Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(4):391-398
(http://derpharmachemica.com/archive.html)

Synthesis of nitric oxide donating acridone derivatives as cytotoxic agents in cancer

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ABSTRACT

In search of new class of cytotoxic molecules we have designed and synthesized novel scaffold by linking nitric oxide donating group to acridone moiety. Synthesized derivatives were evaluated for their cytotoxic potentials in comparison with reference drugs doxorubicin (DX) against Human colon cancer cell line (HT-29) by using Sulphorhodamine-B (SRB) assay. Compound 15 exhibited good cytotoxic activity with an IC_{50} value 8.6 µM. Cytotoxic effects are presumed to be due to the presence of nitric oxide donating group, acetyl group on 2^{nd} position and butyl side chain at N^{10} position. Further, we tested whether the levels of NO production by the indicated compounds in human colon cancer cell line were associated with their cytotoxic effects. Good correlations were observed when cytotoxic data of these molecules compared with nitrite production.

Keywords: Acridone, SRB assay, Breast cancer, nitric oxide.

INTRODUCTION

Major health problem currently faced by mankind is cancer. Different drugs available in the market for the treatment of cancer diseases have serious side effects and hence cannot be used continuously for long time. Limited number of anti-cancer drugs available for the treatment of specific cancer is in the market. It can be concluded that there is an urgent need tosearch new class of anti-cancer agents [2–4].

Several planar molecules containing tricyclic structures with different side chains have been reported to possess significant cytotoxic and/or cytostatic potencies. These tricyclic systems include anthraquinone, acridine and xanthene. Several acridone alkaloids have been isolated from plants of the Rutaceae family, acronycine possesses significant antitumor activity [5, 6]. Glyfoline, another naturally occurring acridone alkaloid, was found to be the most effective compound for inhibition of cellular growth of human leukemia HL-60 cells *in vitro* [7]. Several acridone derivatives with or without an alkyl side chain connected to the N^{10}-position have been found to exhibitanti-cancer properties [8-11].Another imidazo acridone derivative has excellent anti-cancer and anti-MDR property against a number of human cancer cell lines [12, 13]. Studies have shown that the activity is due to DNA intercalation [14]. Further, ithas been found that the cytotoxicity activity of C1311 was related to itseffect on cell cycle progression in various types of solid tumor and leukaemias [13].

Nitric oxide (NO) is naturally generated from L-arginine by the action of NO synthase (NOS) and a key signaling molecule involved in regulating the several physiologic and pathologic processes [15]. High levels of NO and its metabolic derivatives, reactive nitrogen species (RNS) and reactive oxygen species (ROS) can modify functional proteins by S-nitrosylation, nitration, disulfide formation, leading to bio-regulation, inactivation and cytotoxicity, mainly in tumor cells [16]. Indeed, earlier studies have shown that synthesized NO-donating compounds have strong cytotoxic activity against human carcinoma cells *in vitro* and inhibit cancer growth and metastasis *in vivo*. [17-20].

As nitric oxide plays a significant role in various physiologic and pathologic processes, including in nonspecific anti-tumor immune response. New set of NO-releasing compounds need to be developed to get a tissue-specific NO-related response. This makes the opportunity that new molecules can be designed which can supplements nitric oxide exogenously when the body cannot produce enough amounts to permit normal biological functions. As a sequel in our research to discover new anti-cancer agents, we hypothesized that introduction of a NO releasing group into a N^{10}- alkylated acridone moiety would be capable to release NO in a controlled manner and might potentiate the cytotoxic property of acridones. In the present study new set of nitric oxide donating acridone derivatives were designed, synthesized and tested for their cytotoxic property in cancer cells.

MATERIALS AND METHODS

All chemicals and solvents were supplied by Sigma Aldrich, S.D.Fine Chemicals Ltd, Bangalore India. Reactions were monitored by TLC and compounds were purified by using column chromatography with silica gel Merck Grade 60 (230–400 mesh, 60; Merck, Germany). Melting points were recorded on a Tempirol hot-stage with microscope (AGA International, India) and were uncorrected. Nitric oxide donating acridones were characterized by ^{1}H- and ^{13}C-NMR in DMSO-d_6 solution in a 5-mm tube on a Brukerdrx 500 Fourier transform spectrometer (Bruker Bioscience, USA) and tetra methyl silane (TMS) was used as an internal standard. Chemical shifts were expressed as δ (ppm) values. The spectrometer was internally locked to deuterium frequency of the solvent. Acridones were also characterized by ESI-MS spectrometry. Collision-induced dissociation (CID) spectra were acquired in the positive ion mode on an MDS Sciex (Concord, Ont., Canada) API 4000 triple quadrupole mass spectrometry with direct infusion of each acridone at a concentration of 10 µM in 50 % methanol, at flow rate of 25 µL/ min. The instrument was operated with a spray voltage of 5.5 kV, a declustering potential of 50 eV, a source temperature of 100^0 C, a GSI value of 50 and the curtain gas set at 10. Ultra-pure nitrogen was used as both curtain gas and collision gas. MS/MS spectra of the protonated molecule of each drug were acquired and multiple reaction monitoring (MRM) transition for important fragments were monitored as the collision energy was ramped from 5-100 V (step size 0.5 V). The data for the fragment-ion curves represent an average of five consecutive experiments. The nitrate/nitrite release rates from individual derivatives in the cells were determined by a colorimetric assay using the nitrate/nitrite colorimetric assay kit (Sigma Aldrich, India), according to the manufacturer's instructions.

4.2. *In-vitro* cytotoxic studies by SRB assay:

The nitric oxide releasing acridone derivatives were evaluated for their cytotoxic activity against different cancer cells by using the Sulforhodamine B (SRB) assay [16]. In brief, cells were cultured in RPMI 1640 supplemented with 10 % fetal calf serum, and cultures were passed once or twice a week using trypsin EDTA to detach the cells from their culture flasks. The fast-growing cells were harvested, counted and plated at suitable concentrations in 96-well microplates. After incubation for 24 h, the compounds were dissolved in the culture medium then added to the culture wells in triplicate and incubated further for 72 hours at 37^0 C under 5 % CO_2 atmosphere. The cultures were fixed with cold TCA and stained with 0.4% SRB dissolved in 1% acetic acid. After dissolving the bound stain with 150 µl of 10 mM unbufferedTris base (Tris(hydroxymethyl)aminomethane) solution using gyratory shaker, absorbance was measured at 540 nm using a microplate reader (Tecan). Cytotoxicity was assessed by measuring the concentration required to inhibit protein synthesis by 50 % (i.e., IC_{50}) as a comparison. Each value represents the mean of triplicate experiments.

4.3. Nitrite measurement *in-vitro*:

The levels of nitrate/nitrite formed from individual compounds in the cells were determined by a colorimetric assay using the nitrate/nitrite colorimetric assay kit (Sigma Aldrich, India), according to the manufacturer's instructions. Confluent cell monolayers (6 x 10^5/well) in 35 mm diameter Petri dishes were treated in triplicate with 25 µM NO releasing acridone and incubated for 24 hours under the experimental conditions. Washed twice in ice-cold PBS and detached with trypsin/EDTA (0.05/0.02% v/v). Cells were centrifuged for 5 min at 15,000 rpm (4^0 C) and re-suspended in 1 mL of a 1:1 mixture of ethanol/0.3 N HCl. Then nitrite production was measured by mixing 100 µl

of cell lysates with 100 µl of Griess reagent in a 96-well plate, and after a (30-300) minute incubation at 37^0 C in the dark, absorbance was measured at 540 nm with a Tecan microplate reader, then50 µl of cell suspension used for measurement of cellular proteins. A blank was prepared for each experimental condition in the absence of cells, and its absorbance was subtracted from that obtained in the presence of cells. Nitrite concentration was expressed as nanomoles of nitrite per 24 h/mg of cellular protein.

4.5. Synthesis and chemical characterization:

4.5.1. Ullmann Condensation (I):

To a mixture of o- chloro benzoic acid (7.8 g, 0.05 moles), 4-chloro aniline (5.6 g, 0.05 moles) and copper powder (0.2 g) in 60 mL isoamylalcohol, dry potassium carbonate (5 g) was slowly added and the contents were allowed to reflux for 6-8 hours on an oil bath at 160^0 C. The isoamylalcohol was removed by steam distillation and the mixture was poured into one liter of hot water and acidified with concentrated hydrochloric acid. Precipitate formed was filtered, washed with hot water and collected. The crude acid was dissolved in aqueous sodium hydroxide solution, boiled in the presence of activated charcoal and filtered. On acidification of the filtrate with concentrated hydrochloric acid, light yellowish precipitate was obtained which was washed with hot water and recrystallized from aqueous methanol to give light yellow solid (yield 90 %, mp 190^0C).

4.5.2. Synthesis of acridone (II):

Five grams of 4'-chloro diphenylamine-2-carboxylic acid (I) was taken into a round bottom flask and 50 grams of polyphosphoric acid was added to it. The reaction mixture was shaken well and heated on a water bath at 100^0 C for 3 hours. Appearance of yellow colour indicated the completion of the reaction. Then, it was poured into one liter of hot water and made alkaline by liquor ammonia. The yellow precipitate that formed was filtered and collected. The compound2-chloro acridone (II) was recrystallized from acetic acid (yield 88%, mp 312-314^0C). Further, purity of the compound was checked by TLC and the purified product was characterized by spectral methods.

^{1}H NMR (DMSO-d_6) δ ppm:δ 7.32-8.27 (m, 7H, Ar-H); 11.21 (s,1H, NH).**^{13}C NMR (DMSO-d_6) δ ppm:** δ116.52 , 125.01, 117.53, 158.23, 122.03, 144.25, 126.72, 132.34, 178.85, 135.51, 135.02. **ESI-MS (m/z, %):** 230.0 (100), 232.1 (72), 196.2 (15), 195.2 (91), 167.2 (16).

4.5.4. *N*-alkylation of acridone (2):

One gram of substituted acridone was dissolved in 25 ml tetrahydrofuran and then 20 ml of 6 N potassium hydroxide and 0.5 g (0.015 mmol) of tetra butyl ammonium bromide were added. The reaction mixture was stirred at room temperature for 30 min and 1-bromo-3-chloropropane or 1-bromo-4-chloro butane (0.015 moles) added slowly into the reaction mixture and stirred for 24 hours at room temperature. Tetrahydrofuran was evaporated and the aqueous layer was extracted with chloroform. The chloroform layer was washed with water and the organic layer was dried over anhydrous sodium sulfate and rotaevaporated. The crude product was purified by column chromatography using the solvent system chloroform/acetone (8:1) to give a yellow solid of *N*-alkylated acridone.

2: ^{1}H NMR (DMSO-d_6) δ ppm:δ 7.28-8.21 (m, 7H , Ar-H); 3.61 (t, 2H, N-CH_2-CH_2-$\underline{CH_2}$-Cl); 3.52 (t, 2H, N-$\underline{CH_2}$-CH_2-CH_2-Cl); 2.13 (m, 2H, N-CH_2-$\underline{CH_2}$-CH_2-Cl).**^{13}C NMR (DMSO-d_6) δ ppm:** δ 115.27, 126.81 , 117.72, 153.75 , 123.14, 143.64, 126.57, 122.28, 174.50, 135.05, 132.93, 49.37, 42.56, 47.69. **ESI-MS (m/z, %):** 307.2 (55), 309.1 (32).

4.5.5. Nitration of *N*-alkylated acridone (5-18):

A solution of the appropriate chloroalkyl derivative in dry acetonitrile (2 ml) was treated portion wise with a solution of $AgNO_3$ (0.34 g, 2 mmol) in dry acetonitrile (5 ml) and the whole mixture was stirred at room temperature for 3 hours and the reaction was monitored by TLC. The mixture was then filtered, evaporated to dryness then residue was recrystallized from absolute ethanol.

5: ^{1}H NMR (DMSO-d_6) δ ppm:δ 7.21-8.28 (m, 8H , Ar-H); 3.98 (t, 2H, N-CH_2-CH_2-$\underline{CH_2}$-ONO_2); 3.28 (t, 2H, N-$\underline{CH_2}$-CH_2-CH_2- ONO_2); 2.13 (m, 2H, N-CH_2-$\underline{CH_2}$-CH_2- ONO_2).**^{13}C NMR (DMSO-d_6) δ ppm:** δ 115.21, 127.54 , 117.47, 152.56 , 123.15, 143.78, 125.52, 122.85, 173.11, 134.13, 130.17, 53.12, 44.50, 46.62. **ESI-MS (m/z, %):** 299.3 (35).

6: ^{1}H NMR (DMSO-d_6) δ ppm: 7.23-8.30 (m, 7H, Ar-H), 3.93 (t, 2H, N-CH_2-CH_2-$\underline{CH_2}$-ONO_2); 3.24 (t, 2H, N-$\underline{CH_2}$-CH_2-CH_2- ONO_2); 2.16 (m, 2H, N-CH_2-$\underline{CH_2}$-CH_2- ONO_2).**^{13}C NMR (DMSO-d_6) δ ppm:** 172.40,161.06, 157.26, 144.86, 143.02,137.11, 136.43, 135.53, 132.65, 128.14, 127.45, 126.35, 124.55, 59.67, 48.22, 45.15.
ESI-MS (m/z, %): 334.6 (70).

14: ^{1}H NMR (DMSO-d_6) δ ppm:7.20-8.31 (m, 7H, Ar-H), 3.89 (t, 2H, N-CH_2-CH_2-$\underline{CH_2}$-ONO_2); 3.33 (t, 2H, N-$\underline{CH_2}$-CH_2-CH_2- ONO_2); 2.11 (m, 2H, N-CH_2-$\underline{CH_2}$-CH_2- ONO_2), 3.71 (s, 3H, OCH_3) (**^{13}C NMR (DMSO-d_6) δ ppm:** 170.12,162.17, 157.28, 144.52, 143.12,135.45, 135.44, 134.45, 132.47, 128.12, 127.17, 126.47, 124.58, 59.12, 47.47, 46.15, 41.12 **ESI-MS (m/z, %):** 329.1 (66).

15: ^{1}H NMR (DMSO-d_6) δ ppm:7.22-8.34 (m, 7H, Ar-H), 3.87 (t, 2H, N-CH_2-CH_2-$\underline{CH_2}$-ONO_2); 3.28 (t, 2H, N-$\underline{CH_2}$-CH_2-CH_2- ONO_2); 2.08 (m, 2H, N-CH_2-$\underline{CH_2}$-CH_2- ONO_2), 3.61 (s, 3H, $COCH_3$) (**^{13}C NMR (DMSO-d_6) δ ppm:** 171.17,161.18, 158.88, 146.28, 144.10,135.19, 135.72, 134.49, 131.78, 129.11, 127.71, 126.49, 123.18, 58.91, 47.71, 46.45, 41.22 **ESI-MS (m/z, %):** 342.1 (80).

RESULTS AND DISCUSSION

2.1. Chemistry:

2.1.1 Synthesis of compounds (1-18)

Nitric oxide donating acridones (1-18) were synthesized according to Scheme 1. Parent acridone (II) was synthesized by Ullmann condensation reaction of 2-chlorobenzoic acid with substituted aniline to produce diphenylamine-2-carboxylic acid (I). Diphenylamine-2-carboxylic acid (I) was then cyclized with polyphosphoric acid instead of sulfuric acid in a water bath at 100^0 C for three hours to give acridone (II) with excellent yields (90%).

Further, *N*-alkylation of the various substituted acridones (III) was achieved by using a phase transfer catalyst (PTC) because the nitrogen atom of the acridone nucleus is weakly basic nature and usually resistant to undergo *N*-alkylation with alkyl halides.Stirring of substituted acridone with alkylating agents 1-bromo, 3-chloro propane or 1-bromo, 4-chloro butane at room temperature in a two-phase system containing an organic solvent tetrahydrofuran (THF) and 5 N aqueous potassium hydroxide (KOH) solution in the presence of catalyst tetra butyl ammonium bromide (TBAB) to produce good yields of *N*-alkyl substituted acridones. Here, the TBAB transports the OH^- ion from the aqueous phase to the organic phase where the actual reaction takes place. The phenolate stabilized anion formed, which successively undergoes alkylation to form the aromatized system. To introduce the nitric oxide (NO)donating group, *N*-alkylated acridones were treated with silver nitrate in dry acetonitrile for three hours. Physical characterization data of all synthesized compounds was shown in Table 1.

All the acridone derivatives were separated and purified by column chromatography and/or recrystallization and dried under vacuum for more than 10 hours. Purified derivatives were characterized by using ^{1}H-NMR, ^{13}C-NMR and Mass spectrometric studies. The ^{1}H-NMR spectrum of 2-chloro acridone (II) showed seven aromatic protons were resonated at δ 7.32-8.27 ppm as multiplet and proton of NH was resonated at 11.21. Further, ^{1}H-NMR spectrum of *N*-alkylated acridone (5) showed eight aromatic protons at 7.21-8.28 as multiplet. Protons of CH_2-ONO_2 resonated as triplet at δ 3.98 ppm and protons of N-CH_2- resonated as triplet at δ 3.28 ppm. Multiplet at 2.13 was assigned to two protons which are substituted at second carbon of propyl chain. Thus, a combination of chemical shift, spin-spin couplings and integration data permits the identification of individual hydrogen atoms at aromatic ring and alkyl chain. The assignment of protons in all the compounds is fully supported by the integration curves and all the derivatives displayed the characteristic chemical shifts for the acridone nucleus. The ^{13}C-NMR spectrum of NO donating acridone (5) shown14 signals representing 14 magnetically dissimilar environmental carbons.
Synthesized NO releasing acridones were also analyzed by mass spectrometry (MS) under ESI conditions. Molecular ions were observed in the form of M + H. The data indicates that as such there is no difference in the fragmentation pattern among the set of NO releasing acridone derivatives. In overall, mass spectral features of these acridones were similar and straight forward. Most of the compounds yield abundant molecular ions in the form of M + H. All the bonds in the N^{10}-side chain portion are prone to cleavage. This means, the data presented in this article also reveal the usefulness of MS for characterization of acridone derivatives. In conclusion,^{1}H, ^{13}C-NMR and mass spectral data were consistent with the proposed structures of acridones.

Scheme 1: Synthesis of nitric oxide donating acridone derivatives

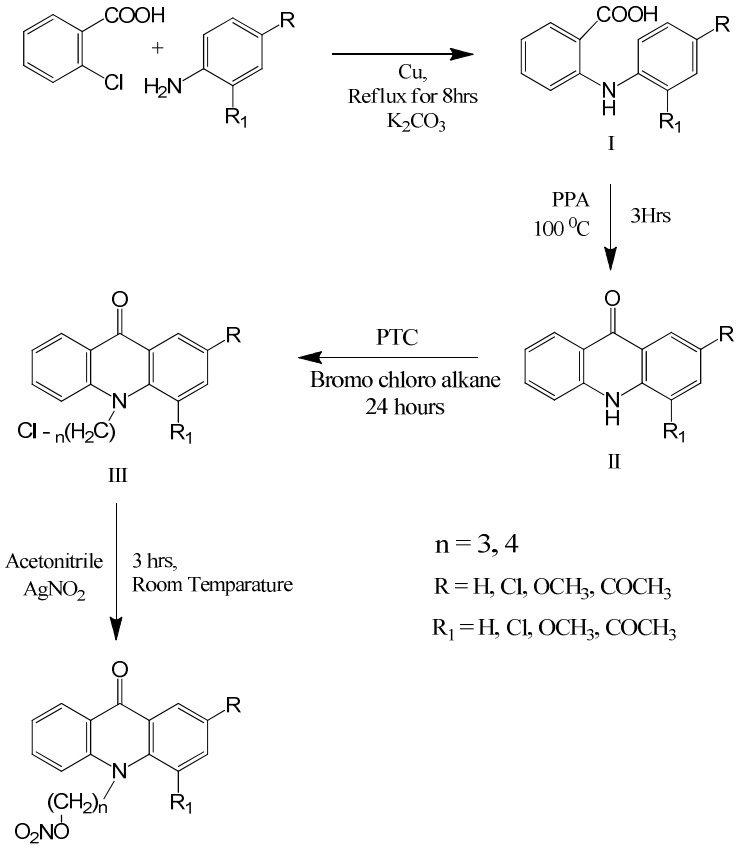
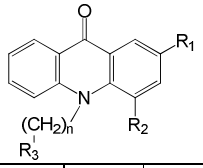


Table 1Physical characterization data of nitric oxide releasing acridone derivatives



Compound No	n	R_1	R_1	R_3	Molecular formula	Molecular weight
1	4	H	H	Cl	$C_{17}H_{16}NO$	285.32
2	4	H	Cl	Cl	$C_{17}H_{15}ClNO$	318.71
3	4	H	OCH_3	Cl	$C_{18}H_{18}NO_2$	315.12
4	4	H	$COCH_3$	Cl	$C_{19}H_{18}NO_2$	327.10
5	3	H	H	ONO_2	$C_{16}H_{14}N_2O_4$	298.29
6	3	H	Cl	ONO_2	$C_{16}H_{13}ClN_2O_4$	332.74
7	3	H	OCH_3	ONO_2	$C_{17}H_{16}N_2O_5$	328.32
8	3	H	$COCH_3$	ONO_2	$C_{18}H_{16}N_2O_5$	340.33
9	3	Cl	H	ONO_2	$C_{16}H_{13}ClN_2O_4$	332.74
10	3	OCH_3	H	ONO_2	$C_{17}H_{16}N_2O_5$	328.32
11	3	$COCH_3$	H	ONO_2	$C_{18}H_{16}N_2O_5$	340.33
12	4	H	H	ONO_2	$C_{17}H_{16}N_2O_4$	312.32
13	4	H	Cl	ONO_2	$C_{17}H_{15}ClN_2O_4$	346.76
14	4	H	OCH_3	ONO_2	$C_{18}H_{18}N_2O_5$	342.35
15	4	H	$COCH_3$	ONO_2	$C_{19}H_{18}N_2O_5$	354.36
16	4	Cl	H	ONO_2	$C_{17}H_{15}ClN_2O_4$	346.76
17	4	OCH_3	H	ONO_2	$C_{18}H_{18}N_2O_5$	342.35
18	4	$COCH_3$	H	ONO_2	$C_{19}H_{18}N_2O_5$	354.36

2.2. Biological:

2.2.1. *In-vitro* cytotoxic activity:

The *in-vitro* cytotoxic effects were studied in comparison with reference drugs doxorubicin (DX) against Human colon cancer cell line (HT-29)by using Sulphorhodamine-B (SRB) assay [21]. Cell lineswere incubated with eight different concentrations (0.5 – 100 µM) for each compound and was used to create compound concentration versus survival fraction curves. The response parameter (IC_{50}) was calculated for each cell line and tabulated in Table 2. The IC_{50} value corresponds to the acridones concentration causing a net 50 % loss of initial cells at the end of the incubation period 72 hours. Screening of selected molecules for anticancer property has been designed to understand the role of nitric oxide releasing moiety in cytotoxic effectiveness of acridones againstHT-29cell lines as well as to correlate the nitric oxide release rate with cytotoxic property.

Among the derivatives tested, compound 15 exhibited relatively good cytotoxic activity with an IC_{50} value 8.6 µM. The activity is presumed to be due to the presence of nitric oxide donating moiety, acetyl group at 2nd position and N^{10}substitutedbutyl chain. Cytotoxicity was slightly reduced in compound 8 with an IC_{50} value 12.5 µM when the butyl chain is replaced with propyl chain. Further, N^{10}- butyl substituted compounds 17 and 18 also shown relatively good cytotoxicity with an IC_{50} value9.5 and 9.1 respectively. Whereas N^{10}-substituted acridone derivatives compounds 1 to 4 without nitric oxide releasing group are fails to show cytotoxic property. The overall cytotoxic evaluation data reveals that nitric oxide releasing group in acridones is essential to enhance the cytotoxic activity.

Furthermore, we tested whether the levels of NO production by the indicated compounds in human colon cancer cell line were associated with their cytotoxic properties. The levels of nitrite/nitrate produced in the cell lysates of cancer cells were characterized by using the Griess assay. Treatment with lower cytotoxic compound 12 produced lesser levels of nitrate/nitrite in tested cells. However, treatment with any of the compounds (15 and 18) with a good cytotoxicity produced higher levels of nitrate/nitrite in these cells. It indicates, levels of nitrate/nitrite produced by individual derivatives were associated with their cytotoxic property against these cancer cells *in vitro*.

Table 2: Cytotoxic activity of acridone derivatives against Human colon cancer cell line

Compound	Cell lines/IC_{50} (µM)[1]
	HT-29[2]
1	>50
2	>50
3	>50
4	>50
5	30.6±2.8
6	27.5±2.1
7	28.5±2.0
8	12.5±1.5
9	25.9±2.1
10	19.5±1.5
11	13.5±1.2
12	21.4±1.9
13	14.6±1.8
14	11.2±1.2
15	8.6±1.1
16	16.8±1.1
17	9.5±1.2
18	9.1±1.0
Doxorubicin (Dx)	0.027 ±0.008

[1]*Inhibitory concentrations are presented in Mean ±SEM*
[2]*HT-29: Human colon cancer cell line*

2.2.2. Nitric oxide release:

Rate of nitric oxide release by nitric oxidereleasing acridone derivatives were determined by measuring nitrite production in HT-29 cell lines, to investigate whether control release of nitric oxide exogenously by NO-releasing acridone derivatives improve the cytotoxic property of acridones.Nitrite production was measured by adding 100 µl of cell lysates with to 100 µl of Griess reagent in a 96-well plate and after 30-300 minute of incubation at 37^0 C in the dark, absorbance was measured at 540 nm with Tecan microplate reader, then 50 µl of cell suspension used for

measurement of cellular proteins. A blank was prepared for each experimental condition in the absence of cells, and its absorbance was subtracted from that measured in the samples. Nitrite concentration was expressed as nanomoles of nitrite per 24 h/mg cellular protein. Compounds 12, 15 and 18 were studied for their nitric oxide release rate and the results were shown in Table 3. Results shown that nitrate levels in cell lines were between 4.48 - 5.12nM/mg proteins per 24 hours. Among the derivatives studied compound 15 is found to be better NO releasing molecule with release rate of 5.12 nM/mg in HT-29 cell lines per 24 hours. Further, good correlations were observed when cytotoxic data of these molecules compared with nitrite production.

Table 3: Exogenous release of nitrite by NO-acridones in HT-29 cancer cells

Compound	Nitrite concentration was expressed as nano moles of nitrite per 24 hours/mg cell protein
	HT-29
12	4.48
15	5.12
18	5.05

CONCLUSION

Current investigations were performed to understand the role nitric oxide releasing group on cytotoxicity properties of N^{10}-alkylated acridone derivatives in HT-29 cell lines and also to elucidate the correlation of the nitric oxide release rate with cytotoxic property. Results clearly indicate that NO donating acridones were shown good cytotoxic effect as well as significant rate of nitrite release. Further, increase in alkyl chain length by one carbon at N^{10}-position enhanced the cytotoxic property of NO-acridones. Moreover, good correlations were observed when cytotoxic data of these molecules compared with nitrite production. It is also clear that presence of NO group could potentiate the cytotoxic effect of the acridone derivatives.

Acknowledgements
Dr.V.V.S.Rajendra Prasad would like to acknowledge the funding support from SERB, Department of Science and Technology (DST), Government of India under “Fast Track Scheme” [SR/FT/LS-175/2009].

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