Synthesis and Structure-activity Relationship of Antifungal Coniothyriomycin Analogues

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The structure of the antifungal metabolite coniothyriomycin was systematically modified by changing the acids of the open chain imide, modification of the hydrophobicity, variation in the degree of saturation, replacement of carbons by nitrogen or oxygen, and incorporation of the open chain molecule into cyclic arrangements. Structure-activity studies showed that antifungal activity was retained by replacement of phenylacetic acids by benzoic acids in the imide structure but diminished by hydrogenation of the fumaric ester part.

In connection with our investigation of fungal metabolites as biologically active agents, we isolated an open chain imide named coniothyriomycin (1) (Fig. 1) with remarkable antifungal activity from an unidentified Coniothyrium fungus1). In this communication, the synthesis of analogues with variation of the substituents on the aromatic ring and their biological activity were also presented. Unfortunately, these open chain mixed amides of phenylacetic and fumaric acid, in spite of excellent short-term antifungal activity, did not show curative effects. One reason for this was the inherent instability of the imide functionality in the presence of nucleophiles such as water. Therefore, in the hope to increase the chemical stability and retain or even increase antifungal activity, we extended our study to the preparation and biological testing of various analogues by (i) replacement of the substituted phenylacetic acids with substituted benzoic acids, (ii) change of hydrophobicity by variation of the alcohol component, (iii) variation in the degree of saturation of the fumaric acid moiety, (iv) replacement of carbon by nitrogen or oxygen in the middle part of the molecule, and (v) incorporation of the open chain part of the molecule into cyclic arrangements.

Results and Discussion

In the previous syntheses of coniothyriomycin analogues1) we used the method of SUHARA et al.2) starting from nitriles via the corresponding ethyl imidates. However, the yields using this method were poor (10~36%) and the workup of the dark brown reaction mixture was tedious. Our first efforts were therefore directed towards a simplification of the procedure. To that end, equivalent amounts of benzamide (2) were heated with the fumaric monoethyl ester chloride (3a) in toluene (Scheme 1). However, starting with a 1:1 ratio, only modest yields of the desired mixed imide 5 resulted and the mixed anhydride 4 was identified as the major side product. Evidently, both theoretically possible reaction pathways a

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and b were realized: attack by the amide nitrogen on the acyl chloride (path a) to form 5a, and attack by the nucleophilic oxygen (path b) to form 4. However, the acyl group transfer potential was still preserved in the mixed anhydride 4 and attack by excess benzamide (path c) on the mixed anhydride carbonyl might ultimately result in the formation of the mixed open chain imide 5a. In fact, using two equivalents of amide 2, pathway c was also followed consuming the mixed anhydride 4, and yields of the mixed imide 5a of 50% and more were achieved in a clean, easily worked-up reaction. Interestingly, the formation of the symmetric benzoic acid imide, resulting from attack of the nitrogen on the benzoic acid part of anhydride 4, was not observed.

The method proved to be general and most of the mixed imides described in this paper were prepared using this simple procedure. However, in some cases it was advantageous to use the anion of amides such as 2. Also, the yields were generally higher starting with benzamide (2) than with phenylacetic acid amide (6a).

The first series of compounds prepared were a number of mixed benzoic acid and fumaric ester open chain imides with variation in the substituents in the aromatic ring and the ester alcohol component. We wanted to see whether omission of the methylene group in 5a by replacement of phenylacetic acid in the coniothriomycin analogues with benzoic acid would preserve their antifungal activity. In addition, variation of the substituents from methoxy to fluoride or nitro groups as in 5b~5g (Fig. 2) would show the influence of electron density on biological activity. Furthermore, the lipophilicity of the fungicide was increased in 5h by linking the n-octanyl ester of fumaric acid chloride to benzamide. The data and substituents of the mixed benzoic acid-fumaric ester imides 5a~5h are listed in Table 1.

The next task was the variation in the degree of saturation in the fumaric ester part, starting from phenylacetic amide as well as benzoic acid amide. Omission of a methine group, e.g. the shift from fumaric ester to malonic ester, or replacement with benzoic acid (incorporating the double bond of fumaric acid into a ring system) was also in line with this type of variation and worth testing for fungicidal activity. The construction of the mixed imides 10~14 by coupling of amides 2 and 6a, b with the acid chlorides 7~9 is shown in Scheme 2, employing about two equivalents of the respective amides as outlined in Scheme 1. The phenolic imide 11c, as present in the natural product coniothyriomycin (1), was prepared by reaction of benzyl ether 6b with 7, followed by hydrogenolysis of benzyl ether 11b to 11c in order to evaluate the influence of a phenolic hydroxy group on activity.

Next, we investigated the replacement of carbon by
nitrogen or oxygen in the middle part of the molecule to study the effect of these exchanges on bioactivity. Three different types of compounds resulted from these exchanges: The bis-acylated hydrazine 16, the acylated hydroxamide 18, and the acylated phenylhydrazines 20a–20c. The compounds were prepared by reaction of the hydrazine anion of 15, the hydroxamic acid 17 or the phenylhydrazines 19a,b with the acid chlorides 3 or 3c, respectively, as outlined in Scheme 3.

Finally, the imide function was incorporated in a cyclic arrangement, as demonstrated by the bold lines in structures 24/25 (Scheme 4). The 4-phenyl-pyrrolidine-2,3,5-trione (23) was prepared by sodium ethoxide mediated condensation of phenylacetic amide (22) with diethyl oxalate (21). The carbonyl group of 23 was then reacted with a number of Wittig ylides to afford the E-olefins 24–27 (compare).

Biological Studies
The tested synthetic coniothyriomycin analogues showed primarily control of plant diseases caused by representative fungi belonging to the class of Oomycetes, e.g. late blight on tomatoes caused by Phytophthora infestans or downy mildew on grape vine caused by Plasmopara viticola. The fungicidal activity was tested in vitro in 96-well microtitre plates and on intact plants in a greenhouse.

The change of the molecule fragment phenylacetic amide...
to benzoic amide in 5a to 5h retained the same good in vitro activity as the lead structure coniothyriomycin. 5a to 5d controlled P. infestans with ED90-values of less than 0.5ppm, 5e even with less than 0.125ppm. Some additional control of the causal organism of rice blast, Pyricularia oryzae, and of the causal organism of leaf blotch on wheat, Septoria tritici was observed for 5a to 5e with ED90-values of less than 2 and 8ppm respectively. The fungicidal in vitro activity could only partially be translated into in vivo activity in greenhouse tests on intact plants. Only 5f showed some initial protective control of P. infestans on tomatoes with additional initial good protective activity against P. viticola on grapes. The compounds 5d, 5g and 5h showed only some moderate control of P. viticola.

The hydrogenation of the double bond in the fumaric acid fragment, which resulted in 50 and 51a, led to total loss of fungicidal activity both in vitro and in vivo. The other structural variations in the compounds 13 and 14
resulted in reduced in vitro fungicidal activity against *P. infestans*, with ED$_{90}$-values of less than 31 and 8 ppm respectively. All other structural variations in the compounds 12, 16, 18, 20a–c and 24 led to a loss of any significant in vitro or in vivo fungicidal activity.

**Experimental**

**Biological Tests**
The in vitro tests were run in 96-well microtitre plates. The wells were filled with aqueous solutions of the compounds in the appropriate concentration prepared from a DMSO-stock solution. Thereafter the spore suspensions were added. The spores of *Botrytis cinerea*, *Pyricularia oryzae* and *Septoria tritici* were suspended in aqueous malt extract at a final concentration of 2%, the spores of *Phytophthora infestans* in a 2% aqueous synthetic medium according to SCHEEPENS and FEHRMANN.

The plates were kept for 7 days at 18°C and constant humidity. Mycelial growth was assessed with a THERMOMax microplate reader from Molecular Devices at 405 nm and the values were related to the untreated check.

The in vivo tests were done on intact plants in a greenhouse. The plants were sprayed to run-off with an aqueous solution of the compound. The air-dried plants were inoculated the following day with aqueous spore suspensions of the appropriate fungus. The inoculated plants were then kept in growth chambers with high humidity and at temperatures favourable for the development of the plant disease. After 4 to 7 days, the disease development on the untreated checks had almost covered the whole leaf area. At this point in time the trials were assessed.

**Chemical Synthesis**

For general methods and instrumentation see6).

**Condensation of Amides with Acid Chlorides, General Procedure A and B**

Procedure A: To a boiling solution of the amide (10 mmol) in dry toluene (20 ml) a solution of the acid chloride (5 mmol) in toluene (5 ml) was added dropwise. The mixture was refluxed overnight (TLC monitoring), the solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel (dichloromethane) and recrystallized from ether. For yields and mp of 5a–5h see Table 1.

Procedure B: Alternatively, the sodium salt of the amide (prepared by reaction of the amide solution with sodium hydride) was employed, with fumaric acid monoethyl ester anhydride.

4-Benzoylamino-4-oxobut-2-enoic Acid Ethyl Ester (5a)

Benzamide (2) (1.23 g, 10 mmol) in dry toluene (20 ml) was reacted according to procedure A with fumaric acid monoethyl ester chloride (3a) (1.78 g, 7.5 mmol) in toluene (5 ml) to yield 5a (1.18 g, 51%), mp: 87–89°C. IR (KBr) $\nu$=3291 cm$^{-1}$ (N–H), 1724 (C=O), 1703 (C=O), 1672 (C=O). $^1$H NMR (200 MHz, CDCl$_3$): $\delta$=1.36 (t, $J$=7 Hz, 3H, CH$_3$), 4.32 (q, $J$=7 Hz, 2H, CH$_2$), 6.90 (d, $J$=2 Hz, 2H, CH), 7.02 (d, $J$=2 Hz, 2H, CH), 7.71 (m, 3H, ArH), 7.96 (m, 2H, 5ArH), 9.43 (s, 1H, NH). $^{13}$C NMR (50 MHz, CDCl$_3$): $\delta$=14.6 (CH$_3$), 61.8 (CH$_2$), 128.5 (CH), 129.4 (CH), 133.3 (ArC), 134.1 (ArC), 134.3 (ArC), 135.2 (ArC), 165.4 (C=O), 166.3 (C=O), 167.3 (C=O). Anal Calcd for C$_{12}$H$_{11}$NO$_4$ (233.22): C 63.09, H 5.26; Found: C 61.66, H 5.29.

4-(2-Fluorobenzoylamino)-4-oxobut-2-enoic Acid Ethyl Ester (5b)

2-Fluorobenzoic acid amide (2.000 g, 14.4 mmol) in dry toluene (15 ml) was reacted with fumaric acid chloride (3a) (1.300 g, 9.6 mmol) as described in procedure A to afford imide 5b (890 mg, 42%), mp: 54–55°C. IR (KBr) $\nu$=3388 cm$^{-1}$ (N–H), 1720 (C=O), 1705 (C=O), 1678 (C=O). $^1$H NMR (200 MHz, CDCl$_3$): $\delta$=1.35 (t, $J$=7 Hz, 3H, CH$_3$), 4.30 (q, $J$=7 Hz, 2H, CH$_2$), 6.94 (d, $J$=14 Hz, 1H, CH), 7.38 (m, 2H, ArH), 7.61 (m, 1H, ArH), 7.88 (d, $J$=14 Hz, 1H, CH), 8.08 (m, 1H, ArH), 9.19 (d, $J$=13 Hz, 1H, NH). $^{13}$C NMR (50 MHz, CDCl$_3$): $\delta$=14.5 (CH$_3$), 61.8 (CH$_2$), 116.9 (CH), 120.4 (ArC), 125.8 (CH), 132.7 (ArC), 134.7 (ArC), 135.4 (ArC), 135.8 (ArC), 158.5 (C=O), 162.5 (C=O), 163.5 (C=O), 165.6 (C=O). Anal Calcd for C$_{13}$H$_{12}$FNO$_4$ (265.24): C 58.87, H 4.56, N 5.28. Found: C 58.39, H 4.46, N 5.24.

4-(4-Nitrobenzoylamino)-4-oxobut-2-enoic Acid Ethyl Ester (5c)

4-Nitrobenzoic acid amide (550 mg, 3.3 mmol) was reacted with fumaric acid chloride (3a) (200 mg, 1.5 mmol) as described in procedure A to afford imide 5c (158 mg, 44%), mp: 135–137°C. IR (KBr) $\nu$=3244 cm$^{-1}$ (N–H), 1720 (C=O), 1713 (C=O), 1678 (C=O). $^1$H NMR (200 MHz, DMSO): $\delta$=1.28 (t, $J$=7 Hz, 3H, CH$_3$), 4.24 (q, $J$=7 Hz, 2H, CH$_2$), 6.75 (d, $J$=16 Hz, 1H, CH), 7.54 (d, $J$=16 Hz, 1H, CH), 8.15 (d, $J$=9 Hz, 2H, ArH), 8.37 (d, $J$=9 Hz, 2H, ArH), 11.73 (s, 1H, NH). $^{13}$C NMR (50 MHz, DMSO): $\delta$=14.8 (CH$_3$), 59.6 (CH$_2$), 134.4 (CH), 137.8
(CH), 123.7 (ArC), 123.8 (ArC), 128.2 (ArC), 128.3 (ArC), 139.4 (ArC), 150.6 (ArC), 150.7 (ArC), 156.1 (C=O), 165.5 (C=O), 166.4 (C=O). Anal Calcd for C13H12N2O6 (292.24): C 53.43, H 4.14, N 9.59; Found: C: 55.29, H 4.53, N 8.56.

4-(2-Methoxybenzoylamino)-4-oxobut-2-enoic Acid Ethyl Ester (5d)

2-Methoxybenzoic acid amide (2.100g, 13.9mmol) was reacted with fumaric acid chloride (3a)(1.000g, 7.4mmol) as described in procedure A to afford imide 5d (785mg, 46%), mp: 98-100°C. IR (KBr) v=3303cm-1 (N-H), 1720 (C=O), 1705 (C=O), 1684 (C=O). 1H NMR (200 MHz, CDCl3): δ=1.38 (t, J=7Hz, 3H, CH3), 4.09 (s, 3H, OCH3), 4.32 (q, J=7Hz, 2H, CH2), 6.94 (d, J=14Hz, 1H, CH), 7.18 (m, 2H, ArH), 7.62 (m, 1H, CH), 8.19 (d, J=14Hz, 1H, CH), 10.44 (s, 1H, NH). 13C NMR (50MHz, CDCl3): δ=14.5 (CH3), 56.7 (OCH3), 61.7 (CH2), 112.2 (CH), 120.1 (ArC), 122.2 (CH), 133.3 (ArC), 135.6 (ArC), 136.3 (ArC), 164.2 (C=O), 165.6 (C=O), 166.4 (C=O). Anal Calcd for C14H15NO5 (277.27): C 60.64, H 5.45, N 5.05; Found: C: 60.47, H 5.52, N 5.19.

4-(4-Methoxybenzoylamino)-4-oxobut-2-enoic Acid Ethyl Ester (5e)

4-Methoxybenzoic acid amide (500mg, 3.3mmol) in dry toluene (8ml) was reacted with fumaric acid chloride (3a)(240mg, 1.8mmol) as described in A to afford 5e (185mg, 45%), mp: 120-122°C. IR (KBr) v=3271cm-1 (N-H), 1726 (C=O), 1709 (C=O), 1668 (C=O). 1H NMR (200MHz, CDCl3): δ=1.38 (t, J=7Hz, 3H, CH3), 3.93 (s, 3H, OCH3), 4.32 (q, J=7Hz, 2H, CH2), 6.98 (d, J=14Hz, 1H, CH), 7.03 (d, J=8Hz, 2H, ArH), 7.89 (d, J=8Hz, 2H, ArH), 8.04 (d, J=14Hz, 1H, CH), 8.70 (s, 1H, NH). 13C NMR (50MHz, CDCl3): δ=14.5 (CH3), 55.9 (OCH3), 61.7 (OCH2), 114.6 (CH), 124.6 (ArC), 130.8 (CH), 133.8 (ArC), 135.4 (ArC), 136.7 (ArC), 136.4 (ArC), 164.2 (C=O), 165.6 (C=O), 166.4 (C=O). Anal Calcd for C14H15NO5 (277.27): C 60.64, H 5.45, N 5.05; Found: C: 60.38, H 5.47, N 5.19.

4-(3,5-Dimethoxybenzoylamino)-4-oxobut-2-enoic Acid Ethyl Ester (5f)

3,4-Dimethoxy-benzoic acid amide (500mg, 2.8mmol) in dry toluene (8ml) was reacted with fumaric acid chloride (3a)(200mg, 1.5mmol) as described in procedure A to afford 5f (362mg, 43%), mp: 162-164°C. IR (KBr) v=3271cm-1 (N-H), 1722 (C=O), 1705 (C=O), 1675 (C=O). 1H NMR (200 MHz, CDCl3): δ=1.38 (t, J=7Hz, 3H, CH3), 3.88 (s, 6H, 2×OCH3), 4.33 (q, J=7Hz, 2H, CH2), 6.72 (d, J=14Hz, 1H, CH), 7.01 (s, 1H, ArH), 7.30 (d, J=14Hz, 1H, CH), 8.02 (d, J=8Hz, 2H, ArH), 8.78 (s, 1H, NH). 13C NMR (50 MHz, CDCl3): δ=14.5 (CH3), 56.1 (OCH3), 61.6 (OCH2), 121.6 (CH), 124.8 (ArC), 130.4 (CH), 133.2 (ArC), 136.8 (ArC), 137.9 (ArC), 164.4 (ArC), 165.8 (C=O), 166.1 (C=O), 167.3 (C=O). Anal Calcd for C15H17NO6 (307.30): C 58.63, H 5.58, N 4.56. Found: C 59.77, H 5.52.

4-(3,4,5-Trimethoxybenzoylamino)-4-oxobut-2-enoic Acid Ethyl Ester (5g)

The sodium salt of the amide was prepared by stirring a mixture 3,4,5-trimethoxy-benzoic acid amide (4g, 18.9 mmol) and NaH (1g, 20.8mmol) in THF (100ml) for 30 minutes, and reacted with fumaric acid monoethyl ester anhydride (5.1g, 18.9mmol) in 30 ml THF (procedure B). After column chromatography, 5g was isolated (4.1g, 65%). mp: 114~116°C. IR (KBr) v=3255cm-1 (N-H), 1729 (C=O), 1707 (C=O), 1672 (C=O). 1H NMR (200 MHz, CDCl3): δ=14.5 (CH3), 56.0 (OCH3), 56.7 (OCH3), 61.3 (OCH3), 106.2 (CH), 124.7 (ArC), 127.1 (CH), 133.6 (ArC), 135.3 (ArC), 153.3 (ArC), 165.2 (ArC), 166.0 (C=O), 168.5 (C=O), 169.2 (C=O). Anal Calcd for C16H19NO7 (337.32): C 56.91, H 6.02. Found: C 56.91, H 6.02.

4-Benzoylamino-4-oxobut-2-enoic Acid Octyl Ester (5h)

The sodium salt of benzamide was prepared by stirring a mixture of benzamide (500mg, 4.1mmol) and NaH (172mg, 4.1mmol) in THF (35ml) for 30 minutes, and reacted with fumaric acid monoethyl ester anhydride (1.82g, 4.1 mmol) in 15 ml THF (procedure B). After column chromatography, 13 was isolated as an oil (190mg, 13%). IR (KBr) v=3292cm-1 (N-H), 1724 (C=O), 1709 (C=O), 1687 (C=O). 1H NMR (200MHz, CDCl3): δ=14.5 (CH3), 56.0 (OCH3), 56.7 (OCH3), 61.3 (OCH3), 106.2 (CH), 124.7 (ArC), 127.1 (CH), 133.6 (ArC), 135.3 (ArC), 143.2 (ArC), 153.3 (ArC), 165.2 (ArC), 166.0 (C=O), 168.5 (C=O), 169.2 (C=O). Anal Calcd for C19H25NO4 (337.32): C 69.77, H 5.68. Found: C 69.11, H 6.02.
4-Oxo-4-phenylacetylaminobutyric Acid Methyl Ester (10)

Phenylacetic acid amide (6a) (1.35 g, 10 mmol) in dry toluene (20 ml) was reacted with succinic acid monomethyl ester chloride (7) (1.65 g, 12.26 mmol) as described in general procedure A to afford 692 mg (28%) of 10. IR (KBr): v = 3260 cm⁻¹, 1735 (C=O), 1705 (C=O), 1657 (C=O). ¹H NMR (200 MHz, CDCl₃): δ = 2.69 (t, J = 2 Hz, 4H, CH₂), 3.69 (s, 2H, PhCH₂), 3.70 (s, 3H, OCH₃), 7.37 (m, 5H, ArH), 9.05 (s, 1H, NH). ¹³C NMR (50 MHz, CDCl₃): δ = 28.7 (CH₂), 28.9 (CH₂), 39.3 (PhCH₂), 50.4 (CH₃), 127.4 (ArC), 129.0 (ArC), 129.8 (ArC), 135.9 (ArC), 170.7 (C=O), 172.0 (C=O), 175.2 (C=O). Anal Calcd for C₁₃H₁₅NO₄ (249.26): C 62.58, H 6.01; Found: C 61.33, H: 5.33.

3-Oxo-3-phenylacetylaminobutyric Acid Methyl Ester (12)

Phenylacetic acid amide (6a) (1.0 g, 7.4 mmol) was reacted with malonic acid monomethyl ester chloride (8) (1.22 g, 8.1 mmol) in toluene (5 ml) to afford 12 (520 mg, 30%) after column chromatography (petroleum ether/ethyl acetate 3/1), mp 93–96°C. IR (KBr) v = 3266 cm⁻¹ (N–H), 1750 (C=O), 1709 (C=O), 1698.2 (C=O). ¹H NMR (200 MHz, CDCl₃): δ = 3.76 (s, 2H, CH₂), 3.78 (s, 3H, OCH₃), 3.85 (s, 2H, ArCH₂), 7.31 (m, 3H, ArH), 7.40 (m, 2H, ArH), 8.62 (s, 1H, NH). ¹³C NMR (50 MHz, CDCl₃): δ = 28.7 (CH₂), 28.9 (CH₂), 39.3 (PhCH₂), 50.4 (CH₃), 127.4 (ArC), 129.0 (ArC), 129.8 (ArC), 135.9 (ArC), 170.7 (C=O), 172.0 (C=O), 175.2 (C=O). Anal Calcd for C₁₃H₁₅NO₄ (249.26): C 62.33, H 5.33.
4-(N'-Benzoylhydrazino)-4-oxobut-2-enoic Acid Ethyl Ester (16)
The potassium salt of benzoylhydrazine was prepared by reaction of benzoylhydrazine hydrochloride (300mg, 2.2 mmol) in a hot methanolic solution (5ml) of KOH (98mg) and evaporation of the methanol at reduced pressure. The residue was dissolved in CH₂Cl₂ (10ml) and fumaric acid chloride ethyl ester (360mg, 2.7mmol) was added. The solution was stirred at room temperature for 1 hour, the precipitate was filtered off and washed with cold CH₂Cl₂ to yield 16 (256mg, 50%), mp: 236-238°C. IR (KBr) ν=3498cm⁻¹ (N-H), 3390 (N-H), 1654 (C=O), 1650 (C=O). ¹H NMR (200MHz, DMSO): δ=1.26 (t, 3H, CH₃), 4.20 (q, 2H, CH₂), 6.72 (d, J=15Hz, 1H, CH), 7.09 (d, J=15Hz, 1H, CH), 7.58 (m, 3H, ArH), 7.92 (m, 2H, ArH), 10.53 (s, 1H, NH), 11.15 (s, 1H, NH). ¹³C NMR (50MHz, DMSO): δ=14.9 (CH₃), 61.7 (CH₂), 128.3 (ArC), 129.4 (ArC), 130.8 (ArC), 131.5 (ArC), 132.7 (ArC), 133.4 (ArC), 135.4 (CH), 137.5 (CH), 161.4 (C=O), 165.6 (C=O), 166.7 (C=O). Anal Calcd for C₁₃H₁₄N₂O₄ (262.26) C: 59.54, H: 5.38; Found: C 59.48, H 5.34.

4-Benzoylaminoxy-4-oxobut-2-enoic Acid Ethyl Ester (18)
A mixture of benzoic acid (6.10g, 50mmol), hydroxylamine (65mmol) and dicyclohexyl carbodiimide (10.30g, 50mmol) in methanol (30ml) was stirred for 1 hour. The methanol was evaporated at reduced pressure and the residue extracted with 10% aqueous NaOH (10ml). The basic phase was acidified with 10% HCl (10.3ml) and extracted with CH₂Cl₂ (30ml). The organic phase was dried (Na₂SO₄) and the solvent removed at reduced pressure. A sample of this crude hydroxamic acid 17 (250mg), fumaric acid ethyl ester (3a) (300mg, 1.8mmol), and triethylamine (184mg, 1.8mmol) was dissolved in toluene (20ml) and stirred for 1 hour at room temperature to yield 18 (290mg, 61%) after column chromatography on silica gel, mp: 76-78°C. IR (KBr): ν=3076cm⁻¹ (N-H), 1796 (C=O), 1720 (C=O). ¹H NMR (200MHz, CDCl₃): δ=1.28 (t, J=7Hz, 3H, CH₃), 4.24 (q, J=7Hz, 2H, CH₂), 6.75 (d, J=14Hz, 1H, CH), 7.16 (d, J=14Hz, 1H, CH), 7.56 (s, 1H, ArH), 8.37 (m, 2H, ArH). ¹³C NMR (50MHz, CDCl₃): δ=14.8 (CH₃), 61.4 (OCH₂), 113.2 (ArC), 113.3 (ArC), 119.1 (ArC), 132.0 (ArC), 131.2 (ArC), 135.1 (CH), 137.8 (CH), 144.6 (ArC), 165.2 (C=O), 166.4 (C=O). Anal Caled for C₁₃H₁₃NO₅ (263.25): C 59.31, H 4.98; Found: C 59.60, H 5.03.

3-(N'-Phenylhydrazinocarbonyl)-acrylic Acid Ethyl Ester (20a)
A mixture of phenylhydrazine (1.08g, 10mmol) and fumaric acid chloride ethyl ester (1.34g, 10mmol) in dichloromethane (25ml) and 2–3 drops of triethyl amine was stirred for 30 minutes. The precipitate was filtered off and washed with CH₂Cl₂ to yield 20a (976mg, 48%), mp: 140–142°C. IR (KBr): ν=3368cm⁻¹ (N–H), 3096 (N–H), 1644 (C=O), 1614 (C=O). ¹H NMR (200MHz, CDCl₃): δ=1.28 (t, J=7Hz, 3H, CH₃), 4.22 (q, J=7Hz, 2H, CH₂), 6.77 (d, J=14z, 1H, CH), 6.96 (m, 2H, ArH), 7.27 (d, J=14Hz, 1H, CH), 7.49 (m, J=8Hz, 3H, ArH), 9.98 (s, 1H, NH), 10.15 (s, 1H, NH). ¹³C NMR (50MHz, CDCl₃): δ=13.8 (CH₃), 61.4 (OCH₂), 113.2 (ArC), 113.3 (ArC), 119.1 (ArC), 132.0 (ArC), 131.2 (ArC), 135.1 (CH), 137.8 (CH), 144.6 (ArC), 165.2 (C=O), 166.4 (C=O). Anal Caled for C₁₂H₁₄N₂O₄ (234.25): C 61.53, H 6.02; Found: C 61.60, 6.08.

3-(N'-Phenylhydrazinocarbonyl)-acrylic Acid Octyl Ester (20b)
A mixture of 2,4-dinitrophenylhydrazine (1.5g, 7.6 mmol) and fumaric acid chloride ethyl ester (1.13g, 8.4 mmol) in dichloromethane (30ml) and 2–3 drops of triethyl amine was stirred for 30 minutes. The precipitate was filtered off, and washed with CH₂Cl₂ to yield 20b (604mg, 78%), mp: 161–163°C. IR (KBr) ν=3278cm⁻¹ (N–H), 3024 (N–H), 1695 (C=O), 1670 (C=O). ¹H NMR (200MHz, CDCl₃): δ=1.28 (t, J=7Hz, 3H, CH₃), 4.24 (q, J=7Hz, 2H, CH₂), 6.75 (d, J=14Hz, 1H, CH), 7.16 (d, J=14Hz, 1H, CH), 7.56 (s, 1H, ArH), 8.37 (m, 2H, ArH). 10.08 (s, 1H, NH), 10.28 (s, 1H, NH). ¹³C NMR (50MHz, CDCl₃): δ=14.8 (CH₃), 61.8 (OCH₂), 110.0 (ArC), 113.5 (ArC), 134.4 (ArC), 135.2 (CH), 137.6 (CH), 143.5 (ArC), 148.9 (ArC), 150.1 (ArC), 165.0 (C=O), 166.1 (C=O). Anal Caled for C₁₂H₁₂N₄O₇ (324.25): C 44.45, H 3.73; Found: C 44.36, 3.69.

3-(N'-Phenyl)-hydrazinocarboxylic Acid Octyl Ester (20c)
A solution of phenylhydrazine (504mg, 4.6mmol) and fumaric acid mono-octyl ester anhydride (1.15g, 5.0mmol) in CH₂Cl₂ (10ml) and 2–3 drops of triethyl amine was stirred at room temperature for 1 hour. The solution was cooled to 0°C, the precipitate was filtered off and washed with cold CH₂Cl₂ to yield hydrazide 20c (800mg, 57%), mp: 130–132°C. IR (KBr): ν=3383cm⁻¹ (N–H), 3283 (N–H), 1675 (C=O), 1594 (C=O). ¹H NMR (200MHz,
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\[ \delta = 0.91 \text{ (t, 3H, CH}_3\text{)}, 1.32 \text{ (m, 10H, 5-CH}_2\text{)}, 4.23 \text{ (t, J=4Hz, 2H, CH}_2\text{)}, 6.73 \text{ (d, J=14Hz, 1H, CH)}, 7.01 \text{ (d, 2H, ArH), 7.21 \text{ (d, J=14Hz, 1H, CH), 7.51 (m, 3H, ArH), 9.88 (s, 1H, NH), 10.11 (s, 1H, NH)}. \]
\[ \delta = 13.9 \text{ (CH}_3\text{)}, 22.8 \text{ (CH}_2\text{)}, 26.1 \text{ (CH}_2\text{)}, 29.8 \text{ (CH}_2\text{)}, 30.2 \text{ (CH}_2\text{)}, 30.5 \text{ (CH}_2\text{)}, 32.1 \text{ (CH}_2\text{)}, 65.4 \text{ (OCH}_2\text{)}, 112.9 \text{ (ArC), 118.7 \text{ (ArC), 129.1 \text{ (ArC), 133.8 \text{ (CH), 134.0 \text{ (CH), 134.2 \text{ (ArC), 134.0 \text{ (ArC), 134.2 \text{ (ArC), 165.3 (C=O), 166.3 (C=O), 170.7 (C=O)}}. Anal Calcd for C}_{12}\text{H}_{12}\text{N}_4\text{O}_7 \text{ (324.25): C 68.65, H 8.49; Found: C 68.05, H 8.43.} \]

(2,5-Dioxo-4-phenylpyrrolidin-3-ylidene)-acetic Acid Methyl Ester (24)

4-Phenylpyrrolidine-2,3,5-trione (23) was prepared by sodium ethoxide-catalyzed condensation of phenylacetamide (22) and ethyl oxalate (21) as described in the literature. A solution of 24 (94.5mg, 0.5mmol) and methoxycarbonylmethylenetriphenyl phosphorane (167mg, 0.5mmol) in dichloromethane (30ml) was refluxed for 3 hours. The solvent was removed under reduced pressure and the crude product purified by column chromatography on silica gel to yield 24 (84mg, 69%), mp: 92-93°C. \[ \delta = 3.52 \text{ (s, 3H, OCH}_3\text{)}, 4.69 \text{ (s, 1H, PhCH)}, 6.70 \text{ (s, 1H, CH)}, 7.37 \text{ (m, 5H, ArH). 13C-NMR (50MHz, CDCl}_3\text{):} \delta = 53.9 \text{ (CH}_3\text{)}, 125.8 \text{ (CH)}, 128.1 \text{ (ArC)}, 130.6 \text{ (ArC), 130.8 (ArC), 134.0 (ArC), 134.2 (ArC), 165.3 (C=O), 166.3 (C=O), 170.7 (C=O). Anal Calcd for C}_{13}\text{H}_{11}\text{NO}_4 \text{ (245.23): C 63.67, H 4.52; Found: C 62.75, H 4.81.} \]

(2,5-Dioxo-4-phenylpyrrolidin-3-ylidene)-acetic Acid Ethyl Ester (25)

A solution of 4-phenylpyrrolidine-2,3,5-trione (23) (510mg, 2.7mmol) and ethoxycarbonylmethylene-triphenyl phosphorane (940mg2.7mmol) in dichloromethane (30ml) was refluxed for 3 hours. The solvent was removed under reduced pressure. The residue was redissolved in CH\(_2\)Cl\(_2\) and filtered and the product was purified by column chromatography on silica gel (dichloromethane/methanol 9/1) to yield the ester 25 (295mg, 40%). \[ \delta = 1.11 \text{ (t, J=7.1Hz, 3H, CH}_3\text{), 3.76 (m, 2H, CH}_2\text{), 4.13 (q, J=7.1Hz, 2H, OCH}_2\text{), 6.86 (s, 1H, CH)}, 7.66 \text{ (m, 5H, ArH). Anal Calcd for C}_{15}\text{H}_{13}\text{NO}_4 \text{ (273.28): C 65.92, H 5.53; Found: C 64.12, H 5.05.} \]

3-Methoxymethylene-4-phenylpyrrolidine-2,5-dione (26)

A solution of methoxymethylene-triphenylphosphonium chloride (911mg, 2.7mmol) in dry THF was treated at -78°C with tert-BuOK (303mg, 2.7mmol). After 15 minutes stirring, trione 23 (500mg, 2.7mmol) was added. The mixture was allowed to warm to room temperature and stirring was continued for 24 hours. The solvent was removed under reduced pressure. The residue was redissolved in CH\(_2\)Cl\(_2\) and filtered and the product was purified by column chromatography on silica gel (dichloromethane/methanol 9/1) to afford the enol ether 26 (283mg, 48%). \[ \delta = 3.51 \text{ (s, 3H, OCH}_3\text{), 4.16 (s, 1H, PhCH)}, 5.25 \text{ (s, 1H, CH)}, 7.37 \text{ (m, 5H, ArH). Anal Calcd for C}_{15}\text{H}_{14}\text{NO}_3 \text{ (272.28): C 65.39, H 5.10; Found: C 65.39, H 4.86.} \]

3-(2,5-Dioxo-4-phenylpyrrolidin-3-ylidene)-propionic Acid Ethyl Ester (27)

A solution of ethoxycarbonyl-ethylene-triphenylphosphorane bromide in dry THF (20ml) was treated at -78°C with tert-BuOK (303mg, 2.7mmol). After 15 minutes stirring, trione 23 (500mg, 2.7mmol) was added. The mixture was allowed to warm to room temperature and stirring was continued for 24 hours. The solvent was removed under reduced pressure. The residue was redissolved in CH\(_2\)Cl\(_2\) and filtered and the product was purified by column chromatography on silica gel (dichloromethane/methanol 9/1) to yield the ester 27 (295mg, 40%). \[ \delta = 1.11 \text{ (t, J=7.1Hz, 3H, CH}_3\text{), 3.76 (m, 2H, CH}_2\text{), 4.13 (q, J=7.1Hz, 2H, OCH}_2\text{), 6.86 (s, 1H, CH)}, 7.66 \text{ (m, 5H, ArH). Anal Calcd for C}_{16}\text{H}_{14}\text{NO}_4 \text{ (275.28): C 65.92, H 5.53; Found: C 64.12, H 5.05.} \]

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