JPP Journal of Pharmacy and Pharmacology

JPP 2003, 55: 1259–1265

Received February 12, 2003
Accepted May 12, 2003
DOI 10.1211/0022357021756
ISSN 0022-3573

Synthesis and evaluation of 5-arylated 2(5*H*)-furanones and 2-arylated pyridazin-3(2*H*)-ones as anti-cancer agents

Eric Lattmann, Washington O. Ayuko, Derek Kinchinaton, Christopher A. Langley, Harjit Singh, Leila Karimi and Michael J. Tisdale

Abstract

Bis-cyclic butenolides, 5-arylated 2(5*H*)-furanones **6a–c, 7a, b** and the 3(2H)-pyridazones **9a–d** were prepared by using the aldehyde form of muco halogen acids in electrophilic substitution reactions and in an aldol-like condensation reaction. The cytotoxicity of these simple and bis-cyclic butenolides have been evaluated in tissue culture studies on MAC 13 and MAC 16 murine colon cancer cell lines. The butyl furanone **3** displayed the highest cytotoxicity of 3 μM, as one selected example of a series of dichlorinated pseudoesters. The 5-arylated 2(5*H*)-furanones **6** and **7** did not show a structure–activity relationship (SAR) depending on the substitution pattern of the aromatic system. An IC50 (concentration inhibiting growth by 50%) was found within a range of 30–50 and 40–50 μM for the MAC 13 and MAC 16 cell lines, respectively. The pyridazine series **9** showed a maximum in-vitro activity for the *p*-methoxydrivative **9b**, having an IC50 of 17 in MAC 13 and 11 μM in MAC 16 cell lines. Selected examples of each series and further novel 2(5*H*)-furanones such as the hydrazone **5** and the hydantoin **8** have been screened in-vivo in mice and the data are presented. For the pyridazines **9a–d**, the in-vitro cytotoxicity correlated with an in-vivo inhibition of tumour growth. The ring expansion of the 5-membered 2(5*H*)-furanone ring system such as **6a** into the 6-membered 3(2*H*)-pyridazone **9b** led to an agent with improved antineoplastic properties. On the resistant MAC 16 cell line the pyridazone **9b** displayed 52% tumour inhibition in mice at a dose of 50 mg kg^{-1} compared with 27% for the 5-FU standard.

Introduction

Many cancer patients have metastatic disease at diagnosis and cannot be cured by modern cancer treatment, though there are tumours (e.g. those of the testes, choriocarcinomas, Hodgkin's disease) that are now curable even at an advanced stage. Some other tumours (e.g. in the lung, breast and prostate) may show considerable benefit from chemotherapy or hormonal manipulation. Cancer suspect agents like epoxides, aziridines and *N*-nitroso compounds (Reynolds et al 2000) served as a starting point for the development of anti-cancer agents in the past. The furanone lead structure of our study and selected anti-cancer agents (Ayuko & Lattmann 1999) are outlined in Figure 1. Alkylating agents such as chlorambucil and cyclophosphamide, which are still in clinical use, contain reactive chlorine atoms as leaving groups (Mutschler 1996). Penicillin acid (Black 1966) and basidalin (Hiyama et al 1987) are butenolide natural products (Coombs et al 1998) exhibiting anti-tumour activity in the micromolar range. Butalactin is an antibiotic containing an epoxide side chain (Franco et al 1991). Cisplatinum compounds, useful agents in the treatment of testicle cancer, display a structural similarity to the dichlorinated derivatives of this research.

Here, we wish to report further findings on the synthesis of novel 3,4-dihalogenated 5-substituted 2(5*H*)-furanones (Lattmann & Hoffmann 1996; Lattmann et al 1999) and novel pyridazin-3(2*H*)-ones, their in-vitro evaluation against MAC 13 and MAC 16 tumour cells and subsequent in-vivo studies in mice of selected compounds of each chemical class on the resistant solid MAC 16 tumour.

The School of Pharmacy, Aston University, Aston Triangle, Birmingham B4 7ET, UK

Eric Lattmann, Washington O. Ayuko, Derek Kinchinaton, Christopher A. Langley, Harjit Singh, Leila Karimi, Michael J. Tisdale

Correspondence: E. Lattmann, The School of Pharmacy, Aston University, Aston Triangle, Birmingham B4 7ET, UK. E-mail: e.lattmann@aston.ac.uk

Acknowledgement and funding: The authors would like to thank Kanvir Research LTD for financial support and Mr M. P. Wynter for in-vivo studies.

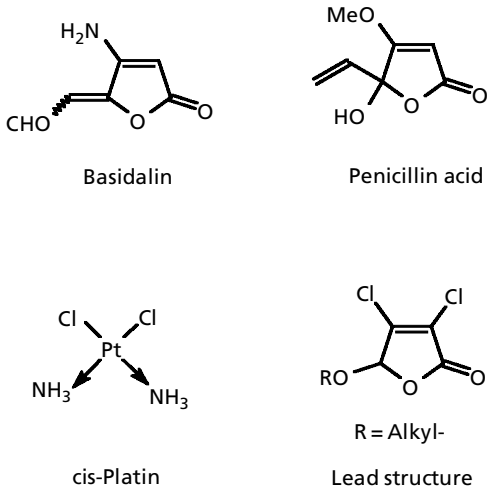


Figure 1 Overview of anti-cancer agents.

Materials and Methods

Chemicals

Muco chloric acid was obtained from Lancaster Ltd (Lancaster, UK). All the other reagents were purchased from Aldrich (UK).

Chemistry

Atmospheric pressure chemical ionisation mass spectrometry (APCI-MS) was carried out on a Hewlett-Packard 5989B quadrupole instrument connected to an APCI accessory. IR spectra were recorded as KBr discs or in chloroform on a Mattson 3000 FT-IR spectrophotometer. Nuclear magnetic resonance spectra were obtained on a Bruker AC 250 instrument operating at 250 MHz, with tetramethylsilane (TMS) as internal standard.

Synthesis of bis-cyclic butenolides was carried out according the scheme shown in Figure 2.

*5-Butoxy-3,4-dichlorofuran-2(5*H*)-one (**3**)*
Muco chloric acid (10 g) was refluxed with 70 mL of n-butanol and a catalytic amount of sulfuric acid for 48 h. The reaction was monitored by TLC with ether–petrolether 1:1. After completion, the reaction mixture was poured onto ice-cold solution of sodium hydrogen carbonate. The desired compound was extracted with ether–petrolether 1:1 and after the removal of the solvent in vacuum the desired compound was obtained as colourless oil.

Yield: 49%; MS-APCI(+): 226/228(M + 1) m/z.; IR ($CHCl_3$) cm^{-1}: 2958, 2873, 2335, 1809, 1637. ^{1}H NMR ($CDCl_3$): δ 5.80 (s, 1H, C<u>H</u>), 3.86–3.59 (m, 2H, OC<u>H</u>$_2$), 1.66–1.35 (m, 4H, OCH$_2$C<u>H</u>$_2$C<u>H</u>$_2$), 0.92 (t, $J = 7.3$ Hz, 3H, C<u>H</u>$_3$) ppm; ^{13}C NMR ($CDCl_3$) δ 163.24 (<u>C</u>=O), 147.48 (<u>C</u>CH), 124.12 (<u>C</u>C=O), 100.94 (<u>C</u>H), 70.05 (O<u>C</u>H$_2$), 31.15 (OCH$_2$<u>C</u>H$_2$), 18.84 (<u>C</u>H$_2$CH$_3$), 13.59 (<u>C</u>H$_3$) ppm.

*3,4-Dichloro-5-(2-oxo-2-phenylethyl) furan-2(5*H*)-one (**4**)*
Muco chloric acid (21.0 g, 0.125 mol) and acetophenone (0.135 mol) were each dissolved in methanol (200 mL) and cooled to 0 °C. A solution of NaOH (8.0 g in 70 mL water, 2.5 M) was added slowly under stirring at 0–5 °C. After the addition of NaOH, the mixture was allowed to stand at room temperature for 3 h. The brown mixture was poured into ice-water, containing an excess of conc. HCl and allowed to stand for 45 min. A yellow oily liquid/solid was decanted and washed with water to give a crude yellow product. It was recrystallized from hot ethanol to give the pure compounds as a white powder (mp 117–118 °C).

Yield: 72%. IR (KBr) cm^{-1}: 3010, 1773, 1683, 1648, 1210, 1033, 767, 692. ^{1}H NMR ($CDCl_3$) δ 3.35–3.61 (m, CH_2), 5.69–5.73 (dd, CH, J = 3.6 Hz), 7.45–7.52 (t, Ar-2H, J = 7.8, 7.2 Hz), 7.56–7.65 (tt, Ar-H, J = 7.4, 7.3 Hz), 7.91–7.95 (d, Ar-2H, J = 7.1 Hz) ppm. ^{13}C NMR ($CDCl_3$) δ 40.0 (CH_2), 78.0 (CH), 121.4 (C-Cl), 128.1 (2 × C), 134.1, 135.7 (2 × C) (Ar-C), 151.9 (C-Cl), 164.8 169.9, 193.7 (C=O) ppm.

*3,4-Dichloro-5-{2-[(Z)-2-(2,4-dinitrophenyl) hydrazono]-2-phenyl ethyl}-furan-Z(5*H*)-one (**5**)*
A solution of the 2(5*H*)-furanone **4** (10.0 g, 0.04 mol) and 2,4-dinitro-phenyl-hydrazine (1.5 eq, 11.1 g, 0.05 mol) were dissolved in ethanol (250 mL) and refluxed in the presence of a catalytic amount of sulfuric acid (8–10 drops). An orange precipitate was formed after 45 min, and the reaction mixture was cooled to room temperature. The solid was collected and recrystallized from ethanol to yield a bright orange solid (mp decomp. > 195 °C).

Yield: 51%; MS-APCI(+): 451 (M+) m/z; IR (KBr) cm^{-1}: 3461, 3295, 3102, 1779, 1590, 1490, 1417, 1328, 1029, 712. ^{1}H NMR (DMSO) δ 3.51–3.79 (m, CH_2), 5.72–5.77 (dd, CH, J = 3.7 Hz), 7.49–7.64 (m, Ar-5H), 8.05–8.08 (d, Ar-H, J = 9.5 Hz), 8.42–8.46 (dd, Ar-H, J = 9.6 Hz), 8.89–8.92 (m, Ar-H), 11.27 (s, NH) ppm; ^{13}C NMR (DMSO) δ 33.6 (CH_2), 76.2 (CH), 90.3 (C=N), 113.6, 120.2, 123.0 (2 × C), 124.1, 128.7, 129.6 (2 × C), 133.6, 133.7, 145.7, 145.9, 150.3, 155.9 (Ar-C), 165.8 (C=O) ppm.

*3,4-Dichloro-5-phenylfuran-2(5*H*)-one (**6a**)*
A solution of aluminium chloride (10 g, 0.075 mol) in benzene (38 g, 0.44 mol) was stirred for 20 min in a conical flask with a drying tube. Muco chloric acid (5 g, 0.03 mol) was added and reacted overnight at room temperature. The mixture was worked up with 6 M hydrochloric acid, poured on ice and was extracted with benzene. The organic phase was dried with magnesium sulfate and evaporated off in a vacuum to give the target as a white solid (mp 69–70 °C).

Yield 69%. MS-APCI(+): 229/231 m/z; IR (KBr) cm^{-1}: 3526, 3027, 1772, 1625, 1454, 1287, 1228. ^{1}H NMR ($CDCl_3$): δ 7.50–7.41 (m, 3H, aryl-H), 7.34–7.28 (m, 2H, aryl-H), 5.86 (s, 1H, CH) ppm; ^{13}C NMR ($CDCl_3$): δ 165.33 (C=O), 152.24 (CHCCl), 131.56 (aryl-CCH), 130.40 & 129.18 & 127.13 (*o*, *m* & *p*-arylC), 121.02 (CC=O), 83.63 (CH) ppm.

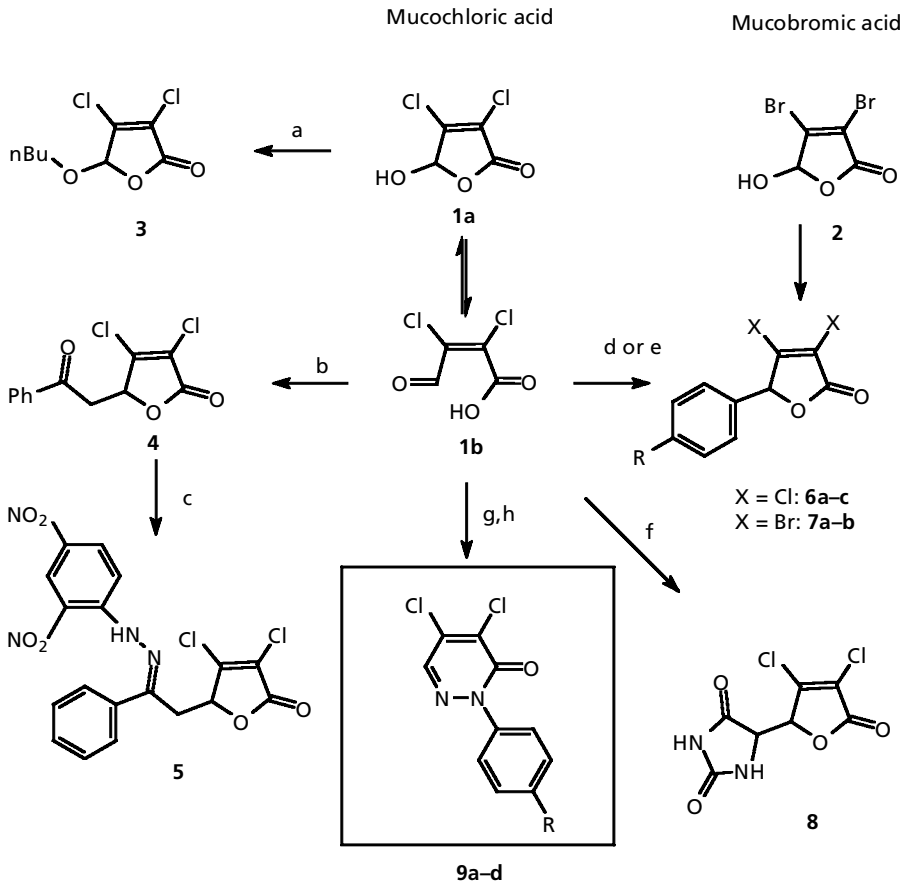


Figure 2 Scheme for synthesis of bis-cyclic butenolides, using the free aldehyde form of muco halogen acids in aldol- and electrophilic substitution reactions. a, I-BuOH, conc. H_2SO_4, reflux; b, acetophenone, KOH, room temperature, MeOH; c, DNPH, H_2SO_4, EtOH, reflux; d, anisol, PPA, room temperature; e, benzene, toluene, $AlCl_3$, room temperature; f, hydantoin, DCM, NaOH–EtOH, room temperature; g, hydrazine, water–MeOH, room temperature; h, hydrazone, acetic acid, reflux.

*3,4-Dichloro-5-(4-methoxyphenyl)furan-2(5*H*)-one (**6c**)*
Phosphorus pentoxide (15.7 g) was added to phosphoric acid (92.8 g) and to this mixture finely powdered muco chloric acid (17.1 g) followed by anisole (10.8 g) was added. This mixture was reacted overnight at ambient temperature. It was worked up with 150 mL of hydrochloric acid and poured onto ice. The mixture was extracted with ether and the combined organic phases were dried with sodium sulfate. The solvent was evaporated off in vacuum to give a yellow oil.

Yield: 76%. MS-APCI(+): 259/260 m/z; IR ($CHCl_3$) cm^{-1}: 3542, 3027, 1797, 1778, 1632, 1604, 1113. ^{1}H NMR ($CDCl_3$): δ 7.33–7.12 (m, 2H, *m-Ar*-H), 6.96–6.90 (m, 2H, *o-Ar*-H), 5.79 (s, 1H, CH), 3.80 (s, 3H, *p*-OCH_3) ppm. ^{13}C NMR ($CDCl_3$): δ 162.63 (C=O), 147.04 (CHCCl), 130.56, 128.40, 127.18, 121.02, 120.11, 82.21(CH), 59.23 (*p*-OCH_3) ppm.

*3,4-Dibromo-5-phenylfuran-2(5*H*)-one (**7a**)*
The preparation is according to the method described for the furanone **6a**.

Mp 74–75 °C; Yield: 61%; MS-APCI(+): 319/321 (M + 1) m/z; IR (KBr) cm^{-1}: 3562, 3029, 3010, 2358, 2339, 1779, 1606. ^{1}H NMR ($CDCl_3$): δ 7.54–7.40 (m, 3H, aryl-H), 7.32–7.29 (m, 2H, aryl-H), 5.86 (s, 1H, CH) ppm. ^{13}C NMR ($CDCl_3$): δ 166.41 (C=O), 148.26 (CHCBr), 131.84 (aryl-CCH), 130.34 & 129.23 & 127.28 (*o*, *m* & *p*-aryl-C), 114.77 (CC=O), 86.61 (CH) ppm.

*5-(3,4-Dichloro-5-oxo-2,5-dihydrofuran-2-yl) imidazolidine-2,4-dione (**8**)*
Muco chloric acid (8.45 g, 0.05 mol) and hydantoin (5.0 g, 0.05 mol) were dissolved in dichloromethane (60 mL) and cooled to 0 °C. A solution of sodium hydroxide (6.0 g in 75 mL ethanol, 2 M) was slowly added, while stirring at 0–5 °C. After the addition of sodium hydroxide, the mixture was allowed to stand at room temperature for 4 h. The solution was poured into ice-water containing an excess of conc. HCl and allowed to stand for 45 min. The precipitate was filtered, washed with water and dried to give a crude light-brown product. Recrystallization from dilute ethanol gave the target compound as pure, white powder (mp 179–181 °C).

Yield: 45%; MS-APCI(+): 251, 253 (M + 1) m/z.; IR (KBr) cm^{-1}: 3286, 3171, 3054, 1787, 1723, 1652, 1419, 1234, 1023, 811. ^1H NMR (DMSO-d_6) δ 4.70 (t, 1H, -CH-furan, J = 1.6, 1.6 Hz), 5.66 (d, 1H, -CH-hydantoin, J = 1.7 Hz), 8.25 (s, -NH), 11.03 (s, -NH) ppm. ^{13}C NMR (DMSO-d_6) δ 57.4 (CH-furan), 80.1 (CH-hydantoin), 121.5 (CH-CCl), 158.2 (C-furan), 165.3 (C=O-furan), 165.3 & 172.4 (C=O-hydantoin) ppm.

*4,5-Dichloro-2-*p*-methoxy-phenyl-3-pyridazone (**9b**)*

A solution of muco chloric acid (2.1 g, 0.012 mol) in methanol–water (1:1, 10 mL) was stirred at room temperature. To this solution a mixture of the *p*-methoxyphenyl-hydrazine hydrochloride (0.016 mol) in methanol–water (1:1, 10 mL) was added drop-wise and stirred at room temperature. The uncyclized hydrazone intermediate was obtained as an orange-brown precipitate, which was collected after 3 h by filtration. Glacial acetic acid was slowly added at 100–110 °C to the hydrazone intermediate and the mixture was subsequently refluxed for 20 min. The solution was diluted with water and cooled on ice. On cooling, the pyridazone adduct was obtained as a beige, coloured powder. After recrystallization from 90% ethanol, a white powder was obtained (mp 176–177 °C).

Yield: 39%. MS-APCI(+): 273/275 (M + 1) m/z; IR (KBr) cm^{-1}: 3049, 2924, 1818, 1655, 1572, 1241, 1136, 943, 807. ^1H NMR ($CDCl_3$) δ 3.80 (s, 3H, OMe); 7.64 + 7.10 (d, 2H + 2H, Ar-H), 8.26 (s, 1H, -CH) ppm; ^{13}C NMR ($CDCl_3$) δ 60.34 (*p*-OCH_3) 126.4, 129.3, 134.3, 136.1 (Ar-C), 136.9, 137.1, 139.6, 154.2 (C=O) ppm.

*4,5-dichloro-2-(4-chlorophenyl)pyridazin-3(2H)-one (**9c**)*

A solution of muco chloric acid (2.1 g, 0.012 mol) in methanol–water (1:1, 10 mL) was stirred at room temperature. To this solution a mixture of 4-chloro-phenyl-hydrazine hydrochloride (2.89 g, 0.016 mol) in methanol–water (1:1, 10 mL) was added drop-wise and stirred for 20 min at room temperature. The resultant orange precipitate was filtered after 3 h and the uncyclized product was obtained. Glacial acetic acid was slowly added at 100–110 °C and refluxed for 20 min. The solution was diluted with water and cooled. On cooling the pyridazone adduct was obtained as a beige powder. After recrystallization from 90% ethanol, a white powder was obtained (mp 186–188 °C).

Yield: 43%. MS-APCI(+): 276/278 (M + 1) m/z; IR (KBr) cm^{-1}: 3215, 3030, 1815, 1633, 1216, 1142, 1035, 816. ^1H NMR ($CDCl_3$) δ 7.78–7.74 (m, 4H, Ar-H), 8.26 (s, 1H, -CH) ppm; ^{13}C NMR ($CDCl_3$) δ 128.4, 134.6, 134.8, 135.3, 136.9, 137.3, 143.6 (N-C-Ar), 159.6 (C=O) ppm.

Biological evaluation

In-vitro cytotoxicity

The cytotoxicity was determined against murine carcinoma cell lines (MAC 13 and MAC 16) using the standard MTT assay (Mosmann 1983).

The culture media used was RPMI 1640 containing HEPES, glutamine and antibiotics, and supplemented with 10% fetal calf serum for MAC 13 cells and 5% fetal calf serum for MAC 16 cells. Cells were counted by the trypan blue exclusion method using plastic Kova counting chambers. The MAC 13 and MAC 16 cells were suspended in appropriate volumes of media and were seeded at 0.5×10^4 and 2×10^4 cells/200 μL, respectively, in flat-bottomed 96-well plates. The test compounds were dissolved in dimethyl sulfoxide (DMSO) to give stock solutions of 100 mM. Dilution series from 10^{-4} to 10^{-9} M were made so that each compound was tested at six concentrations and in triplicate.

5-Fluorouridine (5-FU), a known anti-cancer agent, was used as a control and tested in the 20–0.02 μM range. Plates were then incubated at 37 °C, 5% CO_2 for three days. Compounds were tested on at least two separate occasions. On day three, 20 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) (7.5 mg MTT/mL of phosphate-buffered saline (PBS)) was added to each well and plates were allowed to incubate for a further 2 h. Culture supernatant (120 μL) was carefully removed from each well and 100 μL of acidified isopropanol containing 10% Triton-X100 was then added to each well. Plates were agitated for 10 min at 800 rev min^{-1} on a plate shaker. Following this solubilisation step, all plates were then read, within 15 min, on an Anthos AW200 plate reader at 540 nm with a reference wavelength of 590 nm.

In-vivo experiments in mice – assessment of anti-tumour inhibition

Pure strain NMRI mice, aged 6–8 weeks, from our inbred colony were used for transplanting MAC (murine colon cancer) tumours. Mice had free access to RM3E diet (Lillco-England) and water. Approximately 2 mm cubes containing 2×10^5 cells of MAC 16 tumour fragments were transplanted subcutaneously in the inguinal region via a trocar in a volume of 0.2 mL. Tumour-bearing mice were randomised in groups of 5–7 and the treatment was started 2 days after transplantation. The test compounds were administered in propylene glycol. The effect of chemotherapy was assessed 7 days after transplantation. Mice were killed and the effects were measured by the differences in tumour weight (% inhibition = treated weight/control weight × 100).

The body weight changes were recorded to assess toxicity. The procedure was approved by the Home Office and the bioethics committee of Aston University.

The results are expressed as mean ± s.d. and the data were subjected to repeated measures of the one-way analysis of variance. If the probability level (*P*-value) is less than 0.05, a statistical significance was attained.

Results and Discussion

Chemistry

The pseudoester **3** was synthesised by refluxing muco chloric acid (**1**) in n-butanol in the presence of a catalytic amount of sulfuric acid. A series of pseudoesters, pseudoanhydrides and pseudoamides were synthesised as described

in Lattmann et al (2001), reacting the hydroxy-tautomeric form (**1a**) of muco chloric acid. The free aldehyde form of muco chloric acid (**1b**) (Mowry 1950) and muco bromic acid (**2**) was reacted in aldol-like reactions and electrophilic aromatic substitutions reactions via ring-opened hydroxy-intermediates into 5-substituted 2(5*H*)-furanones.

Muco chloric acid (**1**) was reacted with acetophenone in an alcoholic alkaline solution of sodium hydroxide at ambient temperature, via a 4-hydroxy acrylic acid intermediate, to give the ketone **4** in 72% yield (Mowry 1953). The ketone **4** was reacted further with dinitrophenylhydrazine in ethanol and a catalytic amount of sulfuric acid at room temperature to furnish the bisarylated furanone **5**. Attempts to react muco bromic acid (**2**), analogue to muco chloric acid, with acetophenones in condensation reactions were unsuccessful.

The reaction of muco chloric acid (**1**) and muco bromic acid (**2**) with reactive, electron rich aromatics, such as benzene, toluene and anisole, furnished the 5-arylated-3,4-dichlorinated furanones **6a–c** and 5-arylated-3,4-dibrominated furanones **7a**, **b**, in good yields at ambient temperature in the presence of a Lewis acid (Semonsky et al 1962). Benzene and toluene were reacted with the muco halogen acids **1** and **2** with aluminium chloride at room temperature. Anisole was converted into **6c** using a mixture of phosphorus pentoxide and phosphoric acid at ambient temperature. Indol reacted with muco chloric acid in dichloromethane in a very low yield into the desired furanone derivative. Chlorobenzene did not react with muco chloric acid and aluminium chloride, even under reflux conditions.

Aldehyde **1a** was reacted with hydantoin to give the novel biscyclic furanone **8** in a condensation reaction. The reaction of hydantoin with muco chloric acid (**1**) at room temperature in dichloromethane and an alcoholic sodium hydroxide solution furnished the desired biscyclic compound **8** in 45% yield, as a white precipitate.

The desired pyridazones **9a–d** were synthesised in two steps from muco chloric acid (Schroeter et al 1966) and the parent hydrazines such as phenylhydrazine, *p*-methoxy-phenylhydrazine, *p*-chloro-phenylhydrazine and *p*-nitro-phenyl-hydrazine. The substituted hydrazines gave the hydrazone intermediates of the dichloro acrylic acid derivatives in methanol at ambient temperature. Subsequent cyclisation of these reaction intermediates under reflux conditions in acetic acid furnished the crystalline pyridazone **9a–d**. The 1,4-dinitrohydrazone intermediate did not cyclise into the desired pyridazone structure (Cho et al 1996).

Pharmacology, structure–activity relationship (SAR) studies

The in-vitro screening results, based on the standard colorimetric MTT assay for the MAC 16 cell lines and the selected murine colon adenocarcinoma 13, are outlined in Tables 1 and 2.

The pseudo halogen acids themselves did not show in-vitro activity (IC50 > 100 μM). The pseudo-ester (Farina et al 1983) series have shown a maximum cytotoxicity for agent **3** containing a butyl substituent in the 5-position. The 5-butoxyfuranone **3** was used to compare the anti-cancer activity of previous work (Lattmann et al 2001) and this series of antineoplastic agents.

Unfortunately, the furanone **3** showed an extreme acute toxicity, assessed as a percentage weight loss of above 10% of lean body mass at a dose of 20 mg kg^{-1}.

The ketone **4** was biologically inactive in the cytotoxicity assays (IC50 > 100 μM) and the bisarylated hydrazone **5** ought to be ideal for DNA intercalations. Additionally, it was supposed that the nitro groups

Table 1 In-vitro activity/cytotoxicity.

Entry	Structure	Yield (%)	MAC 13 IC50 (μM)	MAC 16 IC50 (μM)
1a, b		Muco chloric acid	> 100	> 100
2		Muco bromic acid	> 100	> 100
3		85	3 ± 0.4	3 ± 0.2
4		72	> 100	> 100
5		51	80 ± 6	60 ± 4
6a	X = Cl R = H	69	50 ± 2	50 ± 3
6b	X = Cl R = Me	62	40 ± 6	50 ± 6
6c	X = Cl R = MeO	76	30 ± 2	40 ± 2
7a	X = Br R = H	61	30 ± 4	40 ± 4
7b	X = Br R = Me	69	30 ± 3	50 ± 4
8		45	50 ± 3	70 ± 5
9a	H	92	18 ± 3	34 ± 4
9b	MeO	39	17 ± 1	11 ± 1
9c	Cl	43	22 ± 2	19 ± 2
9d	PNO_2	32	33 ± 2	50 ± 3

Table 2 In-vivo data of selected molecules in tumour-bearing mice (transplanted with MAC 16 cell lines).

Entry	Administration (mg kg^{-1})	Tumour inhibition (%)	Body-weight (g)	Treatment (days)
Water	0	0	$+0.1 \pm 0.05$	1–5
3	10	18 ± 2.1	-1.5 ± 0.2	1–4
	20	25 ± 2.6	-2.7 ± 0.3	1
5	50	12 ± 2.3	-1.4 ± 0.1	1–5
6a	50	24 ± 1.5	-1.6 ± 0.1	1–5
8	50	6 ± 1.7	-1.5 ± 0.1	1–5
9b	50	52 ± 3.1	-1.5 ± 0.09	1–5
Sulfonyl urea	300	35 ± 4.8	-0.9 ± 0.05	1–5
5-FU	50	27 ± 2.1	-1.1 ± 0.1	1–5

Route of administration: intraperitoneally in solution of propylene glycol.

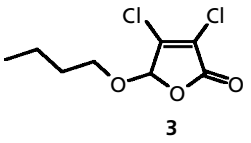
could be easily metabolised to nitroso groups in mice. However, **5** did only show a small inhibition of tumour growth in-vivo.

Derivatives of barbituric acid exhibit cancer-modulating properties and here, instead of barbituric acid, the closely related hydantoin system was connected in the dichlorinated butenolide **8**. The hydantoin derivative **8** was moderately cytotoxic in-vitro and was inactive in animal studies.

5-FU and Lilly's urea (Howbert et al 1990) were used as standards to compare our results. The sulfonyl ureas, which have shown up to 95% of inhibition of tumour growth on leukaemia cell lines, displayed only 35% inhibition of tumour growth on the MAC 16 cell line.

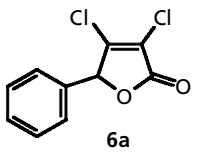
The 5-arylated 3,4-dichloro-furanones **6a**–**c** and dibromo-furanones **7a**, **b** showed a narrow range of cytotoxicity (IC50 30–50 μM) in cell line experiments. As the 2(5*H*)-furanone **6a** (Figure 3) was the only white, crystalline compound of this series, it was selected for testing in-vivo. The 5-arylated derivative **6a** displayed, at the dose of 50 mg kg^{-1}, 24% inhibition of growth. This represents an antineoplastic activity in-vivo in the same range as the 5-FU standard for this cell line.

As varying the substituents on the 5-phenyl ring resulted overall in a very similar cytotoxicity, the ring size of the unsaturated dichlorinated moiety was varied. Starting with muco chloric acid, as a C4-building block, the pyridazones **9a**–**d** were easily obtained in high yields. The nitrophenylpyridazine **9d** displayed a low solubility in organic solvents such as dichloromethane and was poorly soluble in DMSO. The donor substituted pyridazine **9b** (Figure 3) showed the lowest IC50 values (17 and 11 μM for the MAC 13 and 16 cells, respectively), with the right selectivity towards the MAC 16 cell line. Here, compared with the



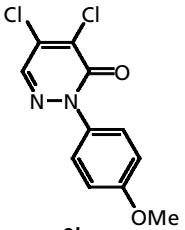
3

Pseudoester



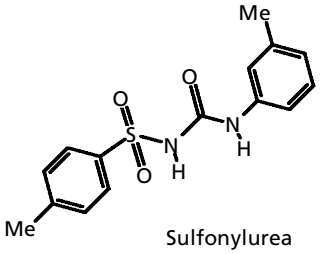
6a

5-Arylated 2(5*H*)-furanone



9b

Arylated pyridazin-3(2*H*)-one



Sulfonylurea

Figure 3 Selection of active molecules.

other selected examples of a series, a correlation of in-vitro cytotoxicity and in-vivo inhibition of tumour growth was found. The pyridazone **9b** displayed an inhibition of 52% of tumour growth at a dose of 50 mg kg^{-1} in mice when administered intraperitoneally.

Conclusions

The cytotoxicity of a series of pseudo-esters (**3**) has been optimised and reported previously (Lattmann et al 2001). The pseudoester **3**, having an IC50 of about 3 μM, displayed a small tumour inhibition in-vivo. The 5-arylated furanone **6a** displayed an antineoplastic activity in the same range as 5-FU, which is used as standard agent in cancer therapy.

Finally, the ring enlargement of the 2(5*H*)-furanone template, with muco chloric acid as C4 building block, furnished the 2-arylated pyridazine-3(2*H*)-ones with improved antineoplastic properties. The *p*-methoxy-dichloro-pyridazone **9b** displayed a good inhibition of tumour growth in mice for the resistant MAC 16 cell line and novel prodrugs of these promising agents are currently under investigation.

References

Ayuko, W. O., Lattmann, E. (1999) Butenolide derivatives as anti-cancer agents. PCT/GB99GB 01074 UK patent, WO99/52888

Black, D. K. (1966) Synthesis of penicillin acid from l-cysteine *J. Chem. Soc.* **23**: 1123–1127

Cho, S. D., Choi, W. Y., Yoon, Y. J. (1996) Alkylation of 4,5-dihalopyridazin-6-ones and synthesis of 5-halo4-hydroxy-pyridazin-6-ones, *J. Heterocyclic. Chem.* **33**: 1579–1582

Coombs, J., Lattmann, E., Hoffmann, H. M. R. (1998) Total synthesis of manoalide. *Synthesis* 1367–1371

Farina, F., Martin, M. F., Sanchez, F. (1983) Pseudoesters and derivatives: synthesis of 2(5H)-furanones. *Synthesis* 397–400

Franco, C. M., Borde, U. P., Chatterjee, S., Bulmbach, J., Ganguli, B. N. (1991) Butalactin a new butenolide antibiotic, *J. Antibiotics* **44**: 225–231

Hiyama, T., Oishi, H., Nishide, K., Saimoto, H. (1987) Synthesis of 4-aminofuranones through intra- and intermolecular nitrile addition of ester enolates. Construction of Basidalin. *Bull. Chem. Soc. Jpn* **60**: 2139–2150

Howbert, J. J., Crossman, C. S., Crowell, T. A., Rieder, B. J., Harper, R. W., Kramer, K. E., Grindey, G. B., Shaw, W. N., Todd, G. C. (1990) Novel effective agents against solid tumors: the diarylsulfonylureas. *J. Med. Chem.* **33**: 2393–2407

Lattmann, E., Hoffmann, H. M. R. (1996) From tetronic acid and furfural to C(4)-halogenated, vinylated and formylated furan-2(5H)-ones and their 5-alkoxy derivatives. *Synthesis* 155–163

Lattmann, E., Billington, D. C., Langley, C. A. (1999) Synthesis of combinatorial libraries of 3,4,5-substitutes 2(5H)-furanones. Part two: construction of a library of 4-amino-5-alkoxy-2(5H)-furanones. *Drug Des. Discov.* **16**: 243–250

Lattmann, E., Kinchington, D., Singh, H., Merino, I., Begum, A., Ayuko, W. O., Tisdale, M. J. (2001) Synthesis of simple and bis-cyclic 3,4-dihalogenated 2(5H)-furanones and their evaluation as novel cytotoxic agents. *Pharm. Pharm. Lett.* **11**: 5–8

Mosmann, T. (1983) Anti-neoplastic assays. *J. Immunol. Meth.* **65**: 55–63

Mowry, D. T. (1950) Mucochloric acid. I. Reactions of the pseudo acid group. *J. Am. Chem. Soc.* 2535–2537

Mowry, D. T. (1953) Mucochloric acid. II. Reactions of the aldehyde group. *J. Am. Chem. Soc.* 1909–1910

Mutschler, E (1996) *Arzneimittelwirkungen, Pharmacology*. 7th edn, Wiss. Verl. Ges, Stuttgart, p. 749

Reynolds, R. C., Tiwary, A., Harwell, J. E., Struck, R. F. (2000) Synthesis and evaluation of several new 92-chloroethyl)nitro-socarbamates as potential anti cancer agents. *J. Med. Chem.* **43**: 1484–1488

Schroeter, S. H., Apple, A., Brammer, R., Schenk, G. O. (1966) Zur Darstellung einiger pyridazine. *Liebigs Annalen* **42**: 697–703

Semonsky, M., Rockowa, E., Cerny, A., Kakak, B., Macek K. (1962) Einige aryl-substituierte crotonlactone, Coll. Czechoslov. *Chem. Comm.* **27**: 1939–1955