mmol) and sodium acetate trihydrate (2.94 g) in ethanol was added and the reaction mixture was heated under reflux up to completeness (TLC). After cooling, the reaction mixture was filtered and the filtrate was evaporated at reduced pressure. To the residue, ice and water were added and the resulting solution was acidified with HCl 6 M. The solid obtained was filtered and dried.

*Data for {[(4,4-Dimethyl-2,6-dioxocyclohexylidene)methyl]-amino}(phenyl)acetic Acid (**9b**).* This compound was obtained from reaction of **8b** with phenylglycine after 1 h and 30 min. Pale-yellow solid; yield 95%; mp 186−187 °C. IR (cm^{-1}) 3408 (NH), 2954 (OH), 1720 (CO), 1651 (CO), 1585 (CO) cm^{-1}. ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.95 (s, 6H, 2 × CH_3), 2.23 (s, 2H, CH_2), 2.33 (s, 2H, CH_2), 3.67 (s, 1H, OH), 5.70 (d, 1H, *J* = 7.3 Hz, CH), 7.36−7.50 (m, 5H, Ar), 8.07 (d, 1H, *J* = 14.1 Hz, CH), 11.60−11.70 (m, 1H, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ 27.8 (q), 28.2 (q), 50.5 (t), 50.8 (t), 63.4 (d), 107.5 (s), 127.3 (2 × d), 128.8 (d), 129.2 (2 × d), 136.8 (s), 156.6 (d), 170.6 (s), 194.5 (s), 194.6 (s), 198.3 (s). Anal. Calcd for $C_{17}H_{19}NO_4$: C, 67.76; H, 6.36; N, 4.65. Found: C, 67.57; H, 6.71; N, 4.36.

*Data for {[(2,6-Dioxocyclohexylidene)methyl]amino}(3,4,5-trimethoxyphenyl)acetic Acid (**9c**).* This compound was obtained from reaction of **8a** with 3,4,5-trimethoxyphenylglycine after 2 h. Pale-yellow solid; yield 95%; mp 183−184 °C. IR (cm^{-1}) 3182 (NH), 2939 (OH), 1714 (CO), 1697 (CO), 1651 (CO). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.72−1.89 (m, 2H, CH_2), 2.32−2.40 (m, 4H, 2 × CH_2), 3.52 (s, 1H, OH), 3.67 (s, 3H, CH_3), 3.78 (s, 6H, 2 × CH_3), 5.55−5.58 (m, 1H, CH), 6.68 (s, 2H, H-2′, H-6′), 8.09 (d, 1H, *J* = 13.8 Hz, CH), 11.59−11.69 (m, 1H, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ 19.38 (t), 37.0 (t), 37.3 (t), 55.9 (2 × q), 60.0 (q), 63.6 (d), 104.7 (2 × d), 108.6 (s), 132.3 (s), 137.7 (s), 153.3 (2 × s), 156.9 (d), 170.6 (s), 195.2 (s), 198.9 (s). Anal. Calcd for $C_{18}H_{21}NO_7$: C, 59.50; H, 5.83; N, 3.85. Found: C, 59.35; H, 5.99; N, 4.01.

*Data for {[(2,6-Dioxocyclohexylidene)methyl]amino}(4-hydroxyphenyl)acetic Acid (**9d**).* This compound was obtained from reaction of **8a** with 4-hydroxyphenylglycine after 1 h. Light-yellow solid; yield 90%; mp 210−211 °C. IR (cm^{-1}) 3252 (NH), 2943 (OH), 2883 (OH), 1730 (CO), 1664 (CO), 1606 (CO). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.76−1.88 (m, 2H, CH_2), 2.30 (t, 2H, *J* = 6.2 Hz,

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CH_2), 2.39 (t, 2H, J = 6.2 Hz, CH_2), 3.49 (s, 1H, OH), 5.54 (d, 1H, J = 6.9 Hz, CH), 6.81 (d, 2H, J = 8.5 Hz, H-3′, H-5′), 7.16 (d, 2H, J = 8.5 Hz, H-2′, H-6′), 8.04 (d, 1H, J = 14.2 Hz, CH), 9.70 (s, 1H, OH), 11.58−11.68 (m, 1H, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ 19.3 (t), 37.0 (t), 37.3 (t), 62.9 (d), 108.5 (s), 115.9 (2 × d), 126.9 (s), 128.7 (2 × d), 156.8 (d), 157.8 (s), 171.0 (s), 195.1 (s), 198.8 (s). Anal. Calcd for $C_{15}H_{15}NO_5$: C, 62.28; H, 5.23; N, 4.84. Found: C, 62.01; H, 5.42; N, 4.28.

*General Procedure for the Synthesis of 2-Acetyl-1-phenyl-6,7-tetrahydro-2H-isoindol-4-yl Acetate (**10b−d**).* To a solution of **9b−d** (10 mmol) in acetic anhydride (30 mL), triethylamine was added (7.17 mmol, 10 mL). The reaction mixture was heated under reflux up to completeness (TLC). After cooling, the reaction mixture was poured into water and ice. It formed a rubbery solid which was decanted and then stirred with a saturated solution of Na_2CO_3 (50 mL). The solid obtained was filtered and dried.

*Data for 2-Acetyl-6,6-dimethyl-1-phenyl-6,7-dihydro-2H-isoindol-4-yl Acetate (**10b**).* This compound was obtained from reaction of **9b** after 15 min. Yellow oil; yield 90%. IR (cm^{-1}) 1747 (CO), 1722 (CO). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.03 (s, 6H, 2 × CH_3), 2.24 (s, 3H, CH_3), 2.31 (s, 2H, CH_2), 2.39 (s, 3H, CH_3), 5.33 (s, 1H, CH), 7.25−7.42 (m, 6H, H-3, Ar). ^{13}C NMR (50 MHz, DMSO-d_6) δ 20.7 (q), 24.2 (2 × q), 28.6 (q), 33.9 (t), 114.4 (d), 118.4 (s), 122.9 (s), 123.0 (s), 124.6 (d), 127.1 (d), 127.7 (2 × d), 129.4 (2 × d), 129.7 (s), 132.6 (s), 140.2 (s), 168.5 (s), 168.6 (s). Anal. Calcd for $C_{20}H_{21}NO_3$: C, 74.28; H, 6.55; N, 4.33. Found: C, 74.02; H, 6.79; N, 4.07.

*Data for 2-Acetyl-1-(3,4,5-trimethoxyphenyl)-6,7-dihydro-2H-isoindol-4-yl Acetate (**10c**).* This compound was obtained from reaction of **9c** after 30 min. Brown solid; yield 75%; mp 147−148 °C. IR (cm^{-1}) 1708 (CO), 1658 (CO). ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.25 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 2.38−2.47 (m, 4H, 2 × CH_2), 3.69 (s, 3H, CH_3), 3.76 (s, 6H, 2 × CH_3), 5.50 (t, 1H, J = 4.1 Hz, CH), 6.61 (s, 2H, H-2′, H-6′), 7.31 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 18.5 (t), 20.7 (q), 22.9 (t), 24.3 (q), 55.9 (2 × q), 56.0 (d), 60.0 (q), 107.1 (2 × d), 114.0 (d), 119.0 (s), 123.5 (s), 128.0 (s), 128.9 (s), 136.8 (s), 141.7 (s), 152.4 (2 × s), 168.6 (s), 168.7 (s). Anal. Calcd for $C_{21}H_{23}NO_6$: C, 65.44; H, 6.02; N, 3.63. Found: C, 65.32; H, 6.13; N, 3.77.

*Data for 4-[2-Acetyl-4-(acetyloxy)-6,7-dihydro-2H-isoindol-1-yl]-phenyl Acetate (**10d**).* This compound was obtained from reaction of **9d** after 20 min. Brown solid; yield 81%; mp 86−87 °C. IR (cm^{-1}) 1749 (CO), 1712 (CO), 1662 (CO). ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.25 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.34−2.47 (m, 7H, CH_3, 2 × CH_2), 5.51 (t, 1H, J = 4.3 Hz, CH), 7.12 (d, 2H, J = 8.6 Hz, H-3′, H-5′), 7.10−7.35 (m, 3H, H-2′, H-6′, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 18.4 (t), 20.7 (q), 20.9 (q), 22.9 (t), 24.1 (q), 114.0 (d), 114.7 (d), 119.2 (s), 121.0 (2 × d), 123.8 (s), 128.1 (s), 130.1 (s), 130.4 (2 × d), 141.6 (s), 149.4 (s), 168.6 (s), 168.7 (s), 169.1 (s). Anal. Calcd for $C_{20}H_{19}NO_5$: C, 67.98; H, 5.42; N, 3.96. Found: C, 68.15; H, 5.64; N, 3.62.

*General Procedure for the Synthesis of 1-Substituted-2,5,6,7-tetrahydro-4H-isoindol-4-one (**11b,c**).* To a solution of **10b** or **10c** (10 mmol) in AcOH (80%, 62 mL), HCl (37%, 5 mL) was added dropwise. The reaction mixture was heated at 60 °C up to completeness (TLC). After cooling, the reaction mixture was poured into water and ice. The solid obtained was filtered and dried.

*Data for 6,6-Dimethyl-1-phenyl-2,5,6,7-tetrahydro-4H-isoindol-4-one (**11b**).* This compound was obtained from reaction of **10b** after 10 min. Brown solid; yield 80%; mp 235−236 °C. IR (cm^{-1}) 3245 (NH), 1656 (CO). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.00 (s, 6H, 2 × CH_3), 2.27 (s, 2H, CH_2), 2.73 (s, 2H, CH_2), 7.25 (t, 1H, J = 7.1 Hz, Ar), 7.39−7.55 (m, 5H, H-3, Ar), 11.90 (s, 1H, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ 28.2 (2 × q), 36.3 (t), 52.3 (t), 119.9 (d), 121.2 (s), 121.3 (s), 121.6 (s), 125.6 (2 × d), 126.2 (d), 127.2 (s), 128.7 (2 × d), 132.3 (s), 193.7 (s). Anal. Calcd for $C_{16}H_{17}NO$: C, 80.30; H, 7.16; N, 5.85. Found: C, 80.52; H, 6.95; N, 6.01.

*Data for 1-(3,4,5-Trimethoxyphenyl)-2,5,6,7-tetrahydro-4H-isoindol-4-one (**11c**).* This compound was obtained from reaction of **10c** after 15 min. Brown solid; yield 70%; mp 185−186 °C. IR (cm^{-1}) 3251 (NH), 1658 (CO). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.92−2.04 (m, 2H, CH_2), 2.38 (t, 2H, J = 6.3 Hz, CH_2), 2.87 (t, 2H, J = 6.3 Hz, CH_2), 3.68 (s, 3H, CH_3), 3.83 (s, 6H, 2 × CH_3), 6.79 (s, 2H, H-2′, H-6′), 7.46 (s, 1H, H-3), 11.88 (s, 1H, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ 22.4 (t), 24.7 (t), 38.8 (t), 55.8 (2 × q), 60.0 (q), 103.0 (2 × d), 119.8 (d), 122.3 (s), 122.5 (s), 126.7 (s), 127.9 (s), 135.8 (s), 153.0 (2 × s), 194.2 (s). Anal. Calcd for $C_{17}H_{19}NO_4$: C, 67.76; H, 6.36; N, 4.65. Found: C, 67.64; H, 6.22; N, 4.76.

*General Procedure for the Synthesis of 1-Substituted-2,5,6,7-tetrahydro-4H-isoindol-4-one (**11d−g**).* To a solution of **10d** (10 mmol) in AcOH (80%, 62 mL), HCl (37%, 5 mL) was added dropwise. The reaction mixture was stirred at room temperature for 55 min. The reaction mixture was poured into water and ice. The solid obtained was filtered and dried.

*Data for 1-(4-Hydroxyphenyl)-2,5,6,7-tetrahydro-4H-isoindol-4-one (**11d**).* Pale-yellow solid; yield 9%; mp 243−244 °C. IR (cm^{-1}) 3356 (NH), 2951 (OH), 1643 (CO). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.89−2.02 (m, 2H, CH_2), 2.36 (t, 2H, J = 6.1 Hz, CH_2), 2.76 (t, 2H, J = 6.1 Hz, CH_2), 6.84 (d, 2H, J = 8.6 Hz, H-3′, H-5′), 7.30−7.34 (m, 3H, H-2′, H-6′, H-3), 9.49 (s, 1H, OH), 11.70 (s, 1H, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ 22.8 (t), 25.3 (t), 39.3 (t), 116.0 (2 × d), 119.5 (d), 121.3 (s), 122.8 (s), 124.0 (s), 127.4 (s), 127.5 (2 × d), 156.4 (s), 194.7 (s). Anal. Calcd for $C_{14}H_{13}NO_2$: C, 73.99; H, 5.77; N, 6.16. Found: C, 74.07; H, 5.99; N, 5.92.

*Data for 4-(4-Oxo-4,5,6,7-tetrahydro-2H-isoindol-1-yl)phenyl Acetate (**11e**).* Pale-brown solid; yield 21%, mp 217−218 °C. IR (cm^{-1}) 3289 (NH), 1736 (CO), 1655 (CO). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.91−2.03 (m, 2H, CH_2), 2.28 (s, 3H, CH_3), 2.38 (t, 2H, J = 6.1 Hz, CH_2), 2.82 (t, 2H, J = 6.1 Hz, CH_2), 7.20 (d, 2H, J = 8.7 Hz, H-3′, H-5′), 7.44 (s, 1H, H-3), 7.54 (d, 2H, J = 8.7 Hz, H-2′, H-6′), 11.92 (s, 1H, NH). ^{13}C NMR (50 MHz, DMSO-d_6) δ 20.8 (q), 22.3 (t), 24.7 (t), 39.3 (t), 120.1 (d), 122.1 (2 × d), 122.6 (s), 122.7 (s), 125.9 (s), 126.5 (2 × d), 130.0 (s), 148.6 (s), 169.3 (s), 194.1 (s). Anal. Calcd for $C_{16}H_{15}NO_3$: C, 71.36; H, 5.61; N, 5.20. Found: C, 71.22; H, 5.98; N, 5.01.

*Data for 2-Acetyl-1-(4-hydroxyphenyl)-2,5,6,7-tetrahydro-4H-isoindol-4-one (**11f**).* Light-brown solid; yield 5%; mp 184−185 °C. IR (cm^{-1}) 2950 (OH), 1718 (CO), 1645 (CO). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.84−1.96 (m, 2H, CH_2), 2.41−2.52 (m, 7H, 2 × CH_2, CH_3), 6.77 (d, 2H, J = 8.5 Hz, H-3′, H-5′), 7.10 (d, 2H, J = 8.5 Hz, H-2′, H-6′), 7.95 (s, 1H, H-3), 9.59 (s, 1H, OH). ^{13}C NMR (50 MHz, DMSO-d_6) δ 20.8 (t), 22.3 (t), 24.7 (q), 38.7 (t), 115.2 (2 × d), 122.3 (d), 122.2 (s), 122.7 (s), 125.9 (s), 130.0 (s), 131.2 (2 × d), 148.6 (s), 169.3 (s), 194.2 (s). Anal. Calcd for $C_{16}H_{15}NO_3$: C, 71.36; H, 5.61; N, 5.20. Found: C, 71.44; H, 5.51; N, 5.31.

*Data for 4-(2-Acetyl-4-oxo-4,5,6,7-tetrahydro-2H-isoindol-1-yl)-phenyl Acetate (**11g**).* Pale-yellow solid; yield 51%; mp 160−161 °C. IR (cm^{-1}) 1734 (CO), 1674 (CO), 1576 (CO). ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.85−1.97 (m, 2H, CH_2), 2.29 (s, 3H, CH_3), 2.43−2.55 (m, 4H, 2 × CH_2), 2.60 (s, 3H, CH_3), 7.14 (d, 2H, J = 8.6 Hz, H-3′, H-5′), 7.34 (d, 2H, J = 8.6 Hz, H-2′, H-6′), 8.05 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 20.9 (q), 21.0 (t), 23.7 (t), 24.1 (q), 39.2 (t), 121.1 (2 × d), 122.9 (d), 123.0 (s), 127.8 (s), 127.9 (s), 129.6 (s), 130.3 (2 × d), 149.5 (s), 169.1 (s), 169.8 (s), 194.9 (s). Anal. Calcd for $C_{18}H_{17}NO_4$: C, 69.44; H, 5.50; N, 4.50. Found: C, 69.19; H, 5.87; N, 4.31.

*General Procedure for the Synthesis of 2,5,6,7-Tetrahydro-4H-isoindol-4-one (**11e, 12t**).* To a solution of **11g**/**12s** (1 mmol) in ethanol (40 mL), an aqueous solution of K_2CO_3 (10%, 0.7 mL) was added dropwise. The reaction mixture was stirred at room temperature for 2 min for **11e** and 16 h for **12t**. Then to the reaction mixture water was added and the resulting solution was extracted with ethyl acetate (3 × 10 mL). The organic phase was dried over Na_2SO_4 and the solvent evaporated at reduced pressure. The crude product was purified by chromatography column using dichloromethane/ethyl acetate (95/5) as eluent.

Derivative 4-(4-oxo-4,5,6,7-tetrahydro-2*H*-isoindol-1-yl)phenyl acetate (**11e**) was prepared also according to this procedure in 93% yield.

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*Data for 1-(4-Hydroxyphenyl)-2-(4-methoxybenzyl)-2,5,6,7-tetrahydro-4H-isoindol-4-one (**12t**).* This compound was obtained from reaction of **12s**. Yellow oil; yield 96%. IR (cm^{-1}) 2956 (OH), 1641 (CO). ^1H NMR (200 MHz, $CDCl_3$) δ 1.84−1.99 (m, 2H, CH_2), 2.34 (t, 2H, J = 6.4 Hz, CH_2), 2.53 (t, 2H, J = 6.4 Hz, CH_2), 3.69 (s, 3H, CH_3), 5.03 (s, 2H, CH_2), 6.79−6.90 (m, 6H, Ar), 7.09 (d, 2H, J = 8.5 Hz, Ar), 7.42 (s, 1H, H-3), 9.67 (s, 1H, OH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.4 (t), 24.7 (t), 39.0 (t), 49.8 (t), 55.0 (q), 113.8 (2 × d), 115.3 (2 × d), 120.5 (s), 121.4 (s), 122.2 (d), 123.7 (s), 128.4 (2 × d), 129.0 (s), 129.6 (s), 131.0 (2 × d), 156.9 (s), 158.5 (s), 193.8 (s). Anal. Calcd for $C_{22}H_{21}NO_3$: C, 76.06; H, 6.09; N, 4.03. Found: C, 75.94; H, 6.30; N, 4.16.

*General Procedure for the Synthesis of 2-Substituted-2,5,6,7-tetrahydro-4H-isoindol-4-ones (**12**).* To a solution of **11a−c,e** (1.35 g, 10 mmol) in anhydrous DMF (15 mL), NaH (0.24 g, 10 mmol) was added at 0 °C and the reaction was stirred for 1 h at room temperature. The suitable benzyl chloride (15 mmol) was added at 0 °C, and the reaction mixture was stirred at room temperature up to completeness (TLC). Then the reaction mixture was poured into ice and brine. The aqueous solution was extracted with dichloromethane (3 × 50 mL). The organic phase was dried over Na_2SO_4 and the solvent evaporated at reduced pressure. The crude product was purified by chromatography column using dichloromethane/ethyl acetate (98/2) as eluent.

*Data for 2-(3-Methoxybenzyl)-1-phenyl-2,5,6,7-tetrahydro-4H-isoindol-4-one (**12d**).* This compound was obtained from reaction of **11a** with 3-methoxybenzyl chloride after 2 h and 30 min. Yellow oil; yield 71%. IR (cm^{-1}) 1651 (CO). ^1H NMR (200 MHz, DMSO-d_6) δ 1.84−1.99 (m, 2H, CH_2), 2.37 (t, 2H, J = 6.2 Hz, CH_2), 2.56 (t, 2H, J = 6.2 Hz, CH_2), 3.63 (s, 3H, CH_3), 5.16 (s, 2H, CH_2), 6.46−6.51 (m, 2H, Ar), 6.75−6.80 (m, 1H, Ar), 7.12−7.48 (m, 6H, Ar), 7.55 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 21.4 (t), 24.7 (t), 38.9 (t), 50.5 (t), 54.9 (q), 112.6 (d), 112.8 (d), 119.0 (d), 120.8 (s), 123.3 (d), 124.6 (s), 127.5 (d), 128.6 (2 × d), 128.8 (s), 129.5 (2 × d), 129.6 (d), 131.0 (s), 139.2 (s), 159.2 (s), 193.8 (s). Anal. Calcd for $C_{22}H_{21}NO_2$: C, 79.73; H, 6.39; N, 4.23. Found: C, 79.57; H, 6.12; N, 4.52.

*Data for 2-(2-Methoxybenzyl)-1-phenyl-2,5,6,7-tetrahydro-4H-isoindol-4-one (**12e**).* This compound was obtained from reaction of **11a** with 2-methoxybenzyl chloride after 1 h and 30 min. Yellow oil; yield 64%. IR (cm^{-1}) 1651 (CO). ^1H NMR (200 MHz, DMSO-d_6) δ 1.85−1.99 (m, 2H, CH_2), 2.37 (t, 2H, J = 5.8 Hz, CH_2), 2.57 (t, 2H, J = 5.8 Hz, CH_2), 3.69 (s, 3H, CH_3), 5.11 (s, 2H, CH_2), 6.60−6.64 (m, 1H, Ar), 6.81−6.97 (m, 2H, Ar), 7.20−7.46 (m, 7H, H-3, Ar). ^{13}C NMR (50 MHz, DMSO-d_6) δ 21.5 (t), 24.7 (t), 38.9 (t), 45.8 (t), 55.2 (q), 110.7 (d), 120.4 (d), 120.7 (s), 123.1 (d), 124.3 (s), 125.4 (s), 127.5 (d), 127.6 (d), 128.5 (2 × d), 129.0 (d), 129.1 (s), 129.5 (2 × d), 130.9 (s), 156.0 (s), 193.8 (s). Anal. Calcd for $C_{22}H_{21}NO_2$: C, 79.73; H, 6.39; N, 4.23. Found: C, 79.51; H, 6.61; N, 4.45.

*Data for 2-(2,3-Dimethoxybenzyl)-1-phenyl-2,5,6,7-tetrahydro-4H-isoindol-4-one (**12f**).* This compound was obtained from reaction of **11a** with 2,3-dimethoxybenzyl chloride after 24 h. Yellow oil; yield 62%. IR (cm^{-1}) 1653 (CO). ^1H NMR (200 MHz, DMSO-d_6) δ 1.87−1.99 (m, 2H, CH_2), 2.37 (t, 2H, J = 6.0 Hz, CH_2), 2.57 (t, 2H, J = 6.0 Hz, CH_2), 3.51 (s, 3H, CH_3), 3.77 (s, 3H, CH_3), 5.15 (s, 2H, CH_2), 6.28 (m, 1H, Ar), 6.97 (d, 2H, J = 4.8 Hz, Ar), 7.29−7.47 (m, 6H, H-3, Ar). ^{13}C NMR (50 MHz, DMSO-d_6) δ 21.5 (t), 24.7 (t), 38.9 (t), 45.9 (t), 55.6 (q), 59.6 (q), 112.5 (d), 119.3 (d), 120.7 (s), 123.2 (d), 124.1 (d), 124.4 (s), 127.5 (d), 128.2 (2 × d), 129.0 (s), 129.5 (2 × d), 130.9 (s), 131.0 (s), 145.5 (s), 152.1 (s), 193.8 (s). Anal. Calcd for $C_{23}H_{23}NO_3$: C, 76.43; H, 6.41; N, 3.88. Found: C, 76.75; H, 6.72; N, 3.52.

*Data for 2-(2,5-Dimethoxybenzyl)-1-phenyl-2,5,6,7-tetraydro-4H-isoindol-4-one (**12g**).* This compound was obtained from reaction of **11a** with 2,5-dimethoxybenzyl chloride after 2 h. Light-brown solid; yield 93%; mp 125−126 °C. IR (cm^{-1}) 1651 (CO). ^1H NMR (200 MHz, DMSO-d_6) δ 1.86−1.99 (m, 2H, CH_2), 2.37 (t, 2H, J = 6.0 Hz, CH_2), 2.57 (t, 2H, J = 6.0 Hz, CH_2), 3.59 (s, 3H, CH_3), 3.63 (s, 3H, CH_3), 5.08 (s, 2H, CH_2), 6.19 (s, 1H, Ar), 6.76−6.90 (m, 2H, Ar), 7.30−7.48 (m, 6H, H-3, Ar). ^{13}C NMR (50 MHz, DMSO-d_6) δ 21.4 (t), 24.7 (t), 38.9 (t), 45.9 (t), 55.2 (q), 55.7 (q), 111.6 (d), 112.7 (d), 114.5 (d), 120.7 (s), 123.1 (d), 124.4 (s), 126.4 (s), 127.5 (d), 128.5 (2 × d), 129.0 (s), 129.5 (2 × d), 131.0 (s), 150.2 (s), 153.0 (s), 193.8 (s). Anal. Calcd for $C_{23}H_{23}NO_3$: C, 76.43; H, 6.41; N, 3.88. Found: C, 76.72; H, 6.75; N, 3.57.

*Data for 2-(3,4-Dimethoxybenzyl)-1-phenyl-2,5,6,7-tetrahydro-4H-isoindol-4-one (**12h**).* This compound was obtained from reaction of **11a** with 3,4-dimethoxybenzyl chloride after 24 h. Yellow oil; yield 62%. IR (cm^{-1}) 1660 (CO). ^1H NMR (200 MHz, DMSO-d_6) δ 1.85−1.97 (m, 2H, CH_2), 2.35 (t, 2H, J = 6.0 Hz, CH_2), 2.54 (t, 2H, J = 6.0 Hz, CH_2), 3.57 (s, 3H, CH_3), 3.68 (s, 3H, CH_3), 5.09 (s, 2H, CH_2), 6.46−6.50 (m, 2H, Ar), 6.82 (d, 1H, J = 8.7 Hz, Ar), 7.32−7.51 (m, 5H, Ar), 7.54 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 21.8 (t), 25.2 (t), 39.4 (t), 50.9 (t), 55.7 (q), 55.9 (q), 111.7 (d), 112.1 (d), 120.1 (d), 121.1 (s), 123.6 (d), 124.0 (s), 125.1 (s), 128.0 (d), 129.1 (2 d), 130.2 (2 d), 130.3 (s), 131.7 (s), 148.6 (s), 148.9 (s), 194.3 (s). Anal. Calcd for $C_{23}H_{23}NO_3$: C, 76.43; H, 6.41; N, 3.88. Found: C, 76.69; H, 6.28; N, 3.39.

*Data for 2-(3,5-Dimethoxybenzyl)-1-phenyl-2,5,6,7-tetrahydro-4H-isoindol-4-one (**12i**).* This compound was obtained from reaction of **11a** with 3,5-dimethoxybenzyl chloride after 2 h. Brown solid; yield 76%; mp 110−111 °C. IR (cm^{-1}) 1653 (CO). ^1H NMR (200 MHz, DMSO-d_6) δ 1.85−1.99 (m, 2H, CH_2), 2.37 (t, 2H, J = 6.0 Hz, CH_2), 2.56 (t, 2H, J = 6.0 Hz, CH_2), 3.62 (s, 6H, 2 × CH_3), 5.10 (s, 2H, CH_2), 6.06 (d, 2H, J = 2.2 Hz, Ar), 6.34 (t, 1H, J = 2.2 Hz, Ar), 7.29−7.49 (m, 5H, Ar), 7.55 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 21.4 (t), 24.7 (t), 38.9 (t), 50.5 (t), 55.0 (2 q), 98.9 (d), 105.1 (2 d), 120.8 (s), 123.4 (d), 124.6 (s), 127.5 (d), 128.6 (2 × d), 129.8 (s), 129.6 (2 × d), 131.0 (s), 140.0 (s), 160.5 (2 × s), 193.8 (s). Anal. Calcd for $C_{23}H_{23}NO_3$: C, 76.43; H, 6.41; N, 3.88. Found: C, 76.79; H, 6.71; N, 3.59.

*Data for 1-Phenyl-2-(3,4,5-trimethoxybenzyl)-2,5,6,7-tetrahydro-4H-isoindol-4-one (**12j**).* This compound was obtained from reaction of **11a** with 3,4,5-trimethoxybenzyl chloride after 16 h. White solid; yield 84%; mp 151−152 °C. IR (cm^{-1}) 1651 (CO). ^1H NMR (200 MHz, DMSO-d_6) δ 1.85−1.97 (m, 2H, CH_2), 2.35 (t, 2H, J = 6.6 Hz, CH_2), 2.54 (t, 2H, J = 6.6 Hz, CH_2), 3.58 (s, 3H, CH_3), 3.59 (s, 6H, 2 × CH_3), 5.09 (s, 2H, CH_2), 6.23 (s, 2H, H-2′, H-6′), 7.37−7.52 (m, 5H, Ar), 7.62 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 21.4 (t), 24.7 (t), 39.9 (t), 50.8 (t), 55.6 (2 × q), 59.9 (q), 105.0 (2 × d), 120.7 (s), 123.2 (d), 124.7 (s), 127.5 (d), 128.6 (s), 128.7 (2 × d), 129.8 (2 × d), 131.3 (s), 132.9 (s), 136.7 (s), 152.7 (2 × s), 193.8 (s). Anal. Calcd for $C_{24}H_{25}NO_4$: C, 73.64; H, 6.44; N, 3.58. Found: C, 73.77; H, 6.29; N, 3.74.

*Data for 2-(4-Methoxy-3-nitrobenzyl)-1-phenyl-2,5,6,7-tetrahydro-4H-isoindol-4-one (**12k**).* This compound was obtained from reaction of **11a** with 3-nitro-4-methoxybenzyl chloride after 1 h and 30 min. Brown solid; yield 83%; mp 196−197 °C. IR (cm^{-1}) 1647 (CO), 1529 (NO_2). ^1H NMR (200 MHz, DMSO-d_6) δ 1.86−1.98 (m, 2H, CH_2), 2.37 (t, 2H, J = 6.6 Hz, CH_2), 2.54 (t, 2H, J = 6.6 Hz, CH_2), 3.86 (s, 3H, CH_3), 5.21 (s, 2H, CH_2), 7.11−7.49 (m, 8H, Ar), 7.63 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 21.3 (t), 24.6 (t), 38.9 (t), 49.3 (t), 56.6 (q), 114.5 (d), 120.9 (s), 123.3 (d), 123.8 (d), 124.9 (s), 127.7 (d), 128.5 (2 × d), 128.6 (s), 129.6 (2 × d), 129.8 (s), 130.8 (s), 133.2 (d), 138.6 (s), 151.3 (s), 193.9 (s). Anal. Calcd for $C_{22}H_{20}N_2O_4$: C, 70.20; H, 5.36; N, 7.44. Found: C, 70.09; H, 5.48; N, 7.32.

*Data for 2-Benzyl-6,6-dimethyl-1-phenyl-2,5,6,7-tetrahydro-4H-isoindol-4-one (**12n**).* This compound was obtained from reaction of **11b** with benzyl bromide after 24 h. Light-brown solid; yield 76%; mp 122−123 °C. IR (cm^{-1}) 1655 (CO). ^1H NMR (200 MHz, DMSO-d_6) δ 0.96 (s, 6H, 2 × CH_3), 2.25 (s, 2H, CH_2), 2.46 (s, 2H, CH_2), 5.21 (s, 2H, CH_2), 6.87−6.92 (m, 2H, Ar), 7.15−7.46 (m, 8H, Ar), 7.56 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 28.2 (2 × q), 35.3 (t), 50.7 (t), 52.6 (t), 119.8 (s), 123.2 (d), 123.3 (s), 123.4 (s), 126.7 (2 × d), 127.4 (d), 127.5 (d), 128.4 (2 × d), 128.5 (2 × d), 129.5 (2 × d), 129.6 (s), 130.9 (s), 137.7 (s), 193.4 (s). Anal. Calcd for $C_{23}H_{23}NO$: C, 83.85; H, 7.04; N, 4.25. Found: C, 84.03; H, 6.84; N, 4.49.

*Data for 2-(4-Methoxybenzyl)-6,6-dimethyl-1-phenyl-2,5,6,7-tetrahydro-4H-isoindol-4-one (**12o**).* This compound was obtained from

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reaction of **11b** with 4-methoxybenzyl chloride after 1 h and 30 min. White solid; yield 88%; mp 107−108 °C. IR (cm^{-1}) 1655 (CO). ^1H NMR (200 MHz, DMSO-d_6) δ 0.95 (s, 6H, 2 × CH_3), 2.24 (s, 2H, CH_2), 2.45 (s, 2H, CH_2), 3.68 (s, 3H, CH_3), 5.11 (s, 2H, CH_2), 6.79 (d, 2H, J = 8.9 Hz, H-3′, H-5′), 6.86 (d, 2H, J = 8.9 Hz, H-2′, H-6′), 7.29−7.49 (m, 5H, Ar), 7.50 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 28.0 (2 × q), 35.3 (t), 50.1 (t), 52.6 (t), 55.0 (q), 113.9 (2 × d), 119.7 (s), 122.9 (d), 123.3 (s), 123.4 (s), 127.5 (d), 128.3 (2 × d), 128.6 (2 × d), 129.4 (s), 129.5 (s), 129.6 (2 × d), 131.0 (s), 158.5 (s), 193.3 (s). Anal. Calcd for $C_{24}H_{25}NO_2$: C, 80.19; H, 7.01; N, 3.90. Found: C, 80.38; H, 6.82; N, 4.19.

*Data for 2-(4-Methoxybenzyl)-1-(3,4,5-trimethoxyphenyl)-2,5,6,7-tetrahydro-4H-isoindol-4-one (**12p**).* This compound was obtained from reaction of **11c** with 4-methoxybenzyl chloride after 1 h. White solid; yield 88%; mp 160−161 °C. IR (cm^{-1}) 1691 (CO). ^1H NMR (200 MHz, DMSO-d_6) δ 1.87−1.99 (m, 2H, CH_2), 2.36 (t, 2H, J = 6.1 Hz, CH_2), 2.59 (t, 2H, J = 6.1 Hz, CH_2), 3.68 (s, 6H, 2 × CH_3), 3.69 (s, 3H, CH_3), 3.70 (s, 3H, CH_3), 5.12 (s, 2H, CH_2), 6.50 (s, 2H, H-2′, H-6′), 6.84 (d, 2H, J = 8.9 Hz, H-3″, H-5″), 6.92 (d, 2H, J = 8.9 Hz, H-2″, H-6″), 7.49 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 21.5 (t), 24.7 (t), 38.9 (t), 50.3 (t), 55.1 (q), 55.8 (2 × q), 60.0 (q), 107.0 (2 × d), 113.9 (2 × d), 120.5 (s), 122.6 (d), 124.4 (s), 126.4 (s), 128.3 (2 × d), 128.9 (s), 129.8 (s), 136.8 (s), 152.7 (2 × s), 158.5 (s), 193.8 (s). Anal. Calcd for $C_{25}H_{27}NO_5$: C, 71.24; H, 6.46; N, 3.32. Found: C, 71.32; H, 6.34; N, 3.46.

*Data for 2-(4-Methoxy-3-nitrobenzyl)-1-(3,4,5-trimethoxyphenyl)-2,5,6,7-tetrahydro-4H-isoindol-4-one (**12q**).* This compound was obtained from reaction of **11c** with 4-methoxy-3-nitro-benzyl chloride after 1 h. Brown solid; yield 74%; mp 192−193 °C. IR (cm^{-1}) 1697 (CO), 1531 (NO_2). ^1H NMR (200 MHz, DMSO-d_6) δ 1.86−1.99 (m, 2H, CH_2), 2.36 (t, 2H, J = 6.0 Hz, CH_2), 2.57 (t, 2H, J = 6.0 Hz, CH_2), 3.69 (s, 3H, CH_3), 3.71 (s, 6H, 2 × CH_3), 3.86 (s, 3H, CH_3), 5.21 (s, 2H, CH_2), 6.50 (s, 2H, H-2′, H-6′), 7.27 (s, 2H, Ar), 7.37 (s, 1H, Ar), 7.62 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 21.3 (t), 24.6 (t), 39.0 (t), 49.5 (t), 55.8 (2 × q), 56.7 (q), 60.0 (q), 107.1 (2 × d), 114.5 (d), 120.7 (s), 123.0 (d), 123.9 (d), 124.7 (s), 126.2 (s), 128.7 (s), 130.1 (s), 133.4 (d), 137.0 (s), 138.6 (s), 151.3 (s), 152.9 (2 × s), 193.8 (s). Anal. Calcd for $C_{25}H_{26}N_2O_7$: C, 64.37; H, 5.62; N, 6.01. Found: C, 64.44; H, 5.79; N, 5.93.

*Data for 4-[2-(4-Methoxybenzyl)-4-oxo-4,5,6,7-tetrahydro-2H-isoindol-1-yl]phenyl Acetate (**12s**).* This compound was obtained from reaction of **11e** with 4-methoxybenzyl chloride after 24 h. Light-brown solid; yield 64%; mp 84−85 °C. IR (cm^{-1}) 1655 (CO), 1620 (CO). ^1H NMR (200 MHz, DMSO-d_6) δ 1.85−1.98 (m, 2H, CH_2), 2.29 (s, 3H, CH_3), 2.36 (t, 2H, J = 6.1 Hz, CH_2), 2.55 (t, 2H, J = 6.1 Hz, CH_2), 3.68 (s, 3H, CH_3), 5.10 (s, 2H, CH_2), 6.79 (d, 2H, J = 8.9 Hz, Ar), 6.87 (d, 2H, J = 8.9 Hz, Ar), 7.20 (d, 2H, J = 8.6 Hz, Ar), 7.35 (d, 2H, J = 8.6 Hz, Ar), 7.50 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 20.9 (q), 21.4 (t), 24.7 (t), 39.3 (t), 50.1 (t), 55.0 (q), 113.9 (2 × d), 120.6 (s), 122.0 (2 × d), 123.1 (d), 124.8 (s), 127.9 (s), 128.5 (2 × d), 129.3 (s), 129.6 (s), 130.7 (2 × d), 149.7 (s), 158.6 (s), 169.1 (s), 193.8 (s). Anal. Calcd for $C_{24}H_{23}NO_4$: C, 74.02; H, 5.95; N, 3.60. Found: C, 74.33; H, 5.61; N, 3.73.

*Synthesis of 2-(4-Methoxyphenyl)-1-phenyl-2,5,6,7-tetrahydro-4H-isoindol-4-one (**12m**).* To a solution of **11a** (20 mmol) in *N*-methyl-2-pyrrolidone (40 mL), anhydrous K_2CO_3 (20 mmol) was added under nitrogen atmosphere and the reaction mixture was stirred at room temperature for 1 h. Then Cu(I)Br (40 mmol) was added and the reaction mixture was stirred at room temperature for 1 h, and finally 4-iodoanisole (70 mmol) was added. The reaction mixture was heated under reflux for 48 h. After cooling, HCl (5%, 20 mL) was added and the mixture was stirred for 1 h, then ethyl acetate (20 mL) was added and the mixture was stirred for further 30 min. Then the resulting solution was filtered through Celite and washed with ethyl acetate (20 mL). The organic layer was stirred for 1 h with ice and brine, separated, dried over Na_2SO_4, and the solvent evaporated at reduced pressure. The crude product was purified by chromatography column using dichloromethane/ethyl acetate (95/5) as eluent. Light-brown solid; yield 60%; mp 159−160 °C. IR (cm^{-1}) 1657 (CO). ^1H NMR (200 MHz, DMSO-d_6) δ 1.92−2.05 (m, 2H, CH_2), 2.44 (t, 2H, J = 6.2 Hz, CH_2), 2.69 (t, 2H, J = 6.2 Hz, CH_2), 3.75 (s, 3H, CH_3), 6.91 (d, 2H, J = 8.9 Hz, H-3′, H-5′), 7.04−7.34 (m, 7H, Ar), 7.53 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 21.8 (t), 24.5 (t), 39.0 (t), 55.3 (q), 114.3 (2 × d), 121.6 (s), 123.8 (d), 125.2 (s), 125.7 (d), 125.8 (2 × d), 128.2 (2 × d), 128.6 (s), 129.2 (2 × d), 130.9 (s), 131.9 (s), 158.3 (s), 194.1 (s). Anal. Calcd for $C_{21}H_{19}NO_2$: C, 79.47; H, 6.03; N, 4.41. Found: C, 79.55; H, 5.88; N, 4.54.

*Procedure for the Synthesis of 2-(4-Methoxybenzyl)-1-(4-methoxyphenyl)-2,5,6,7-tetrahydro-4H-isoindol-4-one (**12u**).* To a solution of **12t** (10 mmol) in THF (15 mL) NaH (0.24 g, 10 mmol) was added at 0 °C, and the reaction was stirred for 1 h at room temperature. Iodomethane (0.3 mL, 10 mmol) was added at 0 °C, and the reaction mixture was stirred at room temperature for 20 h. Then the reaction mixture was poured into ice and brine. The aqueous solution was extracted with dichloromethane (3 × 50 mL). The organic phase was dried over Na_2SO_4 and the solvent evaporated at reduced pressure. The crude product was purified by chromatography column using dichloromethane/ethyl acetate (98/2) as eluent. Yellow oil; yield 88%. IR (cm^{-1}) 1647 (CO). ^1H NMR (200 MHz, DMSO-d_6) δ 1.85−1.99 (m, 2H, CH_2), 2.35 (t, 2H, J = 6.4 Hz, CH_2), 2.51 (t, 2H, J = 6.4 Hz, CH_2), 3.69 (s, 3H, CH_3), 3.79 (s, 3H, CH_3), 5.05 (s, 2H, CH_2), 6.81 (d, 2H, J = 9.0 Hz, Ar), 6.87 (d, 2H, J = 9.0 Hz, Ar), 6.99 (d, 2H, J = 8.7 Hz, Ar), 7.22 (d, 2H, J = 8.7 Hz, Ar), 7.45 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 21.4 (t), 24.7 (t), 38.9 (t), 49.8 (t), 55.0 (q), 55.1 (q), 113.9 (2 × d), 114.0 (2 × d), 120.6 (s), 122.4 (d), 123.1 (s), 124.1 (s), 128.4 (2 × d), 128.6 (s), 129.5 (s), 131.0 (2 × d), 158.5 (s), 158.6 (s), 193.8 (s). Anal. Calcd for $C_{23}H_{23}NO_3$: C, 76.43; H, 6.41; N, 3.88. Found: C, 76.34; H, 6.59; N, 3.73.

*Procedure for the Synthesis of 2-(3-Amino-4-methoxybenzyl)-1-substituted-2,5,6,7-tetrahydro-4H-isoindol-4-one (**12l,r**).* To a solution of **12k,q** (1.5 mmol) in ethanol, palladium 10% on carbon was added and the reaction mixture was stirred under hydrogen atmosphere for 24 h. The solution was filtered, and the filtrate was evaporated at reduced pressure. The crude product was purified by chromatography column using dichloromethane/ethyl acetate (90/10) as eluent.

*Data for 2-(3-Amino-4-methoxybenzyl)-1-phenyl-2,5,6,7-tetrahydro-4H-isoindol-4-one (**12l**).* This compound was obtained from reaction of **12k**. White solid; yield 98%; mp 168−169 °C. IR (cm^{-1}) 3458−3375 (NH_2), 1649 (CO). ^1H NMR (200 MHz, DMSO-d_6) δ 1.87−1.99 (m, 2H, CH_2), 2.36 (t, 2H, J = 6.6 Hz, CH_2), 2.56 (t, 2H, J = 6.6 Hz, CH_2), 3.70 (s, 3H, CH_3), 4.72 (s, 2H, NH_2), 4.96 (s, 2H, CH_2), 6.10 (dd, 1H, J = 8.1, 2.1 Hz, Ar), 6.32 (d, 1H, J = 2.1 Hz, Ar), 6.65 (d, 1H, J = 8.1 Hz, Ar), 7.29−7.44 (m, 6H, H-3, Ar). ^{13}C NMR (50 MHz, DMSO-d_6) δ 21.5 (t), 24.7 (t), 38.9 (t), 50.4 (t), 55.2 (q), 110.2 (d), 112.1 (d), 114.8 (d), 120.6 (s), 123.0 (d), 124.3 (s), 127.4 (d), 128.5 (2 × d), 128.9 (s), 129.6 (2 × d), 129.7 (s), 131.0 (s), 137.7 (s), 145.7 (s), 193.7 (s). Anal. Calcd for $C_{22}H_{22}N_2O_2$: C, 76.28; H, 6.40; N, 8.09. Found: C, 76.39; H, 6.61; N, 7.92.

*Data for 2-(3-Amino-4-methoxybenzyl)-1-(3,4,5-trimethoxyphenyl)-2,5,6,7-tetrahydro-4H-isoindol-4-one (**12r**).* This compound was obtained from reaction of **12q**. White solid; yield 95%; mp 164−165 °C. IR (cm^{-1}) 3452−3373 (NH_2), 1650 (CO). ^1H NMR (200 MHz, DMSO-d_6) δ 1.90−1.99 (m, 2H, CH_2), 2.35 (t, 2H, J = 5.8 Hz, CH_2), 2.61 (t, 2H, J = 5.8 Hz, CH_2), 3.67 (s, 6H, 2 × CH_3), 3.68 (s, 3H, CH_3), 3.71 (s, 3H, CH_3), 4.76 (s, 2H, NH_2), 4.97 (s, 2H, CH_2), 6.15−6.20 (m, 1H, Ar), 6.36 (s, 1H, Ar), 6.51 (s, 2H, H-2′, H-6′), 6.70 (d, 1H, J = 8.1 Hz, Ar), 7.41 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 21.5 (t), 24.8 (t), 39.2 (t), 50.7 (t), 55.3 (q), 55.7 (2 × q), 60.0 (q), 106.9 (2 × d), 110.9 (d), 111.4 (d), 111.9 (d), 112.1 (s), 114.5 (d), 114.7 (s), 120.6 (s), 124.2 (s), 130.4 (s), 136.9 (s), 137.8 (s), 145.7 (s), 152.7 (2 × s), 194.0 (s). Anal. Calcd for $C_{25}H_{28}N_2O_5$: C, 68.79; H, 6.47; N, 6.42. Found: C, 68.65; H, 6.39; N, 6.60.

*Procedure for the Synthesis of 5-(Hydroxymethylidene)-2-substituted-2,5,6,7-tetrahydro-4H-isoindol-4-ones (**13**).* To a suspension of *t*-BuOK (3.7 g, 36 mmol) in anhydrous toluene (30 mL), a solution of the suitable derivative **12** (12 mmol) in anhydrous toluene (40 mL) was added dropwise under N_2 at 0 °C. After 3 h stirring at room temperature, the reaction was cooled at 0 °C and a solution of

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ethyl formate (2.91 mL, 36 mmol) in anhydrous toluene (20 mL) was added and the mixture was kept stirring at room temperature for 24 h, then the solvent was removed at reduced pressure. The residue was dissolved in water, and the solution was washed with diethyl ether. The acqueous solution was then acidified with HCl 6 M and the precipitate was filtered and dried, in absence the solution was extracted with dichloromethane (3 × 30 mL). The organic phase was dried over Na_2SO_4 and the solvent evaporated at reduced pressure. The crude product was purified by chromatography column using dichloromethane as eluent.

*Data for 5-(Hydroxymethylidene)-2-(3-methoxybenzyl)-1-phenyl-2,5,6,7-tetrahydro-4H-isoindol-4-one (**13d**).* This compound was obtained from reaction of **12d**. Brown solid; yield 72%; mp 110−111 °C. IR (cm^{-1}) 2935 (OH), 1633 (CO). ^1H NMR (200 MHz, $CDCl_3$) δ 2.49 (t, 2H, J = 6.3 Hz, CH_2), 2.62 (t, 2H, J = 6.3 Hz, CH_2), 3.73 (s, 3H, CH_3), 5.02 (s, 2H, CH_2), 6.51−6.61 (m, 2H, Ar), 6.76−6.81 (m, 1H, Ar), 7.16−7.42 (m, 7H, H-3, Ar), 7.53 (s, 1H, CH), 14.39 (bs, 1H, OH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.2 (t), 25.8 (t), 51.4 (t), 55.2 (q), 109.5 (s), 112.9 (d), 113.1 (d), 119.3 (d), 120.5 (s), 123.1 (d), 123.3 (s), 127.9 (d), 128.6 (2 × d), 128.7 (s), 129.9 (d), 130.0 (2 × d), 131.0 (s), 138.6 (s), 159.9 (s), 166.1 (d), 186.6 (s). Anal. Calcd for $C_{23}H_{21}NO_3$: C, 76.86; H, 5.89; N, 3.90. Found: C, 76.72; H, 6.12; N, 4.19.

*Data for 5-(Hydroxymethylidene)-2-(2-methoxybenzyl)-1-phenyl-2,5,6,7-tetrahydro-4H-isoindol-4-one (**13e**).* This compound was obtained from reaction of **12e**. Yellow oil; yield 71%. IR (cm^{-1}) 2933 (OH), 1637 (CO). ^1H NMR (200 MHz, $CDCl_3$) δ 2.49 (t, 2H, J = 6.7 Hz, CH_2), 2.63 (t, 2H, J = 6.7 Hz, CH_2), 3.77 (s, 3H, CH_3), 5.05 (s, 2H, CH_2), 6.73−6.89 (m, 3H, Ar), 7.21−7.45 (m, 7H, H-3, Ar), 7.50 (s, 1H, CH), 14.42 (bs, 1H, OH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.2 (t), 25.9 (t), 46.6 (t), 55.3 (q), 109.6 (s), 110.3 (d), 120.2 (s), 120.7 (d), 123.1 (s), 123.3 (d), 125.2 (s), 127.7 (d), 128.5 (2 × d), 128.7 (d), 129.3 (d), 130.0 (2 × d), 130.1 (s), 131.2 (s), 156.7 (s), 165.6 (d), 186.9 (s). Anal. Calcd for $C_{23}H_{21}NO_3$: C, 76.86; H, 5.89; N, 3.90. Found: C, 76.52; H, 6.24; N, 4.15.

*Data for 2-(2,3-Dimethoxybenzyl)-5-(hydroxymethylidene)-1-phenyl-2,5,6,7-tetrahydro-4H-isoindol-4-one (**13f**).* This compound was obtained from reaction of **12f**. Brown solid; yield 70%; mp 115−116 °C. IR (cm^{-1}) 2935 (OH), 1633 (CO). ^1H NMR (200 MHz, $CDCl_3$) δ 2.49 (t, 2H, J = 6.2 Hz, CH_2), 2.64 (t, 2H, J = 6.2 Hz, CH_2), 3.62 (s, 3H, CH_3), 3.85 (s, 3H, CH_3), 5.06 (s, 2H, CH_2), 6.47 (d, 1H, J = 7.5 Hz, Ar), 6.84−7.02 (m, 2H, Ar), 7.27−7.45 (m, 6H, H-3, Ar), 7.51 (s, 1H, CH), 14.39 (bs, 1H, OH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.1 (t), 25.8 (t), 46.7 (t), 55.7 (q), 60.2 (q), 109.5 (s), 112.4 (d), 120.2 (s), 120.5 (d), 123.1 (d), 124.3 (d), 127.8 (d), 128.5 (2 × d), 128.6 (s), 130.1 (2 × d), 130.2 (s), 130.7 (s), 131.1 (s), 146.4 (s), 152.6 (s), 165.7 (d), 186.8 (s). Anal. Calcd for $C_{24}H_{23}NO_4$: C, 74.02; H, 5.95; N, 3.60. Found: C, 74.32; H, 6.12; N, 3.39.

*Data for 2-(2,5-Dimethoxybenzyl)-5-(hydroxymethylidene)-1-phenyl-2,5,6,7-tetrahydro-4H-isoindol-4-one (**13g**).* This compound was obtained from reaction of **12g**. Yellow oil; yield 63%. IR (cm^{-1}) 2929 (OH), 1641 (CO). ^1H NMR (200 MHz, $CDCl_3$) δ 2.49 (t, 2H, J = 6.3 Hz, CH_2), 2.63 (t, 2H, J = 6.3 Hz, CH_2), 3.66 (s, 3H, CH_3), 3.72 (s, 3H, CH_3), 5.02 (s, 2H, CH_2), 6.32 (s, 1H, Ar), 6.76 (s, 2H, Ar), 7.25−7.44 (m, 6H, H-3, Ar), 7.52 (s, 1H, CH), 14.40 (bs, 1H, OH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.1 (t), 25.8 (t), 46.6 (t), 55.7 (q), 55.8 (q), 109.5 (s), 111.3 (d), 113.2 (d), 115.2 (d), 120.2 (s), 123.1 (s), 123.4 (d), 126.3 (s), 127.7 (d), 128.5 (2 × d), 128.6 (s), 130.1 (2 × d), 131.2 (s), 150.9 (s), 153.6 (s), 165.8 (d), 186.8 (s). Anal. Calcd for $C_{24}H_{23}NO_4$: C, 74.02; H, 5.95; N, 3.60. Found: C, 74.22; H, 6.22; N, 3.35.

*Data for 2-(3,4-Dimethoxybenzyl)-5-(hydroxymethylidene)-1-phenyl-2,5,6,7-tetrahydro-4H-isoindol-4-one (**13h**).* This compound was obtained from reaction of **12h**. Brown solid; yield 65%; mp 136−137 °C. IR (cm^{-1}) 2933 (OH), 1635 (CO). ^1H NMR (200 MHz, $CDCl_3$) δ 2.48 (t, 2H, J = 6.1 Hz, CH_2), 2.62 (t, 2H, J = 6.1 Hz, CH_2), 3.74 (s, 3H, CH_3), 3.84 (s, 3H, CH_3), 4.98 (s, 2H, CH_2), 6.45 (s, 1H, Ar), 6.55−6.60 (m, 1H, Ar), 6.76 (d, 1H, J = 8.2 Hz, CH_2), 7.22−7.46 (m, 6H, H-3, Ar), 7.51 (s, 1H, CH), 14.41 (bs, 1H, OH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.1 (t), 25.8 (t), 51.4 (t), 55.8 (q), 55.9 (q), 109.5 (s), 110.4 (d), 111.2 (d), 119.8 (d), 120.3 (s), 122.9 (d), 123.4 (s), 127.8 (d), 128.6 (2 × d), 129.2 (s), 129.9 (s), 130.1 (2 × d), 131.1 (s), 148.6 (s), 149.0 (s), 165.8 (d), 186.7 (s). Anal. Calcd for $C_{24}H_{23}NO_4$: C, 74.02; H, 5.95; N, 3.60. Found: C, 74.38; H, 6.20; N, 3.41.

*Data for 2-(3,5-Dimethoxybenzyl)-5-(hydroxymethylidene)-1-phenyl-2,5,6,7-tetrahydro-4H-isoindol-4-one (**13i**).* This compound was obtained from reaction of **12i**. Yellow oil; yield 60%. IR (cm^{-1}) 2933 (OH), 1633 (CO). ^1H NMR (200 MHz, $CDCl_3$) δ 2.49 (t, 2H, J = 6.1 Hz, CH_2), 2.63 (t, 2H, J = 6.1 Hz, CH_2), 3.71 (s, 6H, 2 × CH_3), 4.97 (s, 2H, CH_2), 6.14 (s, 2H, Ar), 6.33−6.35 (m, 1H, Ar), 7.21−7.40 (m, 6H, H-3, Ar), 7.53 (s, 1H, CH), 14.37 (bs, 1H, OH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.1 (t), 25.8 (t), 51.5 (t), 55.3 (2 × q), 99.5 (d), 105.2 (2 × d), 109.5 (s), 113.6 (s), 120.5 (s), 123.2 (d), 123.3 (s), 127.9 (d), 128.6 (2 × d), 130.1 (2 × d), 131.0 (s), 139.4 (s), 161.1 (2 × s), 166.1 (d), 186.6 (s). Anal. Calcd for $C_{24}H_{23}NO_4$: C, 74.02; H, 5.95; N, 3.60. Found: C, 74.33; H, 6.22; N, 3.38.

*Data for 5-(Hydroxymethylidene)-1-phenyl-2-(3,4,5-trimethoxybenzyl)-2,5,6,7-tetrahydro-4H-isoindol-4-one (**13j**).* This compound was obtained from reaction of **12j**. Light-brown solid; yield 88%; mp 146−147 °C. IR (cm^{-1}) 2935 (OH), 1633 (CO). ^1H NMR (200 MHz, $CDCl_3$) δ 2.50 (t, 2H, J = 6.1 Hz, CH_2), 2.63 (t, 2H, J = 6.1 Hz, CH_2), 3.73 (s, 6H, 2 × CH_3), 3.81 (s, 3H, CH_3), 4.98 (s, 2H, CH_2), 6.17 (s, 2H, H-2′, H-6′), 7.23−7.47 (m, 6H, CH, Ar), 7.52 (s, 1H, H-3), 14.38 (d, 1H, J = 5.2 Hz, OH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.1 (t), 25.8 (t), 51.9 (t), 56.0 (2 × q), 60.9 (q), 104.4 (2 × d), 109.5 (s), 120.4 (s), 122.9 (d), 123.6 (s), 127.9 (d), 128.6 (2 × d), 129.9 (s), 130.1 (2 × d), 131.1 (s), 132.3 (s), 137.5 (s), 153.4 (2 × s), 165.8 (d), 186.8 (s). Anal. Calcd for $C_{25}H_{25}NO_5$: C, 71.58; H, 6.01; N, 3.34. Found: C, 71.67; H, 5.89; N, 3.18.

*Data for 2-Benzyl-5-(hydroxymethylidene)-6,6-dimethyl-1-phenyl-2,5,6,7-tetrahydro-4H-isoindol-4-one (**13n**).* This compound was obtained from reaction of **12n**. Brown solid; yield 72%; mp 117−118 °C. IR (cm^{-1}) 2958 (OH), 1624 (CO). ^1H NMR (200 MHz, $CDCl_3$) δ 1.19 (s, 6H, 2 × CH_3), 2.51 (s, 2H, CH_2), 5.05 (s, 2H, CH_2), 6.96−7.00 (m, 2H, Ar), 7.18−7.42 (m, 9H, H-3, Ar), 7.62 (s, 1H, CH), 15.19 (bs, 1H, OH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 29.1 (2 × q), 36.1 (t), 51.4 (t), 118.4 (s), 119.5 (s), 122.3 (s), 122.4 (s), 123.2 (d), 126.9 (2 × d), 127.8 (d), 127.9 (d), 128.5 (2 × d), 128.8 (2 × d), 130.0 (2 × d), 130.8 (s), 130.9 (s), 137.0 (s), 165.5 (d), 187.1 (s). Anal. Calcd for $C_{24}H_{23}NO_2$: C, 80.64; H, 6.49; N, 3.92. Found: C, 80.29; H, 6.20; N, 4.14.

*Data for 5-(Hydroxymethylidene)-2-(4-methoxybenzyl)-6,6-dimethyl-1-phenyl-2,5,6,7-tetrahydro-4H-isoindol-4-one (**13o**).* This compound was obtained from reaction of **12o**. Brown solid; yield 60%; mp 150−151 °C. IR (cm^{-1}) 2958 (OH), 1624 (CO). ^1H NMR (200 MHz, $CDCl_3$) δ 1.18 (s, 6H, 2 × CH_3), 2.50 (s, 2H, CH_2), 3.77 (s, 3H, CH_3), 4.97 (s, 2H, CH_2), 6.80 (d, 2H, J = 8.8 Hz, H-3′, H-5′), 6.93 (d, 2H, J = 8.8 Hz, H-2′, H-6′), 7.18−7.45 (m, 6H, H-3, Ar), 7.61 (s, 1H, CH), 15.18 (bs, 1H, OH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 29.1 (2 × q), 36.1 (t), 51.0 (t), 55.3 (q), 114.2 (2 × d), 118.5 (s), 119.4 (s), 122.3 (s), 122.4 (s), 123.0 (d), 127.8 (d), 128.5 (2 × d), 128.6 (2 × d), 128.9 (s), 130.1 (2 × d), 130.8 (s), 131.0 (s), 159.2 (s), 165.4 (d), 187.1 (s). Anal. Calcd for $C_{25}H_{25}NO_3$: C, 77.49; H, 6.50; N, 3.61. Found: C, 77.11; H, 6.27; N, 3.86.

*Data for 5-(Hydroxymethylidene)-2-(4-methoxybenzyl)-1-(3,4,5-trimethoxyphenyl)-2,5,6,7-tetrahydro-4H-isoindol-4-one (**13p**).* This compound was obtained from reaction of **12p**. Yellow oil; yield 65%. IR (cm^{-1}) 2956 (OH), 1622 (CO). ^1H NMR (200 MHz, $CDCl_3$) δ 2.51 (t, 2H, J = 6.7 Hz, CH_2), 2.64 (t, 2H, J = 6.7 Hz, CH_2), 3.72 (s, 6H, 2 × CH_3), 3.78 (s, 3H, CH_3), 3.88 (s, 3H, CH_3), 5.00 (s, 2H, CH_2), 6.38 (s, 2H, H-2′, H-6′), 6.83 (d, 2H, J = 8.8 Hz, H-3″, H-5″), 6.96 (d, 2H, J = 8.8 Hz, H-2″, H-6″), 7.35 (s, 1H, H-3), 7.53 (s, 1H, CH), 14.37 (s, 1H, OH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.2 (t), 25.8 (t), 51.1 (t), 55.3 (q), 56.0 (2 × q), 60.9 (q), 100.1 (s), 107.2 (2 × d), 109.4 (s), 114.2 (2 × d), 120.3 (s), 122.9 (d), 123.1 (s), 126.4 (s), 128.2 (2 × d), 129.2 (s), 129.9 (s), 153.1 (2 × s), 159.2 (s), 165.9 (d), 186.7 (s). Anal. Calcd for $C_{26}H_{27}NO_6$: C, 69.47; H, 6.05; N, 3.12. Found: C, 69.59; H, 5.92; N, 3.26.

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*Data for 5-(Hydroxymethylidene)-2-(4-methoxybenzyl)-1-(4-methoxyphenyl)-2,5,6,7-tetrahydro-4H-isoindol-4-one (**13u**).* This compound was obtained from reaction of **12u**. Yellow oil; yield 60%. IR (cm^{-1}) 2965 (OH), 1626 (CO). ^1H NMR (200 MHz, $CDCl_3$) δ 2.45−2.63 (m, 4H, 2 × CH_2), 3.78 (s, 3H, CH_3), 3.84 (s, 3H, CH_3), 4.94 (s, 2H, CH_2), 6.80 (d, 2H, J = 6.7 Hz, Ar), 6.93 (dd, 4H, J = 11.4, 2.4 Hz, Ar), 7.15 (d, 2H, J = 8.9 Hz, Ar), 7.30 (s, 1H, H-3), 7.52 (d, 1H, J = 5.8 Hz, CH), 7.52 (d, 1H, J = 5.8 Hz, OH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.1 (t), 25.8 (t), 50.9 (t), 55.3 (q), 55.4 (q), 109.5 (s), 114.0 (2 × d), 114.2 (2 × d), 120.3 (s), 122.5 (d), 123.0 (s), 123.3 (s), 128.6 (2 × d), 128.9 (s), 129.8 (s), 131.4 (2 × d), 159.2 (s), 159.3 (s), 165.7 (d), 186.8 (s). Anal. Calcd for $C_{24}H_{23}NO_4$: C, 74.02; H, 5.95; N, 3.60. Found: C, 74.33; H, 5.61; N, 3.73.

*Procedure for the Synthesis of 5-[(Dimethylamino)methylidene]-2-substituted-2,5,6,7-tetrahydro-4H-isoindol-4-ones (**14**).* To a solution of the suitable ketone **12** (5.3 mmol) in anhydrous toluene (10 mL), the Bredereck reagent, *t*-butoxy-bis-(dimethylamino)-methane (TBDMAM) (3.31 mL, 16 mmol) was added under nitrogen atmosphere and the reaction mixture was heated under reflux for 24 h. After cooling, the solvent was evaporated at reduced pressure. The crude product was used in the next step without any further purification.

*General Procedure for the Synthesis of [1,2]Oxazolo[5,4-e]isoindoles (**15**).* To a solution of the suitable hydroxymethyleneketones **13** or enaminoketones **14** (5 mmol) in ethanol (15 mL), hydroxylamine hydrochloride (0.38 g, 5.5 mmol) was added and the reaction mixture was heated under reflux for 50 min. After cooling, the solvent was evaporated at reduced pressure. The crude product was purified by chromatography column using dichloromethane as eluent.

*Data for 7-Methyl-6-phenyl-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]-isoindole (**15a**).* This compound was obtained from reaction of **13a**. White solid; yield 62%; mp 145−146 °C. ^1H NMR (200 MHz, DMSO-d_6) δ 2.50−2.74 (m, 4H, 2 × CH_2), 3.59 (s, 3H, CH_3), 7.29 (s, 1H, H-8), 7.33−7.52 (m, 5H, Ar), 8.40 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 19.9 (t), 20.9 (t), 35.4 (q), 109.1 (s), 109.6 (s), 118.0 (d), 118.7 (s), 127.8 (d), 129.0 (2 × d), 129.9 (2 × d), 130.5 (s), 131.5 (s), 149.5 (d), 163.0 (s). Anal. Calcd for $C_{16}H_{14}N_2O$: C, 76.78; H, 5.64; N, 11.19. Found: C, 77.09; H, 5.78; N, 10.83.

*Data for 7-Benzyl-6-phenyl-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]-isoindole (**15b**).* This compound was obtained from reaction of **13b**. White solid; yield 60%; mp 139−140 °C. ^1H NMR (200 MHz, DMSO-d_6) δ 2.60−2.78 (m, 4H, 2 × CH_2), 5.17 (s, 2H, CH_2), 6.92 (d, 2H, J = 7.4 Hz, H-2′, H-6′), 7.19−7.47 (m, 9H, H-8, Ar), 8.42 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 19.8 (t), 20.7 (t), 50.7 (t), 109.5 (s), 110.2 (s), 117.5 (d), 119.2 (s), 127.0 (2 × d), 127.7 (d), 128.0 (d), 128.9 (2 × d), 129.0 (2 × d), 130.0 (2 × d), 130.6 (s), 131.6 (s), 138.9 (s), 149.6 (d), 162.9 (s). Anal. Calcd for $C_{22}H_{18}N_2O$: C, 80.96; H, 5.56; N, 8.58. Found: C, 81.21; H, 5.87; N, 8.30.

*Data for 7-(4-Methoxybenzyl)-6-phenyl-5,7-dihydro-4H-[1,2]-oxazolo[5,4-e]isoindole (**15c**).* This compound was obtained from reaction of **13c**. White solid; yield 67%; mp 115−116 °C. ^1H NMR (200 MHz, DMSO-d_6) δ 2.51−2.65 (m, 4H, 2 × CH_2), 3.68 (s, 3H, CH_3), 5.07 (s, 2H, CH_2), 6.78 (d, 2H, J = 8.2 Hz, H-3′, H-5′), 6.88 (d, 2H, J = 8.2 Hz, H-2′, H-6′), 7.30−7.49 (m, 6H, H-8, Ar), 8.41 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 19.3 (t), 20.2 (t), 49.7 (t), 55.0 (q), 108.9 (s), 109.7 (s), 113.8 (2 × d), 116.7 (d), 118.6 (s), 127.5 (d), 128.1 (2 × d), 128.5 (2 × d), 129.6 (2 × d), 130.0 (s), 130.1 (s), 131.2 (s), 149.1 (d), 158.4 (s), 162.4 (s). Anal. Calcd for $C_{23}H_{20}N_2O_2$: C, 77.51; H, 5.66; N, 7.86. Found: C, 77.75; H, 6.00; N, 7.56.

*Data for 7-(3-Methoxybenzyl)-6-phenyl-5,7-dihydro-4H-[1,2]-oxazolo[5,4-e]isoindole (**15d**).* This compound was obtained from reaction of **13d**. White solid; yield 78%; mp 153−154 °C. ^1H NMR (200 MHz, DMSO-d_6) δ 2.64−2.67 (m, 4H, 2 × CH_2), 3.64 (s, 3H, CH_3), 5.13 (s, 2H, CH_2), 6.46−6.53 (m, 2H, Ar), 6.75−6.80 (m, 1H, Ar), 7.16 (t, 1H, J = 7.9 Hz, Ar), 7.29−7.49 (m, 6H, H-8, Ar), 8.41 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 19.3 (t), 20.2 (t), 50.12 (t), 54.9 (q), 109.0 (s), 109.7 (s), 112.3 (d), 112.6 (d), 117.0 (d), 118.7 (d), 127.5 (d), 128.6 (2 × d), 129.5 (2 × d), 129.6 (d), 130.1 (s), 131.1 (s), 139.9 (s), 149.1 (d), 159.2 (s), 159.3 (s), 162.3 (s). Anal. Calcd for $C_{23}H_{20}N_2O_2$: C, 77.51; H, 5.66; N, 7.86. Found: C, 77.69; H, 5.35; N, 8.10.

*Data for 7-(2-methoxybenzyl)-6-phenyl-5,7-dihydro-4H-[1,2]-oxazolo[5,4-e]isoindole (**15e**).* This compound was obtained from reaction of **13e**. White solid; yield 80%; mp 133−134 °C. ^1H NMR (200 MHz, DMSO-d_6) δ 2.64−2.68 (m, 4H, 2 × CH_2), 3.71 (s, 3H, CH_3), 5.10 (s, 2H, CH_2), 6.56−6.61 (m, 1H, Ar), 6.80−6.96 (m, 2H, Ar), 7.18−7.46 (m, 7H, H-8, Ar), 8.42 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 19.3 (t), 20.2 (t), 45.5 (t), 55.2 (q), 108.9 (s), 109.6 (s), 110.5 (d), 116.9 (d), 118.4 (s), 120.4 (d), 126.2 (s), 127.0 (d), 127.5 (d), 128.5 (2 × d), 128.7 (d), 129.4 (2 × d), 130.3 (s), 131.1 (s), 149.1 (d), 155.8 (s), 162.4 (s). Anal. Calcd for $C_{23}H_{20}N_2O_2$: C, 77.51; H, 5.66; N, 7.86. Found: C, 77.75; H, 5.39; N, 8.03.

*Data for 7-(2,3-Dimethoxybenzyl)-6-phenyl-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindole (**15f**).* This compound was obtained from reaction of **13f**. White solid; yield 70%; mp 142−143 °C. ^1H NMR (200 MHz, DMSO-d_6) δ 2.60−2.68 (m, 4H, 2 × CH_2), 3.54 (s, 3H, CH_3), 3.77 (s, 3H, CH_3), 5.13 (s, 2H, CH_2), 6.22−6.31 (m, 1H, Ar), 6.92−7.01 (m, 2H, Ar), 7.27−7.47 (m, 6H, H-8, Ar), 8.42 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 19.3 (t), 20.2 (t), 45.6 (t), 55.6 (q), 59.6 (q), 109.0 (s), 109.6 (s), 112.2 (d), 116.9 (d), 118.4 (s), 119.0 (d), 124.1 (d), 127.5 (d), 128.5 (2 × d), 129.5 (2 × d), 130.3 (s), 131.0 (s), 131.8 (s), 145.3 (s), 149.1 (d), 152.1 (s), 162.3 (s). Anal. Calcd for $C_{24}H_{22}N_2O_3$: C, 74.59; H, 5.74; N, 7.25. Found: C, 74.69; H, 5.42; N, 7.37.

*Data for 7-(2,5-Dimethoxybenzyl)-6-phenyl-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindole (**15g**).* This compound was obtained from reaction of **13g**. White solid; yield 60%; mp 120−121 °C. ^1H NMR (200 MHz, DMSO-d_6) δ 2.62−2.67 (m, 4H, 2 × CH_2), 3.57 (s, 3H, CH_3), 3.66 (s, 3H, CH_3), 5.07 (s, 2H, CH_2), 6.13 (d, 1H, J = 2.9 Hz, Ar), 6.74−6.89 (m, 2H, Ar), 7.29−7.48 (m, 6H, H-8, Ar), 8.42 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 19.3 (t), 20.2 (t), 45.6 (t), 55.2 (q), 55.6 (q), 109.0 (s), 109.6 (s), 111.4 (d), 112.2 (d), 114.0 (d), 116.9 (d), 118.5 (s), 127.3 (s), 127.5 (d), 128.5 (2 × d), 129.4 (2 × d), 130.3 (s), 131.1 (s), 149.1 (d), 150.0 (s), 153.1 (s), 162.3 (s). Anal. Calcd for $C_{24}H_{22}N_2O_3$: C, 74.59; H, 5.74; N, 7.25. Found: C, 74.44; H, 5.99; N, 7.54.

*Data for 7-(3,4-Dimethoxybenzyl)-6-phenyl-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindole (**15h**).* This compound was obtained from reaction of **13h**. White solid; yield 83%; mp 122−123 °C. ^1H NMR (200 MHz, DMSO-d_6) δ 2.59−2.65 (m, 4H, 2 × CH_2), 3.58 (s, 3H, CH_3), 3.68 (s, 3H, CH_3), 5.07 (s, 2H, CH_2), 6.46−6.51 (m, 2H, Ar), 6.82 (d, 1H, J = 8.1 Hz, Ar), 7.34−7.51 (m, 6H, H-8, Ar), 8.41 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 19.8 (t), 20.6 (t), 50.6 (t), 55.7 (q), 55.9 (q), 109.4 (s), 110.1 (s), 111.4 (d), 112.1 (d), 117.3 (d), 119.2 (s), 119.7 (d), 128.0 (d), 129.1 (2 × d), 130.1 (2 × d), 130.5 (s), 130.9 (s), 131.8 (s), 148.5 (s), 148.9 (s), 149.7 (d), 162.9 (s). Anal. Calcd for $C_{24}H_{22}N_2O_3$: C, 74.59; H, 5.74; N, 7.25. Found: C, 74.34; H, 5.69; N, 7.59.

*Data for 7-(3,5-Dimethoxybenzyl)-6-phenyl-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindole (**15i**).* This compound was obtained from reaction of **13i**. White solid; yield 69%; mp 158−159 °C. ^1H NMR (200 MHz, DMSO-d_6) δ 2.63−2.69 (m, 4H, 2 × CH_2), 3.62 (s, 6H, 2 × CH_3), 5.08 (s, 2H, CH_2), 6.08 (d, 2H, J = 2.2 Hz, Ar), 6.34 (t, 1H, J = 2.2 Hz, Ar), 7.31−7.50 (m, 6H, H-8, Ar), 8.41 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 19.3 (t), 20.2 (t), 50.2 (t), 55.0 (2 × q), 98.7 (d), 104.7 (2 × d), 109.0 (s), 109.7 (s), 117.0 (d), 118.7 (s), 127.5 (d), 128.6 (2 × d), 129.6 (2 × d), 130.1 (s), 131.1 (s), 140.7 (s), 149.1 (d), 160.5 (2 × s), 162.3 (s). Anal. Calcd for $C_{24}H_{22}N_2O_3$: C, 74.59; H, 5.74; N, 7.25. Found: C, 74.40; H, 5.82; N, 7.52.

*Data for 7-(3,4,5-Trimethoxybenzyl)-6-phenyl-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindole (**15j**).* This compound was obtained from reaction of **13j**. White solid; yield 76%; mp 186−187 °C. ^1H NMR (200 MHz, DMSO-d_6) δ 2.62−2.67 (m, 4H, 2 × CH_2), 3.57 (s, 3H, CH_3), 3.60 (s, 6H, 2 × CH_3), 5.06 (s, 2H, CH_2), 6.22 (s, 2H, H-2′, H-6′), 7.36−7.53 (m, 6H, H-8, Ar), 8.42 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 19.3 (t), 20.1 (t), 50.4 (t), 55.6 (2 × q), 59.9 (q), 104.5 (2 × d), 108.9 (s), 109.6 (s), 116.9 (d), 118.8 (s), 127.6 (d), 128.7 (2 × d), 129.7 (2 × d), 129.9 (s), 131.4 (s), 133.6 (s), 136.5

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(s), 149.1 (d), 152.7 (2 × s), 162.4 (s). Anal. Calcd for $C_{25}H_{24}N_2O_4$: C, 72.10; H, 5.81; N, 6.73. Found: C, 72.19; H, 5.68; N, 6.65.

*Data for 7-(4-Methoxy-3-nitrobenzyl)-6-phenyl-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindole (**15k**).* This compound was obtained from reaction of **14k**. Pale-yellow solid; yield 62%; mp 174−175 °C. IR (cm^{-1}) 1532 (NO_2). ^1H NMR (200 MHz, DMSO-d_6) δ 2.63−2.67 (m, 4H, 2 × CH_2), 3.85 (s, 3H, CH_3), 5.17 (s, 2H, CH_2), 7.12−7.49 (m, 9H, H-8, Ar), 8.43 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 19.3 (t), 20.1 (t), 49.0 (t), 56.6 (q), 109.1 (s), 109.9 (s), 114.5 (d), 116.9 (d), 119.0 (s), 123.5 (d), 127.7 (d), 128.6 (2 × d), 129.6 (2 × d), 130.0 (s), 130.5 (s), 130.9 (s), 132.9 (d), 138.7 (s), 149.2 (d), 151.2 (s), 162.2 (s). Anal. Calcd for $C_{23}H_{19}N_3O_4$: C, 68.82; H, 4.77; N, 10.47. Found: C, 69.01; H, 4.65; N, 10.56.

*Data for 2-Methoxy-5-[(6-phenyl-4,5-dihydro-7H-[1,2]oxazolo-[5,4-e]isoindol-7-yl)methyl]aniline (**15l**).* This compound was obtained from reaction of **14l**. White solid; yield 60%; mp 157−158 °C. IR (cm^{-1}) 3455−3372 (NH_2). ^1H NMR (200 MHz, DMSO-d_6) δ 2.64−2.69 (m, 4H, 2 × CH_2), 3.70 (s, 3H, CH_3), 4.71 (s, 2H, NH_2), 4.94 (s, 2H, CH_2), 6.11 (d, 1H, J = 7.9 Hz, Ar), 6.34 (s, 1H, Ar), 6.65 (d, 1H, J = 7.9 Hz, Ar), 7.26−7.54 (m, 6H, H-8, Ar), 8.41 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 19.3 (t), 20.2 (t), 50.0 (t), 55.2 (q), 108.8 (s), 109.6 (s), 110.2 (d), 112.0 (d), 114.6 (d), 118.3 (s), 127.5 (d), 127.8 (d), 128.5 (2 × d), 128.7 (s), 129.5 (2 × d), 130.1 (s), 130.5 (s), 131.1 (s), 137.4 (s), 145.7 (d), 162.4 (s). Anal. Calcd for $C_{23}H_{21}N_3O_2$: C, 74.37; H, 5.70; N, 11.31. Found: C, 74.30; H, 5.85; N, 11.48.

*Data for 7-(4-Methoxyphenyl)-6-phenyl-5,7-dihydro-4H-[1,2]-oxazolo[5,4-e]isoindole (**15m**).* This compound was obtained from reaction of **13m**. White solid; yield 76%; mp 146−147 °C. ^1H NMR (200 MHz, $CDCl_3$) δ 2.77 (t, 2H, J = 6.5 Hz, CH_2), 2.89 (t, 2H, J = 6.5 Hz, CH_2), 3.80 (s, 3H, CH_3), 6.81 (d, 2H, J = 8.9 Hz, H-3′, H-5′), 7.03−7.28 (m, 8H, H-8, Ar), 8.13 (s, 1H, H-3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.0 (t), 20.9 (t), 55.4 (q), 109.6 (s), 111.4 (s), 114.2 (2 × d), 117.3 (d), 120.0 (s), 126.8 (2 × d), 126.9 (d), 128.1 (2 × d), 129.7 (2 × d), 130.0 (s), 131.6 (s), 132.9 (s), 148.8 (d), 158.3 (s), 163.1 (s). Anal. Calcd for $C_{22}H_{18}N_2O_2$: C, 77.17; H, 5.30; N, 8.18. Found: C, 77.26; H, 5.09; N, 8.32.

*Data for 7-Benzyl-4,4-dimethyl-6-phenyl-5,7-dihydro-4H-[1,2]-oxazolo[5,4-e]isoindole (**15n**).* This compound was obtained from reaction of **13n**. White solid; yield 84%; mp 149−150 °C. ^1H NMR (200 MHz, DMSO-d_6) δ 1.19 (s, 6H, 2 × CH_3), 2.54 (s, 2H, CH_2), 5.18 (s, 2H, CH_2), 6.87−6.97 (m, 2H, Ar), 7.15−7.47 (m, 9H, H-8, Ar), 8.52 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 28.2 (2 × q), 36.2 (t), 50.2 (t), 109.9 (s), 117.0 (d), 118.1 (s), 118.9 (s), 119.0 (s), 126.3 (2 × d), 127.2 (d), 127.5 (d), 128.5 (2 × d), 128.6 (2 × d), 129.5 (2 × d), 130.8 (s), 131.0 (s), 138.4 (s), 147.6 (d), 160.7 (s). Anal. Calcd for $C_{24}H_{22}N_2O$: C, 81.33; H, 6.26; N, 7.90. Found: C, 81.53; H, 6.15; N, 8.08.

*Data for 7-(4-Methoxybenzyl)-4,4-dimethyl-6-phenyl-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindole (**15o**).* This compound was obtained from reaction of **13o**. White solid; yield 75%; mp 166−167 °C. ^1H NMR (200 MHz, DMSO-d_6) δ 1.18 (s, 6H, 2 × CH_3), 2.52 (s, 2H, CH_2), 3.68 (s, 3H, CH_3), 5.09 (s, 2H, CH_2), 6.88 (d, 2H, J = 9.0 Hz, H-3′, H-5′), 6.80 (d, 2H, J = 9.0 Hz, H-2′, H-6′), 7.28−7.49 (m, 6H, H-8, Ar), 8.51 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 28.2 (2 × q), 36.2 (t), 49.7 (t), 55.0 (q), 109.0 (s), 113.8 (2 × d), 116.7 (d), 118.2 (s), 118.8 (s), 118.9 (s), 127.5 (d), 127.9 (2 × d), 128.6 (2 × d), 129.6 (2 × d), 130.2 (s), 130.7 (s), 131.1 (s), 147.6 (d), 158.4 (s), 160.7 (s). Anal. Calcd for $C_{25}H_{24}N_2O_2$: C, 78.10; H, 6.29; N, 7.29. Found: C, 78.45; H, 5.99; N, 7.43.

*Data for 7-(4-Methoxybenzyl)-6-(3,4,5-trimethoxyphenyl)-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindole (**15p**).* This compound was obtained from reaction of **13p**. White solid; yield 60%; mp 136−137 °C. ^1H NMR (200 MHz, DMSO-d_6) δ 2.67−2.71 (m, 4H, 2 × CH_2), 3.68 (s, 6H, 2 × CH_3), 3.69 (s, 3H, CH_3), 3.70 (s, 3H, CH_3), 5.09 (s, 2H, CH_2), 6.52 (s, 2H, H-2″, H-6″), 6.85 (d, 2H, J = 8.7 Hz, H-3′, H-5′), 6.94 (d, 2H, J = 8.7 Hz, H-2′, H-6′), 7.35 (s, 1H, H-8), 8.41 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 19.3 (t), 20.2 (t), 50.0 (t), 55.1 (q), 55.8 (2 × q), 60.0 (q), 107.0 (2 × d), 108.8 (s), 109.5 (s), 113.9 (2 × d), 116.7 (d), 118.5 (s), 126.5 (s), 128.0 (2 × d), 130.1 (s), 130.5 (s), 136.9 (s), 149.1 (d), 152.7 (2 × s), 158.4 (s), 162.4 (s). Anal. Calcd for $C_{26}H_{26}N_2O_5$: C, 69.94; H, 5.87; N, 6.27. Found: C, 70.08; H, 5.75; N, 6.40.

*Data for 7-(4-Methoxy-3-nitrobenzyl)-6-(3,4,5-trimethoxyphenyl)-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindole (**15q**).* This compound was obtained from reaction of **14q**. White solid; yield 62%; mp 184−185 °C. IR (cm^{-1}) 1535 (NO_2). ^1H NMR (200 MHz, DMSO-d_6) δ 2.65−2.69 (m, 4H, 2 × CH_2), 3.69 (s, 3H, CH_3), 3.71 (s, 6H, 2 × CH_3), 3.86 (s, 3H, CH_3), 5.18 (s, 2H, CH_2), 6.52 (s, 2H, Ar), 7.28 (s, 2H, Ar), 7.39 (s, 1H, H-8), 7.46 (s, 1H, Ar), 8.42 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 19.2 (t), 20.0 (t), 49.2 (t), 55.8 (2 × q), 56.7 (q), 60.0 (q), 107.1 (2 × d), 109.0 (s), 109.7 (s), 114.5 (d), 116.6 (d), 118.9 (s), 123.6 (d), 126.4 (s), 130.0 (s), 130.8 (s), 133.1 (d), 137.0 (s), 138.7 (s), 149.2 (d), 151.2 (s), 152.9 (2 × s), 162.3 (s). Anal. Calcd for $C_{26}H_{25}N_3O_7$: C, 63.54; H, 5.13; N, 8.55. Found: C, 63.42; H, 5.22; N, 8.61.

*Data for 2-Methoxy-5-{[6-(3,4,5-trimethoxyphenyl)-4,5-dihydro-7H-[1,2]oxazolo[5,4-e]isoindol-7-yl]methyl}aniline (**15r**).* This compound was obtained from reaction of **14r**. White solid; yield 58%; mp 145−146 °C. IR (cm^{-1}) 3453−3371 (NH_2). ^1H NMR (200 MHz, DMSO-d_6) δ 2.67−2.72 (m, 4H, 2 × CH_2), 3.66 (s, 6H, 2 × CH_3), 3.68 (s, 3H, CH_3), 3.71 (s, 3H, CH_3), 4.87−5.02 (m, 4H, CH_2, NH_2), 6.22 (d, 1H, J = 8.1 Hz, Ar), 6.40 (s, 1H, Ar), 6.52 (s, 2H, Ar), 6.71 (d, 1H, J = 8.1 Hz, Ar), 7.29 (s, 1H, H-8), 8.42 (s, 1H, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ 19.3 (t), 20.3 (t), 50.3 (t), 55.4 (q), 55.7 (2 × q), 60.0 (q), 107.0 (2 × d), 108.7 (s), 109.3 (s), 110.4 (d), 111.9 (d), 114.4 (d), 116.7 (d), 118.2 (s), 126.5 (s), 130.1 (s), 131.0 (s), 136.8 (s), 137.4 (s), 145.6 (s), 149.1 (d), 152.7 (2 × s), 162.5 (s). Anal. Calcd for $C_{26}H_{27}N_3O_5$: C, 67.66; H, 5.90; N, 9.10. Found: C, 67.81; H, 5.77; N, 9.01.

*Data for 4-[7-(4-Methoxybenzyl)-5,7-dihydro-4H-[1,2]oxazolo-[5,4-e]isoindol-6-yl]phenyl Acetate (**15s**).* This compound was obtained from reaction of **13s**. Yellow oil; yield 69%. IR (cm^{-1}) 1625 (CO). ^1H NMR (200 MHz, $CDCl_3$) δ 2.67−2.72 (m, 4H, 2 × CH_2), 3.78 (s, 3H, CH_3), 3.82 (s, 3H, CH_3), 5.01 (s, 2H, CH_2), 6.78−6.84 (m, 2H, Ar), 6.90−7.02 (m, 3H, H-8, Ar), 7.14−7.22 (m, 2H, Ar), 7.34−7.42 (m, 2H, Ar), 8.08 (s, 1H, H-3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.0 (t), 20.6 (t), 55.2 (q), 55.3 (q), 69.8 (t), 109.1 (s), 110.6 (s), 114.0 (2 × d), 115.6 (d), 118.7 (s), 128.2 (2 × d), 128.7 (s), 129.3 (2 × d), 129.9 (s), 130.4 (s), 131.3 (2 × d), 148.8 (d), 158.4 (s), 159.0 (s), 159.5 (s), 163.4 (s). Anal. Calcd for $C_{25}H_{22}N_2O_4$: C, 72.45; H, 5.35; N, 6.76. Found: C, 72.65; H, 5.12; N, 6.54.

*Data for 4-[7-(4-Methoxybenzyl)-5,7-dihydro-4H-[1,2]oxazolo-[5,4-e]isoindol-6-yl]phenol (**15t**).* This compound was obtained from reaction of **13t**. Yellow oil; yield 57%. IR (cm^{-1}) 2962 (OH). ^1H NMR (200 MHz, $CDCl_3$) δ 2.65−2.72 (m, 4H, 2 × CH_2), 3.77 (s, 3H, CH_3), 4.94 (s, 2H, CH_2), 6.77−6.97 (m, 7H, H-8, Ar), 7.08−7.13 (m, 2H, Ar), 7.28 (s, 1H, OH), 8.10 (s, 1H, H-3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.0 (t), 20.6 (t), 50.3 (t), 55.3 (q), 109.2 (s), 110.4 (s), 114.0 (2 × d), 114.1 (2 × d), 115.5 (2 × d), 118.7 (s), 123.2 (s), 128.3 (2 × d), 130.0 (s), 130.7 (s), 131.4 (d), 148.7 (d), 156.2 (s), 159.0 (s), 163.7 (s). Anal. Calcd for $C_{23}H_{20}N_2O_3$: C, 74.18; H, 5.41; N, 7.52. Found: C, 74.39; H, 5.13; N, 7.69.

*Data for 7-(4-Methoxybenzyl)-6-(4-methoxyphenyl)-5,7-dihydro-4H-[1,2]oxazolo[5,4-e]isoindole (**15u**).* This compound was obtained from reaction of **13u**. White solid; yield 75%; mp 136−137 °C. ^1H NMR (200 MHz, $CDCl_3$) δ 2.66−2.73 (m, 4H, 2 × CH_2), 3.78 (s, 3H, CH_3), 3.84 (s, 3H, CH_3), 4.95 (s, 2H, CH_2), 6.81 (d, 2H, J = 8.8 Hz, Ar), 6.91−6.98 (m, 5H, H-8, Ar), 7.18 (d, 2H, J = 8.8 Hz, Ar), 8.08 (s, 1H, H-3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.0 (t), 20.6 (t), 50.3 (t), 55.2 (q), 55.3 (q), 109.1 (s), 110.6 (s), 113.9 (2 × d), 114.1 (2 × d), 115.6 (d), 118.7 (s), 123.8 (s), 128.2 (2 × d), 130.0 (s), 130.5 (s), 131.3 (2 × d), 148.8 (d), 159.0 (s), 159.1 (s), 163.4 (s). Anal. Calcd for $C_{24}H_{22}N_2O_3$: C, 74.59; H, 5.74; N, 7.25. Found: C, 74.72; H, 5.89; N, 7.12.

*Synthesis of 4-[(6-Phenyl-4,5-dihydro-7H-[1,2]oxazolo[5,4-e]-isoindol-7-yl)methyl]phenol (**15v**).* To a solution of **15c** (0.42 mmol) in anhydrous dichloromethane (10 mL) BBr_3 (1.26 mmol) was added at −78 °C. Then the reaction mixture was stirred at room temperature for 24 h. Then the reaction mixture was added with

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methanol and the solvent was evaporated at reduced pressure. The crude product was purified by chromatography column using dichloromethane/ethyl acetate (98/2) as eluent. White solid; yield 84%; mp 205−206 °C. IR (cm^{-1}) 3161 (OH). ^1H NMR (200 MHz, $CDCl_3$) δ 2.72−2.75 (m, 4H, 2 × CH_2), 4.96 (s, 2H, CH_2), 5.69 (s, 1H, OH), 6.76 (d, 2H, J = 8.7 Hz, H-3′, H-5′), 6.90 (d, 2H, J = 8.7 Hz, H-2′, H-6′), 7.00 (s, 1H, H-8), 7.24−7.41 (m, 5H, Ar), 8.10 (s, 1H, H-3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.0 (t), 20.7 (t), 50.5 (t), 108.8 (s), 109.3 (s), 110.6 (s), 115.7 (2 × d), 116.1 (d), 119.1 (s), 127.7 (d), 128.5 (2 × d), 128.6 (2 × d), 129.7 (2 × d), 130.1 (s), 130.8 (s), 131.6 (s), 148.8 (d), 155.6 (s). Anal. Calcd for $C_{22}H_{18}N_2O_2$: C, 77.17; H, 5.30; N, 8.18. Found: C, 77.30; H, 5.44; N, 7.95.

Biology. *Drugs.* For in vitro studies, vinorelbine (purchased from Pierre Fabre Pharma), taxol (Paclitaxel; purchased from Santa Cruz Biotechnology), and **15r** were completely dissolved in 1% dimethyl sulfoxide (DMSO), stored at −20 °C, and then diluted in complete culture medium immediately before use at the appropriate concentration. For in vivo studies, **15r** was dissolved in DMSO 10% and diluted in saline solution.

Cell Culture. Human DMPM cell lines (STO, MP4, and MP8) were established from surgical specimens of patients admitted to Fondazione IRCCS Istituto Nazionale dei Tumori of Milan. The normal human lung fibroblast cell line (WI38) was obtained from the American Type Culture Collection (ATCC). Cells were maintained in the logarithmic growth phase as a monolayer in DMEM F12 (STO, MP4, and MP8) and DMEM (WI38) media (Lonza) supplemented with 10% heat-inactivated fetal bovine serum, in a humidified incubator at 37 °C with a supply of 5% CO_2/95% air atmosphere. Cell lines are tested fortnightly for the absence of Mycoplasma and periodically (every six months) monitored for DNA profile of short tandem repeats analysis by the AmpFISTR Identifiler PCR amplification kit (Applied Biosystems).

Cell Proliferation Assay. After harvesting in the logarithmic growth phase, 4500 cells/50 μL were plated in 96-well flatbottomed microtiter plates (EuroClone) for 24 h and then treated with increasing concentrations of derivative **15r** (0.1−100 μM) for 72 h. Control cells received vehicle alone (0.01% DMSO). Studies were performed in eight replicates and repeated at least three times independently. At the end of drug exposure, the antiproliferative potential was determined with the CellTiter 96 AQueous One Solution Cell Proliferation Assay (MTS, purchased from Promega) according to the manufacturer's protocols. Optical density was read at 490 nm on a microplate reader (POLARstar OPTIMA, BMG Labtech GmbH), and the results were expressed as a percentage relative to DMSO-treated cells. Dose−response curves were created, and IC_{50} and IC_{80} values (i.e., concentrations able to inhibit cell growth by 50% and 80%, respectively) were determined graphically from the curve for each compound.

Tubulin Polymerization Assays. Cells were seeded in 6-well plates (Eppendorf) and were exposed the next day to the **15r** derivative for 24 h at concentrations corresponding to the IC_{50} at 72 h. Samples were then processed for the tubulin polymerization assay.[50,51] To separate cytosolic and cytoskeletal-associated proteins, cells were rinsed twice in PIPES−EGTA−$MgCl_2$ (PEM) buffer (85 mmol/L PIPES, pH 6.94; 10 mmol/L EGTA; 1 mmol/L $MgCl_2$; 2 mol//L glycerol; 1 mmol/L phenylmethylsulfonyl fluoride; 0.1 mmol/L leupeptin; 1 μmol/L pepstatin; 2 μg/mL aprotinin), lysed at room temperature for 10 min with PEM buffer supplemented with 0.1% v/v Triton X-100, and rinsed in PEM buffer. The Triton X-100-soluble fractions were then diluted 3:1 with 4× SDS-PAGE sample buffer. The insoluble material that remained attached to the dish was scraped into SDS-PAGE sample buffer containing protease inhibitors. Proteins were separated by SDS-PAGE, and tubulin distribution was analyzed by immunoblotting using anti α-tubulin antibody (Sigma-Aldrich).

The effect of **15r** on the microtubule assembly/disassembly process was determined using the tubulin polymerization assay (Cytoskeleton), according to the manufacturer's instruction. Briefly, purified porcine brain tubulin was resuspended in ice-cold buffer (80 mmol/L PIPES at pH 6.9, 2 mmol/L $MgCl_2$, 0.5 mmol/L EGTA, 1 mmol GTP, 10% glycerol) in the presence of 10 μmol/L **15r** derivative, taxol, and vinorelbine. Tubulin polymerization was started by incubation at 37 °C and followed by absorbance readings at 340 nm (POLARstar OPTIMA) every min up to 60 min.

Cell Cycle Distribution Analysis. Both adherent and floating cells were fixed in 70% EtOH and incubated at 4 °C for 30 min in staining solution containing 50 μg/mL of propidium iodide, 50 mg/mL of RNase, and 0.05% Nonidet-P40 in PBS. Samples were analyzed with BD Accuri c6 flow cytometer (Becton Dickinson). At least 30000 events were read, and histograms were analyzed using the CellQuest software according to the Modfit model (Becton Dickinson).

Fluorescence Microscopy Analysis. Cells seeded on glass coverslips were treated for 72 h with equitoxic (IC_{50}) concentrations of **15r**, taxol, and vinorelbine and then fixed in 2% paraformaldehyde for 30 min and permeabilized with ice-cold methanol for 20 min at −20 °C. After blocking with PBS containing 1% bovine serum albumin, cells were probed overnight with a mouse anti-MPM-2 antibody (Upstate Biotechnology) at room temperature, followed by a 1 h incubation at room temperature with goat antimouse AlexaFluor594 dye secondary antibody (Life Technologies). Images were acquired using a Leika fluorescence microscope (Leica). The percentage of mitotic cells was determined by scoring at least 300 cells for each sample run in duplicate.

Apoptosis Analysis. The catalytic activity of caspase-3 was measured in the same cellular samples as the ability to cleave the specific substrate *N*-acetyl-Asp-Glu-Val-Asp-pNA (DEVD-pNA) by means of the APOPCYTO/caspase-3 kit (MBL), according to manufacturer's instructions. Briefly, cells were washed, pelleted, and lyzed according to the manufacturer's instructions. Total protein and the specific fluorogenic substrate *N*-acetyl-Asp-Glu-Val-Asp-pNA (DEVD-pNA) were mixed for 1 h at 37 °C and transferred to 96-well microtiter plates. The hydrolysis of the specific substrates was monitored by a spectrofluorometer (POLARstar OPTIMA) with 380 nm excitation and 460 nm emission filters. Results were expressed as relative fluorescence units (rfu).

In Vivo Experiments. Female nude mice (6−8 weeks-old, purchased from Charles River) were maintained in laminar flow rooms, keeping temperature and humidity constant. The mice had free access to food and water. Experimental protocols were approved by the Ethics Committee for Animal Experimentation of the Fondazione IRCCS Istituto Nazionale Tumori of Milan according to institutional guidelines that are in compliance with national and international laws and policies. Exponentially growing STO cells were subcutaneously injected into the right flank mice (10^7 cells/flank). Eight mice for each experimental group were used. Compound **15r** was dissolved in a mixture of DMSO (10%) and saline solution (90%) and delivered ip every 7 days for 4 weeks (q7dx4) starting from the tenth day after cell inoculum at a dose of 5 mg/kg. Tumor growth was followed by weekly measurements of tumor diameters with a Vernier caliper, and tumor volume (TV) was calculated according to the formula: TV (mm^3) = $d^2 \times D/2$ where d and D are the shortest and the longest diameter, respectively. The efficacy of the drug treatment was assessed as tumor volume inhibition percentage (TVI %) in treated versus control mice, calculated as TVI% = 100 − (mean TV treated/mean TV control × 100). The toxicity of the drug treatment was determined as body weight loss and lethal toxicity.

To evaluate the proliferation rate, at the end of the experiment, tumor specimens were fixed in 10% buffered formalin and paraffin-embedded (FFPE). Five-micrometer sections of FFPE samples were used and stained, according to standard protocols, with the primary antibodies anti-Ki67.

Statistical Analysis. Statistical evaluation of data was done with the two-tailed Student's t test. Ps < 0.05 was considered statistically significant.

■ ASSOCIATED CONTENT

Supporting Information

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Overview of the results of the NCI in vitro human tumor cell line screen for derivatives **15b,c,e,g,h,l,o−r,t,u**; in vitro activity of compounds **15b,c,e,g,h,1,o−r,t,u** in individual tumor cell lines; dose response curves and mean graph of compounds **15b,c,e,g,l,o,p,r,t,u** (PDF)
Molecular formula strings (CSV)

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

CRS, cytoreductive surgery; DMPM, diffuse malignant peritoneal mesothelioma; HIPEC, hyperthermic intraperitoneal chemotherapy; MPM-2, mitotic protein monoclonal 2; NCI, National Cancer Institute

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