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Challenges in the development of mGluR5 positive allosteric modulators: The discovery of CPPHA

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Abstract—This Letter describes, for the first time, the synthesis and SAR, developed through an iterative analog library approach, that led to the discovery of the positive allosteric modulator (PAM) of the metabotropic glutamate receptor mGluR5 CPPHA. Binding to a unique allosteric binding site distinct from other mGluR5 PAMs, CPPHA has been the focus of numerous pharmacology studies by several laboratories.

Our laboratory recently disclosed the structures and pharmacology of two series of mGluR5 positive allosteric modulators (PAMs), represented by **DFB** (**1**) and **CPPHA** (**2**), and one account of the synthesis, SAR, and in vivo efficacy of the first centrally active mGluR5 PAM, **CDPPB** (**3**) (Fig. 1).[1–4] Data acquired with all three series of mGluR5 PAMs, by indirect activation of NMDA receptors, strongly support the NMDA hypofunction hypothesis of schizophrenia.[5] Since these initial reports, **CPPHA** has proven to be an important pharmacological tool for both our laboratory and a number of other pharmaceutical and academic laboratories.[2,6,7] Of particular note is the fact that **DFB** and **CDPPB** bind at the same allosteric binding site as the mGluR5 negative allosteric modulator, MPEP; in contrast, **CPPHA** does not bind at the MPEP site and therefore interacts with an as yet unknown and distinct allosteric binding site.[1–4] The novel allosteric binding site of **CPPHA** has engendered unique pharmacology to **CPPHA** (vide infra) as well as significant challenges in the lead optimization program that led to the discovery of **CPPHA**. This Letter will detail the screening leads and subsequent synthesis and SAR that ultimately led to the discovery of **CPPHA**, as well as an overview of the pharmacological profile of **CPPHA**.

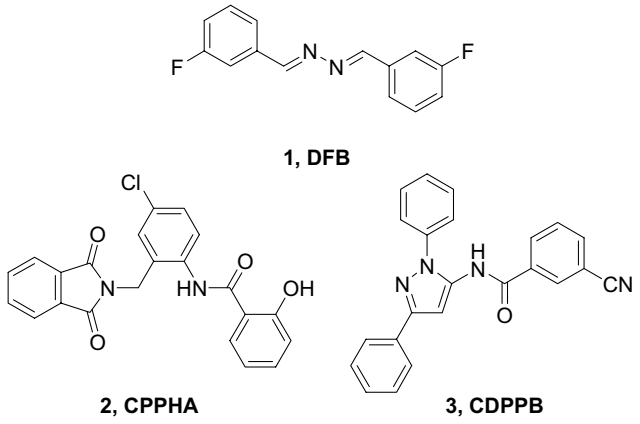

Figure 1. mGluR5 positive allosteric modulators (PAMS).

We screened a portion of our sample collection searching for compounds that selectively potentiated the mGluR5 response to 300 nM glutamate (an EC_{20} concentration

Keywords: MGluR5; Allosteric modulator; Potentiation.
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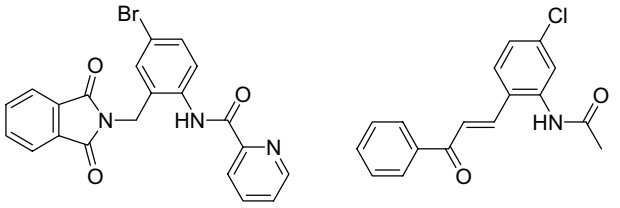


Figure 2. mGluR5 PAM screening leads.

of glutamate).[1–4] Hits from this screen were then titrated in a FLIPR assay at a single, low concentration of agonist to evaluate potency (EC_{50}) and fold potentiation.[8] This led to the identification of **4** (human mGluR5 EC50 = 750 nM, 7.6-fold potentiation) and **5** (human mGluR5 EC50 = 2.7 μM, 5-fold potentiation) as selective mGluR5 PAMs (Fig. 2). Moreover, 4 alone (up to 100 μM) caused no response by human mGluR5 in this assay and both 4 and 5 were selective of mGluR5 (selective vs mGluR1–4, 7, and 8).[2] Note that fold-potentiation is established at a single agonist concentration to provide a convenient and rapid metric to compare compounds, and all values for fold potentiation presented in this Letter were determined in this way. More detailed studies were performed on selected compounds of interest. In this case, agonist concentration–response curves were determined in the presence of several fixed concentrations of test compound. Figure 3 shows the concentration dependent potentiation of the response of CHO cells expressing mGluR5 to glutamate, which is manifested as increased sensitivity to agonist.

Since **4** was modular, an iterative analog library approach was employed to rapidly develop SAR following the synthetic route shown in Scheme 1. In the event, commercially available functionalized 2-methyl anilines **6** were acylated with acid chlorides to deliver amides **7**. Benzylic bromination provided **8** which could be treated with various phthalimides or succinimides to provide analogs represented by general structure **9**, or **8** could be treated with a secondary amine followed by acylation to provide analogs **10**. Yields were generally

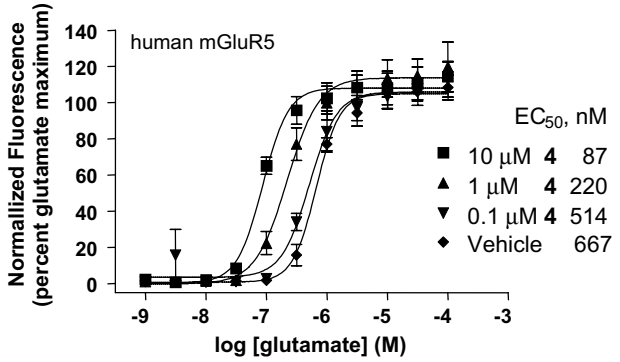
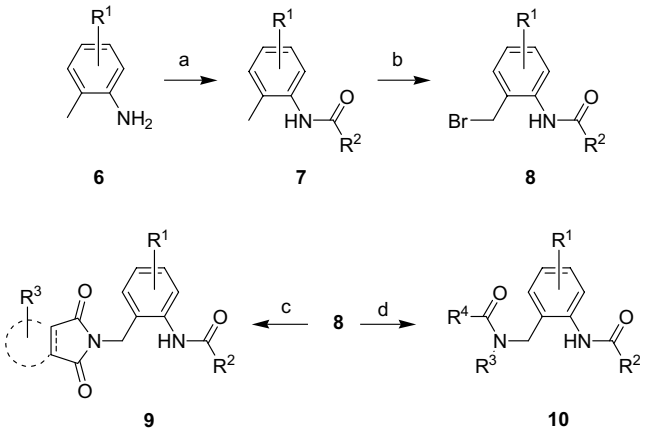


Figure 3. Compound **4** potentiation of response to glutamate is manifested as increased mGluR5 sensitivity. The glutamate EC_{50} value is shifted from 667 nM to 87 nM with the addition of **4**. Similar data acquired with quisqualate and 3,5-DHPG.[2]

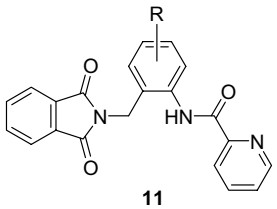


Scheme 1. Synthesis of PAMs **9** and **10**. Reagents and conditions: (a) i—R^2COCl, PS–DIEA, DCM, rt, 90–95%, ii—PS–trisamine, 70–95%; (b) NBS, Bz_2O_2, CCl_4, 90 °C, 45–69%; (c) functionalized phthalimide/succinimide, K_2CO_3, DMF, 50 °C, 70–85%; (d) i—R^3NH_2, PS–DIEA, DCM, rt, 80–90%, ii—R^4COCl, PS–DIEA, DCM, rt, 90–95%. All compounds purified by mass-directed HPLC.[9]

good, with all final products being purified by mass-directed preparative HPLC.[9] We first explored substitutions on the central phenyl ring (Table 1). Replacement of the bromine atom with hydrogen, as in **11a**, led to an ~19-fold loss in potency; in contrast, replacement with a chlorine atom, as in **11c**, afforded a 250 nM mGluR5 PAM with 6.8-fold potentiation. After surveying 48 diverse substituents, we found that only 4-position halogen and OMe were tolerated; all other substituents were inactive as well as substitutions at all other positions on the central phenyl ring. Interestingly, **11e**, a 6-F analog, was devoid of activity at mGluR5, but displayed PAM of mGluR7 (EC_{50} = 7 μM).

Having identified the optimal substituents for the central phenyl ring, the next rounds of library synthesis focused on incorporating functionalized succinimide and phthalimide moieties, as the phthalimide group engendered poor physical properties and aqueous solubility to the series.

Table 1. EC_{50} and fold potentiation of analogs **11**



Compound	R	mGluR5 EC_{50}[a](μM)	Fold potentiation
4	4-Br	0.75	7.6
11a	H	13.9	3.4
11b	4-F	1.5	2.6
11c	4-CI	0.25	6.8
11d	4-OMe	1.4	3.5
11e	6-F	>100	0.9

[a] Human mGluR5.

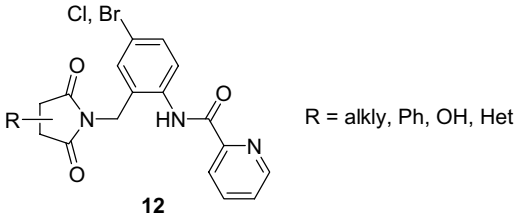
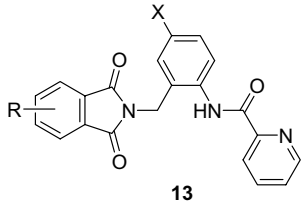


Figure 4. Succinimide analogs **12**.

All attempts to replace the phthalimide moiety with functionalized succinimides (Fig. 4) were unproductive, leading to inactive molecules (mGluR5 EC_{50} > 100 μM). Thus far, SAR for this series was 'flat', with slight structural changes leading to a complete loss of activity. However, substitution was tolerated on the phthalimide moiety (Table 2), with a general trend toward the 4-Cl analogs being more efficacious. Despite affording PAMs of comparable activity to **11c**, none of the analogs **13** displayed improvements in physical properties.

Based on the data generated thus far, subsequent efforts held the unsubstituted phthalimide and the 4-Cl functionality constant, and explored a diverse array of amide analogs **14** (Table 3). From several libraries, totaling over 500 analogs, only ~10% retained mGluR5 PAM activity. Again, on this PAM program and other allosteric modulator programs, SAR tends to be very flat which makes series development difficult.[3,4,10] However, this effort did produce several compounds worthy of further study; notably, **4**, **2** (also known as **CPPHA**), and **14h**. None of these analogs potentiated rat mGluR5 with EC_{50}s of <1 μM; therefore, they were not viable for in vivo proof of concept experiments in rat behavioral models, but could serve as tools for in vitro experiments.[2]

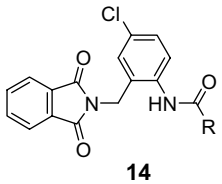
Table 2. EC_{50} and fold potentiation of analogs **13**

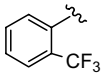
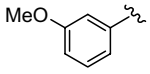
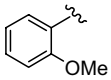
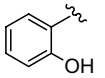
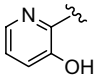
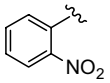


Compound	R	X	mGluR5 EC_{50}[a](μM)	Fold potentiation
13a	5-F	Br	0.93	11.1
	5-F	Cl	0.33	4.6
13b	3-F	Br	0.17	4.5
	3-F	Cl	0.61	4.8
13c	4-OEt	Br	0.72	10.7
	4-OEt	Cl	0.28	4.5
13d	4,5-diOMe	Br	>100	0.6
	4,5-diOMe	Cl	>100	0.6
13e	4,5-diMe	Br	>100	0.6
	4,5-diMe	Cl	2.2	3.7
13f	3,5-diCI	Br	2.1	7.2
	3,5-diCI	Cl	0.89	8.9

[a] Human mGluR5.

Table 3. EC_{50} and fold potentiation of analogs **14**



Compound	R	mGluR5 EC_{50}[a](μM)	Fold potentiation
4		0.25	6.8
14a		35	1.8
14b		2.1	1.3
14c		>100	0.6
14d		>100	0.6
14e		5.7	3.5
14f		1.5	1.6
14g		>100	0.6
2		0.25	7.1
14h		0.26	11.3
14i		5.7	2.9
14j		1.5	4.1
14k		2.3	6.2
14l		1.9	9.2

[a] Human mGluR5.

While **4**, **2**, and **14h** were being screened in ancillary assays, synthetic efforts focused on identifying an alternative for the phthalimide group. According to Scheme 1, benzyl bromide **8** was treated with various primary amines and subsequently acylated to deliver tertiary amide analogs **10** (Fig. 5). This effort produced 150 analogs; however, the majority of these analogs were inactive or had EC_{50}s > 5 μM with <5-fold potentiation for human mGluR5 and EC_{50}s > 1 μM for rat mGluR5.

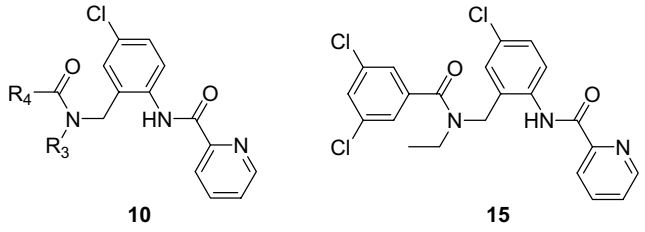
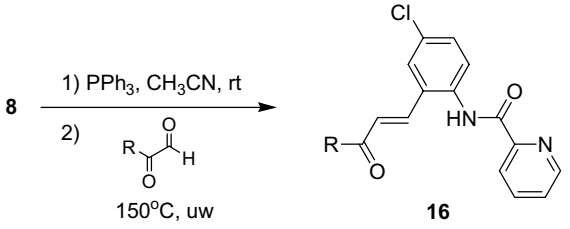


Figure 5. Tertiary amide analogs **10** and **15**.

The most potent compound from this effort was **15** (EC_{50} = 470 nM, 5.9-fold potentiation for human mGluR5); unfortunately, **15** offered no advantage over phthalimide analogs in terms of physical properties or efficacy for rat mGluR5.

Concurrently, synthetic effort was applied to the other screening lead **5**, with the aim of merging the two series into a hybrid molecule, recognizing that the unsaturated phenyl ketone moiety could be a phthalimide equivalent. A number of analogs were prepared from intermediate **8** by conversion to the phosphonium salt, and a subsequent microwave-assisted Wittig reaction to deliver analogs **16** (Scheme 2). Of these analogs, the unsubstituted phenyl analog **17** proved to be the most active with a human mGluR5 EC_{50} of 1.7 μM and 8-fold potentiation (Fig. 6). Unexpectedly, as DMSO stock solutions of **17**, and related analogs of general structure **16**, were stored at room temperature and then assayed again, the EC_{50} diminished to >13 μM. NMR analysis confirmed that **17** quantitatively underwent a hetero-inverse electron demand Diels–Alder reaction to generate the highly functionalized pyran **18**. To avoid this unwanted reaction, the unsaturated analog of **17** was prepared and evaluated. In the event, iodide **19** was subjected to stan-



Scheme 2. Synthesis of analogs **16**. Yields range from 22% to 86%; All compounds purified by mass-directed HPLC.[9].

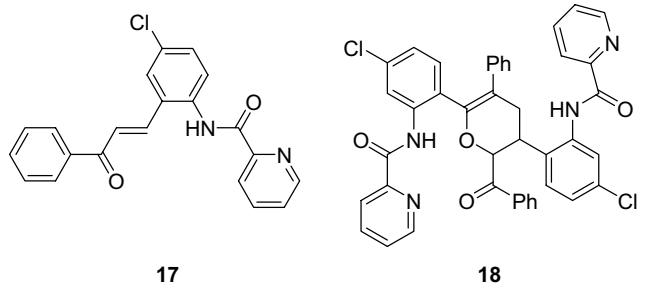
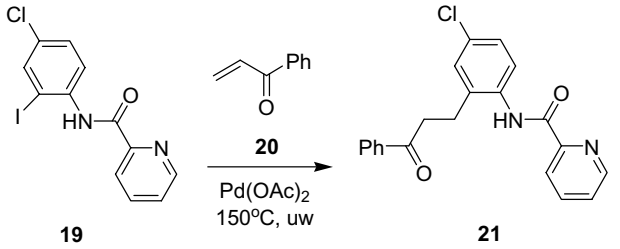


Figure 6. Structure of **17** and cycloaddition product **18**.



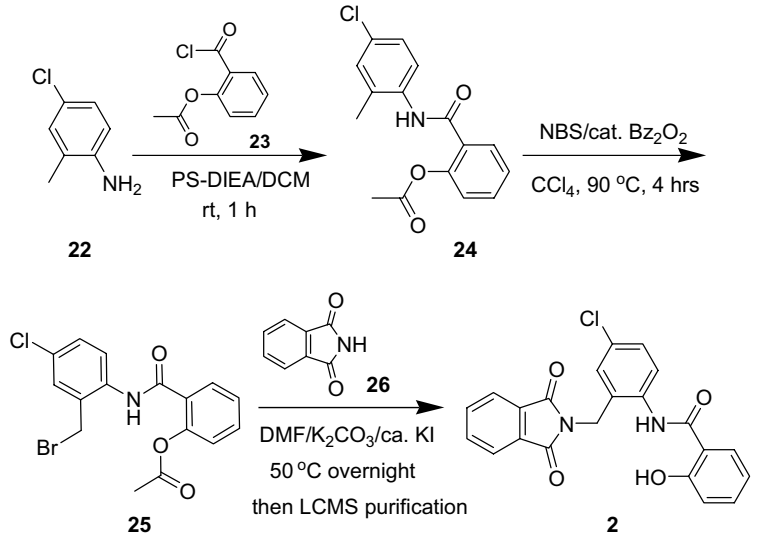
Scheme 3. Synthesis of unsaturated analog **21**. Yields range from 79% to 88%; Compounds purified by mass-directed HPLC.[9]

dard Heck conditions with unsaturated ketone **20**; surprisingly, the desired saturated product **21** resulted instead of the expected unsaturated Heck product **17**. Unfortunately, **21** was only a weak mGluR5 PAM with an EC_{50} of 4.5 μM (Scheme 3).

After evaluation in CYP assays, a panel of 120 enzymes, ion channels, and transporters, and physiochemical property measures, **2** (**CPPHA**) proved to be the best mGluR5 PAM in the series for further proof of concept studies. As we reported previously, **CPPHA** was selective for mGluR5 (no PAM of mGluRs 1, 2, 3, 4, 7 or 8) and the only detected ancillary pharmacology was weak negative allosteric modulation of mGluR4 and 8.[2] In radioligand binding assays, **CPPHA** did not affect binding of the orthosteric ligand ^{3}[H]quisqualate, nor did **CPPHA** affect negative allosteric ligand ^{3}[H]PEPy.[2] This was in sharp contrast to **DFB** and **CDPPB** which compete for ^{3}[H]PEPy binding, and therefore share the same allosteric binding site as MPEP.[1–4] It is interesting to note that **CDPPB** displayed more tractable SAR than **CPPHA** which could be the result of the novel allosteric binding site utilized by **CPPHA**. In total, 985 analogs of **4** and **5** were prepared and evaluated; only 45 analogs (4.5%) possessed mGluR5 EC_{50}s below 5 μM and no tractable SAR could ever be established in these series, highlighting the power and utility of library synthesis to identify **CPPHA**.

Despite the medicinal chemistry challenges, **CPPHA** proved to be an important small molecule tool for in vitro experiments. In electrophysiological studies of brain slice preparations, **CPPHA** (10 μM) potentiated NMDA currents in hippocampal slices induced by subthreshold levels of DHPG and also potentiated mGluR5-mediated, DHPG-induced depolarization of subthalmic nucleous neurons. In the absence of DHPG, **CPPHA** had no effect.[2]

After our initial publication, Conn and Liu disclosed the effects of **CPPHA** on signaling pathways in brain slices.[6,7] Importantly, Conn's study demonstrated that **CPPHA** has qualitatively different effects on mGluR5-mediated calcium responses and ERK1/2 phosphorylation in rat cortical astrocytes, whereas, **DFB**, an mGluR5 PAM that binds at the MPEP allosteric site, had the same effects on both signaling pathways.[6] Liu reported that **CPPHA** potentiated (*R*,*S*)-3,5-dihydroxyphenylglycol (DHPG)-induced ERK and CREB phosphorylation in rat cortical and hippocampal slices, as well as potentiated



Scheme 4. Synthesis of **CPPHA 2**.[11]

DHPG-induced phosphorylation of the NR1 (Ser 897) subunit of NMDA receptors.[7]

In summary, we have disclosed the synthesis and SAR of the mGluR5 positive allosteric modulator **CPPHA**, **2**, with a unique allosteric binding site, distinct from that of MPEP, **DFB**, and **CDPPB**. SAR was flat and developing SAR was virtually impossible through traditional medicinal chemistry strategies. Even with an iterative analog library synthesis approach, only 4.5% of 985 analogs based on screening leads **4** and **5** possessed mGluR5 PAM activity below 5 μM. In general PAMs have proved far more challenging to develop robust, tractable SAR for standard GPCR agonists/antagonists. Despite these challenges, **CPPHA** has become an important tool to probe the pharmacology of mGluR5, and demonstrated that different PAMs can differentially modulate coupling of a single receptor to different signaling pathways.

Acknowledgments

The authors wish to thank Dr. Sandor Varga for extensive NMR work to confirm the structural assignments of the analogs represented by general structure **9**, **11**, **13**, and **14**, and Dr. Charles W. Ross III for obtaining HRMS data.

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8. EC_{50} of potentiation and fold potentiation were determined from concentration–response curves of test compound at a single, low (EC_{20}) concentration of agonist. Fluo-4 (calcium sensitive dye) was loaded into CHO cells expressing human or rat mGluR5. These cells were then pretreated with a range of concentrations of test compound, and finally challenged with an approximate EC_{20} concentration of agonist (in this case, 300 nM glutamate). The inflection point of the concentration–response curve of the test compound was taken as the EC_{50} for potentiation, and the ratio of the maximum agonist response (at the agonist EC_{20}) in the presence of test compound versus the response to the same concentration of agonist in the absence of test compound is taken as the fold potentiation. Ref. 2 provides additional experimental details and a curve (Fig. 2) showing how EC_{50} and fold potentiation are determined. The referenced figure provides an illustration of the variability for each data point. Variability of EC_{50} for potentiation (ranging from 9.4% to 32.8%), is detailed in Table of Ref. 2. Note that Figure 3 in the present paper represents the effect of several fixed concentrations of test compound on the agonist concentration–response curve.

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11. The synthesis of general structure represented by **9**, **11**, **13**, and **14** is exemplified by the synthesis of **CPPHA** (**2**) as shown in Scheme 4.
2-{[(4-chloro-2-methylphenyl)amino]carbonyl}phenyl acetate (**24**): to a mixture solution of *N*,*N*-(diisopropyl)aminomethylpolystyrene (PS-DIEA, loading 3.6 mmol/g) (7.1 g, 26 mmol)[12] and 4-chloro-2-methylaniline **22** (1.42 g, 10 mmol) was slowly added acetylsalicyloyl chloride **23** (1.99 g, 10 mmol) with shaking. The resulting mixture was shaken until the reaction completion (1 h) by LCMS. The resin was filtered and washed with DCM (2×). The collected DCM solution was dried under vacuum to afford **24** as a slightly yellow solid (2.64 g, 87%). Analytical LC/MS: (CH_3CN/H_2O/0.1% TFA, 4 min gradient), 93% pure, *m*/*e* 304 (M+1).
2-({[(2-Bromomethyl)-4-chlorophenyl]amino}carbonyl) phenyl acetate (**25**): compound **24** (2.64 g, 8.7 mmol) obtained from the fist step was immediately taken up in a solution of CCl_4 with fresh-recrystallized NBS (1.62 g, 9.1 mmol) and benzoyl peroxide (Bz_2O_2)[13] (218 mg, 0.9 mmol). The reaction mixture was heated at 90 °C, along with a light source, until completion (4 h) by LCMS. After cooling down to room temperature, the mixture solution was filtered through a small plug of silica gel, yielding **25** as a yellow solid (2.29 g, 74%). Analytical LC/MS: (CH_3CN/H_2O/0.1% TFA, 4 min gradient), 89% pure, *m*/*e* 384 (M+1).
CPPHA (**2**): a mixture of **25** (120 mg, 0.31 mmol), phthalimide **26** (67 mg, 0.45 mmol), K_2CO_3 (207 mg, 1.5 mmol), and KI (17 mg, 0.01 mmol) in 1.2 mL DMF was allowed to stir at 50 °C overnight.[14] Next morning, the reaction mixture was filtered and the collected DMF solution of crude product was purified by LC/MS[9] to afford the pure product **CPPHA** (**2**) as a slightly yellow solid (66.5 mg, 53%). ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 4.83, (s, 2 H), 7.02–7.07 (m, 2H), 7.36 (dd, J = 2.5 Hz, 8.8 Hz, 1H), 7.50 (ddd, J = 1.4 Hz, 8.0 Hz, 8.0 Hz, 1H), 7.59 (d, J = 2.5 Hz, 1H), 7.73 (d, J = 8.8 Hz, 1H), 7.76–7.79 (m, 2H), 7.90–7.93 (m, 2H), 8.18 (dd, J = 1.4 Hz, 8.0 Hz, 1H), 10.17 (s, 1H), 12.26 (s, 1H); Analytical LC/MS: (CH_3CN/H_2O/0.1% TFA, 4 min gradient), single peak (214 nm), *m*/*e* 407 (M+1) at 3.633 min; HRMS: calculated for $[C_{22}H_{15}N_2O_4Cl + H]^+$, 407.0799; found407.0793.
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