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Synthesis and Biological Evaluation of *m*-PEG Attached Acridinedione Derivatives as Antimicrobial, Antioxidant and Anticancer Agents

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Among heterocycles, a wide variety of nitrogen heterocycles have been exploited to develop pharmaceutically important molecules. Particularly, acridines are clinically used potential drug candidates showing various pharmacological activities. Some of the novel *m*-PEG attached acridinediones **4(a-j)** were synthesized with the readily available starting materials such as dimedone, glycine and aldehydes. Structures of the synthesized compounds were characterized by spectral techniques. The synthesized compounds (**4a-j)** were evaluated for *in vitro* antimicrobial, antioxidant and anticancer activities. Compounds **4d**, **4e and 4g** were found to produce potent antimicrobial activities against Gram-positive and Gram-negative organisms while **4c** active against only Gram-positive organisms when compared with the standard, ciprofloxacin. On the other side, **4d** and **4i** produced potent antioxidant activity and **4a**, **4d and 4g** exhibited comparable anticancer properties.

Keywords: Acridinedione, Antimicrobial, Antioxidant, Anticancer.

INTRODUCTION

Acridine derivatives commonly exist in three forms, such as acridine, acridane and acridone (Fig. 1). These three forms are the sources to increases the number of synthetic routes for the development of various acridine scaffolds [1]. The polycyclic planer structure of acridine has nitrogen along with π-electron, which makes acridine derivatives as interesting chemo-therapeutic agents. Acridine derivatives have unique physical and chemical properties along with pharmacological activities and industrial applications [2]. Acridine derivatives have a long history in the treatment of human diseases [3,4]. Pharmacologically acridine derivatives were first used as antibacterial and antiparasitic agents [5]. A large number of synthetic acridine derivatives and natural alkaloids have been tested as antimalarial [6], antioxidant [7], anticancer [8,9], topoisomerase I & II inhibitors [10], anti-inflammatory [9] and antiherpes [11] agents.

Now cancer is a major public health problem worldwide [12]. According to the history of the growth of cancer is ranked as the first or second leading cause of death in 91 of 172 countries. At present, cancer is the second leading cause of adult death in urban and fourth leading causes of adult death in the rural area of India [13-17]. In India, cancer mortality has become double from 1990 to 2016 [18]. Statistical data reveals that in 2020, 19.3 million peoples are suffering from cancer and it is predicted to rise by 47% (28.4 million cases) as demographic changes alone by 2040 [19]. Cancers are mainly caused by mutations, that may be inherited, induced by environmental factors, or by DNA replication errors [20]. The planar structure of acridine confers to the molecule's ability to bind DNA by intercalation and therefore to interfere with metabolic processes. Still now, a smaller number of molecules have entered into clinical trials and have been approved for chemotherapy. The mechanisms by which acridines exert their pharmacological actions are closely related to their capacity to reversibly bind with DNA [8,21], to interact with DNA regulatory enzymes and to disrupt DNA functions in the cell [22]. The resulting cytotoxic activity may be related to potent enzyme inhibition [23,24]. Based on the recent results, outlook on antioxidant and antitumor acridine chemotherapy will be proposed. Some of the reported pharma-cologically active

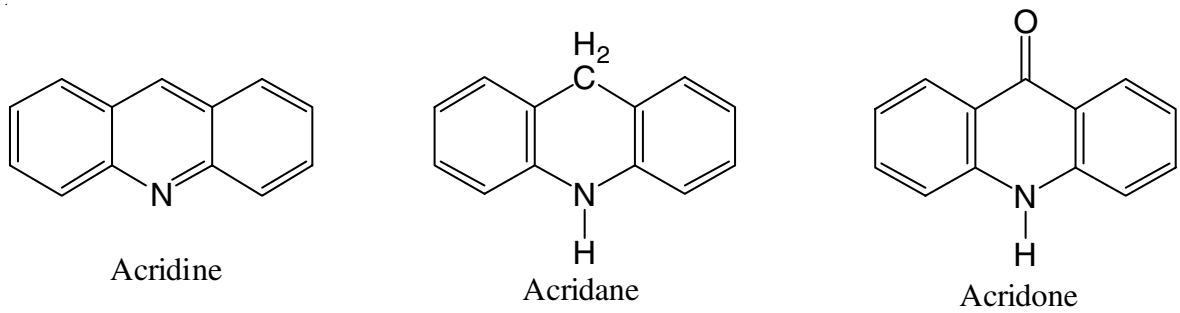


Fig. 1. Different stages of acridine compounds

acridine and acridinedione related compounds (**A-F**) are shown in Fig. 2.

EXPERIMENTAL

All the research-grade chemicals were purchased from Aldrich, Fluka and S.D. Fine Chemicals. Melting points were determined by open capillaries and are uncorrected. The completion of reactions was monitored with the help of thin-layer chromatography (TLC), performed on Merck Kieselgel 60 F_{254} pre-coated aluminium backed and spots were visualized by iodine vapour or by irradiation with ultraviolet light. ^1H & ^{13}C NMR spectra were recorded on Bruker model DRX 400 NMR spectrometer in acetone-d_6, using tetramethylsilane (TMS) as an internal standard. The chemical shift values are reported in parts per million (δ, ppm) from internal standard TMS. Fourier transform infrared (FT-IR) spectra were recorded on Perkin Elmer FT-IR spectrometer using KBr pellet. Mass spectra were recorded with Jeol-JMS-DX 303 HF Shimadzu instrument.

General procedure for the synthesis of 2-(3,3,6,6-tetramethyl-1,8-dioxo-9-substituted-1,2,3,4,5,6,7,8-octahydroacridin-10(9*H*)-yl) acetic acid (3a-j): A mixture of dimedone (**1**) (1 equiv.) and glycine (2 equiv.) was added to various aromatic/aliphatic aldehydes (**2**) (1 equiv.) in acetic acid (20 mL) and the mixture was refluxed for 6 h. The reaction was monitored through TLC at regular intervals until the complete abolition of starting material from the reaction mixture. Later, the reaction mixture was cooled and poured into crushed ice. Then, the solid obtained was filtered, dried and recrystallized from the mixture of methanol and chloroform (1:4).

2-(1,2,3,4,5,6,7,8-Octahydro-3,3,6,6-tetramethyl-1,8-dioxoacridin-10(9*H*)-yl)acetic acid (3a): Light yellow crystal, m.f.: $C_{19}H_{25}NO_4$, m.w.: 331.41, yield 56%, m.p.: 221-223 ºC, IR (KBr, ν_{max}, cm^{-1}): 3463 (OH), 1728.71 (C=O) of carboxyl, 1612.66 (C=O), 1368.98 (CN). ^1H NMR (acetone 400 MHz): δ 1.032 & 1.079 (2s, 12H, C_3 & C_6-dimethyl), 2.034 (s, 2H, C_4-CH_2), 2.773 (s, 2H, C_2-CH_2), 3.102 (s, 2H, C_9-CH_2), 11.560 (s, 1H, -COOH).

2-(9-Ethyl-1,2,3,4,5,6,7,8-octahydro-3,3,6,6-tetramethyl-1,8-dioxoacridin-10(9*H*)-yl)acetic acid (3b): Dark yellow crystal, m.f.: $C_{20}H_{27}NO_4$, m.w.: 345.19, yield 63%, m.p.: 227-229 ºC, IR (KBr, ν_{max}, cm^{-1}): 3440.8 (OH), 2869.9 (alkane-CH), 1651 (C=O) of carboxyl, 1627.8 (C=O), 1354.2 (hetero-

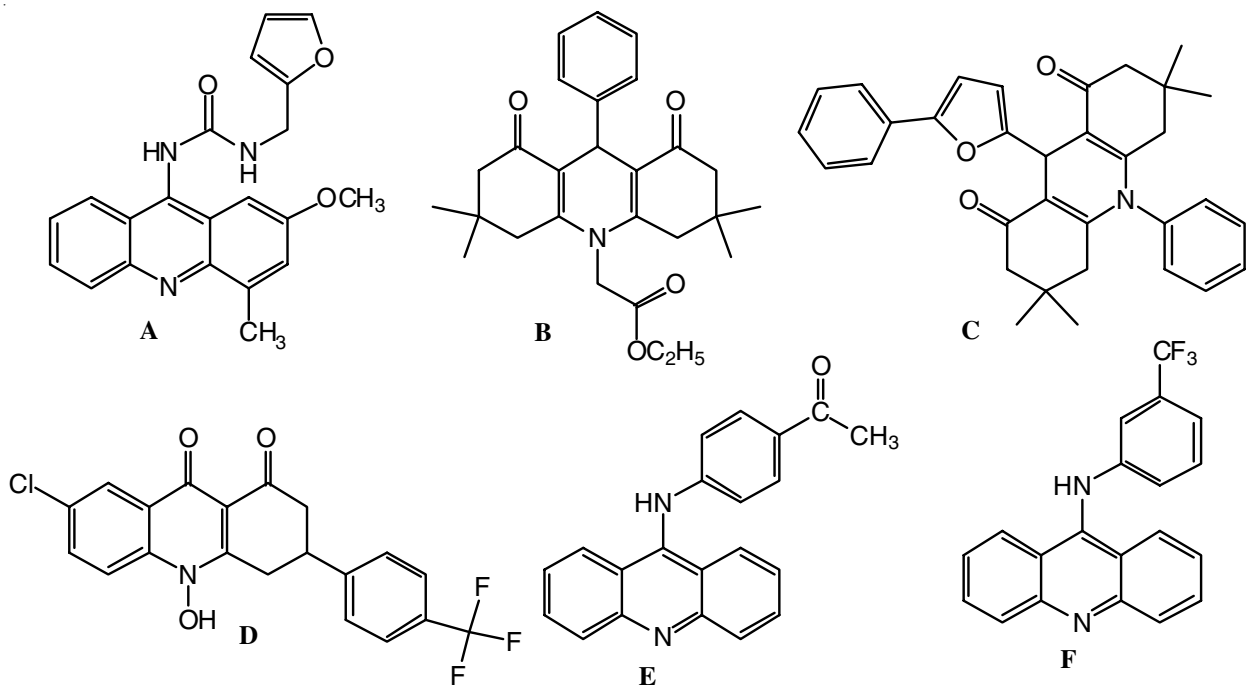


Fig. 2. Reported pharmacologically active acridine and their derivatives (**A-F**)

C-N). ^{1}H NMR (acetone 400 MHz): δ 1.035 & 1.055 (2s, 12H, C_3 & C_6-dimethyl), 1.104 (s, 3H, C_9-CH_3), 2.034 (s, 2H, C_4-CH_2), 2.773 (s, 2H, C_2-CH_2), 3.302 (s, 1H, C_9-H).

2-(9-Ethyl-1,2,3,4,5,6,7,8-octahydro-3,3,6,6-tetramethyl-1,8-dioxoacridin-10(9*H*)-yl)acetic acid (3c): Yellow crystal, m.f.: $C_{21}H_{29}NO_4$, m.w.: 359.46, yield 71%, m.p.: 238-240 ºC, IR (KBr, ν_{max}, cm^{-1}): 3455.74 (OH), 2875.07 (alkane-CH), 1725.60 (C=O) of carboxyl, 1616.60 (C=O), 1372.18 (hetero-C-N). ^{1}H NMR (acetone 400 MHz): δ 0.637-0.666 (t, 3H, -aliph. CH_3), 1.036 & 1.074 (2s, 12H, C_3 & C_6-dimethyl), 1.221-1.292 (m, 2H, -aliph. CH_2), 2.051 (s, 2H, C_4-CH_2), 2.633 (s, 2H, C_2-CH_2), 3.312 (s, 1H, C_9-H), 11.621 (s, 1H, -COOH).

2-(9-Ethyl-1,2,3,4,5,6,7,8-octahydro-3,3,6,6-tetramethyl-1,8-dioxoacridin-10(9*H*)-yl)acetic acid (3d): Dark yellow crystal, m.f.: $C_{26}H_{31}NO_4$, m.w.: 421.53, yield 55%, m.p.: 245-247 ºC, IR (KBr, ν_{max}, cm^{-1}): 3420.02 (OH), 2922.49 (arom. -CH), 1731.24 (C=0) of carboxyl, 1634.85 (C=O), 1356.80 (hetero-C-N). ^{1}H NMR (acetone 400 MHz): δ 0.948-0.969 (2s, 12H, C_3 & C_6-dimethyl), 2.143 (d, 2H, C_4-CH_2), 2.256 (d, 3H, Ar-CH_3), 2.658 (s, 2H, C_2-CH_2), 4.684 (s, 1H, C_9-H), 6.898-7.235 (d, 4H, Ar-H).

2-(1,2,3,4,5,6,7,8-Octahydro-9-(4-methoxyphenyl)-3,3,6,6-tetramethyl-1,8-dioxoacridin-10(9*H*)-yl)acetic acid (3e): Light yellow crystal, m.f.: $C_{26}H_{31}NO_5$, m.w.: 437.53, yield 71%, m.p.: 238-240 ºC, IR (KBr, ν_{max}, cm^{-1}): 3463.08 (OH), 2999.52 (arom. -CH), 1728.71 (C=O) of carboxyl, 1612.66 (C=O), 1368.98 (hetero-C-N). ^{1}H NMR (acetone 400 MHz): δ 0.948-0.974 (d, 12H, C_3 & C_6-dimethyl), 2.143 (s, 2H, C_4-CH_2), 2.653 (d, 2H, C_2-CH_2), 3.680 (s, 3H, -OCH_3), 4.691 (s, 1H, C_9-H), 7.138-7.160 (d, 4H, Ar-H). MS, *m/z* (%): 437.53 (M, 31), 438.37 (M+1, 15), 436.34 (M-1, 100), 873.72 (30), 874.71 (15), 875.76 (9).

2-(9-(4-Chlorophenyl)-1,2,3,4,5,6,7,8-octahydro-3,3,6,6-tetramethyl-1,8-dioxoacridin-10(9*H*)-yl)acetic acid (3f): Dark yellow crystal, m.f.: $C_{25}H_{28}NO_4Cl$, m.w.: 441.95, yield 72%, m.p.: 258-260 ºC, IR (KBr, ν_{max}, cm^{-1}): 3439.59 (OH), 2957.99 (arom. -CH), 1737.26 (C=O) of carboxyl, 1655.35 (C=O), 1373.41 (hetero-C-N). ^{1}H NMR (acetone 400 MHz): δ 0.939-0.971 (2d, 12H, C_3 & C_6-dimethyl), 2.155 (s, 2H, C_4-CH_2), 2.66 (d, 2H, C_4-CH_2), 4.709 (s, 1H, C_9-H), 7.202-7.274 (d, 4H, Ar-H). MS, *m/z* (%): 441.95 (M), 442.31 (M+1, 43), 440.32 (M-1, 100), 443.31 (18), 318.18 (45), 320.18 (28), 321.19 (3), 759.53 (32), 881.63 (32).

2-(1,2,3,4,5,6,7,8-Octahydro-9-(4-hydroxyphenyl)-3,3,6,6-tetramethyl-1,8-dioxoacridin-10(9*H*)-yl)acetic acid (3g): Yellow crystal, m.f.: $C_{25}H_{29}NO_5$, m.w.: 423.5, yield 76%, m.p.: 238-240 ºC, IR (KBr, ν_{max}, cm^{-1}): 3390.87 (OH), 2959.83 (arom. -CH), 1729.89 (C=O) of carboxyl, 1647.68 (C=O), 1370.01 (hetero-C-N). ^{1}H NMR (acetone 400 MHz): δ 0.953-0.976 (2s, 12H, C_3 & C_6-dimethyl), 2.136 (s, 2H, C_4-CH_2), 2.652 (d, 2H, C_2-CH_2), 4.674 (s, 1H, C_9-H), 5.106 (s, Ar-OH), 7.047-7.069 (d, 4H, Ar-H). MS *m/z* (%): 423.50 (M, 34), 425.29 (M+1, 15), 422.33 (M-1, 100), 731.61 (12), 788.62 (38), 845.67 (20), 902.64 (10).

2-(1,2,3,4,5,6,7,8-Octahydro-3,3,6,6-tetramethyl-9-(3-nitrophenyl)-1,8-dioxoacridin-10(9*H*)-yl)acetic acid (3h): Yellow crystal, m.f.: $C_{25}H_{28}N_2O_6$, m.w.: 452.5, yield 76%, m.p.: 222-224 ºC, IR (KBr, ν_{max}, cm^{-1}): 3456.46 (OH), 2963.19 (arom. -CH), 1737.26 (C=O) of carboxyl, 1655.41 (C=O), 1373.35 (hetero-C-N). ^{1}H NMR (Acetone 400 MHz): δ 0.939-0.971 (2d, 12H, C_3 & C_6-methyl), 2.155(s, 2H, C_4-CH_2), 2.662 (d, 2H, C_2-CH_2), 4.707 (s, 1H, C_9-H), 7.196-7.274 (d, 4H, Ar-H).

2-(1,2,3,4,5,6,7,8-Octahydro-3,3,6,6-tetramethyl-1,8-dioxo-9-(thiophen-2-yl)acridin-10(9*H*)-yl)acetic acid (3i): Light yellow crystal, m.f.: $C_{25}H_{29}NO_4S$, m.w.: 439.57, yield 68%, m.p.: 230-232 ºC, IR (KBr, ν_{max}, cm^{-1}): 3435.74 (OH), 2959.97 (arom. -CH), 1722.50 (C=O) of carboxyl, 1630.96 (C=O), 1377.53 (hetero-C-N). ^{13}C NMR (acetone 400 MHz): 27.241 (C_3 & C_6), 33.150, 40.041, 41.048, 47.732 (C_2, C_4, C_5 & C7-CH_2), 123.472, 123.968 (2-C=C), 151.089-152.477 (aromatic carbon), 171.058 (acid group), 206.062 (ketonic group). MS *m/z* (%): 413.53 (M, 22), 414.26 (M+1, 10), 412.29 (M-1, 90), 825.64 (100), (60), 827.57 (20), 828.58 (10).

2-(1,2,3,4,5,6,7,8-Octahydro-3,3,6,6-tetramethyl-1,8-dioxo-9-phenylacridin-10(9*H*)-yl)acetic acid (3j): Dark yellow crystal, m.f.: $C_{25}H_{31}NO_4$, m.w.: 409.52, yield 71%, m.p.: 180-182 ºC, IR (KBr, ν_{max}, cm^{-1}): 3436.09 (OH), 2960.68 (arom. -CH), 1725.28 (C=O) of carboxyl, 1662.31 (C=O), 1363.63 (hetero-C-N). ^{13}C NMR (acetone 400 MHz): 27.184 (C_3 & C_6), 29.80, 29.992, 30.185, 30.377 (C_2, C_4, C_5 & C7-CH_2), 126.289, 126.919 (2-C=C), 151.861 (aromatic carbon), 171.218 (acid group), 206.084 (ketonic group). MS *m/z* (%): 407.50 (M, 31), 408.43 (M+1, 5), 406.34 (M-1, 100), 813.70 (50), 814.68 (28), 815.66 (10).

Synthesis of *m*-PEG attached acridinediones (4a-j): A mixture of acridinedione (**3a-j**, 1 equiv.) in 30 mL of dichloromethane was taken, to which triethylamine (3 equiv.) and thionyl chloride (1.2 equiv.) was added and stirred on a magnetic stirrer for 4 h, followed by addition of mPEG (1 equiv.) and the stirring was continued for another 8 h. The mixture was filtered and heated on a water bath to recover the solid *m*-PEG attached acridinediones (**4a-j**).

***m*-PEG attached 2-(1,2,3,4,5,6,7,8-octahydro-3,3,6,6-tetramethyl-1,8-dioxoacridin (4a):** Dark brown crystal, yield 61.5%, m.p.: 197-199 ºC, IR (KBr, ν_{max}, cm^{-1}): 3171 (arom. -CH), 2926 (alkane-CH), 1400 (alicyclic-CH), 1153 (hetero-C-N), 1634 (C=O). ^{1}H NMR (acetone 400 MHz): δ 1.093-1.104 (2d, 12H, C_3 & C_6-dimethyl), 2.046 (s, 2H, C_4-CH_2), 2.255 (d, 2H, C_2-CH_2), 2.464 (s, 1H, C_9-H). ^{13}C NMR (acetone 400 MHz): 28.83 (C_3 & C_6-CH_3), 40.87 (C_9-CH_2), 40.93, 46.11, 49.66, 50.72 (C_2, C_4, C_5 & C_7-CH_2), 111.38, 111.47 (2-C=C), 189.66 (ketonic group).

***m*-PEG attached 2-(1,2,3,4,5,6,7,8-octahydro-3,3,6,6,9-pentamethyl-1,8-dioxoacridin (4b):** Dark brown crystal, yield 66.5%, m.p.: 203-205 ºC, IR (KBr, ν_{max}, cm^{-1}): 3127 (arom. -CH), 2926 (alkane-CH), 1401 (Alicyclic-CH), 1344 (hetero-C-N), 1595 (C=O). ^{1}H NMR (acetone 400 MHz): δ 1.032-1.079 (2d, 12H, C_3 & C_6-methyl), d1.079 (s, 1H, C_9 -CH_3), 2.046 (s, 2H, C_4-CH_2), 2.287 (d, 2H, C_2-CH_2), 3.104 (s, 1H, C_9-H). ^{13}C NMR (acetone 400 MHz): 27.29 (C_9-CH_3), 29.21 (C_3 & C_6-CH_3), 40.80, 46.04, 46.91, 50.63 (C_2, C_4, C_5 & C_7-CH_2), 121.66, 122.75 (2-C=C), 196.50 (ketonic group).

***m*-PEG attached 2-(9-ethyl-1,2,3,4,5,6,7,8-octahydro-3,3,6,6-tetramethyl-1,8-dioxoacridin (4c):** Dark brown crystal,

yield 66.2%, m.p.: 208-210 ºC, IR (KBr, ν_{max}, cm^{-1}): 3150 (arom. -CH), 1400 (alicyclic-CH), 1312 (hetero-C-N), 1680 (C=O). ^{1}H NMR (acetone 400 MHz): δ 1.035-1.104 (2d, 12H, C_3 & C_6-dimethyl), 1.384 (s, 1H, C_9 -CH_3), 2.076 (s, 2H, C_4-CH_2), 2.244 (d, 2H, C_2-CH_2), 4.584 (s, 1H, C_9-H). ^{13}C NMR (acetone 400 MHz): 10.18, 25.71 (C_9-CH_2, CH_3), 29.21 (C_3 & C_6-CH_3), 32.17, 32.73, 40.08, 41.03 (C_2, C_4, C_5 & C_7-CH_2), 114 (C=C), 197.46 (ketonic group).

***m*-PEG attached 2-(9-ethyl-1,2,3,4,5,6,7,8-octahydro-3,3,6,6-tetramethyl-1,8-dioxoacridin (4d):** Dark brown crystal, yield 61%, m.p.: 215-217 ºC, IR (KBr, ν_{max}, cm^{-1}): 3154 (arom. -CH), 1400 (alicyclic-CH), 1280 (hetero-C-N), 1595 (C=O). ^{1}H NMR (acetone 400 MHz): δ 0.948-1.085 (2d, 12H, C_3 & C_6-dimethyl), 2.177 (s, 2H, C_4-CH_2), 2.549 (d, 2H, C_2-CH_2), 4.684 (s, 1H, C_9-H), 6.898-7.235 (d, 4H, Ar-H). ^{13}C NMR (acetone 400 MHz): 26.963 (C_9 Ar-CH_3), 27.241, 27.459 (C_3 & C_6-CH_3), 33.150, 40, 041, 40.048, 47.732 (C_2, C_4, C_5 & C_7-CH_2), 50.539 (C_9-CH), 114.809 (C=C), 123.472-126.963 (C_9-Ar), 206.062 (ketonic group).

***m*-PEG attached 2-(1,2,3,4,5,6,7,8-octahydro-9-(4-methoxyphenyl)-3,3,6,6-tetramethyl-1,8-dioxoacridin (4e):** Dark brown crystal, yield 64.4%, m.p.: 225-227 ºC, IR (KBr, ν_{max}, cm^{-1}): 3147 (arom. -CH), 1400 (alicyclic-CH), 1239 (hetero-C-N), 1595 (C=O). ^{1}H NMR (acetone 400 MHz): δ 0.974-1.084 (2d, 12H, C_3 & C_6-dimethyl), 2.046 (s, 2H, C_4-CH_2), 2.545 (d, 2H, C_2-CH_2), 4.691 -OCH_3, 5.125 (s, 1H, C_9-H), 6.639-7.268 (d, 4H, Ar-H). ^{13}C NMR (acetone 400 MHz): 27.46, 29.40 (C_3 & C_6-CH_3), 32.80 (-OCH_3), 40.22, 40.99, 49.82, 50.86 (C_2, C_4, C_5 & C_7-CH_2), 113.51, 113.61, 115.78, 115.93 (C_9-Ar), 129.09, 129.43 (2C=C), 196.88 (ketonic group).

***m*-PEG attached 2-(9-(4-chlorophenyl)-1,2,3,4,5,6,7,8-octahydro-3,3,6,6-tetramethyl-1,8-dioxoacridin (4f):** Dark brown crystal, yield 60.6%, m.p.: 188-190 ºC, IR (KBr, ν_{max}, cm^{-1}): 3148 (arom. -CH), 1400 (alicyclic-CH), 1303 (hetero-C-N), 1594 (C=O). ^{1}H NMR (acetone 400 MHz): δ 0.939-0.998 (2d, 12H, C_3 & C_6-dimethyl), 2.126 (s, 2H, C_4-CH_2), 2.562 (d, 2H, C_2-CH_2), 5.150 (s, 1H, C_9-H), 7.109-7.273 (d, 4H, Ar-H). ^{13}C NMR (acetone 400 MHz): 27.184 (C_3 & C_6-CH_3), 40.264, 41.061, 47.608, 50.562 (C_2, C_4, C_5 & C_7-CH_2), 51.141 (C_9-CH) 126.289, 126.919 (2C=C), 128.335-129.289 (C_9-Ar), 206.084 (ketonic group).

***m*-PEG attached 2-(1,2,3,4,5,6,7,8-octahydro-9-(4-hydroxyphenyl)-3,3,6,6-tetramethyl-1,8-dioxoacridin (3g):** Dark brown crystal, yield 63%, m.p.: 194-196 ºC, IR (KBr, ν_{max}, cm^{-1}): 3412.42 (arom. -CH), 1361.19 (alicyclic-CH), 1115.86 (hetero-C-N), 1653.87 (C=O). ^{1}H NMR (acetone 400 MHz): δ 0.967-1.087 (2d, 12H, C_3 & C_6-dimethyl), 2.076 (s, 2H, C_4-CH_2), 2.556 (d, 2H, C_2-CH_2), 4.645 (s, 1H, C_9-H), 5.607 (s, Ar-OH), 7.04-7.10 (d, 4H, Ar-H). ^{13}C NMR (acetone 400 MHz): 45.69, 45.96 (C_3 & C_6-CH_3), 76.57, 76.84, 77.16, 77.16 (C_2, C_4, C_5 & C_7-CH_2), 128.28 (C=C).

***m*-PEG attached 2-(1,2,3,4,5,6,7,8-octahydro-3,3,6,6-tetramethyl-9-(3-nitrophenyl)-1,8-dioxoacridin (3h):** Dark brown crystal, yield 65.9%, m.p.: 242-244 ºC, IR (KBr, ν_{max}, cm^{-1}): 3128 (arom. -CH), 1401 (alicyclic-CH), 1342 (hetero-C-N), 1595 (C=O). ^{1}H NMR (acetone 400 MHz): δ 0.939-0.971 (2d, 12H, C_3 & C_6-dimethyl), 2.229 (s, 2H, C_4-CH_2), 2.559 (d, 2H, C_2-CH_2), 5.150 (s, 1H, C_9-H), 7.273-7.557 (d, 4H, Ar-H). ^{13}C NMR (acetone 400 MHz): 27.32 (C_3 & C_6-CH_3), 40.87, 46.10, 47.12, 50.75 (C_2, C_4, C_5 & C_7-CH_2), 115.64 (2-C=C), 126.39-128.38 (aromatic carbon), 196.60 (ketonic group).

***m*-PEG attached 2-(1,2,3,4,5,6,7,8-octahydro-3,3,6,6-tetramethyl-1,8-dioxo-9-(thiophen-2-yl)acridin-10(9*H*)-yl)acetic acid (3i):** Dark brown crystal, yield 67.2%, m.p.: 231-233 ºC, IR (KBr, ν_{max}, cm^{-1}): 3404.77 (aromatic-CH), 1472.50 (Alicyclic-CH), 1357.80 (hetero-C-N), 1634.85 (C=O). ^{1}H NMR (acetone 400 MHz): δ 1.032-1.198 (2d, 12H, C_3 & C_6-dimethyl), 2.200 (s, 2H, C_4-CH_2), 2.207-2.300 (d, 2H, C_2-CH_2), 3.555 (s, 1H, C_9-H), 7.260-7.732 (d, 4H, Ar-H). ^{13}C NMR (acetone 400 MHz): 27.241 (C_3 & C_6-CH_3), 40.04, 41.047, 47.732, 50.539 (C_2, C_4, C_5 & C_7-CH_2), 114.807 (2-C=C), 123.6, 126.9 (aromatic carbon), 195.171 (ketonic group).

***m*-PEG attached 2-(1,2,3,4,5,6,7,8-octahydro-3,3,6,6-tetramethyl-1,8-dioxo-9-phenylacridin (4j):** Dark brown crystal, yield 68.8%, m.p.: 211-213 ºC, IR (KBr, ν_{max}, cm^{-1}): 3450.91 (arom. -CH), 1381.96 (hetero-C-N), 1634.38 (C=O). ^{1}H NMR (acetone 400 MHz): δ 0.953-0.973 (2d, 12H, C_3 & C_6-dimethyl), 2.136 (s, 2H, C_4-CH_2), 2.652 (s, 2H, C_2-CH_2), 4.674 (s, 1H, C_9-H), 5.106 (s, Ar-OH), 7.047-7.069 (d, 4H, Ar-H). ^{13}C NMR (acetone 400 MHz): 27.922 (C_3 & C_6-CH_2), 32.665, 32.896, 33.083, 40.264 (C_2, C_4, C_5 & C_7-CH_2), 115.985 (2-C=C), 126.288, 126.919 (aromatic carbon), 196.235 (ketonic group).

Biological screening

***in vitro* Antimicrobial studies** [25]**:** The *in vitro* antibacterial activity of the synthesized compounds (**4a-j**) was evaluated by a disc diffusion test against four pathogenic bacteria, *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. The stock solution was prepared by weighing 10 mg of each compound by dissolving in DMSO. All the subsequent dilutions of the stock solutions were prepared with the same solvent. Sabouraud Agar media obtained from Himedia for bacteria subculture was used for determining the zone of inhibition of synthesized compounds. The inoculum was prepared by using a loop or swab, transfer the colonies to equal that of a McFarland 0.5 turbidity standard that has been vortexes. Dip a sterile cotton swab into the inoculum and rotate it against the wall of the tube above the liquid to remove the excess inoculums. Swab enters the surface of the agar plate three times by rotating the plates approximately 60 ºC between streaking to ensure even distribution. Allow the inoculated plate to stand for 3 min before making the wall. Five walls were made by using a hollow tube of 5 mm diameter and then with the help of micropipette 75, 50, 25, 10 and 5 µL sample solution was added in each wall. Then plates were incubated for 18-24 h at 37 ºC in an incubator.

***in vitro* Antioxidant assay by DPPH method:** The present study describes the 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity of the synthesized compounds (**4a-j**). All the synthesized compounds were evaluated for antioxidant activity by the DPPH assay method [26]. In brief, sample stock solutions (1.0 mg/mL) were diluted to final concentrations of 25, 50, 75, 100 µg/mL, in ethanol. An ethanolic solution of 1 mL of

DPPH (0.3 mM) was added to 0.5 mL of compound and allowed to react at room temperature in a dark place for 0.5 h. After 0.5 h, the absorbance values were measured at 517 nm. All the measurements were taken as triplicate values from the average of the absorbance values; lower absorbance of the reaction mixture indicates higher free radical scavenging activity. All the antioxidant scavenging activities of the DPPH radical was calculated according to the following equation:

$$\text{Inhibition (\%)} = \frac{ABS_{control} - ABS_{test}}{ABS_{control}} \times 100$$

where, control = absorbance of ethanol + DPPH, test = absorbance of DPPH + compound/standard.

All the experiments were repeated to check their accuracy. The percentage antioxidant activity of the tested compounds was calculated.

***in vitro* Anticancer studies by MTT assay:** The MTT assay was determined by using human cervical carcinoma cell line (HeLa) [27] and it was obtained from the National Cancer Institute (NCI), Pune, India. The cells were seeded 96-well flat-bottom microplate and maintained at 37 ºC in 95% humidity and 5% CO_2 overnight. Different concentration (400, 200, 100, 50, 25, 12.5 µg/mL) of sample were treated. The cells were incubated for another 48 h and the wells were washed twice with PBS to removed the unblended dyes. Then 20 µL of MTT staining solution was added to each well and the plate was incubated at 37 ºC. After 4 h, 100 µL of DMSO was added to each well to dissolve the formazan crystals and absorbance was measured with a 570 nm using a microplate reader. The IC_{50} of the compounds were calculated using Graphpad Prism software version 5.1. The mean cell viability was calculated by using the formula:

$$\text{Surviving cell (\%)} = \frac{\text{Mean OD of test compound}}{\text{Mean OD of negative control}} \times 100$$

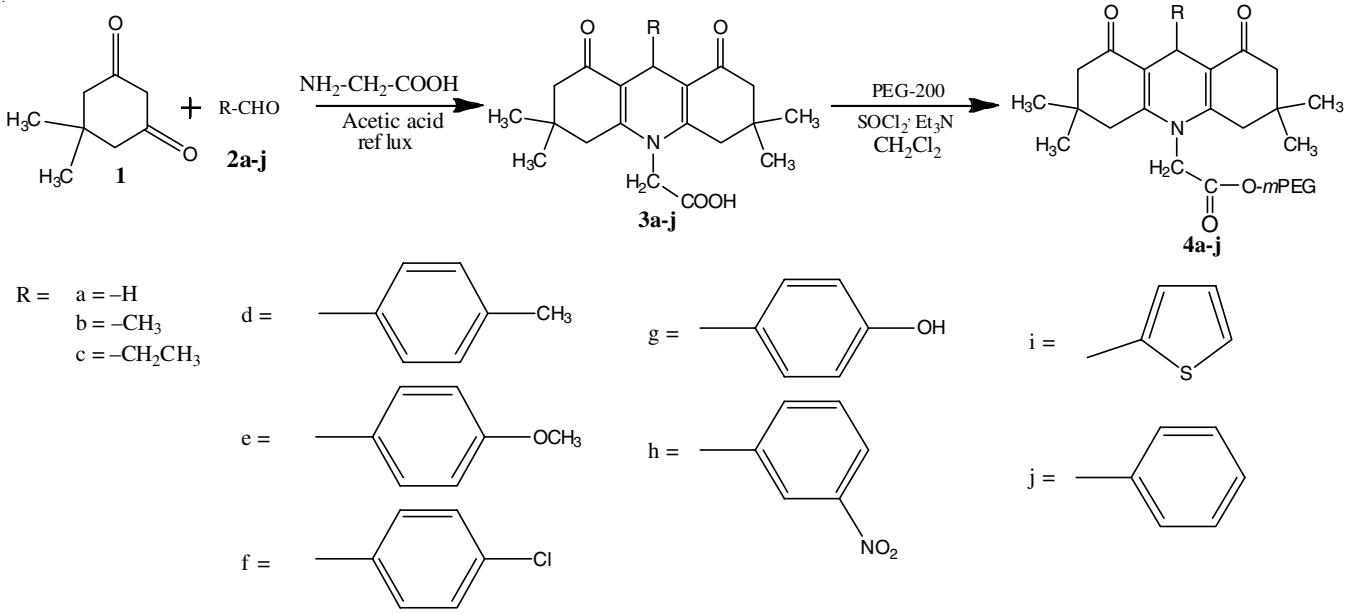
RESULTS AND DISCUSSION

A range of novel acridine substituted derivatives were synthesized using a multistep synthetic methodology in the present study (**Scheme-I**). Initially, 5,5-dimethyl cyclohexane-1,3-dione (**1**) and different substituted aldehydes were treated with glycine to produce acridinediones (**3a-j**). Further reaction *m*-PEG in alkaline medium afforded *m*-PEG attached acridinedione derivatives (**4a-j**).

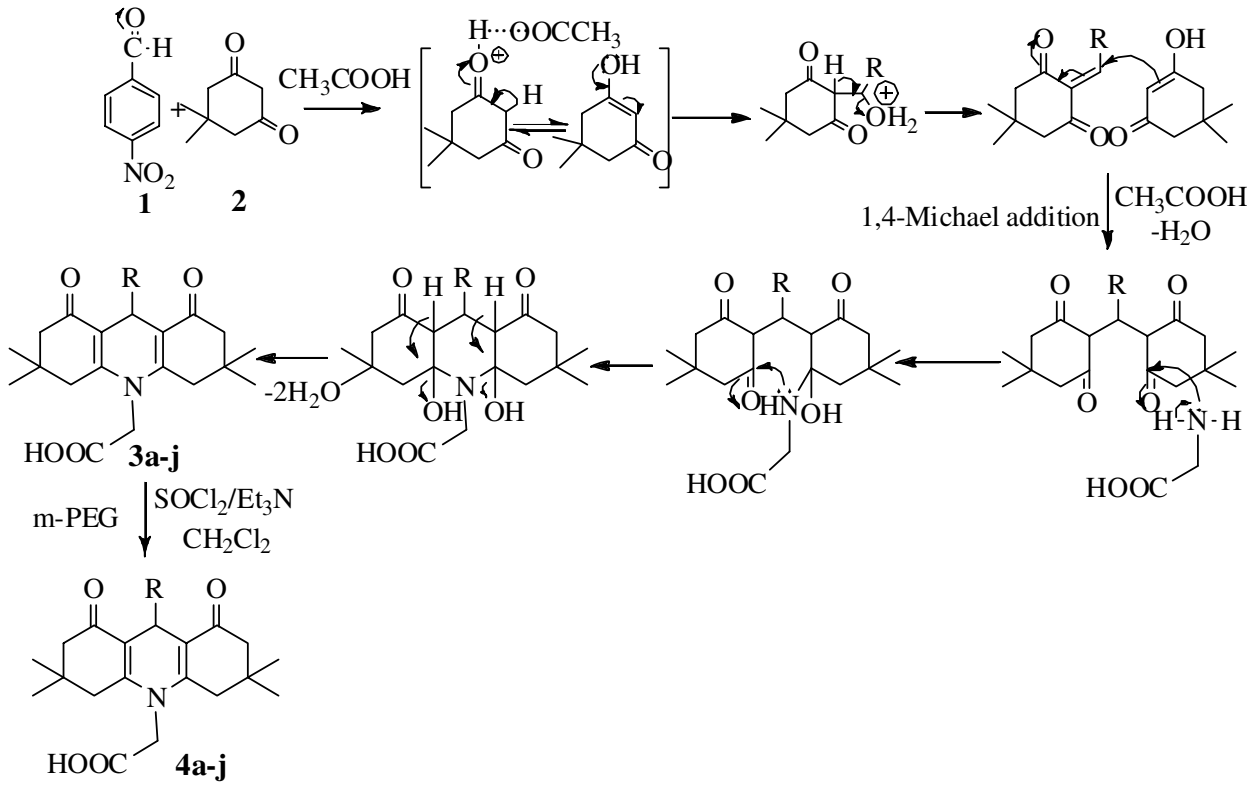
The IR spectra of all the synthesized compounds showed some characteristic peaks indicating the presence of various anticipated functional groups, namely the stretching frequency observed in the range of 3150-3050 cm^{-1} due to aromatic-CH, 3000-2850 cm^{-1} due to alkane-CH, 1465 cm^{-1} due to alicyclic-CH, 1350-1000 cm^{-1} due to hetero-C-N and 1725-1705 cm^{-1} due to C=O group. Similarly, the appearance of peaks in ^{1}H NMR spectra at δ 1.2-1.8 was attributed to *gem*-dimethyl protons; δ 2.0-4.0 due to methylene protons and δ 7.1-7.9 due to aromatic protons. Additionally, ^{13}C NMR spectra of the compounds confirmed the structure of synthesized acridinediones. Elemental analysis of all synthesized compounds was found to be within ± 0.4 % concerning the calculated value which confirmed the purity of synthesized compounds. A probable mechanism for the formation of acridinediones is given in **Scheme-II**.

Antimicrobial activity: The antimicrobial screening of newly synthesized acridinedione derivatives (**4a-j**) showed significant antimicrobial activity especially at the dose of 75 and 50 µg/mL as evidenced from their zone of inhibition against standard drug ciprofloxacin. Among all the synthesized compounds **4d**, **4e** and **4g** were significantly active against Gram-positive and Gram-negative microorganisms, whereas **4c** showed effective action against Gram-positive organisms. The detailed results are shown in Table-1.

Antioxidant activity: All the newly synthesized *m*-PEG attached acridinedione (**4a-j**) were screened for *in vitro* antioxidant activity by DPPH method at the concentration of 25,



Scheme-I: Proposed mechanism for the development of acridine derivatives (**3a-j** & **4a-j**)



Scheme-II: Mechanistic pathway of tetrahydro acridinedione compounds (**4a-j**)

TABLE-1
in vitro ANTIMICROBIAL ACTIVITIES OF THE SYNTHESIZED COMPOUNDS (**4a-j**)

Comp.	Zone of inhibition (mm)																			
	Staphylococcus epidermidis (Gram-positive)					*Staphylococcus aureus* (Gram-positive)					*Escherichia coli* (Gram-negative)					*Pseudomonas aeruginosa* (Gram-negative)				
	µL/mL					µL/mL					µL/mL					µL/mL				
	75	50	25	10	5	75	50	25	10	5	75	50	25	10	5	75	50	25	10	5
4a	12	R	R	R	R	10	R	R	R	R	12	R	R	R	R	12	10	R	R	R
4b	13	R	R	R	R	12	R	R	R	R	15	R	R	R	R	12	R	R	R	R
4c	10	R	R	R	R	15	10	R	R	R	13	R	R	R	R	12	10	R	R	R
4d	13	10	R	R	R	12	10	R	R	R	12	10	R	R	R	R	R	R	R	R
4e	12	R	R	R	R	10	8	R	R	R	10	8	R	R	R	13	R	R	R	R
4f	12	R	R	R	R	10	R	R	R	R	13	10	R	R	R	10	R	R	R	R
4g	12	10	R	R	R	12	R	R	R	R	12	R	R	R	R	13	10	R	R	R
4h	10	R	R	R	R	10	R	R	R	R	15	13	R	R	R	12	R	R	R	R
4i	12	R	R	R	R	12	R	R	R	R	10	R	R	R	R	10	R	R	R	R
4j	10	R	R	R	R	10	R	R	R	R	12	R	R	R	R	12	R	R	R	R
Cip.	32					38					25					21				

Cip. = Ciprofloxacin; S = sensitive, R = Resistant.

50, 75 and 100 µg/mL. DMSO was used as a solvent and the results are summarized in Table-2. Compounds **4d** and **4i** showed significant radical scavenging activity, due to the presence of electron-donating *p*-tolyl group (**4d**) and thiophene (**4i**) at 9th position of acridine skeleton and results were compared with the standard drug ascorbic acid. Compounds **4a-c**, **4e-g** and **4j** showed moderate to mild antioxidant activity while compound **4h** showed the minimum antioxidant activity. In general, it was observed that acridinediones with an electron-donating group at 9th position greater antioxidant activity.

Anticancer activity: The newly synthesized *m*-PEG attached acridinedione compounds (**4a-j**) were screened for their anticancer activity against cervical HeLa cells by using the MTT assay. The different concentrations (400, 200, 100, 50, 25, 12.5 µg/mL) were used to test anticancer activity and the relation between the surviving fraction and drug concentration was plotted to get the survival curve of each cancer cell line after a specified time. The concentration required for 50% inhibition of cell bility (IC_{50}) was calculated and compared with the reference drug paclitaxel and the results are given in Table-3.

TABLE-2
in vitro[ab] ANTIOXIDANT ACTIVITY BY DPPH ASSAY METHOD FOR COMPOUNDS (**4a-j**)

Compound		Concentration (µg/mL)				
		25	50	75	100	IC_{50}
4a	Moderate –	43.12 ± 0.12	47.22 ± 0.24	48.12 ± 0.23	50.23 ± 0.15	23.13 ± 4.1
4b	Moderate (-CH_3)	40.40 ± 0.33	48.20 ± 0.25	49.41 ± 0.34	51.12 ± 0.16	24.10 ± 2.1
4c	Moderate (-CH_3CH_2)	43.12 ± 0.21	47.31 ± 0.33	48.12 ± 0.23	50.24 ± 0.15	27.13 ± 4.1
4d	Significant (Ar-*p*CH_3)	53.13 ± 0.31	55.5 ± 0.51	63.53 ± 0.30	67.17 ± 0.17	19.11 ± 1.3
4e	Moderate (Ar-*p*OCH_3)	43.12 ± 0.21	47.31 ± 0.33	48.12 ± 0.23	50.24 ± 0.15	27.13 ± 4.1
4f	Moderate (Ar-*p*Cl)	40.40 ± 0.33	48.20 ± 0.25	49.41 ± 0.34	51.12 ± 0.16	24.10 ± 2.1
4g	Moderate (Ar-*p*OH)	43.12 ± 0.21	47.31 ± 0.33	48.12 ± 0.23	50.24 ± 0.15	27.13 ± 4.1
4h	Least (Ar-*m*NO_2)	24.40 ± 0.33	48.20 ± 0.24	49.41 ± 0.34	51.12 ± 0.16	24.10 ± 2.1
4i	Significant (Hetero)	52.11 ± 0.21	54.9 ± 0.41	61.51 ± 0.40	66.41 ± 0.11	18.11 ± 1.5
4j	Moderate (Ar)	42.11 ± 0.20	48.20 ± 0.21	49.41 ± 0.30	51.12 ± 0.10	24.10 ± 2.0
Blank		–	–	–	–	–
Standard (ascorbic acid)		77.15 ± 0.14	84.33 ± 0.32	89.19 ± 0.11	91.20 ± 0.61	17.09 ± 1.1

[a]Values are expressed as mean ± SEM; [b]Data represent the mean ± SEM values of these independent determinations.

TABLE-3
IC_{50} VALUE OF THE SYNTHESIZED COMPOUNDS (**4a-j**) IN µg/mL

Sample code	HeLa
4a	98.32
4b	159.30
4c	343.40
4d	99.72
4e	155.30
4f	456.20
4g	93.85
4h	157.80
4i	203.20
4j	207.40
Paclitaxel (PTX)	0.23
	196.98 µg/mL

Table-4 reveals that the compounds (**4a**, **4d** & **4g**) showed significant activity towards the HeLa cell lines with the IC_{50} value of 98.32, 99.72 and 93.85 µg/mL. Compounds (**4a**, **4d** and **4g**) showed that the activity was reasonably good when compared to NO_2, halogen group substituted derivatives. The observed data concluded that both aliphatic side chain substitution and heterocyclic ring such as thiophene on acridinone showed excellent enhanced anticancer activity. So, it was considered as a most potent analog.

Conclusion

The *m*-PEG attached acridinedione derivatives were designed and synthesized while remembering the fact that a majority of clinically active antimicrobial, antioxidant and anticancer compounds possess a nitrogen heteroaromatic system with various substitutions, at least one carbonyl group in their structure and the presence of hydrogen donor/acceptor unit. The structure of these compounds (**4a-j**) satisfied all the pharmacophoric structural requirements that are, presence of phenyl moiety as a hydrophobic portion, "*N*" as an electron donor system, responsible for controlling the pharmacokinetic properties.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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TABLE-4
CALCULATED MEAN CELL VIABILITY OF COMPOUNDS (**4a-j**)

Concentration (µg/mL)	Mean cell viability (HeLa)									
	4a	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**	**4h**	**4i**	**4j**
400	30.07	38.77	44.93	25.54	28.26	45.47	29.71	26.63	30.27	32.72
200	35.69	42.94	58.15	44.75	41.12	65.22	34.42	53.99	42.45	44.29
100	47.65	59.24	81.16	58.88	62.14	66.12	47.83	63.59	62.64	68.68
50	69.93	68.30	82.97	60.33	79.17	71.20	62.32	66.67	79.21	80.43
25	70.83	69.57	85.87	63.95	84.78	76.99	72.10	69.02	83.73	84.16
12.5	70.65	77.90	87.50	66.67	91.12	78.44	78.80	80.25	87.54	88.18
Negative control	100									

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