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# Cu-mediated 1,3-dipolar cycloaddition of azomethine ylides with dipolarophiles: a faster access to spirooxindoles of potential pharmacological interest

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ABSTRACT

CuI facilitated three-component reaction of isatin derivatives, L-proline and terminal alkynes containing an amide or ester functional group. The multi-component reaction (MCR) afforded a faster and practical synthesis of spirooxindole derivatives. A range of novel spirooxindoles were synthesized by using this straightforward and one-pot efficient methodology. A representative compound showed significant inhibition of PDE4B enzyme in vitro and good interactions with this protein in silico.



The unique structural features of spiro heterocycles and the presence of spiro linkage as a basic skeleton in several natural products have increased their importance to a great extent. The naturally occurring spiro heterocycles for example spirooxindoles (with five membered nitrogen containing ring) present in horsfiline[1] (analgesic activity), spirotryprostatins **A**[2] (inhibitors of mammalian cell cycle at G2/M phase), and elacomine[3] showed interesting biological properties (Fig. 1). Mitraphylline (Fig. 1), a natural compound, containing the spirooxindole framework possesses anti-tumor activity against human brain cancer cell lines and malignant glioma GAMG.[4] Rhynchophylline, another natural product is used as antipyretic, anti-hypertensive, and anti convulsant medications for the treatment of headache, vertigo, and epilepsy.[5] The oxindole class of compounds attracted our particular attention due to their impressive PDE4 inhibitory properties reported earlier. For example, the PDE4 inhibitor **A** (Fig. 2) significantly reduced antigen-induced bronchoconstriction in animal models and in asthmatic patients.[6] This prompted us to synthesize and assess the PDE4 inhibitory properties of novel spiro derivatives of oxindoles **B** (Fig. 2) possessing an amide/ester at C-2′. We anticipated that this group may facilitate the interactions of **B** with PDE4B. To the best of our knowledge PDE4 inhibitory properties of **B** have not been reported in the literature earlier.

The most common synthetic routes reported so far for the synthesis of spiro cyclic compounds include alkylation methods, rearrangement based approaches, ring closure of geminally substituted compounds, radical cyclizations, organometallic processes (metals include Rh, Pd, Cu, Ir etc.,), organocatalytic approaches, cleavage of bridged ring systems, or cycloaddition reactions.[7] Recently, the

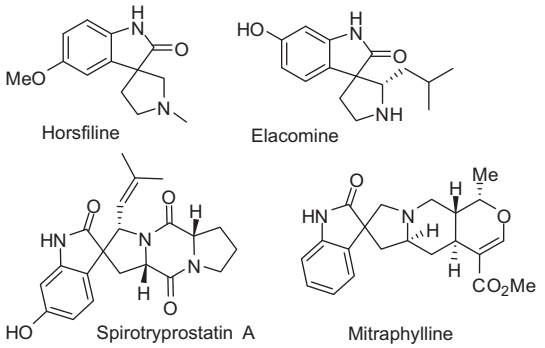


**Figure 1.** Structure of natural products containing spirooxindole framework.

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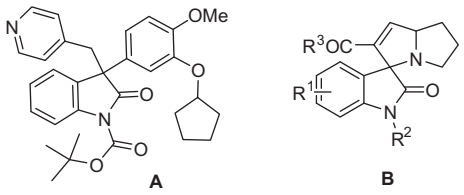
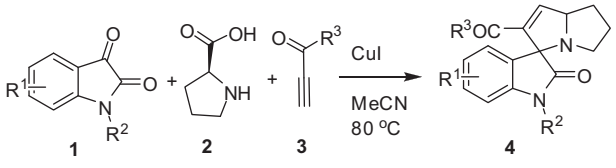



**Figure 2.** Known PDE4 inhibitor **A** and our target spirooxindoles **B**.

multi component reaction or MCR strategy has been successfully adopted by many researchers for the synthesis of spirooxindoles. This reaction involved 1,3-dipolar cycloaddition of azomethine ylide generated in situ with different dipolarophiles in a single pot. The azomethine ylides were efficiently generated by the reaction of isatin with L-proline. Chen et al. have reported the synthesis of the spirooxindole pyrrolidine ring by three-component coupling of isatin, L-proline, and 1,4-naphthoquinone in methanol under ultrasound.[8] Kang and co-workers reported a similar type of coupling in 1,4-dioxane by varying the dipolarophile for the synthesis of spiropyrrolidine oxindoles.[9] They used dimethyl maleate instead of 1,4-naphthoquinone. The synthesis of spiropyrrolidine was also reported by using terminal and internal alkynes as dipolarophiles in acetonitrile by Pardasani et al.[10] that required a relatively longer reaction time for example 20 h. Moreover, the use of only one terminal alkyne that is phenyl acetylene was examined in their study. Herein, we report a faster and efficient method leading to our target spiropyrrolidine oxindoles **B** or **4** (Scheme 1) using isatin (**1**), L-proline (**2**), and terminal alkynes (**3**) containing ester or amide substituents in the presence of catalytic CuI in acetonitrile. Notably the use of alkyne **3** in a similar MCR is not common in the literature.

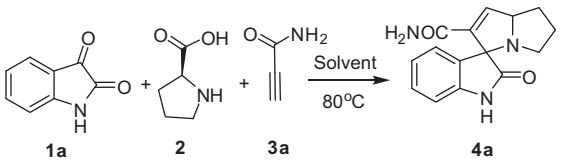
Initially, the coupling reaction was performed by heating a mixture of isatin (**1a**), L-proline (**2**), and propiolamide (**3a**) in DMF at 80 °C. After 24 h, the spiro compound **4a** was obtained in 60% yield (Table 1, entry 1). To improve the product yield the reaction was carried out in different solvents like ethanol (Table 1, entry 2) and acetonitrile (Table 1, entry 3). In case of ethanol, the yield decreased to 55%, but improved in acetonitrile (70%). However, the duration of the reaction was not satisfactory for a quick access to the compound library related to **4a**. After screening a range of catalysts a major improvement in yield (92%) as well as reaction time (2 h) was observed when catalytic amount of CuI was added to the reaction mixture in acetonitrile (Table 1, entry 4). While the reaction proceeded in the presence of other copper salts for example CuBr and CuCl, the yield of product **4a** was poor (Table 1, entries 5 and 6) in these cases. The MCR was found to be less effective in an aqueous media for example in 1:1 *i*-PrOH/$H_2O$ when **4a** was obtained only in 32% yield (Table 1, entry 7). Thus based on the observation that CuI in acetonitrile decreased the reaction time from 20 to 2 h, the combination of CuI and acetonitrile was used for our further study.

The scope and generality of the reaction were further tested by performing the reactions using a range of isatin derivatives (**1**) and terminal alkynes (**3**) containing various carbonyl functionalities (Table 2).[11] Substituents such as Br, F, $NO_2$, and aryl group on the isatin ring were well tolerated. The terminal alkynes employed




**Scheme 1.** Cu-mediated faster synthesis of spirooxindoles **4**.

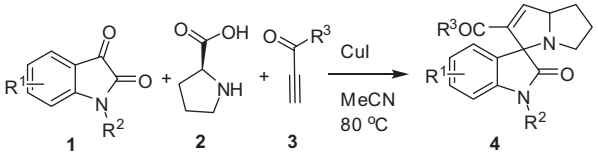
**Table 1**
Effect of reaction conditions on three-component reaction of **1a**, **2**, and **3a**[a]




| Entry | Solvent | Catalyst | Time (h) | Temp (°C) | Yield[b] (%) |
|---|---|---|---|---|---|
| 1 | DMF | No catalyst | 24 | 80 | 60 |
| 2 | EtOH | No catalyst | 24 | 80 | 55 |
| 3 | $CH_3CN$ | No catalyst | 24 | 80 | 70 |
| 4 | $CH_3CN$ | CuI | 2 | 80 | 92 |
| 5 | $CH_3CN$ | CuBr | 8 | 80 | 78 |
| 6 | $CH_3CN$ | CuCl | 12 | 80 | 75 |
| 7 | *i*-PrOH:$H_2O$ (1:1) | CuI | 6 | 80 | 32 |

[a] Reactions were carried out using **1a** (1.0 mmol), **2** (1.0 mmol), and propiolamide (**3a**) (1.0 mmol) in a solvent (15 mL) under nitrogen.
[b] Isolated yield.

**Table 2**
Synthesis of spirooxindoles **4** via Cu-mediated MCR of **1**, **2**, and **3**[a]




| Entry | Isatin derivative ($R^1$, $R^2$=) **1** | Alkyne ($R^3$=) **3** | Time (h) | Product **4** | Yield[b] (%) |
|---|---|---|---|---|---|
| 1 | H, H **1a** | $NH_2$ **3a** | 2.0 | **4a** | 92 |
| 2 | **1a** | NHMe **3b** | 2.0 | **4b** | 90 |
| 3 | **1a** | NHEt **3c** | 2.0 | **4c** | 91 |
| 4 | 5-Br, H **1b** | **3a** | 2.5 | **4d** | 85 |
| 5 | **1b** | **3b** | 2.5 | **4e** | 82 |
| 6 | **1b** | **3c** | 2.0 | **4f** | 85 |
| 7 | 5,7-Di-$NO_2$, H **1c** | **3a** | 2.0 | **4g** | 90 |
| 8 | 5-F, H **1d** | **3a** | 2.5 | **4h** | 80 |
| 9 | **1a** | OEt **3d** | 3.0 | **4i** | 81 |
| 10 | **1b** | **3d** | 2.5 | **4j** | 80 |
| 11 | **1c** | **3d** | 2.5 | **4k** | 85 |
| 12 | H, $-CH_2C_6H_4Cl$-*m* **1e** | **3a** | 3.0 | **4l** | 75 |

[a] Reactions were carried out using isatin (**1**) (1.0 mmol), proline (**2**) (1.0 mmol), alkyne (**3**) (1.0 mmol), and CuI (0.01 mmol) in acetonitrile (15 mL) at 80 °C under nitrogen.
[b] Isolated yield.

contained an N-unsubstituted or substituted amide (e.g. $NH_2$, NHMe, or NHEt) or an ester moiety. The reaction proceeded well in all these cases affording desired spirooxindoles **4** in good to excellent yield.

All the spirooxindole derivatives (**4a–l**) synthesized were characterized by their $^1H$ and $^{13}C$ NMR, IR, and mass spectral data. The sharp peak at ~3300 $cm^{-1}$ in IR, a singlet at 10.0 ppm in $^1H$ NMR correspond to the spirooxindole NH group. Moreover, a sharp IR absorption at ~1640 $cm^{-1}$ and appearance of a quaternary carbon signal at ~178.0 ppm in the $^{13}C$ NMR indicated the presence of the C=O group. While a sharp IR absorption at ~1735 $cm^{-1}$ and a signal at ~161.0 ppm in the $^{13}C$ NMR indicated the presence of the ester carbonyl group (e.g. **4i–k**), the amide derivatives (**4a–h**)

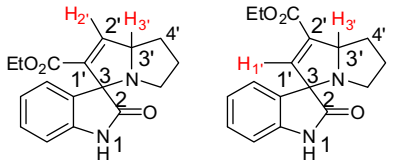



**Figure 3.** The possible regioisomers of compound **4i**.

showed IR absorption at ~1640 $cm^{-1}$ and a carbonyl signal at ~163.0 ppm in the $^{13}C$ NMR. Based on the fact that the methodology may lead to the formation of regioisomeric products for example **4i**–**I** and **4i**–**II** of a representative compound **4i** (Fig. 3), the regioselectivity of the reaction was addressed by NOE experiment using **4i** (Fig. 4). The ring juncture proton of **4i** that is $H_{3'}$ that appeared as a triplet of doublet at 4.43 ppm in the $^1H$ NMR spectra was irradiated to examine its relationship with other nearby protons. It was found that the intensity of the proton at 7.21 ppm and the multiplet at ~2.0 ppm for the C-4′ aliphatic protons was increased (Fig. 4). This observation suggested that (i) the proton at 7.21 ppm is the olefinic one and (ii) the regioisomer isolated was **4i**–**I** and not **4i**–**II** (because in case of **4i**–**II** the intensity of the olefinic proton ($H_{1'}$) was not expected to be affected during the NOE experiment as it was not adjacent to $H_{3'}$).

A plausible mechanism that reconciles the structure of the synthesized spirooxindole derivatives **4a**–**1** has been outlined in Scheme 2. The reaction proceeds via the formation of an imine

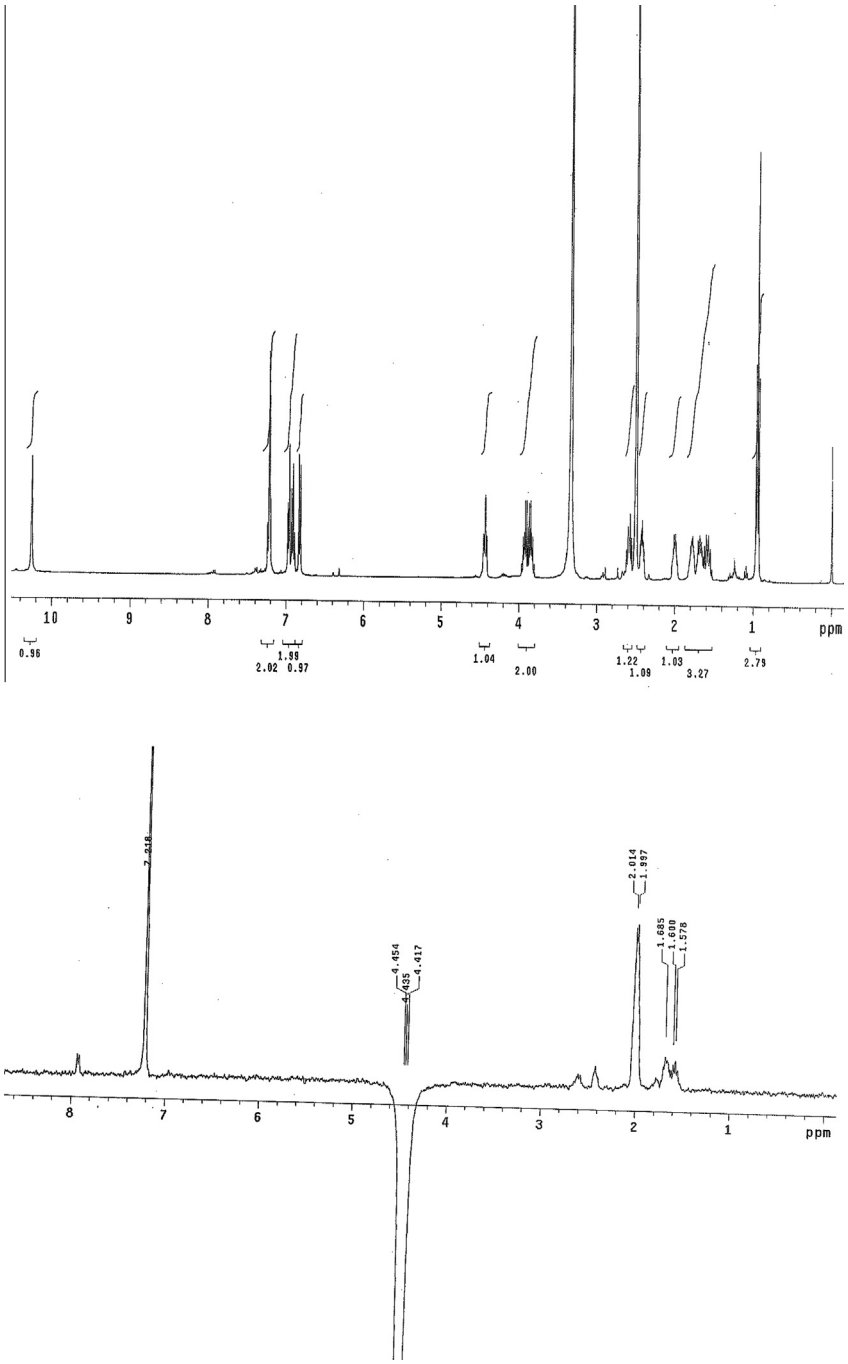
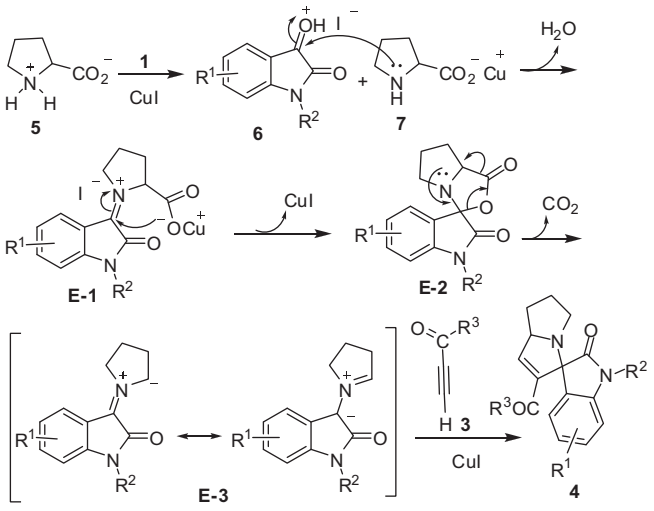
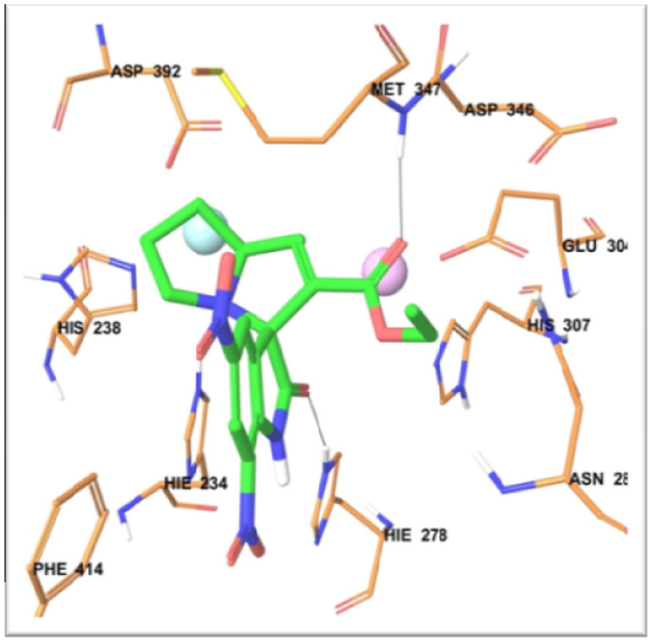



**Figure 4.** $^1H$ NMR spectra and NOE experiment of compound **4i**.




**Scheme 2.** Proposed mechanism for the Cu-mediated MCR leading to **4**.



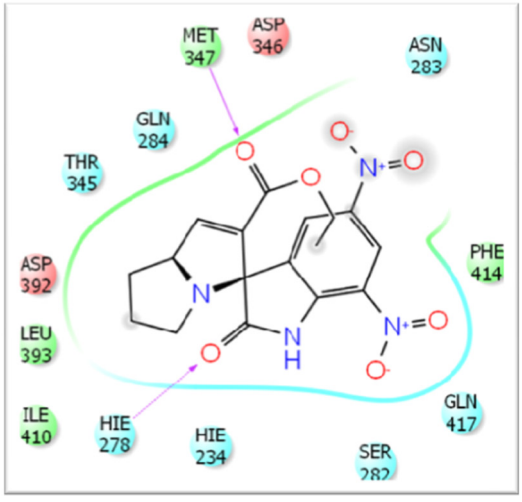




**Figure 5.** Binding modes and interactions of molecule **4k** with the inhibitor binding site of PDE4B.

intermediate **E-1** as a result of the reaction between zwitterionic L-proline **5** and isatin **1**. This step was assisted by CuI which was regenerated after the conversion of **E-1** to a spirocyclic intermediate **E-2**. Elimination of $CO_2$ from **E-2** generated the dipolar azomethine ylide **E-3** which on cycloaddition with dipolarophile that is the terminal alkyne **3** in the presence of CuI afforded the desired spirooxindole **4**. A theoretical study[10] on azomethine ylide **E-3** ($R^1 = R^2 = H$) indicated that it has a planar structure where the proline ring is planar instead of having an envelope shape, and co-planar with the isatin moiety. Thus, the cycloaddition of alkyne **3** on the planar azomethine ylide can occur from either side leading to the formation of product **4** having three chiral centers that is the possibility of generation of $2^3 = 8$ isomers. Once again based on earlier theoretical studies[10] the generation of four isomers as a result of front side attack was ruled out. Similarly, two isomers arising from the back side attack can also be ruled out as their formation does not follow a concerted mechanism. Moreover, further theoretical calculations favored an *endo* approach leading to the (*3S*)-**4** isomer instead of (*3R*)-**4**.

All the spirooxindoles (**4**) synthesized were tested for their PDE4 inhibitory properties in vitro using PDE4B enzyme assay.[12] Notably, recent studies have indicated that among the four subtypes of PDE4 for example A, B, C, and D, the PDE4B subtype is linked to inflammatory cell regulation.[13] It was therefore hypothesized that inhibition of the PDE4B may provide a means to achieve efficacy while potentially mitigating the adverse effects.[14] We have used a known inhibitor rolipram[15] as a reference compound in our assay. Among all the compounds tested, **4k** showed >40% inhibition of PDE4B when tested at 30 μM. To understand its interaction with the PDE4B protein, docking studies were performed using co-crystal structural coordinates of PDE4B from the protein data bank (PDB) and the (*3S*)-isomer of **4k**. The in silico study showed that both C=O groups of **4k** were involved in two strong hydrogen bonds with His 278 and Met 347 residues of the active site. Additionally, a few hydrophobic interactions with hydrophobic clamp residue of Q-pocket were also observed. Both nitro groups of the molecule facilitate the deep insertion in the enzyme pocket (Fig 5). The glide score of −5.6 obtained for **4k** (see Supplementary data, Table S-1) indicates its good interaction with the PDE4B protein. Notably, **4k** showed selectivity toward PDE4B over D in silico as indicated by its docking score −3.3 obtained during its interaction with PDE4D (see Supplementary data, Fig. S4 and Table S-2).

In conclusion, we have reported the first Cu-mediated one-pot three-component reaction of isatin derivatives, L-proline, and terminal alkynes leading to spiropyrrolidine oxindoles in good yields. This remarkably faster and operationally simple one-pot methodology seemed to have advantages over the previously reported methods. This also demonstrates the first use of terminal alkynes containing an ester/amide group in the synthesis of a range of spirooxindoles. A representative compound **4k** showed significant inhibition of PDE4B when tested in vitro. Docking studies indicated that both the carbonyl and nitro groups of this molecule played key roles in the interactions with the PDE4B protein. Overall, the spirooxindole framework described here represents a new template for the identification of novel inhibitors of PDE4 and the synthetic methodology could be useful in constructing a library of molecules related to spirooxindoles of potential pharmacological interest.

## Acknowledgments

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.07.126.

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11. *General procedure for the preparation of* **4**: CuI (0.01 mmol) was added to a mixture of isatin (1.0 mmol), L-proline (1.0 mmol) and alkyne (1.0 mmol) in acetonitrile (15 mL) and the reaction mixture was stirred at 80 °C for 2–3 h under nitrogen. After completion of the reaction (monitored by TLC) the solvent was then removed under reduced pressure and the residue was purified by column chromatography on silica gel using 50% hexane-ethyl acetate to afford the desired compound. Spectral data of selected compounds: compound **4a**; off white solid; mp: 260–263 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 10.0 (s, 1H), 7.50 (s, 1H), 7.20–7.15 (m, 1H), 7.00–6.70 (m, 5H), 4.45–4.35 (m, 1H), 2.50–2.40 (m, 2H), 2.05–1.95 (m, 1H), 1.80–1.45 (m, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 178.5, 163.8, 143.2, 139.1, 137.7, 128.8, 127.3, 125.4, 120.4, 109.4, 76.9, 71.6, 47.8, 30.6, 26.8; IR (KBr): 3275, 2873, 1663, 1598, 1472, 1384, 1205, 745 cm$^{-1}$; *m*/*z* (ES): 270.12 (M+1, 100%); HRMS: *m*/*z* [M+H] calcd for $C_{15}H_{16}N_3O_2$: 270.1243; found: 270.1240. Compound**4b**; orange solid; mp: 266-269 °C; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 10.0 (s, 1H), 8.00 (d, *J* = 4.9 Hz, 1H), 7.20–7.15 (m, 1H), 6.95–6.85 (m, 3H), 6.75 (d, *J* = 7.3 Hz, 1H), 4.45–4.35 (m, 1H), 2.50–2.30 (m, 5H), 2.00–1.95 (m, 1H), 1.80–1.60 (m, 2H), 1.65–1.45 (m, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 178.5, 162.4, 143.3, 138.1, 137.6, 128.9, 127.2, 125.4, 120.5, 109.4, 77.0, 71.6, 47.9, 30.7, 26.7, 25.2; IR (KBr): 3310, 2879, 1650, 1611, 1546, 1467, 1321, 1200, 755 cm$^{-1}$; *m*/*z* (ES): 284.14 (M+1, 100%); HRMS: *m*/*z* [M+H] calcd for $C_{16}H_{18}N_3O_2$: 284.1399; found: 284.1406.
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