SUPPLEMENTARY MATERIAL for

Anti-cancer Activity of Phenyl and Pyrid-2-yl 1,3-Substituted Benzo[1,2,4]triazin-7-ones and Stable Free Radical Precursors

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INDEX

Figures S1-S4 ¹H and ¹³C-NMR Spectra for Compounds 4a and 4b

Figure S5-S6 NCI-60 One-Dose Mean Graph Data for Compounds 4a and 4b

Figures S7-S8 NCI-60 Five-Dose Mean Graph Data for Compounds 4a and 4b

Figures S9-S13 Viability of DU145 and MCF-7 cell lines as determined using the MTT assay for Compounds **TEMPO**, **3a**, **3b**, **4a** and **4b**



Figure S1. ¹H NMR (500 MHz) spectrum in CDCl₃ of 3-phenyl-1-(pyrid-2-yl)-benzo[*e*][1,2,4]triazin-7(1*H*)-one (**4a**).



Figure S2. ¹³C NMR (125 MHz) spectrum in CDCl₃ of 3-phenyl-1-(pyrid-2-yl)benzo[e][1,2,4]triazin-7(1H)-one (**4a**).



Figure S3. ¹H NMR (500 MHz) spectrum in CDCl₃ of 1-phenyl-3-(pyrid-2-yl)-benzo[*e*][1,2,4]triazin-7(1*H*)-one (**4b**).



Figure S4. ¹³C NMR (125 MHz) spectra in CDCl₃ of 1-phenyl-3-(pyrid-2-yl)-benzo[*e*][1,2,4]triazin-7(1*H*)-one (**4b**).

Developmental Therapeutics Program		NSC: D-780816/1	Cono: 1.00E-5 Molar	Test Date: May 27, 2014	
One Dose Mean Graph		Experiment ID: 14050893		Report Date: Nov 30, 2017	
Panel/Cell Line	Growth Percent	Mean Growth Percent - Growth Percent			
Panel/Cell Line Leukemia CCRF-CEM HL-50(TB) K-562 MOLT-4 RPMI-8226 SR Non-3mail Cell Lung Cancer A549/ATCC EKVX HOP-92 NCH-H226 NCH-H226 NCH-H226 NCH-H322M NCH-H322M NCH-H322M NCH-H322M NCH-H322 NCH-H322 Colon Cancer COLO 205 HCC-2998 HCT-116 HCT-15 HT29 KM12 SW-520 CNS Cancer SF-258 SF-258 SF-258 SF-251 Melanoma LOX IMVI MALME-3M M14 MDA-MB-435 SK-MEL-5 UACC-257 UACC-52 Ovcara:a Cancer IGROV1 OvCAR-5 <t< td=""><td>Growth Percent 1.08 -24.28 -24.28 -10.55 35.83 1.20 102.62 108.64 95.95 55.81 91.87 90.80 102.10 105.80 -59.67 -68.75 102.77 42.71 33.88 -46.47 103.96 -46.19 96.60 102.98 99.50 102.24 106.01 94.41 49.57 50.12 105.45 94.63 96.79 87.21 76.64 72.28 93.38 88.99 -14.88 83.52 116.74 80.05 101.49 83.87 63.46 88.25</td><td>Mean Growth</td><td>Percent - Growth Perc</td><td></td></t<>	Growth Percent 1.08 -24.28 -24.28 -10.55 35.83 1.20 102.62 108.64 95.95 55.81 91.87 90.80 102.10 105.80 -59.67 -68.75 102.77 42.71 33.88 -46.47 103.96 -46.19 96.60 102.98 99.50 102.24 106.01 94.41 49.57 50.12 105.45 94.63 96.79 87.21 76.64 72.28 93.38 88.99 -14.88 83.52 116.74 80.05 101.49 83.87 63.46 88.25	Mean Growth	Percent - Growth Perc		
CAKI-1 RXF 393 SN12C TK-10 UO-31 Prostate Cancer PC-3 DU-145 Breast Cancer MCF7 MDA-MB-231/ATCC	-37.40 26.81 93.09 -83.43 20.51 83.51 105.45 -37.05 91.13				
HIS 578T BT-549 T-47D MDA-MB-458 Mean Delta Range	101.23 108.14 -40.87 -65.24 53.32 145.17 208.59				
	150	100 50	0 -50	-100 -150	

Figure S5. One dose mean graph data for 3-phenyl-1-(pyrid-2-

yl)benzo[*e*][1,2,4]triazin-7(1*H*)-one (**4a**) towards the NCI-60 cell line panel.

Developmental Therapeutics Program		NSC: D-780815 / 1 Conc: 1.00E-5 Molar		Test Date: May 27, 2014
One Dose Mean Graph		Experiment ID: 1405OS93		Report Date: Nov 30, 2017
Panel/Cell Line	Growth Percent	Mean Growth Percent - Growth Percent		
Panel/Cell Line Laskemia CCRF-CEM HL-80(TB) K-562 MOLT-4 RPMI-8228 SR Nor-Small Cell Lung Cancer A549AATCC EKVX HOP-42 HOP-42 HOP-42 HOP-42 HOP-42 NCI-H228 NCI-H228 NCI-H228 NCI-H228 NCI-H229 NCI-H229 NCI-H229 Colon Cancer COLO 205 HCC-2598 HCC-2598 HCC-2598 HCC-2598 SF-285	Growth Percent	Mean Growth	Percent - Growth Perc	
HS 578T BT-549 T-47D MDA-MB-468	93.86 86.58 -28.41 -32.06	-		_
MUA-MD-465 Mean Delta Range	-32.06 50.31 135.03 199.96			—
	150	100 50	0 -50	-100 -150

Figure S6. One dose mean graph data for 1-phenyl-3-(pyrid-2-

yl)benzo[*e*][1,2,4]triazin-7(1*H*)-one (4**b**) towards the NCI-60 cell line panel.



Figure S7. Five dose data for 3-phenyl-1-(pyrid-2-yl)benzo[*e*][1,2,4]triazin-7(1*H*)-one (**4a**) towards the NCI-60 cell line panel.



Figure S8. Five dose data for 1-phenyl-3-(pyrid-2-yl)benzo[*e*][1,2,4]triazin-7(1*H*)-one (**4b**) towards the NCI-60 cell line panel.

Cell Viability Graphs



TEMPO



Figure S9. Viability of DU-145 (•) and MCF-7 (•) cell lines determined using MTT assay following treatment with TEMPO under aerobic conditions for 72 h at 37 °C. Each data point is the mean of at least two independent experiments. The lines shown are trend lines.





Figure S10. Viability of DU-145 (•) and MCF-7 (•) cell lines determined using MTT assay following treatment with 3-phenyl-1-(pyrid-2-yl)-1,4-dihydrobenzo[*e*][1,2,4]triazin-4-yl (**3a**) under aerobic conditions for 72 h at 37 °C. Each data point is the mean of at least two independent experiments. The lines shown are trend lines.





Figure S11. Viability of DU-145 (•) and MCF-7 (•) cell lines determined using MTT assay following treatment with 1-phenyl-3-(pyrid-2-yl)-1,4-dihydrobenzo[*e*][1,2,4]triazin-4-yl (**3b**) under aerobic conditions for 72 h at 37 °C. Each data point is the mean of at least two independent experiments. The lines shown are trend lines.



Figure S12. Viability of DU-145 (•) and MCF-7 (•) cell lines determined using MTT assay following treatment with for 3-phenyl-1-(pyrid-2-yl)benzo[*e*][1,2,4]triazin-7(1*H*)-one (**4a**) under aerobic conditions for 72 h at 37 °C. Each data point is the mean of at least two independent experiments. The lines shown are trend lines.







Figure S13. Viability of DU-145 (•) and MCF-7 (•) cell lines determined using MTT assay following treatment with 1-phenyl-3-(pyrid-2-yl)benzo[*e*][1,2,4]triazin-7(1*H*)- one (**4b**) under aerobic conditions for 72 h at 37 °C. Each data point is the mean of at least two independent experiments. The lines shown are trend lines.