INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

# SYNTHESIS AND BIOLOGICAL EVALUATION OF CHALCONE DERIVATIVES OF ACRIDINES AS ANTICANCER AGENTS

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Article Received on: 26/08/19 Approved for publication: 27/09/19

**DOI: 10.7897/2230-8407.1010305**

**ABSTRACT**

A novel series of chalcone fused acridine derivatives (10a-j) were synthesized and their structures were confirmed by $^{1}$HNMR, $^{13}$CNMR and mass spectral data. Further, these derivatives were screened for their biological activities towards three different human cancer cell lines such as MCF-7, A549 and MDA MB-231. Among the tested derivatives, five compounds, 10a (MCF-7 = 1.89 ± 0.17 µM, A549 = 0.12 ± 0.01 µM and MDA MB-231 = 2.89 ± 1.78 µM), 10b (MCF-7 = 0.24 ± 0.018 µM, A549 = 0.35 ± 0.02 µM and MDA MB-231 = 0.11 ± 0.01 µM), 10g (MCF-7 = 2.45 ± 1.60 µM and A549 = 2.78 ± 1.66 µM), 10h MCF-7 = 1.22 ± 0.11 µM, A549 = 1.76 ± 0.16 µM and MDA MB-231 = 1.34 ± 0.13 µM) and 10j (MCF-7 = 2.67 ± 1.63 µM, A549 = 1.67 ± 0.14 µM, MDA MB-231 = 0.56 ± 0.015 µM) were demonstrated more potent anticancer activity than positive control (MCF-7 = 3.12 ± 2.56 µM, A549 = 2.10 ± 2.11 µM, MDA MB-231= 3.41 ± 2.59 µM).

**Keywords:** *m*-AMSA, acridine, chalcone, anticancer activity.

## INTRODUCTION

Acridines are a nitrogen atom containing bioactive agents that exhibits various biological activities such as fungicidal, antimicrobial, anti-parasitic, anti-inflammatory, anticancer, antiviral,[1-4] DNA topoisomerases-I,II, telomerase, protein-kinase,[5,6] analgesic[7] and anticonvulsant.[8] It interact with DNA base pairs due to its unique planner ring structure.[9,10] Among them, amsacrine (1, Figure 1, *m*-AMSA) is a acridine nucleus possessing anti leukemic property that inhibits the human DNA topoisomerase-II enzyme.[11]

On the other hand, chalcones (2) are structurally divergent molecules and consist of two aromatic rings linked by a three carbon α, β-unsaturated carbonyl system,[12] and are precursors for aurones, isoflavones, flavones and anthocyanins. Chalcones exhibits a wide range of pharmacological activities including antitumor,[13] induce apoptosis,[14] antioxidant[15] antimalarial,[16] antioncogenic,[17] antituberclosis,[18] antibacterial,[19] antifungal,[20] anti-inflammatory,[21] antiviral,[22] cytotoxic,[23] antileishmanial,[24] cardiovascular,[25] hyperglycemic[26] and antimitotic.[27]

In view of the above a fore mentioned biological importance of Acridines and Chalcones, the present research embodiment describes the synthesis, characterization ($^{1}$H NMR, $^{13}$CNMR and mass spectral analysis) and anticancer activity of a novel chalcone fused acridine derivatives.

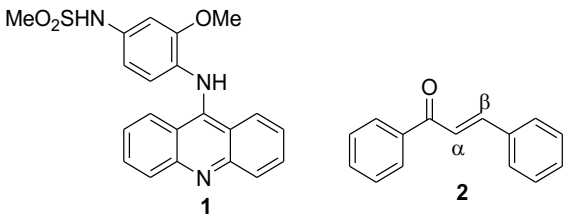



**Figure 1**

## MATERIALS AND METHODS

All chemicals and reagents were obtained from Aldrich (Sigma–Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) and were used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 F-254, and visualization on TLC was achieved by UV light or iodine indicator. $^{1}$H and $^{13}$C NMR spectra were recorded on Bruker, Bruker UXNMR/XWIN-NMR (500 MHz, 400 MHz, 300 MHz) instrument. Chemical shifts (d) are reported in ppm downfield from internal TMS standard. ESI spectra were recorded on Micro mass, Quattro LC using ESI + software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. Melting points were determined with an electro thermal melting point apparatus and are uncorrected.

### 2-(1H-Benzo[d]imidazol-6-ylamino)benzoic acid (5)

To a mixture of *o*-chlorobenzoic acid (3) (10 g, 63.8 mmol), 5-aminobenzimidazole (4) (8.5 g, 63.8 mmol) and copper powder (10 g, 76.5 mmol) in 70 mL isoamyl alcohol, dry potassium

carbonate (26 g, 191.4 mmol) in several portions was heated to reflux for 12 hours. The isoamyl alcohol was removed by steam distillation and the mixture poured into 500 mL of hot water and filtered. The filtrate was acidified with concentrated hydrochloric acid and the precipitated solid formed was filtered, washed with hot water and collected. The crude product 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 yellow precipitates were obtained and was washed with hot water and re-crystallized from aqueous methanol to give 5, Pale yellow solid 12.6 g, with 78% yield. IR (neat): 3354, 2928, 2862, 1613, 1414, 1326 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$7.17-7.26 (m, 3H), 7.61 (t, 1H, $J$ = 7.9, 7.1 Hz), 7.75-7.81 (m, 2H), 8.11 (d, 1H, $J$ = 8.12 Hz), 8.17 (s, 1H), 9.50 (s, 1H), 9.53 (s, 1H), 12.96 (bs, 1H); MS (ESI): 254 $[M + H]^+$.

**10-Chloro-3H-imidazo[4,5-b]acridine (6)**

In a 500 mL round bottom flask fitted with a water-cooled condenser, (11 g, 43.4 mmol) of 2-(1H-benzo[d] imidazol-6-ylamino) benzoic acid (5) is mixed with (40 mL) of phosphorus oxychloride. The mixture was slowly heated for about 15 minutes to 90°C on a water bath. After 20 minutes, when the boiling subsides somewhat, the flask was heated on a heating mantle for 3 hours at 150°C. The excess phosphorus oxychloride was removed by distillation. The residue, after cooling was poured into a well-stirred mixture of 20 mL of concentrated ammonia solution, 50 g of ice and 20 mL of chloroform. The flask was rinsed by shaking with a little chloroform-ammonia mixture (about 25-30 mL). When no more un-dissolved solid remains (about 30 minutes is required), the chloroform layer is separated, and the aqueous layer is extracted with an additional 10 mL of chloroform. The united chloroform extracts are dried over 1 g of calcium chloride and filtered and the solvent was removed by evaporation to afford pure compound 6, 9.2 g with 84% yield.IR (neat): 3224, 1601, 1511, 1404, 1311 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ 7.75 (t, 1H, $J$ = 8.4, 7.2 Hz), 8.09 (t, 1H, $J$ = 8.1, 7.2 Hz), 8.21 (d, 1H, $J$ = 8.13 Hz), 8.24 (s, 1H), 8.29 (d, 1H, $J$ = 8.14 Hz), 8.43 (s, 1H), 8.49 (s, 1H), 9.54 (s, 1H); MS (ESI): 254 $[M + H]^+$.

**1-(4-(3H-Imidazo[4,5-b]acridin-10-ylamino)phenyl)ethanone (8)**

10-Chloro-3H-imidazo[4,5-b]acridine (6) (8 g, 31.6 mmol) and 4-aminoacetophenone (**7**) (4.3 g, 31.6 mmol) were dissolved in NMP (10 mL) containing one drop of concentrated hydrochloric acid and stirred at reflux for 30 minutes. The reaction was monitored by TLC and when complete, the mixture was poured into ethyl acetate (100 mL). The resulting precipitates were collected by suction filtration. The solids were dissolved in hot methanol and poured into ethyl acetate (100 mL) to re-precipitate. The solids formed were collected by suction filtration and dried under vacuum to yield the product 8, 10.2 g with 92% yield. IR (neat): 3224, 1601, 1511, 1404, 1311 $cm^{-1}$; Mp: 213-215 °C, $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$2.78(s, 3H), 7.24 (d, 2H, $J$ = 7.89 Hz), 7.54-7.60 (m, 2H), 7.71 (d, 1H, $J$ = 8.13 Hz), 7.85 (d, 2H, $J$ = 7.89 Hz), 8.11 (d, 1H, $J$ = 8.17 Hz), 8.28 (s, 1H), 8.34 (s, 1H), 8.53 (s, 1H), 9.55 (s, 1H), 11.09 (s, 1H); MS (ESI): 353 $[M + H]^+$.

**(E)-1-(4-(3H-Imidazo[4,5-b]acridin-10-ylamino)phenyl)-3-phenylprop-2-en-1-one (10a)**

The compound 1-(4-(3H-imidazo[4,5-b]acridin-10-ylamino)phenyl)ethanone (8) (400 mg, 1.13 mmol) was dissolved in 5 mL of ethanol, followed by addition of benzaldehyde (9a) (0.12 mL, 1.13 mmol) and 3 drops of piperidine. The reaction mixture was heated under reflux for 6 hours. After cooling water (20 mL) was added slowly. The crystalline precipitate was separated by filtration and purified by re-crystallization from ethanol to afford pure compound 10a, 280 mg in 56% yield. Mp: 219-221 °C; IR (neat): 3382, 3033, 2978, 2361, 1653, 1611, 1573, 1528, 1429, 1335, 1216, 1182, 1031 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ 6.86 (d, 1H, $J$ = 14.7 Hz), 7.27 (t, 2H, $J$ = 7.94, 6.8 Hz), 7.32 (d, 2H, $J$ = 7.90 Hz), 7.40-7.54 (m, 5H), 7.60 (d, 1H, $J$ = 14.7 Hz), 7.70 (d, 1H, $J$ = 8.15 Hz), 7.83 (d, 2H, $J$ = 7.90 Hz), 8.11 (d, 1H, $J$ = 8.13 Hz), 8.27 (s, 1H), 8.35 (s, 1H), 8.57 (s, 1H), 9.56 (s, 1H), 11.10 (s, 1H); $^{13}C$ NMR (100 MHz, DMSO-d6): $\delta$116.8, 117.9, 122.7, 123.6, 124.6, 125.7, 126.4, 128.3, 129.3, 129.6, 130.4, 131.4, 132.5, 134.6, 134.8, 135.6, 135.9, 138.6, 145.6, 145.7, 147.6, 147.9, 148.4, 150.4, 165.7; MS (ESI): 441 $[M + H]^+$.

**(E)-1-(4-(3H-Imidazo[4,5-b]acridin-10-ylamino)phenyl)-3-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (10b)**

This compound 10b was prepared by the method described for 10a, employing 8 (400 mg, 1.13 mmol) was dissolved in 5 mL of ethanol, followed by addition of 3, 4, 5-trimethoxybenzaldehyde (9b) (221 mg, 1.13 mmol) and 3 drops of piperidine to afford pure compound 10b, 271 mg in 45% yield. Mp: 243-245°C, IR (neat): 3380,3039, 2975, 2360, 1652, 1613, 1572, 1529, 1459, 1315, 1211, 1182, 1031 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ 3.87 (s, 3H), 3.93 (s, 6H), 6.85-6.89 (m, 3H), 7.32 (d, 2H, $J$ = 7.89 Hz), 7.43-7.56 (m, 3H), 7.74 (d, 1H, $J$ = 8.15 Hz), 7.82 (d, 2H, $J$ = 7.89 Hz), 8.11 (d, 1H, $J$ = 8.14 Hz), 8.27 (s, 1H), 8.34 (s, 1H), 8.56 (s, 1H), 9.56 (s, 1H), 11.10 (s, 1H); $^{13}C$ NMR (100 MHz, DMSO-d6): $\delta$ 57.4, 62.9, 109.5, 116.4, 117.8, 122.4, 123.4, 124.5, 125.6, 126.7, 128.3, 129.6, 132.4, 132.6, 134.5, 134.8, 135.5, 138.6, 145.6, 145.8, 146.3, 147.5, 148.4, 150.3, 151.4, 151.8, 165.6; MS (ESI): 531 $[M + H]^+$.

**(E)-1-(4-(3H-Imidazo[4,5-b]acridin-10-ylamino)phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (10c)**

This compound 10c was prepared by the method described for 10a, employing 8 (400 mg, 1.13 mmol) was dissolved in 5 mL of ethanol, followed by addition of 4-methoxybenzaldehyde (9c) (0.14 ml, 1.13 mmol) and 3 drops of piperidine to afford pure compound 10c, 285 mg in 53% in yield. Mp: 221-223°C, IR (neat): 3344,3031, 2958, 2364, 1654, 1613, 1570, 1522, 1455, 1319, 1211, 1182, 1031 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ 3.87 (s, 3H), 6.86 (d, 1H, $J$ = 14.3 Hz), 7.10 (d, 2H, $J$ = 7.84 Hz), 7.32 (d, 2H, $J$ = 7.89 Hz), 7.44-7.53 (m, 4H), 7.56 (d, 1H, $J$ = 14.5 Hz), 7.73 (d, 1H, $J$ = 8.15 Hz), 7.82 (d, 2H, $J$ = 7.89 Hz), 8.11 (d, 1H, $J$ = 8.13 Hz), 8.27 (s, 1H), 8.34 (s, 1H), 8.57 (s, 1H), 11.09 (s, 1H); $^{13}C$ NMR (100 MHz, DMSO-d6): $\delta$ 57.5, 115.6, 116.7, 117.9, 120.4, 122.4, 123.5, 125.5, 126.7, 128.5, 129.5, 131.4, 132.4, 134.5, 134.8, 135.6, 138.7, 145.5, 145.8, 146.5, 147.6, 148.6, 152.8, 156.7, 165.7; MS (ESI): 471 $[M + H]^+$.

**(E)-1-(4-(3H-Imidazo[4,5-b]acridin-10-ylamino)phenyl)-3-(4-chlorophenyl)prop-2-en-1-one (10d)**

This compound 10d was prepared by the method described for 10a, employing 8 (400 mg, 1.13 mmol) was dissolved in 5 mL of ethanol, followed by addition of 4-chlorobenzaldehyde (9d) (0.13 mL, 1.13 mmol) and 3 drops of piperidine to afford pure compound 10d, 311 mg in 58% yield. Mp: 240-242°C, IR (neat): 3324, 3035, 2952, 2344, 1653, 1618, 1575, 1526, 1456, 1320, 1211, 1182, 1031 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ 6.87 (d, 1H, $J$ = 14.7 Hz), 7.32 (d, 2H, $J$ = 7.90 Hz), 7.44-7.54 (m, 4H), 7.56 (d, 1H, $J$ = 14.6 Hz), 7.73-7.78 (m, 3H), 7.82 (d, 2H, $J$ = 7.90 Hz), 8.11 (d, 1H, $J$ = 8.13 Hz), 8.27 (s, 1H), 8.34 (s, 1H), 8.57 (s,

1H), 11.10 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-d6): $\delta$ 116.7, 117.9, 122.4, 123.5, 123.8, 125.7, 126.4, 128.4, 129.4, 130.3, 130.8, 132.4, 134.4, 134.8, 135.3, 135.8, 137.3, 138.4, 144.5, 145.5, 145.7, 147.6, 148.3, 151.6, 165.8; MS (ESI): 475 $[M + H]^+$.

**(E)-1-(4-(3H-Imidazo[4,5-b]acridin-10-ylamino)phenyl)-3-(4-bromophenyl)prop-2-en-1-one (10e)**

This compound 10e was prepared by the method described for 10a, employing 8 (400 mg, 1.13 mmol) was dissolved in 5 mL of ethanol, followed by addition of 4-bromobenzaldehyde (9e) (209 mg, 1.13 mmol) and 3 drops of piperidine to afford pure compound 10e, 320 mg in 54% yield. Mp: 244-246ºC, IR (neat): 3322, 3037, 2951, 2345, 1650, 1610, 1570, 1526, 1456, 1320, 1211, 1182, 1031 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 6.88 (d, 1H, $J$ = 14.7 Hz), 7.13 (d, 2H, $J$ = 7.78 Hz), 7.33-7.42 (m, 4H), 7.43-7.48 (m, 2H), 7.57 (d, 1H, $J$ = 14.6 Hz), 7.73 (d, 1H, $J$ = 8.14 Hz), 7.83 (d, 2H, $J$ = 7.90 Hz), 8.12 (d, 1H, $J$ = 8.13 Hz), 8.27 (s, 1H), 8.35 (s, 1H), 8.57 (s, 1H), 11.10 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-d6): $\delta$ 116.6, 117.8, 122.4, 123.6, 123.7, 125.6, 125.8, 126.7, 128.6, 129.7, 130.6, 132.4, 133.5, 134.6, 134.8, 135.6, 135.8, 138.6, 144.5, 145.5, 145.7, 147.6, 148.7, 152.6, 165.7; MS (ESI): 520 $[M + H]^+$.

**(E)-1-(4-(3H-Imidazo[4,5-b]acridin-10-ylamino)phenyl)-3-(4-fluorophenyl)prop-2-en-1-one (10f)**

This compound 10f was prepared by the method described for 10a, employing 8 (400 mg, 1.13 mmol) was dissolved in 5 mL of ethanol, followed by addition of 4-fluorobenzaldehyde (9f) (0.12 ml, 1.13 mmol) and 3 drops of piperidine to afford pure compound 10f, 305 mg in 59% yield. Mp: 228-230 ºC, IR (neat): 3329, 3036, 2957, 2348, 1652, 1619, 1575, 1528, 1458, 1320, 1211, 1182, 1031 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 6.87 (d, 1H, $J$ = 14.6 Hz), 7.33-7.44 (m, 6H), 7.45-7.49 (m, 2H), 7.56 (d, 1H, $J$ = 14.5 Hz), 7.72 (d, 1H, $J$ = 8.11 Hz), 7.82 (d, 2H, $J$ = 7.90 Hz), 8.12 (d, 1H, $J$ = 8.13 Hz), 8.27 (s, 1H), 8.35 (s, 1H), 8.57 (s, 1H), 11.10 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-d6): $\delta$ 116.7, 117.8, 117.9, 122.3, 122.6, 123.5, 125.6, 126.7, 128.6, 129.6, 131.5, 132.4, 132.7, 134.5, 134.8, 135.7, 138.6, 144.5, 145.6, 145.9, 147.6, 148.4, 152.4, 158.4, 165.8; MS (ESI): 459 $[M + H]^+$.

**(E)-1-(4-(3H-Imidazo[4,5-b]acridin-10-ylamino)phenyl)-3-(4-nitrophenyl)prop-2-en-1-one (10g)**

This compound 10g was prepared by the method described for 10a, employing 8 (400 mg, 1.13 mmol) was dissolved in 5 mL of ethanol, followed by addition of 4-nitrobenzaldehyde (9g) (170 mg, 1.13 mmol) and 3 drops of piperidine to afford pure compound 10g, 335 mg in 61% yield. Mp: 250-252ºC, IR (neat): 3324, 3038, 2959, 2349, 1654, 1610, 1573, 1546, 1454, 1320, 1211, 1182, 1031 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 6.88 (d, 1H, $J$ = 14.6 Hz), 7.33 (d, 2H, $J$ = 7.90 Hz), 7.44-7.49 (m, 2H), 7.57 (d, 1H, $J$ = 14.8 Hz), 7.71-7.78 (m, 3H), 7.82 (d, 2H, $J$ = 7.90 Hz), 8.10-8.17 (m, 3H), 8.27 (s, 1H), 8.35 (s, 1H), 8.57 (s, 1H), 11.10 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-d6): $\delta$ 116.7, 117.9, 121.3, 122.6, 123.5, 125.5, 125.8, 126.4, 128.5, 129.5, 129.9, 132.4, 134.5, 134.6, 135.5, 138.7, 142.4, 142.6, 145.5, 145.7, 147.6, 148.4, 149.7, 152.8, 165.8; MS (ESI): 486 $[M + H]^+$.

**(E)-1-(4-(3H-Imidazo[4,5-b]acridin-10-ylamino)phenyl)-3-(3,5-dimethoxyphenyl)prop-2-en-1-one (10h)**

This compound 10h was prepared by the method described for 10a, employing 8 (400 mg, 1.13 mmol) was dissolved in 5 mL of ethanol, followed by addition of 3, 5-dimethoxbenzaldehyde (9h) (187 mg, 1.13 mmol) and 3 drops of piperidine to afford pure compound 10h, 291 mg in 51% yield. Mp: 231-233ºC, IR (neat): 3329, 3044, 2979, 2344, 1658, 1619, 1577, 1546, 1454, 1320, 1211, 1182, 1031 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 3.89 (s, 6H), 6.45 (s, 1H), 6.86 (d, 1H, $J$ = 14.6 Hz), 7.09 (s, 2H), 7.32 (d, 2H, $J$ = 7.89 Hz), 7.44-7.55 (m, 3H), 7.72 (d, 1H, $J$ = 8.11 Hz), 7.82 (d, 2H, $J$ = 7.89 Hz), 8.11 (d, 1H, $J$ = 8.13 Hz), 8.27 (s, 1H), 8.35 (s, 1H), 8.56 (s, 1H), 11.10 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-d6): $\delta$ 57.8, 101.7, 110.6, 116.7, 117.9, 122.4, 123.6, 124.5, 125.7, 126.5, 128.4, 129.5, 132.5, 134.6, 134.8, 135.6, 138.4, 138.8, 145.4, 145.7, 145.9, 147.5, 148.5, 152.7, 158.5, 165.7; MS (ESI): 501 $[M + H]^+$.

**(E)-1-(4-(3H-Imidazo[4,5-b]acridin-10-ylamino)phenyl)-3-p-tolylprop-2-en-1-one (10i)**

This compound 10i was prepared by the method described for 10a, employing 8 (400 mg, 1.13 mmol) was dissolved in 5 mL of ethanol, followed by addition of 4-methylbenzaldehyde (9i) (0.133 ml, 1.13 mmol) and 3 drops of piperidine to afford pure compound 10i, 261 mg in 51% yield. Mp: 236-238ºC, IR (neat): 3349, 3024, 2971, 2324, 1651, 1615, 1574, 1543, 1453, 1320, 1211, 1182, 1031 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.47 (s, 3H), 6.88 (d, 1H, $J$ = 14.6 Hz), 7.32 (d, 2H, $J$ = 7.89 Hz), 7.43-7.54 (m, 6H), 7.56 (d, 1H, $J$ = 14.7 Hz), 7.72 (d, 1H, $J$ = 8.12 Hz), 7.82 (d, 2H, $J$ = 7.89 Hz), 8.11 (d, 1H, $J$ = 8.14 Hz), 8.27 (s, 1H), 8.35 (s, 1H), 8.56 (s, 1H), 11.09 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-d6): $\delta$ 27.6, 116.6, 117.8, 122.4, 122.5, 123.6, 125.6, 126.7, 128.6, 129.5, 129.8, 130.6, 132.4, 133.5, 134.5, 134.7, 135.6, 138.6, 141.5, 145.6, 145.8, 145.9, 147.6, 148.7, 152.5, 165.7; MS (ESI): 455 $[M + H]^+$.

**4-((E)-3-(4-(3H-Imidazo[4,5-b]acridin-10-ylamino)phenyl)-3-oxoprop-1-enyl)benzonitrile (10j)**

This compound 10j was prepared by the method described for 10a, employing 8 (400 mg, 1.13 mmol) was dissolved in 5 mL of ethanol, followed by addition of 4-cyanobenzaldehyde (9j) (148 mg, 1.13 mmol) and 3 drops of piperidine to afford pure compound 10j, 278 mg in 53% yield. Mp: 267-269ºC; IR (neat): 3341, 3026, 2970, 2328, 1653, 1613, 1579, 1542, 1458, 1325, 1211, 1182, 1031 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 6.88 (d, 1H, $J$ = 14.7 Hz), 7.32 (d, 2H, $J$ = 7.90 Hz), 7.44-7.49 (m, 2H), 7.56-7.63 (m, 3H), 7.72 (d, 1H, $J$ = 8.13 Hz), 7.82-7.90 (m, 4H), 8.12 (d, 1H, $J$ = 8.14 Hz), 8.27 (s, 1H), 8.35 (s, 1H), 8.57 (s, 1H), 11.10 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-d6): $\delta$ 116.5, 117.8, 118.5, 122.4, 123.4, 123.8, 124.5, 125.6, 126.8, 128.5, 129.5, 131.5, 132.5, 134.5, 134.7, 135.6, 138.4, 140.4, 145.3, 145.8, 145.9, 147.4, 148.4, 152.5, 165.8; MS (ESI): 466 $[M + H]^+$.

**MTT Assay**

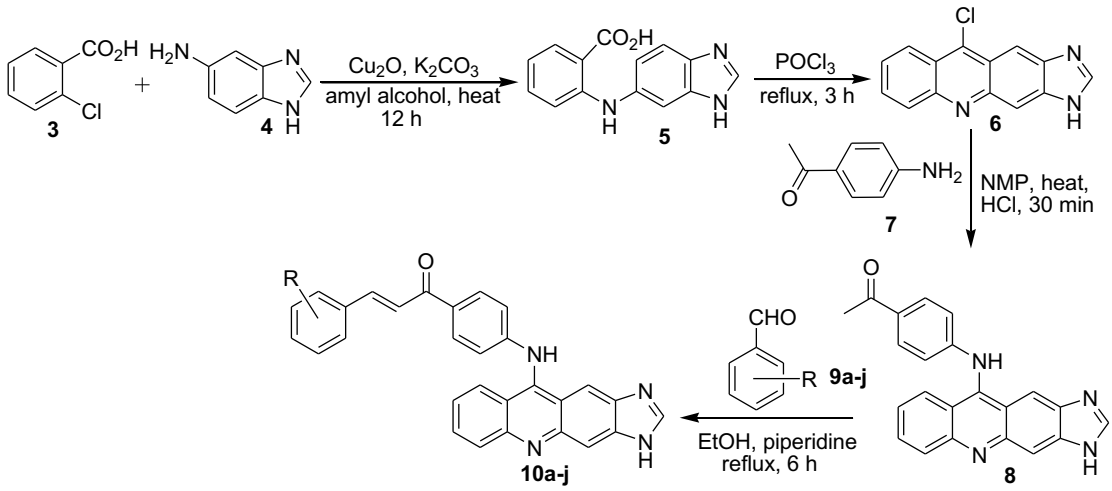
The cytotoxic activity of the compounds was determined using MTT assay. 1 × $10^4$ cells/well were seeded in 200 ml DMEM, supplemented with 10% FBS in each well of 96-well micro culture plates and incubated for 24 hours at 37ºC in a $CO_2$ incubator. Compounds, diluted to the desired concentrations in culture medium, were added to the wells with respective vehicle control. After 48 hours of incubation, 10 ml MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) (5 mg/ml) was added to each well and the plates were further incubated for 4 hours. Then the supernatant from each well was carefully removed, formazan crystals were dissolved in 100 ml of DMSO and absorbance at 540 nm wavelength was recorded.

## RESULTS AND DISCUSSION

### Chemistry

The synthetic sequence for the title compounds (10a-j) is illustrated in Scheme 1. The condensation of 2-aminobenzoic acid (3) with 5-aminobenzimidazole (4) in the presence of $Cu_2O$ (cuprous oxide) and $K_2CO_3$ in amyl alcohol at reflux for 12 hours yielded 2-(1H-Benzo[d]imidazol-6-ylamino)benzoic acid 5. Cyclization of 2-(1H-Benzo[d]imidazol-6-ylamino) benzoic acid 5 in presence of $POCl_3$ at reflux for 3 hours produced 10-Chloro-3H-imidazo [4,5-b]acridine (6) in 84% yield. Condensation of 10-Chloro-3H-imidazo[4,5-b] acridine (6) with 4-aminoacetophenone (7) in presence of catalytic quantity of conc.; HCl in *N*-methyl-2-pyrrolidone (NMP) at 100°C for 30 minutes yielded 1-(4-(3H-Imidazo[4,5-b]acridin-10-ylamino)phenyl) ethanone (8) in 92% yield. The Claisen-Schmidt condensation of 1-(4-(3H-Imidazo[4,5-b]acridin-10-ylamino)phenyl) ethanone (8) with commercially available aromatic aldehydes (9a-j) in the presence of piperidine in ethanol resulted in the formation of chalcone fused acridine derivatives (10a-j).

The structural elucidation of these of these compounds was confirmed by $^1$H NMR, $^{13}$ C NMR, Mass and IR spectroscopy data.




**9a**; R = H
**9b**; R = 3,4,5-trimethoxy
**9c**; R = 4-methoxy
**9d**; R = 4-chloro
**9e;** R = 4-bromo
**9f**; R = 4-fluoro
**9g**; R = 4-nitro
**9h**; R = 3,5-dimethoxy
**9i;** R = 4-trifluoromethyl
**9j;** R = 4-cyano

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**10j;** R = 4-cyano

**Scheme 1**

### Biological evaluation

#### *In vitro* Cytotoxicity

The anticancer effect of ten synthetic chalcone fused acridine derivatives (10a-j) was evaluated on three human cancer cell lines such as MCF-7 (Beast), A549, MDA MB-231 (lung) by using the MTT assay and these results are summarized in Table 1. Adriamycin was used as reference drug. Most of these compounds showed good anticancer activity with $IC_{50}$ values in low micromole range. Within the series of chalcone fused acridine derivatives 10a, 10b, 10g, 10h and 10j were exhibited promising anticancer activity. Further, all these derivatives studied for structure activity relationship (SAR) and it revealed that compound 10a without substituent on the phenyl ring was demonstrated potent anticancer activity in three cell lines with 7 ± 0.14 µM, MDA MB-231= 0.56 ± 0.015 µM). $IC_{50}$ values ranges (MCF-7 = 1.89 ± 0.17 µM, A549 = 0.12 ± 0.01 µM and MDA MB-231= 2.89 ± 1.78 µM). Compound 10b having electron donating group 3, 4, 5-trimethoxy on the phenyl ring was displayed more potent activity in three cell lines (MCF-7 = 0.24 ± 0.018 µM, A549 = 0.35 ± 0.02 µM and MDA MB-231= 0.11 ± 0.01 µM). When compound 10c with 4-methoxy functional group on the phenyl ring was showed lower activity in all cell lines (MCF-7 = 3.90 ± 3.01 µM and A549 = 8.12 ± 4.78 µM). While compound 10h with 3, 5-dimethoxy substitution on the phenyl ring was showed moderate activity in two cell lines (MCF-7 = 2.45 ± 1.60 µM and A549 = 2.78 ± 1.66 µM). Interestingly, compound 10g and 10j with electron withdrawing 4-nitro and 4-cyano group son the phenyl ring were exhibited good activity in three cell lines (MCF-7 = 1.22 ± 0.11µM, A549 = 1.76 ± 0.16 µM and MDA MB-231= 1.34 ± 0.13 µM) and (MCF-7 = 2.67 ± 1.63 µM, A549 = 1.6

**Table 1: Cytotoxicity ($IC_{50}$ μM) data of chalcone fused acridine derivatives (10a-j)[a]**

| Compound | MCF-7[b] | A549[c] | MDA MB-231[c] |
|---|---|---|---|
| **10a** | 1.89 ± 0.17 | 0.12 ± 0.01 | 2.89 ± 1.78 |
| **10b** | 0.24 ± 0.018 | 0.35 ± 0.02 | 0.11 ± 0.01 |
| **10c** | 3.90 ± 3.01 | 8.12 ± 4.78 | - |
| **10d** | 3.67 ± 2.65 | 4.89 ± 3.12 | - |
| **10e** | - | 13.78 ± 4.27 | - |
| **10f** | 12.89 ± 4.10 | 14.8 ± 5.19 | 7.67 ± 4.11 |
| **10g** | 1.22 ± 0.11 | 1.76 ± 0.16 | 1.34 ± 0.13 |
| **10h** | 2.45 ± 1.60 | 2.78 ± 1.66 | 11.8 ± 4.09 |
| **10i** | 16.8 ± 5.23 | - | - |
| **10j** | 2.67 ± 1.63 | 1.67 ± 0.14 | 0.56 ± 0.015 |
| **Adriamycin** | 3.12 ± 2.56 | 2.10 ± 2.11 | 3.41 ± 2.59 |

"-" Not active. [a] 50% Growth inhibition and the values are mean of three determinations. [b] Breast cancer. [c] Lung cancer.

## CONCLUSION

In conclusion, a series of novel chalcone fused acridine analogues (10a-j) were synthesized and were confirmed by $^{1}H$ NMR, $^{13}CNMR$ and mass spectral analysis. Most of them showed promising cytotoxicity towards three human cancer cell lines such as MCF-7 (Beast), A549, MDA MB-231 (lung). Among them, five compounds 10a (MCF-7 = 1.89 ± 0.17 μM, A549 = 0.12 ± 0.01 μM and MDA MB-231= 2.89 ± 1.78 μM), 10b (MCF-7 = 0.24 ± 0.018 μM, A549 = 0.35 ± 0.02 μM and MDA MB-231 = 0.11 ± 0.01 μM), 10g (MCF-7 = 2.45 ± 1.60 μM and A549 = 2.78 ± 1.66 μM), 10h (MCF-7 = 1.22 ± 0.11 μM, A549 = 1.76 ± 0.16 μM and MDA MB-231 = 1.34 ± 0.13 μM) and 10j (MCF-7 = 2.67 ± 1.63 μM, A549 = 1.67 ± 0.14 μM, MDA MB-231 = 0.56 ± 0.015 μM) were exhibited more potent anticancer activity than adriamycin (MCF-7 = 3.12 ± 2.56 μM, A549 = 2.10 ± 2.11 μM, MDA MB-231= 3.41 ± 2.59 μM).

## ACKNOWLEDGEMENT

The author is thankful to JNT University-Hyderabad for constant encouragement during this research program. The author is also thankful to Green Evolution Laboratories for providing necessary facility for research work.

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**Cite this article as:**

Sanivarapu Sumalatha *et al*. Synthesis and Biological evaluation of Chalcone derivatives of Acridines as anticancer agents. Int. Res. J. Pharm. 2019;10(10):93-98 http://dx.doi.org/10.7897/2230-8407.1010305

Source of support: Nil, Conflict of interest: None Declared