Synthesis of 2-Methyl N^{10}-Substituted Acridones as Selective Inhibitors of Multidrug Resistance (MDR) Associated Protein in Cancer Cells

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Abstract: A series of N^{10}-substituted-2-methyl acridone derivatives are synthesized and are examined for its ability to reverse P-glycoprotein (P-gp) mediated multidrug resistance (MDR) in breast cancer cell lines MCF-7 and MCF-7/Adr. The structural requirement of *in-vitro* anti-cancer and reversal of drug resistance are studied. The results showed that compound **16** with four carbon spacer exhibited promising *in-vitro* anti-cancer and reversal of drug resistance in comparison to the other analogues.

Key Words: Acridones, anti-cancer, P-glycoprotein (P-gp), multidrug resistance (MDR).

INTRODUCTION

The appearance of tumor cells resistance to a range of cytotoxic drugs is a serious problem in cancer chemotherapy. Circumvention of P-gp and MRP transport is important for high efficacy of anticancer drugs. One form of MDR can be caused by members of the ATP-binding cassette (ABC) family of transport proteins [1]. These are large polytopic membrane proteins that actively transport drugs out of cells, resulting in a decreased intracellular drug concentration. In humans, two ABC transporters have been identified that cause resistance in tumor cells: P-glycoprotein (P-gp) (*MDR1*) [2] and the multidrug resistance associated protein (*MRP1*) [3]. P-gp transports drugs in an unmodified form, whereas *MRP1* transports drugs conjugated to the anionic ligands glutathione (GSH), glucuronide, or sulfate [4] or transports them in an unmodified form, probably together with GSH [5]. Among those cytotoxic drugs transported by *MRP1* are various natural product oncolytics, such as vinca alkaloids, epipodophyllotoxins, anthracyclines, and camptothecins, [6] most of which are also substrates for P-gp transport, [7] although taxanes are apparently not subject to *MRP1* mediated resistance [8]. Furthermore, *MRP1* transports leukotriene C4 (LTC4) as substrate in an ATP-dependent fashion with high efficiency [9].

The differential expression and tissue/tumor specificities of Pgp and *MRP1* have been reviewed recently [10. 11] although it is also known that P-gp and *MRP* can be overexpressed at the same time in drug-resistant cells [12]. The correlation between drug resistance and expression of the drug efflux pumps, Pgp and *MRP1*, has spurred considerable efforts in the development of inhibitors Pgp and *MRP1* [13]. A number of compounds, so called chemosensitizers are able to reverse the effect of P-gp on MDR [14]. However, some MDR modulators such as verapamil were associated with many side effects [15] and several of these agents have been evaluated in clinical trials [16, 17], but currently none are in clinical use. Unfortunately, most of these agents suffer clinically from their intrinsic toxicity. Clinical studies with prospective MDR drugs have helped to unravel the complex nature of clinical drug resistance and the problem associated with combination chemotherapy of anticancer drugs together with MDR inhibitors. These limitations have spurred considerable efforts in the developments of new and more effective, less toxic MDR inhibitors.

An acridonecarboximide derivative [18] has already been shown as a P-gp mediated MDR inhibitor. Furthermore, it has also been demonstrated that a novel acridone derivative (1,3-bis (9-oxoacridin-10-yl) propane, as a potent and poorly reversible modulator of P-glycoprotein mediated vinblastine transport [19]. Previously, we have reported dual inhibitory activities of compounds containing heterocyclic rings and basic side chains, like N^{10}-substituted acridone analogues against Pgp and *MRP1* [20-23]. With an intention of discovering selective anti-MDR agents, the authors have prepared a series of seventeen novel 2-methylacridone derivatives and screened for anticancer and reversal of drug resistance in cancer cells.

The compounds showed significant activity at the given concentration levels. The tricyclic N^{10}-substituted acridone with a methyl group at position C-2 and a secondary amine side chain containing a tertiary amino group at a distance of at least three to four carbon atoms from the tricyclic ring is responsible for marked anti-MDR activity. Hence these compounds appear to be promising anticancer and reversers of drug resistance in cancer cells.

CHEMISTRY

2-methylacridone (**1**) and its derivatives **2-17** were synthesized by the Scheme **1**, Parent 2-methylacridone (**1**) was synthesized by the Ullmann condensation reaction of 2-chlorobenzoic acid and 4-methyl aniline to 4'-methyldi-

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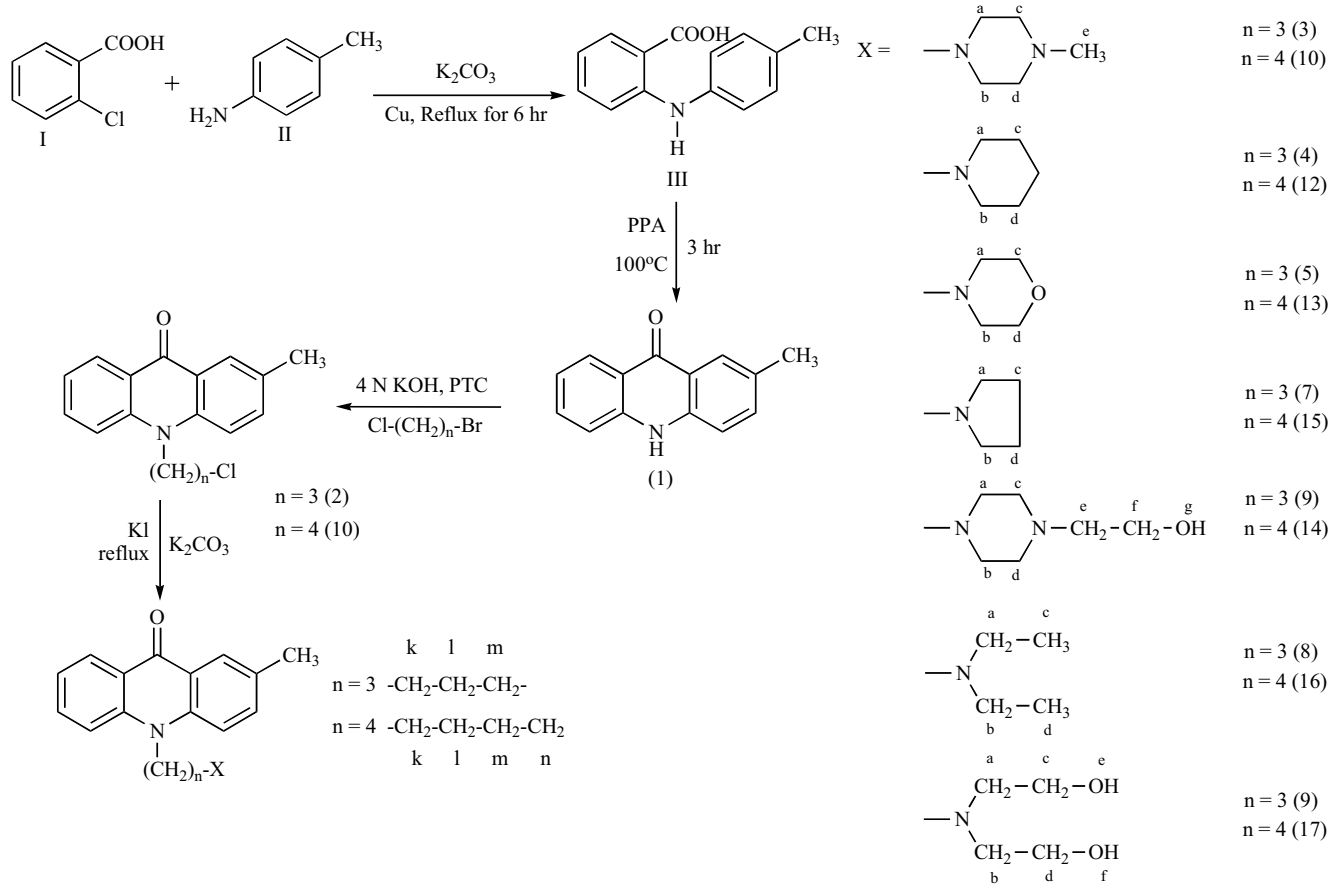


Scheme 1.

phenylamine-2-carboxylic acid (**III**). The 4'-methyldiphenylamine-2-carboxylic acid (**III**) was cyclized with polyphosphoric acid instead of sulfuric acid on water bath at 100^0 C to get only single product of 2-methylacridone (**1**) with better yield (95%) was obtained.

N-alkylation of the 2-methylacridone was achieved by using Phase transfer catalyst (PTC) because nitrogen atom of the acridone nucleus is generally resistant to undergo *N*-alkylation with alkyl halides due to their weakly basic in nature. Stirring of 2-methylacridone (**1**) at room temperature with alkylating agents 1-bromo-3-chloro propane or 1-bromo-4-chloro butane in a two phase system consisting of an organic solvent (tetrahydrofuran) and 4N aqueous potassium hydroxide solution in the presence of tetra butyl ammonium bromide (PTC) to synthesize the compounds **2** and **10** respectively in good yields. Here, catalyst (PTC) transports the OH^- ion from the aqueous phase to organic phase where actual reaction takes place. The ion formed may be regarded as phenolate stabilized anion, which subsequently undergoes alkylation to form the aromatized system.

Iodide catalyzed nucleophilic substitution reaction of the N^{10}-chloropropyl or N^{10}-chlorobutyl 2-methylacridone is carried out with various secondary amines (*N*-methylpiperazine, piperidine, morpholine, (β-hydroxyethyl) piperazine), *N,N*-diethylamine, pyrrolidine, *N,N*-diethanolamine by refluxing for different time intervals in the presence of anhydrous potassium carbonate in acetonitrile gave the free bases **3–9** and **11–17**.

All the molecules were separated and purified by column chromatography or recrystallization method and dried under high vacuum for more than 12 h. The purified compounds were characterized by ^{1}H-NMR and ^{13}C-NMR and mass spectral methods and elemental analysis. The assignment of protons is fully supported by the integration curves and all the derivatives showed the characteristic chemical shifts for the acridone nucleus. The assignment of the ^{13}C-NMR spectra of the derivatives are in close agreement with an analogous compounds N^{10}-alkyl substituted acridone. The mass spectra of all the acridone derivatives were analyzed under ESI conditions. Molecular ions were observed either in the form of $M^{+\cdot}$ and M+H in the spectra of these 2-methyl acridone derivatives. From the mass spectral data, it was clear that as such there is no difference in fragmentation pattern among the set of acridone series compounds. In general, mass spectral features of these compounds were similar and straight forward. Most of the compounds yield abundant molecular ions in the form of M+H. All bonds in the N^{10}-side chain portion are prone to cleavage. In conclusion, the data presented in this article demonstrate the usefulness of MS for characterization of acridone derivatives.

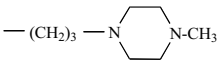
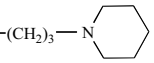
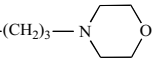
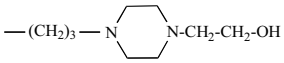
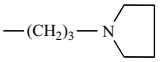
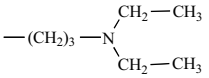
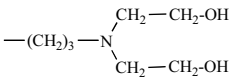
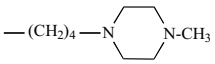
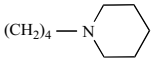
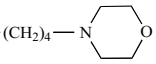
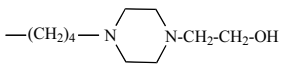
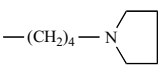
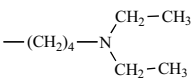
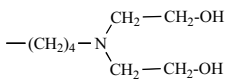
BIOLOGICAL ACTIVITY

Lipophilicity

The compounds lipophilicity was determined using the software ALOGPS (Virtual Computational Chemistry Laboratory Project, Institute for Bioinformatics, GSF - For-

schungszentrum fu¨r Umwelt und Gesundheit, GmbH, Ingolsta¨dter Landstrasse 1, D-85764 Neuherberg, Germany). The efficacy of an MDR modulator will depend in part on its ability to accumulate in cells. The acridone derivatives are weak bases and able to exist in both charged (protonated) and uncharged (unprotonated) forms. The lipophilicity data varying from 2.18 to 5.20, expressed in $\log_{10} P$, are given in Table **1**. In order to elucidate the role played by the $-CH_3$ group at position C-2 of the acridone ring, the lipophilicity data of **1-17**. Substitution of hydrogen by methyl group in position C-2 of the acridone ring has resulted in a slight enhancement in the $\log_{10} P$ values. Additionally, it is speculated that the acridone nucleus with methyl group at position C-2 may exhibit higher affinity for membranes or be more

Table 1. Lipophilicity Values of Different N^{10}-substituted Acridone Derivatives

Compound No.	R	$\log_{10}P$[a]
1	-H	3.36
2	$-CH_2-CH_2-CH_2-Cl$	4.14
3		3.31
4		4.55
5		3.21
6		2.77
7		4.05
8		4.58
9		2.18
10	$-CH_2-CH_2-CH_2-CH_2-Cl$	4.57
11		3.69
12		4.98
13		3.68
14		3.12
15		4.45
16		5.20
17		2.67

[a]Lipophilicity was determined using the ALOGPs Software.

Table 2. Cytotoxicity of Acridone Derivatives in Human MCF-7 Cells

Comp. No	IC_{50} (μM)	Comp. No	IC_{50} (μM)
1	71.10	10	61.60
2	62.20	11	36.60
3	48.80	12	32.80
4	52.50	13	31.66
5	50.20	14	11.16
6	20.10	15	12.17
7	22.66	16	08.16
8	18.12	17	06.10
9	16.20		

readily taken up into cells than that with a hydrogen atom. Analysis of the relationship between $\log_{10} P$ values and the effectiveness of the modulators to reversing drug resistance in cancer cells showed a poor correlation ($R^2 = 0.0019$). The major outlier in this analysis was the parent molecules (**1, 2** and **10**) are comparatively having higher $\log_{10} P$ values than any of its substituted derivatives, yet these were not very effective at increasing anticancer activity. In contrast, compound **16** with the highest $\log_{10} P$ value (5.20) did not show the maximal activity. Therefore, the degree of lipophilicity of each drug would seem to be important, but it is not the sole determinant of potency for the Pgp-modulating activity of acridone derivatives.

Cytotoxicity

The cytotoxicity of seventeen acridones was examined on MCF-7 breast cancer cell line by SRB assay with several concentrations of acridones. The IC_{50} was determined by SRB assay and the concentration percent survival curves and the results are given in Table **2**. The IC_{50} values for N^{10}-chloropropyl substituted-2-methyl acridone and N^{10}-chlorobutyl substituted derivatives against MCF-7 cells revealed that anti-proliferative activity relatively increased as the chain length increased from 3 to 4 suggesting that hydrophobicity plays an important role in biological activity. The increase of distance between the ring nucleus and amino group increased the anti-proliferative activity of these compounds. It is clear from the data that the comparison of the cytotoxicity of the butyl derivatives has shown that the cell killing potency follows the order **17>16>14>15>13>12>11>10** and propyl derivatives **9>8>6>7>3>5>4>2>1**.

Therefore from this study we can tentatively conclude that the structural features required within the series to cause a maximum anti-proliferative activity in MCF-7 cells include hydrophobic acridone ring nucleus with a side chain tertiary cationic amino group that is separated from the aromatic ring by atleast three to four carbon atom. However, it is not possible to draw conclusion about the correlation between structure and anti-proliferative activity from these studies.

Sensitization of Drug-Resistant MCF-7 Cells by 2-methyl-N^{10}-substituted Acridones

All the newly synthesized seventeen N^{10}–substituted-2-methyl acridone derivatives were evaluated for drug resistance activity. Their ability to modulate the cytotoxicity of vinblastine in drug-resistant MCF-7/Adr cell line. Also, an attempt has been made to correlate the structure and anti-MDR activity among the set of compounds. Cells (MCF-7/Adr) were exposed continuously to 0-100 nM vinblastine for 7-days in the absence or presence of IC_{10} concentrations of acridone modulators. At IC_{10} of modulators the fold-potentiation of vinblastine cytotoxicity for the drug resistant MCF-7/Adr summarized in Table **3**.

The IC_{50} value of vinblastine against MCF-7/Adr cells in the presence of IC_{10} of modulators (**1-17**) lie in the range of 4.10 - 28.12 nM. Comparative study of the abilities of the modulator to potentiate the cytotoxicity of vinblastine in the presence of acridone modulators revealed that the modulators (**17, 15** & **13**) demonstrated the greatest effect followed by **16 > 8 > 9 > 14 > 7 > 12 > 11 > 10 > 3 > 6 > 5 > 2 > 4 > 1**. Only three acridone derivatives (**17, 15** & **13**), like verepamil, were able to completely reverse the 25-fold resistance of MCF-7/Adr cells to doxorubicin. The IC_{50} values for continuos exposure to vinblastine was 3.0 nM in MCF-7 and 69.0 nM in MCF-7/Adr cells in the absence of modulating agent.

The influence of alkyl bridge length connecting the acridone ring nucleus to the amino group was examined. Increasing the distance between the ring nucleus and the amino group from three to four carbons increased the antiproliferative and anti-MDR effects of these compounds. The influence of the side chain amino group on anti-MDR activity has created a great deal of interest. To determine the importance of the side chain amino group, the anti-MDR activity of acridone derivatives having different tertiary amino groups and side chains of different length like $(-CH_2-)_3$ or $(-CH_2-)_4$ were studied.

Over time, these experiments were refined and features common to most active modulating compounds are highly

Table 3. Cytotoxicity of Acridone Derivatives on the Potentiation of Doxorubicin Cytototoxicity in Drug resistant MCF-7/Adr Cells

Comp. No.	Vinblastine IC^{b}_{50} (nM)	Fold Potentiation	Comp. No	Vinblastine IC^{b}_{50} (nM)	Fold Potentiation
1	40.20	3.21	**10**	21.20	8.80
2	31.10	4.20	**11**	20.16	7.20
3	22.20	7.10	**12**	18.17	17.20
4	32.10	5.60	**13**	08.16	[a]Complete
5	30.20	4.20	**14**	16.60	19.00
6	26.20	8.80	**15**	20.20	[a]Complete
7	18.10	9.0	**16**	08.80	20.00
8	14.20	12.0	**17**	06.20	[a]Complete
9	15.10	13.0	_		

Note: [a]At IC_{10} concentration of the modulators they are able to reverse drug resistance completely in MCF-7/Adr cancer cells.

lipophilic aromatic planar ring system substituted with a preferably cyclic, tertiary amino group. However, the attributes of an optimal modulator remains to be elucidated and much can be learned from the study of structure-activity relationships within such a series.

To Investigate Whether 2-Methylacridones Interact with P-glycoprotein by Photolabeling this Protein with [^{3}H] Azidopine

It has been previously demonstrated that P-gp is specifically labeled with vinblastine analogues and that VRP blocked the specific labeling [24]. To confirm the binding of acridones to P-gp the authors have examined the competition between [^{3}H] azidopine and seventeen 2-methylacridones. At their IC_{50} concentrations, 2-methylacridones inhibited the labeling of P-gp by [^{3}H] azidopine. The binding of [^{3}H] azidopine to P-gp after inhibition by acridones expressed in percentage of control (no competitor) is as follows: **1** by 64%, **2** by 81%, **3** by 63%, **4** by 61%, **5** by 45%, **6** by 33%, **7** by 57%, **8** by 62%, **9** by 31%, **10** by 52%, **11** by 70%, **12** by 31%, **13** by 28%, **14** by 24%, **15** by 31%, **16** by 18%, **17** by 20% and VRP by 61%. Comparison of the data on the competition between [^{3}H] azidopine and each acridone modulator at its IC_{50} concentration revealed that the ability of all the compounds, except compounds **2,** and **11,** to inhibit the [^{3}H] azidopine labeling of P-gp, is greater than that of the standard modulator verapamil. If a modulator inhibits labeling by the probe of interest, then it is said that this modulator probably functions by competing for the drug-binding site on the protein. The fact that all the compounds have reduced the photoaffinity labeling of azidopine appreciably, the results predict that the modulators compete for azidopine for binding to P-gp. Careful evaluation of the data of the modulators showed that butyl derivatives have exhibited greater competition than that of propyl derivatives suggesting that this is due to enhanced lipophilicity of the compounds have exhibited greater competition for azidopine labeling, suggesting that the activity of 2-methylacridones may be mediated through P-gp-dependent mechanism.

CONCLUSIONS

From the synthesized 17 acridone derivatives screened for anticancer and reversers of drug resistance in cancer cells. From anticancer and anti-MDR activity screening, the compounds showed that significant activity at the given concentration levels. The results show that these compounds reverse the drug resistance in MCF-7/Adr cancer cell lines by P-gp inhibition. The tricyclic N^{10}- substituted acridone with a methyl group at position C-2 and a secondary amine side chain containing a tertiary amino group at a distance of at least three to four carbon atoms from the tricyclic ring is responsible for marked anti cancer and reversal of drug resistance activity. Hence these compounds appear to be promising anticancer and reversers of drug resistance in cancer cells.

EXPERIMENTAL SECTION

Reactions were monitored by TLC. Column Chromatography utilized silica gel Merck Grade 60 (230–400 mesh, 60 Å). Melting points were recorded on a Tempirol hot-stage with microscope and are uncorrected. Elemental analyses were performed and found values are within 0.4% of theoretical values unless otherwise noted. ^{1}H- and ^{13}C-NMR spectra were recorded in DMSO solution in a 5-mm tube on a Bruker drx 500 Fourier transform spectrometer with tetramethylsilane as internal standard. Chemical shifts are expressed as δ (ppm) values. The spectrometer was internally locked to deuterium frequency of the solvent. To obtain molecular weight information, acridone derivatives were analyzed by ESI mass spectrometry. Collision-induced dissociation (CID) spectra were acquired in the positive ion mode on a MDS Sciex (Concord, Ont., Canada) API 4000 triple quadrupole mass spectrometer 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°C, a GSI value and the curtain gas set at 10. Ultra-pure nitrogen was value of 50 and the 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.

Synthesis of 4'-methyl diphenylamine-2-carboxylic Acid

Ullmann Condensation

To a mixture of *o*-chlorobenzoic acid (5 g, 0.032 mol), 4-methylaniline (6.68 ml, 0.032 mol) and copper powder (0.2 g) in 30 ml isoamylalcohol, anhydrous potassium carbonate (5 g) was slowly added and the contents were allowed to reflux for 8 h on an oil bath. The isoamylalcohol was removed by steam distillation and the mixture poured into 1: l 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, precipitate was obtained which was washed with hot water and recrystallized from methanol to give a yellow solid (yield 85%), m.p. 185° C.

Synthesis of 2-methylacridone (1)

Five grams of 4'-methyl diphenylamine-2-carboxylic acid was taken in a flask to which was added 50 g of polyphosphoric acid. Shaken well and heated on a water bath at 100 °C for 3 h. Appearance of yellow color indicated the completion of the reaction. Then, it was poured into 1: l of hot water and made alkaline by liquor ammonia and the yellow precipitate formed was filtered, washed with hot water and collected. The sample of 2-methyl 9(10-H) acridone (**1**) was recrystallized from acetic acid (yield 95%). M.p. 334° C, ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 7.05-8.20 (m, 7H, Ar-H); 1.21 (s, 3H, CH_3) and 11.25 (s, 1H, NH); ^{13}C-NMR (150 MHz, DMSO-d_6) δ = 112.62, 115.11, 115.70, 116.07, 117.06, 119.55, 120.71, 121.23, 125.12, 131.50, 132.83, 144.31 (Ar' 12-C), 179.10 (C=O), 18.09 (CH_3); ESIMS *m/z* (%): 209 ($M^{+\cdot}$, 45). Anal. Calcd for $C_{14}H_{11}NO$: C, 80.36; H, 5.30; N, 6.69. Found: C, 80.20; H, 5.12; N, 6.61.

Synthesis of N^{10}-alkylated Acridones *via* Phase Transfer Catalysis

Synthesis of 10-(3'-Chloropropyl)-2-methylacridone (2)

One gram (0.0048 mol) of 2-methylacridone was dissolved in 25 mL tetrahydrofuran and then 20 mL of 4 N potassium hydroxide and 0.5 g (0.015 mol) of tetrabutylammonium bromide was added to it. The reaction mixture was stirred at room temperature for 30 min and added 1-bromo-3-chloropropane (0.015 mol) slowly into the reaction mixture and stirred for 24 h at room temperature. Tetrahydrofuran was evaporated and the aqueous layer was extracted with chloroform. The chloroform layer was washed with water and organic layer dried over anhydrous sodium sulfate and rotavaporated. The crude product was purified by column chromatography by using the solvent system chloroform/acetone (8:1) to give a yellow solid of 10-(3′-chloropropyl)-2-methylacridone (**2**) (yield 50%).

M.p. 106° C, ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 7.20–8.37 (m, 7H, Ar-H); 1.21 (s, 3H, CH_3); 3.69–3.73 (t, 2H, H_m), 3.99 (t, 2H, H_k), 2.19 (m, 2H, H_l); ^{13}C-NMR (150 MHz, DMSO-d_6) δ = 112.62, 115.11, 115.70, 116.07, 117.06, 119.55, 120.71, 121.23, 125.12, 131.50, 132.83, 144.31 (Ar' 12-C), 179.78 (C=O), 48.70 (C_m), 41.83 (C_k), 34.76 (C_l), 19.90 (CH_3); ESIMS *m/z* (%): 286 ($M^{+\cdot}$, 100) Anal. Calcd for $C_{17}H_{16}ClNO$: C, 71.45; H, 5.64; N, 4.90. Found: C, 71.40; H, 5.49; N, 4.72.

10-(3'-[N-Methylpiperazino] propyl)-2-methylacridone (3)

10-(3′-chloropropyl)-2-methylacridone 1.42g (0.005 mol) was dissolved in 60 ml of anhydrous acetonitrile and 1.68 g potassium iodide and 3.3 g of potassium carbonate were added and refluxed for 30 min. Then added 0.9 ml (0.009 mol) of *N*-methyl piperazine into it slowly and refluxed for 15 h until a substantial amount of the product was formed as evidenced by TLC. The contents were cooled, diluted with water and extracted with chloroform. The chloroform layer was washed with water thrice, dried over anhydrous sodium sulfate and evaporated to give an oily product. The semi solid residue was purified by column chromatography using the solvent system chloroform/acetone (8:1) to give a light yellow product of 10-[3′-*N*-(methylpiperazino) propyl]-2-methylacridone (**3**). An acetone solution of the free base was treated with ethereal hydrochloride to give the hydrochloride salt, which was dried over high vacuum to get pure solid (**3**) (yield 56%). M.p. 207° C, ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 7.23–8.27 (m, 7H, Ar-H); 1.22 (s, 3H, CH_3), 2.30 (s, 3H, H_e), 3.21-3.50 (m, 12H, H_a, H_b, H_c, H_d, H_k and H_m) and 2.16–2.25 (m, 2H, H_l). ^{13}C-NMR (150 MHz, DMSO-d_6) δ = 111.22, 115.31, 115.89, 116.23, 117.45, 119.12, 120.00, 121.22, 125.23, 131.58, 132.90, 143.30 (Ar' 12-C), 178.08 (C=O), 47.34 (C_k), 42.95 (C_m), 52.03 (C_a and C_b), 48.07 (C_c and C_d), 26.53 (C_e), 24.50 (C_l), 18.54 (CH_3); ESIMS *m/z* (%): 350 (M + $H^{+\cdot}$, 55), Anal. Calcd for $C_{22}H_{27}N_3O$: C, 75.61; H, 7.79; N, 12.02. Found: C, 75.31; H, 7.33; N, 11.91.

10-[3'-N-Piperidinopropyl]-2-methyl Acridone (4)

The experimental steps used for (**3**) were repeated by taking 1.42 g (0.005 mol) of (**2**), 1.6 g of KI, 3.3 g K_2CO_3 and 0.76 ml (0.009 mol) of piperidine. The crude product was purified to give a pale yellow oily product, which was converted into hydrochloride salt (yield 40%). M.p. 125° C, ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 7.23 – 8.30 (m, 7H, Ar-H); 1.11 (s, 3H, CH_3), 3.15 (m, 8H, H_k, H_m, H_a and H_b), 1.73–1.97 (m, 8H, H_l, H_c and H_d), 1.31–1.44 (p, 2H, H_e); ^{13}C-NMR (150 MHz, DMSO-d_6) δ = 110.12, 115.34, 116.09, 116.63, 117.49, 119.00, 120.09, 121.67, 125.73, 131.56, 132.96, 143.37 (Ar' 12-C), 178.78 (C=O), 55.45 (C_m), 53.23 (C_k), 51.56 (C_a and C_b), 25.67 (C_l), 23.68 (C_c and C_d), 22.34 (C_e), 18.85 (CH_3); ESIMS *m/z* (%): 335 (M + H^+, 65). Anal. Calcd for $C_{22}H_{26}N_2O$: C, 79.00; H, 7.84; N, 8.38. Found: C, 78.98; H, 7.80; N, 8.33.

10-[3'-N-Morpholinopropyl]-2-methylacridone (5)

The procedure used for **3** was followed with 1.42 g (0.005 mol) of **2**, 1.6 g of KI, 3.3 g of K_2CO_3 and 0.78 ml

(0.009 mol) of morpholine. The purified oily product was converted into hydrochloride salt (yield 40%). M.p. 126° C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 7.26–8.19 (m, 7H, Ar-H); 1.20 (s, 3H, CH_3), 3.06–3.25 (t, 4H, H_c and H_d), 2.56–2.89 (t, 4H, H_a and H_b), 2.20 (m, 4H, H_k and H_m), 1.63–1.96 (t, 2H, H_l); ^{13}C-NMR (150 MHz, DMSO-d_6) δ = 111.02, 115.84, 116.09, 116.93, 117.49, 119.78, 120.09, 121.98, 125.89, 131.56, 132.65, 143.39 (Ar' 12-C), 177.67 (C=O), 61.80 (C_c and C_d), 53.67 (C_a and C_b), 49.45 (C_k), 48.12 (C_m), 26.56 (C_l), 18.90 (CH_3); ESIMS *m/z* (%): 337 (M + H$^+$, 35). Anal. Calcd for $C_{21}H_{24}N_2O_2$: C, 74.97; H, 7.19; N, 8.33. Found: C, 74.85; H, 7.11; N, 8.12

10-(3'-N-[(β-Hydroxyethyl)piperazino]propyl)-2-methylacridone (6)

Compound **6** (yield 58%) in the pure form was prepared by following the procedure used for **3** with 1.44 g (0.005 mol) of **2**, 1.6 g of KI, 3.3 g of K_2CO_3 and 1.17 ml (0.009 mol) of (β-hydroxyethyl)piperazine. M.p. 215° C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 7.25–8.31 (m, 7H, Ar-H); 1.11 (s, 3H, CH_3), 4.11 (t, 1H, H_g), 3.57–3.85 (m, 2H, H_f), 3.06–3.34 (m, 14H, H_k, H_m, H_a, H_b, H_c, H_d and H_e), 2.43 (t, 2H, H_l); ^{13}C-NMR (150 MHz, DMSO-d_6) δ = 111.23, 115.85, 116.23, 116.94, 117.40, 119.12, 120.34, 121.90, 125.86, 131.76, 132.95, 143.99 (Ar' 12-C), 177.23 (C=O), 57.20 (C_f), 54.23 (C_e), 52.34 (C_k), 49.56 (C_m), 48.56 (C_a and C_b), 47.67 (C_c and C_d), 26.78 (C_l), 17.09 (CH_3) ; ESIMS *m/z* (%): 379 (M + H$^+$, 12). Anal. Calcd for $C_{24}H_{30}N_2O_2$: C, 72.79; H, 7.70; N, 11.07. Found: C, 72.65; H, 7.72; N, 10.68.

10-(3'-N-Pyrrolidinopropyl)-2-methylacridone (7)

The procedure used for **3** was repeated with 1.42 g (0.005 mol) of **2**, 1.6 g of KI, 3.3 g of K_2CO_3 and 0.63 ml (0.009 mol) of pyrrolidine. M.p. 118° C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 7.29–8.21 (m, 7H, Ar-H); 1.14 (s, 3H, CH_3), 3.60–3.89 (m, 8H, H_k, H_m, H_a and H_b), 1.69–1.95 (m, 6H, H_l, H_c and H_d); ^{13}C-NMR (150 MHz, DMSO-d_6) δ = 111.27, 115.78, 116.76, 117.04, 117.49, 119.89, 120.98, 121.98, 125.87, 131.76, 132.95, 143.90 (Ar' 12-C), 178.03 (C=O), 54.56 (C_k), 48.34 (C_m), 48.56 (C_a and C_b), 25.89 (C_c and C_d), 27.56 (C_l), 19.12 (CH_3); ESIMS *m/z* (%): 321 (M + H$^+$, 34), Anal. ($C_{21}H_{24}N_2O$) C, H, N.

10,10-[3'-(N-Diethyl amino) propyl]-2-methylacridone (8)

The procedure used for **3** was repeated with 1.42 g (0.005 mol) of **2**, 1.6 g of KI, 3.3 g of K_2CO_3 and 0.65 ml (0.009 mol) of Diethyl amine. M.p. 133° C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 7.20–8.22 (m, 7H, Ar-H); 1.19 (s, 3H, CH_3), 3.23 (m, 2H, H_k), 2.70-2.99 (m, 6H, H_a, H_b and H_m), 1.37–1.45 (m, 2H, H_l), 1.16-1.19 (m, 6H, H_c and H_d); ^{13}C-NMR (150 MHz, DMSO-d_6) δ = 111.23, 115.58, 116.12, 117.04, 117.69, 119.80, 120.92, 121.96, 125.81, 131.70, 132.23, 143.45 (Ar' 12-C), 178.34 (C=O), 54.30 (C_k), 48.95 (C_m), 48.05 (C_a and C_b), 25.12 (C_c and C_d), 27.20 (C_l), 17.29 (CH_3); ESIMS *m/z* (%): 323 (M + H$^+$, 23). Anal. Calcd for $C_{21}H_{26}N_2O$: C, 78.22; H, 8.13; N, 8.69. Found: C, 78.02; H, 8.10; N, 8.49.

10-[3'-[N-Bis(hydroxyethyl)amino]propyl]-2-methylacridone (9)

The procedure used for **3** was repeated with 1.42 g (0.005 mol) of **2**, 1.6 g of KI, 3.3 g of K_2CO_3 and 0.94 ml (0.009 mol) of *N*,*N*-diethanolamine. M.p. 129° C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 7.26–8.23 (m, 7H, Ar-H); 1.15 (s, 3H, CH_3), 4.70 (s, 2H, H_e and H_f), 3.72 (m, 6H, H_k, H_c and H_d), 3.45 (m, 2H, H_m), 2.75 (m, 4H, H_a and H_b), 1.60–1.76 (m, 2H, H_l); ^{13}C-NMR (150 MHz, DMSO-d_6) δ = 111.20, 115.57, 116.23, 117.67, 118.69, 119.88, 120.90, 121.93, 125.34, 131.72, 132.22, 143.43 (Ar' 12-C), 178.34 (C=O), 54.34 (C_k), 48.45 (C_m), 53.35 (C_a and C_b), 51.67 (C_c and C_d), 19.34 (C_l), 16.34 (CH_3); ESIMS *m/z* (%): 355 (M + H$^+$, 45). Anal. Calcd for $C_{22}H_{26}N_2O_3$: C, 71.16; H, 7.39; N, 7.90. Found: C, 71.00; H, 7.19; N, 7.23.

10-(4'-Chlorobutyl)-2-methylacridone (10)

The experimental steps used for **2** were followed by taking 1 g (0.0048 mol) of (**1**), 0.5 g of (0.015 mol) of tetrabutylammonium bromide and 0.015 mol of 1-bromo-4-chloro butane. The crude product was purified by column chromatography using chloroform/acetone (8:2) to give a solid (yield 52%). Mp: 108^{0}C ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 7.26–8.22 (m, 7H, Ar-H); 1.25 (s, 3H, CH_3), 3.45–3.56 (t, 2H, H_n), 3.20– 3.34 (t, 2H, Hk), 1.85–1.98 (m, 4H, H_l and H_m); ^{13}C-NMR (150 MHz, DMSO-d_6) δ = 111.23, 115.22, 116.23, 117.62, 118.55, 119.28, 120.20, 121.23, 125.32, 131.22, 132.22, 143.55 (Ar' 12-C), 178.84 (C=O), 48.59 (C_n), 40.82 (C_k), 34.22 (C_l and C_m), 18.38 (CH_3); ESIMS *m/z* (%): 299 (M$^+$, 23)$^{\cdot}$. Anal. Calcd for C_{18} H_{18}Cl N O: C, 71.16; H, 7.39; N, 7.90. Found: C, 71.13; H, 7.32; N, 7.85.

10-[4'-N-(Methylpiperazino) butyl]-2-methylacridone (11)

Amounts of 1.49 g (0.005 mol) of 10, 1.6 g of KI, 3.3 g of K_2CO_3 and 0.9 ml (0.009 mol) of *N*-methylpiperazine in acetonitrile were refluxed and worked up according to protocol used for **3**. The crude semi solid product was subjected to column chromatography for purification and then converted into hydrochloride salt (yield 57%). M.p. 131° C, ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 7.25–8.56 (m, 7H, Ar-H); 1.27 (s, 3H, CH_3), 2.67–2.85 (m, 8H, H_a, H_b, H_c and H_d), 2.44–2.44 (m, 6H, H_k, H_n and H_e), 1.16–1.16 (m, 4H, H_l and H_m); ^{13}C-NMR (150 MHz, DMSO-d_6) δ = 111.27, 115.27, 116.27, 117.62, 118.67, 119.28, 120.70, 121.03, 125.38, 131.82, 132.89, 143.89 (Ar' 12-C), 178.94 (C=O), 59.01 (C_f), 57.81 (C_e), 55.89 (C_k), 48.69 (C_m), 46.19 (C_a and C_b), 45.94 (C_c and C_d), 23.18 (C_l) , 17.34 (CH_3) ; ESIMS *m/z* (%): 364 (M + H$^+$, 34). Anal. Calcd for $C_{23}H_{29}N_3O$: C, 76.00; H, 8.04; N, 11.56. Found: C, 75.87; H, 8.09; N, 11.52.

10-(4'-N-Piperidinobutyl)-2-methylacridone (12)

The procedure used for (**3**) was repeated with 1.49 g (0.005 mol) of **10**, 1.6 g of KI, 3.3 g of K_2CO_3 and 0.76 ml (0.009 mol) of piperidine. The light yellow oil was purified by column chromatography and finally converted into hydrochloride salt (yield 46%). M.p. 106° C, ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 7.20–8.35 (m, 7H, Ar-H); 1.27 (s, 3H, CH_3), 3.35–3.98 (m, 8H, H_a, H_b, H_k and H_n), 1.70–1.78 (m, 8H, H_c, H_d, H_l and H_m), 1.10–1.29 (m, 2H, H_e); ^{13}C-NMR (150 MHz, DMSO-d_6) δ = 112.25, 115.56, 116.67, 117.62, 118.45, 119.67, 120.70, 121.34, 125.68, 131.56, 132.78, 143.45 (Ar' 12-C), 178.45 (C=O), 59.61 (C_a and C_b), 50.71 (C_k), 48.78 (C_n), 24.71 (C_l), 22.84 (C_m), 21.86 (C_c and C_d), 21.85 (C_e), 17.08 (CH_3). ESIMS *m/z* (%): 349 (M + H$^+$, 45). Anal. Calcd for $C_{23}H_{28}N_2O$: C, 79.27; H, 8.10; N, 8.14. Found: C, 79.29; H, 8.11; N, 8.11.

10-(4'-N-Morpholinobutyl)-2-methylacridone (13)

Amounts of 1.49 g (0.005 mol) of **10**, 1.6 g of KI, 3.3 g of K_2CO_3 and 0.78 ml (0.009 mol) of morpholine were refluxed and processed according to the procedure used for **3**. The product was purified by column chromatography and converted to hydrochloride salt (yield 42%). M.p. 109° C, ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 7.29–8.19 (m, 7H, Ar-H); 1.20 (s, 3H, CH_3), 3.45–3.55 (m, 4H, H_c and H_d), 3.29–3.43 (m, 8H, H_k, H_n, H_a and H_b), 1.69–1.90 (p, 2H, H_m) and 1.79–1.98 (p, 2H, H_l); ^{13}C-NMR (150 MHz, DMSO-d_6) δ = 112.20, 115.59, 116.78, 117.67, 118.05, 119.56, 120.80, 121.78, 124.08, 131.98, 132.89, 143.95 (Ar' 12-C), 178.95 (C=O), 68.96 (C_c and C_d), 56.60 (C_a and C_b), 51.78 (C_k), 50.10 (C_n), 26.50 (C_l), 21.78 (C_m), (CH_3) ; ESIMS *m/z* (%): 351 (M + H^+, 45). Anal. Calcd for $C_{22}H_{26}N_2O_2$: C, 75.40; H, 7.48 N, 7.99. Found: C, 75.12; H, 7.23; N, 7.56.

10-(4'-N-[(β-Hydroxyethyl)piperazino]butyl)-2-methylacridone (14)

Compound **14** (yield 50%) as hydrochloride salt was prepared according to the procedure used for **3** with 1.49 g (0.005 mol) of (**10**), 1.6 g of KI, 3.3 g of K_2CO_3 and 1.17 ml (0.009 mol) of (β-hydroxyethyl) piperazine. It was purified by column chromatography.

M.p. 152° C, ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 7.24–8.39 (m, 7H, Ar-H), 1.27 (s, 3H, CH_3), 3.08–3.47 (m, 2H, H_f), 3.10–3.57 (m, 14H, H_a, H_b, H_c, H_d, H_e, H_k and H_n), 1.16–1.27 (m, 4H, H_l and H_m); ^{13}C-NMR (150 MHz, DMSO-d_6) δ = 112.45, 115.79, 116.08, 117.98, 118.95, 119.96, 120.87, 121.88, 124.09, 131.08, 132.89, 143.99 (Ar' 12-C), 178.96 (C=O), 55.48 (C_f), 50.27 (C_e), 48.27 (C_k), 40.39 (C_n), 38.47 (C_c and C_d), 34.46 (C_a and C_b), 25.44 (C_l), 22.30 (C_m), 17.45 (CH_3). ESIMS *m/z* (%): 394 (M + H^+, 23). Anal. Calcd for $C_{25}H_{32}N_2O_2$: C, 73.25; H, 7.94; N,10.68. Found: C, 73.21; H, 7.64; N, 10.63.

10-(3'-N-Pyrrolidinobutyl)-2-methylacridone (15)

The procedure used for **3** was repeated with 1.49 g (0.005 mol) of **10**, 1.6 g of KI, 3.3 g of K_2CO_3 and 0.63 ml (0.009 mol) of Pyrrolidine. M.p. 119°C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 7.20–8.23 (m, 7H, Ar-H); 1.15 (s, 3H, CH_3), 3.57 (m, 2H, H_k), 2.80 (m, 6H, H_a, H_b, and H_n), 1.57–1.68 (m, 8H, H_l, H_m, H_c and H_d); ^{13}C-NMR (150 MHz, DMSO-d_6) δ = 112.40, 115.69, 116.07, 117.98, 118.45, 119.66, 120.77, 121.72, 124.79, 131.48, 132.87, 143.49 (Ar' 12-C), 178.96 (C=O), 54.09 (C_k), 48.88 (C_n), 23.09 (C_m), 51.28 (C_a and C_b), 25.47 (C_c and C_d), 27.67 (C_l), 18.08 (CH_3); ESIMS *m/z* (%): 335 (M + H^+, 100). Anal. Calcd for $C_{22}H_{26}N_2O$: C, 79.00; H, 7.84; N, 8.38. Found: C, 78.96; H, 7.74; N, 8.32.

10-[3'-(N-Diethylamino)butyl]-2-methylacridone (16)

The procedure used for **3** was repeated with 1.49 g (0.005 mol) of **10**, 1.6 g of KI, 3.3 g of K_2CO_3 and 0.65 ml (0.009 mol) of diethyl amine. M.p. 135° C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 7.24–8.29 (m, 7H, Ar-H); 1.17 (s, 3H, CH_3), 3.85 (m, 2H, H_k), 2.86-2.99 (m, 6H, H_a, H_b, and H_n), 1.30–1.43 (m, 4H, H_l and H_m), 1.16-1.10 (m, 6H, H_c and H_d); ^{13}C-NMR (150 MHz, DMSO-d_6) δ = 111.89, 115.56, 116.08, 117.90, 118.98, 119.06, 120.90, 121.92, 124.09, 131.08, 132.86, 143.45 (Ar' 12-C), 178.00 (C=O), 54.73 (C_k), 43.86 (C_n), 23.95 (C_m), 48.97 (C_a and C_b), 12.12 (C_c and C_d), 24.28 (C_l), 17.99 (CH_3); ESIMS *m/z* (%): 337 (M + H^+, 60). Anal. Calcd for $C_{22}H_{28}N_2O$: C, 78.53; H, 8.39; N, 8.33. Found: C, 78.52; H, 8.34; N, 8.33.

10-[3'-[N-Bis(hydroxyethyl)amino]butyl]-2-methylacridone (17)

The procedure used for **3** was repeated with 1.49 g (0.005 mol) of **10**, 1.6 g of KI, 3.3 g of K_2CO_3 and 0.94 ml (0.009 mol) of *N*,*N*-diethanolamine. M.p. 122° C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 7.23–8.29 (m, 7H, Ar-H); 1.39 (s, 3H, CH_3), 4.78 (s, 2H, H_e and H_f), 3.70 (m, 6H, H_k, H_c and H_d), 3.46 (m, 2H, H_n), 2.33 (m, 4H, H_a and H_b), 1.45–1.70 (m, 4H, H_l and H_m); ^{13}C-NMR (150 MHz, DMSO-d_6) δ = 112.09, 115.45, 116.58, 117.94, 118.96, 119.56, 120.96, 121.72, 124.34, 131.56, 132.86, 143.48 (Ar' 12-C), 178.65 (C=O), 54.29 (C_k), 43.32 (C_n), 19.98 (C_m), 55.21 (C_a and C_b), 57.89 (C_c and C_d), 20.29 (C_l), 18.55 (CH_3); ESIMS *m/z* (%): 370 (M + H^+, 45). Anal. Anal. Calcd $C_{22}H_{28}N_2O_3$: C, 71.71; H, 7.66; N, 7.60. Found: C, 71.52; H, 7.34; N, 7.33.

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