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Design, synthesis and evaluations of acridone derivatives using *Candida albicans*—Search for MDR modulators led to the identification of an anti-candidiasis agent

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ABSTRACT

In order to search for MDR modulators, rationally designed acridone derivatives were investigated for their effect on influx or efflux of Rhodamine6G (R6G) in CAI4 cells. Results of these investigations indicate that in presence of compound **12**, inhibition of growth of CAI4 cells and also an increased influx/efflux of R6G in CAI4 cells have been observed. This seems to be occurring due to the cell wall rupturing of *Candida albicans*. Compound **12** may be a suitable candidate for candidiasis therapy.

1. Introduction

Multi drug resistance (MDR),[1–3] emanating due to the decrease in the intracellular drug concentration, is a great hurdle in the successful practice of chemotherapy of various diseases like cancer, AIDS and even malaria. The role of *p*-glycoprotein (*p*-gp),[4,5] a plasma membrane protein and a member of ABC family of proteins, is well established in causing MDR due to the effluxing of the drug molecules out of the cell. Similar to the role of *p*-gp, cdrp1 and cdrp2[6] are responsible for drug efflux in *Candida albicans* and hence provide a hurdle in the anti-candidasis therapy. Accordingly, a number of chemical entities have been investigated for their *p*-gp[7–13] and cdrp1, cdrp2[14] modulating activities.

Since *p*-gp/cdrp1, cdrp2 interact with drugs (while effluxing) as well as MDR modulators, combination of structural features of the drug and modulator in a single molecule may enhance the interactions of new molecule with these transporting proteins and hence modulate their drug effluxing behaviour, better than the parent modulator. Therefore, in order to find a potential candidate for modulating the drug effluxing activities of transporting proteins, in the present investigations, we have designed acridones (part of many anti-tumour,[15–17] anti-protozoan,[18–20] anti-viral[21] drugs and MDR modulators[22,23]) substituted with a hydroxyl amine (pharmacophore of MDR modulators[24]) and investigated for their drug influx/efflux behaviour over *C. albicans*.

2. Results

2.1. Chemistry

The synthesis of the target molecules have been achieved from the commercially available, cheaper materials. The Ullmann type arylation[25–27] of aniline with *o*-chlorobenzoic acid provided the acridone skeleton of the molecule after the treatment of **1a** and **1b** with acid. Treatment of acridone **1** with NaH in DMSO followed by stirring with epichlorohydrin gave N-substituted acridone **2**. NMR and mass spectral data confirm the formation of this compound. Irradiating an equimolar mixture of acridone **2** and piperidine in microwave oven for 5 min results in the formation of compound **4**. Likewise the reactions of acridone **2** with other amines provided compounds **3**, **5** and **6** in 5–7 min (Scheme 1). A similar sequence of reactions of *o*-chlorobenzoic acid with anthranilic acid provided compounds **9**–**12** (Scheme 2). Therefore, epoxy ring opening with secondary amines under microwave irradiations[28,29] constitutes a versatile approach to the synthesis of target compounds.

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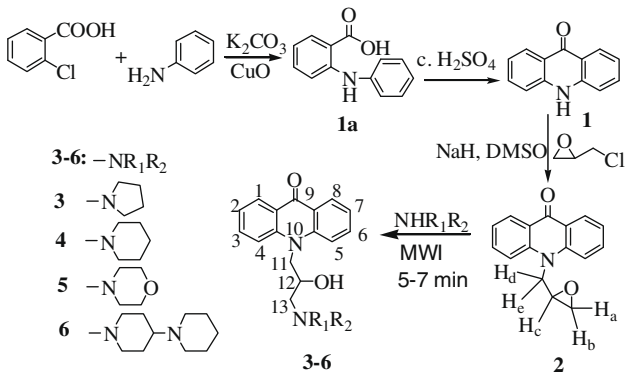


Scheme 1. Synthesis of acridone derivatives.

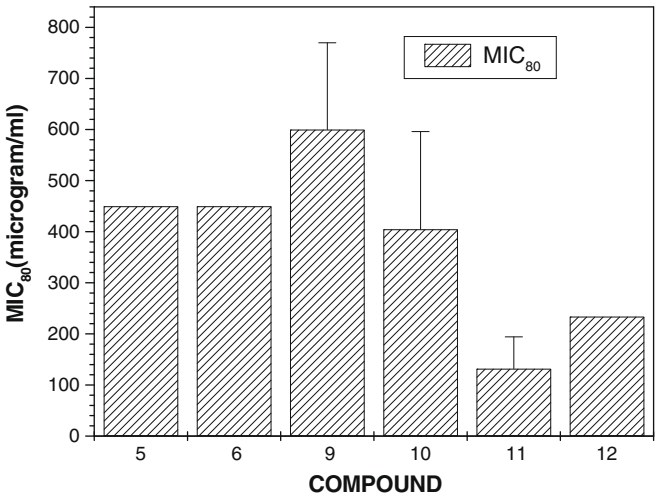


Figure 1. MIC_{80} for compounds **5**, **6**, **9**, **10**, **11** and **12**. (Note: For compounds **5**, **6** and **12** the duplicate values are identical and hence no error bar is visible.)

2.2. Biology

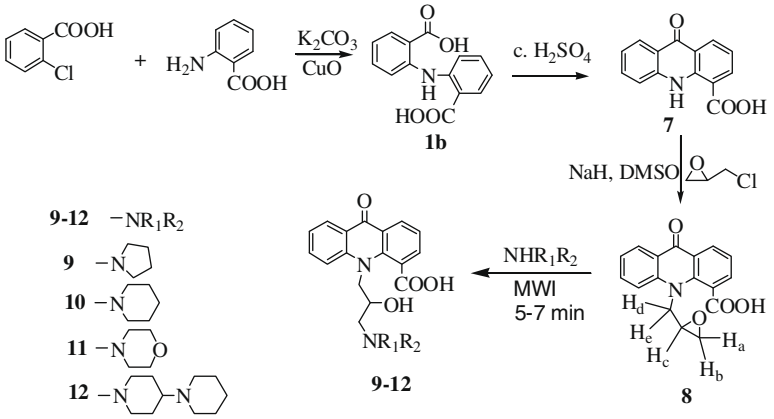
Biological investigations of these molecules were performed on the strain CAI4,[30] which is the derivative of wild type *C. albicans* SC5314. Strain CAI4 is similar to wild type except for one mutation at URA3 locus, that is, it is not able to grow on uridine deficient medium.

2.2.1. MIC_{80} assays

To test the sensitivity of the test compounds to *C. albicans*, MIC_{80} (Minimum Inhibitory Concentration-80) assays, that is, the concentration of the toxin at which 80% of the cells are killed, were performed. For this purpose, 96 well (8 row × 12 column) flat bottom transparent cell culture plates were used. Serial dilution of the drugs was done in a 96-well ELISA plate. *Candida* cells which were already grown in rich medium (YEPD) for 24 h, at 30 °C were resuspended in a 0.9% normal saline solution to give an optical density of 0.1 at 600 nm (OD_{600}). The cells were then diluted 100 times in YEPD media. 100 μl of diluted cells was added to each well except A11, which was our media control to monitor contamination during the study. A12 did not contain any of the test compounds and was our control for comparison of growth. The plate was incubated at 30 °C for 48 h and OD_{600} was monitored using a microplate reader. Based on OD_{600}, we obtained the concentration of the compound which gave only 20% growth as compared to the control in the last well. This concentration of the test compound corresponds to the MIC_{80} value (Fig. 1). The MIC_{80} assays were performed twice for each compound and then the MIC_{80} value was estimated. Compounds **10**, **11** and **12** for which the MIC_{80} was estimated to be 405 μg/ml, 132 μg/ml, and 234 μg/ml, respectively, were selected for spot assays. These compounds (**3–12**) also exhibit appreciable interactions with *p*-gp (evaluated using 'Drug-*p*-glycoprotein interaction' assay kit;[31] compound **12** exhibits 33% increase in the basal activity of *p*-gp at 0.05 μM concentration). In comparison to the reported acridone derivatives[22,23] compound **12** carries piperidino-piperidine moiety as a part of hydroxyl amine substituent present at *N*-1 and a COOH group on the benzene ring of acridone. Compounds **3** and **4** exhibit MIC_{80} values in the range of mg/ml (not shown in Fig. 1).

2.2.2. Spot assays

Spot assays were performed in small Petriplates, requiring 7.5 ml of YEPD-Agar medium per plate. Concentrations of compounds **10**, **11** and **12** were taken in the plates in the range of their MIC. The solvent control plates were made corresponding to each compound by taking the same volume of solvent, as the volume of compound used in the corresponding plates. One control plate was also made with YEPD alone. The plates were incubated overnight at 30 °C to check for any contamination. The primary culture was grown by taking CAI4 streak in 10 ml YEPD medium and growing for 24 h at 30 °C. Secondary culture was grown in 10 ml of YEPD with 4% inoculums from primary culture, and grown at 30 °C for around 5 h. The cells were counted in 200-fold diluted secondary culture using haemocytometer. Spots (labelled 1, 2, 3, 4, 5, 6) of 5 μl cell suspension were spotted on the plates corresponding to 10^5, 10^4, 10^3, 10^2, 10^1, 10^0 cells. The plates were



Scheme 2. Synthesis of acridone derivatives.

incubated at 30 °C for around 48 h and then checked for growth. No significant difference in growth was observed in the plates for compounds **10** and **11** (Fig. 2A and B) with their corresponding solvent control plates despite the fact that they had shown inhibition of *Candida* growth in liquid medium (as seen from MIC_{80}). Such differences between solid and liquid medium growth of *Candida* cells have been previously reported as well.[32] However, there was no growth in plate of compound **12** (Fig. 2C) but visible growth in its solvent control plate, suggesting that compound **12** is inhibiting *Candida* growth in solid phase as well.

Since compound **12** had an MIC_{80} value of 234 μg/ml on CAI4 strain of *C. albicans*, we used ~300 μg/ml of this compound in YEPD plates and found that the compound greatly inhibited growth of CAI4 in our spot assays (Fig. 2C). As it was clearly seen in Figure 2C, the inhibition in growth was a result of the compound and not due to the solvent background.

2.2.3. Disc diffusion assay

Disc diffusion assay was performed to confirm the effect of compound **12** on *Candida* growth inhibition in solid medium. CAI4 cells were grown overnight in YEPD medium at 30 °C. 10^7 cells were spread on a 90 mm diameter YEPD-agar plate and the plate was left to dry for about 10 min. Sterile filter paper discs of 6 mm diameter were placed on this plate with the help of sterile forceps. Compound **12** (200 μg) dissolved in 20 μl of methanol was spotted on one of the paper discs. As a solvent control 20 μl of methanol alone was spotted on one paper disc. Two known antifungal agents, fluconazole and miconazole, were also spotted on similar size paper discs at a known inhibitory amount of 6 μg per disc. The plate was incubated at 30 °C and the inhibition zone diameters were measured after 24 h. As seen in Figure 3, compound **12** clearly inhibits the *Candida* growth, though at a concentration higher than the other antifungals. A derivative of this compound could probably have improved antifungal activity.

2.2.4. Rhodamine 6G (R6G) influx/efflux assay (screening for MDR modulation)

Since compound **12** was the best inhibitor of growth of CAI4 cells in both solid and liquid medium, we carried out influx/efflux assays for this compound to understand what might be its effect on

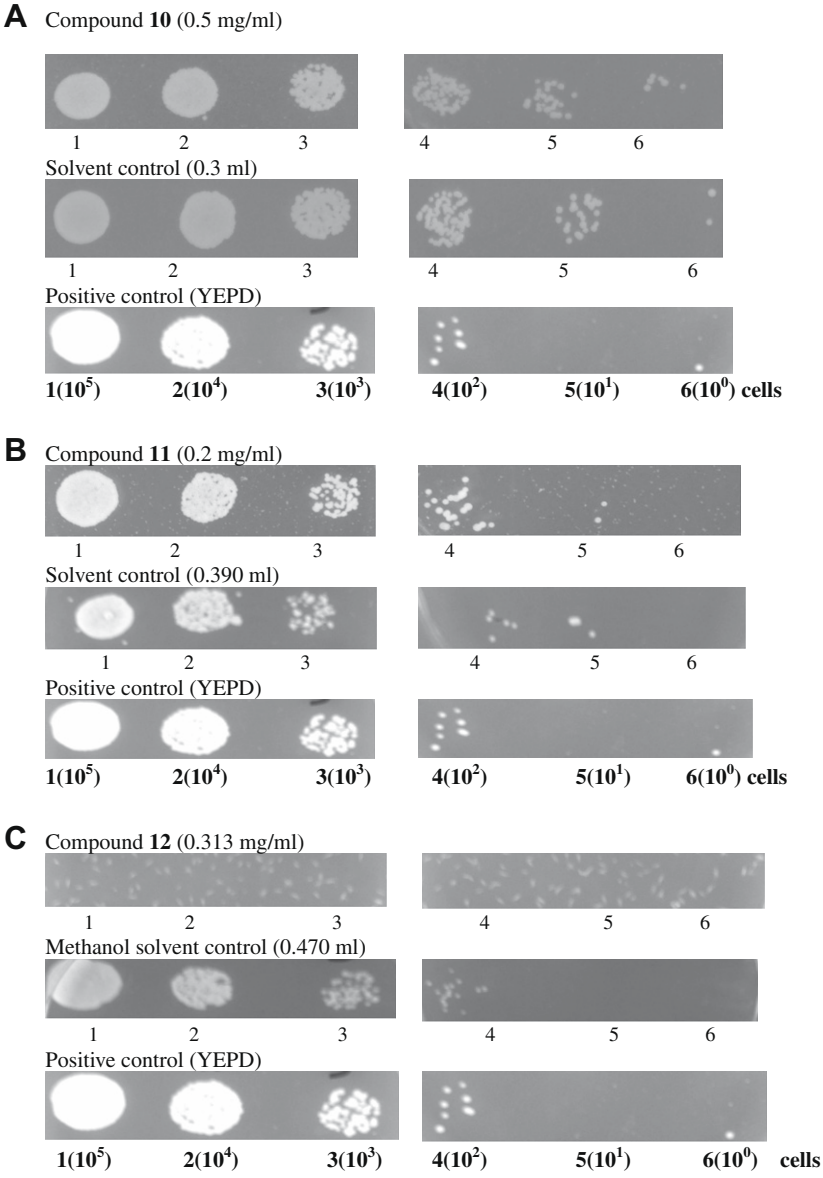
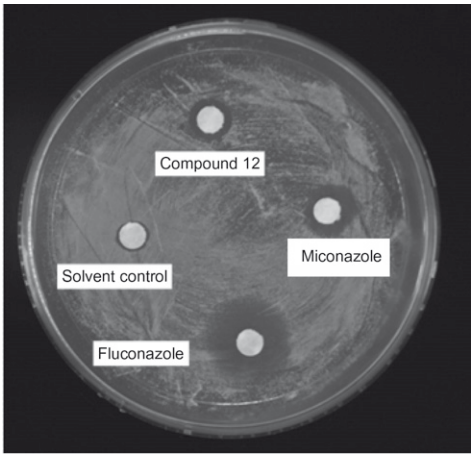


Figure 2. Plate assays on compounds: (A) **10**, (B) **11**, (C) **12** compared with solvent and medium controls. (Note: The white specks observed in plate for compound **12** are due to precipitation of the drug upon long incubation and not fungal cell colonies.)



	Compound 12 (200 μg)	Solvent control (20 μl)	Miconazole (6 μg)	Fluconazole (6 μg)
Inhibition zone diameter (mm)	10	7	12	15

Figure 3. Disc diffusion assay.

the drug influx/efflux ability of the cells. CAI4 primary culture (10 ml) was grown in YEPD medium at 30 °C for 24 h. Secondary culture was grown in 600 ml YEPD with 2% inoculum from primary culture, and grown at 30 °C for around 5 h. The cells were then pelleted at 4000 rpm and washed twice with sterile water. The cell pellet was divided into four sets, 2% cell suspension of each was made in PBS with 2 mM 2-deoxyglucose and then kept on a rocker for 2 h. After 2 h 230 μg/ml of compound **12** was added to two of the sets while 10 μM/ml of R6G was added to each set. Aliquots (700 μl) were taken from each set at this time point (considered as 0 min), and then kept back at rocker. Aliquots were taken out at different time points, spun at 2000 rpm for 2 min and the supernatant was transferred into a fresh microcentrifuge tube. The amount of R6G remaining in the medium was monitored by measuring $Abs_{527\ nm}$. The data thus obtained corresponds to influx of R6G.[33] A higher $Abs_{527\ nm}$ value corresponds to lesser amount of the drug having entered the cell.

After 60 min incubation with R6G, the cells were again pelleted at 2000 rpm and washed twice with water. The cell pellets were further divided into two sets each. To each paired set of cells, after making 2% cell suspensions made in PBS, 2% glucose was added to one while PBS alone was added to the other. The cell suspensions were then kept back at rocker and aliquots taken out at different time points. The aliquots were spun at 2000 rpm for 2 min and the supernatant was used for monitoring the amount of drug effluxed out from the cells in the absence and presence of glucose.[33] The fluorescence intensity was monitored on a Cary-Varian spectrofluorimeter in these cases using excitation wavelength of 529 nm and emission wavelength of 553 nm (5 nm excitation and emission slit widths). Higher fluorescence intensity corresponded to higher efflux of R6G from the cells.

Influx of R6G in cells takes place by a passive diffusion, while efflux is either passive or ATP dependent (mediated by MDR pumps). As can be seen from Figure 4, compound **12** had a significant effect on *Candida* cells, increasing the influx of R6G into the cells in its presence. Likewise, the efflux of R6G in the absence of glucose (passive or ATP independent efflux) too appears to be higher. The relative ratio of glucose stimulated or ATP driven efflux of R6G to the efflux in the absence of glucose is the same in the control versus compound **12** treated cells. Hence, it seems that the higher influx and efflux of R6G in the compound **12** treated cells is because of the effect of compound **12** on the *Candida* cell wall, which leads to more influx and more efflux passively, and the higher efflux is not because of the function of the MDR pumps. It would therefore appear that compound **12** does not directly affect the multi drug pumps in *C. albicans*, although it appears to alter the cell wall integrity.

In order to verify this, we studied the effect of compound **12** in combination with cell wall toxins CFW and CR that are known to

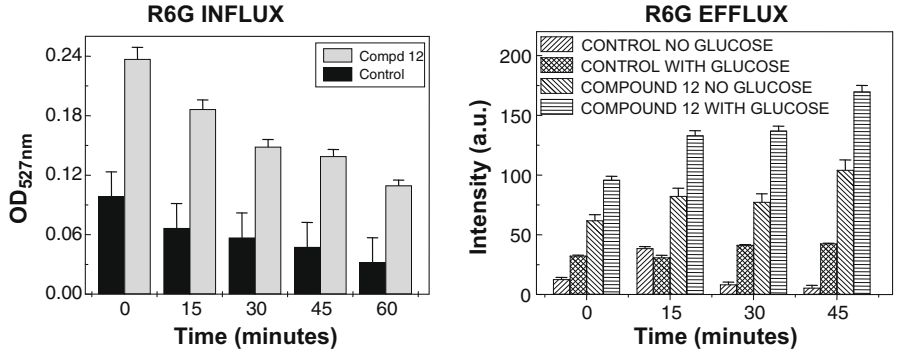


Figure 4. R6G influx/efflux in CAI4 cells in presence of compound **12**.

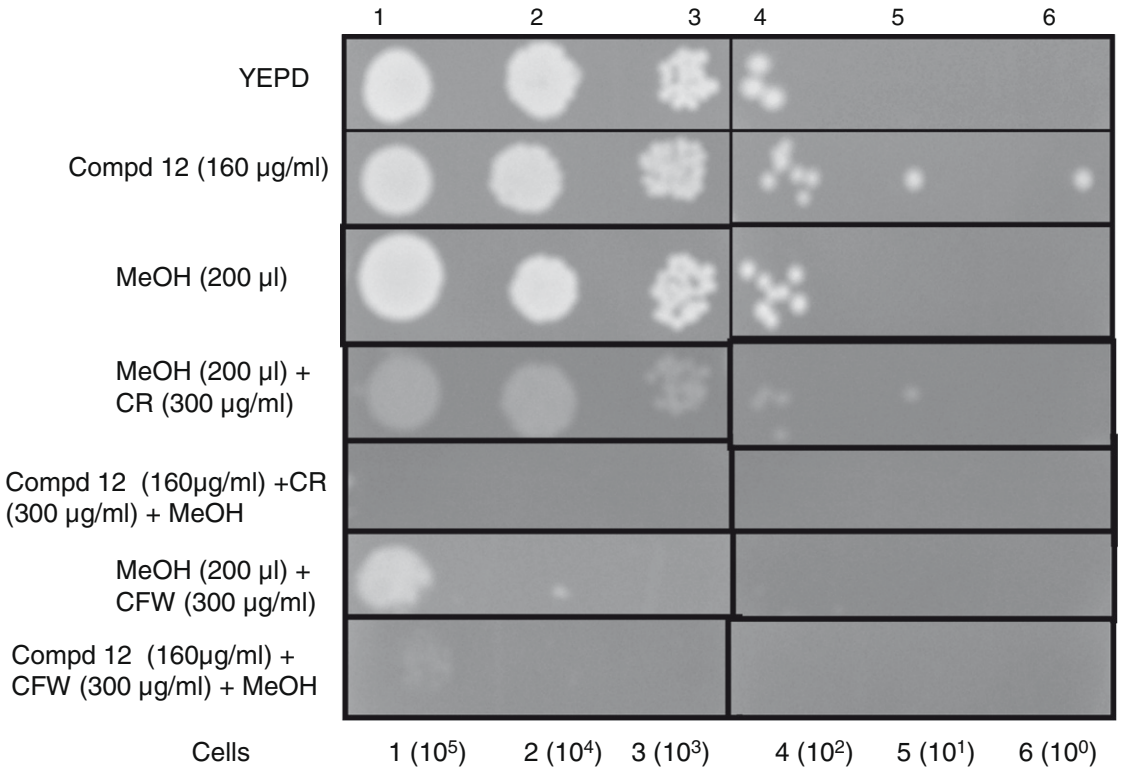


Figure 5. Effect of compound **12** on CAI4 cells in combination with CFW and CR.

inhibit chitin polymerization and thereby affect the cell wall.[34] For this purpose, a concentration of compound **12** (160 μg/ml) which was not toxic to the cells was chosen (Fig. 5). As can be seen from Figure 5 the presence of compound **12** alters the toxicity of CFW as well as CR on CAI4. A combination of compound **12** and CFW/CR is far more effective in inhibiting the *Candida* growth than any of these acting alone, suggesting a possible synergistic mechanism, although further detailed analysis will need to be done in order to understand the mechanism of this action.

Thus it appears that compound **12** affects *Candida* cell wall and is therefore toxic for this pathogenic fungus. It may be pointed out that one of the major features of several anti-fungals currently being used against candidiasis involves targeting and weakening of the *Candida* cell wall.[35] Since the cell wall is a distinct element in fungal cells that does not exist in mammalian cells, such selective targeting is a particularly important strategy for therapy against *C. albicans*.

3. Conclusions

In conclusion, during our attempt to find out suitable MDR modulators from rationally designed acridone derivatives, we found that compound **12** weakens the cell wall of *C. albicans*. Further refinement of this molecule may develop it into an effective agent for candidiasis therapy.

4. Experimental

4.1. General note

Melting points were determined in capillaries and are uncorrected. ^{1}H and ^{13}C NMR spectra were run on JEOL JNM AL 300 MHz and 75 MHz NMR spectrometer, respectively, using $CDCl_3$ as solvent. Chemical shifts are given in ppm with TMS as an internal reference. *J* values are given in hertz. (+) Signs in DEPT spectra correspond to signals due to CH_3, CH groups while (−) signs denote signals of CH_2 groups. Chromatography was performed with silica 100–200 mesh and reactions were monitored by thin layer chromatography (TLC) with silica plates coated with silica gel HF-254.

4.2. General procedure for the syntheses of 1 and 7 (procedure A)

A mixture of 2-chlorobenzoic acid (1.56 g, 10 mmol), aniline/anthranillic acid (10 mmol), powdered CuO (25 mg) and K_2CO_3 (1.5 g, ~11 mmol) in isoamyl alcohol (10 ml) was heated at 160 °C for 10 h. After cooling the alcohol was evaporated under vacuum and the residue was dissolved in hot water (120 ml) and acidified with concd HCl. The precipitates were washed with hot water, dissolved in ethyl acetate and the solution was dried over anhydrous Na_2SO_4 and then evaporated. The crude product was purified by column chromatography over silica gel. This product was taken in concd H_2SO_4 (just to dissolve the compound) and heated on water bath for 1.5 h. Reaction mixture was added to hot water and the resulting precipitates were filtered to get acridone **1**/**7**.

4.3. General procedure for the syntheses of compounds 2 and 8 (procedure B)

Sodium hydride (3.0 mmol) was washed with dry hexane and taken in 15 ml of dimethyl sulfoxide. To this solution, acridone **1**/**7** (2.5 mmol) and epichlorohydrin (3.0 mmol) were added and the reaction mixture was stirred for 17–18 h at 70–80 °C (TLC monitoring). The reaction mixture was extracted with ethyl acetate. The organic phase was dried over anhydrous Na_2SO_4. The solvent was distilled off and the residue was column chromatographed using ethyl acetate and hexane as eluents to isolate pure compound. Spectral data for compound **2** has already been reported.[31]

4.3.1. 10-Oxiranylmethyl-9-oxo-9,10-dihydro-acridine-4-carboxylic acid (8)

Compound **8** was synthesized using compound **7** according to the synthetic procedure B as a yellow solid in a yield of 65%. Mp 105 °C; IR (KBr, cm^{-1}): 1605 (C=O), 1680 (C=O), 3400 (OH); ^1H NMR (300 MHz, $CDCl_3$) δ 2.78–2.80 (dd, 1H, J^2 = 4.8 Hz, J^3 = 2.7 Hz, H_b), 2.95–2.97 (dd, 1H, J^2 = 4.8 Hz, J^3 = 3.9 Hz, H_a), 3.39–3.42 (m, 1H, H_c), 4.25–4.31 (dd, 1H, J^2 = 12.3 Hz, J^3 = 6.3 Hz, H_e), 4.72–4.77 (dd, 1H, J^2 = 12.3 Hz, J^3 = 3.0 Hz, H_d), 7.23–7.39 (m, 3H, ArH), 7.65–7.70 (m, 1H, ArH), 8.41–8.71 (m, 2H, ArH), 8.74 (d, 1H, J = 1.8 Hz, ArH), 11.59 (b, 1H, COOH); ^{13}C (normal/DEPT-135) δ 42.61 (−ve, CH_2), 44.64 (−ve, CH_2), 49.26 (+ve, CH), 113.02 (+ve, ArCH), 119.88 (+ve, ArCH), 122.39 (+ve, ArCH), 126.95 (+ve, ArCH), 136.75 (+ve, ArCH), 139.87 (+ve, ArCH), 167.37 (C=O), 178.15 (C=O), MS (FAB) 296 (M^++1). Anal. Calcd for $C_{17}H_{13}NO_4$: C, 69.15; H, 4.44; N, 4.74. Found: C, 69.18; H, 4.48; N, 4.79.

4.4. General procedure for the syntheses of compounds 3–6, 9–12 (procedure C)

A mixture of acridone **2**/**8** (1 mmol) and appropriate amine (1 mmol) was irradiated in microwave oven for 5–7 min. On completion of the reaction (TLC), it was washed with diethyl ether to isolate pure product. Spectral data for compounds **3–6** has already been reported.[31]

4.4.1. 10-(2-Hydroxy-3-pyrrolidin-1-yl-propyl)-9-oxo-9,10-dihydroacridine-4-carboxylic acid (9)

Compound **9** was synthesized using compound **8** according to the synthetic procedure C as a yellow solid in a yield of 63%. Mp 65 °C; IR (KBr, cm^{-1}): 1620 (C=O), 1680 (C=O), 3210 (OH), 3320 (OH); ^1H NMR (300 MHz, $CDCl_3$) δ 1.60–1.69 (m, 4H, 2 × CH_2), 2.35–2.41 (m, 2H, NCH_2), 3.74–3.78 (m, 4H, 2 × NCH_2), 4.15–4.23 (m, 1H, $C_{12}H$), 4.36–4.41 (dd, 1H, J^2 = 12.0 Hz, J^3 = 6.0 Hz, $C_{11}H$), 4.51–4.56 (dd, 1H, J^2 = 12.0 Hz, J^3 = 3.0 Hz, $C_{11}H$), 7.21–7.39 (m, 3H, ArH), 7.65–7.70 (m, 1H, ArH), 8.42–8.45 (m, 2H, ArH), 8.69–8.73 (dd, 1H, J^2 = 7.8 Hz, J^3 = 1.8 Hz, ArH), 11.61 (b, 1H, COOH); ^{13}C (normal/DEPT-135) δ 25.32 (−ve, CH_2), 53.22 (−ve, CH_2), 64.76 (+ve, CH), 66.86 (−ve, CH_2), 67.13 (−ve, CH_2), 117.49 (+ve, ArCH), 119.84 (+ve, ArCH), 122.37 (+ve, ArCH), 126.93 (+ve, ArCH), 133.94 (+ve, ArCH), 136.66 (+ve, ArCH), 167.15 (C=O), 177.15 (C=O); MS (FAB) m/z 367 (M^++1). Anal. Calcd for $C_{21}H_{22}N_2O_4$: C, 68.84; H, 6.05; N, 7.65. Found: C, 68.89; H, 6.10; N, 7.69.

4.4.2. 10-(2-Hydroxy-3-piperidin-1-yl-propyl)-9-oxo-9,10-dihydroacridine-4-carboxylic acid (10)

Compound **10** was synthesized using compound **8** according to the synthetic procedure C as a yellow solid in a yield of 74%. Mp 115 °C; IR (KBr, cm^{-1}): 1610 (C=O), 1680 (C=O), 3240 (OH), 3320 (OH); ^1H NMR (300 MHz, $CDCl_3$) δ 1.25–1.50 (m, 2H, CH_2), 1.95–2.08 (m, 4H, 2 × CH_2), 2.96 (m, 2H, CH_2), 3.32–3.58 (m, 2H CH_2), 3.83–3.98 (m, 2H, CH_2), 4.48–4.82 (m, 3H, $C_{11}H_2/C_{12}H$), 7.36–7.44 (m, 2H, ArH), 8.34–8.61 (m, 4H, ArH), 8.69–8.75 (m, 1H, ArH), 12.02 (b, 1H, COOH); ^{13}C NMR (normal/DEPT-135) δ 23.14 (−ve, CH_2), 54.10 (−ve, CH_2), 59.96 (−ve, CH_2), 64.06 (−ve, CH_2), 66.50 (+ve, CH), 117.68 (+ve, ArCH), 120.41 (+ve, ArCH), 122.95 (+ve, ArCH), 126.46 (+ve, ArCH), 133.89 (+ve, ArCH), 134.76 (+ve, ArCH), 167.19 (C=O), 177.93 (C=O); MS (FAB) m/z 381 (M^++1). Anal. Calcd for $C_{22}H_{24}N_2O_4$: C, 69.46; H, 6.36; N, 7.36. Found: C, 69.49; H, 6.40; N, 7.38.

4.4.3. 10-(2-Hydroxy-3-morpholin-4-yl-propyl)-9-oxo-9,10-dihydroacridine-4-carboxylic acid (11)

Compound **11** was synthesized using compound **8** according to the synthetic procedure C as a yellow solid in a yield of 88%. Mp 110 °C; IR (KBr, cm^{-1}): 1610 (C=O), 1680 (C=O), 3200 (OH), 3280 (OH); ^1H NMR (300 MHz, $CDCl_3$) δ 2.48–2.57 (m, 4H, 2 × CH_2), 2.68–2.75 (m, 2H, $C_{13}H_2$), 3.73–3.77 (m, 4H, 2 × OCH_2), 4.19 (m, 1H, $C_{12}H$), 4.36–4.42 (dd, 1H, J^2 = 12.0 Hz, J^3 = 6.0 Hz, $C_{11}H$), 4.51–4.56 (dd, 1H, J^2 = 12.0 Hz, J^3 = 3.0 Hz, $C_{11}H$), 7.23–7.41 (m, 3H, ArH), 7.65–7.71 (m, 1H, ArH), 8.43–8.47 (m, 2H, ArH), 8.71–8.74 (dd, J^2 = 8.1 Hz, 1H, J^3 = 1.5 Hz, ArH), 11.63 (b, 1H, COOH); ^{13}C (normal/DEPT-135) δ 53.62 (−ve, CH_2), 60.65 (−ve, CH_2), 64.77 (+ve, CH), 66.83 (−ve, CH_2), 67.15 (−ve, CH_2), 117.49 (+ve, ArCH), 119.83 (+ve, ArCH), 122.37 (+ve, ArCH), 126.92 (+ve, ArCH), 133.94 (+ve, ArCH), 136.65 (+ve, ArCH), 167.58 (C=O), 177.84 (C=O); MS (FAB) m/z 383 (M^++1). Anal. Calcd for $C_{21}H_{22}N_2O_5$: C, 65.96; H, 5.80; N, 7.33. Found: C, 65.98; H, 5.84; N, 7.37.

4.4.4. 10-(3-[1,4]Bipiperidinyl-1′-yl-2-hydroxypropyl)-9-oxo-9,10-dihydroacridine-4-carboxylic acid (12)

Compound **12** was synthesized using compound **8** according to the synthetic procedure C as a light yellow solid in a yield of 78%. Mp 100 °C; IR (KBr, cm^{-1}): 1604 (C=O), 1670 (C=O), 3240 (OH), 3355 (OH); ^1H NMR (300 MHz, $CDCl_3$) δ 1.43–1.45 (m, 2H, CH_2), 1.53–1.65 (m, 6H, 3 × CH_2), 1.83–1.87 (m, 2H, CH_2), 1.98–2.06 (m, 1H, CH), 2.23–2.38 (m, 2H, CH_2), 2.45–2.52 (m, 6H, 3 × CH_2) 2.89 (m, 1H, $C_{13}H$), 3.07 (m, 1H, $C_{13}H$), 4.11–4.13 (m, 1H, $C_{12}H$), 4.32–4.38 (dd, 1H, J^2 = 11.4 Hz, J^3 = 6.0 Hz, $C_{11}H$), 4.48–4.53 (dd, 1H, J^2 = 9.0 Hz, J^3 = 3.0 Hz, $C_{11}H$), 7.22–7.40 (m, 3H, ArH), 7.65–7.70 (m, 1H, ArH), 8.41–8.46 (m, 2H, ArH), 8.70–8.73 (d, 1H, J = 8.1 Hz, ArH), 11.63 (b, 1H, COOH); ^{13}C NMR (normal/DEPT-135) δ 24.50 (−ve, CH_2), 25.86 (−ve, CH_2), 27.82 (−ve, CH_2), 50.28 (−ve, CH_2), 51.84 (−ve, CH_2), 55.36 (−ve, CH_2), 59.75 (−ve, CH_2), 62.39 (+ve, CH), 65.03 (+ve, CH), 117.47 (+ve, ArCH), 119.91 (+ve, ArCH), 122.36 (+ve, ArCH), 126.84 (+ve, ArCH), 133.88 (+ve, ArCH), 136.81 (+ve, ArCH), 167.00 (C=O), 178.00 (C=O); MS (FAB) m/z 464 (M^++1). Anal. Calcd for $C_{27}H_{33}N_3O_4$: C, 69.95; H, 7.18; N, 9.06. Found: C, 69.98; H, 7.120; N, 9.10.

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