

Electronic Supplementary Information

**Facile assembly of two 6-membered fused *N*-heterocyclic rings: A rapid access to novel small molecules via Cu-mediated reaction**

Raju Adepu,<sup>a</sup> Rajnikanth Sunke,<sup>a</sup> Chandana Lakshmi,<sup>a</sup> D. Rambabu,<sup>a</sup> G. Rama Krishna,<sup>b</sup> C. Malla Reddy,<sup>b</sup> Girdhar Singh Deora,<sup>a</sup> Kishore V. L. Parsa<sup>a</sup> and Manojit Pal<sup>a,\*</sup>

<sup>a</sup>*Institute of Life Sciences, University of Hyderabad Campus, Gachibowli, Hyderabad 500 046, India*

<sup>b</sup>*Department of Chemical Sciences, Indian Institute of Science Education and Research, Kolkata, West Bengal 741252, India*

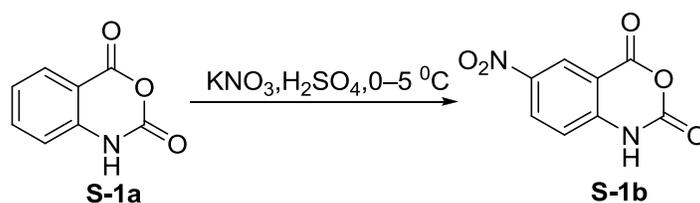
E-mail: manojitpal@rediffmail.com

## Experimental

### Chemistry

**General methods:** Unless stated otherwise, reactions were performed under nitrogen atmosphere using oven dried glassware. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualizing with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (230-400 mesh) using distilled hexane, ethyl acetate.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded in  $\text{CDCl}_3$  or  $\text{DMSO}-d_6$  solution by using 400 or 100 MHz spectrometers, respectively. Proton chemical shifts ( $\delta$ ) are relative to tetramethylsilane (TMS,  $\delta = 0.00$ ) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublet), td (triplet of doublet), t (triplet) and m (multiplet) as well as b (broad). Coupling constants ( $J$ ) are given in hertz. Infrared spectra were recorded on a FT-IR spectrometer. MS spectra were obtained on a Agilent 6430 series Triple Quad LC-MS / MS spectrometer. High-resolution mass spectra (HRMS) were recorded using a Waters LCT Premier XE instrument. Melting points (mp) were by using Buchi B-540 melting point apparatus and are uncorrected. Chromatographic purity by HPLC (Agilent 1200 series Chem Station software) was determined by using area normalization method and the condition specified in each case: column, mobile phase (range used), flow rate, detection wavelength, and retention times.

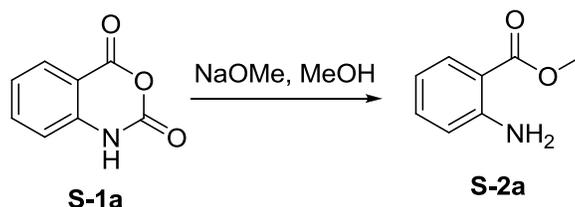
### Typical procedure for preparation of 6-nitro-1*H*-benzo[*d*][1,3]oxazine-2,4-dione<sup>1</sup> (S-1b)



To a solution of isatoic anhydride **S-1a** (2 g, 12.27 mmol) in con. $\text{H}_2\text{SO}_4$  (3 mL) at 0 °C was added potassium nitrate (1.24 g, 12.27 mmol) in small batches over a period of 30min and the stirring was continued for an additional 15min. Then, the reaction mixture was poured into crushed ice with stirring and the precipitate formed was filtered. The solid obtained was washed with water and dried under vacuum to afford the nitro compound **S-1b**.

Yield: 92% (2.4 g); light brown solid; mp: 259-261 °C (lit<sup>2</sup> 260 °C); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ: 12.33 (bs, 1H), 8.56 (d, *J* = 2.4 Hz, 1H), 8.51 (dd, *J* = 8.9, 2.5 Hz, 1H), 7.30 (d, *J* = 9.0 Hz, 1H).

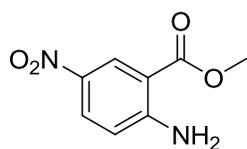
#### Typical procedure for preparation of methyl 2-amino benzoate (S-2a)



To the solution of sodium methoxide (1.36 g, 24.52 mmol) in methanol (20 mL), **S-1a** (2 g, 12.26 mmol) was slowly added at 0 °C and stirred at 75 °C for 1h. After completion of the reaction, the excess of sodium methoxide was quenched with ice cold water and methanol was removed under reduced pressure. The residue was diluted with water (50 mL) and extracted with ethyl acetate (2 x 25 mL). The organic layers were collected, combined, washed with brine solution (30 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated under reduced pressure to give desired compound **S-2a**.

Yield: 95% (1.76 g); color less liquid; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 7.85 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.31-7.23 (m, 1H), 6.66-6.62 (m, 2H), 5.70 (bs, 2H), 3.87 (s, 3H).

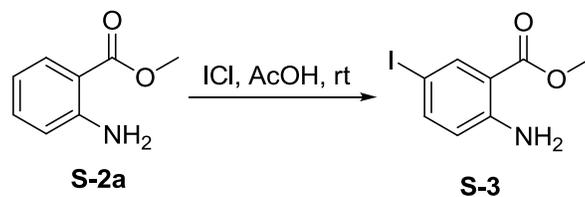
#### Methyl 2-amino-5-nitrobenzoate (S-2b)



Compound **S-2b** was synthesized from **S-1b** following a procedure similar to that of compound **S-2a**.

Yield: 87% (1.64 g); yellow solid; mp: 165-167 °C (lit<sup>3</sup> 166-168 °C); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 8.84 (d, *J* = 2.6 Hz, 1H), 8.13 (dd, *J* = 9.1, 2.6 Hz, 1H), 6.66 (d, *J* = 9.1 Hz, 1H), 3.93 (s, 3H).

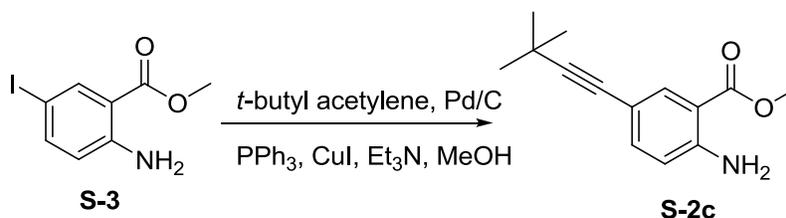
#### Typical procedure for preparation of methyl 2-amino-5-iodobenzoate (S-3)<sup>4</sup>



To a solution of methyl 2-aminobenzoate (1 g, 6.62 mmol) in glacial acetic acid (10 mL), iodine monochloride (1 g, 6.62 mmol) in glacial acetic acid (10 mL) was added over 10 min. The resulting mixture was stirred at room temperature for 24 h. The ensuing precipitate was filtered, washed with glacial acetic acid followed by diethyl ether, and dried to provide the title compound.

Yield: 90% (1.65 g); white solid; mp: 188-190 °C (lit<sup>4</sup> 188-192 °C); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ: 7.91 (d, *J* = 2.1 Hz, 1H), 7.47 (dd, *J* = 8.7, 2.1 Hz, 1H), 6.64 (d, *J* = 8.7 Hz, 1H), 3.77 (s, 3H).

#### Typical procedure for preparation of methyl 2-amino-5-(3,3-dimethylbut-1-ynyl)benzoate (**S-2c**)

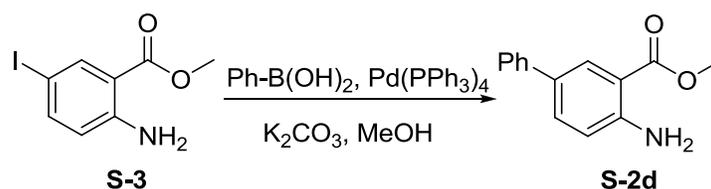


To a solution of methyl 2-amino-5-iodobenzoate (300 mg, 1.08 mmol) in methanol (5.0 mL), 5% Pd/C (1.0 mg, 0.01 mmol), CuI (1.9 mg, 0.01 mmol), PPh<sub>3</sub> (5.2 mg, 0.02 mmol) and triethyl amine (0.18 mL, 2.71 mmol) was added under nitrogen atmosphere. The reaction mixture was allowed to stir at room temperature for 15 min, and then added *t*-butyl acetylene (0.16 mL, 1.29 mmol). The mixture was refluxed for 3 hr. Upon completion, the reaction mixture was diluted with saturated NH<sub>4</sub>Cl solution (15 ml) and the product was extracted with ethyl acetate (3 x 15 ml). The organic layers were collected, combined, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under a reduced pressure. The residue was purified by column chromatography using ethyl acetate – hexane to give desired compound **S-2c**.

Yield: 94% (234 mg); light red solid; mp: 141-143 °C; *R*<sub>f</sub> = 0.6 (10% EtOAc/ *n*-hexane); IR (KBr, cm<sup>-1</sup>): 3336, 3172, 3053, 2219, 1713; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 7.92 (d, *J* = 1.8 Hz,

1H), 7.28-7.26 (m, 1H), 6.56 (d,  $J = 8.5$  Hz, 1H), 5.79 (bs, 2H), 3.87 (s, 3H), 1.30 (s, 9H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 168.0, 149.6, 137.0, 134.6, 116.5, 111.7, 110.4, 95.9, 78.4, 51.6, 31.1 (3C), 27.8; MS (ES mass): 231.9 (M+1); HPLC: 92.6%, column: X-Bridge C-18 150 x 4.6 mm 5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water, mobile phase B:  $\text{CH}_3\text{CN}$ , gradient (T/%B): 0/10, 2/10, 9/95, 16/95, 17/10, 20/10; flow rate: 1.0 mL/min; UV 225 nm, retention time 10.34 min.

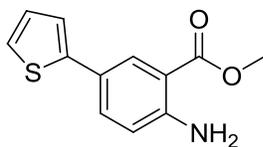
### Typical procedure for preparation of Methyl 4-aminobiphenyl-3-carboxylate (**S-2d**)



To a solution of methyl 2-amino-5-iodobenzoate (**S-3**) (300 mg, 1.08 mmol) in methanol (5.0 ml),  $\text{Pd(PPh}_3)_4$  (37 mg, 0.03 mmol),  $\text{K}_2\text{CO}_3$  (298 mg, 2.16 mmol) and phenyl boronic acid (157 mg, 1.29 mmol) was added under nitrogen atmosphere. The reaction mixture was allowed to stir at 70 °C for 8 h. Upon completion of the reaction, solvent was removed under reduced pressure. The residue was diluted with water (10 mL) and extracted with ethyl acetate (15 mL). The organic layer was washed with brine solution (10 mL), dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and concentrated under reduced pressure. The residue was purified by column chromatography using ethyl acetate – hexane to give desired compound **S-2d**.

Yield: 85% (254 mg); white solid; mp: 75-77 °C (lit<sup>5</sup> 78.1-80.3 °C);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 8.13 (d,  $J = 2.1$  Hz, 1H), 7.58-7.52 (m, 3H), 7.41 (t,  $J = 7.6$  Hz, 2H), 7.29-7.27 (m, 1H), 6.75 (d,  $J = 7.9$  Hz, 1H), 6.12 (bs, 2H), 3.90 (s, 3H).

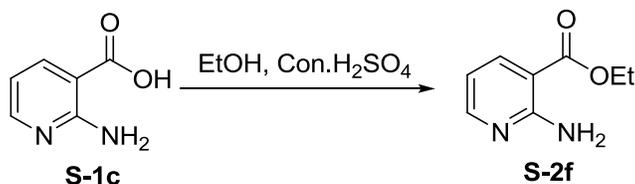
### Methyl 2-amino-5-(thiophen-2-yl)benzoate (**S-2e**)



Compound **S-2e** was synthesized from the reaction of **S-3** and thiophen-2-ylboronic acid following a procedure similar to that of compound **S-2d**.

Yield: 88% (221 mg); light green solid; mp: 159-161 °C;  $R_f = 0.5$  (10% EtOAc/ *n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3421, 3327, 3100, 2948, 1702;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 8.11 (d,  $J = 2.1$  Hz, 1H), 7.53 (dd,  $J = 8.5, 2.1$  Hz, 1H), 7.19-7.17 (m, 2H), 7.03 (d,  $J = 4.6$  Hz, 1H), 6.69 (d,  $J = 8.5$  Hz, 1H), 5.70 (bs, 2H), 3.91 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 168.3, 149.7, 144.1, 131.9, 128.4, 127.8, 123.2, 123.1, 121.5, 117.1, 110.7, 51.6; MS (ES mass): 233.9 (M+1); HPLC: 96.3%, column: X-Bridge C-18 150 x 4.6 mm 5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water, mobile phase B:  $\text{CH}_3\text{CN}$ , gradient (T/%B): 0/10, 2/10, 9/95, 16/95, 17/10, 20/10; flow rate: 1.0 mL/min; UV 220 nm, retention time 10.02 min.

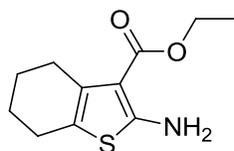
### Typical procedure for preparation of ethyl 2-aminonicotinate (S-2f)



To a solution of 2-aminonicotinic acid **S-1c** (1g, 7.24 mmol) in ethanol (20 mL) was added con. Sulfuric acid (3 mL). The reaction mixture was heated at reflux for 16 hours, and then cooled to room temperature. The solvent was removed under reduced pressure. Then, water was added and the crude basified to pH 8.0 with 1N NaOH solution. The product was extracted into ethyl acetate (50 mL), dried over anhydrous sodium sulphate and concentrated to afford compound **S-2f**.

Yield: 95% (1.14 g); light brown solid; mp: 90-92 °C (lit<sup>6</sup> 97-99 °C);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 8.21 (dd,  $J = 4.7, 1.7$  Hz, 1H), 8.14 (dd,  $J = 7.8, 1.7$  Hz, 1H), 6.62 (dd,  $J = 7.8, 4.7$  Hz, 1H), 6.34 (bs, 2H), 4.34 (q,  $J = 7.2$  Hz, 2H), 1.38 (t,  $J = 7.2$  Hz, 3H).

### Typical procedure for the synthesis of ethyl 2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (S-4a)

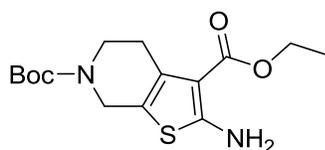


A mixture of cyclo hexanone (1.06 mL, 10 mmol), ethyl cyanoacetate (1.15 mL, 10 mmol), morpholine (0.90 mL, 10 mmol), sulphur (0.32 g, 10 mmol) in ethanol (10 mL) was stirred and

refluxed for overnight. After completion of the reaction, the reaction mixture was cooled to room temperature and the solvent was removed under vacuum. The crude solid was washed with cold ethanol and filtered through sintered funnel, dried under vacuum. The crude product was dissolved in dichloromethane and washed with brine. The organic layer was collected and concentrated under low vacuum to give the title compound.

Yield: 73% (1.83 g); brown solid; mp: 115-117 °C (lit<sup>7</sup> 116.2-117.2 °C); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 5.93 (s, 2H), 4.25 (q, *J* = 7.3 Hz, 2H), 2.68-2.71 (m, 2H), 2.47-2.51 (m, 2H), 1.74-1.80 (m, 4H), 1.33 (t, *J* = 7.3 Hz, 3H).

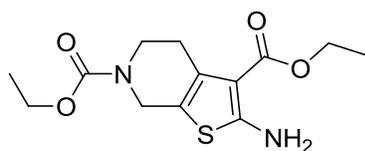
#### 6-*tert*-Butyl 3-ethyl 2-amino-4,5-dihydrothieno[2,3-*c*]pyridine-3,6(7*H*)-dicarboxylate (S-4b)



Compound **S-4b** was synthesized from N-Boc-4-piperidone and Et<sub>3</sub>N as a base following a procedure similar to that of compound **S-4a**.

Yield: 80% (1.31 g); light yellow solid; mp: 156-158 °C (lit<sup>7</sup> 157-158 °C); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 6.05 (s, 2H), 4.35 (bs, 2H), 4.26 (q, *J* = 7.2 Hz, 2H), 3.62 (t, *J* = 4.8 Hz, 2H), 2.78 (bs, 2H), 1.48 (s, 9H), 1.34 (t, *J* = 7.2 Hz, 3H).

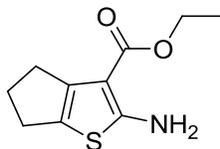
#### Diethyl 2-amino-4,5-dihydrothieno[2,3-*c*]pyridine-3,6(7*H*)-dicarboxylate (S-4c)



Compound **S-4c** was synthesized from ethyl 4-oxopiperidine-1-carboxylate and Et<sub>3</sub>N as a base following a procedure similar to that of compound **S-4a**.

Yield: 76% (1.32 g); brown solid; mp: 143-145 °C (lit<sup>8</sup> 144-146 °C); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 6.02 (bs, 2H), 4.40 (s, 2H), 4.26 (q, *J* = 7.1 Hz, 2H), 4.17 (q, *J* = 7.1 Hz, 2H), 3.66 (bs, 2H), 2.82 (bs, 2H), 1.33 (t, *J* = 7.1 Hz, 3H), 1.27 (t, *J* = 7.1 Hz, 3H).

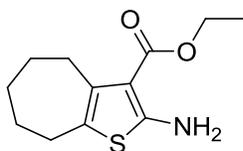
#### Ethyl 2-amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-carboxylate (S-4d)



Compound **S-4d** was synthesized from cyclo pentanone following a procedure similar to that of compound **S-4a**.

Yield: 78% (1.79 g); brown solid; mp: 182-184 °C (lit<sup>7</sup> 182.5-183.5 °C); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 5.89 (s, 2H), 4.23 (q, *J* = 7.3 Hz, 2H), 2.81-2.83 (m, 2H), 2.68-2.70 (m, 2H), 2.26-2.30 (m, 2H), 1.40 (t, *J* = 7.3 Hz, 3H).

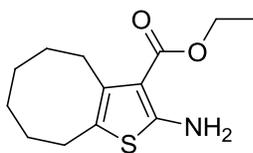
#### **Ethyl 2-amino-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene-3-carboxylate (S-4e)**



Compound **S-4e** was synthesized from cyclo heptanone following a procedure similar to that of compound **S-4a**.

Yield: 69% (1.47 g); light yellow solid; mp: 88-90 °C (lit<sup>7</sup> 89.5-90.5 °C); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ ppm: 5.77 (s, 2H), 4.27 (q, *J* = 6.9 Hz, 2H), 2.97 (t, *J* = 5.5 Hz, 2H), 2.57 (t, *J* = 5.6 Hz, 2H), 1.77-1.83 (m, 2H), 1.58-1.66 (m, 4H), 1.34 (t, *J* = 6.9 Hz, 3H).

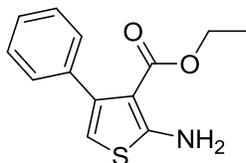
#### **Ethyl 2-amino-4,5,6,7,8,9-hexahydrocycloocta[b]thiophene-3-carboxylate (S-4f)**



Compound **S-4f** was synthesized from cyclo octanone following a procedure similar to that of compound **S-4a**.

Yield: 77% (1.52 g); brown solid; mp: 48-50 °C (lit<sup>9</sup> 50-51 °C); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 5.91 (bs, 2H), 4.29 (q, *J* = 7.1 Hz, 2H), 2.85-2.81 (m, 2H), 2.68-2.64 (m, 2H), 1.68-1.44 (m, 6H), 1.35 (t, *J* = 7.1 Hz, 3H), 1.30-1.28 (m, 2H).

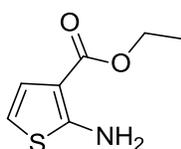
#### **Ethyl 2-amino-4-phenylthiophene-3-carboxylate (S-4g)**



Compound **S-4f** was synthesized from acetophenone following a procedure similar to that of compound **S-4a**.

Yield: 68% (1.15 g); light green solid; mp: 97-99 °C (lit<sup>7</sup> 97.5-98.5 °C); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 7.28-7.31 (m, 5H), 6.08 (bs, 2H), 6.06 (s, 1H), 4.10 (q, *J* = 6.9 Hz, 2H), 0.94 (t, *J* = 6.9 Hz, 3H),

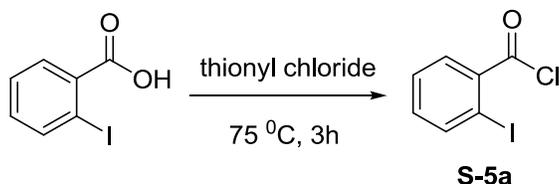
#### Ethyl 2-aminothiophene-3-carboxylate (**S-4h**)



A mixture of 1,4-dithiane-2,5-diol (1.0 g, 6.45 mmol), ethyl cyanoacetate (1.45 mL, 12.90 mmol), triethylamine (1.80 mL, 12.90 mmol) in DMF was heated to 60 °C for 1 h. After completion of reaction, reaction mixture was cooled to room temperature. The mixture was then diluted with ethyl acetate (25 mL), washed with water (3 x 20 mL) followed by brine solution (20 mL). The organic layers were collected, combined, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under a reduced pressure. The residue was purified by column chromatography using ethyl acetate–hexane to give desired compound.

Yield: 71% (0.81 g); off white solid; mp: 46-48 °C (lit<sup>7</sup> 47-48 °C); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 6.97 (d, *J* = 5.7 Hz, 1H), 6.24 (d, *J* = 5.7 Hz, 1H), 6.06 (bs, 2H), 4.30 (q, *J* = 6.9 Hz, 2H), 1.38 (t, *J* = 6.3 Hz, 3H).

#### Typical procedure for preparation of 2-iodo benzoyl chloride (**S-5a**)

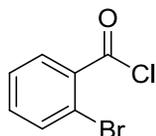


Thionyl chloride (10 equiv.) was slowly added to the 2-iodo benzoic acid (1 equiv.) at 0 °C and the reaction mixture was heated to 75 °C for 3h. Then, the reaction mixture cooled to room

temperature and excess of thionyl chloride removed under reduced pressure to give desired product which was used further without any purification.

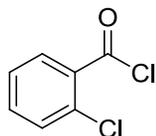
### 2-bromo benzoyl chloride (S-5b)

Compound **S-5b** was synthesized from 2-bromo benzoic acid following a procedure similar to that of compound **S-5a**.



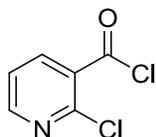
### 2-chloro benzoyl chloride (S-5c)

Compound **S-5c** was synthesized from 2-chloro benzoic acid following a procedure similar to that of compound **S-5a**.

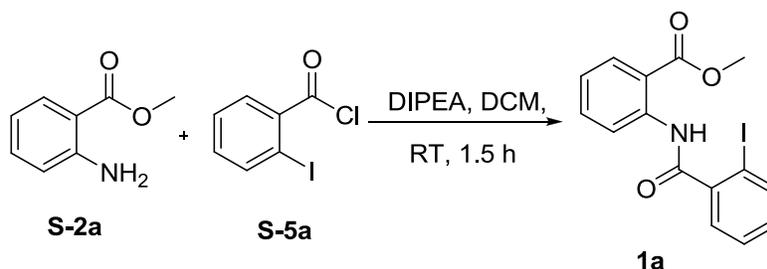


### 2-chloro nicotinoyl chloride (S-5d)

Compound **S-5d** was synthesized from 2-chloro nicotinic acid following a procedure similar to that of compound **S-5a**.



### Typical procedure for preparation of methyl 2-(2-iodobenzamido)benzoate (1a)

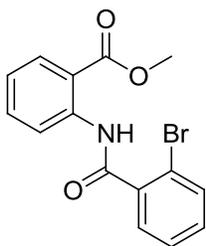


To a solution of compound **S-2a** (100 mg, 0.66 mmol) in dry DCM (5 mL), DIPEA (0.23 mL, 1.32 mmol) was added at 0 °C under nitrogen atmosphere. To this 2-iodo benzoyl chloride (0.11

mL, 0.79 mmol) was slowly added and the reaction mixture stirred at room temperature for 1.5 h. After completion of reaction, the reaction mixture diluted with DCM (5 mL), washed with saturated NaHCO<sub>3</sub> solution (15 mL), followed by brine solution (10 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated under reduced pressure. The residue was purified by column chromatography using ethyl acetate – hexane to give desired compound **1a**.

Yield: 95% (239 mg); white solid; mp: 102-104 °C (lit<sup>10</sup> 102-103 °C); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 11.41 (bs, 1H), 8.89 (d, *J* = 8.2 Hz, 1H), 8.10-8.07 (m, 1H), 7.95 (d, *J* = 7.9 Hz, 1H), 7.66-7.59 (m, 1H), 7.56 (dd, *J* = 7.6, 1.5 Hz, 1H), 7.49-7.42 (m, 1H), 7.18-7.13 (m, 2H), 3.91 (s, 3H).

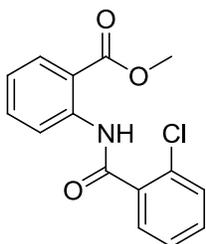
### Methyl 2-(2-bromobenzamido)benzoate (**1b**)



Compound **1b** was synthesized from the reaction of **S-2a** and **S-5b** following a procedure similar to that of compound **1a**.

Yield: 95% (209 mg); white solid; mp: 80-82 °C (lit<sup>10</sup> 80-81 °C); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 11.49 (bs, 1H), 8.90 (d, *J* = 8.0 Hz, 1H), 8.08 (dd, *J* = 7.8, 1.3 Hz, 1H), 7.70-7.58 (m, 3H), 7.42 (t, *J* = 7.6 Hz, 1H), 7.34-7.30 (m, 1H), 7.18-7.11 (m, 1H), 3.91 (s, 3H).

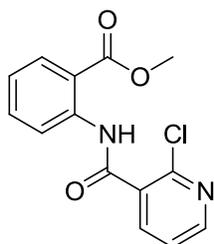
### Methyl 2-(2-chlorobenzamido)benzoate (**1c**)



Compound **1c** was synthesized from the reaction of **S-2a** and **S-5c** following a procedure similar to that of compound **1a**.

Yield: 92% (176 mg); light red solid; mp: 75-77 °C (lit<sup>10</sup> 81-82 °C); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 11.52 (bs, 1H), 8.91 (d, *J* = 8.5 Hz, 1H), 8.07 (d, *J* = 7.3 Hz, 1H), 7.69-7.58 (m, 2H), 7.47 (d, *J* = 7.3 Hz, 1H), 7.45-7.34 (m, 2H), 7.15 (t, *J* = 7.6 Hz, 1H), 3.90 (s, 3H).

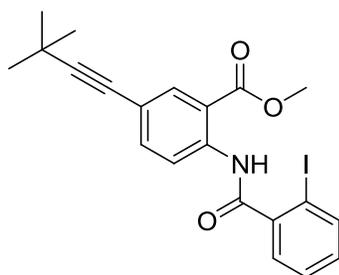
### Methyl 2-(2-chloronicotinamido)benzoate (**1d**)



Compound **1d** was synthesized from the reaction of **S-2a** and **S-5d** following a procedure similar to that of compound **1a**.

Yield: 88% (168 mg); light brown solid; mp: 144-146 °C; *R*<sub>f</sub> = 0.6 (20% EtOAc/ *n*-hexane); IR (KBr, cm<sup>-1</sup>): 3276, 2956, 1704, 1657; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 11.67 (bs, 1H), 8.85 (d, *J* = 8.3 Hz, 1H), 8.53 (d, *J* = 1.2 Hz, 1H), 8.09 (d, *J* = 8.0 Hz, 1H), 8.01 (dd, *J* = 7.7, 1.1 Hz, 1H), 7.63 (t, *J* = 7.2 Hz, 1H), 7.38 (dd, *J* = 7.4, 4.9 Hz, 1H), 7.18 (t, *J* = 7.6 Hz, 1H), 3.91 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ: 168.6, 163.8, 150.9, 140.7, 138.4, 134.8, 132.6, 130.9, 129.4, 123.5, 122.6, 120.7, 115.6, 52.5; MS (ES mass): 290.8 (M+1); HPLC: 93.2%, column: Symmetry C-18 75 x 4.6 mm 3.5μ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: CH<sub>3</sub>CN, gradient (T/%B): 0/50, 1.0/50, 9/98, 16/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 230 nm, retention time 5.43 min.

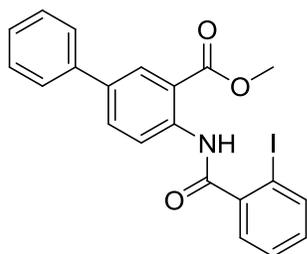
### Methyl 5-(3,3-dimethylbut-1-ynyl)-2-(2-iodobenzamido)benzoate (**1e**)



Compound **1e** was synthesized from the reaction of **S-2c** and **S-5a** following a procedure similar to that of compound **1a**.

Yield: 85% (199 mg); white solid; mp: 135-137 °C;  $R_f$  = 0.6 (10% EtOAc/ *n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3294, 2964, 2217, 1683, 1654;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 11.39 (bs, 1H), 8.82 (d,  $J$  = 8.4 Hz, 1H), 8.13-8.07 (m, 1H), 7.95 (d,  $J$  = 7.9 Hz, 1H), 7.61 (dd,  $J$  = 8.5, 1.8 Hz, 1H), 7.58-7.53 (m, 1H), 7.47-7.43 (m, 1H), 7.20-7.11 (m, 1H), 3.91 (s, 3H), 1.33 (s, 9H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 168.1, 167.5, 161.2, 142.1, 140.5, 137.5, 134.1, 132.2, 128.2, 120.3, 119.1, 115.2, 98.9, 95.3, 92.7, 77.8, 52.5, 30.1 (3C), 27.9; MS (ES mass): 461.7 (M+1); HPLC: 93.6%, column: X-Bridge C-18 150 x 4.6 mm 5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water, mobile phase B:  $\text{CH}_3\text{CN}$ , gradient (T/%B): 0/20, 2/20, 9/98, 16/98, 17/20, 20/20; flow rate: 1.0 mL/min; UV 230 nm, retention time 11.86 min.

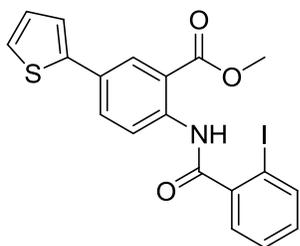
### Methyl 4-(2-iodobenzamido)biphenyl-3-carboxylate (**1f**)



Compound **1f** was synthesized from the reaction of **S-2d** and **S-5a** following a procedure similar to that of compound **1a**.

Yield: 88% (177 mg); white solid; mp: 134-136 °C;  $R_f$  = 0.5 (10% EtOAc/ *n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3275, 2987, 1697, 1654;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 11.42 (bs, 1H), 8.32 (d,  $J$  = 2.1 Hz, 1H), 7.96 (d,  $J$  = 7.8 Hz, 1H), 7.87 (dd,  $J$  = 8.4, 2.1 Hz, 1H), 7.65-7.56 (m, 3H), 7.50-7.43 (m, 4H), 7.37 (t,  $J$  = 7.2 Hz, 1H), 7.17 (t,  $J$  = 7.6 Hz, 1H), 3.94 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 168.2, 167.3, 141.8, 140.1, 139.1, 135.7, 133.7, 131.9, 131.2, 128.9, 128.5 (2C), 128.0, 127.8, 127.7, 126.4 (2C), 120.7, 115.4, 92.3, 52.2; MS (ES mass): 457.7 (M+1); HPLC: 93.2%, column: Symmetry C-18 75 x 4.6 mm 3.5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water, mobile phase B:  $\text{CH}_3\text{CN}$ , gradient (T/%B): 0/50, 1.0/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 210 nm, retention time 4.89 min.

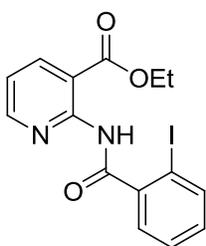
### Methyl 2-(2-iodobenzamido)-5-(thiophen-2-yl)benzoate (**1g**)



Compound **1g** was synthesized from the reaction of **S-2e** and **S-5a** following a procedure similar to that of compound **1a**.

Yield: 85% (168 mg); light green solid; mp: 131-133 °C;  $R_f = 0.5$  (10% EtOAc/ *n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3267, 2976, 1698, 1657;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 11.39 (bs, 1H), 8.31 (d,  $J = 2.0$  Hz, 1H), 7.96 (d,  $J = 8.0$  Hz, 1H), 7.86 (d,  $J = 8.3, 2.0$  Hz, 1H), 7.57 (d,  $J = 7.4$  Hz, 1H), 7.50-7.44 (m, 3H), 7.34 (d,  $J = 3.6$  Hz, 1H), 7.30 (d,  $J = 3.6$  Hz, 1H), 7.16 (t,  $J = 7.6$  Hz, 1H), 3.95 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 167.9, 167.2, 141.8, 140.1, 133.7, 131.9, 131.5, 131.2, 128.0, 127.8 (2C), 127.7, 127.6, 124.7, 122.9, 120.7, 115.4, 92.3, 52.3; MS (ES mass): 463.7 (M+1); HPLC: 95.3%, column: Symmetry C-18 75 x 4.6 mm 3.5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water, mobile phase B:  $\text{CH}_3\text{CN}$ , gradient (T/%B): 0/50, 1.0/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 230 nm, retention time 8.34 min.

#### Ethyl 2-(2-iodobenzamido)nicotinate (**1h**)

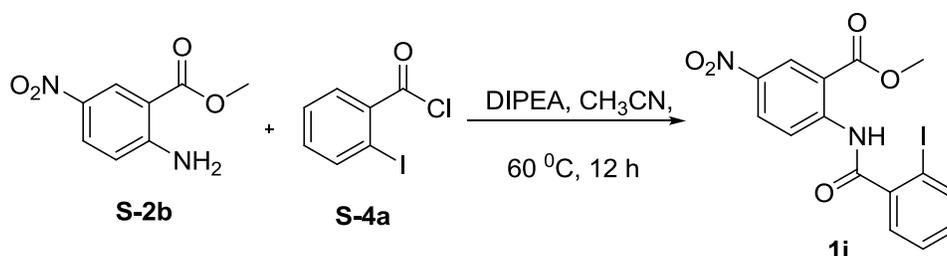


Compound **1h** was synthesized from the reaction of **S-2f** and **S-5a** following a procedure similar to that of compound **1a**.

Yield: 82% (195 mg); brown liquid;  $R_f = 0.3$  (40% EtOAc/*n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3169, 3071, 2983, 1703, 1651;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 11.16 (bs, 1H), 8.69 (d,  $J = 3.7$  Hz, 1H), 8.36 (d,  $J = 8.0$  Hz, 1H), 7.93 (d,  $J = 7.9$  Hz, 1H), 7.56 (d,  $J = 7.8$  Hz, 1H), 7.44 (t,  $J = 7.6$  Hz, 1H), 7.19-7.10 (m, 2H), 4.39 (q,  $J = 7.2$  Hz, 2H), 1.40 (t,  $J = 7.2$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 166.6, 153.1, 152.2, 142.0, 140.2, 139.9, 131.4 (2C), 128.3, 128.2, 118.8, 112.1, 92.3,

62.1, 14.1; MS (ES mass): 396.7 (M+1); HPLC: 92.8%, column: Symmetry C-18 75 x 4.6 mm 3.5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water, mobile phase B: CH<sub>3</sub>CN, gradient (T/%B): 0/30, 1/30, 3/98, 10/98, 10.5/30, 12/30; flow rate: 1.0 mL/min; UV 210 nm, retention time 4.01 min.

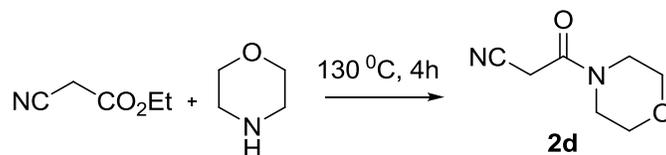
#### Typical procedure for preparation of methyl 2-(2-iodobenzamido)-5-nitrobenzoate (**1i**)



To a solution of compound **S-2b** (100 mg, 0.51 mmol) in CH<sub>3</sub>CN (5 mL), DIPEA (0.18 mL, 1.02 mmol) was added at 0 °C under nitrogen atmosphere. To this 2-iodo benzoyl chloride (0.09 mL, 0.61 mmol) was slowly added and the reaction mixture heated 60 °C for 12 h. Then, solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate (15 mL). The organic layer was washed with water (15 mL), saturated NaHCO<sub>3</sub> solution (15 mL), followed by brine solution (10 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated under reduced pressure. The residue was purified by column chromatography using ethyl acetate – hexane to give desired compound **1i**.

Yield: 35% (75 mg); white floppy solid; mp: 159-161 °C;  $R_f$  = 0.4 (10% EtOAc/ *n*-hexane); IR (KBr, cm<sup>-1</sup>): 3170, 2960, 1696, 1611; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 11.74 (bs, 1H), 9.12 (d,  $J$  = 9.3 Hz, 1H), 8.99 (d,  $J$  = 2.3 Hz, 1H), 8.47 (dd,  $J$  = 9.3, 2.2 Hz, 1H), 7.99 (d,  $J$  = 7.7 Hz, 1H), 7.58 (d,  $J$  = 7.6 Hz, 1H), 7.50 (t,  $J$  = 7.5 Hz, 1H), 7.21 (t,  $J$  = 7.8 Hz, 1H), 3.99 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$ : 167.8, 167.2, 146.2, 142.2, 140.8, 140.7, 132.1, 129.5, 128.5, 128.2, 126.9, 120.7, 115.3, 92.6, 53.2; MS (ES mass): 424.7 (M-1); HPLC: 99.0 %, column: X-Bridge C-18 75 x 4.6 mm 3.5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water, mobile phase B: CH<sub>3</sub>CN, gradient (T/%B): 0/30, 0.5/30, 4/98, 10/98, 10.5/30, 12/30; flow rate: 1.0 mL/min; UV 230 nm, retention time 5.16 min.

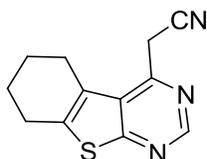
#### Typical procedure for preparation of 3-morpholino-3-oxopropanenitrile<sup>11</sup> (**2d**)



A mixture of ethyl cyanoacetate (1 g, 8.89 mmol) and morpholine (0.87 g, 8.89 mmol) was heated to 130 °C for 4 h and cooled to room temperature. The solid obtained was washed with ethyl acetate and hexane and filtered off to afford the desired compound **2d**.

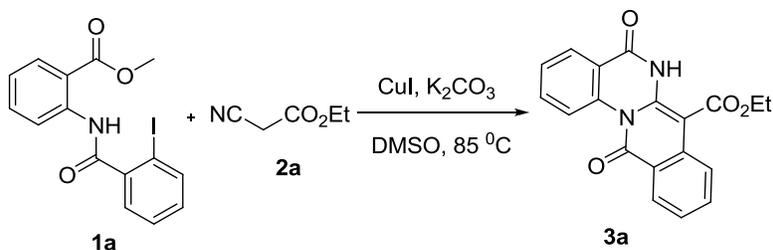
Yield: 75% (1.02 g); brown solid; mp: 81-83 °C; (lit<sup>12</sup> 82-84 °C); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ: 4.01 (s, 2H), 3.60-3.47 (m, 4H), 3.47-3.39 (m, 2H), 3.33-3.27 (m, 2H).

### Preparation of 2-(5,6,7,8-tetrahydrobenzo[*b*]thieno[2,3-*d*]pyrimidin-4-yl)acetonitriles (**2e**).



Compound **2e** was prepared in 4 steps according to the procedure described in lit.<sup>13</sup>

### Typical procedure for preparation of Ethyl 5,12-dioxo-6,12-dihydro-5*H*-isoquinolino[2,3-*a*]quinazoline-7-carboxylate (**3a**)

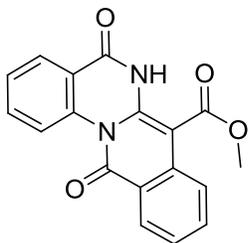


A mixture of compound **1a** (100 mg, 0.26 mmol), K<sub>2</sub>CO<sub>3</sub> (107 mg, 0.78 mmol), ethyl cyanoacetate (**2a**) (0.04 mL, 0.31 mmol) and CuI (4.9 mg, 0.026 mmol) in DMSO (2 mL) was heated to 85 °C under anhydrous conditions (CaCl<sub>2</sub> filled guard tube) for 3 h. After completion of the reaction, reaction mixture was cooled to RT, diluted with ethyl acetate (15 mL) and passed through celite. The resulting solution was washed with water (3 x 15 mL) followed by brine solution (25 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated under reduced pressure. The

residue was purified by column chromatography using ethyl acetate–hexane to give desired compound **3a**.

White solid; mp: 153-155 °C;  $R_f = 0.5$  (20% EtOAc/ *n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 2956, 1704, 1634, 1587;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 13.12 (bs, 1H), 8.77 (d,  $J = 8.8$  Hz, 1H), 8.42-8.38 (m, 2H), 8.30 (dd,  $J = 7.6, 1.6$  Hz, 1H), 7.76-7.67 (m, 2H), 7.50 (t,  $J = 7.6$  Hz, 1H), 7.42 (t,  $J = 7.8$  Hz, 1H), 4.52 (q,  $J = 7.1$  Hz, 2H), 1.49 (t,  $J = 7.2$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 168.6, 161.8, 157.8, 144.5, 137.1, 133.8, 133.6, 133.4, 128.5, 127.3, 126.9, 125.4, 125.2, 122.4, 121.9, 119.4, 86.8, 61.9, 14.2; MS (ES mass): 332.9 (M-1); HPLC: 99.2%, column: X-Bridge C-18 150 x 4.6 mm 5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water, mobile phase B:  $\text{CH}_3\text{CN}$ , gradient (T/%B): 0/30, 2/30, 8/98, 16/98, 17/30, 20/30; flow rate: 1.0 mL/min; UV 240 nm, retention time 9.12 min. HRMS (ESI): calcd for  $\text{C}_{19}\text{H}_{15}\text{N}_2\text{O}_4$  (M+H) $^+$  335.1032, found 335.1016.

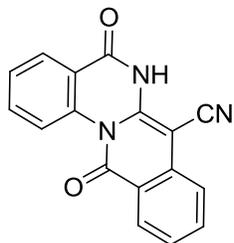
### Methyl 5,12-dioxo-6,12-dihydro-5H-isoquinolino[2,3-a]quinazoline-7-carboxylate (**3b**)



Compound **3b** was synthesized from the reaction of **1a** and methyl cyano acetate (**2b**) following a procedure similar to that of compound **3a**.

White solid; mp: 189-191 °C;  $R_f = 0.5$  (15% EtOAc/ *n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 2965, 1712, 1634, 1590;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 13.06 (bs, 1H), 8.76 (d,  $J = 8.4$  Hz, 1H), 8.39 (d,  $J = 7.9$  Hz, 1H), 8.33 (d,  $J = 8.5$  Hz, 1H), 8.29 (dd,  $J = 7.8, 1.4$  Hz, 1H), 7.77-7.66 (m, 2H), 7.51 (t,  $J = 7.5$  Hz, 1H), 7.42 (t,  $J = 7.5$  Hz, 1H), 4.03 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 169.1, 161.9, 157.8, 144.7, 137.2, 133.9, 133.7, 133.3, 128.6, 127.4, 127.1, 125.5, 125.4, 122.4, 122.0, 119.4, 86.8, 52.4; MS (ES mass): 320.8 (M+1); HPLC: 99.6%, Symmetry C-18 75 x 4.6 mm 3.5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water, mobile phase B:  $\text{CH}_3\text{CN}$ , gradient (T/%B): 0/30, 0.5/30, 4/98, 10/98, 10.5/30, 12/30; flow rate: 1.0 mL/min; UV 240 nm, retention time 4.83 min.

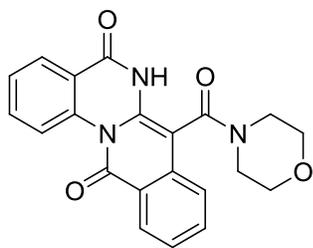
### 5,12-dioxo-6,12-dihydro-5H-isoquinolino[2,3-a]quinazoline-7-carbonitrile (**3c**)



Compound **3c** was synthesized from the reaction of **1a** and malano nitrile (**2c**) following a procedure similar to that of compound **3a**.

Brown solid; mp: 292-294 °C;  $R_f = 0.5$  (30% EtOAc/ *n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3145, 2209, 1691, 1606;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$ : 12.05 (bs, 1H), 8.86 (d,  $J = 8.8$  Hz, 1H), 8.25 (d,  $J = 8.0$  Hz, 1H), 8.13 (dd,  $J = 7.8, 1.6$  Hz, 1H), 7.87-7.79 (m, 2H), 7.80-7.55 (m, 2H), 7.49 (t,  $J = 7.8$  Hz, 1H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$ : 161.7, 159.2, 146.5, 137.6, 133.9, 128.9, 126.9, 126.8, 126.7, 121.9, 121.8 (2C), 121.3, 121.2, 119.8, 115.7, 72.3; MS (ES mass): 285.9 (M-1); HPLC: 99.2%, column: X-Bridge C-18 150 x 4.6 mm 5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water, mobile phase B: CH<sub>3</sub>CN, gradient (T/%B): 0/20, 2/20, 9/98, 16/98, 17/20, 20/20; flow rate: 1.0 mL/min; UV 230 nm, retention time 8.48 min; HRMS (ESI): calcd for C<sub>17</sub>H<sub>10</sub>N<sub>3</sub>O<sub>2</sub> (M+H)<sup>+</sup> 288.0845, found 288.0854.

### 7-(morpholine-4-carbonyl)-5H-isoquinolino[2,3-*a*]quinazoline-5,12(6H)-dione (**3d**)

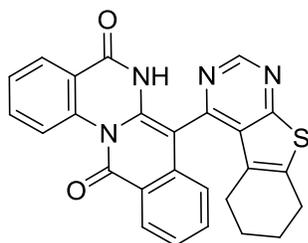


Compound **3d** was synthesized from the reaction of **1a** and **2d** following a procedure similar to that of compound **3a**.

White solid; mp: 236-238 °C;  $R_f = 0.4$  (40% EtOAc/ *n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3206, 3087, 1682, 1628, 1562;  $^1\text{H}$  NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 9.75 (bs, 1H), 9.15 (d,  $J = 8.8$  Hz, 1H), 8.45 (d,  $J = 8.0$  Hz, 1H), 8.28 (dd,  $J = 7.8, 1.4$  Hz, 1H), 7.77-7.69 (m, 2H), 7.50-7.39 (m, 3H), 3.98-3.18 (m, 8H);  $^{13}\text{C}$  NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$ : 165.7, 161.7, 157.9, 137.8, 136.5, 134.2, 134.0, 132.7, 129.3, 127.7, 126.7, 125.6, 122.1, 121.9, 121.5, 118.7, 92.7, 66.7 (2C), 44.4, 42.9; MS

(ES mass): 374.0 (M-1); HPLC: 96.2%, column: X-Bridge C-18 150 x 4.6 mm 5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water, mobile phase B: CH<sub>3</sub>CN, gradient (T/%B): 0/10, 2/10, 9/95, 16/95, 17/10, 20/10; flow rate: 1.0 mL/min; UV 230 nm, retention time 8.15 min. HRMS (ESI): calcd for C<sub>21</sub>H<sub>18</sub>N<sub>3</sub>O<sub>4</sub> (M+H)<sup>+</sup> 376.1297, found 376.1280.

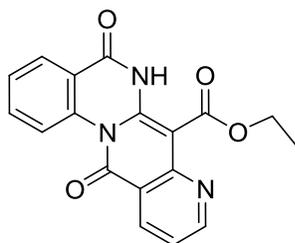
**7-(5,6,7,8-tetrahydrobenzo[b]thieno[2,3-*d*]pyrimidin-4-yl)-5*H*-isoquinolino[2,3-*a*]quinazoline-5,12(6*H*)-dione (3e)**



Compound **3e** was synthesized from the reaction of **1a** and **2e** following a procedure similar to that of compound **3a**.

Light brown solid; mp: 329-331 °C; R<sub>f</sub> = 0.4 (25% EtOAc/ *n*-hexane); IR (KBr, cm<sup>-1</sup>): 3224, 2940, 1697, 1678, 1619; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 9.27 (d, *J* = 8.8 Hz, 1H), 9.18 (s, 1H), 8.53-8.50 (m, 2H), 8.21 (dd, *J* = 7.8, 1.4 Hz, 1H), 7.78 (t, *J* = 8.6 Hz, 1H), 7.53-7.41 (m, 3H), 6.73 (d, *J* = 7.8 Hz, 1H), 2.89-2.86 (m, 2H), 2.29-2.22 (m, 1H), 1.87-1.80 (m, 2H), 1.75-1.66 (m, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$ : 170.3, 162.2, 158.0, 153.1, 152.7, 140.7, 138.2, 135.5, 134.5, 134.1, 133.9, 131.9, 129.2, 127.9, 126.7, 126.6, 125.8, 122.6, 122.2, 121.8, 118.7, 96.8, 26.0, 24.8, 22.3, 22.1; MS (ES mass): 449.0 (M-1); HRMS (ESI): calcd for C<sub>26</sub>H<sub>19</sub>N<sub>4</sub>O<sub>2</sub>S (M+H)<sup>+</sup> 451.1229, found 451.1225.

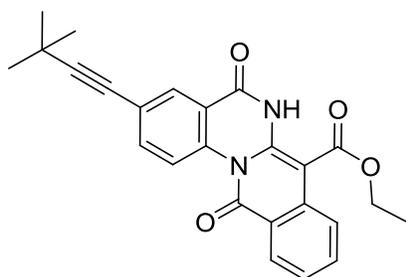
**Ethyl 5,12-dioxo-6,12-dihydro-5*H*-[1,6]naphthyridino[6,7-*a*]quinazoline-7-carboxylate (3f)**



Compound **3f** was synthesized from the reaction of **1d** and **2a** following a procedure similar to that of compound **3a**.

Light brown solid; mp: 134-136 °C;  $R_f = 0.4$  (40% EtOAc/ *n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3078, 2923, 1701, 1646, 1590;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 12.65 (bs, 1H), 8.98-8.97 (m, 1H), 8.89 (d,  $J = 8.4$  Hz, 1H), 8.64 (dd,  $J = 8.2, 1.4$  Hz, 1H), 8.33 (dd,  $J = 8.0, 1.4$  Hz, 1H), 7.77 (t,  $J = 7.8$  Hz, 1H), 7.54 (t,  $J = 7.8$  Hz, 1H), 7.38-7.35 (m, 1H), 4.55 (q,  $J = 7.1$  Hz, 2H), 1.46 (t,  $J = 7.2$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 168.2, 161.9, 157.6, 154.9, 150.5, 145.6, 137.1, 136.9, 134.3, 127.8, 127.5, 121.8, 120.4, 119.3, 117.9, 89.9, 62.2, 14.2; MS (ES mass): 335.8 (M+1); HPLC: 92.5%, column: X-Bridge C-18 150 x 4.6 mm 5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water, mobile phase B:  $\text{CH}_3\text{CN}$ , gradient (T/%B): 0/10, 3/10, 9/95, 16/95, 17/10, 20/10; flow rate: 1.0 mL/min; UV 220 nm, retention time 8.43 min. HRMS (ESI): calcd for  $\text{C}_{18}\text{H}_{14}\text{N}_3\text{O}_4$  (M+H) $^+$  336.0984, found 336.0988

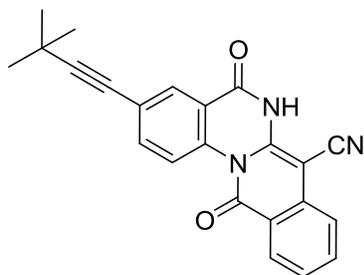
**Ethyl 3-(3,3-dimethylbut-1-ynyl)-5,12-dioxo-6,12-dihydro-5H-isoquinolino[2,3-a]quinazoline-7-carboxylate (3g)**



Compound **3g** was synthesized from the reaction of **1e** and **2a** following a procedure similar to that of compound **3a**.

White solid; mp: 183-185 °C;  $R_f = 0.5$  (15% EtOAc/ *n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 2968, 2218, 1700, 1654, 1599;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 13.12 (bs, 1H), 8.72 (d,  $J = 8.8$  Hz, 1H), 8.40-8.37 (m, 2H), 8.28 (d,  $J = 2.0$  Hz, 1H), 7.71-7.67 (m, 2H), 7.42 (t,  $J = 7.6$  Hz, 1H), 4.52 (q,  $J = 7.2$  Hz, 2H), 1.49 (t,  $J = 7.2$  Hz, 3H), 1.33 (s, 9H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 168.6, 161.7, 157.3, 144.3, 136.4, 135.8, 133.7, 133.4, 130.2, 128.6, 125.5, 125.3, 123.4, 122.3, 121.9, 119.3, 101.3, 87.1, 77.3, 61.9, 30.8 (3C), 28.0, 14.3; MS (ES mass): 414.9 (M+1); HPLC: 99.3%, column: X-Bridge C-18 150 x 4.6 mm 5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water, mobile phase B:  $\text{CH}_3\text{CN}$ , gradient (T/%B): 0/30, 2/30, 8/98, 16/98, 17/30, 20/30; flow rate: 1.0 mL/min; UV 240 nm, retention time 11.2 min. HRMS (ESI): calcd for  $\text{C}_{25}\text{H}_{23}\text{N}_2\text{O}_4$  (M+H) $^+$  415.1658, found 415.1657.

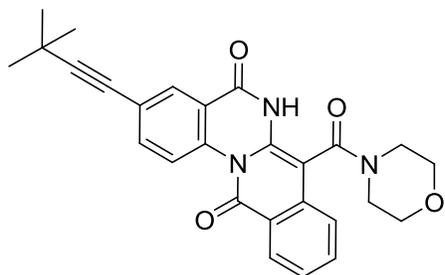
**3-(3,3-dimethylbut-1-ynyl)-5,12-dioxo-6,12-dihydro-5H-isoquinolino[2,3-a]quinazoline-7-carbonitrile (3h)**



Compound **3h** was synthesized from the reaction of **1e** and **2c** following a procedure similar to that of compound **3a**.

Brown solid; mp: 318-320 °C;  $R_f = 0.2$  (30% EtOAc/ *n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3194, 2964, 2223, 1703, 1617, 1559;  $^1\text{H}$  NMR (400 MHz, DMSO  $d_6$ )  $\delta$ : 8.88 (d,  $J = 8.8$  Hz, 1H), 8.26 (d,  $J = 8.0$  Hz, 1H), 7.99 (d,  $J = 1.8$  Hz, 1H), 7.86 (t,  $J = 8.0$  Hz, 1H), 7.76 (dd,  $J = 8.8, 1.8$  Hz, 1H), 7.61 (d,  $J = 8.0$  Hz, 1H), 7.51 (t,  $J = 7.8$  Hz, 1H), 1.31 (s, 9H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$ : 161.8, 160.2, 136.9, 135.3, 135.0, 129.9, 129.2, 128.8 (2C), 122.1 (2C), 121.9, 121.7, 120.7, 119.4, 109.8, 100.8, 79.5, 77.7, 30.9 (3C), 29.3; MS (ES mass): 366.0 (M-1); HPLC: 98.8%, column: X-Bridge C-18 150 x 4.6 mm 5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water, mobile phase B:  $\text{CH}_3\text{CN}$ , gradient (T/%B): 0/20, 2/20, 9/98, 16/98, 17/20, 20/20; flow rate: 1.0 mL/min; UV 240 nm, retention time 11.03 min.

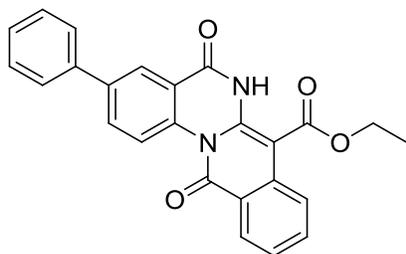
**3-(3,3-dimethylbut-1-ynyl)-7-(morpholine-4-carbonyl)-5H-isoquinolino[2,3-a]quinazoline-5,12(6H)-dione (3i)**



Compound **3i** was synthesized from the reaction of **1e** and **2d** following a procedure similar to that of compound **3a**.

Light brown solid; mp: 233-235 °C;  $R_f = 0.3$  (45% EtOAc/ *n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3198, 2970, 2215, 1697, 1623, 1572;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 9.83 (bs, 1H), 9.12 (d,  $J = 8.4$  Hz, 1H), 8.44 (d,  $J = 7.6$  Hz, 1H), 8.28 (d,  $J = 2.0$  Hz, 1H), 7.76-7.68 (m, 2H), 7.48-7.37 (m, 2H), 3.94-3.41 (m, 8H), 1.34 (s, 9H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 165.8, 161.7, 157.5, 136.9, 136.6, 136.5, 134.2, 132.8, 130.6, 129.4, 125.9, 125.8, 123.1, 122.2, 121.9, 121.6, 118.7, 101.2, 93.0, 66.8 (2C), 45.6, 43.8, 30.8 (3C), 29.7; MS (ES mass): 454.0 (M-1); HPLC: 93.6%, column: X-Bridge C-18 150 x 4.6 mm 5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water, mobile phase B:  $\text{CH}_3\text{CN}$ , gradient (T/%B): 0/30, 2/30, 8/98, 16/98, 17/30, 20/30; flow rate: 1.0 mL/min; UV 230 nm, retention time 9.18 min. HRMS (ESI): calcd for  $\text{C}_{27}\text{H}_{26}\text{N}_3\text{O}_4$  (M+H) $^+$  456.1923, found 456.1909.

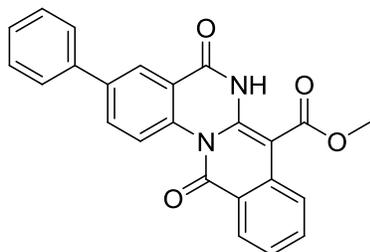
**Ethyl-5,12-dioxo-3-phenyl-6,12-dihydro-5H-isoquinolino[2,3-*a*]quinazoline-7-carboxylate (3j)**



Compound **3j** was synthesized from the reaction of **1f** and **2a** following a procedure similar to that of compound **3a**.

White solid; mp: 209-211 °C;  $R_f = 0.5$  (15% EtOAc/ *n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 2995, 1694, 1643, 1595;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 13.21 (bs, 1H), 8.90 (d,  $J = 8.9$  Hz, 1H), 8.55 (d,  $J = 2.1$  Hz, 1H), 8.44 (t,  $J = 9.0$  Hz, 2H), 8.00 (dd,  $J = 9.0, 2.3$  Hz, 1H), 7.75-7.69 (m, 3H), 7.55-7.41 (m, 4H), 4.56 (q,  $J = 7.1$  Hz, 2H), 1.52 (t,  $J = 7.1$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 168.7, 161.9, 157.9, 144.4, 139.8, 138.3, 136.3, 133.8, 133.6, 132.3, 129.1 (2C), 128.7, 128.3, 126.9 (2C), 125.6, 125.4, 125.2, 122.6, 122.5, 119.8, 87.1, 61.9, 14.3; MS (ES mass): 408.9 (M-1). HPLC: 92.8%, column: Symmetry C-18 75 x 4.6 mm 3.5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water, mobile phase B:  $\text{CH}_3\text{CN}$ , gradient (T/%B): 0/20, 5/20, 5/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 240 nm, retention time 6.75 min. HRMS (ESI): calcd for  $\text{C}_{25}\text{H}_{19}\text{N}_2\text{O}_4$  (M+H) $^+$  411.1345, found 411.1337.

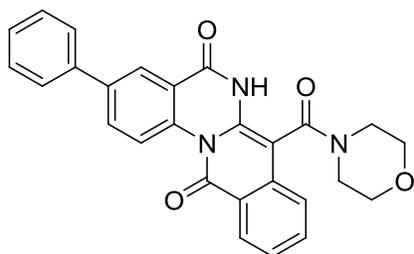
**Methyl-5,12-dioxo-3-phenyl-6,12-dihydro-5H-isoquinolino[2,3-a]quinazoline-7-carboxylate (3k)**



Compound **3k** was synthesized from the reaction of **1f** and **2b** following a procedure similar to that of compound **3a**.

Light yellow floppy solid; mp: 209-211 °C;  $R_f = 0.6$  (15% EtOAc/*n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3010, 1699, 1642, 1589;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 13.13 (bs, 1H), 8.89 (d,  $J = 8.8$  Hz, 1H), 8.54 (d,  $J = 2.4$  Hz, 1H), 8.44 (d,  $J = 8.0$  Hz, 1H), 8.37 (d,  $J = 8.2$  Hz, 1H), 7.99 (dd,  $J = 8.8, 2.3$  Hz, 1H), 7.73-7.71 (m, 3H), 7.52-7.48 (m, 2H), 7.48-7.41 (m, 2H), 4.06 (s, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 169.1, 161.9, 157.9, 144.6, 139.9, 138.3, 136.2, 133.8, 133.4, 132.3, 129.1 (2C), 128.7, 128.3, 127.1, 126.9 (2C), 125.7, 125.5, 125.2, 122.6, 119.9, 87.0, 52.5; MS (ES mass): 396.7 (M+1); HPLC: 98.6%, column: X-Bridge C-18 150 x 4.6 mm 5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water, mobile phase B:  $\text{CH}_3\text{CN}$ , gradient (T/%B): 0/20, 5/20, 5/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 240 nm, retention time 5.43 min.

**7-(morpholine-4-carbonyl)-3-phenyl-5H-isoquinolino[2,3-a]quinazoline-5,12(6H)-dione (3l)**

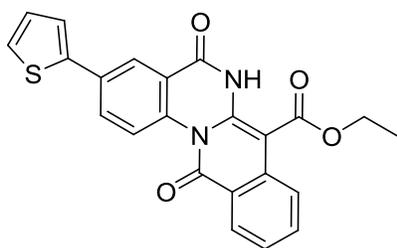


Compound **3l** was synthesized from the reaction of **1f** and **2d** following a procedure similar to that of compound **3a**.

Light brown solid; mp: 366-368 °C;  $R_f = 0.4$  (45% EtOAc/ *n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3208, 2889, 1689, 1620, 1570;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 9.98 (bs, 1H), 9.27 (d,  $J = 9.0$  Hz, 1H), 8.54 (d,  $J = 2.2$  Hz, 1H), 8.48 (d,  $J = 7.9$  Hz, 1H), 7.99 (dd,  $J = 9.0, 2.3$  Hz, 1H), 7.74-7.71 (m,

3H), 7.51-7.38 (m, 5H), 4.01-3.24 (m, 8H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 168.5, 165.8, 163.4, 139.2, 138.3, 134.6, 134.1, 132.9, 132.5, 129.4, 129.0 (2C), 128.6, 128.2, 127.1, 126.9 (2C), 126.6, 125.7, 122.2, 121.9, 114.0, 95.2, 66.8 (2C), 43.8, 41.5; MS (ES mass): 449.9 (M-1); HPLC: 92.8%, column: Symmetry C-18 75 x 4.6 mm 3.5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water, mobile phase B:  $\text{CH}_3\text{CN}$ , gradient (T/%B): 0/20, 5/20, 5/98, 10/98, 10.5/20, 12/20; flow rate: 1.0 mL/min; UV 210 nm, retention time 5.36 min. HRMS (ESI): calcd for  $\text{C}_{27}\text{H}_{22}\text{N}_3\text{O}_4$  (M+H) $^+$  452.1610, found 452.1627

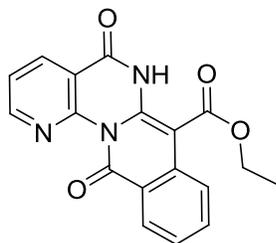
**Ethyl-5,12-dioxo-3-(thiophen-2-yl)-6,12-dihydro-5H-isoquinolino[2,3-a]quinazoline-7-carboxylate (3m)**



Compound **3m** was synthesized from the reaction of **1g** and **2a** following a procedure similar to that of compound **3a**.

White solid; mp: 184-186 °C;  $R_f$  = 0.5 (20% EtOAc/ *n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3023, 2975, 1712, 1691, 1643, 1599;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 13.18 (s, 1H), 8.83 (d,  $J$  = 9.0 Hz, 1H), 8.49 (d,  $J$  = 2.3 Hz, 1H), 8.41 (t,  $J$  = 8.6 Hz, 2H), 7.94 (dd,  $J$  = 9.0, 2.3 Hz, 1H), 7.73-7.66 (m, 1H), 7.48 (d,  $J$  = 2.6 Hz, 1H), 7.43 (t,  $J$  = 7.5 Hz, 1H), 7.38 (d,  $J$  = 5.6 Hz, 1H), 7.14-7.12 (m, 1H), 4.53 (q,  $J$  = 7.2 Hz, 2H), 1.50 (t,  $J$  = 7.2 Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 168.6, 161.7, 157.6, 144.2, 141.5, 135.8, 133.6, 133.3, 133.2, 130.7, 128.6, 128.3, 126.1, 125.5, 125.3, 124.4, 123.4, 122.6, 122.3, 119.7, 87.0, 61.9, 14.2; MS (ES mass): 414.9 (M-1); HPLC: 93.5%, column: Symmetry C-18 75 x 4.6 mm 3.5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water, mobile phase B:  $\text{CH}_3\text{CN}$ , gradient (T/%B): 0/50, 1/50, 3/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 240 nm, retention time 5.19 min.

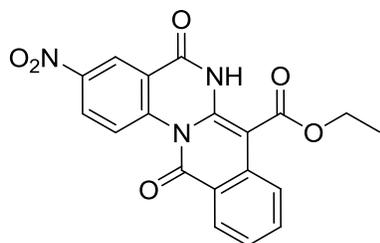
**Ethyl-5,12-dioxo-6,12-dihydro-5H-isoquinolino[2,3-a]pyrido[2',3'-d]pyrimidine-7-carboxylate (3n)**



Compound **3n** was synthesized from the reaction of **1h** and **2a** following a procedure similar to that of compound **3a**.

White solid; mp: 214-216 °C;  $R_f = 0.3$  (40% EtOAc/*n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3071, 2983, 1703, 1651, 1606;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 13.16 (bs, 1H), 8.87 (dd,  $J = 4.8, 1.9$  Hz, 1H), 8.56 (dd,  $J = 7.7, 1.9$  Hz, 1H), 8.40 (dd,  $J = 7.9, 1.2$  Hz, 1H), 8.35 (d,  $J = 8.4$  Hz, 1H), 7.73-7.65 (m, 1H), 7.49 (dd,  $J = 7.7, 4.7$  Hz, 1H), 7.43 (t,  $J = 7.3$  Hz, 1H), 4.52 (q,  $J = 7.1$  Hz, 2H), 1.49 (t,  $J = 7.1$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 168.6, 161.1, 157.8, 153.1, 149.9, 144.3, 136.6, 133.7, 133.3, 128.7, 125.8, 125.7, 124.1, 122.8, 115.3, 87.3, 61.9, 14.3; MS (ES mass): 335.8 (M+1); HPLC: 97.4%, column: Symmetry C-18 75 x 4.6 mm 3.5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water, mobile phase B:  $\text{CH}_3\text{CN}$ , gradient (T/%B): 0/30, 1/30, 4/98, 10/98, 10.5/30, 12/30; flow rate: 1.0 mL/min; UV 245 nm, retention time 4.42 min.

**Ethyl-3-nitro-5,12-dioxo-6,12-dihydro-5H-isoquinolino[2,3-*a*]quinazoline-7-carboxylate**  
**(3o)**

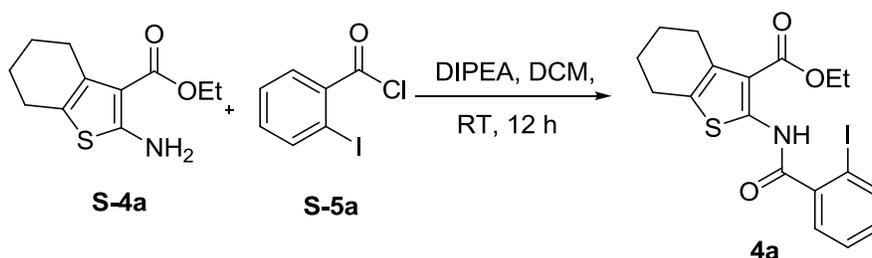


Compound **3o** was synthesized from the reaction of **1i** and **2a** following a procedure similar to that of compound **3a**.

Light yellow solid; mp: 179-181 °C;  $R_f = 0.4$  (15% EtOAc/ *n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 2990, 1696, 1644, 1598;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 13.25 (bs, 1H), 9.12 (d,  $J = 2.5$  Hz, 1H), 8.98 (d,  $J = 9.5$  Hz, 1H), 8.53 (dd,  $J = 9.5, 2.5$  Hz, 1H), 8.40 (t,  $J = 8.7$  Hz, 2H), 7.74 (t,  $J = 7.6$  Hz, 1H), 7.49 (t,  $J = 7.2$  Hz, 1H), 4.55 (q,  $J = 7.2$  Hz, 2H), 1.51 (t,  $J = 7.2$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 168.5, 167.2, 161.8, 156.2, 155.0, 143.7, 141.4, 133.9, 133.2, 128.6, 127.4,

126.1, 123.6, 123.0, 120.4, 116.3, 92.2, 62.4, 14.2; MS (ES mass): 377.9 (M-1); HPLC: 98.6%, column: X-Bridge C-18 150 x 4.6 mm 5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water, mobile phase B: CH<sub>3</sub>CN, gradient (T/%B): 0/30, 2/30, 8/98, 16/98, 17/30, 20/30; flow rate: 1.0 mL/min; UV 230 nm, retention time 9.07 min.

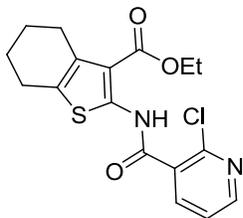
**Typical procedure for preparation of ethyl 2-(2-iodobenzamido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (4a)**



To a solution of compound **S-4a** (100 mg, 0.44 mmol) in dry DCM (5 mL), DIPEA (0.15 mL, 0.88 mmol) was added at 0 °C under nitrogen atmosphere. To this 2-iodo benzoyl chloride (0.09 mL, 0.66 mmol) was slowly added and the reaction mixture stirred at room temperature for 12 h. After completion of reaction, the reaction mixture diluted with DCM (5 mL), washed with saturated NaHCO<sub>3</sub> solution (15 mL), followed by brine solution (10 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated under reduced pressure. The residue was purified by column chromatography using ethyl acetate – hexane to give desired compound **4a**.

Yield: 82% (165 mg); Light yellow solid; mp:109-111 °C;  $R_f$  = 0.2 (20% EtOAc/*n*-hexane); IR (KBr, cm<sup>-1</sup>): 3228, 2933, 1725, 1663; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 11.63 (bs, 1H), 7.94 (d,  $J$  = 8.0 Hz, 1H), 7.54 (d,  $J$  = 7.8 Hz, 1H), 7.43 (t,  $J$  = 7.4 Hz, 1H), 7.16 (t,  $J$  = 7.4 Hz, 1H), 4.31 (q,  $J$  = 7.0 Hz, 2H), 2.79 (t,  $J$  = 5.4 Hz, 2H), 2.69 (t,  $J$  = 5.4 Hz, 2H), 1.86-1.78 (m, 4H), 1.36 (t,  $J$  = 7.0 Hz, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$ : 166.5, 165.3, 146.9, 140.6, 140.5, 139.9, 131.9, 131.5, 128.5, 127.5, 112.5, 92.8, 60.6, 26.4, 24.4, 22.9, 22.8, 14.2; MS (ES mass): 455.7 (M+1); HPLC: 93.3%, column: X Bride C-18 150\*4.6mm 5 $\mu$ , mobile phase A: 5mm Ammonium acetate in water, mobile phase B: CH<sub>3</sub>CN, gradient T/B%: 0/20,2/20, 9/95, 15/95, 17/20,20/20; flow rate: 1.0 mL/min; UV 210 nm, retention time 12.2 min.

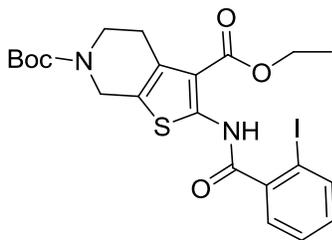
**Ethyl-2-(2-chloronicotinamido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (4b)**



Compound **4b** was synthesized from the reaction of **S-4a** and **S-5d** following a procedure similar to that of compound **4a**.

Yield: 77% (124 mg); Yellow solid; mp: 112-114 °C;  $R_f$  = 0.2 (40% EtOAc/*n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3239, 2945, 1730, 1658;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 12.22 (bs, 1H), 8.51 (s, 1H), 8.26-8.10 (m, 1H), 7.40-7.36 (m, 1H), 4.35-4.29 (m, 2H), 2.79 (bs, 2H), 2.68 (bs, 2H), 1.80 (bs, 4H), 1.37-1.34 (m, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 166.0, 161.0, 151.4, 147.4, 145.9, 139.8, 131.2, 129.7, 127.6, 122.5, 112.9, 60.5, 26.2, 24.2, 22.7, 22.6, 14.1; MS (ES mass): 364.8 (M+1); HPLC: 95.1%, column: Symmetry C-18 75\*4.6 mm 3.5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water, mobile phase B:  $\text{CH}_3\text{CN}$ , gradient T/B%: 0/50, 1/50, 4/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 210 nm, retention time 5.56 min.

**6-tert-butyl-3-ethyl-2-(2-iodobenzamido)-4,5-dihydrothieno[2,3-c]pyridine-3,6(7H)-dicarboxylate (4c)**

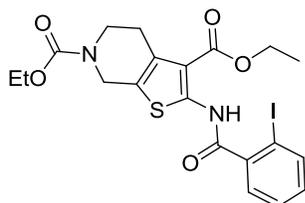


Compound **4c** was synthesized from the reaction of **S-4b** and **S-5a** following a procedure similar to that of compound **4a**.

Yield: 81% (138 mg); Light yellow solid; mp: 157-159 °C;  $R_f$  = 0.2 (35% EtOAc/*n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3252, 2924, 1731, 1687;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 11.62 (s, 1H), 7.96 (d,  $J$  = 7.9 Hz, 1H), 7.55 (dd,  $J$  = 7.5, 1.0 Hz, 1H), 7.45 (d,  $J$  = 7.7 Hz, 1H), 7.18 (d,  $J$  = 7.6 Hz, 1H), 4.55 (s, 2H), 4.33 (q,  $J$  = 7.2 Hz, 2H), 3.67 (t,  $J$  = 5.6 Hz, 2H), 2.91 (bs, 2H), 1.49 (s, 9H), 1.37 (t,  $J$  = 7.1 Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 166.0, 165.4, 154.6, 147.7, 140.7, 140.5, 139.5, 132.2, 128.8, 128.6, 128.3, 112.0, 92.7, 80.2, 60.8, 42.8, 42.7, 28.5 (3C), 28.3, 14.1; MS

(ES mass): 554.9 (M-1); HPLC: 94.1%, column: X-Bridge C-18 150\*4.6 mm 5 $\mu$ , mobile phase A: 5mm ammonium acetate in water, mobile phase B: CH<sub>3</sub>CN, gradient T/B%: 0/20, 2/20, 9/95, 15/95, 17/20, 20/20; flow rate: 1.0 mL/min; UV 210 nm, retention time 11.8 min.

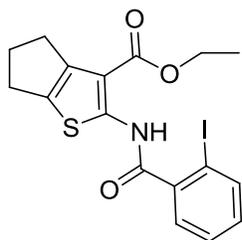
**Diethyl-2-(2-iodobenzamido)-4,5-dihydrothieno[2,3-*c*]pyridine-3,6(7*H*)-dicarboxylate (4d)**



Compound **4d** was synthesized from the reaction of **S-4c** and **S-5a** following a procedure similar to that of compound **4a**.

Yield: 80% (141 mg); White solid; mp: 112-114 °C;  $R_f$  = 0.2 (35% EtOAc/*n*-hexane); IR (KBr, cm<sup>-1</sup>): 3213, 2935, 1736, 1669; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 11.55 (s, 1H), 7.89 (d,  $J$  = 7.9 Hz, 1H), 7.48 (dd,  $J$  = 7.6, 1.4 Hz, 1H), 7.39 (t,  $J$  = 7.5 Hz, 1H), 7.11 (dt,  $J$  = 7.7, 1.4 Hz, 1H), 4.53 (s, 2H), 4.26 (q,  $J$  = 7.1 Hz, 2H), 4.12 (q,  $J$  = 7.1 Hz, 2H), 3.65 (t,  $J$  = 5.1 Hz, 2H), 2.86 (s, 2H), 1.30 (t,  $J$  = 7.1 Hz, 3H), 1.22 (t,  $J$  = 7.1 Hz, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$ : 165.9 (2C), 165.3, 155.3, 147.7, 140.5 (2C), 139.3, 131.9, 128.5 (2C), 128.2, 92.6, 61.5, 60.7, 42.5 (2C), 29.5, 14.5, 14.1; MS (ES mass): 528.7 (M+1); HPLC: 95.5%, column: Symmetry C-18 75\*4.6mm 3.5 $\mu$ , mobile phase A: 0.1% Formic acid in water, mobile phase B: CH<sub>3</sub>CN, gradient T/B%: 0/50, 1/50, 3/98, 10/98, 10.5/50,12/50; flow rate: 1.0 mL/min; UV 210 nm, retention 4.73 min.

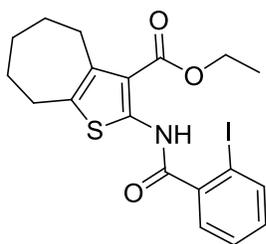
**Ethyl-2-(2-iodobenzamido)-5,6-dihydro-4*H*-cyclopenta[b]thiophene-3-carboxylate (4e)**



Compound **4e** was synthesized from the reaction of **S-4d** and **S-5a** following a procedure similar to that of compound **4a**.

Yield: 80% (141 mg); Light brown liquid;  $R_f = 0.4$  (25% EtOAc/*n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3230, 2935, 1736, 1670;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 11.7 (bs, 1H), 7.81-7.78 (m, 1H), 7.49-7.35 (m, 3H), 4.30 (q,  $J = 7.1$  Hz, 2H), 2.94-2.86 (m, 4H), 2.43-2.36 (m, 2H), 1.35 (t,  $J = 7.1$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 165.9, 165.1, 150.8, 142.2, 141.6, 140.5, 139.6, 132.9, 132.4, 128.4, 109.2, 92.9, 60.6, 30.3, 28.9, 28.0, 14.3; MS (ES mass): 441.7 (M+1); HPLC: 91.2%, column: X Bristle C-18 150\*4.6mm 5 $\mu$ , mobile phase A: 5mM Ammonium acetate in water, mobile phase B:  $\text{CH}_3\text{CN}$ , gradient T/B%: 0/20,2/20, 9/95, 15/95, 17/20,20/20; flow rate: 1.0 mL/min; UV 210 nm, retention time 1.69 min.

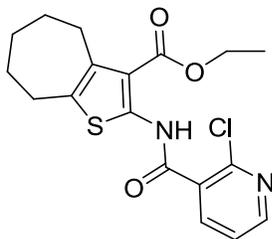
**Ethyl-2-(2-iodobenzamido)-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene-3-carboxylate (4f)**



Compound **4f** was synthesized from the reaction of **S-4e** and **S-5a** following a procedure similar to that of compound **4a**.

Yield: 76% (149 mg); Light brown solid; mp: 96-98 °C;  $R_f = 0.2$  (20% EtOAc/*n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3236, 2986, 1732, 1658;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 11.52 (bs, 1H), 7.95 (d,  $J = 7.6$  Hz, 1H), 7.53 (d,  $J = 7.4$  Hz, 1H), 7.44 (t,  $J = 7.6$  Hz, 1H), 7.17 (t,  $J = 7.6$  Hz, 1H), 4.33 (q,  $J = 7.2$  Hz, 2H), 3.07 (t,  $J = 5.6$  Hz, 2H), 2.77 (t,  $J = 5.6$  Hz, 2H), 1.89-1.84 (m, 2H), 1.72-1.61 (m, 4H), 1.37 (t,  $J = 7.2$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 166.5, 165.3, 144.8, 140.7, 140.5, 139.9, 136.8, 131.8, 131.7, 128.4, 113.8, 92.9, 60.8, 32.2, 28.7, 28.3, 27.8, 26.9, 14.2; MS (ES mass): 469.7 (M+1); HPLC: 94.2%, column: Symmetry C-18 75\*4.6mm 3.5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water, mobile phase B:  $\text{CH}_3\text{CN}$ , gradient T/B%: 0/50, 1/50, 3/98, 10/98, 10.5/50,12/50; flow rate: 1.0 mL/min; UV 210 nm, retention time 5.4 min.

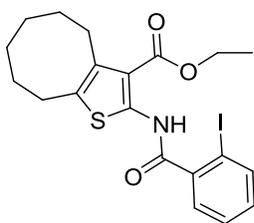
**Ethyl-2-(2-chloronicotinamido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (4g)**



Compound **4g** was synthesized from the reaction of **S-4e** and **S-5d** following a procedure similar to that of compound **4a**.

Yield: 72% (113 mg); Light brown liquid;  $R_f = 0.2$  (50% EtOAc/*n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3189, 2919, 1725, 1662;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 12.08 (s, 1H), 8.54 (dd,  $J = 4.7, 1.8$  Hz, 1H), 8.18 (dd,  $J = 7.6, 1.8$  Hz, 1H), 7.40 (dd,  $J = 7.6, 4.7$  Hz, 1H), 4.37 (q,  $J = 7.1$  Hz, 2H), 3.09-3.06 (m, 2H), 2.78-2.76 (m, 2H), 1.89-1.84 (m, 2H), 1.71-1.61 (m, 4H), 1.39 (t,  $J = 7.1$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 166.3, 161.2, 151.5, 147.6, 143.9, 140.0, 137.0, 132.1, 130.0, 122.7, 114.4, 60.9, 32.2, 28.6, 28.2, 27.7, 26.9, 14.2; MS (ES mass): 378.8 (M+1); HPLC: 95.4%, column: Symmetry C-18 75\*4.6mm 3.5 $\mu$ , mobile phase A: 0.1% Formic acid in water, mobile phase B:  $\text{CH}_3\text{CN}$ , gradient T/B%: 0/50, 1/50, 4/98, 10/98, 10.5/50, 12/50; flow rate: 1.0 mL/min; UV 210 nm, retention time 5.56 min.

#### **Ethyl-2-(2-iodobenzamido)-4,5,6,7,8,9-hexahydrocycloocta[b]thiophene-3-carboxylate (4h)**

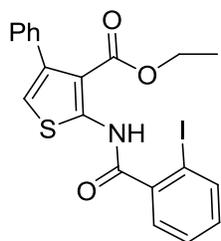


Compound **4h** was synthesized from the reaction of **S-4f** and **S-5a** following a procedure similar to that of compound **4a**.

Yield: 72% (137 mg); Light brown liquid;  $R_f = 0.2$  (10% EtOAc/*n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3242, 2912, 1730, 1669;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 11.70 (s, 1H), 7.95 (d,  $J = 7.9$  Hz, 1H), 7.55 (dd,  $J = 7.6, 1.2$  Hz, 1H), 7.44 (t,  $J = 7.5$  Hz, 1H), 7.19-7.13 (m, 1H), 4.33 (q,  $J = 7.1$  Hz, 2H), 2.92 (t,  $J = 6.2$  Hz, 2H), 2.77 (t,  $J = 6.2$  Hz, 2H), 1.66 (d,  $J = 5.4$  Hz, 4H), 1.52-1.45 (m, 2H), 1.37 (t,  $J = 7.1$  Hz, 3H), 1.34-1.27 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 166.4, 165.2, 146.4, 143.4, 140.6, 139.8, 133.6, 131.9, 128.5, 128.3, 112.7, 92.8, 60.7, 32.3, 29.8, 26.8, 26.4,

25.5, 25.4, 14.1; MS (ES mass): 483.7 (M+1); HPLC: 94.6%, column: X Bridge C-18 150\*4.6 mm, 5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water, mobile phase B: CH<sub>3</sub>CN, gradient T/B%: 0/40, 2/40, 9/98, 16/98, 17/40,20/40; flow rate: 1.0 mL/min; UV 210 nm, retention time 12.28 min.

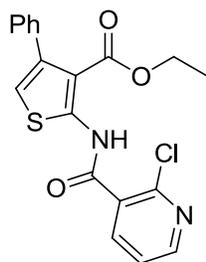
#### Ethyl-2-(2-iodobenzamido)-4-phenylthiophene-3-carboxylate (**4i**)



Compound **4i** was synthesized from the reaction of **S-4g** and **S-5a** following a procedure similar to that of compound **4a**.

Yield: 74% (142 mg); Brown solid; mp:147-149 °C;  $R_f$  = 0.2 (50% EtOAc/*n*-hexane); IR (KBr, cm<sup>-1</sup>): 3198, 2925, 1732, 1655; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$ : 11.68 (bs, 1H), 7.98 (d,  $J$  = 8.1 Hz, 1H), 7.59 (d,  $J$  = 6.9 Hz, 1H), 7.47 (t,  $J$  = 7.3 Hz, 1H), 7.36-7.28 (m, 5H), 7.24-7.16 (m, 1H), 6.70 (s, 1H), 4.06 (q,  $J$  = 7.3 Hz, 2H), 0.92 (t,  $J$  = 7.1 Hz, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$ : 165.8, 165.6, 149.4, 142.0, 140.6, 140.0, 137.5, 132.0, 129.0 (2C), 128.5, 128.3, 127.2 (2C), 126.9, 115.5, 112.4, 92.7, 60.5, 13.3; MS (ES mass): 477.7 (M+1); HPLC: 99.2%, column: Symmetry C-18 75\*4.6mm 3.5 $\mu$ , mobile phase A: 0.1% Tri fluoro acetic acid in water, mobile phase B: CH<sub>3</sub>CN, gradient T/B%: 0/50,0.5/50, 4/98, 10/98, 10.5/50,12/50; flow rate: 1.0 mL/min; UV 220 nm, retention time 5.46 min.

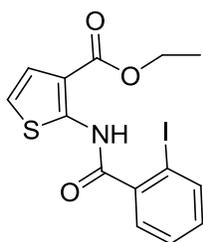
#### Ethyl-2-(2-chloronicotinamido)-4-phenylthiophene-3-carboxylate (**4j**)



Compound **4j** was synthesized from the reaction of **S-4g** and **S-5d** following a procedure similar to that of compound **4a**.

Yield: 75% (117 mg); white solid; mp:165-167 °C;  $R_f$  = 0.2 (70% EtOAc/*n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3211, 2935, 1724, 1663;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 12.28 (bs, 1H), 8.58 (dd,  $J$  = 4.7, 1.9 Hz, 1H), 8.26 (dd,  $J$  = 7.6, 1.9 Hz, 1H), 7.44 (dd,  $J$  = 7.6, 4.7 Hz, 1H), 7.40-7.29 (m, 5H), 6.74 (s, 1H), 4.11 (q,  $J$  = 7.1 Hz, 2H), 0.94 (t,  $J$  = 7.1 Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 165.7, 161.7, 151.8, 148.8, 147.7, 140.3, 140.2, 137.6, 129.6, 129.1 (2C), 127.4 (2C), 127.1, 122.8, 115.9, 113.2, 60.7, 13.4; MS (ES mass): 386.8 (M+1); HPLC: 97.2%, column: Symmetry C-18 75\*4.6mm 3.5 $\mu$ , mobile phase A: 0.1% Formic acid in water, mobile phase B:  $\text{CH}_3\text{CN}$ , gradient T/B%: 0/50,1/50,3/98, 10/98, 10.5/50,12/50; flow rate: 1.0 mL/min; UV 210 nm, retention time 4.53 min.

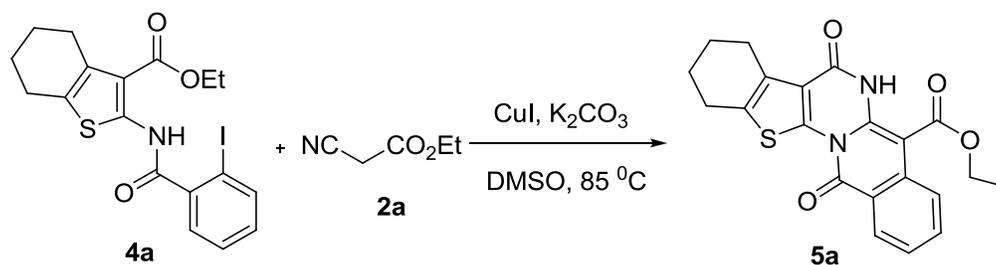
#### Ethyl-2-(2-iodobenzamido)thiophene-3-carboxylate (4k)



Compound **4k** was synthesized from the reaction of **S-4h** and **S-5a** following a procedure similar to that of compound **4a**.

Yield: 76% (178 mg); Light brown liquid;  $R_f$  = 0.2 (50% EtOAc/*n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3269, 2925, 1731,1670;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 11.39 (bs, 1H), 7.97 (d,  $J$  = 7.9 Hz, 1H), 7.57 (dd,  $J$  = 7.6, 1.5 Hz, 1H), 7.48-7.44 (m, 1H), 7.28 (d,  $J$  = 5.7 Hz, 1H), 7.19 (tb,  $J$  = 7.6, 1.6 Hz, 1H), 6.83 (d,  $J$  = 5.7 Hz, 1H), 4.38 (q,  $J$  = 7.2 Hz, 2H), 1.38 (t,  $J$  = 7.1 Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ :165.5, 165.4, 148.1, 142.1, 139.4, 134.1, 128.7, 128.4, 124.0, 116.5, 113.8, 92.8, 60.8, 14.3; MS (ES mass): 401.7 (M+1); HPLC: 98.4%, column: X-Bridge C-18 150\*4.6 mm 5 $\mu$ , mobile phase A: 0.1 % Formic Acid in water, mobile phase B:  $\text{CH}_3\text{CN}$ , gradient T/B%: 0/20, 2/20, 9/98, 16/98, 17/20,20/20; flow rate: 1.0 mL/min; UV 220 nm, retention time 10.5 min.

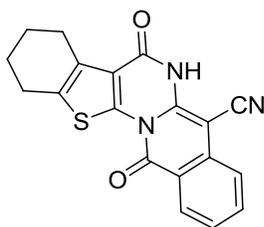
#### Typical procedure for preparation of compound 5a



A mixture of compound **4a** (100 mg, 0.22 mmol), K<sub>2</sub>CO<sub>3</sub> (91 mg, 0.66 mmol), ethyl cyanoacetate (**2a**) (0.03 mL, 0.31 mmol) and CuI (4.1 mg, 0.022 mmol) in DMSO (2 mL) was heated to 85 °C under anhydrous conditions (CaCl<sub>2</sub> filled guard tube) for 6 h. After completion of the reaction, reaction mixture was cooled to RT, diluted with ethyl acetate (15 mL) and passed through celite. The resulting solution was washed with water (3 x 15 mL) followed by brine solution (25 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated under reduced pressure. The residue was purified by column chromatography using ethyl acetate–hexane to give desired compound **5a**.

White solid; mp: 232-234 °C; R<sub>f</sub> = 0.2 (20% EtOAc/*n*-hexane); IR (KBr, cm<sup>-1</sup>): 3096, 2932, 1680, 1638, 1586; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 12.92 (s, 1H), 8.56 (d, *J* = 8.7 Hz, 1H), 8.50 (dd, *J* = 8.3, 1.2 Hz, 1H), 7.73 (t, *J* = 7.2 Hz, 1H), 7.44 (t, *J* = 8.4 Hz, 1H), 4.55 (q, *J* = 7.1 Hz, 2H), 3.05 (dd, *J* = 7.6, 3.5 Hz, 2H), 2.81 (t, *J* = 5.2 Hz, 2H), 1.94-1.82 (m, 4H), 1.51 (t, *J* = 7.1 Hz, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ: 168.4, 158.5, 155.3, 143.6, 141.5, 134.7, 134.1, 134.0, 130.8, 128.6, 125.3, 125.0, 118.8, 118.7, 86.5, 61.9, 24.8, 23.8, 22.6, 22.1, 14.3; MS (ES mass): 394.9 (M+1).

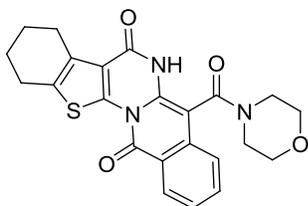
### Compound **5b**



Compound **5b** was synthesized from the reaction of **4a** and **2c** following a procedure similar to that of compound **5a**.

White solid; mp: 303-308 °C;  $R_f = 0.2$  (40% EtOAc/*n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3177, 2934, 2207, 1678, 1606;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$ : 8.29-8.27 (m, 2H), 7.87 (t,  $J = 7.6$  Hz, 1H), 7.66 (d,  $J = 7.8$  Hz, 1H), 7.49 (t,  $J = 7.6$  Hz, 1H), 2.91-2.86 (m, 2H), 2.77-2.74 (m, 2H), 1.83-1.74 (m, 4H); MS (ES mass): 345.9 (M-1).

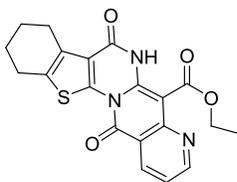
### Compound 5c



Compound **5c** was synthesized from the reaction of **4a** and **2d** following a procedure similar to that of compound **5a**.

Yellow solid; mp: 276-278 °C;  $R_f = 0.2$  (60% EtOAc/*n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3272, 2941, 1680, 1605;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 9.45(s, 1H), 8.52 (d,  $J = 8.4$  Hz, 1H), 7.73 (t,  $J = 7.2$  Hz, 1H), 7.77-7.69 (m, 2H), 4.05-3.15 (m, 8H), 3.08-2.98 (m, 2H), 2.80- 2.04 (m, 2H), 1.95-1.80 (m, 4H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 165.8, 158.2, 155.2, 141.7, 136.6, 134.2, 134.1, 133.1, 131.0, 129.0, 125.3, 122.4, 118.7, 117.8, 91.7, 66.8 (2C), 46.4, 43.7, 24.6, 23.9, 22.7, 22.1; MS (ES mass): 433.9 (M-1).

### Compound 5d

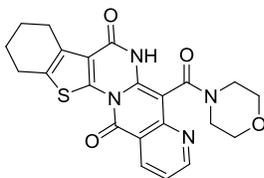


Compound **5d** was synthesized from the reaction of **4b** and **2a** following a procedure similar to that of compound **5a**.

Yellow solid; mp: 247-249 °C;  $R_f = 0.2$  (50% EtOAc/*n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3186, 2932, 1688, 1632, 1589;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 12.43 (s, 1H), 9.02-8.99 (m, 1H), 8.69 (dd,  $J = 7.2, 1.4$  Hz, 1H), 7.37-7.34 (m, 1H), 4.57 (d,  $J = 7.1$  Hz, 2H), 3.04 (t,  $J = 4.8$  Hz, 2H), 2.81 (t,  $J = 4.8$  Hz, 2H), 1.93-1.84 (m, 4H), 1.48 (t,  $J = 7.1$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 168.0, 158.5, 155.6, 155.0, 150.6, 144.6, 141.1, 136.5, 134.8, 131.0, 120.1, 118.8, 114.2, 89.7,

62.1, 24.8, 23.9, 22.6, 22.0, 14.2; MS (ES mass): 395.8 (M+1); HRMS (ESI): calcd for  $C_{20}H_{18}N_3O_4S$  (M+H)<sup>+</sup> 396.1013, found 396.1010.

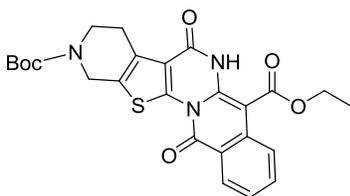
### Compound 5e



Compound **5e** was synthesized from the reaction of **4b** and **2d** following a procedure similar to that of compound **5a**.

Brown solid; mp: 282-284 °C;  $R_f = 0.2$  (80% EtOAc/*n*-hexane); IR (KBr,  $cm^{-1}$ ): 3262, 2951, 1686, 1612;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$ : 9.77 (s, 1H), 8.96-8.89 (m, 1H), 8.72 (d,  $J=8.4$  Hz 1H), 7.38-7.31 (m, 1H), 4.14-3.70 (m, 5H), 3.57-3.30 (m, 2H), 3.28-2.93 (m, 3H), 2.87-2.75 (m, 2H), 1.95-1.83 (m, 4H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$ : 165.8, 158.2, 155.6, 141.7, 136.6, 134.1, 133.1, 131.0, 129.0, 125.3, 122.4, 118.6, 117.7, 91.7, 66.8 (2C), 44.6, 41.9, 24.9, 23.9, 22.7, 22.1; MS (ES mass): 436.8 (M+1); HRMS (ESI): calcd for  $C_{22}H_{21}N_4O_4S$  (M+H)<sup>+</sup> 437.1284, found 437.1278.

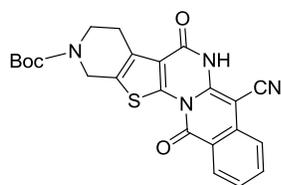
### Compound 5f



Compound **5f** was synthesized from the reaction of **4c** and **2a** following a procedure similar to that of compound **5a**.

White solid; mp: 160-163 °C;  $R_f = 0.2$  (50% EtOAc/*n*-hexane); IR (KBr,  $cm^{-1}$ ): 3091, 2928, 1689, 1648, 1593;  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$ : 12.98 (s, 1H), 8.57 (d,  $J = 8.0$  Hz, 1H), 8.48 (d,  $J = 7.9$  Hz, 1H), 7.79-7.71 (m, 1H), 7.50-7.41 (m, 1H), 4.68 (s, 2H), 4.56-4.52 (m, 2H), 3.73 (bs, 2H), 3.14 (bs, 2H), 1.57 (bs, 3H), 1.51 (s, 9H);  $^{13}C$  NMR (100 MHz,  $CDCl_3$ )  $\delta$ : 168.4, 158.5, 155.1 (2C), 143.4, 138.7 (2C), 138.6, 137.2, 134.3, 133.9, 128.7, 125.4, 125.3, 118.6, 87.0, 80.4, 62.1, 42.1, 42.0, 29.6, 28.4 (3C), 14.3; MS (ES mass): 494.0 (M-1).

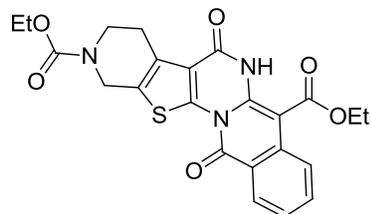
### Compound 5g



Compound **5g** was synthesized from the reaction of **4c** and **2c** following a procedure similar to that of compound **5a**.

Brown solid; mp: 145-147 °C;  $R_f = 0.2$  (50% EtOAc/*n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3186, 2925, 2215, 1665, 1569;  $^1\text{H}$  NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$ : 8.32-8.30 (m, 1H), 7.91 (t,  $J = 7.9$  Hz, 1H), 7.83 (s, 1H), 7.70 (d,  $J = 7.6$  Hz, 1H), 7.57-7.50 (m, 1H), 4.64 (s, 2H), 3.67-3.60 (m, 2H), 3.00-2.94 (m, 2H), 1.44 (s, 9H); MS (ES mass): 447.0 (M-1);

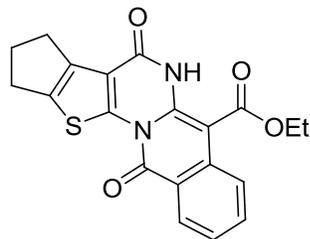
### Compound 5h



Compound **5h** was synthesized from the reaction of **4d** and **2a** following a procedure similar to that of compound **5a**.

Brown solid; mp: 190-192 °C;  $R_f = 0.2$  (50% EtOAc/*n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3095, 2935, 1682, 1648, 1589;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 12.96 (s, 1H), 8.55 (d,  $J = 8.6$  Hz, 1H), 8.46 (dd,  $J = 8.1, 1.2$  Hz, 1H), 7.77-7.69 (m, 1H), 7.43 (t,  $J = 7.5$  Hz, 1H), 4.72 (s, 2H), 4.55 (q,  $J = 7.1$  Hz, 2H), 4.21 (q,  $J = 7.1$  Hz, 2H), 3.78 (t,  $J = 5.2$  Hz, 2H), 3.15 (s, 2H), 1.52 (t,  $J = 7.1$  Hz, 3H), 1.32 (d,  $J = 7.1$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 168.3, 158.3, 155.5, 154.9, 143.3, 142.3, 134.2 (2C), 133.8, 128.6, 125.4 (2C), 125.2, 118.5, 118.1, 86.9, 62.1, 61.8, 42.2 (2C), 29.6, 14.6, 14.3; MS (ES mass): 395.9 (M-CO<sub>2</sub>Et+1).

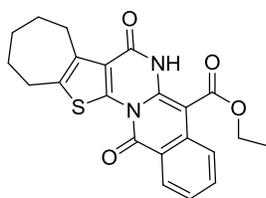
### Compound 5i



Compound **5i** was synthesized from the reaction of **4e** and **2a** following a procedure similar to that of compound **5a**.

Light brown solid; mp: 216-219 °C;  $R_f = 0.2$  (30% EtOAc/*n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3081, 2993, 1682, 1650;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 13.00 (s, 1H), 8.55 (d,  $J = 8.7$  Hz, 1H), 8.48 (d,  $J = 7.9$  Hz, 1H), 7.75-7.68 (m, 1H), 7.43 (t,  $J = 7.5$  Hz, 1H), 4.56 (q,  $J = 7.1$  Hz, 2H), 3.11 (t,  $J = 7.2$  Hz, 2H), 3.00 (t,  $J = 7.2$  Hz, 2H), 2.57-2.48 (m, 2H), 1.52 (t,  $J = 7.1$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 168.4, 158.4, 155.0, 145.4, 143.7, 141.5, 140.2, 133.9, 133.8, 128.5, 125.3, 125.0, 118.6, 116.0, 86.8, 62.0, 28.9, 28.7 (2C), 14.3; MS (ES mass): 378.9 (M-1).

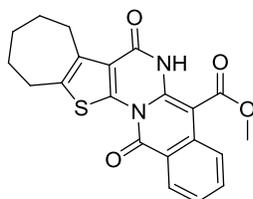
### Compound **5j**



Compound **5j** was synthesized from the reaction of **4f** and **2a** following a procedure similar to that of compound **5a**.

White fluffy solid; mp: 188-189 °C;  $R_f = 0.2$  (30% EtOAc/*n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3141, 2974, 1682, 1632, 1588;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 12.91 (s, 1H), 8.54 (d,  $J = 8.6$  Hz, 1H), 8.45 (d,  $J = 8.5$  Hz, 1H), 7.69 (t,  $J = 7.8$  Hz, 1H), 7.40 (t,  $J = 7.6$  Hz, 1H), 4.54 (q,  $J = 7.1$  Hz, 2H), 3.42-3.35 (m, 2H), 2.91-2.82 (m, 2H), 1.98-1.88 (m, 2H), 1.80-1.64 (m, 4H), 1.52 (t,  $J = 7.1$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 168.4, 158.5, 155.8, 143.4, 139.7, 139.1, 136.3, 134.0, 133.9, 128.5, 125.2, 124.9, 119.1, 118.6, 86.2, 61.9, 32.5, 28.6, 27.8, 27.2, 27.0, 14.3; MS (ES mass): 408.9 (M+1); HRMS (ESI): calcd for  $\text{C}_{22}\text{H}_{21}\text{N}_2\text{O}_4\text{S}$  (M+H) $^+$  409.1222, found 409.1204.

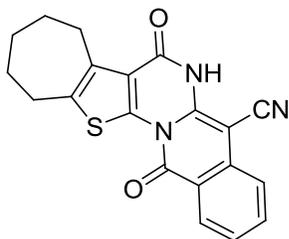
### Compound **5k**



Compound **5k** was synthesized from the reaction of **4f** and **2b** following a procedure similar to that of compound **5a**.

Light yellow; mp: 239-243°C;  $R_f = 0.2$  (30% EtOAc/*n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3081, 2926, 1682, 1650;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 12.89 (s, 1H), 8.48 (t,  $J = 8.5$  Hz, 2H), 7.71 (t,  $J = 7.6$  Hz, 1H), 7.42 (t,  $J = 7.6$  Hz, 1H), 4.06 (s, 3H), 3.41-3.35 (m, 2H), 2.91-2.84 (m, 2H), 1.95-1.89 (m, 2H), 1.77-1.67 (m, 4H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 168.8, 158.4, 155.7, 143.5, 139.7, 139.2, 136.3, 134.0, 133.7, 128.5, 125.2, 125.0, 119.1, 118.5, 86.0, 52.4, 32.5, 28.6, 27.8, 27.2, 27.0; MS (ES mass): 392.9 (M-1); HRMS (ESI): calcd for  $\text{C}_{21}\text{H}_{19}\text{N}_2\text{O}_4\text{S}$  (M+H) $^+$  395.1066, found 395.1068.

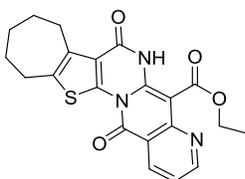
### Compound 5l



Compound **5l** was synthesized from the reaction of **4f** and **2c** following a procedure similar to that of compound **5a**.

White solid; mp: 311-313 °C;  $R_f = 0.2$  (40% EtOAc/*n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3185, 2916, 2849, 2209, 1661, 1548;  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$ : 8.32 (bs, 1H), 8.25 (d,  $J = 8.0$  Hz, 1H), 7.87 (t,  $J = 7.8$  Hz, 1H), 7.65 (d,  $J = 8.0$  Hz, 1H), 7.50 (t,  $J = 7.8$  Hz, 1H), 3.28-3.26 (m, 2H), 2.87-2.82 (m, 2H), 1.89-1.82 (m, 2H), 1.64-1.59 (m, 4H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO-}d_6$ )  $\delta$ : 158.3, 156.9, 144.5, 144.3, 139.9, 138.5, 135.9, 134.3, 128.6, 128.6, 118.8, 118.1 (2C), 115.3, 70.9, 32.4, 28.2, 27.9, 27.2, 26.8; MS (ES mass): 359.9 (M-1); HRMS (ESI): calcd for  $\text{C}_{20}\text{H}_{16}\text{N}_3\text{O}_2\text{S}$  (M+H) $^+$  362.0963, found 362.0949.

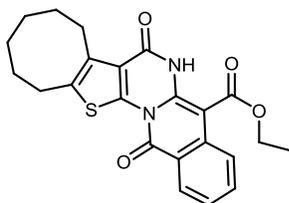
### Compound 5m



Compound **5m** was synthesized from the reaction of **4g** and **2a** following a procedure similar to that of compound **5a**.

Brown solid; mp: 165-167 °C;  $R_f = 0.2$  (60% EtOAc/*n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3075, 2989, 1689, 1643, 1590;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 12.47 (bs, 1H), 9.05-8.97 (m, 1H), 8.73 (d,  $J = 6.8$  Hz, 1H), 7.40-7.33 (m, 1H), 4.57 (q,  $J = 6.8$  Hz, 2H), 3.43-3.35 (m, 2H), 2.93-2.85 (m, 2H), 1.93 (bs, 2H), 1.80-1.65 (m, 4H), 1.48 (t,  $J = 7.1$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 167.7, 158.3, 155.3, 144.3, 139.2, 138.3, 136.7, 136.5, 126.2, 120.0, 119.1, 114.1, 113.8, 89.0, 63.4, 31.7, 28.5, 27.6, 27.0, 26.9, 14.1; MS (ES mass): 409.9 (M+1); HRMS (ESI): calcd for  $\text{C}_{21}\text{H}_{20}\text{N}_3\text{O}_4\text{S}$  (M+H) $^+$  410.1175, found 410.1162.

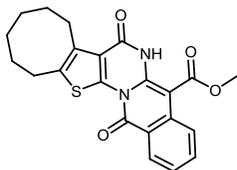
### Compound 5n



Compound **5n** was synthesized from the reaction of **4h** and **2a** following a procedure similar to that of compound **5a**.

Light brown; mp: 200-205 °C;  $R_f = 0.2$  (30% EtOAc/*n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3082, 2986, 1680, 1651, 1589;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 12.91 (s, 1H), 8.53 (d,  $J = 8.6$  Hz, 1H), 8.46 (d,  $J = 7.2$  Hz, 1H), 7.69 (t,  $J = 7.2$  Hz, 1H), 7.40 (t,  $J = 7.4$  Hz, 1H), 4.54 (q,  $J = 7.1$  Hz, 2H), 3.20 (t,  $J = 6.0$  Hz, 2H), 2.88 (t,  $J = 5.6$  Hz, 2H), 1.80-1.67 (m, 4H), 1.52-1.47 (m, 5H), 1.37-1.27 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 168.4, 158.5, 155.3, 143.5, 141.0, 137.8, 134.0, 133.9, 133.5, 128.6, 125.3, 125.0, 118.7 (2C), 86.4, 61.9, 32.2, 29.7, 26.0, 25.9, 25.6, 24.2, 14.3; MS (ES mass): 422.8 (M+1); HRMS (ESI): calcd for  $\text{C}_{23}\text{H}_{23}\text{N}_2\text{O}_4\text{S}$  (M+H) $^+$  423.1379, found 423.1382.

### Compound 5o

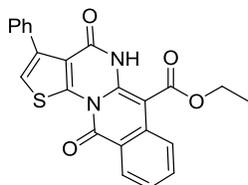


Compound **5o** was synthesized from the reaction of **4h** and **2b** following a procedure similar to that of compound **5a**.

Light yellow; mp: 224-229 °C;  $R_f = 0.2$  (30% EtOAc/*n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3081, 2993, 1682, 1650;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 12.91 (s, 1H), 8.57-8.44 (m, 2H), 7.73 (t,  $J = 7.2$  Hz,

1H), 7.43 (t,  $J = 7.2$  Hz, 1H), 4.08 (s, 3H), 3.19 (t,  $J = 6.4$  Hz, 2H), 2.91 (t,  $J = 6.4$  Hz, 2H), 1.85-1.67 (m, 4H), 1.65-1.43 (m, 2H), 1.41-1.22 (m, 2H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 168.9, 158.6, 155.4, 143.6, 139.2, 137.9, 134.1, 133.8, 133.6, 128.6, 125.4, 125.1, 118.8, 118.7, 86.3, 52.4, 32.2, 29.8, 26.0, 25.9, 25.6, 24.3; MS (ES mass): 408.9 ( $\text{M}+1$ ); HRMS (ESI): calcd for  $\text{C}_{22}\text{H}_{21}\text{N}_2\text{O}_4\text{S}$  ( $\text{M}+\text{H}$ ) $^+$  409.1222, found 409.1223.

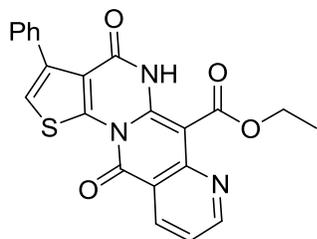
### Compound 5p



Compound **5p** was synthesized from the reaction of **4i** and **2a** following a procedure similar to that of compound **5a**.

Yellow solid; mp: 230-232 °C;  $R_f = 0.2$  (30% EtOAc/*n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3087, 2919, 1689, 1644, 1592;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 12.99 (s, 1H), 8.63-8.57 (m, 1H), 8.57-8.50 (m, 1H), 7.79-7.74 (m, 1H), 7.57-7.54 (m, 2H), 7.49-7.41 (m, 4H), 7.21 (s, 1H), 4.57 (d,  $J = 7.1$  Hz, 2H), 1.52 (t,  $J = 7.1$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 168.2, 158.7, 154.5, 144.8, 143.4, 138.7, 134.6, 134.2, 133.9, 129.4 (2C), 128.6, 127.9, 127.7 (2C), 125.3, 125.1, 122.5, 118.5, 117.5, 86.5, 62.0, 14.2; MS (ES mass): 414.9 ( $\text{M}-1$ ).

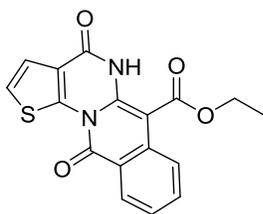
### Compound 5q



Compound **5q** was synthesized from the reaction of **4j** and **2a** following a procedure similar to that of compound **5a**.

Brick red solid; mp: 177-179 °C;  $R_f = 0.2$  (60% EtOAc/*n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3096, 2926, 1680, 1646, 1588;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 12.37 (s, 1H), 9.03 (d,  $J = 2.9$  Hz, 1H), 8.71 (dd,  $J = 8.0, 1.5$  Hz, 1H), 7.54-7.51 (m, 2H), 7.45-7.37 (m, 4H), 7.20 (s, 1H), 4.56 (q,  $J = 7.1$  Hz, 2H), 1.47 (t,  $J = 7.1$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 167.6, 158.8, 155.7, 154.1, 150.5, 144.4, 144.3, 138.9, 136.5, 134.3, 130.8, 129.4 (2C), 127.9, 127.7 (2C), 122.4, 120.3, 117.6, 89.7, 62.1, 14.1; MS (ES mass): 417.8 ( $\text{M}+1$ ).

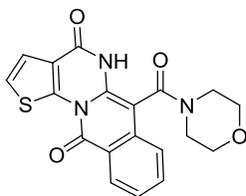
### Compound 5r



Compound **5r** was synthesized from the reaction of **4k** and **2a** following a procedure similar to that of compound **5a**.

White fluffy solid; mp: 194-197° C;  $R_f = 0.2$  (40% EtOAc/*n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3109, 2924, 1681, 1632, 1588;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 13.07 (s, 1H), 8.55 (d,  $J = 8.5$  Hz, 1H), 8.49 (dd,  $J = 8.0, 1.0$  Hz, 1H), 7.74-7.70 (m, 1H), 7.59 (d,  $J = 5.8$  Hz, 1H), 7.44 (t,  $J = 7.2$  Hz, 1H), 7.32 (d,  $J = 5.8$  Hz, 1H), 4.56 (q,  $J = 7.1$  Hz, 2H), 1.53 (t,  $J = 7.1$  Hz, 3H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$ : 168.3, 158.2, 154.8, 143.6, 143.2, 134.2, 133.7, 128.5, 125.3, 125.2, 124.6, 121.1, 121.0, 118.5, 87.0, 62.1, 14.3; MS (ES mass): 338.9 (M-1); HRMS (ESI): calcd for  $\text{C}_{17}\text{H}_{13}\text{N}_2\text{O}_4\text{S}$  (M+H) $^+$  341.0596, found 341.0594.

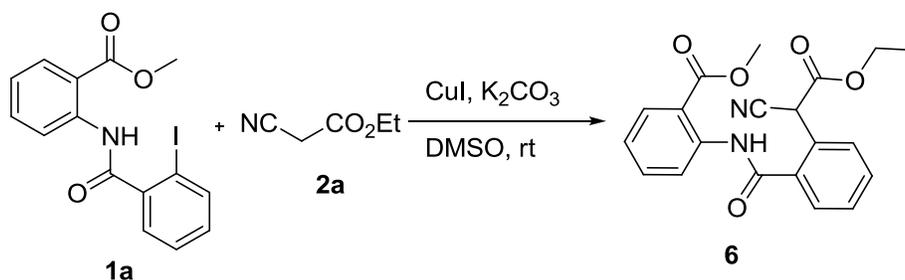
### Compound 5s



Compound **5s** was synthesized from the reaction of **4k** and **2d** following a procedure similar to that of compound **5a**.

Yellow solid; mp: 206-210 °C;  $R_f = 0.2$  (80% EtOAc/*n*-hexane); IR (KBr,  $\text{cm}^{-1}$ ): 3086, 2915, 1678, 1613, 1559;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 8.51 (d,  $J = 8.2$  Hz, 1H), 7.77 (t,  $J = 7.6$  Hz, 1H), 7.57 (d,  $J = 6.0$  Hz, 1H), 7.51-7.45 (m, 2H), 7.30 (d,  $J = 5.8$  Hz, 1H), 4.02-3.38 (m, 8H);  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$ : 163.8, 158.4 (2C), 143.6, 134.6 (2C), 134.1, 128.3 (2C), 125.4, 124.5, 123.2, 121.1, 118.5, 95.1, 66.5, 66.1, 46.9, 42.4; MS (ES mass): 379.9 (M-1); HRMS (ESI): calcd for  $\text{C}_{19}\text{H}_{16}\text{N}_3\text{O}_4\text{S}$  (M+H) $^+$  382.0862, found 382.0846.

### Methyl 2-(2-(1-cyano-2-ethoxy-2-oxoethyl)benzamido)benzoate (**6**)



A mixture of compound **1a** (100 mg, 0.26 mmol), K<sub>2</sub>CO<sub>3</sub> (36 mg, 0.26 mmol), ethyl cyanoacetate (**2a**) (0.04 mL, 0.31 mmol) and CuI (4.9 mg, 0.026 mmol) in DMSO (2 mL) was stirred at room temperature under anhydrous conditions for 2h. Then the reaction mixture was diluted with ethyl acetate (15 mL) and passed through celite. The resulting solution was washed with water (3 x 15 mL) followed by brine solution (25 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated under reduced pressure. The residue was purified by column chromatography using ethyl acetate–hexane to give desired compound **6**.

Light brown liquid; R<sub>f</sub> = 0.4 (20% EtOAc/ *n*-hexane); IR (KBr, cm<sup>-1</sup>): 3265, 2949, 2252, 1748, 1682, 1593; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ: 11.84 (bs, 1H), 8.80 (d, *J* = 8.0 Hz, 1H), 8.09 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.87 (dd, *J* = 7.5, 1.6 Hz, 1H), 7.73 (d, *J* = 7.6 Hz, 1H), 7.65-7.57 (m, 3H), 7.16 (t, *J* = 7.1 Hz, 1H), 6.09 (s, 1H), 4.27-4.19 (m, 2H), 3.94 (s, 3H), 1.24 (t, *J* = 7.15 Hz, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ: 168.9, 166.7, 165.0, 141.2, 134.8, 134.5, 131.8, 130.9, 130.4, 130.2, 129.7, 127.7, 123.2, 120.4, 116.1, 115.5, 63.1, 52.5, 40.3, 13.8; MS (ES mass): 366.9 (M+1); HPLC: 94.5 %, Symmetry C-18 75 x 4.6 mm 3.5μ, mobile phase A: 0.1 % Formic Acid in water, mobile phase B: CH<sub>3</sub>CN, gradient (T/%B): 0/30, 0.5/30, 4/98, 10/98, 10.5/30, 12/30; flow rate: 1.0 mL/min; UV 230 nm, retention time 4.36 min.

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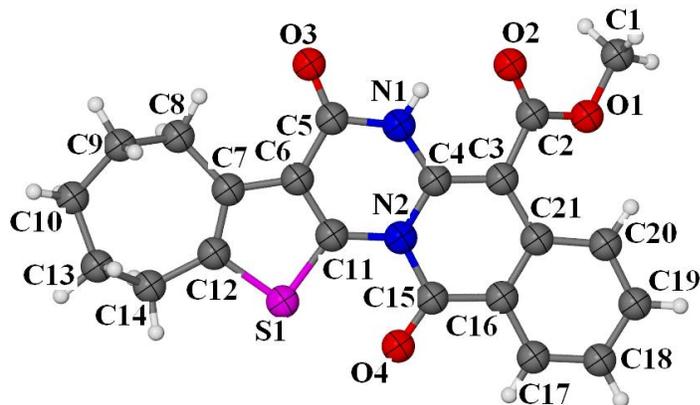
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### Single crystal X-ray data for compound **5k**.

Single crystals suitable for X-ray diffraction of **5k** was grown from methanol. The crystals were carefully chosen using a stereo zoom microscope supported by a rotatable polarizing stage. The data were collected at room temperature on Bruker's KAPPA APEX II CCD Duo with graphite monochromated Mo-K $\alpha$  radiation (0.71073 Å). The crystals were glued to a thin glass fibre using FOMBLIN immersion oil and mounted on the diffractometer. The intensity data were processed using Bruker's suite of data processing programs (SAINT), and absorption corrections were applied using SADABS.<sup>1</sup> The crystal structures were solved by direct methods using SHELXS-97 and refined by full matrix least-squares refinement on  $F^2$  with anisotropic displacement parameters for non-H atoms, using SHELXL-97.<sup>2</sup>

Crystal data of **5k**: Molecular formula = C<sub>21</sub>H<sub>18</sub>N<sub>2</sub>O<sub>4</sub>S, formula weight = 394.44, crystal system = monoclinic, space group =  $P2_1/c$ ,  $a = 7.09$  (1) Å,  $b = 12.017$  (16) Å,  $c = 20.97$  (3) Å,  $V = 1783$  (4) Å<sup>3</sup>,  $T = 296$  K,  $Z = 4$ ,  $D_c = 1.469$  Mg m<sup>-3</sup>,  $\mu(\text{Mo-K}\alpha) = 0.21$  mm<sup>-1</sup>, 17226 reflections

measured, 4329 independent reflections, 2496 observed reflections [ $I > 2.0 \sigma(I)$ ],  $R_{1\_obs} = 0.071$ , Goodness of fit = 1.04. Crystallographic data (excluding structure factors) for **5k** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC 902761.



**Figure 1.** ORTEP representation of the **5k**. Thermal ellipsoids are drawn at 50% probability level.

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## Pharmacology

### PDE4B protein production and purification

PDE4B1 cDNA was sub-cloned into pFAST Bac HTB vector (Invitrogen) and transformed into DH10Bac (Invitrogen) competent cells. Recombinant bacmids were tested for integration by PCR analysis. Sf9 cells were transfected with bacmid using Lipofectamine 2000 (Invitrogen) according to manufacturer's instructions. Subsequently, P3 viral titer was amplified, cells were infected and 48 h post infection cells were lysed in lysis buffer (50 mM Tris-HCl pH 8.5, 10 mM 2-mercaptoethanol, 1 % protease inhibitor cocktail (Roche), 1 %

NP40). Recombinant His-tagged PDE4B protein was purified as previously described elsewhere.<sup>19a</sup> Briefly, lysate was centrifuged at 10,000 rpm for 10 min at 4 °C and supernatant was collected. Supernatant was mixed with Ni-NTA resin (GE Life Sciences) in a ratio of 4:1 (v/v) and equilibrated with binding buffer (20 mM Tris-HCl pH 8.0, 500 mM-KCl, 5 mM imidazole, 10 mM 2-mercaptoethanol and 10 % glycerol) in a ratio of 2:1 (v/v) and mixed gently on rotary shaker for 1 hour at 4 °C. After incubation, lysate-Ni-NTA mixture was centrifuged at 4,500 rpm for 5 min at 4 °C and the supernatant was collected as the flow-through fraction. Resin was washed twice with wash buffer (20 mM Tris-HCl pH 8.5, 1 M KCl, 10 mM 2-mercaptoethanol and 10% glycerol). Protein was eluted sequentially twice using elution buffers (Buffer I: 20 mM Tris-HCl pH 8.5, 100 mM KCl, 250 mM imidazole, 10 mM 2-mercaptoethanol, 10% glycerol, Buffer II: 20 mM Tris-HCl pH 8.5, 100 mM KCl, 500 mM imidazole, 10 mM 2-mercaptoethanol, 10% glycerol). Eluates were collected in four fractions and analyzed by SDS-PAGE. Eluates containing PDE4B protein were pooled and stored at -80 °C in 50% glycerol until further use.

#### **PDE4 enzymatic assay**

The inhibition of PDE4 enzyme was measured using PDElight HTS cAMP phosphodiesterase assay kit (Lonza) according to manufacturer's recommendations. Briefly, 10 ng of in house purified PDE4B1 or 0.5 ng commercially procured PDE4D2 enzyme was pre-incubated either with DMSO (vehicle control) or compound for 15 min before incubation with the substrate cAMP (5 μM) for 1 hour. The reaction was halted with stop solution and reaction mix was incubated with detection reagent for 10 minutes in dark. Dose response studies were performed at 13 different concentrations ranging from 200 μM to 0.001 μM. Luminescence values (RLUs) were measured by a Multilabel Plate Reader (PerkinElmer 1420 Multilabel Counter). The percentage of inhibition was calculated using the following formula and the IC<sub>50</sub> values were determined by a nonlinear regression analysis from dose response curve using Graphpad Prism software (San Diego, U.S.A). IC<sub>50</sub> values are presented as mean ± SD.

$$\% \text{ inhibition} = \frac{(\text{RLU of vehicle control} - \text{RLU of inhibitor})}{\text{RLU of vehicle control}} \times 100$$

Some of the synthesized compounds were tested for their PDE4B inhibitory potential *in vitro* at 30  $\mu$ M using PDE4B enzyme<sup>7</sup> and rolipram as a reference compound.

**Table 1.** In vitro PDE4B inhibition by compound **3** and **5**

Entry	Compound	% inhibition @ 30 $\mu$ M <sup>a</sup>
1	<b>3a</b>	38.9 $\pm$ 1.32
2	<b>3c</b>	41.1 $\pm$ 2.63
3	<b>3d</b>	14.9 $\pm$ 5.82
4	<b>3f</b>	50.7 $\pm$ 3.46
5	<b>3g</b>	15.1 $\pm$ 1.11
6	<b>3h</b>	27.9 $\pm$ 2.51
7	<b>3i</b>	9.5 $\pm$ 2.52
8	<b>3l</b>	39.6 $\pm$ 1.74
9	<b>3m</b>	15.9 $\pm$ 2.08
10	<b>3n</b>	56.7 $\pm$ 2.12
11	<b>5e</b>	62.3 $\pm$ 1.76

<sup>a</sup>results are average of three experiments.

### Molecular Modeling Studies

**Docking Method:** The docking studies of molecules were performed using the Maestro, version 9.2.<sup>1</sup> The compounds were sketched in 3D format using build panel and LigPrep module was used to produce low-energy conformers and to refine the structural parameters of molecules. The crystal structure of PDE4B (PDB ID: 3D3P)<sup>2</sup> was obtained from the protein data bank. The protein was prepared by giving preliminary treatment like adding hydrogen, adding missing residues, refining the loop with prime and finally minimized by using OPLS-2005 force field. Grids for molecular docking were generated with bound co-crystallized ligand. Compounds were docked using Glide in extra-precision mode,<sup>3</sup> with up to three poses saved per molecule. Ligands were kept flexible by producing the ring conformations and by penalizing non-polar amide bond conformations, whereas the receptor was kept rigid throughout the docking studies. All other parameters of the Glide module were maintained at their default values. The lowest energy

conformation was selected and the ligand interactions (hydrogen bonding and hydrophobic interaction) with the active sites of PDE4B were determined.

**Table 1:** Glide score and contributing parameters

Molecules	GScore	LipophilicEvdW	PhobEn	HBond	LowMW
<b>5e</b>	-7.4	-4.28	-1.90	-0.90	-0.05
<b>3n</b>	-7.3	-4.32	-1.70	-0.70	-0.20

LipophilicEvdW: Chemscore lipophilic pair term and fraction of the total protein-ligand vdw energy

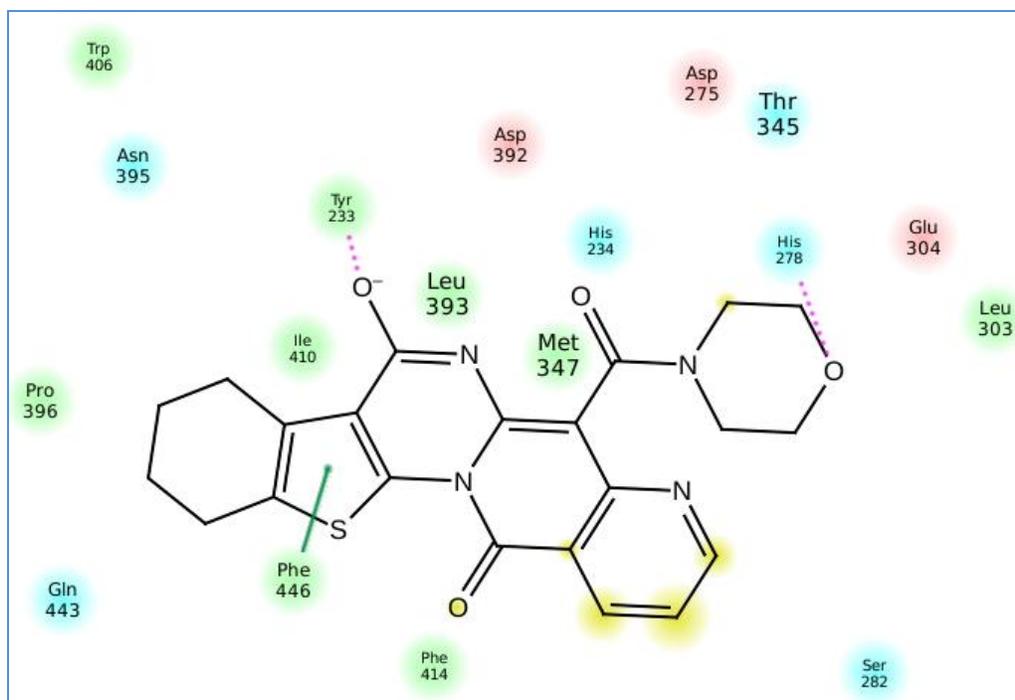
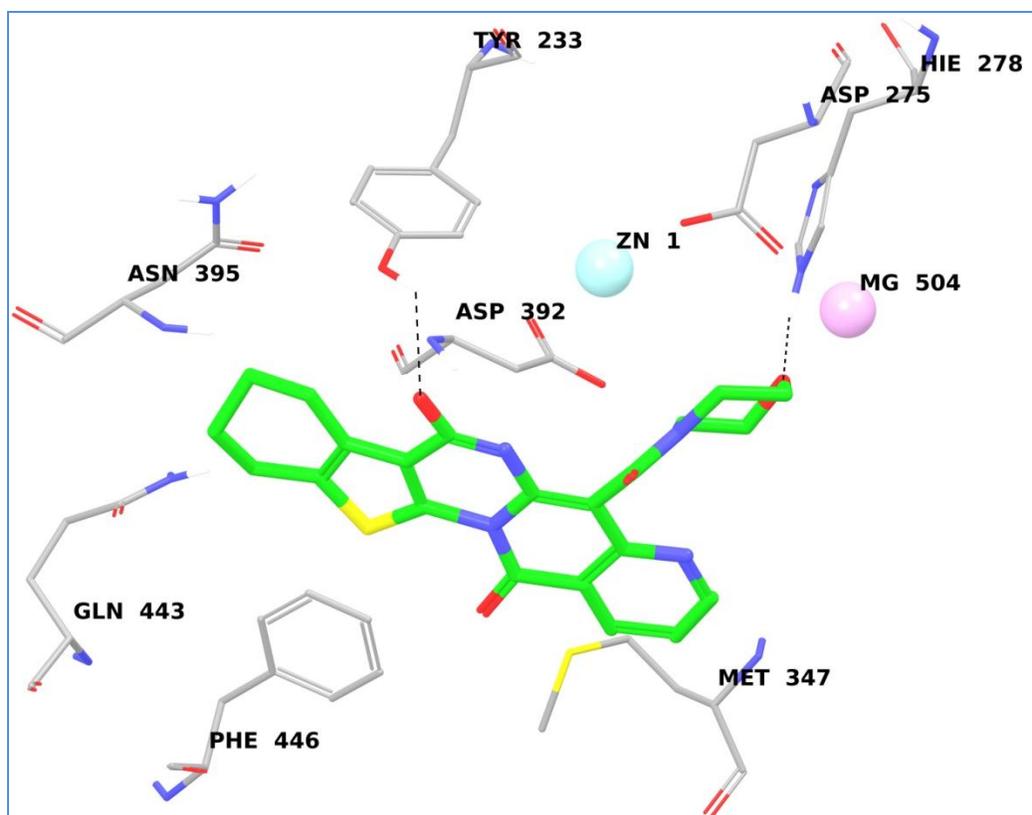
PhobEn: Hydrophobic enclosure reward

HBond: Rewards for hydrogen bonding interaction between ligand and protein

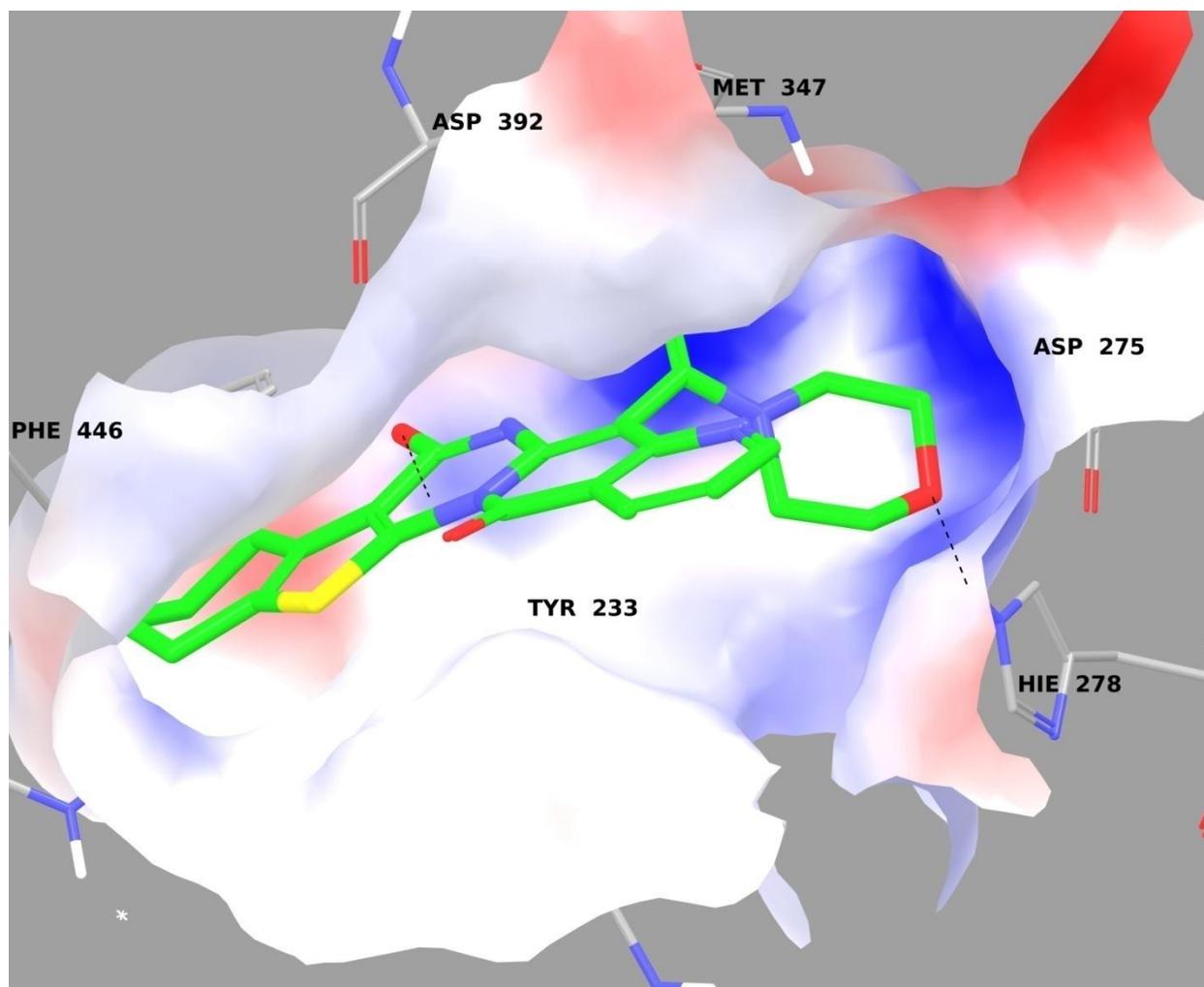
Electro: Electrostatic reward

Penalty: Polar atom burial and desolvation penalties, and penalty for intra-ligand contacts

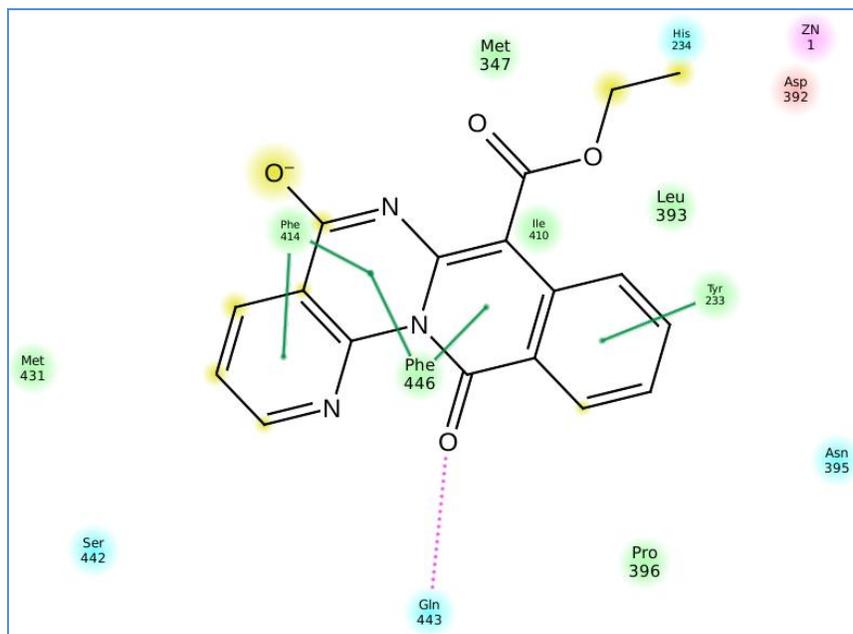
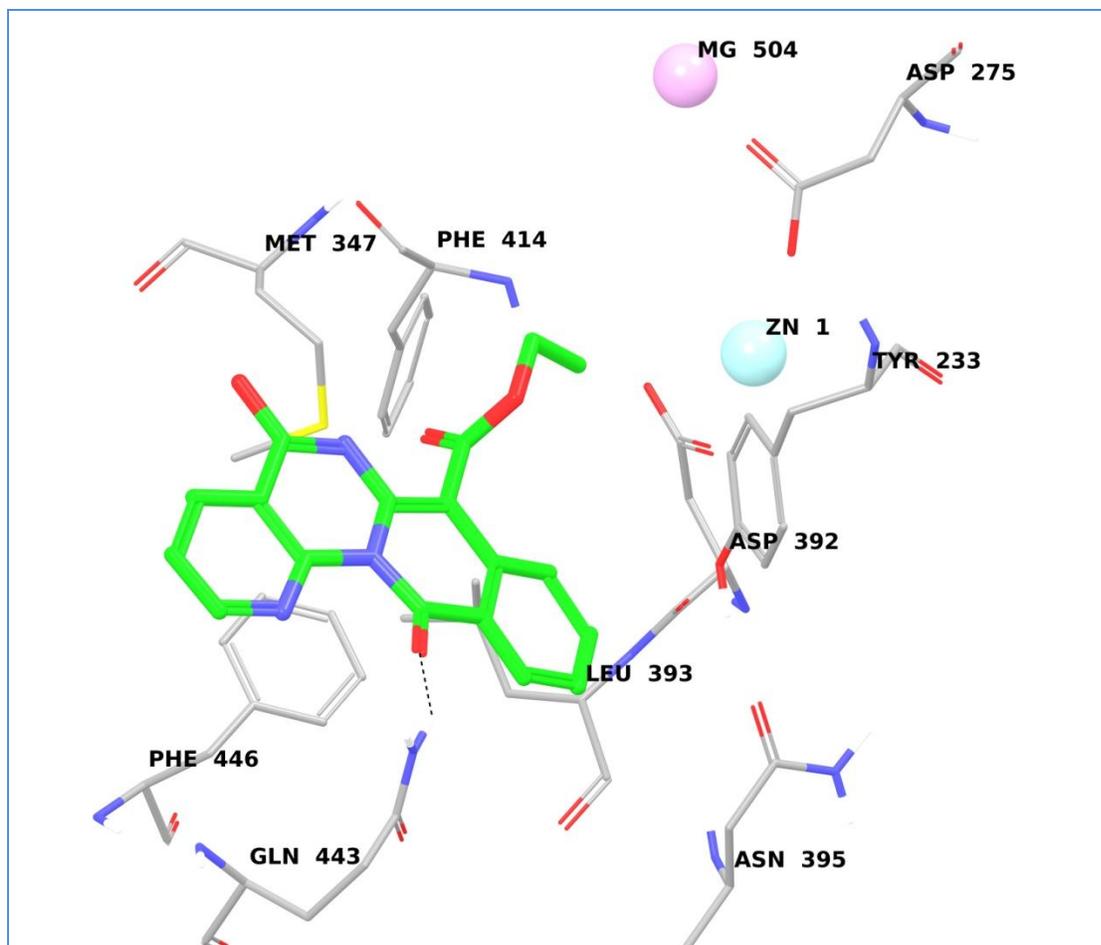
**Docking Discussion:** The molecular docking was performed using most potent molecules against PDE4B (Table 1). Docking studies predicted good binding interaction with the PDE4B enzyme. In case of molecule **5e** oxygen atom of morpholine ring and CO group participated in H-bonding with NH of His-278 and OH of Tyr 233 respectively. A pi-pi stacking between the molecule **5e** and Phe-446 was also observed (Figure 1). The morpholine ring of molecule **5e** was found to be well occupied in the partially charged pocket of active site (Figure 2). In case of molecule **3n**, the hydrophobic interactions were found to be the primary binding interactions. Aromatic rings of molecule **3n** participated in good pi-pi stacking with active site residues (Tyr-233, Phe-414, and Phe-446) of the protein. In addition a hydrogen bond interaction between molecule **3n** and amino group of Gln-443 was also observed (Figure 3 and 4).



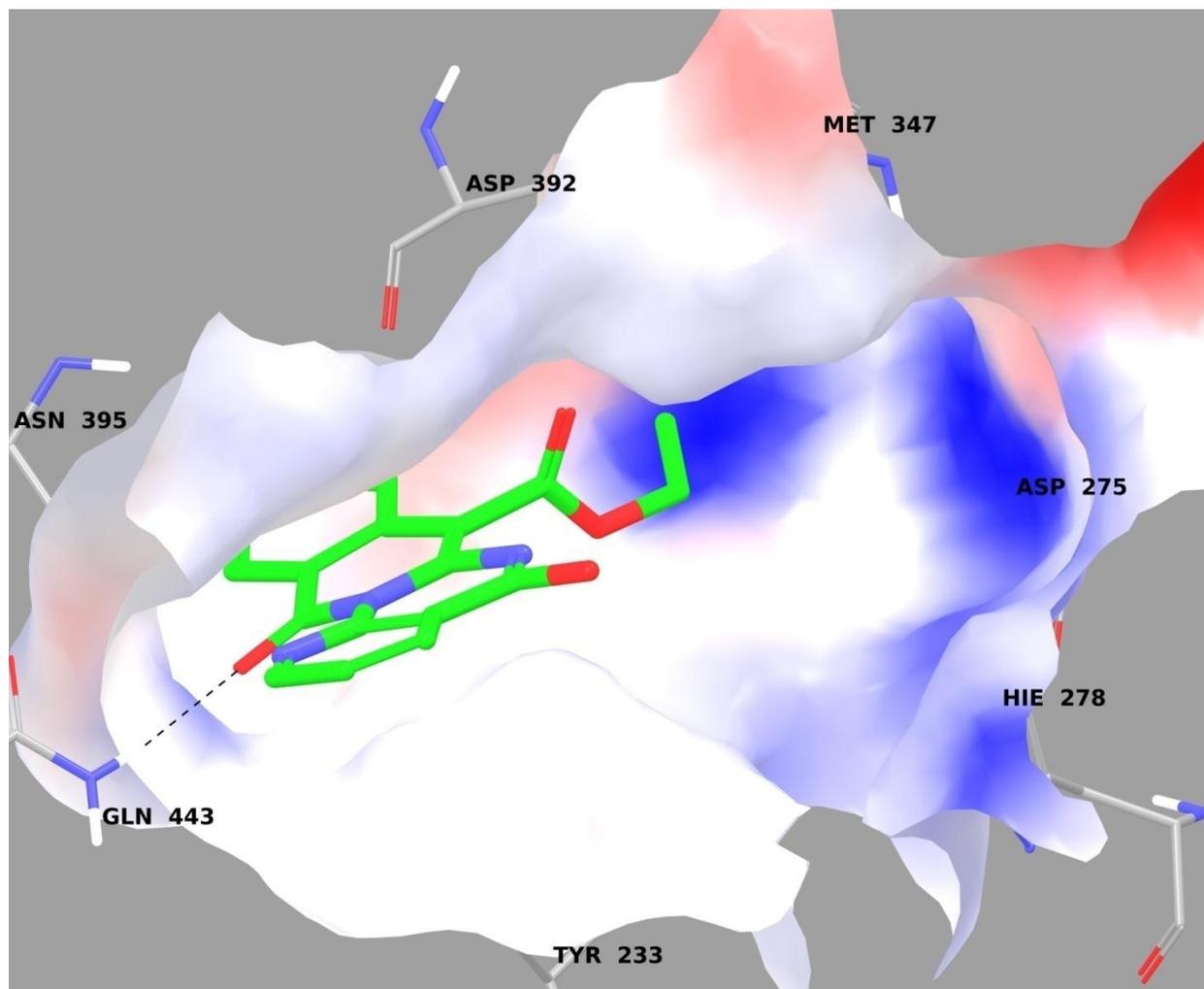
**Figure 1:** Binding mode and interactions molecule **5e** with PDE4B.



**Figure 2:** Binding orientation of molecule **5e** at the active site pocket of PDE4B.



**Figure 3:** Binding mode and interactions of molecule **3n** with PDE4B.



**Figure 4:** Binding orientation of molecule **3n** at the active site pocket of PDE4B.

**Reference:**

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