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Fluorinated 2′-hydroxychalcones as garcinol analogs with enhanced antioxidant and anticancer activities

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ABSTRACT

Chalcones are involved in the synthesis of flavonoids and are themselves known to exhibit multiple pharmacological properties. However, compared to other structurally similar phytochemicals like garcinol and curcumin, the therapeutic use of chalcones is limited because of their lower bioavailability and rapid metabolic clearance from biological system. In the present work, we have attempted to overcome these limitations in case of 2′-hydroxychalcones through bioisosteric substitution of fluoro groups in place of phenolic hydroxyls. The fluorinated chalcones were found to be more potent antioxidant and anti-proliferative compounds than their hydroxyl counterparts indicating the influence of metabolically stable C–F bonds towards bioavailability. The difluoro derivatives were found to be most effective against human pancreatic BxPC-3 cancer cells which possess up-regulated COX-2 expression and also showed activity against human breast cancer BT-20 cells with triple negative phenotype, suggesting that these compounds will have broader application in the future.

Chalcones are the natural precursors of flavonoid compounds that are produced in plants by the action of enzyme chalcone synthase. Compounds belonging to both these classes have been shown to possess several pharmacological activities including antioxidant, anti-inflammatory, and anticancer, respectively.[1–4] Chemically, chalcones are 1,3-diphenylpropenones and benzylideneacetophenones which are made up of two aromatic rings in trans configuration separated by three carbon α,β-unsaturated carbonyl system. The compounds can be broadly classified based on substitution pattern of the parent chalcone as: hydroxylated chalcones, methoxylated chalcones, aminochalcones, N-containing chalcones, etc.[5] Our interest in following structural and biological chemistry of 2′-hydroxychalcones stems from the remarkable anticancer property exhibited by tri-isoprenylated hydroxychalcone, viz. garcinol, which is the active principle compound from *Garcinia indica*.[6] The compound structurally resembles with well-known antioxidant and anticancer compound of plant origin, viz. curcumin (Fig. 1), whose chemical and biological properties have been reviewed recently by us.[7] The presence of the isoprenyl groups in garcinol is known to enhance its lipophilicity and help promote cell internalization. Both the compounds are known to exhibit antioxidant and anti-inflammatory activities. Since preparation of tri-isoprenylated chalcone like garcinol is difficult to achieve through synthetic route with good yields, we have been interested in mimicking its biological

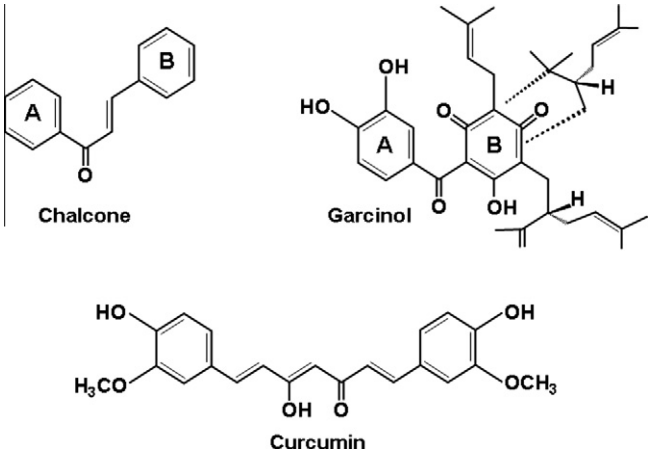

Figure 1. Structures of chalcone, garcinol, and curcumin.

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properties through corresponding hydroxychalcone (without the isoprenyl groups) by addition of hydroxyl/fluoro groups in the B ring since appending these groups has led to highly potent anticancer compounds in our lab in case of curcumin.[8]

The bioisosteric substitution of fluorine in place of hydrogen in many biologically active molecules has led to potent compounds without extensive stereochemical changes due to its small size.[9] However, such substitution is also known to modulate overall reactivity and stability of the compounds due to resistance of the carbon–fluorine bond toward metabolic transformations and changes in acidity due to electronegativity differences between the two atoms.[10] There have been a few reports on the improved biological activities of fluorinated methoxy chalcones[11] and chromones[12] than their non-fluorinated analogs in the past. Following our work on fluorocurcumins, Dou and co-workers have recently described enhanced antitumor and proteasome-inhibitory activities for epigallocatechins in breast cancer xenograft model.[13]

We were thus motivated to examine whether such effects can also be brought about in structurally similar chalcone group of compounds. For this purpose we selected 2′-hydroxychalcone as the parent compound since it is one of the biologically active chalcone with moderate anticancer activity. Several reports suggested that 2-hydroxyl substitution on A ring is crucial for its antioxidant,[14] anti-inflammatory,[15] and anticancer activities.[16] There is also an evidence suggesting that these chalcones can modulate antioxidant systems leading to mitochondrial oxidative stress which can result in apoptosis.[17] Hence in our synthetic strategy we have kept this part of the molecule untouched and have used only the B ring for substitution of hydroxy/fluoro groups. Our literature search revealed that B ring of the chalcone has been substituted with chloro, bromo, methoxy as well as hydroxyl groups[17,18] but not so frequently with the fluoro groups. In the present work, we describe preparation, characterization, and molecular docking studies on 2′-hydroxychalcones appended with hydroxy/fluoro groups in the B ring along with evaluation of their radical scavenging potential and anti-proliferative activities against human pancreatic and breast cancer cells. Our results suggest that chalcones fluorinated in the B ring exhibit enhanced anti-proliferative activities than their hydroxylated counterparts against these cell lines and bear a good correlation with their antioxidant activities.

The chalcones were synthesized by procedures reported earlier.[19,20] All synthesized hydroxychalcones (Fig. 2) **1**, **3**, **5** were reddish brown while the corresponding fluoro derivatives, **2**, **4**, **6**, were yellow in color. The IR spectra of all synthesized chalcones showed a medium intensity band in the region 3300–3200 cm^{-1} due to stretching frequency of intra-molecularly H-bonded hydroxyl group at C-2 in ring A. The broad band attributable to the carbonyl stretch of α,β-unsaturated ketone was observed in all compounds at 1700–1650 cm^{-1}.[21] The aromatic ν(C=C) stretches could be located at 1620–1580 cm^{-1}. The hydroxy- and fluorochalcones can be differentiated by the presence of strong C–F stretching absorption at 1350–1150 cm^{-1} and weaker peak in the region 800–400 cm^{-1} (Table 1).[22]

The electronic spectra of all synthesized chalcones in methanol show intra-ligand transitions for the hydroxyl compounds in the range 400–220 nm which undergoes shift towards lower wavelength in their fluoro counterparts. NMR spectra for the compounds **3** and **4** reveal the aromatic protons in ring B at δ 6.90–7.64 ppm while the ethylene proton is observed as a doublet at δ 7.39 and δ 8.17 ppm, respectively. The three phenolic hydroxyl groups in compound **3** appear as a singlet at δ 5.0 ppm while only one appears in case of **4**.[23] The compositional and spectral data on all synthesized compounds is summarized in Table 1.

Since anti-inflammatory property is one of the important characteristics of chalcone compounds, we evaluated the COX-2 selectivity of the new chalcone derivatives by performing molecular docking studies on them in the COX-2 protein cavity.[24] All hydroxyl and fluorochalcone compounds were found to dock into the active site of COX-2 (Fig. 3), confirming that hydroxy and fluoro substitution does not introduce any major steric changes in the parent 2-hydroxychalcone moiety **7** except for allowing additional hydrogen bonding interactions (Table 2). The higher log *P* values

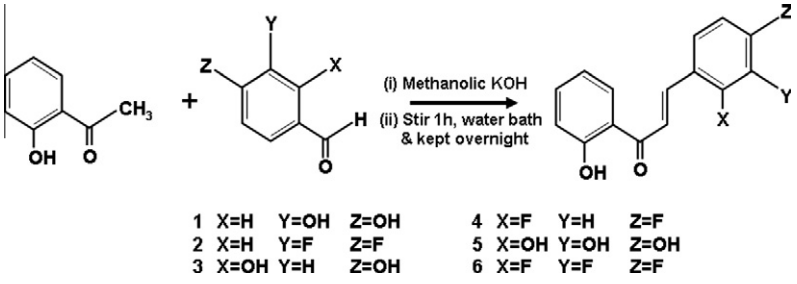

Figure 2. Schematic representation of synthesis of fluoro- and hydroxyl-substituted chalcone.

Table 1
Compositional and spectral data on fluorinated 2′-hyrdroxycalcones

Compounds	Molecular formula	Molecular weight	Electronic absorptions (λ, nm)		IR frequencies (KBr, ν, cm^{-1})				^{1}H NMR (300.40 MHz, $CDCl_3$, δ, ppm)
			$\pi \rightarrow \pi^*$	$n \rightarrow \pi^*$	O–H	C=O	C=C	C–F	
1	$C_{15}H_{12}O_4$	256.25	282	323	3228	1675	1596	–	–
2	$C_{15}H_{10}F_2O_2$	260.24	254	321	3236	1689	1612	1280	–
3	$C_{15}H_{12}O_4$	256.25	289	324	3258	1682	1619	–	5.0 (s, –3OH), 6.90–7.64 (benzylic), 7.39 (d, =CH), 8.17 (d, =CH)
4	$C_{15}H_{10}F_2O_2$	260.24	254	322	3222	1693	1612	1277	5.0 (s, –OH), 6.90–7.64 (benzylic), 7.39 (d, =CH), 8.17 (d, =CH)
5	$C_{15}H_{12}O_5$	272.25	295	325	3232	1680	1612	–	–
6	$C_{15}H_9F_3O_2$	278.23	254	321	3251	1693	1600	1274	–

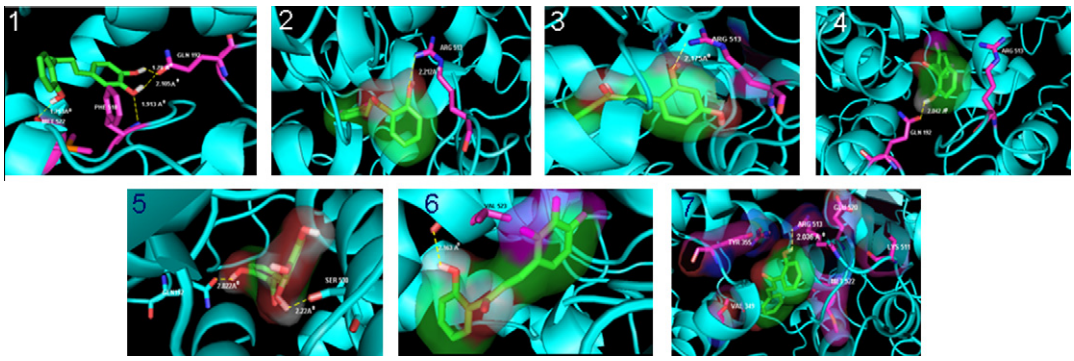

Figure 3. Binding of chalcone analogs into the active site of COX-2 as assessed by computer modeling studies.

Table 2
Docking results and consensus scores of synthesized chalcone analogs

Lig. no.	Binding energy (kcal/mol)	Docking energy (kcal/mol)	log *P*	No of hydrogen bonds	Hydrogen bonding residues	Distance (Å)
1	−9.05	−10.12	2.42	4	PHE 518, GLN 192, MET 522, GLN 192	1.913, 1.780, 1.753, 2.105
2	−9.10	−9.96	3.51	1	ARG 513	2.212
3	−8.57	−9.60	2.42	1	ARG 513	2.175
4	−8.48	−9.56	3.51	1	GLN 192	2.042
5	−8.88	−9.93	2.03	2	GLN 192, SER 530	2.022, 2.220
6	−8.63	−9.62	3.67	1	VAL 523	2.163
7	−8.10	−8.73	3.20	1	ARG 513	2.036

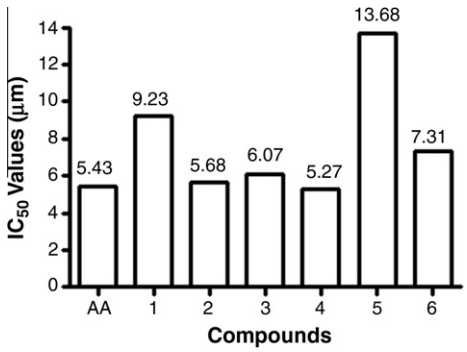

Figure 4. IC_{50} values of superoxide dismutase activity of the synthesized chalcone (**1**–**6**) and ascorbic acid (**AA**) using NBT spectrometric assay.

observed for the fluorochalcone compounds **2**, **4**, and **6** clearly reflect their higher lipophilicities. Subsequently their binding energies were found to be in the range of −8.48 to −9.10 kcal/mol compared to −8.10 kcal/mol observed for the parent compound **7** indicating their tight binding in the active site of COX-2 than parent compound. Similarly, the parent compound exhibited only one hydrogen bonding interaction involving ARG 513 residue, while compounds **1** and **5** exhibited four and two hydrogen bonding interactions, respectively. The hydroxyl groups on rings A and B of compound **1** favored Van der Waals interactions with PHE 518 (1.913 Å), GLN 192 (1.780 Å). GLN 192 (2.105 Å), MET 522 (1.753 Å) residues, while for the compounds **2**, **4**, **5**, **6**, and **7** the hydroxyl group on ring A undergoes similar interactions with ARG 513 (2.212 Å), GLN 192 (2.042 Å), GLN 192 (2.022 Å), VAL 523 (2.163 Å), and ARG 513 (2.036 Å), respectively. Thus, it can be concluded that the hydroxyl groups on either ring A or B favor hydrogen bonding interactions with amino acid residues in COX-2 protein which contribute in stabilizing the ligand–enzyme complexes (Fig. 3).

The IC_{50} values calculated for the SOD scavenging activity of present compounds by the NBT method[25,26] are in the range 5.27–13.68 µM (Fig. 4) where fluoro derivatives are found to be more potent reflecting higher metabolic stability of the C–F bond. Compounds **2** and **4** were found to be the most potent compounds whose activity is comparable with that of ascorbic acid used as a reference antioxidant compound[27] in the studies (Fig. 4). Since the synthesized chalcones exhibited significant antioxidant activity, we checked for their efficacy in inhibiting the growth of COX-2 positive human pancreatic cancer cell line BxPC-3 as well as against the estrogen receptor-negative BT-20 breast cancer cell line (Fig. 5).[28]

We found that in case of pancreatic BxPC-3 cells the IC_{50} values[29] for the fluorochalcones were three to seven times lower than their

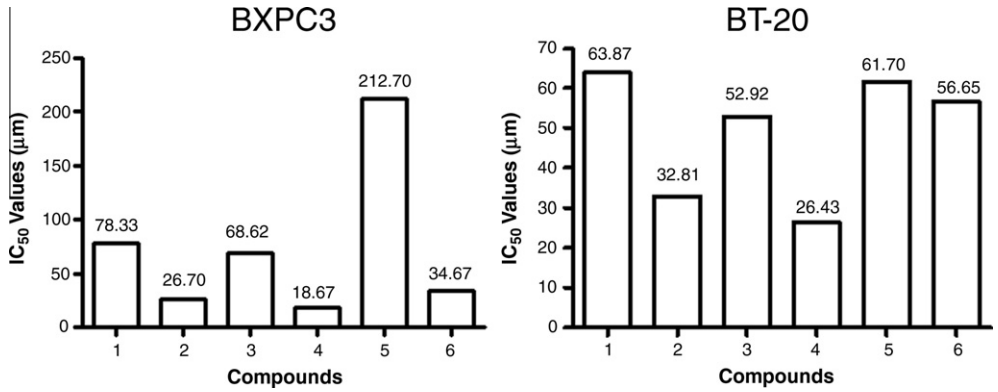

Figure 5. IC_{50} values of the hydroxy and fluoro chalcones (**1**–**6**) against human pancreatic cancer cell line, BxPC-3, and breast cancer cell line, BT-20.

hydroxy counterparts (Fig. 5). The most substantial lowering was observed for the trifluoro compound than that of trihydroxychalcone. The lowest IC_{50} value was observed for the compound **4**. A similar trend was observed in breast cancer BT-20 cells as well (Fig. 5), although differences between hydroxyl and fluoro-substituted analogs were not as dramatic as in pancreatic cells. Additionally, potency of anticancer activity of these compounds correlates well with their antioxidant property.

Our research group has been exploring different natural products containing novel classes of active phytochemicals which can effectively modulate inflammatory pathways.[6,30,31] As pointed out by Hong et al.[32] most of natural antioxidants exhibit biphasic action in modulating cell growth in vitro. At low concentrations (<1 μM), they stimulate cell proliferation possibly by activating ERK1/2 and AKT through generation of hydrogen peroxide, but at higher concentrations they inhibit cell growth possibly by inducing apoptosis. The net effects of these compounds, therefore, depend upon the tissue levels of these compounds and their metabolites. Interestingly three structurally resembling phytochemicals, viz. garcinol, chalcones, and curcumin (Fig. 1) have very low bioavailability and rapid metabolic clearance from biological system. In order to make them useful as therapeutic agents, appropriate structural modifications are necessary which can address these two problems. Recently, we have shown that bioisosteric substitution of fluoro groups in place of phenolic hydroxyls in such phytochemicals can not only enhance their bioavailability but improve metabolic rates as well.[8] In the present work we have successfully extended such strategy to chalcones which are garcinol mimics for all practical purposes; however, their bioavailability must be tested in future studies.

The modified chalcone derivatives exhibited potent antioxidant activities and are capable of blocking the inflammatory pathway as revealed from the molecular modeling studies although further biochemical studies would be required to prove the point. These compounds also exhibited potent inhibitory activities against human pancreatic BxPC-3 cancer cells which possess up-regulated COX-2 expression as well as against human breast cancer BT-20 cells with triple negative phenotype. Both these cancers are known for their insensitivities to conventional chemotherapeutics, resulting in poor prognosis. The results of the current study indicates that fluorochalcones might be able to provide a starting point for building effective strategy to overcome many limitations especially the bioavailability and improve cell growth inhibitory activity against aggressive cancers and, thus, further studies are warranted.

References and notes

1. Dimmock, J. R.; Elias, D. W.; Beazely, M. A.; Kandepu, N. M. *Curr. Med. Chem.* **1999**, *6*, 1125.
2. Go, M. L.; Wu, X.; Liu, X. L. *Curr. Med. Chem.* **2005**, *12*, 481.
3. Nowakowska, Z. *Eur. J. Med. Chem.* **2007**, *42*, 125.
4. Kontogiorgis, C.; Mantzanidou, M.; Hadjipavlou-Litina, D. *Mini Rev. Med. Chem.* **2008**, *8*, 1224.
5. Boumendjel, A.; Ronot, X.; Boutonnat, J. *Curr. Drug Targets* **2009**, *10*, 363.
6. Ahmad, A.; Wang, Z.; Ali, R.; Maitah, M. Y.; Kong, D.; Banerjee, S.; Padhye, S.; Sarkar, F. H. *J. Cell Biochem.* **2010**, *109*, 1134.
7. Padhye, S.; Chavan, D.; Pandey, S.; Deshpande, J.; Swamy, K. V.; Sarkar, F. H. *Mini Rev. Med. Chem.* **2010**, *10*, 372.
8. Padhye, S.; Banerjee, S.; Chavan, D.; Pandye, S.; Swamy, K. V.; Ali, S.; Li, J.; Dou, Q. P.; Sarkar, F. H. *Pharm. Res.* **2009**, *26*, 2438.
9. Smart, B. E. Characteristics of C–F Systems. In *Organofluorine Chemistry: Principles and Commercial Applications*; Banks, R. E., Smart, B. E., Tatlow, J. C., Eds.; Plenum Press: New York, 1994; p 57.
10. Kirk, K. L. Fluorine Substitution as a Modulator of Biological Processes. In *Biomedical Chemistry: Applying Chemical Principles to the Understanding and Treatment of Disease*; Torrence, P. F., Ed.; John Wiley & Sons: New York, 2000; p 247.
11. Nakamura, C.; Kawasaki, N.; Miyataka, H.; Jayachandran, E.; Kim, I. H.; Kirk, K. L.; Taguchi, T.; Takeuchi, Y.; Hori, H.; Satoh, T. *Bioorg. Med. Chem.* **2002**, *10*, 699.
12. Ishar, M. P.; Singh, G.; Singh, S.; Sreenivasan, K. K.; Singh, G. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1366.
13. Yang, H.; Sun, D. K.; Chen, D.; Cui, Q. C.; Gu, Y. Y.; Jiang, T.; Chen, W.; Wan, S. B.; Dou, Q. P. *Cancer Lett.* **2010**, *292*, 48.
14. Cheng, J. H.; Hung, C. F.; Yang, S. C.; Wang, J. P.; Won, S. J.; Lin, C. N. *Bioorg. Med. Chem.* **2008**, *16*, 7270.
15. Tran, T. D.; Park, H.; Kim, H. P.; Ecker, G. F.; Thai, K. M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1650.
16. Ban, H. S.; Suzuki, K.; Lim, S. S.; Jung, S. H.; Lee, S.; Ji, J.; Lee, H. S.; Lee, Y. S.; Shin, K. H.; Ohuchi, K. *Biochem. Pharmacol.* **2004**, *67*, 1549.
17. Guzy, J.; Vaskova-Kubalkova, J.; Rozmer, Z.; Fodor, K.; Marekova, M.; Poskrobova, M.; Perjesi, P. *FEBS Lett.* **2010**, *584*, 567.
18. Yang, H. M.; Shin, H. R.; Cho, S. H.; Bang, S. C.; Song, G. Y.; Ju, J. H.; Kim, M. K.; Lee, S. H.; Ryu, J. C.; Kim, Y.; Jung, S. H. *Bioorg. Med. Chem.* **2007**, *15*, 104.
19. Synthesis of chalcones: the chalcones were synthesized by condensing equimolar quantities of *o*-hydroxyacetophenone with corresponding hydroxy/fluoro benzaldehyde in methanol with a drop of aqueous potassium hydroxide solution (50%) ensuring neutral pH. Reaction mixture was stirred overnight after which it was poured on the crushed ice and acidified with hydrochloric acid. The resulting precipitates were filtered, washed with cold methanol and re-crystallized from acetone. The final products were characterized by detailed spectroscopic and micro analytical data.
20. Li, R.; Kenyon, G. L.; Cohen, F. E.; Chen, X.; Gong, B.; Dominguez, J. N.; Davidson, E.; Kurzban, G.; Miller, R. E.; Nuzum, E. O. *J. Med. Chem.* **1995**, *38*, 5031.
21. Hergert, H. L.; Kurth, E. F. *J. Am. Chem. Soc.* **1953**, *75*, 1622.
22. Liu, H. Q.; Jiang, F. C. *Guang Pu Xue Yu Guang Pu Fen Xi* **2007**, *27*, 2243.
23. Silva, A. M. S.; Cavaleiro, J. A. S.; Tarrago, G.; Marzin, C. *New J. Chem.* **1999**, *23*, 329.
24. Docking studies: AutoDock 4.0 software was used to analyze ligand interactions with the crystal structure binding site of COX-2 obtained from PDB ID (6COX). Autodock calculates a rapid energy evaluation through precalculated grids of affinity potentials with a variety of search algorithms to find appropriate binding positions. The 3-D grid box has been generated a grid center co-ordinates 21.804 Å, 21.72 Å, 49.4 Å (*X*, *Y*, and *Z* axis) with grid spacing 0.37 Å considering active site residues included within it. Stable docking conformation of compounds achieved by implementing energy minimization parameter AMBER force field until the gradient convergence value of 0.05 kcal/mol was reached with distance-dependent dielectric function ($e = 4r$). New designed compounds were placed in grid box of COX-2 for docking process. Customized docking parameters were set in Autodock for best results for understanding interaction studies with new designed compounds. Parameter settings were set to 1500 iterations, 50 population sizes, 100.0 kcal/mol of energy threshold for pose generation, 300 simplex evolution steps, and 1.0 neighbor distance factor. For preparing the AutoDock docking parameter file we used default settings (genetic algorithm parameters: population size = 150, number of energy evaluations = 2,500,000, rate of gene mutation = 0.02, rate of crossover = 0.8, maximum number of generations = 27,000, number of GA runs = 10, initial dihedrals were randomly specified, elitism value was set to 1). Prior to docking, total Kollman and Gasteiger charges were added to the protein and the ligand.
25. Nitroblue tetrazolium (NBT) assay: the hydroxy/fluoro derivatives of 2′-hydroxychalcone were evaluated for the SOD like activities by the NBT method in triplicate following the lab protocol established in our lab. Briefly, the test solution (400 μl) consisted of 2.1 ml of 0.2 M Tris buffer and 1 ml of 56 μM NBT. The tubes were kept in ice for 15 min followed by addition of 1.5 ml of KO_2 with stirring and measuring the absorbance of solution at 560 nm with an interval of 30 s for 5 min. Ascorbic acid was used as a standard.
26. Bhirud, R. G.; Srivastava, T. S. *Inorg. Chim. Acta* **1990**, *173*, 121.
27. Nishikimi, M. *Biochem. Biophys. Res. Commun.* **1975**, *63*, 463.
28. Cell culture: pancreatic cancer cell line BxPC-3 was maintained in RPMI culture medium (Invitrogen) while the breast cancer cell line BT-20 was maintained in DMEM medium (Invitrogen). Both the culture media contained penicillin (50 U/ml), streptomycin (50 μg/ml) and 10% fetal calf serum. All cells were cultured in a 5% CO_2-humidified atmosphere at 37 °C. The cell lines have been tested and authenticated in core facility Applied Genomics Technology Center at Wayne State University. The method used for testing was short tandem repeat profiling using the PowerPlex 16 System from Promega.
29. Cell growth inhibition studies by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay: cells (BxPC-3/BT-20—3×10^3) were seeded in a 96-well culture plate. Each treatment had eight replicate wells and, moreover, each experiment was repeated at least three times. Test compounds were dissolved in DMSO and added to cells 24 h after seeding. At the end of treatment, MTT (0.5 mg/ml) was added and plates incubated at 37 °C for 2 h followed by replacement of media with DMSO at room temperature for 30 min. Ultra Multifunctional Microplate Reader (TECAN) was used to record the absorbance.
30. Banerjee, S.; Azmi, A. S.; Padhye, S.; Singh, M. W.; Baruah, J. B.; Philip, P. A.; Sarkar, F. H.; Mohammad, R. M. *Pharm. Res.* **2010**, *27*, 1146.
31. Ali, S.; Ahmad, A.; Banerjee, S.; Padhye, S.; Dominiak, K.; Schaffert, J. M.; Wang, Z.; Philip, P. A.; Sarkar, F. H. *Cancer Res.* **2010**, *70*, 3606.
32. Hong, J.; Kwon, S. J.; Sang, S.; Ju, J.; Zhou, J. N.; Ho, C. T.; Huang, M. T.; Yang, C. S. *Free Radic. Biol. Med.* **2007**, *42*, 1211.