Supporting Information

Reactive Oxygen Species-mediated Apoptosis and Cytotoxicity of Newly Synthesized Pyridazin-3-ones in p815 (Murin Mastocytoma) Cell Line

Cytotoxic Activity of Newly Synthesized Pyridazin-3-Ones Derivatives Against The Murine Mastocytoma Cell Line (P815)

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Experimental chemistry, supplemented materials

MATERIALS AND METHODS

Synthesis

Melting points were determined on a Büchi SMP 20 apparatus and are not corrected. Infrared (IR) spectra were recorded with an IR VERTEX 70 FT-IR (Bruker Optics) spectrometer. \(^1\)H Nuclear magnetic resonance (\(^1\)H NMR) spectra were recorded on a Bruker Avance (300 MHz) spectrometer, using tetramethylsilane (TMS) as internal standard and, CDCl\(_3\) and DMSO\(_d_6\) as solvent. Mass spectra was recorded on a API 3200 LC/MS/MS mass spectrometer using electrospray ionization (ESI) in positive polarity.

General procedures for the formylation of phenols 2a-f, the synthesis of 2-formylphenoxyacetdehyde diethyl acetals 3a-f, the synthesis of substituted benzo[b]furan-2-carbaldehydes 4a-f, the synthesis of substituted 3-benzo[b]furan-2-ylmethylene-levulinic acids 5a-f, the synthesis of substituted 5-(benzo[b]furan-2-ylmethyl)-6-methylpyridazin-3(2H)-ones 6a-f have been described in our paper (Bouchmaa et al., 2018).

**General procedures for the formylation of phenols 2a-f. Method A**

A solution of the substituted phenols 1 (0.5 mol) in 300 mL of 10 N NaOH (3 mol) was heated to 65°C. Then 80 mL of CHCl\(_3\) was added in three portions over 15 min. The mixture was heated at reflux in chloroform for 2 h. After cooling, the mixture was acidified to pH 1 with 12 N HCl, the organic layer collected and the aqueous layer extracted with chloroform. The combined chloroform solution was dried and evaporated to give a crude product which was distilled or recrystallized from an appropriate solvent.

3-Chloro-2-hydroxybenzaldehyde 2c. This compound was obtained as white solid, yield 25%, mp 49-50°C (petroleum ether); IR (KBr \(v\) max cm\(^{-1}\), 1650 (C=O), 3100-3600 (OH); \(^1\)H NMR (CDCl\(_3\), 300 MHz, J Hz), \(\delta=7.04\) (dd, 1H, J5-4=6.30, J5-6=6.30, H5), 7.69 (d, 1H, J4-5=6.30, H4), 7.69 (d, 1H, J6-5=6.30, H6), 10.10 (s, 1H, -CHO), 11.08 (s, 1H, exch D\(_2\)O, -OH).

3-Chloro-2-hydroxy-5-methylbenzaldehyde 2d. This compound was obtained as white solid, yield 30%, mp 50-52°C (hexan); IR (KBr \(v\) max cm\(^{-1}\), 1650 (C=O), 2900-3000 (C-H), 3100-3600 (OH); \(^1\)H NMR (CDCl\(_3\), 300 MHz), \(\delta=2.25\) (s, 3H, CH\(_3\)), 7.49 (s, 1H, H4), 7.49 (s, 1H, H6), 7.57 (s, 1H, H6), 10.08 (s, 1H, -CHO), 10.84 (s, 1H, exch D\(_2\)O, -OH).

3-Bromo-5-chloro-2-hydroxybenzaldehyde 2f. This compound was obtained as white solid, yield 21%, mp 77-78°C (hexan); IR (KBr \(v\) max cm\(^{-1}\), 1650 (C=O), 310-3600 (OH); \(^1\)H NMR (CDCl\(_3\), 300 MHz, J Hz), \(\delta=7.77\) (d, 1H, J=2.70, H4), 7.98 (d, 1H, J=2.70, H6), 10.03 (s, 1H, -CHO), 11.50 (s, 1H, exch D\(_2\)O, -OH).

5-Chloro-2-hydroxybenzaldehyde 2a, 5-bromo-2-hydroxybenzaldehyde 2b and 3,5-dichloro-2-hydroxybenzaldehyde 2e products commercially available.

**General procedures for the synthesis of 2-formylphenoxyacetdehyde diethyl acetals 3a-f. Method B**

To a stirred suspension containing substituted 2-hydroxybenzaldehydes 2 (0.15 mol) and potassium carbonate (0.16 mol) in 100 mL of DMF, bromoacetaldehyde diethyl acetal (0.16 mol) was added drop
wise. The mixture was refluxed for 4 h. After cooling, the precipitate was filtered off and the solvent evaporated under reduced pressure. The oily residue was distilled.

(4-Chloro-2-formylphenoxy)acetaldehyde diethylacetal 3a. This compound was obtained as yellow oil, yield 90%, bp 140-143°C (0.3 mmHg); IR (KBr vmax cm⁻¹), 1690 (C=O), 2900-3000 (C-H); ¹H NMR (CDCl₃, 300 MHz), δ=1.25 (t, 6H, j=7.60 Hz, (-OCH₂-CH₃)₂), 3.50-4.00 (m, 4H, (-OCH₂-CH₃)₂), 4.10 (d, 2H, j=5.71 Hz, -CH₂-CH), 4.87 (t, 1H, j=5.71 Hz, -CH₂-CH₂), 6.95 (d, 1H, J₆.₅=8.50 Hz, H₆), 7.48 (dd, 1H, J₅.₃=2.81, J₅.s=8.50 Hz, H₅), 7.79 (d, 1H, J₃.₅=2.81 Hz, H₃), 10.43 (s, 1H, -CHO).

(4-Bromo-2-formylphenoxy)acetaldehyde diethylacetal 3b. This compound was obtained as yellow oil, yield 81%, bp 160-162°C (0.15 mmHg); IR (KBr vmax cm⁻¹), 1680 (C=O), 2900-3000 (C-H); ¹H NMR (CDCl₃, 300 MHz, J Hz), δ=1.11 (t, 6H, J=6.90, (-OCH₂-CH₃)₂), 3.47-3.64 (m, 4H, (-OCH₂-CH₃)₂), 4.11 (d, 2H, J=5.10, -CH₂-CH), 4.84 (t, 1H, J=5.10, -CH₂-CH₂), 7.24 (d, 1H, J₆.₅=9.02, H₆), 7.71 (dd, 1H, J₅.s=9.02, J₅.₃=2.70, H₅), 7.75 (d, 1H, J₃.₅=2.70, H₃), 10.28 (s, 1H, -CHO).

(6-Chloro-2-formylphenoxy)acetaldehyde diethylacetal 3c. This compound was obtained as yellow oil, yield 82%, bp 140-142°C (0.3 mmHg); IR (KBr vmax cm⁻¹), 1700 (C=O), 2900-3000 (C-H); ¹H NMR (CDCl₃, 300 MHz, J Hz), δ=1.06 (t, 6H, J=6.90, (-OCH₂-CH₃)₂), 3.47-3.64 (m, 4H, (-OCH₂-CH₃)₂), 4.11 (d, 2H, J=5.10, -CH₂-CH), 4.85 (t, 1H, J=5.10, -CH₂-CH₂), 7.29 (dd, 1H, J₄.₃=7.50, J₄.₅=7.50, H₄), 7.68 (dd, 1H, J₄.₃=7.80, J₃.s=1.50, H₃), 7.79 (dd, 1H, J₃.₅=7.50, J₃.s=1.50, H₃), 10.32 (s, 1H, -CHO).

(6-Chloro-4-methyl-2-formylphenoxy)acetaldehyde diethylacetal 3d. This compound was obtained as yellow oil, yield 83%, bp 155-156°C (1.5 mmHg); IR (KBr vmax cm⁻¹), 1700 (C=O), 2900-3000 (C-H); ¹H NMR (CDCl₃, 300 MHz, J Hz), δ=1.18 (t, 6H, J=6.90, (-OCH₂-CH₃)₂), 2.46 (s, 3H, CH₃), 3.47-4.75 (m, 6H, (-OCH₂-CH₃)₂, -OCH₂-CH), 4.50 (t, 1H, J=5.40, -OCH₂-CH), 7.26 (s, 1H, H₅), 7.96 (s, 1H, H₃), 10.34 (s, 1H, -CHO).

(4,6-Dichloro-2-formylphenoxy)acetaldehyde diethylacetal 3e. This compound was obtained as yellow oil, yield 68%, bp 154-156°C (0.3 mmHg); IR (KBr vmax cm⁻¹), 1700 (C=O), 2900-3000 (C-H); ¹H NMR (CDCl₃, 300 MHz, J Hz), δ=1.24 (t, 6H, J=7.30, (-OCH₂-CH₃)₂), 3.50-4.00 (m, 4H, (-OCH₂-CH₃)₂), 4.18 (d, 2H, J=4.6, -CH₂-CH), 4.87 (t, 1H, J=4.6, -CH₂-CH₂), 7.58 (d, 1H, J₅.₃=2.70, H₅), 7.94 (d, 1H, J₃.s=2.70, H₃), 10.42 (s, 1H, -CHO).

(6-Bromo-4-chloro-2-formylphenoxy)acetaldehyde diethylacetal 3f. This compound was obtained as yellow oil, yield 67%, bp 180-183°C (0.5 mmHg); IR (KBr vmax cm⁻¹), 1680 (C=O), 2900-3000 (C-H); ¹H NMR (CDCl₃, 300 MHz, J Hz), δ=1.07 (t, 6H, J=7.20, (-OCH₂-CH₃)₂), 3.47-3.64 (m, 4H, (-OCH₂-CH₃)₂), 4.10 (d, 2H, J=5.10, -CH₂-CH), 4.68 (t, 1H, -CH₂-CH₂), 7.67 (d, 1H, J₃.s=2.40, H₃), 8.10 (d, 1H, J₃-5=2.40, H₅), 10.22 (s, 1H, -CHO).

General procedures for the synthesis of substituted benzo[b]furan-2-carbaldehydes 4a-f. Method C

A stirred solution of compounds 3 (0.1 mol) in 35 mL of concentrated acetic acid was refluxed for 24 h. After cooling, the solution was evaporated to dryness. The crude product was distilled or recrystallized from an appropriate solvent.
5-Chlorobenzo[b]furan-2-carbaldéhyde 4a. This compound was obtained as yellow solid, yield 88%, mp 105°C (hexan); IR (KBr v max cm⁻¹), 1670 (C=O); ¹H NMR (CDCl₃, 80 MHz), δ=7.25-7.75 (m, 4H, H₃, H₄, H₆, H₇), 9.86 (s, 1H, CHO).

5-Bromobenzo[b]furan-2-carbaldehyde 4b. This compound was obtained as yellow solid, yield 82%, mp 106-107°C (ethylacetate); IR (KBr ν max cm⁻¹), 1680 (C=O); ¹H NMR (CDCl₃, 300 MHz, J Hz), δ=7.29 (d, 1H, J₆=1.80, H₆), 7.70 (s, 1H, H₃), 7.88 (d, 1H, J₆,₇=1.80, J₆,₄=0.60, H₄), 8.09 (dd, 1H, J₆,₇=1.80, J₆,₄=0.60, H₆), 9.87 (s, 1H, CHO).

7-Chlorobenzo[b]furan-2-carbaldehyde 4c. This compound was obtained as yellow oil, yield 70%, mp 90-91°C (hexan); IR (KBr ν max cm⁻¹), 1700 (C=O); ¹H NMR (CDCl₃, 80 MHz, J Hz), δ=7.29-8.20 (m, 3H, H₃, H₄ and H₆), 9.95 (s, 1H, CHO).

7-Chloro-5-methylbenzo[b]furan-2-carbaldehyde 4d. This compound was obtained as yellow solid, yield 70%, mp 59-60°C (hexan); IR (KBr ν max cm⁻¹), 1685 (C=O); ¹H NMR (CDCl₃, 300 MHz, J Hz), δ=7.36 (d, 1H, J₆,₇=8.70, H₇), 7.42 (d, 1H, J₆,₇=1.50, H₆), 7.51 (s, 1H, H₃), 9.90 (s, 1H, CHO).

5,7-Dichlorobenzo[b]furan-2-carbaldehyde 4e. This compound was obtained as yellowish oil, yield 87%, mp 127°C (hexan); IR (KBr ν max cm⁻¹), 1700 (C=O); ¹H NMR (CDCl₃, 80 MHz, J Hz), δ=7.29-7.75 (m, 4H, H₃, H₄, H₆ and H₇), 9.86 (s, 1H, CHO).

7-Bromo-5-chlorobenzo[b]furan-2-carbaldehyde 4f. This compound was obtained as yellowish oil, yield 73%, mp 96°C (hexan); IR (KBr ν max cm⁻¹), 1700 (C=O); ¹H NMR (CDCl₃, 300 MHz, J Hz), δ=7.29-8.01 (m, 3H, H₃, H₄ and H₆), 9.88 (s, 1H, CHO).

**General procedures for the synthesis of substituted 3-benzo[b]furan-2-ylmethylene-levulinic acids 5a-f.**

**Method D.**

A stirred solution of compounds 4 (0.1 mol) in 35 mL of concentrated acetic acid was refluxed for 24 h. After cooling, the solution was evaporated to dryness. The crude product was recrystallized from an appropriate solvent.

3((5-Chlorobenzo[b]furan-yl)methylene)-4-oxopentanoic acid 5a. This compound was obtained as yellow oil, yield 53%, mp 184-186°C (ethyl acetate); IR (KBr v max cm⁻¹), 1700 (C=O acid), 1650 (C=O ketone); ¹H NMR (DMSOd₆, 300 MHz, J Hz), δ=2.43 (s, 3H, H₃-C-CO), 3.73 (s, 2H, CH₂-COOH), 7.33 (s, 1H, H₃), 7.41 (dd, 1H, J₆,₇=8.70 and J₆,₄=1.80, H₆), 7.62 (d, 1H, J₆,₇=8.70, H₇), 7.71 (s, 1H, CH=C), 7.82 (d, 1H, J₆,₇=1.80, H₆).

3((5-Bromobenzo[b]furan-yl)methylene)-4-oxopentanoic acid 5b. This compound was obtained as yellow oil, yield 45%, mp 181-184°C (ethyl acetate); IR (KBr v max cm⁻¹), 1700 (C=O acid), 1650 (C=O ketone); ¹H NMR (DMSOd₆, 300 MHz, J Hz), δ=2.43 (s, 3H, H₃-C-CO), 3.73 (s, 2H, CH₂-COOH), 7.33 (s, 1H, H₃), 7.51 (dd, 1H, J₆,₇=8.70 and J₆,₄=2.10, H₆), 7.57 (d, 1H, J₆,₇=8.70, H₇), 7.70 (s, 1H, CH=C), 7.96 (d, 1H, J₆,₇=2.10, H₆).
**3((7-Chlorobenzo[b]furan-yl)methylene)-4-oxopentanoic acid 5c.** This compound was obtained as yellow oil, yield 12%, mp 180-182°C (ethyl acetate); IR (KBr vmax cm⁻¹), 1700 (C=Oacid), 1665 (C=Oacetone); ¹H NMR (DMSOᴅ₆, 300 MHz), δ=2.45 (s, 3H, ᴢC-CO), 3.76 (s, 2H, ᴢH₂-COOH), 7.28-7.76 (m, 4H, ḸH₃, ḸH₄, ḸH and ḸH₆), 7.99 (s, 1H, ḸH=Ĉ=C).

**3((7-Chloro-5-methylbenzo[b]furan-yl)methylene)-4-oxopentanoic acid 5d.** This compound was obtained as yellow oil, yield 10%, mp 203°C (ethyl acetate); IR (KBr νmax cm⁻¹), 1690 (C=Oacid), 1655 (C=Oacetone); ¹H NMR (DMSOᴅ₆, 300 MHz), δ=2.38 (s, 3H, ḸH₃-C-Ar), 2.43 (s, 3H, ḸH₃-C=CO), 3.73 (s, 2H, ḸH₂-COOH), 7.35-7.71 (m, 3H, ḸH₃, ḸH₄, and ḸH₆), 7.94 (s, 1H, ḸH=Ĉ=C).

**3((5,7-Dichlorobenzo[b]furan-yl)methylene)-4-oxopentanoic acid 5e.** This compound was obtained as yellow oil, yield 15%, mp 185-186°C (ethylacetate); IR (KBr νmax cm⁻¹), 1700 (C=Oacid), 1650 (C=Oacetone); ¹H NMR (DMSOᴅ₆, 300 MHz), δ=2.44 (s, 3H, ḸH₃-C=CO), 3.74 (s, 2H, ḸH₂-COOH), 7.40-7.98 (m, 4H, ḸH₃, ḸH₄, ḸH₆ and ḸH=Ĉ=C).

**3((5-Chloro-7-bromobenzo[b]furan-yl)methylene)-4-oxopentanoic acid 5f.** This compound was used crude for the continuation.

**General procedures for the synthesis of substituted 5-(benzo[b]furan-2-ylmethyl)-6-methylpyridazin-3(2H)-ones 6a-g. Method E**

The mixture of acids 5 and hydrazine hydrate solution in ethanol was refluxed for 2h; the precipitate formed is filtered and recrystallized from an appropriate solvent.

5-[(5-Chlorobenzo[b]furan-2-yl)methyl]-6-methylpyridazin-3(2H)-one 6a. This compound was obtained as yellow solid, yield 82%, mp 198-200°C (ethanol); IR (KBr vmax cm⁻¹), 1666 (C=O), 1602 (C=N); ¹H NMR (DMSOᴅ₆, 300 MHz, ḸH, ḸH₃, ḸH₄, ḸH₆ and ḸH=Ĉ=C).
2H, -CH₂-), 6.55 (s, 1H, H₃'), 7.18 (d, 1H, J₄'-₆'=1.50, H₄'), 7.32 (d, 1H, J₆'-₄'=1.50, H₆'), 12.77 (ls, 1H, NH). MS: m/z; 289.3 [M-H]+, 311.3 [M-Na]+.

5-[(5,7-Dichlorobenzo[b]furan-2-yl)methyl]-6-methylpyridazin-3(2H)-one 6e. This compound was obtained as yellow solid, yield 68%, mp 240-243°C (ethanol); IR (KBr v max cm⁻¹), 1666 (C=O), 1600 (C=N); ¹H NMR (DMSO-d₆, 300 MHz, J Hz), δ=2.22 (s, 3H, -N=C-CH₃), 4.14 (s, 2H, -CH₂-), 6.58 (s, 1H, H₄), 6.81 (s, 1H, H₃'), 7.50 (d, 1H, J₄'-₆'=2.10, H₄'), 7.65 (d, 1H, J₆'-₄'=2.10, H₆'), 12.79 (ls, 1H, NH).

5-[(7-Bromo-5-chlorobenzo[b]furan-2-yl)methyl]-6-methylpyridazin-3(2H)-one 6f. This compound was obtained as yellow solid, yield 66%, mp 256-258°C (ethanol); IR (KBr v max cm⁻¹), 1680 (C=O), 1605 (C=N); ¹H NMR (DMSO-d₆, 300 MHz, J Hz), δ=2.23 (s, 3H, -N=C-CH₃), 4.15 (s, 2H, -CH₂-), 6.58 (s, 1H, H₄), 6.83 (s, 1H, H₃'), 7.60 (d, 1H, J₄'-₆'=1.80, H₄'), 7.69 (d, 1H, J₆'-₄'=1.80, H₆'), 12.79 (ls, 1H, NH). MS: m/z; 290.7 [M-H]+, 313.10 [M-Na]+.

General procedures for the synthesis of substituted 5-(benzo[b]furan-2-ylmethyl)-6-methylpyridazin-3(2H)-thiones 7g-h. Method F

The mixture of substituted 6-methylpyridazin-3(2H)-ones 6 and diphosphore pentasulfure solution in pyridin was refluxed for 4h; the precipitate formed is filtered and recrystallized from an appropriate solvent.

5-[(5-Chlorobenzo[b]furan-2-yl)methyl]-6-methylpyridazin-3(2H)-thione 7g. This compound was obtained as yellow solid, yield 65%, mp 165-167°C (ethanol); IR (KBr v max cm⁻¹), 1605 (C=N), 1080 (C=S); ¹H NMR (DMSO-d₆, 300 MHz, J Hz), δ=2.30 (s, 3H, -N=C-CH₃), 4.15 (s, 2H, -CH₂-), 6.76 (s, 1H, H₃'), 7.28 (dd, 1H, J₆'-₇'=8.70 and J₆'-₄'=2.40, H₆'), 7.32 (s, 1H, H₄), 7.58 (d, 1H, J₇'-₆'=8.70, H₇'), 7.65 (d, 1H, J₄'-₆'=2.40, H₄'), 14.51 (ls, 1H, NH).

5-[(7-Chlorobenzo[b]furan-2-yl)methyl]-6-methylpyridazin-3(2H)-thione 7h. This compound was obtained as yellow solid, yield 62%, mp 188-190°C (ethanol); IR (KBr v max cm⁻¹), 1606 (C=N), 1082 (C=S); ¹H NMR (DMSO-d₆, 300 MHz, J Hz), δ=2.33 (s, 3H, -N=C-CH₃), 4.19 (s, 2H, -CH₂-), 6.86 (s, 1H, H₃'), 7.40-7.57 (m, 4H, H₄, H₅, H₆ and H₇'), 14.51 (ls, 1H, NH). MS: m/z; 290.7 [M-H]⁺, 313.10 [M-Na]⁺.

Results

Chemistry

Supplementary Fig 1 Synthesis of target compounds 6a–f, 7g and 7h. Reagents: a CHCl$_3$/NaOH 10 N, reflux 2h; b BrCH$_2$CH(OC$_2$H$_5$)$_2$/K$_2$CO$_3$/DMF, reflux 4h; c CH$_3$COOH, reflux 24h; d H$_2$CCOCH$_2$COOH/CH$_3$COOH, reflux 24h; e H$_2$N-NH$_2$/EtOH, reflux 2h; f P$_2$S$_5$/pyridin, reflux 4h.