Functionalization of photochromic dithienylmaleimides†

D. Wutz, C. Falenczyk, N. Kuzmanovic and B. König*

Photochromic dithienylmaleimides are well known molecular switches, but for applications the suitable functionalization of the photochromic scaffold is required. We report here synthetic routes to dithienylmaleimides, which are functionalized at three different positions: at each of the thiophene moieties and the maleimide nitrogen. A Perkin-type condensation of two thiophene precursors is used as the key step to assemble the maleimide core, which allows the synthesis of non-symmetrically substituted dithienylmaleimides, such as photochromic amino acids. A different approach to the maleimide core is provided by the reaction of a dithienylmaleic anhydride with amines or hydrazides leading to maleimide protected dithienylmaleimides and photochromic labeled natural amino acids. The photochromic properties of the new photoswitches were investigated showing reversible photochromism in polar organic solvents.

Introduction

Photochromism has attracted great attention in materials science and as a tool in molecular biology. A variety of applications are found in molecular optoelectronics and optical data storage. In the field of life sciences, molecular switches have been used to control enzyme activity, Watson–Crick base pairing, the regulation of neuronal activity by photochromic ligands for ion channels and receptors, antibody effects and even the agility of a living organism by light. This broad applicability is one of the reasons why photopharmacology has evolved into a vibrant field of research. Various photochromic molecules, like azobenzenes, spiropyran, spirooxazines, fulgides and diarylethenes have been developed. All these photoswitches can be reversibly toggled between two isomers using light. The well investigated dithienylethenes (DTEs), including dithienylmaleimides, are characterized by a nearly quantitative photochemical conversion between the photoisomers, which are often thermally stable. Irradiation with light of a specific wavelength switches the DTEs between their open and closed photoisomers, which differ in conformational flexibility and electronic conjugation (Fig. 1).

Many DTEs show high fatigue resistance. Despite their outstanding photophysical properties the synthesis of DTEs, in particular of non-symmetric derivatives, is laborious. Different synthetic routes for the preparation of dithienylmaleimides were established. Starting from 3,4-dibromomaleimides and 3,4-diiodomaleimides, respectively, both thiophene moieties can be attached by palladium catalyzed Suzuki coupling. However, only nitrogen protected maleimides can be used and the synthesis of non-symmetric compounds is challenging. Another route uses the reaction of a dithienylmaleic anhydride with amines to the corresponding maleimide. The synthesis of diarylmaleimides by intramolecular Perkin condensation of two independently prepared precursors gives selective access to non-symmetric diarylmaleimides. Compared to diarylperfluorocyclopentenes and diarylecyclopentenes, diarylmaleimides are more hydrophilic and better water soluble, which is valuable for applications in biology and pharmacy. The absorption maxima of diarylmaleimides are shifted to higher wavelengths and thus the photosomerization can be induced by light with lower energy reducing potential cell damage. Moreover, the biocompatibility of diarylmaleimides is known from bisindolylmaleimides, for instance arcyriarubins and arcyriaflavins with antibiotic activities, several other potent protein kinase

Fig. 1 Reversible photochemical isomerization of a dithienylmaleimide between the open and closed photoisomer by irradiation with light of different wavelength.
Results and discussion

Synthesis

Functionalization of the maleimide nitrogen atom. The transformation of diarylmaleic anhydrides into their corresponding diarylmaleimides provides an easy access to compounds with a functionalized maleimide nitrogen atom. Complex functionalities or protecting groups can be introduced at the maleimide nitrogen by reaction with amines or hydrazides. We used the adapted synthetic approach of Scandola et al. for the synthesis of anhydride 4 as precursor (Scheme 1).

Methyl ester 2 was converted to its potassium salt 3 and condensed in a Perkin reaction with carboxylic acid 1 yielding the photochromic maleic anhydride 4. The anhydride moiety allows the subsequent functionalization with hydrazides or amines (Scheme 2). Therefore maleic anhydride 4 was treated with α-Cbz protected γ-glutamic acid γ-hydrazide (5) and α-Cbz protected γ-lysine to give amino acids 6 and 7 with a photochromic dithienylmaleimide on each sidechain. Photochromic tripeptides forming hydrogels with different aggregation modes mainly depending on the switch moiety were recently reported. The reaction of hydrazine hydrate in acetic acid as solvent and 1,2-dimethylhydrazine dihydrochloride, respectively, with maleic anhydride afforded the maleimide nitrogen protected dithienylmaleimides 8 and 9 in good yields (Scheme 2). Remarkably, the formation of any maleic hydrazide or other tautomers was not observed. The protected maleimides 8 and 9 could be used for further functionalizations on the thiophene moieties by palladium-catalyzed cross coupling reactions or other reactions using the reactivity of the heteroaryl chlorides.

Functionalization as photochromic amino acid. Recently, DTE-based non-natural amino acids were synthesized and successfully introduced into small peptides. However, their water-solubility is limited due to the diaryl perfluorocyclopentene core and therefore we developed a more polar dithienylmaleimide amino acid. Compounds 13a and 13b were prepared by a Perkin condensation of the thiophene precursors 10 and 11 bearing a protected primary amino or carboxyl group, respectively (Scheme 3). The Alloc group was chosen as a suitable protection for the amine as it is stable during the synthesis of compound 12. Diester thiophene 11 provides in 4-position the carboxylic ester giving the maleimide core in the Perkin condensation. The ester in 2-position will serve as carboxylate of the amino acid. Both carboxylic acids were protected as methyl ester. Alloc group and methyl ester of 12 were cleaved simultaneously with boron tribromide giving amino acid 13a in 47% yield, accompanied by 20% of the Alloc.

Scheme 1 Synthesis of dithienylmaleic anhydride 4.

Scheme 2 Synthesis of the functionalized photochromic dithienylmaleimides 6–9 starting from maleic anhydride 4.
amino acid 13b as byproduct. A selective non-hydrolytic deprotection of the methyl ester of 12 is possible in low yield using lithium iodide in a polar aprotic solvent.\(^{47,48}\) A large excess of lithium iodide and reflux were necessary to achieve conversion; several solvents were tested with best yields in acetone (see ESI, Table S1†). Standard basic hydrolytic conditions for the deprotection of the methyl ester afforded the deprotected maleic anhydride (see ESI, Scheme S2†). The synthesis of thiophene 10 is depicted in Scheme 4. Bromination\(^{49}\) of 2-methylthiophene (14) and subsequent Rosenmund–von Braun reaction\(^{50}\) giving nitrile 16 were performed according to literature procedures.

The reduction of nitrile 16 with lithium aluminum hydride followed by immediate protection with allyl chloroformate afforded carbamate 17 in good yield. Using Fmoc chloride instead led to the respective Fmoc derivative in lower yields and caused the formation of side products in the subsequent Friedel–Crafts acylation. The yield of glyoxylester 18 in the Friedel–Crafts acylation depends critically on the sequence of the reagent addition. Best results were obtained by mixing 17 and methyl chloroacetate before adding aluminum chloride in small portions. Quenching the reaction with saturated sodium hydrogen carbonate solution avoids the addition of hydrochloric acid to the allyl double bond. Aminolysis with aqueous ammonia converted the glyoxylester 18 in high yield into compound 10. The overall yield for 10 after six steps is 22%.

Thiophene 11 was prepared by esterification\(^{51}\) of methylthiophene acid 19 in the presence of thionyl chloride followed by Friedel–Crafts acylation and finally a thallium trinitrate (TTN) mediated oxidative rearrangement\(^{52}\) (Scheme 5). All intermediates were isolated in good to excellent yields with an overall yield of 68% for three steps. Initial moderate yields for the Friedel–Crafts acylation of around 40% significantly increased to 77% after rigorous removal of stabilizers from the solvent chloroform.

**Functionalization by Suzuki coupling.** Dithienylmaleimides are conveniently synthesized by the Perkin-type condensation. The reaction of two precursors yields the maleimide core without the need for protection of the maleimide nitrogen. Scheme 6 shows the intramolecular Perkin condensation of the
two chlorosubstituted precursors 22 and 23. Both precursors can be differently functionalized by Suzuki coupling before used in the Perkin condensation yielding non-symmetric dithienylmaleimides.

Recently, we described the synthesis of symmetric diarylmaleimides, with thiophene moieties functionalized by palladium-catalysis prior to the condensation reaction. Based on this strategy we prepared a small series of non-symmetric diarylmaleimides (Scheme 7).

The Perkin condensation to the maleimide core was performed under basic conditions combining the different thiophenes. Scheme 8 summarizes the synthesis of the non-symmetric photoswitches 35–37.

**Photochromic properties.** The dithienylmaleimide core structure can be toggled reversibly between a ring-open and ring-closed photoisomer (Fig. 1). The photochemical properties of photochromic compounds 4, 6–9, 12, 13a, 13b, 24 and 35–37 were investigated by UV-Vis spectroscopy. Despite of reports that diarylmaleimides are not able to undergo photoisomerization in polar solvents due to a twisted intramolecular charge transfer (TICT), we could observe reversible photoisomerization of the dithienylmaleimides 6–9, 12, 13a, 13b, 24 and 35–37 in methanol or dimethylsulfoxide, respectively. Fig. 2 shows the changes of the UV-Vis spectra of compound 12 upon irradiation with light of 312 nm (Herolab, 6 W).

Upon irradiating a methanol solution of the ring-open form of compound 12 with UV light (312 nm), the absorption band at...
250 nm immediately decreases. Simultaneously, new absorption maxima at 232 nm, 378 nm and 550 nm arise (Fig. 2) causing the color change of the sample from slightly yellow to purple. The isosbestic points indicate a clean conversion between two components. Compared to typical DTE-cyclopentenes the absorption maxima are red shifted. The photostationary state was reached after 42 s of irradiation (Herolab, 312 nm, 6 W) and the open form can be regained by irradiation with visible light (>420 nm) for 5 min. The photoswitchable amino acid 12 is stable over at least seven closing/opening cycles (Fig. 3).

The absorption maxima and their corresponding extinction coefficients for the open and closed form of all synthesized photochromic compounds are summarized in Table 1. Interestingly, the long wavelength absorption maximum of compound 13a is blue shifted to 537 nm compared to photoswitches 12 and 13b, which may indicate an interaction of the Alloc group with the dithienylmaleimide core. In contrast the selective removal of the methyl ester has almost no influence on the photochromic properties. In comparison to bischloro
dithienylmaleimide 24 the functionalized maleimides 35–37 show a bathochromic shift in their absorption maxima of the closed photosomer. The enlarged π-system of the substituted thiophenes can explain this shift to higher wavelengths.

Conclusions

In summary, we have prepared several photochromic dithienylmaleimides. Maleimide nitrogen atom functionalized derivatives were obtained by the reaction of dithienylethylmaleic anhydride with different hydrazides and amines. Using a Perkin-type condensation non-symmetric dithienylmaleimides were synthesized including a photochromic amino acid and dithienylmaleimides with different aromatic substituents on each thiophene moiety. Reversible photoisomerization in dimethylsulfoxide and methanol was observed for all synthesized photochromic compounds.

Experimental section

General information

Commercial reagents and starting materials were purchased from Acros Organics, Alpha-Aesar, Fluka, Sigma Aldrich or VWR and used without further purification. Solvents were used in p.a. quality and dried according to common procedures, if necessary. To purify the chloroform for Friedel–Crafts acylations, it was washed with sulfuric acid (1 M), dried over calcium chloride, filtered through silica, subsequently refluxed with phosphorus pentoxide (5–10 g L⁻¹) and distilled under nitrogen atmosphere. Compounds 1, 2, 5, 15, 16, 20, 22, 25, 28, 29 and 32 were prepared according to previously reported procedures. Flash column chromatography was performed on a Biotage Isolera One automated flash purification system with UV/Vis detector using Sigma Aldrich MN silica gel 60 M (40–63 μm, 230–400 mesh) for normal phase or pre-packed Biotage SNAP cartridges (KP-C18-HS) for reversed phase chromatography. Reaction monitoring via TLC was performed on alumina plates coated with silica gel (Merck silica gel 60 F₂₅₄, 0.2 mm). Melting points were determined using a Stanford

Table 1 UV-Vis spectroscopic data of the open and closed (PSS) form of the synthesized photochromic compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Solvent</th>
<th>Conc. [μM]</th>
<th>λ_{max} open (e)</th>
<th>λ_{max} closed (e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>MeOH</td>
<td>100</td>
<td>242 (26.0), 298 (9.5)</td>
<td>359 (16.0), 523 (3.7)</td>
</tr>
<tr>
<td>6</td>
<td>DMSO</td>
<td>50</td>
<td>387 (4.6)</td>
<td>359 (17.4), 510 (3.2)</td>
</tr>
<tr>
<td>7</td>
<td>DMSO</td>
<td>50</td>
<td>381 (5.0)</td>
<td>355 (20.8), 500 (4.1)</td>
</tr>
<tr>
<td>8</td>
<td>DMSO</td>
<td>50</td>
<td>386 (6.0)</td>
<td>359 (22.7), 508 (4.3)</td>
</tr>
<tr>
<td>9</td>
<td>DMSO</td>
<td>50</td>
<td>380 (3.3)</td>
<td>351 (12.9), 498 (2.7)</td>
</tr>
<tr>
<td>12</td>
<td>MeOH</td>
<td>50</td>
<td>250 (18.5)</td>
<td>232 (16.2), 378 (13.6), 550 (3.9)</td>
</tr>
<tr>
<td>13a</td>
<td>MeOH</td>
<td>50</td>
<td>252 (13.0)</td>
<td>231 (11.3), 375 (10.0), 537 (2.7)</td>
</tr>
<tr>
<td>13b</td>
<td>MeOH</td>
<td>50</td>
<td>250 (14.3)</td>
<td>232 (12.6), 378 (10.3), 549 (2.8)</td>
</tr>
<tr>
<td>24</td>
<td>MeOH</td>
<td>50</td>
<td>240 (20.2), 370 (4.5)</td>
<td>234 (20.6), 352 (13.8), 497 (2.5)</td>
</tr>
<tr>
<td>35</td>
<td>MeOH</td>
<td>100</td>
<td>264 (18.5), 292 (17.1)</td>
<td>369 (9.7), 543 (3.0)</td>
</tr>
<tr>
<td>36</td>
<td>MeOH</td>
<td>100</td>
<td>255 (28.8), 291^b (14.3)</td>
<td>369 (10.1), 540 (3.7)</td>
</tr>
<tr>
<td>37</td>
<td>MeOH</td>
<td>100</td>
<td>262 (21.6), 297 (20.7)</td>
<td>391 (11.5), 586 (5.7)</td>
</tr>
</tbody>
</table>

^a UV-Vis spectroscopic data are reported for solutions at 25 °C and reported in nm (λ_{max}) and 10^3 cm⁻¹ M⁻¹ (ε). The PSS were obtained by irradiation of solutions of the open isomer with light of 312 nm (Herolab, 6 W). ^b Shoulder.
Research Systems OptiMelt MPA 100. NMR spectra were recorded on a Bruker Avance 300 (1H 300.13 MHz, 13C 75.48 MHz), Bruker Avance 400 (1H 400.13 MHz, 13C 100.61 MHz) or Avance III 600 (1H 600.25 MHz, 13C 150.95 MHz) instrument. The spectra are referenced against the NMR-solvent, chemical shifts are reported in ppm and coupling constants \( J \) are given in Hz.

Resonance multiplicity is abbreviated as: s (singlet), d (doublet), t (triplet), m (multiplet) and b (broad). Carbon NMR signals are reported with (+) for primary/tertiary, (−) for secondary and (q) for quaternary carbons. The assignment resulted from DEPT, HSQC and HMBC experiments. Mass spectra were recorded on a Finnigan MAT95 (EI-MS), Agilent Q-TOF 6540 UHD (ESI-MS, APIC-MS), Finnigan MAT SSQ 710 A (EI-MS, CI-MS) or ThermoQuest Finnigan TSQ 7000 (ES-MS, APIC-MS) spectrometer. UV/Vis absorption spectroscopy was performed using a Varian Cary BIO 50 UV/Vis/NIR spectrometer. IR-spectra were recorded on a Bruker Hyperion 2000 FT-IR spectrometer. Mass spectra were recorded on a Bruker Micro TOF-Q (ESI-MS) or Agilent 6540 UHD (ESI-MS, APCI-MS), Finnigan MAT SSQ 710 A (EI-MS, CI-MS) or Thermonet Finnigan TSQ 7000 (ES-MS, APIC-MS) spectrometer. UV/Vis absorption spectroscopy was performed using a Varian Cary BIO 50 UV/Vis/NIR spectrometer. IR spectra were recorded on a Bruker Hyperion 2000 FT-IR spectrometer.

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15–50% EtOAc) yielded compound 8 (81 mg, 72%) as orange solid. Rf: 0.23 (PE/EtOAc: 2/1); m.p.: 131 °C; IR (neat) νmax 3238, 3088, 2924, 2359, 1717, 1510, 1429, 1255, 1193, 988; 1H-NMR (400 MHz, DMSO-d6): δ = 1.95 (s, 6H, thiophene-CH3), 2.03 (s, 3H, CO-CH3), 7.01 (s, 2H, thiophene-H), 10.58 (s, 1H, NH); 13C-NMR (101 MHz, DMSO-d6): δ = 14.2 (+), 20.0 (+), 125.0 (q), 125.8 (q), 127.6 (+), 131.3 (q), 140.6 (q), 166.9 (q), 168.5 (q); HRMS (ESI): calcld for C16H13Cl2N2O3S2 (M + H)+ 414.9739; found 414.9741.

3,4-Bis(5-chloro-2-methylthiophen-3-yl)-1-methyl-1H-pyrrole-2,5-dione (9)
Maleic anhydride 4 (54 mg, 0.15 mmol) and 1,2-dimethylhydrazine dihydrochloride (60 mg, 0.45 mmol) were heated to 160 °C for 12 h in PEG400 (2 mL) in a crimp top vial. Then water (15 mL) was added and the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic phases were washed with brine (50 mL), dried over MgSO4 and the solvent was removed under reduced pressure. Purification by automated flash column chromatography (PE/EtOAc, 3–10% EtOAc) yielded compound 9 (48 mg, 86%) as orange foam. Rf: 0.32 (PE/EtOAc: 19:1); IR (neat) νmax 3098, 2924, 2851, 1765, 1697, 1435, 1386, 1250, 1174, 980; 1H-NMR (300 MHz, CDCl3): δ = 1.93 (s, 6H, thiophene-CH3), 3.13 (s, 3H, N-CH3), 6.89 (s, 2H, thiophene-H); 13C-NMR (75 MHz, CDCl3): δ = 14.9 (+), 24.4 (+), 126.0 (q), 127.2 (+), 127.2 (q), 132.7 (q), 140.2 (q), 170.2 (q); HRMS (ESI): calcld for C16H14Cl2N2O3S2 (M + H)+ 373.9649; found 373.9652.

Allyl (4-(2-amino-2-oxoethyl)-5-methylthiophen-2-yl)methyl carbamate (10)
To a solution of oxoacetate 18 (282 mg, 0.95 mmol) in THF (5 mL) was added a NH4OH solution (32% in H2O) (1.18 mL, 9.50 mmol) at 0 °C. The reaction was stirred for 90 min at room temperature and then quenched with water (5 mL). The aqueous phase was extracted with EtOAc (3 × 10 mL). The combined organic phases were dried over MgSO4 and the solvent was removed under reduced pressure. Compound 10 (253 mg, 94%) was obtained as yellow solid and used without further purification. Rf: 0.21 (PE/EtOAc: 1:1); m.p.: 108 °C; IR (neat) νmax 3402, 3301, 3167, 2962, 1750, 1684, 1535, 1460, 1254, 1047, 796; 1H-NMR (400 MHz, CDCl3): δ = 2.70 (s, 3H, thiophene-CH3), 4.44 (d, J = 6.1 Hz, 2H, thiophene-CH2NH), 4.59 (d, J = 5.1 Hz, 2H, CH2=CHCH2O), 5.21 (dd, J = 10.4, 0.5 Hz, 1H, CH2=CHCH2), 5.24–5.43 (m, 2H, CH2=CHCH2 and NH), 5.90 (ddt, J = 16.2, 10.7, 5.5 Hz, 1H, CH=CHCH2), 6.05 (bs, 1H, NH2), 7.06 (bs, 1H, NH2), 7.86 (s, 1H, thiophene-H); 13C-NMR (101 MHz, CDCl3): δ = 16.7 (+), 39.8 (−), 65.9 (−), 117.9 (−), 129.0 (+), 130.8 (q), 132.7 (+), 137.0 (q), 155.7 (q), 166.1 (q), 164.4 (q), 182.1 (q); HRMS (ESI): calcld for C21H14N2O5S (M + H)+ 461.0838; found 461.0836.

Methyl 4-(4-(5-(((allyloxy)carbonyl)amino)methyl)-2-methylthiophen-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-5-methylthiophene-2-carboxylate (12)
KOtBu (1 M in THF, 0.88 mL, 0.88 mmol) was added to a solution of glyoxylamide 10 (206 mg, 0.73 mmol) in anhydrous THF (5 mL) at 0 °C under nitrogen atmosphere. After stirring for 90 min at 0 °C, diester 11 (200 mg, 0.88 mmol) in THF (2 mL) was added at 0 °C and stirred for 3 days at room temperature. Then the reaction was quenched with 1 M aqueous HCl solution (3 mL) and diluted with EtOAc (10 mL). The organic phase was washed with water (2 × 10 mL), brine (10 mL) and dried over MgSO4. The solvent was removed under reduced pressure and purification of the crude product by automated flash column chromatography (PE/ EtOAc, 25–50% EtOAc) yielded 12 (148 mg, 44%) as yellow foam. Rf: 0.20 (PE/ EtOAc: 2:1); IR (neat) νmax 3289, 3070, 2952, 1703, 1540, 1458, 1339, 1248, 994, 909, 727; 1H-NMR (400 MHz, CDCl3): δ = 1.90 (s, 3H, thiophene-CH3), 1.98 (s, 3H, thiophene-CH3), 3.87 (s, 1H, OCH3), 4.45 (d, J = 6.0 Hz, 2H, thiophene-CH2NH), 4.60 (d, J = 4.9 Hz, 2H, CH2=CHCH2O), 5.13–5.27 (m, 2H, CH2=CHCH2 and CH2=CH2); 13C-NMR (101 MHz, CDCl3): δ = 15.0 (+), 15.3 (+), 39.9 (−), 52.3 (+), 65.9 (−), 117.9 (−), 125.8 (q), 126.7 (+), 127.5 (q), 130.9 (q), 132.7 (+), 134.8 (q), 134.9 (+), 142.1 (q), 148.6 (q), 156.0 (q), 162.1 (q), 170.0 (q), 170.2 (q); HRMS (ESI): calcld for C23H16N2O6S (M + H)+ 482.1038; found 482.1032.

4-(4-(5-(Aminomethyl)-2-methylthiophen-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-5-methylthiophene-2-carboxylic acid (13a) and 4-(4-(5-(((allyloxy)carbonyl)amino)methyl)-2-methylthiophen-3-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl)-5-methylthiophene-2-carboxylic acid (13b)
A solution of BBr3 (1 M in CH2Cl2, 2.0 mL, 2.00 mmol) was added to a solution of compound 12 (92 mg, 0.20 mmol) in anhydrous CH2Cl2 (6 mL) in a crimp top vial. The mixture was heated to 40 °C for 5 h. Then water (4 mL) was added via syringe and the suspension was stirred at 40 °C for additional 30 min. After cooling to room temperature the solvent was removed at
the rotary evaporator. Purification by automated reversed phase flash column chromatography (MeCN/H2O with 0.05% TFA, 3–100% MeCN) yielded compound 13a (34 mg, 47%) as yellow solid and compound 13b (18 mg, 20%) as yellow solid.

**Analytical data of 13a**

𝑅_\text{c} = 0.02 (PE/EtOAc: 1/1); m.p.: 173 °C; IR (neat) 𝜈_{\text{max}}: 3008, 2924, 1766, 1712, 1618, 1545, 1463, 1344, 1188, 1137, 1001, 839, 799, 756, 723; 1H-NMR (600 MHz, MeOD): δ = 2.00 (s, 3H, thiophene-CH3); 2.08 (s, 3H, thiophene-CH3); 2.47 (s, 2H, thiophene-CH2-NH); 7.18 (s, 1H, thiophene-H); 7.64 (s, 1H, thiophene-H); 13C-NMR (151 MHz, MeOD): δ = 14.7 (+), 15.3 (+), 38.5 (−), 128.5 (q), 129.3 (q), 132.2 (+), 132.8 (q), 133.1 (q), 135.0 (q), 135.7 (q), 136.3 (+), 144.6 (q), 149.7 (q), 164.6 (q), 172.4 (q), 172.6 (q); HRMS (ESI): calcd for C18H13N3O2S2{M + H}+: 363.0469; found 363.0468.

**Analytical data of 13b**

𝑅_\text{c} = 0.04 (PE/EtOAc: 1/1); m.p.: 94 °C; IR (neat) 𝜈_{\text{max}}: 3026, 2926, 1781, 1769, 1709, 1544, 1545, 1344, 1246, 1185, 1150, 1049, 991, 849, 762; 1H-NMR (300 MHz, CD3CN): δ = 1.93 (s, 3H, thiophene-CH3); 1.97 (s, 3H, thiophene-CH3); 4.33 (d, 𝐽 = 6.3 Hz, 2H, thiophene-CH2-NH); 4.52 (d, 𝐽 = 5.3 Hz, 2H, CH2=CHCH2O); 5.18 (dd, 𝐽 = 10.5, 1.4 Hz, 1H, CH2=CHCH2O); 5.27 (dd, 𝐽 = 17.3, 1.6 Hz, 1H, CH2=CHCH2O); 5.74–6.05 (m, 1H, CH2=CHCH2O), 6.14 (bs, 1H, CH2=NHCO), 6.79 (s, 1H, thiophene-H); 7.60 (s, 1H, thiophene-H); 8.80 (bs, 1H, COOH); 13C-NMR (75 MHz, CD3CN): δ = 14.8 (+), 15.2 (+), 40.1 (−), 66.0 (−), 117.5 (−), 127.2 (q), 127.4 (+), 129.1 (q), 131.4 (q), 134.1 (q), 134.4 (+), 136.2 (+), 136.2 (q), 141.2 (q), 141.8 (q), 149.6 (q), 157.1 (q), 162.9 (q), 171.6 (q), 171.7 (q); HRMS (ESI): calcd for C20H18N3O2S2{M + H}+: 447.0679; found 447.0676.

**Alternative procedure to obtain 13b**

Compound 12 (40 mg, 0.09 mmol) was dissolved in acetone (10 mL) and LiI (350 mg, 2.60 mmol) was added. The mixture was heated to 100 °C overnight. After cooling to room temperature it was quenched with 1 M aqueous HCl solution (5 mL) and diluted with CH2Cl2 (5 mL). The phases were separated and the aqueous phase was extracted with CH2Cl2 (3 × 5 mL). The combined organic phases were dried over Na2SO4 and the solvent was removed at the rotary evaporator. Automated reversed phase flash column chromatography (MeCN/H2O with 0.05% TFA, 3–100% MeCN) yielded compound 13b (14 mg, 35%) as yellow solid.

**Allyl (5-methylthiophen-2-yl)methylcarbamate (17)**

LAH (2.78 g, 73.2 mmol) was added in portions to a solution of nitride 16 (3.01 g, 24.4 mmol) in anhydrous Et2O (250 mL) at 0 °C under nitrogen atmosphere. After stirring for 4 h at room temperature the reaction was quenched with water (80 mL) and saturated aqueous NaHCO3 solution (50 mL) at 0 °C. The suspension was filtered and the aqueous phase was extracted with Et2O (3 × 80 mL). The combined organic phases were dried over MgSO4 and concentrated in vacuo. Then the residue was dissolved in anhydrous THF (100 mL) and pyridine (2.47 mL, 30.50 mmol) was added at 0 °C. Within 1 h allyl chloroformate (4.02 mL, 37.82 mmol) in anhydrous THF (5 mL) was dropped to the solution via a syringe pump at 0 °C. After stirring for 14 h at room temperature the reaction was quenched cautiously with water (50 mL) and extracted with EtOAc (3 × 30 mL). The combined organic phases were dried over MgSO4 and the solvent was removed under reduced pressure. Purification of the crude product by automated flash column chromatography (PE/EtOAc, 8–15% EtOAc) yielded 17 (3.40 g, 66%) as yellow oil.

**Methyl 2-((allyloxy)carbonyl)amino)methyl)-2-methylthiophen-3-yl)-2-oxoacetate (18)**

Carbamate 17 (169 mg, 0.80 mmol) and methyl chlorooxocacetate (81 μL, 0.88 mmol) were dissolved in anhydrous CH2Cl2 (6 mL) under nitrogen atmosphere. Then aluminum chloride (427 mg, 3.20 mmol) was added in portions at 0 °C and the suspension was stirred for 20 h at room temperature. The reaction was quenched with saturated aqueous NaHCO3 solution (1 mL) at 0 °C and diluted with water (5 mL). The aqueous phase was extracted with CH2Cl2 (3 × 5 mL), the combined organic layers were washed with brine (10 mL) and dried over MgSO4. After evaporation of the solvent the crude product was purified by automated flash column chromatography (PE/EtOAc, 15–40% EtOAc) to obtain 18 (117 mg, 49%) as brown oil.

**Methyl 4-acetyl-5-methylthiophene-2-carboxylate (21)**

Thiophene 20 (800 mg, 5.12 mmol) and acetyl chloride (550 μL, 7.68 mmol) were dissolved in purified anhydrous CHCl3 (10 mL) under nitrogen atmosphere. After cooling to 0 °C aluminum chloride (2.05 g, 15.4 mmol) was added in small portions. The yellow suspension was heated to 45 °C overnight upon turning bright red, then the reaction was quenched with ice/water and the aqueous phase was extracted with EtOAc (3 × 10 mL). The combined organic phases were washed with a saturated
aqueous solution of NaHCO₃ (10 mL) and brine (10 mL). The organic phase was dried over MgSO₄ and the solvent was evaporated. The crude product was purified by automated flash column chromatography (PE/ EtOAc, 5–25% EtOAc) and compound 21 (781 mg, 77%) was obtained as colorless solid. Rf: 0.41 (PE/EtOAc: 3/1); m.p.: 84 ºC; IR (neat) νmax: 3007, 2957, 1717, 1678, 1539, 1457, 1439, 1254, 1233, 1074, 1021, 745; ¹H-NMR (300 MHz, CDCl₃): δ = 2.52 (s, 3H, thiophene-CH₃), 2.76 (s, 3H, CH₃), 3.88 (s, 3H, OCH₃), 8.03 (s, 1H, thiophene-H); ¹³C-NMR (75 MHz, CDCl₃): δ = 168.1 (+), 29.6 (+), 52.3 (+), 128.5 (q), 135.0 (+), 136.3 (q), 155.8 (q), 162.0 (q), 193.7 (q); HRMS (APCI): calcd for C₇H₁₀ClN₂O₂S (M+H)⁺ 221.0146; found 221.0144.

General procedure A: Suzuki coupling

To a suspension of Pd₂(dba)₃ (5 mol%), XPhos (10 mol%), the appropriate boronic acid (1.5 eq.) and K₃PO₄ (1.5 eq.) in 1,4-dioxiane (0.5 M) the appropriate ester (1.0 eq.) was added. The resulting mixture was heated to 100 ºC and stirred overnight. After cooling to room temperature the reaction mixture was diluted with ETOAc and the organic phase was washed twice with water. The organic phase was dried over MgSO₄, filtered and the solvent was removed under reduced pressure.

General procedure B: aminolysis

An NH₄OH solution (25% in H₂O) (10.0 eq.) was added to a solution of the appropriate oxoacetate (1.0 eq.) in THF (0.3 M) at 0 ºC. The reaction was stirred for 1 h at room temperature and then quenched with water. The aqueous phase was extracted with ETOAc. The organic phases were combined and dried over MgSO₄, filtered and the solvent was removed under reduced pressure.

General procedure C: Perkin condensation

KOtBu (1 M in THF) (1.2 eq.) was added to a solution of the appropriate amide (1.0 eq.) in THF (0.2 M) at 0 ºC. After 90 min stirring at 0 ºC the appropriate ester (1.0 eq.) was added at 0 ºC and stirred overnight at room temperature. The reaction was quenched with 1 M HCl and diluted with ETOAc. The organic phase was washed three times with water and one time with brine. The organic phase was dried over MgSO₄, filtered and the solvent was removed under reduced pressure.

2-(5-Chloro-2-methylthiophen-3-yl)-2-oxoacetamide (23)

Compound 23 was prepared from 28 (800 mg, 3.66 mmol) according to general procedure B. The amide 23 (640 mg, 85%) was obtained as light yellow solid and used without further purification. m.p.: 183 ºC; IR (neat) νmax: 3446, 3252, 1996, 1670, 1618, 1296, 1221, 1153; ¹H-NMR (300 MHz, DMSO-d₆): δ = 2.64 (s, 3H, thiophene-CH₃), 7.49 (s, 1H, thiophene-H), 7.94 (bs, 1H, NH), 8.25 (bs, 1H, NH); ¹³C-NMR (75 MHz, DMSO-d₆): δ = 15.2 (+), 123.9 (q), 128.4 (+), 131.4 (q), 150.9 (q), 166.1 (q), 184.2 (q); HRMS (APCI): calcd for C₇H₁₆ClN₂O₂S (M+H)⁺ 221.0146; found 221.0144.

3,4-Bis(5-chloro-2-methylthiophen-3-yl)-1H-pyrrole-2,5-dione (24)

Compound 24 was prepared from amide 23 (600 mg, 2.95 mmol) and ester 22 (720 mg, 3.54 mmol) according to general procedure C. Purification by automated flash column chromatography (heptane/EtOAc: 5/1) yielded 24 (660 mg, 63%) as orange solid. Rf: 0.18 (heptane/EtOAc: 5/1); m.p.: 237 ºC; IR (neat) νmax: 3381, 2939, 2818, 1653, 1437, 1002; ¹H-NMR (400 MHz, DMSO-d₆): δ = 1.87 (s, 6H, thiophene-CH₃), 6.97 (s, 2H, thiophene-H), 11.25 (bs, 1H, NH); ¹³C-NMR (101 MHz, DMSO-d₆): δ = 14.6 (+), 125.0 (q), 127.0 (q), 128.4 (+), 133.6 (q), 140.0 (q), 171.4 (q); HRMS (ESI): calcd for C₁₄H₁₀Cl₂N₂O₅ (M+H)⁺ 357.9525; found 357.9523.

Methyl 2-(5-(4-(tert-butyl)phenyl)-2-methylthiophen-3-yl)-acetate (26)

Compound 26 was prepared from 22 (200 mg, 0.98 mmol) according to general procedure A. Purification by automated flash column chromatography (PE/EtOAc, 0–25% EtOAc) yielded 26 (163 mg, 55%) as yellow liquid. Rf: 0.63 (PE/EtOAc: 5/1); IR (neat) νmax: 2961, 1736, 1609, 1520, 1435, 1364, 1239, 1018, 825; ¹H-NMR (300 MHz, CDCl₃): δ = 1.33 (s, 9H, tBu), 2.41 (s, 3H, thiophene-CH₃), 3.55 (s, 2H, thiophene-CH₂C(O)OCH₃), 3.71 (s, 3H, thiophene-CH₂C(O)OCH₃), 7.08 (s, 1H, thiophene-H), 7.32–7.40 (m, 2H, Ph), 7.43–7.52 (m, 2H, Ph); ¹³C-NMR (75 MHz, CDCl₃): δ = 13.3 (+), 31.3 (+), 34.1 (+), 34.6 (q), 52.1 (+), 124.7 (+), 125.2 (+), 125.7 (+), 130.1 (q), 131.6 (q), 134.8 (q), 140.1 (q), 150.2 (q), 171.6 (q); HRMS (ESI): calcd for C₁₄H₁₆O₂S (M+H)⁺ 303.1413; found 303.1418.

Methyl 2-(5-(1,1'-biphenyl)-3-yl)-2-methylthiophen-3-yl)acetate (27)

Compound 27 was prepared from 22 (500 mg, 2.44 mmol) according to general procedure A. Purification by automated flash column chromatography (PE/EtOAc, 0–15% EtOAc) yielded 27 (569 mg, 72%) as yellow liquid. Rf: 0.50 (PE/EtOAc: 5/1); IR (neat) νmax: 1707, 1597, 1449, 1262, 1174, 755, 696; ¹H-NMR (300 MHz, CDCl₃): δ = 2.44 (s, 3H, thiophene-CH₃), 3.58 (s, 2H, thiophene-CH₂C(O)OCH₃), 3.72 (s, 3H, thiophene-C(=O)OCH₃), 7.19 (s, 1H, thiophene-H), 7.37–7.45 (m, 3H, Ph), 7.46–7.54 (m, 3H, Ph), 7.59–7.66 (m, 2H, Ph), 7.74–7.76 (m, 1H, Ph); ¹³C-NMR (75 MHz, CDCl₃): δ = 13.3 (+), 34.1 (+), 52.1 (+), 124.3 (+), 124.4 (+), 125.3 (+), 126.0 (+), 127.2 (+), 127.5 (+), 128.8 (+), 129.2 (+), 130.3 (q), 134.8 (q), 135.5 (q), 139.9 (q), 140.9 (q), 141.9 (q), 171.5 (q); HRMS (ESI): calcd for C₁₄H₁₀Cl₂NO₈S (M+H)⁺ 322.1028; found 322.1032.
Compound 35 was prepared from amide 23 (100 mg, 0.49 mmol) and ester 26 (178 mg, 0.59 mmol) according to general procedure C. Purification by automated flash column chromatography (PE/EtOAc, 0–25% EtOAc) yielded 35 (62 mg, 28%) as dark green solid. Rf 0.47 (PE/EtOAc: 5/1); m.p.: 145 °C; IR (neat) νmax: 3055, 2961, 1707, 1459, 1338, 1181, 1018, 987, 825; 1H-NMR (300 MHz, DMSO-d6): δ = 1.28 (s, 9H, tBu), 1.88 (s, 3H, thiophene-CH3), 1.99 (s, 3H, thiophene-CH3), 7.02 (s, 1H, thiophene-H), 7.28 (s, 1H, thiophene-H), 7.40–7.49 (m, 4H, Ph), 11.27 (s, 1H, NH); 13C-NMR (75 MHz, DMSO-d6): δ = 14.0 (19+), 30.9 (19+), 124.2 (q), 124.4 (q), 124.8 (q), 126.9 (q), 127.2 (q), 127.7 (q), 129.8 (q), 132.3 (q), 133.5 (q), 140.2 (q), 140.6 (q), 142.2 (q), 154.0 (q), 164.0 (q), 180.1 (q); HRMS (ESI): calcd for C24H23ClN2O2S2 (M + H+) 473.1119; found 473.1116.

2-(5-(4-(tert-Butyl)phenyl)-2-methylthiophen-3-yl)-2-oxoacetamide (33)

Compound 33 was prepared from 28 (301 mg, 0.99 mmol) according to general procedure B. The amide 33 (250 mg, 84%) was obtained as light yellow solid and used without further purification. m.p.: 202 °C; IR (neat) νmax: 3395, 2955, 1712, 1651, 1454, 1191, 575; 1H-NMR (300 MHz, DMSO-d6): δ = 1.29 (s, 9H, tBu), 2.71 (s, 3H, thiophene-CH3), 3.76–7.48 (m, 2H, Ph), 7.51–7.59 (m, 2H, Ph), 7.74 (s, 1H, thiophene-H), 7.91 (s, 1H, NH), 8.26 (s, 1H, NH); 13C-NMR (75 MHz, DMSO-d6): δ = 15.4 (+), 30.9 (+), 34.3 (q), 124.4 (+), 125.0 (+), 126.0 (+), 133.0 (q), 138.9 (q), 150.5 (q), 167.0 (q), 185.5 (q); HRMS (ESI): calcd for C17H14NO4S (M + H+) 319.0787; found 319.0787.

2-(5-(1,1′-Biphenyl)-3-yl)-2-methylthiophen-3-yl)-2-oxoacetamide (34)

Compound 34 was obtained from amide 31 (250 mg, 0.74 mmol) according to general procedure B. The amide 34 (224 mg, 94%) was obtained as light yellow solid and used without further purification. m.p.: 151 °C; IR (neat) νmax: 3393, 3302, 3184, 1721, 1652, 1597, 1456, 1352, 1196, 747, 689, 608; 1H-NMR (300 MHz, DMSO-d6): δ = 2.73 (s, 3H, thiophene-CH3), 3.79–7.44 (m, 1H, Ph), 7.47–7.56 (m, 3H, Ph), 7.58–7.66 (m, 2H, Ph), 7.71–7.75 (m, 2H, Ph), 7.83–7.85 (m, 1H, NH), 7.93 (bs, 2H, Ph, thiophene-H), 8.28 (bs, 1H, NH); 13C-NMR (75 MHz, DMSO-d6): δ = 15.4 (+), 123.4 (+), 124.4 (+), 125.5 (+), 126.4 (+), 127.7 (+), 128.9 (+), 129.9 (+), 133.1 (q), 133.2 (q), 138.7 (q), 139.5 (q), 141.1 (q), 151.1 (q), 166.9 (q), 185.6 (q); HRMS (ESI): calcd for C19H16NO2S (M + H+) 322.0896; found 322.0893.

3-(5-(4-(tert-Butyl)phenyl)-2-methylthiophen-3-yl)-4-(5-chloro-2-methylthiophen-3-yl)-1H-pyrrrole-2,5-dione (35)
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Notes and references
