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## Novel derivative of aminobenzenesulfonamide (3c) induces apoptosis in colorectal cancer cells through ROS generation and inhibits cell migration (Article)

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### Abstract

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Background: Colorectal cancer (CRC) is the 3<sup>rd</sup> most common type of cancer worldwide. New anti-cancer agents are needed for treating late stage colorectal cancer as most of the deaths occur due to cancer metastasis. A recently developed compound, 3c has shown to have potent antitumor effect; however the mechanism underlying the antitumor effect remains unknown. Methods: 3c-induced inhibition of proliferation was measured in the absence and presence NAC using MTT in HT-29 and SW620 cells and xCELLigence RTCA DP instrument. 3c-induced apoptotic studies were performed using flow cytometry. 3c-induced redox alterations were measured by ROS production using fluorescence plate reader and flow cytometry and mitochondrial membrane potential by flow cytometry; NADPH and GSH levels were determined by colorimetric assays. Bcl2 family protein expression and cytochrome c release and PARP

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activation was done by western blotting. Caspase activation was measured by ELISA. Cell migration assay was done using the real time xCELLigence RTCA DP system in SW620 cells and wound healing assay in HT-29. Results: Many anticancer therapeutics exert their effects by inducing reactive oxygen species (ROS). In this study, we demonstrate that 3c-induced inhibition of cell proliferation is reversed by the antioxidant, N-acetylcysteine, suggesting that 3c acts via increased production of ROS in HT-29 cells. This was confirmed by the direct measurement of ROS in 3c-treated colorectal cancer cells. Additionally, treatment with 3c resulted in decreased NADPH and glutathione levels in HT-29 cells. Further, investigation of the apoptotic pathway showed increased release of cytochrome c resulting in the activation of caspase-9, which in turn activated caspase-3 and -6. 3c also (i) increased p53 and Bax expression, (ii) decreased Bcl2 and BclxL expression and (iii) induced PARP cleavage in human colorectal cancer cells. Confirming our observations, NAC significantly inhibited induction of apoptosis, ROS production, cytochrome c release and PARP cleavage. The results further demonstrate that 3c inhibits cell migration by modulating EMT markers and inhibiting TGFβ-induced phosphorylation of Smad2 and Smad3. Conclusions: Our findings thus demonstrate that 3c disrupts redox balance in colorectal cancer cells and support the notion that this agent may be effective for the treatment of colorectal cancer. © 2017 The Author(s).

## Author keywords

Apoptosis Cell migration Colorectal cancer NAC ROS

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