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Synthesis, Characterization and Screening of Novel 5,6-Dihydroacridine Derivatives as Potent Antidiabetic and Antioxidant Agent

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Abstract: Acridine is an important nucleus in heterocyclic compounds which possess a wide spectrum of pharmacological activities. In this present work 10-Chloro-5,6-dihydro-12-phenylpyrimido[4,5-a]acridin-2-amine derivatives (PD-1 to PD-5) and 2-Amino-10-chloro-1,4,5,6-tetrahydro-12-phenylbenzo-[1,7]phenanthroline derivatives (PD-6 to PD-10) were synthesized from *(E)*-2-Benzylidene-7-chloro-3,4-dihydro-9-phenylacridin-1(2H)-one with guanidine carbonate and malononitrile. All the synthesized compounds have been characterized by using elemental analysis, FT-IR, ^{1}H NMR, ^{13}C NMR spectroscopy and further supported by mass spectroscopy. Purity of all the compounds has been checked on thin layer chromatographic plate and HPLC technique. All the synthesized compounds were tested for *in vitro* anti-diabetic and anti-oxidant activities. These compounds can be further exploited to get the potent lead compounds. The detailed synthesis and the anti-diabetic and anti-oxidant screening of the new compounds are reported.

INTRODUCTION

The synthesis of heterocyclic compounds has always drawn the attention of chemists over the years mainly because of their important biological properties. The heterocyclic compound acridine is one of the most attractive frameworks with a wide range of biological and pharmacological activities. This physiologically important nucleus is abundantly found in therapeutic agents. Many researchers have described synthesis of acridine and its derivatives along with its applications in literature. A large number of heterocyclic compounds containing the acridine ring are associated with diverse pharmacological properties such as Analgesic (Sham M. Sondhi, 2004), anti-amyloid (Antosova A, 2011), Antibacterial (Nezar L, 2013), Antimalarial (Shibnev VA, 1998), Antifungal (Mehul M. Patel, 2010), Anti-inflammatory (Chandra T, 2010 & M.Sondhi, Sham, 2002), Anticancer (Surbhi Arya, 2015), Antiviral (Lyakhov SA, 2010 & Tonelli M, 2011), Anthelminthic (Chengpao Cao, 2013), Antihypertensive (Sangita Makone, 2015), Cardiovascular (Wang J, 2013), Antioxidant (R. Kalirajan, 2012). Considering the above observations and in connection to previous publications involving the synthesis of new biologically active heterocycles. Thus the efficient synthesis novel series of 5,6-dihydroacridine fused with pyridine and pyrimidine derivatives still represent highly pursued target.

MATERIAL AND METHODS

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV light. All compounds were purified by recrystallization with suitable organic solvents. IR spectra were recorded on Brooker-ALPHA FT-IR instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using direct inlet probe technique. ^{1}H NMR and ^{13}C NMR was determined in $CDCl_3$ solution on a Bruker Ac 400 MHz spectrometer. Purity of the synthesized compounds was checked by HPLC Agilent. The results are in agreements with the structures assigned. Elemental analysis of the all the synthesized compounds was carried out on Euro EA 3000 elemental analyzer and the results are in agreements with the structures assigned.

Preparation of *(E)*-2-benzylidene-7-chloro-3,4-dihydro-9-phenylacridin-1(2H)-one derivatives: The reaction involves condensation of equimolar quantities of 7-chloro-3,4-dihydro-9-phenylacridin-1(2H)-one with a substituted aromatic aldehydes in the presence of alcoholic potassium hydroxide (KOH) solution, resulting in the formation of α, β-unsaturated ketones. The substituted α, β-unsaturated ketones were prepared by stirring a solution of 2.75 g KOH in 20 ml ethanol. The round bottom flask was immersed in crushed ice and 1 m.mol (0.307 g) of 7-chloro-3,4-dihydro-9-phenylacridin-1(2H)-one were added. Exactly 1 m.mol of substituted aromatic aldehydes was added

with stirring at below 25^{0}C for 7–8 hours. Cool the reaction mixture and poured into crushed ice and neutralized with dilute HCl solution. Filter and left to dry. The crude product was separated by column chromatography with ethyl acetate-petroleum ether (1:9) as eluent.

Preparation of 10-Chloro-5,6-dihydro-12-phenylpyrimido[4,5-a]acridin-2-amine derivatives (PD-1 TO PD-5):10-Chloro-5,6-dihydro-12-phenylpyrimido[4,5-a]acridin-2-amine derivatives were synthesized by the mixing of *(E)*-2-benzylidene-7-choloro-3,4-dihydro-9-phenylacridin-1(2*H*)-ones (2.08 gm, 0.01 mol) with guanidine carbonate (0.9008 g, 0.01 mol) and 10 % alc. NaOH (1 g in 10 ml ethanol) and then heated under reflux condition for 5 h. After the completion of the reaction, reaction mixture was cooled and poured into crushed ice. The crude product was separated by column chromatography using ethyl acetate and petroleum ether to get the target derivatives. All synthesized derivatives and their physical data were summarized in Table 1.

10-Chloro-4-(3,4-dimethoxyphenyl)-12-phenyl-5,6-dihydropyrimido[4,5-*a*]acridin-2-amine (PD-1): Yellow solid; M.F: $C_{29}H_{23}ClN_4O_2$; Yield 80 %; M.P: 218-220^{0}C; FT-IR (KBr) ν_{max} (cm^{-1}): 3446 (-NH2), 2931-2958 (-OCH_3); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm), 3.03-3.06 (dd, *J* = 5.6 Hz, *J* = 4.8 Hz, 2H, -CH_2), 3.18-3.21 (dd, *J* = 4.4 Hz, *J* = 5.6 Hz, 2H, -CH_2), 3.92 (s, 3H, -OCH_3), 3.93 (s, 3H, -OCH_3), 4.34 (s, 2H, -NH_2), 6.93-6.95 (d, *J* = 8 Hz, 1H), 7.11 (d, *J* = 2 Hz, 1H), 7.13 (d, *J* = 2 Hz, 1H), 7.15 (d, *J* = 2 Hz, 1H), 7.23-7.26 (m, 2H), 7.44-7.50 (m, 2H), 7.61 (d, *J* = 2.4 Hz, 1H), 7.64 (d, *J* = 2.4 Hz, 1H), 7.66 (d, *J* = 2.4 Hz, 1H); ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm), 24.23, 34.07, 56.11, 56.15, 110.69, 112.12, 118.59, 121.97, 125.24, 126.33, 2×127.43, 128.02, 128.90, 2×129.49, 130.37, 130.68, 131.20, 132.18, 137.94, 146.27, 146.87, 149.02, 150.16, 160.50, 160.78, 161.03, 165.35; Exact Mass: 494.15; Found ESI-MS m/z: 495.52[M+1].

10-chloro-4-(2,5-dimethoxyphenyl)-12-phenyl-5,6-dihydropyrimido[4,5-*a*]acridin-2-amine (PD-2): White solid; M.F: $C_{29}H_{23}ClN_4O_2$; Yield 76 %; M.P: 212-214^{0}C; FT-IR (KBr) ν_{max} (cm^{-1}): 3485 (-NH_2), 2931-2953 (-OCH_3); 1H NMR (400 MHz, $CDCl_3$): δ (ppm), 2.64 (d, *J* = 5.4 Hz, 1H, -CH_2), 2.87 (d, *J* = 4.8 Hz, 1H, -CH_2), 3.14-3.22 (dd, *J* = 6.8 Hz, *J* = 17.2 Hz, 2H, -CH_2), 3.75 (s, 3H, -OCH_3), 3.79 (s, 3H, -OCH_3), 4.30 (s, 2H, -NH_2), 6.89-6.97 (m, 3H), 7.31-7.32 (d, *J* = 4.8 Hz, 1H), 7.46 (m, 3H), 7.57-7.58 (d, *J* = 2.4 Hz, 1H), 7.62-7.63 (d, *J* = 2.4 Hz, 1H), 7.65 (d, *J* = 2.4 Hz, 1H), 7.99-8.01 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm), 23.01, 33.62, 55.80, 56.00, 112.18, 115.43, 115.82, 120.54, 124.98, 126.27, 127.19, 2×127.89, 128.86, 3x130.17, 131.05, 131.98, 138.08, 146.06, 146.95, 150.66, 153.76, 159.41, 2×160.40, 161.29, 163.73; Exact Mass: 494.15; Found ESI-MS m/z 495.35 [M+1].

10-Chloro-4-(3-methoxyphenyl)-12-phenyl-5,6-dihydropyrimido[4,5-*a*]acridin-2-amine (PD-3): Yellow solid; M.F: $C_{28}H_{21}ClN_4O$; Yield 70 %; M.P: 168-170^{0}C; FT-IR (KBr) ν_{max} (cm^{-1}): 3475 (-NH_2), 2924-2960 (-OCH_3); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm), 2.39- 2.40 (m, 2H, -CH_2), 2.71-2.83 (m, 2H, -CH_2), 3.82 (s, 3H, -OCH_3), 4.83 (s, 2H, - NH_2), 6.91 (d, *J* = 1.2 Hz, 1H), 6.92-6.93 (s, 1H), 6.97-6.99 (d, *J* = 7.2 Hz, 1H), 7.02- 7.04 (d, *J* = 8.4 Hz, 1H), 7.12-7.14 (m, 1H), 7.15 (m, 1H), 7.20-7.22 (m, 2H), 7.24 (m, 2H), 7.31-7.33 (d, *J* = 8.4 Hz, 1H), 7.34-7.36 (d, *J* = 7.2 Hz, 1H); ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm), 23.79, 33.85, 55.38, 114.13, 115.03, 118.52, 121.11, 124.98, 126.23, 127.29, 2×127.90, 128.79, 129.35, 2×129.41, 130.26, 131.11, 132.07, 137.87, 139.30, 146.17, 146.89, 159.58, 160.40, 160.69, 160.93, 165.48; Exact Mass: 464.14; Found ESI-MS m/z: 467.31 [M+3].

10-Chloro-4-(2-chlorophenyl)-12-phenyl-5,6-dihydropyrimido[4,5-*a*]acridin-2-amine (PD-4):Light yellow; M.F: $C_{27}H_{18}Cl_2N_4$; Yield 70 %; M.P: 238-240^{0}C; FT-IR (KBr) ν_{max} (cm^{-1}): 3481 (-NH_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm), 3.12-3.16 (m, 4H, 2-CH_2), 5.72 (s, 2H -NH_2), 7.26-7.27 (d, *J* = 7.2 Hz, 2H), 7.37 (s, 1H), 7.41-7.44 (m, 1H). 7.46-7.51 (m, 5H), 7.56-7.58 (d, *J* = 7.6 Hz, 1H), 7.79-7.81 (dd, *J* = 2 Hz, *J* = 2 Hz, 1H), 8.04-8.06 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm), 23.81, 33.75, 118.43, 124.85, 126.25, 127.33, 2×127.93, 128.60, 128.76, 3×129.30, 2x130.23, 131.22, 132.14, 135.42, 136.36, 137.76, 146.18, 147.00, 160.41, 2×160.70, 160.91, 164.31; Exact Mass: 468.09; Found ESI-MS m/z: 469.23 [M+1].

10-Chloro-4-(4-chlorophenyl)-12-phenyl-5,6-dihydropyrimido[4,5-*a*]acridin-2-amine (PD-5): White solid; M.F: $C_{27}H_{18}Cl_2N_4$; Yield 72 %; M.P: 210-212^{0}C; FT-IR (KBr) ν_{max} (cm^{-1}): 3495 (-NH_2); 1H NMR (400 MHz, $CDCl_3$): δ (ppm), 2.97-3.00 (dd, *J* = 6 Hz, *J* = 4.8 Hz, 2H, -CH_2), 3.18-3.21 (dd, *J* = 4.4 Hz, *J* = 6 Hz, 2H, -CH_2), 4.35 (s, 2H -NH_2), 7.23-7.26 (m, 2H), 7.43-7.51 (m, 7H), 7.60 (d, *J* = 2 Hz, 1H), 7.64-7.65 (d, *J* = 2.4 Hz, 1H), 7.66-7.67 (d, *J* = 2.4 Hz, 1H); ^{13}C NMR (400 MHz, $CDCl_3$): δ (ppm), 23.80, 33.76, 118.42, 124.84, 126.25, 127.34, 2×127.93, 128.61, 128.77, 3×129.30, 2×130.22, 131.22, 132.14, 135.43, 136.37, 137.77, 146.19, 147.00, 160.40, 2x160.71, 160.92, 164.31; Exact Mass: 468.09; Found ESI-MS m/z: 470.03 [M+2].

Preparation of 2-Amino-10-chloro-1,4,5,6-tetrahydro-12-phenylbenzo-[1,7]phenanthroline

derivatives (PD-6 TO PD-10): *(E)*-2-Benzylidene-7-chloro-3,4-dihydro-9-phenylacridin-1(2H)-one derivatives were subjected with malononitrile (0.66 gm, 0.01 mol) and ammonium acetate (6.16 gm, 0.08 mol) were dissolved in absolute ethanol (25 ml) was heated under reflux for 5 h. After completion of the reaction, the reaction mixture was cooled and poured into crushed ice. The crude products were separated by column chromatography using ethyl acetate and petroleum ether to get the target derivatives. All synthesized derivatives and their physical data were summarized in Table 1.

2-Amino-10-chloro-4-(3,4-dimethoxyphenyl)-12-phenyl-1,4,5,6-tetrahydrobenzo[*j*] [1,7]phenanthroline-3-carbonitrile (PD-6): Yellow solid; M.F: $C_{31}H_{25}ClN_4O_2$; Yield 80 %; M.P: 230-232^{0}C; FT-IR (KBr) ν_{max} (cm^{-1}): 3450 (-NH2), 3330 (-NH sym), 2192 (- C=N); ^{1}H NMR (DMSO-d6): δ (ppm), 2.04- 2.12 (m, 1H, $-CH_2$), 2.34-2.40 (m, 1H, CH_2), 2.91-2.97 (m, 1H, $-CH_2$), 3.06-3.12 (m, 1H, $-CH_2$), 3.45 (s, 2H, NH_2), 3.68 (s, 3H, $-OCH_3$), 3.72 (s, 3H, $-OCH_3$), 4.10 (s, 1H, -CH), 4.90 (s, 1H, -NH), 6.74-6.76 (d, *J* = 7.6 Hz, 2H), 6.88-6.94 (d, *J* = 8 Hz, 1H), 7.23-7.23 (d, *J* = 2.4 Hz, 1H), 7.35-7.41 (m, 2H), 7.54-7.59 (m, 3H), 7.71-7.75 (dd, *J* = 2.4 Hz, *J* = 7.2 Hz, 1H), 7.91-7.99 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (DMSO-d6): δ (ppm), 23.93, 32.82, 42.76, 55.93, 55.98, 60.22, 110.97, 111.91, 117.91, 119.38, 120.20, 120.62, 125.38, 127.57, 127.93, 128.19, 128.43, 128.97, 129.04, 130.16, 130.35, 132.32, 135.03, 138.59, 140.30, 140.71, 144.92, 148.65, 149.38, 157.88, 158.47; Exact Mass: 520.17; Found ESI-MS m/z: 522.1 [M+2].

2-Amino-10-chloro-4-(2,5-dimethoxyphenyl)-12-phenyl-1,4,5,6-tetrahydrobenzo[*j*] [1,7]phenanthroline-3-carbonitrile (PD-7): White solid; M.F: $C_{31}H_{25}ClN_4O_2$; Yield 76 %; M.P: 238-240^{0}C; FT-IR (KBr) ν_{max} (cm^{-1}): 3452 ($-NH_2$), 3329 (-NH sym), 2198 (- C=N); ^{1}H NMR (DMSO-d_6): δ (ppm), 2.04- 2.11 (m, 1H, $-CH_2$), 2.33-2.39 (m, 1H, - CH_2), 2.88-2.96 (m, 1H, $-CH_2$), 3.07-3.14 (m, 1H, $-CH_2$) 3.33 (s, 2H, $-NH_2$), 3.73 (s, 3H, $-OCH_3$), 3.79 (s, 3H, $-OCH_3$), 4.49 (s, 1H, -CH), 4.86 (s, 1H, -NH), 6.63 (d, *J* = 3.2 Hz, 1H), 6.80-6.83 (dd, *J* = 3.2 Hz, *J* = 2.8 Hz, 1H), 6.95-6.98 (d, *J* = 9.2 Hz, 1H), 7.20-7.21 (d, *J* = 2.4 Hz, 1H), 7.36-7.37 (d, *J* = 3.2 Hz, 2H), 7.54-7.60 (m, 3H), 7.71- 7.73 (dd, *J* = 2 Hz, *J* = 2 Hz, 1H), 7.97-7.99 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (DMSO-d_6): δ (ppm), 23.93, 32.82, 42.76, 55.93, 55.98, 60.22, 110.97, 111.91, 117.91,119.38, 120.20, 120.62, 125.38, 127.57, 127.93, 128.19, 128.43, 128.97, 129.04, 130.16, 130.35, 132.32, 135.03, 138.59, 140.30, 140.71, 144.92, 148.65, 149.38, 157.88, 158.47; Exact Mass: 520.17; Found ESI-MS m/z: 522.1 [M+2].

2-Amino-10-chloro-4-(3-methoxyphenyl)-12-phenyl-1,4,5,6-tetrahydrobenzo[*j*][1,7] phenanthroline-3-carbonitrile (PD-8): White solid; M.F: $C_{30}H_{23}ClN_4O$; Yield 80 %; M.P: 140-142^{0}C; FT-IR (KBr) ν_{max} (cm^{-1}): 3446 ($-NH_2$), 3325 (-NH sym), 2193 (- C=N); ^{1}H NMR (DMSO-d_6): δ (ppm), 2.03- 2.11 (m, 1H, $-CH_2$), 2.35-2.43 (m, 1H, $-CH_2$), 2.89-2.96 (m, 1H, $-CH_2$), 3.08-3.14 (m, 1H, $-CH_2$), 3,33 (s, 2H, $-NH_2$), 3.74 (s, 3H, $-OCH_3$), 4.11 (s, 1H, -CH), 4.93 (s, 1H, - NH), 6.77-6.95 (m, 3H), 7.21-7.22 (d, *J* = 2.4 Hz, 2H), 7.25-7.30 (t, *J* = 7.8 Hz, 1H), 7.35-7.39 (t, *J* = 8 Hz, 1H), 7.56-7.58 (m, 3H), 7.70-7.73 (dd, *J* = 2.4 Hz, *J* = 2 Hz, 1H), 7.96-7.98 (d, *J* = 8.8 Hz 1H); ^{13}C NMR (DMSO-d_6): δ (ppm), 23.55, 32.09, 42.45, 54.96, 57.02, 112.41, 113.53, 118.05, 119.56, 119.87, 120.59, 124.48, 127.67, 127.87, 128.04, 128.17, 128.58, 128.70, 129.77, 129.87, 130.53, 130.88, 137.47, 139.61, 139.99, 144.28, 144.88, 158.18, 158.86, 159.44; Exact Mass: 490.16; Found ESI-MS m/z: 494.0 [M+4].

2-Amino-10-chloro-4-(2-chlorophenyl)-12-phenyl-1,4,5,6-tetrahydrobenzo[*j*][1,7] phenanthroline-3-carbonitrile (PD-9): Orange solid; M.F: $C_{29}H_{20}Cl_2N_4$; Yield 75 %; M.P: 210-212^{0}C; FT-IR (KBr) ν_{max} (cm^{-1}): 3446 ($-NH_2$), 3322 (-NH sym), 2191 (-C=N); ^{1}H NMR (DMSO-d_6): δ (ppm), 2.13-2.21 (m, 1H, $-CH_2$), 2.37-2.43 (m, 1H, $-CH_2$), 2.97-3.06 (m, 1H, $-CH_2$), 3.13-3.20 (m, 1H, $-CH_2$), 3.38 (s, 1H, -CH), 4.69 (s, 1H, -NH), 5.00 (s, 2H $-NH_2$), 7.29-7.29 (d, *J* = 2 Hz, 1H), 7.32-7.36 (d, *J* = 6.8 Hz, 3H), 7.40-7.40 (d, *J* = 1.2 Hz, 1H), 7.44 (s, 1H), 7.46-7.46 (d, *J* = 1.6 Hz, 1H), 7.55- 7.59 (m, 3H), 7.72-7.74 (dd, *J* = 2.4 Hz, *J* = 2.4 Hz, 1H), 7.97-7.99 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (DMSO-d_6): δ (ppm), 23.40, 31.95, 55.59, 116.71, 119.20, 120.46, 124.52, 127.70, 2×127.86, 128.04, 128.13, 128.70, 129.18, 129.73, 3×129.87, 130.55, 2×130.82, 130.93, 132.48, 137.36, 139.78, 140.52, 144.32, 158.61, 158.82; Exact Mass: 494.11; Found ESI-MS m/z: 496.1 [M+2].

2-Amino-10-chloro-4-(4-chlorophenyl)-12-phenyl-1,4,5,6-tetrahydrobenzo[*j*][1,7] phenanthroline-3-carbonitrile (PD-10): White solid; M.F: $C_{29}H_{20}Cl_2N_4$; Yield 71 %; M.P: 255-257^{0}C; FT-IR (KBr) ν_{max} (cm^{-1}): 3442 ($-NH_2$), 3315 (-NH sym), 2205 (-C=N); ^{1}H NMR (DMSO-d6): δ (ppm), 1.95-2.03 (m, 1H, $-CH_2$), 2.33-2.41 (m, 1H, $-CH_2$), 2.51 (s, 2H, $-NH_2$), 2.89-2.96 (m, 1H, $-CH_2$), 3.08-3.15 (m, 1H, $-CH_2$) , 4.69 (s, 1H, -CH), 5.00 (s, 2H $-NH_2$), 7.23-7.23 (d, *J* = 2 Hz, 1H), 7.29- 7.33 (m, 2H), 7.36-7.38 (d, *J* = 6.8 Hz, 2H). 7.44 (s, 1H), 7.45-7.45 (s, 1H), 7.46 (d, *J* = 1.6 Hz, 1H), 7.54-7.62 (m, 1H), 7.72-7.74 (dd, *J* = 2.4 Hz, *J* = 2.4 Hz, 1H), 7.97- 7.99 (d, *J* = 8.8 Hz, 1H); ^{13}C NMR (DMSO-d_6): δ (ppm), 23.89, 32.68, 42.59, 59.59, 117.12, 119.08, 120.38, 125.43,

2×127.57, 128.00, 128.18, 128.41, 3×129.05, 129.18, 129.27, 130.18, 130.46, 132.36, 133.60, 138.55, 140.50, 140.93, 141.04, 144.98, 158.07, 158.24; Exact Mass: 494.11; Found ESI-MS m/z: 496.1 [M+2].

BIOLOGICAL EVALUATION

GLUCOSE DIFFUSION INHIBITORY TEST:

Sample preparation: Four different concentrations (100, 200, 300, 400 µg/ml) of 10-chloro-4,12-diphenyl-5,6-dihydropyrimido[4,5-*a*]acridin-2-amines were prepared. About 1 ml of the sample was placed in a dialysis membrane (12000 MW, Himedia laboratories, Mumbai) along with a glucose solution (0.22 mM in 0.15 M NaCl). Then it was tied at both ends and immersed in a beaker containing 40 ml of 0.15 M NaCl and 10 ml of distilled water. The control contained 1 ml of 0.15 M NaCl containing 0.22 mM glucose solution and 1 ml of distilled water. The beaker was then placed in an orbital shaker. The external solution was monitored every half an hour up to 3 h. Three replications of this test were performed. The results are summarized in Table 2 and 3. The diffused glucose concentration was given in Table 2 and percentage of relative movement was given in Table 3.

Scheme 1: Synthesis of novel 5,6-dihydroacridine derivatives (PD-1 to PD-10)

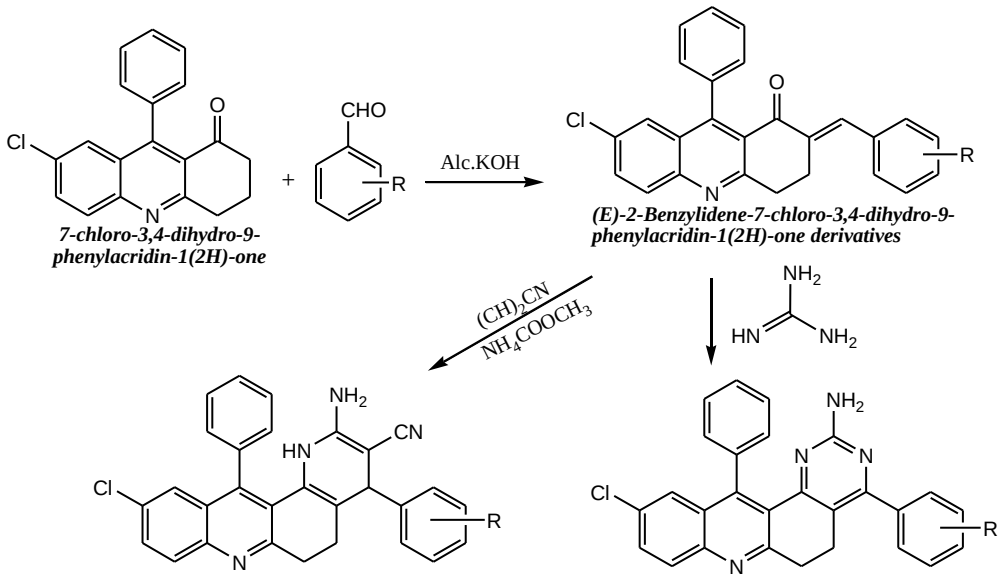

Table 1: Physical data of novel 5,6-dihydroacridine derivatives (PD-1 to PD-10)

Comp no	R	M.F	M.W	M.P (^{0}C)	Yield (%)
PD-1	3,4-dimethoxy	$C_{29}H_{23}ClN_4O_2$	494.97	218-220	80
PD-2	2,5-dimethoxy	$C_{29}H_{23}ClN_4O_2$	494.97	212-214	76
PD-3	3-methoxy	$C_{28}H_{21}ClN_4O$	464.95	168-170	70
PD-4	2-chloro	$C_{27}H_{18}Cl_2N_4$	469.36	238-240	70
PD-5	4-chloro	$C_{27}H_{18}Cl_2N_4$	469.36	210-212	72
PD-6	3,4-dimethoxy	$C_{31}H_{25}ClN_4O_2$	521.01	230-232	80
PD-7	2,5-dimethoxy	$C_{31}H_{25}ClN_4O_2$	521.01	238-240	76
PD-8	3-methoxy	$C_{30}H_{23}ClN_4O$	490.98	140-142	80
PD-9	2-chloro	$C_{29}H_{20}Cl_2N_4$	495.40	210-212	75
PD-10	4-chloro	$C_{29}H_{20}Cl_2N_4$	495.40	255-257	71

Table 2: Release of glucose through dialysis membrane to external solution (mg/dl)

	Time					
Compd	**30min**	**60 min**	**90 min**	**120 min**	**150 min**	**180 min**
PD-1	1.661±0.00	1.831±0.00	1.831±0.01	2.162±0.03	2.511±0.04	2.830±0.00
PD-2	1.830±0.00	2±0.01	2.160±0.00	2.515±0.00	2.831±0.08	2.671±0.00
PD-3	1.5±0.00	1.83±0.01	2.16±0.01	2.5±0.00	2.66±0.00	2.831±0.06
PD-4	2±0.00	2.16±0.00	2.332±0.07	2.5±0.01	2.671±0.07	2.83±0.03
PD-5	1.830±0.00	2±0.01	1.831±0.01	2±0.00	1.662±0.00	1.331±0.00
Control	1.833±0.00	2.670±0.03	2.500±0.00	2.667±0.01	2.833±0.07	2.833±0.07

Values are mean ± SEM for groups of 3 observations; *P < 0.01; **P < 0.05

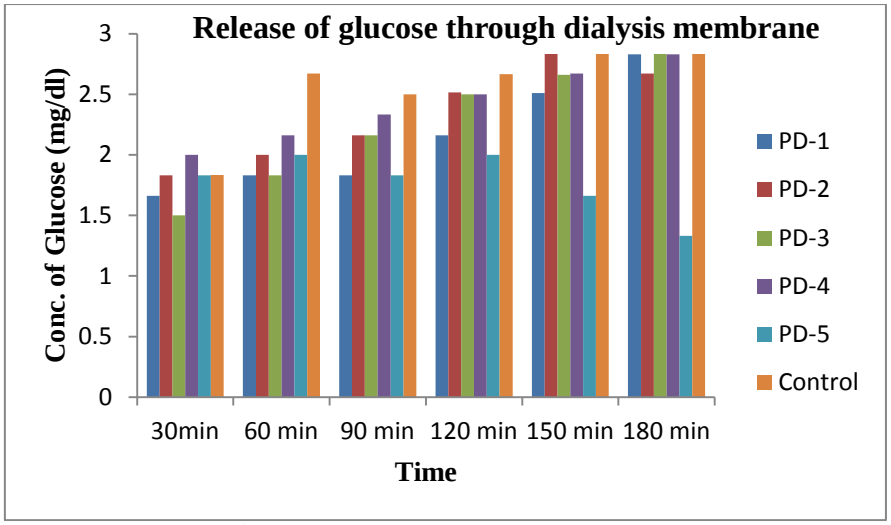

Figure 1: Release of glucose through dialysis membrane to external solution (mg/dl)

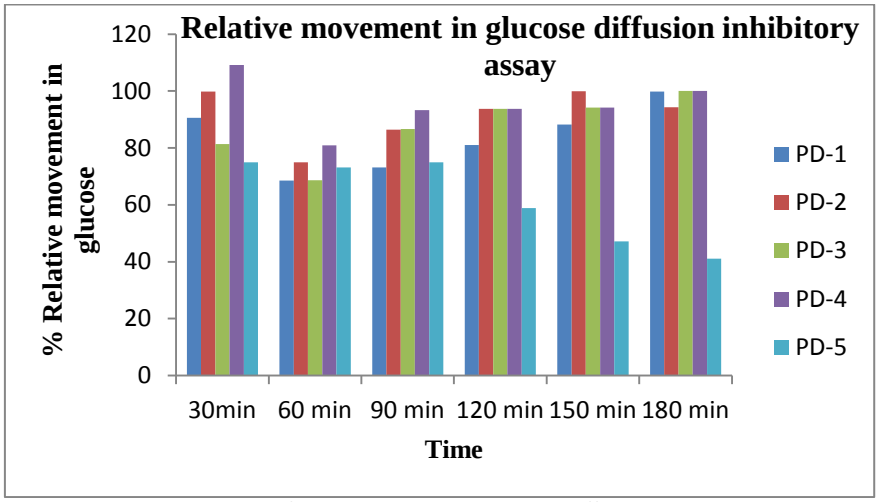

Figure 2: Percentage of relative movement in glucose diffusion inhibitory assay

Table 3: Percentage of relative movement in glucose diffusion inhibitory assay

Compd	Time					
	30min	**60 min**	**90 min**	**120 min**	**150 min**	**180 min**
PD-1	90.56±0.03	68.53±0.00	73.20±0.03	80.99±0.05	88.24±0.06	99.84±0.07
PD-2	99.83±0.00	74.91±0.07	86.40±0.06	93.74±0.05	99.89±0.03	94.24±0.02
PD-3	81.33±0.00	68.66±0.01	86.67±0.02	93.74±0.05	94.13±0.06	100±0.07
PD-4	109.11±0.01	80.90±0.02	93.33±0.03	93.74±0.07	94.13±0.08	100±0.06
PD-5	74.90±0.00	73.20±0.02	74.99±0.03	58.83±0.05	47.18±0.07	41.06±0.06

Values are mean ± SEM for groups of 3 observations; *P < 0.01; **P < 0.05

α-Amylase inhibitory activity (DNSA method): Four different concentrations (100, 200, 300, 400 µg/ml) of 10-chloro-4,12-diphenyl-5,6-dihydro pyrimido[4,5-*a*]acridin-2-amines were prepared and made up to 1 ml with DMSO. A total of 500 µl of sample and 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing α-amylase solution (0.5 mg/ml) were incubated for 10 minutes, at 25 ^{0}C. After pre-incubation, 500 µl of 1 % starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to each tube. This reaction mixture was then incubated for 10 minutes at 25 ^{0}C. About 1 ml of DNSA colour reagent was added to stop the reactions. These test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. Finally this reaction mixture was again diluted by adding 10 ml of distilled water. Percentage inhibition by α-amylase can be calculated using the following formula.

Percentage inhibition = [(Ac-As)/Ac] × 100

Where, Ac = absorbance of control, As = absorbance of sample.

Absorbance was measured at 540 nm. Triplicates were done for each sample at different concentrations. The results are given in Table 4

α-Glucosidase inhibitory activity: Various concentrations of 10-Chloro-4,12-diphenyl-5,6-dihydropyrimido[4,5-*a*]acridin-2-amines were prepared. α-glucosidase (0.075 units) was premixed with compounds. About 3 mM *p*-nitrophenyl glucopyranoside used as a substrate was added to the reaction mixture to start the reaction. The reaction was incubated at 37^0C for 30 min and stopped by adding 2 ml of Na_2CO_3. The α-glucosidase activity was measured by *p*-nitrophenol release from PNPG at 400 nm. Percentage inhibition can be calculated by using the following formula.

Percentage inhibition = [(Ac-As)/Ac] × 100
Where, Ac = absorbance of control, As = absorbance of sample.

Triplicates are done for each sample at different concentrations. The results are given in Table 5.

Table 4: Percentage inhibition of α-amylase assay

Compd	Concentration (µg/ml)			
	100	**200**	**300**	**400**
PD-1	5.43±0.06	5.43±0.02	6.52±0.04	6.52±0.04
PD-2	3.26±0.00	4.34±0.01	5.070±0.08	6.16±0.06
PD-3	18.83±0.02	20.65±0.03	21.73±0.01	23.55±0.01
PD-4	12.23±0.00	13.77±0.01	14.85±0.02	16.66±0.03
PD-5	19.20±0.01	21.37±0.00	53.62±0.03**	57.96±0.04
Acarbose	32.01±0.09	48.15±0.11	70.03±0.25	80.02±0.71

Values are mean ± SEM for groups of 3 observations; *P < 0.01; **P < 0.05

Table 5: Percentage inhibition of α-glucosidase assay

Compd	Concentration (µg/ml)			
	100	**200**	**300**	**400**
PD-1	6.29±0.00	7.31±0.02	8.01±0.03	8.25±0.06
PD-2	4.29±0.01	5.02±0.01	6.09±0.02	9.19±0.02
PD-3	20.91±0.00	21.65±0.01	22.71±0.02	23.01±0.04
PD-4	14.02±0.02	10.77±0.03	15.85±0.04	17.71±0.03
PD-5	21.45±0.01*	24.62±0.09	56.85±0.01	60.25±0.01**
Acarbose	30.08±0.05	45.02±0.12	68.25±0.21	78.01±0.07

Table 6: Percentage inhibition for DPPH radical scavenging

Compd	Concentration (mM)				
	0.02	**0.04**	**0.06**	**0.08**	**0.10**
PD-6	00	18	50	75	80
PD-7	28	30	75	79	88
PD-8	18	27	30	40	55
PD-9	00	16	48	35	45
PD-10	10	15	36	38	44
Ascorbic acid	20	35	70	80	95

IN-VITRO ANTI-OXIDANT ACTIVITY:

The antioxidant activity of 2-Amino-10-choloro-1,4,5,6-tetrahydro-4-(3,4-dimethoxy phenyl)-12-phenylbenzo[*j*][1,7]phenanthroline-3-carbonitriles were determined by different *in vitro* methods such as, DPPH free radical scavenging assay, hydrogen peroxide scavenging activity and hydroxyl free radical scavenging methods. All the assays were carried out in triplicate and average values were considered.

DPPH free radical scavenging activity (Gollapalli Naga Raju, 2015):

Antioxidant assay was based on the measurements of scavenging ability of compounds towards the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). About 1 ml of the sample solutions containing different concentrations were mixed with 3 ml of 0.1mM solution of DPPH. The mixture was kept in dark for 30 minutes. The absorbance was measured after incubation at 517 nm, against a blank of ethanol without DPPH. The control solution consisted of a mixture of 1 ml ethanol and DPPH. Ascorbic acid was used as a standard. Results were expressed as percentage of inhibition of the DPPH radical. Percentage of inhibition of the DPPH radical was calculated according to the following equation (1) and recorded in Table 6.

Percentage Inhibition = $[A_C - A_S/A_C] \times 100$ % (1)
Where, A_C - Absorbance of Control, A_S - Absorbance of Sample.

Hydrogen Peroxide scavenging activity:

The principle of this method is that there is a decrease in absorbance of H_2O_2 upon oxidation. A solution of 43 mM H_2O_2 was prepared in 0.1 M phosphate buffer (pH 7.4). Different concentration of 2-amino-10-choloro-1,4,5,6-tetrahydro-4-(3,4-dimethoxyphenyl)-12-phenylbenzo[*j*][1,7] phenanthroline-3-carbonitriles were mixed well with 3.4 ml phosphate buffer, 0.6 ml of H_2O_2 solution (43 mM). Absorbance of the reaction mixture was recorded at 230 nm. A blank solution contained the sodium phosphate buffer without H_2O_2 were recorded. Ascorbic acid was used as standard. Further the inhibition was calculated using above equation (1) and recorded in Table 7.

Table 7: Percentage inhibition for Hydrogen Peroxide scavenging activity

Compd	Concentration (mM)				
	0.02	**0.04**	**0.06**	**0.08**	**0.10**
PD-6	20	30	50	65	75
PD-7	17	38	68	85	85
PD-8	18	20	35	65	65
PD-9	17	28	32	50	50
PD-10	10	18	30	48	48
Ascorbic acid	38	68	78	96	96

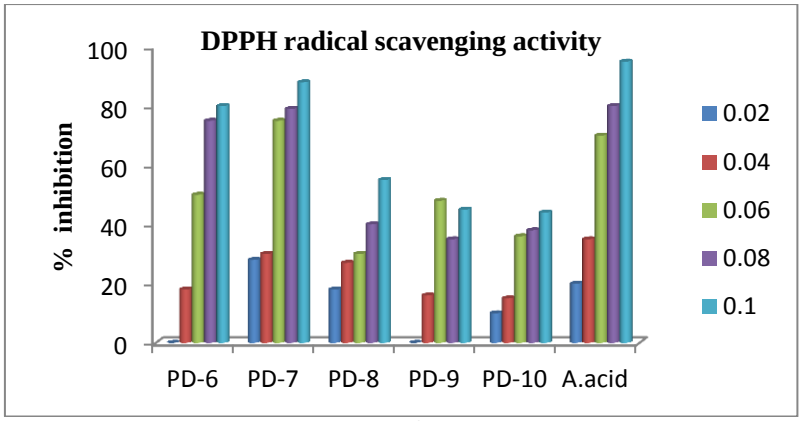

Figure 3: Percentage inhibition for DPPH radical scavenging

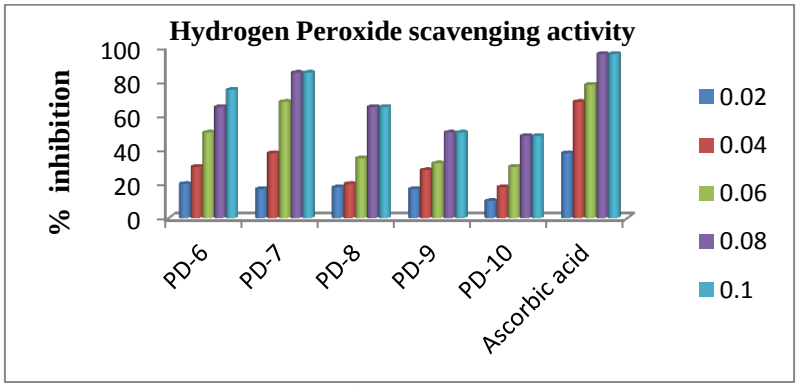

Figure 4: Percentage inhibition for Hydrogen Peroxide scavenging activity

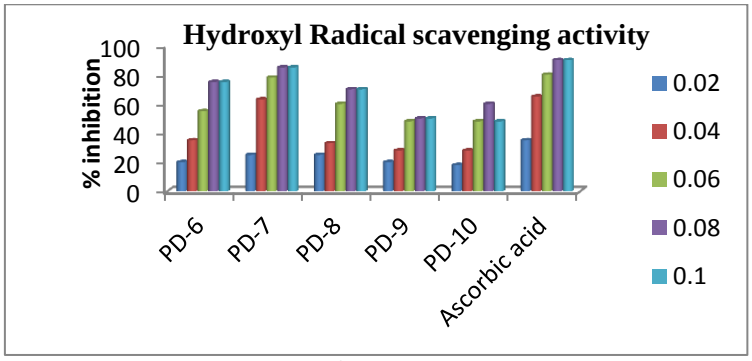

Figure 5: Percentage inhibition for Hydroxyl Radical scavenging activity

Hydroxyl Radical scavenging activity:

About 1 ml of iron – EDTA (0.13 % ferrous Ammonium Sulphate and 0.26 % EDTA), 0.5 ml of 0.018 % EDTA, 1 ml of DMSO, (0.85 % v/v in 0.1 M phosphate buffer, pH 7.4) and 0.5 ml 0.22 % ascorbic acid were added to each tube. The tube were capped tightly and heated in water bath at 80-90 ^{0}C for 15 min. The reaction was terminated by adding 1 ml of ice cold TCA (17.5 % w/v), 3 ml of Nash reagent (75 g of ammonium acetate, 3 ml of glacial acetic acid and 2 ml of acetyl acetone were mixed and distilled water was added to make up total volume of 1 litre) were added to each tube, which were left at RT for 15 min for colour development. The absorbance of the control and test compounds was noted. The percentage inhibition was calculated as per above equation (1) and recorded in Table 8

Table 8: Percentage inhibition for Hydroxyl Radical scavenging activity

Compd	Concentration (mM)				
	0.02	**0.04**	**0.06**	**0.08**	**0.10**
PD-6	20	35	55	75	75
PD-7	25	63	78	85	85
PD-8	25	33	60	70	70
PD-9	20	28	48	50	50
PD-10	18	28	48	60	48
Ascorbic acid	35	65	80	90	90

REULTS AND DISCUSSION

All the chemicals and reagents provided by Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur. All the chemicals are purchased in National Scientific Products (Guntur). All the chemicals used were of laboratory grade and procured from E. Merck (Germany) and S.D. Fine Chemicals (India). All chemicals and solvents used for the synthesis were of analytical reagent grade. In this work 10-Chloro-5,6-dihydro-12-phenyl pyrimido[4,5-a]acridin-2-amine derivatives (PD-1 to PD-5) and 2-Amino-10-chloro-1,4,5,6-tetrahydro -12-phenylbenzo-[1,7]phenanthroline derivatives (PD-6 to PD-10) were synthesized from *(E)*-2-Benzylidene-7-chloro-3,4-dihydro-9-phenylacridin-1(2H)-one with guanidine carbonate and malononitrile. Melting points were determined by open capillary method and were uncorrected. All the synthesized compounds have been characterized by using elemental analysis, FT-IR, ^{1}H NMR, ^{13}C NMR spectroscopy and further supported by mass spectroscopy. 10-Chloro-5,6-dihydro-12-phenylpyrimido[4,5-a]acridin-2-amine derivatives (PD-1 to PD-5) were evaluated for anti-diabetic activity. The diffused glucose concentration was given in Table 2 and percentage of relative movement was given in Table 3. The movement of glucose from inside of membrane to external solution monitored and compared in Table 4. All the samples showed highest percentage of relative movement in glucose diffusion from 30-180 min. 2-Amino-10-chloro-1,4,5,6-tetrahydro-12-phenylbenzo-[1,7]phenanthroline derivatives (PD-6 to PD-10) were evaluated for anti-oxidant activity based on the measurements of scavenging ability of compounds towards the stable DPPH. Percentage inhibition was plotted against various concentrations 0.01, 0.02, 0.04, 0.06, 0.08, and 0.1 mM. A lower IC_{50} value of compounds, PD-6 and PD-7 indicates high in antioxidant activity. In compound, PD-7 having two methoxy functional groups are para to each other, showed good antioxidant activity. H_2O_2 scavenging activity was performed with 2-Amino-10-chloro-1,4,5,6-tetrahydro-12-phenylbenzo-[1,7]phenanthroline derivatives (PD-6 to PD-10) and ascorbic acid used as a standard drug. The IC_{50} value for all compoundswas summarized in Table Band graphically represented in Fig 5. From results, it was found that the compound, PD-7 showed potent free radical scavenging activity compared to all other derivatives. These 1,4-dihydropyridines are electron donors due to the presence of electron donating substituent groups like $-OCH_3$, $-NH_2$ at different positions of acridine scaffold.

CONCLUSION

In this study, few of novel 10-Chloro-4,12-diphenyl -5,6-dihydropyrimido[4,5-*a*]acridin-2-amine derivatives and 2-Amino-10-chloro-1,4,5,6-tetrahydro-4-(3,4-dimethoxyphenyl)-12-phenylbenzo[*j*][1,7]phenanthroline-3-carbonitrile derivatives were synthesized. All compounds were confirmed by spectroscopic techniques. 10-Chloro-4,12-diphenyl-5,6-dihydropyrimido[4,5-*a*]acridin-2-amine derivatives were evaluated *in-vitro* α-amylase and α-glucosidase inhibitory activity and glucose diffusion test of samples. Among all the other derivatives, compound 10-chloro-4-(4-chlorophenyl)-12-phenyl-5,6-dihydropyrimido[4,5-*a*]acridin-2-amine (PD-5) and 10-chloro-4-(3-methoxyphenyl)-12-phenyl-5,6-dihydropyrimido [4,5-*a*]acridin-2-amine (PD-3) shows good inhibitory activity for α-amylase and α-glucosidase with the value of 57.96, 60.27, 23.55 and 23.01 % compounds, (PD-5) and (PD-3) respectively. 2-Amino-10-chloro-1,4,5,6-tetrahydro-4-(3,4-dimethoxyphenyl)-12-phenylbenzo[*j*][1,7] phenanthroline-3-carbonitrile derivatives were evaluated for *in vitro* antioxidant activity. Radical scavenging activity results that compound (PD-2) shows better results compare with all other derivatives.

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