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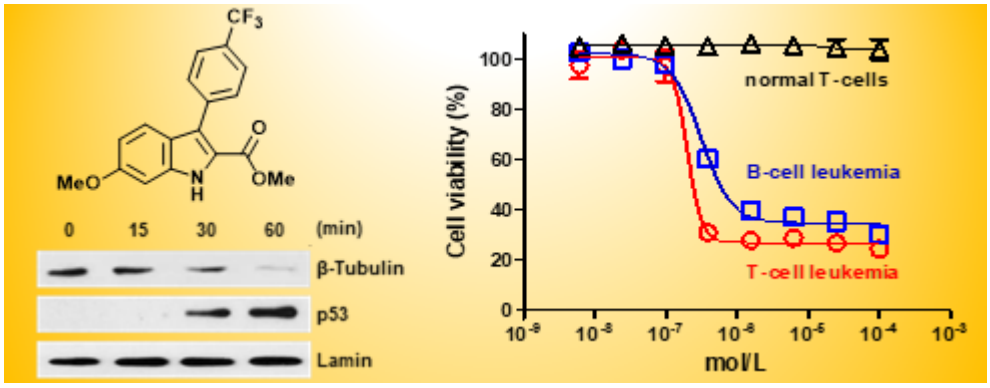

Synthesis and Evaluation of 2-Carboxy Indole Derivatives as Potent and Selective Anti-leukemic Agents

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ABSTRACT

Despite the success achieved in the treatment of acute lymphoblastic leukemia (ALL), the search for new drugs featuring selectivity against leukemia cells and effectiveness to prevent relapsed ALL is still highly desirable. Here, we described the synthesis of several novel 3-substituted and 3,6-disubstituted-2-carboalkoxy indoles followed by the elucidation of their mechanism of action and *in vivo* anti-leukemia efficacy. The synthesis of 3-substituted-2-carboalkoxy indoles relied on two Heck arylations of methyl acrylate and methyl cinnamates respectively, to generate *β,β*-disubstituted acrylates followed by an efficient Cadogan-Sundberg reaction of these latter intermediates. The method developed led to the synthesis of twenty-one novel functionalized indoles. Of these, indole **20** showed selective cytotoxicity against leukemia cells at the nanomolar scale, and, therefore, it was selected for the investigation of its mechanism of action. Indole **20** was found to target tubulin leading to G2/M cell cycle arrest, DNA damage and apoptosis. Indole **20** decreased β-tubulin protein in leukemia cells in a time-dependent manner and induced depolymerization of the microtubule network in Hela cells, thus fully characterizing its microtubule destabilizer activity. The connectivity map analysis of HL60 promyelocytic leukemia cells treated with indole **20** revealed a transcriptional profile similar to that of cells treated with prostaglandins, apparently due to the induction of cellular differentiation as addressed by the expression of CD11 and CD14 markers. Finally, indole **20** given intraperitoneally, at 10 mg/kg, 5x/week significantly prolonged the overall survival of NOD/SCID mice transplanted with RS4;11 B-ALL cells.

Keywords: Indoles; Heck arylations; disubstituted acrylates; acute lymphoblastic leukemia; tubulin inhibitor; apoptosis.

1. Introduction

The indole nucleus is a privileged heteroaromatic framework, which forms a vast and important class of natural and synthetic biologically active compounds. Indole containing compounds show a diversity of pharmacological activities including, antimicrobials, antifungal, antivirals, anti-inflammatory, antidepressants, anticholinergics, anti-migraine, anti-hypertensive, and in particular anticancer activity, mostly linked to targeting tubulin [1–3]. The tubulin polymerization inhibition activity [4] results in the microtubule network disruption, which interferes with the correct mitotic spindle formation and consequently in the cell division process.

The vinca alkaloids vincristine and vinblastine, are examples of indolic compounds used as anticancer agents since 1965 [5]. Vincristine has been used in the treatment of acute lymphoblastic leukemia (ALL) and many other cancers for decades. However, its neurotoxic effects, presumably caused by the disturbance of neurons' microtubule dynamics, limit the dosage and administration regimen of this drug. Recently, the use of liposomal vincristine to overcome this drawback has been demonstrating promising results [6,7]. Despite the successful and consolidated chemotherapy regimen of vincristine for the treatment of childhood ALL, 20% of patients still relapse [8,9]. Moreover, the non-selectivity of currently available chemotherapeutics to malignant cells leads to the undesirable adverse effects observed in ALL patients under chemotherapy. Accordingly, new anti-leukemia agents are still needed to seek better therapeutic indexes as well as selectivity to leukemia cells.

Due to the wide applicability of the indole nucleus in medicinal chemistry, many methods have been developed over the last few decades for its synthesis [10,11]. Some of the classical methods for the indole synthesis employ the reductive cyclization of nitro aromatics intermediates, which include: the Bartoli indole synthesis [12], the Reissert indole synthesis [13], and the Leimgruber and Batcho indole synthesis [14]. Regarding more recent synthetic methods, previous work from our group [15] investigating a Cu-catalyzed 1,4-reduction of *o*-nitro substituted 3,3-diaryl acrylates by chiral copper hydride cleanly provided 2-carboxymethyl-3-aryl indoles as the major product. Due to the importance of aryl indoles as potential pharmacological leads, we decided to investigate this transformation even further. From the onset of this research, we realized that other reducing agents could be applied for the reduction of the *o o*-nitro substituted 3,3-diaryl acrylates as well. Therefore, in an attempt to improve the indoles synthesis (e.g. lower costs, higher yields) from 3,3-diaryl acrylates, and having in mind

the potential evaluation of the biological activity of novel 2-carboalkoxy-3-aryl indoles as anticancer compounds, we explored new methods for their synthesis. An effective method for indole synthesis, the Cadogan-Sundberg reaction [16], employs $P(OEt)_3$ to promote the deoxygenative cyclization of *o*-nitro cinnamates [17] to furnish 2-substituted indoles. In view of its effectiveness and low cost, we decided to investigate the Cadogan-Sundberg reaction for the synthesis of 3-aryl substituted indoles starting from 3,3-diaryl acrylates. To the best of our knowledge, this is the first report of the use of the Cadogan-Sundberg reaction to synthesize 3-substituted indoles (aryl, alkyl or carboxymethyl) starting from *β*,*β*-substituted acrylates.

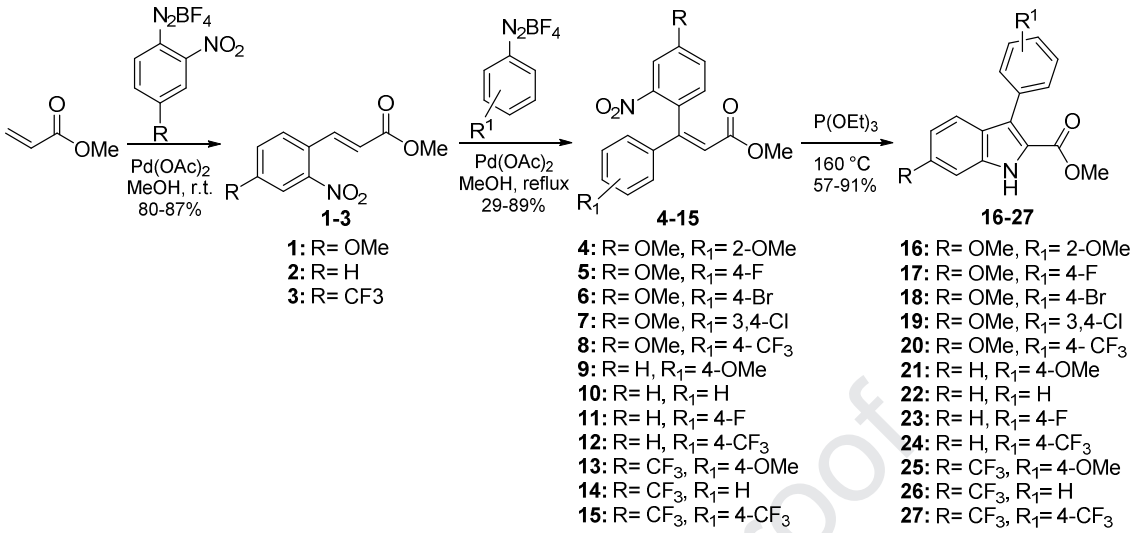
The method developed herein has allowed the synthesis of thirty 2-carbomethoxy-3-substituted indoles, with twenty-one of them being novel compounds. With this library available, their cytotoxic activity against leukemia cells was evaluated *in vitro*. The indole displaying the highest efficiency and selectivity against leukemia cells was selected as representative for a detailed investigation of its mechanisms of action and *in vivo* activity.

2. Results and discussion

2.1. Chemistry

The synthesis of 2-carbomethoxy-3-arylindoles began with the Heck-Matsuda arylation of methyl acrylate with arenediazonium salts containing an *o*-nitro substituent employing 10 mol% of palladium acetate as catalyst to provide the corresponding cinnamates **1-3** (Scheme 1) in 80–87% yield. The nitro cinnamates were then used as starting material to a second Heck-Matsuda reaction employing 2 equivalents of a distinct arenediazonium salt and 7.5 mol% palladium acetate as catalyst in methanol to furnish the *β*,*β*-diaryl acrylate **4-15** in yields ranging from 29% to 89%. Then, the Cadogan-Sundberg reductive cyclization of these *β*,*β*-diaryl acrylates, promoted by $P(OEt)_3$, allowed the effective synthesis of the 2-carbomethoxy-3-arylindoles **16-27** in yields varying from 57% to 91%.

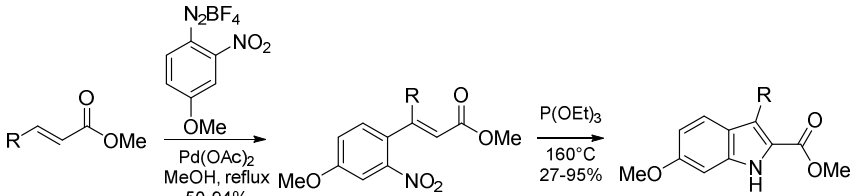
Scheme 1. Synthesis of the 2-carbomethoxy-3-aryl-indoles **16**–**27**.



A similar synthetic strategy has also allowed the synthesis of C-3 alkyl and carboxy substituted indoles by employing 3-alkyl or 3-carboxyl substituted methyl acrylates (Scheme 2) as starting materials. The initial conditions for the Heck-Matsuda arylation employing the 3-substituted methyl acrylates were carried out using 1.5 equivalents of the 4-methoxy-2-nitro arenediazonium tetrafluoroborate, 7.5 mol% of palladium acetate under refluxing MeOH. Optimizations were performed for the synthesis of some 3-alkyl cinnamates (**32**, **33**, **34**) in order to improve their yields. For the 3-cyclohexyl cinnamate **32,** it was necessary to use two equivalents of the 4-methoxy-2-nitro benzenediazonium tetrafluoroborate. For cinnamate **33** (R = benzyl), it was also necessary to use two equivalents of the aryl diazonium salt and 10 mol% of $Pd(OAc)_2$. Finally, for cinnamate **34** (R= CO_2CH_3) it was necessary to use two equivalents of dimethyl fumarate to achieve reasonable yields of the Heck product.

The Cadogan-Sundberg reductive cyclization reaction of cinnamates **28**-**35** was carried out as before to furnish the substituted indoles **36**-**40** and **43** in yields varying from 50% to 95%. For indoles **41** (R = benzyl) and **42** (R = carboxymethyl) only moderated to low yields were obtained (44% and 25%, respectively).

Scheme 2. Synthesis of 2-carbomethoxy-3-alky and 3-carboxyalkyl indoles.



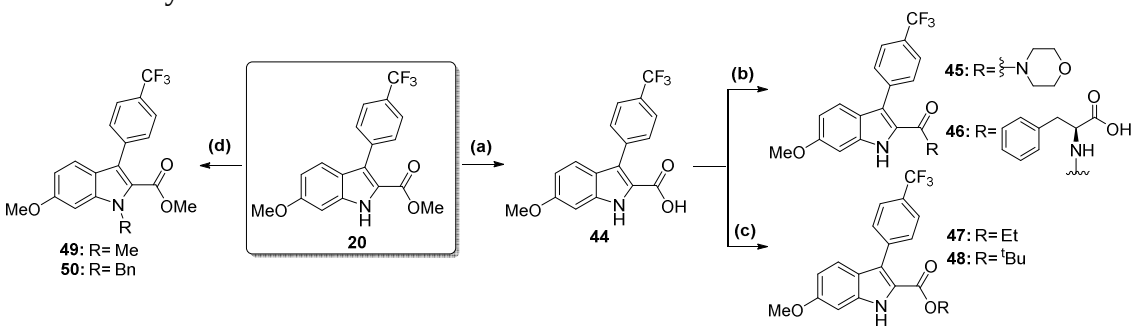
28: R= CH_3
29: R= CH_2CH_3
30: R= $CH(CH_3)_2$
31: R= $CH_2CH(CH_3)_2$
32: R= C_6H_{11}
33: R= $CH_2C_6H_5$
34: R= CO_2CH_3
35: R= $CH_2CO_2CH_3$

36: R= CH_3
37: R= CH_2CH_3
38: R= $CH(CH_3)_2$
39: R= $CH_2CH(CH_3)_2$
40: R= C_6H_{11}
41: R= $CH_2C_6H_5$
42: R= CO_2CH_3
43: R= $CH_2CO_2CH_3$

As many of the synthesized indoles have functionalities, which allow significant structural modifications, new analogues were constructed for biological evaluation besides the indoles **16**-**27** and **36**-**43** (a total of 20 indoles). Derivatives of indole **20** were also synthesized by functionalization of its carboxyl group or by alkylation of the indole nitrogen to provide the new indoles **44**-**50** (Scheme 3).

The synthesis of the 2-carboxyindole **44** was carried out by reacting the ester **20** with LiOH in a mixture of THF/MeOH/H_2O at room temperature (pathway a). The synthesis of amides **45** and **46** was performed by reacting 2-carboxyindole **44** with the amines morpholine or phenylalanine in the presence of 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC), hydroxybenzotriazole (HOBt), and triethylamine as base (pathway b). It was also possible to generate new esters from 2-carboxyindole **44** through its conversion to the corresponding acyl chloride and reacting it with the appropriate alcohol to furnish the novel esters **47** and **48** (pathway c). *N*-alkylations of indole **20** were also performed using K_2CO_3 as base and methyl and benzyl iodides as alkylating agents to provide indoles **49** and **50** respectively (pathway d).

Scheme 3. Synthesis of new derivatives from indole **20**.[a]

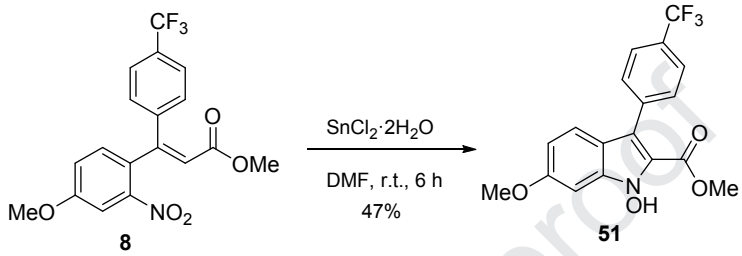


[a]Reagents and conditions: (a) LiOH·H_2O, MeOH/H_2O/THF, 48h, rt; (b) anhydrous DCM, HOBt, EDC·HCl, amine, Et_3N, overnight; (c) anhydrous THF, oxalyl chloride/DMF, alkali metal alkoxide

(sodium etoxide or potassium *tert*-butoxide); (d) anhydrous DMF, K_2CO_3, alkyl halide (methyl iodide or benzyl bromide).

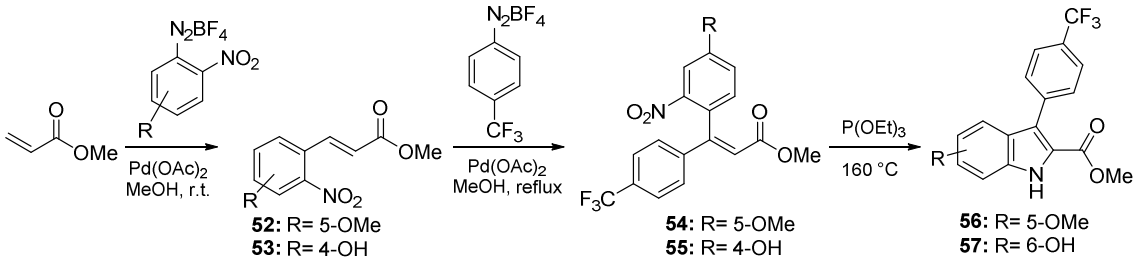
The *N*-hydroxy indole **51** (Scheme 4) was prepared from 3,3-diaryl acrylate **8** by reductive cyclization with $SnCl_2$ in DMF. The product was obtained in a moderate yield of 47%.

Scheme 4. Synthesis of *N*-hydroxy indole **51**.



Finally, two other analogues of indole **20** were synthesized for evaluation (Scheme 5). Cinnamate **52** possessing a methoxy group at position 5 of the indole ring was prepared from 5-methoxy-2-nitro arenediazonium salt and methyl acrylate to evaluate the influence of a methoxy group at that position. Cinnamate **53** (hydroxy group at position 6) was prepared from 4-hydroxy-2-nitro arenediazonium salt and methyl acrylate to evaluate the effect of a phenolic group in the antileukemic activity when compared to a methoxy group. The synthesis of the indole **56** (methoxy group at position 5) and **57** (hydroxy group at position 6) were performed as previously described in Scheme 1 from 3,3-diaryl acrylate **54** and **55**.

Scheme 5. Synthesis of 2-carbomethoxy-3-aryl-indoles **56** and **57**.

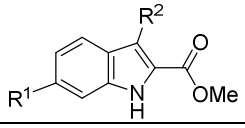


2.2. Biological assays

2.2.1 Indoles cytotoxicity against leukemia cell lines

Initially, the 2-carbomethoxy-3-aryl-indoles **16**-**27** (Table 1) were tested for their cytotoxic activity against two different leukemia cell lines. Aryl indoles **18**, **19**, and **20** showed activity, in a submicromolar range, against CEM cells with EC_{50} values of 0.33, 0.58, and 0.22 μM respectively. Aryl indoles **18** and **20** also showed a submicromolar activity against the RS4;11 cell line with EC_{50} values of 0.20 and 0.30 μM. The influence of different substituents at the C6 position of indole bearing distinct electronics (OMe, H, CF_3) was also tested. With the exception of indole **22** (Entry 7, Table 1), all other indoles bearing a hydrogen atom or a CF_3 group at the position 6 showed poor cytotoxicity (>100μM) against both CEM and RS4;11 cells (Entry 6, and entries 8-12, Table 1). The presence of a methoxy group at the C-6 position (Entries 1-5, Table 1) of aryl indoles **16**-**20** proved beneficial when compared to aryl indoles **21**-**24**, which exhibited low cytotoxicity, with the exception of aryl indole **22** with modest *in vitro* anti-leukemia activity. The next step was to evaluate aryl indoles bearing a methoxy group at the C6 position and to test the effect of other substituents at the C3 position of the indole moiety. This study was carried out with the C-3 alkyl and C-3 ester indoles **36**-**43**. However, C-3 alkyl or C-3 esters groups at position 3 of the indole nucleus did not improve the anti-leukemia activity, showing cytotoxicity at the micromolar range (Entries 13-20, Table 1).

Table 1. The antileukemic activity of the 3-substituted-indoles.

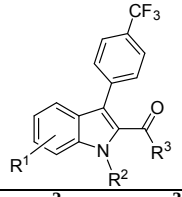


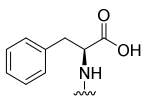
Entry	Compound	R^1	R^2	CEM EC_{50} (μM)	RS4;11 EC_{50} (μM)
1	**16**	OMe	2-OMe-Ph	1.90	10.5
2	**17**	OMe	4-F-Ph	10.0	85.6
3	**18**	OMe	4-Br-Ph	0.33	0.20
4	**19**	OMe	3,4-Cl-Ph	0.58	3.80
5	**20**	OMe	4-CF_3-Ph	0.20	0.30
6	**21**	H	4-OMe-Ph	>100	>100
7	**22**	H	Ph	35.8	95.2
8	**23**	H	4-F-Ph	>100	>100
9	**24**	H	4-CF_3-Ph	>100	>100
10	**25**	CF_3	4-OMe-Ph	>100	>100
11	**26**	CF_3	Ph	>100	>100
12	**27**	CF_3	4-CF_3-Ph	>100	>100
13	**36**	OMe	Me	>100	>100
14	**37**	OMe	Et	>100	>100
15	**38**	OMe	^{i}Pr	>100	81.5

16	**39**	OMe	^{i}Bu	16.6	37.8
17	**40**	OMe	Cy	80.2	>100
18	**41**	OMe	Bn	8.70	61.6
19	**42**	OMe	CO_2Me	>100	>100
20	**43**	OMe	CH_2CO_2Me	>100	>100

In view of the promising *in vitro* anti-leukemia activity displayed by the carboxymethyl aryl indole **20**, we synthesized the aryl indole derivatives **44-48,** the *N*-alkyl and *N*-hydroxy indoles **49-51**, and the aryl indoles **59** and **60** (methoxy at C-5, and a hydroxy group at C-6 respectively). The 2-carboxyl analog **44** (Entry 1, Table 2) and the amides analogs **45** and **46** (Entries 2 and 3, Table 2) showed moderate activity for both CEM and RS4;11 cells (19.8 to 52.0 µM; Table 2). However, these compounds were much less active when compared to indole **20** (EC_{50} = 0.20 µM for CEM, and 0.30µM for RS4;11). Both esters **47** and **48** (Entries 4 and 5, Table 2), and the *N*-methyl indole **49** and *N*-benzyl indole **50** (Entries 6 and 7) displayed decreased cytotoxicity against both CEM and RS4;11 cells. However, the *N*-hydroxy indole derivative **51** exhibited potency similar to that of aryl indole **20** with EC_{50} values of 0.39 nM and 0.30 nM against CEM and RS4;11 cells (Entry 8, Table 2). Finally, the change in the position of the methoxy group was deleterious to the cytotoxic activity with aryl indole **56** (OMe at C-5) showing an EC_{50} higher than 100 µM (Entry 9, Table 2). The aryl indole **57** bearing a hydroxyl group at position 6 showed a moderate activity of ~10 µM for both CEM and RS4;11 cells (entry 10, Table 2) indicating that, in comparison to aryl indole **20**, demethylation was deleterious to the anti-leukemia activity.

Table 2. Evaluation of the new analogs of aryl indole **20.**



Entry	Compound	R^1	R^2	R^3	CEM EC_{50} (µM)	RS4;11 EC_{50} (µM)
1	**44**	6-OMe	H	OH	19.8	24.4
2	**45**	6-OMe	H		29.7	47.6
3	**46**	6-OMe	H		52.0	43.1
4	**47**	6-OMe	H	OEt	>100	>100
5	**48**	6-OMe	H	O^{t}Bu	>100	>100
6	**49**	6-OMe	Me	OMe	>100	>100

7	**50**	6-OMe	Bn	OMe	>100	>100
8	**51**	6-OMe	OH	OMe	0. 39	0.30
9	**56**	5-OMe	H	OMe	>100	>100
10	**57**	6-OH	H	OMe	9.90	10.3

Among the compounds with the highest cytotoxic activity against leukemia cell lines, we chose indole **20** for further investigation including its mechanisms of action since it showed no cytotoxicity against healthy T-lymphocytes up to a dose of 100 μM, indicating selective cytotoxicity against leukemia cells (Figure 1).

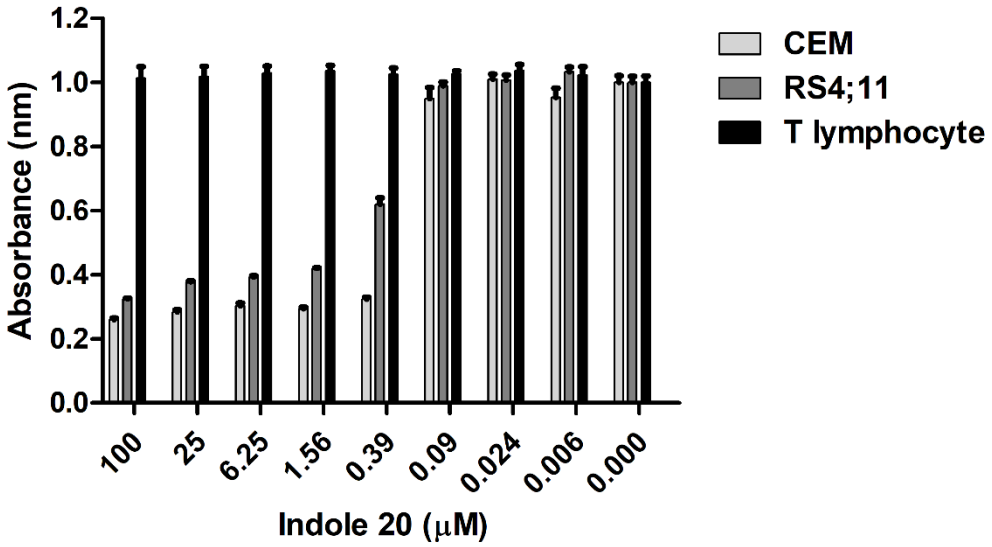


Figure 1. Indole 20 presented selective cytotoxicity against leukemia cells. CEM, RS4;11 cell lines and T-lymphocytes stimulated with phytohemagglutinin and interleukin-2 overnight were incubated with decreasing doses of indole **20** for 48h and cell viability was assessed using the MTT method.

2.2.2 Indole 20 targets tubulin leading to cell cycle arrest and apoptosis

To shed light on the mode of action of indole **20**, we performed gene expression profiling analysis in HL60 cells treated with 1 μM of indole **20** (EC_{90} dose - data not shown) for 6 h. The HL60 acute promyelocytic leukemia cell line was chosen for the gene expression assay to enable Connectivity Map (CMap) analysis [18]. Treatment of HL60 cells with indole **20** resulted in the upregulation of 22 genes and downregulation of 23 genes ($-1.5 \leq FC \geq 1.5$, p-value$\leq$0.01, Supplemental Table 1). Gene Set Enrichment Analysis (GSEA) [19] revealed a significant enrichment of G2/M Checkpoint, Mitotic Spindle and NOTCH Signaling gene sets as well as a decrease in the cholesterol homeostasis gene set as a result of indole **20** treatment (Figure 2).

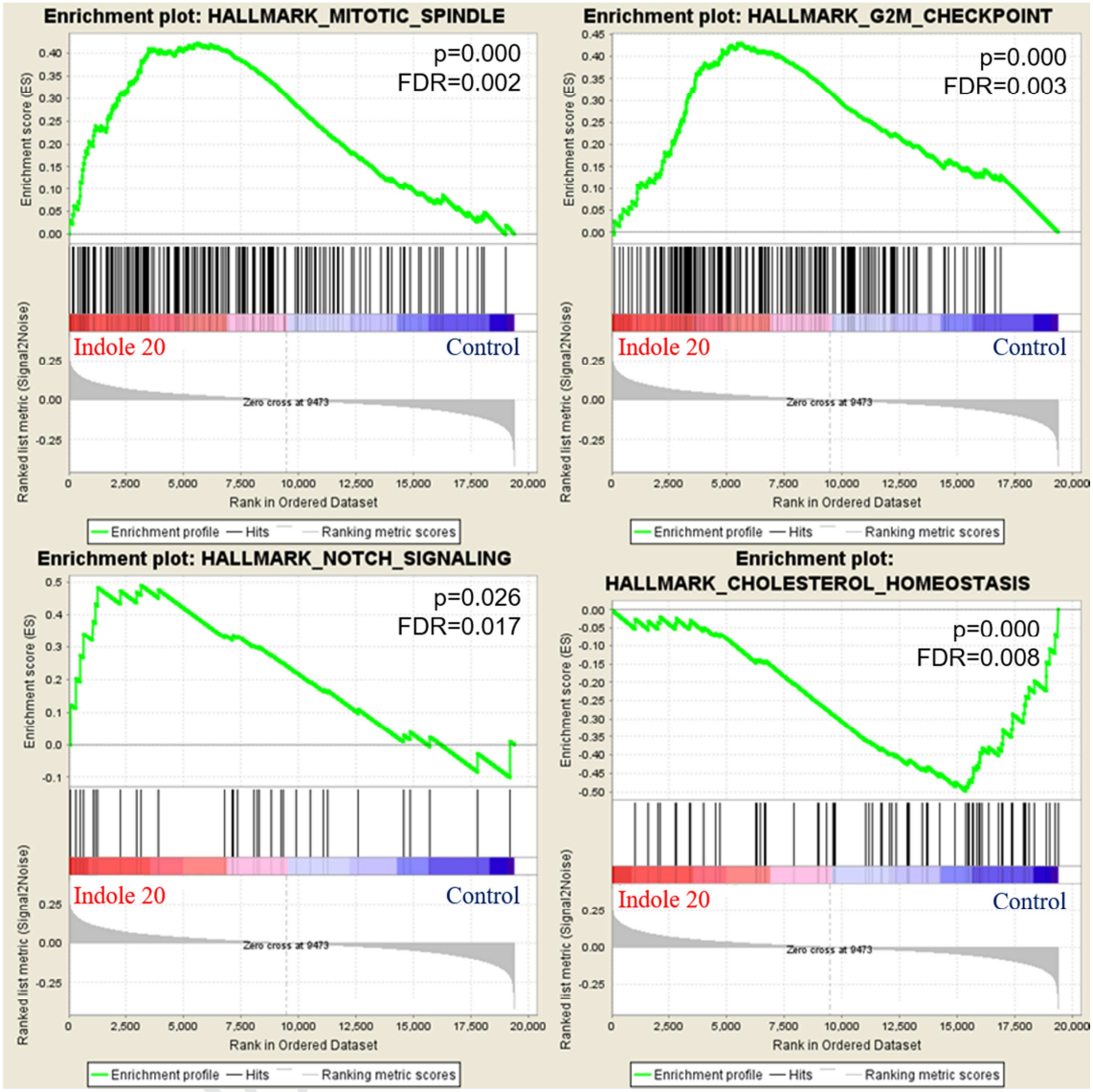


Figure 2. GSEA enrichment plots. HL60 cells were treated with 1 μM of indole **20** for 6 h and gene expression data was analyzed using the GSEA platform. All the probe sets/transcript clusters annotated with a Gene Symbol in the array were used in the analysis. p, p-value; FDR, False Discovery Rate after 1,000 permutations by gene-set.

As expected, cell cycle analysis of HL60 cells treated with indole **20** manifested G2/M arrest in a time-dependent manner, as well as the rising of a sub-G1 population of cells indicative of apoptosis (Figure 3A). Apoptosis was further confirmed by an assay detecting both phosphatidylserine exposure and cell membrane integrity. Indole **20** induced apoptosis in HL60, CEM, and RS4;11 leukemia cells after 18 hours of treatment (Figure 3B).

To investigate whether cell cycle arrest induced DNA damage and then apoptosis, RS4;11 cells were treated with indole **20** (Figure 4A) for 18 hours followed by BrdU/PI (cell cycle), H2AX (DNA damage) and PARP (apoptosis) labeling. As

shown in Figure 4B, indole **20** treatment had no effect on S-phase proportion but caused a significant increase in G2/M and the corresponding decrease in G1. Although it is not well known how anti-mitotic drugs kill cancer cells, prolonged mitotic arrest triggers cell death. Some of the cells divide unequally, i.e., producing aneuploid daughter cells [20]. Indole **20** treatment resulted in a population of cells in between G1 and G2 (hereafter called sub-G2-like) which may be suggestive of unequal division.

Staining with anti-H2AX antibody allowed us to verify that DNA damage occurred in 6.9%, 2.3% and 6.9% of each G1, sub-G2-like and G2 populations respectively, while staining with PARP antibody revealed that apoptosis occurred in 7%, 3.5% and 1.5% of each G1, sub-G2-like and G2 populations respectively (data not shown).

One possible interpretation of these results is that indole **20** induces DNA damage in G2. Some of these cells die (PARP labeled) right away but some of them proceed with division or unequal division, dyeing afterwards in G1 or sub-G2-Like. That is why there is higher PARP labeling in G1 and sub-G2 than in G2.

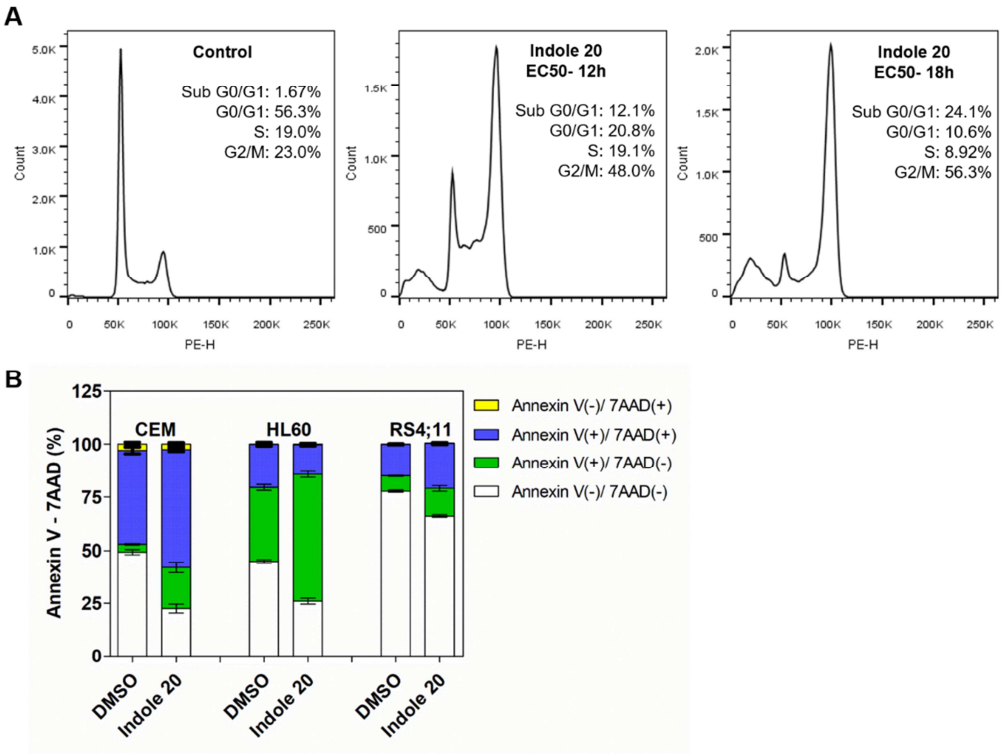


Figure 3. Indole 20 promotes G2/M cell cycle arrest and apoptosis in leukemia cells. In a time-dependent manner. For cell cycle analysis, HL60 cells were treated with 586 nM (EC_{50}) and 1 µM (EC_{90}) for 12 and 18 hours followed by PI staining. Cell cycle phases were represented as sub-G0/G1 (apoptotic cells), G0/G1, S, and G2/M. Apoptosis induced by indole **20**. For apoptosis analysis, CEM, HL60, and RS4;11 cells were treated with 300 nM of indole **20** for 18 hours followed by staining with Annexin V and PI and flow cytometry analysis.

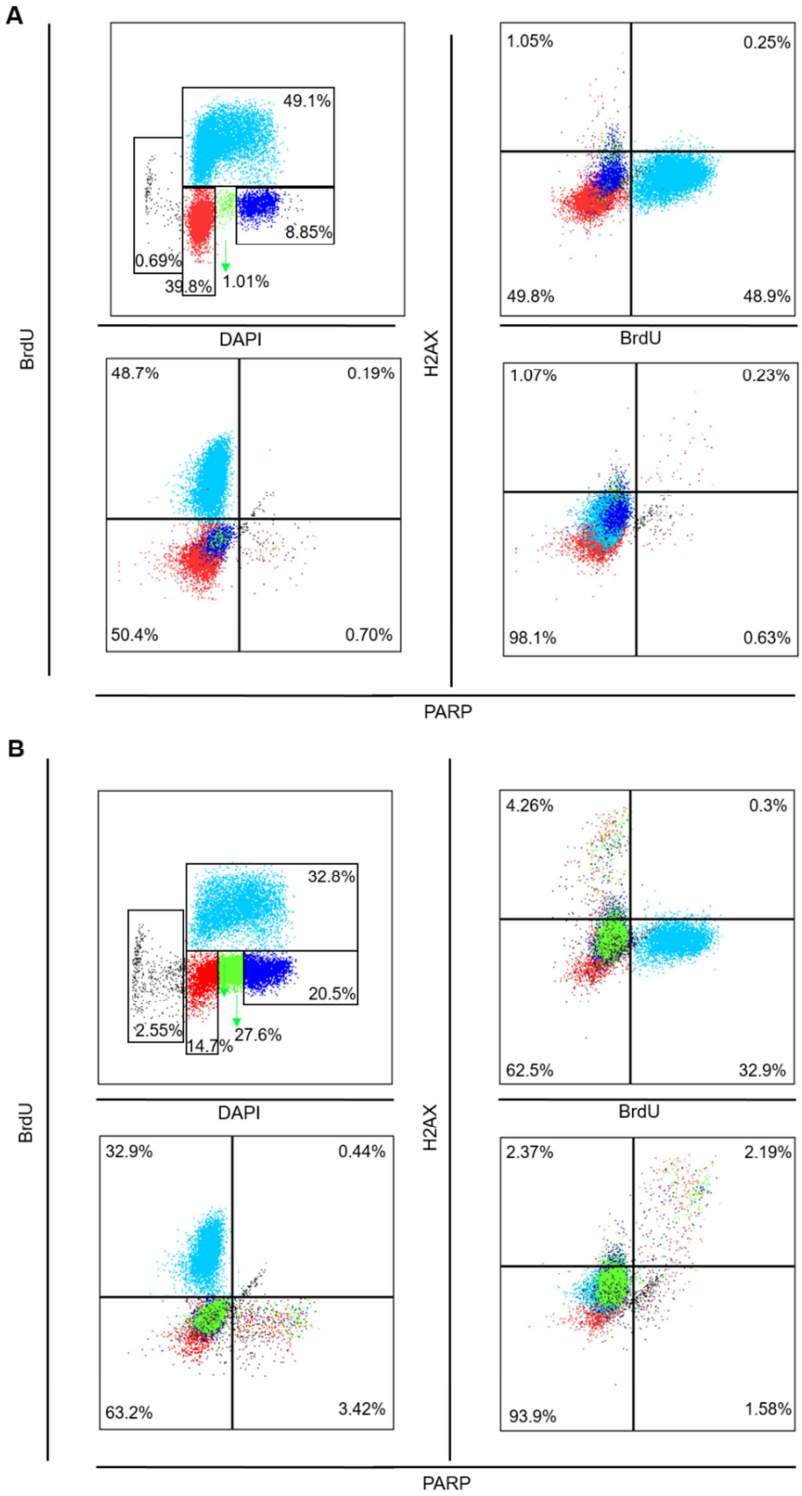


Figure 4. Indole 20 induces apoptosis of RS4;11 cells at G2/M and G0/G1 phases. Cells were treated with **(A)** DMSO (vehicle) or **(B)** 300 nM of indole **20** for 18 hours followed by labeling with 10 μM BrdU for 1 hr. The cells were then harvested and analyzed by immunofluorescent staining and multicolor flow cytometric analysis. BrdU-positive cells are color-gated light blue whereas BrdU-negative cells at G1 phase and G2/M phase of the cell cycle are colored red and dark blue respectively. Cells between G1 and G2 phases (sub-G2-like cells) and sub-G0/G1 are colored light green and dark gray respectively.

It is well established that some microtubule-interacting agents, like vincristine and vimblastine, promote cell cycle arrest by inhibiting tubulin polymerization. Free tubulin, i.e. tubulin that is not incorporated into microtubules, negatively regulates the levels of tubulin mRNA and tubulin protein [21]. Western blot analysis of RS4;11, CEM and HL60 cells treated with indole **20** showed a markedly tubulin decrease along time (Figure 6), indicating that this compound acts through inhibition of microtubule polymerization. In addition, we could also observe an increase on p53 protein during time (Figure 6). These results are in agreement with our previous data evidencing the induction of cell cycle arrest in G2/M (Figure 3A), DNA damage (Figure 4B) and apoptosis (Figures 3B and 4B), respectively.

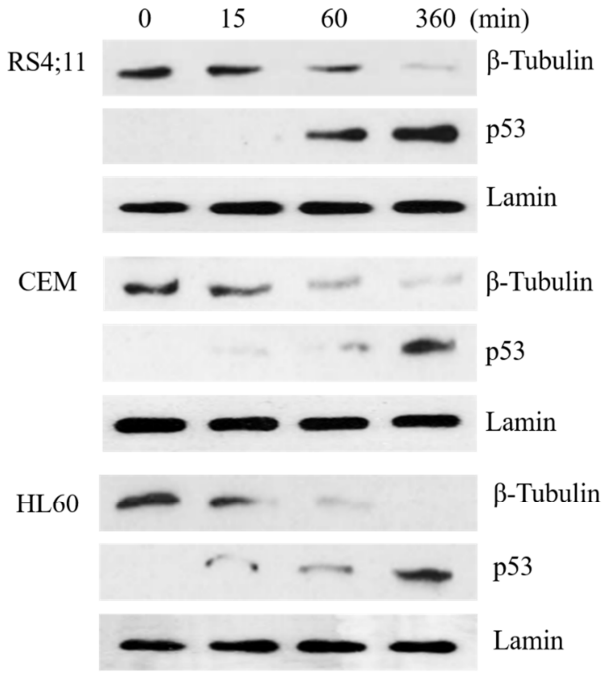


Figure 6. Indole 20 decreases β-tubulin and increases p53 proteins in leukemia cell lines. Western blotting analysis for β-tubulin and p53 proteins in RS4;11, CEM and HL60 cells treated with 300 nM of indole **20** for, 0, 15, 30, 60 and 360 min. Lamin was used as loading control.

To further confirm tubulin as the main target of indole **20,** we performed the immunofluorescence assay using HELA cells stained with anti β-tubulin antibody after 20 hours of treatment with indole **20** (Figure 7). Compared to vehicle-treated cells, 500 nM of indole **20** clearly induced the microtubule network depolymerization and the cell cycle arrest, evidenced by the condensed chromosomes. In addition, we could observe abnormal chromosomal segregation and fragmented centrosomal material which are

characteristic of microtubules degradation. At the dose of 1 μM of indole **20**, we could barely observe tubulin staining in some of the cells (Figure 7).

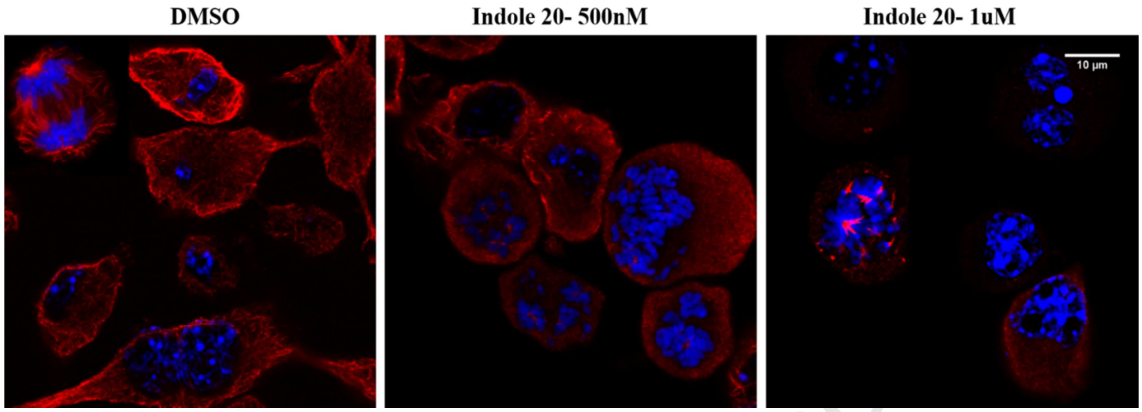


Figure 7. Effects of indole 20 on microtubule cytoskeleton of Hela cells. Hela cells were incubated for 18 hours with DMSO (Control), 500 nM and 1 μM of indole **20**. Anti-β-tubulin antibodies and DAPI were used to stain microtubules (red), and DNA (blue), respectively.

2.2.3 Indole 20 promotes leukemia cells differentiation

The gene expression profile of HL60 cells treated with indole **20** was compared to the gene expression profiles obtained from hundreds of known drugs, available in the Connectivity Map platform [22]. Indole **20** showed a gene expression signature similar to that of prostaglandins (C01EA; Figure 8A). Prostaglandin J2 was shown to inhibit microtubule polymerization [23]. Moreover, prostaglandins were shown to promote rapid *in vitro* differentiation of HL60 cells along the granulocyte pathway [24]. Consistent with this fact, treatment of HL60 with indole **20** during 48 hours resulted in an increased expression of the differentiation markers CD11b and CD14, in a dose-dependent manner (Figure 8B). Retinoic acid and vitamin D3 treatments were used as positive controls for CD11b and CD11b/CD14, respectively. CD11b levels, which is a marker of the granulocyte pathway, begins to increase after 24h of indole **20** treatment (Supplemental Figure 1), while cells positive for both CD11b and CD14 levels began to appear later, after 48 hours of treatment (Figure 8B).

Of note, CMap analysis evidenced that indole **20** induced a gene expression profile opposite to both apigenin, a well-known as an autophagy inducer [25], and HDAC (histone deacetylases) inhibitors which also act inducing autophagy [26] (Supplemental Figure 2). These results suggest that indole **20** may decrease or inhibit

the cellular self-degradative process of autophagy. Additional experiments are warranted to investigate the role of indole **20** in autophagy.

A

ATC Code	Enrichment Score	p-value	Cmap plot
C01EA	0.699	0.0005	

B

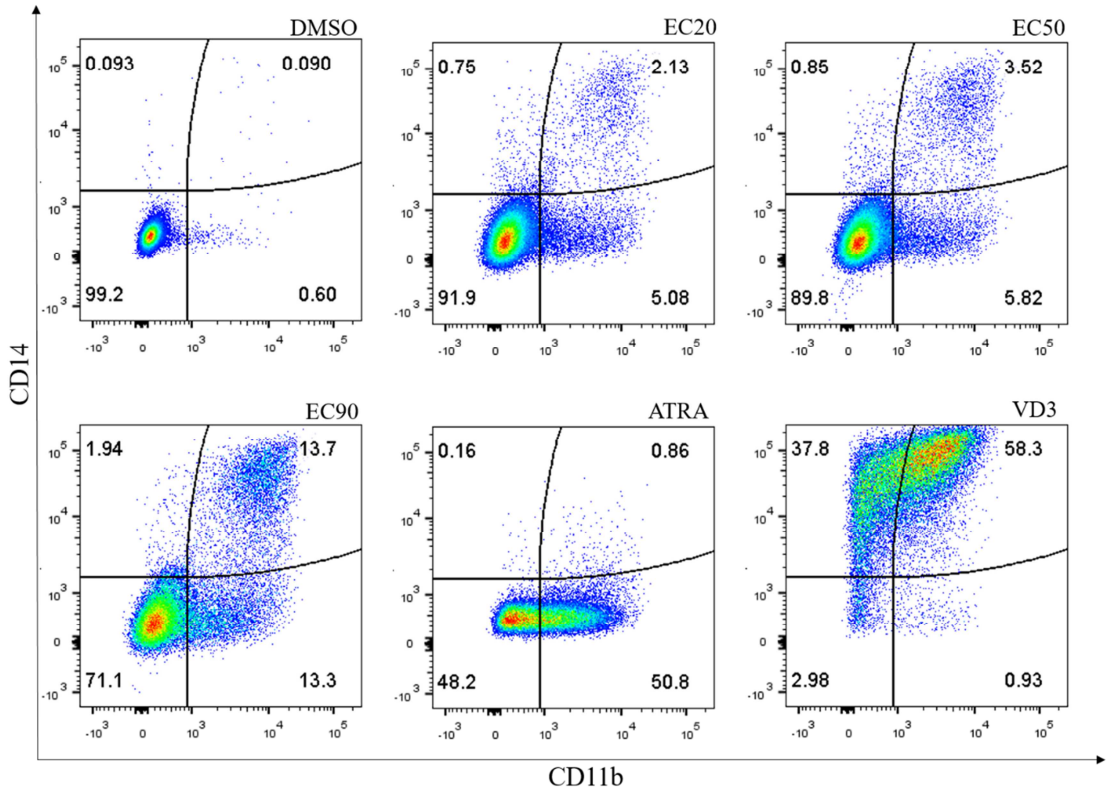


Figure 8. Indole 20 induces differentiation of HL60 leukemia cells. A. Cmap analysis based on ATC code showed that indole **20** presented a drug-induced gene expression profile similar to prostaglandins. **B.** HL60 cells were treated with vehicle (DMSO), EC_{20}, EC_{50} and EC_{90} doses of indole **20**, 2 µM of ATRA or 100 nM of VD3 for 48 hours. ATRA, Trans retinoic acid; VD3, vitamin D3.

2.2.4 Indole 20 anti-leukemic activity in ALL engrafted NOD/SCID mice

The *in vivo* anti-leukemia effect of indole **20** was evaluated in NOD/SCID mice transplanted with RS4;11 leukemia cells. Ten animals were randomized in control (vehicle-treated) and indole **20**-treated groups. Treatment initiated when the human ALL percentage in the peripheral blood of half of the animals reached >1,5%, featuring an advanced stage of the disease. Animals were treated with vehicle (1% PVP, 4% DMSO and 95% PBS) or indole **20** at 10 mg/kg, given intraperitoneally once a day, 5x per week. Indole **20** was well tolerated by mice during the whole experiment. As shown

in Figure 9, NOD/SCID mice treated with indole **20** showed a significant increase in survival compared to controls.

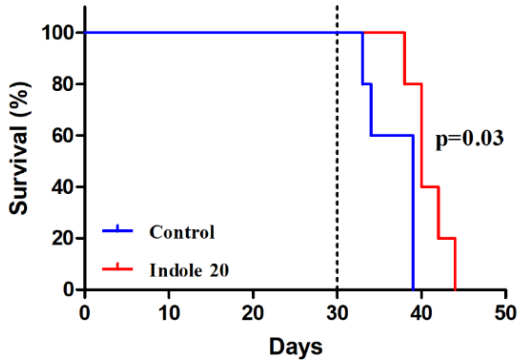


Figure 9. Indole-20-prolonged survival of RS4;11 engrafted NOD/SCID mice. Kaplan Meyer survival curves of mice following the treatment with 10 mg/kg of indole **20** (n=5) or vehicle (n=5) administrated 5x per week, intraperitoneally. Curves were compared by the log-rank test. Dashed line marks the time when leukemia engraftment at ≥ 1.5% in peripheral blood was documented and treatment was initiated.

2.2.5 Discussion

It is a well-known fact that indole molecules acting as inhibitors of tubulin polymerization are considered potential new anticancer agents. Here, we show the synthesis of the new indole **20**, which showed anti-proliferative, and pro-apoptotic activity, at the nanomolar range, against acute leukemia cells. Indole **20** promoted microtubule depolymerization and cell cycle arrest at G2/M suggesting that our structural modifications in the indole ring did not affect its well-known molecular target in cancer cells. However, an attractive feature of indole **20** was its selectivity against leukemia cells, showing no apparent cytotoxicity against normal T-lymphocytes, even at 100 μM. On the contrary, vincristine, one of the main drugs used in the treatment of ALL, presents a narrow therapeutic window regimen due to its high toxicity [6,7]. The selective cytotoxicity of indole **20** against leukemia cells in comparison to T-lymphocytes may be due to a particular tubulin isoform composition of leukemia cell [27,28].

Microarray expression analysis of HL60 cells treated with indole **20** confirmed the upregulation of mitotic spindle and G2/M checkpoints gene sets, as expected for a tubulin-binding agent. However, indole **20** transcriptional profile showed also similarity to prostaglandins, probably due to upregulation of NOTCH signaling [24] and induction of cell differentiation.

CMap analysis also showed a transcriptional profile opposite to apigenin and HDAC inhibitors. Both classes of drugs are known to induce autophagy in TF1 and AML1-ETO leukemia cells, respectively [25,26]. Thus, we speculated that indole **20** may have an autophagy repression activity. It is well known that autophagy has cytoprotective roles to guarantee cell survival [29]. Autophagy activation has been described as a mechanism of resistance to dexamethasone [30] and its inhibition can overcome this resistance in lymphoid malignant cells [31]. Moreover, inactivation of autophagy potentiated the efficacy of AraC treatment as well as reduced functional leukemia initiation cells in murine myeloid leukemia [32]. Further studies are warranted to verify the effects of indole **20** on autophagy.

Indole **20** prolonged the overall survival of NOD/SCID mice transplanted with a pre B-ALL cell line when given at a dose of 10 mg/kg/5x per week. Of note, indole **20** treatment started when leukemia cells were ≥ 1.5% on peripheral blood, which in this model corresponds to a high stage leukemia burden in the bone marrow and spleen [33]. These preliminary results revealed that indole **20** had a positive effect on survival, even when used as a single agent against ALL. However, further *in vivo* studies are necessary to better establish the correct schedule of indole **20** administration and its combination with other chemotherapeutic agents.

3. Conclusions

We have developed a versatile and high yielding method for the synthesis of several new 2-carboxyindoles starting from *β*,*β*-acrylates. This synthetic method allowed the construction of a library of over thirty different 2-carbomethoxy-3-substituted indoles with a diversity of groups at the C3 position such as aryl, alkyl, and carboxymethyl groups. The new active compound methyl 6-methoxy-3-(4-(trifluoromethyl) phenyl)-1H-indole-2-carboxylate (indole **20**) was found to target tubulin leading to G2/M cell cycle arrest, DNA damage and apoptosis featuring selectivity against leukemia cells. The Connectivity map analysis of HL60 promyelocytic leukemia cells treated with indole **20** revealed a transcriptional profile similar to that of cells treated with prostaglandins. Moreover, indole **20** given intraperitoneally, at 10 mg/kg, 5x/week significantly prolonged the overall survival of NOD/SCID mice transplanted with RS4;11 B-ALL cells. These results indicate that indole **20** is a promising lead for the development of new agents for the treatment of acute lymphoblastic leukemia.

4. Experimental section

4.1 Chemistry

General Information. Solvents used for chromatography were distilled prior to use. The other reagents were used without further purification unless otherwise stated. The purification of reaction products was performed by flash chromatography using silica gel (220−440 mesh) and EtOAc/hexane mixtures as the eluent. Analytical thin-layer chromatography (TLC) was performed employing silica gel 60 F_{254} plates. Visualization was accomplished with UV light. NMR analyses were performed on 250, 400, 500 or 600 MHz NMR spectrometers. Chemical shifts (δ) for ^{1}H and ^{13}C-NMR spectra are given in ppm. The residual solvent signals were used as references for ^{1}H and ^{13}C-NMR spectra and the chemical shifts converted to the TMS scale. Data were reported as follows: chemical shift (δ), multiplicity, coupling constant (J) in Hertz and integrated relative intensity. High-resolution mass spectrometry (HRMS) was performed by direct infusion in the mass spectrometer using electrospray ionization with quadrupole time-of-flight or orbitrap as an analyzer (ESI-Q-TOF or ESI-orbitrap, respectively). Melting points (Mp) were determined on a capillary melting point apparatus and are uncorrected. The arenediazonium tetrafluoroborates were prepared according to the literature [34]. Methyl (*E*)-4-phenylbut-2-enoate and methyl (*E*)-3-cyclohexyl acrylate starting materials were synthesized according to the literature and the spectroscopic data matches the values reported in the literature [35,36]. Other acrylate derivatives were commercially available in its pure *E* configuration and have been used without previous purification.

General Procedure A: Synthesis of of Cinnamates. To a round-bottom flask equipped with a magnetic stir bar, a solution of methyl acrylate (15 mmol) in methanol (60 mL) was kept under stirring. Next, half of the *o*-nitro substituted arenediazonium tetrafluoroborate (5 mmol), and palladium acetate (0.5 mmol) were added. After stirring at room temperature for 15 minutes, the other half (5 mmol) of the *o*-nitro substituted arenediazonium tetrafluoroborate (5 mmol) and additional palladium acetate (0.5 mmol) were added. The mixture was stirred at r.t. until complete consumption of the arenediazonium salt. The reaction mixture was then filtered through a short pad of silica

and purified by flash column chromatography using hexane/ethyl acetate (80:20) as eluent.

General Procedure B: Heck Matsuda of Cinnamates. A round-bottom flask equipped with a magnetic stir bar was charged with the cinnamate ester (1 mmol), $Pd(OAc)_2$ (7.5 mol%, 0.075 mmol), and methanol (6 mL). After vigorous stirring at room temperature, the corresponding arenediazonium tetrafluoroborate (2 mmol) was added in one portion. The reaction mixture was immersed in oil bath and heated to reflux until TLC indicated complete consumption of the cinnamate. After cooling, the mixture was filtered through a short pad of silica and purified by flash column chromatography using hexane/ethyl acetate (80:20) as an eluent.

General Procedure C: Synthesis of indoles. In a sealed tube were added the *β*,*β*-substituted acrylate (1 mmol) and triethyl phosphite (0.79 mL, 4 mmol). The reaction was heated at 160°C in an oil bath for 24 hours. After cooling to rt, the crude reaction mixture was mixed with silica gel, and the resulting slurry was place on the top of a flash column chromatography (silica gel), and purified using hexane/ethyl acetate (80:20) as eluent to provide the corresponding indoles.

Methyl (*E*)-3-(4-methoxy-2-nitrophenyl)acrylate (1). Prepared using general procedure A from methyl acrylate and 4-methoxy-2-nitrobenzenediazonium tetrafluoroborate to provide the compound **1** as a yellow solid (80% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (d, J = 15.8 Hz, 1H), 7.58 (d, J = 8.7 Hz, 1H), 7.50 (d, J = 2.7 Hz, 1H), 7.17 (dd, J = 8.8, 2.6 Hz, 1H), 6.31 (d, J = 15.8 Hz, 1H), 3.91 (s, 4H), 3.82 (s, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.5, 160.9, 149.4, 139.5, 130.0, 122.5, 121.1, 120.0, 109.5, 56.1, 51.9. CAS No.: 103986-96-5.

Methyl (*E*)-3-(2-nitrophenyl)acrylate (2). Prepared using general procedure A from methyl acrylate and 2-nitrobenzenediazonium tetrafluoroborate to provide the compound **2** as a light yellow solid (87% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (d, J = 15.8 Hz, 1H), 8.05 (d, J = 8.0 Hz, 1H), 7.69–7.61 (m, 2H), 7.55 (ddd, J = 8.6, 6.6, 2.2 Hz, 1H), 6.37 (d, J = 15.8 Hz, 1H), 3.83 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.2, 148.4, 140.1, 133.5, 130.3, 129.1, 124.9, 122.9, 52.0. CAS No.: 612-43-1.

Methyl (*E*)-3-(2-nitro-4-(trifluoromethyl)phenyl)acrylate (3). Prepared using general procedure A from methyl acrylate and 4-trifluoromethyl-2-nitrobenzenediazonium tetrafluoroborate to provide the compound **3** as a light yellow solid (86% yield). Mp 58-59 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.33 (s, 1H), 8.12 (d, J =15.8 Hz, 1H), 7.91 (d, J = 8.1 Hz, 1H), 7.78 (d, J = 8.1 Hz, 1H), 6.43 (d, J = 15.9 Hz, 1H), 3.85 (s, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.7, 148.1, 138.7, 134.1, 132.7, 132.5, 130.2, 130.0, 124.9, 123.6, 122.4, 121.5, 52.2. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{11}H_9F_3NO_4$: 276.1907; found 276.1909.

Methyl (*E*)-3-(4-methoxy-2-nitrophenyl)-3-(2-methoxyphenyl)acrylate (4). Prepared using general procedure B from compound **1** and 2-methoxybenzenediazonium tetrafluoroborate to provide the compound **4** as a light yellow solid (78% yield). Mp 118-119 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.62 (d, J = 2.6 Hz, 1H), 7.29 (ddd, J = 8.4, 7.4, 1.8 Hz, 1H), 7.20 (d, J = 8.5 Hz, 1H), 7.14 (dd, J = 8.5, 2.6 Hz, 1H), 7.09 (dd, J = 7.7, 1.8 Hz, 1H), 6.90 (d, J = 8.8 Hz, 1H), 6.87 (dd, J = 7.5, 1.1 Hz, 1H), 6.59 (s, 1H), 3.89 (s, 3H), 3.75 (s, 3H), 3.60 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 166.3, 159.2, 157.6, 150.7, 148.3, 132.2, 131.0, 130.6, 128.0, 120.7, 120.5, 119.4, 111.7, 108.6, 55.8, 55.6, 51.4. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$calcd for $C_{18}H_{18}NO_6$: 344.1129; found 344.1122.

Methyl (*Z*)-3-(4-fluorophenyl)-3-(4-methoxy-2-nitrophenyl)acrylate (5). Prepared using general procedure B from compound **1** and 4-fluorobenzenediazonium tetrafluoroborate to provide the compound **5** as a light yellow solid (55% yield). Mp 98-100 °C.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, J = 2.5 Hz, 1H), 7.32–7.29 (m, 2H), 7.22 (dd, J = 8.5, 2.6 Hz, 1H), 7.15 (d, J = 8.5 Hz, 1H), 7.01 (t, J = 8.6 Hz, 2H), 6.41 (s, 1H), 3.92 (s, 3H), 3.60 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.0, 163.4 (d, J = 251 Hz), 159.9, 153.0, 148.6, 135.0, 134.9, 132.0, 129.6, 129.5, 126.6, 120.2, 116.4 (d, J = 21.9 Hz), 115.9, 115.7, 109.4, 56.0, 51.6. HRMS (ESI-Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{17}H_{15}FNO_5$: 332.09288; found 332.09285. Also, $[M + Na]^+$ calcd for $C_{17}H_{14}FNO_5Na$: 354.07482; found 354.07489.

Methyl (*Z*)-3-(4-bromophenyl)-3-(4-methoxy-2-nitrophenyl)acrylate (6). Prepared using general procedure B from compound **1** and 4-bromobenzenediazonium tetrafluoroborate to provide the compound **6** as a light yellow solid (57% yield). Mp

114-115 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, J = 2.6 Hz, 1H), 7.46 (d, J = 8.7 Hz, 1H), 7.24 (dd, J = 8.5, 2.6 Hz, 1H), 7.21 (d, J = 8.6 Hz, 2H), 7.17 (d, J = 8.5 Hz, 1H), 6.44 (s, 1H), 3.92 (s, 3H), 3.60 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.7, 159.9, 152.8, 148.4, 137.6, 131.9, 131.8, 129.0, 126.1, 124.3, 120.0, 116.9, 109.26, 55.9, 51.6. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{17}H_{15}BrNO_5$: 392.0128; found 392.0142.

Methyl (*Z*)-3-(3,4-dichlorophenyl)-3-(4-methoxy-2-nitrophenyl)acrylate (7). Prepared using general procedure B from compound **1** and 3,4-dichlorobenzenediazonium tetrafluoroborate to provide the compound **7** as a white solid (56% yield). Mp 133-134 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73 (d, J = 2.7 Hz, 1H), 7.41 (d, J = 8.4 Hz, 1H), 7.38 (d, J = 2.2 Hz, 1H), 7.23 (dd, J = 8.5, 2.6 Hz, 1H), 7.17 (dd, J = 8.5, 2.2 Hz, 1H), 7.14 (d, J = 8,5 Hz, 1H), 6.43 (s, 1H), 3.94 (s, 3H), 3.61 (s, 3H).^{13}C NMR (126 MHz, $CDCl_3$):δ 165.5, 160.0, 151.6, 148.4, 138.7, 134.0, 133.1, 131.8, 130.6, 129.2, 126.6, 125.5, 120.2, 117.8, 109.4, 55.9, 51.7. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{17}H_{14}Cl_2NO_5$: 382.0244; found 382.0241.

Methyl (*Z*)-3-(4-methoxy-2-nitrophenyl)-3-(4-(trifluoromethyl)phenyl)acrylate (8). Prepared using general procedure B from compound **1** and 4-trifluoromethyl benzenediazonium tetrafluoroborate to provide the compound **8** as a light yellow solid (65% yield). Mp 114-115 °C.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.62 (d, J = 8.1 Hz, 2H), 7.48 (d, J = 8.2 Hz, 2H), 7.28 (m, 1H), 7.21 (d, J = 8.6 Hz, 1H), 6.53 (s, 1H), 3.97 (s, 3H), 3.65 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.7, 160.2, 152.6, 148.6, 142.3, 132.0, 131.6 (q, J = 32.8 Hz), 128.0, 126.0, 125.7 (q, J = 3.5 Hz), 124.0 (q, J = 272 Hz), 120.2, 118.6, 109.5, 56.0, 51.7. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{18}H_{15}F_3NO_5$: 382.0897; found 382.0888.

Methyl (*Z*)-3-(4-methoxyphenyl)-3-(2-nitrophenyl)acrylate (9). Prepared using general procedure B from compound **2** and 4-methoxybenzenediazonium tetrafluoroborate to provide the compound **8** as a light yellow solid (57% yield). Mp 148-149 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.20 (d, J = 8.2 Hz, 1H), 7.67 (m, 1H), 7.57 (m, 1H), 7.26 (m, 3H), 6.84 (d, J = 9.0 Hz, 2H), 6.42 (s, 1H), 3.80 (s, 3H), 3.57 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.1, 161.1, 153.6, 147.8, 135.2, 133.3, 131.0, 130.5, 129.0, 128.8, 124.6, 114.2, 114.1, 55.4, 51.4. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{17}H_{16}NO_5$: 314.1023; found 314.1020.

Methyl (*Z*)-3-(2-nitrophenyl)-3-phenylacrylate (10). Prepared using general procedure B from compound **2** and benzenediazonium tetrafluoroborate to provide the compound **10** as a light yellow solid (39% yield). Mp 115-116 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14 (dd, J = 8.3, 1.3 Hz, 1H), 7.60 (td, J = 7.5, 1.3 Hz, 1H), 7.50 (td, J = 8.7, 7.4, 1.5 Hz, 1H), 7.28–7.25 (m, 2H), 7.25-7.23 (m, 3H), 7.21 (dd, J = 7.6, 1.4 Hz, 1H), 6.42 (s, 1H), 3.51 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.9, 154.0, 147.8, 138.1, 134.9, 133.3, 131.1, 129.9, 128.9, 128.7, 127.5, 124.7, 116.4, 51.5. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{16}H_{14}NO_4$: 284.0917; found 284.0906.

Methyl (*Z*)-3-(4-fluorophenyl)-3-(2-nitrophenyl)acrylate (11). Prepared using general procedure B from compound **2** and 4-fluorobenzenediazonium tetrafluoroborate to provide the compound **11** as a light yellow solid (89% yield). Mp 98-99 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.22 (dd, J = 8.3, 1.0 Hz, 1H), 7.69 (td, J = 7.5, 1.2 Hz, 1H), 7.62–7.55 (m, 1H), 7.34–7.29 (m, 2H), 7.27 (dd, J = 7.6, 1.3 Hz, 1H), 7.06–6.99 (m, 2H), 6.43 (s, 1H), 3.58 (s, 3H).^{13}C NMR (126 MHz, $CDCl_3$) δ 165.8, 163.8 (d, J = 251 Hz), 153.0, 147.8, 134.8, 134.5, 134.4, 133.5, 131.0, 129.6 (d, J = 8.5 Hz), 129.2, 124.9, 116.4, 115.9 (d, J = 21.9 Hz), 51.6. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{16}H_{13}FNO_4$: 302.0823; found 302.0825.

Methyl (*Z*)-3-(2-nitrophenyl)-3-(4-(trifluoromethyl)phenyl)acrylate (12). Prepared using general procedure B from compound **2** and 4-trifluoromethylbenzenediazonium tetrafluoroborate to provide the compound **12** as a light yellow solid (40% yield). Mp 114-115 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (dd, J = 8.2, 1.3 Hz, 1H), 7.72 (td, J = 7.5, 1.3 Hz, 1H), 7.63–7.59 (m, 3H), 7.45 (d, J = 8.0 Hz, 2H), 7.29 (dd, J = 7.6, 1.5 Hz, 1H), 6.52 (s, 1H), 3.60 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.5, 152.6, 147.8, 141.8, 134.2, 133.7, 131.7 (q, J = 32.8 Hz), 131.1, 129.5, 127.9, 125.8 (q, J = 3.7 Hz), 125.0, 123.9 (q, J = 272 Hz), 118.5, 51.8. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{17}H_{13}F_3NO_4$: 352.0791; found 352.0788.

Methyl (*Z*)-3-(4-methoxyphenyl)-3-(2-nitro-4-(trifluoromethyl)phenyl)acrylate (13). Prepared using general procedure B from compound **3** and 4-methoxybenzenediazonium tetrafluoroborate to provide the compound **13** as a light yellow solid (49% yield). Mp 142-144°C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.46 (d, J =

1.7 Hz, 1H), 7.91 (dd, *J* = 8.1, 1.7 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 1H), 7.22 (d, *J* = 8.9 Hz, 2H), 6.86 (d, *J* = 8.9 Hz, 2H), 6.46 (s, 1H), 3.81 (s, 3H), 3.59 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.0, 161.4, 152.3, 147.9, 139.0, 132.0, 131.4 (q, *J* = 34.2 Hz), 129.7 (q, *J* = 3.9 Hz), 129.0, 122.9 (q, *J* = 273 Hz), 122.0 (q, *J* = 3.8 Hz), 114.7, 114.3, 55.4, 51.6. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{18}H_{15}F_3NO_5$: 382.0877; found 382.0880.

Methyl (*Z*)-3-(2-nitro-4-(trifluoromethyl)phenyl)-3-phenylacrylate (14). Prepared using general procedure B from compound **3** and benzenediazonium tetrafluoroborate to provide the compound **14** as a light yellow solid (29% yield). Mp 132-134 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.52(d, *J* = 1.7 Hz, 1H), 7.96 (dd, *J* = 8,0, 1.0 Hz, 1H), 7.48 (d, *J* = 8.0 Hz, 1H), 7.42–7.37 (m, 3H), 7.34-7.32 (m, 2H), 6.57 (s, 1H), 3.65 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.85, 152.8, 148.0, 138.8, 137.5, 132.2, 131.7 (q, *J* = 34.3 Hz), 130.5, 129.8 (q, *J* = 3.3 Hz), 129.0, 127.6, 125.1 (q, *J* = 273 Hz), 122.2 (q, *J* = 3.8 Hz), 117.1, 51.8. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{17}H_{13}F_3NO_4$: 352.0791; found 352.0802.

Methyl (*Z*)-3-(2-nitro-4-(trifluoromethyl)phenyl)-3-(4(trifluoromethyl)phenyl) acrylate (15). Prepared using general procedure B from compound **3** and 4-trifluoromethylbenzenediazonium tetrafluoroborate to provide the compound **15** as a light yellow solid (44% yield). Mp 1221-124 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.50 (d, *J* = 1.9 Hz, 1H), 7.96 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.62 (d, *J* = 8.2 Hz, 2H), 7.46 (d, *J* = 8.0 Hz, 1H), 7.43 (d, *J* = 8.2 Hz, 2H), 6.57 (s, 1H), 3.63 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.4, 151.3, 147.8, 137.9, 132.1 (q, *J* = 32.8 Hz), 132.1, 130.1 (q, *J* = 3.2 Hz), 127.9, 126.0 (q, *J* = 3.6 Hz), 123.8 (q, *J* = 272 Hz), 122.4 (q, *J* = 3.8 Hz), 122.8 (q, *J* = 272 Hz), 119.1, 52.0. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{18}H_{12}F_6NO_4$: 420.0665; found 420.0669.

Methyl 6-methoxy-3-(2-methoxyphenyl)-1*H*-indole-2-carboxylate (16). Prepared using general procedure C from compound **4** to provide the compound **16** as a white solid (83% yield). Mp 162-165 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.89 (s, 1H), 7.40-7.38 (m, 2H), 7.37 (d, *J* = 8.9 Hz, 1H), 7.08 (dd, *J* = 7.4, 1.1 Hz, 1H), 7.05 (dd, *J* = 8.5, 1.1 Hz, 1H), 6.87 (d, *J* = 2.2 Hz, 1H), 6.82 (dd, *J* = 8.9, 2.3 Hz, 1H), 3.90 (s, 3H), 3.79 (s, 3H), 3.78 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.5, 159.1, 157.4, 136.8, 132.1,

128.9, 122.9, 122.7, 122.6, 122.5, 120.5, 120.2, 112.1, 110.9, 93.5, 55.6, 51.6. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{18}H_{18}NO_4$: 312.1236; found 312.1235.

Methyl 3-(4-fluorophenyl)-6-methoxy-1*H*-indole-2-carboxylate (17). Prepared using general procedure C from compound **5** to provide the compound **17** as a white solid (64% yield). Mp 184-186 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.85 (s, 1H), 7.52–7.50 (m, 2H), 7.45 (d, *J* = 8.8 Hz, 1H), 7.19–7.11 (m, 2H), 6.85 (d, *J* = 1.8 Hz, 1H), 6.83 (dd, *J* = 8.8, 2.2 Hz, 1H), 3.88 (s, 3H), 3.81 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.4 (d, *J* = 246 Hz), 162.3, 159.6, 136.9, 132.2 (d, *J* = 8.0 Hz), 129.5, 124.0, 122.6, 122.4, 121.4, 115.0 (d, *J* = 22.0 Hz), 112.7, 93.6, 55.7, 51.8. HRMS (ESI-Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{17}H_{15}FNO_3$: 300.10305; found 300.10278. Also $[M + Na]^+$ calcd for $C_{17}H_{14}FNO_3Na$: 322.08499; found 322.08441.

Methyl 3-(4-bromophenyl)-6-methoxy-1*H*-indole-2-carboxylate (18). Prepared using general procedure C from compound **6** to provide the compound **18** as a white solid (57% yield). Mp 193-195 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.93 (s, 1H), 7.60 (d, *J* = 8.4 Hz, 1H), 7.47 (d, *J* = 8.8 Hz, 1H), 7.44 (d, *J* = 8.4 Hz, 1H), 6.87 (d, *J* = 2.1 Hz, 1H), 6.85 (dd, *J* = 8.8, 2.3 Hz, 1H), 3.90 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 162.1, 159.4, 136.8, 132.4, 132.1, 131.1, 123.5, 122.3, 122.0, 121.4, 121.3, 112.7, 93.5, 55.6, 51.7. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{17}H_{15}BrNO_3$: 360.0230; found 360.0210.

Methyl 3-(3,4-dichlorophenyl)-6-methoxy-1*H*-indole-2-carboxylate (19). Prepared using general procedure C from compound **7** to provide the compound **19** as a white solid (91% yield). Mp 227-230 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.87 (s, 1H), 7.64 (d, *J* = 2.0 Hz, 1H), 7.51 (d, *J* = 8.2 Hz, 1H), 7.44 (d, *J* = 9.5 Hz, 1H), 7.38 (dd, *J* = 8.3, 2.0 Hz, 1H), 6.85 (d, *J* = 3.9 Hz, 1H), 6.84 (d, *J* = 3.4 Hz, 1H), 3.88 (s, 3H), 3.82 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.9, 159.5, 136.7, 133.6, 132.2, 131.9, 131.3, 129.9, 129.8, 122.1, 121.9, 121.5, 113.0, 93.5, 55.6, 51.8. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{17}H_{14}Cl_2NO_3$: 350.0345; found 350.0349.

Methyl 6-methoxy-3-(4-(trifluoromethyl)phenyl)-1*H*-indole-2-carboxylate (20). Prepared using general procedure C from compound **8** to provide the compound **20** as a white solid (75% yield). Mp 196-197 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.96 (s, 1H),

7.73 (d, *J* = 8.2 Hz, 2H), 7.70 (d, *J* = 8.1 Hz, 2H), 7.47 (d, *J* = 8.8 Hz, 1H), 6.89 (d, *J* = 2.2 Hz, 1H), 6.87 (dd, *J* = 8.8, 2.3 Hz, 1H), 3.91 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 162.1, 159.6, 137.5, 136.9, 130.9, 129.4 (q, *J* = 32.2 Hz), 125.0 (q, *J* = 3.6 Hz), 124.5 (q, *J* = 272 Hz), 123.37, 122.36, 122.15, 121.67, 113.07, 93.65, 55.7, 51.9. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{18}H_{15}F_3NO_3$: 350.0999; found 350.0976.

Methyl 3-(4-methoxyphenyl)-1*H*-indole-2-carboxylate (21).[37] Prepared using general procedure C from compound **9** to provide the compound **21** as a white solid (82% yield). Mp 161-162 °C (lit. 162–164 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.01 (s, 1H), 7.67 (dd, *J* = 8.2, 1,0 Hz, 1H), 7.55–7.51 (m, 2H), 7.46 (m, 1H), 7.39 (ddd, *J* = 8.2, 6.8, 1.1 Hz, 1H), 7.18 (ddd, *J* = 8.1, 6.8, 1.1 Hz, 1H), 7.04 (m, 2H), 3.91 (s, 3H), 3.86 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.4, 158.9, 135.8, 131.7, 128.0, 125.9, 125.6, 124.3, 122.2, 121.8, 120.8, 113.4, 111.7, 55.3, 51.8. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{17}H_{16}NO_3$: 282.1125; found 282.114.

Methyl 3-phenyl-1*H*-indole-2-carboxylate (22).[37] Prepared using general procedure C from compound **10** to provide the compound **22** as a white solid (95% yield). Mp 132-135 °C (lit. 132-135 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.06 (s, 1H), 7.67 (dd, *J* = 8.2, 1.0 Hz, 1H), 7.59 (m, 2H), 7.52–7.46 (m, 3H), 7.44–7.38 (m, 2H), 7.18 (ddd, *J* = 8.1, 6.9, 1.1 Hz, 1H), 3.85 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.4, 135.8, 133.4, 130.6, 127.9, 127.3, 125.9, 124.4, 122.4, 121.8, 120.9, 111.7, 51.8. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{16}H_{14}NO_2$: 252.1019; found 252.1020.

Methyl 3-(4-fluorophenyl)-1*H*-indole-2-carboxylate (23). Prepared using general procedure C from compound **11** to provide the compound **23** as a white solid (78% yield). Mp 154-155 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ 9.11 (s, 1H), 7.60 (d, *J* = 8.1 Hz, 1H), 7.56 – 7.50 (m, 2H), 7.45 (d, *J* = 8.3 Hz, 1H), 7.41 – 7.34 (m, 1H), 7.20 – 7.13 (m, 3H), 3.84 (s, 3H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 162.5, 162.3 (d, *J* = 246 Hz), 135.9, 132.3 (d, *J* = 8.0 Hz), 129.4, 129.4, 128.0, 126.2, 123.5, 122.6, 121.6, 121.2, 115.0 (d, *J* = 22.0 Hz), 111.9, 52.0. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{16}H_{13}FNO_2$: 270.0925; found 270.0925.

Methyl 3-(4-(trifluoromethyl)phenyl)-1*H*-indole-2-carboxylate (24).[37] Prepared using general procedure C from compound **12** to provide the compound **24** as a white solid (87% yield). Mp 174-175 °C (lit. 175-177 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.12 (s, 1H), 7.75 (d, J = 8.7 Hz, 2H), 7.70 (d, J = 8.1 Hz, 2H), 7.62 (dd, J = 8.2, 1.0 Hz, 1H), 7.50 (d, J = 8.0 Hz, 1H), 7.42 (ddd, J = 8.3, 6.9, 1.1 Hz, 1H), 7.21 (ddd, J = 8.1, 6.9, 1.1 Hz, 1H), 3.87 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.2, 137.4, 135.8, 131.0, 129.4 (q, J = 32.3 Hz), 127.7, 126.3, 125.0 0 (q, J = 3.6 Hz), 124.5 (q, J = 272 Hz), 123.2, 122.9, 121.5, 112.0, 52.1. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{17}H_{13}F_3NO_2$: 320.0893; found 320.0872.

Methyl 3-(4-methoxyphenyl)-6-(trifluoromethyl)-1*H*-indole-2-carboxylate (25). Prepared using general procedure C from compound **13** to provide the compound **25** as a white solid (83% yield). Mp 225-226 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.16 (s, 1H), 7.76 (m, 2H), 7.49 (m, 2H), 7.39 (dd, J = 8.6, 1.5 Hz, 1H), 7.05 (m, 2H), 3.92 (s, 3H), 3.88 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.1, 159.4, 134.6, 131.7, 130.2, 127.79, 126.16, 124.8 (q, J = 272 Hz), 124.21, 122.75, 117.4 (q, J = 3.0 Hz), 113.7, 109.5 (q, J = 4.5 Hz), 55.5, 52.2. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{18}H_{15}F_3NO_3$: 350.0999; found 350.0979.

Methyl 3-phenyl-6-(trifluoromethyl)-1*H*-indole-2-carboxylate (26).[38] Prepared using general procedure C from compound **14** to provide the compound **26** as a white solid (80% yield). Mp 189-190 °C (lit. 192-193 °C). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.23 (s, 1H), 7.75 (s, 1H), 7.72 (d, J = 8.6 Hz, 1H), 7.53 (d, J = 6.7 Hz, 2H), 7.48 (t, J = 7.6 Hz, 2H), 7.48–7.42 (m, 1H), 7.37 (dd, J = 8.6,1.5 Hz, 1H), 3.84 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.0, 134.4, 130.5, 130.0, 128.1, 127.7, 124.8, 124.7 (q, J = 273 Hz), 124.20, 122.6, 117.4 (q, J = 3.3 Hz), 109.5 (q, J = 4.6 Hz), 52.1. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{17}H_{13}F_3NO_2$: 320.0893; found 320.0880.

Methyl 6-(trifluoromethyl)-3-(4-(trifluoromethyl)phenyl)-1*H*-indole-2-carboxylate (27). Prepared using general procedure C from compound **15** to provide the compound **27** as a white solid (71% yield). Mp 169-170 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.35 (s, 1H), 7.80 (s, 1H), 7.76 (m, 2H), 7.72 (d, J = 8.0 Hz, 1H), 7.69–7.67 (m, 2H), 7.43 (dd, J = 8.5, 1.5 Hz, 1H), 3.89 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.8, 136.6, 134.5, 131.0, 129.9, 129.8 (q, J = 32.3 Hz), 128.3 (q, J = 32.3 Hz), 125.2 (q, J = 3.6 Hz), 124.7

(q, *J* = 272 Hz), 124.5 (q, *J* = 272 Hz), 122.7, 122.26, 118.0 (q, *J* = 3.4 Hz), 109.8 (q, *J* = 4.5 Hz), 52.42. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{18}H_{12}F_6NO_2$: 388.0767; found 388.0774.

Methyl (*E*)-3-(4-methoxy-2-nitrophenyl)but-2-enoate (28). Prepared using general procedure B from methyl (*E*)-but-2-enoate and 4-methoxy-2-nitrobenzenediazonium tetrafluoroborate to provide the compound **28** as a light yellow solid (90% yield). Mp 50-52 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (d, *J* = 4.0 Hz, 1H), 7.19 (d, *J* = 8.0 Hz, 1H), 7.12 (dd, *J* = 8.0, 4.0Hz, 1H), 5.77 (q, *J* = 4Hz, 1H), 3.88 (s, 3H), 3.74 (s, 3H), 2.42 (d, *J* = 4Hz, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 166.4, 159.6, 155.0, 147.8, 131.6, 130.8, 119.7, 118.6, 109.4, 56.0, 51.2, 20.5. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{12}H_{14}NO_5$: 252.0866; found 252.0852.

Methyl (*E*)-3-(4-methoxy-2-nitrophenyl)pent-2-enoate (29). Prepared using general procedure B from methyl (*E*)-pent-2-enoate and 4-methoxy-2-nitrobenzenediazonium tetrafluoroborate to provide the compound **29** as a light yellow solid (77% yield). Mp 59-60 °C. ^{1}H NMR (500 MHz, CDCl3) δ 7.54 (d, *J* = 2.4 Hz, 1H), 7.16 (d, *J* = 8.5 Hz, 1H), 7.14–7.12 (m, 1H), 5.70 (s, 1H), 3.88 (s, 3H), 3.73 (s, 3H), 2.90 (q, *J* = 7.5 Hz, 2H), 0.97 (t, *J* = 7.6 Hz, 3H).^{13}C NMR (126 MHz, $CDCl_3$) δ 166.3, 161.2, 159.8, 148.0, 131.7, 130.2, 119.6, 118.1, 109.6, 56.1, 51.4, 26.8, 12.8. HRMS (ESI-Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{13}H_{16}NO_5$: 266.10230; found 266.10233. Also, $[M + Na]^+$ calcd for $C_{13}H_{15}NO_5Na$: 288.08424; found 288.08423.

Methyl (*E*)-3-(4-methoxy-2-nitrophenyl)-4-methylpent-2-enoate (30). Prepared using general procedure B from methyl (*E*)-4-methylpent-2-enoate and 4-methoxy-2-nitrobenzenediazonium tetrafluoroborate to provide the compound **30** as an orange oil, (50% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (s, 1H), 7.11 (d, *J* = 1.3 Hz, 2H), 5.63 (s, 1H), 4.14-4.03 (m, 1H), 3.88 (s, 3H), 3.72 (s, 3H), 1.00 (d, *J* = 6.9 Hz, 6H).^{13}C NMR (126 MHz, CDCl3) δ166.0, 164.0, 159.4, 148.7, 131.3, 127.9, 119.2, 118.8, 109.2, 56.0, 51.3, 31.0, 21.5. HRMS (ESI-Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{14}H_{18}NO_5$: 280.11795; found 280.11789. Also $[M + Na]^+$ calcd for $C_{14}H_{17}NO_5Na$: 302.09989; found 302.09979.

Methyl (*E*)-3-(4-methoxy-2-nitrophenyl)-5-methylhex-2-enoate (31). Prepared using general procedure B from methyl (*E*)-5-methylhex-2-enoate and 4-methoxy-2-nitrobenzenediazonium tetrafluoroborate to provide the compound **31** as an orange oil, (72% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 (d, J = 2.6 Hz, 1H), 7.21 (d, J = 8.5 Hz, 1H), 7.12 (dd, J = 8.5, 2.6 Hz, 1H), 5.84 (s, 1H), 3.88 (s, 3H), 3.74 (s, 3H), 2.79 (d, J = 7.1 Hz, 2H), 1.60-1.52 (m, 1H), 0.85 (d, J = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.4, 159.8, 158.4, 148.3, 131.8, 130.7, 120.0, 119.5, 109.7, 56.1, 51.3, 41.2, 28.3, 22.7. HRMS (ESI-Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{15}H_{20}NO_5$: 294.13360; found 294.13354. Also $[M + Na]^+$ calcd for $C_{15}H_{19}NO_5Na$: 316.11554; found 316.11542.

Methyl (*E*)-3-cyclohexyl-3-(4-methoxy-2-nitrophenyl)acrylate (32). Prepared using general procedure B from methyl (*E*)-3-cyclohexylacrylate and two equivalents of 4-methoxy-2-nitrobenzenediazonium tetrafluoroborate to provide the compound **32** as an orange oil (60% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (t, J = 1.4 Hz, 1H), 7.11 (d, J = 1.4 Hz, 2H), 5.62 (d, J = 0.6 Hz, 1H), 3.88 (s, 3H), 3.72 (s, 3H), 1.81–1.61 (m, 6H), 1.42–1.33 (m, 2H), 1.06–0.94 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.1, 163.7, 159.3, 148.5, 131.3, 128.4, 119.2, 118.6, 109.1, 56.0, 51.3, 41.5, 26.5, 26.0. HRMS (ESI-Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{17}H_{22}NO_5$: 320.14925; found 320.14844. Also $[M + Na]^+$ calcd for $C_{17}H_{21}NO_5Na$: 342.13119; found 342.13123.

Methyl (*E*)-3-(4-methoxy-2-nitrophenyl)-4-phenylbut-2-enoate (33). Prepared using general procedure B from methyl (*E*)-4-phenylbut-2-enoate and two equivalents of 4-methoxy-2-nitrobenzenediazonium tetrafluoroborate and 10 mol% of $Pd(OAc)_2$ to provide the compound **33** as a light brown solid (58% yield). Mp 80-82 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51 (d, J = 2.6 Hz, 1H), 7.18–7.12 (m, 3H), 7.04 (d, J = 7.8 Hz, 2H), 6.92 (dd, J = 8.5, 2.6 Hz, 1H), 6.71 (d, J = 8.5 Hz, 1H), 5.81 (s, 1H), 4.32 (s, 2H), 3.84 (s, 3H), 3.80 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.4, 159.7, 157.5, 147.8, 137.7, 132.4, 129.6, 129.5, 128.4, 126.6, 119.4, 119.1, 109.4, 56.0, 51.6, 38.7. HRMS (ESI-Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{18}H_{18}NO_5$: 328.11795; found 328.11795. Also, $[M + Na]^+$ calcd for $C_{18}H_{17}NO_5Na$: 350.09989; found 350.09967.

Dimethyl 2-(4-methoxy-2-nitrophenyl)maleate (34). Prepared using general procedure B from two equivalents of dimethyl fumarate and 4-methoxy-2-nitrobenzenediazonium tetrafluoroborate to provide the compound **34** as a red oil (50%

yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.63 (d, J = 2.6 Hz, 1H), 7.36 (d, J = 8.5 Hz, 1H), 7.18 (dd, J = 8.5, 2.7 Hz, 1H), 6.29 (s, 1H), 3.91 (s, 3H), 3.85 (s, 3H), 3.73 (s, 3H).^{13}C NMR (126 MHz, CDCl3) δ 166.0, 164.8, 160.8, 148.5, 139.4, 133.0, 128.4, 123.6, 120.0, 110.1, 56.2, 52.8, 52.5. HRMS (ESI-Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{13}H_{14}NO_7$: 296,07648; found 296.07648. Also, $[M+ Na]^+$ calcd for $C_{13}H_{13}NO_7Na$: 318.05842; found 318.05829.

Dimethyl (*E*)-3-(4-methoxy-2-nitrophenyl)pent-2-enedioate (35). Prepared using general procedure B from dimethyl (*E*)-pent-2-enedioate and 4-methoxy-2-nitrobenzenediazonium tetrafluoroborate to provide the compound **35** as an orange oil (94% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, J = 2.6 Hz, 1H), 7.41 (d, J = 8.5 Hz, 1H), 7.18 – 7.15 (dd, J = 8.5, 2.5 Hz 1H), 5.93 (s, 1H), 3.96 (s, 2H), 3.89 (s, 3H), 3.75 (s, 3H), 3.65 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.6, 166.1, 160.1, 150.7, 147.4, 132.5, 130.0, 121.6, 120.2, 109.6, 56.1, 52.1, 51.7, 39.0. HRMS (ESI-Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{14}H_{16}NO_7$: 310.09213; found 310.09192. Also $[M + Na]^+$ calcd for $C_{14}H_{15}NO_7Na$: 332.07407; found 332.07385.

Methyl 6-methoxy-3-methyl-1*H*-indole-2-carboxylate (36).[39] Prepared using general procedure C from compound **28** to provide the compound **36** as a beige solid (85% yield). Mp 162-164 °C (lit. 146–148 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (s, 1H), 7.52 (d, J = 4.0 Hz, 1H), 6.81 (dd, J = 8.0, 4.0 Hz, 1H), 6.78 (d, J = 4.0 Hz, 1H), 3.93 (s, 3H), 3.85 (s, 3H), 2.57 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.0, 159.2, 134.0, 123.0, 122.2, 121.7, 120.9, 111.4, 93.5, 55.5, 51.5, 10.0. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ $C_{12}H_{14}NO_3$: 220.0968; found 220.0954.

Methyl 3-ethyl-6-methoxy-1*H*-indole-2-carboxylate (37).[40] Prepared using general procedure C from compound **29** to provide the compound **37** as a white solid (62% yield). Mp 154-155°C (lit. 150–152 °C). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.55 (s, 1H), 7.56 (d, J = 8.7 Hz, 1H), 6.81 (dd, J = 8.7, 2.2 Hz, 1H), 6.78 (d, J = 1.9 Hz, 1H), 3.93 (s, 3H), 3.85 (s, 3H), 3.08 (q, J = 7.5 Hz, 2H), 1.27 (t, J = 7.5 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.9, 159.3, 137.2, 127.8, 122.2, 121.8, 121.5, 111.5, 93.7, 55.7, 51.7, 18.3, 15.6. HRMS (ESI-Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{13}H_{16}NO_3$: 234.11247; found 234.1120. Also $[M + Na]^+$ calcd for $C_{13}H_{15}NO_3Na$: 256.09441; found 256.09399.

Methyl 3-isopropyl-6-methoxy-1*H*-indole-2-carboxylate (38). Prepared using general procedure C from compound **30** to provide the compound **38** as a yellow solid (77% yield). Mp 138-139 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.54 (s, 1H), 7.74 (d, J = 8.6 Hz, 1H), 6.79 – 6.75 (m, 2H), 4.04 (hept, J = 7.1 Hz, 1H), 3.92 (s, 3H), 3.85 (s, 3H), 1.45 (s, 3H), 1.44 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.9, 158.9, 137.6, 132.0, 123.8, 121.0, 120.7, 111.1, 93.8, 55.6, 51.7, 25.9, 23.0. HRMS (ESI-Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{14}H_{18}NO_3$: 248.12812; found 248.12799. Also $[M + Na]^+$ calcd for $C_{14}H_{17}NO_3Na$: 270.11006; found 270.11008.

Methyl 3-isobutyl-6-methoxy-1*H*-indole-2-carboxylate (39). Prepared using general procedure C from compound **31** to provide the compound **39** as a white solid (71% yield). Mp 122-124 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.58 (s, 1H), 7.53 (d, J = 8.6 Hz, 1H), 6.80–6.78 (m, 2H), 3.92 (s, 3H), 3.85 (s, 3H), 2.94 (d, J = 7.2 Hz, 2H), 2.06–1.95 (m, 1H), 0.94 (d, J = 6.6 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.0, 159.2, 137.0, 125.5, 123.1, 122.3, 122.3, 111.5, 93.6, 55.6, 51.6, 33.9, 30.4, 22.9. HRMS (ESI-Orbitrap) *m/z* $[M + Na]^+$ calcd for $C_{15}H_{19}NO_3Na$: 284.12571; found 284.12579.

Methyl 3-cyclohexyl-6-methoxy-1*H*-indole-2-carboxylate (40). Prepared using general procedure C from compound **32** to provide the compound **40** as a yellow solid (72% yield). Mp 165-166 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (s, 1H), 7.78 (d, J = 9.7 Hz, 1H), 6.77–6.74 (m, 2H), 3.92 (s, 3H), 3.84 (s, 3H), 3.71–3.63 (m, 1H), 1.99–1.79 (m, 7H), 1.50–1.35 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.8, 158.9, 137.5, 131.4, 124.0, 121.3, 120.8, 111.1, 93.7, 55.6, 51.6, 36.5, 33.0, 27.3, 26.5. HRMS (ESI-Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{17}H_{22}NO_3$: 288.15942; found 288.15939. Also, $[M + Na]^+$ calcd for $C_{17}H_{21}NO_3Na$: 310.14136; found 310.14111.

Methyl 3-benzyl-6-methoxy-1*H*-indole-2-carboxylate (41).[41] Prepared using general procedure C from compound **33** to provide the compound **41** as a white solid (44% yield). Mp 175-176 °C (lit. 176– 178 °C). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.63 (s, 1H), 7.45 (d, J = 8.8 Hz, 1H), 7.26 (t, J = 6.4 Hz, 3H), 7.22 (d, J = 7.8 Hz, 1H), 7.15 (t, J = 7.1 Hz, 1H), 6.79 (d, J = 2.0 Hz, 1H), 6.76 (dd, J = 8.8, 2.2 Hz, 1H), 4.47 (s, 2H), 3.92 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.8, 159.3, 141.1, 137.2, 128.6, 128.4, 126.0, 123.6, 122.6, 122.5, 122.3, 111.9, 93.7, 55.6, 51.8, 30.8. HRMS (ESI-

Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{18}H_{18}NO_3$: 296.12812; found 296.12814. Also, $[M + Na]^+$ calcd for $C_{18}H_{17}NO_3Na$: 318.11006; found 318.10992.

Dimethyl 6-methoxy-1*H*-indole-2,3-dicarboxylate (42).[42] Prepared using general procedure C from compound **34** to provide the compound **42** as a white solid (27% yield). Mp 139-140 °C (lit. 134–137 °C). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.51 (s, 1H), 7.89 (d, J = 9.0 Hz, 1H), 6.91 (dd, J = 9.0, 2.0 Hz, 1H), 6.82 (d, J = 1.7 Hz, 1H), 3.97 (s, 3H), 3.94 (s, 3H), 3.82 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.8, 161.6, 159.2, 136.1, 126.8, 123.7, 121.3, 114.2, 112.4, 93.7, 55.6, 52.6, 52.0. HRMS (ESI-Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{13}H_{14}NO_5$: 264.08665; found 264.08664. Also, $[M + Na]^+$ calcd for $C_{13}H_{13}NO_5Na$: 286.06859; found 286.06818.

Methyl 6-methoxy-3-(2-methoxy-2-oxoethyl)-1*H*-indole-2-carboxylate (43). Prepared using general procedure C from compound **35** to provide the compound **43** as a white solid (95% yield). Mp 138-140 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (s, 1H), 7.50 (d, J = 8.8 Hz, 1H), 6.82 (dd, J = 8.9, 1.6 Hz, 1H), 6.76 (d, J = 1.6 Hz, 1H), 4.14 (s, 2H), 3.90 (s, 3H), 3.84 (s, 3H), 3.70 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.9, 162.4, 159.4, 137.0, 123.2, 122.4, 121.6, 116.5, 112.3, 93.8, 55.6, 52.2, 51.8, 30.7. HRMS (ESI-Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{14}H_{16}NO_5$: 278.10230; found 278.10229. Also, $[M + Na]^+$ calcd for $C_{14}H_{15}NO_5Na$: 300.08424; found 300.08414.

6-methoxy-3-(4-(trifluoromethyl)phenyl)-1*H*-indole-2-carboxylic acid (44). Prepared by the following procedure: Indole **20** (1 mmol) and $LiOH \cdot H_2O$ (4 mmol) were dissolved in a solution of THF/MeOH/H_2O (3/1/1, 1 mL/mmol) and stirred for 48 h at room temperature. The solvent was then evaporated under vacuum, and the crude mixture was taken up in H_2O, acidified with HCl 1N and extracted with ethyl acetate. The organic layer was separated, washed with brine, dried over anhydrous Na_2SO_4, and filtered. The solvent was evaporated *in vacuo* and the crude product was purified by flash column chromatography using ethyl acetate /hexane (40 to 100%) as an eluent. Compound **44** was obtained as a white solid in 88% yield. Mp 224-225 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.77 (s, 1H), 11.78 (s, 1H), 7.78 (d, J = 5.0 Hz, 2H), 7.71 (d, J = 10.0, 2H), 7,36 (d, J = 10.0 Hz, 1H), 6.94 (d, J = 5.0 Hz, 1H), 6.76 (dd, J = 10.0, 5.0 Hz, 1H), 3.80 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.5, 158.1, 138.4, 137.0, 131.1, 128.0, 127.0 (q, J = 31.6 Hz), 124.6 (q, J = 3.6 Hz), 124.5 (q, J = 272 Hz), 122.8,

121.1, 120.7, 112.3, 94.0, 55.2. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{17}H_{13}F_3NO_3$: 336.0847; found 336.0827.

General Procedure D: Synthesis of Amides 45 and 46. To a stirred solution of indole **44** (1 mmol) in dry dichloromethane (5 mL/mmol), it was added hydroxybenzotriazole (HOBt, 1 equiv) and EDC·HCl (1 equiv), under nitrogen atmosphere at room temperature. After 10 minutes, 1 equiv of amine (morpholine or phenylalanine) and 3 equiv of triethylamine were added and the reaction mixture stirred overnight. Water was then added, the reaction mixture was transferred to a separatory funnel, and extracted with EtOAc. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was then purified by silica gel flash column chromatography using hexane/ethyl acetate (80:20) as eluent to provide amides **45** and **46**.

(6-methoxy-3-(4-(trifluoromethyl)phenyl)-1*H*-indol-2-yl)(morpholino)methanone (45). Prepared using general procedure D from compound **44** and morpholine to provide the compound **45** as a white solid (72% yield). Mp 209-211 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.39 (s, 1H), 7.76 (d, J = 10.0 Hz, 2H), 7.64 (d, J = 10.0 Hz, 2H), 7.58 (d, J = 5.0 Hz, 1H), 6.93 (d, J ~ 5.0 Hz, 1H), 6.88 (dd, J = 10.0 Hz, 5Hz), 3.88 (s, 3H), 3.40 (s, 8H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.0, 158.3, 138.2, 136.9, 129.6, 129.4 (q, J = 32.3 Hz), 125.9 (q, J = 3.7 Hz), 125.3, 124.2 (q, J = 272 Hz), 120.7, 120.2, 116.8, 112.3, 94.3, 66.1, 55.6. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{21}H_{20}F_3N_2O_3$: 405.1426; found 405.1452.

(6-methoxy-3-(4-(trifluoromethyl)phenyl)-1*H*-indole-2-carbonyl)phenylalanine (46). Prepared using general procedure D from compound **44** and phenylalanine to provide the compound **46** as a white solid (28% yield). Mp 207-209 °C. ^{1}H NMR (500 MHz, Acetone-d_6) δ 10.95 (s, 1H), 7.70 (d, J = 5.0 Hz, 2H), 7.60 (d, J = 10.0 Hz, 2H), 7.31 (d, J = 10.0 Hz, 1H), 7.23 (m, 3H), 7.10 (d, J < 5.0 Hz, 1H), 7.05 (m, 2H), 6.78 (dd, J = 10.0, <5.0 Hz, 1H), 6.52 (d, J = 10.0 Hz, 1H), 4.90 (m, 1H), 3.84 (s, 3H), 3.25 (dd, J = 15.0, 5.0 Hz, 1H), 3.08 (dd, J = 15.0, 10.0 Hz). ^{13}C NMR (126 MHz, Acetone-d_6) δ 210.0, 172.5, 161.8, 159.6, 138.9, 137.7, 137.5, 131.9, 130.0, 129.7 (q, J = 32.3 Hz), 129.2, 127.6 (q, J = 3.4 Hz), 126.7, 125.4 (q, J = 271 Hz), 122.9, 121.7, 118.0,

113.2, 95.0, 55.7, 54.3, 37.6. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{26}H_{22}F_3N_2O_4$: 483.1532; found 483.1503.

General Procedure E: Synthesis of esters 47 and 48. To a stirred solution of indole **44** (1 mmol) in dry THF (3 mL/mmol) under N_2, oxalyl chloride (2.3 mmol) was added, followed by the addition of DMF (16 µL/mmol) (caution: vigorous evolution of gas). After stirring for 40 min, the reaction mixture was evaporated to dryness. The resulting solid was dissolved in dry THF (2.5 mL/mmol) and cooled to r.t. under N_2. A solution of 1.0 M in THF (2.3 mmol) of potassium *tert*-butoxide or sodium ethoxide was then added slowly, and the reaction mixture stirred for an additional 45 min. The reaction was then quenched with water, neutralized with a few drops of H_3PO_4, and extracted with ethyl acetate. The organic extracts were combined, washed with saturated aqueous $NaHCO_3$, water, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by silica gel flash column chromatography using hexane/ethyl acetate (80:20) as an eluent to provide the esters **47** or **48**.

Ethyl 6-methoxy-3-(4-(trifluoromethyl)phenyl)-1*H*-indole-2-carboxylate (47). Prepared using general procedure E from compound **44** and sodium ethoxide to provide the compound **47** as a white solid (27% yield). Mp 201-203 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.93 (s, 1H), 7.72– 7.64 (m, 4H), 7.45 (d, J = 8.7 Hz, 1H), 6.85 (dt, J = 8.7, 1.9 Hz, 2H), 4.29 (q, J = 7.1 Hz, 2H), 1.24 (t, J = 7.1 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.7, 159.6, 137.6, 136.84, 131.0, 129.4 (q, J = 32.4 Hz), 125.6, 124.8 (q, J = 3.7 Hz), 124.5 (q, J = 272 Hz), 123.2, 122.3, 122.2, 113.0, 93.7, 61.0, 55.7, 14.2. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{26}H_{22}F_3N_2O_4$: 483.1532; found 483.1503.

Tert-butyl 6-methoxy-3-(4-(trifluoromethyl)phenyl)-1*H*-indole-2-carboxylate (48). Prepared using general procedure E from compound **44** and *tert*-butoxide to provide the compound **48** as a white solid (61% yield). Mp 204-206 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.92 (s, 1H), 7.69 (d, J = 8.2 Hz, 2H), 7.63 (d, J = 8.1 Hz, 2H), 7.40 (d, J = 8.8 Hz, 1H), 6.87 (d, J = 2.2 Hz, 1H), 6.82 (dd, J = 8.9, 2.2 Hz, 1H), 3.88 (s, 3H), 1.43 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 161.2, 159.3, 138.2, 136.5, 131.1, 129.2 (q, J = 32.1 Hz), 124.8 (q, J = 3.6 Hz), 124.6 (q, J = 272 Hz), 123.7, 122.3, 122.2, 112.7, 93.7, 82.1, 55.7, 28.3. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{26}H_{22}F_3N_2O_4$: 483.1532; found 483.1503.

General Procedure F: *N*-alkylation of indole. To a stirred solution of indole **20** (1 mmol) in dry DMF (0.5 mL/mmol), under N_2, it was added K_2CO_3 (1.5 mmol). The reaction mixture was stirred for 5 min, followed by the addition of the corresponding alkyl halide (4.5 mmol of methyl iodide or benzyl bromide). The reaction was warmed up to 90 °C and magnetically stirred for 5 hours. The reaction was then quenched with water (5 mL) and extracted with ethyl acetate (3 x 10 mL). The organic extracts were combined, washed with saturated aqueous $NaHCO_3$, water, dried over $MgSO_4$, filtered, and then concentrated *in vacuo*. The crude product was purified by silica gel flash column chromatography using hexane/ethyl acetate (80:20) as an eluent to provide the esters **49** or **50**.

Methyl 6-methoxy-1-methyl-3-(4-(trifluoromethyl)phenyl)-1*H*-indole-2-carboxylate (49). Prepared using general procedure F from compound **20** and methyl iodide to provide the compound **49** as a white solid (79% yield). Mp 132-134 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.69 (d, J = 8.1 Hz, 2H), 7.53 (d, J = 8.0 Hz, 2H), 7.37 (dd, J = 8.6, 0.5 Hz, 1H), 6.87 – 6.76 (m, 2H), 4.05 (s, 3H), 3.92 (s, 3H), 3.69 (s, 3H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 162.8, 159.4, 139.8, 138.9, 130.8, 129.0 (q, J = 32.3 Hz), 124.8 (q, J = 3.7 Hz), 124.6 (q, J = 272 Hz), 123.8, 123.7, 122.3, 120.9, 112.7, 92.3, 55.8, 51.4, 32.3. HRMS (ESI-Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{19}H_{16}F_3NO_3$: 364.11550; found 364.11548. Also, $[M + Na]^+$ calcd for $C_{19}H_{16}F_3NO_3Na$: 386.09745; found 386.09735.

Methyl 1-benzyl-6-methoxy-3-(4-(trifluoromethyl)phenyl)-1*H*-indole-2-carboxylate (50). Prepared using general procedure F from compound **20** and benzyl bromide to provide the compound **50** as a white solid (45% yield). Mp 90-92 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.71 (d, J = 8.2 Hz, 2H), 7.58 (d, J = 8.1 Hz, 2H), 7.41 (d, J = 8.8 Hz, 1H), 7.33–7.25 (m, 3H), 7.17–7.11 (m, 2H), 6.85 (dd, J = 8.8, 2.2 Hz, 1H), 6.78 (d, J = 2.0 Hz, 1H), 5.80 (s, 2H), 3.82 (s, 3H), 3.62 (s, 3H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 162.6, 159.5, 139.8, 138.8, 138.1, 130.8, 129.9, 129.0 (q, J = 32.3 Hz), 128.8, 127.4, 126.5, 124.9 (q, J = 3.7 Hz), 124.6 (q, J = 272 Hz), 123.4, 121.2, 112.7, 92.9, 55.7, 51.4, 48.5. HRMS (ESI-Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{25}H_{21}F_3NO_3$: 440.14680; found 440.14700. Also, $[M + Na]^+$ calcd for $C_{25}H_{20}F_3NO_3Na$: 462.12875; found 462.12878.

Methyl 1-hydroxy-6-methoxy-3-(4-(trifluoromethyl)phenyl)-1H-indole-2-carboxylate (51). Prepared by the following procedure: To a solution containing 2 mmol of $SnCl_2 \cdot 2H_2O$ in 2 mL of DMF, 3,3-diaryl cinnamate **8** (1 mmol) was added at room temperature and the reaction was stirred for 6 hours. Next, the solution was poured into a mixture of ice and water (10 mL), followed by the addition of AcOEt (5 mL) and EDTA (0.58 g, 1 equivalent to Sn). The reaction mixture was then filtered through a short pad of Celite, the solvent removed in vacuo, and the crude product purified by silica gel flash column chromatography using hexane/ethyl acetate (80:20) as an eluent to provide compound **51**. The *N*-hydroxy-indole **51** was obtained as a white solid in 53% yield**.** Mp 150-151 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.56 (s, 1H), 7.80 (d, J = 8.0 Hz, 2H), 7.66 (d, J = 8.0 Hz, 2H), 7.43 (d, J = 8.0 Hz, 1H), 6.95 (d, J = 4.0 Hz, 1H), 6.82 (dd, J = 8.0, 4.0 Hz, 1H), 3.86 (s, 3H), 3,73 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 160.9, 159.2, 138.1, 136.9, 131.1, 129.0, 127.7 (q, J = 31.8 Hz), 125.4 (q, J = 3.7 Hz), 124.9 (q, J = 272 Hz), 123.6, 122.9, 121.8, 117.8, 115.7, 113.7, 91.8, 55.9, 52.1. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{18}H_{15}F_3NO_4$: 366.0953; found 366.0971.

Methyl (*E*)-3-(5-methoxy-2-nitrophenyl)acrylate (52). Prepared using general procedure A from methyl acrylate and 5-methoxy-2-nitrobenzenediazonium tetrafluoroborate to provide the compound **52** as a light yellow solid (82% yield). Mp 123-124 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (d, J = 15.8 Hz, 1H), 8.15–8.11 (m, 1H), 6.99–6.95 (m, 2H), 6.29 (d, J = 15.8 Hz, 1H), 3.92 (s, 3H), 3.83 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.6, 163.8, 141.8, 141.5, 134.1, 128.0, 123.0, 115.2, 114.5, 56.4, 52.3. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{11}H_{12}NO_5$: 238.0710; found 238.0708.

Methyl (*E*)-3-(4-hydroxy-2-nitrophenyl)acrylate (53). Prepared using general procedure A from methyl acrylate and 4-hydroxy-2-nitrobenzenediazonium tetrafluoroborate to provide the compound **53** as a light yellow solid (65% yield). Mp 192-193 °C. ^{1}H NMR (500 MHz, Acetone-d_6) δ 9.68 (s, 1H), 7.93 (d, J = 15.8 Hz, 1H), 7.83 (d, J = 8.6 Hz, 1H), 7.46 (d, J = 2.1 Hz, 1H), 7.24 (dd, J = 8.5, 2.0 Hz, 1H), 6.45 (d, J = 15.8 Hz, 1H), 3.76 (s, 3H). ^{13}C NMR (126 MHz, Acetone) δ 167.0, 160.3, 150.9,

139.9, 131.2, 121.6, 121.4, 120.9, 111.9, 51.9. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{10}H_{10}NO_5$: 224.0553; found 224.0553.

Methyl (*E*)-3-(5-methoxy-2-nitrophenyl)-3-(4-(trifluoromethyl)phenyl)acrylate (54). Prepared using general procedure B from compound **52** and 4-trifluoromethyl benzenediazonium tetrafluoroborate to provide the compound **54** as a light yellow solid (62% yield). Mp 101-104 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.29 (d, *J* = 9.2 Hz, 1H), 7.59 (d, *J* = 8.4 Hz, 2H), 7.46 (d, *J* = 8.2 Hz, 2H), 7.03 (dd, *J* = 9.2, 2.6 Hz, 1H), 6.72 (d, *J* = 2.6 Hz, 1H), 6.50 (s, 1H), 3.91 (s, 3H), 3.62 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.7, 163.8, 153.1, 141.7, 140.9, 137.0, 131.9 (q, *J* = 33 Hz), 128.0, 127.9, 126.0 (q, *J* = 3.7 Hz), 124.1 (q, *J* = 272 Hz), 125.5, 122.8, 118.3, 116.5, 113.9, 56.4, 52.0, 27.8. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{18}H_{15}F_3NO_5$: 382.0897; found 382.0896.

Methyl (*E*)-3-(4-hydroxy-2-nitrophenyl)-3-(4-(trifluoromethyl)phenyl)acrylate (55). Prepared using general procedure B from compound **53** and 4-trifluoromethyl benzenediazonium tetrafluoroborate to provide the compound **55** as a white solid (60% yield). Mp 182-184 °C. ^{1}H NMR (250 MHz, Acetone-d_6) δ 9.45 (s, 1H), 7.80 – 7.57 (m, 5H), 7.29 (dd, *J* = 8.4, 2.4 Hz, 1H), 7.22 (d, *J* = 8.4 Hz, 1H), 6.63 (s, 1H), 3.56 (s, 3H). ^{13}C NMR (63 MHz, Acetone) *δ* 166.0, 158.9, 152.9, 149.6, 143.82, 133.3, 131.4 (q, *J* = 32 Hz), 129.1, 126.4 (q, *J* = 3.8 Hz), 125.6, 125.1 (q, *J* = 272 Hz), 121.8, 119.5, 111.9, 51.7. HRMS (ESI/Q-TOF) *m/z* $[M + H]^+$ calcd for $C_{17}H_{13}F_3NO_5$: 368.0740; found 368.0740.

Methyl 5-methoxy-3-(4-(trifluoromethyl)phenyl)-1*H*-indole-2-carboxylate (56).[37] Prepared using general procedure C from compound **54** to provide the compound **56** as a white solid (70% yield). Mp 193-194°C (lit 195-197). ^{1}H NMR (250 MHz, $CDCl_3$) *δ* 9.18 (s, 1H), 7.70 (q, *J* = 8.3 Hz, 4H), 7.36 (d, *J* = 8.9 Hz, 1H), 7.07 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.95 (d, *J* = 2.2 Hz, 1H), 3.83 (s, 3H), 3.80 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) *δ* 162.1, 155.5, 137.7, 131.2, 130.9, 129.3 (q, *J* = 32.5 Hz), 128.0, 125.0 (q, *J* = 3.7 Hz), 124.5 (q, *J* = 272 Hz),123.3, 122.4, 118.1, 113.0, 101.1, 55.9, 52.0. HRMS (ESI-Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{18}H_{15}F_3NO_3$: 350.09985; found 350.09955. Also, $[M + Na]^+$ calcd for $C_{18}H_{14}F_3NO_3Na$: 372.08180; found 372.08155.

Methyl 6-hydroxy-3-(4-(trifluoromethyl)phenyl)-1*H*-indole-2-carboxylate (57). Prepared using general procedure C from compound **55** to provide the compound **57** as a white solid (65% yield). Mp 200-202 °C. ^{1}H NMR (250 MHz, Acetone) δ 10.79 (s, 1H), 8.48 (s, 1H), 7.78 (s, 4H), 7.41 (d, J = 8.8 Hz, 1H), 7.01 (d, J = 2.1 Hz, 2H), 6.79 (dd, J = 8.8, 2.1 Hz, 2H), 3.76 (s, 7H). ^{13}C NMR (63 MHz, Acetone) δ 161.5, 156.7, 138.6, 137.9, 131.2, 128.2 (q, J = 31.8 Hz), 124.8 (q, J = 271 Hz), 124.6 (q, J = 3.9 Hz), 122.1, 121.6, 121.5, 121.2, 112.7, 96.5, 50.8. HRMS (ESI-Orbitrap) *m/z* $[M + H]^+$ calcd for $C_{17}H_{13}F_3NO_3$: 336.08420; found 336.08389. Also, $[M + Na]^+$ calcd for $C_{17}H_{12}F_3NO_3Na$: 358.06615; found 358.06584.

4.2. Biology

Cell lines culture and viability assay. Pre-B ALL RS4;11, T-ALL CCRF-CEM and AML HL60 cell lines were grown in RPMI-1640 supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 µg /ml streptomycin. Post-ficoll lymphocytes obtained from healthy individuals were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum and stimulated overnight with phytohemagglutinin (25 µL/mL) and Interleukin 2 (50 U/mL). All cells were maintained in a 5% CO_2-humidified incubator at 37 °C.

Cell viability experiments were performed in 96-well micro-titer plates using the MTT reduction assay (0.5mg/ml final concentration, 4 hrs incubation) after 48 h of treatment. The formazan dye formed by the viable cells was dissolved by the addition of acid sodium dodecyl sulfate solution (10% SDS, 0.01 mol/L HCl). Following overnight incubation, absorbance was measured at 570 nm and 620 nm. Percentage of cell survival was calculated in relation to controls. EC_{50} values were calculated from dose-response curves using the GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA)

software. EC_{20} and EC_{90} doses were calculated from EC_{50} dose using "http: www.graphpad.com/quickcalcs/ECanything1/".

Flow Cytometry Analysis. For cell cycle analysis, 200,000 HL60 cells in 1 mL were incubated with different concentrations of the drugs for 12h and 18h then, fixed in 70% ethanol, washed with PBS, and incubated at 37 °C for 15 min in 1 mL PI Buffer (0.1% Triton X-100, 0.2 mg/mL RNAse, and 20 μg/mL propidium iodide, in PBS). Thirty thousand events were analyzed in the BD FACSVerse™flow cytometer (BD Biosciences, Franklin Lakes, NJ). For apoptosis analysis, RS4,11, CEM and HL60 cells (5×10^5) treated with their respective EC_{50} and EC_{90} doses of indole **20** or vehicle for 18h were incubated at 37°C for 15 min with FITC-conjugated Annexin-V (Invitrogen Corporation) in Annexin-V binding buffer followed by PI (5 μg/mL) staining for 3 min. Cells were acquired in the BD FACSVerse™ flow cytometer (BD Biosciences, Franklin Lakes, NJ). For the differentiation analysis of HL60 cells, $2x10^5$ cells were treated with EC_{20}, EC_{50} and EC_{90} doses of indole **20** or vehicle for 24h and 48h. Treatment with vitamin D3 (1α,25-Dihydroxyvitamin D3; Sigma-Aldrich) at the dose of 100nM and ATRA (Retinoic acid; Sigma-Aldrich) at the final concentration of 2uM were used as positive controls. Harvested cells were stained with APC anti-CD11b and PE anti-CD14 antibodies (BioLegend, San Diego, CA), for 30 min at 4°C. The final wash was followed by 7-AAD (BioLegend, San Diego, CA) staining and acquisition of twenty thousand events in the BD FACSVerse™ flow cytometer (BD Biosciences, Franklin Lakes, NJ). All data were acquired using FACSDiva software (BD Biosciences, San Jose, CA) and analyzed using FlowJo software (TreeStar, San Carlos, CA).

Gene expression signature. Treatments were performed in triplicates. HL60 cells treated with indole **20** at the dose of 1.085 µM, (EC_{90}) or vehicle for 6h followed by total RNA extraction using the IllustraRNAspin Mini RNA Isolation Kit (GE Healthcare), including an on column DNAse treatment step. Total RNA samples were converted to labeled RNA probes using the Clariom S assay human kit (ThermoFisher) followed by hybridization on human Clariom™ S Arrays according to the manufacturer's recommendations. Transcriptome Analysis Console (Affymetrix, Santa Clara, CA, USA) was used for scaling and normalization using the RMA algorithm, and to obtain gene expression values in a log 2 scale. For the identification of differential expressed genes, we used the following criteria: FDR adjusted p-value ≤ 0.01 and -1.5 ≤ Fold-change ≥ 1.5. Gene Set Enrichment Analysis (GSEA) [19] was conducted using the GSEA platform (fabrocanyte) with default settings and permutation based on gene set. All the probe sets/transcript clusters annotated with a Gene Symbol in the array were used in the analysis. CMap analysis (http://www.broadinstitute.org/cmap/)[18] was performed using only probesets identical in HG-U133A and Clariom S arrays. Gene sets with -1.5 ≤ Fold-change ≥ 1.5, $p < 0.05$, and FDR < 0.25 were considered differentially expressed.

Immunofluorescence Analysis. Hela cells were plated (100,000 cells/mL) and cultured overnight in Cell View ^{TM} cell culture dish with a glass bottom. Cells were treated with 500nM and 1uM of indole **20** or vehicle (DMSO) for 18h. Cells were permeabilized with 0.5% Triton X100 and fixed with 3.7% formaldehyde, as previously described [43]. Then, cells were incubated with the unconjugated monoclonal Anti-β-Tubulin antibody (Sigma-Aldrich) overnight at 4° C, washed 5 times in PBS and incubated with Alexa Fluor 647-conjugated secondary goat anti-mouse IgG H&L antibody (Abcam,

ab150115) for 1 hour at room temperature. The coverslips were washed, and the nuclei of the remaining cells were labeled with 1 μg/mL of DAPI for 10 min. Finally, samples were washed, examined and photographed using a Zeiss Axioplan epifluorescence microscope. Images were overlaid using Imaje J Fiji software (Schindelin, J.; Arganda-Carreras, I. & Frise, E. et al. (2012), "Fiji: an open-source platform for biological-image analysis", Nature methods 9(7): 676-682, PMID 22743772, doi:10.1038/nmeth.2019).

Western Blot. Western blotting analysis of RS4;11, CEM and HL60 cells treated with indole **20** at the correspondent EC_{50} dose for 0, 15, 60 and 360 min was performed using antibodies against Beta-tubulin (#2146S Cell Signaling Technology, Beverly, MA, USA), p53 (#2527S Cell Signaling Technology, Beverly, MA, USA) and Lamin B1H90 (SC20682,Santa Cruz Biotechnology, Santa Cruz, CA, USA), which was used as the loading control. The Super Signal West Pico Chemiluminescent Substrate detection reagent (Thermo-Scientific) was used for immunodetection and visualization of autoradiography.

***In vivo* drug treatment of ALL xenografts mice.** The experiment was approved by the Ethics Committee in Animal Experimentation of the State University of Campinas under Protocol #3624-1. Ten million of human pre-B ALL RS4;11 cells were washed with PBS and injected in the tail vein of unconditioned NOD/SCID (NOD.CB17-Prkdcscid/J) mice (The Jackson Laboratory, Bar Harbor, ME) obtained from the central vivarium of the State University of Campinas. Animals were monitored for ALL engraftment [44]. Briefly, mice blood collected by retro-orbital bleeding into tubes containing EDTA were submitted to ficoll centrifugation for the isolation of mononuclear cells. Labeling of these cells with anti-human CD45-PE (clone HI30, BD

Pharmingen,San Diego, CA or EXBIO, Prague, Czech Republic) and anti-mouse CD45-FITC (clone 30F-11, BD Pharmingen) antibodies, was followed by the evaluation of the presence and quantification of human ALL cells by flow cytometry using the BD FACSVerse™(Becton Dickinson, Franklin Lakes, NJ). When human CD45(+) cells reached at least 1.5% of total CD45 peripheral blood cells in half of the mice, they were randomly distributed between control (1% PVP, 4% DMSO and 95% PBS) and indole **20** (1 mg/kg; 1% PVP, 4% DMSO and 95% PBS) 5x per week, intraperitoneally) groups, which received treatment 5x per week, intraperitoneally, until death. Every week, blood was collected to measure the percentage of leukemic cells (hCD45+ cells) by flow cytometry. Kaplan-Meier survival curves were compared using the log-rank test with the GraphPad Prism 5 software.

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CONFLICTS OF INTERESTS

The authors declare that they have no conflicts of interests.

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Highlights

- Synthesis of several new 2-carbomethoxy-3-substituied indoles from *β*,*β*-disubstituted acrylates.
- *β*,*β*-disubstituted acrylates from two sequential Heck-Matsuda reaction.
- Indole **20** showed selective cytotoxicity against leukemia cells at the nanomolar scale.
- Indole **20** promoted microtubule depolymerization and cell cycle arrest at G2/M.