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Inhibitors of the Tissue Factor/Factor VIIa-Induced Coagulation: Synthesis and In Vitro Evaluation of Novel 2-Aryl Substituted Pyrido[3,4-d][1,3]-, Pyrido[2,3-d][1,3]-, Pyrazino[2,3-d][1,3]-, Pyrimido[4,5-d][1,3]-, Pyrazolo[3,4-d][1,3]-, Thieno[3,2-d][1,3]- and Thieno[2,3-d][1,3]-oxazin-4-ones

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Abstract—The synthesis of a series of 2-aryl substituted hetero annulated 1,3-oxazin-4-ones and their evaluation as specific inhibitors of the tissue factor (TF)/factor VIIa (FVIIa)-induced pathway of coagulation is reported. Inhibitory activities (IC_{50} values) in the range 0.64 to $>40\,\mu M$ on the activation of factor X (FX) by the TF/FVIIa complex were found for compounds having one or two electronegative substituents such as F and NO_2 in the 2-aryl substituent. Some of the compounds showed a selectivity ratio towards FX and thrombin of >50, thus being similar in specificity to 2-aryl substituted 4*H*-3,1-benzoxazin-4-ones described as potential drugs for oral antithrombotic treatment without side effects, such as bleeding, which is observed especially with thrombin inhibitors.

Introduction

Blood coagulation is a complex process involving various blood components or factors that eventually give rise to a fibrin clot. Activated factor X (FXa) is required to convert prothrombin to thrombin, which then converts fibrinogen to fibrin as a final stage in forming a fibrin clot.

2-Aryl substituted 4*H*-3,1-benzoxazin-4-ones[1] have been shown to act as specific inhibitors of the activation of factor X (FX) through the 'extrinsic pathway' in which factor VIIa (FVIIa) and its cofactor, tissue factor (TF), exert the proteolysis of FX to FXa.

TF is a membrane-bound protein and does not normally circulate in plasma. Upon vessel disruption, however, it is exposed and forms a complex with FVIIa to catalyse FX or factor IX activation in the presence of Ca^{2+} and phospholipid. FVIIa and TF appear to be the principal initiators of blood coagulation in vivo.[2] It is often desirable to inhibit the coagulation cascade in a patient. Anticoagulants such as heparin, coumarin and its derivatives, indandione derivatives, low-molecular-weight thrombin or FXa inhibitors, or other agents may be used. Treatment with heparin and other anticoagulants may, however, have undesirable side effects, for example bleedings. At the initial stage of blood coagulation, i.e., of the FVIIa/TF activity, inhibition, results in significantly less bleeding.[3] In addition, clinically available anticoagulants act throughout the body, rather than acting specifically at the site of injury where the coagulation cascade is active. Other known anticoagulants comprise thrombin and factor Xa inhibitors derived from bloodsucking organisms. Antithrombins, hirudin, hirulog and hirugen are recombinant proteins or peptides derived from the leach *Hirudo medicinalis*, whereas the factor Xa inhibitor antistatin and the recombinant derivative rTAP are tick-derived proteins. Inhibitors of platelet aggregation such as monoclonal antibodies or synthetic peptides, which interfere with the platelet receptor gpIIb/IIIa, are also effective as anticoagulants. These agents are, however, not suitable for oral administration. Specific protein inhibitors of the FVIIa/TF activity are the physiological inhibitor

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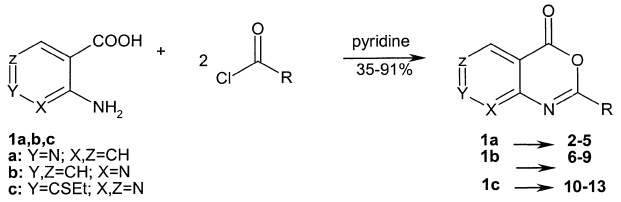
(tissue factor pathway inhibitor) and the inactivated FVII (FVIIai), which binds to TF and competes with FVIIa.

In connection with our investigations of 2-aryl substituted 4*H*-3,1-benzoxazin-4-ones,[1] we found it of interest to investigate the influence of alterations of ring size and the presence of heteroatoms in the 'benzo'-part of the molecule. Heteroatoms which change basicity as well as charge distribution and spatial arrangement in the ring system might influence the activity of the active ester (the lactone) and thereby cause change in activity and specificity.

We report here the synthesis of some hetero annulated 1,3-oxazin-4-one analogues of 2-aryl substituted 4*H*-3,1-benzoxazin-4-ones in which the benzo ring is changed to 5-membered or 6-membered heterocycles such as thiophen, pyrazol, pyrimidine, pyridine and pyrazine, and their SAR in in vitro systems of TF/FVIIa-catalysed activation of FX, as well as their effects on the amidolytic activity of FXa and thrombin and the prolongation of clotting time caused by selected compounds.

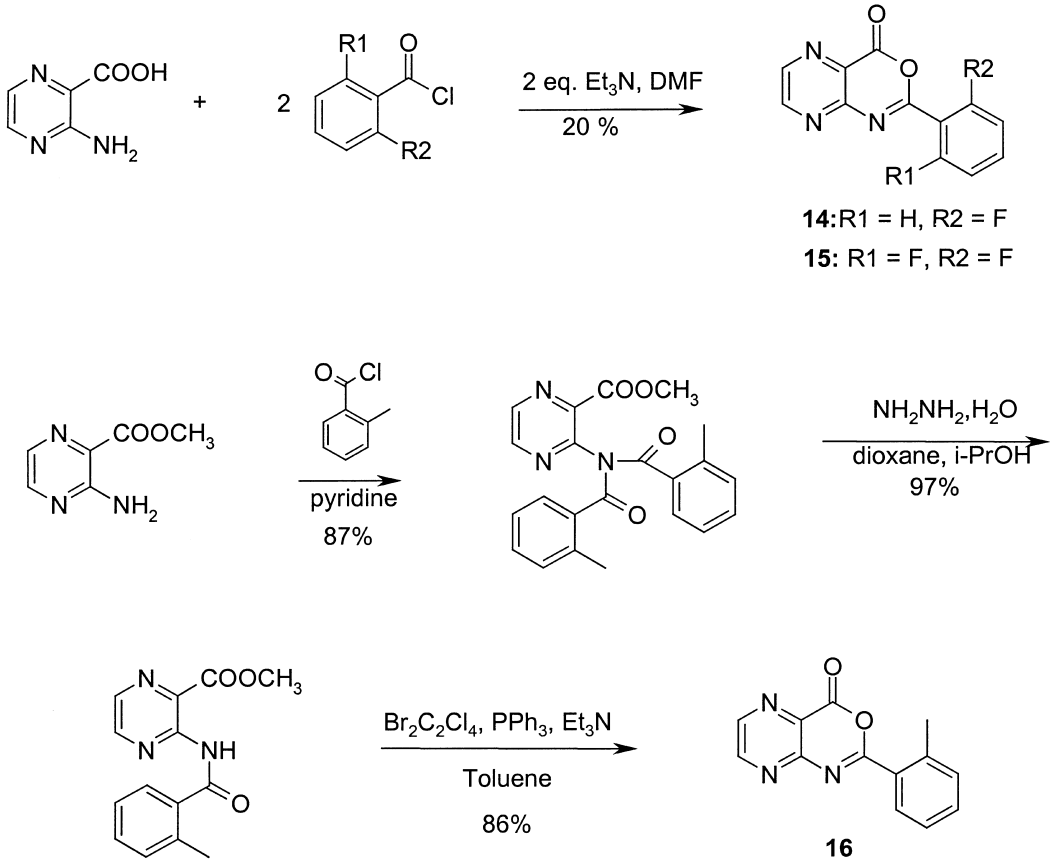
Hetero annulated 1,3-oxazin-4-ones have been reported as anti-inflammatory agents,[4,5] human leukocyte elastase inhibitors,[6,7] herpes proteases inhibitors,[8,9] and anti-allergia.[10] Different synthetic pathways to this group of compounds have been reported;[11–16] however, only few of the reported compounds contain an aryl group directly connected to the ring system, and none of the previously described compounds has been reported to specifically inhibit the TF/FVIIa-induced coagulation pathway.



Scheme 1. Reaction pathway for pyridines and pyrimidines. (Compound: R): **2**, **7**: 2,6-F_2Ph; **3**, **6**, **10**: 2-FPh; **4**, **8**: 2-thienyl; **5**, **9**: 2-furanyl; **11**: 2-NO_2Ph; **12**: 2-MePh; **13**: 2-ClPh.

Chemistry

The target compounds were prepared from optionally substituted *ortho* amino substituted heterocyclic carboxylic acids by means of reaction with an aryl carboxylic acid chloride in toluene or DMF using triethylamine or pyridine as base. The substitution pattern on the aroyl chlorides was mainly chosen as electronegative groups like F and NO_2 in the 2- or 2,6-positions, based on the knowledge from the benzoxazinone series.[1] However, few other substituents were also used. In some cases this methodology gave very low yields, and we used the method described by Wamhoff and Kroth[14] in which



Scheme 2. Reaction pathway to pyrazino[2,3-d][1,3]oxazin-4-ones **14**–**16**.

the *ortho* amino carboxylic acid esters were used, giving a diaroylamino intermediate which was treated with hydrazine followed by ring closure with $Br_2C_2Cl_4$ and PPh_3. The reactions are depicted in Schemes 1–4.

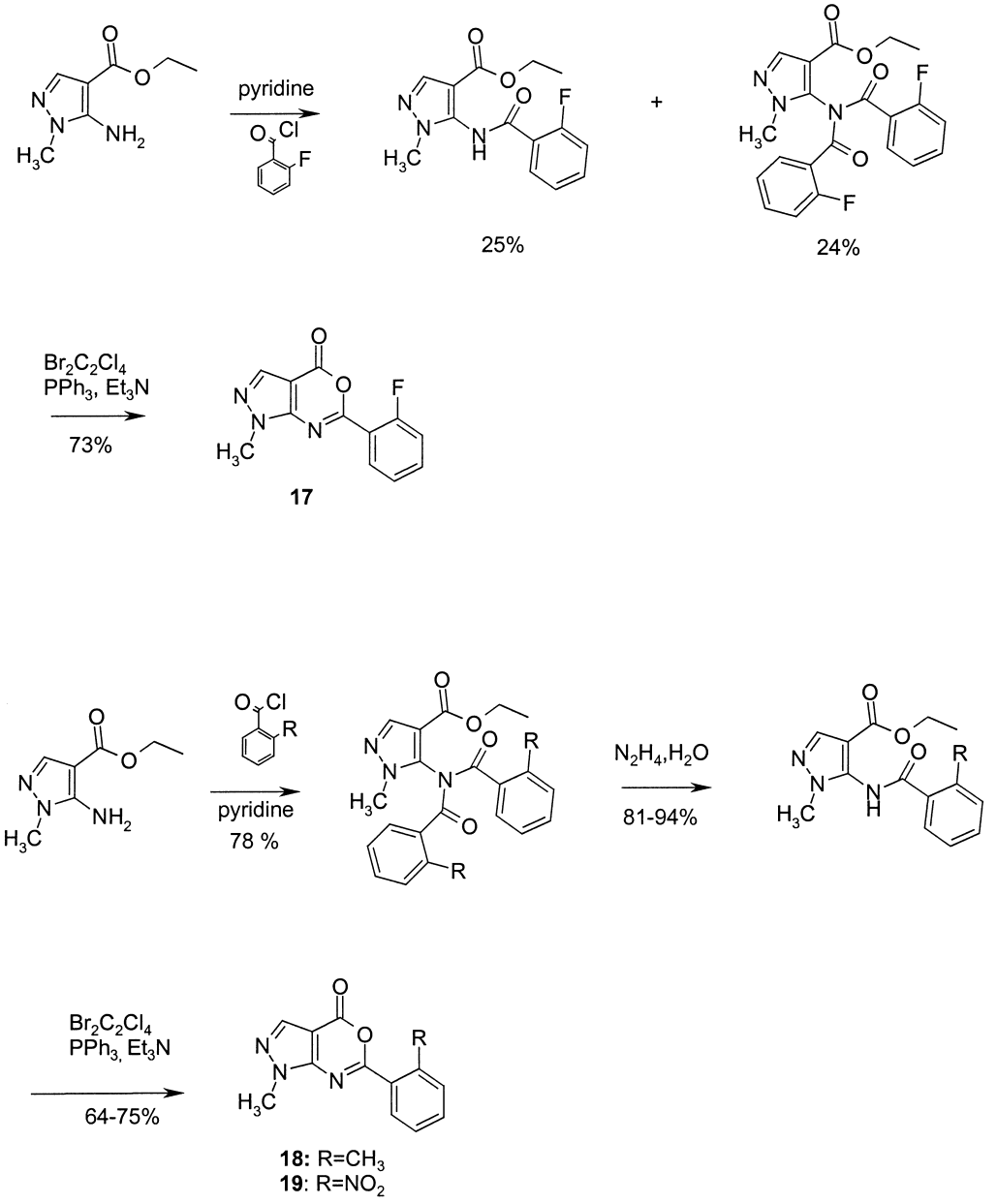
For the pyridine and pyrimidine systems the ring closure proceeded straightforwardly in pyridine solution giving fair to good yields as reported for most benzo analogues[1] (Scheme 1).

In the pyrazine series this reaction also proceeded smoothly when 2-fluoro or 2,6-difluorobenzoyl chloride was used as reagent; however, for the 2-methylbenzoyl chloride this gave low yield and the *N,N*-disubstituted intermediate was formed. This was subsequently treated with hydrazine followed by ring closure by means of $Br_2C_2Cl_4$ and PPh_3 (Scheme 2).

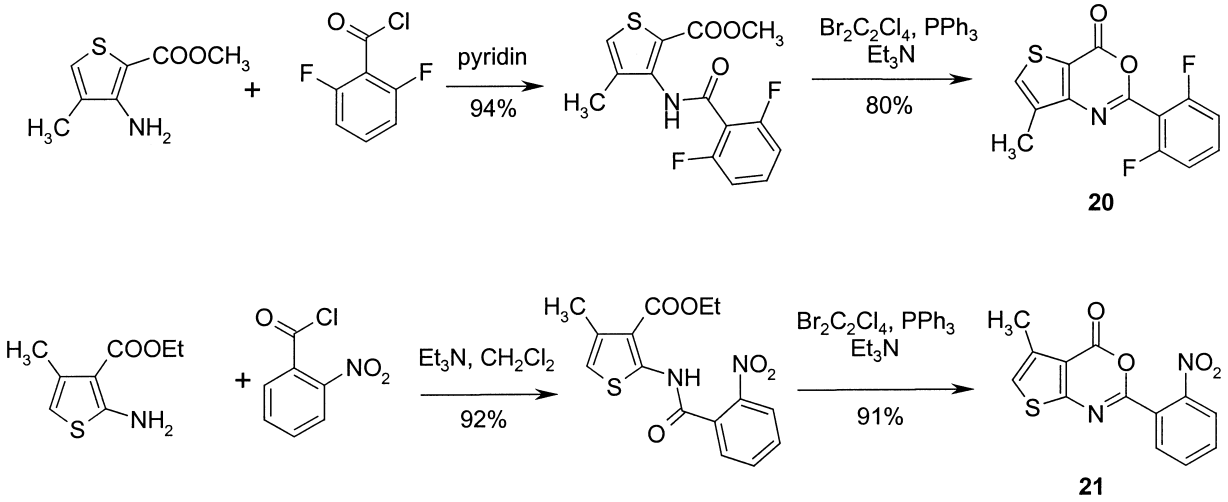
The same methodology was used for the preparation of pyrazolo[2,3-d][1,3]oxazin-4-one series. 2-Fluorobenzoyl chloride reacted with ethyl 5-amino-1-methylpyrazole-4-carboxylate giving a 1:1 mixture of the *N*-mono-*N,N*-disubstituted intermediate which on ring closure gave good yield of the hetero annulated oxazinone **17**. For 2-methyl- and 2-nitrobenzoyl chloride only the *N,N*-disubstituted intermediate was identified, leading to compounds **18** and **19** on hydrazine treatment and ring closure (Scheme 3). The same reaction sequence was used in the thienyl series (Scheme 4).

Biology

The compounds were evaluated for inhibitory activities on the TF/FVIIa-induced activation of FX using a two-



Scheme 3. Synthetic pathway to pyrazolo[2,3-d][1,3]oxazin-4-ones **17**–**19**.



Scheme 4. Synthetic pathway to thieno[3,2-d][1,3]- and thieno[2,3-d][1,3]-oxazin-4-ones **20** and **21**.

step amidolytic assay. Compounds showing good inhibitory activities in that assay were further tested for activities on FXa and thrombin in order to sort out structures with the best selectivity. Selected compounds were further tested for their abilities to prolong clotting time in a standard in vitro clotting assay. Selected data are depicted in Table 1.

Discussion

In the pyrazine series **14**–**16**, no inhibition of the FX activation was observed even when the best 2-substituents were used. For the rest of the investigated compounds all the heterocycles were active as inhibitors when the optimal 2-aryl substituents were chosen. Thienyl and furanyl groups in the 2-position (**4**, **5**, **8** and **9**) likewise exerted no inhibitory effects on the FX activation. The best selectivity for a heterocyclic structure taken as a group was seen in the pyrimidine series (**10**–**13**); however, only **12** gave some prolongation in clotting ratio. The strongest inhibitors were found in the pyridine series **2**, **3**, **6** and **7**, but only compound **2** had a reasonable specificity, the effect on FX and thrombin being $>200\,\mu M$, with a slight effect on the clotting ratio. The strongest effect on the clotting time was observed for **18**, **19** and **21** (1.84, 1.82, and 2.18, respectively); for **20** and **21** this might be due partly to the inhibitory effect on thrombin resp. FX. It is of interest that compounds **12** and **18**, which both have a 2-methylphenyl substituent, exerted a good inhibition, selectivity as well as prolongation of clotting time; this was not seen in the benzoxazinone series.[1]

Table 1. IC_{50} values (μM) for the inhibition of TF/FVIIa-catalysed activation of FX, FXa and thrombin, and effects on TF/FVIIa-induced clotting. The ratio of the clotting times with (330 μM) and without test compound is given

Compound no.	FX Activation IC_{50} (μM)	FXa IC_{50} (μM)	Thrombin IC_{50} (μM)	Clotting ratio
2	1.3	>200	>200	1.24
3	2.6	90		1.04
4	>40			
5	>40			
6	2.8	32	14.4	1.37
7	0.64	5.6	126.5	1.1
8	30	>200	36.9	1.2
9	25	>200	46.5	1.34
10	15	>200	>200	1.06
11	5.6	139	>200	0.96
12	1.1	78	125	1.23
13	12	182	>200	0.95
14	>200			
15	>200			
16	>200			
17	>40			
18	18	>200	129	1.84
19	7.7	>200	35	1.48
20	5.3	>200	16	1.82
21	3.5	42	141	2.18

The expected mechanism of action for the serine protease inhibition by benzoxazinone-type compounds is deactivation by means of reaction of the benzoxazinone lactone with the active site serine, probably under the formation of a drug-enzyme ester followed by its hydrolysis.[17,18] This mechanism would expectedly be much influenced by the presence of electronegative groups influencing the polarity of the lactone carbonyl group and thereby changing the reactivity. Also the presence of bulky groups or rings would be expected to have influence on the reactivity. Obviously, the heterocyclic structure exhibiting the most negative effect on the activity as a serine protease inhibitor was the pyrazine group, the proximity of the nitrogen atom to the lactone diminishing the polarity of the C=O. The same effect was not seen in the pyrimidine or pyridine series. This seems to be in accordance with data for the benzoxazinone series in which electronegative substituents in the 5 and 6 positions gave good inhibitory effects in the FX activation assay, while amino substitution at the same positions gave compounds with less inhibitory effects in compounds carrying one of the best 2-substituents, namely 2,6-difluorophenyl. The orientation of the thieno group in **20** and **21** seemed to influence the activity on FX and thrombin, while the effect on FVIIa/TF-induced FX activation was almost the same for the two compounds.

Conclusion

The synthetic approach described easily yielded a series of 2-aryl substituted hetero annulated 1,3-oxazin-4-ones in fair to good yields by standard synthetic methods. The compounds described comprise several compounds with excellent activity and selectivity, thus supporting the information obtained for similar benzo compounds. Good inhibition of the FVIIa/TF induced activation of FX seems to require the presence of at least one electron-attracting substituent in the *ortho* position of the 2-aryl substituent, while reasonable inhibitory effect was also obtained with a methyl substituent when the annulated heterocycle was a pyrazole or pyrimidine. Annulated pyrazine, however, gave no inhibitory effect when the same 2-aryl substituents were used. Simple heterocycles like thienyl or furanyl as the 2-substituents caused disappearance of inhibitory effect.

Experimental

General procedures

Melting points (uncorrected) were measured on a Büchi 535. NMR spectra were recorded on a Bruker Avance DPX 200 or 300 MHz instrument operating at room temperature. Mass spectra were obtained on a Finnigan MAT TSQ 70 apparatus using a direct inlet system. The HPLC-MS analyses were performed on a PE Sciex API 100 LC/MS system using a Waters™ 3 mm × 150 mm, 3.5 μ, C-18 Symmetry column and positive ion spray with a flow rate at 20 μL/min. The column was eluted with a linear gradient of 5–90% A, 85–0% B and 10% C for 15 min at a flow rate of 1 mL/min (solvent A = acetonitrile, solvent B = water, and solvent C = 0.1% trifluoroacetic acid in water). Microanalyses were performed by Novo Nordisk Analytical Department.

FX activation assay

The compounds were dissolved in DMSO and mixed with FVIIa (produced in-house) in 50 mM Hepes, pH 7.4, containing 0.1 M NaCl, 5 mM $CaCl_2$, and 1 mg/mL bovine serum albumin (1 + 5). 30 μL of this mixture was then mixed with 45 μL TF (American Diagnostica, relipidated in PC/PS vesicles) and 25 μL of FX (Enzyme Research Laboratories), all in a Ca^{2+}-containing buffer. This gave final concentrations of 100 pM FVIIa, 5 pM TF, 175 nM FX, and various concentrations of the compounds. After a 5-min incubation, the FVIIa/TF-catalysed activation of FX was terminated by an addition of 50 μL buffer containing enough EDTA to give an excess of the Ca^{2+} ions present. 50 μL of a 2-mM solution of S-2765 (Chromogenix, 2 mM in water) was then added and the FXa formed was allowed to hydrolyse the substrate for 10 min, during which the absorbance at 405 nm was continuously monitored in a SPECTRAmax™ 340 plate reader. The slope of the absorbance curve was compared to that of a control where only DMSO was added to FVIIa/TF/FX.

Thrombin activity assay

170 μL thrombin (Boehringer Mannheim, 3.5 nM) in the buffer described above, 10 μL test compound in DMSO (or DMSO alone in the control) and 20 μL S-2238 (Chromogenix, 10 mM in water) were mixed. The hydrolysis of S-2238 was monitored as described for the FX activation assay.

FXa activity assay

170 μL FXa (Enzyme Research Laboratories, 1.2 nM in buffer) in the buffer described above, 10 μL test compound and 20 μL S-2765 (Chromogenix, 10 mM in water) were mixed. The hydrolysis of S-2765 was monitored as described for the FX activation assay.

FVIIa/TF-initiated clotting assay

The test compounds, 20 mM in DMSO, were diluted in citrated normal human plasma just before the analysis (1 + 19) and placed in the sample carousel of an ACL 300 Research coagulometer. A 55-μL sample (compound in plasma) was mixed with 55 μL of thromboplastin (Innovin, Dade; diluted 1:500 after dissolution) and incubated for 5 min. The clotting reaction was started by adding 55 μL of a 25-mM $CaCl_2$ solution, yielding a final compound concentration of 0.33 mM. The ratio between the clotting time in the presence and absence of test compounds was used to quantify the anticoagulant effect.

2-(2,6-Difluoro-phenyl)-pyrido[3,4-d][1,3]oxazin-4-one (2). 3-Aminopyridine-4-carboxylic acid (0.28 g, 2.0 mmol) was dissolved in dry pyridine (8 mL), and 2,6-difluorobenzoyl chloride (0.56 mL, 4.5 mmol) was added dropwise under stirring and cooling. The reaction was stirred at room temperature for 24 h, after which it was concentrated in vacuo. The crude product was purified by flash chromatography on silica-gel using EtOAc:heptane 1:4 and EtOAc:MeOH 10:1 as the eluent. Two compounds were isolated: impure **2**, 0.39 g, R_f 0.50 EtOAc:heptane 1:1, and a yellow crystalline compound, 0.11 g, R_f 0.50 EtOAc:MeOH 10:1. Impure **2** was dissolved in CH_2Cl_2, washed with aq $NaHCO_3$ (5×10 mL), dried (Na_2SO_4) and concentrated to give **2** as colourless crystals, 0.22 g (44%): mp 137–140 °C; EI/SP MS: M^+ 260; ^{1}H NMR ($CDCl_3$) 9.30 (1H, s), 9.00 (1H, d), 8.05 (1H, d), 7.55 (1H, p), 7.10 (1H, t); ^{13}C NMR ($CDCl_3$) 163.8, 163.7, 158.7, 158.6, 157.8, 153.1, 150.9, 150.1, 140.8, 134.3, 134.1, 133.9, 123.2, 120.4, 113.0, 112.9, 112.5, 112.4, 110.7, 110.4, 110.0.

2-(2-Fluoro-phenyl)-pyrido[3,4-d][1,3]oxazin-4-one (3). 3-Aminopyridine-4-carboxylic acid (0.28 g, 2.0 mmol) was dissolved in dry pyridine (10 mL), cooled in an ice bath, and 2-fluorobenzoyl chloride (0.53 mL, 4.5 mmol) was added dropwise. The solution was stirred at room temperature for 16 h, and the solvent was evaporated in vacuo. The solid was purified by flash chromatography on silica-gel using EtOAc:heptane 1:1 as the eluent, to give **3** as colourless crystals, 0.44 g (91%): mp 130–135 °C; R_f 0.50 (EtOAc:heptane 1:1); ^{1}H NMR ($CDCl_3$) 9.18

(1H, s), 8.83 (1H, d), 8.11 (1H, dt), 8.03 (1H, dd), 7.6-7.5 (1H, m), 7.35–7.11 (2H, m); ^{13}C NMR ($CDCl_3$) 164, 159.3, 158.0, 157.2, 157.1, 150.8, 149.2, 141.4, 135.2, 135.0, 131.6, 124.9, 124.8, 123.0, 120.4, 118.8, 118.6, 118.0, 117.6.

2-Thiophen-2-yl-pyrido[3,4-d][1,3]oxazin-4-one (4). 3-Aminopyridine-4-carboxylic acid (0.28 g, 2.0 mmol) was dissolved in dry pyridine (8 mL), and 2-thiophenecarbonyl chloride (0.47 mL, 4.5 mmol) was added dropwise under cooling and stirring. The solution was stirred at room temperature for 30 h and subsequently concentrated in vacuo. Purification by flash chromatography on silica-gel using CH_2Cl_2:acetone 50:1 as the eluent gave crude **3**, 0.50 g. The crude product was dissolved in CH_2Cl_2 (40 mL) and washed with aqueous $NaHCO_3$ (5×20 mL), dried (Na_2SO_4) and concentrated to give **4**, 0.35 g (76%). The compound was recrystallized from EtOAc; mp 167–168 °C; EI/SP MS: M^+ 230; ^{1}H NMR ($CDCl_3$) 9.09 (1H, d), 8.74 (1H, d), 8.00 (2H, dd), 7.68 (1H, dd), 7.21 (1H, dd); ^{13}C NMR ($CDCl_3$) 158.0, 155.8, 150.4, 148.5, 141.8, 133.9, 133.0, 128.9, 122.5, 120.4. Anal. calcd for: C, 57.38%; H, 2.63%; N, 12.17%; found: C, 57.38%; H, 2.59%; N, 12.00%.

2-Furan-2-yl-pyrido[3,4-d][1,3]oxazin-4-one (5). 3-Aminopyridine-4-carboxylic acid (0.28 g, 2.0 mmol) was dissolved in dry pyridine (8 mL), and 2-furan-carbonyl chloride (0.45 mL, 4.5 mmol) was added under stirring and cooling. The solution was stirred at room temperature for 30 h, the solvent was evaporated and the residue was dissolved in CH_2Cl_2 (40 mL). The organic phase was washed with saturated $NaHCO_3$ (5×20 mL) and concentrated in vacuo to give **5**, 0.32 g (76%). The compound was dissolved in EtOAc and filtered through activated charcoal, followed by recrystallization from EtOAc to give **5** as colourless crystals: mp 173–174 °C; EI/SP MS: M^+ 214; ^{1}H NMR ($CDCl_3$) 9.15 (1H, d), 8.78 (1H, d), 7.98 (1H, dd), 7.76 (1H, dd), 7.43 (1H, dd), 6.67 (1H, dd). Anal. calcd for: C, 61.69%; H, 2.28%; N, 13.08%; found: C, 61.67%; H, 2.78%; N, 12.81%.

2-(2-Fluoro-phenyl)-pyrido[2,3-d][1,3]oxazin-4-one (6). 2-Aminopyridine-3-carboxylic acid (0.28 g, 2.0 mmol) was dissolved in dry pyridine (8 mL) and 2-fluorobenzoyl chloride (0.53 mL, 4.5 mmol) was added under stirring and cooling. Stirring was maintained for 18 h at room temperature, whereupon the solvent was evaporated. The residue was dissolved in CH_2Cl_2 (40 mL), the organic phase was washed with saturated $NaHCO_3$ (5×20 mL) and concentrated. The crude product was purified by flash chromatography on silica-gel using EtOAc:heptane 1:1 as the eluent. This gave **6** as a colourless solid, 0.40 g (83%): R_f 0.20 (EtOAc:heptane 1:1); mp 146–147 °C; EI/SP MS: M^+ 242; ^{1}H NMR ($CDCl_3$) 9.04 (1H, dd), 8.60 (1H, dd), 8.28 (1H, dd), 7.68–7.50 (2H, m), 7.38–7.18 (2H, m); ^{13}C NMR ($CDCl_3$) 164.7, 159.5, 159.4, 159.0, 158.0, 157.8, 138.2, 135.5, 135.3, 132.1, 124.8, 124.7, 124.4, 118.7, 118.5, 118.0, 117.6, 113.2. Anal. calcd for: C, 64.47%; H, 2.91%; N, 11.57%; found: C, 64.33%; H, 2.91%; N, 11.44%.

2-(2,6-Difluoro-phenyl)-pyrido[2,3-d][1,3]oxazin-4-one (7). 2-Aminopyridine-3-carboxylic acid (0.28 g, 2.0 mmol) was dissolved in dry pyridine (8 mL) and 2,6-difluorobenzoyl chloride (0.52 mL, 4.5 mmol) was added under stirring and cooling. Stirring was maintained for 18 h at room temperature, whereupon the solvent was evaporated. The residue was dissolved in CH_2Cl_2 (40 mL), the organic phase was washed with saturated $NaHCO_3$ (5×20 mL) and concentrated. The solid was purified by flash chromatography on silica-gel using EtOAc:heptane 1:1–EtOAc:MeOH 10:1 as the eluent. This gave two products. Compound **7** was isolated as a colourless crystalline compound, 0.18 g (35%): R_f 0.30 (EtOAc:heptane 1:1). Furthermore, a yellow crystalline compound was isolated, 0.11 g, R_f 0.25 (EtOAc:MeOH 10:1); this compound was not fully characterized. Compound **7** had mp 209–210 °C; EI/SP MS: M^+ 260; ^{1}H NMR ($CDCl_3$) 9.08 (1H, dd), 8.63 (1H, dd), 7.51–7.40 (2H, m), 7.08 (2H, t); ^{13}C NMR ($CDCl_3$) 163.9, 163.8, 159.3, 158.8, 158.7, 158.0, 157.4, 154.9, 138.3, 134.4, 134.2, 133.9, 125.1, 113.6, 112.9, 112.8, 112.5, 112.4, 110.8, 110.4, 110.1.

2-Thiophen-2-yl-pyrido[2,3-d][1,3]oxazin-4-one (8). 2-Aminopyridine-3-carboxylic acid (0.28 g, 2.0 mmol) was dissolved in dry pyridine (8 mL) and 2-thiophenecarbonyl chloride (0.47 mL, 4.5 mmol) was added under stirring and cooling. Stirring was maintained for 20 h at room temperature, whereupon the solvent was evaporated. The residue was dissolved in CH_2Cl_2 (40 mL), the organic phase was washed with saturated $NaHCO_3$ (5×20 mL) and concentrated. The solid was purified by flash chromatography on silica-gel using EtOAc:heptane 1:1 as the eluent. This gave compound **8** as colourless crystals, 0.28 g (61%): R_f 0.25 (EtOAc:heptane 1:1); mp 199–200 °C; EI/SP MS: M^+ 230; ^{1}H NMR ($CDCl_3$) 8.98 (1H, dd), 8.54 (1H, dd), 8.10 (1H, dd), 7.72 (1H, dd), 7.47 (1H, dd), 7.24 (1H, dd); ^{13}C NMR ($CDCl_3$) 159.3, 158.2, 158.0, 157.3, 138.3, 134.7, 133.8, 133.7, 129.0, 123.6, 112.9. Anal. calcd for: C, 57.38%; H, 2.63%; N, 12.17%; found: C, 57.50%; H, 2.60%, N, 11.92%.

2-Furan-2-yl-pyrido[2,3-d][1,3]oxazin-4-one (9). 2-Aminopyridine-3-carboxylic acid (0.28 g, 2.0 mmol) was dissolved in dry pyridine (8 mL) and 2-furan-carbonyl chloride (0.45 mL, 4.5 mmol) was added under stirring and cooling. Stirring was maintained for 20 h at room temperature, whereupon the solvent was evaporated. The residue was dissolved in CH_2Cl_2 (40 mL), the organic phase was washed with saturated $NaHCO_3$ (10×20 mL) and concentrated. The solid was purified by flash chromatography on silica-gel using EtOAc:heptane 3:2 as the eluent. Compound **9** was isolated as colourless crystals, 0.28 g (65%): R_f 0.28 (EtOAc:heptane 3:2); mp 147–148 °C; EI/ SP MS: M^+ 214; ^{1}H NMR ($CDCl_3$) 8.98 (1H, dd), 8.52 (1H. dd), 7.74 (1H, dd), 7.52 (1H, dd), 7.46 (1H, dd), 6.65 (1H, dd); ^{13}C NMR ($CDCl_3$) 159.0, 158.0 (d), 152.9, 148.3, 144.3, 138.2, 123.8, 119.4, 113.4, 113.0.

7-Ethylthio-2-(2-fluoro-phenyl)-pyrimido[4,5-d][1,3]oxazin-4-one (10). 4-Amino-5-carboxy-2-ethyl mercaptopyrimidine (100 mg, 0.50 mmol) was dissolved in dry DMF (5 mL) and Et_3N (0.14 mL, 1.0 mmol), and subsequently 2-fluorobenzoyl chloride (0.12 mL, 1.0 mmol) was added dropwise. The solution was stirred at room

temperature for 17 h and the solvent was evaporated. The solid was stirred with 0.2 M HCl (5 mL) followed by addition of saturated $NaHCO_3$ (10 mL). The aqueous phase was extracted with CH_2Cl_2 (3×10 mL), and the organic phase was washed with saturated $NaHCO_3$ (10 mL) and concentrated. Purification by flash chromatography on silica-gel using EtOAc:heptane 1:1 as the eluent gave **10**, crystalline compound, 80 mg (53%). The compound was recrystallized from EtOAc. ^{1}H NMR ($CDCl_3$) showed traces of impurities: 9.27 (1H, s), 8.28 (1H, dt), 7.72–7.58 (1H, m), 7.39–7.20 (2H, m), 3.30 (2H, q), 1.47 (3H, t); ^{13}C NMR ($CDCl_3$) 181.0, 163,5, 162.9, 161.7, 158.3, 156.1, 135.0, 134.8, 131.0, 123.4, 123.3, 116.8, 116.7, 116.2, 104.1, 24.8, 12.8; mp 155–156 °C; EI/SP MS: M^+ 303.

7-Ethylthio-(2-nitro-phenyl)-pyrimido[4,5-d][1,3]oxazin-4-one (11). 4-Amino-5-carboxy-2-ethyl mercaptopyrimidine (120 mg, 0.60 mmol) was dissolved in dry DMF (5 mL), and Et_3N (0.17 mL, 1.2 mmol) and subsequently 2-nitrobenzoyl chloride (0.16 mL, 1.2 mmol) were added dropwise. The solution was stirred at room temperature for 18 h and the solvent was evaporated. The solid was stirred with 0.2 M HCl (5 mL) followed by addition of saturated $NaHCO_3$ (10 mL). The aqueous phase was extracted with CH_2Cl_2 (3×10 mL), and the organic phase was washed with saturated $NaHCO_3$ (3×10 mL) and concentrated. Purification by flash chromatography on silica-gel using EtOAc:heptane 1:2 as the eluent gave **11** as a crystalline compound, 120 mg (60%). The compound was recrystallized from EtOAc:heptane. ^{1}H NMR ($CDCl_3$) 9.28 (1H, s), 8.20–8.12 (1H, m), 8.03–7.93 (1H, m), 7.86–7.72 (2H, m), 3.31 (2H, q), 1.46 (3H, t). ^{13}C NMR ($CDCl_3$) 183.3, 165.8, 163.1, 160.4, 157.3, 148.7, 134.2, 133.8, 131.9, 126.7, 125.6, 106.0, 26.8, 14.6. R_f 0.45 (EtOAc:heptane 1:1); mp 113–114 °C; EI/SP MS: M^+ 330.

7-Ethylthio-2-*O*-tolyl-pyrimido[4,5-d][1,3]oxazin-4-one (12). 4-Amino-5-carboxy-2-ethyl mercaptopyrimidine (120 mg, 0.60 mmol) was dissolved in dry DMF (5 mL), and Et_3N (0.17 mL, 1.2 mmol) and subsequently 2-methylbenzoyl chloride (0.16 mL, 1.2 mmol) were added dropwise. The solution was stirred at room temperature for 24 h and the solvent was evaporated. The solid was stirred with 0.2 M HCl (5 mL) followed by addition of saturated $NaHCO_3$ (10 mL). The aqueous phase was extracted with CH_2Cl_2 (3×10 mL), and the organic phase was washed with saturated $NaHCO_3$ (3×10 mL) and concentrated. Purification by flash chromatography on silica-gel using EtOAc: heptane 1:6–1:4 as the eluent gave **12** as a crystalline compound, 60 mg (33%). The compound was recrystallized from EtOAc:heptane. ^{1}H NMR ($CDCl_3$) 9.23 (1H, s), 8.16 (1H, dd, b), 7.52 (1H, dt, b), 7.36 (2H, t, b), 3.30 (2H, q), 2.78 (3H, s), 1.47 (3H, t); ^{13}C NMR ($CDCl_3$) 180.2, 16.5, 161.2, 157.7, 156.1, 138.9, 131.7, 130.6, 129.6, 126.9, 124.6, 103.4, 24.2, 20.9, 12.3. R_f 0.45 (EtOAc:heptane 1:3); mp 127–128 °C; EI/SP MS: M^+ 299. Anal. calcd for: C, 60.19%; H, 4.38%; N, 14.04%; found: C, 60.44%; H, 4.33%; N, 13.90%.

2-(2-Chloro-phenyl)-7-ethylthio-pyrimido[4,5-d][1,3]oxazin-4-one (13). 4-Amino-5-carboxy-2-ethyl mercaptopyrimidine (120 mg, 0.60 mmol) was dissolved in dry DMF (5 mL), and Et_3N (0.17 mL, 1.2 mmol) and subsequently 2-chlorobenzoyl chloride (0.15 mL, 1.2 mmol) were added dropwise. The solution was stirred at room temperature for 24 h and the solvent was evaporated. The solid was stirred with 0.2 M HCl (5 mL) followed by addition of saturated $NaHCO_3$ (10 mL). The aqueous phase was extracted with CH_2Cl_2 (3×10 mL), and the organic phase was washed with saturated $NaHCO_3$ (3×10 mL) and concentrated. Purification by flash chromatography on silica-gel using EtOAc:heptane 1:3 as the eluent gave impure **13** (100 mg). The solid was dissolved in CH_2Cl_2 (10 mL), washed with saturated $NaHCO_3$ (3×5 mL), dried (Na_2SO_4) and concentrated to give compound **13** as colourless crystals, 80 mg (42%). The compound was recrystallized from EtOAc: heptane. ^{1}H NMR ($CDCl_3$) 9.24 (1H, s), 8.02 (1H, dt), 7.56 (2H, m), 7.49–7.38 (1H, m), 3.30 (2H, q), 1.44 (3H, t); ^{13}C NMR ($CDCl_3$) 181.9, 165.3, 162.3, 159.1, 156.9, 133.7, 133.4, 131.9, 131.1, 128.7, 126.6, 104.9, 25.6, 13.6. R_f 0.20 (EtOAc:heptane 1:3); mp 154–155 °C; EI/SP MS: M^+ 319. Anal. calcd for: C, 52.59%; H, 3.15%; N, 13.14%; found: C, 52.71%; H, 3.14%; N, 12.97%.

2-(2-Fluoro-phenyl)-pyrazino[2,3-d][1,3]oxazin-4-one (14). 2-Aminopyrazine-3-carboxylic acid (0.14 g, 1.0 mmol) was dissolved in dry DMF (5 mL), Et_3N (0.28 mL, 2.0 mmol) was added, followed by addition of 2-fluorobenzoyl chloride (0.25 mL, 2.1 mmol). The suspension was stirred at room temperature for 17 h, after which the solvent was evaporated. The residue was suspended and stirred with 0.2 M HCl (5 mL). Saturated $NaHCO_3$ (10 mL) was added, and the aqueous phase was extracted with CH_2Cl_2 (3×10 mL). The organic phase was washed with saturated $NaHCO_3$ (2×10 mL) followed by concentration. The solid was purified by flash chromatography on silica-gel using EtOAc:heptane 1:3–1:0 as the eluent. The crude product was isolated, 50 mg (21%). The product was dissolved in CH_2Cl_2 (10 mL), washed with saturated $NaHCO_3$ (5×5 mL), and the solvent was evaporated to give a solid (45 mg) which was recrystallized from EtOAc to give compound **14** as colourless crystals: mp 218–220 °C (decomposition); EI/SP MS: M^+ 243; ^{1}H NMR ($CDCl_3$) 9.05 (1H, d), 8.89 (1H, d), 8.29 (1H, dt), 7.71–7.51 (1H, m), 7.39–7.11 (2H, m); ^{13}C NMR ($CDCl_3$) 169.0, 165.2, 159.9, 159,4, 159.3, 136.5, 136.3, 136.3, 132.9, 124.5, 117.6, 117.2. Anal. calcd for: C, 59.27%; H, 2.48%; N, 17.28%; found: C, 59.32%; H, 2.46%, N, 16.94%.

2-(2,6-Difluoro-phenyl)-pyrazino[2,3-d][1,3]oxazin-4-one-(15). 2-Aminopyrazine-3-carboxylic acid (0.14 g, 1.0 mmol) was dissolved in dry DMF (5 mL), Et_3N (0.27 mL, 2.0 mmol) was added, followed by addition of 2,6-difluorobenzoyl chloride (0.25 mL, 2.0 mmol). The suspension was stirred at room temperature for 16 h, after which the solvent was evaporated. The residue was suspended and stirred with 0.2 M HCl (5 mL). Saturated $NaHCO_3$ (10 mL) was added, and the aqueous phase was extracted with CH_2Cl_2 (3×10 mL). The organic phase was washed with saturated $NaHCO_3$ (10×10 mL) followed by concentration. The solid was purified by flash chromatography on silica-gel using EtOAc:heptane 1:2–1:0 as the eluent. Impure **15** was isolated,

70 mg (21%). The product was dissolved in CH_2Cl_2 (10 mL), washed with saturated $NaHCO_3$ (5×5 mL), and the solvent was evaporated to give **15**, 50 mg (19%), as a solid. The compound was recrystallized from EtOAc to give compound **10** as colourless crystals: ^1H NMR ($CDCl_3$) 9.05 (1H, d), 8.95 (1H, d), 7.65–7.48 (1H, m), 7.10 (2H, dd); EI/SP MS: M^+ 261.

2-*O*-Tolyl-pyrazino[2,3-d][1,3]oxazin-4-one (16). Compound **16** was prepared in three steps starting from 3-aminopyrazine-2-carboxylic acid methyl ester as described by Wamhoff.[14] ^1H NMR ($CDCl_3$) 9.80 (1H, d), 8.87 (1H, d), 8.19 (1H, dd), 7.58–7.45 (1H, m), 7.5 (2H, dt, b), 2.80 (3H, t); ^{13}C NMR ($CDCl_3$) 162.7, 157.8, 155.0, 151.9, 146.2, 141.1, 133.5, 132.8, 131.5, 139.5, 128.5, 126.8, 23.2. R_f 0.70 (EtOAc); mp 174–175 °C (lit. 166 °C); EI/SP MS: M^+ 271. Anal. calcd for: C, 65.27%; H, 3.79%; N, 17.56%; found: C, 65.26%; H, 3.77%; N, 17.39%.

6-(2-Fluoro-phenyl)-1-methyl-1*H*-pyrazolo[3,4-d][1,3]oxazin-4-one (17). Ethyl 5-amino-1-methylpyrazol-4-carboxylate (0.51 g, 3.0 mmol) was dissolved in dry pyridine (15 mL) and 2-fluorobenzoyl chloride (0.44 mL, 3.6 mmol) was added dropwise. The solution was stirred at 50 °C for 5 h, and the solvent was evaporated. The residue was dissolved in CH_2Cl_2 (20 mL) and washed with saturated $NaHCO_3$ (3×10 mL). The organic phase was dried (Na_2SO_4) and concentrated, and the crude product was purified by flash chromatography on silica-gel using EtOAc:heptane 1:3 as the eluent. This gave two products: the bis-acylated compound, 0.3 g, R_f 0.52 (EtOAc:heptane 1:1), and 5-(2-fluoro-benzoylamino)-1-methyl-1*H*-pyrazole-4-carboxylic acid ethyl ester, 0.22 g (25%) as colourless crystals: R_f 0.38 (EtOAc:heptane 1:1); mp 106–108 °C; EI/ SP MS: M^+ 291. Anal. calcd for: C, 57.73%; H, 4.84%; N, 14.43%; found: C, 58.16%; H, 4.87%; N, 14.07%.

5-(2-Fluoro-benzoylamino)-1-methyl-1*H*-pyrazole-4-carboxylic acid ethyl ester (0.16 g, 0.55 mmol) was dissolved in dry toluene (6 mL). Triphenylphosphine (0.16 g, 0.60 mmol) was added, followed by addition of Et_3N (0.23 mL, 1.65 mmol). $Br_2C_2Cl_4$ (0.20 g, 0.60 mmol) was dissolved in dry toluene (1 mL) and added dropwise. The reaction was stirred at 80 °C for 24 h, after which the solvent was evaporated. Purification by flash chromatography on silica-gel using EtOAc:heptane 1:3 as the eluent gave **17** as colourless crystals, 95 mg (73%). R_f 0.30 (EtOAc:heptane 1:2); ^1H NMR ($CDCl_3$) 8.16 (1H, dt), 8.09 (1H, s), 7.66–7.52 (1H, m), 7.36–7.18 (2H, m), 4.07 (3H, s); ^{13}C NMR ($CDCl_3$) 164.6, 160.0, 159.9, 159.4, 154.8, 151.2, 137.2, 135.2, 135.0, 131.7, 124.8, 124.7, 118.9, 118.7, 118.0, 117.6, 99.6, 35.1. The compound was recrystallized from EtOAc:heptane; mp 134–135 °C; EI/SP MS: M^+ 245. Anal. calcd for: C, 58.78%; H, 3.29%; N, 17.14%; found: C, 58.85%; H, 3.22%; N, 16.89%.

1-Methyl-6-(2-methyl-phenyl)-1*H*-pyrazolo[3,4-d][1,3]oxazin-4-one (18). Ethyl 5-amino-1-methylpyrazol-4-carboxylate (0.85 g, 5.0 mmol) was dissolved in dry pyridine (25 mL), and 2-methylbenzoyl chloride (1.96 mL, 15.0 mmol) was added dropwise. The suspension was stirred at 40 °C for 17 h. The suspension was subsequently stirred with $NaHCO_3$ (1.26 g, 15.0 mmol), and the solvent was evaporated. The solid was suspended in CH_2Cl_2 (40 mL) and washed with H_2O (20 mL) and aq $NaHCO_3$ (3×15 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated, and the crude product was crystallized from EtOAc:heptane to give 5-[bis-(2-methyl-benzoyl)-amino]-1-methyl-1*H*-pyrazole-4-carboxylic acid ethyl ester as slightly coloured crystals, 1.60 g (79%): mp 170–171 °C; R_f 0.51 (EtOAc:heptane 1:1); EI/SP MS: M^+ 405.

5-[Bis-(2-methyl-benzoyl)-amino]-1-methyl-1*H*-pyrazole-4-carboxylic acid ethyl ester (1.49 g, 3.7 mmol) was dissolved in dioxane (10 mL) and 2-propanol (6 mL), and hydrazine hydrate (0.18 mL, 3.7 mmol) was added dropwise. The solution was refluxed for 1.5 h and the solvent was evaporated. Purification by flash chromatography on silica-gel using EtOAc:heptane 2:3–1:1 as the eluent gave 1-methyl-5-(2-methyl-benzoylamino)-1*H*-pyrazole-4-carboxylic acid ethyl ester as colourless crystals, 1.0 g (94%): R_f 0.40 (EtOAc:heptane 1:1); mp 119–120 °C; EI/SP MS: M^+ 287. Anal. calcd for: C, 62.71%; H, 5.96%; N, 14.62%; found: C, 62.84%; H, 6.00%; N, 14.58%.

1-Methyl-5-(2-methyl-benzoylamino)-1*H*-pyrazole-4-carboxylic acid ethyl ester (0.52 g, 1.88 mmol) was dissolved in dry toluene (15 mL) at 80 °C. Triphenylphosphine (0.54 g, 2.07 mmol) was added, followed by addition of Et_3N (0.78 mL, 5.64 mmol). $Br_2C_2Cl_4$ (0.67 g, 2.07 mmol) was dissolved in dry toluene (3 mL) and added dropwise. The reaction was stirred at 80 °C for 18 h, after which the suspension was filtered and the filtrate was concentrated. Purification by flash chromatography on silica-gel using EtOAc:heptane 1:3 as the eluent gave **18** as colourless crystals, 0.40 g (89%): R_f 0.25 (EtOAc:heptane 1:3); ^1H NMR ($CDCl_3$) 8.08 (1H, dd), 8.04 (1H, s), 7.50–7.28 (3H, m), 4.03 (3H, s), 2.72 (3H, s); ^{13}C NMR ($CDCl_3$) 163.5, 155.2, 151.4, 140.1, 137.0, 132.6, 131.0, 129.3, 126.6, 99.3, 35.0, 23.0. The compound was recrystallized from EtOAc:heptane; mp 130–131 °C; EI/SP MS: M^+ 241. Anal. calcd for: C, 64.72%; H, 4.60%; N, 17.42%; found: C, 64.60%; H, 4.63%; N, 17.38%.

1-Methyl-6-(2-nitro-phenyl)-1*H*-pyrazolo[3,4-d][1,3]oxazin-4-one (19). Ethyl 5-amino-1-methylpyrazol-4-carboxylate (0.85 g, 5.0 mmol) was dissolved in dry pyridine (25 mL), and 2-nitrobenzoyl chloride (2.0 mL, 15.0 mmol) was added dropwise. The suspension was stirred at 40 °C for 17 h. The suspension was subsequently stirred with $NaHCO_3$ (1.26 g, 15.0 mmol) and the solvent was evaporated. The solid was suspended in CH_2Cl_2 (40 mL) and washed with H_2O (20 mL) and aq $NaHCO_3$ (3×15 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated, and the crude product was crystallized from EtOAc:heptane to give 5-[bis-(2-nitro-benzoyl)-amino]-1-methyl-1*H*-pyrazole-4-carboxylic acid ethyl ester as slightly coloured crystals, 1.83 g (78%): mp 167–168 °C; R_f 0.32 (EtOAc:heptane 1:1); EI/SP MS: M^+467.

5-[Bis-(2-nitro-benzoyl)-amino]-1-methyl-1*H*-pyrazole-4-carboxylic acid ethyl ester (1.71 g, 3.7 mmol) was dissolved in dioxane (10 mL) and 2-propanol (6 mL), and hydrazine hydrate (0.18 mL, 3.7 mmol) was added dropwise. The solution was refluxed for 1.5 h and the solvent was evaporated. Purification by flash chromatography on silica-gel using EtOAc:heptane 1:1 as the eluent gave 1-methyl-5-(2-nitro-benzoylamino)-1*H*-pyrazole-4-carboxylic acid ethyl ester as colourless crystals, 0.95 g (81%): R_f 0.20 (EtOAc:heptane 1:1); mp 204–205 °C; EI/SP MS: M^+ 318. Anal. calcd for: C, 52.83%; H, 4.43%; N, 17.60%; found: C, 52.89%; H, 4.44%; N, 17.54%.

1-Methyl-5-(2-nitro-benzoylamino)-1*H*-pyrazole-4-carboxylic acid ethyl ester (0.37 g, 1.16 mmol) was dissolved in dry toluene (75 mL) at 80 °C. Triphenylphosphine (0.34 g, 1.28 mmol) was added, followed by addition of Et_3N (0.48 mL, 3.49 mmol). $Br_2C_2Cl_4$ (0.42 g, 1.28 mmol) was dissolved in dry toluene (2 mL) and added dropwise. The reaction was stirred at 80 °C for 18 h, after which the suspension was filtered and the filtrate was concentrated. Purification by flash chromatography on silica-gel using EtOAc:heptane 2:3 as the eluent gave **19** as colourless crystals, 0.24 g (75%): R_f 0.37 (EtOAc:heptane 1:1); ^{1}H NMR ($CDCl_3$) 8.12 (1H, s), 8.04–7.92 (2H, m), 7.80–7.70 (2H, m), 4.00 (3H, s); ^{13}C NMR ($CDCl_3$) 160.2, 154.2, 150.6, 149.3, 137.4, 133.2, 131.4, 125.6, 124.9, 99.6, 35.2. The compound was recrystallized from EtOAc:heptane; mp 175–176 °C; EI/SP MS: M^+ 272. Anal. calcd for: C, 52.95%; H, 2.96%; N, 20.58%; found: C, 52.94%; H, 2.95%; N, 20.48%.

2-(2,6-Difluoro-phenyl)-7-methyl-thieno[3,2-d][1,3]oxazin-4-one (20). Methyl 3-amino-4-methylthiophene-2-carboxylate (0.68 g, 4.0 mmol) was dissolved in dry pyridine (10 mL) and 2,6-difluorobenzoyl chloride (0.53 mL, 4.2 mmol) was added. The suspension was stirred at room temperature for 7 days, after which the solvent was evaporated. The solid was dissolved in CH_2Cl_2 (50 mL) and the organic phase was washed with saturated $NaHCO_3$ (3×30 mL), dried (Na_2SO_4), filtered and concentrated. This gave 3-(2,6-difluoro-benzoylamino)-4-methyl-thiophene-2-carboxylic acid methyl ester as a colourless crystalline compound (1.17 g, 94%): R_f 0.33 (EtOAc:heptane 1:2). The compound was recrystallized from EtOAc; mp 159–160 °C; EI/SP MS: M^+ 311. Anal. calcd for: C, 54.02%; H, 3.56%; N, 4.50%; found: C, 53.97%; H, 3.53%; N,4.45%.

3-(2,6-Difluoro-benzoylamino)-4-methyl-thiophene-2-carboxylic acid methyl ester (0.45 g, 1.45 mmol) was dissolved in dry toluene (25 mL) at 80 °C. Then PPh_3 (0.42 g, 1.59 mmol) was added followed by addition of Et_3N (0.60 mL, 4.34 mmol). $C_2Br_2Cl_4$ (0.52 g, 1.59 mmol) was dissolved in dry toluene (2 mL) and added to the reaction mixture. The suspension was stirred at 80 °C for 7 h and at 50 °C for 90 h, after which it was filtered and the filtrate was concentrated. The crude product was purified by flash chromatography on silica-gel using EtOAc:heptane 1:4 as the eluent. This gave **20** as a colourless crystalline compound (0.32 g, 80%): R_f 0.50 (EtOAc:heptane 1:2); ^{1}H NMR ($CDCl_3$) 7.62 (1H, d), 7.58–7.41 (1H, m), 7.08 (2H, t), 2.44 (3H, s); ^{13}C NMR ($CDCl_3$) 163.8, 163.7, 158.7, 158.6, 155.6, 155.3, 153.7, 135.3, 133.7, 133.5, 133.3, 118.0, 112.8, 112.7, 112.4, 112.3, 110.8, 13.1. The compound was recrystallized from EtOAc; mp 149–151 °C; EI/SP MS: M^+ 279. Anal. calcd for: C, 55.91; H, 2.53%; N, 5.02%; found: C, 55.95%; H,2.49%; N, 4.98%.

5-Methyl-2-(2-nitro-phenyl)-thieno[2,3-d][1,3]oxazin-4-one (21). Ethyl 2-amino-4-methylthiophene-3-carboxylate (0.74 g, 4.0 mmol) was dissolved in dry CH_2Cl_2 (10 mL). 2-Nitrobenzoyl chloride (0.58 mL, 4.4 mmol) was added followed by the addition of Et_3N (0.61 mL, 4.4 mmol). The suspension was stirred at room temperature for 72 h, after which the organic phase was washed with saturated $NaHCO_3$ (3×30 mL), dried (Na_2SO_4), filtered and concentrated. This gave 4-methyl-2-(2-nitro-benzoylamino)-thiophene-3-carboxylic acid ethyl ester as slightly coloured crystals (1.23 g, 92%): R_f 0.25 (EtOAc:heptane 1:2). The compound was recrystallized from EtOAc; mp 135–137 °C; EI/SP MS: M^+ 334.

4-Methyl-2-(2-nitro-benzoylamino)-thiophene-3-carboxylic acid ethyl ester (0.38 g, 1.1 mmol) was dissolved in dry toluene (25 mL) at 80 °C. PPh_3 (0.33 g, 1.3 mmol) was added followed by the addition of Et_3N (0.47 mL, 3.4 mmol). $C_2Br_2Cl_4$ (0.41 g, 1.3 mmol) was dissolved in dry toluene (2 mL) and added to the reaction mixture. The suspension was stirred at 80 °C for 24 h, after which it was filtered and the filtrate was concentrated. The crude product was purified by flash chromatography on silica-gel using EtOAc:heptane 1:4 as the eluent. This gave **21** as a slightly yellow crystalline compound (0.29 g, 91%): R_f 0.31 (EtOAc:heptane 1:2); ^{1}H NMR ($CDCl_3$) 8.02–7.92 (2H, m), 7.68–7.55 (2H, m), 6.97 (1H, m), 2.57 (3H, s); ^{13}C NMR ($CDCl_3$) 163.4, 157.2, 155.0, 149.1, 136.2, 133.3, 132.7, 131.4, 125.9, 125.0, 120.7, 117.7, 16.4. The compound was recrystallized from EtOAc to give slightly coloured crystals; mp 155–165 °C; EI/SP MS: M^+ 288. Anal. calcd for: C, 54.16%; H, 2.80%; N, 9.72%; found: C, 54.11%; H, 2.75%; N, 9.67%.

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