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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

SnO_2 nanoparticles mediated nontraditional synthesis of biologically active 9-chloro-6,13-dihydro-7-phenyl-5*H*-indolo [3,2-*c*]-acridine derivatives

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Abstract An efficient, practical, and eco-friendly method of preparation of 9-chloro-6,13-dihydro-7-phenyl-5*H*-indolo[3,2-*c*]-acridines (**3a–c**) is reported by Friedlander condensation of 2-amino-5-chlorobenzophenone **1** and active methylene compound 3,4-dihydro-2*H*-carbazol-1(9*H*)-ones (**2a–c**) in the presence of SnO_2 nanoparticles under microwave irradiation. Indoloacridines, **3a–c**, were screened for their in vitro hemolytic activity, on human erythrocytes and cytotoxicity, on HeLa and Vero cells.

Keywords Friedlander reaction · SnO_2 nanoparticles · Erythrocytes · HeLa · Vero cells

Introduction

Nitrogen heterocyclics are common in animal and plant cells (Morton, 1965) and play a prominent role in biological functions such as bioenergetic transport agents (Gupta *et al*., 1979). The pharmacological, biochemical properties, and therapeutic applications of *N*-heterocyclics depend on substituents in basic structure, i.e., alterations in the chemical structure of *N*-heterocyclic compounds could influence their biological properties such as anticancer property (Fig. 1). Among the various synthetic methods in quinoline synthesis, Friedlander condensation was extremely useful (Zolfigol *et al*., 2008). The solvent-free microwave-assisted quinoline syntheses are reported (Roopan *et al*., 2009; Chaudhuri and Hussain, 2006). In addition, quinolines are valuable synthons for the preparation of nano–meso structures and polymers that combine enhanced electronic, optoelectronic, or non-linear optical properties with different mechanical properties (Agarwal and Jenekhe, 1991; Zhang *et al*., 1999, 2000). A number of catalysts have been developed for the synthesis of quinolines such as $AuCl_3{\cdot}3H_2O$, $FeCl_3$, $ZnCl_2$, $SnCl_2$, and $ZnCl_2$/triethylamine has been used for Friedlander reaction (De and Gibbs, 2005; Hu *et al*., 2003).

Solvent-free microwave-assisted chemical reactions are gaining importance due to the advantages and environmentally friendly processes they offer, as compared to conventional reactions (Roopan *et al*., 2008). In recent years organic reactions in the solid state have been attracting the synthetic organic chemists because of their simplicity and synthetic value (Roopan *et al*., 2009). Furthermore, the solid state reaction has many advantages including, reduced pollution, low costs and simplicity in process and handling. The applications of SnO_2 nanoparticles in the organic transformations as explored in the present report. To the best of our knowledge, there is no report on the use of SnO_2 in Friedlander condensation reaction.

Recently, major health problems faced by mankind are cancer diseases. Limited numbers of anticancer drugs for the treatment of cancer are available in the market. It can be concluded that there is urgent need to search new anticancer agents (Da *et al*., 1994). Acridine derivatives are one of the more studied chemotherapeutic compounds, widely used as antimalarial, antiprotozoal (Gamage *et al*., 1997), antibacterial (Mauel *et al*., 1993), and antitumor agents (Sugaya *et al*., 1994). Many modifications have been made to the acridine skeleton in the search for anticancer compounds.

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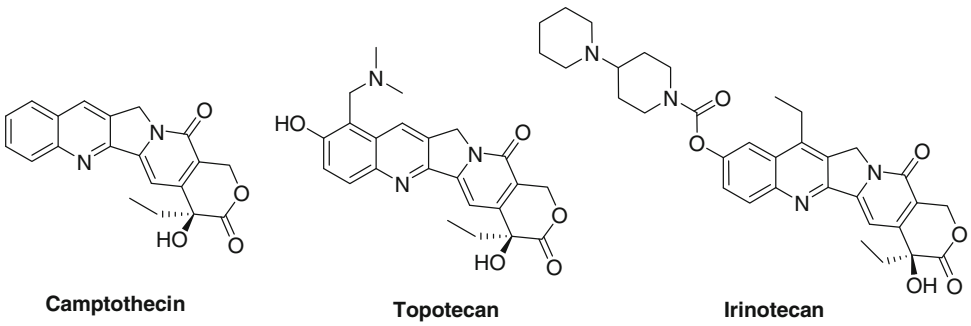
Fig. 1 Quinoline fused biologically important compounds



In continuation of our interest in synthesis of biologically active heterocyclics (Roopan *et al*., 2008; Manivel *et al*., 2008), a number of 9-chloro-6,13-dihydro-7-phenyl-5H-indolo [3,2-*c*] acridine analogues has been synthesized and examined for their bioactivities. In the present investigation, the hemolytic and cytotoxicity potential of 9-chloro-6,13-dihydro-7-phenyl-5*H*-indolo [3,2-*c*] alcidine analogues against human erythrocytes, HeLa and Vero cells were explored.

Materials and methods

Structural investigation

The 9-chloro-6,13-dihydro-7-phenyl-5*H*-indolo [3,2-*c*] alcidines were denoted by specific codes for identity (**3a**, **3b**, and **3c**). SnO_2 nanoparticles were prepared by the reported method (Jie *et al*., 2006). Synthesized tin oxide nanoparticles size of 25 ± 0.5 nm was confirmed by HRSEM image. TLC was performed using silica gel-TLC, mobile phase: ethyl acetate: Pet. ether (1:9). Domestic microwave SANYO EM-G3586W was used for the synthesis. Melting points were measured in open capillary tubes and are corrected with reference to benzoic acid. IR spectra in KBr pellets were recorded on Nucon Infrared spectrophotometer. Nuclear Magnetic Resonance (^{1}H) spectra were recorded on a 300 MHz spectrometer. Chemical shifts are reported in parts per million (δ) downfield from an internal tetramethylsilane reference. High Resolution Scanning Electron Microscope (HRSEM) spectra were recorded on FEI Quanta FEG 200.

General procedure for the synthesis of 9-chloro-6,13-dihydro-7-phenyl-5*H*-indolo [3,2-*c*] acridine (**3a**–**c**)

Under solid-phase MWI conditions

A mixture of 2-amino-5-chlorobenzophenone (1 mmol) (**1**), 3,4-dihydro-2*H*-carbazol-1(9*H*)-one (1 mmol) (**2**), tin oxide nanoparticles (5 mol%), and drop of Conc. H_2SO_4 was ground in a beaker until a homogeneous powder was formed. The reaction flask was held inside an alumina bath, microwave irradiated for 2–4 min at 500 W. The reproducibility of the whole experiment in the microwave condition was ensured by carrying out every reaction three times on reproducible positions marked in the microwave oven. The progress of the reaction was monitored by thin-layer chromatography (TLC). The reaction mixture was diluted with chloroform and then centrifuged to remove the nanoparticles. Evaporation of the organic solvent gives the titled compound (**3**) (Scheme 2).

Under solid-phase grinding conditions

The title compounds (**3**) were also synthesized using grind stone methodology. A mixture of 2-amino-5-chlorobenzophenone (1 mmol) (**1**), 3,4-dihydro-2*H*-carbazol-1(9*H*)-one (1 mmol) (**2a**–**c**), tin oxide nanoparticles (5 mol%), and drop of Conc. H_2SO_4 was ground by a mortar and pestle at room temperature for 15 min and was kept aside overnight. The completion of the reaction was monitored by TLC. The reaction mixture was work up as before.

Spectral data of synthesized compounds as follows

*9-Chloro-6,13-dihydro-1-methyl-7-phenyl-5H-indolo [3,2-c]acridine (**3a**)*

Yield, 82.5% as orange needles; m.p., 282°C; FTIR υmax (cm^{-1}, KBr): 3453 (NH); ^{1}H NMR (300 MHz; DMSO-d_6): 11.51 (s, 1H, –NH indole ring), 8.08–8.11 (d, J = 9.2 Hz, 1H, ArH), 7.60–7.54 (m, 4H, ArH), 7.37–7.45 (m, 3H), 7.16 (s, 1H), 6.94–7.01 (m, 2H), 2.92(s, 3H, –CH_3), 2.46–2.58 (m, 4H, 2*X*–CH_2); LCMS (m/z): calc. mass = 394, actual mass = 395.0 (M + 1), 396.0 (M + 2).

*9-Chloro-6,13-dihydro-2-methyl-7-phenyl-5H-indolo [3,2-c]acridine (**3b**)*

Yield, 81.0% as orange needles; m.p., 250°C; FTIR υmax (cm^{-1}, KBr): 3433 (NH); ^{1}H NMR (300 MHz; DMSO-d_6): 11.55 (s, 1H, –NH indole ring), 8.00–8.03 (d, J = 8.8 Hz, 1H, ArH), 7.54–7.70 (m, 4H, ArH), 7.39–7.44 (m, 2H, ArH), 7.27–7.31 (m, 1H), 7.14–7.28 (m, 1H), 6.72–7.07

(m, 2H), 3.17–3.21 (t, 2H, $-CH_2$), 2.90 (s, 3H, $-CH_3$), 2.40–2.57 (t, 2H, $-CH_2$); LCMS (m/z): calc. mass = 394, actual mass = 395.0 (M + 1).

*9-Chloro-6,13-dihydro-3-methyl-7-phenyl-5H-indolo[3,2-c]acridine (**3c**)*

Yield, 84.4% as orange needles; m.p., 231°C; FTIR υmax (cm^{-1}, KBr): 3421 (NH); ^1H NMR (300 MHz; DMSO-d_6): 11.58 (s, 1H, –NH indole ring), 8.00–8.03 (d, J = 8.9 Hz, 1H, ArH), 7.56–7.69 (m, 4H, ArH), 7.32–7.41 (m, 4H, ArH), 7.15 (s, 1H), 7.01–7.03 (d, J = 8.6 Hz, 1H), 2.91 (s, 3H, $-CH_3$), 1.89–2.49 (m, 2H, 2*X*–CH_2); LCMS (m/z): calc. mass = 394, actual mass = 395.0 (M + 1).

Biological activity

The 9-chloro-6,13-dihydro-7-phenyl-5*H*-indolo [3,2-*c*] acridines such as **3a**, **3b**, and **3c** (1 mg) were dissolved in 1 ml of DMSO solution. The appropriate concentrations of the compounds were made by serial dilution in culture medium.

In vitro hemolytic assay

Hemolytic effect of the compounds on human erythrocytes was evaluated by using washed erythrocytes (RBCs). For the preparation of mouse and human erythrocytes (Suthindhiran and Kannabiran, 2009; Roopan *et al*., 2009) Blood samples from the rats were collected from Charles foster strain (each weighing 130–180 g) in citrated tubes. The cells were then washed three times with 20 mM Tris–HCl containing 144 mM NaCl (pH 7.4) and 2% erythrocyte suspension was prepared. Human erythrocytes were obtained from the peripheral blood (O^+) of healthy volunteer. The blood was used within 24 h after bleeding and washed three times in 9 volumes of sterile 0.9% NaCl saline solution. After each washing, cells were centrifuged 150×*g* for 5 min and the supernatant was discarded. The final pellet was diluted 1:9 (v/v) in sterile 0.9% NaCl saline solution then 1:24 (v/v) in sterile Dulbecco's phosphate buffer saline (D-PBS), pH 7.0 containing 0.5 mM boric acid and 1 mM calcium chloride.

The hemolytic activities of the compounds were tested by the method of Malagoli under in vitro conditions in 96-well plates. Each well received 100 µl of 0.85% NaCl solution containing 10 mM $CaCl_2$. The first well served as negative control contained only water, and from the second well 100 µl of compound of various concentrations (5–500 µg/ml) were added. The last well served as positive control containing 20 µl of 0.1% Triton X-100 in 0.85% saline. Then, each well received 100 µl of a 2% suspension of mouse and human erythrocytes in 0.85% saline containing 10 mM $CaCl_2$. After 30 min incubation at room temperature, centrifuged, and the supernatant was used to measure the absorbance of the liberated hemoglobin at 540 nm. The average value was calculated from triplicate assay.

Cell culture

HeLa and Vero cell lines were obtained from ATCC and maintained in DMEM and RPMI 1640 (Himedia, Mumbai, India) medium supplemented with 10% FBS (v/v) and 100 mg/l streptomycin and 100 IU/ml penicillin (Himedia, India) at 37°C in a CO_2 incubator with 5% CO_2.

MTT cell proliferation assay

The cytotoxic activity of the compounds (diluted in DMSO 0 to 100 µg/ml) on HeLa and Vero cells (1×10^5 cells/well) were tested by using the CellQuanti-MTT cell viability assay kit (Bioassay Systems). The wells with only culture medium or cells treated with 0.1% of DMSO served as control. The graph was plotted with cell viability against the time period in hours at increasing concentrations of secondary metabolite. The mean and the IC_{50} value was calculated by non-linear regression analysis using the data analysis software (Prism) from three independent experiments.

Trypan blue dye exclusion assay

Cell viability assay was done by Trypan blue dye exclusion assay. The cells were incubated with or without compound. After 24 h of incubation cells were trypsinised, centrifuged for 5 min at 100×*g*, and the pellet was resuspended in 1 ml PBS. Trypan blue (0.4%) 10 µl was added with 10 µl of cell suspension and incubated for 3–5 min. Trypan blue/cell mixture (10 µl) was placed in a haemocytometer and a total of 100 cells were counted and the number of viable and non-viable cells was recorded. The assay was done in triplicates.

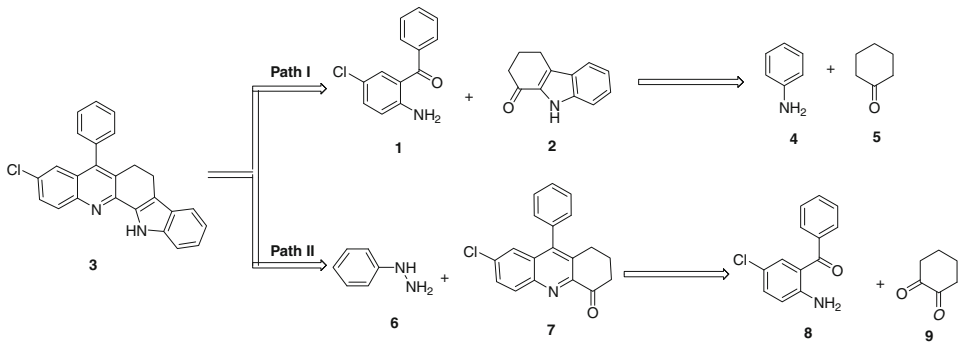
Results and discussion

Chemistry

Retro synthetic analysis

A retro synthetic analysis (Scheme 1) approach has been employed in designing a multistep synthesis of the target

Scheme 1 Retrosynthetic pathway to synthesis 9-chloro-6,13-dihydro-7-phenyl-5*H*-indolo [3,2-*c*] acridine (**3**)



molecule such as 9-chloro-6,13-dihydro-7-phenyl-5*H*-indolo [3,2-*c*] acridine (**3**). Two pathways can be utilized for the construction of target molecule. However, based on the availability and cost of the materials we proposed path I for the target compound (**3**). The quinoline ring 'AB' can be formed through Friedlander reaction of ring 'C' of the carbazole 'CDE' (**2**). Similarly the carbazole ring 'CDE' (**2**) can be obtained from aniline (**4**) and cyclohexanone (**5**) by reported method (Roopan *et al*., 2008) (Scheme 1).

*Synthesis of 9-chloro-6,13-dihydro-7-phenyl-5H-indolo [3,2-c] acridine compounds (**3a–c**)*

A green chemical method for the synthesis of biologically active indoloacridine is reported. In the present study, synthesis of 9-chloro-6,13-dihydro-7-phenyl-5*H*-indolo [3,2-*c*] acridine using tin oxide nanoparticles under mild condition has been reported and compared the results to the reported literature (Sridharan *et al*., 2009) Initially, efforts were focused on optimizing the microwave conditions for the formation of substituted 9-chloro-6,13-dihydro-7-phenyl-5*H*-indolo [3,2-*c*] acridine analogues (**3a–c**) by condensing 2-amino-5-chlorobenzophenone (**1**) and 3, 4-dihydro-2*H*-carbazol-1(9*H*)-one (**2a–c**) in the presence of SnO_2 nanoparticles as catalyst (Scheme 2). The title compounds (**3a–c**) were formed in good yield (>80%) in less time (5 min) (Table 1). The preparation of 9-chloro-6,13-dihydro-7-phenyl-5*H*-indolo [3,2-*c*] acridine analogues (**3a–c**) on solid support has not been reported so far. Then, the condensation of 2-amino-5-chlorobenzophenone (**1**) with 3,4-dihydro-2*H*-carbazol-1(9*H*)-one (**2a–c**) was also attempted under grinding conditions using SnO_2 nanoparticles as solid support (Table 1). To our delight, the product was formed in comparable yields (>80%). The desired product in significant purity was obtained by simple aqueous work-up.

These conditions were applied for the preparation of a series of substituted 9-chloro-6,13-dihydro-7-phenyl-5*H*-indolo[3,2-*c*]-acridine (**3a–c**). To optimize the reaction conditions, reaction was carried out in the presence and absence of nanoparticles, H_2SO_4 for the synthesis of titled compounds **3a** (Table 2). The results suggested that, mixture of nanoparticles and H_2SO_4 provided the products **3a** (Table 2, Entry 4), whereas in the absence of nanoparticles the reaction was not proceeded (Table 2, Entry 1, 3). Same reaction when carried in the presence of nanoparticles and absence of H_2SO_4 (Table 2, Entry 2) was not futile. Further an optimized amount of 5 mol% tin oxide nanoparticles were required for the reaction.

The diverse hemolytic and cytotoxic activity profile of the synthesized title compounds has also been obtained with a view to seek the structural requirements for potential drug candidates with a high therapeutic index.

In vitro hemolytic assay

As described earlier quinoline ring appears to be essential for both cytotoxic and hemolytic activities, suggesting that the mechanism of action is connected with the redox

Scheme 2 Synthesis of 9-chloro-6,13-dihydro-7-phenyl-5*H*-indolo [3,2-*c*] acridine compounds (**3a–c**)

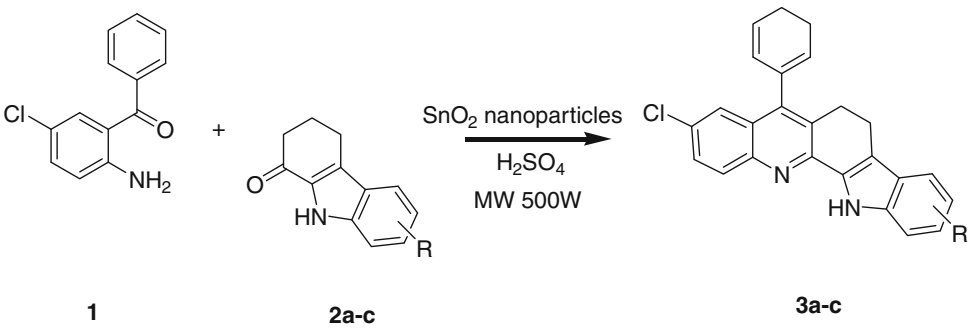
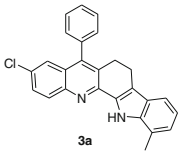
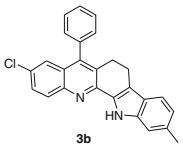
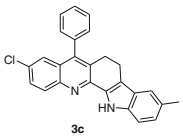


Table 1 Synthesis of 9-chloro-6,13-dihydro-7-phenyl-5*H*-indolo [3,2-*c*] acridine (**3a–c**) under solvent free, SnO_2 nanoparticles as catalyst

Sl.no.	Reactants	Reaction carried out in the presence of SnO_2 nanoparticles				
		Time taken (min)		Product, 3a–c	Yield[a] %	
		MW	Grinding		MW	Grinding
1	1, 2a	3 min	30 min	 3a	82.5	85
2	1, 2b	4 min	30 min	 3b	47	81
3	1, 2c	4 min	30 min	 3c	64	84.4

1 (1 mmol), **2** (1 mmol), tin oxide nanoparticles (5 mol%), drop of Con.H_2SO_4, at RT for the specified period of time

[a] Isolated yields

Table 2 In vitro hemolytic on human erythrocytes and cytotoxicity on HeLa and vero cells of compounds (**3a–c**)

Compounds	Hemolytic activity EC_{50} (μg/ml)	Cytotoxicity on HeLa IC_{50} (μg/ml)	Cytotoxicity on Vero IC_{50} (μg/ml)
3a	458.0	>100	>100
3b	408.6	>100	>100
3c	212.0	>100	>100
Camptothecin	429.9	0.28	58.0

properties of quinines. It is known that the most important reaction of quinones is their reversible reduction to the corresponding hydroquinones via semiquinone free radicals (Bentley and Campbell, 1974). These semiquinone radicals cause one electron reduction of oxygen (O_2) to superoxide radical anions, which interact with H_2O to form H_2O_2 and highly reactive hydroxyl radicals. Hydroxyl free radicals generated may cause damage to oxidative cells, particularly erythrocytes (or red blood cells) (Papas, 1999). Evidence has been presented that none of the synthetic quinoline has showed remarkable hemolytic and cytotoxic activity when compared to the standard. But it has seen that the compound **3c** has shown hemolytic activity to some extend but not much enough for the formation of cytotoxic oxidation products to accumulate and show cytotoxic activity. It can be suggested that substances able to suppress peroxidation chain reactions, neutralizing active forms of oxygen and thus protecting cell membranes from the damaging actions of free radicals (Muller *et al*., 1987).

The total hemolysis was obtained with 20 μl of Triton X-100 (0.1%) and 1 h incubation. The EC_{50}, IC_{50}, and 95% confidence interval (CI 95%) was obtained by non-linear regression analyses. EC_{50} value lower than 250 μg/ml was considered as active for hemolytic activity. As shown in Table 2, compound (**3c**) showed good hemolytic activity while other compounds (**3a** and **3b**) displayed moderate hemolytic activities.

Cytotoxic activity

IC_{50} value lower than 50 μg/ml was considered as active for cytotoxicity on HeLa and Vero cells. As shown in Table 2, compounds (**3a**, **3b**, and **3c**) have inactive on both cells.

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References

Agarwal AK, Jenekhe SA (1991) New conjugated polyanthrazolines containing thiophene moieties in the main chain. Macromolecules 24:6806–6808

Bentley R, Campbell IM (1974) Biological reactions of quinones. In: Patai S (ed) The chemistry of the quinonoid compounds. John Wiley & Sons, London, pp 683–736, Part 2

Chaudhuri MK, Hussain S (2006) An efficient synthesis of quinolines under solvent-free conditions. J Chem Sci 118(2):199–202

Da SA, Da SF, Marini AM, Primofiore G, Salerno S, Viola G, Dalla VL, Gamage SA, Tepsiri N, Wilairat P, Wojcik SJ, Figgit DP, Ralph R, Denny WA (1994) Synthesis and in vitro evaluation of 9-anilino-3-,6-diaminoacridines active against a multidrug resistant strain of the malaria parasite *Plasmodium falciparum*. J Med Chem 37:1486–1494

De SK, Gibbs RA (2005) A mild and efficient one-step synthesis of quinolines. Tetrahedron Lett 46:1647–1649

Gamage SA, Figgit DP, Wojcik SJ, Ralph R, Ransijn A, Mauel J (1997) Structure activity relationships for the antileishmanial and antitrypanosomal activities of 1-substituted-9-anilinoacridines. J Med Chem 40:2634–2642

Gupta RR, Jain SK, Ojha KG (1979) A simple and convenient synthesis of [1,4] benzoxazino [2,3-b] phenoxazines. Indian J Chem 17B:278–280

Hu YZ, Zang G, Thummel RP (2003) Friedländer approach for the incorporation of 6-bromoquinoline into novel chelating ligands. Org Lett 5:2251–2253

Jie Z, Li-Hua H, Shan G, Hui Z, Jing-Gui Z, Jie Z, Li-Hua H, Shan G, Hui Z, Jing-Gui Z (2006) Alcohols and acetone sensing properties of SnO_2 thin films deposited by dip-coating. Sens Actuators B 115:460–464

Manivel P, Roopan SM, Khan FN (2008) Synthesis of O-substituted benzophenones by grignard reaction of 3-substituted isocoumarins. J Chil Chem Soc 53(3):1609–1610

Mauel J, Denny WA, Gamage S, Ransijn A, Wojcik S, Figgit D, Ralph R (1993) 9-Anilinoacridines as potential antileishmanial agents. Antimicrob Agents Chemother 37(5):991–996

Morton RA (1965) Introductory account of quinones. In: Biochemistry of quinones. Academic Press, New York, pp 183–206

Muller WEG, Sladic D, Zahn RK, Bassler KH, Dogovic N, Gerner H, Gacic MJ, Schrfder HC (1987) Avarol-induced DNA strand breakage in vitro and in friend erythroleukemia. Cancer Res 47:6565–6571

Oxford Diffraction (2009) CrysAlisPro CCD and CrysAlis RED. Versions 1.171.33.34d. Oxford Diffraction Ltd., Abingdon, Oxfordshire, England

Papas AM (1999) In: Papas AM (ed) Antioxidant status, diet, nutrition, and health. CRC Press, Boca Raton, pp 21–36

Roopan SM, Khan FN (2008) Free radical scavenging activity of nitrogen heterocyclics-Quinazolinones and tetrahydrocarbazolones. Indian J Heterocycl chem 18:183–184

Roopan SM, Khan FN (2009) Synthesis, antioxidant, hemolytic and cytotoxic activity of AB ring cores of mappicine. ARKIVOC xiii:161–169

Roopan SM, Maiyalagan T, Khan FN (2008) Solvent free syntheses of some quinazolin-4(3H)-ones derivatives. Can J Chem 86:1019–1025

Roopan SM, Sakthi Sri TR, Ramakrishna Reddy B, Khan FN (2009) Synthesis and antibacterial activity of some 2- and 3-substituted isoquinolinones. Indian J Heterocycl chem 19:77–78

Sridharan M, Prasad KJR, Ngendahimana A, Zeller M (2009) Structural investigations of novel indoloacridines. J Chem Crystallogr 39:270–278

Sugaya T, Mimura Y, Shida Y, Osawa Y, Matsukuma I, Ikeda S, Akinaga S, Morimoto M, Ashizawa T (1994) 6H-pyrazolo[4, 5, 1-de]acridin-6-ones as a novel class of antitumour agents, synthesis and biological activity. J Med Chem 37(7):1028–1032

Suthindhiran K, Kannabiran K (2009) Hemolytic activity of Streptomyces VITSDKI spp. isolated from marine sediments in Southern India. J de Mycologie Médicale 19(12):77–86

Zhang X, Shetty AS, Jenekhe SA (1999) Electroluminescence and photophysical properties of polyquinolines. Macromolecules 32:7422–7429

Zhang X, Shetty AS, Jenekhe SA (2000) Electroluminescence of multicomponent conjugated polymers. 1. Roles of polymer/polymer interfaces in emission enhancement and voltage-tunable multicolor emission in semiconducting polymer/polymer heterojunctions. Macromolecules 33:2069–2082

Zolfigol MA, Salehi P, Shiria M, Faal Rastegara T, Ghaderia A (2008) Silica sulfuric acid as an efficient catalyst for the Friedlander quinoline synthesis from simple ketones and *ortho*-aminoaryl ketones under microwave irradiation. J Iran Chem Soc 5:490–497