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## Design, Synthesis and Characterization of 3-(Benzylidene)indolin-2-one Derivatives as Ligands for $\alpha$ -Synuclein Fibrils

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### Abstract

A series of 3-(benzylidene)indolin-2-one derivatives were synthesized and evaluated for their *in vitro* binding to alpha synuclein ( $\alpha$ -syn), beta amyloid (A $\beta$ ), and tau fibrils. Compounds with a single double bond in the 3-position had only a modest affinity for  $\alpha$ -syn and no selectivity for  $\alpha$ -syn versus A $\beta$  or tau fibrils. Homologation to the corresponding diene analogs yielded a mixture of *Z,E* and *E,E* isomers; substitution of the indoline nitrogen with an *N*-benzyl group resulted in increased binding to  $\alpha$ -syn and reasonable selectivity for  $\alpha$ -syn versus A $\beta$  and tau. Introduction of a para-nitro group into the benzene ring of the diene enabled separation of the *Z,E* and *E,E* isomers and led to the identification of the *Z,E* configuration as the more active regioisomer. The data described here provide key structural information in the design of probes which bind preferentially to  $\alpha$ -syn versus A $\beta$  or tau fibrils.

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### ASSOCIATED CONTENT

Methods for the 2D NMR study.

Figure S1. NOE in *E,E* configuration of **41b** and 3D structure of **41a** and **41b**.

Figure S2. <sup>1</sup>H NMR spectrum of **41b**.

Figure S3. 2D NMR spectrum of **41b**.

Figure S4. Stability of **41a** and **41b** in dichloromethane/methanol solution.

Figure S5. Saturation binding studies to determine binding affinity of Thio- T for  $\alpha$ -syn, A $\beta$  and tau fibrils

Figure S6. Analytical HPLC of [<sup>18</sup>F]**46a**

<sup>1</sup>H NMR of compounds: **5**, **6**, **7**, **8**, **9**, **10**, **11**, **12**, **13**, **14**, **18**, **19**, **20**, **21**, **22**, **23**, **24**, **25**, **27**, **28**, **29**, **30**, **31**, **32**, **33**, **35**, **36**, **38a**, **38b**, **39a**, **39b**, **41a**, **41b**, **42a**, **42b**, **43a**, **43b**, **44a**, **44b**, **45a**, **45b**, **46a**, **46b**, **47**, **48**, **49**

## INTRODUCTION

It is generally accepted that many neurodegenerative disorders are characterized by the accumulation of misfolded proteins which lead to the formation of insoluble protein aggregates in the CNS.<sup>1, 2</sup> For example, Alzheimer's disease (AD) is characterized by the formation of two different insoluble protein aggregates: 1) beta amyloid (A $\beta$  plaques, which consists of aggregated beta amyloid protein, A $\beta$ <sub>1-42</sub>; and 2) neurofibrillary tangles (NFTs), which consist of aggregates of hyperphosphorylated tau protein. The diagnosis of AD historically relied on the cognitive assessment of patients with moderate to severe memory deficits. Although a progressive cognitive decline resulting in a severe impairment of daily function was considered consistent with "Alzheimer-like dementia", the clinical diagnosis of AD could not be confirmed until postmortem analysis demonstrated the presence of A $\beta$  plaques and NFTs in the temporal and parietal cortex. A major breakthrough in the study of patients presenting with AD-like symptoms emerged with the development of [<sup>11</sup>C]PiB, a positron-labeled analog of thioflavin-T (Thio-T), the fluorescent dye used to visualize A $\beta$  plaques in postmortem samples of AD brain.<sup>3, 4</sup> Initial PET studies in AD patients and healthy controls demonstrated increased [<sup>11</sup>C]PiB retention in the frontal cortex in the patients with a clinical diagnosis of a progressive loss of cognitive function in comparison with normal controls.<sup>5</sup> More recent studies in patients with familial AD have clearly shown that PiB(+) A $\beta$  plaque formation occurs early in the disease process, and suggest amyloid plaques may represent an antecedent biomarker of AD.<sup>6, 7</sup> The subsequent emergence of <sup>18</sup>F-labeled A $\beta$  imaging agents including [<sup>18</sup>F]florbetapir,<sup>8, 9</sup> [<sup>18</sup>F]florbetaben,<sup>10</sup> and [<sup>18</sup>F]flumetamol<sup>11</sup> has enabled the expansion of PET studies in AD patients to imaging centers without an on-site cyclotron facility. Recently, a number of <sup>18</sup>F- and <sup>11</sup>C-labeled agents for imaging tau deposits in NFTs have been reported.<sup>12-14</sup> These agents are expected to provide valuable information on the temporal separation between the formation of A $\beta$  plaques (a possible antecedent biomarker) and NFT formation (thought to reflect neuronal loss in AD).<sup>6</sup> This ability to measure the neuropathological time course of A $\beta$  plaque and NFT formation in patients will be important in the evaluation of new disease-modifying therapeutics aimed at slowing the clinical progression of AD.

Another protein capable of forming insoluble aggregates in brain is alpha synuclein ( $\alpha$ -syn), which is the principal species found in Lewy bodies (LBs) and Lewy neurites (LNs).<sup>15, 16</sup> LBs are a dense core of insoluble aggregates of  $\alpha$ -syn found in the cell bodies of neurons, principally located in the brain stem and subcortical regions of the CNS. LNs are abnormal axons or dendrites that contain insoluble aggregates of  $\alpha$ -syn and have a more diffuse morphology than LBs. The formation of LBs and LNs are the primary pathological features of a collection of neurological disorders referred to as "synucleinopathies", which include Parkinson's disease (PD), Dementia with Lewy Bodies (DLB), multiple system atrophy (MSA), and Picks' disease.<sup>17</sup> In addition, 50% of familial and sporadic AD patients show evidence of LBs at autopsy.<sup>18</sup> Furthermore, AD patients with concomitant LB pathology demonstrate an accelerated cognitive decline and may represent a subset of AD patients.<sup>18</sup> These data suggest that a PET radiotracer for imaging insoluble  $\alpha$ -syn aggregates should be useful in the study of the formation of LBs and LNs in a diverse panel of CNS disorders.

Recently, a number of phenothiazine analogs including the tricyclic ligands **SIL23**, **SIL5**, and **SIL26**, the fluorescent dyes **LDS 798** and **LDS 730** and the indolinone **5** (Figure 1) have shown reasonable *in vitro* selectivity for  $\alpha$ -syn versus A $\beta$  and tau fibrils.<sup>19–24</sup> Although these compounds are noteworthy as the first analogs shown to label insoluble  $\alpha$ -syn aggregates, their low *in vitro* stability and moderate affinity for  $\alpha$ -syn aggregates limits their utility as PET imaging agents. Therefore, there is a need to identify new chemical scaffolds which can serve as lead compounds for PET radiotracer development. In this study, we describe the synthesis and *in vitro* evaluation of a novel class of compounds, the indolinone-diene analogs, having high affinity and selectivity for  $\alpha$ -syn aggregates which could serve as a second generation lead compound for PET radiotracer development.

## RESULTS

### Chemistry

The first step in the design of a probe for imaging  $\alpha$ -syn aggregates involved identifying a suitable lead compound for our structure-activity relationship (SAR) studies. An earlier publication reported that compound **5** (Figure 1) had a modest affinity for  $\alpha$ -syn fibrils but low selectivity versus A $\beta$  and tau fibrils.<sup>20</sup> We synthesized a number of indolinone analogs to determine if it would be possible to improve the affinity of compound **5** for  $\alpha$ -syn and reduce its affinity for A $\beta$  and tau fibrils by exploring substituents in the benzylidene aromatic ring and alkylation the indolin-2-one nitrogen atom. A recent publication additionally demonstrated that the fluorescent dyes **LDS 798** and **LDS 730** labeled Lewy bodies in postmortem PD cases.<sup>21</sup> Therefore, the diene fragment of **LDS 798** and **LDS 730** was incorporated into compound **5** to determine if these groups would improve the affinity and selectivity of the new analogs for  $\alpha$ -syn fibrils versus A $\beta$  and tau fibrils.

The synthesis of the 3-benzylidene-indolin-2-one analogs is shown in Scheme 1. 3-(4-(dimethylamino)benzylidene)indolin-2-one (**5**), 3-(4-(dimethylamino)benzylidene)-1*H*-pyrrolo[2,3-*b*]pyridin-2(3*H*)-one (**6**), and 3-(4-(dimethylamino)benzylidene)-5-hydroxyindolin-2-one (**7**) were prepared by condensing indolin-2-one (**1**), 1,3-dihydro-2*H*-pyrrolo[2,3-*b*]pyridin-2-one (**2**), 5-hydroxyindolin-2-one (**3**) and dimethylaminobenzaldehyde (**4**) in acetic acid with refluxing for 3 h. The nitrogen of **5** and **6** was alkylated by treatment of **5** or **6** with sodium hydride in DMF at 0 °C, followed by addition of select alkyl halides to give compounds **8–14**. Similarly, indolin-2-one (**1**), 1,3-dihydro-2*H*-pyrrolo[2,3-*b*]pyridin-2-one (**2**) or 5-hydroxyindolin-2-one (**3**) was condensed with a 4-substituted cinnamaldehyde (**15–17**) in acetic acid with refluxing to give 3-(4-substituted-phenyl)allylidene)indolin-2-one (**18–20**), 3-(3-(4-substituted phenyl)allylidene)-1*H*-pyrrolo[2,3-*b*]pyridin-2(3*H*)-one (**21**) or 3-(3-(4-(dimethylamino)phenyl)allylidene)-5-hydroxyindolin-2-one (**22**) respectively. The nitrogen of compound **18–21** were alkylated by treatment of **18**, **19**, **20** or **21** with sodium hydride in DMF at 0 °C, followed by the addition of select alkyl halides to give compounds **23–32**. The nitro group of **28** was reduced to an amino group with iron powder and HCl in methanol to afford compound **33**. 1-Phenylindolin-2-one (**34**) was condensed with **4** or **17** in acetic acid with refluxing to afford **35** or **36**, respectively (Scheme 1).

Compounds **5**, **6**, **7** and their nitrogen alkylated analogs (**8–14**, and **35**) exist as either *Z* or *E* geometric isomers depending on the characteristics of the substituent at the C-3 position of the 3-substituted indolin-2-one or 3-substituted 1*H*-pyrrolo[2,3-*b*]pyridin-2(3*H*)-one. The 3-(4-substituted-phenyl)allylideneindolin-2-one (**18–20**), 3-(3-(4-substituted phenyl)allylidene)-1*H*-pyrrolo[2,3-*b*]pyridin-2(3*H*)-one (**21**) and 3-(3-(4-(dimethylamino)phenyl)allylidene)-5-hydroxyindolin-2-one (**22**) and their analogs (**23–33**, and **36**), should theoretically have 4 geometric isomers for the double bond of each compound with *Z,Z*, *E,E*, *Z,E*, and *E,Z* configuration. Because only the trans (*E* isomer) of 4-substituted cinnamaldehyde was used for the synthesis, most of the compounds have only two geometric isomers: *E,E* or *Z,E*. We first attempted to separate the *E,E* and *Z,E* isomers by HPLC for binding assays. As an example, although the two isomers **20** and **27** could be separated by HPLC, the isolated pure *Z,E* and *E,E* isomers each isomerized within 20 min to a mixture of *Z,E* and *E,E* isomers. Because we were unable to obtain pure *Z,E* and *E,E* isomers for the binding assay, a mixture of the *Z* and *E* isomers for compounds **5–7**, and their analogs (**8–14** and **35**), and mixtures of *Z,E* and *E,E* isomers of **18–22**, and their analogs (**23–32** and **36**) were used to measure binding affinities; the ratio of the *Z* and *E*, or *Z,E* and *E,E* isomers was measured with HPLC and determined by <sup>1</sup>H NMR spectra.

Similarly, compounds **39** and **42–46** were synthesized from condensation of **1** with 4-nitrobenzaldehyde (**37**) and (*E*)-3-(4-nitrophenyl)acrylaldehyde (**40**) to give 3-(4-nitrobenzylidene)indolin-2-one (**38**) and 3-(3-(4-nitrophenyl)allylidene)indolin-2-one (**41**) followed by alkylation of **38** and **41** with various alkyl halides (Scheme 2). Unlike other 4-substituted-benzaldehyde and 4-substituted cinnamaldehyde condensed analogs, the isomers of condensed products of 4-nitrobenzaldehyde (**37**) and (*E*)-3-(4-nitrophenyl)acrylaldehyde (**40**) and their derivatives could be separated by flash chromatography with CH<sub>2</sub>Cl<sub>2</sub>-ethyl ether (3:1) to afford **38a–39a** and **38b–39b**, and **41a–46a** and **41b–46b**, respectively. The stability of compounds **41a** and **41b** in solution was measured by HPLC. In a solvent mixture of CH<sub>2</sub>Cl<sub>2</sub>-MeOH (1:1), **41a** was stable for 20 h as evidenced by the HPLC peak and retention time. In contrast, **41b** was unstable under the same conditions, showing 33% isomerization to an un-identified isomer different from **41a** (Supporting Information, Figure 4–7).

The two isomers, forms *Z,E* and *E,E* of compound **41–46** could be distinguished by 2D NMR spectra analysis. Compounds **41a** and **41b** were used to determine configuration structure by 2D NMR. The *E,E* configured compounds should show a nuclear Overhauser effect (NOE) between the proton at the C-4 position and the vinyl proton, like H<sub>4</sub> and H<sub>b</sub>, whereas the *Z,E* configured compounds should not show a NOE effect between the proton at the C-4 position and the vinyl proton(s) in the C-3 substitution of the 3-substituted indolin-2-ones (Figure 2). The proton <sup>1</sup>H shift of compound **41b** was assigned by <sup>1</sup>H NMR. In DMSO solution, the peak of H<sub>4</sub> in C-4 position of indole overlapped with the peak of H<sub>a</sub> in the nitrophenyl ring. After adding 10% D<sub>6</sub>-benzene, the two peaks is separated very well (H<sub>4</sub>, 8.15 ppm and H<sub>a</sub>, 8.07 ppm, respectively). The H<sub>4</sub> and H<sub>b</sub> show a strong NOE effect in the 2D NMR spectra of **41b**. Therefore, compound **41b** is *E,E* configure, and **41a** is *Z,E* configure (supporting information, Figure 1–3). The configurations of the remaining compounds **42a–46a** and **42b–46b** were assigned by comparison of their <sup>1</sup>H NMR spectrum

with **41a** and **41b**. The chemical shifts of its particular proton H<sub>b</sub> (dd couple) is 8.81 ppm for *Z,E* configuration and 7.78 ppm for *E,E* configure. Similarly, configurations of the compounds **5–14**, and **35** were assigned based on the chemical shifts of particular protons, H<sub>a</sub>, H-2 and/or H-6, by comparing the reported chemical shifts for compound **5**;<sup>25</sup> configurations of compounds **18–33** and **36** were assigned based on the chemical shifts of particular proton, H<sub>b</sub>.

### Radiosynthesis

The synthesis of the mesylate precursor **49** and the <sup>18</sup>F-labeling are shown in Scheme 3. Radiosynthesis of [<sup>18</sup>F]**46a** was carried out manually starting from **49** under conventional conditions using *tert*-amyl alcohol as the solvent to afford [<sup>18</sup>F]**46a** with a yield of 19.3 ± 6.3 mCi (n = 6) (decay corrected) with specific activity ranging from 800 to 5000 mCi/μmol at the end of synthesis. The radiochemical purity was > 99%.

### Fibril Binding Assays

Thio-T competitive binding assays were utilized to measure affinity and selectivity for α-syn fibrils. Thio-T is a weakly fluorescent dye that displays a dramatic increase in fluorescence when bound to amyloid fibrils. Thio-T assays for α-syn fibrils were modified from our previous studies<sup>22</sup> to improve the consistency of binding results for different classes of compounds. Furthermore, we developed additional competitive binding assays with Aβ and tau fibrils in order to evaluate selectivity. Under the conditions used for these studies, we determined that the affinity constants (*K<sub>d</sub>*) of Thio-T for α-syn, Aβ and tau fibrils were 1850 nM, 32 nM, and 2700 nM respectively (Supplemental Figure S5). The competitive binding assays measured displacement of Thio-T binding by increasing concentrations of each indolinone compound, using fixed concentrations of fibrils and Thio-T. The Thio-T concentration was set at 150% of the *K<sub>d</sub>* value determined for each fibril species).

All final compounds in the manuscript possess a purity of at least 95% as determined by elemental analysis or HPLC analysis. The competitive binding results for indolinone and aza-indolinone analogs having a single double bond are shown in Table 1. In our assay, lead compound **5**, with an *E:Z* ratio of 75:25, had a similar affinity for α-syn and Aβ fibrils (*K<sub>i</sub>* ~ 85 nM) and a 3-fold lower affinity for tau fibrils (*K<sub>i</sub>* = 262 nM). The corresponding aza-indolinone analog **6** had a reversed *E:Z* ratio and a higher affinity for Aβ (*K<sub>i</sub>* = 40 nM) versus α-syn and tau fibrils (*K<sub>i</sub>* = 125 – 190 nM). Substitution of the 5-position of **5** with a hydroxyl group **7** resulted in a reduction in affinity for α-syn fibrils (*K<sub>i</sub>* = 407 nM) and no change in affinity for Aβ and tau fibrils. Substitution of the indolinone nitrogen atom (**8–14**, **35**) had only a minor effect on the affinity of this series for all three fibrils. Replacement of the dimethyl amino group of **5** and **9** with a nitro group permitted the isolation of *Z* and *E* regioisomers. The *Z* isomer **38a** had a higher affinity for α-syn (*K<sub>i</sub>* = 347 nM) than the corresponding *E* isomer **38b** (*K<sub>i</sub>* = 620 nM), but the affinity was too low to be useful as a PET probe for imaging α-syn, a feature observed in all of the analogs having a single double bond in the indolinone ring system (Tables 1 and 3).

The results of the *in vitro* binding data for the indolinone-diene analogs are shown in Tables 2 and 3. As with the analogs of **5**, indolinone-diene analogs having either a hydrogen, methoxy, or dimethylamino group in the pendant aromatic ring consisted of a mixture of regioisomers, in this case *E,E* and *Z,E* isomers. Substitution of the para-position of the pendant phenyl group of **18** with a methoxy group (**19**) resulted in an increase in affinity for  $\alpha$ -syn ( $K_i = 62$  nM versus 206 nM), A $\beta$  ( $K_i = 125$  nM versus 267 nM), and tau fibrils ( $K_i = 169$  nM versus 522 nM), with a slight preference in affinity for  $\alpha$ -syn. However, a similar substitution with a dimethylamino group resulted in a compound (**20**) having a higher affinity for A $\beta$  ( $K_i = 27$  nM) versus  $\alpha$ -syn ( $K_i = 41$  nM) and tau ( $K_i = 54$  nM). The corresponding aza-indolinone-diene analog of **20** (i.e., **21**) had high affinity ( $K_i \sim 12 - 35$  nM), for all three fibrils. Substitution of the indolinone nitrogen atom of **18** with a benzyl group resulted in **23**, which was primarily in the *Z,E* configuration, and did not lead to an improvement in affinity for  $\alpha$ -syn ( $K_i = 227$  nM), A $\beta$  ( $K_i = 700$  nM) or tau ( $K_i = 680$  nM) fibrils. However, substitution of the para position of **24** with either a methoxy group (**27**) or nitro group (**28**) resulted in an improvement in affinity for  $\alpha$ -syn fibrils versus A $\beta$  and tau fibrils, but it is not clear if this improvement in affinity was due to the substituent effect or the preference of these analogs for the *E,E* isomer. The other analogs in Table 2, which consisted of a mixture of *E,E* and *Z,E* isomers had modest affinity for  $\alpha$ -syn, A $\beta$  and tau fibrils. The most interesting compound in this series was **28**, which has a 4 nM affinity for  $\alpha$ -syn fibrils and 20-fold lower affinity for A $\beta$  and tau fibrils.

Introduction of a *para* nitro group into the benzene ring of the diene moiety enabled the isolation of both the *E,E* and *Z,E* regioisomers. In general, the *Z,E* regioisomers were more potent than the corresponding *E,E* configuration (Table 3). Although compound **41a** had a similar affinity for  $\alpha$ -syn, A $\beta$  and tau fibrils ( $K_i \sim 15 - 35$  nM), substitution of the indolinone nitrogen with a benzyl group **42a** resulted in increase in selectivity for  $\alpha$ -syn ( $K_i = 40$  nM) versus A $\beta$  ( $K_i = 214$  nM) and tau ( $K_i = 121$  nM) fibrils. The most potent and selective compound in the series was **46a** ( $K_i = 2$  nM) and a 70-fold selectivity versus Ab and 40-fold selectivity versus tau fibrils. The 2-fluoroethoxy-group in the benzyl moiety enabled  $^{18}\text{F}$ -labeling for direct binding assays with  $\alpha$ -syn, A $\beta$  and tau fibrils.

### Fluorescent tissue staining

The fluorescent properties of compound **20**, and its high affinity for  $\alpha$ -syn, A $\beta$ , and tau fibrils (Figure 3) encouraged us to investigate *in vitro* fluorescent staining of postmortem brain samples from PD/DLB and PD cases. Sections from midbrain tissue of PD/DLB and temporal lobe tissue sections from neuropathologically verified AD cases were incubated with 5  $\mu\text{M}$  of compound **20** and imaged using fluorescence microscopy. Antibody labeling was performed on adjacent sections to determine the protein species labeled by the fluorescent probe. Compound **20** was found to label both Lewy bodies and A $\beta$  plaques, which was anticipated given the high affinity of this compound for  $\alpha$ -syn and A $\beta$  fibrils. In addition, the clear staining of LBs and A $\beta$  plaques showed good agreement between the *in vitro* binding studies using the fibrillary protein and insoluble protein aggregates found in postmortem samples of PD/DLB and AD brain samples.

### Saturation binding assays to measure the affinity of **46a** for $\alpha$ -syn fibrils

To further confirm the binding affinity determined in Thio-T assays, we synthesized [ $^{18}\text{F}$ ]**46a** and directly measured its binding affinity in saturation binding assays with  $\alpha$ -syn fibrils. We observed specific binding of [ $^{18}\text{F}$ ]**46a** to  $\alpha$ -syn fibrils with an average  $K_d$  of 8.9 nM (Figure 4a). We observed consistent binding values for three independently prepared fibril batches. Scatchard analysis indicates that the binding fits a one-site model (Figure 4b). We also determined binding affinity of **46a** for A $\beta$  and tau fibrils. The average  $K_d$  values for A $\beta$  and tau fibrils were 271 nM and 50 nM, respectively, in two independent assays (Figure 5).

## DISCUSSION

The past decade has witnessed significant advances in the field of the development of PET-based radiotracers for imaging insoluble protein aggregates which occur in many neurodegenerative disorders. Radiotracers such as [ $^{11}\text{C}$ ]PiB, [ $^{18}\text{F}$ ]florbetapir, [ $^{18}\text{F}$ ]florbetaben and [ $^{18}\text{F}$ ]flutemetamol are widely used in clinical PET studies, and it is currently believed that imaging A $\beta$  plaques with these radiotracers is capable of identifying patients for risk for developing AD.<sup>6, 7</sup> More recent studies have focused on imaging tau; clinical PET studies conducted with [ $^{18}\text{F}$ ]**T807** (7-(6- $^{18}\text{F}$ -fluoropyridin-3-yl)-5H-pyrido[4,3-b]indole), [ $^{18}\text{F}$ ]**THK-5105** (6-[(3- $^{18}\text{F}$ -fluoro-2-hydroxy)propoxy]-2-(4-dimethylaminophenyl)quinoline), [ $^{18}\text{F}$ ]**THK-5117** (6-[(3- $^{18}\text{F}$ -fluoro-2-hydroxy)propoxy]-2-(4-methylaminophenyl)quinoline), and [ $^{11}\text{C}$ ]**PBB3** (2-((1E,3E)-4-(6-( $^{11}\text{C}$ -methylamino)pyridin-3-yl)buta-1,3-dienyl)benzo[d]thiazol-6-ol) have shown promise in imaging NFTs in AD patients.<sup>26</sup> A logical next step in the evolution of PET radiotracers for imaging neurodegeneration is the development of a probe capable of imaging  $\alpha$ -syn aggregates in Lewy bodies and Lewy neurites in PD.

A key step in the process of developing PET radioligands is the identification of a suitable lead compound for structure-activity relationship studies. The lead compound for the A $\beta$  imaging agent [ $^{11}\text{C}$ ]PiB, was Thio-T, a fluorescent dye used for staining A $\beta$  plaques in postmortem samples of AD brain tissue.<sup>3, 27, 28</sup> The  $^{18}\text{F}$ -labeled compounds [ $^{18}\text{F}$ ]florbetapir and [ $^{18}\text{F}$ ]florbetapen were developed using X-34, a structural analog of the fluorescent dye Congo Red as the lead compound.<sup>29, 30</sup> Thio-T also labels  $\alpha$ -syn aggregates in Lewy bodies and Lewy neurites, but its low fluorescent staining of  $\alpha$ -syn and higher affinity for A $\beta$  plaques (which led to the development of [ $^{11}\text{C}$ ]PiB) suggest that it would not be a good lead compound for a PET radiotracer to image insoluble  $\alpha$ -syn aggregates in PD. Although several tricyclic analogs (**SIL23**, **SIL26**, **SIL5**) were recently reported as ligands that bind aggregated  $\alpha$ -syn in postmortem PD brain tissue assays,<sup>22, 23</sup> their affinity and selectivity is not optimal for imaging  $\alpha$ -syn aggregates *in vivo*.

The indolinone analog **5** (Figure 1) was previously identified as having modest binding affinity to  $\alpha$ -syn in a rapid throughput screening assay potential lead compounds as a tau imaging agent for PET.<sup>20</sup> We used this compound as the basis of the SAR studies described in this study. The first series of compounds focused on direct analogs of **5** and explored the effect of different substitutions in the indolinone ring system. Most of the compounds within

this series had only modest affinity for  $\alpha$ -syn and no selectivity towards  $\alpha$ -syn, A $\beta$  or tau fibrils. Although most of the compounds in this series consisted of a mixture of *E* and *Z* regioisomers, it was possible to separate compounds **38** and **39** into their *E* and *Z* isomers; the *Z* configuration was found to have a higher affinity for  $\alpha$ -syn, A $\beta$  and tau fibrils than the corresponding *E* isomer.

The presence of a diene moiety in the structure of the dyes **LDS 798** and **LDS 730** encouraged us to add a second double bond into the structure of **5**. This change in structure resulted in improved affinity for  $\alpha$ -syn fibrils; this indolinone-diene compound (**20**), which was also a mixture of *E,E* and *Z,E* regioisomers, had a high affinity for both A $\beta$  and tau fibrils. Fortunately the strong fluorescence of compound **20** enabled us to confirm that the indolinone-dienes could label both Lewy bodies and A $\beta$  plaques in postmortem samples of PD and AD brain. It was difficult to identify clear structure-activity relationships because of the *EE:ZE* mixture for each compound, which varied depending on the nature of the substitution of the indolinone nitrogen. However, it was possible to reach some conclusions by comparing structural congeners having a similar *EE:ZE* ratio. Substitution of the indolinone nitrogen with bulky substituents such as a benzyl or substituted benzyl group versus an *N*-methyl group appeared to improve affinity and selectivity for  $\alpha$ -syn versus A $\beta$  and tau. The 4-nitrobenzyl analog **28** was the most potent compound in this series, having an affinity for  $\alpha$ -syn of 4 nM and 20-fold selectivity for  $\alpha$ -syn versus A $\beta$  and tau.

Introduction of the 4-nitrobenzene ring into the diene moiety enabled the separation of stable *Z,E* and *E,E* regioisomers, and led to the identification of the *Z,E* configuration as being the more active of the regioisomers. A number of compounds were identified having a high affinity for  $\alpha$ -syn, and good selectivity for  $\alpha$ -syn versus A $\beta$  and tau fibrils. The most noteworthy compound in this series was **46a**, which had a high affinity (2 nM) and excellent selectivity for  $\alpha$ -syn versus A $\beta$  and tau fibrils. This was also confirmed via direct binding studies with the corresponding  $^{18}\text{F}$ -labeled compound in  $\alpha$ -syn, A $\beta$  and tau fibrils. Unfortunately, the high log *P* value of this compound (4.18) made it difficult to obtain reliable and reproducible binding data from insoluble  $\alpha$ -syn obtained from PD brain (results not shown). The high log *P* and potential of the nitro group to be reduced to the corresponding amino group *in vivo* indicates that [ $^{18}\text{F}$ ]**46a** will not be a useful *in vivo* PET tracer for imaging Lewy bodies and Lewy neurites in PD brain. However, our SAR results are noteworthy since they identified structural features leading to preferential binding to  $\alpha$ -syn versus A $\beta$  and tau fibrils. Consequently, compound **46a** will serve as a secondary lead compound for further SAR studies. Other analogs within this series can serve as potential radioligands for *in vitro* binding assays for  $\alpha$ -syn and represent an improvement to the current assay which uses Thio-T as a screening ligand, which binds more potently to A $\beta$  versus  $\alpha$ -syn, and tau fibrils (Supplemental Figure S5).

In summary, a series of indolinone and indolinone-diene analogs were synthesized and *in vitro* binding assays were conducted for  $\alpha$ -syn, A $\beta$  and tau fibrils. In general, the indolinone-diene analogs had a higher affinity for all three fibrils versus the corresponding indolinone congeners. The presence of two different regioisomers made it difficult to identify clear structure-activity relationships for the two series of compounds, but this was overcome by introduction of a para-nitro substituent into the benzene ring of the ene and diene fragment.



Within the indolinone-diene series, the *Z,E* configuration was the more stable of the two regioisomers; substitution of the indolinone nitrogen with a benzyl moiety improved both the  $\alpha$ -syn affinity and selectivity versus A $\beta$  and tau fibrils. The results of this study have identified clear structural requirements for preparing compounds having a preferential affinity for  $\alpha$ -syn versus A $\beta$  and tau fibrils.

## EXPERIMENTAL SECTION

### General methods and materials

All chemicals were obtained from standard commercial sources and used without further purification. All reactions were carried out using standard air-free and moisture-free techniques under an inert nitrogen atmosphere with dry solvents unless otherwise stated. Flash column chromatography was conducted using Scientific Adsorbents, Inc. silica gel, 60A, "40 Micron Flash" (32–63  $\mu$ m). Melting points were determined by using MEL-TEMP 3.0 apparatus and are uncorrected. Routine  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded at 400 MHz and 100 MHz on Agilent Technologies spectrometers. All chemical shifts were reported as a part per million (ppm) downfield from tetramethylsilane (TMS). All coupling constants (*J*) are given in Hertz (Hz). Splitting patterns are typically described as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Elemental analysis (C, H, N) was determined by Atlantic Microlab, Inc., Norcross, GA. Purities of final products were determined to be >95% by elemental analysis. [ $^{18}\text{F}$ ]Fluoride was produced at Washington University by the  $^{18}\text{O}(\text{p,n})^{18}\text{F}$  reaction through proton irradiation of enriched (95%) [ $^{18}\text{O}$ ] water in the RDS111 cyclotron. HPLC analysis was performed with an UV detector and a scintillation NaI (TI) detector and associated electronics for radioactivity detection. An Agilent SB-C18 250 $\times$ 9.4 mm 5  $\mu$ m semi-preparative column and an Agilent SB-C18 250 $\times$ 4.6 mm 5  $\mu$ m analytical column were used for preparative purification and for post-synthesis analysis of chemical and radiochemical purity and specific activity, respectively. The semi-preparative HPLC conditions were: 62% acetonitrile and 38% ammonium formate buffer (0.1 M, pH 4.5) with a flow rate of 4 mL/min and UV at 264 nM for purification; the mobile phase for QC analysis was 80% acetonitrile and 20% ammonium formate buffer with a flow rate of 1.5 mL/min. Radio-TLC was accomplished using a Bioscan AR-2000 imaging scanner (Bioscan, Inc. Washington DC).

**Synthesis method 1**—The 2-oxindole (20 mmol) and aldehyde (20 mmol) in acetic acid (50 mL) and 37% HCl (1 mL) were heated at reflux for 3 h, then water (500 mL) was added at rt. The solids were filtered out and recrystallized with methanol to afford the 3-(substituted) indolin-2-one condensation product.

**Synthesis method 2**—The 3-(substituted) indolin-2-one (1.0 mmol) in THF 5 mL) was added to 60% NaH (1.5 mmol) at 0  $^\circ\text{C}$ . After 15 min, the alkyl halide (3 mmol) was added. The reaction mixture was stirred for 8 h, ethyl acetate (75 mL) was added, washed with water (50 mL  $\times$  2), saturated NaCl (50 mL) and dried over  $\text{Na}_2\text{SO}_4$ . After evaporation of the ethyl acetate, the crude product was purified by silica gel column chromatography eluting with hexane- $\text{CH}_2\text{Cl}_2$ -EtOAc (10:10:3, v/v/v) to afford the nitrogen alkylated compound.

**3-(4-(Dimethylamino)benzylidene)indolin-2-one (5)** was prepared by method 1 with compound **1** and **4** to afford the desired product, (**5**), as a red solid (90%), mp 190.5–191.9 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 9.20 and 8.74 (s, 1H), 8.40, and 7.69 (d, *J* = 8.8 Hz, 2H), 7.90 and 7.48 (d, *J* = 7.6 Hz, 1H), 7.79 and 7.47 (s, 1H), 7.18 and 7.00 (t, *J* = 7.6 Hz, 1H), 7.15 and 6.92 (t, *J* = 8.0 Hz, 1H), 6.94 and 6.87 (d, *J* = 7.6 Hz, 2H), 3.07 (s, 6H). (<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 171.4, 168.6, 151.4, 141.0, 139.1, 138.9, 138.7, 134.9, 132.2, 128.5, 127.0, 126.6, 122.6, 122.4, 122.3, 122.1, 121.4, 121.2, 120.4, 118.1, 111.4, 111.2, 110.1, 109.3, 40.1, 40.0. Anal. C<sub>17</sub>H<sub>16</sub>N<sub>2</sub>O Calcd. C: 77.25, H: 6.10, N: 10.60. Found C: 77.24, H: 6.29, N: 10.60.

**3-(4-(Dimethylamino)benzylidene)-1H-pyrrolo[2,3-b]pyridin-2(3H)-one (6)** was prepared by method 1 with compound **2** and **4** to afford the desired product, (**6**), as a red solid (47%), mp 247.4–248.6 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.62 (s, 1H), 8.40 and 7.64 (d, *J* = 8.8 Hz, 2H), 8.10 and 7.67 (d, *J* = 6.4 Hz, 2H), 7.87 and 7.42 (s, 1H), 6.95 and 6.88 (t, *J* = 7.2 Hz, 1H), 6.76 (d, *J* = 8.4 Hz, 2H), 3.10 (s, 6H). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>) δ 169.5, 167.7, 156.6, 154.2, 152.6, 152.2, 147.0, 145.8, 140.6, 139.8, 135.6, 132.9, 128.8, 125.6, 122.3, 121.2, 121.1, 120.4, 118.4, 117.6, 117.4, 116.7, 112.1, 111.6, 40.2. Anal. C<sub>16</sub>H<sub>15</sub>N<sub>3</sub>O.0.25H<sub>2</sub>O Calcd. C: 71.82, H: 5.75, N: 15.70. Found C: 71.89, H: 5.83, N: 15.58.

**3-(4-(Dimethylamino)benzylidene)-5-hydroxyindolin-2-one (7)** was prepared by method 1 with compound **3** and **4** to afford the desired product, (**7**), as a red solid (77%), mp 221.1–222.4 °C. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 10.10 (s, 1H), 8.89 (s, 1H), 7.58 (d, *J* = 8.0 Hz, 2H), 7.43 (s, 1H), 7.26 (s, 1H), 6.78 (d, *J* = 8.8 Hz, 2H), 6.62 (d, *J* = 8.0 Hz, 1H), 6.57 (d, *J* = 7.6 Hz, 1H), 2.99 (s, 6H). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>) δ 169.9, 152.1, 151.7, 137.4, 135.0, 132.2, 123.6, 122.9, 121.6, 115.7, 111.9, 110.4, 109.8, 40.1. Anal. C<sub>17</sub>H<sub>16</sub>N<sub>2</sub>O<sub>2</sub>.0.125H<sub>2</sub>O Calcd. C: 72.26, H: 5.80, N: 9.91. Found C: 72.31, H: 5.80, N: 9.80.

**3-(4-(Dimethylamino)benzylidene)-1-methylindolin-2-one (8)** was prepared by method 2 with compound **5** and CH<sub>3</sub>I to afford the desired product, (**8**), as a red solid (55%), mp 100.3–101.5 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.42 and 7.67 (d, *J* = 8.0 Hz, 2H), 7.91 and 7.49 (d, *J* = 7.6 Hz, 1H), 7.80 and 7.46 (s, 1H), 7.24 (t, *J* = 7.2 Hz, 1H), 7.03 and 6.94 (t, *J* = 7.2 Hz, 1H), 6.83 (d, *J* = 8.0 Hz, 1H), 6.75 (d, *J* = 8.0 Hz, 2H), 3.29 (s, 3H), 3.07 (s, 6H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 169.4, 151.3, 143.5, 138.8, 138.3, 134.8, 132.0, 128.4, 122.3, 122.2, 122.1, 122.0, 121.4, 121.3, 111.4, 111.1, 107.9, 107.5, 40.1, 26.1. Anal. C<sub>18</sub>H<sub>18</sub>N<sub>2</sub>O Calcd. C: 77.67, H: 6.52, N: 10.06. Found C: 77.45, H: 6.77, N: 9.83.

**1-Benzyl-3-(4-(dimethylamino)benzylidene)indolin-2-one (9)** was prepared by method 2 with compound **5** and benzyl bromide to afford the desired product, (**9**), as an orange solid (82%), mp 111.0–112.4 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.44 and 7.69 (d, *J* = 8.8 Hz, 2H), 7.92 and 7.50 (d, *J* = 7.6 Hz, 1H), 7.87 and 7.51 (s, 1H), 7.35–7.22 (m, 5H), 7.11 (t, *J* = 7.6 Hz, 1H), 6.90 (t, *J* = 7.2 Hz, 1H), 6.74 (d, *J* = 8.4 Hz, 2H), 6.71 (m, 1H), 5.01 (s, 2H), 3.06 (s, 6H). (<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 169.4, 152.0, 151.4, 142.6, 140.3, 139.2, 138.6, 136.7, 136.4, 134.9, 132.1, 128.7, 128.6, 128.3, 127.4, 127.3, 126.9, 122.5, 122.2, 122.1, 122.0, 121.5, 121.4, 117.8, 111.4, 111.2, 108.9, 108.4, 43.7, 40.1, 40.0. Anal. C<sub>24</sub>H<sub>22</sub>N<sub>2</sub>O Calcd. C: 81.33, H: 6.26, N: 7.90. Found C: 81.22, H: 6.34, N: 7.96.

**3-(4-(Dimethylamino)benzylidene)-1-(4-methoxybenzyl)indolin-2-one (10)** was prepared by method 2 with compound **5** and 4-methoxybenzyl chloride to afford the desired product, (**10**), as an orange solid (61%), mp 154.8–155.5 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.44 and 7.68 (d, *J* = 8.8 Hz, 2H), 7.91 (d, *J* = 7.6 Hz, 1H), 7.86 and 7.49 (s, 1H), 7.48 and 7.26 (d, *J* = 5.2 Hz, 1H), 7.28 (d, *J* = 8.8 Hz, 2H), 7.10 (t, *J* = 7.6 Hz, 1H), 6.99 (t, *J* = 7.6 Hz, 1H), 6.82 (d, *J* = 8.8 Hz, 2H), 6.73 (d, *J* = 8.0 Hz, 2H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 169.3, 166.6, 158.8, 151.9, 151.4, 142.6, 140.4, 139.1, 138.5, 134.9, 132.1, 128.9, 128.7, 128.5, 128.3, 125.7, 122.5, 122.1, 121.4, 121.3, 112.0, 117.8, 114.1, 114.0, 111.4, 111.2, 108.9, 108.4, 55.2, 43.1, 42.9, 40.1, 40.0. Anal. C<sub>25</sub>H<sub>24</sub>N<sub>2</sub>O<sub>2</sub> Calcd. C: 78.10, H: 6.29, N: 7.29. Found C: 77.85, H: 6.47, N: 7.24.

**3-(4-(Dimethylamino)benzylidene)-1-(pyridin-4-ylmethyl)indolin-2-one (11)** was prepared by method 2 with compound **5** and 4-(bromomethyl)pyridine to afford the desired product, (**11**), as a red solid (56%), mp 153.0–153.7 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.55 (d, *J* = 4.8 Hz, 2H), 8.43 and 7.97 (d, *J* = 8.0 Hz, 1H), 7.89 and 7.55 (s, 1H), 7.71 (d, *J* = 8.4 Hz, 2H), 7.22 (d, *J* = 5.2 Hz, 2H), 7.14 (t, *J* = 7.6 Hz, 1H), 6.95 (t, *J* = 7.6 Hz, 1H), 6.76 (d, *J* = 9.2 Hz, 2H), 6.63 (d, *J* = 8.0 Hz, 1H), 5.02 (s, 2H), 3.09 (s, 6H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 169.4, 151.5, 150.2, 150.1, 145.5, 141.8, 139.9, 135.0, 132.3, 128.3, 122.3, 122.2, 122.0, 121.9, 121.8, 121.3, 118.0, 111.4, 111.2, 108.5, 42.6, 40.1, 40.0. Anal. C<sub>23</sub>H<sub>21</sub>N<sub>3</sub>O Calcd. C: 77.72, H: 5.96, N: 11.82. Found C: 77.64, H: 6.12, N: 11.74.

**Ethyl 3-(4-(dimethylamino)benzylidene)-2-oxoindoline-1-carboxylate (12)** was prepared by method 2 with compound **5** and ethyl chloroformate to afford the desired product, (**12**), as a red solid (59%), mp 121.7–123.1 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.36 and 7.67 (d, *J* = 8.4 Hz, 2H), 7.98 and 7.89 (d, *J* = 7.6 Hz, 2H), 7.82 and 7.50 (s, 1H), 7.52 and 7.29 (t, *J* = 7.6 Hz, 1H), 7.17 and 7.07 (t, *J* = 7.6 Hz, 1H), 6.74 (d, *J* = 8.4 Hz, 2H), 4.51 (q, *J* = 7.2 Hz, 2H), 3.09 (s, 6H), 1.48 (t, *J* = 7.2 Hz, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 167.3, 151.7, 140.5, 140.1, 135.5, 132.3, 128.6, 123.7, 122.8, 121.7, 121.4, 117.5, 115.0, 114.8, 111.3, 111.1, 63.1, 40.1, 14.3. Anal. C<sub>20</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub> Calcd. C: 71.41, H: 5.99, N: 8.33. Found C: 71.57, H: 6.15, N: 8.33.

**1-Benzyl-3-(4-(dimethylamino)benzylidene)-1H-pyrrolo[2,3-b]pyridin-2(3H)-one (13)** was prepared by method 2 with compound **6** and benzyl bromide to afford the desired product, (**13**), as a red solid (70%), mp 144.7–145.4 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.41 and 7.61 (d, *J* = 8.8 Hz, 2H), 8.10 and 8.05 (d, *J* = 7.6 Hz, 1H), 7.90 and 7.41 (s, 1H), 7.49 (d, *J* = 7.6 Hz, 2H), 7.31–7.21 (m, 4H), 6.91 and 6.83 (dd, *J* = 7.6 Hz, 5.6 Hz, 1H), 6.73 and 6.70 (d, *J* = 8.8 Hz, 2H), 5.13 and 5.11 (s, 2H), 3.06 (s, 6H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 168.9, 166.2, 155.7, 153.6, 152.3, 151.7, 146.5, 145.5, 140.7, 140.3, 137.4, 137.2, 135.3, 132.4, 128.5, 128.4, 128.3, 127.3, 127.2, 124.3, 122.1, 121.7, 120.4, 112.0, 117.5, 117.3, 116.9, 111.5, 111.2, 42.5, 42.3, 40.1, 40.0. Anal. C<sub>23</sub>H<sub>21</sub>N<sub>3</sub>O Calcd. C: 77.72, H: 5.96, N: 11.82. Found C: 77.86, H: 6.05, N: 11.81.

**3-(4-(Dimethylamino)benzylidene)-1-(4-methoxybenzyl)-1H-pyrrolo[2,3-b]pyridin-2(3H)-one (14)** was prepared by method 2 with compound **6** and 4-methoxybenzyl chloride to afford the desired product, (**14**), as a red solid (70%), mp 123.5–124.2 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.41 and 8.04 (d, *J* = 8.4 Hz, 1H), 8.11 (d, *J* = 7.6

Hz, 1H), 7.89 and 7.41 (s, 1H), 7.61 (d,  $J = 8.8$  Hz, 2H), 7.47 (d,  $J = 8.8$  Hz, 2H), 6.93–6.80 (m, 3H), 6.73 and 6.71 (d,  $J = 9.2$  Hz, 2H), 5.06 and 5.04 (s, 2H), 3.75 (s, 3H), 3.07 (s, 6H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  168.8, 166.1, 158.9, 158.8, 155.7, 153.6, 152.3, 151.7, 146.4, 145.4, 140.5, 140.1, 135.2, 132.3, 129.8, 129.7, 129.5, 128.3, 124.2, 122.1, 121.7, 120.4, 120.0, 117.6, 117.2, 116.8, 113.8, 111.4, 111.2, 55.2, 41.9, 41.7, 40.1, 40.0. Anal.  $\text{C}_{24}\text{H}_{23}\text{N}_3\text{O}_2$  Calcd. C: 74.78, H: 6.01, N: 10.90. Found C: 75.04, H: 6.10, N: 10.86.

**3-(3-Phenylallylidene)indolin-2-one (18)** was prepared by method 1 with compound **1** and **15** to afford the desired product, (**18**), as an orange solid (91%), mp 203.9–205.2 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.60–8.53 (m, 1H), 8.30 (s, 1H), 7.73–7.58 (m, 3H), 7.51–7.30 (m, 4H), 7.26–7.15 (m, 2H), 7.09–6.85 (m, 2H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  170.3, 169.1, 144.5, 143.1, 141.0, 139.8, 136.5, 136.4, 136.0, 129.7, 129.3, 129.0, 128.8, 128.7, 127.8, 127.7, 125.7, 124.8, 124.4, 124.2, 123.7, 123.4, 123.0, 122.1, 121.8, 119.5, 110.2, 109.7. Anal.  $\text{C}_{17}\text{H}_{13}\text{NO}$ .0.125 $\text{H}_2\text{O}$  Calcd. C: 81.82, H: 5.33, N: 5.61. Found C: 81.73, H: 5.47, N: 5.48.

**3-(3-(4-Methoxyphenyl)allylidene)indolin-2-one (19)** was prepared by method 1 with compound **1** and **16** to afford the desired product, (**19**), as an orange solid (85%), mp 226.1–226.8 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  10.46 and 10.41 (s, 1H), 7.93 (d,  $J = 7.2$  Hz, 1H), 7.72 (d,  $J = 7.6$  Hz, 1H), 7.60–7.50 (m, 2H), 7.29–7.07 (m, 3H), 6.97 (m, 3H), 6.81 (d,  $J = 7.2$  Hz, 1H), 3.77 (s, 3H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  169.4, 168.8, 161.1, 160.8, 145.0, 142.7, 142.3, 141.2, 136.5, 135.6, 130.3, 129.5, 129.3, 129.2, 129.1, 129.0, 124.9, 124.5, 124.4, 124.3, 122.7, 122.3, 121.8, 121.5, 121.4, 120.1, 115.1, 114.8, 110.0, 109.8, 55.8. Anal.  $\text{C}_{18}\text{H}_{15}\text{NO}_2$  Calcd. C: 77.96, H: 5.45, N: 5.05. Found C: 77.66, H: 5.60, N: 5.02.

**3-(3-(4-(Dimethylamino)phenyl)allylidene)indolin-2-one (20)** was prepared by method 1 with compound **1** and **17** to afford the desired product, (**20**), as an orange solid (82%), mp 227.8–229.4 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  10.37 and 10.33 (s, 1H), 8.18 and 7.45 (dd,  $J = 15.6$  Hz, 11.6 Hz, 1H), 7.87 (d,  $J = 7.6$  Hz, 1H), 7.60 and 7.39 (d,  $J = 8.8$  Hz, 2H), 7.47 and 7.24 (d,  $J = 12.0$  Hz, 1H), 7.20 and 7.04 (d,  $J = 15.6$  Hz, 1H), 7.14 and 7.09 (t,  $J = 7.6$  Hz, 1H), 6.96 and 6.89 (t,  $J = 7.6$  Hz, 1H), 6.80 and 6.75 (d,  $J = 7.2$  Hz, 1H), 6.72 and 6.71 (d,  $J = 8.8$  Hz, 2H), 2.97 and 2.95 (s, 6H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  169.5, 168.9, 151.8, 151.6, 146.4, 144.3, 141.9, 140.7, 136.6, 130.3, 129.5, 128.5, 124.7, 124.2, 124.0, 123.2, 122.8, 122.4, 121.6, 121.6, 121.2, 119.8, 112.5, 112.3, 109.9, 109.7, 40.2. Anal.  $\text{C}_{19}\text{H}_{18}\text{N}_2\text{O}$ .0.125 $\text{H}_2\text{O}$  Calcd. C: 77.99, H: 6.29, N: 9.57. Found C: 77.95, H: 6.32, N: 9.56.

**3-(3-(4-(Dimethylamino)phenyl)allylidene)-1H-pyrrolo[2,3-b]pyridin-2(3H)-one (21)** was prepared by method 1 with compound **2** and **17** to afford the desired product, (**21**), as a purple solid (74%), mp 273.6–274.4 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  10.96 and 10.92 (s, 1H), 8.22 and 7.79 (d,  $J = 7.2$  Hz, 1H), 7.99 and 7.96 (d,  $J = 4.8$  Hz, 1H), 7.62 (d,  $J = 8.4$  Hz, 2H), 7.44–7.34 (m, 2H), 7.26 and 7.08 (d,  $J = 15.2$  Hz, 1H), 6.95 and 6.91 (d,  $J = 7.4$  Hz, 5.2 Hz, 1H), 6.70 (d,  $J = 8.8$  Hz, 2H), 2.97 and 2.96 (s, 6H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$  169.1, 156.0, 155.1, 152.0, 151.8, 147.9, 146.4, 145.8, 139.7, 138.6, 130.7, 130.4, 129.9, 126.3, 123.9, 123.8, 120.8, 119.7, 119.0, 118.5, 117.7, 117.5, 117.4, 112.5, 112.2,

40.1. Anal. C<sub>18</sub>H<sub>17</sub>N<sub>3</sub>O Calcd. C: 74.20, H: 5.88, N: 14.42. Found C: 73.92, H: 5.84, N: 14.32.

**3-(3-(4-(Dimethylamino)phenyl)allylidene)-5-hydroxyindolin-2-one (22)** was prepared by method 1 with compound **1** and **15** to afford the desired product, (**22**), as a purple solid (81%), mp >280 °C. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 10.06 and 10.02 (s, 1H), 8.93 and 8.91 (s, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.38-6.88 (m, 5H), 6.71 (d, *J* = 7.2 Hz, 2H), 6.59-6.54 (m, 2H), 2.96 and 2.94 (s, 6H). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>) δ 169.5, 169.1, 152.5, 152.4, 151.7, 151.5, 145.7, 144.0, 137.0, 136.0, 134.5, 133.4, 129.9, 129.4, 125.5, 124.3, 124.0, 123.8, 123.7, 123.2, 119.8, 118.5, 115.0, 112.6, 112.4, 111.1, 110.3, 110.1, 110.0, 106.9, 40.1. Anal. C<sub>19</sub>H<sub>18</sub>N<sub>2</sub>O<sub>2</sub>·0.25H<sub>2</sub>O Calcd. C: 73.41, H: 6.00, N: 9.01. Found C: 73.50, H: 6.09, N: 8.72.

**1-Benzyl-3-(3-phenylallylidene)indolin-2-one (23)** was prepared by method 2 with compound **18** and benzyl bromide to afford the desired product, (**23**), as an orange solid (90%), mp 124.6–125.1 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.67 (dd, *J* = 15.8 Hz, 11.6 Hz, 1H), 7.62 (d, *J* = 7.2 Hz, 2H), 7.46 (d, *J* = 7.6 Hz, 1H), 7.39-7.25 (m, 9H), 7.14 (t, *J* = 7.6 Hz, 1H), 7.05 (d, *J* = 16.0 Hz, 1H), 6.99 (t, *J* = 7.6 Hz, 1H), 6.71 (d, *J* = 7.6 Hz, 1H), 4.97 (s, 2H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 167.4, 143.0, 141.6, 136.4, 136.3, 129.3, 128.8, 128.7, 128.6, 127.8, 127.5, 127.3, 124.5, 124.4, 123.5, 121.9, 119.3, 108.9, 43.3. Anal. C<sub>24</sub>H<sub>19</sub>NO Calcd. C: 85.43, H: 5.68, N: 4.15. Found C: 85.22, H: 5.88, N: 4.13.

**1-Benzyl-3-(3-(4-methoxyphenyl)allylidene)indolin-2-one (24)** was prepared by method 2 with compound **19** and benzyl bromide to afford the desired product, (**24**), as an orange solid (78%), mp 167.3–169.3 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.72 (d, *J* = 7.2 Hz, 1H), 7.59-7.51 (m, 3H), 7.35-7.25 (m, 5H), 7.17-7.11 (m, 2H), 7.04 and 6.99 (t, *J* = 7.6 Hz, 1H), 6.94 (d, *J* = 8.8 Hz, 2H), 6.90 (d, *J* = 9.2 Hz, 1H), 6.72 and 6.70 (d, *J* = 8.0 Hz, 1H), 4.99 (s, 2H), 3.86 and 3.84 (s, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 168.8, 161.0, 160.8, 144.3, 143.1, 142.5, 136.9, 136.7, 136.4, 136.2, 129.5, 129.3, 128.9, 128.7, 128.4, 128.2, 127.4, 127.3, 127.2, 123.9, 123.2, 122.7, 122.6, 122.0, 121.7, 121.4, 119.0, 114.5, 114.3, 109.1, 108.8, 55.4, 43.6. Anal. C<sub>25</sub>H<sub>21</sub>NO<sub>2</sub> Calcd. C: 81.72, H: 5.76, N: 3.81. Found C: 81.57, H: 5.93, N: 3.82.

**3-(3-(4-(Dimethylamino)phenyl)allylidene)-1-methylindolin-2-one (25)** was prepared by method 2 with compound **20** and CH<sub>3</sub>I to afford the desired product, (**25**), as an orange solid (67%), mp 170.5–171.5 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.71 (d, *J* = 7.2 Hz, 1H), 7.53-7.41 (m, 3H), 7.30 (d, *J* = 11.6 Hz, 1H), 7.24 and 7.21 (t, *J* = 7.6 Hz, 1H), 7.09 and 6.97 (d, *J* = 14.8 Hz, 1H), 7.07 and 7.00 (t, *J* = 8.0 Hz, 1H), 6.83 and 6.79 (d, *J* = 8.0 Hz, 1H), 6.70 and 6.68 (d, *J* = 8.8 Hz, 2H), 3.27 (s, 3H), 3.02 and 3.02 (s, 6H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 168.9, 167.7, 151.4, 151.2, 145.5, 144.3, 143.0, 141.8, 137.6, 137.4, 129.6, 129.4, 127.8, 127.6, 124.6, 124.1, 124.0, 122.9, 122.8, 122.2, 121.7, 121.5, 120.3, 119.0, 118.5, 112.0, 111.9, 107.8, 107.6, 40.2, 26.0, 25.6. Anal. C<sub>20</sub>H<sub>20</sub>N<sub>2</sub>O Calcd. C: 78.92, H: 6.62, N: 9.20. Found C: 78.73, H: 6.72, N: 9.29.

**1-Benzyl-3-(3-(4-(dimethylamino)phenyl)allylidene)indolin-2-one (26)** was prepared by method 2 with compound **20** and benzyl bromide to afford the desired product, (**26**), as an

orange solid (85%), mp 131.5–133.1 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.11 (d, *J* = 5.6 Hz, 1H), 7.84 and 7.58 (d, *J* = 7.6 Hz, 1H), 7.62 (d, *J* = 12.4 Hz, 1H), 7.53–7.64 (m, 4H), 7.36–7.20 (m, 5H), 7.12 and 7.01 (d, *J* = 14.8 Hz, 1H), 6.95 and 6.90 (t, *J* = 5.6 Hz, 1H), 6.69 (d, *J* = 8.8 Hz, 2H), 5.08 (s, 2H), 3.05 and 3.03 (s, 6H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 168.4, 155.3, 151.6, 151.5, 146.9, 146.0, 139.7, 139.3, 137.3, 137.2, 130.0, 129.7, 129.0, 128.5, 128.4, 128.2, 127.3, 123.7, 120.3, 120.0, 118.3, 117.6, 117.5, 111.9, 42.4, 40.1. Anal. C<sub>26</sub>H<sub>24</sub>N<sub>2</sub>O Calcd. C: 78.71, H: 6.08, N: 11.02. Found C: 78.27, H: 6.16, N: 10.87.

**3-(3-(4-(Dimethylamino)phenyl)allylidene)-1-(4-methoxybenzyl)indolin-2-one (27)** was prepared by method 2 with compound **20** and 4-methoxybenzyl chloride to afford the desired product, (**27**), as an orange solid (47%), mp 169.5–170.3 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.46 and 7.46 (dd, *J* = 15.4 Hz, 11.6 Hz, 1H), 7.70 and 7.41 (d, *J* = 7.6 Hz, 1H), 7.57 and 7.33 (d, *J* = 12.0 Hz, 1H), 7.52–7.46 (m, 2H), 7.28–7.24 (m, 2H), 7.14–6.94 (m, 3H), 6.84–6.81 (m, 2H), 6.74–6.65 (m, 3H), 4.91 (s, 2H), 3.75 (s, 3H), 3.02 and 3.01 (s, 6H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 169.0, 167.7, 158.9, 151.4, 151.2, 145.7, 144.6, 142.1, 140.9, 137.9, 137.8, 129.7, 129.5, 128.7, 128.6, 128.5, 127.7, 127.5, 124.6, 124.1, 124.0, 123.1, 122.9, 122.0, 121.7, 121.5, 121.2, 120.3, 118.9, 118.6, 114.0, 112.0, 111.9, 108.9, 108.6, 55.2, 43.0, 42.7, 40.2, 40.1. Anal. C<sub>27</sub>H<sub>26</sub>N<sub>2</sub>O<sub>2</sub> Calcd. C: 79.00, H: 6.38, N: 6.82. Found C: 79.08, H: 6.51, N: 6.75.

**3-(3-(4-(Dimethylamino)phenyl)allylidene)-1-(4-nitrobenzyl)indolin-2-one (28)** was prepared by method 2 with compound **20** and 4-nitrobenzyl bromide to afford the desired product, (**28**), as an orange solid (83%), mp 204.2–205.6 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.17 (d, *J* = 8.0 Hz, 2H), 7.76 (d, *J* = 7.2 Hz, 1H), 7.61 and 7.40 (d, *J* = 12.4 Hz, 1H), 7.54–7.45 (m, 5H), 7.17–7.00 (m, 3H), 6.71–6.61 (m, 3H), 5.08 (s, 2H), 3.06 and 3.04 (s, 6H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 169.0, 167.6, 151.6, 147.4, 146.6, 145.5, 144.0, 138.8, 138.6, 137.7, 129.8, 129.7, 128.0, 127.9, 127.7, 127.5, 124.0, 123.9, 123.1, 122.3, 122.1, 121.1, 120.1, 118.8, 118.7, 112.0, 111.9, 108.4, 108.1, 43.0, 42.7, 40.2. Anal. C<sub>26</sub>H<sub>23</sub>N<sub>3</sub>O<sub>3</sub> Calcd. C: 73.39, H: 5.45, N: 9.88. Found C: 73.48, H: 5.63, N: 9.81.

**3-(3-(4-(Dimethylamino)phenyl)allylidene)-1-(pyridin-4-ylmethyl)indolin-2-one (29)** was prepared by method 2 with compound **20** and 4-(bromomethyl)pyridine to afford the desired product, (**29**), as a purple solid (51%), mp 194.1–195.1 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.53 (d, *J* = 4.4 Hz, 2H), 7.76 (d, *J* = 7.2 Hz, 1H), 7.61 (d, *J* = 12.4 Hz, 1H), 7.54–7.39 (m, 3H), 7.20–7.02 (m, 5H), 6.71 (d, *J* = 8.8 Hz, 2H), 6.62 (d, *J* = 7.6 Hz, 1H), 5.00 (s, 2H), 3.06 and 3.03 (s, 6H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 169.0, 151.5, 150.1, 146.4, 145.5, 145.3, 141.4, 138.5, 129.8, 129.6, 127.8, 123.9, 123.1, 122.2, 122.0, 121.3, 118.7, 112.0, 111.9, 108.5, 42.6, 40.2. Anal. C<sub>25</sub>H<sub>23</sub>N<sub>3</sub>O Calcd. C: 78.71, H: 6.08, N: 11.02. Found C: 78.76, H: 6.27, N: 10.95.

**Ethyl 3-(3-(4-(dimethylamino)phenyl)allylidene)-2-oxoindoline-1-carboxylate (30)** was prepared by method 2 with compound **20** and ethyl chloroformate to afford the desired product, (**30**), as a red solid (57%), mp 169.9–170.5 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.39 and 7.53 (dd, *J* = 15.2 Hz, 12.0 Hz, 1H), 7.98 and 7.91 (d, *J* = 8.0 Hz, 1H), 7.77 and 7.46 (d, *J* = 7.2 Hz, 1H), 7.52 and 7.50 (d, *J* = 9.2 Hz, 2H), 7.37–7.02 (m, 4H), 6.70 and 6.67 (d, *J* = 8.8 Hz, 2H), 4.51 (q, *J* = 7.2 Hz, 2H), 3.05 and 3.04 (s, 6H), 1.49 (t, *J* = 7.2 Hz, 3H). <sup>13</sup>C

NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  165.5, 151.5, 147.3, 146.4, 139.3, 139.1, 137.3, 130.0, 129.8, 128.0, 127.9, 124.7, 124.3, 124.1, 124.0, 123.8, 122.4, 120.2, 119.2, 118.5, 118.1, 115.1, 115.0, 112.0, 111.9, 63.1, 40.1, 14.4. Anal. C<sub>22</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub> Calcd. C: 72.91, H: 6.12, N: 7.73. Found C: 73.18, H: 6.04, N: 7.80.

**1-Benzyl-3-(3-(4-(dimethylamino)phenyl)allylidene)-1H-pyrrolo[2,3-b]pyridin-2(3H)-one (31)** was prepared by method 2 with compound **21** and benzyl bromide to afford the desired product, (**31**), as a purple solid (72%), mp 203.1–204.0 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.11 (d, *J* = 5.2 Hz, 1H), 7.83 (d, *J* = 7.6 Hz, 1H), 7.61 (d, *J* = 12.0 Hz, 1H), 7.52–7.34 (m, 4H), 7.31–7.20 (m, 4H), 7.11 (d, *J* = 15.2 Hz, 1H), 6.94 (dd, *J* = 7.4 Hz, 5.2 Hz, 1H), 6.68 (d, *J* = 9.2 Hz, 2H), 5.01 (s, 2H), 3.03 and 3.02 (s, 6H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  168.4, 155.3, 151.6, 151.5, 146.9, 146.0, 145.9, 139.6, 139.3, 137.3, 137.2, 130.0, 129.7, 129.0, 128.5, 128.4, 128.2, 127.3, 125.0, 124.2, 123.7, 120.3, 120.0, 119.0, 118.7, 118.3, 117.6, 117.4, 111.9, 42.4, 42.1, 40.1. Anal. C<sub>25</sub>H<sub>23</sub>N<sub>3</sub>O Calcd. C: 78.71, H: 6.08, N: 11.02. Found C: 78.88, H: 6.21, N: 10.93.

**3-(3-(4-(Dimethylamino)phenyl)allylidene)-1-(4-methoxybenzyl)-1H-pyrrolo[2,3-b]pyridin-2(3H)-one (32)** was prepared by method 2 with compound **21** and 4-methoxybenzyl chloride to afford the desired product, (**32**), as a purple solid (81%), mp 189.7–191.9 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.11 (d, *J* = 5.6 Hz, 1H), 7.82 (d, *J* = 7.6 Hz, 1H), 7.60 (d, *J* = 12.8 Hz, 1H), 7.46 (d, *J* = 8.8 Hz, 2H), 7.44 (d, *J* = 8.0 Hz, 2H), 7.30 (dd, *J* = 14.8 Hz, 12.4 Hz, 1H), 7.11 (d, *J* = 14.8 Hz, 1H), 6.93 (dd, *J* = 7.2 Hz, 5.6 Hz, 1H), 6.81 (d, *J* = 8.8 Hz, 2H), 6.67 (d, *J* = 8.8 Hz, 2H), 5.01 (s, 2H), 3.75 (s, 3H), 3.03 (s, 6H). (<sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  168.3, 158.8, 155.3, 151.6, 146.8, 145.9, 139.2, 129.8, 129.7, 129.5, 129.0, 123.7, 120.1, 118.4, 117.6, 117.4, 113.8, 111.9, 55.2, 41.8, 40.1. Anal. C<sub>26</sub>H<sub>25</sub>N<sub>3</sub>O<sub>2</sub> Calcd. C: 75.89, H: 6.12, N: 10.21. Found C: 75.97, H: 6.13, N: 10.18.

**1-(4-Aminobenzyl)-3-(3-(4-(dimethylamino)phenyl)allylidene)indolin-2-one (33)** A mixture of **28** (425 mg, 1 mmol) and Fe powder (560 mg, 10 mmol) in methanol (30 mL) and 37% HCl (1 mL) was heated to reflux for 3 h. The solid was filtered out, the filtrate was evaporated, ethyl acetate (75 mL) was added, washed with saturated Na<sub>2</sub>CO<sub>3</sub> (50 mL), water (50 mL), NaCl (50 mL), and dried over Na<sub>2</sub>SO<sub>4</sub>. After removed of the solvent, the crude product was purified by silica gel column chromatography eluting with EtOAc-MeOH (10:1, v/v) to afford 263 mg (66%) of **33** as a red solid, mp 221.0–222.1 °C. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  7.91 (d, *J* = 7.6 Hz, 1H), 7.61 (d, *J* = 8.8 Hz, 2H), 7.51–7.36 (m, 2H), 7.26 (d, *J* = 14.4 Hz, 1H), 7.14 (t, *J* = 7.6 Hz, 1H), 7.00 (t, *J* = 8.0 Hz, 1H), 6.97 (d, *J* = 8.4 Hz, 2H), 6.89 (d, *J* = 7.6 Hz, 1H), 6.70 (d, *J* = 8.4 Hz, 2H), 6.46 (d, *J* = 8.4 Hz, 2H), 4.98 (s, 2H), 4.71 (s, 2H), 2.96 (s, 6H). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>)  $\delta$  168.1, 151.9, 148.4, 147.2, 142.2, 137.5, 130.4, 128.8, 128.3, 124.0, 123.9, 123.6, 122.6, 122.1, 121.5, 118.6, 114.3, 112.3, 109.4, 42.7, 40.1. Anal. C<sub>26</sub>H<sub>25</sub>N<sub>3</sub>O.0.5H<sub>2</sub>O Calcd. C: 77.20, H: 6.48, N: 10.39. Found C: 77.74, H: 6.34, N: 10.31.

**3-(4-(Dimethylamino)benzylidene)-1-phenylindolin-2-one (35)** was prepared by method 1 with compound **4** and **34** to afford the desired product, (**35**), as a yellow solid (88%), mp 171.1–172.8 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.99 (d, *J* = 6.8 Hz, 1H), 7.89 (s, 1H), 7.72 (d, *J* = 8.4 Hz, 2H), 7.53–7.40 (m, 5H), 7.16 (t, *J* = 7.2 Hz, 1H), 6.97 (t, *J* = 7.2 Hz, 1H),

6.84 (d,  $J = 6.8$  Hz, 1H), 6.77 (d,  $J = 8.8$  Hz, 2H), 3.01 (s, 6H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  168.7, 151.4, 143.3, 139.6, 135.0, 132.2, 129.5, 128.3, 127.7, 126.9, 122.2, 122.1, 121.9, 111.4, 109.2, 40.1. Anal.  $\text{C}_{23}\text{H}_{20}\text{N}_2\text{O}$  Calcd. C: 81.15, H: 5.92, N: 8.23. Found C: 81.01, H: 6.04, N: 8.18.

**3-(3-(4-(Dimethylamino)phenyl)allylidene)-1-phenylindolin-2-one (36)** was prepared by method 1 with compound **17** and **34** to afford the desired product, (**36**), as a purple solid (77%), mp 206.8–207.9 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.48 (dd,  $J = 15.2$  Hz, 11.6 Hz, 1H), 7.54–7.45 (m, 7H), 7.39 (d,  $J = 12.0$  Hz, 1H), 7.38 (t,  $J = 7.6$  Hz, 1H), 7.12 (t,  $J = 7.6$  Hz, 1H), 7.04 (d,  $J = 7.6$  Hz, 1H), 7.00 (d,  $J = 15.6$  Hz, 1H), 6.82 (d,  $J = 8.0$  Hz, 1H), 6.64 (d,  $J = 8.8$  Hz, 2H), 2.99 (s, 6H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  167.1, 151.4, 145.0, 141.6, 138.5, 135.0, 129.8, 129.5, 127.6, 127.5, 126.7, 124.6, 124.2, 122.1, 121.0, 120.5, 110.8, 112.0, 109.1, 40.2. Anal.  $\text{C}_{25}\text{H}_{22}\text{N}_2\text{O}$  Calcd. C: 81.94, H: 6.05, N: 7.74. Found C: 81.92, H: 6.25, N: 7.59.

**(Z)-3-(4-Nitrobenzylidene)indolin-2-one (38a)** and **(E)-3-(4-nitrobenzylidene)indolin-2-one (38b)** were prepared by method 1 with compound **1** and **37**. The crude product was purified by silica gel column chromatography eluting with  $\text{CH}_2\text{Cl}_2$ -EtOAc (100:5, v/v), compound **38a** eluted first and was afforded as a red solid (41%), mp 247.5–248.6 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.34 (d,  $J = 8.8$  Hz, 2H), 8.28 (d,  $J = 8.8$  Hz, 2H), 7.55 (d,  $J = 8.0$  Hz, 1H), 7.53 (s, 1H), 7.42 (s, 1H), 7.29 (t,  $J = 7.2$  Hz, 1H), 7.08 (t,  $J = 7.2$  Hz, 1H), 6.86 (d,  $J = 8.0$  Hz, 1H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO-d}_6$ )  $\delta$  167.2, 147.8, 142.0, 140.7, 133.9, 132.9, 131.5, 130.9, 130.6, 124.6, 124.4, 123.6, 121.8, 121.1, 110.1. Anal.  $\text{C}_{15}\text{H}_{10}\text{N}_2\text{O}_3 \cdot 0.125\text{H}_2\text{O}$  Calcd. C: 67.10, H: 3.85, N: 10.43. Found C: 67.08, H: 3.81, N: 10.47. Compound **38b** eluted second as a red solid (48%), mp 252.6–252.9 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO-d}_6$ )  $\delta$  10.68 (s, 1H), 8.31 (d,  $J = 8.4$  Hz, 2H), 7.93 (d,  $J = 8.0$  Hz, 2H), 7.63 (s, 1H), 7.37 (d,  $J = 8.0$  Hz, 1H), 7.23 (t,  $J = 8.0$  Hz, 1H), 6.85 (d,  $J = 7.6$  Hz, 1H), 6.81 (t,  $J = 7.6$  Hz, 1H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO-d}_6$ )  $\delta$  168.6, 147.9, 143.9, 141.9, 133.4, 131.5, 130.9, 130.5, 124.4, 123.3, 121.8, 120.7, 110.8. Anal.  $\text{C}_{15}\text{H}_{10}\text{N}_2\text{O}_3$  Calcd. C: 67.67, H: 3.79, N: 10.52. Found C: 67.54, H: 3.71, N: 10.60.

**(Z)-1-Benzyl-3-(4-nitrobenzylidene)indolin-2-one (39a)** was prepared by method 2 with compound **38a** and benzyl bromide to afford the desired product, (**39a**), as a red solid (76%), mp 200.4–201.1 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.39 (d,  $J = 9.2$  Hz, 2H), 8.28 (d,  $J = 8.4$  Hz, 2H), 7.57 (s, 1H), 7.56 (d,  $J = 6.8$  Hz, 1H), 7.32–7.22 (m, 6H), 7.06 (t,  $J = 7.2$  Hz, 1H), 6.75 (d,  $J = 7.6$  Hz, 1H), 4.98 (s, 2H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  165.7, 148.0, 142.2, 139.8, 135.7, 133.5, 132.3, 130.2, 129.4, 128.8, 127.7, 127.3, 123.5, 123.4, 122.3, 119.8, 109.2, 43.7. Anal.  $\text{C}_{22}\text{H}_{16}\text{N}_2\text{O}_3 \cdot 0.25\text{H}_2\text{O}$  Calcd. C: 73.22, H: 4.61, N: 7.76. Found C: 72.93, H: 4.50, N: 7.75.

**(E)-1-Benzyl-3-(4-nitrobenzylidene)indolin-2-one (39b)** was prepared by method 2 with compound **38b** and benzyl bromide to afford the desired product, (**39b**), as red solid (66%), mp 161.9–162.2 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.34 (d,  $J = 8.4$  Hz, 2H), 7.87 (s, 1H), 7.81 (d,  $J = 8.4$  Hz, 2H), 7.44 (d,  $J = 7.2$  Hz, 1H), 7.35–7.26 (m, 5H), 7.20 (t,  $J = 7.6$  Hz, 1H), 6.86 (t,  $J = 7.6$  Hz, 1H), 6.75 (d,  $J = 7.6$  Hz, 1H), 5.00 (s, 1H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  167.9, 147.9, 143.9, 141.7, 135.6, 133.7, 130.9, 130.0, 129.7, 128.8, 127.7, 127.3,



124.0, 123.0, 122.2, 120.4, 109.6, 43.9. Anal. C<sub>22</sub>H<sub>16</sub>N<sub>2</sub>O<sub>3</sub> Calcd. C: 74.15, H: 4.53, N: 7.86. Found C: 74.14, H: 4.61, N: 7.84.

**(Z)-3-((E)-3-(4-Nitrophenyl)allylidene)indolin-2-one (41a) and (E)-3-((E)-3-(4-Nitrophenyl)allylidene)indolin-2-one (41b)** were prepared by method 1 with compound **1** and **40**, the crude product was purified by silica gel column chromatography eluting with CH<sub>2</sub>Cl<sub>2</sub>-EtOAc (100:5, v/v). Compound **41a** eluted first and was afforded as a red solid (41%), mp 256.2–257.2 °C. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 10.56 (s, 1H), 8.58 (dd, *J* = 15.6 Hz, 11.2 Hz, 1H), 8.22 (d, *J* = 8.8 Hz, 2H), 7.77 (d, *J* = 8.8 Hz, 2H), 7.55 (d, *J* = 7.6 Hz, 1H), 7.54 (d, *J* = 11.2 Hz, 1H), 7.22 (d, *J* = 15.6 Hz, 1H), 7.17 (t, *J* = 7.2 Hz, 1H), 6.93 (t, *J* = 7.6 Hz, 1H), 6.77 (d, *J* = 8.0 Hz, 1H). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>) δ 168.6, 147.5, 143.1, 142.0, 139.5, 134.6, 130.1, 128.6, 128.3, 128.0, 124.7, 123.7, 121.7, 120.9, 110.1. Anal. C<sub>17</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub> Calcd. C: 69.86, H: 4.14, N: 9.58. Found C: 69.59, H: 4.03, N: 9.51. Compound **41b** eluted second and was afforded as a red solid (46%), mp 263.8–264.4 °C. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 10.49 (s, 1H), 8.19 (d, *J* = 8.4 Hz, 2H), 8.00 (d, *J* = 8.4 Hz, 3H), 7.87 (dd, *J* = 15.0 Hz, 12.8 Hz, 1H), 7.42 (d, *J* = 14.8 Hz, 1H), 7.24 (d, *J* = 12.4 Hz, 1H), 7.21 (t, *J* = 8.0 Hz, 1H), 6.98 (t, *J* = 7.6 Hz, 1H), 6.2 (d, *J* = 8.0 Hz, 1H). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>) δ 169.1, 147.6, 143.1, 142.8, 141.8, 133.6, 130.2, 129.3, 128.4, 127.8, 125.1, 124.4, 122.2, 121.9, 110.3. Anal. C<sub>17</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>·0.50H<sub>2</sub>O Calcd. C: 67.77, H: 4.35, N: 9.30. Found C: 67.94, H: 4.73, N: 8.87.

**(Z)-1-Benzyl-3-((E)-3-(4-nitrophenyl)allylidene)indolin-2-one (42a)** was prepared by method 2 with compound **41a** and benzyl bromide to afford the desired product, (**42a**), as a red solid (76%), mp 224.5–225.8 °C. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 8.66 (dd, *J* = 15.6 Hz, 11.6 Hz, 1H), 8.25 (d, *J* = 8.8 Hz, 2H), 7.83 (d, *J* = 8.8 Hz, 2H), 7.70 (d, *J* = 12.0 Hz, 1H), 7.65 (d, *J* = 7.6 Hz, 1H), 7.33–7.18 (m, 7H), 7.00 (t, *J* = 7.6 Hz, 1H), 6.92 (d, *J* = 8.0 Hz, 1H), 4.93 (s, 2H). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>) δ 166.8, 143.0, 140.3, 137.1, 135.5, 131.3, 130.1, 129.1, 128.7, 128.2, 127.8, 126.7, 124.7, 122.5, 120.8, 109.6, 40.6. Anal. C<sub>24</sub>H<sub>18</sub>N<sub>2</sub>O<sub>3</sub>·0.125H<sub>2</sub>O Calcd. C: 74.94, H: 4.78, N: 7.28. Found C: 74.83, H: 4.85, N: 7.18.

**(E)-1-Benzyl-3-((E)-3-(4-nitrophenyl)allylidene)indolin-2-one (42b)** was prepared by method 2 with compound **41b** and benzyl bromide to afford the desired product, (**42b**), as a red solid (67%), mp 237.1–237.9 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.27 (d, *J* = 8.4 Hz, 2H), 7.79 (dd, *J* = 15.2 Hz, 12.4 Hz, 1H), 7.72 (d, *J* = 8.4 Hz, 3H), 7.54 (d, *J* = 12.0 Hz, 1H), 7.323–7.17 (m, 7H), 7.07 (t, *J* = 7.6 Hz, 1H), 6.75 (d, *J* = 7.6 Hz, 1H), 4.99 (s, 2H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 168.3, 147.8, 143.3, 142.2, 140.6, 135.9, 134.1, 129.7, 128.7, 128.0, 127.7, 127.6, 127.3, 127.2, 124.3, 123.8, 122.3, 122.0, 109.5, 43.7. Anal. C<sub>24</sub>H<sub>18</sub>N<sub>2</sub>O<sub>3</sub> Calcd. C: 75.38, H: 4.74, N: 7.33. Found C: 75.30, H: 6.78, N: 7.28.

**(Z)-1-(4-Methoxybenzyl)-3-((E)-3-(4-nitrophenyl)allylidene)indolin-2-one (43a)** was prepared by method 2 with compound **41a** and 4-methoxybenzyl chloride to afford the desired product, (**43a**), as a red solid (55%), mp 182.6–183.3 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.82 (dd, *J* = 15.6 Hz, 11.6 Hz, 1H), 8.21 (d, *J* = 8.0 Hz, 2H), 7.72 (d, *J* = 8.4 Hz, 2H), 7.46 (d, *J* = 7.6 Hz, 1H), 7.30 (d, *J* = 12.8 Hz, 1H), 7.28 (d, *J* = 9.2 Hz, 2H), 7.19 (t, *J* = 8.0 Hz, 1H), 7.03 (d, *J* = 16.8 Hz, 1H), 7.01 (t, *J* = 7.8 Hz, 1H), 6.85 (d, *J* = 8.0 Hz, 2H), 6.75 (d, *J* = 7.6 Hz, 1H), 4.90 (s, 2H), 3.77 (s, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 167.1,

159.1, 147.5, 142.7, 142.2, 139.0, 134.2, 129.6, 128.7, 128.5, 128.1, 128.0, 127.1, 124.1, 123.0, 122.1, 119.8, 114.2, 109.1, 55.2, 42.9. Anal. C<sub>25</sub>H<sub>20</sub>N<sub>2</sub>O<sub>4</sub> Calcd. C: 72.80, H: 4.89, N: 6.79. Found C: 72.52, H: 5.03, N: 6.67.

**(E)-1-(4-Methoxybenzyl)-3-((E)-3-(4-nitrophenyl)allylidene)indolin-2-one (43b)** was prepared by method 2 with compound **41b** and 4-methoxybenzyl chloride to afford the desired product, (**43b**), as a red solid (47%), mp 237.7–238.5 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.27 (d, *J* = 8.0 Hz, 2H), 7.78 (dd, *J* = 15.0 Hz, 12.0 Hz, 1H), 7.71 (d, *J* = 8.0 Hz, 2H), 7.53 (d, *J* = 12.0 Hz, 1H), 7.26–7.21 (m, 4H), 7.18 (d, *J* = 14.8 Hz, 1H), 7.06 (t, *J* = 7.6 Hz, 1H), 6.84 (d, *J* = 8.0 Hz, 2H), 6.77 (d, *J* = 8.4 Hz, 1H), 4.91 (s, 2H), 3.77 (s, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 168.2, 159.1, 153.8, 147.8, 143.3, 142.2, 140.5, 134.0, 129.7, 128.7, 128.0, 127.8, 127.4, 124.3, 123.7, 122.2, 122.0, 114.1, 109.5, 55.2, 43.2. Anal. C<sub>25</sub>H<sub>20</sub>N<sub>2</sub>O<sub>4</sub> Calcd. C: 72.80, H: 4.89, N: 6.79. Found C: 72.75, H: 5.01, N: 6.73.

**(Z)-1-(3-Methoxybenzyl)-3-((E)-3-(4-nitrophenyl)allylidene)indolin-2-one (44a)** was prepared by method 2 with compound **41a** and 3-methoxybenzyl bromide to afford the desired product, (**44a**), as a red solid (52%), mp 193.3–194.6 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.81 (dd, *J* = 16.0 Hz, 11.6 Hz, 1H), 8.21 (d, *J* = 8.4 Hz, 2H), 7.72 (d, *J* = 8.4 Hz, 2H), 7.47 (d, *J* = 7.6 Hz, 1H), 7.32 (d, *J* = 12.0 Hz, 1H), 7.24 (t, *J* = 7.6 Hz, 1H), 7.18 (t, *J* = 8.0 Hz, 1H), 7.03 (d, *J* = 17.2 Hz, 1H), 7.01 (t, *J* = 7.2 Hz, 1H), 6.91 (d, *J* = 7.6 Hz, 1H), 6.86 (s, 1H), 6.80 (d, *J* = 8.8 Hz, 1H), 6.73 (d, *J* = 8.0 Hz, 1H), 4.94 (s, 2H), 3.77 (s, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 167.2, 159.9, 147.5, 142.7, 142.2, 139.1, 137.6, 134.3, 129.9, 129.6, 128.5, 128.0, 127.0, 124.1, 123.0, 122.1, 119.8, 119.5, 113.2, 112.7, 109.1, 55.2, 43.3. Anal. C<sub>25</sub>H<sub>20</sub>N<sub>2</sub>O<sub>4</sub> Calcd. C: 72.80, H: 4.89, N: 6.79. Found C: 72.80, H: 5.06, N: 6.66.

**(E)-1-(3-Methoxybenzyl)-3-((E)-3-(4-nitrophenyl)allylidene)indolin-2-one (44b)** was prepared by method 2 with compound **41b** and 3-methoxybenzyl bromide to afford the desired product, (**44b**), as a red solid (59%), mp 237.7–239.0 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.28 (d, *J* = 8.0 Hz, 2H), 7.82–7.67 (m, 3H), 7.62–7.45 (m, 1H), 7.33–7.18 (m, 3H), 7.08–7.04 (m, 2H), 6.91–6.75 (m, 4H), 4.95 (s, 2H), 3.77 (s, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 140.6, 134.1, 129.8, 128.0, 127.3, 124.3, 124.2, 123.7, 122.3, 119.5, 113.1, 112.8, 109.5, 55.2, 43.7. Anal. C<sub>25</sub>H<sub>20</sub>N<sub>2</sub>O<sub>4</sub> Calcd. C: 72.80, H: 4.89, N: 6.79. Found C: 72.87, H: 4.91, N: 6.72.

**(Z)-1-(3,4-Dimethoxybenzyl)-3-((E)-3-(4-nitrophenyl)allylidene)indolin-2-one (45a)** was prepared by method 2 with compound **41a** and 3,4-dimethoxybenzyl bromide to afford the desired product, (**45a**), as a red solid (55%), mp 199.6–200.2 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.81 (dd, *J* = 15.4 Hz, 11.6 Hz, 1H), 8.21 (d, *J* = 8.0 Hz, 2H), 7.72 (d, *J* = 8.0 Hz, 2H), 7.47 (d, *J* = 7.2 Hz, 1H), 7.32 (d, *J* = 12.0 Hz, 1H), 7.19 (t, *J* = 7.2 Hz, 1H), 7.05–7.01 (m, 2H), 6.87 (m, 2H), 6.80 (d, *J* = 7.6 Hz, 1H), 6.76 (d, *J* = 7.6 Hz, 1H), 4.90 (s, 2H), 3.85 (s, 6H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 167.2, 149.3, 148.5, 147.5, 142.7, 142.2, 139.1, 134.3, 129.6, 128.5, 128.0, 127.1, 124.1, 123.0, 122.1, 119.8, 119.7, 111.1, 110.6, 109.1, 56.0, 55.9, 43.3. Anal. C<sub>26</sub>H<sub>22</sub>N<sub>2</sub>O<sub>5</sub> Calcd. C: 70.58, H: 5.01, N: 6.33. Found C: 70.70, H: 5.14, N: 6.26.

**(E)-1-(3,4-Dimethoxybenzyl)-3-((E)-3-(4-nitrophenyl)allylidene)indolin-2-one (45b)** was prepared by method 2 with compound **41b** and 3,4-methoxybenzyl bromide to afford the desired product, (**45b**), as a red solid (51%), mp 201.3–201.9 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.27 (d, *J* = 7.2 Hz, 2H), 7.82–7.71 (m, 2H), 7.72 (d, *J* = 6.8 Hz, 2H), 7.44 (d, *J* = 12.0 Hz, 1H), 7.26–7.17 (m, 2H), 7.08 (t, *J* = 7.2 Hz, 1H), 6.86 (m, 2H), 6.80 (d, *J* = 7.2 Hz, 2H), 4.92 (s, 2H), 3.84 (s, 6H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 168.3, 149.3, 148.5, 147.8, 143.3, 142.2, 140.6, 134.1, 129.7, 128.4, 128.0, 127.7, 127.3, 124.3, 123.8, 122.3, 122.0, 119.7, 111.1, 110.6, 109.5, 55.9, 55.8, 43.6. Anal. C<sub>26</sub>H<sub>22</sub>N<sub>2</sub>O<sub>5</sub> Calcd. C: 70.58, H: 5.01, N: 6.33. Found C: 70.76, H: 5.26, N: 6.26.

**(Z)-1-(4-(2-Fluoroethoxy)benzyl)-3-((E)-3-(4-nitrophenyl)allylidene)indolin-2-one (46a)** was prepared by method 2 with compound **41a** and 4-(2-fluoroethoxy)benzyl bromide to afford the desired product, (**46a**), as a red solid (61%), mp 187.8–188.1 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.81 (dd, *J* = 16.0 Hz, 11.6 Hz, 1H), 8.21 (d, *J* = 8.4 Hz, 2H), 7.72 (d, *J* = 8.4 Hz, 2H), 7.47 (d, *J* = 8.0 Hz, 1H), 7.31 (d, *J* = 11.6 Hz, 1H), 7.28 (d, *J* = 8.8 Hz, 2H), 7.19 (t, *J* = 7.6 Hz, 1H), 7.03 (d, *J* = 16.0 Hz, 1H), 7.01 (t, *J* = 7.6 Hz, 1H), 6.88 (d, *J* = 8.4 Hz, 2H), 6.74 (d, *J* = 7.6 Hz, 1H), 4.91 (s, 2H), 4.73 (dt, *J* = 47.2 Hz, 4.0 Hz, 2H), 4.17 (dt, *J* = 28.4 Hz, 4.0 Hz, 2H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 167.2, 157.9, 147.5, 142.7, 142.1, 139.1, 134.3, 129.6, 128.8, 128.7, 128.5, 128.0, 127.1, 124.1, 123.0, 122.1, 119.8, 114.9, 109.1, 81.8 (d, *J* = 169.7 Hz), 67.1 (d, *J* = 20.1 Hz), 42.8. Anal. C<sub>26</sub>H<sub>21</sub>FN<sub>2</sub>O<sub>4</sub> Calcd. C: 70.26, H: 4.76, N: 6.30. Found C: 70.46, H: 4.91, N: 6.25.

**(E)-1-(4-(2-Fluoroethoxy)benzyl)-3-((E)-3-(4-nitrophenyl)allylidene)indolin-2-one (46b)** was prepared by method 2 with compound **41b** and 4-(2-fluoroethoxy)benzyl bromide to afford the desired product, (**46b**), as a red solid (55%), mp 225.4–226.1 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.27 (d, *J* = 8.4 Hz, 2H), 7.78 (dd, *J* = 15.0 Hz, 12.0 Hz, 1H), 7.72 (d, *J* = 8.4 Hz, 3H), 7.53 (d, *J* = 12.0 Hz, 1H), 7.27–7.17 (m, 4H), 7.07 (t, *J* = 7.6 Hz, 1H), 6.87 (d, *J* = 8.8 Hz, 2H), 6.77 (d, *J* = 8.0 Hz, 1H), 4.92 (s, 2H), 4.73 (dt, *J* = 47.6 Hz, 4.0 Hz, 2H), 4.18 (dt, *J* = 28.0 Hz, 4.0 Hz, 2H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 168.2, 157.9, 147.8, 143.3, 142.2, 140.6, 134.1, 129.7, 128.7, 128.6, 128.0, 127.8, 127.3, 124.3, 123.8, 122.3, 122.0, 114.9, 109.4, 81.8 (d, *J* = 169.7 Hz), 67.1 (d, *J* = 20.1 Hz), 43.2. Anal. C<sub>26</sub>H<sub>21</sub>FN<sub>2</sub>O<sub>4</sub> Calcd. C: 70.26, H: 4.76, N: 6.30. Found C: 70.22, H: 4.68, N: 6.31.

**3-(3-(4-Aminophenyl)allylidene)indolin-2-one (47)** A mixture of **41a** (146 mg, 0.5 mmol) and Fe powder (280 mg, 5 mmol) in methanol (15 mL) and 37% HCl (0.5 mL) was heated to reflux for 3 h. The solid was filtered out, the filtrate was evaporated, ethyl acetate (75 mL) was added, washed with saturated Na<sub>2</sub>CO<sub>3</sub> (50 mL), water (50 mL), NaCl (50 mL), and dried over Na<sub>2</sub>SO<sub>4</sub>. After removed of the solvent, the crude product was purified by silica gel column chromatography eluting with EtOAc-MeOH (10:1, v/v) to afford 98 mg (67%) of **47** as a red solid, mp 263.3–264.4 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 10.36 and 10.31 (s, 1H), 8.14 and 7.39 (dd, *J* = 15.6 Hz, 12.0 Hz, 1H), 7.86 and 7.44 (d, *J* = 7.2 Hz, 1H), 7.46 (d, *J* = 8.8 Hz, 2H), 7.26 and 7.15 (d, *J* = 11.2 Hz, and 12.8 Hz, 1H), 7.13 and 7.08 (t, *J* = 8.0 Hz, 1H), 6.94 and 6.88 (t, *J* = 8.0 Hz, 1H), 6.80 and 6.75 (d, *J* = 7.6 Hz, 1H), 6.57 (d, *J* = 8.0 Hz, 2H), 5.79 and 5.75 (s, 2H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 169.6, 169.0, 151.7, 151.4, 147.0, 144.8, 141.8, 140.6, 137.7, 136.8, 130.6, 129.9, 128.4, 128.1, 124.7, 124.1, 123.9, 123.8, 123.2, 122.4, 122.0, 121.6, 121.2, 119.5, 119.1, 118.0, 114.4, 114.2,

109.8, 109.6. Anal. C<sub>17</sub>H<sub>14</sub>N<sub>2</sub>O.0.125H<sub>2</sub>O Calcd. C: 77.18, H: 5.43, N: 10.59. Found C: 77.34, H: 5.58, N: 10.37.

**(Z)-1-(4-(2-Bromoethoxy)benzyl)-3-((E)-3-(4-nitrophenyl)allylidene)indolin-2-one (48)** was prepared by method 2 with compound **41a** and 4-(2-bromoethoxy)benzyl bromide to afford the desired product, (**48**), as a red solid (78%), mp 192.3–193.1 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.81 (dd, *J* = 15.8 Hz, 11.6 Hz, 1H), 8.22 (d, *J* = 8.8 Hz, 2H), 7.73 (d, *J* = 8.8 Hz, 2H), 7.47 (d, *J* = 7.6 Hz, 1H), 7.32 (d, *J* = 12.0 Hz, 1H), 7.28 (d, *J* = 8.8 Hz, 2H), 7.194 (t, *J* = 7.6 Hz, 1H), 7.04 (d, *J* = 17.2 Hz, 1H), 7.02 (t, *J* = 7.4 Hz, 1H), 6.86 (d, *J* = 8.8 Hz, 2H), 6.75 (7.6 Hz, 1H), 4.91 (s, 2H), 4.25 (t, *J* = 6.2 Hz, 2H), 3.61 (t, *J* = 6.2 Hz, 2H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 167.2, 157.6, 147.5, 142.7, 142.1, 139.1, 134.3, 129.6, 128.9, 128.8, 128.5, 128.0, 127.1, 124.1, 123.0, 122.1, 119.8, 115.0, 109.1, 67.8, 42.8, 29.0.

**2-(4-(((Z)-3-((E)-3-(4-Nitrophenyl)allylidene)-2-oxoindolin-1-yl)methyl)phenoxy)ethyl methanesulfonate (49)** A solution of **48** (127 mg, 0.5 mmol) and silver methanesulfonate (500 mg, 5 mmol) in acetonitrile (15 mL) was heated to reflux for 20 h. After evaporation of the solvent, the crude product was purified by silica gel column chromatography eluting with EtOAc: MeOH (10: 1, v/v) to afford 89 mg (68%) of **49** as red solid, mp 186.4–187.1 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.80 (dd, *J* = 15.4 Hz, 11.4 Hz, 1H), 8.21 (d, *J* = 8.8 Hz, 2H), 7.72 (d, *J* = 8.4 Hz, 2H), 7.47 (d, *J* = 7.6 Hz, 1H), 7.31 (d, *J* = 11.6 Hz, 1H), 7.29 (d, *J* = 8.8 Hz, 2H), 7.19 (t, *J* = 7.6 Hz, 1H), 7.03 (d, *J* = 16.4 Hz, 1H), 7.01 (t, *J* = 7.6 Hz, 1H), 6.85 (d, *J* = 8.4 Hz, 2H), 6.73 (d, *J* = 7.6 Hz, 1H), 4.90 (s, 2H), 4.54 (t, *J* = 6.2 Hz, 2H), 4.20 (t, *J* = 6.2 Hz, 2H), 3.06 (s, 3H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 167.1, 157.5, 147.5, 142.7, 142.0, 139.2, 134.4, 129.6, 129.2, 128.9, 128.4, 128.0, 127.0, 124.1, 123.0, 122.2, 119.9, 114.8, 109.0, 67.9, 65.8, 42.8, 37.8.

**Radiosynthesis of [<sup>18</sup>F]46a**—[<sup>18</sup>F]Fluoride (50–200 mCi) in [<sup>18</sup>O]water (without treatment by trapping on quaternary methyl ammonia cartridge) was transferred into a Pyrex screw cap tube (10 mL) containing K<sub>2</sub>CO<sub>3</sub> (0.3 mg, 2.2 μmol) and Kryptofix 222 (K<sub>222</sub>) (2.2 mg, 5.9 μmol); the activity was dried by azeotropic distillation at 105 °C using MeCN (3 × 1 mL) under a gentle flow of N<sub>2</sub> gas. To the dried activity was added the mesylate precursor **49** (1.8 mg, 3.46 μmol) as solid with *tert*-amyl alcohol (300 μL) using a transfer pipette. The tube was capped and shaken in an oil bath of 105 °C to dissolve the precursor. The reaction mixture was heated at 105 °C for 10 min. After cooling to room temperature, the mixture was treated by passing through a silica column (5×20 mm); MeCN (6×0.5 mL) was used to rinse the tube and the column. The combined eluents were concentrated at 105 °C under a flow of N<sub>2</sub> to almost dryness, and HPLC mobile phase (3 mL) was added to the above residue for HPLC purification. Under the specified conditions, [<sup>18</sup>F]**46a** eluted at 40 min. The HPLC fraction was collected and diluted with water (50 mL), the diluted solution was passed through a Waters Oasis HLB plus cartridge. The cartridge was rinsed with water (10 mL), and the final product was eluted with DMF for binding studies.

## Binding Assay Methods

**Preparation of recombinant α-syn and tau protein**—Recombinant proteins were produced in *E. coli* and purified as previously described.<sup>23</sup> Briefly, α-syn was extracted

from *E. coli* using an osmotic shock method then purified by a combination of heat-precipitation and DEAE (diethylaminoethyl) ion exchange chromatography. Purified  $\alpha$ -syn protein was dialyzed overnight in 10 mM Tris-HCl pH 7.6, 50 mM NaCl, 1 mM DTT. Preparations contained greater than 95%  $\alpha$ -syn protein as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and bicinchoninic acid (BCA) protein assay (Thermo Scientific, Rockford, IL), with a typical yield of 30 mg protein per 250 ml culture.

Recombinant tau protein was produced in *E. coli*. BL21(DE3)RIL The bacterial cultures were transformed with a pRK172 bacterial expression plasmid encoding a human tau fragment containing the four microtubule binding repeats (amino acids 243–375), provided by Marc Diamond at Washington University. Cultures were inoculated and grown overnight as above for  $\alpha$ -syn protein production. Purified tau protein was prepared using the previously described method and dialyzed overnight in 100 mM sodium acetate pH 7.0.<sup>31</sup>

**Preparation of recombinant  $\alpha$ -syn fibrils**—Purified recombinant  $\alpha$ -syn monomer (2 mg/ml) was incubated in 20 mM Tris-HCl, pH 8.0, 100 mM NaCl for 72 h at 37 °C with shaking at 1000 rpm in an Eppendorf Thermomixer as previously described.<sup>23</sup> Fibril concentration was determined by BCA protein assay to determine monomer concentration in the supernatant obtained by centrifuging the fibril reaction mix at 18,000 g for 15 min to separate fibrils from monomer. The measured decrease in  $\alpha$ -syn monomer concentration was used to determine the concentration of fibrils in the 72 h fibril reaction mixture.

**Preparation of A $\beta$ <sub>1-42</sub> fibrils**—Synthetic A $\beta$ <sub>1-42</sub> peptide (1 mg) (Bachem, Torrance, CA) was first dissolved in 50  $\mu$ l DMSO. An additional 925  $\mu$ l of ultrapure water was added. Finally, 25  $\mu$ l 1M Tris-HCl pH 7.6 was added to bring the final peptide concentration to 222  $\mu$ M (1 mg/ml). The dissolved peptide was incubated for 30 h at 37 °C with shaking at 1000 rpm in an Eppendorf Thermomixer. Fibril formation was confirmed by Thio-T fluorescence. To determine the concentration of fibrils, the fibril reaction mix was centrifuged at 18,000  $\times$  g for 15 min to separate fibrils from monomer. The concentration of A $\beta$  monomer in the supernatant was determined in a BCA protein assay using a BSA standard curve that contained DMSO at a percentage equivalent to the samples.

**Preparation of recombinant tau fibrils**—Purified recombinant tau monomer (300  $\mu$ g/ml) was incubated in 20 mM Tris-HCl pH 8.0, 100 mM NaCl, 25  $\mu$ M low molecular weight heparin, 0.5 mM DTT for 48 h at 37 °C with shaking at 1000 rpm in an Eppendorf Thermomixer. To determine the concentration of fibrils, the fibril reaction mix was centrifuged at 18,000  $\times$  g for 15 min to separate fibrils from monomer. The concentration of tau monomer in the supernatant was determined in a BCA protein assay along with a BSA standard curve. The measured decrease in monomer concentration was used to determine the concentration of tau fibrils in the 48 h fibril reaction mixture.

**Preparation of  $\alpha$ -syn, A $\beta$ <sub>1-42</sub>, and tau fibrils for binding and competition assays**—The prepared fibril mixture was centrifuged at 18,000  $\times$  g for 15 min to prepare fibrils for binding assays. The supernatant was discarded and the fibril pellet was

resuspended in 30 mM Tris-HCl pH 7.4, 0.1% BSA to achieve the desired concentration of fibrils for use in the assay.

### ***In vitro* saturation binding studies to determine binding affinity of Thio-T for fibrils**

A fixed concentration (1  $\mu\text{M}$ ) of  $\alpha$ -syn, A $\beta$ , or tau fibrils were incubated for 2 h at 37  $^{\circ}\text{C}$  with increasing concentrations of Thio-T in 30 mM Tris-HCl pH 7.4, 0.1% BSA in a reaction volume of 150  $\mu\text{L}$ . Nonspecific binding was determined in a duplicate set of binding reactions containing the same concentration of Thio-T with no fibrils. Fluorescence was determined in a Biotek plate reader using a 440/30 excitation filter and a 485/20 emission filter. All data points were performed in triplicate. The dissociation constant ( $K_d$ ) and the maximal number of binding sites ( $B_{\text{max}}$ ) values were determined by fitting the data to the equation  $Y=B_{\text{max}}*X/(X+K_d)$  by nonlinear regression using Graphpad Prism software (version 4.0).

### **Thio-T competition studies**

Competition assays used a fixed concentration of  $\alpha$ -syn, A $\beta$ , or tau fibrils (1  $\mu\text{M}$ ), consisting of 3  $\mu\text{M}$ , 50 nM and 4  $\mu\text{M}$  of Thio-T respectively. The competitor reaction was diluted in 30 mM Tris-HCl pH 7.4, 0.1% BSA and added to the reactions in varying concentrations. To optimize the determination of selectivity, assays for all three fibril species were set up in parallel using a common set of intermediate compound dilutions for each analog. Reactions were incubated at room temperature for 1.5 h before quantifying bound ligand as described above for the saturation binding assay. Fluorescence was determined in a Biotek plate reader using a 440/30 excitation filter and a 485/20 emission filter. All data points were performed in triplicate. Nonspecific fluorescence was measured in parallel reactions containing Thio-T plus each concentration of competitor but no fibrils, and these measurements were subtracted from the reactions with fibrils to yield fibril-specific fluorescence. Data were analyzed using Graphpad Prism software (version 4.0) to obtain  $\text{EC}_{50}$  values by fitting the data to the equation  $Y=\text{bottom}+(\text{top}-\text{bottom})/(1+10^{(x-\log\text{EC}_{50})})$ .  $K_i$  values were calculated from  $\text{EC}_{50}$  values using the equation  $K_i=\text{EC}_{50}/(1+[\text{radioligand}]/K_d)$ .

### ***In vitro* saturation binding studies of [ $^{18}\text{F}$ ]46a**

$\alpha$ -Syn, A $\beta$ , or tau fibrils (1  $\mu\text{M}$ ) were incubated for 1 h at 37  $^{\circ}\text{C}$  with increasing concentrations of [ $^{18}\text{F}$ ]46a in 30 mM Tris-HCl pH 7.4, 0.1% BSA in a reaction volume of 150  $\mu\text{L}$ . A fixed ratio of labeled: unlabeled 46a was used for all radioligand concentrations. The exact labeled: unlabeled 46a ratio was measured in each experiment by counting a 2  $\mu\text{L}$  sample of the radioligand preparation in a scintillation counter. Nonspecific binding was determined in the parallel binding reactions containing 20 as the competitor or in reactions containing no fibrils, both of which yielded similar nonspecific binding measurements. Bound and free radioligand were separated by vacuum filtration through 1.0  $\mu\text{m}$  glass fiber filters in 96-well filter plates (Millipore), followed by three 200  $\mu\text{L}$  washes with ice-cold assay buffer. Filters containing the bound ligand were mixed with 150  $\mu\text{L}$  of Optiphase Supermix scintillation cocktail (PerkinElmer) and counted immediately. All data points were performed in triplicate. The dissociation constant ( $K_d$ ) and the maximal number of binding

sites ( $B_{\max}$ ) values were determined by fitting the data to the equation  $Y = B_{\max} * X / (X + K_d)$  by nonlinear regression using Graphpad Prism software (version 4.0).

### Human tissue acquisition

Post-mortem midbrain tissue from DLB and PD cases was obtained from the Harvard Brain Tissue Resource Center at McLean Hospital. Paraffin-embedded formalin-fixed tissue from the temporal lobe of AD cases was obtained through the Massachusetts Alzheimer's Disease Research Center Brain Bank. The human brain tissue provided was neuropathologically defined as DLB tissue using standard criteria.

### Labeling of synuclein pathology in human DLB and PD cases

Blocks of midbrain tissue from human PD/DLB cases were fixed with 4% paraformaldehyde and stored at  $-80^{\circ}\text{C}$ . In preparation for immunohistochemistry, the tissue block was deparaffinized with xylene and rehydrated. Midbrain tissue was sliced into 40  $\mu\text{m}$  thick sections with a freezing sledge Leica SM 2000R microtome. Free-floating tissue was permeabilized with 0.5% Triton-X 100, then incubated with normal goat serum (NGS) at room temperature for 1 h with shaking. Primary H3C antibody (1:5000 in 1.5% NGS) to  $\alpha$ -syn was incubated overnight at  $4^{\circ}\text{C}$  with shaking, and then washed with  $1 \times \text{PBS}$  three times. The tissue was then incubated for 2 h at room temperature with a fluorescent secondary antibody labeled with Alexa Fluor 488 (1:200 in PBS). Tissue was then treated with the compound to be tested. Each tissue section was incubated at room temperature for 30 min with 5  $\mu\text{M}$  of test compound dissolved in PBS. The tissue was washed with  $1 \times \text{PBS}$  three times, and then mounted onto Adhesion SuperFrost Plus slide and cover slipped. The tissue was imaged with an Olympus BX51 upright microscope (Olympus America Inc., Melville, NY) using standard excitation/emission filters for Alexa Fluor 488 and Cy5.

### Staining of plaques in human tissue from AD cases

Paraffin-embedded paraformaldehyde (4%) fixed sections of temporal lobe of neuropathologically verified human AD cases were deparaffinized. Tissue sections were stained with 0.05% Thioflavin S Thio-T (Sigma, St. Louis, MO) in 50% ethanol for 5 min to visualize senile plaques. Tissue was then incubated with 5  $\mu\text{M}$  of compound in PBS for 20 min at room temperature. Slides were rinsed in PBS, coverslipped, and then imaged on an Olympus BX51 microscope using a 20x Plan Neofluar objective (NA=0.7) using standard excitation/emission filters for AlexaFluor488 and Cy5.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## ABBREVIATIONS USED

<b>α-syn</b>	alpha synuclein
<b>BCA assay</b>	bicinchoninic acid assay
<b>DEAE ion exchange</b>	diethylaminoethyl ion exchange
<b>DLB</b>	dementia with Lewy bodies
<b>LB</b>	Lewy body
<b>LN</b>	Lewy neurite
<b>MSA</b>	multiple system atrophy
<b>NFT</b>	neurofibrillary tangles
<b>QC</b>	quality control
<b>Thio-T</b>	thioflavin-T

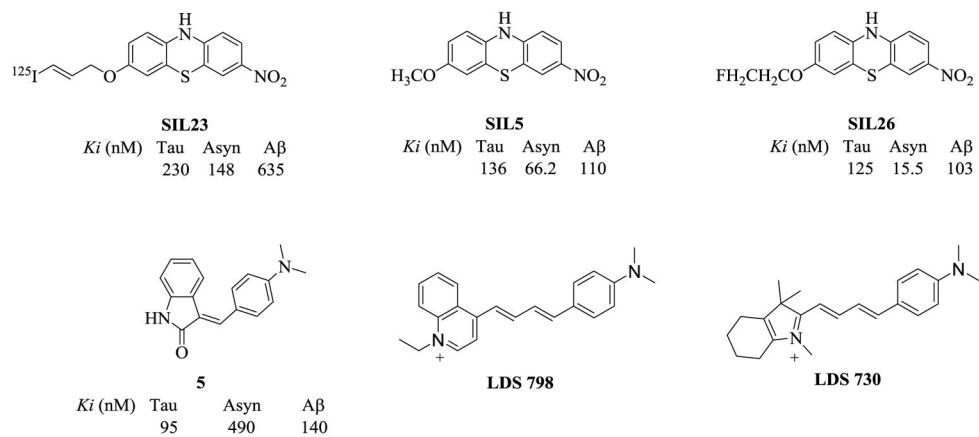
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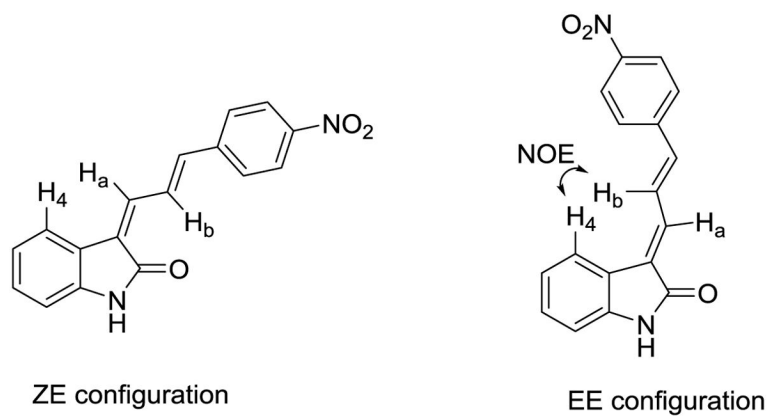


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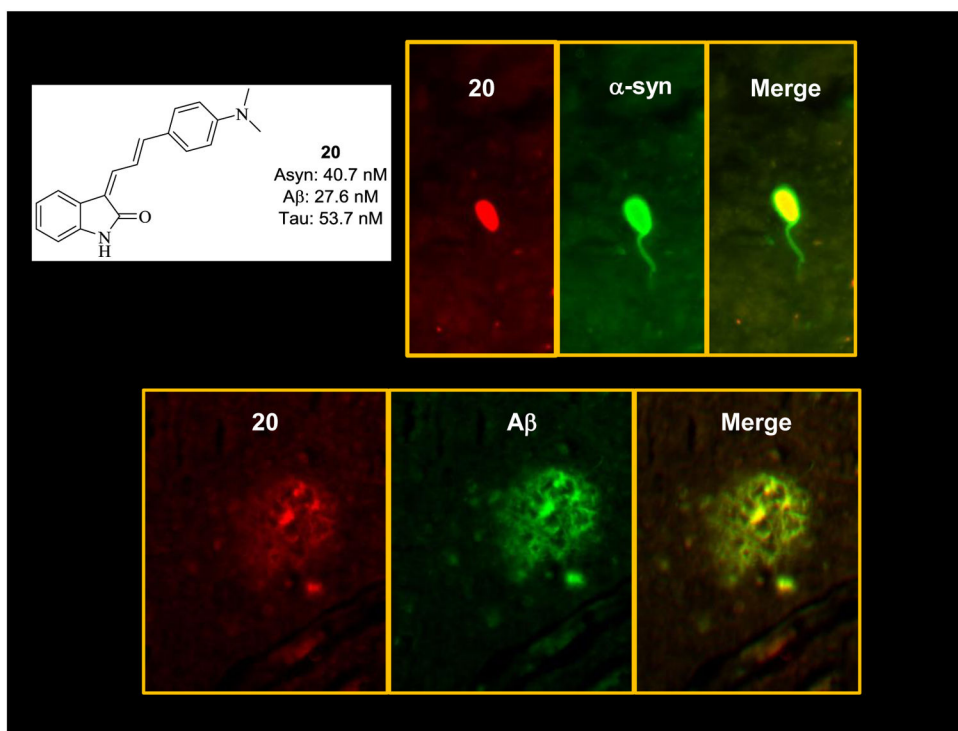
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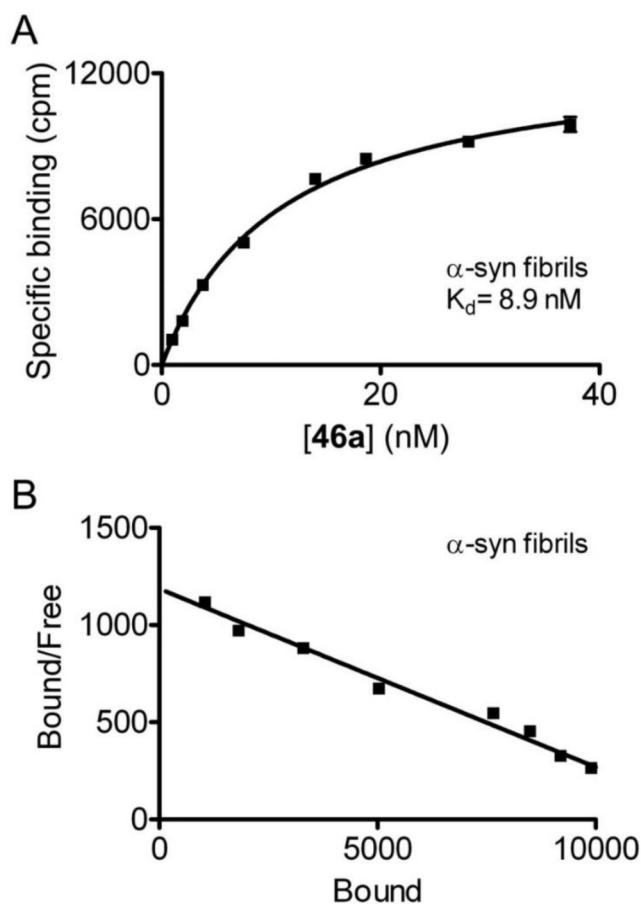
**Figure 1.**  
Reported ligands for tau, α-synuclein, and Aβ.



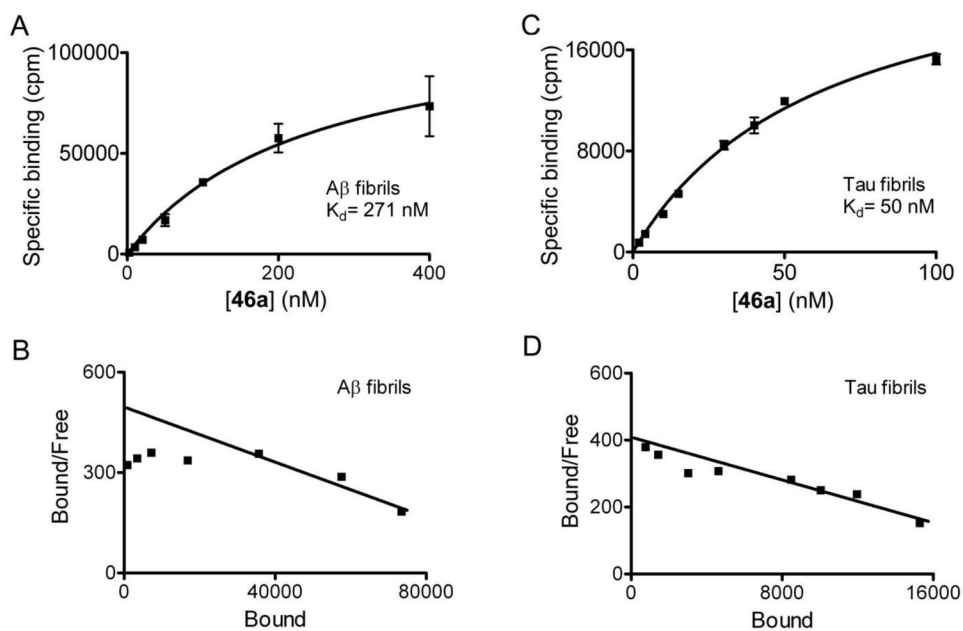
**Figure 2.**  
Nuclear Overhauser effect in *E,E* configuration of compound **41**.



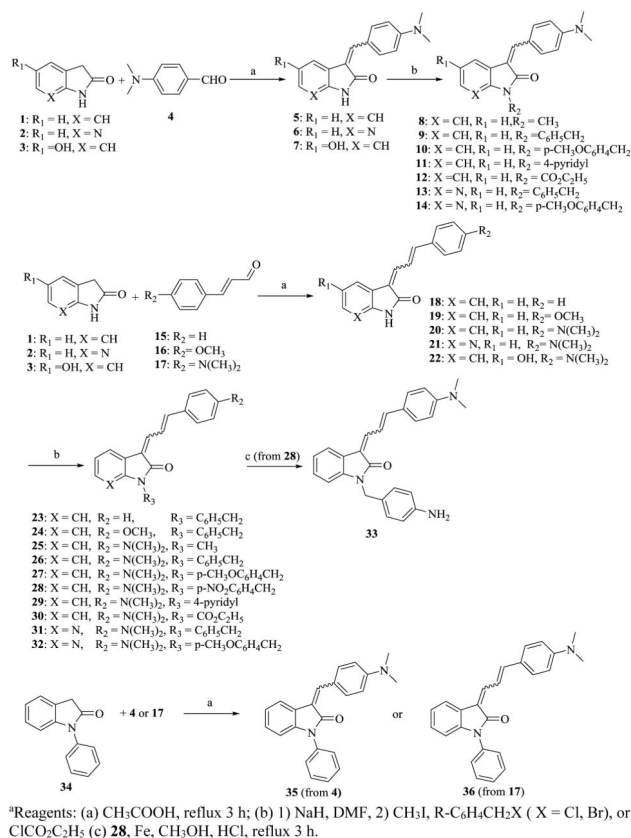
**Figure 3.** Fluorescent microscopy studies of compound 20 in postmortem samples of PD/DLB and AD brain. Note the high labeling of both Lewy Bodies and Aβ plaques, which was confirmed with appropriate immunohistochemistry labeling.



**Figure 4.  $[^{18}\text{F}]46\text{a}$  binds recombinant  $\alpha$ -syn fibrils with an average  $K_d$  of 8.9 nM**  
Fibrils prepared from recombinant  $\alpha$ -syn were incubated with increasing concentrations of  $[^{18}\text{F}]46\text{a}$ . Nonspecific binding was determined in parallel reactions utilizing **20** as the competitor or in reactions containing no fibrils. A representative plot of specific binding versus  $[^{18}\text{F}]46\text{a}$  concentration is shown in A. Data points represent mean  $\pm$  s.d. ( $n = 3$ ). The data were analyzed by curve fitting to a one-site binding model using nonlinear regression.  $K_d$  values were determined by fitting the data to the equation  $Y = B_{\text{max}} * X / (X + K_d)$ . Scatchard analysis of binding is shown in B. Similar results were obtained in more than three independent experiments.



**Figure 5. Radioligand binding studies demonstrate that [ $^{18}\text{F}$ ]46a binds with lower affinity to synthetic A $\beta_{1-42}$  fibrils or recombinant tau fibrils compared to  $\alpha$ -syn fibrils**  
Binding affinities of [ $^{18}\text{F}$ ]46a to A $\beta$  (A) and tau (C) fibrils were determined in saturation binding studies. The average  $K_d$  values of A $\beta$  and tau fibrils were 271 nM and 50 nM, respectively. Data points represent mean  $\pm$  s.d. ( $n = 3$ ). Scatchard plots of binding values are shown in (B) for A $\beta$  and (D) for tau. Similar results were obtained in two independent experiments.

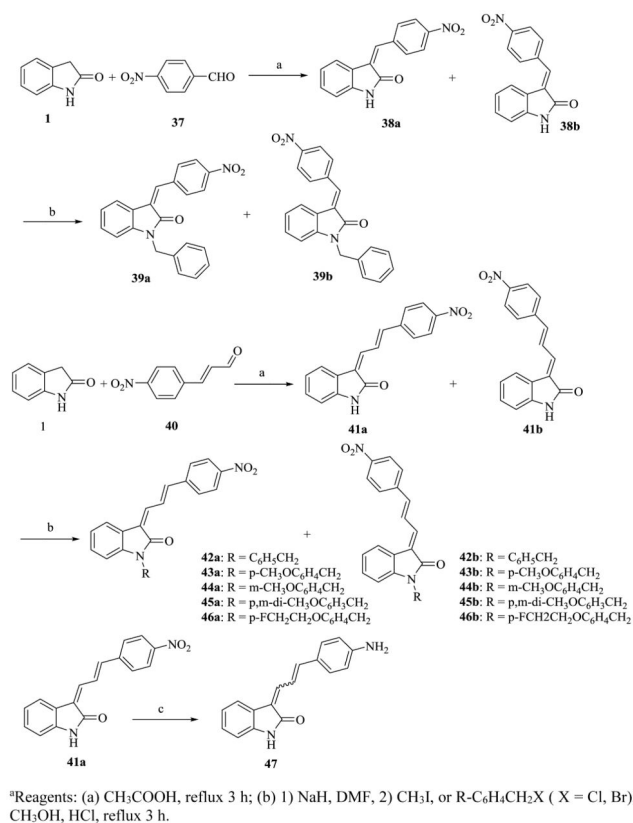


**Scheme 1.**

Synthesis of 3-(Benzylidene)indolin-2-one Derivatives<sup>a</sup>

<sup>a</sup>Reagents: (a) CH<sub>3</sub>COOH, reflux 3 h; (b) 1) NaH, DMF, 2) CH<sub>3</sub>I, R-C<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>X (X = Cl, Br), or ClCO<sub>2</sub>C<sub>2</sub>H<sub>5</sub> (c) **28**, Fe, CH<sub>3</sub>OH, HCl, reflux 3 h.

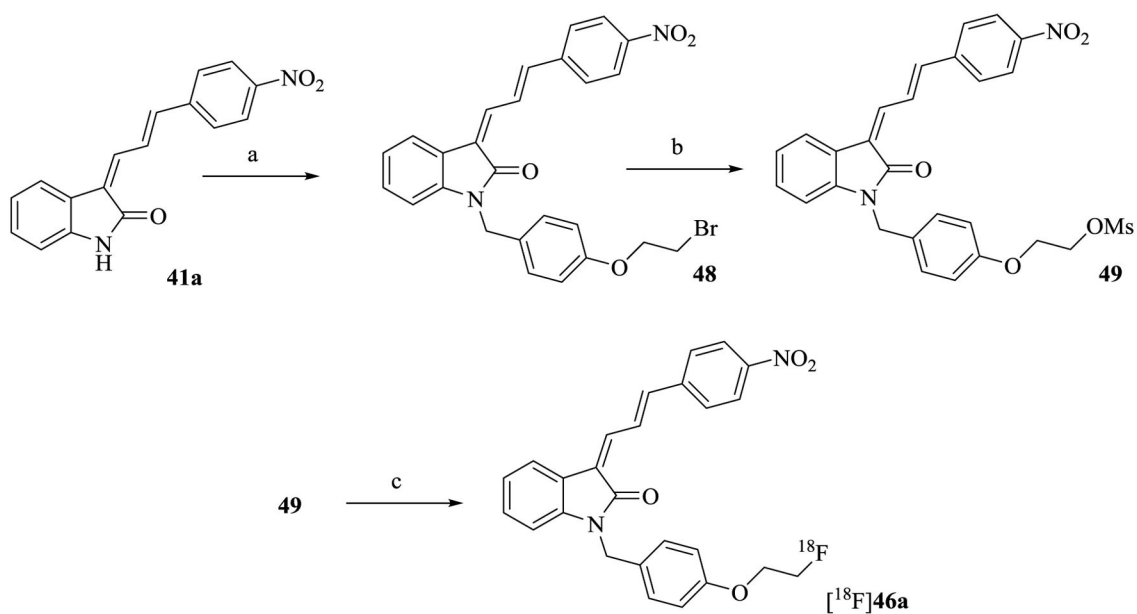




### Scheme 2.

#### Synthesis of 3-(Benzylidene)indolin-2-one Derivatives<sup>a</sup>

<sup>a</sup>Reagents: (a) CH<sub>3</sub>COOH, reflux 3 h; (b) 1) NaH, DMF, 2) CH<sub>3</sub>I, or R-C<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>X (X = Cl, Br); (c) Fe, CH<sub>3</sub>OH, HCl, reflux 3 h.



Reagents: (a) 1) NaH, DMF, 2) BrCH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>OCH<sub>2</sub>CH<sub>2</sub>Br; (b) AgOMs, CH<sub>3</sub>CN; (c) [<sup>18</sup>F]KF, K<sub>2</sub>CO<sub>3</sub>, K<sub>222</sub>, *tert*-amyl alcohol.

**Scheme 3.**

Synthesis of Labeling Precursor **48** and Radiosynthesis of [<sup>18</sup>F]**46a**

Reagents: (a) 1) NaH, DMF, 2) BrCH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>OCH<sub>2</sub>CH<sub>2</sub>Br; (b) AgOMs, CH<sub>3</sub>CN; (c) [<sup>18</sup>F]KF, K<sub>2</sub>CO<sub>3</sub>, K<sub>222</sub>, *tert*-amyl alcohol.

**Table 1**

$K_i$  Values of 3-(Benzylidene)indolin-2-one Derivatives for  $\alpha$ -Syn, A $\beta$  and Tau

Compd	<i>E</i> configuration					<i>Z</i> configuration				
	R <sub>1</sub>	X	R	<i>E</i> : <i>Z</i>	$\alpha$ -Syn (nM)	A $\beta$ (nM)	Tau (nM)	Log $P^a$		
5	H	CH	H	75:25	84.4±13.5	91.6±18.5	261.5±4.9	3.0		
6	H	N	H	26:74	124.3±46.1	40.0±22.3	191.3±39.5	2.4		
7	OH	CH	H	67:33	407.1±98.6	94.8±22.4	212.4±110.9	2.6		
8	H	CH	CH <sub>3</sub>	84:16	507.6±10.0	155.1±8.8	617.6±182.0	3.2		
9	H	CH	PhCH <sub>2</sub>	83:17	144.4±53.2	260.4±78.5	797.8±70.2	4.9		
10	H	CH	<i>p</i> -CH <sub>3</sub> OC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	52:48	114.5±53.9	251.5±52.0	431.7±123.3	4.8		
11	H	CH	<i>p</i> -PyridylCH <sub>2</sub>	77:23	149.8±45.7	87.1±34.1	364.0±121.1	3.6		
12	H	CH	CO <sub>2</sub> C <sub>2</sub> H <sub>5</sub>	62:38	342.6±114.4	139.9±70.1	1116.2±69.1	3.8		
13	H	N	PhCH <sub>2</sub>	66:34	350.2±201.3	190.2±84.8	858.8±172.0	4.3		
14	H	N	<i>p</i> -CH <sub>3</sub> OC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	69:31	79.5±22.1	113.3±2.6	853.5±158.6	4.2		
35	H	CH	Ph	94:6	290.2±120.2	179.2±71.3	789.8±153.7	4.9		

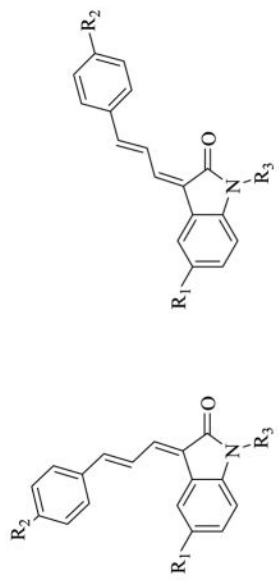
<sup>a</sup> Calculated by ChemBioDraw Ultra 13.0.

**Table 2**

$K_i$  Values of 3-(Benzylidene)indolin-2-one Derivatives for  $\alpha$ -Syn, A $\beta$  and Tau

#	<i>E, E</i> configuration					<i>Z, E</i> configuration				
	R <sub>1</sub>	X	R <sub>2</sub>	R <sub>3</sub>	<i>EE:ZE</i>	$\alpha$ -Syn (nM)	A $\beta$ (nM)	Tau (nM)	Log <i>p</i> <sup>d</sup>	
18	H	CH	H	H	77:23	206.7±8.7	266.8±136.5	522.1±152.6	3.2	
19	H	CH	OCH <sub>3</sub>	H	78:22	61.1±9.6	125.8±42.6	169.0±22.3	3.1	
20	H	CH	N(CH <sub>3</sub> ) <sub>2</sub>	H	41:59	40.7±8.7	27.6±4.8	53.7±9.7	3.5	
21	H	N	N(CH <sub>3</sub> ) <sub>2</sub>	H	38:62	11.5±2.0	15.3±5.5	35.1±12.3	2.9	
22	OH	CH	N(CH <sub>3</sub> ) <sub>2</sub>	H	47:53	54.0±17.9	37.1±13.6	48.0±17.0	3.1	
23	H	CH	H	CH <sub>2</sub> Ph	0:100	226.9±64.0	703.3±214.7	682.4±120.2	5.2	
24	H	CH	OCH <sub>3</sub>	CH <sub>2</sub> Ph	84:16	72.5±22.6	741.0±254.6	717.6±295.3	5.0	
25	H	CH	N(CH <sub>3</sub> ) <sub>2</sub>	CH <sub>3</sub>	63:37	63.7±29.6	56.0±14.8	229.1±121.4	3.7	
26	H	CH	N(CH <sub>3</sub> ) <sub>2</sub>	CH <sub>2</sub> Ph	65:35	40.9±25.2	357.9±161.7	374.0±209.3	5.5	
27	H	CH	N(CH <sub>3</sub> ) <sub>2</sub>	<i>p</i> -CH <sub>3</sub> OC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	52:48	54.6±11.4	129.6±41.4	239.7±157.6	5.3	
28	H	CH	N(CH <sub>3</sub> ) <sub>2</sub>	<i>p</i> -NO <sub>2</sub> C <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	65:35	4.2±0.8	92.4±21.9	90.0±48.8	5.5	
29	H	CH	N(CH <sub>3</sub> ) <sub>2</sub>	<i>p</i> -PyridylCH <sub>2</sub>	50:50	92.0±11.2	45.9±19.4	188.0±68.0	4.1	
30	H	CH	N(CH <sub>3</sub> ) <sub>2</sub>	CO <sub>2</sub> C <sub>2</sub> H <sub>5</sub>	51:49	45.3±9.0	84.0±35.0	263.2±110.5	4.4	
31	H	N	N(CH <sub>3</sub> ) <sub>2</sub>	CH <sub>2</sub> Ph	80:20	68.3±31.2	85.1±23.0	257.0±116.7	4.8	
32	H	N	N(CH <sub>3</sub> ) <sub>2</sub>	<i>p</i> -CH <sub>3</sub> OC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	87:13	29.8±6.6	107.0±18.9	204.3±26.7	4.7	
33	H	CH	N(CH <sub>3</sub> ) <sub>2</sub>	<i>p</i> -NH <sub>2</sub> C <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	55:45	43.6±20.0	98.9±12.1	131.1±51.5	4.7	
36	H	CH	N(CH <sub>3</sub> ) <sub>2</sub>	Ph	29:71	68.1±33.0	286.8±142.2	465.1±94.6	5.4	

		<i>E, E</i> configuration				<i>Z, E</i> configuration			
#	R <sub>1</sub>	X	R <sub>2</sub>	R <sub>3</sub>	EE:ZE	α-Syn (nM)	Aβ (nM)	Tau (nM)	Log p <sup>a</sup>
47	H	H	NH <sub>2</sub>	H		44.8±15.6	50.8±18.5	38.3±28.7	2.4



<sup>a</sup> Calculated by ChemBioDraw Ultra 13.0.

**Table 3**

$K_i$  Values of 3-(Benzylidene)indolin-2-one Derivatives for  $\alpha$ -Syn, A $\beta$  and Tau

Compd	<i>E</i> configuration		<i>Z</i> configuration		Log $P^d$	
	R	E or Z	$\alpha$ -Syn (nM)	A $\beta$ (nM)		Tau (nM)
<b>38a</b>	H	Z	347.2 $\pm$ 88.9	325.2 $\pm$ 73.5	362.5 $\pm$ 21.4	2.7
<b>38b</b>	H	E	621.8 $\pm$ 149.0	439.2 $\pm$ 162.8	528.5 $\pm$ 77.0	2.7
<b>39a</b>	PhCH <sub>2</sub>	Z	482.9 $\pm$ 139.8	459.5 $\pm$ 49.9	1756.7 $\pm$ 104.5	3.8
<b>39b</b>	PhCH <sub>2</sub>	E	959.3 $\pm$ 376.5	694.7 $\pm$ 72.0	1410.0 $\pm$ 298.4	3.8

Compd	<i>E, E</i> configuration		<i>Z, E</i> configuration		Log $P^d$	
	R	<i>E, E</i> or <i>Z, E</i>	$\alpha$ -Syn (nM)	A $\beta$ (nM)		Tau (nM)
<b>41a</b>	H	<i>Z, E</i>	14.6 $\pm$ 7.5	36.2 $\pm$ 12.9	27.1 $\pm$ 3.7	3.4
<b>41b</b>	H	<i>E, E</i>	211.4 $\pm$ 22.7	60.0 $\pm$ 19.1	208.7 $\pm$ 110.3	3.4

Compd	R	E configuration		Z configuration		Log P <sup>a</sup>
		E or Z	α-Syn (nM)	Aβ (nM)	Tau (nM)	
42a	C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub>	Z,E	25.0±12.7	214.2±52.1	121.5±18.8	4.5
42b	C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub>	E,E	192.7±57.4	235.4±143.0	590.5±74.7	4.5
43a	<i>p</i> -CH <sub>3</sub> OC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	Z,E	12.9±4.9	130.8±64.4	72.4±24.1	4.7
43b	<i>p</i> -CH <sub>3</sub> OC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	E,E	81.9±31.3	138.5±40.2	318.1±104.3	4.7
44a	<i>m</i> -CH <sub>3</sub> OC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	Z,E	3.8±0.6	109.7±0.4	228.6±113.7	4.7
44b	<i>m</i> -CH <sub>3</sub> OC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	E,E	64.7±14.6	264.8±64.4	354.6±211.7	4.7
45a	<i>p,m</i> -di-CH <sub>3</sub> OC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	Z,E	3.5±0.2	73.6±27.3	151.7±64.7	4.8
45b	<i>p,m</i> -di-CH <sub>3</sub> OC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	E,E	53.3±15.0	129.5±16.0	630.3±250.7	4.8
46a	<i>p</i> -FCH <sub>2</sub> CH <sub>2</sub> OC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	Z,E	2.1±0.3	142.4±36.9	80.1±12.0	4.7 <sup>b</sup>
46b	<i>p</i> -FCH <sub>2</sub> CH <sub>2</sub> OC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub>	E,E	76.1±41.2	125.3±40.1	455.7±66.1	4.7

<sup>a</sup> Calculated by ChemBioDraw Ultra 13.0.

<sup>b</sup> Measured: 4.18 ± 0.02 (n = 3).